Safety Assessment of Transgenic Organisms

OECD CONSENSUS DOCUMENTS

Volume 1

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ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

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The OECD is a unique forum where the governments of 30 democracies work together to address the economic, social and environmental challenges of globalisation. The OECD is also at the forefront of efforts to understand and to help governments respond to new developments and concerns, such as corporate governance, the information economy and the challenges of an ageing population. The Organisation provides a setting where governments can compare policy experiences, seek answers to common problems, identify good practice and work to co-ordinate domestic and international policies.

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This book is published on the responsibility of the Working Group on Harmonisation of Regulatory Oversight in Biotechnology, which is a subsidiary group of the Chemicals Committee and Working Party on Chemicals, Pesticide and Biotechnology of the OECD.

FOREWORD

Genetically engineered crops (also known as transgenic crops) such as maize, soybean, rapeseed and cotton have been approved for commercial use in an increasing number of countries. During the period from 1996 to 2005, for example, there was more than fifty-fold increase in the area grown with transgenic crops worldwide, reaching 90 million hectares in 2005.[1] Such approvals usually follow a science-based risk/safety assessment.

The environmental safety/risks of transgenic organisms are normally based on the information on the characteristics of the host organism, the introduced traits, the environment into which the organism is introduced, the interaction between these, and the intended application. The OECD's Working Group on Harmonisation of Regulatory Oversight in Biotechnology decided at its first session, in June 1995, to focus its work on identifying parts of this information, which could be commonly used in countries for environmental safety/risk assessment to encourage information sharing and prevent duplication of effort among countries. Biosafety Consensus Documents are one of the major outputs of its work.

Biosafety Consensus Documents are intended to be a "snapshot" of current information on a specific host organism or trait, for use during regulatory assessments. They are not intended to be a comprehensive source of information on everything that is known about a specific host or trait; but they do address the key or core set of issues that member countries believe are relevant to risk/safety assessment. This information is said to be mutually acceptable among member countries. To date, 25 Biosafety Consensus Documents have been published. They include documents which address the biology of crops, trees and micro-organisms as well as those which address specific traits which are used in transgenic crops.

This book is a compilation of those Biosafety Consensus Documents published before February 2006. It also includes two recently published texts: the first, entitled *An Introduction to the Biosafety Consensus Document of OECD's Working Group for Harmonisation in Biotechnology*, explains the purpose of the consensus documents and how they are relevant to risk/safety assessment. It also describes the process by which the documents are drafted using a "lead country" approach. The second text is a *Points to Consider for Consensus Documents on the Biology of Cultivated Plants.* This is a structured checklist of "points to consider" for authors when drafting or for those evaluating a consensus document. Amongst other things, this text describes how each point is relevant to risk/safety assessment.

This book offers ready access to those consensus documents which have been published thus far. As such, it should be of value to applicants for commercial uses of transgenic crops, regulators in national authorities as well as the wider scientific community. As each of the documents may be updated in the future as new knowledge becomes available, users of this book are encouraged to provide any information or opinions regarding the contents of this book or indeed, OECD's other harmonisation activities. If needed, a short pre-addressed questionnaire is attached at the end of this book that can be used to provide such comments.

The published Consensus Documents are also available individually from OECD's website (http://www.oecd.org/biotrack) at no cost.

1 . Clive James (2005), International Service for the Acquisition of Agri-biotech Applications (http://www.isaaa.org/)

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INTRODUCTION TO THE BIOSAFETY CONSENSUS DOCUMENTS

1. About OECD's Working Group

OECD's Working Group comprises delegates from the 30 Member countries of OECD and the European Commission. Typically, delegates are from those government ministries and agencies, which have responsibility for the environmental risk/safety assessment of products of modern biotechnology. The Working Group also includes a number of observer delegations and invited experts who participate in its work. They include: Argentina; Russia; Slovenia; the United Nations Environment Programme (UNEP); the Secretariat of the Convention on Biological Diversity (SCBD); the United Nations Industrial Development Organisation (UNIDO); and the Business and Industry Advisory Committee to OECD (BIAC).

2. Regulatory Harmonisation

The Working Group was established in 1995[2] at a time when the first commercial transgenic crops were being considered for regulatory approval in a number of OECD Member countries. From the beginning, one of its primary goals was to promote international regulatory harmonisation in biotechnology among member countries. Regulatory harmonisation is the attempt to ensure that the information used in risk/safety assessments, as well as the methods used to collect such information, are as similar as possible. It could lead to countries recognising or even accepting information from one anothers' assessments. The benefits of harmonisation are clear. It increases mutual understanding among member countries, which avoids duplication, saves on scarce resources and increases the efficiency of the risk/safety assessment process. This in turn improves safety, while reducing unnecessary barriers to trade (OECD 2000). Many delegates have said that the process of working towards harmonisation, and the resulting discussions among member countries, is almost as important as the products produced.

3. The Need for Harmonisation Activities at OECD

The establishment of the Working Group and its programme of work followed a detailed analysis by member countries of whether there was a need to continue work on harmonisation in biotechnology at OECD, and if so, what that work should entail. This analysis was undertaken by the Ad Hoc Group for Environmental Aspects of Biotechnology (established by the Joint Meeting[3]), which was active, mainly during 1994.

The Ad Hoc Group took into consideration, and built upon, the earlier work at OECD, which began in the mid-1980s. Initially, these previous activities at OECD concentrated on the environmental and agricultural implications of field trials of transgenic organisms, but this was soon followed by a consideration of their large-scale use and commercialisation. (A summary of this extensive body of work is found in Annex I.)

4. Key Background Concepts and Principles

The Ad Hoc Group took into account (amongst other things) previous work on risk analysis that is summarised in *Safety Considerations for Biotechnology: Scale-up of Crop Plants* (OECD 1993a). The following quote gives the flavour: "*Risk/safety analysis is based on the characteristics of the organism, the*

2 . The original title of the Working Group was the Expert Group for the Harmonisation of Regulatory Oversight in Biotechnology. It became an OECD Working Group in 1998.

3 . The Joint Meeting was the supervisory body of the Ad Hoc Group and, as a result of its findings, established the Working Group as a subsidiary body. Today, its full title is the Joint Meeting of the Chemicals Committee and the Working Party on Chemical, Pesticides and Biotechnology.

introduced trait, the environment into which the organism is introduced, the interaction between these, and the intended application." This body of work has formed the basis for environmental risk/safety assessment that is now globally accepted. So in considering the possibilities for harmonisation, the attention of the Ad Hoc Group was drawn to these characteristics and the information used by risk/safety assessors to address them.

This was reinforced by the concept of familiarity, which is also elaborated in the "Scale-up" document (OECD 1993a). This concept "…is based on the fact that most genetically engineered organisms are developed from organisms such as crop plants whose biology is well understood". "Familiarity allows the risk assessor to draw on previous knowledge and experience with the introduction of plants and micro-organisms into the environment..." For plants, familiarity takes account of a wide-range of attributes including, for example, knowledge and experience with "the crop plant, including its flowering/reproductive characteristics, ecological requirements, and past breeding experiences" (OECD 1993a – see also Annex I for a more detailed description). This illustrates the role of information related to the biology of the host organism as a part of an environmental risk/safety assessment.

The Ad Hoc Group also took into account the document "Traditional Crop Breeding Practices: An Historical Review to Serve as a Baseline for Assessing the Role of Modern Biotechnology" (OECD 1993b) which also focuses on host organisms. It presents information on 17 different crop plants, which are used (or are likely to be used) in modern biotechnology. It includes sections on phytosanitary considerations in the movement of germplasm and on current uses of these crop plants. There is also a detailed section on current breeding practices.

5. A Common Approach to Risk/Safety Assessment

An important additional point for the Ad Hoc Group was to identify the extent to which member countries address the same questions and issues during risk/safety assessment. If there are big differences it would mean that attempts to work towards harmonisation would be difficult. On the other hand, a high level of similarity would suggest that harmonisation efforts would be more feasible.

This point was resolved by two studies, which the Ad Hoc Group was able to consider. The first covered crop plants (OECD 1995a, 1995b) while the second concerned micro-organisms (OECD 1995c, 1996). Both studies involved a survey targeted at those national authorities that are responsible for risk/safety assessment. The aim was to identify the questions which are addressed by them during the assessment process (as outlined in national laws/regulations/guidance documents) in order to establish the extent of similarity among national authorities. Both these studies used the information provided in OECD's *"Blue Book"* (OECD 1986) as a reference point, in particular, the sections of the book (appendices b, c and d) which cover: i) General Scientific Considerations; ii) Human Health Considerations; and iii) Environmental and Agricultural Considerations. Both studies identified a remarkably high degree of similarity among member countries in the questions/issues addressed in risk/safety assessment.

6. The Emergence of the Concept of Consensus Documents

So the Working Group was established in the knowledge that national authorities have much in common, in terms of the questions/issues addressed, when undertaking risk/safety assessment. It also took into account those characteristics identified as part of risk/safety assessment (*i.e. the organism*, *the introduced trait* and *the environment*) around which harmonisation activities could focus.

It was further recognised that much of the information used in risk/safety assessment that relates to the biology of organisms (both crop plants and micro-organisms) would be similar or virtually the same in

all assessments involving the same organism. In other words, the questions addressed during risk/safety assessment which relate to the biology of the host organism - for example, the potential for gene transfer within the crop plant species, and among related species, as well as the potential for weediness – remain the same for each application involving the same host species. This also applies to some extent to information related to introduced traits.

Consequently, the Working Group evolved the idea of compiling information common to the risk/safety assessment of a number of transgenic products, and decided to focus on two specific categories: the biology of the host species or crop; and traits used in genetic modifications. The aim of this compilation was to encourage information sharing and prevent duplication of effort among countries by avoiding the need to address the same common issues in each application involving the same organism or trait. It was recognized that biology and trait consensus documents could be agreed upon quickly by the member countries (within one or two years). This compilation process was quickly formalised in the drafting of Consensus Documents.

7. The Purpose of Consensus Documents

The Consensus Documents are not intended to be a substitute for a risk/safety assessment, because they address only a part of the necessary information. Nevertheless, they should make an important contribution to environmental risk/safety assessment.

As originally stated by the Working Group, Consensus Documents are intended to be a "snapshot" of current information, for use during the regulatory assessment of products of biotechnology. They are not intended to be a comprehensive source of information on everything that is known about a specific host organism or trait; but address – on a consensus basis – the key or core set of issues that member countries believe are relevant to risk/safety assessment.

The aim of the documents is to share information on these key components of an environmental safety review in order to prevent duplication of effort among countries. The documents were envisaged as being used: a) by applicants as information in applications to regulatory authorities; b) by regulators as a general guide and reference source in their reviews; and c) by governments for information sharing, research reference and public information.

Originally, it was said that the information in the Consensus Documents is intended to be *mutually recognised* or *mutually acceptable* among OECD Member countries, though the precise meaning of these terms, in practice, is still open for discussion. During the period of the Ad Hoc Group and the early days of the Working Group (1993-1995), the phrase *Mutual Acceptance of Data* was discussed. This is a concept borrowed from OECD's Chemicals Programme which involves a system of OECD Council Decisions that have legally binding implications for member countries. In the case of the Consensus Documents there has never been any legally binding commitment to use the information in the documents, though from time to time, the Working Group has discussed whether and how to increase the level of commitment member countries are willing to make in using the information in the documents. Participation in the development of documents, and the intention by member countries to use the information, is done in "good faith." It is expected, therefore, that reference will be made to relevant consensus documents during risk/safety assessments.

8. The Process through which Consensus Documents are Initiated and Brought to Publication

There are a number of steps in the drafting of a specific consensus documents. The first step occurs when a delegation, in a formal meeting of the Working Group, makes a proposal to draft a document on a new topic, typically a crop species or a trait. If the Working Group agrees to the proposal, a provisional

draft is prepared by either a single country or two or more countries working together. This is often called the "lead country approach". Typically, the lead country(ies) has had experience with the crop or trait which is the subject of the new document and is able to draw on experts to prepare a provisional draft.

The provisional draft is first reviewed by the Bureau of the Working Group[4] to ensure that the document addresses range of issues normally covered by Consensus Documents and is of sufficiently high quality to merit consideration by the Working Group as a whole.

Based on the comments of the Bureau, a first draft is then prepared for consideration by the full Working Group. This is the opportunity for each delegation to review the text and provide comments based on their national experiences. The incorporation of these comments leads to a second draft, which is again circulated for review and comment to the Working Group. At this point, the Working Group may be asked to recommend that the document be declassified. Such a recommendation is only forthcoming when all delegations have come to a consensus that the document is complete and ready for publication. Sometimes, however, the text may need a third or even a fourth discussion in the Working Group before a recommendation for declassification is possible.

When the Working Group has agreed that a document can be recommended for declassification, it is forwarded to the supervisory Committee, the Joint Meeting, which is invited to declassify the document. Following the agreement of the Joint Meeting, the document is then published.

It is important to note that the review of Consensus Documents is not limited to formal meetings of the Working Group. Much discussion also occurs through electronic means, especially via the Working Group's Electronic Discussion Group (EDG). This enables a range of experts to have input into drafts.

For a number of documents, it has also been important to include information from non-member countries. This has been particularly true in the case of crop plants where the centre of origin and diversity occurs in a non-member country(ies). In these cases, UNEP and UNIDO have assisted in the preparation of documents by identifying experts from countries which include the centres of origin and diversity. For example, this occurred with the Consensus Document on the Biology of Rice.

9. Current and Future Trends in the Working Group

The Working Group continues its work, not only on the preparation of specific Consensus Documents, but also on the efficiency of the process by which they are developed. At the present time, an increasingly large number of crops and other host species are being modified, for increasing number of traits.

At the OECD Workshop on Consensus Documents and Future Work in Harmonisation, which was held in Washington DC, 21-23 October 2003, the Working Group was able to consider, amongst other things, how to set priorities for drafting future Consensus Documents among the large number of possibilities. The Working Group is currently considering how best to set priorities in the future.

The Workshop also recognised that published Consensus Documents may be in need of review and updating from time to time, to ensure that they include the most recent information. The Working Group is currently considering how best to organise this in the future.

4 . The Bureau comprises the Chair and vice-Chairs of the Working Group. The Bureau is elected by the Working Group once per year. At the time of writing, the Chair is from Austria and the vice-Chairs are from Canada, Japan the Netherlands and the United States.

For the future drafting of new and updated documents, the Workshop identified the usefulness of developing a standardised structure of Consensus Documents, which is called “Points to Consider”. The Working Group is expected to develop, firstly, a Points to Consider document for the biology Consensus Documents and then that of the trait Consensus Documents.

The Workshop also recognised the importance strengthening the input of non-member countries into the future development of Consensus Documents. Once again, the Working Group is considering how best to implement this recommendation.

APPENDIX I

OECD Biosafety Principles and Concepts Developed Prior to the Working Group 1986-1994

Since the mid-1980s the OECD has been developing harmonised approaches to the risk/safety assessment of products of modern biotechnology. Prior to the establishment of the Working Group, OECD published a number of reports on safety considerations, concepts and principles for risk/safety assessment as well as information on field releases of transgenic crops, and a consideration of traditional crop breeding practices. This Annex notes some of the highlights of these achievements that were background considerations in the establishment of the Working Group and its development of Consensus Documents.

Underlying scientific principles

In 1986, OECD published its first safety considerations for genetically engineered organisms (OECD 1986). These included the issues (relevant to human health, the environment and agriculture) that might be considered in a risk/safety assessment. In its recommendations for agricultural and environmental applications, it suggested that risk/safety assessors:

- "Use the considerable data on the environmental and human health effects of living organisms to guide risk assessments.

- Ensure that recombinant DNA organisms are evaluated for potential risk, prior to application in agriculture and the environment by means of an independent review of potential risks on a case-by-case basis.

- Conduct the development of recombinant DNA organisms for agricultural and environmental applications in a stepwise fashion, moving, where appropriate, from the laboratory to the growth chamber and greenhouse, to limited field testing and finally to large-scale field testing.

- Encourage further research to improve the prediction, evaluation, and monitoring of the outcome of applications of recombinant DNA organisms."

The role of confinement in small scale testing

In 1992, OECD published its Good Developmental Principles (GDP) (OECD 1992) for the design of small-scale field research involving GM plants and GM micro-organisms. This document, amongst other things, describes the use of *confinement* in field tests. Confinement includes measures, to avoid the dissemination or establishment of organisms from a field trial, for example, the use of physical, temporal, or biological isolation (such as the use of sterility).

Scale-up of crop-plants – "risk/safety analysis"

By 1993, the focus of attention had switched to the *scale-up* of crop plants as plant breeders began to move to larger-scale production and commercialisation of GM plants. OECD published general principles for, *scale-up* (OECD 1993a), which re-affirmed that, "*safety in biotechnology is achieved by the appropriate application of risk/safety analysis and risk management. Risk/safety analysis comprises hazard identification and, if a hazard has been identified, risk assessment. Risk/safety analysis is based on*

the characteristics of the organism, the introduced trait, the environment into which the organism is introduced, the interaction between these, and the intended application. Risk/safety analysis is conducted prior to an intended action and is typically a routine component of research, development and testing of new organisms, whether performed in a laboratory or a field setting. Risk/safety analysis is a scientific procedure which does not imply or exclude regulatory oversight or imply that every case will necessarily be reviewed by a national or other authority" (OECD 1993a).

The role of familiarity in risk/safety assessment

The issue of *scale-up* also led to an important concept, *familiarity*, which is one key approach that has been used subsequently to address the environmental safety of transgenic plants.

The concept of familiarity is based on the fact that most genetically engineered organisms are developed from organisms such as crop plants whose biology is well understood. It is not a risk/safety assessment in itself (U.S. NAS 1989). However, the concept facilitates risk/safety assessments, because to be familiar, means having enough information to be able to make a judgement of safety or risk (U.S. NAS 1989). Familiarity can also be used to indicate appropriate management practices including whether standard agricultural practices are adequate or whether other management practices are needed to manage the risk (OECD 1993a). Familiarity allows the risk assessor to draw on previous knowledge and experience with the introduction of plants and micro-organisms into the environment and this indicates appropriate management practices. As familiarity depends also on the knowledge about the environment and its interaction with introduced organisms, the risk/safety assessment in one country may not be applicable in another country. However, as field tests are performed, information will accumulate about the organisms involved, and their interactions with a number of environments.

Familiarity comes from the knowledge and experience available for conducting a risk/safety analysis prior to scale-up of any new plant line or crop cultivar in a particular environment. For plants, for example, familiarity takes account of, but need not be restricted to, knowledge and experience with:

- "The crop plant, including its flowering/reproductive characteristics, ecological requirements, and past breeding experiences.
- The agricultural and surrounding environment of the trial site.
- Specific trait(s) transferred to the plant line(s).
- Results from previous basic research including greenhouse/glasshouse and small-scale field research with the new plant line or with other plant lines having the same trait.
- The scale-up of lines of the plant crop varieties developed by more traditional techniques of plant breeding.
- The scale-up of other plant lines developed by the same technique.
- The presence of related (and sexually compatible) plants in the surrounding natural environment, and knowledge of the potential for gene transfer between crop plant and the relative.
- Interactions between/among the crop plant, environment and trait." (OECD, 1993a).

Risk/safety assessment and risk management

Risk/safety assessment involves the identification of potential environmental adverse effects or hazards, and determining, when a hazard is identified, the probability of it occurring. If a potential hazard or adverse affect is identified, measures may be taken to minimise or mitigate it. This is risk management. Absolute certainty or zero risk in a safety assessment is not achievable, so uncertainty is an inescapable aspect of all risk assessment and risk management (OECD 1993a). For example, there is uncertainty in extrapolating the results of testing in one species to identify potential effects in another. Risk assessors and risk managers thus spend considerable effort to address uncertainty. Many of the activities in intergovernmental organisations, such as the OECD, address ways to handle uncertainty (OECD 2000).

APPENDIX II

References Cited in Chronological Order

Recombinant DNA Safety Considerations. Safety considerations for industrial, agricultural and environmental applications of organisms derived by recombinant DNA techniques ("*The Blue Book*"), OECD, 1986.

Field Testing of Genetically Modified Organisms: Framework for Decisions. U.S. NAS - National Academy of Sciences, National Academy Press, Washington DC. USA 1989.

Good Developmental Principles (GDP), OECD 1992.

Safety Considerations for Biotechnology: Scale-up of Crop Plants, OECD, 1993a.

Traditional Crop Breeding Practices: An Historical Review to serve as a Baseline for Assessing the Role of Modern Biotechnology, OECD 1993b.

Commercialisation of Agricultural Products Derived through Modern Biotechnology: Survey Results, OECD 1995a.

Report of the OECD Workshop on the Commercialisation of Agricultural Products Derived through Modern Biotechnology, OECD 1995b.

Analysis of Information Elements Used in the Assessment of Certain Products of Modern Biotechnology, OECD 1995c.

Industrial Products of Modern Biotechnology Intended for Release to the Environment: The Proceedings of the Fribourg Workshop, OECD 1996.

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PRESENTATION DES DOCUMENTS DE CONSENSUS SUR LA BIOTECHNOLOGIE

A propos du Sous-groupe de l'OCDE

Le Sous-groupe comprend des délégués des 30 pays Membres de l'OCDE et de la Commission européenne. En général, les délégués sont des fonctionnaires des ministères et organismes gouvernementaux chargés de l'évaluation des risques pour l'environnement et de la sécurité des produits issus de la biotechnologie moderne. Le Sous-groupe comprend aussi plusieurs délégations et experts invités qui participent à ses travaux en qualité d'observateurs notamment l'Argentine, la Russie, la Slovénie, le Programme des Nations Unies pour l'environnent (PNUE), le Secrétariat de la Convention sur la diversité biologique (SCDB), l'Organisation des Nations Unies pour le développement industriel (ONUDI) et le Comité consultatif économique et industriel auprès de l'OCDE (BIAC).

Harmonisation de la réglementation

Le Sous-groupe a été créé en 1995[5] au moment des premières demandes d'autorisation réglementaires de cultures commerciales transgéniques dans plusieurs pays Membres de l'OCDE. Dès le début, l'un de ses premiers objectifs a été de promouvoir l'harmonisation internationale de la réglementation entre les pays Membres. L'harmonisation réglementaire vise à assurer que les données utilisées pour l'évaluation des risques et de la sécurité, de même que les méthodes utilisées pour les collecter sont aussi uniformes que possible entre les pays. Elle peut permettre aux pays de reconnaître ou même d'accepter les informations des évaluations réalisées par d'autres pays. Les avantages de l'harmonisation sont évidents. Elle accroît la compréhension mutuelle entre les pays Membres, et permet donc d'éviter les recoupements, d'économiser les ressources rares et d'accroître l'efficacité des procédures d'évaluation des risques et de la sécurité. Cela permet d'améliorer la sécurité, tout en éliminant les obstacles inutiles au commerce (OCDE 2000). De l'avis de nombreux délégués la recherche d'une harmonisation, et les débats qui en résultent entre les pays Membres, sont presque aussi importants que les produits obtenus.

Pourquoi mener des activités d'harmonisation à l'OCDE

Le Sous-groupe a été créé et son programme, établi après que les pays Membres aient mené une réflexion approfondie pour déterminer s'il fallait poursuivre ou non les travaux sur l'harmonisation en biotechnologie dans le cadre de l'OCDE, et dans l'affirmative, ce que ces travaux impliqueraient. Cette réflexion a été menée par le Groupe ad hoc sur les aspects environnementaux de la biotechnologie (créé par la Réunion conjointe[6]), qui a mené un grand nombre d'activités principalement pendant l'année 1994.

Le Groupe ad hoc a pris en considération et mis à profit les précédents travaux faits à l'OCDE, à partir du milieu des années 80. Les activités antérieures de l'OCDE se sont initialement concentrées sur les conséquences pour l'environnement et l'agriculture des essais au champ d'organismes transgéniques, mais ont ensuite très vite porté sur leur utilisation à grande échelle et leur commercialisation. (On trouvera un résumé de tous ces travaux à l'annexe I.)

Principaux concepts et principes de base

Le Groupe ad hoc a pris en compte (entre autres éléments) les précédents travaux sur l'analyse des risques dont on trouve un résumé dans le document *Considérations de sécurité relatives à la*

5 . Le Sous-groupe s'appelait à l'origine Groupe d'experts pour l'harmonisation de la surveillance réglementaire en biotechnologie. Il est devenu un Sous-groupe de l'OCDE en 1998.

6 . La Réunion conjointe était l'organe de tutelle du Groupe ad hoc et a fait du Sous-groupe, en raison de ses résultats, un organe subsidiaire. Aujourd'hui, son nom officiel est Réunion conjointe du Comité des produits chimiques et du Groupe de travail sur les produits chimiques, les pesticides et la biotechnologie.

biotechnologie : passage à l'échelle supérieure des plantes cultivées (OCDE 1993a). L'extrait suivant en donne un aperçu : « *l'analyse de risque/de sécurité s'appuie sur les caractéristiques de l'organisme, le caractère introduit, l'environnement dans lequel l'organisme est libéré, les interactions de ces facteurs entre eux et l'utilisation prévue.* » Ces travaux ont servi de point de départ à l'évaluation environnementale des risques et de la sécurité, aujourd'hui acceptée mondialement. Par conséquent en examinant les possibilités d'harmonisation le Groupe ad hoc s'est intéressé à ces caractères et aux informations utilisées par les évaluateurs des risques et de la sécurité pour les examiner.

A cela s'ajoute le concept de familiarité, qui est aussi décrit dans le document « Mise à l'échelle » (OCDE 1993a). Ce concept « est basé sur le fait que la plupart des organismes transformés génétiquement sont développés à partir d'organismes comme les végétaux cultivés, dont la biologie est bien comprise ». « La familiarité permet à l'évaluateur de risques d'appliquer ses connaissances et son expérience de l'introduction des végétaux et des microorganismes dans l'environnement. » S'agissant des végétaux, la familiarité tient compte d'un grand nombre d'éléments, par exemple, des connaissances et de l'expérience concernant « les végétaux cultivés, y compris leurs caractéristiques de floraison et de reproduction, leurs exigences écologiques et les expériences passées en matière de sélection » (OCDE 1993a – une description plus détaillée est proposée dans l'annexe I). Cela montre bien le rôle des informations concernant la biologie de l'organisme hôte lors de l'évaluation des risques pour l'environnement et de la sécurité.

Le Groupe ad hoc a également pris en compte le document intitulé «Méthodes traditionnelles de sélection des plantes : un aperçu historique destiné à servir de référence pour l'évaluation du rôle de la biotechnologie moderne » (OCDE 1993b) qui met aussi l'accent sur les organismes hôtes. On y trouve des informations sur 17 plantes cultivées qui sont (ou pourraient être) utilisées en biotechnologie moderne. Ce document comprend des sections consacrées aux aspects phytosanitaires du transfert de matériel génétique et aux utilisations actuelles de ces plantes. Une section détaillée est également consacrée aux pratiques de sélection actuelles.

Une approche commune de l'évaluation des risques et de la sécurité

L'une des missions importantes du Groupe ad hoc était aussi d'évaluer dans quelle mesure les pays Membres étudient les mêmes questions et problèmes lors de l'évaluation des risques et de la sécurité. En cas de différences importantes la recherche d'une harmonisation aurait pu se révéler difficile ; à l'inverse, en présence de nombreuses similitudes, le travail d'harmonisation serait plus aisé.

Deux études ont permis au Groupe ad hoc de répondre à cette question. La première concernait les plantes cultivées (OCDE 1995a, 1995b) et la seconde les micro-organismes (OCDE 1995c, 1996). Ces deux études étaient basées sur une enquête adressée aux autorités nationales chargées de l'évaluation des risques et de la sécurité. L'objectif était de faire ressortir les questions couvertes par le processus d'évaluation (d'après la législation, la réglementation ou les documents d'orientation nationaux) pour établir le degré de similitude entre les autorités nationales. Ces deux études ont pris pour référence les informations figurant dans le « *Livre bleu* » de l'OCDE (OCDE 1986), en particulier dans les annexes b, c et d) intitulées : i) Considérations scientifiques générales ; ii) Considérations relatives à la santé humaine ; et iii) Considérations relatives à l'environnement et l'agriculture. Ces deux études ont permis de constater que les questions et problèmes traités par les pays Membres pour évaluer les risques et la sécurité présentaient un remarquable degré de similitude.

Apparition du concept de document de consensus

Le Sous-groupe a pu ainsi établir que les questions et problèmes traités par les autorités nationales aux fins de l'évaluation des risques et de la sécurité présentaient de très nombreux points communs. Il a également pris en compte les caractères identifiés dans le cadre de l'évaluation des risques et de la sécurité

(*l'organisme, le caractère introduit et l'environnement*) sur lesquelles pourraient se concentrer les activités d'harmonisation.

Il a ensuite été constaté qu'une grande partie des informations concernant la biologie des organismes (aussi bien des plantes que des micro-organismes) utilisées dans l'évaluation des risques et de la sécurité étaient les mêmes ou pratiquement mes mêmes dans toutes les évaluations portant sur le même organisme. En d'autres termes, les questions concernant la biologie de l'organisme hôte (par exemple, le potentiel de transfert de gènes à l'intérieur d'une espèce de plante cultivée, et entre des espèces apparentées, de même que le caractère adventice potentiel) examinées dans le cadre de l'évaluation des risques et de la sécurité étaient les mêmes pour chaque demande impliquant les mêmes espèces hôtes. Il en allait de même, jusqu'à un certain point, pour les informations relatives aux caractères introduits.

En conséquence, le Sous-groupe a eu l'idée de regrouper les informations communes utilisées dans l'évaluation des risques et de la sécurité d'un certain nombre de produits transgéniques et décidé de se concentrer sur deux catégories particulières : la biologie des espèces ou plantes hôtes ; et les caractères utilisés dans les modifications génétiques. L'objectif était d'encourager le partage de l'information et d'éviter les doublons en permettant aux pays de ne pas traiter les mêmes questions communes pour chaque demande concernant le même organisme ou le même caractère. Il a été décidé que des documents de consensus sur la biologie ou les caractères pouvaient être rapidement adoptés par les pays Membres (en un ou deux ans). Ce processus de compilation a rapidement débouché sur la rédaction de documents de consensus.

Objet des documents de consensus

Les documents de consensus ne prétendent pas se substituer à l'évaluation des risques et de la sécurité, car ils ne concernent qu'une partie de l'information nécessaire. Cependant, ils devraient faciliter grandement l'évaluation environnementale des risques et de la sécurité.

Comme l'a indiqué initialement le Sous-groupe, les documents de consensus visent à fournir un aperçu des données courantes pouvant être utilisées dans le processus d'évaluation réglementaire des produits issus de la biotechnologie. Ils ne prétendent pas offrir une source d'informations exhaustive sur l'ensemble des connaissances concernant un organisme hôte ou un caractère particulier ; ils abordent plutôt le « noyau dur » des questions jugées pertinentes, sur la base d'un consensus, par les pays Membres pour l'évaluation des risques et de la sécurité.

Ces documents visent à faciliter l'échange d'informations sur ces composantes clés des évaluations de la sécurité environnementale afin d'éviter que les activités menées dans les pays ne fassent double emploi. Ils étaient en principe destinés : a) aux pétitionnaires à titre d'informations pour les demandes adressées aux autorités de réglementation ; b) aux autorités chargées de la réglementation comme guide général et source de référence pour leurs examens ; et c) aux gouvernements aux fins de l'échange d'information, comme références de recherche et pour l'information du public.

Il a été décidé initialement que les informations contenues dans les documents de consensus devaient être *mutuellement reconnues ou mutuellement acceptées* par les pays Membres de l'OCDE, bien que le sens de ces expressions reste encore à préciser. L'expression *acceptation mutuelle des données* a été étudiée pendant la période de mandat du Groupe ad hoc et durant les premières années du Sous-groupe (1993-1995). Il s'agit en fait d'une notion empruntée au Programme des produits chimiques de l'OCDE pour désigner un système de Décisions du Conseil de l'OCDE qui ont un caractère contraignant pour les pays Membres. Dans le cas des documents de consensus, il n'a jamais été obligatoire d'utiliser les informations y figurant, même si le Sous-groupe s'interroge de temps à autres sur l'opportunité et la façon d'impliquer davantage les pays Membres qui utilisent les informations fournies dans ces documents. La

participation des pays Membres à l'élaboration des documents et leur intention d'utiliser les informations qu'ils contiennent sont présumées de bonne foi. On peut donc penser que les documents de consensus applicables serviront de référence dans les évaluations des risques et de la sécurité.

Processus d'établissement des documents de consensus débouchant sur leur publication

La rédaction d'un document de consensus se fait en plusieurs étapes. Tout commence lorsqu'une délégation, à l'occasion d'une réunion officielle du Sous-groupe, propose d'établir un document sur un nouveau sujet, en général une espèce végétale ou un caractère. Si le Sous-groupe approuve la proposition, un premier projet est préparé par un, deux ou plusieurs pays en collaboration. Cette étape repose sur le principe de « pilotage ». En général, le ou les pays pilote(s) possèdent une expérience de la plante ou du caractère visés par le nouveau document et peuvent faire appel à des experts pour préparer une première version.

Cette version préliminaire est d'abord examinée par le Bureau du Sous-groupe[7] qui vérifie que le document étudie bien tous les aspects habituellement pris en compte par les documents de consensus et que sa qualité est suffisamment bonne pour le présenter à l'ensemble du Sous-groupe.

Un premier projet est établi à la lumière des commentaires du Bureau puis présenté à l'ensemble du Sous-groupe. De cette façon, chaque délégation peut étudier le texte et formuler des commentaires en fonction de l'expérience de son pays. Après incorporation de ces commentaires on obtient la deuxième mouture, qui est à nouveau diffusée pour examen et commentaires au Sous-groupe. A ce stade, le Sous-groupe peut être invité à recommander la déclassification du document. Cette demande intervient uniquement lorsque toutes les délégations ont décidé d'un commun accord (consensus) que le document était complet et prêt pour publication. Il arrive cependant que le texte nécessite un troisième, voire un quatrième examen au sein du Sous-groupe avant que sa déclassification puisse être recommandée.

Lorsque le Sous-groupe est convenu que le document pouvait être recommandé pour déclassification, le document est transmis à l'organe de tutelle, la Réunion conjointe, qui est invitée à le déclassifier. Une fois approuvé par la Réunion conjointe, le document est publié.

Il importe de noter que l'examen des documents de consensus dépasse le cadre des réunions officielles du Sous-groupe. De nombreux échanges de vues se font aussi par voie électronique, notamment dans le cadre du groupe de discussion électronique du Sous-groupe. Cette formule permet à divers experts de compléter les projets.

Il s'est révélé également important, dans le cas de plusieurs documents, d'inclure des informations de pays non membres. Cela s'est notamment produit pour les plantes cultivées dont les centres d'origine et de diversité se trouvent dans un ou des pays non membre(s). Dans ces cas, le PNUE et l'ONUDI ont contribué à l'établissement des documents en indiquant quels experts contacter dans les pays renfermant les centres d'origine et de diversité. Une telle situation s'est produite par exemple, lors de l'établissement du document de consensus sur la biologie du riz.

Évolutions actuelles et futures au sein du Sous-groupe

Le Sous-groupe poursuit ses travaux, non seulement de préparation des documents de consensus, mais aussi d'étude de l'efficacité du processus d'établissement de ces documents. A l'heure actuelle, un nombre

7 . Le Bureau comprend le Président et les Vice- présidents du Sous-groupe. Le Bureau est élu par le Sous-groupe une fois par an. Au moment où ce document est écrit, le Président est autrichien et les Vice-présidents sont des représentants du Canada, des États-Unis, du Japon et des Pays-Bas.

croissant de plantes cultivées et d'autres espèces sont modifiées par le transfert de caractères transférés de plus en plus nombreux.

Lors de l'atelier de l'OCDE consacré aux documents de consensus et aux travaux futurs sur l'harmonisation, qui s'est tenu à Washington DC, du 21 au 23 octobre 2003, le Sous-groupe a réfléchi, entre autres choses, à la façon d'établir un ordre de priorité pour la préparation des documents de consensus. Il examine actuellement comment améliorer à l'avenir le classement des priorités .

Les participants à l'atelier ont également reconnu qu'il pourrait être nécessaire de revoir ou de mettre à jour périodiquement les documents de consensus, pour veiller à ce qu'ils contiennent les informations les plus récentes. Le Sous-groupe examine actuellement la meilleure façon de procéder pour concrétiser ce projet.

Les participants ont constaté qu'il serait utile d'établir une structure type de document de consensus, sous forme de liste de « points à examiner » pour faciliter l'établissement des prochains documents et leur mise à jour. Le Sous-groupe devrait préparer un document recensant les points à examiner tout d'abord pour les documents de consensus relatifs à la biologie puis pour ceux concernant les caractères.

L'atelier a par ailleurs indiqué qu'il était important d'associer davantage les pays non membres à l'établissement des prochains documents de consensus. Ici encore, le Sous-groupe réfléchit à la meilleure façon de mettre en oeuvre cette recommandation.

ANNEXE I

Principes et concepts relatifs à la biosécurité établis par l'OCDE avant la création du Sous-groupe 1986-1994

Depuis le milieu des années 1980, l'OCDE a développé des approches harmonisées pour l'évaluation des risques et de la sécurité des produits de biotechnologie moderne. Avant la création du Sous-groupe, l'OCDE a publié plusieurs rapports d'experts portant sur des questions de sécurité, les concepts et principes relatifs à l'évaluation des risques et de la sécurité de même que sur la dissémination des cultures transgéniques dans les champs et sur la question des pratiques de croisement des cultures traditionnelles. La présente annexe récapitule les éléments essentiels de ces travaux qui ont servi de point de départ à la création du Sous-groupe et aux documents de consensus établis.

Principes scientifiques sous-jacents

En 1986, l'OCDE a publié ses premières études sur la sécurité des organismes transformés génétiquement (OCDE, 1986). Ceux-ci comprenaient des questions (intéressant la santé humaine, l'environnement et l'agriculture) qui pourraient être prises en compte dans l'évaluation des risques et de la sécurité. Dans ses recommandations pour les applications agricoles et environnementales, il est suggéré que les évaluateurs des risques et de la sécurité :

- « Utilisent des données nombreuses sur les effets au niveau de l'environnement et de la santé humaine des organismes vivants afin de guider les évaluations des risques.

- Assurent que les organismes formés de molécules d'ADN recombiné sont évalués pour déterminer les risques possibles, préalablement à leur application dans l'agriculture et dans l'environnement par un examen distinct des risques potentiels de façon ponctuelle.

- Dirigent le développement d'organismes formés d'ADN recombiné pour des applications agricoles et environnementales d'une manière progressive, allant si approprié, du laboratoire à la chambre de culture et à la serre, puis à des essais limités en conditions réelles, et finalement à des essais au champ à grande échelle.

- Encouragent la recherche pour améliorer les prédictions, l'évaluation et le suivi des résultats des applications d'organismes formés d'ADN recombiné. »

Rôle du confinement dans les essais à échelle réduite

En 1992, l'OCDE a publié son Principe d'élaboration saine (OCDE, 1992) pour la conception de recherche sur le terrain à échelle réduite impliquant des végétaux et microorganismes GM. Ce document, entre autres, décrit l'utilisation du *confinement* dans les tests sur le terrain. Le confinement comprend des mesures pour éviter la dissémination ou l'établissement des organismes d'un terrain faisant l'objet d'une étude, comme par exemple l'isolation physique, temporelle ou biologique (comme l'utilisation de la stérilité).

Mise à l'échelle des végétaux cultivés – « analyse des risques et de la sécurité »

À partir de 1993, l'attention a été transférée à la mise à l'échelle des végétaux cultivés au fur et à mesure que les sélectionneurs de végétaux commençaient à accroître la production et la commercialisation des végétaux GM. L'OCDE a publié les principes généraux pour la *mise à l'échelle* (OCDE, 1993a), lesquels réaffirmaient que, « *La sécurité en biotechnologie est réalisée par l'application appropriée de l'analyse des risques et de la sécurité et de la gestion des risques. L'analyse des risques et de la sécurité comprend l'identification des dangers et, si un danger a été identifié, la gestion du risque. L'analyse des risques et de la sécurité est fondée sur les caractéristiques de l'organisme, le trait caractéristique introduit, l'environnement dans lequel l'organisme est introduit, les interactions entre l'environnement et l'organisme de même que l'application prévue. L'analyse des risques et de la sécurité est menée préalablement à une action visée et est en général une composante de routine de la recherche, du développement et des essais de nouveaux organismes, que ces actions soient effectuées en laboratoire ou sur le terrain. L'analyse des risques et de la sécurité est une procédure scientifique qui n'implique ni n'exclut une surveillance au niveau de la réglementation, et qui n'exige pas que chaque cas soit nécessairement examiné par une autorité nationale ou autre »* (OCDE, 1993)a.

Rôle de la familiarité dans l'évaluation des risques et de la sécurité

La question de la *mise à l'échelle* a également mené à un concept important, la *familiarité*, qui constitue l'une des approches stratégiques utilisées par la suite pour aborder la sécurité environnementale des végétaux transgéniques.

Le concept de la familiarité est basé sur le fait que la plupart des organismes transformés génétiquement sont développés à partir d'organismes comme les végétaux cultivés, dont la biologie est bien comprise. Elle ne constitue pas une évaluation des risques et de la sécurité en elle-même (NAS, 1989). Toutefois, le concept facilite les évaluations des risques et de la sécurité parce que la familiarité suppose que l'on dispose de suffisamment de renseignements pour être en mesure de poser un jugement sur la sécurité ou sur le risque (U.S. NAS, 1989). La familiarité peut aussi être utilisée pour indiquer les pratiques de gestion appropriées, comme par exemple déterminer si les pratiques agricoles standard sont adéquates ou si d'autres pratiques de gestion sont nécessaires pour gérer le risque (OCDE, 1993a). La familiarité permet à l'évaluateur de risques d'appliquer ses connaissances et son expérience de l'introduction des végétaux et des microorganismes dans l'environnement, ce qui lui donne une indication des pratiques de gestion appropriées. Comme la familiarité dépend aussi de la connaissance de l'environnement et de ses interactions avec les organismes introduits, l'évaluation des risques et de la sécurité effectuée dans un pays peut ne pas s'appliquer à un autre pays, Toutefois, au fur et à mesure que les essais en champ sont effectués, des renseignements sur les organismes impliqués de même que sur leurs interactions avec divers environnements seront recueillis.

La familiarité provient des connaissances et de l'expérience disponibles pour analyser les risques et la sécurité préalablement à la mise à l'échelle de toute nouvelle lignée de végétaux ou cultivars dans un environnement particulier. Pour les végétaux par exemple, la familiarité tient compte, sans y être limitée, des connaissances et de l'expérience au niveau :

- « Des végétaux cultivés, y compris leurs caractéristiques de floraison et de reproduction, leurs exigences écologiques et les expériences passées en matière de sélection des végétaux.
- De l'environnement agricole et environnant du site d'essais.
- Du ou des trait(s) caractéristique(s) spécifique(s) transféré(s) à la ou les lignée(s) de végétaux.

- Des résultats des précédents travaux de recherche fondamentale, notamment la recherche en serre et à échelle réduite sur la nouvelle lignée de végétaux ou sur d'autres lignées présentant les mêmes traits caractéristiques.

- De la mise à l'échelle de lignées de végétaux cultivés développés par des techniques plus traditionnelles de sélection des végétaux.

- De la mise à l'échelle d'autres lignées de végétaux développées par la même technique.

- De la présence de végétaux apparentés (et sexuellement compatibles) dans l'environnement naturel et des connaissances au niveau de la possibilité de transfert génique entre la plante cultivée et la plante apparenté.

- Des interactions entre la plante cultivée, l'environnement et les traits caractéristiques et des interactions au sein de la plante cultivée. » (OCDE, 1993a).

Évaluation des risques et de la sécurité et gestion des risques

L'évaluation des risques et de la sécurité suppose l'identification des effets nocifs ou des dangers possibles au niveau de l'environnement et la détermination, lorsqu'un danger est identifié, de la probabilité qu'il se produise. Si un danger ou un effet nocif sur la santé est identifié, des mesures doivent être entreprises pour le minimiser ou l'atténuer. C'est ce que l'on appelle la gestion des risques. La certitude absolue ou l'absence totale de risques est impossible à obtenir en matière d'évaluation de la sécurité. L'incertitude est donc un aspect inévitable de toutes les évaluations des risques et de toute gestion des risques (OCDE, 1993a). Par exemple, l'on retrouve de l'incertitude en extrapolant les résultats des tests effectués sur une espèce pour identifier les effets possibles chez une autre espèce. Les évaluateurs et les gestionnaires de risques déploient donc des efforts considérables à traiter les incertitudes. Plusieurs des activités des organisations gouvernementales, comme l'OCDE, tentent de déterminer des façons de gérer ces incertitudes (OCDE 2000).

ANNEXE II

Références (par ordre chronologique)

Considérations de sécurité relatives à l'ADN recombiné. Considérations de sécurité relatives à l'utilisation d'organismes obtenus par des techniques de recombinaison de l'ADN dans l'industrie, dans l'agriculture et dans l'environnement (« *Livre bleu* »), OCDE, 1986.

Field Testing of Genetically Modified Organisms: Framework for Decisions. U.S. NAS - National Academy of Sciences, National Academy Press, Washington DC. Etats-Unis 1989.

Bons principes de développement (BPD), OCDE 1992.

Considérations de sécurité relatives à la biotechnologie : Passage à l'échelle supérieure des plantes cultivées, OCDE, 1993a.

Méthodes traditionnelles de sélection des plantes : Un aperçu historique destiné à servir de référence pour l'évaluation du rôle de la biotechnologie moderne, OCDE 1993b.

Commercialisation of Agricultural Products Derived through Modern Biotechnology: Survey Results, OCDE 1995a.

Report of the OECD Workshop on the Commercialisation of Agricultural Products Derived through Modern Biotechnology, OCDE 1995b.

Analysis of Information Elements Used in the Assessment of Certain Products of Modern Biotechnology, OCDE 1995c.

Industrial Products of Modern Biotechnology Intended for Release to the Environment: The Proceedings of the Fribourg Workshop, OCDE 1996.

Rapport du Sous-groupe sur l'harmonisation de la surveillance réglementaire en biotechnologie au Sommet du G8 d'Okinawa, OCDE 2000.

PART 1

CONSENSUS DOCUMENTS ON THE BIOLOGY OF CROPS

SECTION 1
POINTS TO CONSIDER FOR CONSENSUS DOCUMENTS ON THE BIOLOGY OF CULTIVATED PLANTS

As most of the Consensus Documents included in this book were published before the "Points to Consider" were drafted, some do not follow closely the recommendations in this text.

Introduction

Most environmental risk/safety assessments of transformed (genetically modified or engineered) plants are based upon a broad body of knowledge and experience with the untransformed species (variety, *etc*.), *i.e.* familiarity with the crop plant. The intent of the biology consensus document is to describe portions of this body of knowledge directly relevant to risk/safety assessment in a format readily accessible to regulators. The document is not an environmental risk/safety assessment of the species. Rather, the consensus document provides an overview of pertinent biological information on the untransformed species to help define the baseline and scope (the comparator against which transformed organisms will be compared), in the risk/safety assessment of the transformed organism. Consensus documents are not detailed crop handbooks or manuals of agricultural or silvicultural practice or economic botany, but rather focus on the biological information and data that may be clearly relevant to the assessment of newly transformed plants.

This *Points to Consider* document is meant as a structured explanatory checklist, regarding both order and contents, of relevant points to consider in preparing or evaluating a consensus document on the biology of a cultivated vascular plant species or other taxonomic group of interest, in relation to biotechnology and environmental risk/safety assessment. The general approach laid out in this document may also be pertinent to non-vascular plants (*e.g.* mosses), and fungi and micro-organisms; however, these groups are biologically and ecologically so different that further adaptation and refinement of the general approach will be necessary.

The biology consensus documents that have been published to date as well as most in preparation [excepting the documents on *Pleurotus* spp. (oyster mushrooms) and several on micro-organisms] are on annual crops, timber trees, and fruit trees. The plants of interest that have been the subject of the documents are primarily row crops, or trees managed silviculturally or grown in plantations or orchards. They are vascular plants, either flowering plants (angiosperms) or conifers (gymnosperms).

The points to consider as covered in the present document create a basic format and scope to be used for writing or reviewing new consensus documents and updating the earlier documents. While this *Points to Consider* document is meant to provide a basic format and scope, it is not intended to be rigid or inflexible. Of the biology consensus documents to date, some have addressed a particular point in depth, others lightly, and some not at all, depending on the relevance of the point to the plant species or other group of interest. Should additional points beyond those covered in this document be needed for a particular plant, the additional information can be included in the body of the consensus document, or in appendices. If a particular point is not covered in a consensus document, the text may briefly explain why the point, in the particular case, is not relevant.

Authors of the draft of a plant biology consensus document should be familiar with this *Points to Consider* document as well as existing consensus documents in the OECD Series on Harmonisation of Regulatory Oversight in Biotechnology (SHROB), in order to develop the appropriate scoping and presentation of information and data and for general editorial style. Existing consensus documents, particularly more recent ones, may provide detailed examples (some noted below) that are helpful models or thought-provoking for particular cases. Those interested in gaining perspective on coverage of pertinent topics as these documents have evolved and been fine-tuned for the varied cultivated plants in relation to environmental risk/safety assessment may consult especially *Analysis of Consensus Document's Section I: Analysis and Comparison of Consensus Documents* [ENV/JM/BIO(2003)16]. This review was presented at the OECD Workshop on Review of Consensus Documents and Future Work in Harmonisation, held in Washington, D.C., USA in October 2003. This document on points to consider for consensus documents on the biology of cultivated plants results from a recommendation of that meeting.

An understanding of the biology of the species or other group of interest will aid in determining the kinds of information pertinent to the environmental risk/safety assessment. This *Points to Consider* document provides an explanation of why the point (as enumerated below) is important in risk/safety assessment of the transformed plant, and presents a rationale for how the information in the point relates to risk/safety assessment. For a particular environmental risk/safety assessment, biological or ecological information in addition to that presented in the consensus document may be needed to address the regional environments into which the genetically engineered plant is proposed to be released.

1. Species or Taxonomic Group

The focus of each biology consensus document has usually been a species, but in some cases the focus has been a group of species or a genus, or just a subspecies or a cultivar group (examples are below). The primary focus of this *Points to Consider* document also is the species of interest, so appropriate adjustments will be necessary if the focus of the consensus document is more broad or narrow.

1.1. Classification and nomenclature

Give the scientific name of the cultivated species of interest, with its authors, and pertinent synonyms (*i.e.* actively used alternative scientific names, if any). If necessary to delimit the plant, also give the horticultural name, *e.g.* the cultivar group (*e.g. Beta vulgaris* subsp. *vulgaris* Sugar Beet Group). Provide main international common name(s) at least in English for the species of interest. Give the taxonomic context of the species (family always, perhaps the order, and perhaps the subfamily, tribe, subgenus or section). If the taxonomy is not settled, be relatively conservative in choosing the taxonomy, and briefly explain the alternative(s). The latest taxonomic or nomenclatural study is not necessarily definitive, and may need time for scientific consensus before it becomes adopted. A common name for the crop species of interest can be introduced here, to be used in much of the document as a more familiar name (aide-memoire).

Describe the taxonomic relationships of the cultivated species: related species, and related genera particularly if there is good potential for spontaneous hybridisation or the generic limits are unsettled. A list of related species (with brief ranges) should be given and include all the relatives with a potential for hybidization (*i.e.* crossable relatives). This topic is dealt with in detail in Section IV. The listing here may provide brief information on chromosome numbers and ploidy if these data are pertinent to the taxonomic differentiation of the species, whereas a more complete coverage of the relevant details is provided in Section III or IV.

Rationale: The scientific name enables an unequivocal understanding (*i.e.* a circumscription) of the plant of interest, at the appropriate level, such as the species or the subspecies. This addresses what the

species (or other group) is and what it is called (*i.e.* circumscription and name). The list of close relatives could help in subsequent analysis to form an idea of the kinds of pertinent traits such as disease resistance or stress tolerance that may already occur in these direct relatives of the cultivated plant, and may help elucidate how genes/traits are shared and may move via gene flow amongst related populations. The list of close relatives may aid in understanding the range of diversity and variability between the species and its natually crossable relatives.

Examples: OECD Series on Harmonisation of Regulatory Oversight in Biotechnology (SHROB) No. 14 (rice, Section II, pp. 12-14); No. 16 (poplars, Section II, pp. 15-18); No. 18 (sugar beet, Section I, pp. 11-12); No. 22 (eastern white pine, Section II, p. 12); and No. 31 (sunflower, Section I, pp. 11-13).

1.2. Description

Give a brief non-technical description of the species of interest, understandable to the non-specialist. Provide the habit and general characteristics of the plant, for example that it is an annual, a long-lived tree, or a biennial cultivated as an annual crop, and that it is, for instance, grown for fibre, fruit, or seeds. Also provide a concise technical (taxonomic) description sufficient to make a positive identification of the plant (or part). Illustration (a line drawing or black-and-white photo) may be useful. To clarify distinctiveness, emphasise the practical diagnostic or distinguishing morphological or other characters. Limit jargon, by the precise use of phrases and familiar words. A table of main differences or taxonomic key may be instructive (*e.g. Oryza sativa* and *O. glaberrima* in SHROB No. 14). If necessary, for example when based on recent information or a new approach, present or reference the analytical methods by which a differential identification of the similar plants (*e.g.* species) is now made.

Rationale: These descriptions provide broad orientation, and as well accurate identification. They briefly explain how the species of interest is actually identified in relation to others. Additionally, the description may give particular characteristics of the plant to aid in defining the scope of a risk/safety assessment. Although an exact identification often is based on experience (*i.e.* recognition) or on regional publications, rigorous or subtle analysis using specialist resources sometimes is required.

Examples: OECD SHROB No. 8 (potato, Section IV, pp. 14-15) and No. 28 (European white birch, Section I, pp. 12-13).

1.3. Geographic distribution, natural and managed ecosystems and habitats, cultivation and management practices, and centres of origin and diversity

This subsection covers the primary or crop species of interest, including the plants that are wild or free-living (whether native or naturalised) or weedy, and as cultivated or managed in the field. Crossable relatives with the relevant information and data on their intraspecific and interspecific crossing are discussed in Sections III and IV.

1.3.1. Geographic distribution

Describe the overall geographic distribution (if helpful including altitudinal range or climatic region), indicating broadly where the species of interest is native (*i.e.* indigenous), where it has been naturalised (introduced but free-living), and where it is in cultivation. A general map may be useful.

Rationale: Knowledge of the geographic distribution sets the context for understanding the potential interaction of the species with its relatives and with the surrounding ecosystems. For example, it is important to make a distinction between the species' native and naturalised occurrence when assessing the potential effects and the importance of gene flow.

Examples: OECD SHROB No. 8 (potato, Sections II & III, pp. 12-13); No. 13 (white spruce, Section III, pp. 15-16); and No. 16 (poplars, Section II, pp. 15-18).

1.3.2. Ecosystems and habitats where the species occurs natively, and where it has naturalised

Indicate the natural and non-cultivated or non-managed ecosystems where populations of the species of interest are native (indigenous) and where introduced and now naturalised (free-living) components of the vegetation. Designated natural areas (*e.g.* protected reserves, parks) where the species may be an invasive problem would be noted here. A species weedy in disturbed waste (*e.g.* abandoned) areas would be included here, whereas the species weedy in intensively managed areas would be discussed in the following subsection. Those ecosystems and habitats in which the species of interest occurs and its abundance are indicated here, whereas its ecological interactions with biotic components of the ecosystems and habitats are developed in Section V.

Rationale: The focus of this subsection is the relatively natural, self-sustaining context, rather than the land areas strongly managed for plant production. Knowledge of where the species occurs indigenously or is free-living provides baseline information for understanding the range of habitats in which the species exists, the range of behaviours exhibited in those habitats, and how characteristics of the species determine the range of habitats where it occurs. This information provides an understanding of the species' potential for interaction with its relatives and surrounding habitats.

Example: OECD SHROB No. 28 (European white birch, Section III, pp. 19-20).

1.3.3. Agronomic, silvicultural, and other intensively managed ecosystems where the species is grown or occurs on its own, including management practices

Describe where the species is dependent on management for survival or persistence over several years of usual conditions. Areas where the plant may be a weed problem would be discussed here. Areas to be discussed could include habitats such as annual row crops or bordering areas, tree plantations, orchards and vineyards, along regularly managed roadsides, rights-of-way, irrigation ditches, etc. Identify the pertinent general agronomic or other practices, and if relevant, regional differences in practices (including various practices within a region). Information might briefly encompass site preparation after clear-cutting, tillage, sowing or planting, weed control, control of volunteers, harvesting, plant protection practices during crop growth and after harvest, transport practices, and the use of harvested materials (*e.g.* for silage). The relevant ecological interactions of the species with particular organisms in these managed ecosystems are discussed in Section V.

Rationale: The focus of this subsection is on the plant's survival in agro-ecological, silvicultural, and other such managed areas, to provide the baseline environmental information on how the plant responds to or is managed by accepted agronomic, silvicultural or similar intensive practices. Identification of significant cultivation or management practices provides an understanding of measures available to manage or control the plant.

Examples: OECD SHROB No. 7 (oilseed rape, Section III, p. 13); No. 14 (rice, Section VII, pp. 26-27); No. 15 (soybean, Sections II & V, pp. 13 & 14); and No. 18 (sugar beet, Sections I & II, pp. 16-17).

1.3.4. Centres of origin and diversity

Describe the known or probable primary centre(s) of origin, as well as secondary centres where additional important variability or biodiversity may occur, whether naturally (*e.g. Beta*) or through the process of domestication (*e.g. Zea mays*, *Solanum tuberosum* subsp. *tuberosum*). The evolutionary centres important for natural biodiversity should be mentioned, and the central areas of domestication and landrace

diversity, with indication of the centres' relative importance. Genetic diversity is covered in Section III. Provide a brief sketch of the history or extent of domestication including mention of relevant domestication traits (*e.g.* non-shattering, loss of seed dormancy).

Rationale: The interaction of the cultivated plant with close relatives especially in a centre of origin is an important consideration because gene flow, varietal competition, or a change in cultivation practices may alter this especially rich and valuable diversity. If the plant is not expected to be grown near a center of diversity, the absence of such relatives would also be important. A brief review of domestication may provide insight showing the continuity of modification of the species and the degree of the crop plant's adaptation to or dependence on the managed environment.

Examples: OECD SHROB No. 9 (bread wheat, Section III, pp. 13-16); No. 27 (maize, Section IV, pp. 18-20); and No. 31 (sunflower, Section I, pp. 14-15.

2. Reproductive Biology

2.1. Generation time and duration under natural circumstances, and where grown or managed

Important aspects of generation time and duration include the time to first flowering and total life cycle of the plant, and time from planting to plow-down. Include the effects of agronomic, silvicultural, and similar practices when describing generation time and duration of the cultivated plant. Important differences within both the natural and the cultivated regions should be noted.

Rationale: The generation time and duration are indications of the terms in which environmental effects may occur. Precocious generation times and shorter durations in agriculture affect the likelihood of outcrossing with free-living (wild) relatives, and give a general indication of when outcrossing may first occur.

Examples: OECD SHROB No. 14 (rice, Sections V & VII, pp. 21 & 26-27) and No. 18 (sugar beet, Section I, pp. 13-14).

2.2. Reproduction (production of flowers or cones, fruits, seeds, and vegetative propagules)

Include a characterisation of the key stages in the life cycle necessary for the plant to survive, reproduce, and disperse. Particular attention is given to any uncommon survival structures or strategies and their importance under natural and cultivation conditions, and to the dependence of survival and reproduction on ecological and geographical factors.

Rationale: The reproductive capabilities of a plant determine the means by which the plant can produce progeny and spread or disperse. Both the plant and its progeny may affect the environment, including other organisms, and thus the time frame and geographic area over which effects might occur.

2.2.1. Floral biology

Describe the general floral dynamics (*e.g.* flowering season, flowering time, anthesis, selfing and/or outcrossing). Relevant genetic details of the outcrossing and/or selfing are addressed in Section III.

Rationale: This information will assist in understanding some of the factors that affect the potential for gene flow, and in assessing particular management strategies for reducing gene flow when outcrossing may occur. Such management strategies may include induced male sterility or asynchronous flowering times.

Examples: OECD SHROB No. 8 (potato, Section VI, p. 17); No. 14 (rice, Section V, p. 21); and No. 21 (Sitka spruce, Section III, p. 15).

2.2.2. Pollination (wind, insects, both, etc.), pollen dispersal, pollen viability

Describe observed modes of pollen dispersal, indicating the most prevalent way. Important insect or other animal pollinators should be indicated. Give data on the range of pollen dispersal through the air and/or by the animal vectors, if known. Note how climatic or regional (*e.g.* geographic) differences can affect pollination. Provide available information or data on the influence of pollen quantity, movement, viability, load and competition on outcrossing, which is discussed in Sections III and IV. The details on pollination as they pertain to the plant are covered here, whereas details particularly pertinent to the pollinator are covered in Section V.

Rationale: Pollen biology is an important component in the assessment of potential for gene flow, and in the evaluation of a need for and the type(s) of pollen confinement strategies such as buffer rows or isolation distances.

Examples: OECD SHROB No. 8 (potato, Section VI, p. 17) and No. 18 (sugar beet, Section IV, pp. 22-23).

2.2.3. Seed production, and natural dispersal of fruits, cones, and/or seeds

Briefly describe the sexual reproductive structures, including relevant morphological characteristics of fruits (or cones) and seeds, and note any inherent means of dispersal (*e.g.* shattering, fruit splitting, ballistic). Note the quantity of seeds produced by a plant (*e.g.* seeds per fruit and number of fruits). Provide information on the means and range of dispersal (*e.g.* by gravity, wind, water, on and/or in animals), and if there are several means indicate their relative importance. Cover apomixis below, in Subsection 2.2.5.

Rationale: The number of seeds and seed/fruit dispersal mechanisms are factors to consider in understanding the potential for establishment of free-living plants or populations, and thus the time and geographic area over which environmental effects might occur. The range of variability of these factors is also an important consideration.

Examples: OECD SHROB No. 15 (soybean, Section IV, p. 14) and No. 28 (European white birch, Section IV, p. 23).

2.2.4. Seed viability, longevity and dormancy, natural seed bank; germination, and seedling viability and establishment

Discuss factors in the establishment of any seed bank, including its transience or persistence, and the viability, longevity and dormancy of seeds under natural conditions. Note any special conditions that affect dormancy and/or germination (*e.g.* depth of burial, light and/or temperature, passage through an animal's digestive tract, or need for fire) that might be particularly relevant. Note any special requirements for the establishment and survival of seedlings (*e.g.* soil qualities or regime), as the organism's fitness may be revealed at this challenging phase in the life cycle.

Rationale: Seed viability is a key factor to consider in assessing the likelihood of survival of non-cultivated plants. Natural seed banks are often the main source of weeds in cultivated fields, whether they are previous-crop volunteers or non-crop weedy relatives. Whether seedlings can establish usually is a primary limiting factor in continuing the life cycle.

Example: OECD SHROB No. 7 (oilseed rape, Section VI, p. 17).

2.2.5. Asexual propagation (apomixis, vegetative reproduction)

Take into account natural vegetative cloning (*e.g.* in grasses and poplars), the kinds of propagules (special structures, and/or fragmented plant pieces), dispersal of the propagules, and their viability. Discuss the relative importance of asexual reproduction for the plant, including any differences dependent on habitat or region. For apomixis (non-sexual production of seeds), similarly consider its relative importance and effectiveness.

Rationale: If a plant has a strategy that includes asexual propagation, this could be a means for considerable or quite different dispersal or spread, and consequently may also affect the time frame and geographic area over which environmental effects might occur.

Example: OECD SHROB No. 16 (poplars, Section IV, p. 23).

3. Genetics

3.1. Relevant detailed genetic information on the species

Give a basic overview of the relevant genetic constitution and genetic dynamics of the species. If more appropriate in a particular case, some basic genetic information (*e.g.* ploidy, ancestral/progenitor genomes) may be more fully or instead discussed in Section IV. In this Section III (including subsections as needed), cover for example and if appropriate cytogenetics (*e.g.* karyology, meiotic behavior), nuclear genome size, possible extent of repetitive or non-coding DNA sequences, main genetic diversity or variability (*e.g.* among or within populations or varieties, and of alleles at a locus), evidence of heterosis or inbreeding depression, maternal and/or paternal inheritance of organellar genomes, and methods of classical breeding (*e.g.* utility from employing mutagenesis with the species). The relevance of the information to the species' variability and the potential effects of transformation are paramount in deciding what to include, as the focus is not to provide this genetic characterisation for plant development.

Intraspecific crossing with both non-cultivated strains (*e.g.* weedy races) and among non-transformed cultivars is appropriately covered here (perhaps with a table or diagram), including any genetic or cytoplasmic constraints or limitations to crossing (*e.g.* cytoplasmic or nuclear sterility, incompatibility systems). Interspecific crosses are addressed in the following section.

Rationale: The information in this section includes genetic and breeding data, such as details of genomic or genetic stability (including gene silencing) and intraspecific outcrossing behaviour and potential, only to the extent that such information describes parameters that influence how genetic material (including new material) behaves in particular genetic backgrounds, and in outcrossing. Interspecific hybridisation is in a separate section (which follows) because intraspecific crossing is more likely (and familiar), and interspecific hybrids may bring in broader or more extensive concerns.

Examples: OECD SHROB No. 9 (bread wheat, Sections III & V, pp. 13-17 & 20-24); No. 12 (Norway spruce, Section VI, pp. 21-23); No. 13 (white spruce, Section V, pp. 22-24); No. 14 (rice, Section VI, pp. 23-25); No. 24 (*Prunus* spp. – stone fruits, Section II, pp. 15-20); and No. 31 (sunflower, Section IV, pp. 27-28).

4. Hybridisation and Introgression

4.1. Natural facility of interspecific crossing (extent, sterility/fertility)

Describe interspecific (including intergeneric) crosses observed under natural conditions. Provide a list and perhaps a diagram of the documented hybrids, *i.e.* the crossings that may occur unaided under

usual environmental conditions — if the crossable relatives (other species) might be present. The information could include a discussion of ploidy (and ancestral/progenitor genomes). Provide an indication or review of the likelihood of first-generation (F_1) hybrids and later generations of these F_1 hybrids, and as well whether the F_1 hybrids may be bridges for genes to cross into other (non-parental) species. Rare plant species are considered here and in the following subsection. Indicate naturally hybridising species that are weedy (including invasive) in the list of hybridising species (detailed discussion of their weediness in a local environment would be covered in an environmental risk/safety assessment).

Rationale: The ability of a cultivated species to hybridise with other cultivated or wild species is a significant factor in determining whether genes or traits could be transferred to other species.

Examples: OECD SHROB No. 7 (oilseed rape, Section VII, pp. 18-21); No. 9 (bread wheat, Section V, pp. 20-24); and No. 16 (poplars, Sections III & VI, pp. 20 & 28-29).

4.2. Experimental crosses

Discuss the experimental data available on outcrossing under controlled conditions, and theoretical possibilities for and barriers to outcrossing. This information is in contrast to that in the previous subsection, which indicates the outcrossing to readily crossable relatives. Experimental data that is the result of forced crosses employing special techniques (*e.g.* embryo rescue) would be relevant only if such studies help to clarify degree of relatedness and likelihood of natural crossing. Theoretical considerations or experimental information might be, for example, on cytogenetic data and meiotic behaviour, or sexual incompatibility systems.

Rationale: Experimental data and theoretical considerations may broaden the understanding of potential (or as yet unknown) unaided (natural) gene transfer. The information and data are only relevant if unaided crossing in the field can occur.

Examples: OECD SHROB No. 8 (potato, Section VII, pp. 19-21); No. 13 (white spruce, Section VI, pp. 25-26); No. 16 (poplars, Section VI, pp. 28-29); and No. 22 (eastern white pine, Section IV, p. 17).

4.3. Information and data on introgression

Provide an indication or review of the likelihood of F_1 hybrids backcrossing into one or both parents. Provide information on both natural and experimental introgression (extensive backcrossing), and on the (types of) genes or the traits for which introgression has been demonstrated. For example, extensive backcrossing and introgression may be only in one direction, rather than into both parental lines or species' populations. Information should include the extent of likely natural (*i.e.* unaided) introgression or generations of experimental backcrossing, and the fertility and fecundity of the resultant plants.

Rationale: Of primary consideration is whether interspecific crossing will lead to the introgression of genes. Interspecific crossing is a necessary but typically not a sufficient step for considerable introgression to occur. Even if introgression occurs, it is not the presence but the expression of the gene or trait that may be of primary importance.

Examples: OECD SHROB No. 7 (oilseed rape, Section VII, pp. 20-21); No. 24 (*Prunus* spp. – stone fruits, Section II, p. 30); and No. 31 (sunflower, Section IV, pp. 28-29).

5. General Interactions with Other Organisms (Ecology)

5.1. Interactions in natural ecosystems, and in agronomic, silvicultural or other ecosystems where the species is cultivated or managed

Provide a general overview (including subsections as needed) of main functional ecological interactions of the species of interest within these natural and managed ecosystems and habitats, for example symbiotic relationships, food webs (*e.g.* fruit and seed consumers or predators), noxious/toxic or other important interactions with insects (*e.g.* chemical defense) and other animals, and with plants (*e.g.* allelopathy). Tritrophic interactions may also be considered. Subsections 1.3.2 and 1.3.3 list and briefly characterise the natural (unmanaged) and managed ecosystems and habitats in which the species of interest occurs. The importance of a pollination system to the animal pollinator is detailed here, whereas the importance to the plant is addressed in Subsection 2.2.2. A listing of pertinent pests and pathogens (and diseases) may be presented as an appendix, with only those that are critically relevant discussed here.

Rationale: The description of the basic general ecology of the species of interest is useful when determining the scope of interactions that may be used as a baseline for understanding the influences the cultivated plant may have on organisms that are in usual close contact. A general understanding of the interactions of the species with other organisms will aid in determining whether any concerns may arise with a change in the genetics of the species.

Examples: OECD SHROB No. 7 (oilseed rape, Section VII & Appendix, pp. 21 & 29) and No. 13 (white spruce, Section VII, pp. 28-31).

Human Health and Biosafety

6.1. Plant characteristics relevant for human health

Provide brief information on major natural toxicants and common allergenic or medicinal properties of the plant. In some cases, it may be relevant to mention similar information from related species (*e.g.* glycoalkaloids in crossable wild relatives of *Solanum tuberosum* subsp. *tuberosum*, potato).

Rationale: This theme can be regarded as human ecology, a subset of Section V that warrants coverage separately. Baseline information is briefly described, relating to human health as it might be affected by cultivation of the plant (*e.g.* levels of latex or psoralen). Potential effects on human health would be thoroughly treated elsewhere, such as in an OECD plant compositional consensus document for dietary issues.

Example: OECD SHROB No. 8 (potato, Section IV, p. 14).

7. Additional Information

The possibility is expressly left open for topics of additional information that is pertinent to environmental risk/safety assessment, as a section in the main text of the document, and/or as appendices.

8. References

As much as possible, the references should be peer-reviewed literature available internationally. After the references directly cited in the text, this section could include a subsection on additional useful references ‘for further reading’.

Example: OECD SHROB No. 7 (oilseed rape, Section IX, pp. 27-28).

Appendix I – Common Pests and Pathogens

Provide a list of causative organisms for diseases (pathogens) and pests that commonly occur in the crop under agronomic, silvicultural, or equivalent conditions.

Rationale: Provide as considered useful for risk/safety assessment rather than usual production management. Critically important organisms and ecological relationships (*e.g.* a virus disease that is a principal management issue) are covered in Section V. The risk/safety assessment would then consider whether the transformation in the crop would be of environmental concern.

Examples: OECD SHROB No. 18 (sugar beet, Appendix, pp. 32-37 and No. 31 (sunflower, Section V & Appendices 1 & 2, pp. 31 & 37-47).

Appendix II – Biotechnological Developments

General information on the kinds of traits being introduced into the species may be included. Provide information directly necessary for defining the scope or detail of biological information that would be useful. For example, transgenes under experimental development for a crop might result in a change in environmental fitness or range and habitats of the plant or its relatives (*e.g.* disease resistance, and drought, frost or salinity tolerance). Other biotechnological developments (*e.g.* to assist in marketing) may not be pertinent to address here.

Rationale: An overview of biotechnological developments may help to assure that the biological information included in a consensus document is pertinent to the environmental risk/safety assessments anticipated. Consensus documents that include the biotechnological developments to bring traits into the crop can be quite useful in explaining the relevance of assessing certain kinds of biosafety information.

Examples: OECD SHROB No. 14 (rice, Appendix III, pp. 42-45) and No. 27 (maize, Appendix A, pp. 39-41).

SECTION 2
SOYBEAN (*GLYCINE MAX* (L.) MARR.)

1. General Description Including Taxonomy and Morphology, and Use as a Crop Plant

Cultivated soybean, *Glycine max* (L.) Merr., is a diploidized tetraploid (2n=40), in the family Leguminosae, the subfamily Papilionoideae, the tribe Phaseoleae, the genus *Glycine* Willd. and the subgenus *Soja* (Moench). It is an erect, bushy herbaceous annual that can reach a height of 1.5 metres. Three types of growth habit can be found amongst soybean cultivars: determinate, semi-determinate and indeterminate (Bernard and Weiss, 1973). Determinate growth is characterised by the cessation of vegetative activity of the terminal bud when it becomes an inflorescence at both axillary and terminal racemes. Determinate genotypes are primarily grown in the southern United States (Maturity Groups V to X). Indeterminate genotypes continue vegetative activity throughout the flowering period and are grown primarily in central and northern regions of North America (Maturity Groups 000 to IV). Semi-determinate types have indeterminate stems that terminate vegetative growth abruptly after the flowering period. None of the soybean varieties are frost tolerant, and they do not survive freezing winter conditions.

The primary leaves are unifoliate, opposite and ovate, the secondary leaves are trifoliolate and alternate, and compound leaves with four or more leaflets are occasionally present. The nodulated root system consists of a taproot from which emerges a lateral root system. The plants of most cultivars are covered with fine trichomes, but glabrous types also exist. The papilionaceous flower consists of a tubular calyx of five sepals, a corolla of five petals (one banner, two wings and two keels), one pistil and nine fused stamens with a single separate posterior stamen. The stamens form a ring at the base of the stigma and elongate one day before pollination, at which time the elevated anthers form a ring around the stigma. The pod is straight or slightly curved, varies in length from two to seven centimetres, and consists of two halves of a single carpel which are joined by a dorsal and ventral suture. The shape of the seed, usually oval, can vary amongst cultivars from almost spherical to elongate and flattened.

Soybean is grown as a commercial crop in over 35 countries. The major producers of soybeans are the United States, China, Democratic People's Republic of Korea and Republic of Korea, Argentina and Brazil. Soybean is grown primarily for the production of seed, has a multitude of uses in the food and industrial sectors, and represents one of the major sources of edible vegetable oil and of proteins for livestock feed use.

A major food use in North America and Europe is as purified oil, utilised in margarines, shortenings and cooking and salad oils. It is also used in various food products, including tofu, soya sauce, simulated milk and meat products. Soybean meal is used as a supplement in feed rations for livestock. Industrial use of soybeans ranges from the production of yeasts and antibodies to the manufacture of soaps and disinfectants.

Soybean is commonly considered one of the oldest cultivated crops, native to North and Central China (Hymowitz, 1970). The first recording of soybeans was in a series of books known as Pen Ts'ao Kong Mu written by the emperor Sheng Nung in the year 2838 B.C., in which the various plants of China are described. Historical and geographical evidence suggests that soybeans were first domesticated in the

eastern half of China between the 17th and 11th century B.C. (Hymowitz, 1970). Soybeans were first introduced into the United States, now a major producer, in 1765 (Hymowitz and Harlan, 1983).

2. Agronomic Practices

Soybean is a quantitative short day plant and hence flowers more quickly under short days (Garner and Allard, 1920). As a result, photoperiodism and temperature response is important in determining areas of cultivar adaptation. Soybean cultivars are identified based on bands of adaptation that run east-west, determined by latitude and day length. In North America, there are thirteen maturity groups (MG), from MG 000 in the north (45° latitude) to MG X near the equator. Within each maturity group, cultivars are described as early, medium or late maturing.

The seed will germinate when the soil temperature reaches 10°C and will emerge in a 5-7 day period under favourable conditions. In new areas of soybean production an inoculation with *Bradyrhizobium japonicum* is necessary, for optimum efficiency of the nodulated root system. Soybeans do not yield well on acid soils and the addition of limestone may be required. Soybeans are often rotated with such crops as corn, winter wheat, spring cereals, and dry beans.

3. Centres of Origin of the Species

Glycine max belongs to the subgenus *Soja*, which also contains *G. soja* and *G. gracilis*. *Glycine soja*, a wild species of soybean, grows in fields, hedgerows, roadsides and riverbanks in many Asian countries. Wild soybean species are endemic in China, Korea, Japan, Taiwan and the former USSR, but do not exist naturally in North America. Cytological, morphological and molecular evidence suggest that *G. soja* is the ancestor of *G. max*. *Glycine gracilis* is considered to be a weedy or semi-wild form of *G. max*, with some phenotypic characteristics intermediate to those of *G. max* and *G. soja*. *Glycine gracilis* may be an intermediate in the speciation of *G. max* from *G. soja* (Fekuda, 1933) or a hybrid between *G. soja* and *G. max* (Hymowitz, 1970).

4. Reproductive Biology

Soybean is considered a self-pollinated species, propogated commercially by seed. Artificial hybridisation is used for cultivar breeding.

The soybean flower stigma is receptive to pollen approximately 24 hours before anthesis and remains receptive 48 hours after anthesis. The anthers mature in the bud and directly pollinate the stigma of the same flower. As a result, soybeans exhibit a high percentage of self-fertilisation, and cross pollination is usually less than one percent (Caviness, 1966).

A soybean plant can produce as many as 400 pods, with two to twenty pods at a single node. Each pod contains one to five seeds. Neither the seedpod, nor the seed, has morphological characteristics that would encourage animal transportation.

5. Cultivated *Glycine max* as a Volunteer Weed

Cultivated soybean seed rarely displays any dormancy characteristics and only under certain environmental conditions grows as a volunteer in the year following cultivation. If this should occur, volunteers do not compete well with the succeeding crop, and can easily be controlled mechanically or chemically. The soybean plant is not weedy in character. In North America, *Glycine max* is not found outside of cultivation. In managed ecosystems, soybean does not effectively compete with other cultivated plants or primary colonisers.

6. Crosses

A. Inter-species/genus

In considering the potential environmental impact following the unconfined release of genetically modified *Glycine max*, it is important to have an understanding of the possible development of hybrids through interspecific and intergeneric crosses with related species. The development of hybrids could result in the introgression of the novel traits into these related species and result in:

- The related species becoming more weedy.
- The introduction of a novel trait, with potential for ecosystem disruption, into related species.

For a trait to become incorporated into a species genome, recurrent backcrossing of plants of that species by hybrid intermediaries, and survival and fertility of the resulting offspring, is necessary.

The subgenus *Soja*, to which *G. max* belongs, also includes *G. soja* Sieb. and Zucc. (2n=40) and *G. gracilis* Skvortz. (2n=40), wild and semi-wild annual soybean relatives from Asia. *Glycine soja* (2n=40) is a wild viny annual with small and narrow trifoliate leaves, purple flowers and small round brown-black seeds. It grows wild in Korea, Taiwan, Japan, Yangtze Valley, N.E. China and areas around the border of the former USSR. *Glycine gracilis*, an intermediate in form between *G. soja* and *G. max*, has been observed in Northeast China (Skvortzow, 1927). Interspecific, fertile hybrids between *G. max*. and *G. soja* (Sieb and Zucc.) (Ahmad *et al*., 1977; Hadley and Hymowitz, 1973; Broich, 1978), and between *G. max* and *G. gracilis* (Karasawa, 1952) have been easily obtained.

In addition to the subgenus *Soja*, the genus *Glycine* contains the subgenus *Glycine*. The subgenus *Glycine* consists of twelve wild perennial species, including *G. clandestina* Wendl., *G. falcata* Benth, *G. latifolia* Benth., *G. latrobeana* Meissn. Benth., *G. canescens* F.J. Herm., *G. tabacina* Labill. Benth., and *G. tomentella* Hayata. These species are indigenous to Australia, South Pacific Islands, China, Papua New Guinea, Philippines, and Taiwan (Hymowitz and Newell, 1981; Hermann, 1962; Newell and Hymowitz, 1978; Grant, 1984; Tindale, 1984, 1986). Hybrids between diploid perennial *Glycine* species show normal meiosis and are fertile.

Early attempts to hybridise annual (subgenus *Soja*) and perennial (subgenus *Glycine*) species were unsuccessful. Although pod development was initiated, these eventually aborted and abscised (Palmer, 1965; Hood and Allen, 1980; Ladizinsky *et al*., 1979). Intersubgeneric hybrids were later obtained in vitro through embryo rescue, between *G. max* and *G. clandestina* Wendl; *G. max* and *G. tomentella* Hayata (Singh and Hymowitz, 1985; Singh *et al*., 1987); and *G. max* and *G. canescens*, using transplanted endosperm as a nurse layer (Broué *et al*., 1982). In all cases, the progeny of such intersubgeneric hybrids was sterile and obtained with great difficulty.

B. Introgression into relatives

Soybean can only cross with other members of *Glycine* subgenus *Soja*. The potential for such gene flow is limited by geographic isolation. Wild soybean species are endemic in China, Korea, Japan, Taiwan and the former USSR. These species are not naturalised in North America, and although they could occasionally be grown in research plots, there are no reports of their escape from such plots to unmanaged habitats.

C. Interactions with other organisms

The table in the Appendix is intended as an identification guide for categories of organisms, which interact with *Glycine max*. This table, representative of North America, is intended to serve as an example only. Environmental safety assessors should, on a country-by-country basis, draw up their own lists as a guide for assessing potential effects of the release of genetically modified plants on interacting organisms in their country.

The intention is not to require comparison data between a plant with novel traits and its *G. max* counterpart(s) for all interactions. Depending on the novel traits, applicants might decide to submit data for only some of the interactions. Sound scientific rationale will be required to justify the decision that data would be irrelevant for the remaining interactions. For example, the applicant might choose not to provide data on the weediness potential of a plant with novel traits if it can be clearly shown that the novel trait will not affect reproductive or survival characteristics of *G. max,* either directly or indirectly. Some of the life forms are listed as categories (*i.e.* pollinators, mychorrhizal fungi, animal browsers, birds, soil microbes, and soil insects). When, because of the novel traits, a concern is perceived for these specific categories, applicants will be required to provide detailed information on interactions with indicator species in each category. Where the impact of a plant with novel traits on another life form (target or non-target organism) is significant, secondary effects may need to be considered.

This section will be revised to include relevant new data as they become available.

7. Summary of Ecology of *Glycine max*

Glycine max (L.) Merr., the cultivated soybean, is a summer annual herb that has never been found in the wild (Hymonitz, 1970). This domesticate is in fact extremely variable, due primarily to the development of soybean "land races" in East Asia. The subgenus *Soja* contains, in addition to *G. max* and *G. soja*, the form known as *G. gracils*, a form morphologically intermediate between the two. This is a semi-cultivated or weedy form, and is known only from Northeast China.

Glycine soja, considered the ancestor of cultivated soybean, is an annual procumbent or slender twiner that is distributed throughout China, the adjacent areas of the former USSR, Korea, Japan and Taiwan. It grows in fields and hedgerows, along roadsides and riverbanks.

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APPENDIX

Examples of Potential Interactions of *G. max* with Other Life Forms During its Life Cycle

Other life forms Common Name	Interaction with *G. max* Pathogen; Symbiont or Beneficial Organism; Consumer; Gene Transfer
Brown spot (*Septoria glycines*)	Pathogen
Downy mildew (*Peronospora trifoliorum var. manshurica*)	Pathogen
Brown stem rot (*Phialophora gregata* or *Acremonium strictum*)	Pathogen
Phytophthora root and stalk rot (*Phytophthora megasperma*)	Pathogen
Stem canker (*Diaporthe phaseolorum var caulivora*)	Pathogen
Rhizoctonia stem and root rot (*Rhizoctonia solani*)	Pathogen
Pythium root rot (*Pythium spp*.)	Pathogen
Fusarium wilt, blight, and root rot (*Fusarium spp*.)	Pathogen
Sclerotinia stem rot (*Sclerotinia sclerotiorum*)	Pathogen
Pod and stem blight (*Diaporthe phaseolorum var. sojae*)	Pathogen
Bacterial Blight (*Pseudomonas syringae*)	Pathogen
Soybean mosaic virus (SMV)	Pathogen
Anthracnose (*Colletotrichum truncatum*)	Pathogen
Purple seed stain (*Cercospora kikuchii*)	Pathogen
Powdery mildew (*Microsphaera diffusa*)	Pathogen
Root knot (*Meloidogyne spp*.)	Pathogen
Spider mite (*Acari: Terranychidae*)	Consumer
Soybean cyst nematode (*Heterodera glycines*)	Consumer
Soybean looper, white fly (*Lepidopterans*)	Consumer
Soil insects	Consumer
Birds	Consumer
Animal browsers	Consumer
Pollinators	Symbiont or Beneficial Organism; Consumer
Mychorrhizal fungi	Symbiont or Beneficial Organism
Soil microbes	Symbiont or Beneficial Organism
Earthworms	Gene Transfer
Other *G. max*	Symbiont or Beneficial Organism
Others	---

SECTION 3
MAIZE (*ZEA MAYS* SUBSP. *MAYS*)

1. General Information

Maize, or corn, is a member of the *Maydeae* tribe of the grass family, *Poaceae*. It is a robust monoecious annual plant, which requires the help of man to disperse its seeds for propagation and survival. Corn is the most efficient plant for capturing the energy of the sun and converting it into food, it has a great plasticity adapting to extreme and different conditions of humidity, sunlight, altitude, and temperature. It can only be crossed experimentally with the genus *Tripsacum*, however member species of its own genus (teosinte) easily hybridise with it under natural conditions.

This document describes the particular condition of maize and its wild relatives, and the interactions between open-pollinated varieties and teosinte. It refers to the importance of preservation of native germplasm and it focuses on the singular conditions in its centre of origin and diversity. Several biological and socio-economic factors are considered important in the cultivation of maize and its diversity; therefore these are described as well.

A. Use as a crop plant

In industrialised countries maize is used for two purposes: 1) to feed animals, directly in the form of grain and forage or sold to the feed industry; and 2) as raw material for extractive industries. "In most industrialised countries, maize has little significance as human food" (Morris, 1998; Galinat, 1988; Shaw, 1988). In the European Union (EU) maize is used as feed as well as raw material for industrial products (Tsaftaris, 1995). Thus, maize breeders in the United States and the EU focus on agronomic traits for its use in the animal feed industry, and on a number of industrial traits such as: high fructose corn syrup, fuel alcohol, starch, glucose, and dextrose (Tsaftaris, 1995). It is also noteworthy to understand how corn is used in the rising consumption of sweet corn and popcorn in developed countries (White and Pollak, 1995; Benson and Pearce, 1987).

In developing countries use of maize is variable; in countries such as Mexico, one of the main uses of maize is for food. In Africa as in Latin America, the people in the sub-Saharan region consume maize as food, and in Asia it is generally used to feed animals (Morris, 1998).

Maize is the basic staple food for the population in many countries of Latin America and an important ingredient in the diet of these people. All parts of the maize plant are used for different purposes: processed grain (dough) to make "tortillas", "tamales" and "tostadas"; grain for "pozole", "pinole" and "pozol"; dry stalks to build fences; a special type of ear cob fungi can be used as food (that is, "corn smut", or *Ustylago maydis*). In general, there are many specific uses of the maize plant depending on the region. Globally, just 21 % of total grain production is consumed as food.

