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Office of the Gene Technology Regulator

The Biology and Ecology of Rice (*Oryza sativa* L.) in Australia

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PREAMBLE

This document addresses the biology and ecology of the species *Oryza sativa* L. (cultivated rice). Information included relates to the taxonomy, genetics and origins of cultivated rice, general descriptions of its morphology, development, reproductive biology, pests and diseases, toxicity, allergenicity and its general ecology. This document also addresses the potential for gene transfer to occur to closely related *Oryza* species.

SECTION 1. BIOLOGY AND ECOLOGY OF RICE (*ORYZA SATIVA* L.)

Section 1.1   Taxonomy and Genetics

The genus *Oryza* belongs to the tribe Oryzeae of the family Poaceae (*http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi*). There are 12 genera within the Oryzeae tribe (Vaughan 1994). The genus *Oryza* contains approximately 22 species of which 20 are wild species and two, *O. sativa* and *O. glaberrima*, are cultivated (Vaughan 1994). There is some confusion in the literature concerning the correct nomenclature of the species most closely related to *O. sativa*, as they often lack clear distinguishing morphological characteristics (Vaughan & Morishima 2003). At various times, more than 100 names have been proposed for the *Oryza* species, including 19 for *O. sativa* alone (Oka 1988; Lu 2004). Recently Vaughan (2003) has proposed a new nomenclature for cultivated and wild rice in Asia: *O. sativa* sensu lacto subspp. *indica* and *japonica*, and *O. rufipogon* sensu lacto subspp. *nivara* (annual) and *rufipogon* (perennial), respectively. In addition, two new wild species have recently been recognised in the genus (*O. glumaepatula* and *O. malapuzhaensis*) (Vaughan et al. 2003). The species names presented in Table 1 will be used throughout this document for consistency with the majority of the literature.

O. sativa is the most widely grown of the two cultivated species. It is grown worldwide, including in Asian, North and South American, European Union, Middle Eastern and African countries. *O. glaberrima* however, is grown solely in West African countries. *O. sativa* and *glaberrima-sativa* hybrids are replacing *O. glaberrima* in many parts of Africa due to higher yields (Linares 2002).

Research has suggested that the progenitors of *O. sativa* are the Asian species. These are *O. rufipogon* and *O. nivara*, which are perennial and annual respectively (Vaughan & Morishima 2003). Within the cultivars that have been developed, there are a range of forms bearing more or less similarity to the wild progenitors. These traits are described in more detail in later Sections. *O. barthii* and *O. longistaminata* are thought to be the progenitors of the African cultivated rice, *O. glaberrima* (Vaughan & Morishima 2003). Other wild *Oryza* species are native to Africa, Central and South America, Asia and Oceania with overlapping distributions between these regions as shown in Table 1.

Four *Oryza* species have been reported to grow naturally in Australia. These are *O. australiensis*, *O. meridionalis, O. officinalis* and *O. rufipogon* (OECD 1999; Lu & Jackson 2004). These wild species grow in the northern regions of Western Australia, the Northern Territory and Queensland (Figures 1 and 2). However, no herbarium records exist for *O. officinalis* in Australia's Virtual Herbarium database (*http://www.anbg.gov.au/avh*). A single herbarium record exists of *O. nivara* collected in Queensland (Vaughan 1994), although no records exist for this species in Australia's Virtual Herbarium database. The normal distribution of *O. nivara* is India, Sri Lanka, Nepal and Thailand. It does not appear to be established in Australia.
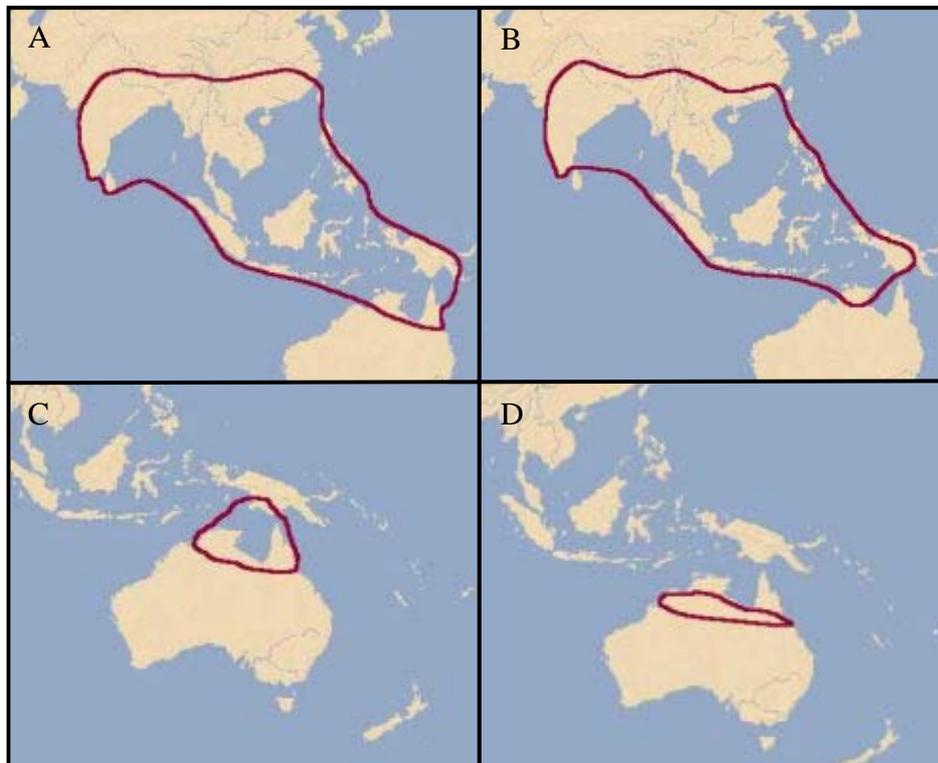
Figure 1. Distributions of wild *Oryza* species occurring in Australia. A. *O. rufipogon*; B. *O. officinalis*; C. *O. meridionalis*; and D. *O. australiensis*. Maps reproduced with permission from Wild Rice Taxonomy (Lu & Jackson 2004)
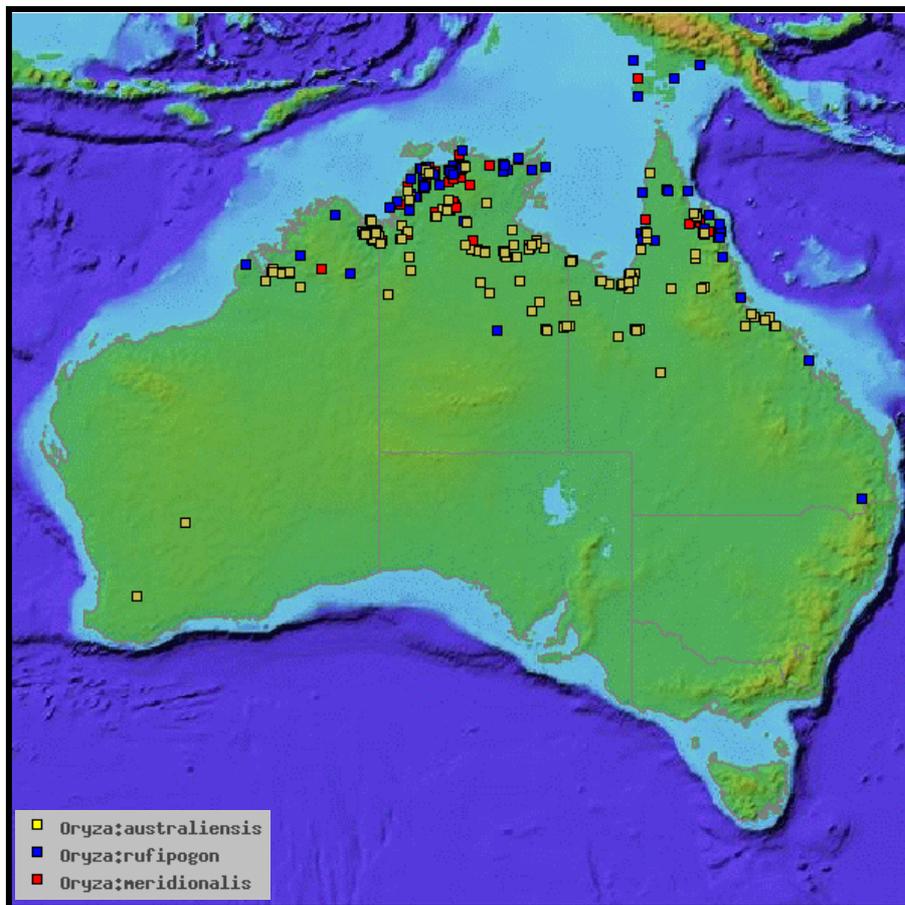
Figure 2. Map of Australia showing locations where herbarium specimens were collected for three of the four wild rice species native to Australia. *O. officinalis* is not shown, as no herbarium records were found for this species. Source: Australia's Virtual Herbarium (*http://www.anbg.gov.au/avh*).

O. sativa has a relatively small (430 million base pairs) diploid genome (2n = 24). This is the smallest genome of all food crops and approximately 50% of the genome is composed of repetitive sequences (Chang 2003). Most other *Oryza* species are also diploid, however some are tetraploid (4n = 48). Species in the *Oryza* genus can be classified into nine groups (with one unclassified species, see Table 1) on the basis of genome compatibility at meiosis in F_1 (first generation) hybrids. These groups, along with the geographic locations of each species, are shown in Table 1 (OECD 1999; Vaughan et al. 2003).

Table 1. The distribution and genomic classification of rice species throughout the world

| *Oryza* species	Genome type	Africa	Central or South America	Asia	Oceania
O. sativa complex					
O. sativa	AA	√	√	√	√
O. glaberrima	AA	√			
O. barthii	AA	√			
O. glumaepatula[1]	AA		√		
O. longistaminata	AA	√			
O. meridionalis	AA				√
O. nivara	AA			√	
O. rufipogon	AA		√	√	√
O. officinalis complex					
O. punctata	BB, BBCC	√			
O. malampuzhaensis[2]	BBCC			√	
O. minuta	BBCC			√	√
O. eichingeri	CC	√		√	
O. officinalis	CC			√	√
O. rhizomatis	CC			√	
O. alta	CCDD		√		
O. grandiglumis	CCDD		√		
O. latifolia	CCDD		√		
O. australiensis	EE				√
O. brachyantha	FF	√			
O. granulata complex					
O. granulata	GG			√	
O. meyeriana	GG			√	
O. ridleyi complex					
O. longiglumis	HHJJ				√
O. ridleyi	HHJJ			√	√
O. schlechteri	??[3]				√

Data in table summarised from (OECD 1999; Vaughan & Morishima 2003).

