THESIS

TRANSFER OF DROUGHT RESISTANT CHARACTER FROM WILD RICE (*Oryza meridionalis* and *Oryza nivara*) TO CULTIVATED RICE (*Oryza sativa* L.) BY BACKCROSSING AND IMMATURE EMBRYO CULTURE

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GRADUATE SCHOOL, KASETSART UNIVERSITY
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Drought is an abiotic stress to be continuing threat to rice production. The characteristics supporting to drought resistance are restricted in cultivars while wild species of *Oryza* are an important reservoir of useful genes for rice improvement. The objective of this study was to transfer drought resistant character from wild species (*O. meridionalis* and *O. nivara*) to cultivated rice varieties, RD23 and CN1, by backcrossing in combination with immature embryo culture. Hybridization between cultivated rice and wild species was made to produce six interspecific hybrid crosses. Crossability between cultivated rice and wild species of all crosses varied from 21.2 to 50% with an average of 35.3%. The F_1 hybrid embryos obtained were rescued by culturing on the half-strength MS medium. Germination ability of the hybrid embryos of all crosses ranged from 36.2 to 61.5% with an average of 51.9%. The F_1 hybrid plants obtained showed low pollen fertility and relatively poor agronomic characters. They were then backcrossed to their cultivated recurrent parents to restore fertility and good agronomic performance from cultivated rice. The 299 BC_1F_1 plants were produced from six backcrosses of which 235 plants could set BC_1F_2 seeds. The 452 BC_1F_2 progenies from six backcrosses were screened for drought resistance at vegetative growth stage by visual scoring of leaf rolling, leaf drying and plant recovery. Selection was made for 39 BC_1F_2 plants resistant to drought and having high seed yield per plant and good agronomic performance. They will be advanced to be BC_1F_3 lines for further evaluation on drought resistance, yield and agronomic performance.

	P. Sripichitt	Mar. / 8 / 2006
Student's signature	Thesis Advisor's signature	

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Pham Thien Thanh
March, 2006

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INTRODUCTION

Three cereal crops – rice, wheat and maize feed the world. These crops supply 49% of calories consumed by the world population; 23% come from rice, 17% from wheat and 9% from maize. Rice is the primary food source for more than a third of the world's population. It is planted on almost 150 million ha annually or 11% of the world's cultivated land. More than 90% of rice are produced and consumed in Asia. It is also an important staple in Latin America, Africa and the Middle East. Rice is grown under a wide range of agroclimatic conditions. Four major ecosystems are generally recognized: irrigated (55%), rain-fed lowland (25%), upland (12%) and flood-prone (8%) (Brar and Khush, 2002). The largest areas of upland rice are grown in Brazil and West Africa. Significant upland areas can still be found in India, Indonesia, and Laos (David, 1991). More than 8.5 million ha of rice are currently grown in highly drought-prone or drought-prone upland rice areas in South and Southeast Asia (Herht, 1991).

Moreover, the world will need more rice than what is produced today to feed the extra billions who will rely on it in the future. Tomorrow's technology and management must enhance the production of rice per unit area with less water and less pressure on the natural resource base. Research must respond to the need for many rice growers in drought conditions to change from subsistence farming to farming for profit to produce the surplus needed to feed rapidly growing population.

Obviously, world rice production has more than doubled, from 257 million tons in 1966 to 600 million tons in 2000. To meet the growing needs of the ever-increasing human population, however, rice production must increase by 40% during the next 25 years (Brar and Khush, 2002). To achieve this, several biotic and abiotic stresses that adversely affect rice productivity must be overcome. Some of the major diseases and pests affecting rice production include bacterial blight, blast and sheath blight diseases and insects such as the brown planthopper and stemborer and abiotic stresses including cold, salinity, acidity, iron toxicity, submergence under water (flooding tolerance) and drought. Evenson *et al.* (1996) estimated average annual production loss due to drought in global-rice is 18 million metric tons. Thus, the solution can be visualized as beginning with changes in farm-level water management and concomitant changes in agronomic management. Ideally, the task of creating new rice varieties adapted to water limited conditions would proceed with drought stress.

Drought is an abiotic stress to be continuing threat to rice production. In recent years, many works have been published on the use of DNA marker assisted selection (MAS) for drought tolerance, but drought tolerance is acknowledged to be a quantitative trait and thus polygenic in nature. The role of MAS in improvement of rice for drought tolerance is still equivocal (O'Toole, 2004). On the other hand, some publications identify DNA molecular markers related to secondary traits which correlated with yield under drought conditions (O'Toole, 2004). The most exhaustively researched traits assessed to date have been root system morphology (Champoux *et al.*, 1995; Yadav *et al.*, 1997; Price and Courtois, 1999; Shen *et al.*, 2001; Toorchi *et al.*, 2001; Zhang *et al.*, 2001; Kamoshita *et al.*, 2002; Babu *et al.*, 2003) and osmotic adjustment of the shoot (Lilley *et al.*, 1996; Robin *et al.*, 2003).

There is thus an urgent need to broaden the rice gene pool by introgression of new genes from diverse sources to meet various challenges affecting rice production. In addition, the wild relatives of cultivated rice constitute a major gene pool for rice improvement. In recent years, interest in the wild relatives of rice has increased as evaluation of this germplasm for new source of resistance to pests, diseases and unfavorable conditions. The techniques to transfer genes from wild rice to cultivated rice have been improved such as a gene for resistance to grassy stunt virus of rice was recovered in *O. nivara* (= *O. rufipogon*) and transferred to *O. sativa* by backcrossing (Khush *et al.*, 1977). Similarly, a gene for resistance to many races of bacterial blight of rice was found in *O. longistamina* and transferred to *O. sativa* by backcrossing (Khush *et al.*, 1990). As new techniques are applied to gene exchange and evaluation of genetic makeup of *Oryza* species, wild species of *Oryza* are an important reservoir of useful genes for rice improvement (Brar and Khush, 2002).

Interspecific hybridization is a prime importance in the breeding programs of many crops, more particularly of some cereals like wheat and oat. Nevertheless, the situation is different in rice where the introduction of wild genes to cultivated varieties has been rare, and the use of hybridization has been restricted to closely related species sharing the same genome.

