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Series on Harmonization of Regulatory Oversight in Biotechnology No. 14

CONSENSUS DOCUMENT ON THE BIOLOGY OF ORYZA SATIVA (RICE)

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No. 1, *Commercialisation of Agricultural Products Derived through Modern Biotechnology: Survey Results* (1995)

No. 2, *Analysis of Information Elements Used in the Assessment of Certain Products of Modern Biotechnology* (1995)

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OECD Environmental Health and Safety Publications

Series on Harmonization of Regulatory Oversight in Biotechnology

No. 14

Consensus Document on the Biology of *Oryza sativa* (Rice)

Environment Directorate

Organisation for Economic Co-operation and Development

Paris 1999

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The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 29 industrialised countries in North America, Europe and the Pacific, as well as the European Commission, meet to co-ordinate and harmonize policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD's work is carried out by more than 200 specialized Committees and subsidiary groups composed of Member country delegates. Observers from several countries with special status at the OECD, and from interested international organisations, attend many of the OECD's Workshops and other meetings. Committees and subsidiary groups are served by the OECD Secretariat, located in Paris, France, which is organised into Directorates and Divisions.

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FOREWORD

The OECD's Working[1] Group on Harmonization of Regulatory Oversight in Biotechnology decided at its first session, in June 1995, to focus its work on the development of **Consensus Documents** that are mutually recognised among Member countries. These consensus documents contain information for use during the regulatory assessment of a particular product.

This Consensus Document addresses the biology of rice (*Oryza sativa*). It contains a general description of rice as a crop plant, as well as its taxonomy, centre of origin/diversity, identification methods, reproductive biology, crosses and ecology. However, its ecology in regard to specific geographic regions is not presented, since this will vary. For intraspecific and interspecific crosses, emphasis has been placed on detailing potential hybridisation between the cultivated species and their close relatives. Emphasis has also been placed on the recent contributions of biotechnology to knowledge of rice biology, including identification and transformation methods. These reflect progress in biotechnology, particularly with respect to rice among monocot crop plants.

Japan served as lead country in the preparation of this document. It is based on materials from scientific research, meetings, conferences and the literature developed in Member countries as well as other important sources including the International Rice Research Institute, the global centre for rice research. It has been revised based on comments received from OECD Member countries. As part of a joint project with the United Nations Industrial Development Organization (UNIDO) on centres of origin and diversity, the document was reviewed by experts in several countries that are centres of origin and diversity for rice.

The Joint Meeting of the Chemicals Committee and Working Party on Chemicals has recommended that this document be made available to the public. It is published on the authority of the Secretary-General of the OECD.

1. In August 1998, following a decision by the OECD Council to rationalise the names of Committees and Working Groups across the OECD, the "Expert Group on Harmonization of Regulatory Oversight in Biotechnology" became the "Working Group".

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Preamble

OECD Member countries are now approving the commercialisation and marketing of agricultural and industrial products of modern biotechnology. They have therefore identified the need for harmonization of regulatory approaches to the biosafety assessment of these products, in order to avoid unnecessary trade barriers.

In 1993, **Commercialisation of Agricultural Products Derived through Modern Biotechnology** was instituted as a joint project of the OECD's Environment Policy Committee and Committee on Agriculture. The objective of this project is to assist countries in their regulatory oversight of agricultural products derived through modern biotechnology - specifically in their efforts to ensure safety, to make oversight policies more transparent and efficient, and to facilitate trade. The project is focused on the review of national policies, with respect to regulatory oversight, that will affect the movement of these products into the marketplace.

The first step in this project was to carry out a survey concentrating on national policies with regard to regulatory oversight of these products. Data requirements for products produced through modern biotechnology, and mechanisms for data assessment, were also surveyed. The results were published in *Commercialisation of Agricultural Products Derived through Modern Biotechnology: Survey Results* (OECD, 1995).

Subsequently, an OECD Workshop was held in June 1994 in Washington, D.C, with the aims of improving awareness and understanding of the various systems of regulatory oversight developed for agricultural products of biotechnology; identifying similarities and differences in various approaches; and identifying the most appropriate role for the OECD in further work towards harmonization of these approaches. Approximately 80 experts in the areas of environmental biosafety, food safety and varietal seed certification, representing 16 OECD countries, eight non-member countries, the European Commission and several international organisations, participated in the Workshop. The *Report of the OECD Workshop on the Commercialisation of Agricultural Products Derived Through Modern Biotechnology* was also published by the OECD in 1995.

As a next step towards harmonization, the Working Group on Harmonization of Regulatory Oversight of Biotechnology instituted the development of ***Consensus Documents*** which are ***mutually acceptable*** among Member countries. The goal is to identify common elements in the safety assessment of a new plant variety developed through modern biotechnology, in order to encourage information sharing and prevent duplication of effort among countries. These common elements fall into two general categories: the biology of the host species, or crop; and the gene product. *Oryza sativa* (Rice) is the fourth crop plant chosen for review; the first three were *Brassica napus* L. (Oilseed Rape), *Solanum tuberosum* subsp. *tuberosum* (Potato) and *Triticum aestivum* (Bread Wheat).