[1.] Vaughan (Vaughan et al. 2003) uses this nomenclature as it is widespread in the literature but recognises no characteristics to distinguish this species from *O. rufipogon*.

[2.] *O. malampuzhaensis* has recently been distinguished from *O. officinalis* on the basis of morphological characters and its tetraploid genome (Li et al. 2000; Thomas et al. 2001). It distribution is restricted to a small region of southern India.

[3.] The genome type of *O. schlechteri* has not been determined yet (Vaughan et al. 2003).

The genus *Oryza* has also been classified into four complexes, grouping species together as a way of reflecting the genetic diversity of the genus (Vaughan 1994). Of these, the *O. sativa*

and *O. officinalis* complexes are the best studied. The *O. sativa* complex species are all diploid, have AA-type genomes and are pantropical. Adaptation to different ecological niches has resulted in a continuum of morphological types from perennial to annual. Intermediate varieties flower more than once during a season but do not live for more than one season.

The structure of the floral organs and the timing of floret receptivity combine to make *O. sativa* largely autogamous and self-pollinating, although out-crossing does occur at a low frequency (see Section 4 for details).

Section 1.2   Origin and Cultivation

Oryza sativa was first cultivated in south-east Asia, India and China between 8 000 and 15 000 years ago (OECD 1999; Normile 2004). *O. glaberrima* has been cultivated since approximately 1 000 BC (Ahn et al. 1992; Murray 2005). Current cultivation for *O. sativa* is worldwide, extending from latitude 35°S (New South Wales and Argentina) to 50°N (Northern China), over 110 countries. Ninety percent of all rice is grown and consumed in Asia. Cultivation areas utilise approximately 10% of all available crop land worldwide (144 million ha), with only wheat covering more surface area. Rice is also grown from sea level to 3 000 m and in both temperate and tropical climates. A variety of water regimes are used, including unsubmerged upland rice (10% of total cultivation), moderately submerged lowland rice (irrigated, 45%, or rain-fed, 30%), and submerged rice (up to six m of water, 11%, or floating, 4%). Rice can grow in a wide range of soil types as well, including saline, alkaline and acid-sulfur soils (Takahashi 1984b; Oka 1988; Ahn et al. 1992; OECD 1999). The chemical properties of the soils do not appear to be as important as the physical ability of the soil to hold a flood (Scott et al. 2003).

A large number of *O. sativa* cultivars have been developed through centuries of rice domestication. The International Rice Gene Bank (*http://www.knowledgebank.irri.org*) holds approximately 100 000 different rice varieties, most of which are *O. sativa*. Cultivars can be distinguished on the basis of many characteristics, including the following: adaptation to different water regimes; growth habit and height; shapes, size and colour of the culm, leaf blade, panicle, hull and grain; degree of pubescence; cold-, disease- and drought-tolerance; and many more (OECD 1999; Takahashi 1984a). Cultivars can be divided into three ecological varieties, Indica (tropical and sub-tropical distribution), Javanica (grown in Indonesia) and Japonica (temperate distribution), however, there are many overlaps between these classes and classification can be difficult. The two most widely grown, and researched, ecotypes are Indica and Japonica. Generally, Japonica grains are shorter and wider than Indica grains and are softer and stickier when cooked. Other characters used to distinguish between the two types include temperature sensitivity, drought resistance and apiculus hair length (OECD 1999).

Low temperature is one of the main limits on crop yield (McDonald 1979; McDonald 1994). Japonica cultivars are grown predominantly in temperate regions and can germinate and grow under lower temperatures (15 to 20°C) than the tropical and sub-tropical Indica cultivars. Temperatures below 18°C at night during pollen formation result in sterile pollen in all cultivars (McDonald 1994).

1.2.1   Effects of Domestication

Through domestication, *O. sativa* has evolved into many different cultivars that are adapted to the wide range of conditions found in rice growing regions. As mentioned in the previous Section, these include adaptation to tropical or temperate climates, a wide range of soils, and a greater or lesser dependence on water during their lifecycle (Takahashi 1984b; Oka 1988; OECD 1999).

The effects of rice domestication are apparent when cultivars are compared to their wild relatives. Wild rice propagates independently of humans, while cultivated rice is much more

dependent on human interventions (Oka 1988). This dependence has come about through selection against survival traits such as seed shatter, dormancy and ratooning. Out-crossing rates have also diminished through changes in the morphology of rice flowers. Wild rice varieties often have longer, exserted (protruding) stigmas that are more exposed to pollen from nearby plants than those of the cultivars, which tend to remain at least partially within the hull and are more protected from non-self pollen. In cultivars, the anthers are also shorter and overhang the stigma, and pollen is released shortly after the florets open. Wild pollen is released later, from longer anthers (Oka 1988). Wild rice can also harbour resistance to common pests and diseases of cultivated rice, a valuable trait for plant breeders (Ikeda et al. 1994; Vaughan 1994).

1.2.2   Use of mutants in rice breeding programs

Information presented in this Section has been summarised from Matsuo et al. (1997) and references cited therein.

During the domestication of rice, natural variation (resulting from natural mutations) has resulted in the development of a large number of distinct cultivars differing in a range of agronomically useful characteristics (see previous Section). Natural mutations have the potential to alter many morphological traits including plant height, tiller number and form (e.g. erect or prostrate) and leaf, grain and panicle shape. Physiological traits can also be affected such as heading time, sterility, shattering habit, waxiness of endosperm and resistance to pests and diseases or environmental stresses.

Mutagens (agents which induce genetic mutations) have been used since the early part of the 20th century to assist in the development of new rice cultivars by increasing genetic diversity available to rice breeding programs. Two types of mutagens have been used, ionizing radiation (e.g. X-rays and gamma rays) and mutagenic chemicals (e.g. DNA base analogues, acridines and alkylating chemicals). The degree of exposure to a mutagen determines the frequency of mutation in the resulting plant lines. At higher doses, the frequency of sterility in the mutant lines increases and decreases the chance of isolating useful mutations. Research conducted by the Institute of Radiation Breeding in Japan has shown that very large amounts of irradiated seed (in the order of 10 000) is required for a practical phenotype to result. Useful phenotypes that have resulted from mutagenesis programs have included plants that mature earlier in the season, semi-dwarf cultivars and plants displaying higher yields, altered grain weight and composition, more tillers, disease resistance or stress tolerance. As theres mutations can only be detected as phenotypic changes, there is a potential for other, undetected, mutations to accumulate in the lines used in the breeding program.

1.2.3   Cultivation in Australia

Rice may have initially been introduced to Australia and grown by Chinese gold miners in Queensland during the 1850s (Ricegrowers' Association of Australia Inc. 2004a). However, a Japanese immigrant grew the first recorded rice crop on 200 acres of flood-prone land donated by the Victorian government. He began cultivating the land in 1906 as a demonstration crop and in 1914 produced enough rice for commercial sale. With the establishment of the Murrumbidgee Irrigation Area, near Leeton in New South Wales, experimental crops of Californian rice were grown from 1922, with commercial harvests produced from 1924 (Sunrice 2004; Ricegrowers' Association of Australia Inc. 2004a).

From the 1950s, commercial rice crops were also trialled in the Northern Territory (Humpty Doo, east of Darwin) and Western Australia (Camballin, Lower Fitzroy River and Kununurra, Ord River). These failed for a variety of reasons including inappropriate yearly rainfall distributions and soil mineral deficiencies (McDonald 1979). An attempt to establish a commercial rice industry in northern Queensland (Burdekin Irrigation Area) also failed in the early 1990s. Currently, commercial rice production in Australia is mainly located in the

Murray and Murrumbidgee valleys of New South Wales, with a small number of farms in northern Victoria. The New South Wales rice industry produces high average yields, using temperate Japonica cultivars. In 2002/2003 280 000 tonnes of milled rice was produced in Australia (USDA FAS 2004). Fifteen percent of this crop is consumed domestically while the remaining 85% is exported. In addition, by-products of rice (rice hulls, rice bran, broken rice and rice straw) are used in a variety of products, including horticultural, livestock, industrial, household, building and food products (Ricegrowers' Association of Australia Inc. 2004a).

Rice is grown in contour bays (levelled using laser-assisted technology) on clay soils, to minimise water drainage and loss. The main methods used for sowing are: aerial sowing, combine sowing and sod seeding (McDonald 1994). Aerial sowing of pre-soaked and germinated seeds, is performed from aeroplanes into pre-flooded bays. During combine sowing, seed and fertiliser are planted into the soil simultaneously, and sod seeding is performed directly into pasture fields. Aerial sowing predominates, providing the best crop establishment in cooler locations. For most Australian cultivars, the end of October is the latest recommended date for sowing, to allow establishment of the crop under satisfactory conditions. The reproductive phases of growth will then occur in January when temperatures are unlikely to be too cold for pollen development, and grain maturation can occur during the milder conditions of early autumn (Lacy et al. 2003). Sod seeding is the method preferred by organic rice growers (McDonald 1994). Rotation cropping is used on New South Wales rice farms, with legume pastures or wheat planted after harvest to utilise residual soil moisture (Sunrice 2004). Until the 1970s, rice was grown in long rotations of one crop every four to five years with pasture crops grown in between. This regime relieved many of the typical problems associated with rice production such as high reliance on pesticides and fertilisers, increasing costs of water and chemicals, and environmental pressures such as salinity, waterlogging and chemical residues. Since then, decreasing returns on animal production have resulted in shorter rotation periods (Lattimore 1994). Australia has one of the highest yields (8-10 tonnes/ha) of rice producing nations in the world (USDA FAS 2004).

Section 1.3   Morphology

The following description is based on (McDonald 1979; OECD 1999). Rice is a typical grass, forming a fibrous root system bearing erect culms and developing long flat leaves. It has a semi-aquatic lifestyle, requiring water particularly during the reproductive growth phase. It forms multiple tillers, consisting of a culm and leaves, with or without a panicle. The panicle emerges on the uppermost node of a culm, from within a flag-leaf sheath and bears the flowers in spikelets. The culm consists of a number of nodes and hollow internodes that increase in length and decrease in diameter up the length of the culm. Primary tillers emerge from nodes near the base of the main culm and secondary and tertiary tillers emerge sequentially from these. Single leaves develop alternately on the culm, consisting of a sheath, which encloses the culm and a flat leaf blade. The leaf forms a collar or junctura between the sheath and blade and a ligule and two auricles develop on the inside of the junctura and base of the leaf blade respectively. Cultivars can vary widely in the length, width, colour and pubescence of the leaves.