There are probably several explanations for such a difference. Domestication of the cultivated rice occurred in several areas of Asia starting from different populations of the same species, *Oryza sativa* (Chang, 1976a). The broad genetic diversity of this species allowed an adaptation to very different growing conditions and spectacular increase in the yield potential without introduction of wild characters. On the other hand, the use of wide hybridization implies more sophisticated techniques which were more frequently available in institutions working on temperate crops.

Nevertheless, the genus *Oryza* includes a number of species more or less related to the cultivated rice, and it seems obvious that characters useful for breeding could be found in some wild genomes. Thus, application of wide hybridization to transfer drought tolerance character from wild rice to cultivated rice is necessary and it can be expected in this thesis.

OBJECTIVE

The objective of this study was to transfer drought resistant character from wild rice (*Oryza meridionalis* and *Oryza nivara*) to cultivated rice (*Oryza sativa* L. cultivars CN1 and RD23) by backcrossing in combination with immature embryo culture.

LITERATURE REVIEW

The Genus *Oryza* and wild rice

Oryza is a modest-sized genus consisting of 20 well-recognized wild species and two advance cultigens, *O. glaberrima* and *O. sativa*. These species, their chromosome numbers, genome symbols, and geographic distributions are summarized in Table 1.

Most of the species are diploid, having 12 pairs of chromosomes. Seven species are tetraploid (2n = 4x = 48). Six basic genomes of 12 chromosomes each have been identified by the meiotic pairing behavior in interspecific F1 hybrids examined under the light microscope. The chromosomes of rice species are small and deficient in morphologic markers, rendering them difficult to discern and identify. Clear figures of pachytene chromosomes are also difficult to obtain which the preparation made by Shastry *et al.* in 1960 remains the prime model (Chang, 2003).

Seventeen species in the genus have one or two haploid chromosome complements (genomes) designated as A, B, C, D, E and F by Japanese, Chinese and U.S. workers through long years of painstaking investigations from 1930s through the early 1960s. At the 1963 Rice Genetics and Cytogenetics Symposium held at the International Rice Research Institute (IRRI), key workers agreed to assign the A genome to the two cultigens and their immediate wild relatives. Thus the cross-fertile taxa in the *O. sativa* primary gene pool (species complex) (i.e., *O. sativa*, *O. nivara*, *O. rufipogon*, and their weed races) are assigned the A genome symbol. Related species that have shown incomplete cross-fertility, detectable aberrations in meiotic pairing and other aberrations in their crosses with *O. sativa* are assigned to subgenomes of A bearing a lowercase superscript letter corresponding to the species name (IRRI, 1964). For instance, the genome symbol of *O. glaberrima* is designed as A^g; for *O. barthii*, A^b. The superscripts have undergone revision following revision in species names (Vaughan and Morishima, 2003). Those taxa less compatible with *O. sativa* are assigned genome symbols such as C (for *O. officinalis*), BC (for *O.*

puntata) or E (for *O. australiensis*). This scheme corresponds to the primary, secondary and tertiary gene pools of Harlan and de Wet (1971).

Following repeated revisions and corrections, the total number of *Oryza* species has been trimmed from a total of 28 listed in the 1940s (Chatterjee, 1948; Chang, 1964, 1975, 1976b, 1985, 1988) to 22 species (Chang, 1985; Vaughan and Morishima, 2003). The number of genomes remains at six.

Table 1 Species of *Oryza*, chromosome numbers, genome symbols and geographical distributions.

Species name (Synonym)	2n for x = 12	Genome group	Distribution
O. alta Swallen	48	CCDD	Central and South America
O. australiensis Domin	24	EE	Australia
O. barthii A. Chev. (*O. breviligulata*)	24	A^bA^b	West Africa
O. brachyantha A. Chev. et Roehr	24	FF	West and Central Africa
O. eichingeri A. Peter	24, 48	CC, BBCC	East and Central Africa
O. glaberrima Steud.	24	A^gA^g	West Africa
O. glumaepatula teud. (*O. perennis* subsp. *cubensis*)	24	$A^{gp}A^{gp}$	South Africa, West Indies
O. grandiglumis (Deoall.) Prod.	48	CCDD	South Africa
O. granulata Nees et Arn. ex. Hook f.	24	_	South and Southeast Asia
O. latifolia Desv.	48	CCDD	Central and South America
O. longiglumis Jansen	48	_	Papua New Guinea
O. longistaminata A. Chev. et Roehr *(O. barthii)*	24	A^lA^l	Africa

Table 1 Continued

Species name (Synonym)	2n for x = 12	Genome group	Distribution
O. meridionalis Ng	24	AA	Australia
O. meyeriana (Zoll. et Morrill ex. Steud.) Baill.	24	_	Southeast Asia, Southern China
O. minuta J.S. Presl. ex. C.B. Presl.	48	BBCC	Southeast Asia
O. nivara Sharma et Shastry *(O. fatua, O. sativa f. spontanea)*	24	AA	South, Southeast Asia and Southern China
O. officinalis Wall. ex. Watt	24	CC	South and Southeast Asia, Southern China, Papua New Guinea
O. punctata Kotschy ex. Steud.	48, 24	BBCC, BB	Africa
O. ridleyi Hook f.	48	_	Southeast Asia
O. rufipogon Griff. (*O. perennis*, *O. fatua*, *O. perennis* subsp. *balunga*)	24	AA	South and Southeast Asia, Southern China
O. sativa L.	24	AA	Asia
O. schlechteri Pilger	48	_	Papua New Guinea

Source: Chang (2003)

Useful traits of wild rice

Evaluation of wild relatives of rice has shown some sources of resistance to be widely scattered among different species on the genus (Table 2). Sources of resistance to green leafhopper have been found in 6 species, to bacterial blight in 6 species, and to brown planthopper in 5 species. Good sources of resistance to sheath blight in cultivated rice are limited, hampering the development of host resistance. *O. minuta* has resistance to sheath blight.

Table 2 Chromosome number, genomic composition and potential useful traits of *Oryza* species.