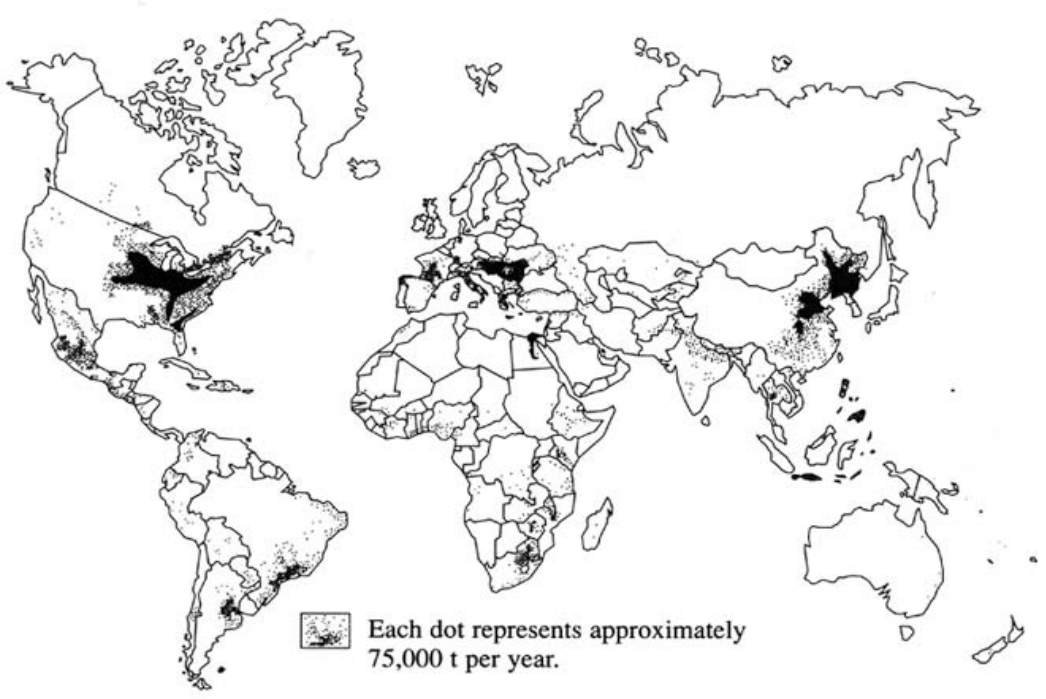
The countries, which have the highest annual maize consumption per capita in the world, are listed in Table 1.1.

Table 1.1 Consumption of maize *per capita* by country

Country	Annual consumption of maize per capita (Kg)
Malawi	137
Mexico	127
Zambia	113
Guatemala	103
Honduras	98
South Africa	94
El Salvador	93
Kenya	93
Zimbabwe	89
Lesotho	87
Venezuela	68
Nicaragua	56

Source : Morris, 1998

Figure 1.1 Maize production worldwide



Source : Morris, 1998

According to Morris (1998), "maize is the world's most widely grown cereal, reflecting its ability to adapt to a wide range of production environments" (Fig. 1).

Transgenic maize is already being used as a crop not only with agricultural purposes in several industrialized countries. Industrialised countries have dominant production of maize, because they possess

advantageous factors that contribute to generate maize surplus. First, "maize production is generally concentrated in zones of abundant rainfall and fertile soils" (Morris, 1998), and, second, the use of many inputs and technology is extensive (Pollak and White, 1995; Rooney and Serna-Saldivar, 1987; Shaw, 1988; White and Pollak, 1995). By contrast, in developing countries the situation is highly variable. From Mexico to the Northern Andean region in South America, maize is a very important staple food in rural areas and the use of technology together with improved varieties is limited. However, Brazil, Argentina and Chile resemble industrialised countries because in these countries maize is a "cash crop grown by large scale commercial producers using extensive mechanisation" (Morris, 1998).

In many countries of Latin America maize is produced on small units of land. For example, in Mexico most of the land planted with maize (77 %) is less than 5 hectares in size, which contributes 67 % of total production (Calva, 1992 in Turrent-Fernández *et al.*, 1997). Only 5 % of the units of land dedicated to the production of maize averaged 12.2 hectares. More recently (Turrent-Fernández *et al.*, 1997), land units of maize production have increased in size but the technology inputs are below average: only 40 % of producers utilised improved seed; 64 % used nitrogen and phosphorous to fertilise the soil; and only 42 % received technical assistance.

In Africa, maize is an important crop mainly in the eastern and southern regions where it is "the dominant food crop and the mainstay of rural diets" (Morris, 1998). Also, maize production in Africa is similar to the production in some Latin American countries because the peasants of less developed rural areas grow maize in small plots, using negligible amounts of inputs or technology and no improved varieties.

In Asia, China dominates maize production. China is the second largest producer of maize closely behind the United States (Morris, 1998). Asian countries produce maize for livestock feed and likewise Meso-America and most African countries; "farms are small, use of improved germplasm and purchased inputs is modest, and yields are generally low" (Morris, 1998).

2. Taxonomic Status of *Zea*

The Western Hemisphere genera *Zea* and *Tripsacum* are included in the tribe Maydeae (Table 1.2). The Asian genera of Maydeae are *Coix* (2n = 10, 20), *Polytoca* (2n = 20), *Chionachne* (2n = 20), *Schlerachne* (2n = 20) and *Trilobachne* (2n = 20).

Based on the morphology of the glumes of the male spikelets, Iltis and Doebley (1980) and Doebley and Iltis (1980) proposed a new classification system of the genus *Zea*. First, *Zea* was separated into two sections: LUXURIANTES and ZEA. The section LUXURIANTES grouped three species: *Z. luxurians*, *Z. diploperennis* and *Z. perennis*, and very recently it has included *Z. nicaraguensis* (Iltis and Benz, 2000). The section ZEA comprises only one species, *Z. mays*, which in turn is sub-divided into three subspecies: ssp. *mays*, for maize, ssp. *mexicana* for the races Nobogame, Central Plateau, Durango and Chalco (Wilkes, 1967; 1977) and ssp. *parviglumis*. This latter in turn is separated into two varieties, var. parviglumis for the race Balsas of Wilkes (1967) and var. huehuetenangensis for the race Huehuetenango of Wilkes (1967). Later on Doebley (1984, 1990) suggested that the var. huehuetenangensis should be elevated to a subspecies level.

Regarding the separation of the genus into sections LUXURIANTES and ZEA there is no controversy since morphological (Doebley, 1983; Smith *et al*., 1981), isoenzymatic (Doebley *et al.*, 1984; Smith *et al.*, 1984), cytoplasm organelle DNA (Doebley *et al.*, 1987a, b; Sederoff *et al*., 1981; Timothy *et al.*, 1979), and cytological (Kato, 1984; Kato and Lopez, 1990) evidence supports it.

The main controversy resides on the classification system within the section ZEA, particularly the grouping of the annual teosintes and maize into a single species, *Z. mays*. There is evidence showing that annual teosintes and maize are completely isolated from each other based on chromosome knob data (Kato, 1984; Kato and Lopez, 1990), and morphological-ecological data (Doebley, 1984). Although the isoenzymatic data suggest a low level of introgression between populations of these two plant types (Doebley, 1984; 1990), they have mainly the same isozyme alleles and the frequencies of these are distinct between most of the races of teosinte and most of the races of maize (Goodman, 1988). If it is accepted that the annual teosintes and maize are genetically isolated, then according to the biological species concept, the classification of the section ZEA made by Iltis and Doebley (1980) and Doebley and Iltis (1980) would not be acceptable, and would support the one proposed by Wilkes (1967).

Wilkes (1967) classified the annual teosintes within six races: Nobogame; Central Plateau; Chalco; Balsas; Huehuetenango; and Guatemala. Bird (1978) raised the race Guatemala into species rank, *Z. luxurians*.

The perennial teosintes from Jalisco in Mexico are separated into two more species (Iltis *et al*., 1979) that have a ploidy difference, *Z. perennis* (2n=40) and *Z. diploperennis* (2n=20).

Doebley and Iltis (1980) and Iltis and Doebley (1980) classified teosinte as two subspecies of *Z. mays*: *mexicana* (Chalco, Central Plateau, and Nobogame) and *parviglumis* (var. parviglumis=Balsas and var. huehuetenangensis=Huehuetenango).

Table 1.2 Classification of the genus Zea within the tribe Maydeae of the Western Hemisphere, and the genus Tripsacum

Family: Poaceae
Subfamily: Panicoideae
Tribe: Maydeae

Western Hemisphere:
Genus *Zea*[1]
Section *ZEA*
- *Zea mays* L. (maize)
- *Zea mays* subsp. *mays* (L.) Iltis (maize, $2n^2 = 20$)
- *Zea mays* subsp. *mexicana* (Schrader) Iltis (teosinte, 2n = 20))
  - race Nobogame[3]
  - race Central Plateau[3]
  - race Durango[4]
  - race Chalco[3]
- *Zea mays* subsp. *parviglumis* Iltis and Doebley (teosinte, 2n = 20)
  - var. parviglumis Iltis and Doebley (=race Balsas)
  - var. *huehuetenangensis* Doebley (=race Huehuetenango)

Section *LUXURIANTES* Doebley and Iltis
- *Zea diploperennis* Iltis, Doebley and Guzman (perennial teosinte, 2n = 20)
- *Zea luxurians* (Durieu) Bird (teosinte, 2n = 20)
- *Zea nicaraguensis*[5] (2n = 20?)
- *Zea perennis* (Hitchc.) Reeves and Mangelsdorf (2n = 40)

Genus *Tripsacum*
- *T. andersonii (2n* = 64)
- *T. australe (2n = 3*6)
- *T. bravum (2n = 3*6, 72)
- *T. cundinamarce* (2n = 36)
- *T. dactyloides (2n* = 72)
- *T. floridanum (2n* = 36)
- *T. intermedium (*2n = 72)
- *T. manisuroides (2*n = 72)
- *T. latifolium (2n* = 36)
- *T. pereuvianum (2*n = 72, 90, 108)
- *T. zopilotense (2n* = 36, 72)
- *T. jalapense (2n* = 72)
- *T. lanceolatum (2*n = 72)
- *T. laxum (2n = 36*?)
- *T. maizar (2n = 3*6, 72)
- *T. pilosum (2n* = 72)

[1] Iltis and Doebley, 1980; Doebley, 1990. [2] diploidy number. [3] Wilkes, 1967. [4] Sánchez-González *et al.*, 1998. [5] Iltis and Benz, 2000.

3. Identification Methods

A. General description of *Zea mays*

Zea mays is a tall, monoecious annual grass with overlapping sheaths and broad conspicuously distichous blades. Plants have pistillate inflorescences enclosed in numerous large foliaceous bracts (ears), from 7 to 40 cm long, with spikelets in 8 to 16 rows on a thickened axis (cob) in the leaf axils and staminate spikelets in long spike-like racemes that form large spreading terminal panicles (tassels).

B. Identification among races of *Zea mays*

To study and classify this huge variation, a system of racial classification was established (Wellhausen *et l*., 1952; Wellhausen *et al*., 1957; Brown, 1953; Sato and Yoshida, 1956; Hateway, 1957; Roberts *et al.,* 1957; Briger *et al*., 1958; Timothy *et al*., 1961, 1963; Grobman *et al.;*1961; Grant *et al*., 1963; Brandolini, 1968; Mochizuki, 1968; Costa-Rodriguez, 1971; Paterniani and Goodman, 1977; Wellhausen, 1988; Avila and Brandolini 1990). Latin American countries, specifically Mexico, possess a great wealth of maize genetic diversity. There have been more than 40 land races of maize in Mexico (Wellhausen *et al*., 1952; Hernández-Xolocotzi and Alanís, 1970; Ortega-Pazcka, 1980; Benz, 1986; Sánchez-González, 1989), and almost 250 land races in the Americas (Goodman and Brown, 1988).

C. Identification among *Zea mays* and wild species

The closest known relative of *Zea* is *Tripsacum*. The genus *Tripsacum* comprises two sections: section FASCICULATA with five species; and section TRIPSACUM with twelve species. The chromosome number varies from 2n=36 to 2n=108. All species are perennials (deWet *et al*., 1982, 1983). Twelve of these are native to Mexico and Guatemala with an extension of *T. dactyloides* throughout the eastern half of the United States, the tetraploids being near the East coast and the diploid in the central region. *T. lanceolatum* occurs in the southwest of the United States and *T. floridanum* is native to South Florida and Cuba. Three species of *Tripsacum* are known in South America.

Species of the section FASCICULATA are mostly and widely distributed in Meso-America, however, *T. lanceolatum* is found along the North of Sierra Madre Occidental, Mexico, up to Arizona. On the other hand, species of the section TRIPSACUM are distributed more extensively than the section FASCICULATA, although different species are found in relatively restricted territories; for example, *T. dactyloides* is found from a latitude about 42° North and 24° South. *T. dactyloides* tetraploid forms are also found in Kansas and Illinois in the United States. *T. manisuroides* is known only from Tuxtla Gutierrez, Chiapas, Mexico (deWet *et al*., 1981, 1982, 1983). *T. andersonii* is of uncertain origin and is mostly sterile, it is an unusual species in that there is cytological (deWet *et al*., 1983) and molecular evidence showing that its 2n=64 chromosomes comprise 54 *Tripsacum* chromosomes and ten *Zea* chromosomes (Talbert *et al*., 1990).

D. Genetics and molecular identification

Maize has been one of the best studied plants in disciplines ranging from classical genetics to molecular biology. The study of maize has contributed to major breakthroughs in science such as the discovery of transposable elements (McClintock, 1929, 1934, 1944a, 1944b, 1944c, 1945; Fedoroff and Botstein, 1992). McClintock first characterised the ten chromosomes of maize using mitotic studies. Presently cytological research is being conducted on chromosome staining techniques, meiotic mutants, examination of the B chromosomes and better understanding of the events involved during synapsis. Transposable elements are very important in maize genetics. Many different transposable element systems have been described for maize, the best characterised has been the Activator (Ac) and Dissociation (Ds)

system. Ac/Ds comprises a family of maize transposable elements. Ac is the autonomous member of the family, capable of producing a transposable factor needed for mobility. Ds elements are not autonomous and capable of transposition only when trans-activated by Ac. Both genes have now been cloned and their mode of action is well characterised (Tsaftaris, 1995). A recent review of transposable elements is found in Federoff (2000).

The genetics of mitochondria and chloroplast in maize are of special importance. The mitochondrial genomes (mtDNAs) of higher plants are larger than those of mammalian or fungal mitochondrial genomes. The higher plant mitochondrial genomes are also more variable in their organization and have a larger coding capacity than mitochondrial genomes in mammals and fungi. Five types of mitochondrial genomes have been identified. Their designations are NA and Nb for the normal male fertile phenotypes, and T, S and C for the three different cytoplasmic male sterile (cms) phenotypes. Physical maps for three of the maize cytotypes have been completed. Mitochondrial genomes of higher plants have integrated DNA sequences that originate from other cell compartments (Tsaftaris, 1995). In contrast to plant mitochondria genomes, the chloroplastic genome is smaller and simpler; thus many chloroplastic genomes have been completely sequenced. The similarities between the genomes of chloroplasts and bacteria are striking. The basic regulatory sequences, such as transcription promoters and terminators, are virtually identical in both cases. Protein sequences encoded in chloroplasts are clearly recognisable as bacterial, and several clusters of genes with related functions are organised in the same way in the genomes of chloroplasts, *E. coli*, and cyanobacteria. In about two-thirds of higher plants, including maize, the chloroplast as well as mitochondrial DNA, is maternally inherited (Tsaftaris, 1995).

There is an abundant literature on the genetics, physiology, cytogenetics and molecular biology of maize and concise, thorough reviews are available (Coe *et al.*, 1988; Carlson, 1988; Walbot and Messing, 1988; Hageman and Lambert, 1988; Freeling and Walbot, 1994).

E. Maize genome maps

The first RFLP map of corn was developed by Helentjaris *et al.*, (1985, 1986a, 1986b). The corn linkage map encompasses approximately 1200 map units. The RFLP markers are not randomly distributed. The corn genome is about 5 X 10^6 kb, then there would be approximately 4 X 10^3 kb per map unit. It includes highly repeated sequences that constitute about 20% of the genome; these sequences are present in about ten superabundant sequence types. There are more than 1000 different moderately repetitive sequence families collectively representing 40% of the genome, this leaves approximately 40% single copy sequences, or more than 10^6 approximately gene size pieces.

Maize has one of the most well saturated genetic maps of any cultivated plant of this genome size. In principle this offers the possibility of easily locating any transgene and/or identifying any specific genotype (Tsaftaris, 1995). Recent maize genome maps and most of the information on the maize genome can be found in the following web addresses: http://www.agron.missouri.edu; http://www.zmdb.iastate.edu; http://w3.aces.uiuc.edu/maize-coop/. An expressed sequence tag (EST) database can also be found at http://www.zmdb.iastate.edu.

4. Centre of Origin /Diversity, Maize Diversity

There are four main hypotheses on the origin of maize.

- **The descent from teosinte hypothesis.** This is the oldest proposal and was advanced by Ascherson in 1895 (Mangelsdorf and Reeves, 1939) and proposes that maize was domesticated from teosinte by human selection. This is the most widely accepted hypothesis at present (Beadle, 1986; deWet and Harlan, 1972; Doebley and Stec, 1991; Doebley, 1990; Galinat, 1977; Iltis and

Doebley, 1980; Goodman, 1988; Kato, 1984; Kato and López, 1990; Timothy *et al.,* 1979). The main problem with this hypothesis was how the distichous small female spike could have been transformed into the polistichous gigantic maize spike (ear) by human selective domestication. However, Doebley *et al.,* (1990) have found five major genes controlling 'key' traits distinguishing maize and teosinte, and more recently Wang *et al.,* (1999) have discussed a gene controlling the inflorescence character in teosinte and maize.

- **The tripartite hypothesis.** The main assumption of this hypothesis is that there existed a wild maize in the past, which is considered extinct at present. This wild maize gave origin to the annual teosintes by crossing with *Tripsacum*. Further crossing of teosinte with wild maize gave rise to the modern races of maize (Mangelsdorf and Reeves, 1939; and Mangelsdorf, 1974). Later on Mangelsdorf *et al.,* (1981) based on experimental crossing between *Z. diploperennis* and the race Palomero Toluqueño of maize and further observations of its progenies, proposed that the annual teosintes are the products of this crossing. The fact that until now no evidence at all has been found about the existence, in the past or at present, of a wild maize, this hypothesis has lost much credence with time (although see Eubanks, 1995).

- **The common origin hypothesis.** This hypothesis proposes that maize, teosinte and *Tripsacum* originated by "ordinary divergent evolution" from a common ancestor. Consequently, it is conceived that there existed a wild maize plant that further was transformed into a cultivated plant by the selection and care of man (Weatherwax, 1955; Randolph, 1955; Randolph, 1959). The postulation that wild maize existed in the past makes this hypothesis not acceptable, as in the case of the tripartite hypothesis.

- **The catastrophic sexual transmutation hypothesis.** This hypothesis proposes that the maize ear evolved from the terminal male inflorescence of teosinte lateral branch by a "… sudden epigenetic sexual transmutation involving condensation of primary branches [and further] genetic assimilation under human selection of an abnormality, perhaps environmentally triggered" (Iltis, 1983). The finding of five mutant genes controlling key characters separating maize from teosinte (Doebley and Stec, 1991; Doebley *et al.,* 1990) seems to make the catastrophic sexual transmutation hypothesis untenable.

Centre of maize domestication

The Meso-American region located within middle South Mexico and Central America is recognised as one of the main centres of origin and development of agriculture as well as centre of origin and diversification of more than one hundred crops (Vavilov, 1951; Smith, 1995; Harlan, 1992). At the present time, there is no agreement about where exactly maize was domesticated and there are several proposals in this regard. Based on the findings of archaeological materials from the maize plant (pollen, cobs, husks, and other remnants) in the United States and Mexico, which are older than those found in South America, Randolph (1959) proposed that maize was domesticated, independently, in the southwestern United States, Mexico, and Central America.

Mangelsdorf (1974) proposed that "corn had not one origin but several in both Mexico and South America", because the archaeological evidences are found in Mexico and several morphological characteristics in extant population found in the maize races of South America (Andes region) in comparison to those races of Meso-America.

The preliminary studies of McClintock (1959, 1960) on the chromosome knob constitutions of several races of maize from South America, Mexico and Central America, led her to conclude "that present-day maize may have derived from several different centres". These chromosome studies were further exploited

(Kato, 1976, 1984; McClintock, 1978; McClintock *et al*., 1981). They confirmed McClintock's previous conclusion and led to the proposal that maize was domesticated, independently, in four centres located in Mexico (two in Oaxaca-Chiapas region, one in the central highlands and one in the mid-highlands of Morelos-northern Guerrero), and one in the highlands of Guatemala. "This conclusion is based on the fact that chromosome knobs are not geographically and racially distributed at random, and that some knobs show restricted distributions following clear-cut pathways through specific territories, dispersion that clearly indicate that they were started in specific regions or centres of distribution. These centres are then considered as the places where original maize germplasm was domesticated from teosinte populations that were already cytogenetically well diversified" (Kato, 1984).

Contrary to the above multicentres origin of maize proposals, the isoenzymatic variation studies of maize and teosinte suggested to Doebley *et al.*, (1987a) that maize was domesticated once in the Balsas basin region because "… all maize races of Mexico are isoenzymatically closer to var. parviglumis than to other teosintes…". Supporting this hypothesis, further molecular genotyping studies also suggest that maize originated from a single domestication in southern Mexico 9000 years ago (Matsuoka *et al.*, 2002).

Maize diversity

From the time of the discovery of America, Columbus noted the presence of corn on the North coast of Cuba and introduced it to Europe through Spain. At that time, corn was grown from Chile to southeastern Canada. Within two generations, after its introduction in Europe, corn became a cultivated crop throughout the world (Goodman, 1988). Germplasm resources are preserved *ex-situ* in many parts of the world, however, only in the Meso-American region there still exists, *in situ*, the original ancient maize that gave rise to improved varieties that are grown in all regions of the world. Most of the maize variation can be found in the Meso-American region and the northern part of South America. The great diversity of environments and conditions have created the basis for the development of maize varieties well adapted to harsh conditions of soil and climate as well as to biotic stresses. There is a close correlation among community culture, production system and the type of consumption of maize, with the diversification and variation of maize (Aguirre *et al*., 1998; Louette and Smale, 1998).

Maize germplasm diversity is threatened by several factors: improved seed adoption; shift to cultivation of cash crops; and change in land use (Aguirre *et al*., 1998; Bellon *et al*., 2000; Louette, 1997). In some areas the adoption of hybrids and improved seed has increased dramatically, which has reduced the production of maize for traditional uses and, consequently, the increase of genetic erosion. Although these factors play an important role in reducing maize germplasm diversity, the persistence of maize land races in the Central American region is evident. Small farmers, peasants and indigenous ethnic groups and communities in many Latin American countries still preserve and select traditional maize.

Some arguments to explain the maize land race survival have been advanced (Ortega-Pazcka, 1973). The paramount importance of native maize for small communities, ethnic groups, small farmers and peasants, resides in the fact that land races of maize have very specific qualities for food and special uses as mentioned in Section I, rather than maize yield itself; therefore, many land races of maize have not been displaced by more productive maize types promoted by governmental agencies. For example, in Mexico after 50 years of maize genetic improvement programs, the adoption of hybrids and improved varieties is low. The research of Hernández-Xolocotzi (1972), Ortega-Pazcka (1973), Benz (1986), and Ortega-Pazcka *et al.*, (1988), on maize diversity and peasant communities, demonstrates that local maize has been preserved by peasants, using traditional methods, basically intact for decades. As the result of a poll carried out in 1992 (CIMMYT, 1994), it was concluded that open pollinated land races of maize cover 42% of arable land dedicated to maize in less developed countries.

The approach for conservation of Latin American maize land races relies on two main criteria: the adaptation to a particular ecological niches and special forms of consumption of specific land races. Native germplasm utilisation has varied depending on the country and the needs of development. In general, the strategy is to identify sources of elite germplasm by means of characterizing and evaluating samples from land race collections, consisting of composite groups, populations and pools. National programs, international institutions, private seed industries and universities use these germplasm materials. Native maize land races have not been widely used for improvement programs and in Mexico, for example, only 10% of Mexican maize land races have been incorporated in specific breeding programs. There are a couple of examples in Mexico where native races of maize were characterised and evaluated for selection to generate improved populations, which were released as new open pollinated varieties: variety V520 (from land race San Luis Potosí-20); and variety Rocamex V7 (from land race Hidalgo-7). However, there is still germplasm in farmers' fields that have not been evaluated for their improvement and utilisation (Márquez-Sánchez, 1993).

Examples of maize land races specifically adapted to special conditions are (Hernández-Xolocotzi, 1988): Gaspe, short growing season (early maturity); Guatemalan Big Butt, long growing season (late maturity); Tuxpeño, Celaya, Chalqueño, Cuban Yellow Flint and Cuzco Gigante, high efficiency and productivity under good rainfed conditions; Chococeño, Enano and Piricinco, tolerance to high temperature and humidity; Cónico norteño, tolerance to semi-dry environments; Palomero Toluqueño, Cónico, Cacahuacintle and Sabanero, well adapted to high elevations, low temperature; Nal-tel, adapted to calcareous soil.

5. Reproductive Biology

A. Sexual reproduction

Zea mays is an allogamous plant that propagates through seed produced predominantly by cross-pollination and depends mainly on wind borne cross-fertilisation. Z. *mays* is a plant with a protandrous inflorescence; however, decades of conventional selection and improvement have produced varieties of maize with protogynous traits. Z. *may*s has staminate flowers in the tassels and pistillate flowers on the ear shoots.

The tassel. The structure and development of the stamens are similar to other grasses. The anther develops four chambers or loculi each one containing a central row of archeosporial cells that gives rise to sporogenous tissue. After seven weeks the microspore mother cells are in the meiosis stage. Microspores are organised around four nuclei and become mature pollen grains. The amount of pollen produced by a tassel is estimated at 18 million pollen grains (Kiesselbach, 1980). Probably the best-improved varieties would produce more than this. On average 21,000 pollen grains could be produced for each kernel on an ordinary ear with 1000 kernels. Kiesselbach (1980) calculated that: "With a stand of three stalks in hills 42 inches apart, an area of 588 square inches is available in the field for each stalk. Thus an average of 42,500 pollen grains are provided for each square inch of the field. If the silks of an ear display a total surface of 4 square inches they will intercept about 170,000 pollen grains. Estimating 1,000 silks per ear, this amounts to 170 pollen grains per silk. Considering that corn in the field sheds pollen for 13 days, each silk receives an average of 13 pollen grains per day."

The ear shoot. At each node of the stem there is an axillary bud enclosed in the prophylum. Only one or two of these axillary buds will develop as ear shoot and reach the fertilisation stage. At first the ear is smooth but protuberances soon form in rows. The basal protuberances are formed first and development advances towards the tip of the ears. Each one becomes two lobed, each lobe developing into a spikelet with two flowers, only one of which commonly persists. The growing point of the upper flower is differentiated to form the functional pistil. The part above the attachment of the carpels develops a single

sessile ovule, which consists of a nucellus with two integuments or rudimentary seed coats. The united carpels, which will form the ovary wall or pericarp of the mature kernel, grow upward until they completely enclose the ovule. Where they meet, the functionless so-called stylar canal is formed. The two anterior carpels, which face the ear tip, form outgrowths, which develop into the style or silk. The surface of the silk becomes covered with numerous hairs, which are developed from cells of the epidermis. At the base of the silk is a growth zone where new cells develop, causing continuous elongation of the silk until it is pollinated and fertilisation takes place. The development of the embryo sac is characteristic of the grass family. One of the three nuclei at the micropylar end enlarges and becomes the nucleus of the egg, while the others become the nuclei of the synergids. At this stage the embryo sac is ready for fertilisation but if pollination is prevented it may remain in this condition for some time, perhaps two weeks, after which the embryo sac and nucellus disorganise and fertilisation is no longer possible.

Fertilisation occurs after the pollen grain is caught by the silk and germinates to create the pollen tube which penetrates up to the micropyle and enters the embryo sac. The pollen is carried mainly by wind, thus it is highlighted that pollination can occur even, although rarely, over long distances measured in kilometers.

B. Asexual reproduction

There is no asexually reproductive maize. Cell/tissue culture techniques can be used to propagate calli and reproduce tissues or plants asexually; however, with maize cells and tissues these techniques are difficult.

6. Crosses

A. Intra-specific crosses

Maize is essentially 100% open-pollinated (cross-fertilising) crop species. Until the 20th century, corn evolved through open pollinated varieties, which are a collection of heterozygous and heterogeneous individuals developed by mass selection of the people from the different civilisations existing in the Americas (Hallauer, 2000). Corn pollen is very promiscuous, lands on any silk, germinates almost immediately after pollination, and within 24 h completes fertilisation. Thus all corns will interpollinate, except for certain popcorn varieties and hybrids that have one of the gametophyte factors of the allelic series *Ga* and *ga* on chromosome four (Kermicle, 1997).

There is a great sexual compatibility between maize and annual teosinte and it is known that they produce fertile hybrids (Wilkes, 1977). In areas of Mexico and Guatemala maize and teosinte freely hybridise when in proximity of each other. Wilkes (1977) reported a frequency of one F1 hybrid (corn x teosinte) for every 500 corn plants or 3 to 5 % of the teosinte population for the Chalco region of the Valley of Mexico. Kermicle and Allen (1990) have shown that maize can introgress to teosinte; however, there is incompatibility between some maize populations and certain types of teosinte resulting in low fitness of some hybrids that prevents a high rate of introgression (Evans and Kermicle, 2001).

B. Inter-specific crosses

Although it is extremely difficult, *Tripsacum* species (*T. dactyloides*, *T. floridanum*, *T. lanceolatum*, and *T. pilosum*) can be crossed with corn; however, hybrids have a high degree of sterility and are genetically unstable (Mangelsdorf, 1974). Galinat (1988) advanced that since *Tripsacum* and *Zea* have different chromosome numbers, the addition of an extra *Tripsacum* chromosome into the maize genome would occur with a low frequency and consequently the rate of crossing-over would be extremely reduced. Despite these arguments, Eubanks (1995, 1998) developed a method for transferring *Tripsacum* genes into maize. In this method two wild relatives of maize, *Tripsacum* and diploid perennial teosinte (*Zea*

diploperennis), are crossed to produce a hybrid, which is called tripsacorn, used to generate maize-tripsacorn hybrids. The use of tripsacorn is intended to confer resistance to pests and disease, drought tolerance and improved uniformity. Recently it has been claimed (Eubanks, 2000) that traits such as apomixis, totipotency, perennialism, adaptation to adverse soil conditions and to carbon dioxide enriched atmosphere can be transmitted to maize via maize x *Tripsacum*-perennial teosinte (and/or its reciprocal).

The cross between maize and *Tripsacum* has been studied since long ago (deWet *et al.*, 1973; Bernard and Jewell, 1985), and recently efforts have been made to transfer genes related to traits like apomixis from *Tripsacum* to maize (Burson *et al.*, 1990; Savidan and Berthaud, 1994; Hanna, 1995; Leblanc *et al.*, 1995; Grimanelli *et al.*, 1998; Grossniklaus *et al.*, 1998). Maize x *Tripsacum* hybrids have been produced and consequently several patents on apomictic maize have been published (Kindiger and Sokolov, 1998; Savidan *et al.*, 1998; Eubanks, 2000).

C. Gene flow

The interaction between domesticated plants and their wild relatives can lead to hybridisation and in many cases to gene flow of new alleles from a novel crop into the wild population (Ellstrand *et al.*, 1999). While gene flow *per se* is not a concern, theoretically, it can lead to the potential for the evolution of aggressive weeds or the extinction of rare species. There has been preliminary documentation of this in some cases although not for maize (Ellstrand *et al.*, 1999).

As mentioned in Section VI-A some teosinte species can produce fertile hybrids with maize. All teosintes, members of the Section LUXURIANTES and subspecies *mexicana* and *parviglumis*, occur only in Mexico and Guatemala (Sánchez-González and Ruiz-Corral, 1997). It has been documented that maize and teosinte often interact, particularly with *Zea mays* ssp. *mexicana* (Wilkes, 1977). Also, the known distribution of teosintes, together with high likelihood of the presence of land races in the maize production areas of Mexico indicates, as shown in Appendix II, that there exist high probabilities of genetic exchange between conventional maize, land races and teosinte (Sánchez-González and Ruiz-Corral, 1997; Serratos-Hernández *et al.*, 1997; Serratos-Hernández *et al.*, 2001). However, there is some evidence of restricted gene flow between *Zea* spp. that occurs predominantly from teosinte into maize (Doebley *et al.*, 1987a). To date, there is no genetic analysis of morphologically intermediate plants that could identify "whether the maize-teosinte intermediates are true hybrids, introgressants or crop mimics" (Ellstrand *et al.*, 1999). Out-crossing of maize with *Tripsacum* species is not known to occur in the wild.

Another factor to take into account regarding gene flow is the exchange of seed and traditional maize improvement practised by peasant communities and small farmers. As observed by Louette (1997), rural communities are open systems where "...there is a constant flow of genetic material among communities over large areas." therefore, as in the case of Mexico, "...a land race variety, an improved variety, or a transgenic variety of maize, can reach any zone of the country, even the most isolated ones, such as those where teosinte grows." The human factor together with the changes in policy and strategies in maize production (Nadal, 1999) may increase several fold the chance of gene flow between improved maize, teosinte and landraces.

7. Agro-ecology

A. Cultivation

Although maize was domesticated and diversified mostly in the Meso-American region, at present it is cultivated mainly in warm temperate regions where the conditions are best suited for this crop (Norman *et al.*, 1995).

Maize is an annual plant and the duration of the life cycle depends on the variety and on the environments in which the variety is grown (Hanway, 1966). Maize cannot survive temperatures below 0˚ C for more than 6 to 8 hours after the growing point is above ground (5 to 7 leaf stage); damage from freezing temperatures, however, depends on the extent of temperatures below 0˚ C, soil condition, residue, length of freezing temperatures, wind movement, relative humidity, and stage of plant development. Light frosts in the late spring in temperate areas can cause leaf burning, but the extent of the injury usually is not great enough to cause permanent damage, although the corn crop will have a ragged appearance because the leaf areas damaged by frost persist until maturity. Maize is typically grown in temperate regions due to the moisture level and number of frost-free days required to reach maturity. The number of frost-free days dictates the latitude at which corn varieties with different life cycle lengths can be grown. Maize having a relative maturity of 100 to 115 days is typically grown in the U.S. corn belt. Maize varieties with different relative maturities do not occur in parallel east-to-west zones because they are also dependent on prevailing weather patterns, topography, large bodies of water, and soil types (Troyer, 1994 in Hallauer, 2000).

In tropical regions, maize maturity increases due to altitude effects. Tropical land races of maize in the tropics characteristically show three to five ears and axillary tillering, as opposed to modern cultivars that suppress lower ears and tillers (Norman *et al.*, 1995). In the tropics Oxisols, Ultisols, Alfisols and Inceptisols are best suited for maize production; however, maize is adapted to a wide variety of soils in the tropics, from sands to heavy clay. Of particular importance is aluminium toxicity for maize on acid tropical soils. Liming can solve this problem, "Deep lime incorporation in the subsoil of some Oxisols has overcome aluminium toxicity, thereby improving rooting depth in maize and tolerance to dry periods" (Norman *et al*., 1995).

The farmland of Mexico covers a wide range of ecological conditions: from sea level to 2800 meters, from very dry to wet climates, well drained to poorly drained soils, flat to severe slopes, shallow to deep soils, low to high solar radiation; drought, wind and frost damage are common.

The poorest farmers are typically Indian farmers that inhabit the Sierras. Dry beans, squash, grain amaranth and several other species were also domesticated by the inhabitants of the region, as complements to their diet. They also developed the typical “milpa cropping system” as a cultivated field that may involve the association, inter-cropping, or relay-cropping of maize, beans, squash, grain amaranth, tree species and several tolerated herbal species. The isolation of these farming communities has caused the development of a great resource of maize germplasm diversity, which is conserved using *in situ* and *ex situ* (germplasm banks) means. Inter-cropping of maize with other crops is practiced in many areas of less developed countries (Norman *et al.*, 1995). These systems imply changes at the level of cultivation and management of maize production which are important in terms of ecological relationships.

B. Volunteers and weediness

Maize has lost the ability to survive in the wild due to its long process of domestication, and needs human intervention to disseminate its seed. Although corn from the previous crop year can over-winter and germinate the following year, it cannot persist as a weed. The presence of corn in soybean fields following the corn crop from the previous year is a common occurrence. Measures are often taken to either eliminate the plants with the hoe or use of herbicides to kill the plants in soybean fields, but the plants that remain and produce seed usually do not persist during the following years. Volunteers are common in many agronomic systems, but they are easily controlled; however, maize is incapable of sustained reproduction outside of domestic cultivation. Maize plants are non-invasive in natural habitats (Gould, 1968). In contrast to weedy plants, maize has a pistillate inflorescence (ear) with a cob enclosed with husks. Consequently seed dispersal of individual kernels does not occur naturally. Individual kernels of corn, however, are distributed in fields and main avenues of travel from the field operations of harvesting the crop and transporting the grain from the harvested fields to storage facilities (Hallauer, 2000).

C. Soil ecology (Microbiology of maize rhizosphere)

Maize root system acts as a soil modifier due to its association with several microbial groups such as bacteria, fungi, actinomycetes (Vega-Segovia and Ferrera-Cerrato, 1996a), protozoa and mites. The highest microbial population usually is bacteria, followed by fungi and actinomycetes. All these microbial groups play a particular role in the soil ecology, such as nutrimental cycling and the availability of nutrients for plant growth. In addition, these microbial organisms contribute to the protection of the root system against soil pathogens.

Some research has been oriented to understand more on microbial activity and its physiology. For instance, the physiology of free nitrogen-fixing bacteria such as Azotobacter, Beijerenckia and Azospirillum which have been found in the rhizosphere of several maize cultivars and teosinte (González-Chávez *et al.,* 1990; González-Chávez and Ferrera-Cerrato, 1995; Vega-Segovia and Ferrera-Cerrato, 1996b).

There is information related to symbiosis with arbuscular mycorrhizal fungi (AMF) which shows that these endophytes associate with specific maize genotypes (González-Chávez, and Ferrera-Cerrato, 1989; González-Chávez and Ferrera-Cerrato. 1996). There are reports related to the capability of a single AMF to establish symbiosis with a wide range of maize land races and teosinte (Santamaría and Ferrera-Cerrato, 1996; Benítez *et al.*,unpublished data). All these materials are used in Mexican agriculture. The role of these symbiosis relationships is to increase root metabolism in order to improve phosphorus uptake.

A great deal of life diversity is associated with maize grown in the milpa system of the Sierras. One example is the adaptation developed by a type of maize race in the Mixe Sierra of Oaxaca. The brace roots are overdeveloped and covered by a mucilaginous material that harbours species of nitrogen fixing free bacteria (R. Ferrera-Cerrato, personal comm.).

Soil ecology studies are undertaken to identify micro-organisms with agricultural value in places where maize is cultivated (Pérez-Moreno and Ferrera-Cerrato, 1997). Nowadays, these micro-organisms are being studied for the potential to augment corn cultivation. Selective breeding and nutrient management are also being evaluated for enhancing maize production.

D. Maize-insect interactions

In Appendix III, a list of common insect pests and pathogens of maize is presented.

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APPENDIX I

Maize Biotechnology

For practical purposes maize biotechnology could be divided into two fields: genetic engineering and molecular genetics.

Molecular genetics refers to the identification and location (genome mapping) of genes within the genome of organisms by means of molecular techniques that make use of the chemical properties of DNA (Hoisington *et al*., 1998). The marker technologies presently available for genomics work are: 1) Restriction Fragment Length Polymorphisms (RFLPs); 2) Random Amplified Polymorphic DNAs (RAPDs); 3) Sequence Tagged Sites (STSs); 4) Simple Sequence Repeats (SSRs); 5) Amplified Fragment Length Polymorphisms (AFLPs); and 6) Single Nucleotide Polymorphisms (SNPs). These technologies have been applied in maize breeding through fingerprinting for identification of genotypes, monitoring genetic diversity and for the efficient management of genetic resources (Hoisington *et al*., 1998). Other applications of molecular genetics and molecular markers are 1) Comparative Mapping, and 2) Marker Assisted Selection.

Genetic engineering methodologies can make possible the insertion of foreign DNA, from organisms of different species, into another individual organism. In maize, at the commercial level, the introduction of foreign DNA has been successfully accomplished through a technique known as biolistics. In this technique, DNA coated microparticles are shot by means of an air compression device, to cells in plant tissue or callus. In the case of maize, embryogenic callus is used for bombardment with foreign DNA. To identify the cells that have taken up the foreign DNA in maize, a herbicide resistant selectable gene has been used. Fertile transgenic maize plants have also been produced using 1) PEG-mediated protoplast transformation; 2) electroporation of intact or partly degraded cells of immature embryos, callus or embryonic suspensions; 3) 'whiskers' technology; and 4) *Agrobacterium*-mediated transformation.

At present there are two types of commercially released transgenic maize produced by means of genetic engineering: 1) Insect pest resistant maize or Bt-maize; and 2) Herbicide resistant maize. However, more research and development in this area is underway. Transgenic maize with elevated (10 KD) zein and methionine has been obtained (Anthony *et al.*, 1997). Antifungal proteins, such as chitinases and beta-1,3-glucanases, have been genetically engineered to attempt expression in the maize kernels with the aim to prevent the growth of *Aspergillus flavus* and the production of aflatoxins (Duncan *et al.*,1985; Wu *et al.*, 1994; Wan *et al*., 1995). Transgenic maize will serve as bioreactors for producing various biomolecules with applications in food, feed and the pharmaceutical industry (Nikolov, 1999).

The complicated and plastic nature of organellar genomes especially those of maize mitochondria, requires special consideration for the stability of the cytoplasmic male sterility genes if they are used for preventing pollen formation. Equally these features of organelle genomes would also apply to any genes cloned into them (since recent developments indicate that organelles could be a better target for generating transgenic plants). Therefore, stable incorporation of a transgene into the plastid genome guarantees amplification of the transgene, potentially resulting in a very high level of foreign gene expression. Since chloroplast (and mitochondrial) genomes resemble the genomes of other organisms and are most probably evolutionarily related, the possible transfer of genes from these organelles to microorganisms should be studied in the future if more and more transgenes are targeted to these organelles

The great similarity between the chloroplastic genome and microbial genomes was one of the reasons for choosing the chloroplast as a target for transferring native microbial genes to plants. For instance since the transcriptional machinery of the plastid is prokaryotic in origin and its genome is relatively A-T rich, it was possible that native Bt toxin genes from *B. thuringiensis* might be efficiently expressed in this organelle without nuclear modification. In addition, plant cells may contain up to 50,000 copies of the circular plastid genome.

Transposable elements are not expected to affect transgenes differently from their reported effects on non-modified genes of maize, unless sequences of the transposable element are contained in the inserted genetic material (Tsaftaris, 1995).

The potential crossing of landrace maize germplasm with transgenic improved maize, hybrids or inbreds should be considered carefully since, for example in Mexico, it is well known the high incidence of transposable elements in landraces of maize (Gutiérrez-Nava *et al.*, 1998).

Several investigations conducted by national and international research instititutions have demonstrated that gene exchange between improved maize and landraces is a continuing process taking place in small farmers' corn fields. The report on the presence of trangenes in peasants' maize fields of Oaxaca (Quist and Chapela, 2001), have been further demontrated by the Mexican government (INE-CONABIO, 2001), confirming that gene movement in traditional agriculture is an open system.

Weediness of transformed corn varieties

Gene transformation is the acquisition by a cell of new gene(s) by the uptake of naked DNA, which in the case of maize can be by direct introduction of DNA. As stated before, the more common applications of gene transfer in corn are insect resistance or tolerance to herbicides. Herbicide tolerance is usually conferred by single genes that interact with key enzymes in important metabolic pathways. Insect resistance is conferred by the expression of an insecticidal protein from *B. thuringiensis*. The overall phenotype of transformed plants with these two types of genes is similar to the original phenotype: the reproductive organs (tassels and ears), duration of plant development, methods of propagation, ability to survive as a weed, will not change with these two types of genes.

Gene exchange between cultivated corn and transformed corn would be similar to that which naturally occurs at the present time. Wind-blown pollen would move about among plants within the same field and among plants in nearby fields. Free flow of genes would be similar to that which occurs in cultivated corn. The transformed plants include individual genes, and depending on the relative expression of the transformed genes (relative levels of dominance for gene expression), plant architecture and reproductive capacities of the inter-crossed plants will be similar to non-transformed corn. With the transgenic maize that is available at this moment in the world, the chance that a weedy type of corn will result from inter-crossing of transgenic maize with cultivated conventional maize is remote.

Out-crossing of transformed corn plants with wild relatives of corn will be the same as for non-transformed corn plants. Out-crossing with teosinte species will only occur where teosinte is present in Mexico, Guatemala and probably in some other places of Central America. Out-crossing with *Tripsacum* species is not known to occur in the wild.

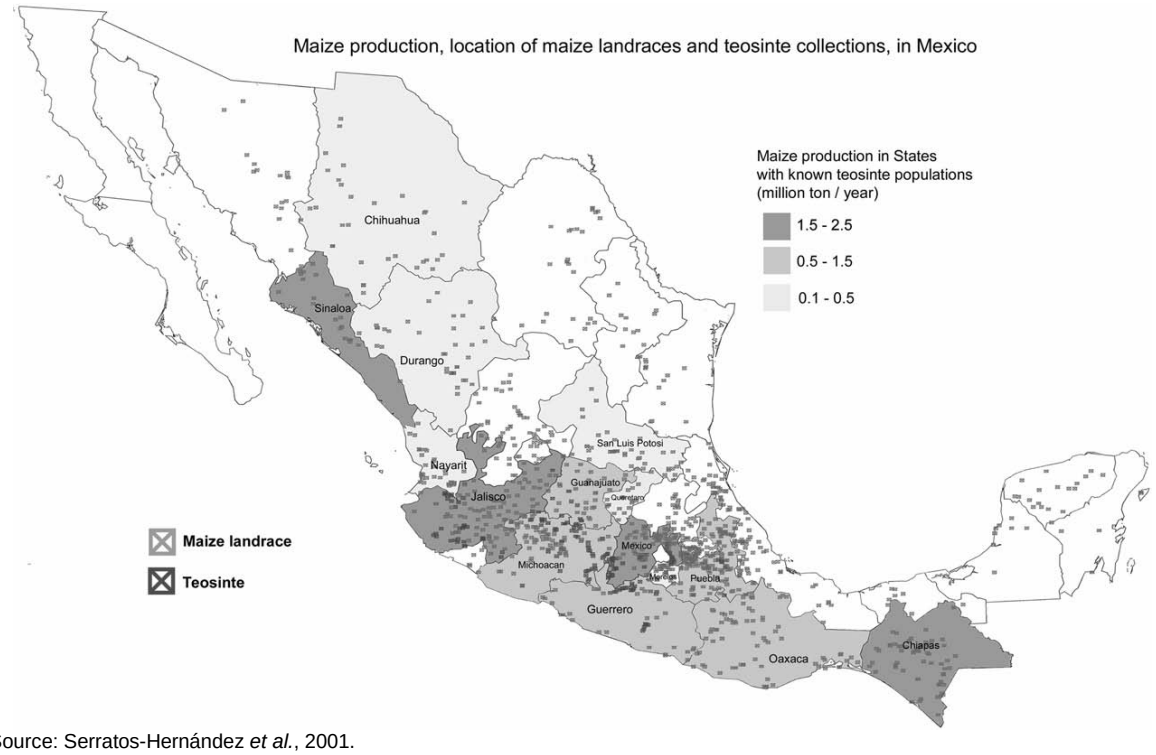
Unintended effects

The commercial release of transgenic maize expressing delta-endotoxin from *Bacillus thuringiensis* has driven the interest of ecologists concerned with the evolution of pest resistance to pesticide plants (Bergvinson *et al.*, 1997; Willcox and Bergvinson, 1997; Marvier, 2001; Obrycki *et al.*, 2001). The evolution of pest resistance is commonly known in any system where negative selection occurs from the

use of traditional chemical pesticides, including plants bred traditionally for pest resistance. Recently, an effect of pollen from transgenic maize on the monarch butterfly larvae, a non-target insect, has preliminarily been described (Losey *et al.*, 1999). However, recent studies in the field have shown a less dramatic effect on non-target organisms (Wraight *et al.*, 2000; Hellmich *et al.*, 2001; Sears *et al.*, 2001; Zangerl *et al.*, 2001).

APPENDIX II

Distribution of Maize Landraces and Teosinte in Mexico



Source: Serratos-Hernández *et al.*, 2001.

APPENDIX III

Common Diseases and Insect Pests of Maize (CIMMYT and DGSV Guides)

Maize	Insect pests	Diseases
Stalk	Termites *(Coptotermes formosanus*), Sugarcane borer (*Diatraea saccharalis*), Southwestern corn borer (*Diatraea grandiosella*), Neotropical corn borer (*Diatraea lineolata*), Asian maize borer (*Ostrinia furnicalis*), Spotted sorghum stem borer (*Chilo partellus*), African maize stem borer (*Busseola fusca*), African pink borer (*Sesamia calamistis*), African sugarcane borer (*Eldona saccharina*), Maize stem weevils (*Cilindrocopturus adpersus*), European corn borer *(Ostrinia nubilaris)*.	Charcoal rot (*Macrophomina phaseoli*), Diplodia stalk rot (*Diplodia maydis*), Gibberella stalk rot and Fusarium stalk rot (*Fusarium* spp), Brown spot (*Physoderma maydis*), Black bundle disease (*Cephalosporium acremonium*), Late wilt (*Cephalosporium maydis*), Maize bushy stunt disease (MBSD), Botryodiplodia stalk rot (*Botryodiplodia theobromae*), Maize lethal necrosis (simultaneous infection of maize chlorotic mottle virus and either maize dwarf mosaic virus or wheat streak mosaic virus), Maize chlorotic mottle virus (MCMV), Corn stunt disease (*Spiroplasma*), Pythium stalk rot (*Pythium aphanidermatum*, *Pythium* spp.), Erwinia stalk rot (*Erwinia carotovora* f. sp. *zeae*)
Leaf	Corn stunt leafhoppers (*Dalbulus maidis*), Maize streak virus leafhoppers (*Dalbulus maidis, D. elimatus*), Fall armyworm (*Spodoptera frugiperda*), Armyworm (*Mythimna unipuncta*), Spider mites (*Oligonychus mexicanus*), Corn leaf aphid (*Rhopalosiphum maidis, R. padi*), Maize Whorl Maggots (*Euxesta* spp.), Sugarcane Froghoppers (*Aeneolamia postica, Prosapia simulans*), Chafers, Grasshoppers (*Sphenarium* spp., *Melanoplus* spp.).	Downy mildew (*Sclerospora* spp., *Sclerophthora* spp), Curvularia leaf spot (*Curvularia lunata* and *Curvularia pallescens*), Cercospora leaf spot (*Cercospora zeae-maydis*), Septoria leaf blotch (*Septoria maydis*), Turcicum leaf blight (*Helminthosporium turcicum*), Diplodia macrospora leaf stripe (*Diplodia macrospora*), Phyllosticta leaf spot (*Phyllosticta maydis*), Helminthosporium carbonum leaf spot (*Helminthosporium carbonum*), Bacterial leaf stripe (*Pseudomonas rubrilineans*), Eyespot of maize (*Kabatiella zeae*), Leptosphaeria leaf spot (*Leptosphaeria michotii*), Maydis leaf blight (*Helminthosporium maydis*), Stewart's wilt (*Erwinia stewartii*), Maize dwarf mosaic (MDMV), Southern rust (*Puccinia polysora*), Common rust (*Puccinia sorghi*), Tropical rust (*Physopella zeae*), Zonate leaf spot (*Gloeocercospora sorghi*), Banded leaf and sheath spot (*Rhizoctonia solani* f. sp. *sasakii*), Tar spot (*Phyllachora maydis*), Brown spot (*Physoderma maydis*) leaf anthracnose (*Colletotrichum graminicola*), Phaeosphaeria leaf spot, Fine stripe virus, Corn streak virus, Bacterial leaf stripe, Maize chlorotic mottle virus, Fine stripe virus, Fine mosaic virus I, Corn stunt disease, Black bundle disease.
Ear	Ear maggots, Corn earworms (*Helicoverpa zea*), Stink bugs (*Euschistus servus, Nezara viridula*), Angoumois grain moth (*Sitotroga cerealella*), Indian meal moth (*Plodia interpunctella*), Grain weevils (*Sitophilus granarius, S. zeamais*), Grain borers (*Prostephanus truncatus*).	Corn stunt disease, Botrydiplodia, Penicillium ear rot, Cladosporium ear rot, Giberella ear rot, Maydis leaf blight (T strain), Nigrospora ear rot, Tar spot, Black bundle disease, Maize dwarf mosaic, Downy mildew, Giberella ear rot, Helminthosporium carbonum ear rot, Banded leaf and sheath spot, Ergot of maize, Head smut, Aspergillus ear rots, Banded leaf and sheath spot, Maize stripe virus, Comon smut, Gray ear rot, Diploidia ear rot, Charcoal ear rot.

Tassel	Corn stunt leafhoppers *(Dalbulus maidis*), Maize streak virus leafhoppers (*Dalbulus maidis, D. elimatus*), Fall armyworm (*Spodoptera frugiperda*), Armyworm (*Mythimna unipuncta*), Spider mites (*Oligonychus mexicanus*), Corn leaf aphid (*Rhopalosiphum maidis, R. padi*), Maize Whorl Maggots, Sugarcane Froghoppers (*Aeneolamia postica, Prosapia simulans*), Chafers, Grasshoppers (*Sphenarium* spp., *Melanoplus* spp.).	Head smut, Downy mildew, Maize chlorotic mottle virus, Bacterial leaf stripe, False head smut, Corn stunt disease, Maize stripe virus.
Seed, Root, and Seedling	Seedcorn maggots (*Hylemya platura*), Wireworms *(Agriotes lineatus*), Flea beetles (*Phyllotreta* spp.), Diabrotica beetles (*Diabrotica* spp.), Maize billbugs (*Sphenophorus maidis*), White grubs (*Phyllophaga* spp., *Anomala* spp.), Cutworms (*Agrotis* spp.), Thrips (*Frankliniella* spp.), Lesser cornstalk borer (*Elasmopalpus lignosellus*).	

APPENDIX IV

Maize World Production

World	Element			
Maize	Seed (Mt)	Area Harvested (Ha)	Yield (Hg/Ha)	Production (Mt)
1961	6,223,099	105,484,151	19,435	205,004,683
1962	6,370,267	103,418,906	19,808	204,856,937
1963	6,193,721	108,384,382	20,319	220,228,333
1964	5,785,022	107,790,032	19,961	215,162,627
1965	5,988,088	106,591,240	21,252	226,524,256
1966	5,944,346	111,157,704	22,096	245,609,160
1967	5,872,917	112,313,038	24,266	272,538,473
1968	5,981,586	111,494,042	22,927	255,620,551
1969	5,838,480	111,242,302	24,226	269,491,068
1970	6,013,828	113,027,431	23,519	265,831,145
1971	6,185,867	118,150,571	26,544	313,622,622
1972	6,137,730	114,910,552	26,875	308,826,290
1973	6,132,362	116,856,034	27,238	318,290,469
1974	6,074,833	119,772,684	25,572	306,287,347
1975	6,429,594	121,442,141	28,133	341,656,971
1976	6,170,127	124,154,181	28,382	352,370,866
1977	6,181,283	125,192,168	29,679	371,561,355
1978	6,235,069	124,664,903	31,570	393,562,091
1979	6,281,256	123,598,634	33,866	418,577,993
1980	6,373,981	125,694,717	31,551	396,573,388
1981	6,440,288	127,816,716	34,950	446,722,107
1982	6,300,922	124,310,829	36,109	448,875,780
1983	6,605,234	117,763,540	29,468	347,024,034
1984	6,711,131	127,703,340	35,269	450,399,992
1985	6,646,135	130,454,042	37,214	485,474,301
1986	6,806,025	131,754,681	36,293	478,178,515
1987	6,623,584	129,888,090	34,880	453,054,894
1988	7,013,976	129,902,556	31,019	402,940,593
1989	7,158,041	131,711,470	36,203	476,833,660
1990	7,090,222	131,315,568	36,801	483,248,513
1991	7,379,181	134,125,220	36,851	494,267,664
1992	5,487,753	136,974,563	38,945	533,443,038
1993	5,497,737	131,500,199	36,242	476,576,466
1994	5,360,864	138,334,591	41,139	569,095,143
1995	5,474,640	136,271,016	37,914	516,655,836
1996	5,691,964	139,856,300	42,127	589,171,299
1997	5,588,723	141,270,173	41,407	584,954,064
1998	5,788,484	138,816,826	44,308	615,063,554
1999	5,765,380	138,460,288	43,786	606,261,782
2000	5,722,092	138,738,942	42,742	592,999,083
2001	5,912,420	137,596,759	44,273	609,181,620

Source : FAOSAT http://apps.fao.org

SECTION 4
OILSEED RAPE (*BRASSICA NAPUS* L.)

1. General Information

This consensus document addresses the biology of the species *Brassica napus* L. Included are general descriptions of this species as a crop plant, its origin as a species, its reproductive biology, its centres of origin, and its general ecology. The ecology of this species is not described in relation to specific geographic regions. Special emphasis has been placed on detailing potential hybridisation between *B. napus* and its close relatives, although this discussion is limited to hybridisation events which do not require intervention through means such as embryo rescue (*i.e.* these events could possibly occur in nature, and could result in fertile offspring).

This document was prepared by a lead country, Canada. It is based on material developed in OECD Member countries – for example, for risk assessments or for presentation at conferences and scientific meetings. It is intended for use by regulatory authorities and others who have responsibility for assessments of transgenic plants proposed for commercialisation, and by those who are actively involved in these plants' design and development.

The table in the Appendix showing potential interactions of *B. napus* with other life forms during its life cycle was developed with respect to Canada. As such, it is intended to serve as an example. Member countries are encouraged to develop tables showing interacting organisms specific to their own geographic regions and environments.

2. General Description and Use as a Crop

Brassica napus L. is a member of the subtribe *Brassicinae* of the tribe *Brassiceae* of the Cruciferous (Brassicaceae) family, sometimes referred to as the mustard family. The name "cruciferous" comes from the shape of its flowers, which have four diagonally opposite petals in the form of a cross. The dark bluish green foliage of *B. napus* is glaucous, smooth or has a few scattered hairs near the margins, and is partially clasping. The stems are well branched, although the degree of branching depends on variety and environmental conditions; branches originate in the axils of the highest leaves on the stem, and each terminates in an inflorescence. The inflorescence is an elongated raceme; the flowers are yellow, clustered at the top but not higher than the terminal buds, and open upwards from the base of the raceme (Musil, 1950).

There are two types of *B. napus*: 1) oil-yielding oleiferous rape, of which one subset with specific quality characteristics is often referred to as "canola" (vernacular name), and 2) the tuber-bearing swede or rutabaga. This document is written for oil-yielding oleiferous rape. The oleiferous type can also be subdivided into spring and winter forms. Sanskrit writings of 2000 to 1500 BC directly refer to oleiferous *B. napus* forms (sarson types) and mustard. Greek, Roman and Chinese writings of 500 to 200 BC refer to rapiferous forms of *B. rapa* (Downey and Röbbelen, 1989). In Europe, domestication is believed to have occurred in the early Middle Ages. Commercial plantings of rapeseed are recorded in the Netherlands as early as the 16th century. At that time rapeseed oil was used primarily as an oil for lamps. Later it came to be used as a lubricant in steam engines.

Although used widely as an edible oil in Asia, only through breeding for improved oil quality, and the development of improved processing techniques, has rapeseed oil become important in western countries. Since the Second World War, rapeseed production in Europe and Canada has increased dramatically as a result of improved oil and meal quality. Modern techniques of plant transformation and genotype identification using isozymes, restriction fragment length polymorphism (RFLP) markers, or random amplified polymorphic DNA (RAPDs) markers will complement classical breeding for the production of other improved lines (Buzza, 1995). China, India, Europe and Canada are now the top producers, although this crop can be successfully grown in the United States, South America and Australia, where annual production has increased sharply over the last few years.

Today, two species of *Brassica* have commercialised varieties with "double low" characteristics, *i.e.* low erucic acid content in the fatty acid profile and very low glucosinolate content in the meal, characteristics desirable for high-quality vegetable oil and high-quality animal feed. In North America these species (*B. napus* and *B. rapa*) are considered to be of "canola" quality. *B. napus* is grown as a winter annual in regions where winter conditions do not result in very low temperatures, which would kill the plants. These biotypes typically require vernalisation before the onset of stem elongation, raceme development, flowering and seed set. In North America and northern parts of Europe, a spring biotype of *B. napus* that requires no vernalisation prior to flowering is grown. These biotypes are typically lower yielding than the winter annual types, but require considerably less time to complete their life cycle.

3. Agronomic Practices for Oleiferous *B. napus*

The spring-type oleiferous *B. napus*, a cool season crop, is not very drought tolerant. It is widely adapted and performs well under a range of soil conditions, provided that moisture and fertility levels are adequate. Air and soil temperatures influence plant growth and productivity. The optimum temperature for maximal growth and development of spring-type oilseed rape is just over 20°C, and it is best grown between 12°C and 30°C. After emergence, seedlings prefer relatively cool temperatures up to flowering; high temperatures at flowering will hasten the plant's development, reducing the time from flowering to maturity. Among cultivated crop plants, *Brassica* species show the highest nutritional demand for sulphur.

Due to increased awareness of soil conservation issues, minimal or no-till *B. napus* production is advised, in which most of the crop residue and stubble are left on the soil surface to trap snow, reduce snow melt run-off, reduce wind and water erosion of the soil, and increase soil water storage. Reduced tillage techniques, however, are only effective when combined with a good systematic weed control programme. Winter oilseed rape covers the soil for ten to eleven months. It has high nutritional demands in autumn and reduces soil erosion in winter.

Weeds can be one of the most limiting parameters in rapeseed production. The closely related cruciferous weeds, for example wild mustard (*Sinapis arvensis*), stinkweed (*Thlaspi arvense*), shepherd's purse (*Capsella bursa-pastoris*), ball mustard (*Neslia paniculata*), flixweed (*Descurainia sophia*), wormseed mustard (*Erysimum cheiranthoides*), hare's ear mustard (*Coringia orientalis*), common peppergrass (*Lepidium densifolium*), etc., are often problematic. Spring-type oilseed rape does not compete well with weeds in the early growth stages, as it is slow-growing and slow to cover the ground. Weeds must be controlled early to avoid yield loss due to competition. Although rapeseed crops can be attacked by a number of insect pests, insect control must be carefully designed to reduce unnecessary and costly pesticide applications, the chances of resistance build-up in insects, and damage to honeybees and native pollinating insects. Diseases can be severe in large production areas, and are greatly influenced by cultivation practices and environmental factors, so that disease management programmes are advisable (refer to the table in the Appendix for examples of *B. napus* pests and diseases in Canada).

When the first siliques begin to shatter, *B. napus* can be cut just below the level of the seed pods and swathed. The use of dessicants allows a reduction of shattering, and possibly allows direct combining.

Generally, oilseed rape should not be grown on the same field more often than once every three to four years in order to prevent the build-up of diseases, insects and weeds. Chemical residues from herbicides and volunteer growth from previous crops (including rapeseed crops grown for different oil types) are also important factors to consider when selecting sites, although suitable soil treatments following harvest may considerably reduce the volunteer problem.

4. Centres of Origin/Diversity[8]

A. Geographic origin of *B. napus*

The origins of *B. napus* (an amphidiploid with chromosome n=19) are obscure, but were initially proposed to involve natural interspecific hybridisation between the two diploid species *B. oleracea (n* = 9) and *B. rapa* (syn. *campestris)*[9] (n = 10) (U, 1935). Recent evidence (Song and Osborn, 1992), through analyses of chloroplast and mitochondrial DNA, suggests that *B. montana (n* = 9) might be closely related to the prototype that gave rise to both cytoplasms of *B. rapa* and *B. oleracea*. It also suggests that *B. napus* has multiple origins, and that most cultivated forms of *B. napus* were derived from a cross in which a closely related ancestral species of *B. rapa* and *B. oleracea* was the maternal donor. In Europe, it is predominantly the winter form which has become a common yellow crucifer found along roadsides, on waste sites and cultivated ground, on docks, in cities and towns, on tips, and on arable fields and along riverbanks. In the British Isles, it has been naturalised wherever oilseed rape is grown. It is a relatively recent introduction into Canada and the United States, and is described as an occasional weed, escape or volunteer in cultivated fields (Munz, 1968, and Muenscher, 1980). It is found typically in crops, fields and gardens, along roadsides, and on waste sites.

B. Geographic origin of *B. oleracea*

The wild form of *B. oleracea*, a suffrutescent (low, shrubby plant with woody lower parts of stems and herbaceous upper parts) perennial, grows along the coast of the Mediterranean from Greece through to the Atlantic coasts of Spain and France, around the coast of England, and to a limited extent in Helgoland (Snogerup *et al*., 1990). Typically the wild type is found on limestone and chalk cliffs in situations protected from grazing. Individuals are often found below cliffs in scree, where they grow among other shrubs, and some populations are found on steep grassy slopes. In Helgoland, populations are found on open rocky ground. In Europe and North America, domesticated types have been reported as escapes but do not form self-sustaining populations outside cultivation. *B. oleracea* is a recent introduction into North America.

C. Geographic origin of *B. rapa*

Wild *B. rapa* (subspecies *sylvestris* L.) is regarded as the species from which the ssp. *rapa* (cultivated turnip) and *oleifera* (turnip rape) originated. It is native throughout Europe, Russia, central Asia and the Near East (Prakash and Hinata, 1980), with Europe proposed as one centre of origin. There is some debate as to whether the Asian and Near Eastern types arose from an independent centre of origin in Afghanistan

8 . This section draws heavily on discussions with, and a review paper prepared by, Dr S.I. Warwick and A. Francis (1994), Centre for Land and Biological Resources Research, Agriculture and Agri-Food Canada.

9 . First described as two species by Linnaeus, with *B. rapa* being the turnip form and *B. campestris* the oleiferous form. Metzger in 1933 concluded that these were the same species and chose the name *B. rapa* (Toxeopus et al., 1984).

and then moved eastward as *B. rapa* became domesticated. Prakash and Hinata (1980) suggest that oleiferous *B. rapa* subspecies developed in two places, giving rise to two different races, one European and the other Asian.

Typically, *B. rapa* is found in coastal lowlands, high montane areas (the slopes of high valleys or mountain ranges), and alpine and high sierras. In Canada, where it is a recent introduction, it is found on disturbed land, typically in crops, fields and gardens, along roadsides, and on waste sites (Warwick and Francis, 1994).

D. Geographic origin of *B. montana*

B. montana, possibly a progenitor species of *B. napus* (see above) and also a suffrutescent perennial, originates in the Mediterranean coastal area between Spain and Northern Italy (Snogerup *et al*., 1990). It is found typically on or below limestone cliffs and rocks, walls, etc., often on disturbed ground. Although usually found in coastal areas and on rocky islets, it has been recorded at an elevation of 1000 m somewhat inland of the coast.

5. Reproductive Biology

Under field conditions the fertilisation of ovules usually results from self-pollination, although outcrossing rates of 5-30 per cent have been reported (Hühn and Rakow, 1979, and Rakow and Woods, 1987). The pollen, which is heavy and sticky, can be transferred from plant to plant through physical contact between neighbouring plants and by wind and insects. Oilseed rape pollen has been detected in the air above rape fields (Williams, 1984) and beyond the borders of a rape crop (Olsson, 1955); however, the concentration decreases rapidly with increasing distance from the source of the pollen and windborne pollen may make no or only a negligible contribution to long-distance pollination of oilseed rape (Mesquida and Renard, 1982, and McCartney and Lacey, 1991). Timmons *et al*., (1995), using pollen traps and "bait" plants whose petals had been removed and which had been emasculated, reported airborne pollen at distances up to 2.5 km from commercial plantings of *B. napus*. The "bait" plants also produced some seed at this distance from the commercial oilseed rape, suggesting the airborne pollen might be capable of successful fertilisation events.

Pollinating insects, in particular honeybees (*Apis mellifera*) and bumblebees (*Bombus* sp.), play a major role in *B. napus* pollination and are believed to be involved in the transfer of pollen over long distances. Oilseed rape is very attractive to bees because it produces large quantities of nectar and pollen. Williams *et al*., (1987) reported that "plants in plots caged with bees had their flowers pollinated faster, shed petals sooner, finished flowering earlier and were shorter than plants caged without bees." *B. napus* pollen is a major food source for bees, and hives are often placed near rapeseed fields during flowering to take advantage of the honey production potential (Marquard and Walker, 1995).

When beehives were placed at the centre of each side of a 1 ha square of non-transgenic *B. napus* plants with a 9 m circle of transgenic plants at the centre, Scheffler *et al*., (1993) reported outcrossing ranging from 1.5 per cent at 1 m to 0.00033 per cent at 47 m. In a later study using 20 x 20 m plots of transgenic and non-transgenic plants, separated by distances of 200 and 400 m, the space separating the plots being either bare ground or planted with barley (*Hordeum vulgare*), Scheffler *et al*., (1995) reported the average frequency of hybridisation to be 0.0156 per cent at 200 m and 0.0038 per cent at 400 m.

The dynamics of bee-mediated pollen movement depend on the quantity of pollen available (size and density of donor population) and the size and location of the receiving populations, as well as on environmental conditions and insect activity (Levin and Kerster, 1969, Ellstrand *et al*., 1989, and Klinger *et al*., 1992). These studies, together with the findings of Scheffler *et al*., (1993 and 1995), suggest that

surrounding an experimental plot of *B. napus* with other plants of the same species flowering synchronously with the experimental plants could decrease the long-distance dispersal of pollen from experimental plants by insects.

6. Cultivated *B. napus* as a Volunteer Weed

As with all crops cultivated and harvested at the field scale, some seed may escape harvesting and remain in the soil until the following season, when it germinates either before or following the seeding of the succeeding crop. In some instances the volunteers may give considerable competition to the seeded crop and cause deterioration in the quality of the crop harvest. In such instances, chemical and/or mechanical control is essential.

The problem of volunteer plants in succeeding crops is common to most field crop species. Much depends on the management practices used in the production of the crop, for example whether the plants have disbursed seed at the time of harvest, the setting of the harvesting equipment, and the speed of the harvesting operation, which will determine whether more or less seed is lost by the harvester. With crops of the *Brassica* family, because of the small seed size and large number of seeds produced by the crop, poor management practices can result in severe volunteer problems in succeeding crops. Suitable soil treatment after the harvest can considerably reduce the volunteer problem.

7. Crosses

A. Inter-species/-genus

In considering potential environmental impact following the unconfined release of genetically modified *B. napus*, it is important to have an understanding of the potential for the development of hybrids through interspecific and intergeneric crosses between the crop and its related species. The development of such hybrids could result in the introgression of the novel traits into these related species, and result in:

- The related species becoming weedy or more invasive of natural ecosystems.
- Altered environmental interactions, potentially causing harm to the environment or to human health and safety.

While many interspecific and intergeneric crosses have been made between *B. napus* and its relatives (Prakash and Hinata, 1980, Warwick and Black, 1993, and Scheffler and Dale, 1994), many have necessitated intervention in the form of ovary culture, ovule culture, embryo rescue and protoplast fusion. Reported in Table 1.3, and ranked in order of relative ability to form hybrid progeny when crossed with *B. napus*, are instances reported by Scheffler and Dale (1994) of sexually obtained interspecific and intergeneric crosses with *B. mapus*.

Flowering periods of *B. napus* and these species are critical. For interhybridisation events to occur, their flowering periods, which are largely environmentally influenced, must overlap at least partially. To evaluate hybridisation potential, it is important to know the flowering chronology of both the cultivated plant and related species; the physical distance between potentially hybridising species; occurrence of vectors for pollination; and how pollination takes place.

The chromosome numbers of the cultivated species and relatives are also important. Many hybrids fail to occur due to lack of development of the endosperm (tissue resulting from the fertilisation of the two polar nuclei of the embryo sac by a male reproductive nucleus). The ratio of maternal and paternal chromosomes must be of 2:1 or higher (Nishiyama and Inomata, 1966). This explains why the direction of

crossing is often important. The pollination of a tetraploid female parent by a diploid male usually produces seeds. The reciprocal cross, on the other hand, is sterile. In order to understand existing exceptions, Johnston *et al*., (1980) proposed the concept of the endosperm balance number (EBN), where the value attributed to a given species is not linked to its chromosome number but to an arbitrary value determined from a successful cross and from the hypothesis that the EBN ratio is 2:1 in the endosperm.

Table 1.3 Sexually obtained interspecific and intergeneric crosses with *B. Napus* (reported by Scheffler and Dale, 1994)

Cross female x male	Progeny	References
B. rapa x *B. napus*	SH, F1, F2, BcP	Morinaga, 1929 U and Nagamatu, 1933 U, 1935 Bing *et al*., 1991 Jørgensen and Andersen, 1994 Mikkelsen *et al*., 1996
B. napus x *B. rapa*	SH, F1, F2, BcP	Morinaga, 1929 U and Nagamatu, 1933 U, 1935 Bing *et al*., 1991 Jørgensen and Andersen, 1994 Mikkelsen *et al*., 1996
B. juncea x *B. napus*	SH, F1, F2, BcP	Morinaga, 1934 Roy, 1980 Bing *et al*., 1991 Fernandez-Serrano *et al*., 1991 Frello *et al*., 1995
B. napus x *B. juncea*	SH, F1, F2, BcP	Morinaga, 1934 Roy, 1980 Bing *et al*., 1991 Fernandez-Serrano *et al*., 1991 Frello *et al*., 1995
B.oleracea x *B. napus*	F1	U, 1935
B. napus x *B. oleracea*	F1, F2, BcP	Roemer, 1935 Röbbelen, 1966 Yamagishi and Takayanagi, 1982
B. carinata x *B. napus*	F1, F2, BcP	Roy, 1980 Fernandez-Escobar *et al*., 1988 Fernandez-Serrano *et al*., 1991
B. napus x B. carinata	F1, F2, BcP	U, 1935 Roy, 1980 Fernandez-Escobar *et al*., 1988 Fernandez-Serrano *et al*., 1991
B. nigra x *B. napus*	SH, F1, BcP	Bing *et al*., 1991
B. napus x *B. nigra*	SH, F1, F2, BcP	Heyn , 1977 Bing *et al*., 1991
B. napus x *Hirschfeldia incana*	SH, SH(BnMS), F1, BcP	Lefol *et al*., 1991 Chevre *et al*., 1992 Eber *et al*., 1994
B. napus x *Raphanus raphanistrum*	SH, SH(BnMS), F1, BcP	Chevre *et al*., 1992 Lefol *et al*., *in press* Eber *et al*., 1994
Diplotaxis erucoides x *B. napus*	F1, BcP	Ringdahl *et al*., 1987
D. muralis x *B. napus*	F1, BcP	Ringdahl *et al*., 1987
B. napus x *Erucastrum gallicum**	F1, BcP	Lefol *et al*., *in press*
B. napus x *Sinapis alba*	F1	Heyn, 1977
B. napus x S. arvensis	F1	Heyn, 1977
B. napus x B. fruticulosa	F1	Heyn, 1977

B. napus x B. tournefortii	F1	Heyn, 1977
B. napus x D. tenuifolia	F1	Heyn, 1977
B. napus x Eruca sativa	F1	Heyn, 1977
B. napus x R. rugosum	F1	Heyn, 1977
B. napus x R. sativus	F1	McNaughton and Ross, 1978

Note:
SH = spontaneous hybrids formed without the aid of emasculation and manual pollination transfer;
SH(BnMS) = spontaneous hybrids with male sterile B. napus *as female parent;*
F1 = F1 hybrids produced through intervention of some sort, i.e. *emasculation and manual pollination;*
F2 = F2 hybrids produced;
BcP = backcross progeny produced.

* *This hybridisation event not reported by Scheffler and Dale (1994)*

Generally, crosses between two species are possible only if the female species has a polyploidy level at least as high as the pollinating male species. Since *B. napus* is tetraploid, it will cross more readily with wild species (diploid) as a female parent (Sikka, 1940, Harberd and McArthur, 1980, and Kerlan *et al*., 1991). In the case of *Raphanus raphanistrum,* no difference was noted in the direction of crosses (Kerlan *et al*., 1991); in the case of *Sinapis alba,* the opposite situation occurs (Ripley and Arnison, 1990).

For a trait to become incorporated into a species genome, recurrent backcrossing of plants of that species by the hybrid intermediaries, and survival and fertility of the resulting offspring, will be necessary.

B. Introgression into relatives

The potential hybridisation events listed are intended to assist the assessment of the potential for introgression of "novel traits" introduced from cultivated *B. napus* into wild relatives. The first step in this assessment is to determine which, if any, of the potential "mates" of *B. napus* are recorded as present in the geographic region where the cultivation is proposed. Should there be potential wild relative "mates" present, the frequency of hybridisation events and the potential for environmental impact should introgression occur would then be considered. Should a trait with positive selective value be introgressed into wild or weedy populations, the gene may become a permanent part of the gene pool of these populations.

The above listed species are all plants of "disturbed land" habitats. Their success will be dependent on their ability to compete for space with other primary colonisers, particularly other successful weedy plant types. This in turn will depend on how well suited they are to the particular climate, soil conditions, etc. of individual sites. Equal ability of the hybrids to compete among wild populations or in cultivated fields has been shown for *B. napus* and hybrids (Lefol *et al*., 1995).

C. Interactions with other organisms

The table in the Appendix is intended as an identification guide for categories of organisms which interact with *B. napus*. This table, representative of Canada, is intended to serve as an example only. Environmental safety assessors should, on a country-by-country basis, draw up their own lists as a guide for assessing potential effects of the release of genetically modified plants on interacting organisms in their country.

8. Ecology

B. napus and its progenitors grow in "disturbed land" habitats. In non-managed ecosystems these species may be considered "primary colonisers," *i.e.* plant species that are the first to take advantage of the

disturbed land, where they compete for space with plants of similar types. Unless the habitats are disturbed on a regular basis, for example along the edges of cliffs, rivers, and pathways, populations of these types of plants will be displaced by intermediaries and finally by plants that form climax ecologies, such as perennial grasses on prairies and tree species and perennial shrubs in forests.

In non-natural ecosystems, including along roadsides and on industrial and waste sites as well as cropland, there is potential, because of their "primary colonising" nature, for ever-present populations of these species to be maintained. It is in such habitats that the species are recorded among the flora of countries where *B. napus* has been introduced as a crop plant. Their success will depend on their ability to compete for space with other primary colonisers, in particular successful weedy types. This, in turn, will depend on how well suited they are to the particular climate, soil conditions, etc. of individual sites.

In crop production systems, poor management practices and insufficient resistance to pod shattering may result in large amounts of *B. napus* seed not being harvested. Especially where there are high crop densities, this may cause volunteer "weed" problems in succeeding crops as well as contamination of such crops with respect to their seed quality.

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APPENDIX

Potential Interactions of *B. napus* with other life forms during its life cycle (Canada)

X indicates the type of interaction between the listed organisms and *B. napus*

	Interaction with *B. napus*			
Other life forms	**Pathogen**	**Symbiont or beneficial organism**	**Consumer**	**Gene transfer**
Albugo candida	X			
Alternaria spp.	X			
Botrytis cinerea	X			
Erysiphe spp.	X			
Leptosphaeria maculans	X			
Peronospora parasitica	X			
Plasmodiophora brassicae	X			
Pseudocercosporella capsellae	X			
Pseudomonas sp.	X			
Pyrenopeziza brassicae	X			
Pythium debaryanum	X			
Rhizoctonia solani	X			
Sclerotinia sclerotiorum	X			
Xanthomonas spp.	X			
Verticillium dahliae	X			
Mychorrhizal fungi		X		
Aster yellow mycoplasma	X			
Cauliflower Mosaic Virus (CaMV)	X			
Beet Western Yellow Virus (BWYV)	X			
Turnip mosaic virus	X			
Soil microbes		X		
Earthworms		X		
Flea beetle			X	
Pollinators		X	X	
Soil insects			X	
Animal browsers (*e.g.* deer, hare, rabbit)			X	
Birds			X	
Other *Brassica napus*				X
Brassica rapa				X
Brassica juncea				X
Brassica nigra				X
Raphanus raphanistrum				X
Erucastrum gallicum				X
Others				X

SECTION 5
RICE (*ORYZA SATIVA L.*)

1. Use as a Crop Plant

Rice is grown worldwide and is a staple food for about a half of the world's population. It is a nutritious grain crop which contains carbohydrates, proteins, lipids, minerals, etc. Rice straw is an important animal feed in many countries.

Rice is now cultivated as far north as the banks of the Amur River (53º N) on the border between Russia and China, and as far south as central Argentina (40º S) (IRRI, 1985). It is grown in cool climates in the mountains of Nepal and India, and under irrigation in the hot deserts of Pakistan, Iran and Egypt. It is an upland crop in parts of Asia, Africa and Latin America. At the other environmental extreme are floating rices, which thrive in seasonally deeply flooded areas such as river deltas - the Mekong in Vietnam, the Chao Phraya in Thailand, the Irrawady in Myanmar, and the Ganges-Brahmaputra in Bangladesh and eastern India, for example. Rice can also be grown in areas with saline, alkali or acid-sulphate soils. Clearly, it is well adapted to diverse growing conditions.

There are two cultivated rice species: *Oryza sativa*, grown worldwide, and *Oryza glaberrima*, grown in West and Central Africa. *O. sativa* has many ecotypes (cultivars) adapted to various environmental conditions. The morphology, physiology, agronomy, genetics and biochemistry of *O. sativa* have been intensively studied over a long period.

2. Taxonomic Status

The genus *Oryza* contains 22 species: two are cultivated and 20 are wild (Table 2.4) (Morishima, 1984; Vaughan, 1994). *O. sativa* is cultivated worldwide, and the word "rice" generally indicates a plant and a crop of this species. *O. glaberrima* is cultivated in West and Central Africa.

The basic chromosome number of the genus *Oryza* is 12. *O. sativa*, *O. glaberrima* and 14 wild species are diploids with 24 chromosomes, and eight wild species are tetraploids with 48 chromosomes. *O. punctata* consists of diploid and tetraploid types. Genome symbols, A to F, are assigned to the species on the basis of meiotic chromosome pairing of F_1 hybrids. Those species with the same genome symbols show no significant disturbance in chromosome pairing in their hybrids. Recently Aggarwal *et al*., (1997) used molecular methods to identify genomes G, H and J.

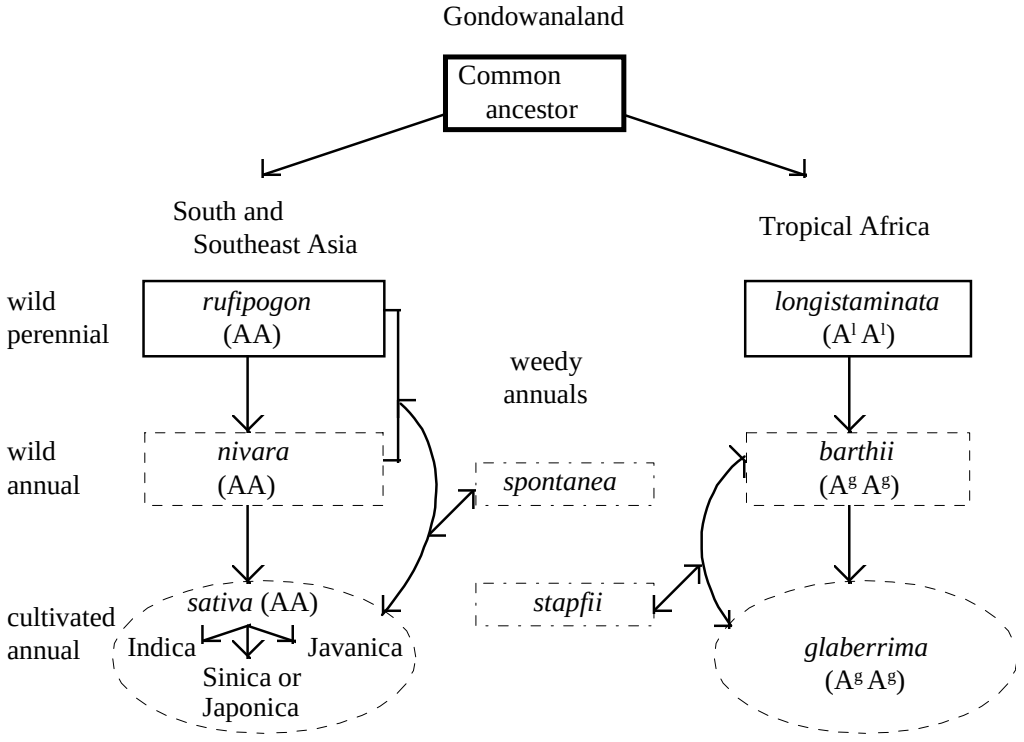
The progenitors of *O. sativa* are considered to be the Asian AA genome diploid species *O. nivara* and *O. rufipogon*, and those of *O. glaberrima* to be the African AA genome diploid species *O. barthii* and *O. longistaminata* (Figure 1.2) (Chang, 1976).

Table 1.4 Species belonging to the genus *Oryza*

Species	Number of chromosomes	Genome	Geographical distribution
Section Oryzae			
[*O. sativa* complex]			
O. sativa L.	24	AA	Worldwide, cultivated
O. nivara Sharma et Shastry	24	AA	Asia
O. rufipogon Griff.	24	AA	Asia, Australia, America (Latin, South)
O. glaberrima Steud.	24	AA	Africa, cultivated
O. barthii A. Chev.	24	AA	Africa
O. longistaminata Chev. et Roehr.	24	AA	Africa
O. meridionalis Ng.	24	AA	Australia
[*O. officinalis* complex]			
O. officinalis Wall. ex Watt	24	CC	Asia, New Guinea
O. minuta Presl. et Presl.	48	BBCC	Asia, New Guinea
O. eichingeri Peter	24	CC	Africa, Asia (Sri Lanka)
O. rhizomatis Vaughan	24	CC	Asia (Sri Lanka)
O. punctata Kotschy ex Steud.	24,48	BB,BBCC	Africa
O. latifolia Desv.	48	CCDD	America (Latin, South)
O. alta Swallen	48	CCDD	America (South)
O. grandiglumis Prod.	48	CCDD	America (South)
O. australiensis Domin	24	EE	Australia
Section Ridleyanae			
O. brachyantha Chev. et Roehr.	24	FF	Africa
O. schlechteri Pilger	48		New Guinea
[*O. ridleyi* complex]			
O. ridleyi Hook. f.	48	HHJJ	Asia, New Guinea
O. longiglumis Jansen	48	HHJJ	New Guinea
Section Granulatae			
[*O. meyeriana* complex]			
O. meyeriana Baill.	24	GG	Asia
O. granulata Nees et Arn. ex Watt	24	GG	Asia

Source : Morishima, 1998; Vaughan, 1994; Aggarwal *et al*., 199

Figure 1.2 Evolutionary pathway of the two cultivated species of rice



Source : Adapted from Chang, 1976

3. Centre of Origin/Diversity

The genetic diversity of various traits in local cultivars of rice is greatest in the area extending from Assam in India and Bangladesh to Myanmar and northern Thailand, and to Yunnan Province in China (Oka, 1988). This area is characterised by topographical and hydrological heterogeneity, and is considered the centre of diversity. Today genetic diversity in this area is being lost, since many rice growers are now growing modern cultivars.

The wild progenitors of *Oryza sativa* are the Asian common wild rices, which show a wide range of variation from perennial to annual types.

Domestication of Asian rice, *O. sativa*, is considered to have occurred in 15,000 to 10,000 BC. Annual forms might have gradually developed in northeastern and eastern India, northern Southeast Asia and southern China (Chang, 1985). They spread and diversified to form two ecological groups, Indica and Japonica (Oka, 1988). There are other studies indicating that the two groups were derived independently from the domestication of two divergent wild rices in Southeast Asia and China, respectively (Second, 1982; 1986).

The wild progenitors of African cultivated rice, *O. glaberrima*, are grasses endemic to West Africa. *O. glaberrima* is considered to have been domesticated in the Niger River delta (Chang, 1976). The primary centre of diversity of *O. glaberrima* is the swampy basin of the upper Niger. In rice fields managed by West African farmers, *O. sativa* and *O. glaberrima* are sometimes grown as mixtures of varying proportions (Chang, 1976; Oka *et al*., 1978; Morishima and Oka, 1979).

4. Identification Methods

A. General description of *Oryza sativa*

Coleoptiles and roots first emerge from the germinating rice seeds. Seedlings differentiate leaves from the growing point of the main culm and tiller buds in the axil of leaves. Panicle primordia differentiate at the top of culms. At heading time, panicles come out of flag-leaf sheaths. Flowering takes place in spikelets on a panicle, followed by pollination on stigmata and fertilisation in ovules. Embryo and endosperm mature in the ovule and become a seed for the next generation. Rice plants are very easily propagated by seeds or tiller buds.

The leaf consists of a blade, a sheath, and a ligule and auricle at the junction between blade and sheath. The culm consists of nodes and hollow internodes. The spikelet has six stamens and the ovary has a two-branched stigma. The seed consists of embryo, endosperm, pericarp and testa enclosed by a palea, and a lemma with an apiculus on the top of the lemma.