The panicle emerges from the flag-leaf sheath and consists of a central rachis with up to four primary branches at each node. Primary and secondary branches bear the flower spikelets. Each spikelet has a single floret and two glumes. It is enclosed by a rigid, keeled lemma, which is sometimes extended to form an awn and partially envelops the smaller palea. The floret contains six stamens and a single plumose ovary with two branches. At anthesis, two lodules at the base of the floret swell and force the lemma and palea apart as the stamens elongate and emerge. The stigma is sometimes exposed as well.

The fertilised ovary is a caryopsis, meaning a small, single-seeded dry fruit with the pericarp and seed coat fused. It is commonly called a grain. The grain consists of an embryo,

endosperm, pericarp and testa, surrounded by the husk or hull (the lemma and palea). Grain length varies with cultivar between 5 and 7 mm, and grains can be round, bold or slender.

Section 1.4   Growth and Development

There are three main developmental stages in rice. These are germination/vegetative growth, reproductive development, and grain ripening. Unless otherwise indicated, the descriptions below are based on those published by the International Rice Research Institution (International Rice Research Institute 2002).

1.4.1   Germination and vegetative growth

After imbibition of the seed, germination begins with the emergence of the coleorhiza and coleoptile from the pericarp. The radicle gives rise to the seminal root system, which has limited branching. Germination can occur under aerobic or anaerobic conditions. Under anaerobic conditions, the coleoptile emerges first, as it is the only part of the embryo that can grow under energy derived solely from fermentation (Moldenhauer & Gibbons 2003). The fibrous roots develop from underground nodes. The coleoptile elongates along with the epicotyl, and when the coleoptile reaches the soil or water surface, it splits open and the primary leaf emerges (McDonald 1979). During this early phase of development, the plant can produce a leaf every four to five days as the primary culm develops. As the rice plant grows, primary tillers begin to emerge from the axial nodes of the lower leaves. These give rise to the secondary tillers, from which tertiary tillers can also develop. The internodes begin to elongate at, or near, panicle initiation (see below) (Moldenhauer and Gibbons 2003).

1.4.2   Reproductive development

The reproductive stage begins with panicle initiation. The timing of this may be linked to specific photoperiods and is highly cultivar-dependent (McDonald 1979). Panicle initiation occurs at the growing tip of the tiller. As the panicle grows inside the flag-leaf sheath, senescence of the lower leaves begins. A further three leaves develop before heading (panicle emergence) occurs. The panicle may emerge partially or fully, and greater emergence is selected for in cultivars as a means of decreasing disease occurrence (Moldenhauer & Gibbons 2003). Flowering typically begins one day after heading and continues down the panicle for approximately seven days until all florets on the panicle have opened. Anthesis begins with the opening of the florets followed by stamen elongation and generally lasts 1.2 to 2.5 hrs between 9 am and 2 pm. However, this is temperature dependent and can take longer and occur later on cooler or cloudy days (Moldenhauer & Gibbons 2003). As pollen shedding generally occurs within nine minutes of floret opening (Oka 1988), pollen is usually shed onto the florets of the same panicle, resulting in self-fertilisation. Fertilisation is completed within six hours. This is the stage when rice is most sensitive to cold temperatures (McDonald 1979).

1.4.3   Grain ripening

Once the florets are fertilised, the ovaries begin to develop into grains. Initially, the grain fills with a white, milky fluid as starch deposits begin to form. The panicle remains green at this stage and begins to bend downwards. Leaf senescence continues from the base of the tillers but the flag-leaf and next two lower leaves remain photosynthetically active. The grain then begins to harden into the soft dough stage. Husks begin to turn from green to yellow and senescence of the leaves and tillers is at an advanced stage. During the final stage the grain matures, becoming hard and dry. The entire plant begins to yellow and dry out, at which point the grain can be harvested.

Section 1.5   Reproduction

1.5.1   Pollination and pollen dispersal

All wild and cultivated rices are wind-pollinated, with a few varieties having scented flowers that attract bees (Oka 1988). Rice breeders are reported to observe greater out-crossing when honeybees are present (Gealy et al. 2003). Although wind-assisted pollen dispersal distances have been estimated at up to 110 m (Song et al. 2004), pollen is short-lived, with most pollen grains losing viability after approximately five minutes under typical environmental conditions (Koga et al. 1969). Pollen grain morphology changes dramatically after shedding from the anther. Initially the grains are spherical but within minutes they begin to collapse. The collapse of the pollen grains coincides with a measured loss of viability. In one study, 90% of pollen grains were found to be viable for up to four minutes, while between 5 and 8 minutes after shedding, viability decreased to approximately 33% (Koga et al. 1969). It is recommended that buffering isolation zones are wider than 110 m or consist of tall crops such as sugarcane to prevent gene flow (den Nijs et al. 2004).

Rice flowers are generally self-pollinated due to a number of factors limiting their receptivity to out-crossing. These factors include a short style and stigma (1.5 to 4 mm in combined length), short anthers, limited pollen viability, and brief period between opening of florets and release of pollen (between 30 seconds and nine minutes) (Morishima 1984; Oka 1988). Wild rices differ from cultivars in all of these characteristics, tending to encourage out-crossing with longer styles, stigmas and anthers, and pollen that remains viable for up to twice as long as in cultivated rice (Oka 1988).

As described in Section 5, *O. sativa*, is predominantly autogamous, but does demonstrate low rates of out-crossing including hybridisation with some wild rice species.

1.5.2   Seed dispersal

The probability of seed dispersal from rice plants varies widely within the *O. sativa* species. Most cultivars have limited dispersal ability, being bred to allow maximal harvest by farmers. In wild rices and some cultivars, mature rice seeds can be shed from the plant through seed shatter. Shattered seed can either be buried in the soil for subsequent germination or be eaten/dispersed by animals. Another cultivar-specific trait is the presence or absence of awns at the tip of the lemma. When present, awns can vary in their rate of development, length, diameter and bristle length. The presence or absence of awns influences the potential for seed dispersal through attachment to passing animals (Oka 1988).

1.5.3   Seed dormancy

Seed dormancy is a mechanism that delays the germination of seeds in the presence of suitable germination conditions. In wild plant species, variable dormancy can be a useful adaptation, preventing competition between seedlings resulting from simultaneous germination (Oka 1988). It has been reported that of the three *O. sativa* ecotypes, Indica cultivars display the greatest degree of dormancy, followed by Javanica and then Japonica cultivars (Ellis et al. 1983). Seed dormancy in rice is also important in tropical species and cultivars as a means of preventing preharvest sprouting (germination of the seed in the panicle) if conditions are warm and wet before harvest (Gu et al. 2004). In one study evaluating rice seed dormancy, unhulled seeds were kept at room temperature after harvest and the time taken for seed germination was recorded (reported in Oka, 1988). Of the 92 different cultivars tested, 67 demonstrated 40% germination within a month of harvest and all cultivars had reached 40% germination within three months. Of 17 annual 'wild varieties', 15 took more than 8 months to reach 40% germination. For some rice varieties, dormancy allows the seeds to remain viable but ungerminated in the soil for several years (Moldenhauer & Gibbons 2003). In temperate, Japonica cultivars, dormancy is far less common. Dormancy is a heritable trait but the environmental conditions during seed maturation also appear to

influence the degree of dormancy present in the seeds. For example, Indica cultivars have stronger dormancy after maturation in rainy weather (Takahashi 1984b). Drying the seed at high temperatures (40°C to 50°C for up to two weeks) after harvest removes dormancy from all rice seeds (Takahashi 1984b).

1.5.4   Vegetative regeneration

Rice plants have the ability to propagate vegetatively, through the production of new growth at internodes after grain maturation. These secondary growths are called ratoons and under favourable conditions, can provide a second harvest (OECD 1999). *O. sativa* cultivars range in growth habit from perennial to strictly annual, with the majority of crops grown as annuals. Some of the South American *Oryza* species form ratoons that can break off the parent plants and disperse by floating down rivers, forming mats of regenerative material that can take root on reaching land (Oka 1988; Vaughan et al. 2003).

Section 1.6   Pests and Diseases

1.6.1   Weeds

In Australia, the main weed species affecting rice crops are Barnyard Grass (*Echinochloa crus-galli* and related species), Dirty Dora (*Cyperus difformis*), Starfruit (*Damasonium minus*) and *Elatine gratioloides* (McIntyre et al. 1991; Jahromi et al. 2001). The first three of these are recognised as serious weeds, needing routine applications of herbicides for control (McIntyre et al. 1991). In addition, *E crus-galli* has been listed among the world's ten worst weeds (Kendig et al. 2003). Despite herbicide-tolerant weed populations developing in California, the use of rotation cropping has so far slowed the development of resistance in Australia (Sanders 1994).

Different methods of crop establishment can influence the occurrence of weeds. Aerial sowing favours the establishment of *C. difformis* and *D. minus*, while suppressing *E. crus-galli*. In contrast, sod and combine sowing favour *E. crus-galli*. These correlations are dependent on the methods used in monitoring weed levels and whether herbicides have been applied or not (McIntyre et al. 1991). Intensity of cropping is another factor determining the abundance of weed species in rice crops. Higher intensity cropping favours all of the major species as well as some more minor weeds, such as *Chara* spp. and *Diplachne fusca* in Australia. Intensive cropping decreased the abundance of some weed species, which could be classified as either pasture species or wetland species. Species from either of these groups are not adapted to growing in the conditions found in rice fields and wetland species are generally only apparent in new rice fields (McIntyre et al. 1991).

Rice itself has developed into a weed, particularly in areas where rice crops show high levels of genetic diversity and grow sympatrically with wild species (Oka 1988). This tendency towards weediness is addressed in Section 4. The geographical isolation of the Australian rice industry from wild rice populations, its relative youth and the sowing of a relatively small number of cultivars from pure seed stocks have limited the occurrence of weedy/red rice in Australia.

1.6.2   Insects and other invertebrate pests

Invertebrate pests mainly attack rice crops during the initial germination and establishment phases of growth. The major pests of Australian rice crops are bloodworms (the larvae of a midge, *Chironomus tepperi*), various aquatic snails (including *Isidorella newcombi*) and aquatic earthworms (*Eukerria saltensis*). Other less damaging pests are leaf miners (*Hydrellia michelae* and possibly other congeneric species), tadpole shrimp (*Triops australiensis australiensis*) and common armyworm (*Leucania convecta*) (Stevens, 2003).

Bloodworms are the most common pest of rice in New South Wales. They destroy the tips of developing roots and can prevent the establishment of the crop in serious infestations (McDonald, 1979). Recent trials of insecticides (Fipronil, α−Cypermethrin) in the field show promise for controlling these pests (Stevens 2003).

Aquatic snails graze on young seedlings and can be effectively controlled by applications of copper sulfate (McDonald, 1979), however this has long-term toxicity effects, particularly for livestock grazing on pastures following rice production. The development of a new, less toxic compound has therefore been a priority for the industry. Chlorothalonil, a horticultural fungicide, is effective against *I. newcombi* and also improves plant growth, presumably through its anti-fungal properties (Stevens 2003).