Species	2n	Genome	Useful or potentially useful traits[*]
O. sativa complex			
O. sativa	24	AA	Cultigen
O. nivara	24	AA	Resistance to grassy stunt virus, blast, drought avoidance
O. rufipogon	24	AA	Elongation ability, resistance to BB, source of CMS
O. breviligulata	24	A^gA^g	Resistance to GLH, BB, drought avoidance
O. glaberrima	24	A^gA^g	Cultigen
O. logistaminata	24	A^gA^g	Resistance to BB, drought avoidance
O. meridionalis	24	A^mA^m	Elongation ability, drought avoidance
O. glumaepatula	24	$A^{gp}A^{gp}$	Elongation ability, source of CMS
O. officinalis complex			
O. punctata	24	BB	Resistance to BPH, zigzag leafhopper
O. minuta	48	BBCC	Resistance to sheath blight, BB, BPH, GLH
O. officinalis	24	CC	Resistance to thrips, BPH, GLH, WBPH
O. rhizomatis	24	CC	Drought avoidance, rhizomatous
O. eichingeri	24	CC	Resistance to yellow mottle virus, BPH, WBPH, GLH
O. latifolia	48	CCDD	Resistance to BPH, WBPH, GLH
O. alta	48	CCDD	Resistance to striped stemborer, high biomass production
O. grandiglumis	48	CCDD	High biomass production
O. australiensis	24	EE	Drought avoidance, resistance to BPH
O. brachyantha	24	FF	Resistance to yellow stemborer, leaf-folder, whorl maggot, tolerance to laterite soil
O. meyeriana complex			
O. granulata	24	GG	Shade tolerance, adaptation to aerobic soil
O. meyeriana	24	GG	Shade tolerance, adaptation to aerobic soil
O. ridleyi complex			
O. longiglumis	48	HHJJ	Resistance to blast, BB
O. ridleyi	48	HHJJ	Resistance to stemborer, whorl maggot, blast, BB
Unknown genome			
O. schlerchteri	48	Unknown	Stoloniferous

Source: Eizenga and Rutger (2003).

[*] BB , bacterial blight; BPH, brown planthopper; CMS, cytoplasmic male sterility; GLH, green leafhopper; WBPH, white-black planthopper.

Some *Oryza* species have a wide array of useful traits. The *O. officinalis* has sources of resistance to thrips, brown planthopper, green leafhopper, and white-backed planthopper. The tetraploid species *O. minuta* has been used as a source of sheath blight, bacterial blight, brown planthopper, and green leafhopper resistance.

A comparison of the percentages of cultivated and wild rice accessions resistance to various pests was shown by Heinrichs (Heinrichs *et al.*, 1985). The result revealed the high proportion of accessions of wild rice with resistance to insect pest. Evaluation of pathogens had revealed similar high levels of resistance in wild germplasm. Sixty-six of 98 accessions of *O. rufipogon* were shown to be resistant to 6 Philippine races of bacterial blight (Ikeda *et al.*, 1990).

Resistance to abiotic stresses and useful special traits are also presented in some wild species. The *O. glaberrima*, *O. nivara*, *O. breviligulata*, *O. longistaminata*, *O. meridionalis* and *O. australiensis* have been as sources of drought avoidance. Sources of cytoplasmic male sterility have been found in *O. rufipogon* and *O. glumaepatula* (Jena and Khush, 2000; Brar and Khush, 2002; Eizenga and Rutger, 2003).

Rice improvement programs have benefited substantially from some traits that come from wild species having the AA genome. The *O. nivara* from India provided the sole source of resistance to grassy stunt virus (Chang *et al.*, 1975). A wild rice, *O. rufipogon*, from Thailand has been found to have a high level of resistance to tungro virus (Ikeda *et al.*, 1994). The *O. longistaminata* has been a source of bacterial blight resistance (Khush *et al.*, 1990). The *O. glumaepatula* has been a new source of cytoplasmic male sterility (Dalmacio *et al.*, 1996). These wild species have helped fuel yield gains and indicated the potential of wild rice in rice improvement.

Species less closely related to rice, not having the AA genome, are now being used in breeding programs. The rapid progress in wide hybridization has occurred in part due to the emphasis given to this work by the Rockefeller program on biotechnology, which was complimented by international efforts to collect and conserve wild rice. At IRRI, hybrids have been successfully produced between cultivated rice and eight species in the genus of *Oryza* (Brar *et al*., 1991).

Wide hybridization

Genetic variability for some traits of rice such as resistance to tungro virus, sheath blight and yellow stem borer, and tolerance of abiotic stresses is limited in the cultivated germplasm. Wild species offer useful sources of genes for rice improvement.

Wide hybrids have been produced through embryo rescue between elite breeding lines or varieties of rice and several accessions of 11 wild species representing BBCC, CC, CCDD, EE, FF, GG and HHJJ genomes (Brar *et al*., 1991). These hybrids have been produced to transfer useful genes for resistance to brown planthopper, bacterial blight, blast, sheath blight, tungro virus and yellow stem borer. Additional hybrids involving rice and AA genome wild species have been produced for the diversification of cytoplasmic male sterility and for the transfer of tolerance to acid sulphate conditions (Khush and Brar, 1998).

A number of useful genes have also been transferred from wild species to rice. Khush (1977) transferred the gene for grassy stunt virus resistance from *O. nivara* to rice through backcrossing. Grassy stunt virus resistance has been bred into several rice cultivars. Recently, useful genes were transferred from other wild species. Jena and Khush (1990) transferred genes for resistance to three Philippine biotypes of brown planthopper from *O. officinalis* into the elite breeding line IR31917-45-3-2. Some of the derived lines have also shown resistance to brown planthopper populations in Bangladesh and India. These lines are free from the undesirable features of wild species such as grain shattering and poor plant type, and are on a par with the recurrent parent for grain yield. They are being used as parents in rice breeding at IRRI and in other national programmes. Of them, three breeding lines resistance to brown planthopper derived from *O. officinalis* have been released as varieties in Vietnam. Genes for blast, bacterial blight and brown planthopper resistance have been transferred to rice from other wild species including *O. minuta*; *O. latifolia*; *O. australiensis* and *O. brachyantha* (Khush and Brar, 1998).