B. Identification among cultivars of *O. sativa*

There are a great number of rice cultivars grown in the world. More than 100,000 accessions are conserved in national and international genebanks such as that of the International Rice Research Institute.

Cultivars can be distinguished by differences in morphological, physiological and ecological characters. Essential characters for identifying cultivars are adaptation to different water regimes; growing habit; plant height; shape, size and colour of culm, leaf blade, panicle, hull, apiculus and dehulled grain; presence or absence of pubescence; grain aroma; growth duration, including time to heading and maturity; resistance or tolerance to disease and insect pests, temperature, lodging, grain shattering, seed germinability and seed dormancy; grain quality, including appearance, starch glutinousness and protein content. For rice growers, the cultivar's adaptation to water regimes is the most important consideration, followed by grain characters such as glutinous or non-glutinous, then whether the cultivar is early or late maturing, and other characteristics.

C. Identification among groups of *O. sativa*

O. sativa has been classified into several groups on the basis of morphological, physiological and ecological characters. Kato *et al*., (l928) reported two subspecies, *japonica* and *indica*, from the sterility of F_1 hybrids between cultivars. Ting (l949, 1957) proposed that the subspecies *indica* and *japonica* corresponded to the *hsien* and *keng* classification in China. Matsuo (l952) classified world rice cultivars into group A, having round grains like those of Japanese cultivars; B, having large grains like some tropical cultivars; and C, having slender grains like Indica cultivars. Oka (1958) classified diverse varietal types into Indica and Japonica. Indica cultivars are distributed mainly in the tropical to subtropical zones, while Japonica cultivars are grown in the tropical to northern temperate zones. The two groups differ in many characters when typical varieties are compared, but they show some overlapping variations in each character. Oka (1988) further classified the Indica group into seven sub-groups (Boro, Aus, Broadcast Aman, Transplanted Aman, Rayada, Ashina and Hill Rice) and the Japonica group into tropical and temperate subgroups. The name Javanica was originally used for tropical Japonica-like varieties from Java, and the morphological and physiological traits of currently cultivated Asian and American Javanica fall exactly in the Japonica group (Glaszmann and Arraudeau, 1986; Sato, 1987; Oka, 1988).

Traditionally, the shape or length/width ratio of the spikelet (unhulled rice), and cereal chemistry characteristics such as the hardness and stickiness of cooked rice, have been regarded as criteria to distinguish between Indica and Japonica cultivars. Indica cultivars have longer grains, and are harder and much less sticky when cooked than Japonica. However, this determining characteristic is occasionally

unreliable because of overlapping variation between the two groups. Indica and Japonica are the group names for cultivars that have been selectively adapted for physiological differences favouring different ecological niches.

A discriminant formula combining the measurements of potassium chlorate resistance, low-temperature sensitivity, drought resistance, apiculus hair length and phenol reaction of unhulled rice can classify those two groups efficiently (Morishima and Oka, 1981). Potassium chlorate resistance has the highest diagnostic power to identify each group, followed by drought resistance, apiculus hair length and cold sensitivity score.

Isozyme patterns are effective for identifying cultivar groups. Glaszmann (l987) grouped local cultivars from different Asian countries into six groups, using 15 isozyme loci for eight enzymes detected in young seedlings. When other classifications were compared with these results, most of the cultivars were classified as Indica rice belonged to groups I and II, while group VI corresponded to the Japonica including both the temperate and tropical types. Further, groups III, IV and V included such cultivars as the Rayada rices of Bangladesh, the Sadri rices of Iran, and the Basmati rices of Pakistan and India, but these groups are not identifiable as Indica or Japonica. Kochko (1987a, b) reported isozyme patterns representative of Indica and Japonica groups in traditional cultivars from most African countries.

D. Differentiation between *O. sativa* and *O. glaberrima*

There are discrete differences between the key characters of *O. sativa* and *O. glaberrima* (Table 1.5), and intermediate type plants rarely exist (Morishima *et al*., 1962). *O. sativa* has more secondary branches on the panicles, and longer and smoother ligules, than *O. glaberrima*. A typical *O. glaberrima* has glabrous (hairless) spikelets and leaf blades, while *O. sativa* cultivars are mostly pubescent, although most cultivars in the United States are glabrous. The seed of *O. glaberrima* has longer dormancy than that of *O. sativa*. *O. sativa* is cultivated as an annual agricultural crop, but botanically it is a perennial plant, while *O. glaberrima* is annual both botanically and agronomically. Alone, any of these traits cannot always be a definite discriminant of the two species.

Table 1.5 Comparison of main characters of domesticated cultivars of *O. sativa* and *O. glaberrima*

Character	*O. sativa*	*O. glaberrima*
Habit	Essentially perennial	Annual
Ligule	Long and soft	Short and tough
Panicle branches	Many	Few
Frequency of glabrous varieties	Low	High
Varietal differentiation	Highly variable	Limited variation
Ecotypes	Many	Few
Distribution	Worldwide	Endemic to West Africa

Source : Modified from Oka, 1991

E. Identification of wild species

The wild progenitors of *O. sativa* are the Asian common wild rices, which show a wide variation from perennial to annual types. Wild species are taxonomically identified by examination of their key characters.

In the field, species are usually identified visually based on a combination of characteristics. On the basis of morphological and ecological data, multivariate analysis has been applied to classify wild plants into appropriate wild species groups (Morishima and Oka, 1960; Morishima, 1969).

Wild species are distinguished from *O. sativa* by such traits as habitat, plant type, colouration of spikelet and anther, length and shape of ligule and auricle, panicle type, and awnedness.

Isozyme patterns are also useful to distinguish wild species from *O. sativa. O. rufipogon,*[10] the wild species very closely related to *O. sativa*, possesses more alleles at different isozyme loci and is more polymorphic than *O. sativa* cultivars (Oka, 1988). However, the isozyme alleles from the Japonica type are found with a high frequency only in Chinese strains of *O. rufipogon*, while the alleles characterising the Indica type are observed predominantly in South Asian strains (Second, 1986). Oceanian *O. rufipogon* and *O. longistaminata* present a large genetic diversity of isozymes distinguishable from *O. sativa* and *O. glaberrima* (Second, 1986).

A general description of the morphology of wild species is included in Appendix I.

F. Genetic and molecular identification

It is possible to distinguish between cultivars of *O. sativa* and between *Oryza* species using genetic, cytological and molecular techniques.

Gene linkage groups

The data on the linkage maps of all identified genes concerning morphological and physiological traits on the 12 rice chromosomes are reported annually in the *Rice Genetic Newsletter* by the Committee of Gene Symbolisation, Nomenclature and Linkage Groups of the Rice Genetics Cooperative (Rice Genetics Cooperative, 1995). Prior to the work of the Rice Genetics Cooperative, it was difficult to compare results from different laboratories. The Rice Genetics Cooperative has developed an international standard for rice genetic studies.

There has been little research on *O. glaberrima* linkage maps, but the important characters are at the same locations as in *O. sativa* (Sano, 1988).

DNA marker linkage maps

More recently, DNA markers such as RFLP (Restriction Fragment Length Polymorphism) and RAPD (Random Amplified Polymorphic DNAs) have been used to detect DNA polymorphism, which enables cultivars to be identified. Progress in mapping genes using DNA markers such as RFLP has also been reported (McCouch and Tanksley, 1991), and is updated and listed in the *Rice Genetics Newsletter*. Cultivars will be identified in the future on the basis of specific genes at defined locations on the rice genomes.

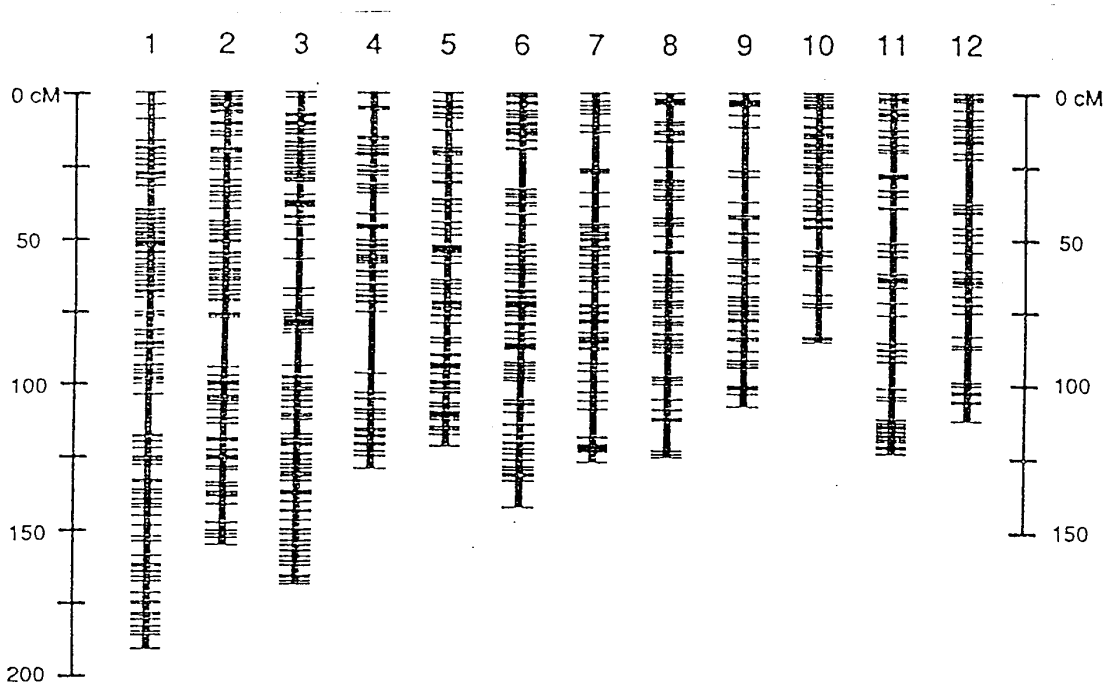
The DNA markers densely mapped on the linkage maps are powerful tools for precise analysis of genotypes of rice plants. Construction of genetic linkage maps using DNA markers such as RFLP and RAPD is in progress not only for *O. sativa*, but also for some wild species. A DNA linkage map has been developed consisting of about 1,400 DNA markers, along with about 1,500 cM over 12 rice chromosomes from an intraspecific cross of *O. sativa* (Figure 1.3) (Kurata *et al*., 1994). From an interspecific backcross

10 . The species name *O. rufipogon* used by Oka and his co-researchers may have included the annual species *O. nivara* and the perennial species *O. rufipogon* in Table 1.

between *O. sativa* and *O. longistaminata*, a molecular map has been constructed consisting of 726 markers for 12 chromosomes (Causse *et al*., 1994). Some of the genes controlling morphologically, physiologically and agronomically important traits have been located on the linkage map.

Mapping of qualitative and quantitative trait loci has progressed rapidly in rice as a consequence, and DNA fingerprinting using RFLP and microsatellite markers will enable identification of individual plants, cultivars and species in the future.

Figure 1.3 Rice RFLP linkage map constrructed with 1,383 DNA markers



Source : Modified by the National Institute of Agrobiological Resources (Japan) from Kurata *et al* (1994).

5. Reproductive Biology

A. Sexual reproduction

Oryza sativa is basically an autogamous plant propagating through seeds produced by self-pollination. Fertilisation occurs in a spikelet, which has six anthers with more than 1,000 pollen grains in each, and an ovule with a branched stigma. Immediately after the spikelet opens at flowering, pollen is dispersed and germinates on the surface of the stigma. Only one pollen tube reaches an ovule to initiate double fertilisation.

The maturation of pollen in an anther is synchronised with the maturation of the ovule within the same spikelet. Pollen can maintain germinability only for several minutes after being shed from the anther under favourable temperature and moisture conditions, while ovules keep their viability to receive pollens for several days after maturation. Pollen of cultivated rice loses its viability within three to five minutes, but wild rice pollen has a longevity of up to nine minutes (Koga *et al*., 1971; Oka and Morishima, 1967).

Most of the wild species have a larger and longer stigma which extends outside the spikelet, increasing the opportunity for outcrossing (Parmer *et al*., 1979; Virmani and Edwards, l983).

The degree of outcrossing is generally higher in Indica cultivars and wild species than in Japonica cultivars (Table 1.6) (Oka, 1988). Cross pollination between wild species and *O. sativa* cultivars has been reported to occur in natural habitats (Oka and Chang, 1961).

B. Asexual reproduction

O. sativa is cultivated annually. However, rice plants can grow vegetatively and continuously under favourable water and temperature conditions, even after they have borne the seeds. This perennial character in *O. sativa* is considered to have been inherited from the ancestral species *O. rufipogon* (Morishima *et al*., 1963).

Under natural conditions, tiller buds on the basal nodes of rice plants start to re-grow after rice grains have been harvested. These new tillers, called the "ratoon", grow best under long-day conditions. In some countries, farmers grow ratoon plants to obtain a second harvest of rice.

Cell/tissue culture techniques can be used to propagate calli and reproduce tissues or plants asexually under the appropriate cultural conditions. Haploid plants are easily obtained through anther culture. They become diploid spontaneously or when artificially treated with chemicals (Niizeki and Oono, 1968).

C. Reproductive barriers

Viable hybrids between *O. sativa* and distantly related varieties or species are difficult to achieve. The postmating barriers are classified into four types, namely F_1 inviability (crossing barrier), F_1 weakness, F_1 sterility and hybrid breakdown (Oka, 1988). All these phenomena have been found in cultivated rice and its wild relatives, although the F_1 plants whose parents have the AA genome in common show no significant disturbances in meiotic chromosome pairing (Chu *et al*., 1969).

In many cases, cross-sterility comes from failure in the development of young F_1 zygotes, particularly the development of endosperm, after fertilisation takes place. The African perennial species *O. longistaminata* showed a stronger crossing barrier with *O. glaberrima* and *O. breviligulata*[11] than with *O. sativa* and *O. rufipogon*[12] (Chu *et al*., l969).

F_1 weakness is controlled by complementary dominant weakness genes (Chu and Oka, 1972) which disturb tissue differentiation or chlorophyll formation. F_1 weakness is rare in crosses between *O. sativa* cultivars (Amemiya and Akemine, 1963). Among strains of *O. glaberrima* and *O. breviligulata*[11], about one-fourth of the crosses examined showed F_1 weakness (Chu and Oka 1972). F_1 weakness was found also in crosses between *O. longistaminata* and *O. glaberrima* or *O. breviligulata*[11], between the American form of *O. perennis*[13] and *O. breviligulata*[11], and between the Asian and Oceanian forms of *O. perennis* complex[14] (Oka, 1988).

11 . The species name *O. breviligulata* used by Oka and his co-researchers is *O. barthii* in Table 1.

12 . The species name *O. rufipogon* may have included the annual species *O. nivara* and the perennial species *O. rufipogon* in Table 1.

13 . The American form of *O. perennis* is *O. rufipogon* in Table 1 and is sometimes called *O. glumaepatula*.

14 . The Asian and Oceanian forms of *O. perennis* complex are *O. rufipogon* and *O. nivara* (in Asia) and *O. meridionalis* (in Australia) in Table 1.

F_1 sterility is frequently found in crosses of cultivated rices and their wild relatives, in which the failure of development of male and female gametes is often observed due to chromosomal disturbance in meiotic pairing or genetic disorders. Cytoplasmic pollen sterility and its fertility-restoration are reported in many crosses (Virmani, 1994).

Partial sterility appears in F_2 plants from crosses between distantly related *O. sativa* cultivars. The sterility is controlled by a set of complementary recessive sterility genes. It seems that there are many sets of complementary or duplicate sterility genes among cultivated and wild species (Kitamura, 1962; Oka, 1964).

The weakness and sterility occurring in the F_2 and later inbred generations are referred to as hybrid breakdown. Hybrid breakdown is controlled by a set of complementary recessive weakness genes (Oka, 1957; Okuno, 1986). Genes for F_2 weakness seem to be distributed occasionally in cultivated and wild rice species.

6. Crosses

A. Intraspecific crosses

Although *Oryza sativa* is basically self-pollinated, natural outcrossing can occur at a rate of up to 5% (Table 1.6) (Oka, 1988). When different cultivars of the same maturity group are planted side by side in a field or in adjacent fields, natural outcrossing can occur between these cultivars. Outcrossing can be avoided by allocating cultivars with sufficiently different maturity time to adjacent fields, or by separating cultivars with the same maturity time.

F_1 plants from crosses within the Indica or Japonica group generally show high fertility in pollen and seedset. Those from crosses between the two groups have lower pollen fertility and lower seedset, with some exceptions, but F_1 fertility is not a good criterion for classifying cultivars into Indica-Japonica groups (Oka, 1988; Pham, 1991).

Hybrid progenies from Indica-Japonica crosses might survive, overcoming various reproductive barriers which are due to genetical and physiological disorders controlled by genic and cytoplasmic factors. Hybridisation between distantly related cultivars of the same species sometimes produces more vigorous hybrid plants than the parental cultivars with more descendant seeds, and establishes new ecotypes which are genetically different from the original population. Artificially selected hybrid plants thus produced may serve an important role in building new cultivars over a long historical period.

B. Interspecific crosses

O. sativa and *O. glaberrima* are often grown as mixtures of various proportions in West African rice fields (Chu *et al*., 1969). The two species resemble each other, perhaps due to co-evolution, but natural hybrids between them are rare, even though experimental hybridisation is easy. The F_1 plants are highly pollen-sterile, but about one-third of the F_1 embryo sacs are normal and functional. Backcrosses can be made with the pollen of either parent. The gene loci that have been examined are identical in the two species (Sano, 1988). Most natural hybrids disappear due to several genetic and physiological disorders, leaving only a very low probability of gene transmission between the two species.

O. rufipogon,[15] the wild progenitor of *O. sativa*, can be crossed with *O. sativa* and sometimes produces hybrid swarms in the field. Their hybrids show no sterility (Oka, 1988). The variation between

15 . The species name *O. rufipogon* used by Oka and his co-researchers may have included the annual species *O. nivara* and the perennial species *O. rufipogon* in Table 1.

perennial and annual types is nearly continuous, and some intermediate perennial-annual populations are most likely to be the immediate progenitor of cultivated rice because they have a high genetic variability, a moderately high seed productivity, and tolerance for habitat disturbance (Sano *et al*., 1980).

O. glaberrima and its wild progenitor *O. breviligulata*[16] produce fertile hybrids and natural hybrid swarms in the fields. They have an annual growth habit and resemble each other in most botanical characters (Oka, 1991).

The common wild rices are distributed throughout the humid tropics and comprise geographical races such as Asian (*O. nivara* and *O. rufipogon*), African (*O. barthii* and *O. longistaminata*), American (*O. glumaepatula*)[17] and Oceanian (*O. meriodinalis*). All these species share the AA genome, but they are separated from one another by F_1 pollen sterility (Chu *et al*., 1969). However, some *O. longistaminata* plants growing in rice fields produce the plants, which are self-compatible and crossable with *O. sativa* (Chu and Oka, 1970; Ghesquiere, 1985). These are probably the result of gene introgression from cultivars across the reproductive barriers.

The relatively high seed-sets (9-73%) can be obtained through the artificial hybridisation of *O. sativa* with these AA genome wild species (Sitch *et al*., 1989). *O. nivara* and *O. rufipogon* have been used in crosses with *O. sativa*. The former gives resistance to grassy stunt virus and the latter donates cytoplasmic male sterility (Khush and Ling, 1974; Lin and Yuan, l980).

Species with the BB, BBCC, CC, or CCDD genome are more crossable with *O. sativa* (0-30% seedset) than the more distantly related EE and FF genome species with *O. sativa* (0.2-3.8% seedset), but their hybrids are highly male and female sterile (Sitch, 1990). Artificial gene transfer has been achieved through a series of backcrosses in crosses between *O. sativa* and *O. officinalis* for brown planthopper (*Nilaparvata lugens*) and white-backed planthopper (*Sogatella furcifera*) resistance (Jena and Khush, 1990) and *O. minuta* for blast and bacterial blight resistance (Amante-Bordeos *et al*., 1992). Artificial crosses between *O. sativa* and more distantly related species such as *O. ridleyi* and *O. meyeriana* have been also reported, but the successful rate of such distant crosses was very low (Katayama and Onizuka, 1979; Sitch *et al*., 1989). Artificial hybridisation in distant crosses is feasible, but requires embryo rescue to obtain F_1 hybrids and first backcross progenies.

16 . The species name *O. breviligulata* used by Oka and his co-researchers is *O. barthii* in Table 1.

17 . *O. glumaepatula* is the American form of *O. rufipogon* in Table 1.

Table 1.6 Outcrossing rates estimated in wild and cultivated rice species by different methods (Oka, 1988)

Taxa/type	Origin	Method	No. of populations	Outcrossing (%)	Reference
Asian *perennis*[18]					
Perennial	Taiwan	Marker gene	1	30.7	Oka, 1956
	Thailand	Marker gene	1	44.0	Oka & Chang, 1961
	Thailand	Isozyme markers	1	50.6	Barbier, 1987
Intermediate	Thailand	Isozyme markers	1	55.9	Barbier, l987
Perennial	India	Variance ratio	1	37.4	Oka & Chang, l959
	Sri Lanka	Variance ratio	2	22.4-26.5	Sakai & Narise, 1959
Annual	India	Variance ratio	1	21.7	Oka & Chang, l959
	India	Variance ratio	3	16.6-33.9	Sakai & Narise, l960
	India	Marker gene	1	7.9	Roy, l921
	Thailand	Isozyme markers	1	7.2	Barbier, l987
Weedy	India	Variance ratio	2	17.3-20.6	Oka & Chang, l959
Breviligulata[19]	Africa	Variance ratio	2	3.2-19.7	Morishima *et al*., 1963
Sativa	India	Marker gene	34	0-6.8	Butany (1957)
Indica	Africa	Marker gene	2	0-1.1	Roberts *et al*., l961
	Taiwan	Marker gene	4	0.1-0.3	Oka (unpubl.)
	Sri Lanka	Variance ratio	1	3.6	Sakai & Narise, l960
Japonica	Taiwan	Marker gene	5	0.6-3.9	Oka (unpubl.)

7. Ecology

A. Cultivation

In rice-growing environments, five water regimes are generally distinguished: irrigated, rain-fed shallow, deepwater, upland, and tidal wetland. Irrigated rice is dominant in Asia, while upland rice is dominant in Africa and Latin America. The proportion of rice culture types varies considerably country by country.

There are two types of rice culture: direct seeding and transplanting. In direct seeding cultivation, dry seeds or seeds that have been pre-soaked and pre-germinated are sown by hand or using seeding machines. With the transplanting method, young seedlings grown in nursery beds are transplanted by hand or transplanting machines to rice fields. In rice fields, plants start in the vegetative phase to make tillers, sheaths and leaves. Then the plants begin the reproductive phase, in which they make panicles and seeds. Seeds are harvested for food. Common diseases and pests are listed in Appendix II.

About 530 million tonnes of rice is harvested annually from plantings of 146 million hectares worldwide (FAO, 1995). More than 91% of world rice production comes from Asia, 5% from the

18 . The species name *O. perennis* used by Oka is *O. nivara* or *O. rufipogon* in the text.

19 . The species name *O. breviligulata* used by Oka is *O. barthii* in the text.

Americas, 3%from Africa, and another 1% from Europe and Oceania. Rice is used for food in various forms. Grains are heated in water to become cooked rice. Rice flour is usually kneaded with water, boiled and used for various rice products. The bran is an important source of oil for food and manufacturing. Husks are used for fertilisers and animal feed, and straw for making various materials for wrapping, mats, etc.

B. Volunteers and weediness

Cultivars vary in the ease with which unhulled grains from panicles are shattered. This characteristic is influenced by the extent of the absciss layer between the hulls and the panicle rachis. Farmers have selected various cultivars, from easy to hard grain shattering, for hand and machine harvesting. Seeds shattered before or during harvesting are allowed to germinate, if the water and temperature regimes are favourable, and act as volunteer weeds both in paddy and upland fields where farmers might grow another cultivar of rice. In general, these shattered seeds and volunteer weeds will be buried or killed by normal agronomic practices such as plowing, drainage or flooding, and rotation. The Indica group has a wider range of grain shattering and greater potential to become a volunteer weed than does the Japonica group.

Seed dormancy enables seeds to remain viable form one season to the next. Non-dormant or weakly dormant seeds can germinate by themselves on the panicle, consequently losing their food grain value. Farmers and breeders have selected cultivars with the dormancy which is suited to the farming cycle. However, shattered seeds with dormancy will keep their longevity for several seasons and germinate sporadically in the fields when a new cultivar is planted. The factors related to seed dormancy exist in the hull, and dormancy enhances the ability of shattered seeds to become volunteer weeds. Indica has a wider range of seed dormancy than Japonica. Either Indica or Japonica red rice easily shatters and has strong dormancy, becoming a weed problem in rice fields. Intraspecific hybridisation between domesticated cultivars and their weedy relatives, including red rice, may occur in many rice-growing areas.

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APPENDIX I

Morphological and Genetic Characteristics of *Oryza* Species (after Vaughan, 1994 with additions from Aggarwal *et al*., 1997)

O. alta

Tall (up to 4m), erect herb with broad leaves (generally >5cm), spikelets >7mm. Teteraploid (2n=48). CCDD genome. Latin and South America.

O. australiensis

Tall (>2m) erect herb, strap-shaped, gray green leaves, pear-shaped spikelets (6.5-9mm) with soft, wispy awn (<5cm) and scabrous panicle axis. Diploid (2n=24). EE genome. Australia.

O. barthii

Erect to semi-erect herb, leaves have short ligule (<13mm); spikelets, large (7.8-11mm), easily shattering, long, strong awns (up to 10cm) usually red; panicle rarely has secondary branching. Diploid (2n=24). AA genome. Africa.

O. brachyantha

Short (<1m),tufted, annual or weakly perennial, with slender culms; small, slender (<1.6mm wide) spikelets with long awns (6-17cm). Diploid (2n=24). FF genome. Africa.

O. eichingeri

Short (usually <1m) with hard, slender culms; glabrous ligule (<3.5mm); chlorophyllous veins the length of the immature spikelet; mature spikelets 4.5-6mm long. Diploid (2n=24). CC genome. Africa, Sri Lanka.

O. glaberrima

Great diversity of morphological characteristics, primary key characters are the lack of secondary and tertiary branching of the panicles, short (usually <10mm) and rounded ligule, spikelets generally awnless and non-shattering. Pubescence on leaves and spikelets usually sparse. Diploid (2n=24). West Africa.

O. grandiglumis

Tall (up to 4m) herb with broad leaves (3-5cm), pubescent ligule; sterile lemma the same length as palea and lemma. Tetraploid (2n=48). CCDD genome. South America.

O. granulata

Short (usually <1m) herb, lanceolate, dark green leaves; spikelets <6.4mm, always awnless, with granulate texture to palea and lemma. Tetraploid (2n=48). GG genome. Asia.

O. latifolia

Short (usually <1m) and tall (2m or more) forms exist. Leaves broad but <5cm; spikelet <7mm. Tetraploid (2n=48). CCDD genome. Latin and South America.

O. longiglumis

Erect to semi-erect tufted herb, usually 1-2m tall; spikelets 7-8mm long and 1.8-2.2mm wide, with trichomes in rows down the length of chartaceous (papery) palea and lemma; sterile lemma narrow and flexous, as long or longer than fertile lemma; awn about 1cm long. Tetraploid (2n=48). HHJJ genome. New Guinea.

O. longistaminata

Tall (usually 2m or more), erect, rhizomatous herb, ligule of lower leaves >15mm, acute or 2-cleft; spikelets with anthers >3mm. Diploid (2n=24). AA genome. Africa.

O. meridionalis

Erect herb usually 1-2m tall; panicle branches tightly adpressed to main panicle axis, rarely having secondary branching; spikelets <2.3mm wide; awns 7.8-10.3cm. Diploid (2n=24). AA genome. Australia.

O. meyeriana

Short (usually <1m) herb, lanceolate, dark green leaves; spikelets >6.4mm long, awnless, with granulate texture to palea and lemma. Diploid (2n=24). GG genome. Asia.

O. minuta

Scrambling, stoloniferous herb; basal panicle branches usually not whorled; spikelets <4.7mm long and <2.0mm wide. Tetraploid (2n=48). BBCC genome. Philippines, New Guinea.

O. nivara

Short or intermediate height (usually <2m) herb; spikelets large, 6-8.4mm long, 1.9-3.0mm wide, 1.2-2.0mm thick; long, strong awn (4-10cm). Diploid (2n=24). AA genome. Asia.

O. officinalis

Erect, usually rhizomatous herb of variable height; basal panicle branches whorled with spikelets inserted half-way or more from base; spikelets <5.4mm long and >2.0mm wide. Diploid (2n=24). CC genome. Asia, New Guinea.

O. punctata

Erect herb of two morphological types, which correspond to two cytological types. Both morphological types have ligule >3mm, which is soft and splits when dried; basal panicle branches widely spreading; spikelets of diploid race >5.5mm long and <2.3mm wide, those of teteraploid race <5.5mm long and >2.3mm wide; awns of both races usully >3cm. Diploid (2n=24) and tetraploid (2n=48). BB and BBCC genome. Africa.

O. rhizomatis

Erect, rhizomatous herb, 1-3m tall; panicle without whorled basal panicle branches; spikelets inserted near base of lowest panicle branch; spikelet length >6mm with extenuated apiculus, often awnless. Diploid (2n=24). CC genome. Sri Lanka.

O. ridleyi

Erect to semi-erect tufted herb, usually 1-2m tall; spikelet 7.6-12.7mm long by 1.6-2.9mm wide, with rows of trichomes down the length of the chartaceous (papery) palea and lemma; sterile lemma narrow and flexuous, shorter than palea and lemma; awn about 1cm long. Tetraploid (2n=48). HHJJ genome. Asia, New Guinea.

O. rufipogon

Tufted and scrambling herb with nodal tillering; spikelets usually 8-9mm long but up to 11mm in Latin American race; anther usually >3mm, reaching 7mm or more; awn usually 6-10cm long but up to 16cm in Latin American race. Diploid (2n=24). AA genome. Asia, New Guinea, Australia, Latin and South America.

O. sativa

Great diversity of forms. Varietal diversity can be categorized into three major groups of the traditional varieties: (1) Indica varieties with usually slender, awnless grains, light green leaves, many tillers; (2) temperate Japonica varieties with usually roundish pubescent grains, dark green leaves, few tillers; (3) tropical Japonicas (Javanicas) usually large, rounded, awned, pubescent spikelets; low shattering; few tillers. Morphological criteria alone are insufficient to distinguish varietal groups. Diploid (2n=24). AA genome. Worldwide.

O. schlechteri

Short (50cm or less), stoloniferous herb with pubescent nodes; short, narrow leaves with pubescent auricle and short ligule; panicle short (<7cm) and spreading; spikelets <2mm long, awnless. Tetraploid (2n=48). Genome unknown. New Guinea.

APPENDIX II

Common Diseases and Pests in *Oryza sativa*

Virus diseases, mycoplasma-like organism diseases

Disease	Vector
Dwarf	*Nephotettix cincticeps* Uhler
	Recilia dorsalis Motschulsky
Black streaked dwarf	*Laodelphax striatellus* Fallen
	Unkanodes sapporonus Matsumura
	Ribautodelphax albifascia Matsumura
Grassy stunt	*Nilaparvata lugens* Stal.
Hoja blanca	*Sogatodes oryzicola* Muir
	Sogatodes cubanus Crawford
Orange leaf	*Recilia dorsalis* Motschulsky
Rugged stunt	*Nilaparvata lugens* Stal.
Stripe	*Laodelphax striatellus* Fallen
	Unkanodes sapporonus Matsumura
	Ribautodelphax albifascia Matsumura
Transitory yellowing	*Nephotettix apicalis* Motschulsky
	Nephotettix cincticeps Uhler
	Nephotettix impicticeps Ishihara
Tungro	*Nephotettix impicticeps* Ishihara
	Nephotettix apicalis Motchulsky
	Nephotettix virescens Distant
	Nephotettix nigropictus Stal.
	Nephotettix parvus Ishihara et Kawase
	Nephotettix malayanus Ishihara et Kawase
	Recilia dorsalis Motschulsky
Yellow dwarf	*Nephotettix virescens* Distant
	Nephotettix cincticeps Uhler
	Nephotettix impicticeps Ishihara
	Nephotettix apicalis Motschulsky
	Nephotettix nigropictus Stal.
Yellow mottle	*Sessilia pusilla* Gerstaeker

Bacterial diseases

Disease	Agent
Bacterial blight	*Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Swings *et al.* =*Xanthomonas campestris* pv. *oryzae* (Ishiyama) Dye
Bacterial leaf streak	*Xanthomonas oryzae* pv. *oryzicola* (Ishiyama) Swings *et al.*
Foot rot	*Erwinia chrysanthemi* Burkholder *et al.*
Grain rot	*Pseudomonas glumae* Kurita & Tabei
Pecky rice (kernel spotting)	Damage by many bacteria and fungi Feeding injury by rice stink bug
Sheath brown rot	*Pseudomonas fuscovaginae* (ex Tanii *et al.*) Miyajima *et al.*

Fungal diseases

Disease	Agent
Aggregate sheath spot	*Ceratobasidium oryzae-sativae* Gunnell & Webster (amorph: *Rhizoctonia oryzae-sativae* (Sawada) Mordue)
Bakanae disease	*Gibberella fujikuroi* (Sawada) Ito *Fusarium moniliforme* Sheldon
Black kernel	*Curvularia lunata* (Wakk.) Boedijin (teleomorph: *Cochliobolus lunatus* R.R. Nelson & Haasis)
Blast (leaf, neck, nodal and collar)	*Pyricularia oryzae* Cavara =*Pyricularia grisea* Sacc. (teleomorph: *Magnaporthe grisea* (Hebert) Barr)
Brown spot	*Cochliobolus miyabeanus* (Ito & Kuribayashi) Dreschler ex Dastur (anamorph: *Bipolaris oryzae* (Breda de Haan) Shoemaker)
Crown sheath rot	*Gaeumannomyces graminis* (Sacc.) Arx & D.Olivier
Downy mildew	*Sclerophthora macrospora* (Sacc.) Thirumalachar *et al*., Eyespot *Drechslera gigantea* (Heald & F.A.Wolf) Ito
False smut (green smut)	*Ustilaginoidea virens* (Cooke) Takahashi
Kernel smut	*Tilletia barclayana* (Bref.) Sacc. & Syd. in Sacc. =*Neovossia horrida* (Takah.) Padwick & A. Khan
Leaf smut	*Entyloma oryzae* Syd. & P. Syd.
Leaf scald	*Microdochium oryzae* (Hashioka & Yokogi) Samuels & I.D. Hallett =*Rhynchosporium oryzae* Hashioka & Yokogi
Narrow brown leaf spot	*Cercospora janseana* (Racib.) O. Const. =*Cercospora oryzae* Miyake (teleomorph: *Sphaerulina oryzina* K. Hara)

Pecky rice (kernel spotting)	Damage by many fungi, including *Cochliobolus miyabeanus* (Ito & Kuribayashi) Drechs. ex Dastur. *Curvularia* spp. *Fusarium* spp. *Microdochium oryzae* (Hashioka & Yokogi) Samuel & I.C. Halett *Sarocladium oryzae* (Sawada) W. Gams & D. Hawksworth and other fungi
Root rots	*Fusarium* spp. *Pythium* spp. *Pythium dissotocum* Drechs. *Pythium spinosum* Sawada
Seedling blight	*Cochliobolus miyabeanus* (Ito & Kuribayashi) Drechs. ex Dastur. *Curuvularia* spp. *Fusarium* spp. *Rhizoctonia solani* Kuhn *Sclerotium rolfsii* Sacc. (teleomorph: *Athelia rolfsii* (Curzi) Tu & Kimbrough and other pathogenic fungi
Sheath blight	*Thanatephorus cucumeris* (A.B. Frank) Donk (anamorph: *Rhizoctonia solani* Kuhn)
Sheath rot	*Sarocladium oryzae* (Sawada) W. Gams & D. Hawksworth =*Acrocylindrium oryzae* Sawada
Sheath spot	*Rhizoctonia oryzae* Ryker and Gooch
Stackburn (Alternaria leaf spot)	*Alternaria padwickii* (Ganguly) M.B. Ellis
Stem rot	*Magnaporthe salvinii* (Cattaneo) R. Krause & Webster (synanamorphs: *Sclerotium oryzae* Cattaneo *Nakataea sigmoidae* (Cavara) K. Hara)
Water-mold (seed-rot and seedling disease)	*Achlya conspicua* Coker *Achlya klebsiana* Pieters *Fusarium* spp. *Pythium* spp. *Pythium dissotocum* Drechs. *Pythium spinosum* Sawada

Nematodes

Pest	**Agent**
Cyst nematode	*Heterodera oryzae* Luc & Briz.
Root-knot nematodes	*Meloidogyne incognita* var. acrita Chitwood
Root nematode	*Hirschmaniella oryzae* Luc & Goodey
Stem nematode	*Ditylenchus angustus* (Butler) Filipjev
White tip (crimp nematode)	*Aphelenchoides besseyi* Christie

Soil pests

Pest	Agent
Mole cricket	*Gryllotalpa orientalis* (=*africana*) Burmeister
Root aphids	*Tetraneura nigriabdominalis* Sasaki
	Geoica lucifuga Zehntner
Root weevils	*Echinocnemus squameus* Billberg
	Lissorhoptrus oryzophilus Kuschel
	Echinocnemus oryzae Marshall
	Hydronomidius molitor Faust

Pests at the vegetative stage

Pest	Agent
Armyworms and cutworms	*Mythimna* (=*Pseudaletia=Leucania=Cirphis*) *separata* (=*unpuncta*) Walker
	Spodoptera mauritia Boisduval
	Spodoptera (=*Prodenia*) *litura* Fabricius
Black bugs	*Scotinophara coarctata* Fabricius
	Scotinophara lurida Burmeister
Caseworm	*Nymphula depunctalis* Guenee
Field crickets	*Hieroglyphus banian*
Gall midge	*Orseolia* (=*Pachydiplosis*) *oryzae* Wood-Mason
Grasshoppers	*Locusta migratoria manilensis*
	Oxya japonica japonica
Green hairy caterpiller	*Rivula atimeta* Swinhoe
Green semilooper	*Naranga aenescens* Moore
Hispa	*Dicladispa* (=*Hispa*) *armigera* Oliver
Leaf beetle	*Oulema* (=*Lema*) *oryzae* Kuwayama
Leafholders	*Cnaphalocrocis medinalis* Guenee
	Marasmia (=*Susumia*) *exigua* Butler
	Marasmia patnalis Bradley
	Marasmia ruralis Walker
Mealybug	*Brevennia* (=*Heterococcus=Ripersia*) *rehi* (=*oryzae*) Lindinger
Seedling maggots	*Atherigona oryzae* Mallock
	Atherigona exigua Stein
Stem bores	
dark-headed stem borer	*Chilo* (=*Chilotraea*) *polychrysus* (=*polychrysa*) Meyrick
pink stem borer	*Sesamia inferens* Walker

striped borer	*Chilo suppressalis* Walker
white stem borer	*Scirpophaga* (=*Tryporyza*=*Schoenobius*) *innotata* Walker
yellow stem borer	*Scirpophaga* (=*Tryporyza*=*Schoenobius*) *incertulas* Walker
Thrips	*Stenchaetothrips* (=*Baliothrips*=*Thrips*) *biformis* (=*oryzae*) Bagnall
Whorl maggots	*Hydrellia philippina* Ferino
	Hydrellia sasakii Yuasa & Ishitani
	Hydrellia griseola Fallen

Pests at the reproductive stage

Pest	**Agent**
Brown planthopper	*Nilaparvata lugens* Stal
Greenhorned caterpillar	*Melanitis leda ismena* Cramer
Green leafhoppers	*Nephotettix nigropictus* (=*apicalis*) Stal
	Nephotettix virescens (=*impicticeps*) Distant
	Nephotettix cincticeps Uhler
	Nephotettix malayanus Ishihara & Kawase
Skippers	*Pelopidas mathius* Fabricius
	Parnara guttata Bremer & Grey
Smaller brown planthopper	*Laodelphax striatellus* Fallen
Whitebacked planthopper	*Sogatella furcifera* Harvath
White leafhopper	*Cofana* (=*Tettigella*=*Cicadella*) *spectra* Distant
Zigzag leafhopper	*Recilia dorsalis* Motschulsky

Pests at the ripening stage

Pest	**Agent**
Rice panicle mite	*Stenotarsonemus spinki* Smiley
Rice seed bugs	*Leptocorisa acuta* Thurnberg
	Leptocorisa oratorius Fabricius
	Leptocorisa chinensis Dallas

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APPENDIX III

Transformation of Rice (*Oryza sativa*)

Rice breeding in rice-growing countries is supported by many breeding technologies that have been developed on the basis of long-accumulated research and experience gained with traditional pratices. Major aspects of traditional rice breeding, including conventional practices and seed multiplication as well as early applications of biotechnology, such as anther culture and somatic mutation through protoplast and tissue culture - are well-described in another OECD publication (Kaneda, 1993)

More recent application of biotechnology to rice breeding, particularly genetic transformation of rice, was started in late 1980s. It was considered more difficult than in other plant species, as rice plant regeneration from protoplast requires special skills. The usual technique of using the Ti plasmid of *Agrobacterium tumefaciens*, which has been very effective for gene introduction in many dicot plant species, was not a useful tool for transferring foreign genes into rice. However, these problems have been overcome through recent progress in transformation techniques.

Currently, the following three methods of gene introduction are reported to have been used among researchers. There is one other method, using polyethylenglycol (PEG), which has had only limited use in recent years.

The first is the electroporation method, which directly introduces foreign genes into protoplasts. Improvement in efficiency and stability of regeneration from protoplasts to plantlets is another factor contributing to the development of this method.

The second is the biolistic (particle gun bombardment) method, which directly introduces foreign genes into regenerable plant cells such as scutellum cells. The main merit of this method is that it eliminates the problems of regeneration from protoplasts and minimises the possibility of the occurrence of somaclonal variation during the regeneration process.

The third is the improved *Agrobacterium*-mediated method, which was initiated a few years ago. Its main merit includes insertion of a more precise gene construction, including promoters and marker genes on the plasmids, which results in improved efficiency of gene introduction as well as more stable expression and inheritance of the transgenes.

After the introduction of foreign genes into rice plant tissue, a suitable selection system is required to select plants that have been successfully transformed. In the case of rice, selection markers usually constitute genes that confer resistance to antibiotics. Among them, kanamycin was used in early stages, but most of the recent successful results of rice transformation have been obtainedusing hygromycin and geneticin (G418) because of their more efficient and stable function in selection procedures.

The types of traits expressed in transformed rice plants are similar to those expressed in the transformation of other plant species. This started with the introduction of marker genes in the early stages and expanded to include genes introduced so that some agronomically or industrially important traits could be expressed. The traits reported in recent successful transformations follow this trend, including pest and disease resistance, herbicide tolerance and specific grain quality.

Table AIII-1 summarises the information presented above in chronological order, to show the progressive development of rice transformation.

In this document, particularly Section IV (Identification Methods), recent progress in basic research on the rice genome is presented. It is expected that transformed rice plants with useful traits will be released for commercialisation in the near future, supported by developments both in basic genome research and in transformation technologies.

Progress in the development of transformed rice

	Method of transformation	Introduced gene	Remarks
Junker *et al*., (1987)	polyethylenglycol	NPT-II	transient expression
Toriyama *et al*., (1988)	electroporation	AMP-II	transformed plant
Shimamoto *et al*., (1989)	electroporation	HPT	transformed plant
Battraw and Hall (1990)	electroporation	NPT-II, GUS	transformed plant
Hayashimoto *et al*., (1990)	polyethylenglycol	HPT	transformed plant
Raineri *et al*., (1990)	*Agrobacterium*	NPT-II	callus formation
Christou *et al*., (1991)	electroporation	*bar*, GUS t	ransformed plant
Meijer *et al*., (1991)	polyethylenglycol	HPT, GUS	transformed plant
Murai *et al*., (1991)	polyethylenglycol	HPT, *Ac*	transformed plant
Battraw and Hall (1992)	electroporation	NPT-II, GUS	transformed plant
Cao *et al*., (1992)	particle gun	*bar*	transformed plant
Datta *et al*., (1992)	polyethylenglycol	HPT, *bar*	transformed plant
Hayakawa *et al*., (1992)	electroporation	HPT, CP of RSV	transformed plant
Li *et al*., (1992a)	polyethylenglycol	HPT, mutant ALS	transformed plant
Li *et al*., (1992b)	polyethylenglycol	HPT	transformed plant
Peng *et al*., (1992)	polyethylenglycol	NPT-II	callus formation
Chan *et al*., (1993)	*Agrobacterium*	NPT-II, GUS	transformed plant
Fujimoto *et al*., (1993)	electroporation	HPT, *cryIA(b)*	transformed plant
Shimamoto *et al*., (1993)	electroporation	HPT, *Ac* transpose non-automonous maize Ds element	transformed plant
Tada and Fujimura (1993)	electroporation	HPT antisense of allergen gene	transformed plant
Uchimiya *et al*., (1993)	electroporation	*bar*	transformed plant
Wang *et al*., (1993)	particle gun	GUS, CAT	transient expression
Hosoyama *et al*., (1994)	electroporation	HPT, Oryzacystatin	transformed plant
Hiei *et al*.(1994)	*Agrobacterium*	HPT, GUS	transformed plant
Xu and Li (1994)	electroporation	NPT-II	transformed plant
Zhu *et al*., (1994)	lipofectin	NPT-II human α -interferon	transformed plant
Christou and Ford (1995)	particle gun	*bar*, GUS	transformed plant
Clough *et al*., (1995)	particle gun	HPT oat phytochome	transformed plant

		a apoprotein	
Cooley *et al*., (1995)	particle gun	*bar*, GUS	transformed plant
Rashid *et al*., (1995)	*Agrobacterium*	HPT, GUS	transformed plant
Li and Murai (1995)	polyethylenglycol	HPT, *Ac*	transformed plant
Lin *et al*., (1995)	electroporation	HPT, chitinase	transformed plant
Lynch *et al*., (1995)	electroporation	NPT-II	field trial
Peng *et al*., (1995)	polyethylenglycol	NPT-II, GUS	transformed plant
Duan *et al*., (1996)	particle gun	*bar*, pin2	transformed plant
Jain *et al*., (1996)	particle gun	HPT, GUS, HVA-1	transformed plant
Sivamani *et al*., (1996)	particle gun	HPT, GUS	transformed plant
Xu *et al*., (1996)	particle gun	*bar*, HVA-1	transformed plant
Wunn *et al*., (1996)	particle gun	HPT, *cryIA(b)*	transformed plant
Zheng *et al*., (1995)	polyethylenglycol	HPT, α -phaseolin	transformed plant
Zhen *et al*., (1996)	particle gun	HPT, GUS	transformed plant
Burkhardt *et al*., (1997)	particle gun	HPT phytoene syntase	transformed plant
Nayak *et al*., (1997)	particle gun	HPT, *cryIA(c)*	transformed plant
Takano *et al*., (1997)	polyethylenglycol	HPT, Luc	transformed plant
Toki (1997)	*Agrobacterium*	HPT, *bar*	transformed plant
Zheng *et al*., (1998)	particle gun	NPT-II Eighth largest segment of RDV	transformed plant

NPT-II: neomycin phosphotransferase,
GUS: β -glucronidase
CAT: chloramphenicol acetyltransferase
RSV: rice stripe virus
Ac: maize transposable element Activator
ALS: acetolactate synthase
Luc: luciferase
HPT: hygromycin phosphotransferase
AMP-: aminoglycoside phosphotransferasea
bar: phosphinothricin acetyltransferase gene
RDV: rice dwarf virus
CP: coat protein
HVA-1: late embryogenesis abundant protein gene
pin2: potato proteinase inhibitor II (PINII) gene

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SECTION 6
POTATO (*SOLANUM TUBEROSUM* SUBSP. *TUBEROSUM*)

1. General Information

This consensus document addresses the biology of the potato (*Solanum tuberosum* subsp. *tuberosum*). It contains general information on the taxonomy, morphology, and centre of diversity of the species which can be of importance during a risk assessment (for example, information on reproductive biology, the possibility of crosses, and ecology). In regard to intra- and interspecific crosses, emphasis has been given to the conditions which make a cross possible rather than listing all successful crosses. Such a list would be very long and subject to frequent changes. Only hybridisation events not requiring human intervention are considered.

The Netherlands served as lead country in the preparation of this document, in collaboration with the United Kingdom.

2. Taxonomic Status

The family *Solanaceae* contains several well known cultivated crops such as tomato (*Lycopersicon esculentum*), eggplant (*Solanum melogena*), tobacco (*Nicotiana tabacum*), pepper (*Capsicum annuum*) and potato (*Solanum tuberosum*). The potato ranks, on a world scale, fourth in food production for starch crops. Around 300,000 metric tonnes are produced (FAO, 1985). About 95 countries produce potatoes, with a total value of around $US 13 billion (Horton *et al*., 1985).

Within the genus *Solanum* over a thousand species have been recognised. According to Burton (1989), there are "well over two thousand species". This genus is subdivided into several subsections, of which the subsection *potatoe* contains all tuber-bearing potatoes. The subsection *potatoe* is divided into series, one of which, *tuberosa*, is relevant to this document. Within the series *tuberosa* about 54 species, both wild and cultivated, are found. One of these is *S. tuberosum* (Hawkes, 1990).

S. tuberosum is divided into two subspecies: *tuberosum* and *andigena*. The subspecies *tuberosum* (Table 1.7) is the cultivated potato widely in use as a crop plant in, for example, North America and Europe. The subspecies *andigena* is also a cultivated species, but cultivation is restricted to Central and South America (Hawkes, 1990; Hanneman, 1994).

Table 1.7 Taxonomic position of S. Tuberosum subsp. Tuberosum

Taxonomic rank	Latin name
family	Solanaceae
genus	*Solanum*
section	*petota*
subsection	*potatoe*
series	*tuberosa*
species	*Solanum tuberosum*
subspecies	*tuberosum*

3. Centre of Diversity

The centre of diversity for wild tuber-bearing potatoes (subsection *potatoe*) lies in Latin America, which is also considered the centre of origin. For the series *tuberosa* (to which *S. tuberosum* belongs) and most other series within the subsection *potatoe*, there are two centres of diversity. One is a long-stretching Andean area in Venezuela, Colombia, Ecuador, Peru, Bolivia and Argentina. The other is in central Mexico. The distribution area of these wild potatoes is much larger: from the southwestern United States to southern Argentina and Chile (Child, 1990; Hawkes, 1990).

Generally the cultivated *Solanum* species are also found within the centres of diversity for wild potatoes. The exception is the cultivated diploid form of *Solanum tuberosum* subsp. *tuberosum*, which is only found in a constricted area of southwestern Chile.

The cultivated tetraploid *Solanum tuberosum* subsp. *tuberosum*, as known in Europe and most other parts of the world, is considered to be a selection from a small introduction of *S. tuberosum* subsp. *andigena* potatoes from Colombia and Peru, and as such has a very narrow genetic basis. The arguments for this thesis are that plants of the original introductions into Europe are known to have been late flowering and tuberising, and that the morphological description of these potatoes matches the *andigena* type (Howard, 1970). Through selection, this introduction was adapted to the longer day lengths and different environmental conditions of Europe. Simmonds (1966) has shown that such transition can take place in a fairly short period of approximately ten years of selection. From Europe, this new type of potato has spread all over the world as a cultivated crop. An alternative theory is that, after the potato blight epidemic in Europe, new germplasm of *S. tuberosum* subsp. *tuberosum* originating from Chile (Hawkes, 1990) was introduced into Europe.

4. Identification Methods

A. Morphology and somaclonal variation

The subsection *potatoe* is distinguished from all other subsections within the genus *Solanum* by "true potatoes whose tubers are borne on underground stolons, which are true stems, not roots" (Hawkes, 1994).

The series *tuberosa* is characterised by "imparipinnate or simple leaves, forked peduncle, rotate to petagonal corolla and round berries" (Hawkes, 1990). The species *S. tuberosum* is characterised by "pedicel articulation placed in the middle third, short calyx lobes arranged regularly, leaves often slightly arched, leaflets always ovate to lanceolate, about twice as long as broad, tubers with well marked dormancy period" (Hawkes, 1990).

The differences between the two subspecies within *S. tuberosum* are very small, the greatest difference being the short day dependence of the subspecies *andigena*. The differences are set out in Table 1.8.

Table 1.8 Distinction between *S. tuberosum* subsp. *tuberosum* and subsp. *Andigena* (Hawkes, 1990)

Characteristic	*tuberosum*	*andigena*
leaves	less dissected	dissected
leaflets	wider	narrow
leaf angle	arched	acute
pedicel	thickened at the apex	not thickened at the apex
tuber formation	long or short days, mostly altitude neutral	short days, higher altitudes

The general description of the morphology of *S. tuberosum* subsp. *tuberosum* is as follows: Herbaceous perennial with weak stems that grow to a maximum of three feet, long pinnate leaves, ovate leaflets with smaller ones disposed along the midrib. The flowers are white, purple, pinkish, or bluish, in clusters, usually with a five-part corolla and exserted stamens with very short filaments. The fruits are yellowish or green, globose, and less than one inch in diameter. Some lack seeds, but others may contain several hundred. The fruits are inedible by humans due to the presence of toxins (Anonymous, 1996; Hawkes, 1990). Tubers are borne at the end of underground stolons. They are round to long oval. The flesh is generally white or cream to yellow, the skin colour light brownish to red. Tubers can contain high levels of solanine, a toxic alkaloid.

Potatoes are very easily regenerated with the use of *in vitro* tissue culture techniques. This form of vegetative propagation normally leads to genetically identical individuals, but considerable heterogeneity is common after tissue culture in which a callus stage is included. This variation is called somaclonal variation. *S. tuberosum* subsp. *tuberosum* is, like all potatoes, quite prone to this kind of variation (Cutter, 1992; Hawkes, 1990).

A. Molecular identification

It is also possible to distinguish between several *Solanum* species with the use of molecular techniques. Restriction Fragment Length Polymorphism (RFLP) of chloroplast DNA, using eight endonucleases (Hosaka *et al*., 1984), showed that 33 tuberous *Solanum* species and hybrids and two *Lycopersicon* species could be distinguished. The four different *Solanum tuberosum* subsp. *tuberosum* accessions showed identical patterns.

RFLP analyses of genomic DNA can also lead to species identification within the genus *Solanum*. Debener *et al*., (1990) showed with the use of 70 probe/enzyme combinations (probes from *S. tuberosum* subsp. *tuberosum*) that 38 accessions representing twelve *Solanum* species and one hybrid could be distinguished. The two accessions from *S. tuberosum* subsp. *tuberosum* were not identical. One of the two accessions was the "true" *S. tuberosum* subsp. *tuberosum* cultivar Bintje, the other was a breeding line in whose pedigree *S. andigena*, *S. demissum* and *S. acaule* were introgressed. It could also be shown that RFLP fingerprinting allows distinction not only between species but also between different cultivars or breeding lines (Weising *et al*., 1992). The use of probes from other *Solanum* species, mostly repetitive sequences, also led to species and cultivar identification (Schweizer *et al*., 1993). Also RAPD markers can be used for potato cultivar and clone fingerprinting (Powell *et al*., 1991; Quiros *et al*., 1993).

In addition, there are indications that the *Solanum* species can be distinguished with the use of the Amplified Fragment Length Polymorphism (AFLP) technique. One primer can generate up to 120 bands per sample, so that discrimination between *Solanum* species is very easy (Kardolus, in press).

It appears that the potato and tomato genomes are so preserved that probes from one can be used to identify the other (Gebhart *et al*., 1991). This is especially important for the construction of a genetic map and the development of molecular markers.

5. Genetic Characteristics: The Genome

The basic chromosome number in the genus *Solanum* is twelve. *S. tuberosum* subsp. *tuberosum* can be diploids (2n=24) or tetraploids (4n=48). The diploids are only found in Chile, while the tetraploids are the most commonly cultivated all over the world. How the tetraploidy originated is unclear. The cultivated *S. tuberosum* subsp. *tuberosum* can be either an autotetraploid (doubling of the chromosomes of a diploid species) or an allotetraploid (doubling of the chromosomes of a diploid hybrid between two related species) (Hawkes, 1990).

The phenomenon of unreduced gametes is common in *Solanum* species. In most *Solanum* species, next to the normal haploid gametes (n), unreduced gametes (2n) can be found, greatly extending the possible number of natural crosses (see section on crosses) (Hanneman, 1995). Watanabe *et al*., (1991) reported that most of the 38 tuber-bearing *Solanum* species examined produced 2n pollen. The frequency varied from 2 up to 10 per cent.

It is also fairly easy to produce dihaploids from cultivated tetraploid *S. tuberosum*. This can be done by pollinating with, for example, *S. phureja*, which leads to the formation of parthenogenetic diploid plants. Anther culture is also in use to produce dihaploids (Howard, 1970; Caligari,1992). It has been shown that, where *S. phureja* is used to produce dihaploids, minor chromosomal fragments are found in these dihaploids originating from *S. phureja* (Clulow *et al*., 1991).

The great value of these diploids is in breeding programmes: species that do not cross readily with the tetraploid potato can cross with a dihaploid (see section on crosses below). These dihaploids are often ovule fertile but pollen sterile.

6. Reproductive Biology

A. Sexual reproduction

Diploid *S. tuberosum* and the other diploid species within the section *petota* are self-incompatible (Kirch *et al*., 1989). This incompatibility is of a gametophytic, multi-allelic nature based on the occurrence of S alleles. In general these species are insect-pollinating, cross-breeding species

The cultivated tetraploid *S. tuberosum* subsp. *tuberosum* is self-compatible. The S alleles occur in this species, but somehow the incompatibility system is weakened. The mechanism behind this is not known. Plaisted (1980) has shown that under field conditions selfing is most likely for tetraploid *S. tuberosum*, with 80-100 per cent of the seeds formed due to selfing.

To facilitate cross-breeding and selfing, the appearance of insects is necessary. In particular, bumblebees (*e.g. Bombus funebris* in Peru and *B. impatiens* in the US) are good pollinators for potatoes (White, 1983). Pollen dispersal is mainly limited by the distance pollinating insects fly. Bumblebees and bees do not fly much further than three kilometres (Reheul, 1987). Normal honeybees (*Apis mellifera*) and *Bombus fervidus* are not pollinators of potato, as the flowers are without any nectar (Sanford and Hanneman, 1981). White (1983) carried out some experiments to determine the importance of pollination by wind for potatoes. Flowers were emasculated, and therefore of no interest to insects. The seedset on these flowers was assessed. No seeds were found, and therefore it was concluded that pollination by wind was of no importance.

Conner *et al*., (1996) collected outcrossing data from several field experiments with genetically modified potatoes, performed in New Zealand, the United Kingdom and Sweden. In each study the outcrossing rate was reduced to 0 per cent where the receiving plants were separated by more than 20 metres from the genetically modified ones.

Although many *Solanum* species are fertile, it appears that a large number of the tetraploid cultivated *S. tuberosum* subsp. *tuberosum* cultivars have a reduced fertility (Ross, 1986). Most cultivars show a reduced pollen fertility or even pollen sterility. Amongst them are well known cultivars like Bintje and King Edward. Although reduced female fertility is not so common, it is noticed that a lot of cultivars flower less profusely than wild material. Another observable phenomenon is that flowers are dropped after pollination, so that no berries are found. The result is that on most *S. tuberosum* subsp. *tuberosum* cultivars few berries and seeds are formed.

Potato seeds cannot be disseminated by birds, but dissemination by small mammals is possible (Hawkes, 1988). Lawson (1983) showed that in Scotland potato seeds could be stored in the ground for up to ten years without losing viability. Love *et al*., 1994 report that potato seeds can survive and germinate for periods of time in excess of seven years.

B. Asexual reproduction

The potato can also multiplicate vegetatively. Tubers are formed under the ground. As the tuber is the product for which potatoes are cultivated, an extensive selection has taken place for good tuber production and quality. These tubers can remain viable for long periods of time as long as there is not a major frost period. The stolons on which the tubers are formed are generally not very long for *S. tuberosum* subsp. *tuberosum* cultivars. Stolons of wild tuber-bearing *Solanum* species are much longer (Hawkes, 1990).

7. Crosses

A. Intraspecific crosses

Solanum tuberosum subspecies *tuberosum* and *andigena* are fully cross-compatible (Plaisted, 1980). Hybrids can occur in nature. The incidence of this cross is not clear, as the morphological distinction between the two subspecies is very small. As both subspecies only occur in southern North America and some parts of South America, natural crosses are only likely to be found there.

B. Interspecific crosses: crosses within the subsection *potatoe* (Tuber-bearing Potatoes)

The gene pool for potato is extremely large. Dale *et al*., (1992) and Evenhuis *et al*., (1991) state that it is likely that all crosses between the tuber-bearing potatoes within the section *petota* may be possible, although in some cases techniques will have to be applied to establish the crosses.

It appears that there are two groups within this section which are very difficult to cross:

- The diploid species in the series morelliformia, bulbocastana, pinnatisecta, polyadenia, commersoniana, lignicaulia, and circaeifoli.

- The diploid species in the other series.

The fertilisation of a diploid plant with normal haploid pollen in fact consists of two fertilisations. The pollen contains two (haploid) generative nuclei; one nuclei fertilises the egg cell, the other fertilises the embryosac nucleus. The result is a diploid embryo with triploid endosperm.

Den Nijs and Peloquin (1977) reported the existence of a "triploid block" where a tetraploid plant was crossed with a diploid plant. This block is due to the imbalance between the endosperm (5x) and the embryo (3x). The endosperm is not formed, and this is followed by embryo abortion (Jacobsen and Rousselle, 1992).

Johnston *et al*., (1980, 1982) also found that some species of the same ploidy level could not cross, whereas crosses between species of different ploidy levels were successful. They introduced the concept of the Endosperm Balance Number (EBN), which is a measure to express the "effective ploidy of a genome in the endosperm". To make the normal development of the endosperm after fertilisation possible, the maternal EBN must be twice the paternal EBN (2:1).

The EBN is independent of the ploidy level of the species, and its behaviour is additive. This means, for instance, that by doubling of the chromosome number the EBN also doubles.

Two situations can occur:

- The EBN of two species is the same: natural crosses are possible;
- The EBN of two species is not the same: natural crosses are not possible.

Where the EBN of two species is not the same, several natural or artificial mechanisms are available to circumvent the incompatibility.

Natural mechanisms:

- The occurrence of unreduced gametes makes it possible that species with a lower EBN can cross with species with a higher EBN. For example:

  A plant with 4x (EBN=4) cannot cross with a 2x (EBN=2) plant, but if the 2x plant produces unreduced gametes the EBN of these gametes becomes 4, which makes the cross possible. The resulting plant is a tetraploid (4x) with an EBN of 4. It is important to notice that, due to the common occurrence of unreduced gametes in most *Solanum* species, crosses of this kind can occur in nature.

Artificial mechanisms:

- Production of dihaploids makes it possible that species with a higher EBN can cross with species with a lower EBN. For example:

  A plant with 4x (EBN=4) cannot cross with a 2x (EBN=2) plant. After dihaploidisation of the 4x (EBN=4) plant a diploid plant with an effective EBN of 2 is formed. This plant can be pollinated by the 2x (EBN=2) plant. The resulting plant is a diploid (2x) with an EBN of 2. It is important to notice that crosses of this kind are not likely to occur in nature, due to the fact that diphaploids are only rarely formed in nature.

- Polyploidisation of plants makes it possible to cross plants with a lower EBN with plants with a higher EBN. For example:

  A plant with 2x (EBN=2) cannot cross with a 4x (EBN=4) plant. After polyploidisation of the 2x (EBN=2) plant a tetraploid plant with an effective EBN of 4 is formed. This plant can cross with the 4x (EBN=4) plant. The resulting plant is a tetraploid (4x) with an EBN of 4. It is important to notice that crosses of this kind are not likely to occur in nature, due to the fact that spontaneous polyploidisation rarely occurs; nevertheless, it cannot be excluded.

Despite the EBN system, potatoes of different groups can be combined by somatic fusion *in vitro*. The application frequency of this method is increasing. Fusion products may be fertile, so somatic hybrids may serve as a bridge for combining incompatible genomes.

In Annex I the ploidy and EBN of the most common potato species within the section *petota* are given. These data can be used as an indication of the possibility of formation of hybrids of *S. tuberosum* subsp. *tuberosum* with these species in nature. It is likely that *Solanum tuberosum* subsp. *tuberosum* can cross readily with all *Solanum* species mentioned in Annex I with the same EBN (=4). Also, due to the occurrence of unreduced gametes, the crosses of *S. tuberosum* subsp. *tuberosum* with all *Solanum* species mentioned in Annex I with an EBN of 2 are possible.

To determine if a cross really is likely to occur in nature, several factors have to be considered. The most important are:

- The EBN of the crossing partners:

  These must be matching, or the EBN of one partner must not be less than half the EBN of the other partner.

- Geographical occurrence of the species involved:

  The species involved must occur in the same area and habitat.

- Flowering period of the species involved:

  The flowering periods must overlap.

- The presence of stylar barriers that prevent the growth of pollen tubes:

  The presence of appropriate pollinators.

In most parts of the world, no *Solanum* species from the section *petota* with an EBN of 2 or 4 will occur next to cultivated tetraploid *S. tuberosum* subsp. *tuberosum.* Crosses are therefore not likely, due to geographical isolation. Only in the southern United States and South America do crossing partners with a suitable EBN occur next to cultivated tetraploid *S. tuberosum* subsp. *tuberosum*. In these areas the characteristics of the species involved and the habitat must be assessed to predict the likelihood of the cross.

C. Interspecific crosses: crosses with species from sections other than *petota*

The occurrence of hybrids with species from sections other than *petota* is not likely, due to strong crossing barriers, although in several areas of the world cultivated tetraploid *S. tuberosum* subsp. *tuberosum* occurs next to indigenous *Solanum* species (not from section *petota*). For instance, *S. nigrum* and *S. dulcamara* occur in the Netherlands. Eijlander and Stiekema (1990) found that the cross of tetraploid *S. tuberosum* subsp. *tuberosum* with *S. dulcamara* did not result in any viable seeds and plants. For the cross of *S. nigrum* with *S. tuberosum* the same is valid. Only after embryo rescue could two hybrids be obtained. These were less vital, male sterile, formed no tubers, and showed less female fertility. After pollination (backcross), no viable seeds were formed.

8. Ecology of *Solanum tuberosum* subsp. *tuberosum*

A. Cultivation

Environmental conditions under which *S. tuberosum* can be successfully grown are very diverse, as can be concluded from the fact that potatoes are cultivated in many parts of the world. A broad spectrum of cultivars are adapted to these different environmental conditions. Some general parameters can be determined for the cultivation of *S. tuberosum* subsp. *tuberosum*:

- The *S. tuberosum* subsp. *tuberosum* tuber cannot survive a temperature of -3°C and lower. The foliage dies at temperatures of -4°C (van Swaaij *et al*., 1987; Vayda, 1994). Dale (1992) reports that potato tubers are destroyed by a frost period of 25 hours at -2°C or a frost period of five hours at -10°C. Latin American *Solanum* species can be much more frost-resistant.

- *S. tuberosum* subsp. *tuberosum* cannot be acclimatised to lower temperatures, whereas other Solanum species (for example, S. acaule) can be (van Swaaij *et al*., 1987; Li and Fennel, 1985).

- Potatoes seem to be very sensitive to soil water deficit (Vayda, 1994).

- A wide range of soil pH can be tolerated by potatoes (normally 5 and higher, but good production at pH 3.7 was observed) (Vayda, 1994).

- S. tuberosum subsp. tuberosum is a daylight neutral crop, which means that tubers are made at a growth stage independent of the day length. But variation for daylight sensitivity can be found among *S. tuberosum* subsp. *tuberosum* cultivars.

- Extreme low or high temperatures, in particular the night temperature, can obstruct tuber formation.

- Short days (-14 hours) and moderate ground temperatures (15-18°) enhance tuber formation. Longer days (14-16 hours) and higher (day) temperatures (20-25°) enhance flowering and seed formation (Beukema and van der Zaag, 1979; Burton, 1989).

- Germplasm from all over the world has been used to improve potato varieties. The main goal is to develop cultivars with resistance to biotic factors (fungal, virus, bacterial and insect resistance). Other goals are: improved starch content, adaptation to tropical growing conditions, herbicide resistance, stress tolerance, and the introduction of anti-bruise genes (Brown, 1995). The species most used to improve potato are *S. demissum*, *S. acaule*, *S. chacoense*, *S. spegazinii*, *S. stoloniferum*, *S. vernei*. Less used are *S. microdontum*, *S. sparsipilum*, *S. verrucosum*, *S. phureja*, *S. tuberosum* subsp. *andigena*, *S. commersonii* and *S. maglia* (Caligari, 1992). This germplasm has been introduced into many cultivars of *S. tuberosum* subsp. *tuberosum*.

In Annex II the most common diseases (insects, mites, viruses, bacteria and fungi) in potato and their spread throughout the world are shown. This annex is not intended to give a complete list of all potato diseases known. Therefore it should be taken into account that locally other diseases can be of great importance. The national phytosanitary service can best be consulted on this subject. Moreover, Annex II does not give any indication of which sanitary or quarantine provisions have to be applied in a country.

B. Volunteers and weediness

In the cultivation of *S. tuberosum* subsp. *tuberosum*, plants from seeds from a previous potato crop can act as a volunteer weed. The tubers can also act as a volunteer weed in cultivation. In general these plants (from seeds and tubers) will be eliminated by normal agronomical practices. In addition, tubers will not survive for a long time in most of the areas of cultivation due to unfavourable environmental conditions (low temperatures).

Outside the field, potato seedlings will have difficulty establishing themselves as they cannot compete with other plants. Love *et al*., 1994 report that these seedlings are limited to cultivated areas for reasons of competition and adaptation. Potato tubers can be spread during transportation and use, but generally these plants will not be established for a long time due to unfavourable environmental conditions.

In general, the potato is not known as a coloniser of unmanaged ecosystems. In climax vegetation it is not able to compete with other species such as grasses, trees and shrubs (Anonymous, 1996).

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APPENDIX I

Ploidy and EBN of Most *Solanum* Species within the Section *petota* (Hawkes, 1990; 1992; 1994)

SUBSECT.	SERIES	PLOIDY	EBN=1	EBN=2	EBN=4	EBN=unknown
estolonifera	–	Diploid	all species			
juglandifolia	–	Diploid				all species
potatoe	*morelliformia*	Diploid				*S. morelliforma*
	bulbocastana	Diploid	*S. bulbocastanum*			*S. clarum*
		Triploid	*S. bulbocastanum*			
	pinnatisecta	Diploid	*S. branchistotrichum* *S. jamesii* *S. cardiophyllum* *S. pinnatisectum* *S. trifidum*			*S. tarnii*
		Triploid	*S. cardiophyllum*			*S. jamesii*
	polyadenia	Diploid				*S. polyadenium* *S. lesteri*
	commersoniana	Diploid	*S. commersonii*			
		Triploid	*S. commersonii*			*S. calvescens*
	circaeifolia	Diploid	*S. capsicibaccatum* *S. cicaeifolium*			
	lignicaulia	Diploid	*S. lignicaule*			
	olmosiana	Diploid				*S. olmosense*
	yungasensa	diploid		*S. chacoense* *S. tarijense*		*S. arnezii* *S. yungasense*
	megistacroloba	diploid		*S. astileyi* *S. megistacrolobum* *S. sanctae-rosae* *S. toralapanum*		*S. boliviense*
	cuneoalata	diploid		*S. infundibuliforme*		

SUBSECT.	SERIES	PLOIDY	EBN=1	EBN=2	EBN=4	EBN=unknown
	conicibaccata	diploid		*S. chomatophilum* *S. violacceimarmoratum* *S. agrimonifolium* *S. colombianum* *S. oxycarpum*		*S. santolalle*
		hexaploid			*S. moscopanum*	
	piurana	diploid				*S. piurae*
	ingifolia	diploid				*S. ingifolium*
	maglia	diploid				*S. maglia*
		triploid				*S. maglia*
	tuberosa	diploid		wild: *S. berthaultii* *S. brevicaule* *S. bukasovii* *S. canasense* *S. gourlayi* *S. kurtzianum* *S. leptophyes* *S. medians* *S. microdontum* *S. multidissectum* *S. multiinteruptum* *S. sparsipilum* *S. spegazzinii* *S. vernei* *S. verrucosum* cultivated: *S. phureja* *S. stenotomum*		wild: *S. alandiae* *S. hondelmanni* *S. neocardenasii* *S. okadae* *S. oplocense* cultivated: *S. ajanhuiri*
		triploid				wild: *S. maglia* *S. microdontum* cultivated: *S. ×chaucha* *S. ×juzepczukii*
		tetraploid			wild: *S. gourlayi* *S. oplocense* *S. sucrense* cultivated: *S. tuberosum* subsp. *tuberosum* *S. tuberosum* subsp. *andigena*	
		pentaploid				*S. ×curtilobum*
		hexaploid			*S. oploscense*	

SUBSECT.	SERIES	PLOIDY	EBN=1	EBN=2	EBN=4	EBN=unknown
	aucalia	tetraploid		*S. acaule*		
		hexaploid			*S. albicans*	
	longipedicellata	triploid				*S. ×vallis-mexci*
		tetraploid		*S. fenderi* *S. hjertingii* *S. papita* *S. polytrichon* *S. stoloniferum*		
SUBSECT.	SERIES	PLOIDY	EBN=1	EBN=2	EBN=4	EBN=unknown
	demissa	triploid				*S. ×semidemissum*
		hexaploid			*S. brachycarpum* *S. demissum* *S. guerreroense* *S. hougasii* *S. iopetalum*	*S. scheckii*

APPENDIX II

The most common diseases in *Solanum tuberosum* subsp. *tuberosum* and their distribution: for each pest/disease category a reference to a more extensive review is given (Hide and Lapwood, 1992; Evans and Trudgill, 1992; Raman and Radcliffe, 1992)

INSECT AND MITE PESTS (review: Hooker, 1986) **Organism**	**Occurrence**
Myzus persicae, Phthorimaea operculella, Agriotes spp.	Worldwide
Macrosiphum euphorbiae, Aphis fabae, Empoasca devastans, Heliothus armigera, Spodoptera exigua	Worldwide except Africa
Plusia orichalcea, Sthenaridea pulsilla, Psylloides plana, Epicauta hiriticornis, Anomala dimidiata, Phyllognathus dionysius, Melolontha spp., *Odontotermes obesus, Eremotermes* spp., *Alcidodes westermanni, Myllocerus subfasciatus, Pyralis farinalis, Nipaecoccus vastator*	Asia
Empoasca fabae, Paratrioza cockerelli, Hypolithus spp.	North America
Diabrotica sp., *Epicauta* spp., *Premnotrypes* spp., *Phylophaga* spp., *Scrobipalpula absoluta, Scrobipalpopis solanivora, Symmetrischema plaeseosema, Feltia experta, Stenotycha* spp., *Copitarsia turbata, Bonthinus mainon, Phenacoccus grenadensis, Liriomyza* spp.	Central and South America
Shistocerca gregaria, Liriomyza trifolii	Africa
Henosepilachna sparsa, H. vigintisexpunctata, Austroasca virigrisea, Listroderes obliquus, Heteronychus arator, Cheiroplatys latipes, Graphognathus leucoloma	Australia
Aphis nasturtii, Limonius spp., *Ctenicera* spp., *Conodorus* spp.	North America and Europe
Aphis gossypii	Central and South America, Asia
Aulacorthum solani	North America, Europe and Africa
Leptinotarsa decemlineata	North America, Europe and Asia
Epitrix spp.	North, Central and South America
Epilachna spp., *Polyphagotarsonemus latus, Thrips palmi, Gryllotalpa africana*	Africa and Asia
NEMATODES (review: Hooker, 1986) **Organism**	**Occurrence**
Globodera rostochiensis, Globodera pallida	Worldwide
Meloidogyne hapla	North America and temperate areas in general
Meloidogyne chitwoodi	Northwest America and parts of Western Europe
Nacobbus aberrans	Peru and Bolivia
Pratylenchus penetrans, Trichodorus and *Paratrichodorus* spp.	North America and Europe

Ditylenchus destructor	North America, Western Europe and the former USSR
Ditylenchus dipsaci	Western Europe
VIRUSES (review: Valkonen, 1994) **Organism**	**Occurrence**
Virus X (mild mosaic), leaf roll virus, Virus Y (severe mosaic), Virus A (mild mosaic), Virus S	Worldwide
Virus M	Europe and North America
Tobacco rattle virus	Europe, North America, Brazil and Japan
Mop top virus	Western Europe and Peru
Yellow dwarf virus	North America
Spindle tuber "viroid"	North America, former USSR and South Africa
Witches' broom (mycoplasma)	Europe, North America, Australia, China
BACTERIA (review: Hooker, 1986) **Organism**	**Occurrence**
Clavibacter michiganensis var. s*epedonicus* (ring rot), *Erwinia carotovora* ssp. *atroseptica* and subsp. *Carotovora* (blackleg, soft rot)	Europe and North America
Erwinia chrysanthemi (blackleg, soft rot)	Tropics and sub-tropics
Streptomyces scabies (common scab)	Worldwide
Pseudomonas solanacearum (brown rot)	Tropics and warm temperate zones
FUNGI (review: Hooker, 1986) **Organism**	**Occurrence**
Alternaria solani (early blight*), Botrytis cinerea* (grey mould*), Collectotrichum coccodes* (black dot*), Helicobasidium purpureum* (violet root rot*), Phytophtora infestans* (late blight), *Pythium ultimun* (watery wound rot), *Rhizoctonia solani* (black scurf), *Spongospora subterranea* (powdery scrab), *Verticillium albo-atrum, V. dahliae* (wilt)	Worldwide
Angiosorus solani (potato smut)	Central and South America
Fusarium spp. (wilt)	North America
Fusarium solani var. *Coeruleum* (dry rot), *Phoma foveata* (gangrene)	Europe
Helminthosporium solani (silver scruf), *Sclerotinia sclerotiorium* (stalk break)	Europe and North America
Macrophomina phaseolina (charcoal rot)	North America and India
Polyscytalum pustulans (skin rot)	Northern Europe, North America, Australia
Synchytrium endobioticum (wart)	Europe, North and South America, South Africa and Asia
Phoma exigua var.*Exigua* (gangrene), *Phytophtora erythroseptica* (pink rot)	Europe, North America and Australia

SECTION 7
BREAD WHEAT (*TRITICUM AESTIVUM*)

1. General Description and Use as a Crop, Including Taxonomy and Morphology

Triticum aestivum, bread wheat, belongs to the order *Poales* (*Glumiflorae*), family *Poaceae* (*Gramineae*), tribe *Triticeae*, genus *Triticum*. The tribe *Triticeae* consists of 18 genera which are divided into two sub-groups, the *Triticinae* and the *Hordeinae*. The major genera in the sub-group *Triticinae* are *Triticum, Aegilops, Secale, Agropyron* and *Haynaldia* (Odenbach 1985, Zeller 1985, Körber-Grohne 1988).

Plants of the genus *Triticum* are annuals with spring or winter forms. They show the following morphological features: short ligule and spikelets that are sometimes hairy, and a smooth, bald, usually hollow culm, 0.7-1.6 metre in height. Pithy filling is less common than a hollow culm. The ears have a brittle or tough rachis. Generally they are four-sided. The spikelets have two to five florets. Each floret can produce one grain (caryopsis), *i.e.* is distichous. The glumes are keeled, on the upper side for example in *T. aestivum*, with serrated lemmas, long and either bearded or unbearded. Grains are loosely enclosed (naked wheat) and easily threshed. The rachilla has thin walls and does not disarticulate on maturity. In case of *T. aestivum* ssp. *spelta* (spelt wheat) the grains are hulled by the spelta. For this reason they cannot be dropped during the process of threshing (Garcke 1972, Geisler 1991).

T. aestivum is a cereal of temperate climates. The northern limit of wheat cultivation in Europe lies in southern Scotland (60° latitude) and occasionally beyond (central Scandinavia up to 64°). In North America wheat is grown to about 55° latitude. Wheat occurrence follows a similar pattern in the southern hemisphere. In the Alps, it is grown to an altitude of 1 500 metres above sea level (Körber-Grohne 1988, Geisler 1991).

The minimum temperature for germination of *T. aestivum* seeds is between 3 and 4°C. Flowering begins above 14°C. The vegetative period is 120 to 145 days for spring wheat and 280 to 350 days for winter wheat. Some varieties of *T. aestivum* need long photoperiods; some, especially those cultivated in southern Europe, are insensitive to day length. The harvested fruit, a grain with the botanical name caryopsis, contains approximately 80 to 84 per cent endosperm, approximately 60 per cent carbohydrate (starch), approximately 10 to 16 per cent protein, approximately 2 per cent fat, and approximately 13 per cent water (Hömmö and Pulli 1993). The starch granules of the *Triticeae* are botanically distinctive. Wheat meal is an important product. Meal from *T. durum* (macaroni wheat), for example, is used for the production of pastas such as spaghetti and semolina. Meal from *T. aestivum* (bread wheat) on the other hand contains a high proportion of gluten. For this reason it is very suitable for baking. Spelt wheat is rich in protein. Overlapping in protein content and high starch content can occur, as there is a wide range of difference due to both genetic variation and variable environmental conditions (Körber-Grohne 1988).

2. Agronomic Practices

In the Northern Hemisphere, depending on the location and the preceding crop, winter wheat can be sown from late August to late December. Sowing usually occurs between mid-September and late October. Seeds of winter wheat need 40 to 70 days vernalisation with a temperature between -1°C and +8°C (Geisler 1970, 1971, Kübler 1994). Hömmö and Pulli (1993) reported a maximum cold tolerance for winter wheat of about -25°C.

Seeds of spring wheat need only 3 to 5 days (Geisler 1970) or 0 to 14 days (Reiner *et al*., 1992) vernalisation. The commencement of growth of shoots is decisively influenced by the photoperiod in the case of spring wheat. The cold tolerance for seedlings of spring wheat is about -5°C (Hömmö and Pulli 1993). The sowing season for spring wheat is from January to May (Kübler 1994).

In normal agricultural practice *T. aestivum* is used in a crop rotation schedule. Sugar beet, grain legumes and corn (*Zea* mays) or fodder maize make good preceding crops (Kübler 1994). Oilseed rape and winter barley occupy large areas and are part of many crop rotation systems that include winter wheat. Wheat/fallow rotations are commonly used in the western Great Plains region of the United States. Problems with plant diseases (see Annex I) may arise from the frequent use of wheat as part of the crop rotation system.

As with all crops cultivated and harvested at the field scale, some seeds may escape and remain in the soil until the following season when they germinate either before or following seeding of the succeeding crop. In some instances these "volunteers" may give considerable competition to the seeded crop and warrant chemical and/or mechanical control. The problem of volunteer plants in succeeding crops is common to most field crop species. Much depends on the management practices used in the production of the crop, *e.g.* the speed of the harvesting operation which will determine whether more or less seed is lost by the harvester. A suitable soil treatment after the harvest can considerably reduce the volunteer problem.

A great number of dicotyledonous and fewer monocotyledonous weeds have been reported to occur in fields used for wheat production. Seeds of some of these, when harvested and mixed with the wheat grain, can reduce flour quality (Wolff 1987).

Isolation of wheat plants for seed multiplication within the context of plant breeding can be done with greaseproof paper or cellophane bags placed over the heads (Mandy 1970, Saatgutverordnung/BGbl 1986). Without these, modest spatial isolation may be required to prevent outcrossing. In Germany, for example, there is no minimum isolation distance for wheat breeding, but there is a requirement for separation from all neighbouring plants that can be threshed, and for a buffer zone of a minimum of 40 cm to prevent mechanical mixing of the seeds (Saatgutverordnung 1986).

3. Centres of Origin/Diversity, Geographic Distribution

A. History of wheat

The oldest archaeological findings of naked wheat (6800 to 5200 B.C.) come from southern Turkey, Israel, Syria, Iraq, Iran and south of the Caucasus Mountains in Georgia. At that time, einkorn, emmer and barley were the staple cereal crops in Asia Minor. Wheat was only grown on a regional basis. There is evidence that naked wheat was cultivated in the southern Caucasus in neolithic settlements between the late fifth and early fourth millennium B.C. Late Bronze Age specimens (approximately 1000 to 900 B.C.) of naked wheat have been found at several sites in the Crimea,

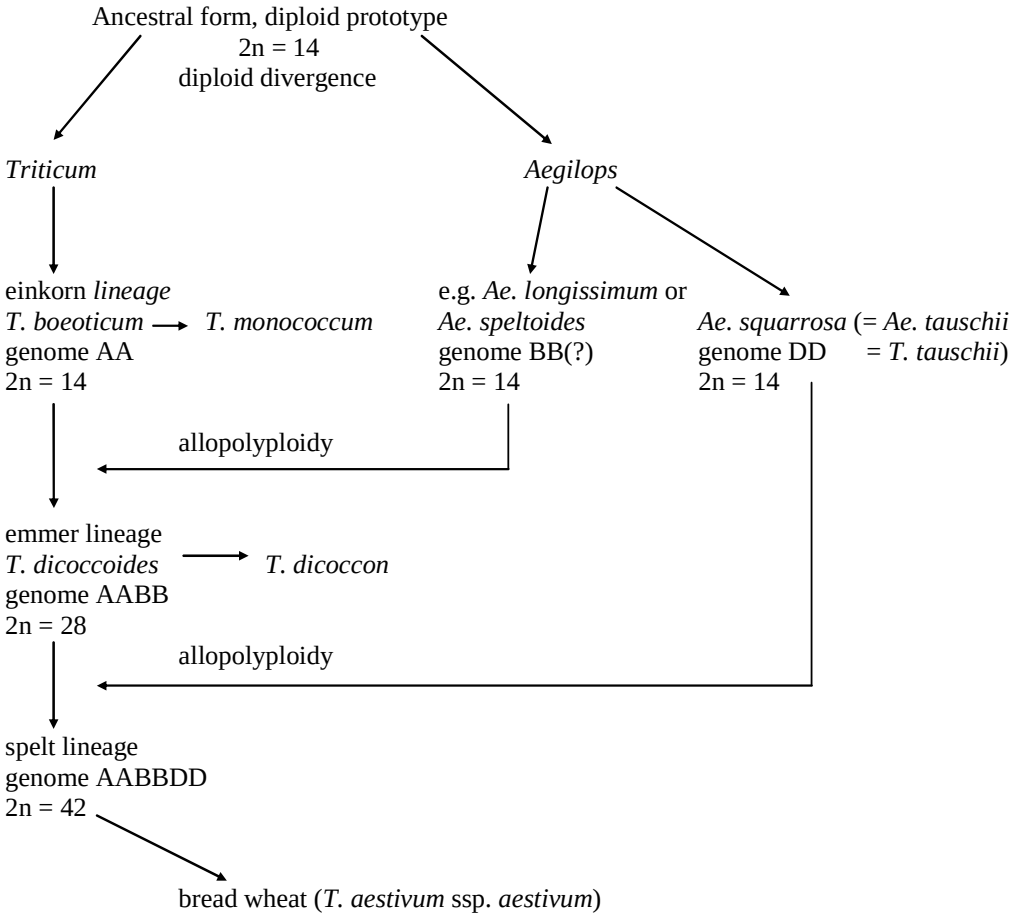
which was an early and significant wheat-growing area. Archaeological findings of wheat in Israel date from the same period (Körber-Grohne 1988).

In Central Europe, the oldest dated findings of wheat grains (a mixture of *T. aestivum*, *T. dicoccon* and *T. monococcum*) were in soil samples from the New Stone Age (4600 to 3800 B.C.). When the late neolithic period began, naked wheat was gaining importance as a crop in some areas along the River Neckar and around riverside and moorland settlements in the northern foothills of the Alps. It was not until the Roman Empire that wheat spread to the lower Rhine regions, the lower Meuse and the Scheldt Estuary, where it became the main cereal crop. Further south, spelt was favoured. Wheat farming declined north of the Alps between the fall of Rome and the Middle Ages. Evidence from excavated sites shows that little wheat was grown in the period 800 to 1200 (Körber-Grohne 1988).