Crop rotations appear to be effective for controlling aquatic earthworms, as the dryland crops (often winter cereals) do not allow these worms to persist in the paddocks. Unlike the other invertebrate pests discussed in this Section, these aquatic earthworms are not native to Australia, having been introduced more than 100 years ago (Stevens 2003).

Table 2 summarises risks relating to the introduction of other invertebrate pests into Australia. The highest risk is associated with the introduction of the Golden Apple snail. The quarantine zone that surrounds the rice growing region of NSW is described in Section 1.6.4. There is a prohibition on the importation or introduction of the Golden Apple snail into this protected zone (NSW Agriculture 1996a).

1.6.3   Vertebrate pests

Rodents are a serious pest of Australian agricultural regions (Brown & Singleton 2002). Mouse plagues occur when mouse densities exceed 1 000 mice/ha and can reach more than 2 500 mice/ha at their peak. There are four stages to a mouse plague. Stage 1 is the low phase with less than 10 mice/ha. During Stage 2, the increase phase, densities increase rapidly to hundreds of mice/ha. Stage 3 is the peak phase with the highest mouse densities and is followed by a population crash where the density falls to less than one mouse/ha. This final phase can last approximately two years (Brown & Singleton 2002). A significant trigger for the start of a mouse plague is the occurrence of higher than average autumn rains, particularly following one or two years of drought (Caughley et al. 1998).

Mouse plagues have been recorded in the grain growing regions of Australia since 1904 and occur somewhere in Australia approximately every four years. The frequency in specific regions can be lower, approximately once every seven years, although this may be increasing (Brown & Singleton 2002). Mouse plagues have a serious economic impact on agricultural regions: the plague of 1993/4 is estimated to have caused losses of US$60 million (Caughley et al. 1994). Mouse densities generally peak in autumn and thus rice crops can be affected at the preharvest stage. In the Murrumbidgee Irrigation Area during autumn 1994, damage to rice crops was estimated at up to 75% on some farms with an average of 6% across the region (Brown & Singleton 2002).

Very high usage of poisons has been reported during Australian mouse plagues. For instance, in South Australia and Victoria during the 1993/4 plague, strychnine baits were distributed over 350 000 ha of crops and in New South Wales, in 1999, 500 000 ha of land was baited with zinc phosphide (summarised in Brown & Singleton (2002)). Rodent management strategies that avoid the heavy use of poisons are being developed both in Asia and Australia. In Asia, a 'Trap Barrier System' has been trialled, in which an early rice crop is planted in or near a rice field and used as bait for rodents, which are trapped as they enter the crop through holes in the barrier surrounding it (Beckmann 2003). Cultural practices, such as clearing vegetation from around rice fields, by spraying with herbicides and/or through grazing, appear to be important in Australia for decreasing the damage by mice (Brown & Croft 2000).

A number of methods have been proposed for monitoring mouse populations in order to better identify the start of a plague. These methods include regular counting of active mouse holes and/or the number of tracks on a selected area of land; placing a weighed amount of grain in a series of bait stations in or around crops and weighing the remaining grain the following day; placing 10 x 10 cm squares of paper, soaked in linseed or canola oil and marked into 1 cm squares, in and around a crop overnight, then counting the number of squares that have been eaten by mice; or setting traps overnight and counting numbers of mice caught (Caughley et al. 1998). There is no known correlation between apparent mouse densities and resulting grain losses however.

In Australia, waterbirds may be responsible for losses to both establishing and established rice crops. The degree to which waterbirds damage Australian rice crops is difficult to estimate and appears to vary from year to year. One study reported that yields in enclosed areas were the same as those in areas exposed to waterbirds (Davey & Roberts 1990), suggesting that waterbirds may not be a major problem for rice crops. Waterbird species known to feed on rice crops are the grey teal (*Anas gibberifrons*), the wood duck (*Chenonetta jubata*) and the black duck (*A. superciliosa*). Ducks feed on flooded rice fields at night, resting during the day at nearby 'refuge' areas (Davey & Roberts 1990). Attempts to scare the birds with loud noises do not offer long-term control of the problem and shooting is most effective before the flocks have landed for the night (Davey 1990). An indirect problem caused by waterbirds is found when rice crops are infested with the aquatic earthworm *E. saltensis*, as large flocks of ibis can congregate on the crop and cause damage through trampling of the young rice plants while they eat the earthworms (Stevens 2003).

In contrast, research in Asia is currently focussed on the feasibility of using ducks to decrease crop damage from snails, insect pests and weeds (Teo 2001; Bui et al. 2002). In one study, the presence of ducks, in the absence of pesticide and herbicide applications, resulted in an 8% decrease in the crop yield but had added economic benefits from sales of the ducks for meat and reduced chemical purchases (Bui et al. 2002). In another study, different species of duck were investigated for control of snail populations in the field and found to be effective biological control agents for these pests as long as the rice seedlings were sufficiently well established to tolerate trampling by the ducks and the duck species was not too large (Teo 2001). Waterbirds are also encouraged to visit winter-flooded fields in the US where they help to control weedy/red rice by eating significant quantities of seeds before they can germinate the following season (Kendig et al. 2003).

Galahs (*Cacatua roseicapilla*) have also been reported to eat freshly sown grain before rice paddies are flooded (Davey & Roberts 1990)

1.6.4   Diseases

A quarantine zone surrounds the rice growing regions of south western NSW. The NSW government prohibits the importation of rice plants and all rice products (including hulls, straw and bran), any machinery that has been used in the production, harvesting or milling of rice, and any packaging, container, covering or goods with which any rice or rice products have come into contact, into the quarantine zone. The local government areas in this quarantine zone are, Balranald, Berrigan, Carrathool, Conargo, Corowa, Deniliquin, Griffith, Hay, Jerilderie, Leeton, Murray, Murrumbidgee, Narrandera, Urana, Wakool and Windouran (NSW Agriculture 1996b). The rice industry of southern Australia remains free of major diseases (McDonald 1979; Sunrice 2004), which is at least partly due to this quarantine zone. However there are a few reports of the following bacterial and fungal diseases in Australia: Stem rot (*Magnaportha* spp.); Narrow Brown Leaf spot (*Cerscospora oryzae*); Bacterial sheath spot and Glume blotch (*Pseudomonas syringae* pv *syringae*); and Crown sheath rot (*Gaeumannomyces graminis*) (Ahn et al. 1992; Lacy et al. 2003). Table 2 summarises the potential diseases that may be imported from other rice growing areas of the world.

Table 2. Summary of potential diseases and pests of Australian rice crops.

Type of Threat	Cause	Perceived Risk
Fungal Disease		
Rice blast	*Magnaporthea griseas*	High
Bakanae	*Gibberella fujikuroi*	High
Kernel smut	*Tilletia barclayana*	Medium
Sheath rot	*Sarocladium oryzae*	Low-Medium
Sheath blight	*Rhizoctonia solani*	Low
Bacterial disease		
Bacterial grain rot/panicle blight	*Burkholderia glumae*	Medium
Viral disease		
Giallume	BYDV virus	Low
Invertebrate pests		
Golden apple snail	*Pomacea canaliculata*	High-Extreme
Rice water weevil	*Lissorhoptrus oryzophilus*	High
Khapra beetle	*Trogoderma granarium*	High
Fall armyworm	*Spodopter frugiperda*	Low
Rice stem maggot	*Chlorops oryzae*	Low
Rice gall midge	*Orseolia oryzae*	Low
Rice hispa	*Dicladispa armigera*	Low
Rice armyworm	*Mythimna unipuncta*	Low
Asiatic pink stemborer	*Sesamia inferens*	Low

Original data published by Plant Health Australia (2003) *www.planthealthaustralia.com.au/project_documents*

1.6.5   Summary

Australia is free from many of the pests and diseases that are prevalent in other rice growing areas of the world. This is reflected in the high yield produced by the Australian rice industry (USDA FAS 2004) and the relatively low rate of chemical application (Ricegrowers' Association of Australia Inc. 2004b). The crop rotation strategy used in the New South Wales rice industry (McDonald 1979; Sunrice 2004), its isolation from other rice growing regions and the region's own quarantine rules (Sunrice 2004), combine to keep New South Wales relatively free of diseases and pests.

SECTION 2. TOXICITY OF RICE

There is no evidence of any toxicity associated with the use of rice grains as a food crop for humans. However, rice straw, which is used as stockfeed in many parts of the world (Jackson 1978; Drake et al. 2002; FAO 2004), has the potential to cause toxicity if fed in large quantities. This occurs through the high levels (1 to 2%) of oxalates present in the straw (Jackson 1978) that can result in calcium deficiencies if supplements are not provided (FAO 2004). Rice straw is neither particularly attractive to stock, nor highly nutritious, and is

mostly fed in combination with other feed as a way of decreasing feed costs (Drake et al. 2002).

SECTION 3. ALLERGENICITY OF RICE

In the general population, rice is considered to be of low allergenicity (Hill et al. 1997). Studies on patients with a predisposition to allergic reactions have found a wide range of incidence (between 1 and 69%) of rice allergy symptoms upon challenge (summarised in Besler 1999). Patients already known to be sensitive to wheat or other cereals had the highest incidence of reactions to rice. A series of studies in Australia, China, Indonesia, Japan, Malaysia, the Philippines, Singapore and Taiwan (Hill et al. 1997) have concluded that the incidence of rice allergies in children is very rare.

Allergenic proteins have been identified in both rice seeds and pollen. The major rice seed allergens are a group of 14-16 kDa proteins with sequence identity to the α-amylase/trypsin inhibitor family, a castor bean storage protein and lipid transfer proteins (Besler 1999). A single pollen protein, Ory s 1, has been identified with sequence identity to pollen allergens from Rye and Burmuda grass (Besler 1999).

SECTION 4. WEEDINESS OF RICE

Rice plants (*O. sativa* or other species) that are grown unintentionally in and around rice growing areas are regarded as weeds (Vaughan & Morishima 2003). Rice has a tendency to become weedy in areas where wild and cultivated rice plants grow sympatrically. In these areas, wild and cultivated rice plants can hybridise, producing plants that compete with the cultivars and produce inferior seed, thus decreasing the yield from the rice crop (Oka 1988). However, weedy rice can also develop in areas without native wild rice populations (Bres-Patry et al. 2001; Vaughan & Morishima 2003). The origins of weedy rice under these conditions are currently under investigation, but they are believed to be derived from hybridisation between different cultivars, selection of weedy traits present in cultivars, relics of abandoned cultivars, or to have been brought into the growing region through contaminated seed stocks (Vaughan & Morishima 2003). Of the *Oryza* species growing in Australia, only *O. rufipogon* and *O. officinalis* are classified as weeds (Randall 2002) but not necessarily in Australia. All known populations of these species occur far from the rice growing regions of Australia (Fig 2, Australia's Virtual Herbarium 2004) and have not become problematic weeds in Australia to date.