Khush and Brar (1998) stated that most of the commercial hybrids of indica rice are based on the wild abortive (WA) source of cytoplasmic male sterility (CMS). More than 95% of the rice hybrids grown in China have WA cytoplasmic male sterile. Such cytoplasmic uniformity increases the vulnerability of hybrid rice to diseases and insects. To overcome this problem, diversification of the cytoplasmic male sterility source is essential. They crossed 45 accessions with the widely grown varieties IR54 and IR64. The results revealed that both IR54 and IR64 could restore the fertility of CMS lines possessing WA cytoplasm. Of all the backcross derivatives, one line with cytoplasm of *O. perennis* (Acc. no. 104823) and the nucleus of IR64 was found to be stable for complete pollen sterility. The newly developed CMS line has been designated IR66707A (Dalmacio *et al*., 1995). Crosses of IR66707A with six restorers of WA cytoplasm also showed almost complete pollen sterility, indicating that this source of CMS is different from that of WA cytoplasm. Hybridization of IR66707A, *O. perennis* and IR66707B with eight mitochondrial DNA-specific probes was carried out. Of 40 combinations, 18 showed a monomorphic pattern, while in 22 polymorphic combinations the banding patterns of IR66707A and *O. perennis* were identical. The results indicated that IR66707A has the same mitochondrial genome as the donor *O. perennis* and that CMS may not be caused by any major rearrangement or modification of mitochondrial DNA (Khush and Brar, 1998).

Barriers to wide hybridization

The first concern in breeding programs involving interspecific hybridization is whether or not a viable F_1 hybrid plant can be obtained. In general, close relationship as indicated by taxonomic status is the most useful criterion of crossability. This correlation is far from complete. For example, the common cabbage, *Brassica oleracea*, will cross with the radish, *Raphanus sativus*, but not with the more closely related turnip, *Brassica rapa*, or with rape, *Brassica napus*. In the genus *Datura*, pollen tubes sometime burst in the styles of foreign species. In *Nicotiana* and in hybrids between *Zea mays* and *Tripsacum* species, failure of fertilization sometimes results from the fact that the style of the maternal species is much longer than the pollen tube of the male parent. Pollen tubes not adapted to traversing the great length between stigma and ovary therefore fails to effect fertilization. In addition, the thick pollen tubes of polyploid species sometimes have difficulty growing in the slender styles of diploids or lower polyploids. This type of barrier to fertilization can sometime be overcome by making the cross in the reciprocal direction or by shortening the style artificially (Allard, 1960a).

Failure to obtain thriving F_1 hybrids can result from genetic or cytoplasmic incompabilities that are expressed either in failure of fertilization or in death of the zygote at any stage between early cleavage divisions and maturity (Allard, 1960a).

Barriers to interspecific hybridization that act by preventing the growth of the F_1 hybrid are common in plants. Allard (1960a) believed that hybrid inviability can be caused by single gene, by general incompability of the genotype of the parents, or by disharmonies between the developing embryo and the endosperm.

Lethality is usually used to explain for incrossability. In an extensive study of the wild American *Gossypium* species, *G. gossypioides*, all but one of the interspecific hybrids that could be obtained with this species died in some stages ranging from early embryogeny until after meiosis had taken place (Allard, 1960b). This behavior was attributed by Silow (1941) to general genotypic disharmony rather than to the action of specific lethal genes. Stephens (1950) and Gerstel (1954), however, have been able to demonstrate that hybrid inviability is controlled by simple genetic mechanism in some of the other interspecific hybrids in cotton. In the other case, F_1 hybrid sterility in interspecific crosses between *O. sativa* and *O. glaberrima* was explained by duplicate gametic lethal of genes (S_1 to S_5) (Sato *et al*., 1987).

Shastry (1964) revealed chromosome arms of the cultivated rice tend to be more symmetrical than those of the wild species. This differentiation has proceeded to the point where chromosome pairing during meiosis of the F_1 hybrid fails completely.

However, several barriers are encountered in transferring useful genes from wild species to cultivated rice (Sitch, 1990). The barrier most commonly encountered is lack of crossability because of chromosomal and genic differences. Biotechnological tools such as embryo rescue and protoplast fusion have become available to overcome the crossability barriers, and several interspecific hybrids have been produced. Molecular techniques have been employed in the precise monitoring of alien gene introgression and the transfer of useful genes to cultivated crop plants.

In most interspecific crosses, the F_1 hybrids are completely sterile. Progenies are advanced through embryo rescue in subsequent backcrosses to the respective recurrent rice parents until plants with $2n = 24$ and 25 chromosomes become available (Khush and Brar, 1998).

Embryo rescue

In a very large number of plant taxa, excision and culture of embryos on a sterile medium have been used to circumvent seed failure and to rescue interspecific and intergeneric hybrids. The application of the technique is easier when the differentiation of the embryonic organs is earlier and more complete. In general, the isolated embryo does not continue its normal growth and differentiation when placed on an artificial culture medium. If it has a radicle and an apical meristem, germination takes place immediately giving directly a small, but normal plantlet. Rescue of younger or less differentiated embryos or proembryos is only possible by using more sophisticated culture media or techniques. The grasses are good material for the application of embryo culture because of the early differentiation of the vital organs. This is probably one of the reasons why very broad diversity of hybrid combinations of species and genera has been obtained in several groups of cereals (Bouharmont, 1991a).

1. Culture media

Very few papers have been published on the mineral composition of the culture media for rice embryos. In fact, the problem did not appear crucial, since positive results have been obtained for various salt combinations. In general, solutions proposed for other species or other plant organs have been used. For example, the medium described by Randolph (1945) for iris embryos was used at the Cuttack experimental station (Central Rice Research Institute Cuttack, 1958). White's medium was used by Nakajima and Morishima (1958) after pollination of diploid and tetraploid plants of *O. sativa* by *O. minuta* or by wild species from Paraguay, and by Wuu *et al.* (1963) for obtaining F1 hybrids between cultivated rice and *O. minuta* or *O. brachyantha*. On the other hand, Kucherenko and Los (1982) obtained good results with MS medium (Murashige and Skoog, 1962). A modified MS medium was used more recently for embryos isolated 15-20 days after pollination (Bouharmont, 1991b). Many papers on the cytogenetics of interspecific hybrids reported the application of embryo culture without giving information on the culture media used; some research institutes have adopted a method which has been routinely used for many years by several workers.