The safety issues identified in the Consensus Documents on the biology of specific crop plants are intended to address the potential for gene transfer within the crop plant species, and among related species, as well as the potential for weediness. They make no attempt to be definitive in this respect,

however, as the many different environments in which the crop species may be grown are not considered individually.

This document is a "snapshot" of current information that may be relevant in a regulatory risk assessment. It is meant to be useful not only to regulatory officials, as a general guide and reference source, but also to industry, scientists and others carrying out research.

In using this document, and others related to the biology of crop plants, reference to two OECD publications which have appeared in recent years will prove particularly useful. *Traditional Crop Breeding Practices: An Historical Review to Serve as a Baseline for Assessing the Role of Modern Biotechnology* presents information concerning 17 different crop plants. It includes sections on phytosanitary considerations in the movement of germplasm and current end uses of the crop plant. There is also a detailed section on current breeding practices. *Safety Considerations for Biotechnology: Scale-Up of Crop Plants* provides a background on plant breeding, discusses scale dependency effects, and identifies various safety issues related to the release of plants with "novel traits".[1]

To ensure that scientific and technical developments are taken into account, OECD countries have agreed that Consensus Documents will be updated regularly. Additional areas relevant to the subject of each Consensus Document will be considered at the time of updating.

Users are therefore invited to provide relevant new scientific and technical information, and to make proposals concerning additional areas that might be considered in the future. ***A short, pre-addressed questionnaire is included at the end of this document. The information requested should be sent to the OECD at one of the addresses shown.***

1. For more information on these and other OECD publications, contact the OECD Publications Service, 2 rue André-Pascal, 75775 Paris Cedex 16, France. Fax: (33) 01.49.10.42.76; E-mail: PUBSINQ@oecd.org; or consult **http://www.oecd.org**

   Also see the BioTrack Online web page at **http://www.oecd.org/ehs/service.htm**

Section I - 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.

Section II - Taxonomic Status

The genus *Oryza* contains 22 species: two are cultivated and 20 are wild (Table 1) (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.

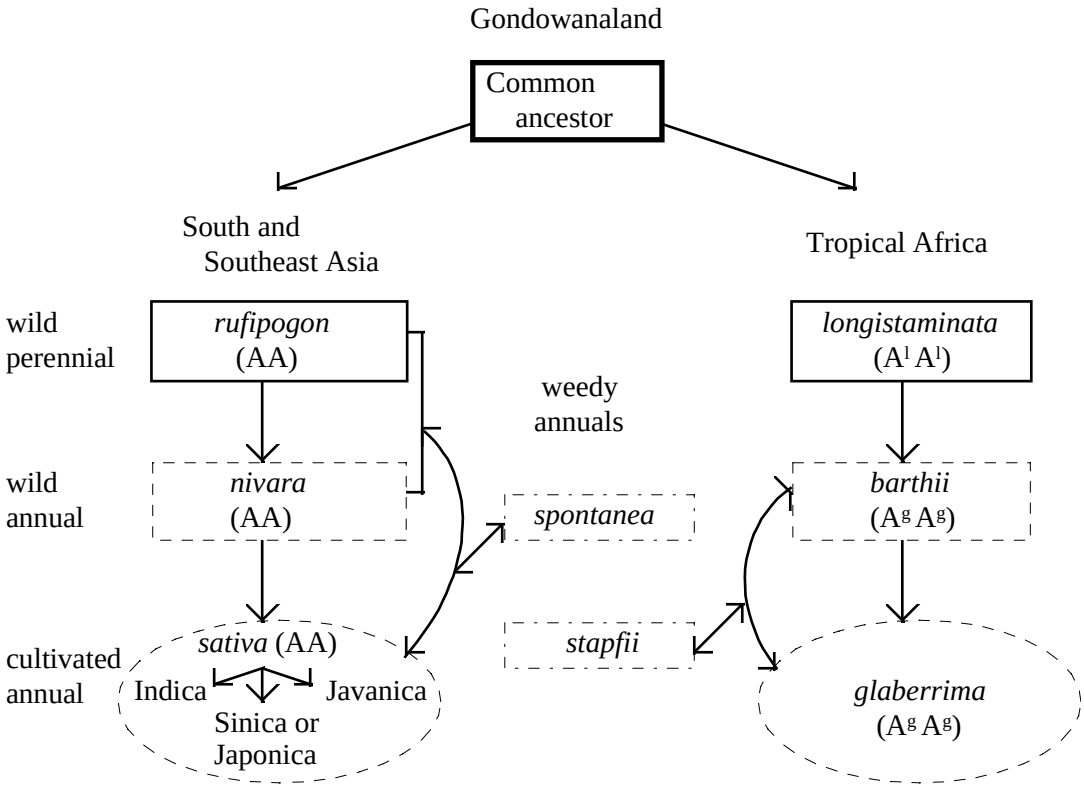
The progenitors of *O. sativa* are considered to be the Asian AA genome diploid species *O. nivara* and *O. rufipogon*, and those of *O. glaberrima* to be the African AA genome diploid species *O. barthii* and *O. longistaminata* (Figure 1) (Chang, 1976).