The literature describing weedy rice identifies both *O. sativa* and other *Oryza* species as weeds. In the case of *O. sativa*, the weeds are known as red rice due to the coloured pericarp associated with these plants. Red rice is viewed as a major economic problem when it occurs in rice fields as it causes losses in yield through competition with the cultivars as well as decreasing the value of the harvested grain through its colour. Other *Oryza* species growing in and around rice fields are known as weedy rice and can also produce red seeds. Both red and weedy rice plants share many characteristics and are treated together as weedy/red rice in this document.

Section 4.1   Characteristics of rice contributing to potential weediness

Weedy/red rice tends to mimic the growth attributes of the cultivars with which it grows, germinating simultaneously and growing at a similar rate, although earlier maturity is not uncommon. This mimicry is likely to be due to their origins in hybridisation between those cultivars and the wild species. Populations of weedy/red rice tend to be genetically diverse and highly heterogeneous (Chang 2003). They have a high seed shedding rate and buried seeds will germinate with the crop the following season. In addition, their seeds can contaminate farmers' seed stocks and be sown with the cultivar each season.

Studies using phenotypic, isozymic and chemical markers have shown that some populations mimic the Indica cultivars, have low levels of seed dormancy and shatter, and rely on farmers for propagation through contamination of the harvest. Other Indica-mimics are able to self-propagate, having high levels of seed dormancy and shatter. The seeds of these varieties self-propagate mainly through soil seed banks. Weedy/red rice can also mimic the Japonica cultivars, in which case they tend to show self-propagating abilities (Tang & Morishima 1996). Other studies using molecular markers have also shown diverse origins for weedy/red rice, with not only the Indica and Japonica cultivars as progenitors, but also *O. rufipogon* (stated in Tang & Morishima 1996). Perhaps as a result of this genetic diversity, weedy/red rice has been reported to be more tolerant to drought, low temperatures and flooding (Vaughan & Morishima 2003). In the past, gene flow has apparently moved predominantly from the in-breeding cultivars to the out-breeding weeds as these are more receptive to non-self pollen, however, weedy/red rice may be a useful source of genes for improving cultivars in the future (Vaughan & Morishima 2003).

A number of general characteristics have been described that can increase the propensity of a plant to become a weed (Baker 1965; Bazzaz 1986). These are discussed below in relation to the *Oryza* species.

4.1.1   Adaptation to different habitats

As described in Section 1, *O. sativa* cultivars exist that are adapted to a wide range of habitats. Different cultivars are grown widely throughout the world, from latitude 50°N in China to 35°S in New South Wales and Argentina (Vaughan 1994), in tropical, temperate, lowland and highland regions and on a wide range of soil types. This is a demonstration of the high level of adaptability within this species that may enable it to colonise new areas quickly. However, individual cultivars do not span this entire geographic and environmental range, being limited to specific ecological niches.

4.1.2   Out-crossing versus selfing ability

The ability to self-fertilise is an important attribute of weeds that allows the plant to propagate without reliance on specialised pollinators and also helps to preserve co-adapted gene complexes. Conversely, out-crossing is also cited as a feature of establishing weed populations as this increases the adaptability of the population (Bazzaz 1986; Noble 1989). Flexibility in the degree of out-crossing versus self-fertilisation could allow the relative benefits of both reproductive systems to be realised (Bazzaz 1986).

As discussed previously (Section 1), cultivated rice is predominantly self-fertilising due to its flower morphology and its brief period of pollen viability. Wild rice species, however, have a greater ability for out-crossing due to longer anthers, greater exsertion of the stigma and longer periods of pollen viability (Oka 1988). Weedy forms of *O. sativa* often have intermediate characteristics between wild and cultivated rice, including the degree of out-crossing versus self-fertilisation (Oka 1988). Out-crossing rates have been reported to be up to 52% in weedy/red rice (Vaughan & Morishima 2003).

4.1.3   Dormancy and persistent seed bank

Variable seed dormancy is important in establishing weed populations as it allows seeds to germinate non-synchronously, thus avoiding adverse environmental conditions that may prevent seedling establishment at different times of the growing season. In general terms, seed dormancy combined with longevity enables the formation of a persistent seed bank in the soil. Seed dormancy is generally weaker in cultivated rice than in wild or weedy/red rice (Oka 1988; Vaughan 1994). The longevity of rice seeds has not been well studied however, wild rice seeds are believed to be long lived (Vaughan 1994) and may be dormant for several years (Moldenhauer & Gibbons 2003). Seed dormancy has been discussed in more detail in Section 1.5.3.

4.1.4   Efficient seed dispersal

Seed shatter is the separation of unhulled grains from the panicle. The ease of seed shatter depends on the extent of the absciss layer between the hull and the panicle rachis and is a trait that has been varied by selection during the cultivation history of rice to suit different harvesting requirements (OECD 1999). A balance has been sought between seed that shatters too easily, falling off the plant before harvest, and seed that is too difficult to remove from the panicle during harvest (McClung 2003). Cultivars from the Indica group demonstrate more seed shatter than do members of the Japonica group (OECD 1999). Combined with dormancy, the occurrence of high seed shatter in some rice cultivars and wild relatives can assist in the spread of weedy/red rice varieties. Seed that shatters before and during harvest can persist in the field and germinate the following season.

In addition to seed shatter, seed dispersal can also be assisted by the presence of awns on the seeds. Awns can assist in seed dispersal by attaching to passing animals. Rice varieties show a continuum between awnless and long-awn varieties (Oka 1988). Indica varieties are mainly awnless, while Japonica has both awned and awnless varieties.

4.1.5   Vegetative regeneration

Rice plants are capable of vegetative regeneration via ratoons, which re-shoot from sub-soil nodes after the first crop has ripened. Where seasonal conditions permit, ratooning results in perennial growth in some cultivars and some wild species. In areas with a long growing season, ratoons can produce a second harvest in a single season (Street & Bollich 2003). Climatic and cultural conditions would not allow ratoon formation in most temperate rice growing regions, such as in Australia.

SECTION 5. POTENTIAL FOR GENE TRANSFER

Gene transfer can occur within a species (i.e. between cultivars and/or weedy varieties of the same species) or between different species of the same, or other genera. These are referred to as intraspecific and interspecific gene transfer respectively. Successful gene transfer requires that three criteria are satisfied: the plant populations must: 1) overlap spatially; 2) overlap temporally (including flowering duration within a year and flowering time within a day); and 3) be sufficiently close biologically that the resulting hybrids are able to reproduce normally (den Nijs et al. 2004).

Section 5.1   Intraspecific gene transfer

Although *O. sativa* is essentially an autogamous plant, low rates of out-crossing can occur when plants with synchronous or overlapping flowering times grow in close proximity (Gealy et al. 2003; den Nijs et al. 2004). Even if flowering periods overlap, the time of day that the flowers open is important as rice flowers often remain open for periods of less than three hours (Moldenhauer & Gibbons 2003).

The use of male sterile mutants has demonstrated the low likelihood of outcrossing occurring in *O. sativa*. Researchers using these mutants, which did not produce viable pollen, were unable to achieve more than 34% out-crossing (described in Gealy et al. 2003).

In one study of cross-pollination between cultivars, ten varieties were grown in four different plot designs. The varieties chosen were paired in order to match, as closely as possible, plant height and flowering time. The pollen acceptor plants had various stigma lengths (ranging from 1.00 to 1.88 mm) and varying degrees of stigma exsertion (32 to 70%). In the first two plot designs, the pollen donor plants (carrying a gene for purple leaf colouration) and pollen acceptor plants were alternated within the rows, so that each acceptor was surrounded by donors. In one of these plots, the panicles of donor and acceptor plants were clipped together in pairs to ensure close contact between the flowers. In the third plot design, alternating rows of the donors and acceptors were planted. In the final plot, paired varieties were planted in

adjacent blocks separated by 1.5 m. The highest rate of gene flow (hybrid progeny identified by leaf pigmentation) found was 0.9%. This occurred between clipped panicles with the pollen acceptors that had the greatest degree of stigma exsertion and the longest stigmas. No hybrids were found amongst the 600-900 seeds tested from the plots that were separated by 1.5 m, indicating that if gene flow was occurring it was occurring at a rate of less than 0.08%, the lowest rate detected (Reano & Pham 1998).

Breeding and seed production programs in the US recommend separation distances of 4.6 to 6.1 m (depending on the agency) for drill-seeded crops to limit pollen flow. Greater distances (15 to 30 m) are recommended when aerial seeding and ground broadcast methods are used, in order to limit seed contamination (summarised in Gealy et al. (2003)). These distances are based on a study performed in 1938 with rice stations across the US participating over four to six years. A glutinous endosperm marker was used, allowing hybrid seeds to be identified immediately after harvest (Beachell et al, 1938, results summarised in Gealy et al. (2003)).

Similarly, under an international seed certification system, all three levels of seed certification (foundation, registered and certified) require an isolation distance of 10 feet (approximately 3 m) for drill-seeded rice. Greater distances are only required for different seeding methods, 50 feet (15 m) for ground broadcast seeding and 100 feet (30 m) for aerial broadcast seed (Association of Official Seed Certifying Agencies 2001). Thus the risk of contamination of breeding stocks appears to be greater during planting (due to human seed dispersal) than during flowering (due to cross-pollination).

Various other gene flow studies have been performed, with reports of low cross-pollination rates influenced by climatic conditions, wind direction and plant proximity. For instance, Messeguer et al (2001) reported rates of less than 1% at 1 m and less than 0.05% at 5 m in concentric circle plots. In these experiments, wind direction was important in pollen transfer at 1 m but at 5 m the more random spread of hybrids around the circular plot indicated that wind direction was no longer important. Gene flow (measured as numbers of herbicide tolerant seedlings) was greater between plants of the same cultivar than between the cultivar and red rice plants, which were taller and flowered slightly earlier, but with a 10 day overlap.

In another study (Zhang et al. 2004), three rice varieties (red seed, purple leaf and herbicide tolerant) were paired and grown in random plots in 50:50 mixes. No hybrid plants were recovered when seeds were collected from the taller red rice plants, indicating a lack of gene flow in that direction. However, hybrids were found in seeds collected from plants grown with the red rice with rates of 0.76% and 0.33% for purple rice and the herbicide tolerant rice respectively. The hybrids formed between the herbicide tolerant and red rice plants were very late in maturing and had to be removed from the field to a glasshouse at the end of the season to reach reproductive maturity. These would not have been able to set seed in the field, and in the glasshouse showed reduced fertility.