In some cases, complex culture media described for other purposes were used for rice embryos. In fact, micronutrients are not necessary and their use is generally not reported. The size of the embryo, the presence of fragments of caryopsis and endosperm, and the short duration of the culture make the addition of other minerals to the macronutrients unnecessary.

Addition of a carbohydrate source is necessary for the synthesis of chlorophyll in the plantlet. Soluble starch and several sugar forms are efficient but the most frequent is sucrose at a concentration of 2 to 5% (Bouharmont, 1991a). In addition to its metabolic role, sucrose also acts as an osmoticum and together with the inorganic nutrition helps to balance the osmotic potential of the culture medium (Evans *et al.*, 2003).

Agar is stable, does not react with medium constituents. It is not normally digested by plant enzymes and remained stable at all feasible incubation temperatures. Normally, concentrations of agar at 0.5 to 1% (Razdal, 2003) and 0.6-1% (Constabel and Shyluk, 1994) are used in the medium to form a firm gel of plant cell culture medium. Agar comprises two fractions, agarose (70%) and agaropectin (30%). Agarose is the gelling fraction. It is used in plant tissue culture in case a high purity support matrix is required for example in protoplast, anther and embryo culture. Constabel and Shyluk (1994) suggested that the concentration of agarose should be used for making solid medium is 0.6 – 0.8%.

Plant cells and tissues culture require optimum pH for growth and development in cultures. The pH affects uptake of irons and for most of the culture media pH 5.0 to 6.0 before sterilization is considered optimal (Razdal, 2003).

2. Excision of the embryo and culture conditions

Dissection of the young ovary is not easy because it is fragile and enveloped by rigid lemma and palea, manipulation is easier when the top of the envelope is cut at pollination time. Excised ovaries can be surface-sterilized with a solution of mercuric chloride (0.05%), chlorox (10%) or hypochlorite (5%) containing a wetting agent. After washing in sterile water, the lower part of the ovary is cut and put on the sterilized culture medium solidified by agar. The pericarp and the endosperm surrounding the embryo should not be discarded (Bouharmont, 1991a).

Ko *et al.* (1983) described a simple method for the isolation of immature embryos involving squeezing through a cut made at the base of caryopsis.

Test tubes or bottles are generally used, because they provide enough space for the leaf elongation. The mode of closure is not important for the evolution of the culture: hermetic sealing dose not prevent a normal development of the plantlets. The test tubes or bottles are generally incubated at about 25^0C with an alternation of light and darkness (Bouharmont, 1991a).

According to Bouharmont (1991a), germination of the embryo is visible after the 1st to 2nd day; the first leaf and the radicle appear and grow taller. The size of the organs, their growth rate, and the number of leaves differ according to the stage of differentiation of the cultured embryos. When the explant is fully differentiated, the plantlet is entirely normal and its growth is rapid. When the embryo is smaller and contains only one or two leaf primordial, the plantlet is slender and weak. It grows slowly but after a few weeks new leaf and adventitious roots enable the development of a normal plantlet.

The plantlets are generally well developed after 2 weeks and they can be planted in soil. Transplantation is not difficult but a plastic cover is useful for maintaining a high humidity until the development of adventitious root is sufficient. If necessary, the plantlets can remain in test tubes for at least 2 additional weeks. Their growth become slower but they remain in a perfect state for transplantation (Bouharmont, 1991a).

3. Culture of younger embryos

Application of simple media does not allow recovery of hybrids when the culture of the embryo occurs before differentiation of the radicle and first leaf. Several experiments have been reported on dicotyledons. For example, Ono and Ikeda (1970) obtained hybrids between different *Mentha* subgenera by adding extracts of *Mentha* ovaries to the culture media. They considered these extracts to contain growth substances importance for embryo development. In other cases, embryo growth was promoted by addition of coconut milk, malt extract, yeast extract, hydrolyzed casein, or fruit juice (Blakeslee and Satina, 1944; Amemiya *et al.*, 1956; Harberd, 1969; Bajaj *et al.*, 1986; Evans *et al.*, 2003). Similar experiments were reported in some cereal species. In barley, differentiation of embryo organs could be induced by an “embryo factor” found in casein hydrolysate and in some other animal or plant extracts. Tomato juice was also very efficient (Kent and Brink, 1947). Likewise, Bajaj (1980) reported the beneficial role of durum wheat endosperm in the development of triticale embryos. In these conditions, the embryos could become larger than the normal ones and gave rise to stronger plantlets after their transfer to a normal medium.

In rice, some successful experiments involving younger embryos (5 days) were reported (Yie and Liaw, 1975; Shome and Bhaduri, 1982). With youngest embryo culture, some organic elements like casein hydrolysate, NAA and kinetin at the concentration of 0.2 mg/l (Ko *et al.*, 1983) are useful. However, embryos excised 7 days after pollination are generally suitable for *in vitro* culture (Bouharmont, 1991a).

Drought resistance

Drought resistance is the ability of a plant to live, grow and yield satisfactorily with limited water supply or under periodic water deficits. Drought resistance is conferred on plants by any or combination of the four mechanisms: escape, avoidance, tolerance and recovery (Arraudeau, 1989a).

Drought escape is the ability of a plant to mature before water stress becomes a serious limiting factor.

Drought avoidance is the ability of a plant to maintain high water status during a drought.

Drought tolerance is the ability of a plant to withstand water deficit as measured by degree and duration of low plant water potential.

Drought recovery is the ability of a plant to resume growth and yield after drought stress with a minimum of irreversible yield loss.

Arraudeau (1989a) believed each of these mechanisms includes several traits and they are considered more detail below.

1. Escape mechanisms

Four main characters can be used. Early maturity is probably the most common and easiest to breed for earliness conferring the capacity to yield before drought onset. Photoperiodic sensitivity with a reproductive phase coincides with high rainfall possibility. Delay of flower initiation and staggered tiller development has to be a quasi-indeterminate growth habit (Arraudeau, 1989a).