The origin of Wheat has been well known since the 1940s, mainly through the work of E. R. Sears at the University of Missouri, Columbia (USA) from 1939 to 1980 (MacFadden and Sears 1946). The evolution of wheat began with an unknown diploid prototype, from which the genera *Triticum* and *Aegilops* were formed by diploid divergence. The development of the genus *Triticum* (see Figure 1.4) began with the einkorn lineage (*T. monococcum* line, genome AA), which developed into the cultured form *T. monococcum* from the wild form *T. boeoticum*. Allopolyploidization with an *Ae. speltoides* descendant (genome BB) led to the tetraploid emmer lineage (*T. turgidum* line, genome AABB) with the wild form *T. dicoccoides* from which the cultured form *T. dicoccon* developed. The origin of the B-genome is more uncertain; *Ae. speltoides*, *Ae. longissimum*, *Ae. bicornis*, *Ae. searsii*, *Ae. sharonense* are suggested as possible progenitors. The spelt lineage[20] with the genome AABBDD resulted from further allopolyploidization with the species *Ae. squarrosa* (= *Ae. tauschii*; genome DD) (Körber-Grohne 1988, Sitte *et al*., 1991, Zeller and Friebe 1991). For the current classification of the genus *Triticum* see the monograph of van Slageren (1994), also available on the home page of the Wheat Genetics Research Center, Kansas State University (http://www.ksu.edu/wgrc, under "Triticum" accessions). More recent references in regard to the issue of wheat origin are Cauderon (1994), Zohary and Hopf (1994) and Feldman *et al*., (1995).

20. Note that the term "lineage" is used to indicate that descendants are related.

Figure 1.4 An overview of the diploid einkorn lineage. (Körber-Grohne, 1988, Sitte *et al*., 1991, Zeller and Friebe, 1991))



B. Origin of einkorn lineage

The einkorn lineage includes the wild species of T. boeoticum and various goat grasses (see Table 219). The latter were formerly considered to belong to the genus Aegilops, but many geneticists now classify them as belonging to the genus Triticum. The only domesticated species in this group is einkorn (T. monococcum). Species have only one grain per floret; however, they may have one or two florets per spikelet. They are diploid (2n = 14, genome AA) (Körber-Grohne 1988, Sitte *et al*., 1991, Zeller and Friebe 1991).

Table 1.9 Geographic distribution of the diploid einkorn lineage (Körber-Grohne, 1988)

Hulled grain
Wild einkorn *T. boeoticum* (AA)
Single-grain var. *aegliopoides* (AA) Balkans, N. Greece, W. Turkey
Double-grain var. *thaoudar* (AA) E. Turkey, N. Iraq, Iran
Progeny of the two varieties (AA) Central Turkey, Transcaucasia
Goat grass *T. tauschii (Aegilops tauschii = Aegilops squarrosa)* (DD) Mediterranean, Central Asia, Iran, Iraq, Transcaucasia
Another five species of *Aegilops* (similar to B) Asia Minor and Central Asia
Einkorn *T. monococcum* (AA)

C. Origin of emmer lineage

The emmer lineage includes only tetraploid hybrids with the genome AABB (see Table 1.10). The cultivated form *T. dicoccon* developed from the wild form *T. dicoccoides*. Three forms of wild emmer are found today in various parts of Asia Minor and Central Asia. Of the six domesticated species, only emmer retains its hull as a mature grain. Species have two to three florets with two grains each (Körber-Grohne, 1988; Sitte *et al*., 1991; Zeller and Friebe, 1991).

Table 1.10 Geographic distribution of the tetraploid emmer lineage (Körber-Grohne, 1988)

Hulled grain	**Naked grain**
Wild emmer *T. dicoccoides* (AABB) S.E. Turkey, Israel, S. Syria, N. Iraq, W. Iran	
Wild emmer *T. timopheevi* (AAGG) Transcaucasia, Armenia, N. Iraq, W. Iran	
Wild emmer *T. araraticum* (AAGG) Transcaucasia	
Emmer *T. dicoccon* (AABB)	Durum wheat *T. durum* (AABB) N.E. Africa, Mediterranean, Spain
	Rivet/cone wheat *T. turgidum* (AABB) Portugal, UK, Spain
	Persian wheat *T. carthlicum* (AABB) Caucasia, Iraq, Iran
	Oriental wheat *T. turanicum* (AABB)
	Polish wheat *T. polonicum* (AABB) S. Europe, Turkey, Iraq, Iran, Armenia, N.W. India

D. Origin of spelt lineage

It is assumed that genome A derives from einkorn (*T. monococcum*) and genome D from goat grass (*T. tauschii = Ae. squarrosa = Ae. tauschii*). The origin of the third genome (B) is still unclear. It possibly belongs to *Ae. speltoides* descendants or ancestors (see Section II: History of Wheat).

The hexaploid wheat group (2n = 42, genome AABBDD) is closely related to spelt, macha and the naked wheats (see Table 1.11). The genetic differences in the gene pool of hexaploid wheat are small, although they exert a considerable influence, yielding both hulled grain (*e.g.* spelt) and naked grain (wheat).

The entire hexaploid lineage (AABBDD) is regarded as a single species. The various grains (*e.g.* bread wheat *T. aestivum* ssp. *vulgare*, spelt *Triticum aestivum* ssp. *spelta*) are considered as subspecies. In practical usage, however, the earlier categories are still frequently applied (Körber-Grohne 1988).

Table 1.11 Geographic distribution of the hexaploid spelt lineage (Körber-Grohne, 1988)

Hulled grain	Naked grain
Macha wheat *T. macha* (AABBDD) Georgia/Transcaucasia	
T. vavilovii (AABBDD) Armenia	
Spelt/dinkel *T. spelta* (AABBDD)	Dwarf/club wheat *T. compactum* (AABBDD) mountains of Afghanistan, Alps
	Cake wheat *(Kugelweizen) T. sphaerococcum* (AABBDD) Afghanistan, Bukhara, N.W. India
	Bread wheat *T. aestivum (aestivum)* (AABBDD) Temperate zones

4. Reproductive Biology

Reproduction of *T. aestivum* is only known in the context of cultivation (Garke 1972). Harvesting and propagation of its seed are entirely dependent on man. Wheat is predominantly self-pollinating. The cross-fertilisation rate may be as high as 1 to 2 per cent, although it can be less than 1 per cent (Poehlmann 1959). Wind-borne cross-fertilisation depends heavily on physical factors. It is minimal (0.1 per cent) where there is high humidity, but higher when there is warm, dry weather. Under such conditions, it has been claimed that the cross-fertilisation rate may be between 3.7 and 9.7 per cent. Cross-fertilisation is considerably more likely in the ears of stem branches (also called tillers) (Mandy 1970). The rate of cross-fertilisation may also depend on the variety (*e.g.* Stoner 24 to 37 per cent). Hucl (1996) shows for 10 Canadian spring wheat cultivars that the cross-pollination frequency varies according to the genotype. The frequency was always lower than 9 per cent. Apomixis is very rare (Mandy 1970).

Wheat's flowering season depends on geographical location. For example, in Germany and Sweden it flowers from late May to late June (Mandy, 1970, Garke, 1972). Flowering times for Mediterranean Europe and the centres of origin and diversity of wheat are late winter, and early spring (Galun, personal communication). Sunny weather and temperatures of at least 11 to 13°C are propitious for flowering (Mandy 1970). The influorescence of wheat is a spike, and the ear on the main culm flowers first. The process begins in the middle third of the ear, spreading towards the tip and base. The spikelets at the top and bottom of the ear are the last to bloom (Mandy, 1970). In cultivated wheat fields, the number of ears is usually between 400 and 650/m². Depending on the proportion of well-developed ears, the average grain count per ear varies between 35 to 40 and 20 to 25. However, the standard number of seeds per head is 30 to 35 (one ear carrying an average of 80 florets) (Kübler 1994; average data in Germany).

When flowering, the lemmas and palaeas open to an angle of 20 to 35°. The pollen sacs appear about four to six minutes later adopting a horizontal position. Under favourable weather conditions a floret will complete the flowering cycle in 13 to 18 minutes. The reproductive organs are slightly protandrous (pollen sacs mature one to three days earlier). An unfertilised spikelet remains open for several hours or even days (Mandy 1970).

Flowering for a full ear takes between 101 and 120 hours, 23 florets a day blooming on average. Blooming begins in the early morning between 4 and 5 a.m. Peak flowering time is between 9 and 10 a.m., with a second peak between 2:30 and 3:30 p.m. By 7 p.m. flowering is usually completed. A wheat plant flowers for four to 15 days (Mandy 1970; average data in Germany).

The quantity of pollen produced by an anther is low, being approximately 2700 pollen grains per sac. It has been established that, on average, 80 per cent of pollen from an anther which protrudes from the spikelet is dispersed into the air. It was assumed from this that a wheat variety with a large number of protruding anthers would make enough pollen available to achieve cross-fertilisation. Under experimental conditions in the laboratory (moderate mass exchange of 10 g/cm per second and moderate wind speed of 3 m/sec), pollen travels about 60 m distance at a height of 1 m (D'Souza 1970). In field experiments Wilson (1968) found 10 per cent seedsetting on male sterile wheat plants that were 30 m from the pollen donor plants.

Pollen begins to germinate 15 minutes after deposition on the stigma (D'Souza 1970) and retains its fertilisation ability for only a very short period. Even under optimum conditions of 5°C and 60 per cent relative atmospheric humidity, this period will not exceed three hours. Under common field conditions of 20°C and 60 per cent relative atmospheric humidity it may remain viable for less than 30 minutes. With temperatures of about 30°C and low relative atmospheric humidity, the pollen is only able to achieve its function for 15 minutes. On hot days, therefore, this short fertilisation period can considerably reduce pollen germination in the event that cross-pollination does occur (D'Souza 1970).

5. Cross-fertilisation

A. Interspecific/genus

Selection breeding, which had been ongoing for centuries, and the more recent methods of classical hybridisation breeding, have led to an enormous improvement of bread wheat traits. Biotechnological methods offer the potential to complement these traditional techniques. It has been 20 years since *in vitro* methods were first used in wheat breeding (Picard and de Buyser 1973). At that time the first variety, "Jinghua", which was produced using anther culture techniques, was licensed in China. In 1985, "Florin" became the first variety developed using *in vitro* methodology to be licensed in Europe (France) (de Buyser *et al*., 1987, Henry and de Buyser 1990).

There are many examples of successful classical cross-breeding within the genome lineage of *T. aestivum*, and between *T. aestivum* and the other lineages described above (see Figure 1.4). Hybridisation is possible with any combination in the hexaploid lineage. The progeny are fertile because the genomes are homologous. Heterosis frequently occurs.

In general, *T. aestivum* has been used as the mother plant in inter-generic and inter-specific crossing. Many crosses have been successful, although techniques such as embryo rescue may be required to obtain viable progeny. Differences have been noted in the receptivity of different varieties of *T. aestivum* to accept cross-fertilisation by other species such as rye (Zeven 1987). One of the reasons for this is the potential control (or lack thereof) by genes Kr1 and Kr2 (Gale and Miller 1987).

Wheat has been the subject of considerable work involving wide crossing, but much of this will have little relevance to crosses that might occur naturally in the environment.

Crosses such as (diploid x hexaploid, tetraploid x hexaploid) reduce the fertility of the F_1 generation substantially. Hybridisation is more successful if the parent with higher chromosome number is used as mother plant, although it should be noted that hybridisation between wheat x barley is efficient when barley (14 chromosomes) is used as the female parent. Most F_1 hybrids from hexaploid x diploid crosses are sterile. Only manual crossing of *T. aestivum* x *T. monococcum* produced F_1 hybrids with grains that germinated. Grains of the reciprocal hybrid did not germinate. When tetraploids were manually crossed with hexaploids, only the crossing of *T. aestivum* with *T. turgidum, T. durum, T. timopheevi* or *T. carthlicum* was successful (Mandy 1970, Sharma and Gill 1983). Hybrids from *T. aestivum* and *T. turgidum* are fertile. So while wheat may be crossed with many related species and some related genera, F_1 plants are often highly sterile, or the embryos abort. Gene transfer occurs only through man's intervention, *e.g.* hand pollination, and through rescue of F_1 embryos or through the use of male-sterile female plants. The chance of gene transfer occurring through such hybrids in nature is minimal. For production of genetically modified *T. aestivum,* and information about technical barriers that were overcome in achieving wheat transformation, see Appendix II.

Triticum species can be crossed by hand with the genera *Aegilops, Secale, Agropyron, Haynaldia Hordeum* and *Elymus* (see Table 1.12). Trigeneric hybrids are formed in some cases (see Table 1.13). Cross-breeding with *Elymus* species has proved least successful (Poehlmann 1959, Sharma and Gill 1983, Zeller 1985, Maan 1987, Jiang *et al*., 1994). Natural wild crosses of *T. aestivum* with the following members of the genera *Aegilops* (*Ae. cylindrica, Ae. triticoides, Ae. neglecta, Ae. triuncalis, Ae. ventricosa, Ae. genicularia, Ae. bluncalis, Ae. crassa, Ae. juvenalis, Ae. speltoides, Ae. tauschii* and *Ae. umbellata*) have been reported (van Slagern 1994). Crosses of *T. aestivum* to tetraploid *Aegilops* species resulted in hybrid seeds from which addition, substitution and translocation lines with introgressed genes for disease resistance have been selected (Spetsov *et al*., 1997, Petrova and Spetsov 1997). For information about cross-breeding of wheat with *Elymus,* see Dewey (1984), Plourde *et al*., (1989) and Koebner *et al*., (1995); with *Thynopyrum,* see Dewey (1984) and Sharma and Baezinger (1986); with *Elytrigia,* see Dewey (1984) and Cauderon (1994); and with *Pseudoroegnaria,* see Dewey (1984). Wheat can also cross with *Sorghum* and *Setaria* (Laurie *et al*., 1990).

Most manual cross-breeding has been carried out with *Secale cereale,* in order to combine the high grain yield and protein quality of wheat with rye's disease resistance and tolerance of poor soil conditions. The resulting generic progeny is called "triticale." There are only a few reports on natural hybridisation between wheat and rye. Müntzing (1979) reports a massive natural hybridisation in 1918, resulting in up to 20 per cent male sterile F_1 wheat x rye hybrids within wheat plots isolated by surrounding rows of rye plants. This spontaneous hybridisation occurred with wheat cultivars exhibiting anemophilic flower characters under dry continental conditions. In most cases, the F_1 hybrids are completely male sterile and have to be pollinated by wheat, rye or fertile tricicale to obtain generic progenies. Another possibility to overcome pollen sterility of wheat x rye hybrids is to double their chromosome number. Modern triticale breeding based on recombination among hexaploid triticales has solved the most important problems with the crop, namely low fertility, poor grain filling, tall stem and late ripening (Wolski *et al*., 1996). Triticale can be exploited as a bridge for the introgression of valuable genes from *Secale cereale, e.g.* by the generation of 1B/1R translocation chromosomes. The first European cultivar of triticale was obtained in France [Clerical since 1982 and on open catalogues since 1983 (Bernard and Guedes Pinto 1980, Cauderon and Bernard 1980)].

Through the use of *in vitro* methods, dihaploid plants have been produced from crosses between wheat and *Hordeum bulbosum* (Blanco *et al*., 1986, Cauderon and Cauderon 1956, Stich and Snape

1987) and wheat and *Zea mays* (Kisana *et al*., 1993). In these cases, the barley and maize chromosomes are eliminated in early stages of embryo development (Barcley 1975, Laurie and Bennett 1988, 1989). After diploidisation of the resulting haploid plants, the homozygous wheat material can be used for RFLP analysis, gene localisation and isolation.

Mandy (1970) reported the first manual intergeneric hybrid between ((*Triticum vulgare* x *Haynaldia villosa*) x *Secale cereale*), with the chromosome number (n = 35). Reciprocal hybridisation has had low success.

Interspecific hybridisation under natural conditions has been reported to occur only rarely (Gotsov and Panayotov 1972).

Table 1.12 Manual intergeneric crossing with *Aegilops (Ae.), Secale (S.), Agropyron (A.), Haynaldia (Ha.), Hordeum (H.) and Elymus (E.)* (Sharma and Gill, 1983)

Wheat parent	**Species of allied genera crossed**
Diploid wheat:	*Ae. bicornis*, *Ae. caudata*, *Ae. columnaris*, *Ae. comosa*, *Ae. cylindrica*,
Triticum monococcum	*Ae. longissima*, *Ae. mutica*, *Ae. ovata*, *Ae. speltoides*, *Ae. squarrosa*,
	Ae. triaristata, *Ae. tripsaccoides*, *Ae. triuncialis*, *Ae. umbellulata*,
	Ae. uniaristata, *Ae. variabilis*, *Ae. ventricosa*
	S. cereale
	A. elongatum, *A. intermedium*
	Ha. villosa
	H. vulgare
Tetraploid wheat:	*Ae bicornis*, *Ae. biuncialis*, *Ae. caudata*, *Ae. clylindrica*,
	Ae. columnaris,
T. turgidum, includes	*Ae. comosa*, *Ae. crassa*, *Ae. dichasians*, *Ae. heldreichii*, *Ae. kotschyi*,
durum, *carthlicum*,	*Ae. longissima*, *Ae. mutica*, *Ae. ovata*, *Ae. sharonensis*, *Ae. speltoides*,
dicoccum and dicoccoides	*Ae. squarrosa*, *Ae. triaristata*, *Ae. tripsaccoides*, *Ae. triunciales*,
	Ae. umbellulata, *Ae. uniaristata*, *Ae. variabilis*, *Ae. ventricosa*
	S. africanum, *S. ancestrale*, *S. cereale*, *S. montanum*, *S. vavilovii*
	A. campestre, *A. dasystachyum*, *A. distichum*, *A. elongatum*,
	A. intermedium, *A. junceum* 4x, *A. obtusiusculum*, *A. repens*
	Ha. hordeace, *Ha. villosa*
	H. brevisubulatum, *H. chilense*, *H. vulgare*
	E. arenarius, *E. giganteus*
Tetraploid wheat:	*Ae. bicornis*, *Ae. caudata*, *Ae. comosa*, *Ae. cylindrica*, *Ae. dichasians*,
T. timopheevi	*Ae. kotschyi*, *Ae. longissima*, *Ae. mutica*, *Ae. ovata*, *Ae. speltoides*,
	Ae. squarrosa, *Ae. triuncialis*, *Ae. umbellulata*, *Ae. uniaristata*,
	Ae. ventricosa
	S. africanum, *S. cereale*, *S. vavilovii*
	A. campestre, *A. cristatum*, *A. elongatum*, *A. intermedium*,
	A. junceum 4x,
	A. repens
	Ha. villosa
	H. bogdanii, *H. vulgare*, *H. vulgare* ssp. *distichon*
Hexaploid wheat:	*Ae. bicornis*, *Ae. biuncialis*, *Ae. caudata*, *Ae. columnaris*, *Ae. comosa*,
	T. aestivumAe. crassa, *Ae. cylindrica*, *Ae. dichasians*, *Ae. juvenalis*,
	Ae. kotschyi,
	Ae. longissima, *Ae. mutica*, *Ae. ovata*, *Ae. sharonensis*, *Ae. speltoides*,
	Ae. squarrosa, *Ae. triaristata*, *Ae. tripsaccoides*, *Ae. truncialis*,
	Ae. umbellulata, *Ae. uniaristata*, *Ae. variabilis*, *Ae. ventricosa*
	S. africanum, *S. ancestrale*, *S. cereale*, *S. montanum*, *S. vavilovii*
	A. caespitosum, *A. distichum*, *A. elongatum*, *A. intermedium*,
	A. junceum 2x, *A. podperae*, *A. scirpeum*, *A. smithi*, *A. trachycaulum*,
	A. yezoense
	Ha. villosa
	H. chilense, *H. pusillum*, *H. spontaneum*, *H. vulgare*, *H. vulgare* var.
	distichum
	E. giganteus

Table 1.13 Trigeneric hybrids from manual crossing *Triticum (T.), Aegilops (Ae.), Hordeum (H.), Agropyron (A.), Haynaldia (Ha.) and Secale (S.)* (Sharma and Gill, 1983)

Trigeneric hybrid	Reference
(*T. timopheevi* x *H. bogdanii*) x *S. cereale*	Kimber & Sallee 1979
(*H. vulgare* x *T. aestivum*) x *S. cereale*	Claus 1980; Fedak & Armstrong 1980
(*H. vulgare* x *T. aestivum*) x *S. montanum*	Claus 1980
(*H. vulgare* x *A. elongatum*) x *Ae. crassa*	Pedigree of Sando's collection, USDA, Beltsville
(*T. aestivum* x *S. cereale*) x *T. aestivum* x *A. elongatum*	USDA, Beltsville
Triticale (6x) x (*T. durum* x *A. intermedium*) amphidiploid	Nowacki *et al*., 1979
(*Ae. ventricosa* x *S. cereale*) x *T. aestivum*	Dosba & Jahier 1981
(*Ae. crassa* x *T. persicum*) x *S. cereale*	Knobloch 1968
(*Ae. ventricosa* x *T. dicoccum*) x *A. intermedium*	Knobloch 1968
(*Ae. ventricosa* x *T. turgidum*) x *S. cereale*	Knobloch 1968
(*Ae. ventricosa* x *T. dicoccum*) x *S. cereale*	Siddiqui 1972
(*T. aestivum* x *Ha. villosa*) x *S. cereale*	Knobloch 1968
(*T. dicoccum* x *Ha. hordeacea*) x *S. cereale*	Knobloch 1968
(*T. dicoccum* x *S. montanum*) x *Ha. villosa*	Knobloch 1968
(*T. turgidum* x *Ha. villosa*) x *S. cereale*	Knobloch 1968

B. Introgression

Interspecific hybridisation under natural conditions has rarely occurred (Gotsov and Panayotov 1972), and the role of environmental conditions must be taken into consideration. For example, weather abnormalities may in some instances contribute to male sterility or in others to overlapping of flowering periods. Both of these factors can result in the breaking down of effective isolation barriers between species. The introgression of a new gene will also be dependent on whether or not that gene confers an ecological advantage on the recipient in specific environments. Even so, data on potential hybridisation events are helpful in assessing the potential for introgression of "novel traits" of transgenic *T. aestivum* into wild relatives. If potential "mates" of *T. aestivum* are occurring in the geographic region of interest, introgression has to be taken into consideration.

Rimpau reported observing volunteer crosses between *T. aestivum* x *S. cereale* in his wheat nursery at the beginning of this century. He called the bastard plants "mule-wheat" because they were infertile and he was not able to collect seed from them. Nevertheless, he continued to make artificial crosses (von Broock, personal communication).

Intra- and interspecific variation exists within the cytoplasms of wheat and related species, and this is important for wheat breeders. Cytoplasmic male sterility (CMS) systems are used successfully in several crops. CMS has been introduced into common wheat through interspecific and intergeneric hybridisation. Today, chloroplasts and mitochondria are subjects of molecular genetic studies and of genetic manipulation, and these techniques may in the future be used in wheat. All genetic information present in the DNA of cytoplasmic organelles is maternally inherited, and therefore the chance for gene transfer in nature is less than for nucleic genes.

C. Interactions with other organisms

Wheat grain yield is decreased by some 50 major diseases which can produce overall crop damage (including storage damage) of 20 per cent (Spaar *et al*., 1989). Fungal diseases are the greatest

problem. Animals, *e.g.* pigeons, crows and pheasants, feed on seeds, dig and tear out plants, or otherwise damage them. Mice, rabbits and deer can also cause considerable damage to wheat plants.

The tables in Appendix I are intended as an identification guide for categories of organisms that interact with *T. aestivum*. Clearly the organisms listed are examples, with their occurrence depending upon the geographic region where *T. aestivum* is grown.

6. Weed Characteristics/Weedness

Wheat is a crop plant species with low competitive ability. It has no natural habitat outside cultivation (Garcke 1972, Tutin *et al*., 1980). Wheat does not have high potential for weediness (Keeler 1989). Wheat plants may sometimes be found in "disturbed" areas where there is little or no competition from other "weed" species (*e.g.* waste places, fallow fields, along roadsides), but their survival at such sites is limited to short periods (Janssen *et al*., 1995). There are no indications that wheat can become established as a self-sustaining population on a long-term basis (Sukopp and Sukopp 1993, Newman 1990).

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APPENDIX I

Most Common Diseases and Pests in *Triticum aestivum*

Potential interactions of *T. aestivum* with other life forms during its life cycle (Wiese 1987, Spaar *et al*., 1989, Wolff and Richter 1989, Chelkowski 1991, Cook and Veseth 1991, Wolff 1992):

Viruses, Mycoplasms (See Brunt *et al*., 1996. For more information, also see the VIDE database: http:\\www.csu.edu.au/viruses/virus.html)

Disease	**Agent**
Agropyron mosaic virus	Agropyron mosaic virus (AgMV), geographic occurrence *e.g.* in Eurasia, Canada and the USA
Barley stripe mosaic hordeivirus	Barley stripe mosaic hordeivirus (BSMV), geographic occurrence *e.g.* in Eurasia, Northern America, Pacific
Barley yellow dwarf virus	Barley yellow dwarf virus (BYDV), geographic occurrence world-wide; wheat varieties show different tolerance level (Baltenberger *et al*., 1987); tolerance level had been increased through cross breeding with resistant *Agropyron* varieties (Ohm *et al*., 1989, Gonlart *et al*., 1993)
Barley yellow streak mosaic virus	Barley yellow streak mosaic virus, geographic occurrence *e.g.* in Canada and USA
Barley yellow striate mosaic cytorhabdovirus	Barley yellow striate mosaic cytorhabdovirus (BYSMV), geographic occurrence *e.g.* in Africa, Eurasia, Middle East and Pacific
Brome mosaic virus	Brome mosaic virus (BMV), geographic occurrence *e.g.* in Eurasia, Australia, South Africa and USA
European striped wheat mosaic	Probably mycoplasms
Wheat American striate mosaic nucleorhabdovirus	Wheat American striate mosaic nucleorhabdovirus (WASMV), geographic occurrence *e.g.* in Canada and USA
Wheat dwarf virus	Wheat dwarf virus (WDV), geographic occurrence *e.g.* in Bulgaria, former Czechoslovakia, Hungary, former USSR, France and Sweden
Wheat European striate mosaic tenuivirus	Wheat European striate mosaic tenuivirus (EWSMV), geographic occurrence *e.g.* in Czech Republic, Poland, Romania,

	Denmark, Finland, Sweden, Germany, UK and Spain
Wheat soilborne mosaic virus	Wheat soilborne mosaic virus, geographic occurrence *e.g.* in China, Japan, Italy and USA
Wheat spindle streak mosaic virus	Wheat spindle streak mosaic virus, (WSSMV), geographic occurrence *e.g.* in France, Germany, Italy, India, Japan, China, and USA
Wheat spindle streak virus	Wheat spindle streak virus
Wheat streak mosaic virus	Wheat streak mosaic virus (WSMV), geographic occurrence *e.g.* in Canada, USA, Romania and Jordan
Wheat striate mosaic virus	Wheat striate mosaic virus
Wheat yellow leaf virus	Wheat yellow leaf virus (WYLV), geographic occurrence *e.g.* in Japan and Italy
Wheat yellow mosaic brymovirus	Wheat yellow mosaic brymovirus, geographic occurrence *e.g.* in China, Japan, Korea, Canada and France
Wheat yellow mosaic virus	

Bacteria

Disease	**Agent**
Basal glume blotch	*Pseudomonas syringae* pv. *atrofaciens* (McCulloch)
Black glume	*Xanthomonas campestris* pv. *translucens* (Jones, Johnson et Reddy) dye Various known forms which differ only in host specificity: *undulosa*, *cerealis*, *hordei*, *secalis*, *orycicola* and *phleipratensis*

Fungi

Disease	**Agent**
Ergot	*Claviceps purpurea*: infects florets and produces grain-like sclerotia containing mycotoxins (ergot alkaloids). The fungal grains are harvested with the wheat grains and, if not removed, mycotoxin contamination of products occurs.
Eyespot, stembreak, straw breaker	*Pseudocerosporella herpotrichoides* (Fron.) Deight., Syn.: *Cerosporella herpotrichoides* (Fron.), breeding for resistance; wheat genotypes with short shoot and good steadiness

Fusarium diseases of shoots (root and culm rots, partial head blight)	Numerous *Fusarium* species play a part in the pathology of the cereal fusaria. The major species are: – *Fusarium nivale* (Ces., Syn.: *Gerlachia nivalis)* – *Fusarium culmorum* (W.G. Smith) Sacc. var. *culmorum* – *Fusarium avenaceum* (Fr.) Sacc. var. *avenaceum* – *Fusarium graminearum* Schwabe (perfect form: *Gibberella zeae* (Schw.) Petch): widespread, especially harmful not only to wheat but also to maize – *Fusarium poae* (Peck) Wollenw.: occurs sporadically, often in conjunction with the grass mite (*Siteroptes graminum* [Reuter]), which feeds on the fungus and helps it to proliferate. – Other species found in wheat include: *Fusarium acuminatum* Ell. et Kellerm. *(Gibberella acuminata* Wollenw.), *Fusarium dimerum* Penzig, *Fusarium equiseti* (Corda) Sacc. *(Gibberella intricans* Wollenw.), *Fusarium porotrichoides* Sherb., *Fusarium tricinctum* (Corda) Sacc. and *Fusarium moniliforme* Sheldon sensu Wollenw. et Reinking, increased resistance breeding in wheat; chemical treatment led to unsatisfactory results (Maurin *et al*., 1996).
Glume blotch (Septoria disease)	*Leptosphaeria nodorum* (E. Müll.), conidial form *Septoria nodorum* Berk., Syn.: *Phaesopheria nodorum* (E. Müll.) Hejarude, only partial resistance in wheat found (Jeger *et al*., 1983, Bostwick *et al*., 1993).
Helminthosporium yellow blotch disease	*Drechslera tritici-repentis* (Died.) Shoem., perfect form: *Pyrenophora trichostoma* (Fr.) Fckl., Syn.: *Pyrenophora tritici-repentis* (Died.) Drechsl.
Mould	*Aspergillus* ssp./*Penicillium* ssp. can proliferate during storage. Both are potential mycotoxin producers (Ochratoxin A).
Phoma leaf spot	*Phoma glomerata* (Cda.) Wr. et Hochaf.
Pointed eyespot (stembreak, straw breaker)	*Rhizoctonia* spp., *Thanatephorus cucumeris* (Frank) Donk.

Powdery mildew of cereals	*Erysiphe graminis* DC. f. sp. *tritici* March, resistance genes, *e.g.* Mlk, Pm1 to Pm9, M1Ax, U1 and U2, can be found in different wheat varieties and related species (Heun and Fischbeck 1987, 1989, Hovmoller 1989, Zeller *et al*., 1993).
Rusts	
Yellow/stripe rust	*Puccinia striiformis* (West., Syn.: *Puccinia glumarum* Erikss. et Henn). Formation of pathotypes which specialise in wheat or barley. In exceptional cases wheat stem rust strains may attack highly susceptible barley varieties or vice versa.
Leaf rust of wheat	*Puccinia recondita* Rob. ex Desm. f. sp. *tritici*, Syn.: *Puccinia triticina* Erikss., Syn.: *Puccinia rubigovera* Wint. Formation of pathotypes, alternate host *Thalictrum* spp.
Black stem rust of wheat	*Puccinia graminis* Pers. f. sp. *tritici* Development of formae speciales specialised in rye, barley, oats, wheat and grasses. Numerous pathotypes formed.
Septoria leaf blotch	*Mycosphaerella graminicola* (Fckl.) Sanderson, conidial form: *Septoria tritici* Rob. ex Desm.
Smuts	
Loose smut of wheat	*Ustilago tritici* (Pers.) Rostr.
Covered smut of wheat	Various *Tilletia* species with different sori, including: – *Tilletia caries* (DC.) Tul. Syn.: *Tilletia tritici* (Bjerk.) Wint. – *Tilletia foetida* (Wallr.) Liro, Syn.: *Tilletia laevis* Kühn or *Tilletia foetens* (Bjerk. et Curt.) Schroet. – *Tilletia intermedia* (Gassner) Savul. Syn.: *Tilletia tritici f. sp. intermedia* Gassner
Dwarf bunt of wheat	*Tilletia controversa* Kühn
Carnal smut	*Neovossia indica* (Mit.) Mund.
Stripe/flag smut	*Urocystis agropyri* (Preuss.) Schroet.
Take-all	*Gaeumannomyces graminis* (Sacc.) v. Arx. et Olivier var. *tritici* Walker Several varieties with overlapping hosts, var. *tritici* attacks wheat, triticale, barley and rye, no resistant varieties in wheat found.

Animals

Pest	**Agent**
Apart from the above-mentioned species of aphid, the following species may cause damage to cereals, maize and grasses:	Bromegrass aphid *(Diuraphis bromicola* [H.R.L.]), cat's-tail aphid *(Diuraphis mühlei* [Börn.]), corn leaf aphid *(Rhopalosiphum maidis* [Fitch.]), yellow cherry/reed canary grass aphid *(Rhopalomyzus lonicerae* [Siebold], *Rhopalomyzus poae* [Gill.], cocksfoot aphid *(Hyalopteroides humilis* [Walk.], *Laingia psammae* (Theob.), *Schizaphis nigerrima* H.R.L., *Metopolophium festucae* (Theob.), green grain aphid *(Schizaphis graminum* [Rond.]), grain aphid *(Sitobion granarium* [Kirby]), cob aphid *(Sipha maydis* [Pass.], *Sipha glyeriae* [Kalt.]), black (bean) aphid *(Aphis fabae* Scop.), green peach aphid *Myzus persicae* [Sulz.])
Aphids:	Aphids arrive from early May (when wheat is shooting), settling first on leaf blades and sheaths, transferring to influorescence as ears extend. Warm and dry conditions encourage generations. The generation cycle lasts 8 to 10 days. Each aphid can lay 30 to 50 larva (parthenogenesis). Around mid-July mass proliferation is briefly interrupted due to poor feeding conditions and the appearance of parasites and predators (ladybirds/ladybugs). The grain aphid undergoes a holocycle, *i.e.* sexual differentiation takes place in autumn, and winter eggs are laid on grasses. More than 10 generations occur in the space of a year.
Grain aphids	*Macrosiphum avenae* (Fabr.), Syn.: *Sitobion avenae* (Fabr.) Also in barley, oats, rye, maize, fodder grasses Aphid species which does not alternate hosts
Oat or bird cherry aphid	*Rhopalosiphum padi* (L.) Alternate-host aphid with broad host plant profile among cereal and grass species, *e.g.* barley, oats, maize, fodder grasses.
Rose grain aphid	*Metopolophium dirhodum* (Walk.) Alternate-host aphid (also in barley, oats, rye, maize, fodder grasses).

Cereal cyst nematodes, cereal stem eelworm	*Heterodera avenae* Woll. Also attacks barley, oats, rye, fodder grasses. Several biotypes distinguished by their host profile. Cysts drop from roots and survive in soil. Larvae hatch in spring and infect roots. Sexual differentiation occurs in the root. Females carry up to 600 eggs. When a female dies, its body turns brown and is transformed into a lemon-shaped cyst, only limited resistance (Cre 1 gene on chromosome No. 2B) found in wheat (Slootmaker *et al.*, 1974).
Cereal leaf beetle	Red-throated cereal leaf beetle *(Oulema melanopus* [L.], Syn.: *Lema melanopa* [L.]), blue cereal leaf beetle *(Oulema lichenis* [Voet], Syn.: *Lema lichenis* [Voet]) Beetles leave winter quarters in mid-April and migrate into cereal fields. Eggs are laid in late May on upper side of leaves. This takes 6 to 8 weeks. Each female lays 50 to 100 eggs. Egg development lasts 7 to 14 days.
Corn beetle	*Zabrus tenebroides* Goeze (corn ground beetle) Beetles appear in late June to early July. Eggs are laid in August and September. Each female lays 80 to 100 eggs in the soil. The first larvae hatch after 14 days and undergo three stages. Overwintering is in the 1st or 2nd larval stage. At soil temperatures of -1°C in spring they resume feeding. The bulk of damage now occurs. Soil pupation takes place in May. The generation cycle of the corn ground beetle lasts one year. Also found in barley, oats, rye, maize, fodder grasses.
Crane-fly larvae	Larvae of the marsh crane-fly *(Pales (Tipula) paludosa* Meig.), common crane-fly *(Pales (Tipula) oleracea* L.), autumn crane-fly *(Pales (Tipula) czizeki* de Jong). Biggest factor: *Pales paludosa*. Also in barley, oats, rye, maize, fodder grasses.

March fly larvae	*Bibio hortulans* (L.), *Bibio marci* (L.), *Bibio johannis* (L.), *Bibio clavipes* (Meig.) Also in barley, oats, rye, maize, fodder grasses.
Myriapods	Various species of myriapods, notably the common millipedes *Cylindroiulus teutonicus* (Pocock) and *Blaniulus guttulatus* (Bosc.) Also in barley, oats, rye, maize, fodder grasses.
Root aphids	*Anoecia corni* (Fabr.), *Anoecia vagans* (Koch), *Aploneura graminis* (Buckt.), *Aploneura lentisci* Pass., *Byrsocrypta personata* Börner, *Forda marginata* Koch, *Forda formicaria* V. Heyden, *Geoica discreta* Börner, *Tetraneura ulmi* (L.) Also in barley, oats, rye, maize, fodder grasses
Slugs	Various species of slug, notably the field slug *(Deroceras reticulatum* O.F. Müll., *Deroceras agreste* L.), the garden/blackfield slug *(Arion hortensis* [Fér.], *Arion rufus* [L.]). Also in barley, oats, rye, maize, fodder grasses.
Wheat and grass bugs	Wheat and grass bugs are a non-homogeneous group of pests. The greatest economic damage is caused by wheat bugs *(Eurygaster* spp.). Also in barley, oats, rye, maize, fodder grasses.
Wheat nematodes	*Anguina tritici* (Steinbuch) Filipjev The larvae which live in the galls can be preserved for years in dried state.

NOTE: A complete list of US wheat pests can be found on the American Phytopathology Society home page: http://www.scisoc.og/resource/common

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APPENDIX II

Transformation of *Triticum aestivum*

The genetic improvement of cereals, including wheat, has been a major focus of plant breeding efforts during the past 50 years. It has resulted in remarkable increases in yield as well as improvements in quality. Nonetheless, plant breeding is a slow process and has biological limitations. In this context the rapidly emerging technologies of plant cell and molecular biology, by permitting access to a much wider gene pool, have attracted much attention, for they provide powerful and novel tools to supplement and complement the traditional methods of plant breeding.

Modern plant biotechnology is based on the delivery, integration and expression of defined foreign genes into plant cells which can then be grown *in vitro* to regenerate plants. The efficient regeneration of normal fertile plants from protoplasts is a basic prerequisite for this technology. For gramineous species, the *in vitro* regeneration of fertile phenotypically normal plants has been very difficult (Vasil and Vasil 1992). The greatest problem to overcome was that of culturing immature and undifferentiated tissue and organ explants at defined development stages in special nutrient media. Now all important cereals, *e.g.* wheat, barley, rice, can be regenerated from cultured tissue as well as single cells (Vasil 1994). Most early attempts to transform cereals were limited to the use of totipotent embryogenic protoplasts, but embryogenic protoplast cultures are difficult to establish and maintain. For wheat, *in vitro* regeneration from immature embryos from young influorescences and microspores (somatic and gametic embryogenesis) has been possible for some time. However, to provide the cells with the greatest access to the transgenes, and in order to obtain cell culture homogeneity, it seems necessary to achieve genetic transformation of cereals using isolated single cells. In this way, it has been thought that the occurrence of chimaeric transformants would also be avoided. This strategy has been successful with many plant species (both dicots and monocots such as rice and maize). Today, normal and fertile plants can be regenerated from all major species of cereals, including wheat (Vasil *et al*., 1990). However, it is still an inefficient, time-consuming procedure (Vasil and Vasil 1992).

There are different methods of delivering foreign genes into plants (see review: Nehra *et al*., 1995). The well known, and often preferred method of *Agrobacterium*-mediated transformation does not work very well with cereals. Like most monocotyledonous species, wheat is generally considered to be outside the natural host range of the *Agrobacterium* pathogen. Experiments with wheat and maize have shown that *Agrobacterium* can transfer viral genomic sequences to cereal cells, resulting in a systemic viral infection called "agroinfection" (Smith and Hood 1995). For this to occur, it is not necessary to achieve integration of the viral genes into the plant genome. Thus it seems that the main difficulty is not the delivery of DNA, but rather its integration (Grimsley *et al*., 1987, Dale *et al*., 1989). Recent data from experiments with rice (Hiei *et al*., 1994), maize (Ishida *et al*., 1996), barley (Tingay *et al*., 1997) and also wheat (Chen *et al*., 1996) showed efficient transformation mediated by *Agrobacterium*, with stable integration, expression and inheritance of the transgenes (Chen *et al*., 1997).

Two methods, involving osmotic (polyethylene glycol treatment) or electric (electroporation) shock, have been used for transformation and have resulted in transient as well as stable expression of the introduced gene (review: Lörz *et al*., 1985), *e.g.* of maize (Fromm *et al*., 1986). For wheat transformation the biolistic method was used (Vasil *et al*., 1992, Weeks *et al*., 1993, Becker *et al*.,

1994, Nehra *et al*., 1994). This procedure is based on the high-velocity bombardment of plant cells with DNA-coated microprojectiles, accelerated by gunpowder discharge or pressurised helium gas (Sanford *et al*., 1991, Klein *et al*., 1992). The main advantage of this method is its ability to deliver DNA into intact regenerable (via the formation of somatic embryos) plant cells, eliminating the need for protoplasts, which thus minimises the potential for tissue culture effects and the resulting abnormalities (Vasil *et al*., 1993, Vasil 1994).

Optimum expression of genes in the target cell is important for achieving a high frequency of stable transformation. In wheat, considerable efforts have been made in developing suitable gene expression vectors for transformation (Nehra *et al*., 1995). The inclusion of an intron between the promoter and the coding region proved useful to achieve enhanced transient gene expression in wheat (Chibbar *et al*., 1991). Furthermore, the isolation of monocot gene promoters, such as the rice actin (Act1) promoter (McElroy *et al*., 1991) or the maize ubiquitin (Ubi1) promoter (Christensen *et al*., 1992) sometimes resulted in higher expression frequency. Transgenic wheat has been produced using both promoters (Weeks *et al*., 1993, Nehra *et al*., 1994).

To obtain transgenic plants from the few stably transformed cells achieved through these transformation techniques, a suitable selection system is required. Selectable marker genes that confer resistance to antibiotics or herbicides are usually used. Among the various antibiotic resistance marker genes in use, the kanamycin resistance gene has proven ineffective for selection of transformed wheat cells because these cells and the wheat tissue itself both have a high level of endogenous tolerance to kanamycin. Another problem is that using this antibiotic as the selection agent interferes with plant regeneration (Hauptmann *et al*., 1988, Peng *et al*., 1992). Geneticin (G 418), however, another member of the aminoglycosides, can be effectively used (Nehra *et al*., 1994). Hygromycin was used by Hauptmann *et al*., (1988) with a positive result, but experiments conducted by Nehra *et al*., (1995) were not successful. As an alternative to antibiotic resistance marker genes, genes conferring resistance to herbicides such as glufosinate ammonium (l-phosphinothricin) can be used (Nehra *et al*., 1995). Detailed descriptions of the available monocot selection marker systems were presented in the following reviews: Wilmink and Dons 1993, McElroy and Brettell 1994.

In recent years there have been releases of transgenic wheat plants (see Table II-1). For more information about this topic in Europe, see RKI, the SNIF database (http://www.rki.de) and the list of "SNIF circulated under article 9 of Directive 90/220/EEC XI/559/94-Rev 6". For the United States, the reviews of James and Krattinger 1996 and de Kathen 1996, and the APHIS ISB environmental release database (http://www.aphis.usda.gov/bbep/bp) provide similar information. The OECD BioTrack database includes information on experimental releases to the environment of genetically modified plants and micro-organisms (http://www.olis.oecd.org/biotrack.nsf).

Future advances in the molecular improvement of wheat, as in that of other plants, will depend upon the limited availability of agronomically important genes more than on any other factor. Attention is being directed to the development of DNA-based maps of wheat for identifying, and then characterising and cloning, genes of importance and interest. Gill *et al*., (1991), for example, provided a standard karyotype and nomenclature system for describing chromosome bands in bread wheat, while Hohmann *et al*., (1994) prepared a genetic/physical map of group 7 chromosomes. Devos and Gale (1992) tested the use of random amplified polymorphic DNA (RAPD) markers. They were unsuccessful because of the non-homologous, non-dose responsive and dominant behaviour of RAPD products. Vaccino and Metakovsky (1995) used RFLP patterns of wheat gliadin alleles as markers, and Devos *et al*., (1995) used microsatellite sequences. Genetic maps, gene markers and QTL are now becoming available or are being developed. This work started in 1985 at the Plant Breeding Institute and the John Innes Centre in the UK, at universities in the United States, and at the INRA in France (Nelson *et al*., 1995a, 1995b, Cadalent *et al*., 1996).

Molecular improvement of wheat for multigenic traits, such as yield, will be a difficult and lengthy process (Vasil 1994). However, the conservation of gene order along chromosomes, as well as the similarity of gene composition and map collinearity in cereals, should be a great advantage in regard to the identification and cloning of important genes (Bennetzen and Freeling 1993, Kurata *et al*., 1994).

Deliberate releases of transgenic wheat

Country	First release	Main trait
UK	1994	marker
UK	1994	herbicide resistance (glufosinate)
UK	1995	herbicide resistance (glufosinate)
UK	1995	improved starch quality
UK	1996	pest resistance (tolerance to leaf fungal disease)
Spain	1996	herbicide resistance (glufosinate), improved starch quality
UK	1997	alteration in baking quality
Belgium	1997	male sterility/restorer
Argentina	1993	improved quality, male sterility, marker
Argentina	1995	herbicide resistance
Chile	1995	herbicide resistance
USA	1994	herbicide resistance
USA	1994	herbicide resistance (glufosinate)
USA	1994	herbicide resistance (glyphosate)
USA	1995	fungal resistance
USA	1995	herbicide resistance
USA	1995	virus resistance
USA	1995	improved quality
USA	1996	fungal resistance
USA	1996	improved quality
USA	1996	fungal resistance
USA	1996	fungal resistance (glyphosate)
USA	1996	improved quality
USA	1996	herbicide resistance
USA	1996	virus resistance (glyphosate)
USA	1996	herbicide resistance
USA	1996	fungal resistance (glyphosate)
USA	1996	fungal resistance

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SECTION 8
SUGAR BEET (*BETA VULGARIS* L.)

1. General Description Including Taxonomy, Morphology, Genetic Characteristics and Use as a Crop Plant

A. Taxonomy

Sugar beet (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima*) belongs to the family *Chenopodiaceae* and the genus. *B. vulgaris* comprises several cultivated forms of *B. vulgaris* subsp. *vulgaris*. Cultivars include leaf beet (var. *cicla*) and beetroot (root beet USA). The genus *Beta* is divided into four sections shown in Table 1.14 below (Ford-Lloyd and Williams, 1975; Campbell, 1976; Tranzschel, 1927 and Ulbrich, 1934):

Table 1.14 Classification of the *Beta* species

Species name	Chromosome number
Section I: *Beta* Tranzschel	
B. vulgaris L. ssp. *vulgaris*	18
B. vulgaris L. ssp. *maritima* (L.) Arcang	18
B. vulgaris L. ssp. *adanensis* (Pam.) Ford-Llyod & Williams	18
B. patula Ait.	18
B. macrocarpa Guss.	18, 36
Section II: *Corollinae* Ulbrich	
B. macrorhiza Stev.	18
B. corolliflora Zoss.	36
B. lomatogona F. et M.	18, 36
B. intermedia *Bunge*	36, 45
B. trigyna *W. et K.*	36, 54
Section III: *Nanae* Ulbrich	
B. nana Boiss. et Heldr.	18
Section IV: Procumbentes Ulbrich	
B. procumbens Chr. Sm.	18
B. webbiana Moq.	18
B. patellaris Moq.	18, 36

In Europe, wild sea beet occurs as a wild plant. Wild *B. vulgaris* species are distributed along the border-zones of the Mediterranean from southern Russia, the Near-East, and Syria to the Canary Islands and Madeira. They are also found along the European Atlantic coasts where they come into contact with the Gulf Stream. *B. vulgaris* has also been introduced into Baltic and Central and South

America. In North America, the species has become naturalised, resulting from the introduction of plants for cultivation. Table 1.16 shows the global distribution of the wild species of *Beta*.

Table 1.15 Distribution and use of cultivated forms of *Beta vulagaris* ssp. *Vulgaris* (according to Mansfeld, 1986)

Species	Var.	Common name	Distribution	Use
Beta vulgaris	*Cicla*	Spinach beet	Central, western and southern Europe Asia	Cooking vegetable
Beta vulgaris	*Flavescens*	Swiss chard	Central, western and southern Europe Asia	Cooking vegetable
Beta vulgaris	*Vulgaris*	Red beet (beetroot)	Central, western and southern Europe; Asia; Western India	Cooking and salad vegetable
Beta vulgaris	*Lutea*	Yellow beet	Central, western and southern Europe; Asia	Salad vegetable
Beta vulgaris	*Rapacea*	Fodder beet	Europe; Commonwealth of independent States (CIS); North America	Fodder plant
Beta vulgaris	*Altissima*	Sugar beet	Europe; CIS; China; Asia; North America; South America	Beet sugar production

Table 1.16 Global distribution of the wild species of the genus Beta (according to Mansfeld, 1986)

Species	Subspecies	Distribution
Beta vulgaris	*maritima*	Mediterranean, Canary Islands, Near East, Madeira, European Atlantic coast to India, North sea, Middle and South America
Beta vulgaris	*adanensis*	Mediterranean, Canary Islands, Near East, Madeira, European Atlantic coast to India, North sea, Middle and South America
Beta macrocarpa		India
Beta patula		Mediterranean, western Europe, North-west Africa
Beta vulgaris	*adanensi*	Mediterranean, western Europe, North-west Africa
Beta intermedia		Asia Minor to Hungary, Persia
Beta corolliflora		Asia Minor, Caucasus, Black sea coast
Beta macrorhiza		Asia Minor, Caucasus, Black sea coast
Beta trygina		Asia Minor, Caucasus, Black sea coast
Beta patellaris		North-west African coast and Islands, southern Spain
Beta procumbens		Canary and Cape Verde Islands, North-west African coast
Beta webbiana		Canary and Cape Verde Islands, North-west African coast

Sugar beet is cultivated world-wide, but primarily in warm and temperate climates with little precipitation. There is an increase in cultivation in subtropical regions (Brouwer *et al*., 1976). The largest areas of cultivated sugar beet are in the U.S.A., C.I.S. (Commonwealth of Independent States, formerly the U.S.S.R. [*e.g.* Russia]), Europe (FAO Yearbook, 1992) and in China.

B. Uses

Sugar beet is used for the production of sugar. By products of sugar production as pulp, molasses, fibre etc, are used as feed.

When sugar beet is grown in areas of livestock production, leaves of the plant may also be used for fodder. More recently, sugar beet has been used for molasses production. Molasses are used for alcohol production and in other forms of fermentation (penicillin production, etc…).

C. Description

Morphology

A glabrous or slightly, hairy annual, biennial or perennial of very varied habit, from 30 to 120 cm (or even 200 cm) in height. The root is stout, sometimes conspicuously swollen forming a beet together with the hypocotyl, and sometimes forming a branched taproot (as in ssp. *maritima*). Stems are decumbent, ascending or erect, and more or less branched. Leaves are very varied in size, shape and colour, often dark green or reddish and rather shiny, frequently forming a radicle rosette. Inflorescences are usually large and more or less branched. The flowers are hermaphrodite arranged in small cymes (Clapham *et al*., 1962; Højland and Pedersen, 1994).

Cultivated forms of sugar beet are essentially biennial and are grown for the swollen roots that develop at the end of the first growing season. Sugar beets is biennial and require a period of vernalisation at the end of the first year before they can flower, although a small proportion of plants flower in their first year and are able to set seeds that persist in the soil. This phenomenon is known as "bolting". A possible source of annual weed beets is the pollination of seed crops by contaminating pollen from annual wild beets (Longden, 1976; Evans and Weir, 1981). In particular, this may have happened in southern Europe during the production of sugar beet seed of triploid monogerm varieties, when the male-sterile diploids used as mother plants are especially susceptible to pollination by contaminating pollen from diploid wild or weed beet plants, rather than by the intended tetraploids pollen bearing plants (Scott R.K. and Longden P.C., 1970). The other possibility is the variability in vernalisation requirements between varieties, some varieties need less vernalisation than the other and can easily flower during the first year.

In Europe, flowering weed beets in sugar beet production areas have, since the early 1970's, become a serious problem. The weed beet is phenotypically different from volunteer sugar beet in that it produces more seed, and in France, this seed has been shown not to require the usual vernalisation period prior to flowering (Harding and Harris, 1994). The weedy form may, in theory, have evolved in parallel with "bolters" *in situ* in sugar beet producing areas, but, molecular evidence suggests that weed beet originated from pollination by wild diploid species in seed producing areas along the Mediterranean (Boudry *et al*., 1992; 1993).

Beta vulgaris ssp. *vulgaris* is customarily divided into two types: fodder beet and sugar beet. Some authors refer to sugar beet as var. *saccharifera*, however the distinction is not clear. The obvious morphological difference is that the beet in fodder beet is formed primarily by the hypocotyl, whereas in the sugar beet a considerable part of the beet is formed by the root. This results in a higher dry matter content in sugar beet, and also the beet itself is placed deeper in the soil. A variety of beet is, as a rule, only accepted as a sugar beet if the dry content matter is 20% or more and the beet is white (Højland and Pedersen, 1994).

The wild *Beta vulgaris* ssp. *maritima* is hardy, has thin, multi-stemmed roots, and low-lying stalks in a rosette-like array.

Seedlings

Sugar beet seeds contain very little perisperm for germination and early growth. This makes seedlings very vulnerable during early growth to competition from weeds and to damage by disease and browsers (Højland and Pedersen, 1994). Weeds emerging within 4 weeks after the sugar beet has reached the two-leaf stage are the most damaging. Weed competition has been estimated to reduce root yields 6 % in Canada and 10% in the USA. Competition from annual grasses also suppress root yields, however, competition from annual grass species is not usually as severe as that from broadleaf weeds because they do not compete for light as effectively as broadleaf weeds (Højland and Pedersen, 1994). Sugar beet seedlings have two, and occasionally three leaves, however, varieties vary in terms of leaf position, leaf number, leaf size, and curling of the leaf edge. Yellow and red pigments are often stored in leaf tissue. After differentiation of the leaves, they become covered with a waxy layer (Gilloly Bystron *et al*., 1968). Unlike fodder beet, the lower leaves of the sugar beet commonly lie in a rosette-form on the ground (Brouwer *et al*., 1976). The crown leaves are spirally arranged in 5/13 position. Leaf development is usually most advanced from the end of July until late August, depending on the area of cultivation. Leaf yellowing and wilting generally begins when temperatures drop below 6°C.

Plant development

Beta vulgaris is hemocryptophytic, that is it is a plant that develops its buds just above, or below the soil-surface where they are protected from drought or cold experienced during very cold winters (Højland and Pedersen, 1994).

Sugar beets generally only show stem elongation in the second growing season although, as previously noted, this may begin in the first year in some plants (bolting). The gene B located on chromosome 2 (Boudry *et al*., 1994) cause shoot elongation and early flowering without vernalisation. The dominant allel fragmentally occur in *B. maritima* populations. Also quantitative genetic variation for bolting has been observed resulting in stem elongation under specific environmental conditions. The most important of these are low temperatures (+1 to +4°C) in the 4-5 leaf stage, the duration of low temperature, day length, and the effects on the phytochrome system of light quality (Lane *et al*., 1965; Lexander, 1981; Smit, 1983). Due to successes in breeding programmes, today's cultivated sugar beet varieties show very little stem elongation ("bolting") in the first year.

Root

The fibrous root system can reach to a depth of 1-2- meters. Numerous secondary roots spread out directly under the soil surface and are highly branched. Secondary roots submerged deeper in the soil are stronger and grow in downward arcs. 70% of the root mass is located in the soil layer from 0-30 cm. The roots of cultivated beets range in colour from white to yellow, orange and red in various shades and intensities. Sugar beets are sometimes "fangy"; this refers to overdeveloped secondary roots alongside the taproot.

D. Genetic characteristics (ploidy number)

The genus *Beta* exists in diploid, tetraploid and hexaploid forms with a chromosome number of x=9 (Walter, 1963) (see also Table 1.14). Dense genetic maps based on molecular marker have been published and linkage groups have been allocated to the 9 chromosomes of beet (Barzen *et al*., 1992;

Pillen *et al*., 1992). also the abundance of repetitive sequence classes has been extensively studied (Schmidt and Heslop-Harrison, 1993). All wild and cultivated *Beta* species are capable of hybridising, and wild beet species represent a valuable gene reservoir and are frequently used in variety breeding programmes.

Most of the sugar beet grown since 1970s has been triploid hybrids, although actually the diploid varieties represent 50 % in France. Triploid plants are produced by crossing a tetraploid male parent, onto a diploid male sterile plant, used as the female parent. The resulting plants are usually doubly sterile because of chromosome imbalance and cytoplasmically inherited male sterility in the same plant. However, small proportions of plants do produce aneuploid pollen, which will give fertile progeny when used to pollinate the diploid male sterile plants.

The development of hybrid sugar beet was made possible by the discovery of cytoplasmic male-sterility (CMS) (OECD, 1993b). As in other plant species, CMS in sugar beet is the result of the interaction between nuclear genes and changes in the mitochondrial genome. To obtain entirely male sterile offspring, CMS plants must be pollinated with so-called maintainer plants, which carry the normal, unchanged mitochondrial genome (OECD, 1993b).

Truly nuclear male-sterility which depends on a single recessive nuclear gene exist but this system does not allow the production of a population that is 100% male-sterile (OECD, 1993b).

The goal of breeding programmes is to develop sugar beet varieties with higher root yield and higher sugar content, better extraction yield (juice purity), higher seed germination percentages; lower tendency to "bolt"; physical attributes of the root well adapted to mechanical harvesting; higher resistance to leaf diseases; and, higher root dry matter content (especially for fodder beet).

E. Survival strategies

Sugar beet possesses long-lived dormant seeds that can become a volunteer weeds in sugar beet fields (Højland and Pedersen, 1994). They tend to germinate in the field 1-3 days later than planted sugar beet seeds (Højland and Pedersen, 1994). Sugar beet seeds may remain in the soil for ten years or more and still retain some germination capacity (OECD, 1993b; Brouwer *et al*., 1976; Lysgaard, 1991). It is generally accepted that six year-old multigerm and four year-old monogerm sugar beet seed exhibit the same germination level of 70%. Eight-year-old sugar beet seeds have been shown to germinate at a level of 59% in laboratory conditions. These germination percentages depend of the quality of the seeds and of the conditions of germination. Thus *Beta vulgaris* has the ability to generate a viable seed bank (Højland and Pedersen, 1994). The seed-balls of *Beta* are resistant to salt water, and ocean currents can move propagules over relatively long distances. Above the high water line, strong winds distribute them over the shoreline, and sometimes even inland (Smart, 1992).

Since commercial sugar producing sugar beet is biennial and is harvested during the first year whilst still in the vegetative phase, sexual reproductive organs (floral parts) never develop. Varieties that tend to bolt in the first year of growth pose some problems and much effort has gone into developing currently cultivated varieties that limit bolting. When *Beta vulgaris* is planted for seed production, some seeds may remain on the field after harvesting the seed crop. Agricultural practices tend to limit those shoots.

F. Isolation measures and distances

As pollen is mainly wind-borne, large isolation distances are necessary to prevent pollination from sources other than the desired male parent. For commercial seed production, isolation distances

are very variable according to the country. In the literature, it varies from 1 to 3.2 (Campbell and Mast, 1971; Smith, 1980; Højland and Pedersen, 1994).

The OECD developed a scheme in order to homogenise the isolation distances (OECD Council Decision of 10th October 1988 (C [88] 66), appendix II).

2. Agronomic Practices

Sugar beets are cultivated all over Europe (including the former USSR) and in the USA (FAO Year book, 1992; Højland and Pedersen, 1994). Various biotypes of beet are found in cultivation throughout Europe (De Bock, 1986) and outside Europe in North Africa, Asia and in North and South America (FAO Yearbook, 1992).

In central Europe sugar beet is usually grown at altitudes below 400m. Climate affects both beet yield and sugar content. To produce high sugar content, sugar beet requires at least 170 growing days and high levels of sunlight (Brouwer *et al*., 1976) but in Nordic countries this growing period is shorter (only 150 days). The crop also requires high amounts of moisture. Sugar beet crop fields are irrigated in regions with low precipitation. Sugar beet roots are slightly tolerant of acidic conditions, although soils with a pH of 7-8 are suitable.

Genetically monogerm seed is used almost exclusively in sugar beet cultivation except in China. Multigerm varieties are still used in Europe, South Africa, Near East and North America. Calibrated and pelleted seed is available. Calibrated seed has been mechanically separated. This process is relatively crude, resulting in fragments of quite different sizes. The monogerm seed for sugar beet on the market is almost exclusively in pellet form. Pellet seed is encased in a coat containing components used to control diseases and pests (Geissler, 1988). In terms of form and size, pelleted seed represents an extremely uniform seed type.

3. Centres of Origin/ Diversity, Geographic Distribution, Close Relatives and Their Geographical Distribution

A. History of cultivated beet

Beet was a well-established vegetable in "classical" ancient Greece and Rome. The earliest documentation comes from eighth century B.C.E. Babylonia. Greek, Roman and Jewish literary sources provide clear information that in the first century BC the crop was represented by several leafy forms (chards). Cultivars with swollen roots appeared later. There are no archaeological records of *Beta vulgaris* from pre-classical times, and it is not known exactly when and where beet was domesticated. The wild forms from which the crop could have been derived are widely distributed over the Mediterranean basin and the Near East (Zohary and Hopf, 1994).

The first known description of beets are of foliage beets (or chards) by Aristotele (c. 350 BC), who described a red chard, and Theophraastos (c. 300 BC) who recognised two different beets, white and black, the colours referring to light and dark green appearance of the leaves. The use of roots of beet are referred to for both culinary and medicinal purposes by Roman writers (Fort-Lloyd and Williams, 1975).

Beet leaves were probably used as potherbs (herbs used in cooking) in prehistoric times. In the sixth to fourth century BC, the first cultivated forms were developed and used as salad vegetables (chards). Red fodder beet has been cultivated since the 15th century, and sugar beets only since the end of the 18th century (Frietema-De Vries, 1996).

Beta vulgaris L. ssp. *maritima*, wild sea beet, is regarded as the mother species of the *Beta* beets (fodder beet, sugar beet, beetroot, yellow beet, Swiss chard). It is indigenous to European coastal regions, particularly the Mediterranean. Beet spinach, convar. *cicla*, has been cultivated in the Mediterranean region since 2000 B.C. In Europe *B. vulgaris* species with distinctly swollen roots were cultivated in the Middle Ages. Central European types are presumed to be descended from those used in Arabian horticulture in Spain. These plants were taken to the Netherlands, where they were cultivated beginning in 1500, and then to the Palatinate region, later spreading throughout Germany as "Burgundy beet". During the sixteenth and seventeenth centuries, red and yellow beets became increasingly common as salad vegetables. Fodder beet cultivation only began to increase during the course of the eighteenth century. The crop was introduced into the USA in 1800 where it became known as a garden beet. Sugar beet was introduced to North America around 1830 and to South America circa 1850 (Mansfeld, 1986).

In 1747, when the pharmacist Markgraf found that the sweet substance in beets was sucrose, efforts to extract sugar from beets began. At this time the sucrose content was 6.2%. Some forty years later in 1786, the breeder Achard selected from 23 local beet varieties a plant from the Halberstadt area for beet-sugar production. Koppy and Sohn selected the local variety "white Silesian Sugar beet." This submerged-root variety became the mother type for all sugar beet varieties. A student of Markgraf built the first factory extracted the sugar from beet in 1801 (Campbell, 1976; OECD, 1993b) and produced the first "variety" White Silesian. In the following 70 years, selection produced a beet variety with sugar contents of 16%. Today's sugar beet has a sugar content of 18-20%. In 1925, the global production of beet sugar represented 50% of the cane sugar production. By 1982, 30% of all sugar produced was from sugar beets.

B. Origin

Sugar beet originates from the cultivated form of beet around the Mediterranean area; region A1 (Near East) (Pernès J., 1984). It is possible that all cultivated beets originated from *B. maritima* (Mc Farlane J.S., 1971).

C. Close relatives and their geographic distribution

Beta vulgaris ssp. *maritima* (wild sea beet), is a common seashore plant of the coasts of Europe and Western Asia, and is perhaps a complex of closely related species. Primitive "superior" forms of this plant have been used as a leaf vegetable since prehistoric times and Root Beets, the ancestors of modern varieties, have been cultivated since the first century (De Rougemont, 1989).

Beta vulgaris ssp. *maritima* has spread from the centre of origin (Near East) to the coastal areas along the Mediterranean Sea to the Canary Islands, the Azores and along the Atlantic coast to Ireland and the southern parts of Scotland. It has been found in Belgium. It is rare in Holland and Germany (only known from the island Helgoland) and there is an isolated area of distribution in Denmark and at the Swedish Kattegar coast (Højland and Pedersen, 1994). It is not known in Switzerland. In Eastern Europe, it is found in Bulgaria and Romania. The area of distribution extends eastward to Iran, India, China and other Asian countries (Højland and Pedersen, 1994). Cultivated beet seed production areas are sometimes adjacent to sea beet populations (Bartsch *et al*., 1999).

Ssp. *maritima* occupies a very narrow coastal niche between high tide level and 10 to 20 meters inland (Doney, 1992). Neither sugar beet nor sea beet is naturalised in habitats away from the coast (Højland and Pedersen, 1994). Ruderal beet from South-western France are very close to Mediterranean *Beta maritima*.

Distribution of the *Beta* species is shown in 1.18:

Table 1.17 Distribution of the five *Beta* species present in Europe

	B. vulgaris L.	*B. macrocarpa* Guss.	*B. patellaris* Moq.	*B. trigyna* Waldst. and Kit.	*B. nana* Boiss. and Heldr.
Albania	X				
Azores	X				
Belgium and Luxembourg	X				
Baleares	X				
Britain	X				
Bulgaria	X			X	
Corsica	X				
Crete	X				
Former Czechoslovakia				X	
Denmark	X				
Finland	X				
France	X	X		X	
Germany	X				
Greece	X	X			X
Ireland	X				
Italy	X	X		X	
Former Yugoslavia	X			X	
Netherlands	X				
Portugal	X	X			
Romania				X	
Former Russia	South western part			Crimea	
Sardinia	X				
Spain	X	X	X		
Sweden	X				
Switzerland	X				
Turkey	X				

(See Tables 2.16 and 2.17. for the general distribution of *Beta* species)

Legend: the presence of an X indicates the presence of the *Beta* species in the country.

4. Reproductive Biology

A. Flower morphology

Flowers of *Beta vulgaris* sspp *vulgaris* are located on the terminal portions of the main axis and on lateral branches subtended from this. Flowers are sessile and occur singly or in clusters of two to eight (Smith, 1980).

Flowers are perfect and consists of a tricarpellate pistil surrounded by five stamens and a perianth of five narrow sepals (Smith, 1980). The flowers, solitary or in clusters of 2-8, are rarely self-pollinating (Free, 1970). The flower has a raised ovary with three or four secure stigmata. Three leaves are fused together into a single gynoecium to form the ovary. The seed arrangement is campylotropous.

B. Compatibility

Beet is a strongly self-incompatible plant (the stigma is not fully mature when the flower opens). Plants set few or no seeds at all when isolated (OECD, 1993b; Smith, 1980; Valdeyron, 1984). Self-fertilising plants exist in nearly every beet population (Barocka, 1985). Their frequency is lower among tetraploids than among diploids. Selecting for the diploid characteristic can significantly increase the potential for self-fertilisation.

The incompatibility system is genetically controlled by a complex gametophytic system governed by at least four loci, each with a number of alleles, and is further influenced by modifying genes. The *Beta* populations found on shores around the North Sea are largely self-incompatible (Dale and Ford-Lloyd, 1985). Mediterranean B. maritima populations are highly self-fertile due to specific gene for self-fertility (Fédération Internationale du commerce des Semences indication).

Sugar beet and sea beet (*Beta vulgaris* ssp. *maritima*) are both protandrous, self-incompatible. Sugar beet is an allogamous species, pollinated by wind and occasionally by insects, the former being the most important. Some cross-pollinations are also achieved by thrips and syrphids (Free J.B. and al., 1975; Valdeyron, 1984).

C. Formation of reproductive parts

The reproductive phase of sugar beet occurs during the second growing season. During the generative growth phase, following vernalisation, the internodes become extended into shoot bearing leaves that are ellipsoid to shape. Leaf axils have buds out of which the flower bearing shoots, panicles, emerge. Often only a single, very strong, unbranched flower shoot develops, although in some instances many flowering stems grow and form an extensive bush. Flowering stems are upright, up to two meters in height, green and coarsely furrowed. The secondary shoots may stretch upward or may hang down.

Temperatures above 21°C favour vegetative growth and temperatures between 4 and 13°C favour initiation of the reproductive phase. Most commercial cultivars of sugar beet require 90 to 110 days of exposure to inductive temperatures for initiation of reproductive development (Smith, 1980). With cultivars that bolt easily, comparatively short exposure to cool temperatures is adequate to induce plants to flower. Cultivars that do not bolt readily require longer periods of cold temperatures for floral induction (Smith, 1980). Photoperiod (short days) has also a strong effect on the vernalisation process.

Flower formation commences on the top shoot and flowers mature from the base upwards, growth of the secondary shoots following afterwards. Individual flowers of the cluster, made up of five thin inwardly curved perianth leaves and five stamens, do not flower synchronously. They are joined by gland-like tissue at the base, and this excretes large amounts of honey. The middle flower of the cluster blooms first followed by the surrounding flowers. Protandry exists at the morphological level. The sugar beet plant flowers for duration of approximately four weeks.

Depending on the cultivar and environmental conditions, mature flowers begin anthesis about 5 to 6 weeks after initiation of reproductive growth and continue for several weeks. Flower opening begins at the base of each stem and continues upward as the stem elongates. Flower open mostly in the morning, but continue throughout the day (Smith, 1980). Stigmas may remain receptive for more than two weeks allowing a good chance for wind blown pollen to effect fertilisation depending on weather conditions (Crane and Walker, 1984). The flowering period for sugar beet in Central European climatic is between June and August (Barocka, 1985).

D. Pollen

Pollen grains are round and have numerous indentations in their wartlike exines. The number of pollen grains per anther is estimated at 17 000. This would correspond to 85 000 grains per flower and, given 10 000 flowers per bush, almost one billion per plant (1 ha sugar beet with circa 25 000 seed plants produced approximately 25 trillion pollen grains) (Schneider, 1942). The pollen ability of survival is limited to maximum 24 hours according to the German experts. This depends on the environmental conditions, especially moisture.

The genus Beta also displays pollen sterility. Nuclear male sterility is under the control nuclear genes restoring male fertility (although cultivated and wild beet may have different [Owen, 1945; Boutin *et al*., 1988 and 1987]). The mechanism can also be cytoplasmic (Owen, 1945, 1952).

E. Pollen dispersal

Pollen is transported primarily in air currents. Insects (honeybees, bees, thrips) play a lesser role in pollen movement. Honeybees may increase seed yields, but seem to visit plants only if no other pollen is available. A study of insect pollination of sugar beet seed crops revealed that most of the visiting insects (129 species) carried sugar beet pollen grains and probably contributed to cross-pollination (Free *et al*., 1975). Because tetraploid plants produce fewer and larger pollen grains than diploid plants, and the pollen is less readily released by the anthers, insect pollination is probably more important for hybrids whose pollen donor is tetraploid than for pure seed crops. This is especially so when relative humidity is high, and little pollen is transported by air movement, yet insect activity is unaffected (Free *et al*., 1975).

Wind-borne pollen can be distributed horizontally at least 4,500 m and has been observed at a height of 5,000 m (Archimowitsch A., 1949). Smith (1980) observed pollen drift of up to 5 km from the originating field, and Gliddon (in Harding and Harris, 1994) assumed that the airborne pollen movement can occur at distances up to 8 km.

F. Pollination

Tetraploid plants release their pollen somewhat later in the day than diploid plants and there is a period in the morning when the stigmata of the male-sterile plants are receptive, but when the pollen donors are not producing an effective pollen cloud. It is during this period that the male-sterile mother plants are most receptive to contamination from background pollen released by wild and weedy forms of diploid *B. vulgaris*, many of which, in continental Europe, are annuals or near annuals. This leads to the production of weedy forms of beets. This contamination is a potentially serious weed problem (Scott and Longden, 1970; Longden, 1976; Hornsey and Arnold, 1979).

G. Seed

The fruit of the sugar beet is a capsule and seed is imbedded in a hollow that remains closed by a small lid that springs open during germination although the seed remains firmly attached to the pericarp. The seed is circular, approximately 1-2 mm in diameter, and has a kidney shaped indentation caused by a small, beak-shaped root. According to Martin and Leonard (1976) and Benjamin and Bell (1985) flowering sugar beet can produce 200 million seeds per hectare. What is generally referred to, as the beet seed is in fact a cluster-like multiple fruit. Sugar beet seed normally consist of a seed-ball formed by two to four true seeds (OCDE, 1993b). 1000 clusters weigh between 10-40 g.

The ovaries are enclosed by the common receptacle of the flower cluster (Smith, 1980). They form a hard and irregular dry body, the so-called seed ball, which usually contains one to four seeds.

A plant with monogerm seed borne in separate flowers was found in the United States in 1948 (Martin and Leonard, 1976). Before the genetically monogerm seed was found, they were made mechanically by grinding seed clusters (Højland and Pedersen, 1994). The characteristic of monocarpy is recessive and determined by one gene. Today most cultivated sugar beet varieties are monogerm and whose seedlings need not be thinned after sowing and germination (Barocka *et al*., 1968; Winner, 1981).

H. Seed dispersal

In wild beet a large proportion of mature seeds produced by the end of the flowering season are shed in the immediate vicinity of the maternal plant. Some of the seeds, however, may be dispersed over greater distances. The major agent of long distance dispersal of the multigerm seedballs of ssp. *maritima* is believed to be tidal movements of the water (Dale and Ford-Lloyd, 1985).

Sugar beet seed do not shatter as easily as some wild *Beta* species, which may drop their seeds as they ripen. Seeds falling on the ground do not usually germinate in the same season, partly because of the presence of germination inhibitors, partly because of poor seed-soil contact (OECD, 1993b; Letscghert, 1993).

5. Cross

A. Intraspecific

The six *Beta vulgaris* subspecies are interfertile although individually they are self-incompatible. The cultivated species *Beta vulgaris* ssp. *vulgaris* is characterised by a great varietal diversity. In addition to sugar and fodder beets, there also exist leaf spinach beet, Swiss chard, and red beet (see Table 1.15). All varieties may cross with one another, a characteristic that must be taken into account in seed production.

Sugar beet and sea-beet (*Beta vulgaris* ssp. *maritima*) hybridise freely and hybrids are spontaneously formed in the wild and in seed-production fields (Bartsch *et al*., 1999). Such hybrids are fertile and do not demonstrate incompatibility at the chromosome level (Evans and Weir, 1981). The most important precondition for hybridisation in natural habitats is the existence of spatially overlapping populations and flowering periods. *Beta vulgaris* ssp. *vulgaris* and *Beta vulgaris* ssp. *maritima*, which hybridise the most frequently in nature, share a common flowering period from May to September. They can occur in the same areas, as ssp. *maritima* is distributed along the Atlantic coastal region (Hanf, 1990). As already noted, these hybrids are an important source of so-called weed beets among the cultivated types of West Europe and North America (Hornsey and Arnold, 1979; Evans and Weir, 1981).

B. Interspecific

Beta *section*

Beta vulgaris belongs to the section *Beta* (syn. *vulgare*) together with *B. maritima*, *B. macrocarpa*, *B. patula* and *B. vulgaris* ssp. *adanensis*, the wild species of the cultivated beet (Valdeyron, 1984; Smart, 1992). All these species are cross compatible (Smith, 1980; Bartsch *et al*., 1999). The hybrids are vigorous and fertile and do not show incompatibility at the chromosome level

(Geyt Van *et al*., 1990). However, hybrids between beet and *B. macrocarpa* are rare due to differing flowering times of the parental species (Mc Farlane, 1975). Wild species of the section *Vulgares* (B. *vulgare* ssp. *maritima*, *B. orientalis*) have been used in beet breeding (Geyt Van *et al*., 1990).

Abe *et al*. (1984) observed that hybridisation between *B. macrocarpa* and *B. vulgaris*, and between *B. maritima* and *B. atripicifolia* result in a certain degree of pollen sterility and seed abortion of the F_1 generation. Lange and De Bock (1989) produced triploid and tetraploid hybrids between tetraploid *B. macrocarpa* and diploid and tetraploid types of *B. vulgaris*. The triploid descendants were sterile although the tetraploid descendants exhibit a better fertility. The F_2 is partially fertile. A number of researchers have reported successful crosses between *B. vulgaris* and species of section *Corollinae* (reference in Geyt *et al*., 1990).

Corollinae *section*

Artificial hybrids can be produced with the species of the *Corollinae* section, but such hybrids are mostly sterile and only set a few seeds when backcrossed to sugar beet (OECD, 1993b).

No evidence of interfertility has been found between the cultivated beet and the Caucasian beet (*Beta trigyna*).

Procumbentes *section*

Artificial hybrids with members of the *Procumbentes* section usually die at the seedling stage. They can be saved by grafting onto sugar beet, and they then develop into vigorous plants. These hybrids are also almost completely sterile and set few seeds upon backcrossing (OECD, 1993b).

The majority of *Beta vulgaris* ssp. can be crossed with wild species of the section *Procumbentes*. Jung and Löptien (1986) achieved crosses between sugar beet and *B. procumbens, B. webbiana* and *B. patellares*. The F1 hybrids were backcross with *B. vulgaris* (See also Højland and Pedersen, 1994) to establish a complete set of monosomic addition lines (2n =19).

Nanae *section*

No hybrids between sugar beet and *B. nana* are known (OCDE, 1993b).

C. Introgression

There is extensive evidence of hybridisation in the wild between, and introgression from wild beet to cultivated sugar beet and vice versa.

D. Interactions with other organisms

The sugar beet leaves contain oxalic acid which can cause problems if fresh, unwilted sugar beet tops are used as cattle feed (OECD, 1993b).

Interactions between *Beta vulgaris* ssp. *vulgaris* with common disease organisms and pests are shown in Appendix I.

6. Weed Characteristics and Weediness

Sugar beet may become a weed through the roots or crowns remaining in the field after harvest. These “volunteer” plants, if left, will flower and produce seed. The offspring of these plants is normally controlled by herbicide treatment or other means in the following crops.

Cultivated beet may possibly run wild but it is difficult to distinguish between cultivated beets and the weed beet. Beet is often found outside cultivation but there is no indication of such plants establishing in the wild (Frietema, 1996).

Sugar beet bolters could produce enough viable seeds to become a weed problem. This “weed beet” is seldom found in winter cereals, sometimes in spring cereals, especially where poor establishment has occurred, but is relatively common in potato and pea crops. This is because of the limited selection of herbicides that can be used, and because of the similar timing of cultivation techniques. It is estimated that one field in four in England will have viable beet seed in the top soil (Højland and Pedersen, 1994).

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APPENDIX

Interactions: Common Diseases and Pests of *Beta vulgaris*

Viral diseases

Beet yellows (BYV and BMYV)

Beta virus 4 causes beet yellow. The disease is transmitted by aphids (primarily by *Myzus persicae* and *Aphis fabae*). Leaf yellowing is the principle symptom. The disease is of considerable economic importance as it leads to significant reduction in beet and sugar yield, particularly in regions where the transmitting aphids appear early (Brouwer *et al*., 1976; Geissler, 1988; Heitefuss *et al*., 1993).

Sugar beet mosaic virus

Beta virus 2 is responsible for this disease which is transmitted by various aphids. Symptoms include whitish or light green mosaic-like patterns on the inner part of young leaves. Sugar beet mosaic virus rarely causes severe economic damage (Brouwer *et al*., 1976; Geissler, 1988).

Beet-leaf curl

Beet leaf curl is caused by *Beta virus 3*. The disease is transmitted by the beet leaf bug *Piesma quadrata*. Symptoms are leaf curling and the mosaic-like lightening of young leaves. Leaf veins swell. Severe epidemics can lead to yield reduction (Brouwer *et al*., 1976; Geissler, 1988; Heitefuss *et al*., 1993).

Rhizomania

Beet necrotic yellow vein virus (BNYVV) is responsible for rhizomania. The soil fungus Polymyxa betae transmits BNYVV. Infected plants display stunted growth, the leaves are smaller, light green, and stand straight up. A strong dense, root network is characteristic of the disease. The disease has been observed in Germany since the mid-1970s. BNYVV represents a serious threat to stricken crops (yield reduction of up to 50%) (Brouwer *et al*., 1976; Barocka, 1985; Geissler, 1988; Heitefuss *et al*., 1993). But it is spreading and is present all over Europe except Ireland and Denmark.

Bacterial diseases

Beet leaf spot

Pseudomonas sytingae is responsible for this bacterial disease known as beet leaf spot. The bacteria enter the beet leaf through the stomata and wounds in the leaf. Brown to black spots of various sizes develop. Infected tissue breaks off. The disease has occurred in central and western Europe with increased frequency in recent years, but has not had significant economic effects (Heitefuss *et al*., 1993).

Crown gall

The disease is caused by *Agrobacterium tumefaciens*. Crown gall can be identified by abnormal tissue growth on the body of the beet near the soil surface. The bacteria enter the beet through small wounds. Damage from crown gall is negligible from an economic point of view (Brouwer *et al*., 1976).

Common beet scab

Actinomyces scabies induces the disease. The scab colours the rind of the beet blackish brown. Infected beets lignify severely. Beet scab does not represent a significant economic threat to beet cultivation (Brouwer *et al*., 1976).

Fungal diseases

Root rot and secondary root rot

The disease is transmitted by fungi contaminating both seeds and soil. *Phoma betae* is transmitted on the beet seed, and infection after cold-weather sowing can cause significant damage during seedling emergence. *Pythium* species and *Aphonomyces* infect the seedling by means of motile zoospores. Damp conditions and cool temperatures increase the likelihood of *Pythium* infection. *Aphanomyces* requires higher temperatures for infection. Symptoms are often discernible on secondary roots. Many fungus-stricken seedlings do not emerge and those that do remain retarded in their development. If the plant survives the disease, growth remains stunted due to secondary root damage and rotting of the root tips. (Brouwer *et al*., 1976; Geissler, 1988; Heitefuss *et al*., 1993).

Beet leaf spot diseases

In warmer areas with high rainfall, beet leaf spot is the fungus that causes the most economic damage in sugar beet production. It is induced by a variety of fungi. The disease is caused by *Cercospora beticola*, for instance. The fungi can be transmitted on beet seed, but can also moved on dead beet leaves or beet tops. The fungus' spores develop at temperatures at or above 17°C (optimum: 27°C) and require high air humidity. Wind and rain carry the spores onto the leaves of the beet plant where they germinate and enter the plant through the stomata. The first spots develop a few days thereafter. The fungus then sporulates once again, leading to renewed infection and the further spread of the disease throughout the crop. Crop damage can be observed in late June with old leaves showing the first symptoms. Initially, 2-3 mm round, reddish spots develop which later turn grey in the centre. As the disease progresses, the spots enlarge and merge and the leaves dry out. If the entire crown dies, the plant responds with new growth.

Beet leaf spot can also be induced by *Phoma betae*. The optimal temperature for development of this disease is 20°C. Diseased sugar beets grown for seed can result in a significant infection of the seed. Symptoms include round leaf spots with lighter centres and concentric dark and light rings. The disease is generally not an economically significant problem.

Ramularia beticola enters the beet through the stomata. Optimal infection temperatures are 18-20°C with a relative humidity of over 95%. After infection, 1 cm leaf grey to brownish spots develop. The disease is primarily of importance in seed beet production.

Powdery mildew

The disease is caused by *Erysiphe betae*. The fungus develops well during dry weather with temperatures around 20°C. Initial infection is induced by spores that may have travelled over long distances. Signs of damage can be observed beginning in mid July. A white, powdery layer develops on the upper side of older leaves and quickly covers the entire leaf. The disease has been identified in central Europe since the early 1970s. Crops infected early can be severely damaged (Brouwer *et al*., 1976; Geissler, 1988; Heitefuss *et al*., 1993).

Downy mildew

Peronospora forimosa requires high air humidity and temperatures below 15°C. A grey mildew layer covers upper and lower leaf surfaces. The disease is generally insignificant in central Europe (Brouwer *et al*., 1976).

Other fungal diseases

Leaf scorch (*Helicobasidium purpureum*), beet rust (*Uromyces betae*), violet root rot (*Helicobasidium purpureum*) and sclerotina rot (*Sclerotinia sclerotiorum, S. fuckeliana*) may infect sugar beet, but generally do not cause significant biological or economic damage in central Europe (Brouwer *et al*., 1976).

Animal pests

"Finger" beetle

The beetle (*Clivinia fossar*) is about 6 mm long and has a small, nearly cylindrical body. Its colouring is reddish brown and it inhabits upper soil layers if sufficient moisture is available. From here the beetle attacks young plants. Occasionally, it causes extensive damage in sugar beet, particularly when fields are sown early and are weed-free after comprehensive herbicide application (Heitefuss *et al*., 1993).

Pygmy mangold beetle

The beetle (*Atomaria linearis*) grow to a length of 1.2-1.7 mm. It is dark in colour and spends the winter in plant remains on field borders and in the ground. The pygmy beetle migrates into beet fields in the spring. The hypocotyls of afflicted plants reveal dark, pin-head-sized bite marks. Plants may die in instances of extreme beetle damage. Considerable economic damage can result when beet crops are planted in succession (Brouwer *et al*., 1976; Geissler, 1988; Heitefuss *et al*., 1993).

Beet cyst nematode

Heterodera schachtii is a "thread worm". In addition to beets, cruciferous plants are among the beet cyst nematodes primary victims. Also known as the "beet eelworm", beet cyst nematodes reach a length of 1.5 mm. Approximately 250 eggs per cyst develop into larvae. A portion of the larvae hatch in the summer and migrate into the roots of the beet. Sexually mature nematodes develop after they have absorbed enough nutrients. The swelling of the females causes the root tissue to break open. After mating, the female hardens and develops into a cyst. Eggs and larvae remain viable in this phase for years. Infested crops mature irregularly, wilt, turn yellow, and die. Plants also develop an unusual number of secondary roots and a so-called "root beard" displaying many cysts. Severely infested soil can result in significant yield reduction. Beet cyst nematodes are considered to be partly responsible

for beet sickness affecting soil (Brouwer *et al*., 1976; Loptien, 1984; Barocka, 1985; Geissler, 1988; Heitefuss *et al*., 1993).

Collembolans

Colembolans (*Onychiurus armatus*) are white, 1-2 mm in length, and live underground, usually using plant detritus in the soil as a food source. Lacking these sources of nutrients, the pest attacks the roots of sugar beet seedlings. Seeds and hypocotyls may be damaged. Plants may die in cases of severe infestation. Colembolans do not account for significant economic losses in sugar beet cultivation (Heitefuss *et al*., 1993).

Wire worm

The brown beetle (*Agriotes ssp.*) is 6-12 mm long. Eggs are laid in the summer and the larvae develop into adults over the course of 3-5 years. They can cause feeding-related damage beginning in their second year of development. The roots of young beets may be partially or completely eaten. The plants wilt and die. Wire worm infestation can result in considerable yield reductions in years with high precipitation or when new land is cultivated (Brouwer *et al*., 1976; Heitefuss *et al*., 1993).

Millipede

Millipedes (*Blaniulus guttulatus*) are light-coloured, approximately 1 mm in diameter, and grow to a length of 20 mm. They have a life span of two years. They occasionally cause feeding-related damage to seeds and seedlings.

Beet-leaf fly

Pegomya betae overwinters as a pupa in the soil. The larvae bore into leaf tissue within 4-10 days after hatching. The larvae exit the leaves after feeding for 2-3 weeks and pupate in the soil. Two to three generations develop each year, but only the first is relevant as a pest. Leaf tissue damaged between the top and bottom sides dries, splits apart, and eventually dies. Crops that are afflicted early in the season may be severely damaged. Damage rarely occurs after the beet has reached the 6-leaf stage. Economic damage due to beet-leaf flies has been on the decline in recent years (Brouwer *et al*., 1976; Heitefuss *et al*., 1993).