Hybrids formed between distantly related *O. sativa* cultivars can also show decreased fertility (Oka, 1988). These include not only Indica-Japonica hybrids but also hybrids formed within each ecotype. Indica-Japonica hybrids in particular have been shown to have decreased fertility (50% abortive pollen in the resulting first hybrid generation (F_1)) (summarised in Gealy et al. (2003)). F_1 pollen fertility varies between 5% and 100% in hybrids of different cultivars. These hybrids can be backcrossed to either parent via fertile female gametes (Vaughan & Morishima 2003)

Section 5.2   Interspecific gene transfer

5.2.1   Gene transfer to other *Oryza* species

Species in the *Oryza* genus can be grouped according to the compatibility of their genomes. *O. sativa* has an AA-type genome, which means that its chromosomes can pair correctly at meiosis with other AA-type species (see Table 1). Despite this, hybrids between AA rice

species can be difficult to obtain and have been reported to show low fertility. However, this does not prevent gene flow between these species, as backcrossing to one of the parents can stabilise the hybrids (Vaughan & Morishima 2003). Successful hybrid formation has resulted in the transfer of resistance to grassy stunt virus from *O. nivara* (Khush 1977), cytoplasmic male sterility from weedy/red rice (*O. sativa* f. *spontanea* (Lin & Yuan 1980)) and resistance to bacterial blight from *O. longistaminata* (Khush et al. 1990).

The post-mating barriers existing between the different *Oryza* species (including non-AA-type species) can be classified into F_1 non-viability, F_1 weakness, hybrid breakdown and F_1 sterility. When F_1 non-viability occurs, the development of F_1 zygotes is affected after fertilisation. Laboratory-based embryo rescue techniques can be used to produce viable hybrids. F_1 weakness affects the growth of the hybrid progeny, preventing the hybrids competing with healthier plants growing nearby. This is most common in crosses involving *O. glaberrima*. Hybrid breakdown occurs in subsequent generations, as the plants become weak and non-competitive. F_1 sterility is the most frequent barrier to interspecific gene transfer between *Oryza* species of the same genotype and can be caused by either non-viable pollen grains or embryos (Oka, 1988).

Naredo et al (1997) measured ranges of pollen viability and seed fertility in the interspecific hybrids formed between the AA-type genome species *O. meridionalis*, *O nivara*, *O. rufipogon* and *O. sativa* at between 1 and 9%. In a subsequent study, *O. meridionalis* consistently showed low crossability with *O. sativa* (Naredo et al. 1998). They also reported decreased viability in the hybrids.

Rates of hybridisation between *O. sativa* and *O. rufipogon* have been studied under field conditions in China (Song et al. 2003). Of 80 000 seeds planted the second season, 23 776 seedlings germinated and 1.2% of these were determined to be hybrids through use of a molecular marker. The frequency of hybrid formation depended on plot design and distance from the pollen source, with the highest frequencies found less than 5 m from the pollen donor in a circular crop design. The maximum gene flow distance was reported to be less than 43.2 m.

In a second hybridisation study between *O. sativa* and *O. rufipogon*, hybrid pollen from crosses was also shown to be less viable than that of the parents (Song 2004). Viability was measured by a standard I_2-KI staining assay (66.2% staining compared with 94.0% and 98.1% in the respective parents) and an *in vitro* germination method (33.9% germination compared with 84.9% and 58.4% in the respective parents). In this experiment it was also noted that although the *O. sativa* cultivar had the highest pollen viability initially (as assessed by *in vitro* germination), it also had the shortest pollen life, losing 50% viability after six minutes. *O. rufipogon* had double the pollen lifespan, losing 50% viability over 12 minutes. The hybrids were intermediate to the parents.

Most recent studies of interspecific gene flow have used *O. rufipogon* as a pollen acceptor, as it exists as a weed species in many rice growing regions. These experiments have generally shown higher frequencies of gene flow than intraspecific gene flow experiments. For instance, Chen et al (2004) reported that gene flow frequencies between *O. sativa* cultivars and weedy varieties were between 0.011 and 0.046% while gene flow to *O. rufipogon* was between 1.21 and 2.19%. Other reports indicate much higher rates of hybridisation. For example, Rates of hybridisation between *O. sativa* and *O. rufipogon* have been reported to be up to 50% but are highly variable (Oka & Morishima 1967) and *O. rufipogon* has been reported to produce hybrid swarms in rice fields with the hybrids showing no decrease in fertility (OECD 1999).

In West Africa, *O. sativa* and *O. glaberrima* are often grown in mixed fields. Natural hybridisation is rare between these species, however artificial hybridisation is possible. The resulting F_1 hybrids are highly pollen sterile and up to 66% of F_1 embryo sacs are

dysfunctional. Pollen of either parent can be successfully used in backcrosses in order to stabilise the hybrids (Oka 1988; OECD 1999).

Hybridisation between *O. sativa* and non-AA-type genome *Oryza* species is also possible with human assistance (Vaughan & Morishima 2003) and has been used to introduce insect and disease resistance into new cultivars. Transfers of genes for resistance to the brown planthopper, whitebacked planthopper, bacterial blight and blast have been made to *O. sativa* from *O. officinalis* (Jena & Khush 1990), *O. minuta* (Amante-Bordeos et al. 1992), *O. australiensis* (Multani et al. 1994) and/or *O. brachyantha* (Brar et al. 1996). These types of interspecific hybridisation do not occur naturally and rely on extensive embryo rescue and backcrossing efforts to obtain fertile hybrids. For example, in one study (Multani et al. 2003), hybrids were obtained from an *O. sativa* x *O. latifolia* cross. Seed set was 19.8% from 910 pollinated spikelets and the germination rate in the rescued embryos was 85.5%. Of the germinated embryos, 79% survived to form F_1 hybrid plants. The overall crossability of these species was calculated as 7.58% (69 hybrids resulting from 910 pollinated spikelets). The surviving hybrids had many of the phenotypic traits of *O. latifolia* and were extremely vigorous but completely male sterile. To overcome the sterility of these hybrids, multiple backcrosses were required. The crossability of each generation remained very low (less than 1%), with few surviving plants in each backcrossed generation.

5.2.2.   Interspecific gene transfer to non-*Oryza* species

By definition, gene flow through conventional sexual hybridisation is limited to *O. sativa* varieties and to the AA-type genome species within this genus. Gene flow between more distantly related species, particularly those outside of the *Oryza* genus, is restricted to artificial breeding methods such as embryo rescue and somatic hybridisation (the regeneration of plants following the fusion of two protoplasts) (Liu et al. 1999; Multani et al. 2003). Examples of the use of these methods are outlined below.

Embryo rescue of hybrids between rice and *Porteresia coarctata* (a member of the Oryzeae tribe) produced male sterile plants (Jena 1994; Brar et al. 1998), which may prove useful in transferring salinity tolerance into cultivated rice.

In an effort to widen the *O. sativa* gene pool for crop improvement, somatic hybridisation has been attempted with a rice weed, barnyard grass (*Echinochloa oryzicola*) without successful production of viable plants (Terada et al. 1987). Carrot-like plants were produced as a result of somatic hybridisation between *O. sativa* and carrot (*Daucus carota*) (Kisaka et al. 1994). Liu et al (1999) produced an asymmetric somatic hybrid between *Zizania latifolia* and *O. sativa*. The single viable and fertile hybrid produced was significantly shorter than both parent varieties, had broader leaves and the seeds ripened earlier. Although *Z. latifolia* seeds are much longer and thinner than rice seeds, the hybrid seeds were smaller and darker than the rice seeds (Liu et al. 1999). No details were supplied in the text to describe if the hybrid was self-fertile, nor if its seeds germinated.

Section 5.3   Gene transfer to other organisms

The only means by which genes could be transferred from plants to non-plant organisms is by horizontal gene transfer (HGT). Such transfers have not been demonstrated under natural conditions (Nielsen et al. 1997; Nielsen et al. 1998; Syvanen 1999) and deliberate attempts to induce them have so far failed (Schlüter et al. 1995; Coghlan 2000).

Transfer of plant DNA to bacteria has been demonstrated under highly artificial laboratory conditions (Nielsen et al. 1998; Gebhard & Smalla 1998; Mercer et al. 1999; Nielsen 1998), but even then only at a very low frequency. Phylogenetic comparison of the sequences of plant and bacterial genes suggests that horizontal gene transfer from plants to bacteria during evolutionary history has been extremely rare, if occurring at all (Nielsen et al. 1998; Doolittle 1999).

Bergthorrson et al. (2003) have examined a number of genes encoded in mitochondria, concluding that HGT between the mitochondria of unrelated plants occurs relatively frequently on a time scale of millions of years and that the mechanism by which gene transfer between sexually incompatible species occurs is unknown. Their main evidence for HGT is three separate genes that appear to have been 'recaptured' from other plant mitochondria following transfer of the original genes to the nucleus. They speculate that HGT may occur through vectors such as viruses, fungi, insects, pollen or meteorites; by transformational uptake of plant DNA released into the soil; or by the occasional grafting of unrelated plants.

Although mutations occur relatively frequently in living organisms, they are unlikely to become fixed and persist in a population unless they confer some advantage to the individual in which the mutation occurs. In a review of the HGT literature, Kurland et al (2003) suggested that HGT and other forms of mutation were likely to have played a major role in the earliest phase of cellular evolution. However, as cellular functions became increasingly well adapted, the probability that a foreign gene (or new mutation) would improve the performance of the recipient cell would have progressively decreased.

Recombination between viral genomes and plant DNA has only been observed at very low levels, and only between homologous sequences under conditions of selective pressure, for example, regeneration of an infectious virus resulted from complementation of a defective virus by viral sequences introduced into a genetically modified plant genome (Greene & Allison 1994; Teycheney & Tepfer 1999).

Thus, gene transfer from rice to organisms other than plants is extremely unlikely. A more detailed review of horizontal gene transfer from rice plants to other organisms is provided in the risk assessment and risk management plan that was prepared in relation to application DIR 052/2004 for the release of GM rice plants into the Australian environment.

SECTION 6. REFERENCES

Ahn, S.-W., Bonman, J.M., Brandon, D.M., Groth, D.E., Gunnell, P.S., Hibino, H., Hollier, C.A., Lee, F.N., Mew, T.W., Prot, J.C., Rush, M.C., Schneider, R.W., Webster, R.K., Whitney, G. (1992). *Compendium of Rice Diseases.* Webster, R.K., Gunnell, P.S. (eds). APS Press,

Amante-Bordeos, A., Sitch, L.A., Nelson, R., Dalmacio, R.D., Oliva, N.P., Aswidinnoor, H., Leung, H. (1992). Transfer of bacterial blight and blast resistance from the tetraploid wild rice *Oryza minuta* to cultivated rice, *Oryza sativa. Theoretical and Applied Genetics* **84**: 345-354.