2. Avoidance mechanisms

Seven main characters need attention: a deep rooting is probably the most important character for increasing the total amount of water available to the plant. Deep rooted upland rice showing very good drought resistance is a clear indication of the interest of such a mechanism. Root density is an important factor of performance under drought conditions. Large root and culm xylem vessels ensure better hydraulic conductivity and reduce axial resistance. Epicuticular wax likes a barrier to non stomatal water losses, although without clear relationship with drought avoidance in rice (O'Toole, 1982). Stomatal behaviour that is under discussion as stomatal closure is the apparent symptom of plant water status degradation. It occurs when the plant is losing its turgescence. Plants showing stomatal closure during a drought spell suffer when stomatal aperture is an adaptive trait. Leaf area reduced tends to minimize water loss through transpiration but also minimize photosynthesis. The large leaf area of drought resistant traditional upland rice cultivars is an indication of the great care to be used in relation to leaf area. Leaf senescence, reducing both leaf area and photosynthesis, is also to be manipulated with precaution. Leaf rolling is not a protecting mechanism but evidences of the degradation of water status, and appears negatively correlated to the relative water content of the plant (Arraudeau, 1989b).

3. Tolerance mechanisms

Some characters can be used in tolerance mechanisms like translocation assimilation, osmotic adjustment, and cellular elasticity. The first is translocation assimilations that the remobilization of assimilations from stem to grains in upland rice is to be analysed in terms of “sink/source” relationship that is specific to a given entry. The efficiency of the photosynthetic source determines uptakes from the translation source. So the key mechanism is not the translocation of assimilations to the grain but the accumulation efficiency of carbohydrates during vegetative phase (Arraudeau, 1989b). The conclusion is that a good plant canopy for photosynthesis efficiency appears to be important as utilization of stored assimilations. The second is osmotic adjustment that may postpone and contribute to lessen tissue death after desiccation. Little genetic diversity of osmotic adjustment has been noted in rice (O’Toole, 1982). The third is cellular elasticity such as cell size which can fluctuate with fluctuation in water availability.

4. Recovery mechanisms

Shallow roots contribute to rapid water uptake as soon as rainfall occurs after a drought spell. Hypersensitivity of plant tissues reacts upon water deficit by entering a dormant stage as soon as water deficit occurs and immediately recover their efficiency as soon as water is available again (Arraudeau, 1989a).

Genetic of drought resistance

Water deficit is one of the most widespread environmental stress limiting plant growth and productivity. Depending on their level of resistance, many varieties can successfully cope with drought by modifying their morphological, physiological and metabolic processes. With the advent of molecular marker technology, research on drought resistance has shifted from simple descriptions of the phenomenon to dissection of the mechanisms involved. Rice, having the smallest genome size among important cereal crops, has received much more attention than ever before. Nguyen *et al*. (1999) mapped quantitative trait loci (QTLs) for various components of drought resistance in four rice mapping populations. Four to ten QTLs for osmotic adjustment (OA) have been detected. For root penetration, 3 to 6 QTLs have been identified. Four to ten QTLs for root thickness have been located. For root pulling force 8 QTLs have been found. Aarti *et al*. (1999) found that the region on chromosome 4 in a DHL (double haploid lines) population of CT9993/IR62266 is responsible for basal root thickness, root penetration index and root pulling force. The region responsive for osmotic adjustment on chromosome 1 was also mapped.

Osmotic adjustment has been shown to be an important adaptive mechanism of drought resistance in rice. Chamarerk *et al*. (1999) did experiment in order to evaluate genetic variation in OA among 42 rice varieties from Vietnam, Thailand, and the International Rice Research Institute. They found that two varieties (Tau Huong and Tam Thom) had OA higher than IR62266-42-6-2 (a standard check variety for high OA), and one variety (LC90-7) had OA lower than CT9993-5-10-1-M (a standard check variety for low OA).

According to Babu *et al*. (1999), the relationship between various physio-morphological traits and plant performance under drought was tested in a preliminary experiment using a subset of 64 double-haploid lines developed from the cross CT9993-5-10-1-M/IR62266-42-6-2. Results indicated significant negative relation between leaf relative water content (RWC) vs leaf rolling, RWC vs leaf drying and leaf drying vs relative biomass under stress condition.

Kamoshita *et al*. (1999) found significance for deep root traits in two populations, double-haploid lines from the cross of upland japonica and lowland indica (CT9993/IR62266) and recombinant inbred lines from a cross of lowland indica (IR58821/IR52561). Three and four QTLs for deep root traits were repeatedly found in CT9993/IR62266 and in IR58821/IR52561, respectively. In CT9993/IR62266, both parents contributed to the alleles for the common QTLs while in IR58821/IR52561 all the alleles for the common QTLs come from IR58821. Transgressive variation for deep root traits was large in CT9993/IR62266 but small in IR58821/IR52561.

With recent biotechnology advances, some varieties have shown as sources for drought resistance but transgressive ability of them is different. So, we need to make a cross to obtain desirable characters.

Breeding for drought resistance

The continuous improvement of rice by pre-science and modern breeding strives to achieve two major ends: (i) the maximization of productivity within narrow agroecological niches and (ii) the stabilization of productivity across variable environments in space and time. In terms of the water regime, rice breeding has the task of stabilizing yield when water supply is unpredictable and plant water deficit develops. According to Nguyen *et al*. (1997), the problem of adaptation to drought conditions in rice is complex and unique. The problem arises from the requirement of the plant to adapt to extremely different soil hydrological conditions, and these conditions which range from soil submergence to severe drying may fluctuate during the growing season.

The ability to design a plant ideotype for drought-prone environment depended on the available database on the physiology of the rice plant, the nature of its environment, and interaction between the plant and the environment (O'Toole and Chang, 1979; Fukai and Cooper, 1995). On one hand, knowledge is still imperfect for the construction of a rice plant ideotype. On the other hand, plant response and adaptation to drought stress are not as complex as they are often presented, at least not when one has to separate major factors that sometime obscure the minor issue. Nguyen *et al.* (1997) defined minor issues to be the reflective properties of leaves or leaf posture in relation to plant water status under drought stress.