Green peach aphid

Myzus persicae spends the winter as an egg in peach (*prunus persica*) and cherry (*Prunus* species) trees. In the spring, the mother aphid hatches and produces wingless aphids. Winged aphids develop beginning in May and these migrate to beet. Here they reproduce asexually for a number of generations. Infested plants can be recognised by their slightly discoloured leaves. The green peach aphid causes significant economic damage as a carrier of viruses responsible for beet yellows (Heitefuss *et al*., 1993).

Blackfly

Aphis fabae spends the winter in egg form in the European Euonymous and the snowball tree (*Viburnum opulus*). Its development is similar to that of the green peach aphid. Infested plants display rolled leaves; young leaves are strongly curled. Severe damage and yield reduction is caused primarily by the sucking activity of the blackfly, although the pest is also a transmitter of viruses (Brouwer *et al*., 1976; Geissler, 1988; Heitefuss *et al*., 1993).

Field slug

Deroceras reticulum attains a length of 50-65 mm. The slugs have a scale-shaped shell and are yellow-white or red-brown in colour. Field slugs damage almost all cultivated plants. They spend the winter as eggs or slugs. Mild winters allow them to multiply more quickly. They eat away at the leaves until only the more sturdy veins remain. The damage caused by field slugs has increased in recent years, and not only with respect to beet cultivation. Rape cultivation, rotational crops, and fallow fields have led to a general increase in the field slug's impact on agriculture (Heitefuss *et al*., 1993).

Common wood mouse

The common wood mouse (*Apodemus sylvaticus*) is grey with a brown-grey to brown-red stomach. It has a short tail and large eyes and ears. Field woods, wood borders, fields and gardens are its habitat. The common wood mouse only gained significance as a pest with the introduction of pelleted beet seed. The mouse causes damage by digging up seeds along the drilled rows, cracking them, and eating the seedlings. This pest repeatedly causes severe damage in some areas (Heitefuss *et al*., 1993).

Some other herbivores attacks the beet:

Agrosetis segetum: roots
Blitophaga linearis: leaves of small plants
Calocoris norvegicus: leaves
Discestra trifolii: leaves
Ditylenchus dipsaci: stems and leaves
Pegonya hyoscyami: leaves
Thrips angusticeps: leaves and stems of small plants.

SECTION 9
SUNFLOWER (*HELIANTHUS ANNUUS* L.)

1. Taxonomy of the Genus *Helianthus,* Natural Habitat and Origins of the Cultivated Sunflower

A. Taxonomy of the genus *Helianthus*

The sunflower belongs to the genus *Helianthus* in the Composite family (Asterales order), which includes species with very diverse morphologies (herbs, shrubs, lianas, etc.). The genus *Helianthus* belongs to the Heliantheae tribe. This includes approximately 50 species originating in North and Central America.

The basis for the botanical classification of the genus *Helianthus* was proposed by Heiser *et al.* (1969) and refined subsequently using new phenological, cladistic and biosystematic methods, (Robinson, 1979; Anashchenko, 1974, 1979; Schilling and Heiser, 1981) or molecular markers (Sossey-Alaoui *et al.*, 1998). This approach splits *Helianthus* into four sections: *Helianthus, Agrestes, Ciliares and Atrorubens.* This classification is set out in Table 1.18.

Section Helianthus

This section comprises 12 species, including *H. annuus*, the cultivated sunflower. These species, which are diploid (2n = 34), are interfertile and annual in almost all cases. For the majority, the natural distribution is central and western North America. They are generally well adapted to dry or even arid areas and sandy soils. The widespread *H. annuus* L. species includes (Heiser *et al.*, 1969) plants cultivated for seed or fodder referred to as *H. annuus* var. *macrocarpus* (D.C), or cultivated for ornament (*H. annuus* subsp. *annuus*), and uncultivated wild and weedy plants (*H. annuus* subsp. *lenticularis, H. annuus* subsp. *Texanus*, etc.).

Leaves of these species are usually alternate, ovoid and with a long petiole. Flower heads, or capitula, consist of tubular and ligulate florets, which may be deep purple, red or yellow.

Section Agrestes

This section includes only the annual species *H. agrestis* (2n = 34), characterised by reddish coloured tubular flowers, yellow styles and glabrous stems bearing leaves that are generally opposite and lanceolate (Bonjean, 1993). Its self-compatibility makes it different from other species (Heiser *et al.*, 1969). It is well suited to the moist soils of central Florida and Georgia.

Section Ciliares

This section includes six perennial species of small size originating in Mexico and the western United States. They are genetically quite distinct from the species in the other sections.

A distinction is made between two series in this section: *Ciliares* and *Pumili*. The first includes three species possessing powerful secondary root systems, making them redoubtable adventives. Their leaves are

usually opposite, bluish in colour, virtually glabrous and with very short petioles. All the species in this series are diploid, with the exception of *H. ciliaris*, which includes tetraploid (2n = 68) and hexaploid (2n=102) populations.

The species in the *Pumili* series have opposite hairy leaves, growing from buds which appear at the crowns of the old roots. They are all diploid (2n = 34).

Section Atrorubens

This section includes thirty species divided arbitrarily into four series, including one cultivated species, the Jerusalem artichoke (*H. tuberosus* L.). Hybridisation between these species and their polyploid forms can make it difficult to classify them precisely.

The natural distribution of these species is the eastern and central United States, with the exception of *H. nuttallii* and *H. californicus*, which are found only in the West.

The *Corona-solis* series contains large species with tuberiform or rhizomatous roots. In some cases, leaves are alternate, large and numerous (*H. giganteus, H. grossesseratus, H. nuttallii*, etc.). Others have opposite, lanceolate leaves with three main veins (*H. divaricatus, H. mollis*). Seven species are diploid. *H. strumosus* and *H. decapetalus* can be found in tetraploid and hexaploid forms. *H. hirsutus* is tetraploid. Six species are hexaploid.

The *Atrorubentes* series comprises four perennial species from the southwest of the United States. Usually without rhizomes and with fibrous roots, they go through a very marked rosette stage. These species were formerly included in the *Divaricati* series in Heiser's classification.

The *Microcephali* series includes four species which have a small capitulum and roots that are fibrous or perhaps slightly rhizomatous. Their stems may be covered with wax and bear leaves that are alternate in most cases. A fifth species, Porter's sunflower (*H. porteri*) has been transferred from the genus *Viguiera* to the genus *Helianthus* (Yates and Heiser, 1979). This is an annual species found only in Georgia.

The species in the *Angustifolii* series are all diploid and located essentially in the southwestern United States. Their stems are hairy and the leaves usually alternate, lanceolate and with a leaf blade that may curl in toward the inside surface. Their roots are fibrous, thick or with rhizomes.

Table 1.18 Classification of the genus Helianthus (Seiler and Rieseberg, 1997)

Section	Series	Species	Ploidy
Helianthus	-	*H. annuus* L. *	2n = 34
		H anomalus Blake *	2n = 34
		H. argophyllus T. & G. *	2n = 34
		H. bolanderi A. Gray *	2n = 34
		H. debilis Nutt. *	2n = 34
		H. deserticola Heiser *	2n = 34
		H. exilis A. Gray *	2n = 34
		H. neglectus Heiser *	2n = 34
		H. niveus (Benth.) Brandegee *	2n = 34
		H. paradoxus Heiser *	2n = 34
		H. petiolaris Nutt. *	2n = 34
		H. praecox Engelm & A. Gray *	2n = 34
Agrestes	-	*H. agrestis* Pollard *	2n = 34
Ciliares	*Ciliares*	*H. arizonensis* R. Jackson	2n = 34
		H. ciliaris DC.	2n = 68, 102
		H. laciniatus A. Gray	2n = 34
Ciliares	*Pumili*	*H. cusickii* A. Gray	2n = 34
		H. gracilentus A. Gray	2n = 34
		H. pumilus Nutt.	2n = 34
Atrorubens	*Corona-solis*	*H. californicus* DC.	2n = 102
		H. decapetalus L.	2n = 68, 102
		H. divaricatus L.	2n = 34
		H. eggertii Small	2n = 102
		H. giganteus L.	2n = 34
		H. grosseserratus Martens	2n = 34
		H. hirsutus Raf.	2n = 68
		H. maximiliani Schrader	2n = 34
		H. mollis Lam.	2n = 34
		H. nuttallii T. & G.	2n = 34
		H. resinosus Small	2n = 102
		H. salicifolius Dietr.	2n = 34
		H. schweinitzii T. & G.	2n = 102
		H. strumosus L.	2n = 68, 102
		H. tuberosus L.	2n = 102
Atrorubens	*Microcephali*	*H. glaucophyllus* Smith	2n = 34
		H. laevigatus T. & G.	2n = 68
		H. microcephalus T. & G.	2n = 34
		H. smithii Heiser	2n = 68
Atrorubens	*Atrorubentes*	*H. atrorubens* L.	2n = 34
		H. occidentalis Riddell	2n = 34
		H. pauciflorus Nutt. (synonym *H. rigidus* Cass.)	2n = 102
		H. silphioides Nutt.	2n = 34
Atrorubens	*Angustifolii*	*H. angustifolius* L.	2n = 34
		H. carnosus Small	2n = 34
		H. floridanus A. Gray ex Chapman	2n = 34
		H. heterophyllus Nutt.	2n = 34
		H. longifolius Pursh	2n = 34
		H. radula (Pursh) T. & G.	2n = 34
		H. simulans E.E. Wats.	2n = 34

* annual species. The others are perennial.

B. The natural distribution of sunflowers *(Helianthus annuus* L.)

The species *H. annuus* comes originally from North America. It is the most diverse North American sunflower species not only in terms of its geographical distribution, but also with respect to its morphology and environmental and physiological adaptation (Seiler, 1984).

It is found at altitudes between sea level and 3,000 metres in areas with a range of different rainfall characteristics, but essentially in the western two-thirds of the United States, southern Canada and northern Mexico. It is usually found in open habitats already disturbed by human activity (Bonjean, 1993).

At maturity, these plants present a high degree of phenotypic variation: their size may vary from less than a metre to more than four metres. They may or may not be branched and may present varying degrees of hairiness. Their leaves, which are generally oval to cordate in shape, are alternate and petiolate, with a size in the range 5 cm to 35 cm wide and 10 cm to 50 cm long. The capitulum is at least 1.5 cm deep and supports relatively broad bracts which may be oval or lanceolate; it is rarely glabrous on the dorsal surface and is usually ciliate at the edges.

The ligulate flowers are approximately 25 mm in length and sterile. There are at least seventeen of these. The tubular flowers are shorter and have corollas with lobes that are purple, reddish or yellow in colour. The achenes are 3 mm to 15 mm in length and are found in a range of colours (for example white, black, black with white stripes, and brown).

In the natural state, the flowering of wild *H. annuus* is a lengthy process involving each capitulum in turn, and lasts from late July to early October. The species is diploid (2n = 34) and hybridises naturally with many other sunflowers.

Wild populations are usually strictly self-incompatible and markedly allogamous, cross pollination being obligate. They are pollinated by insects, first and foremost by bees. Cultivated forms of sunflower generally allow a higher degree of self-compatibility.

C. The origins of the cultivated sunflower

The cultivated sunflower probably comes originally from the western United States. It is certainly the case that wild sunflower seeds were a food resource for Native American populations living in this geographical region.

According to Heiser (1985), the most probable hypothesis as to the domestication of the sunflower is that it was an adventive found at the edges of Native American encampments. Inhabitants of these encampments gathered the achenes of wild sunflowers. In this way, the plant was carried from western to central North America. Based on this hypothesis, it was domesticated there and then introduced in the same period into the eastern and southern parts of the United States.

The fact that cultivated sunflower achenes have been found in several archaeological sites in the eastern and central United States, whereas archaeological digs in the southwest of the country and in Mexico have brought to light only the achenes of wild sunflowers, is one of the strongest pieces of evidence for Heiser's hypothesis.

Using isozyme systems and chloroplasmic DNA, Rieseberg and Seiler (1990) have been able to provide proof at the molecular level that the cultivated sunflower apparently derived from a virtually unique or adventive form of sunflower, which probably grew originally in the central United States.

The cultivated sunflower was introduced into Europe in the late 16th century, probably by Spanish sailors. It was initially grown as an ornamental. No mention is found of its advantages as an oilseed plant before the 18th century, and the sunflower has since seen major genetic advances. It was in Russia that the first such improvements were made to develop single headed, shorter, earlier flowering plants and to increase the oil content of the seeds. More recently, strains with high oleic acid content were developed by Soldatov (1976). For these reasons, Russia is considered as a secondary domestication centre for sunflower.

The cultivated sunflower presents a narrow range of genetic variability, notably with regard to certain agronomic and technical characteristics such as standability and oil content. This is all the more true of hybrids created during the last decade (Bonjean, 1993). Conversely, the wild species of the genus *Helianthus* present a high degree of genetic variability, the exploitation of which has enabled enormous progress to be made in the creation of varieties, especially with regard to increased oil content, as well as resistance to disease, insects and dry conditions (Leclercq *et al.*, 1970; Krauter *et al.*, 1991; Miller *et al.*, 1992; Serieys, 1984; Serieys, 1997). Interspecific hybridisation also enabled identification of many new sources of male cytoplasmic sterility (Serieys, 1999). Partial hybridisation mechanisms frequently observed between perennial *Helianthus* and cultivated sunflower (Faure *et al.*, 2002), represent another way for controlled introgression (Faure *et al.*, 2002).

2. The Botanical Characteristics of the Cultivated Sunflower

The principal morphological and physiological characteristics of the sunflower, such as height, diameter of the capitulum, duration of the growing cycle, size of seeds and oil content, are all highly dependent on the soil climate in which it is cultivated (Merrien, 1986).

Root system

The sunflower's root system is of tap root type. The tap root may go down as far as five metres if conditions are favourable, but it has little real penetrating power. The sunflower also develops extensive superficial root hair growth. If conditions are favourable, root spread in young cultivated plants may amount to as much as 70 kg per hectare per day (Maertens and Bosc, 1981).

Above-ground vegetation

The cultivated sunflower differs from the wild sunflower in that it has a single inflorescence (except male lines cultivated for seed production). The stem is topped by a single capitulum that may, in some cases, be very large.

Germination of the seed is epigeous. The height of the developed plant varies between 0.5 and 5 metres, but is usually 1.6 metres. The diameter of the plant's stem varies in the range of 0.5 cm to 10 cm. The size of a sunflower is related to the number of leaves and the duration of the "seed-to-flower" phase.

The stem has a tendency to bend slightly under the weight of the mature capitulum. The nature of this stem curve is largely under genetic control. The degree of stem curve is of fundamental importance since it determines the angle of the capitulum with respect to the stem and so the capacity to protect the florets and achenes from climatic stress (rain, hail, wind, sun) and birds (Bonjean, 1993; Seiler, 1997).

The leaf blade is continuous, cordate and irregularly toothed; it is frequently covered in short, hard hairs. It has pinnate veins, including three main veins. The first five pairs of leaves are opposite and the others are alternate, following a spiral phyllotaxy. The leaves may have a range of sizes and shapes, with the largest being between the fourth and tenth nodes. These are the intermediate leaves which play the most important part in the formation and accumulation of the seeds' fat reserves. It is worth noting that by the time the capitulum has formed the plant has developed almost half its total leaf surface, and by the beginning of flowering over 75% total leaf surface has developed (Merrien, 1986). The precise number of leaves may vary from 12 to 40, according to variety. However, the range is 20 to 40 in most of the hybrids currently cultivated (Bonjean, 1993).

The reproductive system

The inflorescence is a capitulum the diameter of which may vary on average between 10 and 40 centimetres in most of the hybrids currently cultivated. The capitulum includes a fleshy receptacle which bears two types of flowers: ligulate flowers at the periphery, and tubular flowers in the centre. The edge of the capitulum is surrounded by leafy bracts arranged in overlapping concentric circles (2 to 5 in number).

The ligulate flowers form one or two rows around the periphery of the capitulum. There are never more than approximately thirty of these flowers. They are asexual, or, very rarely, unisexual of female type (Arnaud, 1986).

The tubular flowers or florets make up most of the capitulum. They are arranged in arcs which converge toward the centre of the capitulum. The florets are hermaphrodite and after pollination and fertilisation produce the achenes, which are harvested. The potential number of disk florets varies with capitulum diameter in the range of 60 to 3500.

Flowering

The sunflower's flowering phase lasts between 9 and 15 days on average. The precise duration varies with the size of the capitulum and atmospheric conditions (Merrien, 1986). The flower unfolds centripetally from the periphery of the capitulum toward the centre (Marc and Palmer, 1978). Flowering begins with the ligulate flowers, which unfold their single petal immediately after the capitulum opens, and remain in flower until the florets have finished flowering (tubular flowers). The florets complete their flowering phase in 3-4 days in daily cycles involving one or two concentric rows. The flowering of each floret begins with the initial opening, its anthers projecting above the corolla with the extension of the filaments. The anthers are dehiscent and the pollen spills into the interior of the flower; this is the male stage. On the following day, the style extends through the interior volume of the flower and emerges above the anthers. The two stigmatic lobes separate and curl toward the style; this is the female stage. The stigma may remain receptive for 15 to 20 days (Arnaud, 1986; Bonjean, 1993).

Fertilisation

The sunflower tends to be allogamous, using a complex system of sporophytic self-sterility. Nevertheless, the degree of self-incompatibility of the pollen varies widely and self-fertilisation remains a possibility (Bonjean, 1993), especially in the cultivated material.

The sunflower's pollen grains are relatively large (25 µm to 35 µm). Each pollen grain comprises an outer coating (the exine) covered in sharp spines and a viscous wax. Due to these morphological characteristics, the pollen tends to form caked masses. As a consequence, it is usually carried by insects (Parker, 1981; Freund and Furgala, 1982; Bonjean and Pham-Delegue, 1986). An inventory carried out in France of pollinating fauna in a number of production areas has demonstrated that honey bees and bumble bees are the principal agents of pollination in the sunflower (Lecomte, 1962; Rollier, 1977; Madeuf and Leclercq, 1982). In other countries where sunflowers are cultivated, such as the United States or the Ukraine, surveys of pollinating insects reveal that domesticated bees are the principal pollinators (Bonjean, 1993).

It is unusual for the pollen to be carried on the wind; less than 0.2% of fertilisation occurs by wind pollination at a distance of less than one metre from the pollen source (Madeuf and Leclercq, 1982).

Fructification

Sunflower seeds are achenes (or fruits) that consist of a kernel (true seed) and a pericarp (hull). The kernel consists of an embryo, endosperm, and seed coat. The pericarp (maternal tissue) consists of several layers: cuticle (external layer), epidermis, hypodermis, phytomelanin layer, fibrose tissue, and parenchymal layers adjacent to the kernel (Nassonov, 1940, quoted by Vranceanu, 1977). Pericarp colour is determined by the pigmentation of the epidermis, hypodermis, and phytomelanin layers (Putt, 1940; Mosjidis, 1982). The epidermis can be uniformly unpigmented or have black or dark brown stripes of varying width. The hypodermis is below the epidermis and can also be either completely unpigmented or pigmented (white or purple). The third layer (phytomelanin), if present, has uniform dark brown to black pigments (Putt, 1944).

The achenes located at the periphery of the capitulum are usually larger than those in the centre. While most florets form a shell, they may remain empty. This is because in many cases those in the central area do not produce seeds. As a consequence, there is a "sterile patch" in the centre of the capitulum the diameter of which depends not only on the genotype, but also on the conditions in which the sunflower is cultivated (Arnaud, 1986; Bonjean, 1993).

Sunflower seeds can be of two types:

- Oilseeds with an oil content greater than 40%, and 35% to 38% protein, usually black in colour.
- Edible seeds, which have a lower oil content (approximately 30%) and an outer shell that is usually dark brown or white.

3. The Physiological and Agronomical Characteristics of the Cultivated Sunflower

A. The sunflower growth cycle

The complete growth cycle of the sunflower lasts between 100 and more than 170 days according to the variety and the growing conditions. Given this, and assuming zero growth below 6°C, the required accumulated temperature varies from 1500 °C to over 1700 °C. Flowering usually begins between 65 and 70 days after the emergence of the first shoots, which will occur once the accumulated temperature reaches 850°C, assuming a minimum growing temperature of 6°C (Merrien, 1986). Variations are found according to variety and location of cultivation, which suggests that there is a high degree of interaction with levels of illumination, to which the sunflower is very sensitive.

Sunflower is usually sown in the beginning of spring (February to May in the northern hemisphere) and harvested in late summer. The harvesting period extends from late August to September in the northern hemisphere, varying according to the region concerned.

The growth cycle can be divided into five phases (Rollier, 1972):

- The phase between sowing and the emergence of the first shoots

  This phase lasts between 7 and 20 days. For emergence the temperature must be at least 4°C, the optimum level being around 8°C. This phase is important since it will determine the size of the plot's plant population.

- The phase between the emergence of the first shoots and growth of 4/5 pairs of leaves

This is the phase in which the root system is put in place and it is particularly sensitive to problems of soil structure due to errors in preparing for cultivation. While the rate at which dry matter is accumulated in the part of the plant above ground is high (10 kg per hectare per day), the dry matter in the roots represents approximately 15% of total dry matter in the plant. This is also the stage at which the initial formation of the leaves, and especially the flowers, begins. A lack of water at this point can limit leaf formation, but flower formation will be especially affected by low temperatures, which may lead to malformation of the capitulum.

- The phase between growth of the first five leaf pairs and the beginning of flowering

  This is the most active growth phase for the crop, a phase during which the rate of formation of dry matter may be as high as 200 kg per hectare per day. The most spectacular increase is in leaf surface area, which is at a maximum during this period, as is also the case for the root system. This phase lasts between 40 and 50 days, and it is also the period of maximum intake of minerals such as nitrogen and boron.

- Flowering

  The length of this phase varies slightly according to variety: 15 to 21 days for the plot as a whole, or 10 days for the individual plant. This is a period of limited growth during which the capitulum becomes the main sink for plant assimilates. During this phase, the sunflower is highly sensitive to low levels of moisture and infection of the capitulum by *Sclerotinia* spores.

- The seed-building phase

  Levels of accumulated dry matter increase only very little in this phase, largely because during this period assimilates are reallocated and plant food reserves migrate. This is also the phase typified by active formation of fatty acids and new proteins from the amino acids derived from the breakdown of leaf and stem proteins. The total quantity of dry matter produced varies in the range of 10 to 15 metric tons per hectare (approximately 30 metric hundredweights per hectare). It may be as high as 20 tons per hectare for late hybrids grown in very good conditions. The point of physiological maturity is reached once the seeds have a moisture content of approximately 28%.

Nitrogen compounds, carbohydrates and fat contents vary during the sunflower growth cycle (Table 1.19).

Table 1.19 Qualitative breakdown of plant dry matter (%) over the sunflower growth cycle.

	Growth Stage								
	VE1 – Vegetative Emergence			F1 – Beginning of Flowering			M3 – Maturity		
	Nitrogen Compounds	Carbohydrates	Fats	Nitrogen Compounds	Carbohydrates	Fats	Nitrogen Compounds	Carbohydrates	Fats
Stem	15	15	-	13	37	-	6	25	-
Leaves	38	32	-	16	8	-	2	2	-
Capitulum	-	-	-	7	9	-	4	8	-
Seeds	-	-	-	-	-	-	11	7	35

Source : Merrien, 1986

The marketing norms for sunflower seeds require 9% moisture content and 2% impurities; at these levels, oil content marketing norm is 44%.

B. The functioning of the plant and vegetation cover

The sunflower is characterised by a very high potential for photosynthesis compared with maize and soybean (Table 1.20). This performance can be explained by a number of factors:

- The numerous stomata distributed over the two leaf faces (surface and reverse side).
- Low resistance to diffusion of CO_2 from ambient air toward active photosynthesis sites.
- The very high level of RuBisCO activity, its high percentage in young leaves, and the greater accessibility of CO_2 to this enzyme in sunflowers compared with other crop plants.

Table 1.20 Characteristics of the photosynthesis of selected crop plants

Plant species	Carbon fixation mode	Enzymes involved	Average level of photosynthesis
Helianthus annuus (Sunflower)	C3	RuBisCO[1] + photorespiration losses	40 mg – 50 mg CO_2/h/dm^2
Zea mays (Maize)	C4	RuBisCO + PEPC[2]+PPDK[3] +NADP-ME[4] (little photorespiration)	40 mg CO_2/h/dm^2
Glycine max (Soybean)	C3	RuBisCO + photorespiration losses	20 mg CO_2/h/dm^2

[1] RuBisCO: ribulose-1,5-bisphosphate carboxylase/oxygenase; [2] PEPC: Phosphoenolpyruvate carboxylase; [3] PPDK : Pyruvate orthophosphate dikinase; [4] NADP-ME: NADP-Malic enzyme

Source: Merrien, 1986

The level of photosynthetic activity declines rapidly over time, largely as a result of shade and self-shading due to mutual coverage of vegetation. This decline is accelerated by dry conditions. In adult sunflowers, the best performance is found in the 15-20 leaf rank, which is the largest and therefore captures more illumination.

In the absence of limitations on the water regime, a sunflower will consume a great deal of water. It is capable of extracting large quantities from the soil if its root system is optimal.

Average daily water consumption may be up to 6 mm per day, with extreme daily values of 10 mm and above. This can be explained by the plant's high level of transpiration, at least double that of most species, which in turn is linked to the permeability of its leaves and the plant's overall high conductance.

When water is available in abundance, the sunflower tends to waste it. Conversely, in dry conditions, it is typically capable of regulating its consumption, improving the efficiency of water use. Thus the initial effect of limited water supplies will be reflected in the gradual closure of the stomata, leading to a reduction in water exchange, whereas photosynthesis will continue for some time.

There are two types of assimilate movement in the plant:

- Translocation, involving movements from the leaves (the location of biosynthetic processes) toward sink regions, where assimilates can accumulate (roots, stems, petioles, young growing leaves, capitulum).
- Tedistribution, involving movements of stored assimilates toward other plant organs (from the stem and leaves to the seeds, for example).

As soon as it is formed, the capitulum is the main sink site for assimilates. The biosynthesis of oil in the seeds occurs late and is essentially linked to the potential for post-flowering assimilation. It is preceded by protein synthesis, which uses amino acids previously held in store in the stems and leaves before being redistributed.

The quantity of protein present in the seed is largely dependent on the total quantity of nitrogen mobilised by the vegetation of the plant. Oil content is essentially related to the carbon fixation potential after flowering.

C. Building sunflower yield

Sunflowers are grown in order to produce oil and seed cake. The plant's yield (in terms of oil or protein) can be broken down into a number of distinct components:

- The number of plants per hectare.
- The number of seeds per plant.
- The 1,000 kernel weight.
- The oil (and protein) content of the seeds.

Agronomic and plant physiology research directed at each of the above components of overall yield calls for a number of comments.

It is possible to modulate the "plants per hectare" parameter. This is because where plant density is high, the sunflower capitulum will be reduced in size; there will be more seeds, but each will be smaller. However, high densities increase lodging risks and facilitate the spread of plant diseases. It should also be borne in mind that it is preferable to ensure that the population is spread evenly over the plot, since sunflowers make poor use of free space.

The number of seeds per plant depends on the vigorousness of the plant concerned in its growth phase, total leaf area prior to flowering, and how long the foliage lasts after flowering. Capitulum vascularisation, a limiting factor in the central area, determines a quantitative and qualitative gradient for achene nutrition from the periphery toward the centre (see Table 1.21). A large-diameter capitulum can be seen to be an unsuitable objective in agronomic or genetic terms due to the limiting effect of vascularisation. Conversely, the search for varieties with an even distribution of vessels in the central area is a major goal for research and selection.

Table 1.21 Characteristics of the seed in terms of its location on the capitulum

Achene location	Average achene weight	Protein content		Oil content	
	(mg)	(mg / achene)	(% of seed weight)	(mg / achene)	(% of seed weight)
Periphery	56.4	9.8	17.4	25.4	45.0
Median zone	50.5	9.9	19.6	19.7	39.0
Centre	44.8	9.8	21.8	16.0	35.7

Source : Merrien, 1986

The 1,000 kernel weight is largely dependent on how long the foliage lasts after flowering. It will vary with the position of the achenes on the capitulum, since those at the periphery are larger, although also less dense. The 1,000 kernel weight is always negatively correlated with the number of seeds. It compensates

only imperfectly and unpredictably for a reduction in the number of seeds. To conclude, average seed weight varies according to density (large achenes being associated with low density) as well as the variety concerned.

Yield varies widely according to the growing environment. Water is the main cause of such variation. There is no critical period in the cycle as is the case for maize; it is more the case that the sunflower is sensitive to lack of water throughout its growth cycle. This sensitivity is at its peak around the time of flowering. Water-related stress will affect mainly the number of achenes per plant; seed-filling (1,000 kernel weight) is less affected. Lastly, over-rapid senescence of the leaves following flowering will lead to a lower oil content.

Nitrogen plays a very important role in the phase in which the number of achenes is determined (differentiation), this being a major factor in the yield. However, despite the sunflower's high nitrogen requirement, it is usually fairly unresponsive to nitrogen-based fertilisation. Due to a low utilisation coefficient, nitrogen-based nutrition input to the plant generally takes the form of soil nitrogen.

4 Possibilities of Crosses of Cultivated Sunflower with Wild Species

A. Intraspecific crosses

Wild populations of H. annuus

As has been mentioned in Section I, the cultivated sunflower derives from a wild species (*Helianthus annuus*) originating in North America and domesticated in recent times. In the wild form, the plants are branched, producing large multiple heads that flower over long periods of time, bearing seeds that are small and present varying degrees of dormancy allowing them to remain in the soil for several years.

In the United States, such wild populations are present in the sunflower cultivation area and genes may be easily exchanged between wild and domesticated populations through cross-pollination. The frequency of hybridisation is unknown, but the phenomenon is a recurrent one even when the wild species are several kilometres away from the sunflower fields (Faure *et al.*, 2002).

In Europe, several sub-spontaneous populations of wild *H. annuus* were observed, which are now increasing especially in some places in central Italy and Andalusia, Spain (Faure *et al*., 2002). The origin of these invading populations in Europe is under question (for example, through wild seed importation or de-domestication).

Due to the fact that such exchanges are possible, and in order to maintain purity of commercial and basic seeds, fields used for sunflower seed production in the USA are kept at least 800 metres distance from commercial sunflower fields and wild sunflower populations. For basic seed production, this distance, which was set initially at 3 km, has been increased to 6.4 km from commercial sunflower fields, 3.2 km from seed production locations and 4.8 km from oilseed crops. In Europe, the production of sunflower seeds requires at least 500 metres' separation from all other commercial sunflower crops, and 3 km to 5 km in the case of basic seed production (Faure *et al.*, 2002).

Wild populations of *H. annuus* are also present in Mexico, Canada, Australia and Argentina.

From extensive intraspecific crossing experiments, Heiser (1954) indicates that the *H. annuus* species are cytologically uniform and that intraspecific crossability level is high. In natural conditions, Rieseberg *et al.* (1998) showed that hybrids between cultivated and wild *H. annuus* occurred frequently. As much as 42% of progenies from wild plants near cultivars were hybrids, and cultivar genes have been shown to

persist in wild populations for several generations. The conclusion was that introgression of cultivar loci is widespread in the sympatric wild *H. annuus* populations (Linder *et al.*, 1998).

Volunteer populations

Sunflower seeds may stay in the soil after harvesting and germinate several years later, thus creating quasi-self-sown or volunteer populations along the edges of fields and within later crops in the rotation (fallow, peas, soybean, maize, sunflower).

Unlike the wild populations of *H. annuus*, which have been thoroughly studied in the United States, little information, either in agronomic or genetic terms, is available on this topic in Europe. However, European wild populations of sunflower derive from North America seed import. Therefore, the knowledge of sunflower wild population biology and of management practices accumulated in the United States provides valuable information for Europe as well.

There have been no indications that such self-sown growth is problematic or adventive in relation to crops either in the United States or in Europe. It is usually eliminated over the two years following the harvesting of the sunflower crop (Snow, 1999).

B. Related *Helianthus* species and interspecific hybridisation

In Europe, three wild annual species related to sunflower have been observed (*H. bolanderi*, *H. argophyllus* and *H. debilis*). They tend to be found more in private gardens than in sunflower growing areas.

Considering the annual wild species of the section Helianthus (H. argophyllus, H. petiolaris, H. debilis, H. praecox, H. bolanderi, H. niveus, H. neglectus, H. paradoxus; H. anomalus, H. deserticola), interspecific hybrids may be obtained (more or less easily) in crossings with the cultivated sunflower H. annuus, with (or without) embryo rescue techniques (Whelan, 1978; Christov, 1996). In such interspecific hybrids, semi-sterility is a common trait due to strong genetic barriers: chromosomal translocations, inversions, etc. (Whelan, 1978; Heiser *et al*., 1969). Viable hybrids and fertile interspecific progenies are generally produced. In natural conditions, hybrid zones are frequently observed in the United States, and various experimental procedures have revealed such gene flows between H. annuus and other wild species H. argophyllus, H. bolanderi, H. debilis and H. petiolaris (Rieseberg *et al*., 1998).

Among the perennial species related to the sunflower which are present at a significant level in Europe, there are two hexaploid forms belonging to the *Atrorubens* section, one in the *Corona-solis* series *(H. tuberosus)* and one in the *Atrorubentes* series *(H. rigidus).*

H. tuberosus (Jerusalem artichoke) is a species still grown for its tubers and is used in animal feed. It is found in many places in France, Montenegro and Yugoslavia, as well as in central and eastern Europe.

Today, there are numerous self-sown populations of Jerusalem artichoke in a range of geographical regions but there is little information on their distribution or frequency.

H. rigidus is also a decorative perennial form frequently found in private gardens.

In Europe, the common forms of *H. tuberosus* and *H. rigidus* flower in September and in theory there is no overlap with the flowering stage of large-scale sunflower crops, at least where these are sown in spring. However, in botanical collections wild ecotypes exist that flower early, similarly to cultivated sunflowers.

Artificial F1 hybrids between the cultivated sunflower *H. annuus* L. and many perennial species of the *Atrorubens* section may be obtained, but they are difficult to perform due to the strong genomic and chromosomal divergencies. Successful hybridisation results are reported, with variable sterility levels of their F1 hybrids, for the following perennial species: *H. angustifolius, H. californicus, H. decapetalus, H. divaricatus, H. eggertii, H. floridanus, H. giganteus, H. glaucophyllus, H. grosseserratus, H. hirsutus, H. laevigatus, H. maximiliani, H. microcephalus, H. mollis, H. nuttallii, H. occidentalis, H. resinosus, H. rigidus, H. salicifolius, H. smithii, H. strumosus, H. tuberosus* (Whelan, 1978; Christov, 1996; Gravilova *et al.*, 2000).

In artificial conditions (isolation cages with bees), the hybridisation level of cultivated sunflower with *H. tuberosus* is low and F1 seed set varies in the range of 2-5%, according to accessions (H. Serieys, Pers. comm.).

Partial hybridisation between perennials and sunflower was observed under artificial crossing conditions (Faure *et al.*, 2002). The phenotype and genotype of F1 hybrids was very close to the female parent. This phenomenon, if observed in natural conditions, could be an opportunity for gene-flow from cultivated sunflower to the wild perennial forms.

In natural conditions, interspecific crosses within *Atrorubens* section species frequently occurred (Heiser *et al*., 1969), but little information is available on the natural crossings between sunflower and perennial species. Crosses of sunflower with the species of the *Ciliares* section appeared rather uncommon in natural conditions and the rare hybrids obtained via embryo rescue techniques exhibited strong sterility.

5 Potential Interactions with Other Organisms

A. Sunflower insects

Several insect species attack sunflower (*Helianthus annuus* L.) worldwide. In North America, a large pest complex has evolved on wild sunflower and has moved from wild ancestors to commercial cultivars. In other countries and to a lesser extent in North America, some insects have adapted to utilise sunflower as an alternative host. Many of these insects develop or increase in number on earlier-planted crops and then after senescence move to sunflower.

The table in Appendix 1 is intended as an identification guide for categories of insects which interact with the cultivated and stored *H. annuus*. This table has been established from the article of Charlet *et al.* (1997) and summarises the present state of knowledge on this subject. It is representative of every continent, but should be probably completed by each environmental safety assessor.

B. Sunflower diseases

The distribution of sunflower pathogens around the world has followed the introduction of sunflower into each continent. So, most pathogens of sunflower can be found in every country today. However, with differences in climate and cultural practices among countries, the prevalence and the incidence of specific diseases will vary in each country.

The sunflower diseases related to the causal organisms are presented in Appendix 2.

C. Other sunflower consumers

Rabbits and hares

Some damage can be caused by rabbits and hares at the early sunflower stages, particularly when the fields are planted near woods or set-aside lands. They damage sunflower by cutting the stem of plant from 2 to 5 mm above the soil surface or by eating young leaves. Important losses have already been observed.

Birds

Bird damage is a problem in every sunflower-growing region of the world. It occurs from early maturation to harvest but seems greatest within 18 days after anthesis. Small sparrows (*Passeridae*) to large species such as crows (*Corvidae*) and parrots (*Psittacidae*) eat sunflower achenes (easily obtained) or seeds (Linz and Hanzel, 1997). The losses can be economically severe.

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APPENDIX I

Insect Pests in Sunflower

Insect pests	CONTINENT OR REGION IN WHICH THEY OCCUR						
	USA/CANADA /MEXICO	**SOUTH AMERICA**	**AFRICA**	**WESTERN EUROPE**	**CENTRAL & EASTERN EUROPE**	**ASIA**	**AUSTRALIA**
Cutworms [Lepidoptera : Noctuidae]							
Dark-sided cutworm *Euxoa messoria* (Harris),	X				X		
Euxoa temera (Hübner)					X		
Red-backed cutworm *E. ochrogaster* (Guenee)	X						
Dingy cutworm *Feltia ducens* (Walker)	X						
Sunflower Bud Moth,	X						
Suleima helianthana (Riley)	(Mexico to the						
[Lepidoptera : Tortricidae]	central USA)						
Sunflower Stem Weevil,							
Cylindrocopturus adspersus (LeConte)	X						
[Coleoptera : Curculionidae]							
Black Sunflower Stem Weevil,	X						
Apion occidentale Fall	(North Dakota,						
[Coleoptera: Curculionidae]	Minnesota & Texas)						
Sunflower Root Weevil,	X						
Boris strenua (LeConte)	(Illinois to						
[Coleoptera : Curculionidae]	California & Montana to Guatemala)						
Sunflower Maggot,	X						
Strauzia longipennis (Wiedemann)	(USA, Canada)						
[Diptera : Tephritidae]							
Long-horned Sunflower Stem Girdler,	X						
Dectes texanus LeConte	(North & South						
[Coleoptera: Cerambycidae]	Dakota, Florida)						
Ligyrus gibbosus (DeGreer)	X	X					
[Coleoptera: Scarabaeidae]	(USA, southern Canada, & northern Mexico)						
Ligyrus spp. (Scarabaeidae).		X					

Insect pests	CONTINENT OR REGION IN WHICH THEY OCCUR						
	USA/CANADA /MEXICO	**SOUTH AMERICA**	**AFRICA**	**WESTERN EUROPE**	**CENTRAL & EASTERN EUROPE**	**ASIA**	**AUSTRALIA**
Grasshoppers [Orthoptera: Acrididae] **The most important :** *Melanoplus differentialis* (Thomas) ; Migratory grasshopper, *M. sanguinipes* (Fabricius) ; Twostriped grasshopper, *M. bivittatus* (Say) ; Redlegged grasshopper, M *femurrubrum* (DeGeer) Clearwinged grasshopper, *Camnula pellucida* (Scudder) *Dichroplus platensis* (Burner) ; D. *conspersus* (Burner).	 X X X X X	 X X					
Painted Lady or Thistle Caterpillar, *Vanessa cardui* (L.) [Lepidoptera: Nymphalidae]	X						
Sunflower Beetle, *Zygogramma exclamationis* (Fabricius) [Coleoptera: Chrysomelidae]	X						
Sunflower Moth, *Homoeosoma electellum* (Hulst) [Lepidoptera: Pyralidae] *Homoeosoma nebulellum* Denis and Schiffermiiller *Homoeosoma heinrichi (*Pastrana) *Homoeosoma vinciniae* (Pastrana),	X	 X (Argentina) X (Argentina)		X X	 X	 X (Iran)	
Sunflower Midge, *Contarinia schulzi* (Gagne) [Diptera: Cecidomyiidae]	X						
Sunflower Seed Midge *Neolasioptera helianthis* (Felt) [Diptera: Cecidomyiidae]	X						
Red Sunflower Seed Weevil, *Smicronyx fulvus* (LeConte) [Coleoptera: Curculionidae]	X						
Gray Sunflower Seed Weevil, *Smicronyx sordidus* (LeConte) [Coleoptera: Curculionidae]	X						
Banded Sunflower Moth, *Cochylis hospes* (Walsingham) [Lepidoptera: Cochylidae]	X (USA, Canada)						
Sunflower Receptacle Maggot, *Gymnocarena diffusa* (Snow) [Diptera: Tephritidae]	X Great plains from Montana south to Arizona, east to Missouri						

Insect pests	CONTINENT OR REGION IN WHICH THEY OCCUR						
	USA/CANADA /MEXICO	**SOUTH AMERICA**	**AFRICA**	**WESTERN EUROPE**	**CENTRAL & EASTERN EUROPE**	**ASIA**	**AUSTRALIA**
Sunflower Seed Maggot, *Neotephritis finalis* (Loew) [Diptera : Tephritidae]	X (Southern Canada to northern Mexico; throughout continental North America)						
Sunflower Headclipping Weevil, *Haplorhynchites aeneus* (Boheman) [Coleoptera: Curculionidae]	X (USA, Canada)						
Nymphalid butterfly, *Chlosyne lacinia saundersii*		X (Brazil)					
Noctuid, *Rachiplusia nu* (Guenee)		X (Argentina)					
The black cutworm, *Agrotis ipsilon* (Hufnagel) *Agrotis segetum* (Denis, Schiff.) [Noctuidae] *Agrotis* spp. [Lepidoptera: Noctuidae] brown cutworm, *Agrotis munda* (Walker); bogong moth *A. infusa* (Boisduval); variable cutworm, *A. prophyricollis(* Guenee). Pale western cutworm *Agrotis orthogonia* (Morrison)	X	X (Brazil)	X X	X		X (Iran)	X X X X X
The chrysomelid, *Diabrotica speciosa* (Germar)		X					
The scarab beetle, *Cyclocephala melanocephala* (Fabricius)		X (Brazil)					
Aphids [Homoptera: Aphididae], *Bemisia* sp. ; **The Brazilian leafhopper,** *{Protalebrella brasiliensis* (Baker) ; *Empoasca* sp. (Cicadellidae) The leafhoppers, *Empoasca pteridis* Dhlb. ; *Empoasca devastans (Disi.)* *Liriomyza* sp. (Agromyzidae) ; *Leptocorisa tipuloides* (DeGreer) (Coreidae) ; **The noctuids,** *Pseudoplusia includens* (Walker), **The velvetbean caterpillar,** *Anticarsia gemmatalis* (Hubner), **The fall armyworm,** *Spodoptera frugiperda* (Smith) *Spodoptera exigua* (Hubner)		X X X X X X X X		X	X	X	X

Insect pests	CONTINENT OR REGION IN WHICH THEY OCCUR						
	USA/CANADA /MEXICO	**SOUTH AMERICA**	**AFRICA**	**WESTERN EUROPE**	**CENTRAL & EASTERN EUROPE**	**ASIA**	**AUSTRALIA**
Spodoptera litura (Fabricius)			X (Egypt)		X		
Caterpillar [Nymphalidae], *Actinote pellena pellena* (Hubner)		X (Argentina, Venezuela, Brazil, Paraguay,Uruguay Peru)					
The yellow woolly-bear, *Spilosoma virginica* (Fabricius) *Spilosoma casignetum* (Kollar) *Spilosoma obliqua* (Walker)		X (Argentina)				 X X	
The spotted maïze beetle, *Astylus atromaculatus* Blanchard (Melyridae)		X (Argentina)					
Athaumastus haematicus (Stal) [Coreidae] ; *Edessa meditabunda* (Fabricius) [Pentatomidae]		X X (Argentina)					
Nezara viridula (L.) [Pentatomidae] *Gargaphia torresi* (C.L.) [Tingidae]		X X	X (Egypt)				X
the black cutworm ; **the variegated cutworm,** *Peridroma saucia* (Hubner) (Noctuidae); *Melanagromyza cunctanoides* (Blanchard) [Agromyzidae] ; *Hylemyia* spp. [Anthomyiidae] ; *Disonychodes exclamationis* (Boheman) (Chrysomelidae) ; *Conoderus* spp. (Elateridae) ; *Epicauta leopardina* (Haag-R.) (Meloidae) *Acromyrmex* spp. (Formicidae) ; *Acromyrmex heyeri* (Forel) *Diloboderus abderus* (Sturm.) *Discynetus gagates* (Burmeister)		X X X X X X X X X X X (Uruguay) X X					
A. lundi (Guerin) *B. striatus* (Roger)		X (Uruguay) X					
The Scarabs of the genera, *Phyllophaga, , Dyscinetus Phileurus*		X					
Larvae *of Hylemyia cilicrura* (Rondani) [Anthomyiidae]		X					
Myzus persicae (Sulzer)		X (Uruguay)				X	
Mallocephala deserticola (Bergman) [Noctuidae]		X					
Schizonycha sp. [Scarabaeidae]			X (Nigeria)				
*Gonocephalum simplex (*Fabricius) [Tenebrionidae]			X				

Insect pests	CONTINENT OR REGION IN WHICH THEY OCCUR						
	USA/CANADA /MEXICO	**SOUTH AMERICA**	**AFRICA**	**WESTERN EUROPE**	**CENTRAL & EASTERN EUROPE**	**ASIA**	**AUSTRALIA**
The noctuid, *Plusia orichalcea* (Fabricius) *Plusia* spp. (Noctuidae)			X			X X	
Piercing-sucking Hemiptera			X (Nigeria)				
Macrosteles sp. [Cicadellidae]			X				
Dacus cucurbitae Coquillett [Tephritidae]			X				
The noctuid moth, [Lepidoptera: Noctuidae] *Helicoverpa armigera* (Hubner) *Helicoverpa* spp. *Helicoverpa punctigera* (Wallengren)			X		X	X	X X X
Callidea dregei (Germar) C. *bohemani* (Stal)			X X				
Nysius stall (Evans) [Lygaeidae].			X				
Lygus spp. (Miridae) *Lygus pratensis* L. (Hemiptera: Miridae) *Lygus rugulipennis* (Poppius) [Miridae] *Lygus Gemelatus* (HS)				X X X	 X X		
The aphids, *Brachycaudus helichrysi* (Kaltenbach)				X (Germany)	X (Yugoslavia, Hungary Romania, Bulgaria, former USSR)		
*Aphrodes bicinctus (*Schrank) [Homoptera : Cicadellidae]				X			
Aphids [Homoptera: Aphididae] *Aphis fabae* Scop. *Aphis gossypii* (Glover *Aphis helianthi* (Monell) *Masonaphis masoni* (Knowlton) *Dolycoris baccarum* (L.) (Hemiptera Pentatomidae) ; *Ostrinia nubilalis* (Hilbner) ; *Phlyctaenodes sticticollis* (L.) (Lepidoptera : Pyralidae) ; *Phytomyza geniculata* Macq. (Diptera : Agromyzidae).	 X X			 X X X X X	 X	 X	
Acanthiophilus helianthi (Rossi) [Diptera : Tephritidae]				X (Italy)			
Eurydema ventrale Kolenati [Hemiptera : Pentatomidae]				X (Italy)			
Opatum sabulosum L. (Coleoptera: Tenebrionidae)				X (France)			
Eupteryx atropunctata (Goeze) (Cicidellidae) ; The leafminer, *Phytomyza horticola* (Goureau)				X X			

Insect pests	CONTINENT OR REGION IN WHICH THEY OCCUR						
	USA/CANADA /MEXICO	**SOUTH AMERICA**	**AFRICA**	**WESTERN EUROPE**	**CENTRAL & EASTERN EUROPE**	**ASIA**	**AUSTRALIA**
Acheta deserta Pall. [Orthoptera: Gryllidae]					X (Yugoslavia Hungary Romania and the former USSR)		
Lethrus apterus (Laxm.) [Coleoptera: Scarabaeidae]					X (Bulgaria, former USSR)		
The Wireworms [Coleoptera: Elateridae] *Agriotes* (especially *A. ustulatus* Schall. *A. sputator* L., *A. gurgistanus* Fald, *A. ponticus* Stepanov, *A. lineatus* L., *A. obscurus* L.). *Selatosomus* and *Melanotus* (including *M. fusciceps* Gryll.) *Athus haemorrhoidalis (*Fabricius)					X X X X X X		
Psalidium maxillosum (Fabricius) [Coleoptera: Curculionidae]					X (Yugoslavia, Hungary Bulgaria, former USSR)		
Tanymecus dilaticollis Gyllenhal [Coleoptera: Curculionidae]					X (Yugoslavia, Hungary Bulgaria, former USSR)		
Miscellaneous Coleoptera [Cerambycidae, Mordellidae] *Agapanthia dahli* (Richt)					X		
Beet Webworm, *Loxostege sticticalis* (L.) [Lepidoptera: Pyralidae]					X (Yugoslavia, Hungary Romania, Bulgaria, former, USSR)		
Mamestra brassicae L. [Lepidoptera: Noctuidae]					X		
Adelphocoris lineolatus (Goeze),	X (North America)						
Miscellaneous Plant Bugs [Pentatomidae, Lygaeidae, Coreidae]					X		
Tingidae (Hemiptera), *Galeatus helianthi* (Onder and Lodos) *Galeatus scrophicus* (Saunders)						X (Turkey) X	
*S. littoralis (*Boisduval) (defoliation),						X (Iran)	
Strawberry spider mite, *Tetranychus turkestani* (Ugarov and Nikolski)						X (Iran)	
The western flower thrips, *Frankliniella occidentalis* (Pergande) **Thrips [Thysanoptera],**						X (Israël)	

Insect pests	CONTINENT OR REGION IN WHICH THEY OCCUR						
	USA/CANADA /MEXICO	**SOUTH AMERICA**	**AFRICA**	**WESTERN EUROPE**	**CENTRAL & EASTERN EUROPE**	**ASIA**	**AUSTRALIA**
Thrips tabaci (Linderman), *Frankliniella schultzei* (Trybom), *Desmothrips tenuicornis* (Bagnall), **the plague thrips,** *T. imaginis* (Bagnall).							X X X X
Predators: *Orius spp*.						X	
Ostrinia damoalis Walker (Lepidoptera: Pyralidae)						X	
Rhopalosiphum erysemi (Kaltenbach)						X	
Cirphis unipuncta (Haw.) *Cirphis loreyi* (Dupt.)						X X	
Grasshoppers, *Carpophilus* sp. (Nitidulidae) ; *Disonycha* sp. (Chrysomelidae) ;						X X	
Leafhopper (Homoptera: Cicadellidae), *Amrasca biguttula biguttula* (Ishida)						X	
Phytomyza atricornis (Meigen) (Diptera: Agromyzidae)						X	
Black Scarab Beetles, *Pseudoheteronyx* spp. [Coleoptera: Scarabaeidae]							X
False Wireworms [Coleoptera: Tenebrionidae] **the striate false wireworm,** *Pterohelaeus alternatus* Pascoe, **the eastern false wireworm,** *P. darlingensis* Carter ; **the southern false wireworm,** *Gonocephalum macleayi* (Blackburn) **Another species,** *Celibe* sp. (= *Saragus* sp.)							X (South Australia, New South Wales) X X X
Wingless Cockroaches, *Calolampra* spp. [Orthoptera: Blaberidae] *Calolampra elegans* Roth and Princis and C. *solida* Roth and Princis							X X (Central Highlands of Queensland)
Black Field Earwig, *Nala lividipes* (Dufour) [Dermaptera: Labiduridae]							X
Field Crickets, *Teleogryllus and Lepidogryllus* spp. [Orthoptera: Gryllidae]							X
Black field crickets *Teleogryllus commodus* (Walker) and *T. oceanicus* (Le Guillou), **Brown field crickets,** *Lepidogryllus parvulus* (Walker) and *L. comparatus* (Walker).							X X

Insect pests	CONTINENT OR REGION IN WHICH THEY OCCUR						
	USA/CANADA /MEXICO	**SOUTH AMERICA**	**AFRICA**	**WESTERN EUROPE**	**CENTRAL & EASTERN EUROPE**	**ASIA**	**AUSTRALIA**
Sugarcane Wireworm *Agrypnus variabilis* (Candeze) [Coleoptera: Elateridae]							X
Soybean Looper, *Thysanoplusia orichalcea* (Fabricius) [Lepidoptera: Noctuidae]							X
Greenhouse Whitefly, *Trialeurodes vaporariorum* (Westwood) [Hemiptera: Aleyrodidae]							X
Rutherglen Bug, *Nysius vinitor* (Bergroth) [Hemiptera: Lygaeidae] *N. clevelandensis* (Evans)							X X (Queensland and northern New South Wales)

Insect pests of stored sunflower

the sawtoothed grain beetle *(Oryzaephilus surinamenis* L.) (Coleoptera: Cucujidae),
red flour beetle *(Tribolium castaneum* (Herbst)) (Coleoptera: Tenebrionidae),
Indian meal moth (*Plodia interpunctella* (Hübner)) (Lepidoptera: Pyralidae)

APPENDIX II

The Pathogens of Sunflower (Classification proposed by Gulya *et al*., 1997)

Disease	Causal organism	Country or region in which they occur
Downy mildew	*Plasmopara halstedii* (Farl.) Oomycetes	Every continent with the exception of Australia.
Sunflower rust	*Puccinia helianthi* (Schwein.) *Puccinia xanthii* (Schwein.)	Worldwide. Only in Australia.
Alternaria	*Alternaria helianthi* (Hansf.), syn. *Helminthosporium helianthi* Hansf.) *Alternaria zinnia* (Pape) *A. helianthinficiens* (Simmons) *A. helianthicola* (Rao & Rajagopalan) *A. protenta* (Simmons) *A. tenuis* Nees (Simmons)	Worldwide. Worldwide. North Dakota, Manitoba, Hungary; Yugoslavia. India, Yugoslavia. Uganda, Rhodesia. India, Iran.
Septoria leaf spot Septoria leaf speck	*Septoria helianthi* (Ell & Kell) *Septoria helianthina* (Petrov & arsinijevic)	Worldwide except South America. Yugoslavia.
Bacterial foliar diseases	*Pseudomonas tagetis,* reclassified as *Pseudomonas syringae (Ps)* pv. *tagetis* (Hellmers) Young, Dye, Wilkie ; *Pseudomonas syringae* pv. *Helianthi* (Kawanua) Young, Dye, Wilkie. *Pseudomonas cichorii* (Swingle) *Xanthomonas campestris* pv. *phaseoli*	Every continent. Japan, Canada, Mexico, USA, Europe, Africa, India, New Zealand. Subtropical climates, Brazil. India and Russia.
Powdery mildews	*Erysiphe cichoracearum* DC. ex. Meret	All continents.
	Sphaerotheca fuliginea (Schlecht. ex Fr.) Poll *Leveillula tarucia* (Lev.) Arn.	Africa, Asia, Europe, and South America. China, India, the former Soviet republics, and the Middle East.
White Rust	*Albugo tragopogonis* (DC.) S. F. Gray [Syn.= *Albugo tragopogi* (Pers) Schroet]	Every continent
Virus diseases		
Aster Yellows (rarely observed)	Mycroplamas	USA, Canada, Argentina.
	Other sunflowers diseases with mycoplasmas	France, Israël, India, Sudan.
Cucumber Mosaic	Cucumber Mosaic Virus (CMV)	China, India Once from a nursery in Maryland.
Sunflower Mosaic	Sunflower Mosaic Virus (Potyvirus) (SMV)	Argentina, Texas, Czech Republic.
Sunflower Ringspot	Sunflower Ringspot Virus (Ilarvirus) (SRV)	Queensland, Australia.
Sunflower Yellow Blotch and Leaf Crinkle	Luteovirus	African countries and England.
Tobacco Ringspot	Tobacco Ringspot Virus (Nepovirus) (TRV)	Rio Grande Valley of Texas (on wild *H. annuus*).
Tobacco Streak	Tobacco Streak Virus (Lilarvirus) (TSV)	On garden sunflower in the Netherlands.
Tomato Spotted Wilt	Tomato Spotted Wilt Virus (Tospovirus) (TSWV)	Ukraine.
Minor Foliar Diseases (leaf spots)	*Ascochyta compositarum* (J.J. Davis) – Coelomycetes	USA, Kenya, Japan, Russia.

	Cercospora helianthi (Ell &Ever) – Hyphomycetes ; Cercospora helianthicola (Chupp &Viegas) *Cercospora pachyrus* (Ell & Kellerman)	USA, Brazil, Russia.
	Colletotrichum helianthi J.J. Davis - Coelomycete	-
	Entyloma compositarum Farl - Tilletiaceae	Montana.
	Epicoccum neglectum Desm. - Hyphomycetes	Yugoslavia, Romania.
.	*Itersonilia perplexans* Derx - Basidiomycete	Canada, Uruguay.
	Myrothecium roridum Tode:Fr. (Alb. & Schw.) – Hyphomycetes *M. verrucaria* Ditmar:Fr – Hyphomycetes	Pakistan. Argentina.
	Phialophora asteris (Dowson) Burge & Isaac f. sp. *Helianti* Tirilly & Moreau – (Soilborne fungus)	Canada, Italy.
	Phyllosticta Wisconsinensis H.C. Green - Coelomycete	-
	Sordaria fimicola (Rob. Ex Desm.) Ces & Not. - Ascomycete	Yugoslavia, USA.
Miscellaneous Foliar Pathogens		
	Species of : *Botryodiplodia, Cladosporium, Cornyespora, Curvularia, Helminthoporium, Mycosphaerella,Pyrenophora, Sphaceloma*	Tropical climates.
Sclerotinia Wilt	*Sclerotinia sclerotiorum* (Lib.) de Bary	Worldwide.
	Sclerotinia minor Jagger	Australia, Argentina, Uruguay, Chile, California.
Phomopsis Stem Canker	*Phomopsis helianthi* Munt.-Cvet *et al*.	Worldwide
Phoma Black Stem	*Phoma macdonaldii* (Boerema)	Northern Great Plains of USA, California, Kansas. Countries of Africa, Asia (with the exception of China), Argentina, Europe.
Verticillium Wilt/Leaf Mottle	*Verticillium dahliae (*Klebahnis)	Europe, Argentina, Mexico, former USSR, England, north-central plain in the USA, Canada.
Charcoal Rot	*Macrophomina phaseolina* (Tassi) Goid Synonyms *Sclerotium bataticola* Tabu and *Rhizoctonia bataticola* (Taub.) Briton Jones.	Most around the world but more prevalent in Egypt, India and Pakistan.
Southern blight or collar rot	*Sclerotium rolfsii* Sacc. (syn. *Corticum rolfsii* Curzi*)* - Basidiomycete	Tropical and subtropical climates.
Minor root and stalk pathogens :		
Stem rot Root rots ans seedling damping off Texas root rot Wilt	*Phytophthora cryptogea* Pethyb. & Kaff. Several species of *Pythium* including *P. aphanidermatum* (Edson) Fitzp., *P. debaryanum* Auct. Non-Hesse, *P. irregulare* Buisman, *P. rostratum* Butler *P. splendens* Braun *Phymatotrichum omnivorum* *Fusarium moniliforme* (sheld) *Fusarium oxysporum* (Schlect) *Fusarium tabacinum* (Beyma)	California, Iran. USA, Iran. Texas. India, North America. India, North America. Italy.
Sclerotinia Head rot	*Sclerotinia sclerotiorum*	Argentina, several European countries, Japan, North America.

Rhizopus head rot	*Rhizopus* (at least three species)	Australia, India, South Africa, USA, Canada, the Mediterranean areas of Europe, Egypt and Russia
Botrytis head rot	*Botrytis cinerea* Pers.	All European countries, Egypt, Turkey, India, Pakistan, Russia, Canada, USA
Bacterial head rot	*Erwinia carotovora* Jones, Holland	USA, Mexico, several European countries, several central African countries, Russia
Nematodes	*Meloidogyne* spp. (Volvas & Sassanelli)	California, Florida, Tennessee, Texas, India, South Africa, Italy, Egypt, Serbia.
	Meloidogyne incognita	Brazil, Egypt, India, Italy, Serbia, South Africa, Zambia.
	Meloidogyne javanica	Brazil, Egypt, India, Italy, Serbia, South Africa, Zambia.
	Rotylenchulus (Robinson & Orr)	India.
	Tylenchorhynchus (stunt),	California.
	Helicotylenchus (spiral),	California, Florida, Tennessee, Texas.
	Pratylenchus (pin),	California, Florida, Tennessee, Texas.
	Xiphinema (dagger),	California.
	Hoplolaimus (lance),	
	Quinisulcius (stunt)	
	Trichodorus	Florida, Tennessee, Texas.
	Belonolaimus	Florida, Tennessee, Texas.
	Scutellonema, Paratrichodorus, Rotylenchus	South Africa.

SECTION 10
PAPAYA (*CARICA PAPAYA*)

1. Taxonomy and General Description

A. Taxonomy

Papaya, *Carica papaya* L., is an almost herbaceous (succulently soft-wooded), typically unbranched small tree in the family Caricaceae. Europeans first encountered papaya in the Western Hemisphere tropics by at least the early 1500s (Sauer, 1966), and various interests were soon disseminating it widely (Ferrão, 1992). Papaya is now cultivated worldwide in tropical and subtropical climates mainly for its melon-like fruit.

The Caricaceae is classified in the order Brassicales (sometimes called Capparales), which characteristically express mustard-oil glucosides (glucosinolates) (Jørgensen, 1995; Rodman *et al*., 1998; Olson, 2002). Recently, consensus has been developing that the genus *Carica* L. has only the one species *C. papaya*, and that the Caricaceae may comprise six genera (Aradhya *et al*., 1999; Badillo, 2000; Van Droogenbroeck *et al*., 2002, 2004; Kubitzki, 2003; Manshardt, 2002, Havaii University, pers. com.). Most of the genera are neotropical forest plants, occurring in South America and Mesoamerica or only in Mesoamerica. *Vasconcellea*, the largest genus with 21 species, had usually been considered as a section within *Carica*. The other neotropical genera are *Jacaratia* (7 spp.), *Jarilla* (3 spp.) and *Horovitzia* (1 sp.) (Badillo, 1993). The sixth genus, *Cylicomorpha* (2 spp.), occurs in montane forests in equatorial Africa (Badillo, 1971).

The highland papayas, *Vasconcellea* (not "*Vasconcella*" – see Badillo, 2001; Kubitzki, 2003), are considered the closest relatives to *Carica papaya* (Badillo, 1993; Aradhya *et al*., 1999; Van Droogenbroeck *et al*., *20*02, 2004). *Vasconcellea* has many species with edible fruits (and a few cultivated varieties) (Badillo, 2000; Scheldeman and Van Damme, 2001); commercial cultivation of Caricaceae may be limited to the papaya and chamburo (ababai), babaco, and toronche or higacho (the names vary and sometimes are used locally for more than one species). The chamburo or mountain papaya, *V. cundinamarcensis* (often referred to as *V. pubescens*) is grown in the Americas; the fruits are usually cooked and eaten with sugar. There is commercial-scale cultivation in Chile, where the fruit is known as ababai (Scheldeman and Van Damme 2001). In western South America (particularly Ecuador) local consumers value babaco (often referred to as *V. pentagona*), which is also cultivated somewhat elsewhere, including New Zealand, South Africa, Spain and Italy (Scheldeman and Van Damme, 2001; Villarreal *et al*., 2003). Babaco is generally considered to be an F_1 hybrid (known as *V.* ×*heilbornii* but sometimes still as its var. *pentagona*) (Jiménez *et al*., 1999; Wiersema and León, 1999; Scheldeman and Van Damme, 2001; Morales Astudillo *et al*., 2004). Higacho (or broadly toronche), considered the hybrid *V.* ×*heilbornii* var. *chrysopetala* (sometimes referred to as *V. chrysopetala*) is also found in Ecuador, with a commercial variety also grown in New Zealand (NRC, 1989; Scheldeman and Van Damme, 2001).

The International Plant Genetic Resources Institute (IPGRI) recognises eight different edible fruits from this family. Users from Mexico to South America collect fruits from wild plants or semi-wild plants, or may grow a few (thus incipient domestication). Siglalón silvestre, *V. stipulata*, is a local food in southern Ecuador. Col de monte (*V. monoica*) of Ecuador, Peru and Bolivia has small fruits eaten raw or

cooked. Chungay or mito (*V. candicans*) is a familiar food in Peru (De Feo *et al*., 1999). Papayuelo (*V. goudotiana*) of Colombia is small and apple-like. Other edible *Vasconcellea* include tapaculo (bonete, papayito) or mountain pawpaw, *V. cauliflora*, whose fruit pulp is processed in various ways before consumption (Coppens d'Eeckenbrugge and Libreros Ferla, 2000), and higuera (calasacha), the collected nut *V. quercifolia*. Additionally, Scheldeman and Van Damme (2001) note for their edible fruits *V. crassipetala*, *V. microcarpa* (lechocillo), *V. palandensis* (papaillo), *V. parviflora* (coral) and *V. sphaerocarpa* (higuillo negro). The fruits of *Jacaratia digitata*, *J. mexicana*, *J. spinosa*, and *Jarilla heterophylla* also are eaten locally (Whitmore, 1978; Scheldeman and Van Damme, 2001).

B. Morphology

Carica papaya is a usually unbranched, giant-herb-like tufted tree 2-10 m in height; commercial producers often remove plants if they are reaching a height from which fruits would not be harvested easily. Large, palmately lobed leaves with long stout leaf-stalks (to 125 cm) are attached densely (alternating more or less spirally) near the terminus of the straight trunk, and spreading to form a loose open crown. The leaf-stalks (petioles) end in a leaf blade 20-60 cm (to 75-100 cm) across (Campostrini and Yamanishi, 2001a), with each blade usually 5- or 7-lobed, and each lobe cut pinnately. The trunk tapers from a 10-30 cm wide base to 5-7.5 cm at the crown, and is patterned conspicuously with large leaf-scars; it is thin-barked and often hollow (between nodes) with aging (Elias, 1980). The soft pulpy wood is formed predominantly by phloem, with little secondary xylem (Whitmore, 1978; Carlquist, 1998). This fast-growing plant has *c.* 15-30 mature leaves, with a leaf persisting 2.5-8 months and new leaves arising at the rate of 1.5 to nearly 4 per week (Sippel *et al*., 1989; Allan *et al*., 1997; Mabberley, 1998; Nakasone and Paull, 1998; Fournier *et al*., 2003). Leaf senescence seems to be a function of the leaf's position within the plant's canopy (*i.e.* self-shading) rather than simply increasing age (Ackerly, 1999). All parts of the plant contain a thin, acrid latex, including the unripe fruits. The lifespan of feral trees is some 15-20 years (Anon, 2003). Plants infrequently may develop a forked trunk or a few branches when older or injured; in some places (*e.g.* Kenya) growers may encourage multiple trunks by pinching seedlings or cutting back established plants (Dodson and Gentry, 1978; Rao, 1993; Malo and Campbell, 1994).

The flower-bearing stalks arise in leaf axils. There are three basic flower types in domesticated plants, but with a range of possible variation, resulting overall in about six distinctive kinds (Storey, 1941; 1967; Hsu, 1958a, 1958b; Mosqueda Vázquez and Molina Galán, 1973; Fisher, 1980; Nakasone and Lamoureux, 1982):

- Female flowers (globose-ellipsoid ovary) (sometimes agronomically termed as "type 1").
- Male flowers (of two kinds): morphologically typical (10 stamens, tiny rudimentary pistil) ("type 5"), or functionally male but a somewhat bisexual appearance ("type 4+").
- Bisexual flowers (of three kinds): decandrous (10 stamens, elongate ovary) ("type 4"), pentandrous (5 stamens, deep-furrowed ± ovoid ovary) ("type 2"), or irregular ("type 3") with stamens variably becoming carpel-like (*i.e.* carpellody).

The morphology of inflorescences and flowers varies with the sex of the tree. Varieties typically are either dioecious (with unisexual flowers and exclusively male and female plants), or they are polygamous (with bisexual and unisexual flowers and hermaphrodite and single-sex plants). On female plants, the stalk (peduncle) is just 2.5-6 cm long and has one or a few large bell-shaped flowers with curvy separate petals. On male plants, hanging branching stalks (panicles) 60-100 cm (to over 150 cm) long have many much smaller trumpet-shaped flowers, with the petals (and stamens' filaments) joined in a long narrow tube which has flared lobes (Fisher, 1980; Calif. Rare Fruit Growers, 1997; Nakasone and Paull, 1998; Ronse Decraene and Smets, 1999). On hermaphrodite plants, these structures are intermediate to the unisexual

types, with stalks less than 25 cm long having bisexual flowers that are shortly tubular with a midpoint or lower constriction and the petal lobes larger. Hermaphrodite plants sometimes also bear male flowers (Crop Knowl. Master, 1993). Some plants produce male flowers on short stalks.

The species thus has a richly complex capability in sexual expression and flowering. Some agricultural varieties (*e.g*. Solo and Eksotika) are gynodioecious — their trees are either hermaphrodite or female. Moreover, papaya sexuality can be labile. Bisexual flowers can be influenced by environmental conditions to change to male flowers through reductions in ovary size and function. Male (staminate) plants and andromonoecious plants (with male and hermaphrodite flowers) are phenotypically stable, or mutable. These types may exhibit seasonal sex reversal, developing staminate, bisexual, and pistillate flowers (Storey, 1958, 1976). Young hermaphrodite plants may have male flowers when first flowering, but bisexual flowers with maturity (Stambaugh, 1939). Female (pistillate) plants have stable sex expression; they are not known to develop flowers with masculine structures (Hofmeyr, 1939b; Nakasone and Paull, 1998). For details on conditions that may alter sexual expression and morphology, see Section V.D. Induced alterations to sexual development.

Fruits hang from the stalks attached to the upper trunk, below the old leaves, with the younger fruits above those more mature. Fruit shape is a consequence of selection for the preferences of various local users and markets, but also reflects the flower type; the generally large fruits vary from spherical or ovoid to pear-shaped or elongate, and 10-50 cm in length (Storey, 1969, 1987). Fruit weight can vary substantially (*c.* 0.35-10 kg or even 12 kg) (Font Quer, 1958; Linnell and Arnoult, n.d.), again chiefly dependent on selection by local users and for specific markets. Storey (1969, 1987) reported preference for 2.5-6.0 kg fruits in South America and the South Pacific, 1.25-2.5 kg lobular fruits in South Africa, and just 400-500 g for Solo-type fruits (which were developed in Hawaii). For additional information on varieties and cultivars, see Table 1.22, and Section V.A. Reproductive types and locus of cultivation.

2. Distribution and Centre of Origin

Carica papaya is native in the north-tropical Western Hemisphere. Some have suggested a centre of origin in Central America or the south of Mexico (de Candolle, 1883, 1884, from Singh, 1990; Storey, 1976). Manshardt and Zee (1994) found wild papayas (exclusively dioecious) in the Caribbean coastal lowlands of southern Mexico and northern Honduras. The wild female plants produced golf ball-sized fruits of less than 100 g, which usually were inedible (Manshardt, 1999). The musty bitter fruits (berries) have an extensive investment in seeds, which are about 25% smaller than domesticated papaya seeds and have stronger requirements for breaking dormancy. In experimental testing, wild seeds needed strong light to germinate, but 75% of domesticated seeds germinated in darkness. Also, fluctuating temperatures partially inhibited wild seeds from germinating whereas variable temperature did not affect the domesticated seeds' germination (Paz and Vázquez-Yanes, 1998). On the Caribbean coast of Central America, feral papaya have traits apparently indicating greater introgression from wild papaya compared to the feral papaya on the Pacific coast, which appear to have fewer wild traits (Manshardt and Zee, 1994). Successively greater introgression of domestication traits in the wild plants along with increasing presence of feral domesticated-type plants has been found westward and southward from the known wild papaya region.

The centre of diversity for the relatively large genus *Vasconcellea* (formerly in *Carica*) is South America along the Andes, especially in Ecuador (Badillo, 1993; Morales Astudillo, *et al., 20*04), with outlying species reaching as far as Mexico, Chile, Argentina and Uruguay (Aradhya *et al*., 1999; Van Droogenbroeck *et al*., 2004). This led some to propose South America for the origin for *C. papaya* (Prance, 1984). However, evidence to the contrary is provided by finding only domesticated-type feral *C. papaya* there (Manshardt and Zee, 1994; Morshidi, 1996), but finding unambiguously wild plants in Mexico and Honduras (Moreno, 1980; Manshardt and Zee, 1994; Paz and Vázquez-Yanes, 1998; Manshardt, 1999).

Furthermore, both isozyme and RAPD (random amplified polymorphic DNA) analyses, and RFLP (restriction fragment length polymorphism) analysis of chloroplast and mitochondrial intergenic sequences, show appreciable divergence of *C. papaya* from what is now recognised as the genus *Vasconcellea* (Jobin-Decor *et al*., 1997; Aradhya *et al*., 1999; Kim *et al*., 2002; Van Droogenbroeck *et al*., 2004). This correlates well with the experimental difficulty in forming hybrids of *C. papaya* with other species in Caricaceae (see Section VI.A. Interspecific crosses with *Carica papaya*). Because *C. papaya* is genetically so distinct, and only feral papaya are known in South America, nowadays a South American origin of wild papaya appears hardly tenable.

Papaya was probably domesticated in northern tropical America but a precise region has not been determined (Schroeder, 1958). Feral papayas occur in many tropical habitats of North America, Central America, the Caribbean and South America. In North America, subtropical areas of Mexico and Florida (USA) are the northernmost part of the species' current range; the southern range extends from Colombia and Venezuela to French Guiana, Brazil, Peru, Bolivia and Paraguay. In southern Florida there is evidence indicating pre-Columbian use of papaya by native people (Allen *et al*., 2002). The Spanish and Portuguese encountered cultivated papaya on the Caribbean coasts of Mexico (being used by the Maya) and Panama and Colombia reportedly by at least 1519 and 1526, respectively; cultivated papaya was reported in Jamaica by at least 1756 (Sauer, 1966; Singh, 1990). In the 1500s papaya was transported to the Philippines and India, and it was readily disseminated into tropical Asia, Africa, and Pacific islands (Singh, 1990; Ferrão, 1992). Less widely used names for the fruit, the tree, or both include papaw, paw paw (paw-paw, pawpaw) (but *Asimina* of the Annonaceae is known as pawpaw), papaye, papayer, papayo, lechosa, fruta bomba, melón zapote, mamón, mamonero, mamão and mamoeiro. *Carica papaya* is now well integrated into indigenous culture, agriculture and cuisine in numerous countries, and occurs beyond the cultivated areas as a feral or adventive plant, naturalised to various degrees in the tropics of the New and Old Worlds including Oceania.

3. Use as a Crop, and Agronomic Practices

Extensive non-commercial production of papaya is common, and much of the harvest in some countries is not exported. Instead, growers consume the fruits or trade them locally. For example, Indonesia estimated that it produced 744,000 tons, of which less than 4 tons were exported (Setyobudi and Purnomo, 1999). In Vietnam, 50% of farm households cultivate papaya in home gardens, with 5 to 10 million or more growing 1-10 trees, whereas only 5,000-10,000 farmers produce papaya in monoculture gardens or large fields (Le Tran and Tran, 1999). In the Philippines, as many as 1.5 million farmers derive cash income from the sale of papaya from home garden, monoculture, or multiple-cropping system agriculture (Kositratana *et al*., 1999). Income from a unit of land in papaya cultivation may be two to four times more than the income from rice; papaya's entire value to the small farmer should not be underestimated (The Papaya Biotechnology Network of Southeast Asia-Workshop participants, 1999; ISAAA; *cf.* Cook, 2004).

The commercially reported production of papaya in 52 countries in 2004 reached 6.5 million metric tons (FAO, 2005). The total area harvested was 365, 846 ha. By region, 7 of the production areas are in Middle America (Central America plus North America), 5 in the Caribbean, 10 in South America, 11 in Africa, 4 in the Near East, 10 in Asia plus Australia, and 5 in Oceania. The major producers were especially Brazil (24.6%), Mexico, Nigeria, India and Indonesia, as well as Ethiopia, the Democratic Republic of the Congo, Peru, Venezuela and China.

A. Uses, and adverse effects

Industrial uses

Papaya is primarily a fresh-market fruit, and is used in drinks, jams, pectin, candies and as crystallised fruit. Green fruit may be cooked as a vegetable, as may the leaves, flowers and roots (Duke, 1967; Watson, 1997). Papaya has several well-known industrial uses, notably for the enzyme papain (one of its four major constituent cysteine proteinases) (El Moussaoui *et al*., 2001), which has properties similar to gastric pepsin. Producers induce latex to exude from longitudinal incisions made into unripe fruit; the papain purified from the extract is used in foods, beverages, pharmaceuticals, and other manufacturing (Mabberley, 1998; Wiersema and León, 1999). For example, the food industry uses papain in brewing, manufacturing baby food, and producing proteins for human and animal consumption. Papain is also used to shrinkproof wool and silk, and in the bating process to make leathers more pliable. For some applications however, synthetic enzymes and enzymes from other sources are displacing the use of the natural papain (Watson, 1997; ETA, 2001). The latex from papaya has been used in manufacture of chewing gum (de Wit, 1966). Oil from the fruit's many (200-1000) more or less spheroidal seeds (*c.* 2-5 mm × 3.5-6 mm) (Sharma and Singh, 1975), and other components of fruit and leaves have been used in cosmetics and soap (Quenum, 2001).

Nutritional and medical uses

Papaya constituents contribute to human nutrition and health. Vitamins A and C from one medium papaya (edible portion 350 g) exceed the Dietary Reference Intakes established by the U.S. Food and Nutrition Board (Inst. Medicine, Natl. Acad. Sci.) for adult minimum daily requirements (CRN, 2001; USDA, 2001), and papaya is a good source of the minerals K, Mg and B (Hardisson *et al*., 2001). Papaya has traditional and modern medical and dental uses; fruits, seeds, latex, and extracts have been used for treating at least forty human conditions, and are being investigated for others (*e.g.* Lewis and Elvin-Lewis, 1977; Mezhlumyan *et al*., 2003; Petitto, 2004). The efficacy of some of the uses is well documented (Animal Sci. Dept. Cornell Univ., 2001), including those as an antihelminthic (Satrija *et al*., 1995); an antiamoebic (To and Kyu, 1934), possibly mediated by the alkaloid carpaine (Burdick, 1971); and an enterobacteria antimicrobial (Osato *et al*., 1993). Papain is used in preparation or manufacturing of adjuvants and reagents for antibiotics or vaccines; chymopapain is a biologic used for treatment of herniated disks in the spine (Quenum, 2001; Mezhlumyan *et al*., 2003).

Adverse and other consequences

Hypersensitive or allergic human responses to papaya have been described, including respiratory responses to the pollen (Blanco *et al*., 1998). Consumption of ripe fruit only infrequently produces such adverse consequences (Castillo *et al*., 1996; Iliev and Elsner, 1997). Skin may have such responses to fruit contact (Ezeoke, 1985) or extracts (Banik *et al*., 1992). Contact with latex derived from abraded green fruits and plant parts or extracts that contain papain or other proteinases may harm unprotected skin, but can also be used in healing wounds (Mezhlumyan *et al*., 2003). Tissues of papaya (including leaves and roots) which contain cyanogenic glycosides (Olafsdottir *et al*., 2002; Seigler *et al*., 2002) and tannins may provoke adverse reactions if consumed in quantity.

Papaya enzymes may be injected for medical purposes. However, Moneret-Vautrain *et al*. (1985) have described the allergenic potential of injected chymopapain extracts — up to 1% of the population may have an adverse reaction. Injection may also evoke immune responses to papaya's other known cysteine proteinases, *i.e.* papain, caricain, and glycyl endopeptidase (Dando *et al*., 1995). The reactions to the fruit, pollen, and papain are mediated by an IgE mechanism (Blanco *et al*., 1998; Soto-Mera *et al*., 2000).

Papaya has been investigated for possible effects on pregnancy, since some consider consumption a risk to fetal development, or to cause or alternatively to prevent miscarriage (Eno *et al*., 2000; Adebiyi *et al*., 2002a). In a controlled study of pregnant rats, juice from ripe fruits was considered safe (Adebiyi *et al*., 2002a). No effect of papaya juice was observed on isolated uterine muscle *in vitro*, but crude latex preparations caused spasm (Adebiyi *et al*., 2002a, 2002b). Additional physiological effects have been described from papaya parts not usually consumed, as for example on reproduction in male rodents and monkeys. Mice, rats and Hanuman langur monkeys (*Semnopithecus entellus*) given extracts of papaya seed appear to become infertile reversibly without indication of toxicity (Chinoy *et al*., 1994; Pathak *et al*., 2000; Lohiya *et al*., 2002). A papaya seed extract may offer control of a protozoan parasite that causes a major disease of fish in aquaculture (Ekanem *et al*., 2004).

B. Propagation

Seed

Papaya producers usually grow the crop from seed; agronomic advisers encourage purchasing commercial seed for propagation (Muthukrishnan and Irulappan, 1985). Most commercial seed producers offer inbred selections, but some hybrid lines such as Rainbow and Eksotika II are available. Further discussion on the place of hybrid seed is found below in this section's subsection B.3. Varietal selection, and in Section VII.B.2. Cultivated varieties as sources of genetic variability. A list of frequently encountered varieties in commercial and breeding use is in Table 1.22.

To assure seed quality, growers must take into account the sexual reproductive type and the genetic variability of the variety. Lines heterogeneous for many traits will produce considerable trait diversity in the progeny. Dioecious varieties are open-pollinated and much phenotypic variability can arise in the seeds of on-site fruit-bearing trees. Dioecious lines (described in Section V.A. Reproductive types and locus of cultivation) are more likely to be heterozygous for quality traits; the characteristics of tree and fruit are only maintained exceptionally. To maintain traits for generations, producers strictly control pollination using standard breeding techniques, and careful isolation of multiple lines. Rigorous crossing procedures are preferable (Watson, 1997). A strategy available for maintaining the consistency of some varieties is to obtain seed only from selfed male trees ("ambivalent males"), which produce fruit under limited (such as seasonal) circumstances (Aquilizan, 1987). Traits of superior plants can also be exploited by selection, especially when growers bag and hand-pollinate flowers to produce the seed.

In the hermaphrodite lines self-pollination predominates, but outcrossing is not excluded (see also Section V.C. Pollination). Selection of seeds from only-selfed hermaphrodite plants will provide better trait uniformity (Singh, 1990). Carefully controlled production of hybrid seed is an alternative that may be increasing in importance. Seed producers pollinate selected female trees using pollen from selected hermaphrodite trees.

Seeds, including the outermost layer — a gelatinous sarcotesta (Fisher, 1980), have inhibitors that prevent germination while contained in the fruit or prematurely after release (Yahiro and Hayashi, 1982; Ellis *et al*., 1985; Arumugum and Shanmugavelu, 1975; Tseng, 1992). Seeds freshly harvested from the fruits have very low and variable germination. Removal of the sarcotesta considerably increases germination in the fresh undried seeds. Seed treatment by drying and cool storage and soaking prior to planting can promote viability and the rate and uniformity of germination. Storage below 15ºC for 30-50 days greatly reduces the activity of growth inhibitors and enhances germination (Yahiro 1979; Yahiro and Hayashi, 1982). Soaking (with changes of water) also greatly increases germination (Paz and Vázquez-Yanes, 1998). For improved long-term storage, seed can be dried to moisture levels of 9-12% (Teng and Hor, 1976; Ellis *et al*., 1991); if dry and cool, papaya seeds may retain viability for 3 years (Malo and Campbell, 1994). After desiccation, heat shock can break the dormancy (Wood *et al*., 2000).

Papaya producers either sow seed in the field, or start by germinating seed in a nursery. Germination may occur in 10-21 days after sowing, or in 4-10 days after pretreatment, and may continue intermittently for up to 35-40 days (Chen and Tseng, 1996; Bhattacharya and Khuspe, 2001). Nursery-grown seedlings may be transplanted to the field at about 60 days (Muthukrishnan and Irulappan, 1985). Because the sex of a plant is not known until flowering, growers often plant additional papaya in each mound, and later thin plants of the undesired sex. If growing hermaphrodite plants is intended, two to four (gynodioecious-type) plants may be transplanted per mound. Growers then remove the female saplings when the sex can be determined — in *c.* 4-8 months from sowing. If dioecious plants are grown, then surplus males will be discarded, leaving one male tree to pollinate 10-15 (sometimes more) female trees.

However, several assays have recently been developed that will likely lead to routine molecular tests for determining the sex of seedlings. They include an assay for males and hermaphrodites using a sequence characterised amplified region (SCAR) marker developed from an RAPD marker (Urasaki *et al*., 2002a, 2002b); a pair of SCAR markers whose products are not produced in females (Deputy *et al*., 2002); a male-specific simple sequence repeat (SSR) (or microsatellite) and a SCAR marker (Parasnis *et al*., 1999, 2000); and a hermaphrodite-specific RAPD probe (Lemos *et al*., 2002). Moreover, Chan-Tai *et al*. (2003) are evaluating an exclusively hermaphrodite Sunrise Solo mutant that when selfed appears to be stable in its sexual phenotype, producing only hermaphrodites.

Vegetative propagation

The cultivar Hortus Gold of South Africa is propagated vegetatively using leafy stem cuttings (Allan, 1974). Experimental success in propagating papaya by cuttings was reported by Allan (1964). Large leafy lateral shoots that developed after a winter season, were initially used for the cuttings, and rooted under intermittent mist. Induction and proliferation of suitable-sized lateral shoots (breaking apical dominance) is improved with application of cytokinin and gibberellic acid mixtures to developed plants (Allan, 1995; Ono *et al*., 2004). The cuttings root in about 3 weeks. Exceptionally, some varieties of papaya are seedless (Wettstein *et al*., 1944; de Wit, 1966).

Clonally propagated plants may show greater uniformity, earlier fruiting, lower fruit-bearing height, and improved yield over plants from seed (Drew, 1988; Chan and Teo, 2002). Hawaiian Rainbow transgenic papaya propagated from cuttings flowered 1-3 months earlier and bore fruit 30 cm lower than progeny from seed (Fitch *et al*., 2002). Cloned plants also yielded significantly greater fruit weight, a difference more marked under less favourable environments. The technology for small-scale commercial and experimental micropropagation is well developed (Litz and Conover, 1978; Drew, 1992; Magdalita *et al*., 1997a). Field trials of *in vitro* plantlets have found that they propagate true to sex, without somaclonal reversion.

Papaya can also be propagated by grafting. Airi *et al*. (1986) cleft-grafted scion shoots from cultivars Co-1 and Honey Dew onto uniformly established seedlings. Patch and T budding also can be used, but the success rate is poorer than with cleft grafting. In Malaysia, some growers use grafting in the orchard to supersede female-fruiting trees of the cultivar Eksotika (Cheah *et al*., 1993). As soon as a plant's sex is determined, propagators will side-cleft graft the female trees with scion shoots (basal diameter 2-3 cm) taken from hermaphrodite Eksotika trees; the scions fruit 6 months after the field grafting.

Varietal selection: Inbred lines and hybrid cultivars

Although local papaya varieties are most common in some markets, producers frequently grow other varieties that originated elsewhere. Singh (1990) noted that in terms of phenotypic uniformity and stability, few varieties qualified strictly as cultivars, although Solo came closest. As described in Section VII.B.2. Cultivated varieties as sources of genetic variability, the Solo group of varieties developed in Hawaii has

limited but inherent genetic variability. Other such uniform lines are Eksotika and Eksotika II from Malaysia, and the Tainung series (Formosa group) of hybrids and inbreds distributed by the Known You seed company of Taiwan (Manshardt, 2002, pers. com.). Commercial producers often grow Solo varieties, Khaek Dam from Thailand, and Tainung (Subhadrabandhu and Nontaswatsri 1997; Le Tran and Tran, 1999; Story, 2001); other Solo cultivars include Kapoho and Waimanalo (Watson, 1997; Beltraide, 2000). Larger-type fruits such as Maradol are also planted extensively. Table 1.22 lists some common varieties used in commerce and breeding.