Association of Official Seed Certifying Agencies (2001). Genetic and crop standards of the AOSCA. *ftp://www.aosca.org/opandcs.pdf.*

Australia's Virtual Herbarium (2004). *http://www.cpbr.gov.au/avh.html.*

Baker, H.G. (1965). The genetics of colonizing species: Characteristics and modes of origin of weeds. In: HG Baker, GL Stebbins, eds. *The Genetics of Colonizing Species.* Academic Press, New York and London. pp 147-172.

Bazzaz, F.A. (1986). Life history of colonizing plants: some demographic, genetic and physiological features. Chapter 6. In: HA Mooney, JA Drake, eds. *Ecology of Biological Invasions.* Springer-Verlag, New York. pp 96-110.

Beckmann, R. (2003). Tougher times for raiders of the rice field. *Partners in Research for Development* **16**: 28-33.

Bergthorsson, U., Adams, K.L., Thomason, B., Palmer, J.D. (2003). Widespread horizontal transfer of mitochondrial genes in flowering plants. *Nature* **424**: 197-201.

Besler, M. (1999). Allergen data collection: Rice (*Oryza sativa*). *Internet Symposium on Food Allergens* **1**: 147-160.

Brar, D.S., Dalmacio, R.D., Elloran, R., Aggarwal, R., Angeles, R., Khush, G.S. (1996). Gene transfer and molecular characterization of introgression from wild *Oryza* species into rice. In: GS Khush, ed. *Rice Genetics III.* International Rice Research Institute, Manila, The Philippines. pp 477-485.

Brar, D.S., Elloran, R.M., Talag, J.D., Abbasi, F., Khush, G.S. (1998). Cytogenetic and molecular characterization of an intergeneric hybrid between *Oryza sativa* L. and *Porteresia coarctata* (Roxb.) Tateoka. *Rice Genetics newsletters* **14**: 43-45.

Bres-Patry, C., Lorieux, M., Clement, G., Bangratz, M., Ghesquiere, A. (2001). Heredity and genetic mapping of domestication-related traits in a temperate *japonica* weedy rice. *Theoretical and Applied Genetics* **102**: 118-126.

Brown, P. and Croft, D. (2000). *Mice in the MIA.* CSIRO Wildlife and Ecology, Canberra,

Brown, P.R., Singleton, G.R. (2002). Impacts of house mice on crops in Australia - costs and damage. In: L Clark, J Hone, JA Shivik, RA Watkins, KC VerCauteren, JK Yoder, eds. *Proceedings of the Third NWRC Special Symposium. Human conflicts with wildlife:*

Economic considerations. National Wildlife Research Center, Fort Collins, CO, USA. pp 48-58.

Bui, X.M., Ogle, R.B., Lindberg, J.E. (2002). Studies on integrated duck-rice systems in the Mekong Delta of Vietnam. *Journal of Sustainable Agriculture* **20**: 27-40.

Caughley, J., Bomford, M., Parker, B., Sinclair, R., Griffiths, J., Kelly, D. (1998). *Rodents.* Bureau of Resource Sciences and Grains Research and Development Corporation, Canberra,

Caughley, J., Monamy, V., Heiden, K. (1994). *Impact of the 1993 mouse plague.* Grains Research and Development Corporation, Canberra, Australia.

Chang, T.T. (2003). Origin, Domestication, and Diversification. Chapter 1.1. In: WC Smith, RH Dilday, eds. *Rice. Origin, History, Technology, and Production*. John Wiley and Sons, Inc., Hoboken, New Jersey. pp 3-25.

Chen, L.J., Lee, D.S., Song, Z.P., Suh, H.S., Lu, B.R. (2004). Gene flow from cultivated rice (*Oryza sativa*) to its weedy and wild relatives. *Annals of Botany* **93**: 67-73.

Coghlan, A. (2000). So far so good: for the moment, the gene genie is staying in its bottle. *New Scientist* **2231**: 4.

Davey, C. (1990). Control of waterfowl on rice crops. Chapter Appendix 2. In: P Fleming, I Temby, J Thompson, eds. *National Bird pest Workshop Proceedings* pp 209-224.

Davey, C., Roberts, J. (1990). The Duck Problem on Rice Fields in New South Wales. In: P Fleming, I Temby, J Thompson, eds. *National Bird Pest Workshop Proceedings* pp 42-50.

den Nijs, H.C.M., Bartsch, D., Sweet, J. (2004). *Introgression from genetically modified plants into wild relatives.* CAB International, UK. pp 1-403.

Doolittle, W.F. (1999). Lateral genomics. *Trends in Cell Biology* **9**: 5-8.

Drake, D.J., Nader, G., Forero, L. (2002). Feeding rice straw to cattle. University of California, Division of Agriculture and Natural Resources *http://agronomy.ucdavis.edu/uccerice/STRAW/pub8079.pdf*

Ellis, R.H., Hong, T.D., Roberts, E.H. (1983). Procedures for the safe removal of dormancy in rice seed. *Seed Science & Technology* **11**: 77-112.

FAO (2004). Species description *Oryza sativa* L. *http://www.fao.org/ag/AGP/AGPC/doc/GBASE/data/pf000274.htm*

Gealy, D.R., Mitten, D.H., Rutger, J.N. (2003). Gene flow between red rice (*Oryza sativa*) and herbicide-resistant rice (*O. sativa*): Implications for weed management. *Weed Technology* **17**: 627-645.

Gebhard, F., Smalla, K. (1998). Transformation of *Acinetobacter* sp. strain BD413 by transgenic sugar beet DNA. *Applied and Environmental Microbiology* **64**: 1550-1554.

Greene, A.E., Allison, R.F. (1994). Recombination between viral RNA and transgenic plant transcripts. *Science* **263**: 1423-1425.

Gu, X.Y., Kianian, S.F., Foley, M.E. (2004). Multiple loci and epistases control genetic variation for seed dormancy in weedy rice (Oryza sativa). *Genetics* **166**: 1503-1516.

Hill, D.J., Hosking, C.S., Zhie, C.Y., Leung, R., Baratwidjaja, K., Iikura, Y., Iyngkaran, N., Gonzalez, A.A., Wah, L.B., Hsieh, K.H. (1997). The frequency of food allergy in Australia and Asia. *Environmental Toxicology and Pharmacology* **4**: 101-110.

Ikeda, R., Vaughan, D.A., Kobayashi, N. (1994). Landraces and wild relatives of rice (*Oryza sativa*) as sources of useful genes. *JIRCAS International Symposium Series* **2**: 104-111.

International Rice Research Institute (2002). Growth and Morphology of the Rice Plant. *http://www.knowledgebank.irri.org/pu_growthMorph.htm*

Jackson, M.G. (1978). Rice straw as livestock feed. In: FAO, ed. *Ruminant nutrition: selected articles from the World Animal Review http://www.fao.org/DOCREP/004/X6512E/X6512E00.htm#TOC*

Jahromi, F., Cother, E., and Ash, G. (2001). Weed control in rice crops: Suitability of *Rhynchosporium alismatis* as a mycoherbicide for integrated management of *Damasonium minus* in rice fields. RIRDC Publication No 01/39, Rural Industries and Development Corporation, Canberra.

Jena, K.K. (1994). Production of intergeneric hybrid between *O. sativa* L. and *Porteresia coarctata* T. *Current Science* **67**: 744-746.

Jena, K.K., Khush, G.S. (1990). Introgression of genes from *Oryza officinalis* Well ex Watt to cultivated rice, *O. sativa* L. *Theoretical and Applied Genetics* **80**: 737-745.

Kendig, A., Williams, B., Smith, C.W. (2003). Rice weed control. Chapter 3.7. In: CW Smith, RH Dilday, eds. *Rice. Origin, History, Technology, and Production*. John Wiley and Sons Inc., Hoboken, New Jersey. pp 457-472.

Khush, G.S. (1977). Disease and insect resistance in rice. *Advances in Agronomy* **29**: 265-341.

Khush, G.S., Bacalangco, E., Ogawa, T. (1990). A new gene for resistance to bacterial blight from *O. longistaminata*. *Rice Genetics newsletters* **7**: 121-122.

Kisaka, H., Lee, H., Kisaka, M., Kanno, A., Kang, K., Kameya, T. (1994). Production and analysis of asymmetric hybrid plants between a monocotyledon (*Oryza sativa* L.) and a dicotyledon (*Daucus carota* L.). *Theoretical and Applied Genetics* **89**: 365-371.

Koga, Y., Akihama, T., Fujimaki, H., Yokoo, M. (1969). Studies on the longevity of pollen grains of rice, *Oryza sativa* L. I. Morphological change of pollen grains after shedding. *Cytologia* **36**: 104-110.

Kurland, C.G., Canback, B., Berg, O.G. (2003). Horizontal gene transfer: a critical view. *Proceedings of the National Academy of Science of the United States of America* **100**: 9658-9662.

Lacy, J., Beecher, G., Fleming, M., Lattimore, M.-A., Lewin, L., McRae, M., Murray, A., O'Keefe, K., Reinke, R., Schipp, R., Snell, P., Whitworth, R., Steel, F. (2003). 2003 Ricecheck Recommendations. NSW Agriculture/Rice Research and Development Committee.

Lattimore, M.-A. (1994). Pastures in temperate rice rotations of south-eastern Australia. *Australian Journal of Experimental Agriculture* **34**: 959-965.

Li, C.B., Zhang, D.M., Ge, S., Hong, D.Y. (2000). Identification of genomic constitution of three tetraploid *Oryza* species through two-probe genomic *in situ* hybridization. *International Rice Research Notes (IRRN)* **25**: 19-22.

Lin, S.C., Yuan, L.P. (1980). Hybrid rice breeding in China. In: *Innovative approaches to rice breeding*. International Rice Research Institute, Manila, The Philippines. pp 35-51.

Linares, O.F. (2002). African rice (*Oryza glaberrima*): history and future potential. *Proceedings of the National Academy of Science of the United States of America* **99**: 16360-16365.

Liu, B., Liu, Z.L., Li, X.W. (1999). Production of a highly asymmetric somatic hybrid between rice and *Zizania latifolia* (Griseb): evidence for inter-genomic exchange. *Theoretical and Applied Genetics* **98**: 1099-1103.

Lu, B.-R., Jackson, M. (2004). Wild Rice Taxonomy. International Rice Research Institute. *http://www.knowledgebank.irri.org/wildRiceTaxonomy/default.htm*

Lu, B.R. (2004). Taxonomy of the genus *Oryza* (Poaceae): historical perspective and current status. *International Rice Research Notes (IRRN)* **24**: 4-8.