Recent advances in genetic engineering and genome mapping hold great promise for physiological and genetic research in drought resistance in rice. Drought resistance in rice has been the common experience of many rice breeders that when rice populations are subjected to drying soil conditions, genotypes are very clearly separated into those that wilt and dry readily and those that maintain a measure of turgor and viability as stress conditions (IRRI, 1982; Malabuyoc *et al.*, 1985; De Datta *et al.*, 1988). Furthermore, genotypes differ in their recovery upon dehydration and the level of genotypic recovery is closely related (other factors held constant) to its hydration status prior to recovery (Malabuyoc *et al.*, 1985). Breeding for drought resistance in terms of these responses is most likely dependent mainly on one or more of the following components: (i) moderated water-use through reduced leaf area and shorter growth duration, (ii) the ability of the roots to exploit deep soil moisture to provide for evapotranspirational demand, (iii) the capacity for osmotic adjustment which allows it to retain turgor and protect meristems from extreme desiccation, and (iv) the control over nonstomatal water loss from leaves.

Genome mapping and biotechnology research in recent years have offered two powerful tools to crop improvements: genetic transformation and molecular marker assisted selection. The availability of these two techniques has opened enormous opportunities for meeting the challenges of food production.

Askok *et al.* (1999) evaluated the effectiveness of marker-assisted selection for root thickness using microsatellite. Two QTLs conferring root thickness were identified to be linked to rice microsatellite marker RM60 on chromosome 3 and RM226 on chromosome 4 in the IR64/Azucena doubled haploid mapping population. These markers were tested on three populations viz., CT9993/IR/62266, IR58821/IR52561 and 20 lowland and upland cultivars. The authors suggested that as upland cultivars are known to possess thick roots, this microsatellite marker would enable to screen rice germplasm for upland rice ecosystem and introgression of thickness root system into elite lines for breeding drought resistant cultivars.

Babu *et al*. (2003) used anther culture technique to develop 154 DH lines from the cross C59993-5-10-1-M/IR62266-42-6-2. The *indica* accession, IR62266-42-6-2 contributed most of the alleles for leaf rolling, leaf drying and delay in days to heading under stress. However, favorable alleles from IR62266-42-6-2 also contributed to plant production in terms of biomass, yield, harvest index, and relative yield under stress.

Introgression of QTLs for root morphology was done in a cross IR64/Azucena. The root depth and deep root weight in a double-haploid population of 105 lines derived from this cross were quantitative and the positive alleles at the QTLs came from Azucena, a deep rooted japonica variety (Courtois *et al.*, 2003).

METERIALS AND METHODS

1. Plant materials

1.1 Two wild species having drought avoidance trait (Eizenga and Rutger, 2003) to be used in this experiment were *Oryza meridionalis* Ng (acc. no. w-1625 and w-1629), and *Oryza nivara* (acc. no. 16150). Their taxonomy (Vaughan, 1994) is shown below.

1.1.1 *Oryza meridionalis* Ng

- Morphological characteristics: annual and erect grass usually 1 - 2 m tall, panicles usually compact or seldom open and rarely having secondary branching, spikelets slender 7.2 - 9.6 mm long and 2 - 2.8 mm wide with strong awn (thick at base) about 7 - 13 cm long, anthers 1.5 - 2.5 mm long.
- Chromosome number: 2n = 2x = 24
- Genome: AA
- Distribution: Australia, Indonesia (Irian Jaya), and Papua New Guinea.
- Habitat: found at the edges of freshwater lagoons, rivers, temporary pools, and swamps in 15 – 20 cm of water, growing in black and clay soils in open habitats.

1.1.2 *Oryza nivara* Sharma et Shastry

- Morphological characteristics: annual, short to intermediate high grass (usually ‹ 2m), panicles usually compact and rarely open, spikelets large 6-10.4 mm long and 1.9 - 3.4 mm wide with strong awn (4 - 10 cm long), anthers 1.5 - 3 mm long.
- Chromosome number: 2n = 2x = 24
- Genome: AA
- Distribution: Bangladesh, Cambodia, China, India, Laos, Malaysia, Myanmar, Nepal, Sri Lanka, Thailand, and Vietnam.

- Habitat: found in swampy areas, at edge of pond and tanks, beside streams, in ditches, in or around rice fields, growing in shallow water up to 0.3 m in seasonally dry and open habitats.

1.2 Two cultivated rice varieties to be used as targets for transferring gene pools from wild species were RD23 (RD 7/IR 32//RD 1), and CN1 (IR 13146-158-1/IR 15314-43-2-3-3//BKN 6995-16-1-1-2).

2. Equipments

2.1 For rice cultivation

Pots (30 cm in diameter), paddy soil, and fertilizer (N-P_2O_5-K) were required.

2.2 For hybridization

The equipments needed for hybridization were a pair of scissors, forceps and glassine bag.

2.3 For medium preparation

All the chemicals needed for making MS medium were required. Sucrose and agar were also required. A fully equipped embryo culture laboratory should contain instrumentation for media preparation such as pH meter, centigram balances, autoclave and oven.

2.4 For embryo culture

Materials needed for embryo culture were immature embryos at different ages (8-14 days old after pollination), ethyl alcohol, and Clorox. The equipments needed for embryo culture were petridishes, bottles, burner, and a laminar air flow cabinet for embryo transfer. Growth room should allow for a predetermined light regime (16 hrs of light per day) and temperature control (25^0C).

3. Methods

3.1 Planting method

All materials were grown in pots. Each variety and accession comprised 5-8 plants.

3.2 Hybridization and backcrossing

Hybridization was done between cultivated rice and wild species by emasculation of the female parents. Upper one third of the glume of each spickelet was cut off using scissors after 4 p.m. Then the anthers were removed by using forceps. The emasculated spikelets of panicles were pollinated heavily from the male parents one day afterwards, covered by glassine bag and tagged.

Backcrossing is necessary after wide hybridization. It can solve pollen sterility problem and eliminate undesirable characters. So, the F_1 hybrids between cultivated rice and wild species were backcrossed to cultivated varieties. The crossing process was mentioned above and BC_1F_1 seeds obtained were also cultured on half-strength MS medium for germination.