Table 1.22 Common papaya varieties in commerce and breeding

Variety	Origin	Average fruit size, Notable traits	Fruit characteristics (*e.g.* shape, color)
Bettina	Australia (Florida Betty × Queensland var.)	1.36-2.27 kg	Round-ovoid. Well-colored.
Cariflora	Florida, USA	0.8 kg Tolerant to PRSV.	Round. Dark yellow to light orange flesh.
Coorg Honey Dew [H]	India	2-3.5 kg	Long to ovoid. Yellow.
Eksotika [H]	Malaysia (Sunrise Solo × Subang 6)	0.6-0.9 kg	Elongate (from hermaphrodite). Orange-red flesh.
Eksotika II [H]	Malaysia (Eksotika lines 19 × 20)	0.6-1.0 kg Higher yield than Eksotika.	Fewer freckles on skin, and sweeter than Eksotika.
Sekaki [H]	Malaysia	1.0-2.5 kg	Long, cylindrical, with smooth skin. Red, firm flesh.
Hortus Gold (selection: Honey Gold)	South Africa	1 kg Propagated by cuttings.	Round-ovoid. Golden yellow.
Known You 1 [H]	Taiwan	1.6-3 kg Tolerant to PRSV.	Very long and slender. Yellow flesh.
Maradol	Cuba	2.6 kg	Elongate. Green or yellow skin.
Rainbow [H]	Hawaii, USA (SunUp × Kapoho Solo)	0.65 kg Transgenic resistance to PRSV.	Pear-shaped to ellipsoid. Yellow-orange flesh.
Red Lady 786	Taiwan	1.5-2 kg Tolerant to PRSV.	Elongate. Red flesh.
Red Maradol	Mexico	2.5-2.6 kg	Red flesh; yellow-orange skin.
Solo [H]	Developed in Hawaii, USA; from Barbados originally.	0.5-1 kg Bisexual flowers highly selfing.	Pear-shaped (from hermaphrodites). Orange-yellow skin; golden orange flesh.
Kapoho Solo [H]	Hawaii, USA	0.45 kg	Pear-shaped, but shorter neck than Sunrise Solo. Orange-yellow flesh.
Sunrise Solo [H]	Hawaii, USA	0.57 kg	Pear-shaped. Reddish pink flesh.
Tainung 1 [H]	Taiwan	1.1 kg	Pointed blossom-end (from hermaphrodite). Red flesh.

[H] Hermaphrodite variety (*i.e.* gynodioecious)

Historically, papaya researchers have not found hybrid production necessary for improvement of the crop. More recently, however, hybridisation has demonstrably improved crop potential. The heterosis in F_1 hybrids in some cases has increased plant vigor and yield in agronomic assessments (Subramanyam and Iyer, 1984; Dinesh *et al*., 1992; Chan, 2001) and seed producer trials (Grant, G., 2004, Papaya Seed Australia, pers. com.). In Malaysia, hybridisation of Eksotika Line 20 with its sib-line produced the F_1 hybrid Eksotika II, which has heterosis in vigor and yield (Chan, 1992). In Hawaii, the transgenic cultivar Rainbow was derived as the F_1 progeny of gynodioecious parental lines SunUp (transgenic) and Kapoho (a popular nontransgenic). In Australia, about 65% of commercial plantings over 10 ha are the dioecious

Hybrid 1B (Grant, G., 2004, Papaya Seed Australia, pers. com.) and other hybrids are readily available from commercial sources and commonly planted. The Queensland government maintains parental varieties used for hybrid production and initially had a voluntary seed production scheme with a program for training hybrid seed producers (Dunn, J., 2004, Queensland Dept. Primary Indust. & Fisheries, pers. com.). In diallel crosses, desirable agronomic characters have shown good combining ability (Dinesh *et al.*, 1992; Subhadrabandhu and Nontaswatsri, 1997).

C. Cropping practices

Trees begin bearing within the 1st year of planting (some varieties within 7-9 months). Commercial life of the plant in the large-scale commercial production cycle is usually 3 years, but may be less or more in some areas (Singh, 1990; Watson, 1997). In the U.S. Virgin Islands, early, low-bearing plants have been developed for an annual crop to minimise damage from papaya ringspot virus and seasonal hurricanes (Zimmerman and Kowalski, 2004). In Hawaii production in the 4th year drops off precipitously, thus encouraging replanting after the 3rd year (Younge and Plucknett, 1981). Continuous production is possible even in subtropical regions if winter temperatures may be moderated such as by ocean buffering, but flowering during cooler months in some locations may result in reduced summer harvests (Watson, 1997). Even though fruit production may occur throughout the year in many regions, the month of planting can have an impact on the plant's development, the timing of harvest, and the total yield (Singh and Singh, 1998). Fruit may begin to ripen within 7-9 months, and full-production harvesting may be possible within another 2 months. Depending upon variety, the fruits may need to be thinned. For example, Sunrise Solo may bear up to 5 fruits per node, but is reduced by hand to 2 fruits (Watson, 1997).

Density of planting depends upon the papaya variety and the region where cultivated; typical practice establishes 1160 to 1930 plants per ha (Watson, 1997), with trees spaced from 1.8 to 2.7 m apart in the row and a distance between rows of 2.7 to 3 m. Sometimes double rows are planted, *e.g.* 3.25 × 1.75 × 2.4 m (PROSEA, 1991). Successful practices include provision of optimal soil cover for the orchard space between trees. Younge and Plucknett (1981) showed that clover (*e.g. Trifolium*) or grass reduces return compared to clean cultivation; weedy coverage of the space is similarly disadvantageous. Trash mulching may improve yields, as may a year of rotation with clover between several continuous years of papaya production. Mulching with coarse grass hay may substantially increase yields (Elder *et al*., 2002a).

Papaya can be intercropped if timed appropriately. Before the papaya trees reach bearing age in India, short-term vegetable cropping may be accomplished with tomatoes (*Lycopersicon*), onions (*Allium*), or cabbage or cauliflower (*Brassica*) (Muthukrishnan and Irulappan, 1985), but it was recommended that any competition to the papayas be eliminated during papaya's many months of fruiting to avoid reducing yield. In Nigeria intercropping has been tested with jute (*Corchorus*), sweet potato (*Ipomoea batatas*), okra (*Abelmoschus esculentus*), and watermelon (*Citrullus lanatus*) (Aiyelaagbe and Jolaoso, 1992). However, the cotton or melon aphid (*Aphis gossypii*) can transmit papaya ringspot virus from cucurbits (Cucurbitaceae) (Ali *et al*., 2004). Some growers apparently use papaya itself as a short-term intercrop between rows of mangos (*Mangifera indica*) or litchis (*Litchi chinensis*) (Muthukrishnan and Irulappan, 1985). In Malaysia, sometimes papaya is the intercrop with oil palm (*Elaeis guineensis*) or rubber (*Hevea brasiliensis*) on developing plantations (Chan *et al*., 1999). As papaya is often a 2- or 3-year crop, such interplantings have little impact on the longer lived trees before they reach production age.

D. Cultivation requirements

Adequate irrigation is essential for plant growth and fruit quality. Rainfall in the 1000 to 2500 mm range may be optimal (Watson, 1997), and seasonal variability needs to be taken into account. The pattern of rainfall may lead to soil water deficits, so that supplemental water must be provided for fruit production (Terra de Almeida *et al*., 2003b). Practices include overhead or modified drip or undertree irrigation.

Overhead irrigation may be least suitable, since leaf diseases may increase (Watson, 1997). For Australia, Watson (1997) recommended recharging the root zone twice a week to attain soil water capacity. A pH of 5.0-7.0 is favorable for papaya cultivation (Nakasone and Paull, 1998); lime is commonly used to increase alkalinity.

Balanced nutrition should be provided from the date of planting through harvesting for this fast-growing, heavy-bearing crop (Cunha and Haag, 1980; Watson, 1997). Nitrogen, phosphorus and potassium are important for good growth (Shoji *et al*., 1958; Awada *et al*., 1986; Nakasone and Paull, 1998). Nitrogen deficiency coupled with declining air temperatures can produce carpellodic flowers in some varieties and unmarketable fruits (Awada and Ikeda, 1957). Boron deficiency causes "bumpy" fruit and latex exudation (Chan and Raveendranathan, 1984), which can be remedied by foliar spray of boric acid or ground application of borax.

E. Yield parameters

The yield of papaya fruit varies widely in different countries, and is dependent on soil characteristics, varieties grown, pest and pathogen incidence, and local good management practices (Singh, 1990). Productivity of an orchard is also a function of the number of bearing trees; hermaphrodite and female plant types; average number of fruits per tree, which may be 25-100; and average weight of fruit, which commonly ranges between 350 g and 3000 g. The average yield worldwide in weight of fruit for the 10 years of 1991-2000 was 15,000 kg per ha (FAO, 2001). Optimal production is between 27,000 to 35,000 kg per ha in India (Singh, 1990); 12,500 to 62,500 kg per ha in Trinidad; and experimentally up to 100,000 kg per ha in Hawaii, although the typical Hawaiian yields are 20,000 to 30,000 kg per ha (excluding culled fruit) (Manshardt, 2002, pers. com.). Yield over the lifetime of the tree varies; with intensive cultivation, the highest yield is in the 1st year following planting.

Several thousand metric tons of papaya latex are obtained each year from the unripe fruits. An orchard of not less than 10 ha is usually required to produce one metric ton of dry latex annually (El Moussaoui *et al*., 2001).

Papaya seed production for agriculture varies greatly with variety, growing conditions, cultivation practices (*e.g.* open-pollination in isolated fields, or controlled hand-pollination), and purpose (*e.g.* production of foundation seed for the market, or breeding seed). In India (Bihar) for example, the dioecious Pusa Dwarf and Pusa Giant produced more seeds at lower cost under hand-pollination than the gynodioecious Pusa Delicious and Pusa Majesty. The seed yield of Pusa Dwarf in isolation plots at one site was 579 kg/ha, whereas under controlled pollination at another site the yield was 362 kg/ha. The yield of hand-pollinated Pusa Majesty was just 52 kg/ha (Ram and Majumdar, 1990; Ram, 1996).

4. Pests and Pathogens

Carica papaya's milky latex is stored in a dense network of anastomosing articulated laticifers (joined cells) throughout the plant, but not within the fruit when ripe (Roth and Clausnitzer, 1972; Fisher, 1980; Zeng *et al*., 1994). The latex may provide defense mechanisms by sanitising and healing wounds. The soluble fraction of the latex (which is *c*. 85% water) contains a rich diversity of biomolecules, including some possibly involved or recognised to be directly involved in deterring insects or pathogens — such as glycosyl hydrolases (*e.g.* a class II papaya chitinase), proteinase inhibitors (papaya cystatins), and nine proteinases (El Moussaoui *et al*., 2001; Azarkan *et al*., 2004). Papain (which is not papaya's major cysteine proteinase) is a crucial factor in defense against some lepidopteran larvae (*Samia*, Saturniidae; and *Mamestra* and *Spodoptera*, Noctuidae) (Konno *et al*., 2004). Nonetheless, papaya seedlings experimentally exposed to key damaging mite species (*Tetranychus* and *Calacarus*) and the powdery mildew fungus *Oidium caricae* did not show induced resistance, but instead weak induced susceptibility after being

sanitised and transplanted to the field in Hawaii (Fournier *et al*., 2004). Major pests and pathogens evade defenses, which can also enhance infestation by other such species.

A. Pest species: Mites, insects and nematodes

A small number of mite and insect species are major pests of papaya, though many such species infest the plants (Singh, 1990; Pantoja *et al*., 2002). Aphids often increase in number on weeds surrounding or within the orchards and when the weeds have dried up, attack papaya (Singh, 1990). Green peach aphid (*Myzus persicae*), cotton or melon aphid (*Aphis gossypii*) and cowpea aphid (*A. craccivora*) may transmit papaya ringspot virus. Other insect pests include onion thrips (*Thrips tabaci*) in Hawaii; various scale insects, such as *Aonidiella orientalis* in Queensland; and mealybugs (Pseudococcidae). Fruit flies, such as oriental fruit fly (*Bactrocera dorsalis*) and Mediterranean fruit fly (*Ceratitis capitata*), are principal infesting species in Hawaii (Manshardt, 2002, pers. com.), as are papaya fruit fly (*Toxotrypana curvicauda*) in the Caribbean region (Malo and Campbell, 1994); fruit flies are of most importance in the export market (Nakasone and Paull, 1998). Leafhoppers can be significant pests, including *Empoasca papayae* in Brazil (Firko and Podleckis, 1996) and *E. stevensii* in Hawaii (Manshardt, 2002, pers. com.). Most insect infestations can be treated with appropriate pesticides; aphids may be controlled best by prophylactic removal of host weeds in the vicinity of the orchards (Singh, 1990).

Mite species are likely to be more important pests than insects, with false spider mites (*e.g. Brevipalpus phoenicis*) and spider mites (*e.g.* carmine mite, *Tetranychus cinnabarinus*) occurring in most growing areas (Singh, 1990). Tarsonemid mites (*e.g. Polyphagotarsonemus latus*) are pests in Brazil (Firko and Podleckis, 1996). Suitable acaracides may be employed to control mite damage (Singh, 1990). Root knot nematodes (*Meloidogyne* spp.) and reniform nematode (*Rotylenchulus reniformis*) may limit production in some countries (Singh, 1990). Producers use various halogenated soil fumigants to control nematodes, along with cultural strategies such as removing papaya debris and rotating papaya with other crops.

B. Pathogens: Fungi, viruses and bacteria

More important than mite and insect pests are the pathogens that infect various developmental stages and parts of the plant. The severity of infection depends upon cultural practices and environmental conditions. Singh (1990) reported up to seventeen papaya diseases, of which about six are principal diseases found in many growing areas. Many of the areas are affected by collar rots, damping off, anthracnose, mosaic, and leaf curl diseases. Nishijima (1999) lists a larger number of papaya disease organisms and the associated disease conditions; a recent description of the principal diseases is provided by Persley and Ploetz (2003).

Several fungi produce damping off diseases in nursery plants (younger than 60 days following seeding); the causal agents are *Phytophthora*, *Pythium* and *Rhizoctonia* species. Collar rots are important diseases, affecting seedlings and older plants (stems rot and crack, leading to death); *Pythium* and *Phytophthora* along with *Calonectria* are the causal agents. An anthracnose is in most areas, infesting leaf petioles and fruits; *Colletotrichum gloeosporioides* causes this major disease. Fungal infections of leaf blades and petioles include Corynespora leaf spot, which may be caused by *Corynespora cassiicola* (Carribean region). Powdery mildews affecting the fruit or other parts include *Oidium caricae* (Hawaii), *Sphaerotheca* spp. and *Leveillula taurica* (*Oidiopsis taurica*) (Queensland), and *Ovulariopsis papayae* (East Africa) (Morton, 1987).

Postharvest fungal diseases also cause losses. Phytophthora stem-end rot (*Phytophthora nicotianae* var. *parasitica*), Phomopsis rot (*Phomopsis caricae-papayae*), anthracnose (*C. gloeosporioides*), black stem-end rot (*Phoma caricae-papayae* and *Lasiodiplodia theobromae*) and Alternaria rot (*Alternaria*

alternata) may follow inadequate temperature maintenance or mechanical injury (Kader, 2000). Anthracnoses and Phytophthora blights may be controlled by various fungicides (Pernezny and Litz, 1999). Unspecified replant or yield decline problems (which are probably caused by fungal pathogens) have been treated by replacement of infected soil with virgin soil, or by fumigation (CTAHR, 1985).

Viruses usually impose the most significant limits to papaya cultivation; the importance of some viruses seems to be increasing in many growing areas. Papaya ringspot virus (PRSV), a potyvirus, has produced major crop loss, for example in Hawaii, Mexico, the Caribbean, South America, Africa and Southeast Asia (Persley and Ploetz, 2003). Diagnostic are dark green rings on fruit, and yellow mosaic on leaf lamina accompanied by stunting and shoestring-like leaves (Gonsalves, 1993). PRSV is spread by mechanical means, and also green peach aphid and cotton or melon aphid (Bhargava and Khurana, 1970). Declines in production may occur despite vigorous efforts using cultural strategies to limit spread. Control strategies have included roguing infected plants, but this cannot stem the disease once established (Queensland Dept. Primary Indust. & Fisheries 2003). Treatment with aphicides has been ineffective, because transmission by insertion into the plant occurs before the aphids are killed (Pernezny and Litz, 1999). If non-host crops are interplanted between papaya rows, vectors feed on the non-hosts before feeding on papayas, which can reduce disease transmission and incidence (Gonsalves, 1998). Researchers in Taiwan have developed some tolerant varieties. Seedlings were inoculated with a mild strain of PRSV or a mutated virus to produce cross protection from the more devastating form (Yeh, 1990). However, because of the apparent mutability of the virus, this protection is not completely effective and may not be permanent (Lin *et al*., 1989). Also, the present tolerant varieties (*e.g.* Tainung No. 5) have had poor acceptance because of inferior consumer qualities (Japan Intl. Res. Centre Agric. Sci., 2003).

Collaborators in Cornell University and Hawaii developed transgenic plants to provide resistance to PRSV by expression of viral coat proteins. The newly introduced cultivars brought about a rapid reversal of decline in the papaya industry in Hawaii (Lius *et al*., 1997; Manshardt, 1999; Gonsalves, 2000). Small field trials were conducted in 1992; a scale-up and the release of seeds commercially were completed in 1998. In 2003, nearly half the commercial crop in Hawaii consisted of the PRSV-resistant Rainbow transgenic papaya (Pacific Bus. News 2003). For details about the coat protein-based resistance, see Section VII.B.4. Molecular approaches for agronomic improvement.

Other viruses include papaya mosaic virus (PapMV), a potexvirus identified in parts of South America and Florida (USA) (Malo and Campbell, 1994). PapMV appears to be mechanically transmitted, without a biological vector (Buchen-Osmond and Hiebert, 1988). The incompletely characterised papaya leaf-distortion mosaic virus (PLDMV), a potyvirus, has been analysed experimentally (Maoka *et al*., 1996); it has no more than 59% amino acid sequence homology to PRSV. Chen *et al*. (2001) have shown that the Taiwan isolate DL-1 is antigenically different from PRSV; this virus is also known in Japan (Maoka, 2002). Papaya droopy necrosis virus (PDNV), a rhabdovirus, is found in Florida (Zettler and Wan, 1993). A similar rhabdovirus called papaya apical necrosis virus ("PANV") (but not recognised by the International Committee on the Taxonomy of Viruses) is often a severe pathogen in Venezuela (Zettler and Wan, 1993); it is vectored by a leafhopper (Lastra and Quintero, 1981). Croton yellow vein mosaic virus (CYVMV), a bigeminivirus, causes severe leaf curling and twisting of petioles, leading to death before flowering or fruiting (Singh, 1990; Brunt *et al*., 1996). This can be a devastating pathogen in some areas, such as India; it appears to be transmitted by the whitefly *Bemisia tabaci* even though papaya is not a preferred host. Papaya leaf curl virus (PLCV), a whitefly-transmitted geminivirus, is found in India and Taiwan (Chang *et al*., 2003); it has been analysed for molecular similarity to other viruses (Saxena *et al*., 1998). Papaya lethal yellowing virus (PLYV), with substantial sequence similarity to *Tombusvirus* (Silva *et al*., 1997), can be economically important in Brazil. Tomato spotted wilt virus (TSWV), a tospovirus, has caused sporadic outbreaks in Hawaii; it is hosted by common weed species and vectored by thrips (Gonsalves and Trujillo, 1986; Bautista *et al*., 1995). PRSV type P (papaya) infects papaya and cucurbits; PRSV type W (watermelon) naturally only infects cucurbits, but experimentally it has infected papaya

(Purcifull *et al*., 1986). Also detected have been diseases attributed to distortion ringspot virus, which may actually be a synonym for PRSV (Brunt *et al*., 1996). A regionally important virus in Brazil called papaya meleira virus ("PMeV") (which is currently not ICTV-sanctioned) is a double-stranded DNA virus with no similarities to other viruses (Maciel-Zambolim *et al*., 2003). Control strategies for the other virus diseases of papaya are similar to those for PRSV, but resistance strategies have not yet been developed.

Multiple viruses or other pathogens may occur as coextensive diseases in papaya-growing regions, as do PLYV and PRSV in Venezuela (Marys *et al*., 2000). Evidence for coinfection by PapMV and PRSV has been found in one of ten Mexican states that were assessed (Noa-Carrazana *et al*., 2000). Simultaneous occurrences of zucchini yellow mosaic virus (ZYMV), PRSV, and papaya bunchy top disease have also been detected (Fewerda-Licha, 2002).

Bacterial diseases of papaya are more or less common depending upon the growing region. Species of *Erwinia* initially induce lesions on the lower surface of leaves; yellowing, wilting and death of foliage occur and rotting of the plant follows (Seaver, 2000). This disease is more economically significant in the Caribbean and Venezuela than PRSV (Coppens d'Eeckenbrugge, G, IPGRI Cali, Colombia, pers. com.). To control *Erwinia* in the Virgin Islands, Webb (1985) recommended resistant cultivars and barrier crops that did not support the pathogen, as bactericides and antibiotics were not effective. In the Northern Mariana Islands, disease caused by *Erwinia* is spread by the giant African snail *Achatina fulica* and disease incidence is reduced by snail control.

A severe dieback of papaya in Australia had been attributed to *Phytoplasma australiense* (Liefting *et al*., 1998). Two diseases were indentified, papaya yellow crinkle disease and papaya dieback disease, caused by two different phytoplasma groups (Padovan and Gibb, 2001). A small incidence of a third phytoplasma disease in Australia, a papaya mosaic disease, has been reported (Elder *et al*., 2002b). Leafhoppers (*e.g. Orosius*) infrequently harbored the phytoplasmas, suggesting transmittal but the attribution for causation of the disease is qualified because papaya is not a preferred host. Papaya bunchy top disease appears to be produced by rickettsial bacteria in the laticifers, which are transmitted by the leafhopper *Empoasca papayae* (Davis *et al*., 1998, 1999). Control strategies for bunchy top have included roguing infected plants, topping to allow production of uninfected axillary shoots, and control of vectoring aphids (Davis, 1993).

5. Reproductive Biology

A. Reproductive types and locus of cultivation

Papayas may be cultivated as dioecious varieties (with separate male and female plants), or gynodioecious varieties (having both hermaphrodite and female plants). In subtropical areas dioecious lines are usually planted because the phenotype of gynodioecious plants is unstable under variable and extreme seasonality (Manshardt, 1999). Gynoecious and androecious plants are mixed in the orchard with a ratio of 10 to 25 female plants to 1 male.

Gynodioecious lines are usually confined to tropical areas because flower development in hermaphrodite plants is highly sensitive to climatic stresses and the tropical climate can be more stable and benign for this tropical species. The hermaphrodite plants are susceptible to carpel abortion, or to transformation of stamens into carpel-like fleshy structures (carpellody) so ovarian development is variably expressed. Either female-type or deformed fruits are produced; both are unmarketable. In gynodioecious lines, all plants may be fully productive, as fruit is borne on both the hermaphrodite and the female (gynoecious) plants. In some gynodioecious lines, the hermaphrodite plants frequently produce irregular fruits and their crop also is not as heavy as from the female plants (Shetty, 1953; Persley and Ploetz, 2003). However, in some gynodioecious lines the hermaphrodite fruits are preferred in some areas, and the female

plants are removed. For example, the hermaphrodite plants of the variety Solo produce pear-shaped fruits with more flesh and a smaller seed cavity, which are favored in Hawaii over the spherical fruits on female plants (Arkle and Nakasone, 1984).

B. Sexual reproduction

The categories of unisexual and bisexual flower types occurring variously on plants have been described by many investigators, and have resulted in conflicting descriptions of plant sexual types (Sakai and Weller, 1999). The categories of the papaya sexual types from crosses are complex, but can be summarised simply; Table 1.23 shows the results of such crosses.

Table 1.23 Formation of individual plant sexual types following papaya crosses (adapted from Storey, 1976)

Flowers and plant: S = staminate (male); P = pistillate (female); H = hermaphrodite (male & female)

Mating	S	P	H	(Non-viable zygote)
S × P	1	1	0	0
H × P	0	1	1	0
S × H	1	1	1	1
H × H	0	1	2	1
S × S*	2	1	0	1

*Cross accomplished when sex reversal occurs on staminate (*e.g.* ambivalent male) plants.

The genetic or chromosomal mechanism for this complicated pattern of reproduction is not understood fully; a simple explanation has been used to represent the underlying condition. M is designated as a gene's dominant allele for maleness, M_H the dominant allele for hermaphroditism, and m the recessive allele for femaleness. Zygotes with homozygous dominant alleles (MM, MM_H, M_HM_H) are considered lethal; thus only Mm (male plants), M_Hm (hermaphrodite plants), and mm (female plants) are viable phenotypes (Hofmeyr, 1938a, 1938b, 1939a, Storey, 1938; Muthukrishnan and Irulappan 1985; Ma *et al*., 2004). Storey (1953) furthermore proposed that tightly linked genes on a chromosome, determining sex, lethality, and additionally other sexual characteristics (*e.g.* inflorescence branching and number of flowers, petal fusion, stamen number, ovary shape), would be consistent with the crossing results. Hofmeyr (1967) hypothesised that M (M1) and M_H (M2) represent regions of slightly different length from which vital genes are missing. Sondur *et al*. (1996) accounted for the observations using recent knowledge about development of floral organs in other plants. They proposed that *trans*-acting regulatory proteins induce the sexual forms: an M allele of the sex locus (*Sex1*) induces male floral parts while inhibiting carpel development, an M_H allele induces male parts while only reducing carpel size, whereas the m allele has no ability to induce male parts. Lethality in the dominant homozygotes could result from loss of an essential function when the m allele is lacking. Sex reversal is rare in males, but its occurrence occasionally in hermaphrodites might result from the interaction between M_H and the gene's target (a promoter sequence or another protein factor) being less stable than the interaction between M and the target. Embryos from anther culture have produced only female plantlets (perhaps haploids or polyploids, *e.g.* dihaploids), which presumably originated from microspores, with only the m genotype involved because of the lethality related to the dominant alleles (Rimberia *et al*., 2005).

A mutation recently discovered in a hermaphrodite Sunrise Solo cultivar produces exclusively hermaphrodite plants following self-pollination (*i.e.* no females in the expected 2:1 H:P ratio) (Chan-Tai *et al*., 2003). Pollen of the mutant fertilising typical hermaphrodite plants produced a 3:1 ratio of hermaphrodites to females, indicating that all genotypes survived. Randomly self-pollinating these F_1s produced segregating F_2s that confirmed there were surviving homozygous dominant plants ($M_HM_@$) apparently with a new hermaphrodite allele (a variant of M_H designated as $M_@$). Furthermore, a new recessive lethality gene (l) was inferred that is linked to m, and lethal in female (mm) genotypes when homozygous recessive (mlml).

Based on interspecific hybridisation research in Caricaceae, Horovitz and Jiménez (1967) proposed an XX-XY system of sex determination. Micheletti de Zerpa (1980) studied the meiosis of their BC_2 of *Vasconellea cundinamarcensis* into *V. stipulata*, finding that 2 (only) of the 18 chromosomes had limited pairing and they sometimes behaved as univalents. This was taken as evidence that the Y chromosome of a *V. cundinamarcensis* with a bisexual phenotype had been transferred with its bisexuality genotype to produce the bisexual BC_2, as *V. stipulata* is dioecious. (Both parental species showed no heteromorphic chromosomes.) Liu *et al*. (2004) found that the sex-determining genes in *Carica papaya* are located in a 4.4 Mb region of chromosome LG1 (linkage group 1), *c.* 10% of the chromosome, which shows suppressed recombination (Ma *et al*., 2004). Thus LG1 acts like an incipient Y chromosome, in which a part is Y-like but the rest is autosome-like. This male-specific Y region (equating to *c.* 100-200 genes in average parts of the papaya genome) contains the non-female DNA coding, *i.e.* for male or hermaphrodite characteristics (Viskot and Hobza, 2004). The X-Y sequence divergence may be 10-20% (Charlesworth, 2004).

C. Pollination

Cross-pollination may be common or infrequent, depending upon the papaya variety, flowering behavior (including flower type), and the environment. In some instances, male plants may more effectively pollinate hermaphrodites in adjacent orchards than the hermaphrodites can self pollinate. There may be genotype differences in variety lines, and seasonal changes in flower receptivity affecting pollination (Louw, 2000; A. Louw, 2003, Inst. Trop. & Subtrop. Crops, South Africa, pers. com.; Parés *et al., 20*02; Parés-Martínez *et al*., 2004). In gynodioecious plants, seed set was ten times greater when Coorg Honey Dew plants in India were hand-pollinated after being open-pollinated (Purohit, 1980), but hand-pollination did not increase papaya fruit set in Jamaica (Free, 1975). These results depend upon the pollinators available as well as the papaya variety; for example, bagging of hermaphrodite flowers of the variety Sunrise resulted in 90% fruit set, whereas for the variety Higgins it was only 33% (Rodríguez-Pastor *et al*., 1990). In the dioecious condition the male and female plants are separate, so outcrossing is requisite to fertilisation.

In male flowers, stalked anthers (in an upper whorl) are exserted well beyond the floral tube's opening, whereas nearly sessile anthers (in a lower whorl) are inside the tube and dehisce (open) into it (Wiggins and Porter, 1971). In a dioecious variety (Washington), anther dehiscence was completed 36 to 18 hrs before flower opening (anthesis), and stigmas became receptive a day before anthesis (Khuspe and Ugale, 1977). In gynodioecious varieties, self-fertilisation is possible in hermaphrodite flowers. Anthers dehisce before anthesis, facilitating cleistogamy (Rodríguez Pastor *et al*., 1990; Chan *et al*., 1999; Ronse Decraene and Smets, 1999). The anthers of male, and functionally male (type 4+) flowers have been found to dehisce 2 days before anthesis, whereas the anthers of hermaphrodite flowers dehisce 1 day before anthesis (Parés *et al*., 2002; Parés-Martínez *et al*., 2004). Maximum stigma receptivity has been found to occur on the day of anthesis, although stigmas may become receptive 3 days earlier, and remain so for up to 5 days after anthesis (Subramanyam and Iyer, 1986; Dhaliwal and Gill, 1991). A hermaphrodite flower's pollen may be released before its stigmas' are receptive (protandrous dichogamy), with the stigmas becoming receptive only at anthesis (*e.g.* in the variety Cartagena Amarilla) (Parés *et al*., 2002).

Pollen can be produced year-round. The grains are relative large (32-39 µm diam), and in the subtropics can be larger in local warmer areas (Sippel and Holtzhausen, 1992); the surface is finely reticulate (Allan, 1963a; Fisher, 1980). Viability of pollen (measured by stainability and germination) may vary seasonally, being highest in the rainy season and spring (Singh and Sharma, 1997), and much reduced in winter in subtropical locales such as Australia (Garrett, 1995, in OGTR, 2003a; Allan, 2002). Pollen may be relatively long-lived; in a Petri dish at room temperature, 16% of pollen grains remained viable for 16 days (Sharma and Bajpai, 1969; *cf*. Vahidy and Nafees, 1973).

Some pollen transport may occur by wind transfer, but the detection of pollen near plants seems to be meager (Allan, 1963c). Nontransgenic plants were grown *c.* 396 m (1300 ft) downwind of 0.4 ha of GUS-marked transgenic Rainbow papaya in Kapoho, Hawaii; no GUS expression was detected in the progeny of the nontransgenics (Manshardt, 2002, pers. com.). Purseglove (1968) noted that isolated female trees were pollinated as far as 244 m away from male trees, but Baker (1976) speculated that the observations might be explained by parthenocarpy, which is known to occur in some varieties (*e.g.* Wettstein *et al*., 1944; Free, 1975; Rodríguez Pastor *et al*., 1990; Garrett,1995, in OGTR 2003a). In a study designed to differentiate wind and insect importance, pollination was 38% in open-pollinated plants (Cera-type) but only 26% in controls that were muslin-bagged at the point of anthesis, thus suggesting a substantial contribution by insect pollinators at the suboptimum test site in Veracruz, Mexico (Mateos Sánchez *et al*., 1995). After excluding medium and larger sized insects with wire gauze in South African papaya, no normal fruit set was observed (Allan, 1963c). In some areas, hand-pollination is infrequently undertaken to assist fruit set (Calif. Rare Fruit Growers, 1997).

The flowers open in the early night-time (Mekako and Nakasone, 1975a; Sippel *et al*., 1989; Parés *et al*., 2002), or the morning (Khuspe and Ugale, 1977; Azad and Rabbani, 2004), and since they are strongly dimorphic or polymorphic, provide different cues to potential insect pollinators. Staminate flowers may be more fragrant and open for 24 hrs, and they produce calcium oxalate crystals in the anthers and nectar basally (from the rudimental pistil), thus being an attractant for insects. The pistillate flower has no nectar, but a sweet non-sugar exudate seems available on its flared large antler-like stigmas (or stigmatic lobes) (Ronse Decraene and Smets, 1999; Parés *et al*., 2002), and in these ways it may mimic the male flower (Baker, 1976). The female flowers may remain open for 7 days (Mabberley, 1998).

The main pollinators are somewhat unclear. Details of pollination especially by hawkmoths (Sphingidae), and apparently also mosquitoes, midges and thrips have been described (Heide, 1923; Free, 1975; Baker, 1976; Knudsen and Tollsten, 1993; Garrett, 1995, in OGTR 2003a; Morrisen *et al*., 2003). In the Galápagos Islands, hawkmoths often visit the flowers after dark (McMullen, 1999); in mainland Ecuador, visitors to the male and female flowers include beetles, flies and mosquitoes (Nielsen, 1998, in Ronse Decraene and Smets, 1999). In Venezuela, 17 species were identified as pollinators (or visitors), including *Trigona* and *Xylocopa* bees (Marín Acosta, 1969). In Mexico (Veracruz), 712 insects in 68 families and 12 orders were recovered from 100 flowers, but only 38% pollination was attained (Mateos Sánchez *et al*., 1995). In Central Amazonia (Brazil), papaya pollen is among the preferred foods of the bee *Trigona williana* almost year-round (Marques-Souza *et al*., 1996). Similarly, honeybees were found transporting papaya pollen in South Africa (Allan, 1963c).

In some countries the role of insects in papaya pollination is factored as prominent, whereas in others wind-borne pollen appears to be more the concern. Accordingly, different recommendations for appropriate isolation distances from other papaya may reflect the specific conditions at different locations of production. Recognising both insects and wind as agents for pollen movement, Singh (1990) recommended 2-3 km isolation for production of foundation seed, but cited no experimental observations supporting this distance. The Hawaiian Identity Preservation Protocol for non-GMO papaya seed production specifies at least 1320 ft (400 m) isolation from other varieties (Hawaii Dept. Agric., 2003), based on the transgenic field test reported earlier in this subsection. The Papaya Biotechnology Network of Southeast Asia proposed that nontransgenic papaya should be separated by 400 m from any transgenic papaya plants that could bear anthers in field tests (Anon, 1999). USDA-APHIS approves an isolation distance of 500 m for papaya field tests in Florida. The Gene Technology Regulator (Australia) allows field testing only under conditions of complete insect exclusion by netting and removal of all male inflorescences (OGTR, 2003b).

D. Induced alterations to sexual development

Changes in environmental conditions (temperature, humidity, soil water, nitrogen) can induce various alterations in flowering and reproduction of papaya (Lange, 1961; Singh *et al*., 1963; Rojas *et al*., 1985; Terra de Almeida *et al*., 2003a). When gynodioecious trees develop in hot and dry conditions, the inflorescence at each node may form a terminal bisexual flower but become subtended by male (staminate) axillary flowers (Manshardt, 2002, pers. com.). The male flowers can attain to 80% of all inflorescences on Solo cultivars during such conditions (Nakasone and Paull, 1998). When cooler conditions predominate, the axillary flowers may revert towards a preponderant bisexual morphology.

Sex reversals are also observed in the opposite direction, toward female structures. In both dioecious and gynodioecious lines, high temperatures and increased humidity can cause a shift towards female flowers (Singh, 1990). Lower night-time temperatures in the winter months in Hawaii may occasionally induce carpellody, in which stamens develop to resemble carpels, but associated with a developing fruit (Awada, 1958; Hsu, 1958a). The result is fruits of irregular shape, which are unmarketable (Chandrasekaran *et al*., 1950; Watson, 1997). Carpellody may routinely reach 10-15% in hybrid and inbred lines in subtropical Australia (Grant, 2004).

In dioecious cultivars, where trees bear either male or female flowers, night-time temperatures below 12ºC may induce the formation of bisexual flowers on male plants (Allan *et al*., 1987), and short-day warm conditions may support this change (Aquilizan, 1987). Resistance to sex reversal of male trees is greater in spring compared to the cooler seasons (Allan *et al*., 1987). In subtropical climates, fruit set on male trees (ambivalent males) may occur predictably (Watson, 1997). This conditional reversal is used to insure inbreeding of basically dioecious lines in Queensland (Aquilizan, 1987). In the tropics, the trauma of a few machete slashes to the trunk may stimulate male trees to produce bisexual flowers (Duke, 1967).

The female reproductive structures are stable, in contrast to the bisexual and male structures. Nonetheless, the fertility of female plants can be altered by environmental conditions. Low moisture levels or low nitrogen can induce female sterility (Awada and Ikeda, 1957).

6. Hybridisation

A. Interspecific crosses with *Carica papaya*

By the traditional methods of hybridisation, most attempts to transfer traits from *Vasconcellea* species into *C. papaya* have resulted in endosperm failure (Horovitz and Jiménez, 1958, 1967; Mekako and Nakasone, 1975b; Manshardt and Wenslaff, 1989a). Using embryo rescue and micropropagation techniques, some intergeneric hybrids have been generated (Manshardt and Wenslaff, 1989a, 1989b). Although F_1 plants from crosses with *C. papaya* were produced, they generally were sterile, and produced no F_2s (Manshardt and Drew, 1998). Failure of meiosis resulted in formation of unreduced gametes, which in backcross to *C. papaya* have produced sterile sesquidiploid plants (Manshardt and Drew, 1998). In the Philippines, sterile F_1 hybrids of *C. papaya* with *V. cundinamarcensis* (synonym *V. pubescens*) and as well with *V. quercifolia*, *V. stipulata*, and *V. cauliflora* have been reported (Magdalita *et al*., *19*97b, 1998; Siar *et al*., *19*98; Villegas, 1999). Crossing various *C. papaya* with *V. cauliflora* in Venezuela achieved 0-76% fructification (Vegas *et al*., 2003). All crosses using *V. cundinamarcensis* with *C. papaya* have produced infertile female hybrids (Drew *et al*., 1998; R. Drew, 2001, Griffith, Univ., Australia, pers. com.).

Drew *et al*. (1998) achieved a limited fertile crossability of *C. papaya* with *V. quercifolia*. Large numbers of F_1s were formed following embryo rescue, and backcrossed to *C. papaya*, which produced one male (BC_1) that was fertile and tolerant to papaya ringspot virus; further development by backcrossing it was planned (Drew 2004, pers. com.). Sajise *et al*. (2004) have backcrossed an F_1 (from Drew) with elite

papaya lines in the Philippines and obtained 24 BC_1 plantlets. Also, some *C. papaya* crosses with *V. parviflora* have produced F_1 plants with viable pollen (Drew *et al*., 1998).

Using RAPD techniques (with 14 primers), Jobin-Decor *et al*. (1997) estimated relatedness of some species of Caricaceae. *Carica papaya* had a mean dissimilarity of 69% with six *Vasconcellea* species; it had a dissimilarity of 84% with *Jacaratia spinosa* (and no isozymes in common). There were similar results for *C. papaya* with these *Vasconcellea* species using isozyme analysis — 70% dissimilarity. Using the amplified fragment length polymorphism (AFLP) technique (with 5 primer combinations to generate nearly 500 polymorphic bands), Van Droogenbroeck *et al*. (2002) grouped taxonomic accessions; cluster analysis revealed evidence for strong genetic divergence of *C. papaya* from all eight (plus unidentified) *Vasconcellea* species. In another AFLP study, Kim *et al*., (2002) found that six *Vasconcellea* species were only 43% similar to *C. papaya*, but were 73% similar to one another. Using RFLP analysis of chloroplast and mitochondrial non-coding DNA, Van Droogenbroeck *et al*. (2004) found six *Vasconcellea* species to be more similar to *C. papaya* than to eleven other *Vasconcellea* species, which suggests further possibilities for interspecific crossing with papaya. These more closely related taxa include *V. quercifolia*, *V. weberbaueri* and *V. ×heilbornii*, and less closely also *V. parviflora* and *V. stipulata*, but do not include *V. cundinamarcensis* or *V. cauliflora*.

B. Interspecific crosses within *Vasconcellea*

Workers hope to find a *Vasconcellea* bridge species for crossing with other *Vasconcellea* species, and so interbreeding with *Vasconcellea* known to cross with *C. papaya*. Natural hybrids between some species of *Vasconcellea* occur in the Andes (Badillo, 1971, 1993; Van Droogenbroeck *et al*., 2004). The parentage of the sterile *V. ×heilbornii* is uncertain, as molecular data (Jobin-Decor *et al*., 1997; Aradhya *et al., 1*999; Van Droogenbroeck *et al*., 2002, 2004) do not clearly support the usual interpretation that these wild and semi-domesticated plants (babaco, higacho) came from *V. cundinamarcensis* × *V. stipulata* (Badillo, 1967; NRC, 1989; Jiménez *et al*., 1999). Organellar genome patterns identical with *V. weberbaueri* were found (Van Droogenbroeck *et al*., 2004). *Vasconcellea stipulata* nevertheless has been well documented experimentally to produce fertile hybrids with *V. cundinamarcensis*. Similarly, when *V. stipulata* is the pollen parent in crossing with *V. ×heilbornii*, progeny with 10-20 full seeds are produced (Horovitz and Jiménez, 1967; Micheletti de Zerpa, 1980). Sterile F_1 crosses have been made between *Carica papaya* and *V. ×heilbornii* (as well as *C. papaya* and *V. stipulata*).

7. Genetic Variability

A. Cytology and genome

The diploid (2*n*) number of chromosomes of *C. papaya* is 18 (Meurman, 1925; Asana and Sutaria, 1929; Chen, 1993). No heteromorphic chromosomes have been detected (Datta, 1971; see Section V.B. Sexual reproduction), although differing chromosomal length and constriction morphologies have been found in various varieties. Tetraploids have been induced experimentally (Hofmeyr, 1945).

An analysis of the papaya nuclear genome has been undertaken by constructing a genetic linkage map (Sondur *et al*., 1996). Using RAPD techniques for the analysis of a breeding line and a commercial line, evidence for 11 linkage groups was presented, and a total map distance of *c.* 1000 cM, compared to an expected genome size of *c.* 1350 cM. There was an overall low frequency of polymorphisms per primer (0.16) in comparison to other agronomic plants, suggesting either a relatively low genome size (including polymorphic repetitive DNA) in papaya, or low genetic diversity in the lines. The genome is small, with a 2C of 0.77 picograms and haploid DNA content of 372 Mbp (Arumuganathan and Earle, 1991). Making a map of the entire papaya genome is underway, with a constructed bacterial artificial chromosome (BAC) library of papaya that has nearly 40,000 clones (Ming *et al*., 2001).

B. Genetic variation within *Carica papaya*

The breadth of genetic variation readily available for papaya breeding and improvement is difficult to estimate. Papaya germplasm banks often hold a number of accessions, but the genetic resources in these repositories typically are modestly characterised. A typical collection contains a diverse assortment of *Carica* breeding material, cultivated types and cultivars, and often accessions of some *Vasconcellea* species as well. Surveys or analyses have been carried out to assess agronomic characteristics classically, and increasingly by molecular techniques (*e.g.* Santos *et al*., 2003). Many agronomic descriptors for habit, flower, fruit, seed, etc., have been standardised by IBPGR (1988), including a range of alternative categories for the character. In a conventional analysis of a total of 125 accessions of the Solo group and the Formosa group (Tainung series) and a few intercrosses (holdings in one Brazilian repository), promising variability was found mainly in fruit size and tolerance to *Phytophthora* spp. (Dantas and Firmino de Lima, 2001). An AFLP analysis of 63 accessions from most growing areas (*c.* 17 countries) found an average similarity of 0.880 among them (Kim *et al*., 2002).

Germplasm collections

FAO's Seed and Plant Genetic Resources Service (AGPS) has a list of locations that cultivate papaya germplasm; these include nearly 90 research stations or seed production sites (FAO, 2001; *cf.* Bettencourt *et al*., 1992). However, world germplasm resources for papaya are not organised in an accessible database. CIRAD-FLHOR (Centre de coopération internationale en recherche agronomique pour le développement) and IPGRI have a project for improvement of neotropical fruits which includes establishing a database with limited objectives. A regional effort for collecting and evaluating germplasm holdings of Caricaceae is being developed with a focus on resistance or tolerance traits, particularly for PRSV, bacterial decline, and anthracnoses — the regionally most important pathogens (Coppens, 2001, pers. com.). Other goals for Caricaceae germplasm collections may include development of *Vasconcellea* with potential as commercial fruits, and for new sources of enzymes such as papain (*e.g.* Colombo *et al*., 1989; Villarreal *et al*., 2003).

Breeding programs in various countries have established germplasm collections to co-ordinate with varietal improvement programs. The U.S. National Plant Germplasm System's USDA site in Hilo, Hawaii reports 153 accessions of *C. papaya* and several *Vasconcellea* spp. (GRIN, 2001); agronomic characters associated with specific accessions can be retrieved in a database *via* the Internet. Large holdings include those by Brazil (Coppens, 2001, pers. com.; Dantas and Firmino de Lima 2001) at EBDA-Bahia (82 accessions), EMBRAPA Mandioca e Fruticultura, Cruz das Almas, Bahia (141 accessions) and IAC-Campinas, São Paulo (169 accessions); Colombia at Univ. Nacional Medellín and CORPOICA (83 accessions) with additional accessions at other locations; India (90 *C. papaya* accessions) (Giacometti *et al*., 1987); and Malaysia (72 accessions) (Chan *et al*., 1999).

Most of the germplasm collections consist of living plants, but some include seeds (Giacometti *et al*., 1987). Seed may be stored for up to 12 months at 12 C if capped in a tightly fitting jar, and longer under conditions specified by IBPGR (Giacometti *et al*., 1987). Pollen likewise can maintain viability if stored appropriately, such as for 6 months either at 10ºC and 10% relative humidity (Allan, 1963b) or at -18ºC (Cohen *et al*., 1989), or for 10-16 months cryogenically — even with several thawings and refreezings (Ganeshan, 1986).

Cultivated varieties as sources of genetic variability

Many varieties of papaya are typically cultivated within a country, and each is often quite localised. A catalogue made in one of the high production areas of East Java, Indonesia recorded at least 24 such varieties (Baswarsiati *et al*., 1985, in Setyobudi and Purnomo, 1999). Some papaya varieties have found international acceptance and are grown extensively worldwide. One is Solo, from which other lines have

been selected (such as Sunrise Solo). Eksotika, bred from backcrossing Subang 6 with recurrent parent Sunrise Solo, is the flagship variety in Malaysia for export (Chan *et al*., 1999).

To determine variability among papaya varieties and the degree of relatedness of some cultivars, Stiles *et al*. (1993) used RAPD molecular techniques (with 11 primers amplifying 102 distinct fragments). The comparison among 10 varieties from Malaysia, Mariana Islands, Hawaii and Florida showed their least relatedness was *c.* 70%, and the most closely related cultivars at *c.* 95%. The genetic similarities were generally those expected from knowing the region of origin and breeding history of the variety. Kim *et al*. (2002) analysed 186 AFLPs to estimate genetic diversity within 63 papaya accessions from many international sources; the genetic diversity was quite limited. The average similarity was 0.880, and in a single growing region, such as within Solo-type hermaphrodite cultivars in Hawaii, the average was 0.921. Analysis using isozymes is also possible — a total of 29 alleles have been found in 11 loci that segregate independently (Morshidi, 1998).

Surprisingly, Kim *et al*. (2002) found that the dioecious cultivars (which are open-pollinated) did not have more genetic variability than the hermaphrodite cultivars, which are thought to be mainly self-pollinated and so presumed to be less genetically variable. The literature on papaya breeding includes a considerable number of reviews (Singh, 1990), and no inbreeding depression has been detected (Hamilton, 1954). Indeed, after the initial selection for new traits, inbreeding by sib-mating for about four generations is often the practice to establish new varieties. Hybridisation was typically not used in developing new varieties (Storey, 1953), reinforcing the belief that inbreeding depression is not an important issue in cultivating papaya. Not withstanding, in development hybridisation is increasing, as shown by Australian varieties and Malaysia's success with Eksotika II (see also Section III.B.1. Seed and B.3. Varietal selection).

Genetic variability in resistance to pathogens

Several research programs have looked for plants with the ability to develop tolerance (*i.e.* an ability to be infected but with limited effects) or resistance (lack of susceptibility to infection) to PRSV, one of the most devastating pathogens of papaya. Complete resistance is preferable, but tolerance is a useful option. The dioecious line Cariflora developed in Florida has shown a high level of tolerance (Conover *et al*., 1986). Researchers in the Thailand Department of Agriculture have selected a PRSV-tolerant variety called Thapra 2; the plants may become infected, but have mild symptoms or remain symptom free. A second tolerant Thailand cultivar, Pakchong 1, was developed at Kasetsart University (Kositratana *et al*., 1999). In the Philippines, the variety Sinta has exhibited high tolerance (Villegas *et al*., 1996). In Malaysia, hybrids have been made of the popular variety Eksotika with Tainung No. 5, which has tolerance to PRSV, and the later generations have reasonable or high levels of field tolerance and are under continuing selection (Chan and Ong, 1996; Chan *et al*., 1999; Chan, 2004; Chan, Y.K., pers. com.). See Section IV. Pests and pathogens for details about the results of these approaches. Since the development of transgenic varieties expressing a virus coat protein to confer resistance to PRSV, less effort has been expended to develop tolerant varieties; the genetically engineered resistance is providing more substantial benefits (Ferreira *et al*., 2002).

Molecular approaches for agronomic improvement

Mutagenesis has augmented common breeding practices for improvement, resulting for example in a dwarf papaya with higher fruit yield per unit area (Ram and Majumdar, 1981). An RAPD-based genetic linkage map (Sondur *et al., 19*96) has been used to locate and characterise genes affecting growth (height and stem diameter) and time of first flowering, by an analysis of quantitative trait loci (QTLs) in an F_2 papaya population derived from the cross of a gynodioecious, tall, late-flowering variety and a dioecious, semi-dwarf, early-flowering selection (Sondur *et al., 19*95). Three QTLs affecting rate of height increase

and final height were detected, four QTLs affecting rate of stem diameter increase and final diameter, and two QTLs affecting node at first flowering. This is viewed as the lower limit of major QTLs for these traits. Five of the QTLs were on linkage group 1 (LG1) and one QTL each on LG3, LG4, LG5 and LG10, considered a non-random distribution. The height-influencing QTLs accounted for 64% of the phenotypic variance in height increase, the stem-influencing QTLs accounted for 52% of the variance in diameter increase, and the QTLs influencing node at first flowering accounted for *c.* 30% of the variance in node (in which the first flower-bearing node ranged from the 15th to the 36th). Variance due to environment was estimated to be 20% for height increase and 25% for increase in diameter.

Because practical methods for transforming papaya have been developed and the biotechnology is becoming well refined (Fitch *et al*., 1990; Pinto *et al*., 2002; Zhu *et al*., 2004; Wall *et al*., 2004), and transgenic commercial lines have been approved and available since 1998 (Cai *et al*., 1999; Ying *et al*., 1999; Gonsalves, 2000), papaya is a focus for improvement using genetic engineering approaches. Programs are using such tools to transform various varieties, for example co-ordinated under the Papaya Biotechnology Network of Southeast Asia (ISAAA, 2001a), with a current focus on delayed ripening characteristics and resistance to PRSV. Commercial interests have developed papaya with altered fruit ripening to allow extended marketing. Field testing in Australia has been authorised for papaya transformed with genes (*capacs1* and *capacs2*) that alter expression of ACC synthase, and with an ethylene expression gene (*ETR1*) (OGTR, 2003b). To increase tolerance to aluminum (common in tropical acidic soils), a transgenic papaya has been made in Mexico that overexpresses a citrate synthase gene from *Pseudomonas aeruginosa* (de la Fuente *et al*., 1997). Transgenic papaya also is being researched as a delivery vehicle for an edible vaccine against tuberculosis (Zhang *et al*., 2003).

Genes identified in papaya include some whose expression might be employed to modify various agronomic traits or enhance industrial production. Identified sequences (NCBI, 2001) include those affecting the following (Table 1.24):

Table 1.24 Selected Papaya Genes for which Information is Available

Industrial/Agronomic product	Carbohydrate metabolism	Others
a male-specific SCAR marker	sucrose synthase	arginine decarboxylase (ADC)
chymopapain	cell wall invertase	ATP synthase
papain	β-galactosidase	membrane channel proteins
metallothionein-like protein	α-galactosidase	glutamine cyclotransferase
1-aminocyclopropane-1-carboxylic acid (ACC) synthase	xyloglucan endo-transglycosylase	caricain (proteinase omega) cysteine protease cysteine protease inhibitor
ethylene receptor	pectinesterase	Cu/Zn superoxide dismutase maturase K

Breeders and molecular biologists have a goal of developing resistance to various papaya diseases. Many pathogen-associated sequences have been cloned and identified (NCBI, 2001), which potentially could be employed in transformed papaya to provide endogenous resistance to pathogens that use papaya as host. Large numbers of viral genes have been sequenced, including coat proteins of numerous PRSV biotypes from different locations, a replicase, mRNA products of the virus, and an RNA polymerase gene (*NIb*). The whole PRSV and PapMV genomes have been sequenced. Other genes identified include two genes from PLDMV — an *NIb* gene and a coat protein (capsid protein) gene, a gene from the phytoplasma that causes papaya dieback (tuf) disease, the succinate dehydrogenase gene from the rickettsial bacteria that may cause papaya bunchy top disease; and an ileu tRNA.

Engineered resistance to viral diseases of papaya may require expression of geographically specific viral proteins. Many strains of the widespread PRSV may be virulent to papaya varieties even after they have been transformed with viral capsid sequences. For example some Florida (USA) isolates were

molecularly similar to Mexican and Australian isolates, but dissimilar to those from Asia (Davis and Ying, 1999). The genetically engineered resistance may be less or not effective if the origin of the capsid sequence is from a different region than the local viral strain (Tennant *et al.*, *19*94, 2001, 2002). Recombination involving as few as 5 nucleotides in a virus coat protein gene can cause a susceptible response when a resistant papaya variety expressing the non-mutant coat protein is inoculated with the altered virus (Chiang *et al*., 2000). However, not sequence similarity alone, but also gene dosage, plant stage, and other PRSV genes have important consequences for the expression of field resistance to PRSV (Tennant *et al*., 2001; Tripathi *et al*., 2004).

A consortium of scientists from universities, business and the U.S. Department of Agriculture (USDA-ARS) developed the first resistant papaya, which expressed a Hawaii-specific PRSV coat protein (Fitch *et al*., 1992). Projects to deploy PRSV-resistant transgenic papayas are variously underway for example in Mexico, Guatemala, Jamaica, Venezuela, Brazil, Uganda, Tanzania, Bangladesh, Taiwan, Australia, and the countries that are members of the Papaya Biotechnology Network of Southeast Asia — Malaysia, Thailand, Vietnam, Philippines and Indonesia (Cai *et al*., 1999; Flasinski *et al.*, *20*02; Tennant *et al.*, *20*02). Brazilian researchers working at Cornell University (USA) have transformed five papaya varieties using Brazil-specific PRSV capsid sequences, and planned field tests (ISAAA 2001b; Lima *et al*., 2002). Thailand has developed a PRSV-resistant variety using a sequence from a PRSV strain specific to Southeast Asia; field tests were planned for 2002 (ISAAA, 2001c). Localised research efforts have succeeded in providing coat protein-based immunity in Australian and Venezuelan varieties (Lines *et al*., 2002; Fermin *et al*., 2004).

Several biotechnological solutions have been explored to supply the resistance needed to protect the crop from the prevalent PRSV of various regions. Chiang *et al*. (2001) suggested transforming papaya to express chimeric PRSV coat proteins, which possibly can be protective against these variable viral challenges. Bau *et al*. (2003) showed that a single coat protein sequence from a local Taiwan strain was adequate to provide complete immunity from heterologous strains arising in Mexico, Hawaii and Thailand, and this line did not produce any coat protein. Another approach may be to use the PRSV replicase gene to provide resistance (Chen *et al*., 2001). An approach that produces an untranslatable product, which may result in an RNA-meditated immunity to PRSV, has been successful in protecting Australia cultivars (Lines *et al*., 2002) and Florida cultivars (Davis and Ying, 2002). Also, programs for multiple protections against pathogens are attempting to combine coat proteins from PRSV with coat proteins from PLDMV (Maoka, 2002).

8. Ecology

A. Dispersal

In Cameroon, forest elephants (*Loxodonta cyclotis*) seek papaya fruits beyond their protected reserve and disperse the seeds (Tchamba and Seme, 1993; Barlow, 2000). Wild *Carica papaya* seems to have many characteristics that fit the hypothesised megafaunal dispersal syndrome (Janzen and Martin, 1982; Barlow, 2000). The non-domesticated fruits are fairly large (5-8 cm in diameter) and visually nondescript (greenish unless fully ripened) but with a penetrating aroma, and are held high up on a trunk with suppressed branching. The fruits are indehiscent (without structural opening), and pulpy within but have peppery mustard-tasting seeds (Sharma and Singh, 1975; Passera and Spettoli, 1981) that are grouped centrally. Non-domesticated *Vasconcellea* fruits can be larger. Such unusual species may have evolved in response to consumption of fruits whole and seed dispersal by large (now extinct) mammals such as ground sloths (*Eremotherium*) and mastodon-like gomphotheres (*Cuvieronius*) (Simpson, 1969, 1980; Barlow, 2000).

A great many vertebrates with a wide variety of ecological roles eat papaya fruits and may disperse viable seeds. Coyotes (*Canis latrans*) in coastal western Mexico (Jalisco) habitually seek out papaya as food, sometimes taking fruits directly from the trees and causing important economic loss (Hidalgo-Mihart *et al*., 2001). Pacas (*Agouti paca*), large forest rodents which range from Mexico to Paraguay, when in captivity selectively prefer papaya fruits because of their relatively high energy content (Laska *et al*., 2003). Brow-ridged langur monkeys (*Trachypithecus* spp.) in eastern India (Tripura) raid the crop, and can cause tree mortality (Das, 1998). Arboreal neotropical monkeys consume papayas, such as wild cotton-top tamarins (*Saguinus oedipus*) and captive capuchins (*Cebus*) — which pass the seeds in less than 2 hrs (Wehncke *et al*., 2003). Great fruit-eating bats (*Artibeus lituratus*), which are common from Mesoamerica and the Lesser Antilles to northern Argentina, disperse seeds found viable when tested (Garcia *et al*., 2000). In Papua New Guinea (Madang), papaya was found in fecal samples of lesser bare-backed fruit bats (*Dobsonia minor*) netted in the Kau Wildlife Area (Bonaccorso *et al*., 2002).

In the Yucatán (southern Mexico), the plant is appropriately called papaya de pájaro (bird papaya). Many birds eat the fruits and may disperse seeds, such as Montezuma oropendolas (*Psarocolius montezuma*), a blackbird ranging from southern Mexico to central Panama (Webster, 1997), and Guianan cocks-of-the-rock (*Rupicola rupicola*) (Gilliard, 1962). Papaya was 29% of the diet of West Indian red-bellied woodpeckers (*Melanerpes superciliaris*) studied on Grand Cayman Island (Cruz and Johnston, 1984). Endangered Ouvéa parakeets (*Eunymphicus cornutus uvaeensis*) of New Caledonia preferentially consume papaya which are available throughout the year in Melanesian gardens (Robinet *et al*., 2003). Various bird species in India (Punjab) seek out the fruit (in rind-forming through ripening stages), and can result in crop damage of at least 3.4% (Mahli, 2001).

B. Weediness

Papaya in different regions is variously described as an incidental escapee from cultivated sites, an opportunist, a pioneer species, or sometimes as an invasive or potentially invasive species. Papaya may persist beyond cultivation for indefinite periods. Little and Wadsworth (1964) state that "Through the tropics they grow almost as weeds, bearing fruit the first year from seed and spreading along roadsides and in waste places"; they report that in Puerto Rico papaya is widely cultivated, escaping, and naturalised. In the Galápagos Islands (Santa Cruz), papaya was found along a new road from the coast inland in the arid, transition, and humid zones, but did not persist along the old road (Haro Martínez, 1975). Papaya is usually not characterised as an invasive species (USDA-APHIS, 1997).

Carica papaya is regarded as a pioneer species in fairly natural habitats. Papaya can occur in forest gaps and within the early succession, since it has such characteristics as rapid growth in response to disturbance and high light intensity, and prolific production of seeds and an attractive fruit. Its pioneering ecological strategy includes a short life cycle with seed dormancy and a seed bank.

As an opportunist, papaya has the capacity to establish significant seed banks. In Central Amazonia, Brazil (Santarém region) at some old Dark Earth locations (aboriginally cultivated soils), papaya comes up after the long-standing tropical rain forest has been cleared and burned (Clement *et al*., 2004). In a post-hurricane study of regeneration that compared feral *C. papaya* with a similar-sized native pioneer tree species (*Trema micrantha*) in Florida hammock habitats, papaya had a broader niche for regeneration (Kwit *et al*., 2000). They averred that dormant seed supply seed for population return following natural disturbance. Moist wild papaya seeds kept in total darkness in Petri dishes at room temperature remain viable and dormant (Vázquez-Yanes and Orozco-Segovia, 1996; *cf.* Pérez-Nasser and Vázquez-Yanes, 1989). In Japan's Bonin Islands (Hahajima) in mesic subtropical forest, the seed bank at 16 sites was sampled (0-20 cm) in three layers. Viable papaya seeds occurred in all three, with the most seeds at a depth of 4-10 cm, where their density was *c.* 18 per m^2 (Yamashita *et al*., 2003).

Nakasone and Paull (1998) characterise papaya as "a rapid volunteer in areas where the tree vegetation has been disturbed". Subsequent to major hurricane damage (1992) in southern Florida (USA), papaya recruited abundantly and rapidly in unmanaged and managed areas. In the 1st and 2nd years, it comprised 76% and 40% of all stems respectively in the unmanaged areas (Horvitz and Koop, 2001). In an inventoried natural semi-evergreen forest of southeastern Mexico on the Yucatán Peninsula (Quintana Roo) where no papaya had been recorded, papayas were infrequently present just 5 months after an extreme hurricane had altered the region (Sánchez-Sánchez and Islebe, 1999).

Randall (2002) reported weedy papaya infestations on some tropical islands and in localised areas of New Zealand. On Christmas Island (Indian Ocean) and in the Mariana Islands (Rota) and Samoa (Savaií), papaya is a colonising invader of disturbed or burned habitats (Craig, 1993; Space *et al*., 2000; Elmqvist *et al*,. 2001; Green *et al*., 2004). On Tongatapu, papaya was found in 44% of 52 sample plots in a range of land-cover types (especially fragmented interior forest). Following from these observations, Wiser *et al*. (2002) stated that it should be considered a potentially serious invader. In coastal Queensland (Australia), "small, low-density self-perpetuating populations" may be found (OGTR, 2003a). In the Hawaiian Islands, papaya is sparingly naturalised on four main islands, with some plants even occurring on nearly vertical rock faces (Wagner *et al*., 1999; Oppenheimer and Bartlett, 2000). In a wet-forest region of coastal Ecuador near the Andean foothills, Dodson and Gentry (1978) found papaya to be common in second-growth areas, including a forest regenerating from an agricultural clearing about 18 years previously.

Papayas are sensitive to most herbicides and volunteer plants in agricultural habitats can be eliminated using paraquat, glyphosate, or triclopyr (Lee, 1989; Kline and Duquesnel, 1996). Reports are scarce on efforts to reduce feral *C. papaya* in relatively natural habitats (*e.g.* Horvitz and Koop 2001), which may be important for ecological restoration or to reduce genetic contamination in orchards from feral off-types.

C. Optimal habitats

Carica papaya requires a tropical or semitropical habitat that is always rather warm and provides high illumination. Depending on latitude, cultivated varieties may thrive at elevations from sea level to 600 m and may range up to about 1200 m, being limited by the occurrence of killing frost (Arntzen and Ritter 1994; Bhattarai *et al*., *20*04). Temperatures below 11ºC negatively affect growth and fruit set, and strongly retard fruit maturing and ripening (Shetty, 1953; Allan 2002). At higher elevations fruit tends to be insipid.

Rainfall must attain to at least 350 mm and should not exceed about 2500 mm, as excessive moisture is detrimental to the plant or fruit (Singh 1990). Within its probable native range in Veracruz (central-eastern Mexico) (Moreno, 1980), a study correlated regional parameters with 62 botanical collections (including 25% from scarcely or slightly modified habitats; *cf.* Del Angel-Pérez and Mendoza-Briseno 2004). Most plants were in Köppen's Am(f) subclimate type; the annual estimated total precipitation was 1200-1400 mm, with 100-150 days having considerable rain (an average 30-40 mm possible in 24 hrs), and with 20-40 days per year having essentially no rain (Gómez-C. 2000). Relative humidity of more than 60% may be optimal for papaya (FAO 1986); nevertheless, in South Africa the best-quality fruits are grown in low humidity regions (Malan 1953).

Acceptable growth of papaya can occur in a variety of soils. An optimal soil that promotes growth is well drained, and flooding is not tolerated (Malaysia Dept. Agric. 2001). A pH of 5.0-7.0 is favorable for its cultivation (Nakasone and Paull, 1998); uniform, rich loams of pH 6.5-7.0 are considered optimal (Singh, 1990). For Hawaiian soils, Younge and Plucknett (1981) recommended an optimum pH of 5.8-6.2; if the pH reaches 6.2-6.5, increased damage by *Phytophthora* may occur (Adlan, 1969). Arbuscular mycorrhizal fungi are strongly beneficial in cultivation (Jaizme-Vega and Azcón 1995; Vierheilig *et al., 20*00; Trindade *et al., 20*01). Compacted soils that impede root penetration (Yamanishi *et al., 19*98) will also limit net CO_2 assimilation (Campostrini and Yamanishi 2001b).

Optimal growth is in sheltered locations; strong winds in combination with rain or low temperatures can cause fruit loss as a consequence of lodging that can easily occur in these fairly shallow-rooted plants. Most roots occur in the soil's upper 20 cm and they may extend radially to 80 cm (Fisher and Mueller 1983; Masri 1993; Malaysia Dept. Agric. 2001). Under optimal conditions taproots may reach a depth of about 1 m, and papaya rooting can adapt to slopes (Marler and Discekici 1997). With minimal pre-plant preparation or an absence of fertiliser applications, only a fibrous root system may develop (Younge and Plucknett 1981).

D. Optimal geographic location

The genetic diversity within the present commercial cultivars provides relatively wide adaptability for papaya and permits cultivation in many locations. Production of this world crop is generally found between 30ºN and 40ºS, but commercial production is compressed to a circumferential region around the equator from 25ºN to 25ºS (Singh 1990). When grown outside these tropical latitudes, optimal growth is in well-protected areas near sea level (Nakasone and Paull 1998). Photosynthetic saturation occurs at rather high irradiance, and shade induces major morphological and cellular changes (Imai *et al., 19*82; Buisson and Lee 1993; Marler 1994); papaya has been described as a shade-avoiding species (Grime 1981). Papaya is cold-sensitive, wind-sensitive, flooding-intolerant, and moderately salt-sensitive (Marler 1994; Clemente and Marler 2001). Nevertheless, it has been successfully adapted as a dooryard treelet, and has naturalised in many locations. On most continents and on many islands, the usefulness of papaya's products and its wide range of traits have allowed the papaya a place in many gardens, local markets and commercial enterprises.

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SECTION 11
OYSTER MUSHROOM (*PLEUROTUS* SPP.)

1. General Information

Oyster mushroom is regarded as one of the commercially important edible mushrooms throughout the world. It consists of a number of different species including *Pleurotus ostreatus, Pleurotus sajor-caju, Pleurotus cystidiosus, Pleurotus cornucopiae, Pleurotus pulmonarius, Pleurotus tuber-regium, Pleurotus citrinopileatus* and *Pleurotus flabellatus*. They thrive on most of all hardwoods, wood by-products such as sawdust, paper, pulp sludge, all the cereal straws, corn and corn cobs, coffee residues such as coffee grounds, hulls, stalks, and leaves, banana fronds, and waste cotton often enclosed by plastic bags and bottles. The oyster mushroom is the second most important mushroom in production in the world, accounting for 25% of total world production of cultivated mushrooms. Oyster mushroom is grown world-wide, and China is the major producer. *P. ostreatus* was first cultivated in the USA in 1900 and several other species of the oyster mushroom such as *Pleurotus sajor-caju* were initially cultivated in India after the late of 1940s. The oyster mushroom has been regarded as one of the most profitable cash crops in Korea, accounting for 65% of total domestic mushroom production.

This consensus document which describes the main aspects of the biology of Oyster Mushroom was prepared by the lead country, Korea, to provide background information for science-based decision making in consideration of future release of transgenic mushrooms into the environment. Included are description of the taxonomy and natural habitat of the genus *Pleurotus* and morphological description of *Pleurotus ostreatus,* the agronomic practices, the life cycle and sexual reproduction, and genetics. *Pleurotus ostreatus* is the main focus of this document, but other species of the oyster mushroom are also covered in this consensus document.

2. Taxonomy and Natural Distribution

A. Taxonomy and nomenclature

Oyster mushroom, *Pleurotus* spp., belonging to the genus *Pleurotus* (Quel.) Fr., tribe Lentineae Fayod, family *Polyporaceae* (Fr.) Fr., is widely distributed throughout the Northern Hemisphere, such as Europe, North Africa, Asia and North America (Singer, 1986). To date, approximately as many as 70 species of *Pleurotus* have been recorded and new species are discovered more or less frequently although some of these are considered identical to previously recognised species. The genus *Pleurotus,* which was first recommended as a tribe within genus *Agaricus* by Fries (1821), was proposed as a genus by Quelet (1886). Three genera of this group, *Pleurotus, Lentinus,* and *Panus,* were possible to be separated according to their anatomic characters of the sterile tissues of the hymenophores as being homogeneous taxonomic groups. Hilber (1982) recommended that crossing of monospore cultures is a valuable basis for *Pleurotus* studies. *Pleurotus ostreatus* (Jacq: Fr.) Kummer is the most cultivated species among the oyster mushroom and the type species of the genus *Pleurotus.*

Recently, the majority of mycologists have followed the proposition made by Singer (1986) which divides the genus *Pleurotus* into six sections: Sect. *Lepiotarii* (Fr.) Pilat, Sect. *Calyptrati* Sing., Sect.

Pleurotus Sing., Sect. *Coremiopleurotus* (Hilber), Sect. *Lentodiellum* (Murr.) Sing. and Sect. *Tuberegium* Sing.. *Pleurotus ostreatus* was placed in the Sect. *Pleurotus* based on the absence of veil and with the monomitic hyphal system.

B. Morphological description

Species identification within the genus *Pleurotus* is difficult because of the morphological similarities and possible environmental effects. Mating compatibility studies have demonstrated the existence of eleven discrete intersterility groups in *Pleurotus* to distinguish one species from the others. *P. columbinus, P. florida, P. salignus,* and *P. spodoleucus* are the synonyms or subspecies taxa for the species of *P. ostreatus*.

Macroscopic features of Pleurotus ostreatus (Jacq.: Fr.) Kummer

- Pileus: 40-250mm broad, oyster-shape, spatulate to lingulate when young, convex then later becoming conchate to flabellate, surface smooth, grey lilac, violet-brown to lilac blackish when young later becoming cream-beige, but usually very variable in colour, margin smooth when young, later somewhat undulating and striate. For descriptions of macroscopic features of fruiting bodies, descriptions and illustrations of microscopic characters, and distribution of this taxa, references of Breitenbach and Kranzlin (1991), Donk (1962), Imazeki and Hongo (1987), and Moser (1983) were referred to respectively. Colour names were taken from Kornerup & Wanscher (1983).

- Context: white to grey-white, thin to thick, fleshy, radially fibrous, odour fungoid, taste mild.

- Lamellae: long-decurrent, crowded, whitish to cream or pale greyish, edge smooth, later somewhat undulating, lamellulae 1- or 3-tiers.

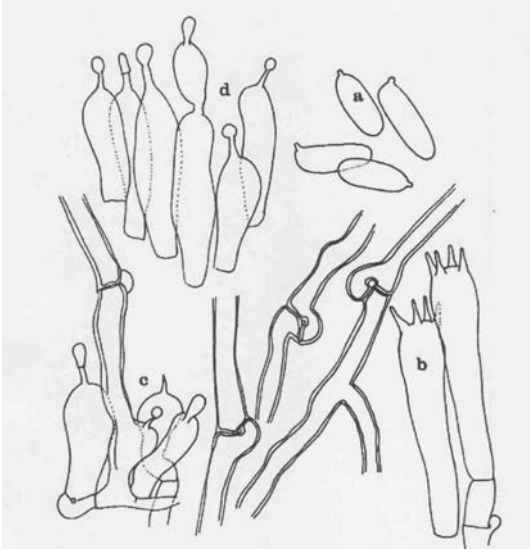
- Stipe: 10-20×10-25mm, rudimentary, usually lateral, severa concrescent, surface longitudinally striate, whitish villose-pilose, context solid.

Figure 1.5 Macroscopic feature of *P. ostreatus*

Microscopic features of Pleurotus ostreatus (Jacq.: Fr.) Kummer

- Spores: 6.5-9×2.8-3.5μm, cylindric to cylindric-ellipsoidal, smooth, hyaline, with vacuoles.
- Spore print dingy grey or pale lilac grey.
- Basidia: 23.6-27×5-7.5μm, slenderly clavate with 4-spored and a basal clamp connection.
- Hymenophoral trama: regular to irregular, trama monomitic.
- Cystidia: absent or cystioid, rarely seen.
- Pileipellis: composed of irregular, densely interwoven, flexuous and branched hyphae, usually 2-4μm across, with brown pigment, somewhat gelatinised, septa with clamp connections.
- Habit & Habitat :Usually gregarious, clustered on the dead hardwood in park and both side of road, rarely on conifers, Suwon, Pochon, Cholwon, Whasong in Kyunggi Province , Gyeryong-san, Chilgap-san in Chungnam Province, Chiak-san in Kangwon Province, Kangjin in Chonnam Province and Hanla-san in Jeju Province in Korea. Spring to autumn.
- Distribution: Europe, America, North Africa, and Asia

Figure 1.6 Microsopic feature of *P. ostreatus* (a: spores, b: basidia, c. cheilocystidia, d: pleurocystidia)



C. Natural habitat

The geographic distribution of the oyster mushroom varies according to its species. For example, *P. pulmonarius* and *P. cystidiosus* are known to be distributed in the tropical and subtropical region, while *P. eryngii* are found in southern Europe, North Africa and central Asia. It has many subspecies and similar taxa such as *P. fuscus* var. *ferulae* from China. *P. ostreatus* is widespread in the temperate zones such as Korea and Japan because it forms fruit-bodies at relatively low temperature compared to other *Pleurotus* species. The geographic distribution of *P. tuber-regium* includes most of equatorial Africa, India, Sri Lanka, Southeast Asia, North Australia, and the southern Pacific countries as well (Table 1.25).

Commonly grown on broad-leaf hardwoods in the spring and fall, especially cottonwoods, oaks, alders, maples, aspens, ash, beech, birch, elm, willows and poplars are favoured natural habitat for oyster mushroom. Although seen on dying trees, *P. ostreatus* is thought to be primarily a saprophyte, but behaves as a facultative parasite at the earliest opportunity. Occasionally, it grows on composting bales of straw and in Mexico, on the pulp residues from coffee production. The most abundant fruiting of this species is in low valley riparian habitats (Stamets, 1993).

Table 1.25 Classification of the genus Pleurotus and its geographical distribution (Singer, 1986)

Sect.	Species	Geographical Distribution
Lepiotarii	*P.dryinus* (Pers: Fr.) Kummer	Japan, USA, Swiss, Germany, Sri Lanka, Portugal
	P.dryinus (Pers: Fr.) Kummer var. *tephrotrichus* (Fr: Secr.) Gill.	
	P.rickii Bres.	
	P.lindquistii Sing.	
Calyptrati	*P.calyptratus* (Lindb.) Sacc.	China
Pleurotus	*P.ostreatus* (Jacqu: Fr.) Kummer	Korea, China, Japan, USA, UK, Switzerland, Netherlands, Germany, Sri-Lanka, Portugal, Slovakia
Pleurotus columbinus Quel.	*P.ostreatus* (Jacqu: Fr.) Kummer var. *columbinus* (Quel. Apud Bres.) Quel.	Japan, USA, Germany, Slovakia
	P.pulmonarius (Fr.) Quel. : Fr.	Korea, China, Japan, Germany, Portugal, New Zealand
	P.citrinopileatus Sing.	Korea, China, Japan
	P.ostreatoroseus Sing.	
	P.opuntiae (Dur. & Lev.) Sacc.	
	P.macropus Bagl.	
	P.laciniatocrenatus (Speg.) Speg.	
	P.euosmus (Berk.) Sacc.	
	P.phellodendri (Sing.) Sing.	
	P.araucariicola Sing.	
	P.pantoleucus (Fr.) Sacc.	
	P.prometheus (Berk. & Curt.) Sacc.	
	P.yuccae Maire	
	P.convivarum Dunal & Delile	
	P.parthenopejus (Comes) Sacc.	
	P.salignus (Schrad.) Quel.	
	P.importatus Henn.	
	P.gemmellari (Inz.) Sacc.	
Coremiopleurotus	*Pleurotus cystidiosus* O.K. Miller	
	P.abalonus Han, Chen & Cheng	
Lentodiellum	*Panus concavus* Berk. *Pleurotus concanvus*(Berk.)Sing.	China, Japan
	P.levis (Berk. & Curt.) Sing.	
	P.strigosus (Berk. & Curt.) Sing.	
	P.fockei (Miquel) Sing.	
	P.calyx (Speg.) Sing.	UK
	P.sajor-caju (Fr.) Sing.	China, USA, Sri-Lanka, Australia
	P.squarrulosus (Mont.) Sing. Ex Pegler	USA, Sri-Lanka
	P.floridanus Sing.	UK
	P.subtilis (Berk.) Sing.	
Tuberregium	*P.tuber-regium* (Rumph.Fr.) Sing.	China, Sri-Lanka, Australia

3. Agronomic Practices

Pleurotus spp. is generally referred as the oyster mushroom because the pileus or cap is shell-like, spatulate and the stipe is eccentric or lateral. *Pleurotus ostreatus* (Jacq.: Fr.) Kummer is one of the best known species among the oyster mushrooms. Other commonly cultivated species include *P. sajor-caju, P. cystidiosus, P. eryngii* and *P.tuberregium* (Chang and Miles, 1989). Various species of these wood-rotting fungi are found all over the world and this mushroom is especially appreciated in Asia for its edibility.

The oyster mushroom has many advantages as a cultivated mushroom: rapid mycelial growth, high ability for saprophytic colonisation, simple and inexpensive cultivation techniques and several kinds of species available for cultivation under different climatic conditions. In addition, oyster mushroom is low in calories, sodium, fat and cholesterol, while rich in protein, carbohydrate, fibre, vitamins and minerals. These nutritional properties make this mushroom as a very good dietary food. In addition, consumption of oyster mushroom has positive effects on the general human health because of a number of special substances (Kues and Liu, 2000). Owing to these attributes during recent years, the production and consumption of this mushroom has increased tremendously and is ranked second to the button mushroom. The high ability to degrade the lignin-cellulose of *Pleurotus* spp. was also used in eliminating of the xenobiotic pollutants such as pentachlorophenol (PCP), dioxin, polycyclic aromatic hydrocarbons (PAHs). This suggests the possibility of new usage of this mushroom for environmental bioremediation (Kubatova *et al*., 2001; Hirano *et al*., 2000).

Despite its usefulness as food and bioconversion materials, three notable disadvantages persist in the cultivation of oyster mushroom. First, the oyster mushroom is quick to spoil and so is presentable to the market for only a few days. Secondly, the spore load generated within the growing room can become a potential health hazard to workers thus pickers can become allergic to the spore. Sporeless strains, which tend to have short gills and are thicker fleshed, prolonging storage, are highly sought after by oyster mushroom growers. Thirdly, the growers must wage a constant battle against the intrusion of mushroom flies. Oyster mushroom attracts Sciarid and Phorid flies to a far greater degree than any other group of mushrooms.

Table 1.26 Production of oyster mushrooms under commercial cultivation in some countries (Chang, 1993; Kues and Liu, 2000)

(Unit: Mt)

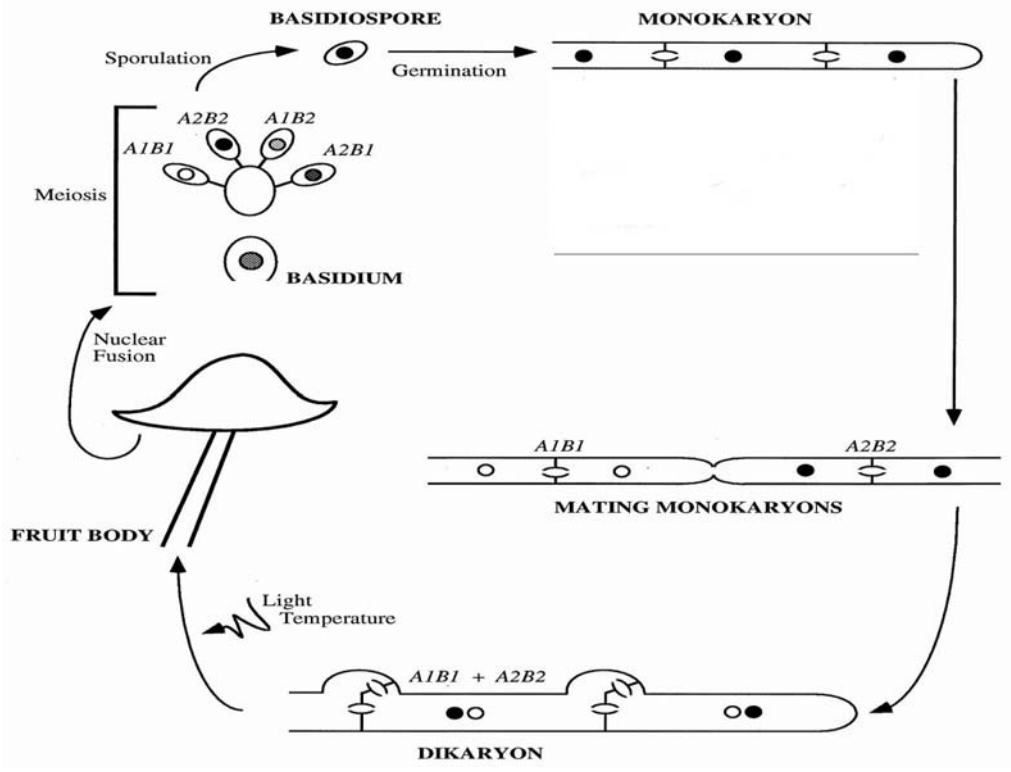
Oyster mushroom production			
Countries	**1991**	**1994**	**1998**
China	800 000	654 000	
Japan	33 475	20 441	
USA	695	900	
Indonesia	15 000	1 000	
Thailand	7 000	15 000	
Spain		100	
Netherlands		150	
Italy		1 500	
UK		150	
Germany		1 000	
France		2 000	
South Korea	51 782	72 810	75 684
Taiwan	3 500		
India	600		
Hungary	2 500		
Total	914 552	696 241	

4. Life Cycle and Growth

A. Life cycle of Pleurotus ostreatus

The major events in the life cycle of *P. ostreatus* could be described as follows (Figure 1.27). A single basidiospore germinates to be a mass of homokaryotic mycelium, each cell of which contains a single haploid nucleus. The homokaryotic mycelia continue to grow until the hypha fuse with the other hyphae which have compatible mating type. After fusion between compatible homokaryotic hyphae, reciprocal nuclear migration occurs and a heterokaryotic mycelium is formed. The subsequent growth involves the synchronous division of the two nuclei in each compartment and their regular distribution as nuclear pair throughout the mycelium via clamp connections. Heterokaryotised mycelia with enough mycelia mass and appropriate environmental stimuli (cooling 10 - 21℃, relative humidity 85-90%, and light requirement 1000-2000lux, CO_2 < 1000 ppm) can form the fruit bodies. During fruit body formation, nuclear fusion and meiosis occur only in the specialised basidia. Haploid nuclei migrate into a tetrad of basidiospores, external to the basidium. Each basidium has commonly four monokaryotic basidiospores. Occasionally five or more have been observed. These spores germinate into homokaryotic hyphae

Figure 1.7 Life cycle of the *Pleurotus ostreatus*



Source : Casselton, 1995

B. Requirement for mycelial growth

The carbon sources suitable for mycelial growth are starch, glucose, fructose, maltose, mannose, sucrose, pectin, cellulose and lignin. Ethanol is also a source of carbon for mycelial growth; however, citrate, oxalate and other organic acids are not beneficial to the growth of the mycelium. The nitrogenous

sources utilised by *Pleurotus* spp. are peptone, corn steep liquor, soybean cake powder, yeast powder, ammonium sulfate, asparagine, serine, alanine and glycine. The utilisation of urea is rather poor.

The optimal temperatures for growth of the mycelium are around 25-28 °C and the range of pH is about 5.5 to 6.5. The tolerance of mycelia for CO_2 is rather strong. The mycelia of *Pleurotus* spp. can still grow flourishingly at the carbon dioxide concentration of 15 to 20%. Only when the concentration of CO_2 is raised to 30% does the growth of mycelia rapidly decrease (Chang and Miles, 1989).

C Requirement for fruit body formation

For fruiting body formation, CO_2, light and temperature is key environmental factors. When the CO_2 concentration in the mushroom house or growing bags is higher than 600 ppm (0.06%), the stipe elongates and the growth of the caps will be prevented. The requirements for light are different for the various stages of growth. The growth of mycelium does not need any light and cultivation of the oyster mushroom in a dark place is better than in a bright place. The formation of primordia and the growth of fruiting bodies require light. The former requires light of 200 lux intensity for over 12 hrs. The growth of the fruiting body requires light of 50 to 500 lux intensity. The colour of the caps is closely related to the intensity of light, and if it is low, then the colour will be pale. The optimal temperatures for the development of fruiting bodies can range from 10 to 18 °C (Chang and Miles, 1989). Growers can choose a suitable strain for their own natural environment. Each *Pleurotus* species needs different environmental conditions for fruitbody development as illustrated in Table 1.27 (Stamets, 1993; Kang, 2004).

Table 1.27 Environmental parameters for fruiting of oyster mushroom

Species	Temp. (°C)	Relative humidity (%)	CO_2 (ppm)	Light (lux)
P. pulmonarius	21-29	90-95	<1,000	500-1,000
P. cystidiosus	21-27	85-90	<2,000	500-1,000
P. djamor	20-30	85-90	500-1,500	750-1,500
P. eryngii	15-21	85-90	<2,000	500-1,000
P. euosmus	21-27	90-95	<1,000	750-1,500
P. ostreatus	10-21	85-90	<1,000	1,000-1,500
P. pulunonarius	18-24	85-90	400-800	1,000-1,500
P.tuberregium	30-35	85-90	<2,000	

5. Sexual Reproduction and Crosses

A. Mating system and gene flow potential

P. ostreatus is heterothallic (self-sterile) and sexual reproduction is governed by the mating type genes. Mating type genes prevent mating between genetically identical cells. *P. ostreatus* has a bifactorial tetrapolar incompatibility mating systems which has two unlinked mating type factors designated A and B (Eugenio and Anderson, 1968). Factor A controls nuclear pairing, clamp cell formation, coordinate cell division and clamp cell septation whereas factor B is responsible for the control of nuclear migration, septa dissolution and clamp cell fusion. Two monokaryotic mycelia are compatible if they have different alleles at both loci. Multiple allelism for mating type genes was first noted by Terakawa (1957) and amply demonstrated in a sample of over 20 dikaryons collected from nature by Eugenio and Anderson (1968). The latter investigators estimated that there are a total number of 63 A types and 190 B types in the natural world-wide population of this species. Because of this multiple allelism of mating type, the out breeding potential is estimated close to 100% in nature and the inbreeding potential can be as low as 25%.

The spore of *P. ostreatus* usually gets off the gill and away from the mushroom cap. Once the spores have cleared the bottom of the cap, air currents carry them away. When the spores are a few millimetres

away from the cap they can be picked up by the faster winds and carried considerable distances thus enabling them to cross with the same species. However, no data are available regarding how far they can travel into the open air. Due to its nature of heterothallism, the spores of *P. ostreatus* behave like open pollinated crops. Therefore, appropriate measures should be taken to avoid unwanted gene flow when *P. ostreatus* is cultivated.