Matsuo, T., Futsuhara, Y., Kikuchi, F., Yamaguchi, H. (1997). *Science of the Rice Plant.* Food and Agriculture Policy Research Center, Tokyo.

McClung, A.M. (2003). Techniques for development of new cultivars. Chapter 2.4. In: CW Smith, RH Dilday, eds. *Rice. Origin, History, Technology, and Production*. John Wiley and Sons Inc., Hoboken, New Jersey. pp 177-202.

McDonald, D.J. (1979). Rice. Chapter 3. In: *Australian field crops vol 2: Tropical cereals, oilseeds, grain legumes and other crops*. Angus and Robertson, London. pp 70-94.

McDonald, D.J. (1994). Temperate rice technology for the 21st century: an Australian example. *Australian Journal of Experimental Agriculture* **34**: 877-888.

McIntyre, S., Finlayson, C.M., Ladiges, P.Y., Mitchell, D.S. (1991). Weed community composition and rice husbandry practices in New South Wales, Australia. *Agriculture, Ecosystems & Environment* **35**: 27-45.

Mercer, D.K., Scott, K.P., Bruce-Johnson, W.A., Glover, L.A., Flint, H.J. (1999). Fate of free DNA and transformation of the oral bacterium *Streptococcus gordonii* DL1 by plasmid DNA in human saliva. *Applied and Environmental Microbiology* **65**: 6-10.

Messeguer, J., Fogher, C., Guiderdoni, E., Marfà, V., Baldi, G., Melé, E., Català, M.M. (2001). Field assessments of gene flow from transgenic to cultivated rice (*Oryza sativa* L.) using a herbicide resistance gene as tracer marker. *Theoretical and Applied Genetics* **103**: 1151-1159.

Moldenhauer, K.A.K., Gibbons, J.H. (2003). Rice Morphology and Development. Chapter 2.1. In: CW Smith, RH Dilday, eds. *Rice. Origin, History, Technology, and Production*. John Wiley and Sons, Inc., Hoboken, New Jersey. pp 103-127.

Morishima, H. (1984). Wild plants and domestication. Chapter 1. In: S Tsunoda, N Takahashi, eds. *Biology of rice*, Volume 7. Elsevier, Amsterdam. pp 3-30.

Multani, D.S., Jena, K.K., Brar, D.S., delos Reyes, B.G., Angeles, E.R., Khush, G.S. (1994). Development of monosomic alien addition lines and introgression of genes from *Oryza australiensis* Domin. to cultivated rice *O. sativa* L. *Theoretical and Applied Genetics* **88**: 102-109.

Multani, D.S., Khush, G.S., delos Reyes, B.G., Brar, D.S. (2003). Alien gene introgression and development of monosomic alien addition lines from *Oryza latifolia* Desv. to rice, *Oryza sativa* L. *Theoretical and Applied Genetics* **107**: 395-405.

Murray, S.S (2005) Searching for the origins of African rice domestication. *http://antiquity.ac.uk/ProjGall/murray*

Naredo, M.E., Juliano, A., Lu, B.-R., Jackson, M.T. (1998). Taxonomic status of *Oryza glumaepatula* Steud. a diploid wild rice species from the new world. II. Hybridization among South American, Asian, and Australian AA genome species. *Genetic Resources and Crop Evolution* **45**: 205-214.

NSW Agriculture (1996a). Prohibition on the importation or introduction of organisms which are snails of the family *Ampullariida* (*Pilidae*) including Golden Apple Snail, *Pomacea caniculata* into the rice growing area of New South Wales. *New South Wales Government Gazette* **117**: 7001.

NSW Agriculture (1996b). Prohibition on the importation or introduction of rice and other things into the south western region of the state. *New South Wales Government Gazette* **117**: 7001.

Nielsen, K.M. (1998). Barriers to horizontal gene transfer by natural transformation in soil bacteria. *APMIS* **106**: 77-84.

Nielsen, K.M., Bones, A.M., Smalla, K., van Elsas, J.D. (1998) Horizontal gene transfer from transgenic plants to terrestrial bacteria - a rare event? *FEMS Microbiology Reviews* **22**: 79-103.

Nielsen, K.M., Gebhard, F., Smalla, K., Bones, A.M., van Elsas, J.D. (1997). Evaluation of possible horizontal gene transfer from transgenic plants to the soil bacterium *Acinobactor calcoaceticus* BD 413. *Theoretical and Applied Genetics* **95**: 815-821.

Noble, I.R. (1989). Attributes of invaders and the invading process: terrestrial and vascular plants. Chapter 12. In: JA Drake, HA Mooney, F di Castri, RH Groves, FJ Kruger, M Rejmanek, M Williamson, eds. *Biological Invasions: A Global Perspective*. John Wiley & Sons, Chichester. pp 301-313.

Normile, D. (2004). Yangtze seen as earliest rice site. *Science* **275**: 309.

OECD (1999). Consensus document on the biology of *Oryza sativa* (rice). Report No. ENV/JM/MONO(99)26, OECD Environmental health and Safety Publications, Paris.

Oka, H.I. (1988). *Origin of cultivated rice.* Elsevier, Amsterdam.

Oka, H.I., Morishima, H. (1967). Variations in the breeding systems of a wild rice, *Oryza perennis*. *Evolution* **21**: 249-258.

Randall, R.P. (2002). *A global compendium of weeds.* R.G. & F.J. Richardson, Meredith, Victoria. pp 1-905.

Reano, R., Pham, J.L. (1998). Does cross-pollination occur during seed regeneration at the International Rice Genebank? *International Rice Research Notes (IRRN)* **23**: 5-6.

Ricegrowers' Association of Australia Inc. (2004a). All about rice. *http://www.rga.org.au/rice/RGA-AllAboutRice.pdf*

Ricegrowers' Association of Australia Inc. (2004b). Our Australian Rice Facts. *http://www.rga.org.au/rice/RGA-AustralianRiceFacts.pdf*

Sanders, B.A. (1994). The life cycle and ecology of *Cyperus difformis* (rice weed) in temperate Australia: a review. *Australian Journal of Experimental Agricultural* **34**: 1031-1038.

Schlüter, K., Fütterer, J., Potrykus, I. (1995). Horizontal gene transfer from a transgenic potato line to a bacterial pathogen (*Erwinia chrysanthemi*) occurs - if at all - at an extremely low frequency. *Bio/Technology* **13**: 1094-1098.

Scott, H.D., Miller, D.M., Renaud, F.G. (2003). Rice soils: Physical and chemical characteristics and behaviour. Chapter 3.3. In: CW Smith, RH Dilday, eds. *Rice. Origin, History, Technology, and Production*. John Wiley and Sons Inc., Hoboken, New Jersey. pp 297-329.

Song, Z.P. (2004). A study of pollen viability and longevity in *Oryza rufipogon*, *O. sativa*, and their hybrids. *International Rice Research Notes (IRRN)* **26**: 31-32.

Song, Z.P., Lu, B.-R., Zhu, Y.G., Chen, J.K. (2003). Gene flow from cultivated rice to wild species *Oryza rufipogon* under experimental field conditions. *New Phytologist* **157**: 657-665.

Song, Z.P., Lu, B.R., Chen, J.K. (2004). Pollen flow of cultivated rice measured under experimental conditions. *Biodiversity and Conservation* **13**: 579-590.

Stevens, M.M. (2003). Improving bloodworm, earthworm and snail control in rice. RIRDC Publication No 03/083, Rural Industries Research and Development Corporation, Canberra.

Street, J.E., Bollich, P.K. (2003). Rice production. Chapter 3.2. In: CW Smith, RH Dilday, eds. *Rice. Origin, History, Technology, and Production*. John Wiley and Sons Inc., Hoboken, New Jersey. pp 271-296.

Sunrice (2004). All about rice. *http://www.sunrice.com.au/rice/index.asp*

Syvanen, M. (1999). In search of horizontal gene transfer. *Nature Biotechnology* **17**: 833.

Takahashi, N. (1984a). Differentiation of ecotypes in *Oryza sativa* L. Chapter 2. In: S Tsunoda, N Takahashi, eds. *Biology of rice*. Elsevier, Amsterdam. pp 31-67.

Takahashi, N. (1984b). Seed germination and seedling growth. Chapter 3. In: S Tsunoda, N Takahashi, eds. *Biology of rice*, Volume 7. Elsevier, Amsterdam. pp 71-88.

Tang, L.H., Morishima, H. (1996). Genetic Characteristics and Origin of Weedy Rice. In: R Chau, B Gordon, G Leir, eds. *Origin and Differentiation of Chinese Cultivated Rice*. China Agricultural university Press, pp 211-218.

Teo, S.S. (2001). Evaluation of different duck varieties for the control of the golden apple snail (*Pomacea canaliculata*) in transplanted and direct seeded rice. *Crop Protection* **20**: 599-604.

Terada, R., Kyozuka, J., Nishibayashi, S., Shimamoto, K. (1987). Plantlet regeneration from somatic hybrids of rice (*Oryza sativa* L.) and barnyard grass (*Echinochloa oryzicola* Vasing). *Molecular and General Genetics* **210**: 39-43.

Teycheney, P. Y. and Tepfer, M. (1999). Gene flow from virus-resistant transgenic crops to wild relatives or to infecting viruses. In "*Gene flow and agriculture: Relevance for transgenic crops, British Crop Protection Council Symposium Proceedings No. 72*", pp. 191-196.

Thomas, G., Joseph, L., Varghese, G., Kalyanaraman, K., Kuriachan, P., Das, M.R. (2001). Discrimination between *Oryza malampuzhaensis* Krish. et Chand. and *Oryza officinalis* Wall ex Watt based on RAPD markers and morphological traits. *Euphytica* **122**: 181-189.

USDA FAS (2004). World Agricultural Production. *http://www.fas.usda.gov/wap/circular/2004/04-07/Wap%2007-04.pdf*

Vaughan, D.A. (1994). *The Wild Relatives of Rice. A Genetic Handbook.* International Rice Research Institute, Manila.

Vaughan, D.A., Morishima, H. (2003). Biosystematics of the genus *Oryza*. Chapter 1.2. In: CW Smith, RH Dilday, eds. *Rice. Origin, History, Technology, and Production*. John Wiley and Sons Inc., Hoboken, New Jersey. pp 27-65.

Vaughan, D.A., Morishima, H., Kadowaki, K. (2003). Diversity in the *Oryza* genus. *Current Opinion in Plant Biology* **6**: 139-146.

Zhang, N., Linscombe, S., Oard, J. (2004). Out-crossing frequency and genetic analysis of hybrids between transgenic glufosinate herbicide-resistant rice and the weed, red rice. *Euphytica* **130**: 35-45.