Table 1. 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, 1988; Vaughan, 1994; Aggarwal et al., 1997.

Figure 1. Evolutionary pathway of the two cultivated species of rice



Source: Adapted from Chang, 1976)

Section III - 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).

Section IV - 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 fertilization 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 2), 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 2. 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*,[1] 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.

1. 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.

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).

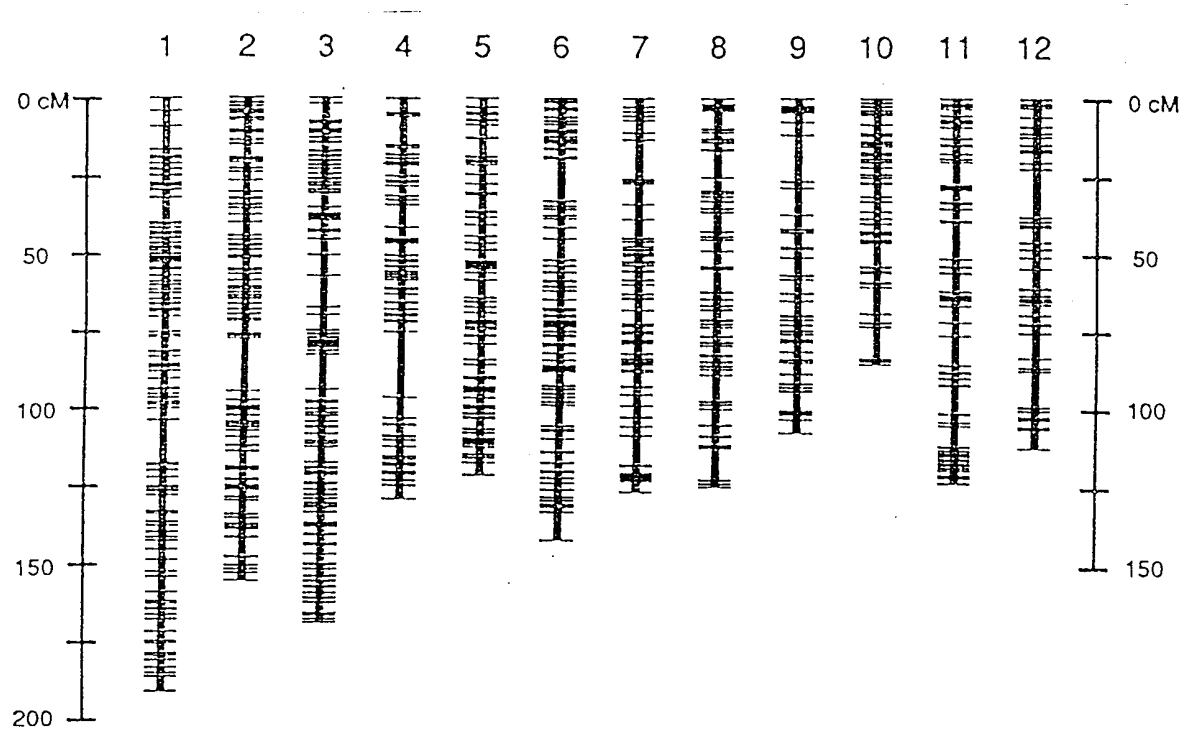
DNA Marker Linkage Maps

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 2) (Kurata et al. 1994). From an interspecific backcross 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 2. Rice RFLP linkage map constructed with 1,383 DNA markers



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

Section V - Reproductive Biology

A. Sexual reproduction

Oryza sativa is basically an autogamous plant propagating through seeds produced by self-pollination. Fertilization 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 fertilization.

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 3) (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 fertilization takes place. The African perennial species *O. longistaminata* showed a stronger crossing barrier with *O. glaberrima* and *O. breviligulata*[2] than with *O. sativa* and *O. rufipogon*[3] (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,*[1] 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*,[1] between the American form of *O. perennis*[4] and *O. breviligulata*,[1] and between the Asian and Oceanian forms of *O. perennis* complex[5] (Oka, 1988).

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.

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

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

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

5. 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.

Section VI - Crosses

A. Intraspecific crosses

Although *Oryza sativa* is basically self-pollinated, natural outcrossing can occur at a rate of up to 5% (Table 3) (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,[6] 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 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).

6. 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.

O. glaberrima and its wild progenitor *O. breviligulata*[7] 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*[8]) 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.

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

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

Table 3. 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*[9]					
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[10]	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.)

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

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

Section VII - 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 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 fertilizers 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 minimizes 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.

Table AIII - 1 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	transformed 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.-interferon	transformed plant
Christou and Ford (1995)	particle gun	*bar*, GUS	transformed plant
Clough et al. (1995)	particle gun	HPT oat phytochome a apoprotein	transformed plant
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, .-phaseolin	transformed plant
Zhen et al. (1996)	particle gun	HPT, GUS	ransformed 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: .-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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