B. Interspecific cross

Interspecific cross was reported among *P. ostreatus, P. florida, P. columbinus* and *P. sapidus* (Peberdy *et al*, 1993). These species are ambiguous in specification of *Pleurotus*. Some scientists said that the species are the same species. There are several reports concerning interspecific crosses involving *Pleurotus* species based on protoplast fusion (Yoo and Cha, 1993).

C. Monokaryotic fruiting

Monokaryotic fruiting has been reported on more than 34 species in basidiomycetes (Stahl and Esser, 1976). *P. ostreatus* has also been found the monokaryotic fruiting (Kim, 2000). Esser *et al.* (1979) proposed that two genes, fi1+ and fi2+, are responsible for initiation of fruiting, and Kim (2000) demonstrated the mating type switching in the homokaryotic fruiting stains.

6. Genetics of *P. Ostreatus*

A. Genome size

The study of genome organisation in *P. ostreatus* has been hampered by the small size of fungal chromosomes. Different authors reported different chromosome numbers and genome sizes for this species (Sagawa and Nagata, 1992, Peberdy *et al*., 1993, Chiu, 1996). Recently, by using Pulse Field Gel Electrophoresis and linkage mapping, eleven chromosomes were resolved per haploid genome which added up to a total genomic size of 35Mb in average as shown in Table 1.28. Each chromosome has size from 1.4Mb to 4.7Mb. The use of chromosome-specific single-copy probes resolved the ambiguities caused by chromosome co-migration (Larraya *et al*., 2000).

Table 1.28 Estimated chromosome size of *Pleurotus* spp

Chromosome	*P.ostreatus*	*P.florida*	*P.sajor-caju*	*P.pulmonarius*	*P.columbinus*	*P.sapidus*
I	4.70	5.1	5.70	5.70	5.70	5.50
II	4.35	4.7	5.10	5.30	4.70	4.60
III	4.55	4.1	3.10	5.10	4.30	4.30
IV	3.55	3.8	2.50	4.50	3.60	3.80
V	3.45	2.7	2.00	3.10	3.10	3.30
VI	3.10	2.2	1.60	2.70	2.50	2.30
VII	3.15	1.6	-	2.00	1.80	1.40
VIII	2.95	1.1	-	1.60	1.40	0.90
IX	2.10	0.7	-	-	-	-
X	1.75	-	-	-	-	-
XI	1.45	-	-	-	-	-
Total genome size (Mb)	**35.1**	**26.00**	**20.00**	**30.00**	**27.10**	**26.10**

Source : Perberdy *et al*., 1993)

B. Linkage map

Using 80 monokaryons derived from one commercial strain, segregation of 196 markers was studied. The linkage analysis allowed to associate the markers into 11 linkage groups which span a total of 1000.7 cM. Also this linkage map was used for QTL mapping associated with growth rate of monokaryon and dikaryon (Larraya *et al*., 2000).

C. Transformation

Although commercial transgenic mushroom strains are not available, molecular breeding studies of the mushrooms have been carried out world-wide. The Netherlands, the United Kingdom, Japan, Spain and the United States are among the leading countries in mushroom biotechnology including the development of transformation systems. Possible target genes for transformation include: senescence genes to improve mushroom quality; substrate utilisation genes to enhance yields; and developmental genes to control mushroom fruiting. There are numerous potential pest and disease resistance targets, including genes involved in response to fungal pathogens, toxicity to insects and natural pest resistance. In addition, transformations with mating type genes that regulate inter-strain compatibility can alter breeding behaviour.

Transformation of *P. ostreatus* was firstly reported by Peng *et al*. (1992). Peng *et al*. transformed the homokaryotic strain using the protoplast and electroporation. They used the pAN7-1 vector which is a common vector used in ascomycetes and has a hygromycin selection marker. Yanai *et al*. (1996) reported the transformation using bialaphos selection marker. Kim *et al.* (1999) developed the transformation system using uracil auxotrophic mutant and the corresponding gene. Honda *et al.* (2000) developed the carboxin resistance gene using *in vitro* mutagenesis of iron-sulfur protein subunit of succinate dehydrogenase gene. Currently, Irie *et al.* (2001) reported the genetically modified *P. ostreatus* strain with an expression system for recombinant genes.

D. Conservation of genetic resources

Storage at ultra low temperatures has proved to be the most successful method for the prevention of degenerative changes in filamentous fungi. Therefore, for long term storage, liquid nitrogen storage is generally used for *P. ostreatus*. International Mycological Institute (IMI) reported the successful storage of *P. ostreatus* mycelia in liquid nitrogen for 23 years (Smith, 1993).

7. Pests and Diseases

Although the mushroom itself is a fungus, it can in turn be affected by a range of fungal pathogens, bacterial diseases, viral diseases and insect pests listed as follows:

A. Fungal pathogens

Pleurotus ostreatus

Bolbitius coprophilous (Peck) Hongo
Chrysonilia sitophila (Mont) Arx: Red Bread Mould
Cladobotryum apiculatum (Tubaki) W. Gams & Hooz.: Brown Spot, White Soft Rot
Cladobotryum dendroides (Bulliard: Merat) W. Gams & Hoozemans: Cobweb, Cobweb Disease, Cobweb Mould, Mildew, Soft Decay, Soft Mildew
Cladobotryum variospermum (Link) Hughes: Cobweb
Cladosporium spp.
Fusarium equiseti (Corda) Saccardo(1886)

Fusarium pallidoroseum (Cooke) Saccardo (1886): Pleurotus Wilt
Fusarium spp.
Gibberella fujikuroi (Sawada) Ito (1931): Pleurotus Wilt
Gibberella zeae (Schweinitz) Petch (1936): Pleurotus Wilt
Gilmaniella humicola G.L. Barron
Mucor spp.
Penicillium spp.: Blue-Green Mould, Green Mould
Rhizomucor spp.
Trichoderma hamatum (Bonord) Bain: Green Mould, Grune Schimmel
Trichoderma spp.: Green Mould, Grune Schimmel
Verticillium fungicola (Preuss) Hassebrauk: Dry Bubble, Fungus Spot, Lamole, Verticillium Brown Spot, Verticillium Disease
Verticillium spp.

Pleurotus

Aphanocladium album (Preuss) W.Gams
Arthrobotrys pleuroti
Calcarisporium spp.: Cobweb Disease
Cephalotrichum sp.: Black Mould
Chaetomium spp.
Cladobotryum spp.
Coprinus spp.: Ink Cap, Inky Cap
Dactylium spp.
Doratomyces sp.: Black Mould
Mucoraceae spp.
Nigrospora spp.
Peziza spp.
Trichurus spp.: Black Mould

B. Bacterial disease

Pleurotus ostreatus

Pseudomonas aeruginosa (Schroeter 1872) Migula 1900: Brown Blotch, Mummy Disease
Pseudomonas agarici Young (1970): Brown Blotch, Drippy Gill, Yellow Blotch
Pseudomonas fluorescens Migula 1895 Biovar: Brown Blotch
Pseudomonas fluorescens Migula 1895 Biotype G (=Biovar V): Bacterial Mummy Disease
Pseudomonas gingeri Preece & Wong 1982 (not validly published): Bacterial Blotch, Ginger Blotch
Pseudomonas tolaasii Paine 1919: Bacterial Blotch, Bacterial Brown Blotch, Brown Blotch, Mushroom Blotch

Pleurotus

Pseudomonas spp.: Pseudomonad

C. Insect pests

Pleurotus ostreatus

Cyllodes biplagiatus Le Conte: Beetle

Hexarthrius davisoni Waterhouse: Beetle
Hypogastrura (Ceratophysella) *armata* (Nicolet, 1842): Mushroom Springtail, 'Gunpowder Mite'
Leiomyza laevigata Meigen: Fly
Leucophenga maculata (Dufour): Vinegar Fly
Lycoriella auripila (Winnertz): Mushroom Sciarid, Black Fungus Gnat
Lycoriella bispinalis Yang and Zhang: Mushroom Sciarid
Lycoriella epleuroti Yang and Zhang: Mushroom Sciarid
Lycoriella jipleuroti Yang and Zhang: Mushroom Sciarid
Lycoriella jingpleuroti Yang and Zhang: Mushroom Sciarid
Lycoriella pleuroti Yang and Zhang: Mushroom Sciarid
Lycoriella yunpleuroti Yang and Zhang: Mushroom Sciarid
Lycoriella spp.: Black Fungus Gnat
Megaselia flavinervis (Malloch): Mushroom Phorid, Scuttle Fly, Humpbacked Fly
Megaselia rubescens (Wood): Mushroom Phorid, Scuttle Fly, Humpbacked Fly
Megaselia spp.: Mushroom Phorid, Scuttle Fly, Humpbacked Fly
Monoclona sp.: Fungus Gnats
Mycetophila oculus Walker: Fungus Gnat
Mycophila spp.: Mushroom Yellow Cecid Fly, Gall Midge
Mycophila speyeri (Barnes): Mushroom Yellow Cecid Fly, Gall Midge
Oxyporus (Pseudoxyporus) lateralis Gravenhorst 1802: Rove Beetle
Oxyporus (Oxyporus) rufipennis Leconte 1863: Rove Beetle
Oxyporus stygicus Say 1834: Rove Beetle
Oxyporus (Oxyporus) vittatus vittatus Gravenhorst 1802: Rove Beetle
Pheidole nodus Smith: Ant
Phorodonta flavipes Meigen: Black Fungus Gnat
Rhymosia domestica Meigen: Fungus Gnat
Scaphisoma convexum Say: Beetle
Scaphisoma stephani Leschen and Lobl, 1990: Beetle
Sciara fenestralis Zetterstedt: Fungus Gnat
Silvicola cinctus (Fabricius, 1787):Fly

Pleurotus

Bleptina sp.: Moth, Cutworms, Armyworms
Cyllodes ater (Herbst, 1792): Beetle
Cyllodes literatus (Reitter): Beetle
Dasyses barbata (Christoph): Fungus Moth
Dasyses rugosella Stainton: Fungus Moth
Heteropezina cathistes Pritchard: Gall Midge
Hydnobioides pubescens Sen Gupta and Crowson: Beetle
Megaselia chaetoneura (Malloch): Mushroom Phorid, Scuttle Fly, Humpbacked Fly
Megaselia frameata Schmitz: Mushroom Phorid, Scuttle Fly, Humpbacked Fly
Megaselia giraudii (Egger): Mushroom Phorid, Scuttle Fly, Humpbacked Fly
Megaselia plurispinulosa (Zetterstedt, 1960): Mushroom Phorid, Scuttle Fly, Humpbacked Fly
Megaselia sylvatica (Wood, 1910): Mushroom Phorid, Scuttle Fly, Humpbacked Fly
Mycomya duplicata Edwards, 1925: Fungus Gnats
Mycetophila ruficollis Meigen: Fungus Gnat
Mycomya marginata (Meigen, 1818): Fungus Gnats
Onthophagus villaneuvai Delgado-Castillo and Deloya, 1990: Scarab Beetle

Phanerota dissimilis (Erichson): Rove Beetle
Phanerota fasciata (Say): Rove Beetle
Pleurotobia tristigmata (Erichson): Rove Beetle
Rondaniella sp.: Fungus Gnat
Sciophila lutea Macquart, 1826: Fungus Gnat
Symbiotes spp.: Beetle
Ulodes spp.: Beetle

D. Nematodes

Pleurotus ostreatus

Species name not given: Gill Knot Disease
Aphelenchoides composticola Franklin (1957): Mycophagous Nematode
Ditylenchus myceliophagus Goodey (1958): Mycophagous Nematode
Paraphalenchus myceliophthorus Goodey (1958): Mycophagous Nematode
Rhabditis axei (Cobbold) Dougherty (1955): Bacterial Feeding Nematode
Rhabditis spp.: Bacterial Feeding Nematode

E. Molluscs

Pleurotus ostreatus

Meghimatium striatum van Hasselt (1823): Slug

F. Mites

Pleurotus ostreatus

Acarus immobilis Griffith, 1964: Acarid Mite
Histiostoma feroniarum (Dufour, 1839): Bacterial Feeding Mite
Proctolaelaps spp.: Ascid Mite
Rhizoglyphus echinopus (Fumouze et Robin, 1868): Bulb Mite
Rhizoglyphus spp.: Acarid Mite
Sancassania spp. indet: Acarid Mite
Tarsonemus spp.: Tarsonemid Mite
Tyrophagus longior (Gervais, 1844): Seed Mite

G. Viruses

Pleurotus ostreatus

Partitiviruses and Totiviruses

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SECTION 12
CAPSICUM ANNUUM COMPLEX

(Chili peppers, Hot peppers and Sweet peppers)

1. Introduction

Capsicum annuum L. is a dicotyledonous flowering plant commonly grown worldwide, with many general names in English, such as hot pepper, chili, chilli or chile pepper, and as well sweet pepper and bell pepper. Sometimes the plant is just called pepper, which however is often reserved for the earlier known Asian *Piper nigrum* (black pepper, white pepper) in the family Piperaceae. The pre-Columbian, indigenous Nahua (Aztec) Amerindian name for the plant was transcribed as chilli or chili, and the usual name in Spanish is chile, which results in the plurals of chillies, chilies, and chiles (Bosland 1996). Other broad names for *C. annuum* relate more to particular varieties or strains, culinary uses, and ripeness, such as jalapeño, Cayenne, pimento (pimiento), paprika, red, and green peppers. Furthermore, four other less commonly cultivated *Capsicum* species are also considered chile peppers, and two of these species are similar and closely related to *C. annuum*.

Capsicum annuum is usually grown as a herbaceous annual in temperate areas. However, ecologically it is a perennial shrub in tropical areas (which may live a few years to a few decades), and it can be grown as a perennial in climate-controlled greenhouses. This species includes the vast majority of the cultivated pungent and non-pungent (sweet) *Capsicum* peppers in temperate as well as some tropical areas. In the species *C. annuum* throughout the world, there is phenotypic diversity in plant habit and especially in shapes, sizes, colours, pungency, and other qualities of the fruits (Andrews 1995, 1998, 1999; DeWitt and Bosland 1996; Greenleaf 1986). This immense horticultural, agricultural and biological diversity has helped to make *C. annuum* globally important as a fresh and cooked vegetable (*e.g.* for salads, warm dishes, pickled) and a source of food ingredients for sauces and powders and as a colourant, which is used as well in cosmetics (Andrews 1995, 1999; Bosland 1994; Bosland and Votava 2000). Moreover, the species is used medicinally and medically, and provides the ingredient for a non-lethal deterrent or repellent to some human and animal behaviours (Krishna De 2003; Cordell and Araujo 1993; Palevitch and Craker 1995; Cronin 2002; Cichewicz and Thorpe 1996; Reilly *et al*. 2001). Chili peppers are also cultivated ornamentally especially for their brightly glossy fruits with a wide range of colors.

Chili pepper comprises numerous chemicals including steam-volatile oil, fatty oils, capsaicinoids, carotenoids, vitamins, protein, fibre, and mineral elements (Bosland and Votava 2000; Krishna De 2003). Many chili pepper constituents have importance for nutritional value, flavour, aroma, texture, and colour. The ripe fruits are especially rich in vitamin C (Osuna-García *et al*. 1998; Marin *et al*. 2004). The two chemical groups of greatest interest are the capsaicinoids and the carotenoids. The capsaicinoids are alkaloids that give hot chili peppers their characteristic pungency. The rich supply of carotenoids contributes to chili peppers' nutritional value and colour (Britton and Hornero-Méndez 1997; Hornero-Méndez *et al*. 2002; Pérez-Gálvez *et al*. 2003).

2. Taxonomy and Cytology

The genus *Capsicum* L. is in the large family Solanaceae, which includes as food the potato (*Solanum tuberosum*), tomato (*Lycopersicon esculentum* or *Solanum lycopersicum*), tree tomato (*Cyphomandra betacea* or *Solanum betaceum*), eggplant (*Solanum melongena*), African eggplants (*Solanum macrocarpon*, *S. aethiopicum*), husk or strawberry tomato (*Physalis pruinosa*) and Cape gooseberry (*P. peruviana*), as well as tobacco (*Nicotiana tabacum*), medicinal plants such as deadly nightshade (*Atropa belladonna*) and *Datura stramonium*, ornamentals such as tree daturas (*Brugmansia*) (which are also hallucinogenic) and *Petunia*, and weeds such as black nightshade (*Solanum nigrum*) (Knapp 2002; Hunziker 2001; George 1985). *Capsicum* is in the subfamily Solanoideae and tribe Capsiceae (Olmstead *et al*. 1999; Knapp 2002; Knapp *et al*. 2004; Hunziker 2001). The genus *Capsicum* consists of about 25 wild and 5 domesticated species (Table 1.30) (IBPGR 1983; Eshbaugh 1993; Bosland and Votava 2000).

The five variously domesticated species are *Capsicum annuum* (Table 1.29), *C. frutescens*, *C. chinense*, *C. baccatum* and *C. pubescens* (Heiser and Smith 1953; Smith and Heiser 1957; Heiser 1985). *Capsicum annuum*, *C. frutescens* and *C. chinense* are grouped in a taxonomic complex which has conventionally three, or perhaps two or one species (Pickersgill 1988), with the three clusters of domesticated plants appearing to be more divergent than their wild progenitors (Heiser 1985; Eshbaugh 1993; Prince *et al*. 1995; Idu and Ogbe 1997; Park *et al*. 1999; Bosland and Votava 2000; Walsh and Hoot 2001; Jarret and Dang 2004; Ryzhova and Kochieva 2004; Baral and Bosland 2004). The remaining two domesticated species are in other taxonomic complexes of the genus (Eshbaugh 1993; Tong and Bosland 1999; Walsh and Hoot 2001). Both are little used beyond Latin America, although *C. baccatum* var. *pendulum* (Willd.) Eshbaugh, the variety that has been extensively domesticated, is much used there. For a while, the name *C. frutescens* instead of *C. annuum* was applied to the domesticated chili peppers (Bailey 1923), so in some literature caution is needed to ascertain whether the plants discussed are actually *C. annuum* (which is more likely), or *C. frutescens* itself (*sensu stricto*, *i.e.* in the narrowly circumscribed sense) or perhaps another of these species (Heiser and Pickersgill 1969; Heiser 1985).

Table 1.29 Classification of Capsicum annuum

Taxonomic placement	Scientific name
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Solanales
Family	Solanaceae
Genus Species	*Capsicum* *annuum*
Botanical varieties	var. *glabriusculum* (synonym var. *aviculare*) var. *annuum*

Capsicum species are diploids, with most having 24 chromosomes ($n = x = 12$), but with several wild species having 26 chromosomes ($n = x = 13$) (Table 1.30) (Pickersgill 1991; Tong and Bosland 2003). *Capsicum annuum* has 24 chromosomes; usually 2 pairs (or sometimes 1) are acrocentric, and 10 (or 11) pairs metacentric or sub-metacentric (Lanteri and Pickersgill 1993). Its nuclear DNA content (determined by flow cytometry and Feulgen densitometry) has been reported to have a mean 1C-value of 3.38 picograms per nucleus, which Moscone *et al*. (2003) discuss in relation to other reports with varying methodology that range from 2.76 to 5.07 pg per nucleus. The total length of the chili pepper genome has been estimated to be between 1498 cM and 2268 cM, which is approximately two to three times larger than the tomato genome (Kang *et al*. 2001; Schreiber 2004).

3. Centres of Origin and Distribution

The centre of diversity for *Capsicum* is in south-central South America (Eshbaugh 1980; Hunziker 1979; D'Arcy and Eshbaugh 1974; Gonzalez and Bosland 1991; WWF and IUCN 1997), with the majority of species having some range in Brazil and/or Bolivia. Some of the non-domesticated species are gathered for occasional use. The primary centre of origin for domesticated *C. annuum* is in semi-tropical Mexico (Hernández-Verdugo *et al*. 1999, 2001a; Andrews 1995; Long-Solís 1998; Whitmore and Turner 2002). The four other domesticated species are usually believed to have originated in South America (Eshbaugh *et al*. 1983; Walsh and Hoot 2001; Denevan 2001). The centres of origin and domestication of the other two species in the *C. annuum* complex are not as clear (*cf*. Clement 1999). Amazonia (in the northern area) is considered the centre for *C. chinense* ("habanero") (Velez 1991; Toquica *et al*. 2003), and western Amazonia is perhaps the centre for *C. frutescens* ("tabasco"), which is more domesticatedly variable in Central America (Heiser 1985; Hernández-Verdugo *et al*. 1999). Bolivia is considered the centre of domestication for *C. baccatum* (ají) (in the subtropical east) and *C. pubescens* (rocoto) (in the mid-elevation Andes, where known only in cultivation) (Eshbaugh *et al*. 1983; Eshbaugh 1993).

By molecular analysis, Loaiza-Figueroa *et al*. (1989) confirmed that the centre of domestication of *C. annuum* var. *annuum*, the cultivated variety, is the upland region (Sierra Madre Oriental) of central-eastern Mexico (in the states of Nuevo León, Tamaulipas, San Luis Potosí, Veracruz and Hidalgo). Its ancestor probably is the wild chiltepín (bird pepper), *C. annuum* var. *glabriusculum* (Dunal) Heiser & Pickersgill, which has a range of unclear natural extent from southern USA through Mesoamerica to Colombia and the Caribbean, and is sometimes wild-harvested still and semi-domesticated as well (Eshbaugh 1993; Hernández-Verdugo *et al*. 2001a; Votava *et al*. 2002; Vásquez-Dávila 1996; Guzmán *et al*. 2005).

The earliest archaeological evidence of *Capsicum* being used dates to 10,500 BP (*C. baccatum*) and 10,000-9,500 BP (*C. chinense*) in the western Central Andes of Peru (Brack 2003; Smith 1980). Substantial evidence in Peruvian dry coastal river valleys, with specimens increasing from rare to moderately abundant through eight millennia, indicates expanding cultivation and domestication of *Capsicum* (Pearsall 2003). *Capsicum frutescens* is recognised in the northwestern area by 4400-3200 BP (Brack 2003). Intriguingly, *Capsicum* seems to be absent from the more recent Chiribaya culture of 1100-600 BP in far southern Peru (Flamini *et al*. 2003), even though earlier (2200-1400 BP) in southwestern Peru *C. frutescens* was being used. The importance of *Capsicum* is suggested by an obelisk from Chavín de Huántar (≈ 2800 BP) in the north-central Peruvian Andes, featuring a foundational Earth-crocodilian associated with (apparently) gourd, chili pepper, manioc (cassava) and peanut (Brotherston 1979; Miller and Burger 1995). Similarly, there is archaeological evidence from about 9000 BP for the use and subsequent domestication of *Capsicum annuum* in central-eastern and south-central Mexico in the states of Tamaulipas (near Ocampo), Puebla (Tehuacán Valley) and Oaxaca (Guilá Naquitz) (Pickersgill 1984; Bosland 1996; *cf*. Smith 2001, 2005).

Capsicum was brought to Europe by Columbus in 1493 as the peppery spice that signified the success of his quest, and the early European voyagers to the Caribbean, Mesoamerica and South America encountered a plethora of variety and landraces of this common food as well as medicinal plant (Sauer 1966; Long-Solís 1998). The ready appeal of *Capsicum* was such that within half a century it had been distributed as far as Asia, and it has been integrated and continues to be diversified in cultures worldwide as it had been originally in the Americas (Ferrão 1992; Andrews 1992, 1995, 1998, 1999; DeWitt and Bosland 1996; Eshbaugh 1983; Yamamoto and Nawata 2004, 2005).

The species of *Capsicum* were listed with their seemingly natural distributions by the International Board for Plant Genetic Resources (IBPGR 1983) and updated by Eshbaugh (1993), Hernández-Verdugo *et al*. (1999) and Bosland and Votava (2000); they are listed in Table 1.30 as currently understood. There is modest uncertainty on the generic limits of *Capsicum* and more uncertainty on its tribal relatives (which at minimum include *Lycianthes*) (Eshbaugh 1993; Hunziker 2001; Knapp 2002), and a lack of consensus on the number and in a few cases the botanical names of the known *Capsicum* species, and on the truly natural distributions of several species (rather than confounding naturalised with native populations).

Table 1.30 The species of Capsicum and their known or apparently natural distributions; those with haploid chromosome number n=13 rather than n=12 are noted (Tong and Bosland 2003). The five domesticated species are grouped into the C. annuum complex (3 spp.) (CA), the C. baccatum complex (CB), and the C. pubescens complex (CP) (Tong and Bosland, 1999; Walsh and Hoot, 2001; Jarret and Dang, 2004; Ryzhova and Kochieva, 2004)

Species	Known or probable natural distribution
C. annuum L. (C_A)	southern USA to Colombia
C. baccatum L. (C_B)	Peru, Bolivia, Paraguay, Argentina and Brazil
C. buforum Hunz.	southern Brazil
C. campylopodium Sendtner; *n* = 13	southern Brazil
C. cardenasii Heiser & P.G. Smith (C_P)	northeastern Bolivia
C. chacoense Hunz. (C_B or C_A)	Argentina, Paraguay and Bolivia
C. chinense Jacq. (C_A)	(northern) Amazonian South America
C. coccineum (Rusby) Hunz.	Bolivia and Peru
C. cornutum (Hiern) Hunz.	southern Brazil
C. dimorphum (Miers) Kuntze	Colombia
C. dusenii Bitter	southeastern Brazil
C. eximium Hunz. (C_P)	Bolivia and northern Argentina
C. flexuosum Sendtner	Argentina, Brazil and Paraguay
C. frutescens L. (C_A)	western Amazon (Colombia to Peru)
C. galapagoense Hunz. (C_A)	Galápagos Islands (Ecuador)
*C. geminifolium (*Dammer) Hunz.	Colombia and Ecuador
C. hookerianum (Miers) Kuntze	Ecuador and northwestern Peru
C. lanceolatum (Greenman) Morton & Standley; *n* = 13	Honduras, Guatemala and Mexico
C. leptopodum (Dunal) Kuntze	Brazil
C. minutiflorum (Rusby) Hunz.	Argentina, Paraguay and Bolivia
C. mirabile Mart. ex Sendtner; *n* = 13	southern Brazil
C. parvifolium Sendtner	northeastern Brazil, Venezuela and Colombia
C. praetermissum Heiser & P.G. Smith (C_B) [synonym *C. baccatum* var. *praetermissum* (Heiser & P.G. Smith) Hunz.]	southern Brazil
C. pubescens Ruiz & Pavón (C_P)	Bolivia to Colombia [only in cultivation]
C. rhomboideum (Dunal) Kuntze [synonym *C. ciliatum* (Kunth) Kuntze]; *n* = 13	Mexico to Peru
C. schottianum Sendtner; *n* = 13	southern Brazil, Paraguay and Argentina
C. scolnikianum Hunz.	northwestern Peru
C. tovarii Eshbaugh, P.G. Smith & Nickrent (C_B)	south-central Peru
C. villosum Sendtner	southern Brazil

4. Morphological Characters and Molecular Markers

A. Morphological characters for identification

The five domesticated species are differentiated by using morphological characters that rely primarily on colour and morphology of flowers and seeds (Andrews 1995; DeWitt and Bosland 1996),

as shown in Table 1.31. However, identifying some plants in the diverse *C. annuum* complex can be problematic (Pickersgill *et al*. 1979; Bosland and Votava 2000; Jarret and Dang 2004; Baral and Bosland 2004). Capsaicinoid profiles are not reliable as unique indicators for identification, though the profile may be useful as a supplementary character. In one study (7-58 accessions per species), the accuracy of identification based solely on capsaicinoid profiles, in the *C. annuum* complex, was 82% of the *C. chinense* accessions, 57% for *C. annuum* and just 20% for *C. frutescens* (but its sample was only 10 accessions), and similarly was 59% for *C. baccatum* and 86% for the distinctive *C. pubescens* (Zewdie and Bosland 2001).

Table 1.31 Morphological characters that generally differentiate the domesticated species of Capsicum

Species	Flowers per node	Calyx	Corolla colour	Corolla lobe basal spots	Anther colour	Seed colour
C. annuum var. *annuum*	1 (-5)	no ring; often teeth	white to dingy white (rarely purple)	none	blue-purple	straw (tan)
C. frutescens	usually 2-4 (1-6)	no ring; usually no teeth	greenish white or greenish	none	blue-purple	straw (tan)
C. chinense	(1-) 2 (-5)	annular ring; no teeth	greenish white or white	none	blue	straw (tan)
C. baccatum var. *pendulum*	1 (-2)	no ring; teeth	white (cream) or greenish-white	yellow-green	white to yellowish	straw (tan)
C. pubescens	1	no ring; teeth	purple or purple-white	none	purple (purple-white)	black (brown/ black)

Source : after Lippert et al., 1966; Heiser, 1985; Greenleaf, 1986; Eshbaugh, 1993; Jarret and Dang, 2004

Cultivated *Capsicum annuum* var. *annuum* is very diverse regionally and worldwide (*e.g.* DeWitt and Bosland 1996; Andrews 1995, 1998), having a wealth of innumerable strains, landraces and varieties that defy both facile description (IPGRI *et al*. 1995; Zewdie *et al*. 2004) and clustering into an inclusive and practicable classification (Bosland and Votava 2000). Sometimes typical characteristics (fruit shape, size, and pungency) have been featured and organised, recognising the Cerasiforme Group (cherry peppers), Conoides Group (conical peppers), Longum Group (*e.g.* Cayenne peppers) and Grossum Group (blocky sweet or bell peppers) (*cf.* Mabberley 1998; Bailey 1923), but as more plants are considered the array of variations and combinations of notable traits increases (*e.g.* fruit sizes and shapes intermediate, whether fruits are erect or pendent), and the groups become less distinct and meaningful.

B. Molecular markers

Various molecular markers have been used for identification of chili peppers, and to evaluate their germplasm diversity. A review is provided by Lefebvre (2005). Rodriguez *et al*. (1999) found diagnostic RAPD (randomly amplified polymorphic DNA) markers for four of the domesticated species (and *C. chacoense*), but not for *C. frutescens*. Use of isozymes has focused predominantly on measuring genetic variability, and clarifying phylogenetic relationships within the genus (Eshbaugh 1993). Conicella *et al.* (1990) analysed esterase isozymes in 15 accessions of *C. annuum* from the Americas; these data plus cytological data also provided evidence that Mexico is the centre of domestication for *C. annuum*.

Prince *et al.* (1995) utilised RAPDs in studying molecular polymorphism in *C. annuum*. Lefebvre *et al*. (2002) developed an integrated intraspecific *C. annuum* molecular linkage map using phenotypic and isozyme markers, known functional genes, RAPDs, RFLPs (restriction fragment length

polymorphisms) and AFLPs (amplified fragment length polymorphisms) from F_1 and F_2 hybrids derived from double-haploid *C. annuum* populations. Using RAPDs and AFLPs on a broad array of *C. annuum* types (from 34 accessions of nine countries), Paran *et al*. (1998) separated large-fruited sweet peppers from small-fruited pungent peppers, and found more genetic variation among the pungent cultivars. Tam *et al*. (2005) found the SSAP (sequence-specific amplification polymorphism) marker system generally more informative than using AFLPs or SSRs (simple sequence repeats or microsatellites). They had similar overall results between large-fruited sweet (bell) types and conical types, but within their pungent as well as sweet conical types the grouping of some cultivars shifted depending on which of the three marker systems was employed.

Tanksley (1984b) developed the first linkage map of *Capsicum* by studying segregating isozymes in an interspecific cross between *C. annuum* and *C. chinense*. Genomic similarities and differences between *Capsicum* and *Lycopersicon* were studied by Tanksley *et al.* (1988) to construct the first RFLP linkage map of chili pepper (with 85 loci). A more complete map was developed by Prince *et al*. (1993), and Livingstone *et al*. (1999) provided a still more comprehensive comparative map with over 1000 loci using mainly AFLP and RFLP markers. Repetitive DNA sequences make up a maximum of 10% of the chili pepper genome, which overall is at least four times more copies than in tomato (Schreiber 2004). Kang *et al*. (2001) constructed a more complete interspecific (*C. annuum* × *C. chinense*) F_2 molecular linkage map using mainly pepper-derived RFLP probes and AFLP markers, and Lee *et al*. (2004a) augmented the map using SSRs. Paran *et al*. (2004) constructed an integrated genetic linkage map of these *Capsicum* spp., consisting of 2262 diverse markers (including several known gene sequences) and covering 1832 cM and 13 linkage groups (with only 15 gaps > 10 cM remaining). An RFLP-based map (92 markers) of an interspecific (*C. annuum* × *C. frutescens*) BC_2 population has also been constructed (Rao *et al*. 2003).

5. Reproductive Biology and Ecology

A. Reproductive organs (morphology, development), fertilisation, dispersal and germination

Flower

Capsicum annuum starts flowering at the axil of the first branching node, with subsequent flowers forming at each additional node (Bosland and Votava 2000). Usually *C. annuum* has a solitary flower at the axil (Bosland and Gonzalez 1994), although some accessions have a few clustered flowers between which there are short internodes (Smith and Heiser 1951) (Table 1.31). Flower differentiation is not affected by daylength (Cochran 1942). The most important factor determining differentiation is air temperature, especially at night (Bosland and Votava 2000; Aloni *et al*. 1999; Rylski 1972).

The *Capsicum* flower is bisexual, hypogynous and usually pentamerous (Bosland and Votava 2000). The flowers are complete, with calyx, corolla, and male and female sex organs. The diameter of a *C. annuum* flower is 9-15 mm. The *Capsicum* calyx is broadly campanulate, ribbed, about 2 mm long, and truncate or undulate to weakly or prominently dentate with 5-7 teeth. The short-tubed corolla is rotate in most *Capsicum* species, with usually 5 but sometimes 6-7 (-8) petals in some species. The number of corolla lobes and stamens is equal. Typically the flowers have 5 stamens; the filaments are white or violet depending on the species (or variety), with the usually connivent to free anthers varying from bluish-purplish to yellow and white depending on the species (*e.g.* Table 1.31) (Dharamadhaj and Prakash 1978). The pistil comprises an ovary of 2-3 (-4) carpels that is 2-5 mm long and 1.5-5 mm in diameter, a style 3.5-6.5 mm long, and a capitate papillate stigma slightly wider

than the style. The style extends well beyond to just beyond the anthers or may be even with them, or it may be slightly exceeded by the anthers.

The daily start of anthesis apparently is controlled by daylength (Aleemullah *et al*. 2000). The corolla typically opens within the first 3 hours after sunrise, and the petals remain open for less than a day; there also can be a smaller peak of anthesis in the afternoon. Hirose (1957) found dehiscence of the anthers to occur late in the morning, between 10 am and noon. The anthers open lengthwise from typically 1 hr after the flower opens to even 10 hrs afterward, but they frequently fail to dehisce entirely, or may dehisce the next morning if the flower opens in late afternoon (Aleemullah *et al*. 2000; *cf.* Horner and Wagner 1992). Depending on the environmental conditions and variety, the period of receptivity of the stigma is 5-8 days, from several days before anthesis to fewer days afterwards, with maximum fertility on the day of anthesis (Cochran and Dempsey 1966; Barai and Roy 1986; Aleemullah *et al*. 2000).

Pollen and fertilisation

The pollen grains of chili pepper are medium to light yellow, subspheroidal, pitted, and tricolporate with longitudinal grooves (Bosland and Votava 2000). The plant has about 1-1.5 mg of pollen per flower (Quagliotti 1979), with 11,000-18,000 pollen grains in a single anther (Hirose 1957). Air temperature has a large effect on pollen formation and viability. Temperatures above 30°C occurring 15 days prior to anthesis cause pollen sterility (Cochran 1938), and night temperatures of 10 ± 2 °C reduce the number and germinability of pollen grains (Shaked *et al*. 2004). The optimal temperature for pollen germination is 20-25°C. Pollen tube growth from the stigma to the egg has been reported to take 6 to 42 hrs. In detailed anatomical studies, Cochran (1938) found that fertilisation occurred 42 hrs after pollination in plants grown at 27°/21°C, whereas Kato (1989) found that 36 hrs were needed for the fertilisation process.

Male sterility is found in *Capsicum*, controlled by cytoplasmic and nuclear genes (Shifriss 1997; Wang *et al*. 2004; Kalloo *et al*. 2002). In plants of both types, the anthers may be small and shrunken and blue-violet, with little or no viable pollen (Wien 1997), or there may be no anthers (Derera *et al*. 2005).

Fruit

There is extensive diversity in fruit shape, size, wall thickness and fleshiness, colour and pungency (Andrews 1995; IPGRI *et al*. 1995), determined by genetic and environmental factors. Among the innumerable varieties of *C. annuum*, the diversification of shapes of the pod (fruit) is striking — *e.g.* blocky (or lantern- or bell-shaped), globose, oblong (sausage-shaped), ovoid, conical, cylindrical, banana-like (curved); and smooth, grooved, lumpy or wrinkled. The length of the pod varies from less than 1 to 32.5 cm. The pedicel length also varies in different pod types (over several cm), and the fruit may be erect to pendent (deflexed). Fruit colours range from green, yellow, orange and red to purple, brown, black, and white as well. Some of the genetics of fruit colour and shape are becoming well understood (Ben Chaim *et al*. 2003; Thorup *et al*. 2000; Huh *et al*. 2001).

Morphologically the *Capsicum* fruit is a berry, sometimes with a few stone cells (sclerified inclusions in the fleshy portion) (Knapp 2002). The pericarp consists of epidermal cells in regular order with a thick-grooved cuticle. Several rows of collenchymatously thickened beaded cells constitute the hypodermis. The mesocarp is formed by thick-walled beaded cells; the inner mesophyll cells are thin-walled ground parenchyma and fibrovascular bundles. Giant cells (perhaps unique to *Capsicum*) occur on the inner wall of the endocarp (Fridvalsky and Nagy 1966). The vascular bundles consist of xylem tissue with spiral vessels and phloem tissue. The pod has two, three or four locules,

with each corresponding wall of the axile placenta having vesicles for production of capsaicinoids (Suzuki *et al*. 1980).

Usually there are many more flowers than fruits (Marcelis *et al*. 2004). The most obvious sign of assimilate competition or dominance among the organs is abscission of flowers and small fruits during the most active fruit-growth period, resulting in a cycling of flowering and fruit set (Hall 1977; Clapham and Marsh 1987; Bhatt and Srinivasa Rao 1997; Marcelis *et al*. 2004). The most actively growing organ of a chili pepper plant after flowering is the fruit (Hall 1977; Beese *et al.* 1982; Marcelis and Baan Hofman-Eijer 1995). Fruit growth is dependent on ovule growth (whether fertilised). The fruit is ordinarily seeded, but parthenocarpic forms exist (Heuvelink and Körner 2001). The seed set affects development and subsequent growth of the fruit; on average there is a direct linear relationship between the number of seeds per fruit and final fruit size, until saturation at perhaps over 200 seeds per fruit (Marcelis and Baan Hofman-Eijer 1997). The number of seeds per fruit ranged from 1 to 34 in wild northwestern Mexico populations of *C. annuum* (Hernández-Verdugo *et al*. 2001b). A low of 50-100 seeds per cultivated fruit (20-30% of maximum) is sufficient for maximal fruit set (Marcelis and Baan Hofman-Eijer 1997); blocky sweet pepper (bell pepper) may average 150-300 seeds per fruit (Aloni *et al*. 1999).

The time from anthesis to a fully grown fruit varies considerably among different pod types (Bosland and Votava 2000). Typically cultivated fruit reaches the mature green stage in 35-50 days after the flower is pollinated. This stage is horticulturally ripe for some uses, but still physiologically immature. Fruit maturity depends on the cultivar, and the environmental conditions before and during maturation (Perry *et al*. 1993; Montes Hernández *et al.* 2004). The fruits are characterised as non-climacteric in ripening (Lownds *et al.* 1993), apparently lacking the typical increase in CO_2 and ethylene production as they ripen (Saltveit 1977).

The fruits of most *Capsicum* are pungent, because the placenta accumulates capsaicinoids (*e.g.* capsaicin) (Zewdie and Bosland 2001; Thompson *et al*. 2005), except in domesticated non-pungent (sweet) varieties which are mostly developed in *C. annuum* (Bosland and Votava 2000). The pungency trait is controlled at a single locus on chromosome 2; when the pungency gene *Pun1* (also called *C*) is homozygous recessive (*i.e.* present as *pun1/pun1* or *cc*), the capacity to make capsaicinoids is lost (Stewart *et al*. 2005; Blum *et al*. 2002). In the pungent chili peppers, other genes variously affect the synthesis of capsaicinoids (Blum *et al.* 2003), and production is also affected by physiological interactions and the environment (Zewdie and Bosland 2000a; Estrada *et al*. 2002; Sung *et al*. 2005). The individual fruit's pungency (content of capsaicinoids) is affected by its node position on the plant, whereas its capsaicinoid profile remains fairly constant (Zewdie and Bosland 2000b; Estrada *et al*. 2002; Kirschbaum-Titze *et al*. 2002). Capsaicinoids increase with fruit growth to a maximum (*e.g.* 40-50 days after fruit set), then decline (Contreras-Padilla and Yahia 1998). Capsaicinoids can be transported within the plant, with different capsaicinoid profiles found in stems and leaves (Estrada *et al*. 2002).

Fruit dispersal

The red fruits of wild *C. annuum* var. *glabriusculum* attract birds, which eat them and disperse viable seeds, but their pungency discourages consumption by wild mammals (Vásquez-Dávila 1996; Tewksbury *et al*. 1999; Tewksbury and Nabhan 2001). Rats experimentally fed hot chili peppers for 2-11 months became desensitised to aversion, but indifferent rather than developing a preference for this spicy food (Rozin *et al*. 1979). Nonetheless, the widespread and common little yellow-shouldered bat (*Sturnira lilium*), which sometimes favors solanaceous fruits (Passos *et al*. 2003; Galindo-González *et al*. 2000), has been reported to consume pungent *Capsicum* in northwestern Argentina and

disperse the seeds — which is favored by local people who recognise this increases the number of wild plants, as they gather the fruits for home seasoning and village marketing (Iudica 1999).

Seed and germination

The seed develops from a campylotropous ovule (Dharamadhaj and Prakash 1978). Within a pod, the many seeds are attached to the placenta walls in close rows, mainly near the calyx end. The seeds are disk-like with a deep chalazal depression. The embryo is surrounded by a well-defined endosperm which makes up the bulk of food reserves for the embryo and young seedling. The endosperm lies directly in front of the radicle and consists of seven to nine thick cells (Watkins *et al*. 1985). *Capsicum annuum* seeds have mainly protein and lipids as storage reserves (Chen and Lott 1992). The seed is covered by a parchment-like seed coat, which does not cause a mechanical restriction to germination (Watkins and Cantliffe 1983b). Seed colour inheritance involves at minimum about three genes (Zewdie and Bosland 2003). Seed size is somewhat dependent on the variety and growing conditions. Seed mass maturity may occur about 50 days after anthesis, with 10-12 more days required for maximum potential longevity but 17-21 days for maximal seedling dry weight (based on variation in time from sowing to emergence) (Demir and Ellis 1992). An average *C. annuum* seed is about 5.3 mm long, 4.3 mm wide and 1 mm thick, with a surface area of 33 mm^2 (Chen and Lott 1992).

Freshly harvested seeds of certain wild *Capsicum* species can exhibit dormancy (Bosland and Votava 2000; Wien 1997; IBPGR 1983). An after-ripening period at room temperature may be required to remove dormancy (Randle and Homna 1981). As *C. annuum* seeds age and lose viability (Ozcoban and Demir 2002) they may become brown. Seed dormancy may be broken by treatment with 0.2 M KNO_3 under white light (750-1250 lux) and alternating temperatures (30°/20°C or 30°/15°C) (*cf.* Hernández-Verdugo *et al*. 2001b). Seeds of cultivated *C. annuum* can be cryopreserved at -196°C and moisture content of 4.7-11.5%, and subjected to rapid or slow freezing and thawing (Quagliotti and Comino 2003).

Capsicum species seeds germinate well in a constant temperature range between 15°C and 30°C (Randle and Homna 1980; *cf.* Dell'Aquila 2004), and do not germinate when exposed to temperatures below 8°C or above 40°C (Choi 1985). No special light requirements are necessary for germination of domesticated chili pepper seeds, whereas seeds of wild *C. annuum* var. *glabriusculum* do not germinate in constant darkness (Hernández-Verdugo *et al*. 2001b).

B. Sexual reproduction

Pollination

Capsicum species are usually self-compatible (Onus and Pickersgill 2004), and *C. annuum* is a partially self-pollinating crop (Allard 1960; Rylski 1986); wind or similar mechanical disturbance may enhance self-pollination (Raw 2000; Kristjansson and Rasmussen 1991). Outcrossing is associated with insect pollinators, less with wind (Odland and Porter 1941; Tanksley 1984a; Raw 2000). The proportion of plants cross-pollinated depends on several factors and can range from 2 to 90% (Pickersgill 1997); in many localities, cross-pollination is predominant. The effect of outcrossing on fruit set of *C. annuum* is significant. Nagarathnam and Rajamani (1963) obtained only 6-11% fruit set when flowers were isolated to self-pollinate. Erwin (1937) found that 46% of self-pollinated flowers set fruit, compared to 71% for flowers left to open-pollinate by bee activity. In field research *Capsicum* should be considered facultative cross-pollinating species (Odland and Porter 1941; Tanksley 1984a). Breeders and seed producers thus need to undertake precautionary measures to prevent uncontrolled cross-pollination (Bosland 1993). To produce large amounts of genetically pure seeds, seed certification programmes employ isolation as the control mechanism. Isolation

requirements may range from 400 m for the Certified class to 1.6 km for the Foundation class (NMCIA 1992) but depend on local conditions, for example being 300 m in Hungary but perhaps requiring 2-3 km or more in Australia (Derera *et al*. 2005).

The odourless flowers are visited by insects both for sugary nectar, which is mostly hexoses and low in daily amount (greatest on the day of anthesis), and also for their pollen (Rabinowitch *et al*. 1993; Vogel 1998; Roldán-Serrano and Guerra-Sanz 2004; Raw 2000). Solitary bees, honeybees, bumblebees, aphids and thrips are likely to transfer the pollen grains, especially those that obtain pollen by buzz pollination, shaking the anthers (Andrews 1995; Raw 2000; de Ruijter *et al*. 1991; Kubišová and Háslbachová 1991; Shipp *et al*. 1994; Dag and Kamer 2001; Kristjansson and Rasmussen 1991).

Crossability and hybridisation

Capsicum species do not hybridise with species in other genera of the Solanaceae (Berke 2000). Pepper breeding continues to be highly rewarding for the improvement of *Capsicum* (Poulos 1994; Berke 2000; Geleta and Labuschagne 2004). Interspecific crossing between many *Capsicum* species has been tried experimentally (often repeatedly) for agronomic and taxonomic purposes (*cf.* Walsh and Hoot 2001; Pickersgill 1991, 1997; Onus and Pickersgill 2004). Fertile hybridisations can occur between taxa within the *Capsicum annuum* complex to varying degrees (Jarret and Dang 2004; Nwankiti 1976; Kumar *et al*. 1987; Panda *et al*. 2004; Baral and Bosland 2004), and also these species with *C. baccatum* but not with *C. pubescens*; Table 1.32 below gives a synopsis (*cf.* Yoon *et al*. 2004). Similar interspecific spontaneous or natural hybrids of these species are difficult to ascertain, but infrequently surmised (Jarret and Dang 2004; Rodriguez *et al*. 1999). Their recognition is confounded by taxonomic uncertainty, the extensive variability from selection within the domesticated species for millennia to decades, and the plasticity of individual plants. Crossings between wild and semi-domesticated *C. annuum* var. *glabriusculum*, and between feral or weedy and domesticated *C. annuum* var. *annuum*, and these two complexes hybridising with each other, are probably a regular occurrence and vary in fertility (Jarret and Dang 2004; Guzmán *et al*. 2005; Hernández-Verdugo *et al*. 2001a; Prince *et al*. 1992; Pickersgill 1971). Crossing also is probable in many regions in the tropics between cultivated and feral *C. frutescens* (*e.g.* Yamamoto and Nawata 2004, 2005; Symon 1981; Wiggins and Porter 1971).

Table 1.32 Crossability (including hybrid viability) of Capsicum annuum with other Capsicum in the three complexes of domesticated species; see Table 1.30

Capsicum annuum (C_A) reciprocal crosses with other *Capsicum* species		
Interspecific parent and species complex		*Capsicum* sp. as female / male
C. frutescens	(C_A)	(+) / +
C. chinense	(C_A)	(+) / (+)
C. galapagoense	(C_A)	† / +
C. chacoense	(C_B or C_A)	0 / +
C. baccatum	(C_B)	+ / +
C. praetermissum	(C_B)	† / †
C. tovarii	(C_B)	0 / 0
C. pubescens	(C_P)	0 / 0
C. cardenasii	(C_P)	0 / †
C. eximium	(C_P)	0 / †

F_1 hybrids produce: viable seeds +, or some viable seeds (+); non-viable seeds † ; or no fruits and/or seeds 0 .

Source : after Pickersgill, 1971; IBPGR, 1983; Zijlstra *et al*., 1991; Tong and Bosland, 1999

C. Asexual reproduction

The chili pepper plant can be propagated asexually by means of cuttings and grafting. Young cut shoots form whole independent plants with roots *in vitro* as well as in the field (Choi *et al*. 1999; Shirai and Hagimori 2004). Scions from chili pepper plants graft successfully on stocks of chili pepper (Chung and Choi 2002) as well as tomato (Deloire and Hébant 1982). The grafted plants can set flowers and fruits. *Capsicum* grafting can induce genetic changes, which may provide variations of breeding value (Taller *et al*. 1998, 1999).

6. Crop Production and Use

Chili peppers are grown worldwide, either outside in fields or in greenhouses. The ability to produce a quality crop in such a wide range of climates and conditions has helped to make chili pepper a globally common crop. Because of the extensive cultivation, adaptation and variability of *C. annuum*, it is difficult to generalise to what is typical, and there is no single method for production (Bosland and Votava 2000).

A. Environmental conditions

Chili pepper is a warm-season crop (Rodríguez-Rey *et al*. 2000), and highly susceptible to frost. Watkins and Cantliffe (1983a) showed that at 25°C radicle emergence required 3.5 days, whereas at 15°C, 9 days were required. Seedling emergence from a soil depth of 1.2 cm took 8-9 days at temperatures from 25-35°C (Lorenz and Maynard 1980), but was prevented below 15°C (Wien 1997). The leaf unfolding rate of seedlings (based on maximum leaf count), which is also a measure of node and internode formation, was optimal at an average daily temperature of ≈ 26°C (Si and Heins 1996).

The plant is usually indeterminate and has continuous sympodial branching, with the individual branch systems apparently functioning as relatively autonomous integrated physiological units (Thomas and Watson 1988; *cf.* de Swart *et al*. 2004). For a high yield of good quality fruit, Bakker and van Uffelen (1988) found that mean air temperatures of 21-23°C were optimal during vegetative growth, followed by 21°C during fruit growth. The minimum temperature for growth and development is 18°C, below which growth is trivial, with plants in the 5-15°C range growing poorly (Sanders *et al*. 1980). The most growth in the vegetative stage occurs at 25-27°C day temperature and 18-20°C night temperature (Dorland and Went 1947; Bakker and van Uffelen 1988). Day temperature lower than

night temperature is detrimental to vegetative growth (as is a low night temperature of 12°C). Nonetheless, to grow compact greenhouse seedlings, higher night temperature is preferable (Si and Heins 1996; Sysoyeva and Kharkina 2000).

Maximum flower set occurs when day and night temperatures are between 21°C and 16°C. Flowers drop when the night temperature is above 24°C. Yields are high when the daily air temperature during fruit set ranges between 18-32°C. Fruits do not set when the mean daily temperature is above 32°C, or is below 16°C — or when cooler, the fruits are malformed (Olareweju 1988; Aloni *et al*. 1999). Productivity is constrained by the adverse effects of high temperature on fruit set, and the detrimental influence of low temperature on fruit shape (Rylski and Spigelman 1982; Rylski 1973).

B. Agricultural practices

The ideal soil for producing chili pepper is deep, well-drained, medium-textured sandy loam or loam that holds moisture and has some organic matter. Plants can be started by direct seeding, or by transplanting after initial growth in trays (Bosland and Votava 2000); the plants are started in greenhouses or hotbeds in many production areas, or in outdoor seedbeds in mild-climate areas. Chili pepper plants are transplanted when they are 6-8 weeks old. Prior to field planting, the plants should be hardened but not excessively.

Whether the field population is established by transplanting or direct seeded, the optimum crop is dependent upon row spacing and between-row spacing of the plants, and the type grown (Bosland and Votava 2000). Chili peppers require adequate amounts of most major and minor nutrients; the most-utilised are normally N and P. Plastic mulch maintains moisture in the soil; increases soil temperature and early yields; reduces weed populations, fertiliser leaching and soil compaction; and protects fruits from soil deposits and soil microorganisms. Competition between weeds and chili peppers for nutrients, light and water is a serious problem in production (Lee and Schroeder 1995). A successful weed control programme is essential for producing a healthy crop. Abiotic stresses include extreme temperatures, moistures, light, nutrients, pH, pollutants and pesticides.

Row covers or tunnel planting systems have been used for production in the field because of their effectiveness to alter microclimates. Chili pepper is sensitive to excessive water (Suh *et al*. 1987). Irrigation is not necessary in areas with regular and ample rain, although it generally is essential in arid and semi-arid regions. Chili pepper is a shallow-rooted crop (González-Cervantes *et al*. 2004). The amount and frequency of irrigation depend on soil type, bed type, plant size, humidity, wind, sunlight and prevailing temperatures. A limited supply of water during the rapid vegetative-growth period reduces the final yield (Beese *et al*. 1982; Srinivasa-Rao and Bhatt 1988; Sato *et al*. 2003). Fruits grown under water deficit may have a higher concentration of capsaicin (Sung *et al*. 2005).

Chili pepper plants can be made to behave perennially under greenhouse conditions, with environmental control carried out by air temperature regulation, supplemental light, and CO_2 enhancement as well. Regular removal of flowers leads to faster vegetative growth (Hall 1977; Clapham and Marsh 1987). In The Netherlands non-pungent chili peppers are greenhouse-grown on 1200 ha, and about a third of the workers develop an allergy to the pollen, which can be alleviated by introducing honeybees to remove pollen (Blacquière *et al*. 2004).

C. Biotic stresses

Production can be diminished by various biotic stresses. Chili pepper is susceptible to diseases and pests that can be primary constraints on cultivation (Bosland and Votava 2000; DeWitt and

Bosland 1993), and their control is one of the most important factors in producing a profitable crop. The diseases and pests usually reduce both quality and quantity of fruits.

Diseases from bacteria infecting the chili pepper plants include bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*), bacterial canker (*Corynebacterium michiganense* or *Clavibacter michiganensis* subsp. *michiganensis*), bacterial soft rot (*Erwinia carotovora* pv. *carotovora*) and bacterial wilt (*Pseudomonas solanacearum* or *Ralstonia solanaceraum*). The plants are susceptible to fungi which cause diseases such as anthracnose (*Colletotrichum* spp.), early blight (*Alternaria solani*), Cercospora leaf spot (*Cercospora capsici*), damping-off/seedling disease (*Pythium, Rhizoctonia, Fusarium, etc.*), Fusarium stem rot (*Fusarium solani*), grey mold (*Botrytis cinerea*), Phytophthora blight and root rot (*Phytophthora capsici*), powdery mildew (*Leveillula taurica* or *Oidiopsis taurica*), Rhizoctonia root rot (*Rhizoctonia solani*), Stemphylium leaf spot (*Stemphylium botryosum* f. sp. *capsicum*), gray leaf spot (*Stemphylium solani* and *S. lycopersici*), southern blight (*Sclerotium rolfsii*), Verticillium wilt (*Verticillium dahliae*) and white mold (*Sclerotinia sclerotirum*). Among the many viruses affecting chili peppers are alfalfa mosaic alfamovirus (AMV), cucumber mosaic cucumovirus (CMV), beet western yellows luteovirus (BWYV), pepper mottle potyvirus (PepMoV), pepper veinal mottle potyvirus (PepVMoV), potato potyvirus Y (PVY), tobacco etch potyvirus (TEV), pepper mild mottle tobamovirus (PepMMoV), pepper ringspot tobravirus (PepRSV), tomato spotted wilt tospovirus (TSWV), pepper golden mosaic bigeminivirus (PepGMV), pepper Huasteco bigeminivirus (PHV or PepHV), Texas pepper bigeminivirus (TPV) and beet curly top hybrigeminivirus (BCTV).

Production is affected by many insect pests such as cutworms, grubs (*Phyllophaga* spp.), flea beetles (*Epitrix* spp.), hornworms (*Manduca sexta* and *M. quinquemaculata*), grasshoppers, leafminers, fruit worms (*Heliothis assulta* and *H. zea*, *Spodoptera* spp. armyworms, *etc.*), European corn borer (*Ostrinia nubilalis*), green peach aphid (*Myzus persicae*), melon or cotton aphid (*Aphis gossypii*), leafhoppers, stink bugs, tarnished plant bug (*Lygus lineolaris*), thrips, whiteflies, chili weevil (*Anthonomus eugenii*) and chili pepper maggot (*Zonosemata electa*), and by spider mites (*Tetranychus* spp.) and nematodes.

Chili pepper production is also influenced by physiological disorders such as flower-bud abscission and flower abscission, blossom-end rot, sunscald, abnormal fruit shape, colour spotting, and fruit cracking.

D. Experience and world statistics

Chili pepper is harvested at different fruit stages, depending on the final use. Fresh chili pods often are harvested at a physiologically immature (but horticulturally mature) stage. The dehydrated and mash industries use physiologically mature fruits, generally showing red colour.

Chili pungency is measured by determining the capsaicinoids content of the fruit, which can be accomplished by several industrial (laboratory) procedures, and as well by a subjective dilution-and-detection test ("taste test") scored as Scoville Heat Units (Scoville 1912; Korel *et al*. 2002; Bosland and Votava 2000; Krishna De 2003; Reilly *et al*. 2001). Physiologically, capsaicinoids cause the heat sensation by activating and then desensitising certain sensory nerve fibres, which is mediated *via* a receptor (VR1) in the pain pathway (Caterina and Julius 2001; Bhave *et al*. 2002). Culinary or medicinal results can be favourable (Rozin 1990; Palevitch and Craker 1995), whereas exposure to excessive amounts can range from avoidance behaviour to severe toxicity (Krenzelok and Provost 1995).

The production of chili pepper for spice, vegetable, and other uses increases every year. It is estimated that it is annually cultivated on more than 1.5 million hectares, in numerous countries (FAO

2001). Forty-six percent of production is in Asia (with China the principal producing country). Southern Europe is the second most important producing region, with 24% of world production. The countries with harvest area of more than 70,000 ha are China, India, Indonesia, Mexico, Korea, Nigeria, Ghana and Turkey.

7. Modern Biotechnology

Modern biotechnology can provide benefit to the chili pepper crop by genetic improvement against diseases and insect pests; for enhanced chemical composition, such as in carotenoids and capsaicinoids; and for improved marketing (Bosland and Votava 2000; Ochoa-Alejo and Ramírez-Malagón 2001). Development of a genetically transformed plant requires two key systems: the genetic transformation itself, *i.e.* transferring gene(s) of interest into host cells; and plant regeneration from the host cells with the inserted gene(s). Some other species in the family Solanaceae, such as tobacco (*Nicotiana tabacum*), tomato (*Lycopersicon esculentum*) and potato (*Solanum tuberosum*), have been used as model systems because of their successful transformation and regeneration. Chili pepper however has been recalcitrant, with application of molecular biotechnology lagging because there was not a reproducible, reliable and efficient system of transformation and regeneration (Ochoa-Alejo and Ramírez-Malagón 2001), but these problems are now being overcome.

A few examples of efforts in the biotechnological development of *Capsicum* follow. Exposure to gamma radiation reduced the efficiency of chili pepper shoot regeneration (Sripichit *et al*. 1988). Streptomycin-resistant shoots and whole plants from cotyledon explants were achieved by a regeneration system and chemical mutagenesis [with ethylmethane sulfonate (EMS)] (Subhash *et al*. 1996). A high frequency of plastid-encoded antibiotic-resistant variants were isolated by Rao *et al*. (1997) from seeds and explants mutagenised with EMS or nitrosomethylurea. Dabauza and Peña (2001) improved the efficiency of organogenesis from seedling explants.

The first genetic transformation in chili pepper using modern molecular biotechnology was insertion of the genes for neomycin phosphotransferase and β-glucuronidase by means of *Agrobaterium tumefaciens* (Liu *et al*. 1990); however, these transformed cells did not regenerate into whole plants. Since then, developments in technique for *C. annuum* have been reported steadily, for example, a stable system of *Agrobacterium*-mediated transformation and *in vitro* plant regeneration (Lee *et al*. 1993), a refined protocol for transformation and regeneration (Manoharan *et al*. 1998), a system for highly efficient transformation (40.8%) along with efficient regeneration (Li *et al*. 2003), and the advances and refinements are continuing (Lee *et al*., 2004b; Mihálka *et al*., 2003).

Traits currently targeted for development of chili pepper include viral resistance to CMV, TEV and TMV (Cai *et al*. 2003), pest resistance against oriental tobacco budworm (*Heliothis assulta*) (Kim *et al*. 2002), altered fruit ripening, and prolonged shelf life. Diminishing cucumber mosaic virus disease has become a reality, after having developed the fertile transgenic plants with CMV resistance (Zhu *et al.* 1996; Kim *et al*., 1997). Genetically transformed *Capsicum annuum* with CMV resistance has been approved for commercialisation in China (Huang *et al*. 2002).

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Part 2

CONSENSUS DOCUMENTS ON TRAITS

SECTION 1

GENERAL INFORMATION CONCERNING THE BIOSAFETY OF CROP PLANTS MADE VIRUS RESISTANT THROUGH COAT PROTEIN GENE-MEDIATED PROTECTION

Summary Note

This document, developed under the auspices of the OECD's Expert Group on Harmonization of Regulatory Oversight in Biotechnology, is intended to provide information that may be of assistance to regulatory officials, developers of virus resistant plants,and other interested parties. Any decision on the use of virus resistant plants at the small- or large-scale stages of product development, or their commercial use, will require a case-by-case review by each Member country, as the specific environment in which such plants will be grown is a component of each of the issues addressed in this document.

The focus of this report is limited to issues that can be discussed in a general fashion without reference to the specific environment in which the transgenic plant is to be introduced. Therefore, any issues relating to the cultivation of the virus resistant plants or to the potential for, or potential effects of, gene transfer from a virus resistant plant to another crop plant or to a wild relative are outside the scope of this document. This document is not intended as an encyclopedic review of all the scientific experimentation on the use of genes to make plant species virus resistant.

The Expert Group identified three topics to be considered in this document as they relate to the use of one specific gene, the viral coat protein, whose expression in plants often results in a resistant phenotype. Two of these topics, transcapsidation and recombination, are known molecular mechanisms through which new virus types may arise; the third topic is the potential for specific synergistic effects that modify symptom development. These are biologically complex phenomena that may sometimes involve at least four different organisms: two viruses, an organism (most often an arthropod) that transmits the virus from plant to plant, and one or more host plant species. These phenomena are not understood in complete detail, and there is considerable research ongoing to address less well-understood aspects.

In some instances, the discussions in this document focus on particular taxa of plant viruses, either those for which the most information is available or those for which the risk issues can be most clearly identified. In addition, the document provides guidance on the biological and molecular information needed to characterise the virus from which the coat protein gene was isolated and the gene inserted into the transgenic plant. Also provided is a list of references that may be helpful in locating such information.

Further research on the basic biology of plant viruses may speed the development of genes for use in virus resistant plants that minimise the potential agronomic or environmental concerns associated with their use, and potentially reduce the likelihood that viral strains will arise that overcome the resistance trait.

1. General Introduction

The following document, developed by a Task Group under the auspices of the Expert Group on Harmonization of Regulatory Oversight in Biotechnology, is intended to be one in a series of documents of use in providing information to regulatory officials, developers of new products produced through biotechnology, and other interested parties.

This document is not intended as a definitive or encyclopaedic review of all the scientific experimentation pertaining to the use of viral coat protein genes to make plant species virus resistant, nor is it intended to dictate to regulatory authorities in any country how they should review requests for field testing, deregulation, or commercialisation of such plants. (For other information on virus resistant transgenic plants, see Hull, 1990, 1994; de Zoeten, 1991; Mansky and Hill, 1993; Falk and Bruening, 1994; Palukaitis, 1991; Tepfer, 1993, 1995; Wilson, 1993.) Rather, the document attempts to describe the current state of experience in Member countries with a particular set of issues pertaining to crop plants made virus resistant through coat protein gene-mediated protection. It draws upon a wide range of information sources, including not only the scientific literature but also risk assessments from Member countries and reports from national conferences and scientific meetings. In an effort to capture the current "state of the art", it also contains preliminary information that may not yet have received full and critical evaluation by the scientific community. Where such information appears, it is indicated as "preliminary".

The issues discussed in this document are a subset of issues that regulatory officials may consider in relation to crop plants made virus resistant through coat protein gene-mediated protection. The focus is limited to issues that can be discussed without reference to the specific environment in which the organism is to be introduced. Therefore, any issues relating to the cultivation of virus resistant plants or to the potential for, or potential effects of, gene transfer from a virus resistant plant to another crop plant or to a wild relative are outside the scope of this document, although these issues are valid considerations that may enter into regulatory deliberations by regulatory authorities in any country. Neither does this document address potential agronomic impacts in any Member country or any other issues that may relate to the potential international marketing of such crop plants.

This document focuses instead on the potential for effects of such genetically modified plants on some natural virus populations or on the severity of viral infections. Specifically, the Expert Group identified three topics to be considered in this document as they relate to the use of one specific viral gene, the gene encoding the viral coat protein, to confer virus resistance. Two of these topics, transcapsidation and recombination, are known molecular mechanisms through which new virus types may arise; the third topic is the potential for specific synergistic effects of particular introduced viral genes on infections with other viruses. These are biologically complex phenomena which may sometimes involve at least four different organisms: two viruses, a viral vector, and a host plant species. These phenomena are not understood in complete detail for all viruses, and there is considerable research ongoing in Member countries to address some of their less well-understood aspects. In some instances, the discussions in this document focus on particular taxa of plant viruses either for which the most information is known, or for which the risk issues can be most clearly identified.

This document represents a consensus of Member countries' positions on factors relevant to addressing the biosafety concerns raised in considering the three identified molecular mechanisms affecting viral populations and viral diseases of plants, as they relate to virus resistant crop plants mediated by CP genes. Current scientific information on these subjects may be sufficient to enable the conduct of case-specific, scientifically sound risk assessments and biosafety evaluations of currently developed varieties. This may enable competent authorities or regulatory officials in countries, after such reviews, to give authorisation for release or commercialisation of particular varieties. The document does not attempt to provide detailed, definitive, or general conclusions on the outcomes of such considerations, nor does it attempt to advise countries on how any such deliberations should be concluded. Member countries have agreed that such deliberations are conducted on a case-by-case basis. Results of particular evaluations of certain issues, as they relate to individual virus resistant crops, are presented for illustrative purposes.

2. Technical Introduction to Plant Viruses

Viral diseases cause significant economic losses to agriculture. Viral infections cause damage to fruits, leaves, seeds, flowers, stems, and roots of many important crop species. Under natural conditions, certain plant viruses are nearly always present on particular crop(s) or weed host(s). The types of symptoms produced in a specific plant vary depending on the virus, the specific strain of the virus, whether the plant is infected with another virus or other viruses, the cultivar of the host plant, and the environment. The severity of infection by a particular virus often varies from location to location and from one growing season to the next, reflecting the importance of the environment on symptom development and vector transmission rates for the virus. Some virus outbreaks have been sufficiently severe that entire plantings of target crops (*e.g.* sugar beet, citrus, and rice) have been destroyed in specific areas. Most crop species are routinely infected with several different viruses. The American Phytopathology Society's Compendium of Plant Disease series lists the important viruses affecting the major crops of the world. *Viruses of Tropical Plants* by Brunt *et al*. (1990) is a useful resource for viral disease of tropical plants.

Plant viruses may be spread in various ways, depending on virus type. Means of spread include: vector-mediated transmission, seed or pollen transmission, and mechanical transmission (whether by transfer of plant sap or by vegetative propagation of infected host tissue). Virus vectors may be nematodes, mites, fungi, or insects. In some environments and for certain viral diseases, substantial inputs of pesticides are needed to control particular vector organisms (typically insects) for serious viral diseases, even though the insects do not themselves cause significant damage to the target crop or could possibly be controlled by biological means. Control of the vector organism does not always result in complete or effective control of the viral disease. In addition, in certain environments particular crop species cannot be grown profitably because of the presence of persistent populations of infected plants and potential vectors. The situation with soil-borne (nematode or fungus transmitted) viruses is even more dire. If these infested vectors become established at a site, eradication or even satisfactory control is usually impossible or environmentally untenable. Unless resistant cultivars are available, cultivation of the susceptible crop at that site may have to be abandoned. Examples include infections of Indian peanut clump furovirus in groundnut in parts of India, and rhizomania disease in sugar beets [caused by beet necrotic yellow vein virus (BNYVV)] in the United Kingdom.

Plant viruses are relatively simple pathogens, in essence protein coats (capsids) wrapped around genomes of either DNA or RNA. Some capsids may also contain carbohydrates and/or lipids. The viral genome encodes at least its own nucleic acid replicating enzyme, (a) protein(s) required for movement of the virus throughout the plant, the viral coat protein(s), and often other necessary proteins. After entering a host plant cell, a virus particle (virion) uncoats, replicates copies of its genome, uses its CP gene to manufacture the protein subunits for the virus protein coat, and then assembles new virions. The new virions or infectious agents may spread to adjacent cells or be transported by vector organisms to other host plants.

Plant viruses are usually named according to the plant species in which they were first detected and the type of symptoms observed in infected plants. The genome of each plant virus is of a characteristic composition, DNA or RNA, either single-stranded or double-stranded depending on the virus. Some plant viruses contain more than one nucleic acid molecule within each virus particle. For other viruses, the genomes consist of more than one nucleic acid molecule, each packaged in a separate virion. Some viral infections are also associated with the production of satellite RNAs or satellite viruses. Satellite RNAs depend on a specific virus (called helper virus) for the replication enzymes needed to replicate their own RNA, are usually smaller in size than their helper viral genome, have no significant sequence homology to the helper virus genome, and affect disease symptoms (at least in some hosts) (Matthews, 1991). In satellite viruses, the satellite codes for its own coat protein, while satellite RNAs are packaged in the coat protein of the helper virus. Plant viruses are taxonomically grouped according to their nucleic acid

composition and other physical properties of the virions. Nucleic acid sequencing of hundreds of animal and plant viral genomes has revealed the evolutionary relationships among many viruses. An important reference for viral taxonomy is Murphy *et al*. (1995), a publication of the International Committee on Taxonomy of Viruses (ICTV).

Plant viruses have traditionally been controlled in agriculture using a variety of strategies with varying effectiveness: exclusion of contaminated material at national or state borders with accompanying virus identification (often by indexing); rogueing of infected crops; plant eradication measures, when appropriate; certification of virus-free stock or seeds (*e.g.* to control plum pox potyvirus in fruit trees and for many viruses in potatoes); use of agronomic practices designed to minimise virus spread or persistence (*e.g.* not planting a particular crop for a specified period of time in a given locality); conventional breeding for virus-resistant cultivars; and conventional cross-protection (*i.e.* pre-inoculation of plants with a mild strain of the virus to protect against severe infection by another strain of the same virus) (used to varying degrees to control tomato mosaic tobamovirus in tomatoes in Europe and Japan and citrus tristeza closterovirus in Brazil). Conventional cross-protection, while commercially important for some crops in certain localities, is only effective for certain viruses. It involves intentional infection of crop plants with a suitable closely related mild virus strain, provided that such a virus strain is available. Two of the main issues associated with the development of useful conventionally-bred, virus resistant crop lines have been 1) identification of breeding stock containing an appropriate resistant trait/gene(s); and 2) potential trade-offs between introgression of the virus resistant trait and other agronomically important traits in the crop itself.

Until 1994, when Whitman *et al*. cloned and sequenced the tobacco N gene conferring resistance to TMV, no plant-derived traditional viral resistance gene had been cloned or sequenced. The exact function of the N gene is still not understood, although recent evidence suggests its involvement in a common signal transduction mechanism for general pathogen resistance (Staskawicz *et al*., 1995). Nonetheless, introduction of traditional resistance genes into agronomically desirable cultivars has been used for decades to protect plants from viral infections even though their mode of action has not been understood. The lack of detailed understanding of the mechanism of traditional resistance genes, or traditional cross protection measures, has not prevented their use.

Another type of strategy for protecting a plant against viral disease involves introduction and expression of a gene encoding the viral CP in the genome of the plant itself. This type of strategy is referred to as "coat protein gene-mediated protection", and its effectiveness was first demonstrated on tobacco mosaic tobamovirus infection of tobacco in 1986 by Powell Abel *et al*. It provides heritable protection of the recipient plant species against the target virus, and frequently against related strains as well. This strategy has been demonstrated in laboratory or field experiments to be effective against at least 50 different viruses to date. Since that time, viral genes other than CP genes [dedicated movement proteins, replicase (polymerase), viral genes modified to contain ribozymes, satellite and defective interfering RNAs] have also been shown to confer a virus resistant phenotype on recipient plants. The growing number of genes used to encode virus resistance is more illustrative of the diversity of the viruses against which resistance is targeted than the plant species they infect. However, this document focuses exclusively on the biosafety of those genetically modified plants made virus resistant through the introduction of a viral CP gene, and on biosafety with respect to interactions of the modified recipient plant with other plant viruses in the environment.

3. Basic Information for Virus Characterisation

The information relevant for a biosafety review of an organism includes that which establishes the identity of the organism in question and that which describes the environments in which the organism is to be used. Any genetically modified plant protected against viral infection through CP gene-mediated

protection will potentially interact with the range of organisms with which the parental plant species can interact within the same environment. Characterisation of the virus which provided the transgene would include information on virus biology, taxonomy, genetics, and known viral interactions in the environment. This necessary information would include:

a. The taxonomic name of the virus, including family, genus, and strain designation, including any synonyms.
b. The type of nucleic acid contained in the virus.
c. Whether the infection is systemic or localised.
d. Whether the virus is restricted to specific tissues (*e.g.* phloem-limited).
e. Whether the virus is associated with any satellite or helper viruses.
f. The natural host range of the virus.
g. How the virus is transmitted.
h. If the virus transmitted by a vector, the identity of the vector including mode of transmission (*e.g.* persistent or non-persistent) and the identity of the viral gene(s) (if known) involved in vector transmission.
i. Whether any synergistic or transcapsidation interactions with other viruses under field situations have been reported in the literature.

In order to evaluate any potential biosafety concerns posed by the use of viral genes, viral sequences engineered into the plant should be well-characterised sequences that are derived from well-characterised viruses, and the specific biological properties of the actual strain utilised should be known. Characterisation of the strain from which the transgene is derived may enable determination of whether that strain is identical or nearly identical to the strain found in other countries. For example, beet necrotic yellow vein furovirus strain A that is widely prevalent in Japan, the United Kingdom, the Netherlands, and other parts of Europe is virtually identical to the strain found in the United States (Kruse *et al.*, 1994). A considerable amount of data on viral strains is readily available in scientific publications and from publicly accessible data bases.

For appropriate designations of most plant viruses, the official taxonomic body for virology is the International Committee on Taxonomy of Viruses (ICTV), which has published the accepted taxonomic names for most plant viruses (Murphy *et al.*, 1995). Relevant types of information to describe the virus in question are the complete name of the virus (including any synonyms), the family and genus names, the strain designation, the name of the disease incited, and the locality where the strain was first isolated. The molecular characteristics of a plant virus most important for describing the properties of viral infections are the type of nucleic acids contained, RNA or DNA, and whether those nucleic acids are single- or double-stranded (Murphy *et al.*, 1995). It is important to describe whether the virus replicates in all cells (*e.g.* tobamoviruses) or is limited to certain cells (*e.g.* phloem cells for luteoviruses).

Although an up-to-date, definitive, and concise list of the host ranges of all plant viruses is not available, several publications and Internet sites have a significant amount of useful information:

a. The Commonwealth Mycological Institute/Association of Applied Biologists' "Description of Plant Viruses" is a series of pamphlets describing the biology of several hundred plant viruses.
b. The USDA's *Plant Pests of Importance to North American Agriculture, Index of Plant Virus Disease* (Agriculture Handbook No. 307, 1966) provides a list of plants and the viruses that infect them.

c. The American Phytopathology Society's series on plant diseases of crops has up-to-date listings of viral diseases for the major crops. The Society also has a list of names of U.S. plant diseases and their causal agents.

d. The Australian Virus Identification Data Exchange (VIDE) is currently being promoted by the ICTV to establish a worldwide database dealing with plant viruses. The World Wide Web site for the database is: http://life.anu.edu.au/viruses/lctv/index.hmtl.

e. The British Society for Plant Pathology's *Names of British Plant Diseases and their Causes*, published in 1984, lists the English and European names of the diseases and the scientific names of the causal organisms, arranged by host plant.

f. The *European Handbook of Plant Diseases* by Smith *et al*. (1988) provides descriptions of the viral, bacterial, and fungal pathogens of European plants.

g. A World Wide Web site maintained by the Garry Laboratory at Tulane University (USA) has as its goal to provide a list of all virology sites on the Word Wide Web. This site can be accessed at http://www.tulane.edu/~dmsander/garryfavweb.html. A mirror site has been established to facilitate access in Europe at the University of Leicester (United Kingdom) (http://www-micro.msb.le.ac.uk/335/garryfavweb.html).

Many publications describe the host range of a particular virus. However, most lists do not describe the host range of specific viral strains. Host range is an important consideration for the three issues with which this document is concerned. Because of the number of different strains of a particular virus, information on the natural host range of the specific viral strain used as a donor organism may be more easily provided by the person who has engineered that plant than by a literature search. Information on the natural host range of a viral strain in managed and unmanaged ecosystems is probably more relevant than information on its "artificial" host range. The natural host range of a virus lists the plants growing in managed and unmanaged ecosystems that are commonly infected with the virus. The artificial host range includes plants that become infected when intentionally inoculated by man under controlled conditions but are not necessarily infected under natural conditions. The artificial host range of a virus includes more plant species than the natural host range (Matthews, 1991).

A definitive worldwide list of the geographical distribution of plant viruses is also unavailable. However, limited information on the geographical distribution of many plant viruses can be found in the references listed above. The United States Department of Agriculture (USDA) has a state-by-state list of occurrences of widely prevalent viruses on its World Wide Web Site (http://www.usda.gov/bbep/bp).

Viruses are transmitted by many vectors, including whiteflies, mites, nematodes, aphids, planthoppers, leafhoppers, beetles, thrips, and fungi. They can also be transmitted mechanically and through seed or pollen. For those viruses that are vector-transmitted, a single virus is transmitted under field conditions by a single vector group. Thus, as an example, three viruses from three different genera of the family Potyviridae, potato Y potyvirus (PVY) (genus *Potyvirus*), ryegrass mosaic virus (genus *Rymovirus*), and barley yellow mosaic virus (genus *Bymovirus*) are transmitted by unrelated types of vectors. In this example several aphid species transmit the first virus, the mite *Aceria tulipae* transmits the second, and the fungus *Polymyxa graminis* transmits the third virus. Each group of vectors transmits particular viruses worldwide (Murphy *et al*., 1995).

Although these three viruses are all in the same family, they are transmitted by only one specific type of vector. The high specificity of this virus-vector relationship is a result of interaction between specific vector-encoded receptors and the specific virus-encoded protein(s) that is unique to each virus (Murphy *et al*., 1995; Murant *et al*., 1988b). Identifying the major vectors of field importance (both scientific and common names) is part of the characterisation of both the virus itself and the recipient environments. In addition, if any viral genes have been identified as being implicated as required for vector transmission, the

nature of the genes and, briefly, how they are believed to be involved in vector transmission should be described.

4. Expression of Coat Protein Gene in Transgenic Plants Results in a Virus Resistant Phenotype

Powell Abel *et al*. (1986) showed that transgenic plants expressing the CP of tobacco mosaic tobamovirus (TMV) imparted resistance to TMV. Since that time, over 30 plants, both monocots and dicots, have been engineered to express more than 50 viral CP genes from ten taxa. Many of these have been field tested. One of the catalysts for this research has been knowledge of the phenomenon of cross protection, in which a plant infected with a mild strain of a virus is often protected from infection by a severe strain of the same virus. Although the exact mechanism by which cross protection works is not clear, evidence suggests that CP is involved with some viruses (Matthews, 1991).

Cross protection has been used in agriculture for many decades worldwide. Currently in Japan, more than half a million tomato plants (for both fresh market and processing uses) are cross protected against cucumber mosaic cucumovirus (CMV) (containing a satellite RNA of Japanese origin) (Sayama *et al*., 1993; Sayama, unpublished data). Tomatoes cross protected with tomato mosaic tobamovirus have been or are being consumed in Europe and Japan (classically-bred resistant cultivars are also widely used), citrus trees have been protected against citrus tristeza closterovirus in Brazil (Fulton, 1986), papaya trees have been protected with papaya ringspot potyvirus (as reviewed by Yeh *et al*., 1988), and zucchini plants have been protected with zucchini yellow mosaic potyvirus. Before indexing was widely used for virus elimination in potato, many potato seed pieces were infected with mild strains of many common viruses, including potato leaf roll luteovirus, potato X potexvirus, and potato Y potyvirus (Hooker, 1981), and therefore were cross protected using traditional techniques. These methods are still used for many vegetatively propagated plants, like strawberries, as well as florist and nursery crops.

Coat protein gene-mediated protection is best understood with TMV and tobacco. A brief summary of the current state of knowledge of this system is summarised below. For protection of tobacco to be effective, TMV CP must accumulate. Development of protection does not seem to involve the induction of the plant's natural disease resistance system. Resistance appears mainly to be based on blocking the uncoating of the CP in the incoming TMV. There is, however, evidence that a later step in infection is also affected (Reimann-Phillip and Beachy, 1993). It has been observed that protection is better when the CP is derived from a viral strain that naturally infects the recipient plant than when the CP is derived from a closely related strain that infects another host plant. Tomato plants expressing tomato mosaic tobamovirus (ToMV) CP gene, the tobamovirus most closely related to TMV, are better protected from ToMV infection in the field than tomato plants expressing tobacco mosaic tobamovirus CP (Sanders *et al*., 1992). Resistance derived from the CP gene of other viruses may have modes of action different from TMV.

Based on the success of CP gene-mediated protection during field testing, most plant virologists believe that CP gene-mediated resistance may be successfully applied for many but not all (Ploeg *et al*., 1993) single-stranded, positive sense RNA viruses, a group which includes over 75 per cent of all plant viruses (Beachy, 1993). More field tests of virus resistant plants have occurred in the U.S. than in any other OECD Member country. In the U.S., most but not all of the CP genes have been derived from viruses that commonly infect the recipient crop. A majority of the viral sequences that have been introduced into transgenic plants and field tested thus far have not been modified from the original sequence found in the parental virus, except for modifications related to cloning of the gene. A few CP genes have been modified so that the ability of the virus to be transmitted by its vector is significantly reduced; others have been isolated from strains that were non-transmissible by the vector under natural conditions. In some cases, expression of CP gene from a viral strain that does not naturally infect the plant can provide resistance to taxonomically related virus that may or may not naturally infect the plant (Stark and Beachy, 1989; Namba *et al*., 1992).

Another approach involves using a CP gene that has been modified by removing some of the nucleotide sequences from the gene, resulting in a truncated CP (Lindbo and Dougherty, 1992 a,b). Depending on how much of the gene is deleted, the CP derived from the truncated transgene may or may not be able to function in virion assembly (Lindbo *et al*., 1993). Dougherty's laboratory (Smith *et al*., 1994; Lindbo and Dougherty, 1992 a,b) has shown that a modified CP transgene that encodes a non-translatable mRNA may also provide protection. This resistance may result from direct interaction of transgene RNA and viral RNA, commonly referred to as RNA-mediated resistance, although host factors may also play a role in resistance (Smith *et al*., 1995).

Antisense expression (the production of complementary, non-coding transcript of a gene) of coat protein gene has generally not been as effective as sense expression in protecting plants against viral infections, although there are some notable exceptions (Hammond and Kamo, 1995; Kawchuk *et al*., 1991; Lindbo and Dougherty, 1992 a,b). This low success rate as compared to sense expression may not be unexpected, since antisense strategies act at the level of gene expression in the nucleus whereas most plant viruses multiply in the cytoplasm (Beachy, 1993). Whether antisense-, truncated sense-, or untranslatable sense-mediated resistance is as effective in providing immunity or resistance as sense CP-gene-mediated protection under field conditions needs further investigation. If the CP-derived transgene produces a CP which cannot encapsidate viral nucleic acid or does not produce a CP, this minimises the issues addressed in Section V(transcapsidation and synergy).

CP-gene mediated resistance will probably not be totally effective against virus strains that have satellite RNAs associated with them. These small RNAs can often modify the symptoms expressed by infected plants. Depending on the genotype of the host plant, the sequence of the small RNA, the helper virus, and environmental conditions, the symptoms may be attenuated or more severe (Matthews, 1991). Although satellites and defective-interfering RNAs have been detected in some viruses, their role in disease development under natural conditions is unclear. For the majority of viruses for which satellite RNAs have been detected, satellites are rarely found in virus-infected plants in the field, nor have they ever been shown to have caused a severe epidemic. There are two major exceptions. One is that of the satellites of CMV, which have caused serious disease epidemics in China, Italy, Japan and Spain in the past decade (Tien and Wu, 1991; Kaper *et al*., 1990). The other is groundnut rosette virus, of which all the isolates that cause rosette symptoms contain satellite RNAs (Murant *et al*., 1988a). Coat protein gene-mediated protection alone does not protect plants against infection if the virus contains satellite RNAs, so that additional measures are likely to be necessary for engineering effective protection against such satellite-containing viruses (Yie and Tien, 1993).

In the Sixth Report of the International Committee on Taxonomy of Viruses (ICTV), the genus *Umbravirus* was recognised with carrot mottle as the type species (Murphy *et al*., 1995). Umbraviruses have worldwide distribution, but have been found only in plants co-infected with luteovirus. Umbraviruses can be distinguished from luteoviruses based on the fact that Umbraviruses are manually transmissible, whereas luteoviruses are only aphid-transmitted. However, in the field Umbraviruses are genomically masked by luteoviral coat protein and thus aphid-transmitted. On the basis of biological properties, four Umbraspecies have been recognised by the ICTV and four additional candidate species have been proposed. No reports have been published regarding transgenic plants engineered to be resistant to Umbraviruses, and the luteovirus resistant plant likely to commercialised within the next few years (see Section V) contains a non-capsid gene as the source of the resistant phenotype. For further information, see the papers cited in the second paragraph of Section I.

5. Issues Related to Potential Effects of CP Gene-mediated Virus Resistance on Virus Infections

Although more than 50 virus resistant plants using CP gene-mediated resistance have been field tested worldwide to date, it is likely that only a limited number of these will be commercially available in the

next few years. Some of the virus resistant plants that may be eligible to be considered for commercialisation in the next few years in OECD Member countries might be:

beet necrotic yellow vein furovirus resistant sugar beets;
tomato mosaic tobamovirus resistant tomatoes;
potato leaf roll luteovirus resistant potatoes; *
potato X potexvirus resistant potatoes;
cucumber mosaic cucumovirus resistant tomatoes, peppers and cucurbits;
zucchini yellow mosaic potyvirus, watermelon mosaic potyvirus 2, and papaya ringspot potyvirus resistant cucurbits;
potato Y potyvirus resistant potatoes;
potato Y potyvirus resistant tobacco;
cucumber mosaic cucumovirus resistant tobacco; and
papaya ringspot potyvirus resistant papayas.

*In North America, Europe and Japan the PLRV resistant lines likely to be commercialised use a non-CP gene as source of the resistance phenotype. PLRV CP-mediated resistance has also been field tested in many countries.

Some plants that may be commercialised could contain combinations of the above resistance genes. An attempt is made in this document to highlight information or data which may be particularly relevant to the above listed viruses.

Three distinct interactions, transcapsidation, synergy, and recombination, have been observed to occur when two plant viruses (or two different strains of the same plant virus) simultaneously infect a cell. A brief description of each of these interactions is provided, followed by an analysis of how each may play a role when transgenic plants are made virus resistant through the use of CP gene-mediated resistance.

A. Transcapsidation

When a single plant cell is simultaneously infected by two different strains of a virus (or two viruses), it may be possible for the genome of one virus to become encapsidated by coat protein of the second virus. If the virus is encapsidated by coat proteins of both viral strains, the phenomenon is called phenotypic mixing (mixed encapsidation). If the virus is encapsidated by only one of the coat proteins, this is termed genomic masking or transcapsidation. (For simplicity, it will be assumed that the terms transcapsidation and genomic masking include the phenotypic mixing phenomenon, since the issues for all are identical). Transcapsidation has been reported to be important in only a few instances in field situations in insect transmission of viruses (Falk *et al*., 1995), even though field grown plants and trees are known to be infected with multiple viruses (Abdalla *et al*., 1985; Falk and Bruening, 1994).

Transcapsidation has been best studied with infections with different strains of the barley yellow dwarf luteovirus, where the phenomenon can be important in field situations in that coat protein determines which specific aphid vector transmits the virus (Matthews, 1991). This phenomenon has also been detected with potyviruses (Bourdin and Lecoq, 1991; Lecoq *et al*., 1993) and tombusviruses (Dalmay *et al*., 1992). (Similar preliminary results have also been reported with nepoviruses (Hiriart, 1995). The result of transcapsidation, a "masked" virion, has a mismatched coat that may or may not be sufficiently functional to allow transmission of the viral genome it contains to another host plant. The "mismatched" or heterologous viral coat is not maintained in subsequent rounds of viral infection, because subsequent production of coat protein subunits is directed by the viral coat protein gene carried in the genome.

Therefore, transcapsidation events are transient and any potential impacts can only persist with the first round of infection of the masked virus if it infects a susceptible host plant.

For some viral taxa, a protein other than CP is the primary determinant of whether a specific organism can successfully transmit a virus. These taxa include potyviruses, caulimoviruses, and waikaviruses (Murphy *et al*. 1995). This vector transmission protein is called a "helper component" in potyviruses and an "aphid helper transmission factor" in caulimoviruses (Murphy *et al*., 1995). Unless the appropriate vector transmission protein is present and functional, transcapsidated virions assembled with CP from a vector transmissible strain will not be efficiently transmitted by the "heterologous" insect vector (Berger *et al*., 1989; Atreya *et al*., 1990). In contrast, viral CPs apparently are the primary determinants for insect-transmissibility for geminiviruses and cucumoviruses (Matthews, 1991). In the fungus-transmitted furoviruses and the aphid-transmitted luteoviruses, the vector transmission protein is synthesised by read-through of the CP termination codon (Zaccomer *et al*., 1995; Schmitt *et al*., 1992; Wang *et al*., 1995). Rice tungro waikavirus is required for aphid-transmission of rice tungro bacilliform badnavirus, and thus probably encodes an aphid-transmission protein (Dasgupta *et al*., 1991). For some taxa, little is yet known about the nature of the protein(s) involved in vector transmission.

Two issues are important to be addressed in considering the likelihood and significance in any potential instance of transcapsidation in transgenic plants. As stated above, if a resistant plant was engineered with a gene that does not produce a CP, or produces one that cannot function in the assembly of virions, these issues need not be addressed:

1. Is there a sufficient amount of coat protein being produced by the transgenic plant to produce a masked virus? Is the CP found in the same or different tissue(s) where the virus is detected in a non-transgenic plant?

2. If a masked virus were produced, would it have any new biological properties (vector transmission and host range) and would any effects resulting from transcapsidation be measurable or significant?

As mixed infections by plant viruses of all taxonomic types are common in nature (Zink and Duffus, 1972; Davis and Mizuki, 1987; Duffus, 1963), it is likely that there are many as yet unrecognised examples of heterologous transcapsidation interaction that naturally occur between plant viruses. However, research thus far indicates that heterologous transcapsidation interactions occur only in specific interactions in most mixed infections. There is evidence for both traditional and transgenic virus resistant plants that transcapsidation may occur (Rochow, 1972; Matthews, 1991; Farnelli *et al*., 1992; Osbourn et al., 1990; Dalmay et al. 1992; Holt and Beachy, 1992; Lecoq et al., 1993; Maiss et al., 1994; Candelier and Hull, 1993).

With the impending commercialisation of transgenic virus resistant plants, an important consideration is whether the use of viral CP-expressing transgenic plants might increase the possibility for heterologous transcapsidation interactions to occur and, if the possibility is increased, whether it poses a significant risk. One way in which scientists have sought to assess potential transcapsidation frequency in transgenic virus resistant plants has been to compare the amount of the engineered coat protein in the transgenic plants with the amount of coat protein in a similar, but susceptible, non-transgenic plant (Issue 1 above). One hypothesis has been that comparable or smaller amounts of coat protein would lead to the prediction that the transcapsidation frequency will be comparable or reduced from the frequency that occurs in naturally occurring mixed infections.

A second consideration would be whether the transgene CP is synthesised in the same tissues that the virus naturally infects in non-transgenic plants. If CP synthesis takes place in the same tissues, then no new

interactions with other viruses that may be limited to other plant tissues can occur. The amount of transgene CP that can be detected in a transgenic plant may increase if the plant is infected by a related virus to which it is susceptible (Farnelli et al., 1992). The increase in detectable CP transgene may be a result of the CP being stabilised in masked virus particles rather than to an increase in transgene mRNA. It may be prudent to ascertain the amount of detectable CP transgene and mRNA in a transgenic plant when inoculated with common viruses with which the transgenic plant would routinely become infected in field situations.

One example of these considerations having entered into the regulatory assessment process in an OECD Member country is the Asgrow Seed Company's ZW20 squash, which is engineered to be resistant to zucchini yellow mosaic potyvirus (ZYMV) and watermelon mosaic potyvirus 2 (WMV2) by the expression of their respective CP genes. The review of ZW20 was conducted by the United States. At the time of preparation of this consensus document, it is the only virus resistant plant that has completed the reviews necessary to allow agricultural use of the plant in an OECD Member country. In ZW20 plants, the review concluded that the CPs are expressed in the same plant tissues in which the corresponding viruses are normally detected, and that the amount of CP produced in ZW20 plants is less than, or equal to, the amount in naturally infected plants. The amount of transgene CP detected increased in ZW20 plants after infection with papaya ringspot potyvirus (PRSV), although transgene RNA concentration did not increase. The amount of transgene CP detected in PRSV-infected ZW20 was still less than that found in PRSV-infected squash plants. In a review of Asgrow's next squash line (CZW-3), which is resistant to cucumber mosaic cucumovirus, ZYMV, and WMV 2, no increase in transgene CPs was detected when the transgenic plants were challenged with PRSV.

It has been demonstrated that heterologous transcapsidation can occur in transgenic plants that express viral CP (Osbourn et al., 1990; Dalmay et al., 1992; Holt and Beachy, 1992). Lecoq et al., 1993 showed that when plants expressing a CP transgene derived from an aphid-transmissible strain were challenged with a non-aphid transmissible strain (defective in CP not aphid transmission factor), a heterologous aphid transmissible strain was detected. Another important question is whether transcapsidation can occur with more distantly related viruses. Candelier-Harvey and Hull (1993) have shown that when plants expressing the CP of alfalfa mosaic alfalmovirus (AlMV) are infected with cucumber mosaic cucumovirus (both members of the family Bromoviridae), the CMV genome is encapsidated in particles that contain AlMV CP. Since AlMV has no known insect vector, it was not possible to evaluate changes in vector specificity. It seems that if heterologous transcapsidation occurs in these plants as a result of virus infection in the field, there are at least two biologically significant outcomes to be considered. These are: 1) that heterologous transcapsidation events in the transgenic plants could alter or facilitate vector transmissibility of the new progeny virions (those generated as a result of heterologous transcapsidation); and 2) that heterologous transcapsidation events in transgenic plants could facilitate systemic movement of the resulting progeny virions within the transgenic plants when they belong to a plant species in which the "normal" virus (that not resulting from heterologous transcapsidation interactions) does not readily move systematically. If the first scenario were to occur, and a virus were to gain vector transmissibility via heterologous transcapsidation with the transgenic plant, would the potential for new disease development be great either within the transgenic crop or in other plants? It is impossible to predict the answer for all situations, because cropping situations, geographic location, type of vector and its abundance, local crops and other factors will vary greatly from one country to another (Falk et al., 1995). Each of these scenarios will be discussed.

Scenario 1A. Altered vector transmission and disease development in the transgenic crop. If vector transmission of a plant virus were altered or facilitated as a result of heterologous transcapsidation interactions resulting from infection of a CP-expressing transgenic plant, it is not known whether this would cause significantly greater virus spread and disease development with the transgenic crop. In this scenario, any virus spread to a new transgenic plant as a result of heterologous transcapsidation would

contain CPs derived from the CP-expressing transgenic plant. If these masked viruses were subsequently vector-transmitted to another CP-expressing plant within the same field (secondary spread), they might or might not be able to infect such a plant. In one experiment, Osbourn et al. (1990) challenged transgenic tobacco plants expressing the functional coat protein derived from U1 strain with a strain (DT1) of TMV that exists only as unencapsidated RNA. (The CP of this strain is defective.) Virions were produced that contained DT1 RNA encapsidated by U1 CP. When the masked virions were inoculated onto transgenic tobacco plants expressing U1 CP, the plants were resistant to infection. Control non-transgenic plants showed the expected symptoms. This supports the notion that secondary spread of masked virions is unlikely to occur within the transgenic crop, as the plants would be resistant to the masked virions.

Although transcapsidation may be detected under laboratory conditions, field tests under natural conditions will indicate whether the secondary spread of heterologous transcapsidated virions is likely. As part of an ongoing multi-year experiment to determine the potential biological impacts of transcapsidation, Dr. Gonsalves and co-workers have been attempting to determine whether there are biological impacts of transcapsidation in a field situation (Gonsalves et al., 1994; Fuchs and Gonsalves, 1995). Melon, squash, and cucurbit plants were developed that express the CP from a highly aphid-transmissible strain of CMV, strain WL. (The CP is known to be the only determinant in aphid-transmission in cucumoviruses.) Depending on the plant line used, the CP transgene may be expressed at relatively high or low concentrations. In the 1993 and 1994 growing season, these plants were grown in the field and challenge inoculated with a strain of CMV (strain C) that was not aphic-transmissible. The researchers looked in their inoculated transgenic plants and healthy, non-inoculated control plants for transcapsidated aphid-transmitted CMV. This transcapsidated CMV would have contained RNA from strain C, encapsidated with CP from WL strain derived from the plant transgene. Thus far, the spread of CMV C from inoculated transgenic to healthy non-transformed plants has not been detected. [Similar indications are also apparently emerging from the 1995 field trial (Fuchs, unpublished data)]. These experiments have been performed in a locality where the crops are routinely grown, the aphid vectors are abundant, and CMV is a serious problem in these crops. Further studies with other virus-crop systems will be useful in trying to confirm these findings (for scenario 1a) for other virus-plant systems and environmental conditions.

Scenario 1B. Altered vector transmission and disease development with another plant. It is also possible that if heterologous transcapsidation were to occur in CP-expressing transgenic plants, the resulting masked virion might be transmitted by the "new" vector to another plant or crop that is not transgenic. In this scenario, the transgenic crop would serve as a new virus reservoir (for the heterologous transcapsidated virus), allowing virus spread to a new plant. However, spread of heterologous transcapsidated virions to the second plant would only be primary spread, *i.e.* spread from the transgenic plants where transcapsidation took place to a different plant species. Once the chimeric transcapsidated virions infect a new, non-transgenic host, they will again resort to the phenotype determined by the viral nucleic acid, as the only capsid protein source in these plants would now be their own genome. For these viruses to spread secondarily through the non-transgenic plant population, they would now have to be spread by their original vector, which may or may not be present (Falk et al., 1995). With respect to the potential effects of any primary spread, for many plant viruses, especially those of annual crops, the most common and economically important form of virus spread is secondary spread (Simons, 1959; Alderz, 1978). Primary spread generally involves few, or a limited number of, plants and in most cases does not result in economically important losses. Secondary spread, in contrast, can be rapid and involves spread from the initial, primary infected plants to the large numbers of remaining healthy plants (Matthews, 1991). Thus, if in the above example the transgenic plants were to serve as sources for primary spread of chimeric transcapsidated virion to another crop, disease and virus incidence from the primary spread would likely be limited in scope. Secondary spread in the non-transgenic plant could only occur if the natural vector(s) of the wildtype virus were already present. However, if the natural vector were already present, then it is possible that the natural vector could provide for primary as well as secondary spread, and both would spread wildtype virus (Falk et al., 1995). Of course this scenario, which deals with epidemiology of

virus spread, depends greatly on the virus, vector, and local-site specific conditions, which might require case-by-case review.

Scenario 2. Disease development resulting from new systemic spread within transgenic plants. The movement of a virus from the initial site of infection throughout a plant, called systemic infection, requires expression of one or more viral genes (a dedicated movement protein, coat protein, and/or viral proteins) and a permissible host plant (Hull, 1989; Maule, 1991; Dawson et al., 1988; Marchoux et al., 1993; Dolja et al., 1995; Cronin et al., 1995; Valkonen and Somersalo, 1995). If a virus is unable to move from the initial site of infection, these infections are called subliminal. In a limited number of cases, viruses that cause subliminal infections in a host species may no longer be restricted when the host is infected by a second virus. In a large number of these studies (Atebekov and Talinsky, 1990) it has not been determined whether the coat protein is solely responsible for this helper dependent movement, but for viral taxa where a dedicated movement protein has not been described, consideration that the coat protein is the primary determinant of movement should be noted. If the coat protein expressed in the transgenic plant can facilitate the movement of viruses that cause subliminal infections, this would be a significant concern only if that CP was from a virus that rarely or never infects the recipient host plant. If CP is derived from a virus that is widely prevalent in the recipient plant, there would be no new novel interactions with subliminally-infecting viruses. This situation is true for the transgenic plants that are likely to be commercialised during the next few years (see Section V). This assumes the transgene is expressed in the same cells as virus. There are several situations in which this type of interaction may need further review.

a. Although the virus that provided the transgene may be widely prevalent in many countries, different strains may be present in different countries. Whether the biological properties of the transgene CP are identical to those of the CP from the viral strains present in another country would require a review.

b. If the virus that provided the CP transgene was not present in a country, then there could be new interactions between the transgene CP and viruses that cause subliminal infections. However, it is unlikely, but not inconceivable, that regulatory agencies in a country would be asked to approve a virus resistant plant where the virus was not an economically important pathogen.

c. If the virus that provided the transgene CP was present in the country, but was usually found in a different plant species from that of the recipient transgenic plant, there could be new interactions between the transgene CP and subliminally-infecting viruses.

In all these cases, if the viruses that cause subliminal infection of the recipient host are known, then easily performed tests can be conducted to determine whether CP facilitates their systemic movement. Whether the movement of the virus in the transgenic plant results in significant disease loss will depend on the virus, plant, and environmental conditions in each locality. Whether the virus can move from the transgenic plant will depend on its mode of transmission, especially whether viral vectors are present and feed on the transgenic crop.

Although not all of the useful experiments regarding the potential effects of heterologous transcapsidation have been completed, reports published in two OECD Member countries have reached certain conclusions about the potential risk concerns posed by heterologous transcapsidation. The conclusions reached in these two countries may not necessarily apply to all Member countries. In its report to the United Kingdom's Ministry of Agriculture, Fisheries, and Food entitled "Risks to the Agricultural Environment Associated with Current Strategies to Develop Virus Tolerant Plants Using Genetic Modification," Henry et al. (1995) state: "The general view is that transcapsidation is not a problem, because it is limited to a single transfer, *i.e.* once a transcapsidated genome is introduced into a new host, it

reverts to using its own CP." In the report of a workshop on virus resistance prepared by the American Institute of Biological Sciences (AIBS) for the U.S. Department of Agriculture, a similar finding is reached (AIBS, 1995): "Transcapsidation of viral RNAs with coat protein produced by transgenic plants should not have long-term effects, since the genome of the infecting virus is not modified."

In conclusion, the potential impacts of transcapsidated viruses from viral CP-gene protected plants is generally expected to be no more serious than the impacts that occur in multiple viral infections of susceptible crops. However, there are a few cases with certain viral taxa where questions remain. Many of these potential impacts can be addressed via currently funded research or during variety development.

B. Synergy

Occasionally, when two viruses simultaneously naturally infect a plant, the symptoms can be more severe than when either of the viruses infects the plant singly. This phenomenon is called synergy (Matthews, 1991). Synergistic infections can often result in severely diseased, unmarketable crops. Synergy was first described and is best studied with PVX and PVY. The majority of synergistic viral combinations include, as one the viruses, a potyvirus (see ***Table 2*.1**, listing some synergistic interactions, which was prepared by Dr. V. Vance, University of South Carolina, U.S.A.). [The discussion here is limited to viral interactions that affect symptom development. Other specific interactions, *e.g.* the ability of TMV to move systemically in barley in the presence of brome mosaic bromovirus (Hamilton and Nichols, 1977), which probably result from movement protein complementation, will not be discussed.]

Will coat protein-mediated resistance produce unintended synergistic symptom expression when the resistant plant is infected by other plant viruses? Since potyvirus CP genes are not involved in synergism, it is unlikely that infection of a transgenic potyvirus resistant plant with any other virus would result in a synergistic interaction. It should also be noted that the specific potyviral gene involved in synergy is likely to be identified within the next few years. The identity of that gene is under investigation, and the search has been narrowed to three potential candidate genes on the 5'-end of the genome, the N-protease, helper component/protease, and the 50 kilodalton protein of unknown function (Vance et al., 1995). [Preliminary indications are that the single gene responsible for the synergism symptom is the helper component-protease gene in PVY and potato X potexvirus and that the *same* gene is responsible for another synergistic symptom between PVY and tobacco mosaic tobamovirus (Vance, unpublished data).]

Because synergy, unlike recombination and transcapsidation, is not related to the potential for creation of new viruses, its effects can in a sense be considered to be agronomic rather than environmental. Evaluating the potential for interactions will be an important part of assessing the agronomic performance of a transgenic crop plant, and potential interactions would likely be assessed during the standard evaluations used in cultivar development.

C. Recombination

Recombination is defined as an exchange of nucleotide sequences between two nucleic acid molecules. Recombination between viral genomes results in heritable, permanent change. The persistence of a recombined viral genome will depend upon its fitness with respect to its ability to replicate within the original host cell, its ability to replicate in the presence of parental viruses, its ability to spread systemically within the host, or its successful transmission to other host plants.

Factors that influence recombination rates and detection of a viable recombinant include: sequence and structural similarity between the nucleic acid molecules, subcellular location and concentration of the nucleic acids, and the number of recombinational events required to form a viable recombinant viral genome (Lai, 1992). The frequency of recombination between two naturally occurring viruses or two viral

strains in field-grown plants in the absence of selection pressure has not been determined (Henry et al., 1995), and is difficult or impossible to measure meaningfully. Recombination is hypothesised as an important mechanism for virus change over evolutionary time frames, during which they may have been quite frequent (Simon and Bujarski, 1994). Recently, the nucleotide sequences of numerous viral strains from many of the known genera have been published. Sequencing data have shown that certain genes in quite different taxa probably arose from recombinational events. In other cases, a single strain of a virus has been found to contain sequences apparently derived from a virus for a different taxa, while all other closely related strains do not have these sequences. [Listing all these events is outside the scope of this document. However, several references can provide readers additional information (Koonin and Dolja, 1993; Murphy et al., 1995; Sano et al., 1992; Edwards et al., 1992; LeGall et al., 1995; Pappu et al., 1994; Goulden et al., 1991; Mayo and Jolly, 1991; Revers et al., 1995; Gibbs and Cooper, 1995]. Currently, it is not possible to determine whether these recombinational events occurred, since for example the development of modern agricultural cropping practices or in much longer time frames. However, there is evidence of virus genome stability in shorter time frames, *i.e.* since the establishment of plant virology as a science. First, the biological properties of TMV have remained remarkably stable over the past century (Ford and Tolin, 1983; Dawson, 1992); and second, the Dutch and Wisconsin (U.S.A.) substrains of alfalfa mosaic alfalmovirus strain 425 have acquired, in approximately 20 years of laboratory use in each country, several nucleotide changes leading to five amino acid changes with apparently no significant changes in biological properties (Jaspars, 1985).

The potential use of virus resistant transgenic plants in agriculture highlights the following questions regarding recombination when transgenic plants are used:

a. Will the overall rate of viral recombination in nature be increased when these transgenic plants are used because there will be increased opportunity for recombination?

b. What factors may affect the rate of recombination, and will that rate be proportional to the concentrations to parent molecules?

c. Are any recombinants thus formed likely to be successful in competition with parental viruses?

Most transgenic plants are likely to be engineered in the near term with CP genes from viruses that regularly infect the host plant, because damage by those viruses poses the most constant potential for loss in the crop species. Sequences from those viruses, when available for recombination, would be unlikely to pose the potential for generating *novel* recombinants in comparison with natural mixed infections in the recipient plant, given certain conditions described below. (Genes from viruses that do not regularly infect the host plant might sometimes be introduced for experimental or other purposes, and the arguments herein would not necessarily apply in those instances.) In most virus resistant plants that have been experimentally engineered to date, transgenes that yield effective resistance to a target virus are usually expressed at very low levels compared with the levels seen in virus-infected plants. For example, in Asgrow's ZW20 squash, infected non-transgenic squash plants had a 100-fold higher concentration of viral RNA than the corresponding CP-transformed ZW20 plants. It is unlikely, though not impossible, that any compelling reason will emerge for scientists or breeders to develop new plant varieties in which high levels of transgene products are expressed, inasmuch as low level expression appears effective in conferring virus resistance. With regard to this issue, the AIBS report notes: "The implications of these low expression levels for recombination are not clear. Even assuming that the higher concentration of transgene RNA the greater the chance for recombination, we do not know what a meaningful range is; what are low and high concentrations of transgene transcript relative to unacceptable recombination rates? Currently, this information (concentration of transgene RNA) is of no use to regulatory agencies because there is no way to factor concentrations of RNA or protein into risk determination in a meaningful manner" (AIBS, 1995).

Even given these quantitative uncertainties, however, the type of background information about virus identity, environment, and disease pressure characterisation indicated in Section III above is helpful.

The use of CP gene-mediated resistance might open the possibility of novel interactions between tissue-specific viruses and other viruses. In cases where the plant is systemically infected (*i.e.* virus can be found in all cell types), the cellular location of the transgene is probably not a major issue. In contrast, if a coat protein transgene from a phloem-limited virus is used for resistance, this might increase the probability of new interactions between the transgene transcript or its gene product and other viruses that replicate only in non-phloem tissues. These new interactions may result in modified symptoms, insect transmission of the infecting virus, or modified movement of the infecting virus within the transgenic plant. However, unless a recombinational event occurred between the transgene and the infecting virus and the resulting recombinant virus was competitive, the effect would be limited and restricted to the transgenic crop. If viral infections that result in subliminal infections are known in this crop, the interactions of the transgene with these viruses in terms of important parameters (movement, symptoms, and insect transmission) can be evaluated experimentally.

Table 2.1 Reported Viral Synergisms

Potyviral Synergistic Interactions		References
Potato Y potyvirus (PVY)	Potato X potexvirus (PVX)	Rochow, W. F., Ross, A. F. 1955. Plant Disease (Reporter) 52:344-358.
Tobacco vein mottling potyvirus	PVX	Vance, V., B. Berger, P. H., Carrington, J. C., Hunt, A. G., Shi, X. M. 1995. Virology 206:583-590.
Tobacco etch potyvirus (TEV)	PVX	see above
Pepper mottle potyvirus	PVX	see above
Blackeye cowpea mosaic potyvirus	Cucumber mosaic cucumovirus (CMV)	Pio-Ribeiro, G., Wyatt, S. D., Kuhn, C.W. 1978. Phytopathology 68: 1260-1265.
Cowpea aphid borne potyvirus	CMV	Fisher, H. U., Lockhart, B. E. 1976. Phytopathology Z. 85:132-138.
Bean yellow mosaic potyvirus	CMV	Harrison, A. N., Gudauskas, R. T. 1968. Plant Disease (Reporter) 52:509-511.
Zucchini yellow mosaic potyvirus	CMV	Poolpol, P., Inouye, T. 1968. Annal Phytopathology Society of Japan 52:22-30.
Soybean mosaic potyvirus	Bean pod mottle comovirus	Calvert, L. A., Ghabrial, S. A. 1983 .Phytopathology 73:992-997. Les, Y-S., Ross, J. P. 1968. Phytopathology 62:839-845. Quiniones, S. S., Dunleavy, J. M. 1971. Phytopathology 763-766. Ross, J. P. 1968. Plant Disease (Reporter) 52:344-348.
SMV	Cowpea mosaic comovirus	Anjos, J. R., Jarlfors, U., Ghabrial, S. A. 1992. Phytopathology 82:17-23.
Maize dwarf mosaic potyvirus	Maize chlorotic mottle virus? (MCMV)	Goldberg, K-B., Brakke, M. K. 1987. Phytopathology 77:162- 177. Niblett, C. I., Claflin, L. E. 977. Plant Disease (Reporter) 62:15-19. Uyemoto, J. K., Claflin, L. E., Wilson, D. L., Raney, R. J. 1981. Plant Disease 65:39-41.
Wheat streak mosaic potyvirus	MCMV	see above
PVY	TMV	Clark, R. L., Hill, J. H., Ellis, M. D. 1980. Phytopathology 70:131-134.
Turnip mosaic potyvirus	Cauliflower mosaic caulimovirus	Kahn, M. A., Demski, J. W. 1982. Plant Disease 66:253-256.
MDMV	Barley yellow dwarf luteovirus	Belli, G., Cinquanta, S., Soneini, C. 1980. Rivista Pathol. Veg. 16:83-86.
TEV	Dodder latent mosaic virus	Bennett, C. W. 1949. Phytopathology 39:637-646.
Non-potyviral Synergistic Interactions		
TMV	PVX	Vanterpool, T. C. 1926. Phytopathology 16:311-331.
TMV	CMV	Garces-Orejuela, C., Pound, G. S. 1957. Phytopathology 47:232-239.
TMV	Tobacco ringspot nepovirus	see above
TMV	Tomato aspermy cucumovirus	Holmes, F. O. 1956. Virology 611-617.
Cowpea chlorotic mottle bromovirus	Southern bean mosaic sobemovirus	Kuhn, C. W., Dawson, W. O. 1973. Phytopathology 63:1380-1385.
Alfalfa mosaic alfamovirus	potato acuba potexvirus	Kassanis, B. 1963. Advances in Virus Research 66:253-256.

There have been attempts to use transgenic plants to estimate experimentally the potential frequency of recombination between the transgene mRNA and the genome of a challenging virus, and/or to determine the rate of recombination between two viruses (or two viral strains). In transgenic plants expressing sequences derived from either a DNA virus (Schoelz and Wintermantel, 1993) or RNA virus (Greene and Allison, 1994), it has been demonstrated in some experiments that recombination between a viral transgene and a defective challenge virus can restore a functional, infective virus under high selection pressure. These results demonstrate that recombinational events can eventually occur in plants expressing viral sequences when inoculated with defective viruses. Because of the great interest in this area, it is expected that in the next several years additional information on the factors that influence recombination will be better understood. The results of all experiments dealing with recombination must be interpreted carefully before conclusions can be drawn, since no single experimental design is ideal to address each potential environmental condition, and each virus taxon, and certain assumptions and conditions are part of each experimental design. Some points to consider in interpreting these experiments are:

a. Are the transgenic plants susceptible or resistant to viral infection? Some scientists have developed experimental systems to study recombination, in which the transgenic plants expressing a viral sequence are susceptible to infection by the virus which provides the transgene. In a susceptible transgenic plant the amount of viral RNA from the infecting virus would be greater than in a resistant plant; thus, higher concentrations of RNA might increase the likelihood of recombination in the experimental system. Most, if not all, transgenic plants containing CP genes that are commercialised are likely to be resistant to infection by the virus (or strain) that provided the transgene sequence.

b. What is the selection pressure in the experiment? The AIBS report (1995) provided the following definition: "[H]igh selection pressure is defined as conditions that favour the recombinant virus, for example, a situation where the virus is not viable unless a recombination event occurs. Low selection pressure would be a situation where the novel phenotype does not confer a competitive advantage to the recombinant under the conditions of the experiment." A clear understanding of selection pressure in the experiment between a viral transgene and an infecting virus is important, since the recombination rate must be compared to natural recombination rates between the two viruses (or strains) to provide a meaningful comparison. The natural recombination rates between two viruses (or strains) may be high or low.

c. Were the experiments performed in the natural hosts for the viruses? If a recombinant virus is formed, is it competitive with wild-type virus? Recombination rates may be affected by the host organism (Lai, 1992). The host plant also affects the mutation rates of the infecting virus (Dawson, 1992). Often, virologists have used *Nicotiana* species as experimental hosts because they are easy to transform and grow, although they are not the natural hosts of the viruses being studied. As one example, the natural host range of cauliflower mosaic virus is limited to the Brassica family (Matthews, 1991), but experiments on this virus have been performed in Solanaceous plants (Takahashi *et al*., 1989; Baughman *et al*., 1989; Schoelz and Shepherd, 1988). However, recombinant viruses can frequently be observed to have increased virulence (*i.e.* more severe symptoms) on model host plants that are not the natural host of either viral parent (infecting virus or virus that the transgene was derived from). If a recombinant virus is generated, determining whether it is competitive with wild type virus in the natural host of the infecting virus and the virus that provided the transgene sequence is most relevant.

d. Do experiments performed in field situations provide additional benefits as compared to laboratory or greenhouse experiments? Whether there is any logistical or conceptual advantage for a field experiment versus a test under contained conditions depends on the experiment. However, in a field test plants are grown under natural stresses that would be found in a

commercialised crop, including inoculation of the plants by vectors containing widely prevalent viral strains of that locality, and the presence of other diseases and pests, including other viruses, etc.

If a recombinant virus is formed in a cell (either in a transgenic plant or during a mixed infection), will that recombinant participate in the replication process in that cell, move systemically in the plant, or cause a new disease? The vast majority of progeny viruses do not apparently function in the replication process. For many viruses, the RNA is encapsidated by CP, viral RNA synthesis in the cell ceases or declines to undetectable levels, and, depending on the virus and whether it is transmitted to another plant or via progeny, is degraded when the plant cell dies (Matthews, 1991). The likelihood of a recombinant becoming established depends on many factors, including its competitiveness with infecting virus and other viruses that naturally infect the plant, and all the additional factors that may affect selection pressure (*e.g.* temperature, vectors, host plants). Thus, to predict the probability of development of new virus disease resulting from recombination of two viruses, or between a virus and a viral derived transgene, requires a considerable level of understanding of the population biology of viruses in cells and virus movement within plants, and a better understanding of the mechanisms of how viruses cause disease.

Much of the discussion of formation of recombinant virus or the detection of new viral strains may leave the impression that a strain of virus is homogenous with respect to plant-induced symptoms or nucleotide sequence. All the single-stranded RNA genomes that have been examined have been found to exist not as a unique nucleotide sequence, but as a collection of related sequence variants around a consensus sequence. This sequence microheterogeneity is always present in natural populations (Holland *et al*., 1982; Domingo *et al*., 1985; Morch *et al*., 1988). This microheterogeneity in viral sequence has led to the concept of "quasi-species" for some viruses (Eigen, 1993). It is thought to be a result of the lack of proof-reading function in the viral replicases and of the large quantity of viral RNA produced per cell.

Most variants have one or two nucleotide changes, although some viruses (*e.g.* soilborne wheat mosaic furovirus) are known to have large deletions in some genes (Matthews, 1991). Variants can also be detected by changes in symptomatology. A PVX strain that produces chlorotic local lesions on tobacco plants frequently gave rise to ring spot local lesion production (Matthews, 1949). A tobacco necrosis necrovirus strain that produced white lesions on cowpea frequently gave rise to strains giving red lesions (Fulton, 1952). Thus, the microheterogeneity of viral RNA may result in sequence variation with no visible differences to major symptom alterations. Of course, even more variability in both sequence and plant-induced symptoms exists in a single virus because many viruses have well-characterised, stable strains that are sufficiently different to have been given a unique identifier (Matthews, 1991).

Although additional research is currently being funded on viral recombination, reports in two OECD Member countries have reached certain conclusions about the potential risk concerns posed by the appearance of new viruses. The conclusions reached in these two countries may not necessarily apply to all Member countries. In a report to Agricultural and Agri-Food Canada, Rochon *et al*. (1995) conclude: "It is likely that current means of detecting and controlling new diseases in this country would be adequate to control any new virus resulting from recombination between a transgene and another virus." The AIBS report to USDA (1995) concludes by stating: "With or without the use of transgenic plants, new plant virus diseases will develop that will require attention."

Undoubtedly, many new crop varieties will need to be developed to resist emerging viruses or new strains of existing viruses. The appropriate application of scientific analysis to ensure the biosafety of new varieties will allow effective control of these diseases while protecting long-term agricultural productivity and the environment.

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SECTION 2

GENERAL INFORMATION CONCERNING THE GENES AND THEIR ENZYMES THAT CONFER TOLERANCE TO GLYPHOSATE HERBICIDE

Summary Note

This document summarises the information available on the source of the genes that have been used to construct glyphosate-tolerant transgenic plants, the nature of the enzymes they encode, and the effects of the enzymes on the plant's metabolism.

Scope of this document: OECD Member countries agreed to limit this document to a discussion of the introduced genes and resulting enzymes that confer glyphosate tolerance to plants. The document is not intended to be an encyclopaedic review of all scientific experimentation with glyphosate-tolerant plants. In addition, this document does not discuss the wealth of information available on the herbicide glyphosate itself or the uses of the herbicide in agricultural and other applications. Food safety aspects of the use of glyphosate on glyphosate-tolerant transgenic plants are not discussed. Such information is available from other sources, including the respective governmental organisations which regulate the use of the herbicide.

While the focus of this document is on the genes and enzymes involved in encoding glyphosate tolerance, reference is not made to specific plant species into which glyphosate tolerance might be introduced. Any issues relating to the cultivation of glyphosate-tolerant plants or to the potential for, or potential effects of, gene transfer from a glyphosate-tolerant plant to another crop plant or to a wild relative are outside the agreed scope of this document. It is intended, however, that this document should be used in conjunction with specific plant species biology Consensus Documents (see list of publications at the front of the document) when a biosafety assessment is made of plants with novel glyphosate herbicide resistance.

1. Herbicide Tolerance

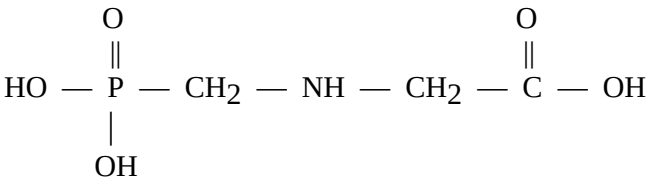
Many herbicides kill plants by interfering with enzyme function in the plant. Most of these herbicides exert their effect on a single enzyme which catalyses a key metabolic reaction in the plant. In general, plants exhibit a range of sensitivities to the herbicides used in agriculture, with some species exhibiting considerable tolerance to a single herbicide. There are several mechanisms by which plants can tolerate exposure to herbicide: (1) the plant produces an enzyme which detoxifies the herbicide; (2) the plant produces an altered target enzyme which is not affected by the herbicide; or (3) the plant produces physical or physiological barriers to uptake of the herbicide into the plant tissues and cells (Devine *et al*. 1993).

2. Glyphosate as a Herbicide

Glyphosate is widely used as a broad-spectrum weed control agent and is registered in many countries (Duke 1996, Shah *et al*. 1986). Even though glyphosate is a reversible competitive inhibitor of the enzyme 5-enolpyruvyl-3-phosphoshikimic acid synthase (EPSPS) with respect to phosphoenolpyruvic acid (PEP), it does not inhibit any other PEP-dependent enzymatic reactions. It is a non-competitive inhibitor of EPSPS with respect to 3-phosphoshikimic acid (Steinrucken *et al*. 1984). Glyphosate is produced by

chemical synthesis. It is not a natural product. Chemically, glyphosate is N-phosphonomethyl-glycine (see *Figure 2*.1). Glyphosate is the active ingredient of the herbicide Roundup® (Monsanto).

Figure 2.1 Glyphosate Structure



The high sensitivity of crop plants to glyphosate has limited its use as a pre-crop emergence herbicide in no-till management strategies, and as a herbicide and crop desiccant when applied shortly before crop harvest. With the development of genetically engineered crop plants that are resistant to glyphosate, this herbicide can instead be applied after both crops and weeds have emerged, with little or no damage to the crop.

Glyphosate interferes with normal plant metabolism through inhibiting the enzyme 5-enolpyruvyl-3-phosphoshikimic acid synthase (EPSPS). In plants and micro-organisms, EPSPS is involved in the biosynthesis of aromatic amino acids, vitamins, and many secondary metabolites. It is not present in animals (Levin and Sprinson 1964, Steinrucken and Amrhein 1980). In plants, EPSPS is localised within plastids. This enzyme condenses phosphoenolpyruvic acid (PEP) and 3-phosphoshikimic acid to 5-enolpyruvyl-3-phosphoshikimic acid. As a consequence of the inhibition of aromatic amino acid biosynthesis, protein synthesis is disrupted, resulting in the plant's death (Kishore and Shah 1988). While some of the downstream products of the EPSPS reaction, amino acids and vitamins, are strictly essential for the growth of all living organisms, some secondary metabolites derived from the shikimate pathway may have specific survival value for the producing organism (Malik 1986). The enzyme has rigid specificity towards its substrates, which are shikimate-3-phosphate and phosphoenolpyruvate (Anderson and Johnson 1990). The reaction product, 5-enolpyruvylshikimate-3-phosphate (EPSP), is further acted upon by other enzymes to yield chorismic acid, which gives anthranilic acid (a precursor of tryptophan) and, on rearrangement, prephenic acid (a precursor of phenylalanine and tyrosine).

Based on the knowledge of the mode of action of glyphosate, several strategies have emerged for developing plants that are tolerant of exposure to the herbicide. The two successful strategies to produce glyphosate-tolerant plants are introduction of glyphosate-tolerant EPSPS and introduction of an enzyme that inactivates glyphosate, glyphosate oxidoreductase (GOX). Recombinant DNA techniques have been used to express genes that encode glyphosate-tolerant EPSPS enzyme alone or a combination of EPSPS and GOX genes in susceptible plants (Nida *et al*. 1996, Padgette *et al*. 1995, 1996).

3. The Development of Glyphosate-Tolerance Plants

Scientists have been unsuccessful in producing glyphosate-tolerant plants using classical techniques. Traditional mutagenesis and selection techniques have to date failed to produce a useful level of tolerance in crop plant species, although such an approach could yield a mutant form of the target enzyme that is tolerant of the herbicide but retains its desirable enzymatic function. Plant breeders also have been unable to develop glyphosate-tolerant crops using the standard techniques in which chemical or radiation exposure of seeds generates mutations in the plant genome. In cases where the desired phenotype is herbicide tolerance, spraying seedlings in the growth chamber or field can sometimes be used with success to select tolerant individual plants from millions of mutagenised individuals. Even though this approach has been used in the commercial development of imidazolinone-tolerant maize and soybean cultivars, it has not been successful in producing glyphosate-tolerant plants. This is because all mutant EPSPS, in parallel to

glyphosate tolerance, has decreased affinity for phosphoenolpyruvate. This has resulted in glyphosate-tolerant plants that have invariably shown reduced biosynthesis of aromatic amino acids.

Recombinant DNA techniques have been used to confer glyphosate tolerance to a variety of crop plant species. In this approach, plants have been transformed with genes that encode a glyphosate-tolerant enzyme that is not inhibited by glyphosate but provides substrates for the biosynthesis of amino acids. In some cases, the tolerance imparted by this gene has been further augmented by expressing a second gene that encodes the enzyme glyphosate oxidoreductase (GOX) to detoxify glyphosate (Padgette *et al*. 1996, Shah *et al*. 1986).

4. Genes and Enzymes that Confer Glyphosate Tolerance

Three genes which provide field-level tolerance to glyphosate, the active ingredient in Roundup® herbicide, have been introduced into commercial cultivars. The first glyphosate-tolerant EPSPS gene was isolated from a soil bacterium, *Agrobacterium* (Barry *et al*. 1994, Duke 1996). The EPSPS synthase from this *Agrobacterium* was highly tolerant to glyphosate. When it is expressed in transgenic plants, the EPSPS encoded by this *Agrobacterium* gene fulfills the aromatic amino acid needs of the plant in the presence of glyphosate, whereas the plant version of this enzyme (ubiquitous in nature) is sensitive to glyphosate. *Agrobacterium* spp. are not human or animal pathogens, but some species are pathogenic to plants (Croon 1996, Holt 1984).

Recently, the EPSPS gene from corn (*Zea mays*) has been mutagenized *in vitro* to obtain a glyphosate-tolerant enzyme. The tolerant version of the enzyme produced by the modified maize gene is 99.3% identical to the parent enzyme (Monsanto 1997).

Also, a gene that encodes for a glyphosate-degrading enzyme called glyphosate oxidoreductase (GOX) was isolated from *Achromobacter* strain LBAA, a soil bacterium ubiquitous in nature (Barry *et al*. 1994). The encoded enzyme deactivates the herbicidal effect of glyphosate. Glyphosate oxidoreductase catalyses the conversion of glyphosate to aminomethylphosphonic acid (AMPA) and glyoxylate. GOX requires flavin adenine dinucleotide (FAD) and magnesium for activity; therefore, it is more appropriately designated an apoenzyme.

EPSPS enzyme, the target of glyphosate action, is synthesised in the cytoplasm and then transported to the chloroplast (Kishore and Shah 1988). The translocation of the protein to the chloroplast is carried out by an N-terminal protein sequence called the chloroplast transit peptide (CTP). CTPs are typically cleaved from a mature protein and degraded following delivery to the plastid (Della-Cioppa *et al*. 1986). A plant-derived coding sequence expressing a chloroplast transit peptide is often linked with each of the genes imparting glyphosate tolerance. This peptide facilitates the import of the newly translated enzymes into the chloroplasts, the site of both the shikimate pathway and glyphosate mode of action.

Use of the technology achieving transgene expression in plants is now routine. In order to achieve efficient expression of bacterial genes within plants, it has been common for researchers to modify the codon usage pattern of genes of bacterial origin prior to introducing them into plants. In this case, the codon usage pattern of the *Agrobacterium* glyphosate-tolerant EPSPS gene and glyphosate oxidase genes of *Achromobacter* have been chemically synthesised for codon optimisation for efficient expression in the plant. The amino acid sequence of the resulting enzymes is not changed. The genes associated with their transit peptide coding sequence are usually linked to other regulatory sequences like promoters, terminators, enhancers and introns. These regulatory sequences do not usually encode for a protein (Croon 1996).

These genes have been engineered (singly or in combination) into many plant species for the development of glyphosate tolerance and for use as selectable markers for identification of transformed plants. Plants field-tested with these genes include: *Beta vulgaris* (beet), *Zea mays* (corn), *Gossypium hirsutum* (cotton), *Lactuca sativa* (lettuce), *Populus* (poplar), *Solanum tuberosum* (potato), *Brassica napus* (oilseed rape, rapeseed, canola), *Glycine max* (soybean), *Nicotiana tabacum* (tobacco), *Lycopersicon esculentum* (tomato), *Triticum aestivum* (wheat).

OECD Member countries have governmental organisations which regulate the field-testing and unrestricted release of genetically engineered plants. Information about these plants is shared among various Member countries. The OECD sponsors an electronic database format for the exchange of this information. The database information is periodically updated to provide information that is both current and accurate (**www.oecd.org/ehs/service.htm**).

5. Effect of Transgene Expression in Plants

During the life cycle of any herbicide-tolerant plant, the plant is exposed only rarely to the herbicide. Except for the production of the enzyme(s) encoding glyphosate tolerance, there should be no other changes in plant metabolism. After glyphosate application, the enzyme activities expressed by the transgenes enable the plant to survive herbicide exposure. In the case of introduced EPSPS, no new metabolic products are formed since the only difference from the native enzyme is its insensitivity to glyphosate. However, if very high expression levels result from the insertion, the levels of downstream metabolites might change. In contrast, GOX will convert glyphosate to aminomethylphosphonic acid (AMPA) and glyoxylate when glyphosate herbicide is applied (Torstensson 1985). Since glyoxylate is a naturally occurring plant metabolite involved in carbon cycling, it will be further metabolised to provide intermediates for the Krebs cycle. Since GOX is highly specific for its substrate, glyphosate, in the absence of glyphosate no metabolites are expected. The United States Environmental Protection Agency has decided that only glyphosate residues are to be regulated in plant and animal commodities, and that the major metabolite AMPA is not of toxicological concern regardless of its level in food (US EPA 1997). Information regarding decisions concerning glyphosate herbicide-tolerant plants can be found at:

http://www.olis.oecd.org/bioprod.nsf
http://www.cfia-acia.agr.ca/english/plant/pbo/home_e.html (Canada)
http://ss.s.affrc.go.jp/docs/sentan/eguide/commerc.htm (Japan)
http://www.aphis.usda.gov/biotech/petday.html (USA)
http://europa.eu.int/comm/dg24/health/sc/scp/outcome_en.html (European Commission)

Western blot and enzymatic activity assays indicate that EPSPS protein from *Agrobacterium* strain CP4 is readily degraded in less than two minutes by incubation in simulated gastric fluid. In simulated intestinal fluid the enzyme activity and immunoreactivity lasts longer, being still detectable at ten minutes but undetectable at 270 minutes. The GOX protein is rapidly degraded in simulated gastric fluid and simulated intestinal fluid. After a 15 second incubation in gastric fluid, GOX has less than 90% of its initial protein epitopes as assayed by Western blot analysis, and enzyme activity loss is also greater than 90% when assayed after one minute incubation in gastric fluid. Similar results are seen in simulated intestinal fluid (US EPA 1996 and 1997).

Expression of GOX and glyphosate-tolerant EPSPS is not detrimental to plant growth, since such crops have agronomic performance similar to their parents. Governmental regulatory agencies in the United States (US Department of Agriculture 1994, 1995, 1997), Canada (Agriculture and Agrifood Canada 1995, 1996), Japan (Ministry of Agriculture, Forestry and Fisheries 1996) and European Union (European Commission 1998a, b) have made decisions that the presence of the EPSPS and GOX proteins in plants does not result in plants that are unsafe in their environments. Several lines of evidence support

the conclusion that these enzymes show low mammalian toxicity: (1) Neither enzyme shows amino acid homology to known allergens or mammalian toxins (Burke and Fuchs 1996); (2) Data from acute oral toxicity tests at high concentration of enzymes showed no toxicity (Harrison *et al*. 1996). In acute oral toxicity tests of bacterially derived CP4 EPSPS protein, no test substance adverse effects occurred at a dose of 572 milligrams per kilogram body weight (mg/kg) of the test animals. The acute toxicity of bacterially derived GOX protein showed no test substance adverse effects at doses of 91.3 mg/kg of the test animals; (3) Both enzymes are readily inactivated by heat or mild acidic conditions and are readily degraded in an *in vitro* digestibility assay which is consistent with the lack of oral toxicity (US EPA 1996, 1997). That the two enzymes show little if any toxicity is consistent with the observation that most enzymes are not considered toxic to vertebrates (Kessler *et al*. 1992). Notable exceptions are diphtheria toxin and certain enzymes in the venom of snakes, with very different exposure scenarios.

Governmental regulatory agencies in the United States (US Food and Drug Administration 1996), Canada (Agriculture and Agrifood Canada 1995, 1996), Japan and the European Union have made decisions that the presence of the EPSPS and GOX proteins in plants released into the environment do not pose a significant allergenicity risk. Two independent lines of evidence support the decision that these enzymes are not potential allergens: (1) Current scientific knowledge suggests that common food allergens tend to be resistant to degradation by heat, acid and proteases, are glycosylated, and are present at high concentrations in food. The EPSPS and GOX proteins are rapidly degraded by gastric fluid *in vitro* and are non-glycosylated. Thus, the potential for these proteins to be food allergens is minimal (Astwood *et al*. 1996, Burke and Fuchs 1996); (2) It is possible to utilise international gene databases to compare the gene sequences of a protein with other genes that encode known allergens. None of the amino acid sequences of known allergens or proteins involved in disease were shown to have similarity to the EPSPS or GOX proteins, as defined by eight identical and contiguous amino acids in a sequence. Likewise, none of the amino acid sequences of known allergens or proteins involved in coleiac disease were shown to have similarity to the GOX protein as defined by eight contiguous amino acids in a sequence (US EPA 1997).

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SECTION 3
GENERAL INFORMATION CONCERNING THE GENES AND THEIR ENZYMES THAT CONFER TOLERANCE TO PHOSPHINOTHRICIN HERBICIDE

Summary Note

This document summarises the information available on the source of the genes that have been used to construct phosphinothricin tolerant transgenic plants, the nature of the enzymes they encode, and the effects of the enzymes on the plant's metabolism.

Scope of this document: OECD Member countries agreed to limit this document to a discussion of the introduced genes and resulting enzymes that confer phosphinothricin tolerance to plants. The document is not intended to be an encyclopaedic review of all scientific experimentation with phosphinothricin tolerant plants. In addition, this document does not discuss the wealth of information available on the herbicide phosphinothricin itself or the uses of the herbicide in agricultural and other applications. Food safety aspects of the use of phosphinothricin on phosphinothricin tolerant transgenic plants are not discussed. Such information is available from other sources, including the respective governmental organisations which regulate the use of the herbicide.

While the focus of this document is on the genes and enzymes involved in encoding phosphinothricin tolerance, reference is not made to specific plant species into which phosphinothricin tolerance might be introduced. Any issues relating to the cultivation of phosphinothricin tolerant plants or to the potential for, or potential effects of, gene transfer from a phosphinothricin tolerant plant to another crop plant or to a wild relative are outside the agreed scope of this document. It is intended, however, that this document should be used in conjunction with specific plant species biology Consensus Documents (see list of publications at the front of the document) when a biosafety assessment is made of plants with novel phosphinothricin herbicide resistance.

1. Herbicide Tolerance

Many herbicides kill plants by interfering with enzyme function in the plant. Enzymes are the proteins which catalyse the diverse reactions which comprise the plant's metabolism. Some herbicides exert their effect on a single enzyme which catalyses a key metabolic reaction in the plant. In general, plants exhibit a range of sensitivities to the herbicides used in agriculture, with some species exhibiting considerable tolerance to a herbicide. There are several mechanisms by which plants can tolerate exposure to herbicide: (1) the plant produces an enzyme which detoxifies the herbicide, (2) the plant produces an altered target enzyme which is not affected by the herbicide, or (3) the plant produces physical or physiological barriers to uptake of the herbicide into the plant tissues and cells (Devine *et al*. 1993).

Phosphinothricin tolerance has been conferred to a variety of plant species (see Section V) by using recombinant DNA techniques to transfer one of two genes (*pat* or *bar*) from bacteria to enable the plant to produce an enzyme (phosphinothricin acetyl transferase; PAT). Expression of PAT within the plant cell detoxifies L-PPT, a herbicide (the L-isomer of phosphinothricin), and thereby makes the plant tolerant to L-PPT. This document summarises the information available on the source of these genes, the nature of the enzymes they encode, and the consequences of transgene expression in the plant. Finally, it is suggested

that the reader visit the OECD BioTrack Online website to see the current status of phosphinothricin tolerant plants that have been released under small-scale experimental field trial conditions (http://www.olis.oecd.org/biotrack.nsf) and those that have been approved for commercial release (http://www.olis.oecd.org/bioprod.nsf).

2. Phosphinothricin as a Herbicide

A. The herbicide phosphinothricin

Phosphinothricin is the amino acid, 4-[hydroxy-(methyl) phosphinoyl]-D,L-homoalanine. The L-isomer of phosphinothricin (L-PPT) is widely used as a broad-spectrum weed control agent and is registered for use as a herbicide in many countries. The D-isomer, D-PPT, exhibits no herbicidal activity. L-PPT is the active ingredient of the herbicide glufosinate ammonium. Glufosinate ammonium is an equimolar, racemic mixture of the D- and L-isomers of PPT. Although D-PPT is not herbicidal, L-PPT inhibits glutamine synthetase of susceptible plants and results in the accumulation of lethal levels of ammonia. L-PPT is considered a broad-spectrum herbicide because it is herbicidal to a wide range of plant species. Some plant species exhibit greater sensitivities than others. Additional information on the properties and use of the herbicide phosphinothricin can be obtained from the governmental authorities which regulate its use. For example, the United States Environmental Protection Agency regulates herbicide use and maintains health assessment information concerning phosphinothricin (glufosinate ammonium) available on the Internet (http://www.epa.gov/ngispgm3/subst/irisbak/0247.htm).

B. Production of L-PPT by micro-organisms

Species of the genera *Streptomyces* and *Kitasatosporia* are the only organisms reported to synthesise the amino acid L-PPT. Species of these genera are Gram-positive, sporulating soil micro-organisms, commonly referred to as actinomycetes (Cross 1989, Locci 1989).

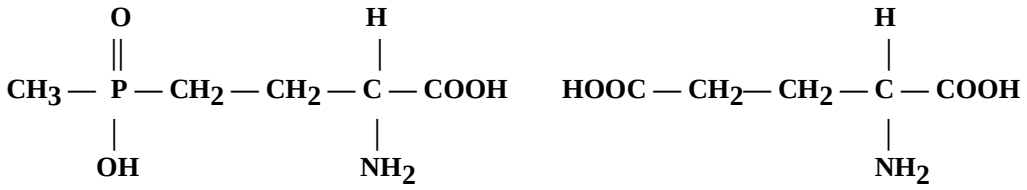
L-PPT has been reported as a component of only two tripeptides, bialaphos and phosalacine (Wild and Ziegler 1989, Omura *et al*. 1984). Bialaphos is a tripeptide (phosphinothricyl-L-alanyl-L-alanine) produced naturally by *Streptomyces hygroscopicus* and *S. viridochromogenes*. Each molecule of bialaphos comprises L-PPT and two residues of alanine. Phosalacine is a tripeptide (phosphinothricyl-L-alanyl-L-leucine) produced by *Kitasatosporia phosalacinea* (Takahashi *et al*. 1984). Peptidase activity readily breaks the peptide bonds, liberating the L-PPT moiety from either bialaphos or phosalacine (Thompson *et al*. 1987, Wild and Ziegler 1989, Omura *et al*. 1984).

L-PPT is the active ingredient in a number of commercial herbicide formulations. The L-PPT can be derived either from fermentation cultures that yield bialaphos, or from chemical synthesis of glufosinate ammonium. Glufosinate ammonium is an equimolar racemic mixture of L-PPT and D-PPT. There are presently no commercial herbicides which use phosalacine.

C. Mode of action of L-PPT herbicides

Herbicides based on L-PPT are active against a broad spectrum of plant species. L-PPT is a structural analogue of glutamate, the substrate of glutamine synthetase (see the side-by-side comparison of L-PPT and glutamate in *Figure 2*.2). L-PPT exerts its herbicidal effect through the inhibition of glutamine synthetase (Bayer *et al*. 1972). In the presence of ATP, L-PPT inhibits glutamine synthetase irreversibly (Devine *et al*. 1993). When L-PPT inhibits glutamine synthetase, phytotoxic levels of ammonia accumulate in the plant (Miflin and Lea 1976, Tachibana *et al*. 1986).

Figure 2.2 L-isomer of phosphinothricin (left) compared to glutamate (right)



Glutamine synthetase is the enzyme responsible for the synthesis of the amino acid glutamine from glutamic acid and ammonia in both eukaryotes and prokaryotes. This is the first reaction in the pathway that assimilates inorganic nitrogen into organic compounds. In plants, glutamine synthetase exists in multiple isozymic forms that can be localised within the cell in the cytosol and plastids. In addition, various isozymic forms are predominately found in certain plant tissues or organs (McNally *et al*. 1983). In plant roots, the primary role of glutamine synthetase is to assimilate ammonia. However, the glutamine synthetase in leaves is primarily responsible for the reassimilation and detoxification of ammonia (Shah *et al*. 1986, Kishore and Shah 1988). Glutamine synthetase is the only enzyme in plants that can detoxify the ammonia released by photorespiration, nitrate reduction and amino acid degradation.

As scientists have increased their understanding of the mode of action of L-PPT, several strategies have emerged for developing plants that are tolerant of exposure to the herbicide. The two most prominent strategies are (1) to identify a variant of glutamine synthetase that is insensitive to inhibition by L-PPT, and (2) to introduce a gene that encodes an enzyme designed to inactivate the herbicidal activity of L-PPT. Despite attempts to utilise the first strategy (AgrEvo 1994), to date only the second strategy has been successful in conferring tolerance to L- PPT.

3. The Development of L-PPT Tolerant Plants

"Traditional" plant breeding techniques. To date, plant breeders have not been successful in using so-called "traditional" plant techniques to develop L-PPT tolerant crop plants. Historically, plant breeders have tried to identify desirable attributes in the germplasm collection of the crop itself or among closely related plant species. The desirable trait(s) would then be bred into the crop via sexual hybridizations, some of which might require some human intervention to achieve success.

Alternatively, in the absence of finding the desired trait in germplasm collections, breeders have used chemical or radiation induced mutagenesis to create variants that would then be evaluated for efficacy and agronomic performance. This technique relies on slightly modifying the plant enzyme which is the "target" of the herbicide (*i.e.* the enzyme(s) which the herbicide inhibits). Thus, the mutagenesis results in a target enzyme that still functions but has lost its sensitivity to a herbicide. This approach has been successful in developing maize and soybean varieties which produce a form of acetolactate synthase that is no longer sensitive to imidazolinone and sulfonylurea herbicides (Saari and Mauvais 1996, Shaner *et al*. 1996). Readers interested in an overview of techniques for producing herbicide tolerant plants may consult Dyer (1996).

Attempts to use such mutagenesis and selection techniques have also failed to produce a useful level of L-PPT tolerance in crop plant species. Included in these efforts has been a decade of failed attempts to obtain maize plants which have a glutamine synthetase that is not inhibited by L-PPT (AgrEvo 1994).

Recombinant DNA techniques. Over the past decade, recombinant DNA techniques have been successfully employed to confer L-PPT tolerance to a variety of crop plant species (see below). Using this approach, plants have been transformed with one of two bacterial genes (*pat* or *bar*) which encode an enzyme, phosphinothricin acetyl transferase (PAT), that detoxifies L-PPT. The expression of the PAT

enzyme in the transgenic plants has been used in three different ways: (1) to confer agronomically useful levels of L-PPT tolerance for crop production, (2) to provide a selectable genetic trait (marker) that can be used in the laboratory or field, or (3) to provide a selectable genetic trait in conjunction with a genetic male sterility system.

- ***L-PPT tolerance for agronomic use.*** In some plants modified to express PAT, the tolerance to L-PPT will be used agronomically in the cultivation of the crop by the grower. An example of such a transgenic L-PPT tolerant plant is the oilseed rape/canola (*Brassica napus* L.) line HCN92, which was the first L-PPT tolerant plant cleared by governmental authorities. Line HCN92 was authorised by Canadian agencies for unconfined release, food and livestock feed use in Canada in 1995 (Agriculture and Agri-Food Canada 1995a, 1995b, 1996a, 1996b). Since then, other transgenic L-PPT tolerant crop plant lines have been cleared through relevant governmental regulatory authorities. The OECD "Biotrack On-line" database (**http://www.olis.oecd.org/bioprod.nsf**) maintains an updated listing of such approvals.

- ***L-PPT tolerance as a selectable marker.*** In some of the plants engineered with the *pat* or *bar* gene, the gene serves as a selectable marker gene. Such plants may not necessarily express agronomically useful levels of tolerance to L-PPT. Marker genes are routinely used in developing transgenic plants because they enable the researches to select successful transformants in the laboratory. In addition, tolerance to L-PPT can be used as a selectable marker in the field. Vasil (1996) states that, in some plant species, expression of L-PPT tolerance has been a more useful selectable marker that the kanamycin resistance that has been used since the inception of recombinant DNA research with plants. Final clearances were granted in the United States in 1995 (USDA 1995) for the first transgenic plant which utilised L-PPT tolerance (conferred by the *bar* gene) as a selectable marker trait.

- ***L-PPT tolerance for selection as part of a male-sterility system.*** Transformation with L-PPT can be used alone or in conjunction with other genes. An example of this is when PAT expression is also part of a genetically engineered male sterility system that can be used in the production of F. hybrid plant varieties (Mariani *et al*. 1990). In this system, plants are transformed with a genetic construct that couples genes that block pollen production, together with the selectable marker gene which confers expression of PAT. Therefore, the PAT expression in the transformed plants makes it possible to use L-PPT as part of a practical system for plant breeders to produce hybrid seed. In 1996, a maize line engineered with this male sterility system was cleared in the United States prior to commercial release (U.S. Department of Agriculture information found at **http://www.aphis.usda.gov/biotech**). Such transgenic male sterility systems are currently being employed for variety development and seed production in canola, chicory and maize.

A variety of plant species have been engineered with either the *pat* or *bar* genes, and many of these plants have been grown in small-scale field tests to evaluate performance under field conditions. As of 1997, these include: *Agrostis palustris* (creeping bentgrass), *Avena sativa* (barley), *Arachis hypogaea* (peanut), *Beta vulgaris* (sugarbeet), *Brassica oleracea* (wild cabbage), *Chichorium intybus* (chicory), *Daucus carota* (carrot), *Festuca arundinacea* (tall fescue), *Gossypium hirsutum* (cotton), *Hordeum vulgare* (barley), *Lycopersicon esculentum* (tomato), *Medicago sativa* (alfalfa), *Gladiolus* sp. (gladiolus), *Cucumis melo* (melon), *Populus* spp. (poplar), *Solanum tuberosum* (potato), *Brassica napus* (rapeseed), *Oryza sativa* (rice), *Glycine max* (soybean), *Sorghum bicolor* (sorghum), *Saccharum officinarum* (sugarcane), *Nicotiana tabacum* (tobacco), *Triticum aestivum* (wheat) and *Zea mays* (maize).

A number of countries have governmental organisations which regulate the field testing and unrestricted release of genetically engineered plants. Information about these plants in OECD Member

countries is available to anyone interested. The database, available on the Internet, is periodically updated to provide information that is both current and accurate (**http//www.oecd.org/ehs/service.htm**).

4. Genes and Enzymes that Confer L-PPT Tolerance

A. Donor organisms for the genes

Two species of actinomycetes, *Streptomyces viridochromogenes* and *S. hygroscopicus*, have been the source of the genes which have been transferred to plants to confer tolerance to L-PPT (Thompson *et al*. 1987, Kumada *et al*. 1988, Hara *et al*. 1991). These species of *Streptomyces* are saprophytic, soil-borne microbes and are not considered pathogens of plants, humans, or other animals (Locci 1989, Cross 1989).

Genes encoding PAT enzymes (PATs) have been isolated from *S. viridochromogenes* and *S. hygroscopicus*. In *S. hygroscopicus*, a PAT is encoded by the *bar* (*b*ial*a*phos-*r*esistance) gene, whereas in *S. viridochromogenes* a PAT is encoded by the *pat* gene (some researchers refer to the PAT encoded by *bar* as BAR). The *pat* and *bar* genes are very similar, sharing 87 per cent homology at the nucleotide sequence level (Wohlleben *et al*. 1988, 1992). The respective PAT enzymes encoded by *pat* and *bar* are also very similar, and share 85 per cent homology at the amino acid level (Wohlleben *et al*. 1988, 1992). Wehrmann and co-workers (1996) recently published results of extensive characterisation of the PATs encoded by *bar* and *pat*. They conclude that the PATs encoded by *pat* and *bar* are so similar as to be functionally equivalent for the purpose of conferring tolerance to L-PPT.

B. Modification of the native gene to enable expression in plants

In order to achieve efficient expression of the *pat* and *bar* genes within plants, it has been common for researchers to modify the codon usage pattern of genes of bacterial origin prior to introducing them into plants. The *bar* and *pat* genes isolated from *Streptomyces* spp. have relatively high G:C content when compared to plant genes, and as a consequence the native microbial genes are inefficiently expressed in plants. In this case, the codon usage pattern of the native *Streptomyces* genes have been modified prior to introduction into the plant. This resulted in increased expression levels. The amino acid sequence of the resultant PAT is not changed (Eckes *et al*. 1989, USDA 1995).

Genes of bacterial origin require modification with appropriate plant-expressible regulatory sequences such as promoters, enhancers, intron and terminators. These regulatory sequences do not encode amino acids and therefore do not affect the coding region of the PAT enzyme. Further discussion on the use of regulatory sequences to achieve expression of transgenes in plants is beyond the scope of this document.

C. Specificity of PAT enzymatic activity

Both PAT enzymes encoded by *bar* and *pat* appear to be: (1) functionally equivalent for the purpose of conferring tolerance to L-PPT, and (2) highly specific for their substrate (Wehrmann *et al*. 1996). In the presence of acetyl-CoA as a co-substrate, PAT catalyses the acetylation of the free amino group of L-PPT to yield *N*-acetyl-L-PPT, a compound that does not inactivate glutamine synthetase. Both of the PAT enzymes are highly specific for L-PPT and do not acetylate other L-amino acids, nor do they acetylate D-PPT (Wehrmann *et al*. 1996, AgrEvo 1994). In the presence of excess concentrations of L-amino acids, both PATs also are unaffected in their ability to acetylate L-PPT (Wehrmann *et al*. 1996).

In L-PPT tolerant plants which express relatively high levels of PAT, the main residue metabolite of L-PPT catabolism is N-acetyl-phosphinothricin (Droege-Laser *et al*. 1994). When PAT expression is low, the degradation pathways of L-PPT can result in the residue metabolites found in L-PPT sensitive plants, namely 4-methyl-phosphinico-2-hydroxy-butanoic acid and 3-methylphosphinico-propionic acid (Droege-Laser *et al*. 1994).

5. Effects of Transgene Expression in Plants

During the life cycle of any herbicide tolerant plant, the plant is only rarely exposed to the herbicide. When the active herbicide L-PPT is applied to the herbicide tolerant plants, the PAT activity will enable the plant to render L-PPT non-toxic to the plant. The PAT enzyme detoxifies phosphinothricin (L-PPT) by acetylation into an inactive compound. Metabolism studies on genetically modified oilseed rape (*Brassica napus* L.) showed a rapid conversion of L-PPT to the non-toxic metabolite, N-acetyl-glufosinate (European Commission 1998). It has also been reported that PAT has extremely high substrate specificity for L-PPT and demethylphosphinothricin (DMPT) (Thompson *et al*. 1987), but experimental data have shown it cannot acetylate L-PPTs analogues L-glutamic acid, D-PPT, nor any protein or amino acid (Wehrmann *et al*. 1996, Agriculture and Agri-Food Canada 1995a, 1995b).

Expression of PAT is not detrimental to plant growth, since such crops have agronomic performance similar to their parents when engineered with either *pat* or *bar* genes. These conclusions have been described in decision documents published by regulatory authorities in Canada, the European Union and the United States prior to the commercialisation of L-PPT tolerant *Chichorium intybus* (chicory), *Brassica napus* (rapeseed, oilseed rape, canola) and *Zea mays* (maize). Information on decisions concerning phosphinotrhicin herbicide tolerant plants can be found at:

http://www.olis.oecd.org/bioprod.nsf
http://www.cfia-acia.agr.ca/english/plant/pbo/home_e.html (Canada)
http://ss.s.affrc.go.jp/docs/sentan/eguide/commerc.htm (Japan)
http://www.aphis.usda.gov/biotech/petday.html (United States)
http://europa.eu.int/comm/dg24/health/sc/scp/outcome_en.html (European Commission)

In recent years, a number of allergenic constituents of plants have been characterised. Allergens usually share a number of characteristics, including the following: (1) they are proteins, (2) they range between 10-70 kiloDaltons in molecular weight, (3) they typically, but not absolutely, are glycosylated, (4) they are stable to digestion (peptic and tryptic conditions of the mammalian digestive system), (5) they are stable to processing, and (6) they are present as the major protein component in the specific food (Metcalfe *et al*. 1996, FAO/WHO 1996, Fuchs and Atwood 1996). The PAT protein is not a known allergen. SDS-PAGE shows a molecular mass of 22-23 kD for *pat* and *bar* gene products, slightly higher than the calculated mass of 20.6 kD. Gel filtration chromatography shows activity at the 43 kD peak (homodimer) (Wehrmann *et al*. 1996). The same authors reported that when PAT and BAR proteins, produced from the *pat* and *bar* genes respectively, were subjected to simulated gastric conditions with pepsin, both proteins were degraded within seconds, and the enzymatic activity dropped to zero within a 5-15 second timeframe.

Other reported studies have shown that the enzyme was inactivated within one minute when subjected to typical mammalian stomach conditions and was inactivated during processing of canola seed (from transgenic *Brassica napus* expressing the PAT enzyme) into feed ingredients (European Commission 1998). The USEPA (1997) reported that experimental data indicated that the PAT protein is rapidly degraded in the gastric environment and is also readily denatured by heat or low pH. Many food allergens have been biochemically characterised, and databases make it possible to compare the amino acid sequence of a protein to those proteins in the database which are known to elicit allergenic responses. The nucleotide sequence of the gene was provided. When subjected to comparative analyses using the GENEBANK DNA database (Agriculture and Agri-Food Canada 1995a) and the FASTDB algorithm of Intelligenetics with three databases of polypeptide sequences (Agriculture and Agri-Food Canada 1995b), the PAT enzyme amino acid sequence did not show significant homology with other proteins present in the databases, except with other phosphinothricin acetyltransferases originating from different organisms. No resemblance with potential toxins or allergens was observed. USEPA (1997a) concluded that “the potential for the PAT protein to be a food allergen is minimal.”

When proteins are toxic, they are known to act via acute mechanisms and at very low dose levels (Sjobald *et al*, 1992). There is no evidence available indicating that the PAT protein is toxic to either humans or other animals. In a 14-day feeding study using bacterially produced purified PAT enzyme, mice gavaged with high levels of the protein (5,050 milligram/kilogram bodyweight) showed no treatment-related significant toxic effects (USEPA 1995). It has also been reported that an avian dietary test was performed with the seed-eating canary bird (*Serinus canaria domestica*), and that a feeding study was performed with the domesticated rabbit (*Oryctolagus cuniculus*); these studies showed no differences in food consumption, behaviour and body weight between birds or rabbits fed with the transgenic PAT producing *Brassica napus* L. (rapeseed, oilseed rape, canola) or non-transgenic counterparts (Agriculture and Agri-Food Canada 1995b).

With respect to the toxicity of PAT, USEPA concludes that "the acute oral toxicity data submitted support the prediction that the PAT protein would be non-toxic to humans." In the United States the EPA, based on submitted toxological data, established an exemption from the requirement of a tolerance for residues of the plant-pesticide ingredients phosphinothricin acetyltransferase (PAT) and the genetic material necessary for its production in all plants (USEPA 1997b).

Governmental regulatory authorities in the United States, Canada, Japan and European Union have made decisions that the presence of the PAT protein in plants does not render them unsafe for consumption as food or feed (see above). Further information on the food safety criteria can be found in published regulations, guidelines and policy statements of various governmental agencies.

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SECTION 4

HERBICIDE BIOCHEMISTRY, HERBICIDE METABOLISM AND THE RESIDUES IN GLUFOSINATE-AMMONIUM (PHOSPHINOTHRICIN) – TOLERANT TRANSGENIC PLANTS

Summary Note

This document summarises the information available on the herbicide biochemistry, the herbicide metabolism and the residues in glufosinate-ammonium (phosphinothricin)-tolerant transgenic plants.

Scope of this document: This document is limited to a condensed discussion of the herbicide biochemistry and metabolism specifically in glufosinate-ammonium (phosphinothricin)-tolerant transgenic plants. It is not intended to be an encyclopaedic review of all scientific experimentation with glufosinate tolerant plants or with the herbicide glufosinate itself. Especially, this document is not to be confused with the type of dossier currently composed for plant pesticides according to directive 91/414/EEC. Moreover, it does not discuss the plentiful information available on the use of the herbicide in agricultural and other applications. Food safety aspects of the use of glufosinate-ammonium on glufosinate-ammonium-tolerant plants are beyond the scope of this document. Such information is available from other sources, including the respective governmental organisations regulating herbicide use.

1. Biochemistry and Physiology of the Herbicide in Non-tolerant and in genetically Modified Glufosinate (Phosphinothricin) – Tolerant Plants

Glufosinate (phosphinothricin; DL-homoalanin-4-yl(methyl)phosphinic acid) is a racemic phosphinico amino acid (Hoerlein, 1994). Its ammonium salt (glufosinate-ammonium) is widely used as a non-selective herbicide and is the active ingredient of the commercial herbicide formulations Basta®, Buster®, Challenge®, Conquest®, Dash®, Final®, Finale®, Liberty® and Ignite®. The L-isomer of glufosinate is a structural analogue of glutamate and, therefore, is a competitive inhibitor of the enzyme glutamine synthetase (GS) of bacteria and plants (Bayer *et al*., 1972; Leason *et al*., 1982). The D-isomer is not a GS inhibitor and is not herbicidally active.

Due to the inhibition of GS, non-tolerant plant cells accumulate large amounts of toxic ammonia produced by nitrate assimilation and photorespiration (Tachibana *et al*., 1986) and the level of available glutamine drops (Sauer *et al*., 1987). Damage of cell membranes and inhibition of photosynthesis are followed by plant cell death. The action of glufosinate is dependent on environmental conditions. Temperatures below 10°C, as well as drought stress, reduce its efficacy because of the limited metabolic activity of the plant (Donn, 1982). Also, light is an important factor for the action of glufosinate (Koecher, 1983).

In genetically modified glufosinate-tolerant plants, the L-isomer of glufosinate is rapidly metabolized by the action of the enzyme phosphinothricin acetyltransferase (PAT) into the non-phytotoxic stable metabolite N-acetyl-L-glufosinate (2-acetamido-4-methylphosphinico-butanoic

acid). N-acetyl-L-glufosinate does not inhibit glutamine synthetase. Therefore, no phytotoxic physiological effects are observed in genetically modified glufosinate-tolerant plants.

Glufosinate is a contact herbicide and is taken up by the plant primarily through the leaves (Haas and Müller, 1986). There is no uptake from the soil through the roots, presumably because of the rapid degradation of glufosinate by soil micro-organisms. There is limited translocation of glufosinate within the plant. After application of L-glufosinate, N-acetyl-L-glufosinate and further metabolites on distinct leaves, a preferential transport into the upper leaves and a low level of translocation into the lower plant parts was observed in both genetically modified and unmodified tobacco plants (Droege, 1991; Droege-Laser *et al*., 1994).

Glufosinate has a wide spectrum of activity encompassing monocotyledonous and dicotyledonous species. Due to its limited systemic action, there is no enduring effect on perennial weeds. Examples of weed species that are not, or only weakly, combated by glufosinate are *Viola arvensis, Bromus spp., Lolium spp., Agropyron repens* and *Urtica urens* (Hoechst, 1991). Weeds emerging after herbicide application are not affected.

Glufosinate is rapidly broken down in soil due to microbial degradation. At 20°C, the soil half-life is less than 10 days (Smith, 1988; Dorn *et al*., 1992). Metabolites arise from oxidative deamination and from acetylation (Dorn *et al*., 1992). L-glufosinate can be used by micro-organisms as a source of nitrogen (Tebbe and Reber, 1989). There are no special reports on the degradation of the D-enantiomer in soil, however, the fast dissipation of the DL-racemic mixture was found in all soils investigated under laboratory, as well as, field conditions (Dorn *et al*., 1992; Smith, 1989). The end products of microbial degradation are CO_2 and natural phosphorus compounds. There is also formation of bound residues which are finally mineralized (Dorn *et al*., 1992).

2. Metabolism of Glufosinate-ammonium in Genetically Modified Plants in Comparison to Non-Transgenic Plants

Because of the widespread use of glufosinate in agricultural practices (non-selective application, as a desiccant, selective application in tolerant crops), the metabolism of glufosinate in sensitive, as well as in glufosinate-tolerant plants, is addressed. If the PAT enzyme is used as part of selectable marker systems of genetically modified plants, lower levels of PAT activity are required compared to glufosinate-tolerant crops for selective field applications of the herbicide.

The metabolism of glufosinate in artificial systems like cell suspension cultures (soybean, wheat, maize) and sterile plants (tobacco, alfalfa, carrot) has been analyzed by Komossa and Sandermann (1992) and by Droege-Laser *et al*. (1994). After treatment of non-transgenic plants with glufosinate, the unstable intermediate 4-methylphosphinico-2-oxo-butanoic acid (PPO) is formed via deamination. A rapid decarboxylation reaction then results in the stable main metabolite 3-methylphosphinico-propionic acid (MPP) which is non-phytotoxic. Within non-transgenic plants, PPO can also be reduced to form 4-methyl-phosphinico-2-hydroxy-butanoic acid, another final and stable product (Droege-Laser *et al*., 1994). In contrast to transgenic PAT-expressing plants, there is no direct proof that in non-tolerant plants only the L-isomer is metabolized.

The metabolism of glufosinate in non-tolerant plants is only limited because plants rapidly die after herbicide application. Moreover, if used as a non-selective herbicide in agricultural practice, glufosinate is not intended to be applied directly, except for desiccation purposes. If crop plants have not emerged at the time of application, residues in the crop plants can only be due to uptake from the soil. Studies evaluating the amount and nature of “indirect” uptake have shown that traces, mainly of the major metabolite 3-methylphosphinico-propionic acid (MPP), can be found (Hoerlein, 1994). This

non-phytotoxic metabolite is also a well known soil metabolite (Tebbe and Reber, 1988) which can be taken up by the roots. It was found to be the only relevant residue following normal weed control in non-transgenic plants (Hoerlein, 1994). In desiccation, residues consist of unchanged glufosinate, with small portions of MPP and a non-relevant portion of 2-methyl-phosphinico-acetic acid.

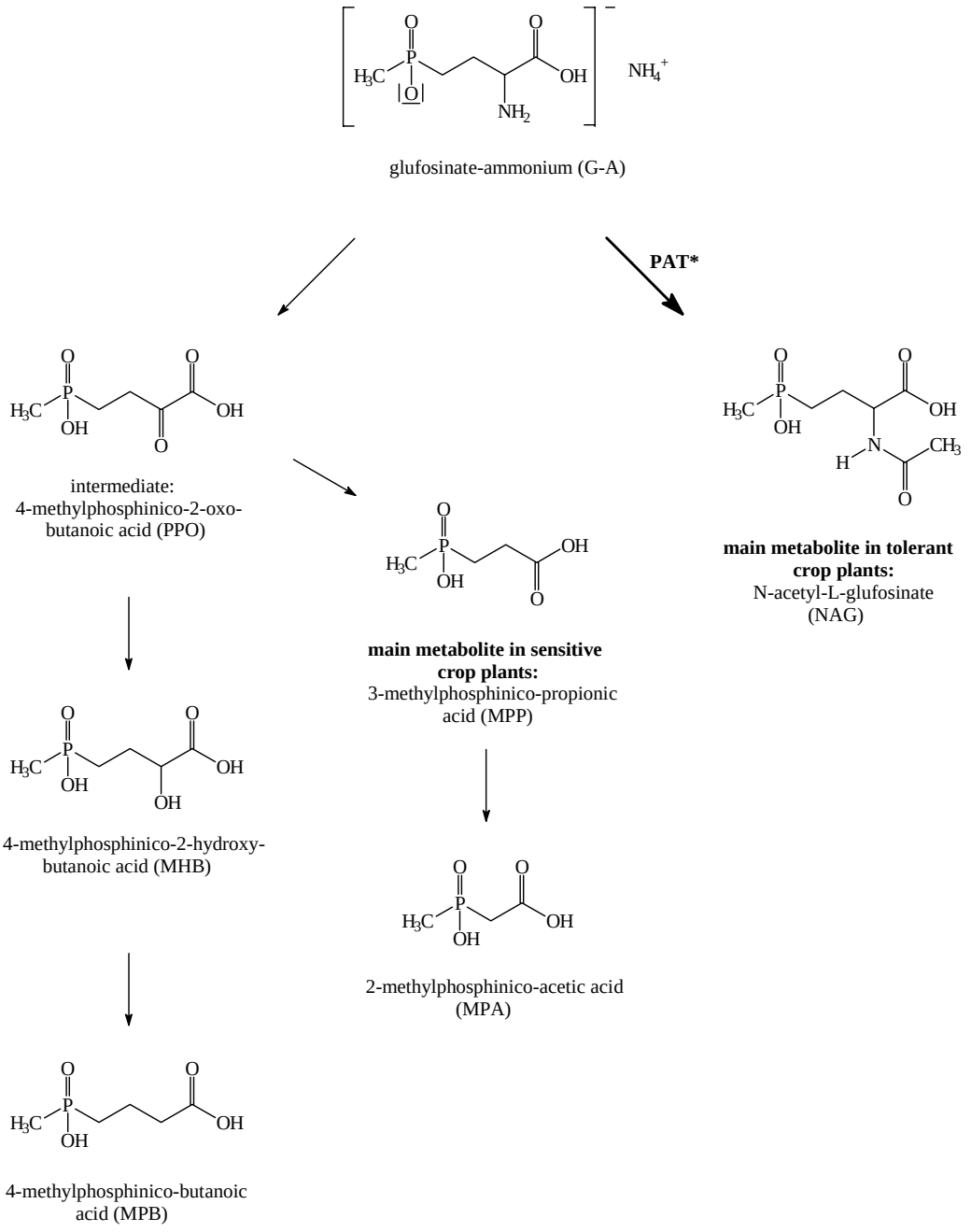
The insertion of genes encoding phosphinothricin acetyltransferase (PAT) enables plants genetically modified in this way to rapidly metabolize the herbicidal active moiety of glufosinate-ammonium into the non-phytotoxic metabolite N-acetyl-L-glufosinate (2-acetamido-4-methylphosphinico-butanoic acid). This metabolite is not found in non-transgenic plants.

The metabolism of glufosinate-ammonium following direct application on genetically modified glufosinate-tolerant corn (maize), oilseed rape (canola), tomato, soybean and sugar beet (*Figure 2*.3) has been investigated with the formulated test substance (Burnett, 1994; Tshabalala, 1993; Thalacker, 1994; Stumpf, 1995; Rupprecht and Smith, 1994; Rupprecht *et al*., 1995; Allan, 1996). In all glufosinate-tolerant crops, the principal residues were N-acetyl-L-glufosinate and - usually with lower concentrations – glufosinate-ammonium and MPP. In corn grain and rape seed, the main residue identified was MPP, with lower concentrations of N-acetyl-L-glufosinate. In corn forage, in soybean seed, in sugar beet roots and in tomato fruit, the main residue was N-acetyl-L-glufosinate. Experiments of Droege *et al*. (1992) and Droege-Laser *et al*. (1994) using transgenic tobacco, carrot, and alfalfa plants also found N-acetyl-L-glufosinate as the major metabolite in glufosinate-tolerant plants. Besides the principal residues, trace levels of other metabolites were also identified in soybean including 2-methylphosphinico-acetic acid (MPA) and 4-methylphosphinico-butanoic acid (MPB). The herbicidally inactive D-glufosinate appears to be stable in plants due to the L-specific acetylation activity of the PAT enzyme (Droege *et al*., 1992).

In genetically modified glufosinate-tolerant plants expressing the PAT enzyme, it appears that two metabolic routes compete: (1) the deamination of glufosinate and subsequent conversion of 4-methyl-phosphinico-2-oxo-butanoic acid (PPO) to 3-methylphosphinico-propionic acid (MPP) or to 4-methyl-phosphinico-2-hydroxy-butanoic acid, and (2) the N-acetylation of L-glufosinate by PAT (Droege-Laser *et al*., 1994). The second of these two routes predominates when PAT specific activity is relatively high.

If genetically modified plants express the PAT enzyme at a low level, the deamination pathway with the formation of MPP predominates. In this case, besides substantial amounts of the acetylated and non-acetylated forms of L-glufosinate, the metabolites 4-methyl-phosphinico-2-oxo-butanoic acid (PPO), 3-methylphosphinico-propionic acid (MPP) and 4-methyl-phosphinico-2-hydroxy-butanoic acid are formed (Droege-Laser *et al*., 1994).

Figure 2.3 **Metabolism of Glufosinate-Ammonium in Non-Transgenic and in Transgenic, Tolerant Crop Plants (Corn, Oilseed rape, Tomato, Soybean, Sugar beet)**



*) PAT = phosphinothricin acetyl - transferase

Source : derived from FAO, 1998

3. Metabolites and Residues in Genetically modified Plants

The FAO's Joint Meeting of Experts on Pesticide Residues (JMPR) suggested, in 1998, a revised residue definition, considering the nature of the residue occurring in conventional and transgenic glufosinate-tolerant plants. This definition was confirmed by the 1999 JMPR as suitable for the establishment of maximum residue levels and for the estimation of dietary intake. For glufosinate-ammonium, residue is defined as the sum of glufosinate-ammonium, MPP and N-acetyl-L-glufosinate (FAO, 1998).

For residue studies, glufosinate-ammonium and the principal metabolites N-acetyl-glufosinate and 3-methylphosphinico-propionic acid (MPP) are extracted from finely ground sample material with water. After cleaning-up of the extracts, the residues are derivatised, resulting in the formation of methylated/acetylated derivatives. These are cleaned up and determined by gas chromatography using a phosphorus-specific flame photometric detector, yielding analytical recoveries which are satisfactory on many substrates. Glufosinate-ammonium and N-acetyl-L-glufosinate are determined as a common derivative and MPP is quantified as a separate derivative. If a differentiation between glufosinate-ammonium and N-acetyl-L-glufosinate is required, the two compounds need to be separated prior to derivatisation.

Using this procedure, the following individual total residues represented as the sum of glufosinate-ammonium, N-acetyl-L-glufosinate and MPP were obtained from genetically modified, glufosinate-tolerant plants while the limit of quantification for each analyte was 0.05 mg/kg. Individual residue data are mainly part of national submissions for glufosinate-ammonium.

A. Oilseed rape

At an application rate of 750 g/ha or 2 x 800 g/ha, the total residue in the seed at harvest encompasses between < 0.05 and 0.24 mg/kg. Rapeseed oil was found to contain below 0.05 mg/kg total residue.

B. Corn

At an application rate of 400 + 500 g/ha or 2 x 800 g/ha, the total residue in corn grain was between < 0.05 and 0.07 mg/kg. Corn oil contained less than 0.05 mg/kg total residue.

C. Soybean

At an application rate of 400 + 500 g/ha, the total residue in soybean seed ranged from 0.32 to 1.88 mg/kg.

D. Sugar beet

At an application rate of 2 x 600 g/ha or 2 x 800 g/ha, the total residue in roots which are relevant to human nutrition as a raw material for sugar production, were found to be between < 0.05 and 0.88 mg/kg. Refined sugar after processing contained no residues (< 0.05 mg/kg).

The lowest NOEL (no observed effect level), established in a chronic (24 months) feeding study in rats, was 2 mg glufosinate-ammonium/kg body weight/day (Ebert *et al*., 1990). This low toxicity is due to the mode of action of glufosinate. In mammals, glufosinate-ammonium competitively inhibits glutamine synthetase (GS). However, contrary to the situation in plants, fixation of ammonia is guaranteed by several metabolic pathways in order to maintain homeostasis of the amino acid pool.

The biosynthesis of glutamine from glutamate forms only one of the possibilities for fixation of ammonia and amino groups. Thus GS is only of minor importance for ammonia fixation in mammals. In this context, Hack *et al*. (1994) found that inhibition of glutamine synthetase by glufosinate did not essentially affect the level of ammonia, glutamate and other amino acids. Since the toxicological data indicated no genotoxic, carcinogenic or teratogenic potential, an acceptable daily intake (ADI) value of 0.02 mg/kg body weight/day was accepted for glufosinate (WHO, 1992). This value has been confirmed as group ADI for glufosinate-ammonium, MPP and N-acetyl-L-glufosinate (WHO, 1999).

Tolerances for combined residues of glufosinate-ammonium and its metabolites (3-methylphosphinicopropionic acid and N-acetyl-L-glufosinate) have been established in the USA for transgenic field corn and transgenic soybean. The tolerances are 0.2 mg/kg and 2.0 mg/kg for corn grain and for soybean seed, respectively (EPA, 1999).

Glufosinate-ammonium is registered for the use in the following transgenic tolerant crops:

Canada	Canola and Corn
USA	Corn and Soybean
Germany	Corn
Portugal	Corn
Argentina	Corn
Romania	Corn

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OECD PUBLICATIONS, 2, rue André-Pascal, 75775 PARIS CEDEX 16
PRINTED IN FRANCE
(97 2006 05 1 P) ISBN 92-64-02258-9 – No. 55155 2006

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ISBN 92-64-02258-9
97 2006 05 1 P

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