3.3 Embryo rescue

Spikelets dated at pollination time were harvested at different stages of 8-14 days old. The husks were removed from the seeds. Whole seeds were immersed in 70% ethyl alcohol for 5 minutes and then sterilized with 10% Clorox containing a wetting agent (2 drops Tween 20/50 ml solution) for 20 minutes. After several rinses with sterilized distilled water, the lower part of the seed with embryo was excised. Two embryos were transferred to the bottle containing 10 ml half-strength MS

(Murashige and Skoog, 1962) medium supplemented with 3% sucrose, 0.6% agar for germination. The pH was adjusted to 5.8. The cultures were incubated at 25°C under dark condition for two days and then moved to the light condition illuminated by 2,000 lux of cool-white fluorescent light with 16 hrs photoperiod until the seedlings reached the two-leaf stage. For acclimatization, the cultured bottles were transferred to a room without direct sunlight until they reached the three-leaf stage and then the F_1 hybrid seedlings were transplanted into soil in pots and grown in the greenhouse. The number of germinated embryos was recorded to determine the optimum age of embryo for culture.

3.4 Male fertility test

3.4.1 Pollen fertility: Five spikelets were collected from individual F_1 hybrid plants before anthesis and fixed in 70% alcohol. Pollens were crushed out from anthers and stained with I_2KI solution. At least three microscopic fields were used to count sterile pollen grains (viz., unstained withered, unstained spherical and partially stained round) and fertile pollen grains (stained round).

3.4.2 Seed fertility: Primary panicles of the F_1 hybrid plants were bagged with glassine bags at heading stage. The number of filled spikelets was counted at grain maturing stage.

3.5 Study of morphological and agronomic characters

Morphological and agronomic characters of the F_1 hybrids and backcross progenies were investigated in accordance with the standard evaluation system for rice (INGER, 1996).

Morphological characters of the F_1 hybrids were recorded including ligule length, panicle number, leaf width, leaf length, flag leaf length and awn color.

Agronomic characters of the F_1 hybrids were determined such as plant height, panicle threshability and seed set ability.

3.6 Screening for drought resistance

The BC_1F_2 progenies were screened for drought resistance in the cement blocks under the greenhouse. The BC_1F_2 seeds were pre-germinated in a room. Ten rows of each BC_1F_2 population were grown in 1.4 m long rows spaced 20 cm apart. Hills were spaced 10 cm apart within each row. Drought susceptible varieties (Taichung Native 1, CN1 and RD23) and resistant varieties (Salumpikit and RD19) were put together as checks after each block to compare the block differences. Sprinkler irrigation was used after transplanting twice a week until 30 days and then the irrigation was stopped.

Withheld sprinkler irrigation was kept until resistant varieties had tightly rolled leaves at midday (10.00 am – 15.30 pm) to cause marked differences in drought sensitivity of the progenies. A rating of leaf rolling score was visually estimated in each block using a 0 – 9 scale, in which a score of 0 indicated no rolling and 9 tightly rolling. To induce recovery condition, sprinkler irrigation was used again. Leaf drying and plant recovery score were collected 10 days afterwards. Rating of leaf drying score from 0 – 9 was estimated based on symptoms of plants. A score of 0 indicated no symptom of stress, with an increasing score when more leaves die due to water deficit. A score of 5 indicated that ½ of all leaves fully dried. The maximum score of 9 indicated that all plants were apparently dead. Rating of recovery score from 1 – 9, in which a score of 1 indicated 90 – 100% plants recovered and 9 indicated 0 – 19% plants recovered (INGER, 1996).

3.7 Data analysis

Analysis of embryo culture was done by using IRRISTAT program with significant differences at $P \leq 0.01$ or $P \leq 0.05$.

Percentage of pollen sterility was calculated as below:

$$\frac{\text{Number of (unstained withered + unstained spherical + partially stained round) pollen grains}}{\text{Total number of pollen grains (including fertile)}} \times 100$$

Degrees of pollen sterility, filled and unfilled spikelet were evaluated following the rule of standard evaluation system for rice (INGER, 1996).

The experiments were conducted at Agronomy Department, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand. All studies were undertaken from June 2004 to March 2006.

RESULTS AND DISCUSSION

Crossability of interspecific hybridization

In all cross combinations, 9,790 cultivated rice spikelets of cultivated rice (RD23 and CN1) were pollinated with the pollens of wild species (*O. meridionalis* and *O. nivara*) and 3,452 seeds were obtained (Table 3). Seed set of all crosses ranged from 21.2 to 50.0% with an average of 35.3%. The two rice cultivars (RD23 and CN1) when pollinated with the wild rice *O. nivara* showed seed set rate of 48.0 – 50.0% which was higher than those crossed with *O. meridionalis* which exhibited the crossability of 21.2 – 44.6%. Particularly, cultivated rice variety RD23 when hybridized with the two wild species (*O. meridionalis* acc. no. w-1625 and w-1629 and *O. nivara* acc. no. 16150) presented higher percentages of seed set (34.6, 44.6 and 50.0%) than CN1 crossed with both wild species which exhibited the seed set rates of 30.6, 21.2 and 48.0%, respectively. These data showed that crossability depended on the wild species and rice cultivars used in hybridization. Similar result was observed when thirteen cultivated rice varieties were pollinated with eight wild species in the experiment of Brar *et al.* (1991).

Crossability may be related to pollen quality of male parent. Partial pollen sterility is a common phenomenon for wild species of rice. Tao and Sripichitt (2000) showed that there was relationship between pollen fertility of male species and crossability and the higher fertility of pollen caused the higher percentage of seed set. In this experiment the results once again indicated that the higher pollen fertility (88%) of the *O. nivara* (Table 7) induced higher crossability than the lower pollen fertility (83 – 86%) of the *O. meridionalis*. However, this observation revealed that crossability was also associated with the maternal effect of cultivated rice.

Table 3 Crossability of interspecific hybridization between cultivated rice and wild species.

Crosses	No. of spikelets pollinated	Set seeds	
		No.	%
RD23/*O.meridionalis* (w-1625)	1647	570	34.6
CN1/*O.meridionalis* (w-1625)	1775	543	30.6
RD23/*O.meridionalis* (w-1629)	1363	608	44.6
CN1/*O.meridionalis* (w-1629)	2594	550	21.2
RD23/*O.nivara* (16150)	1192	596	50.0
CN1/*O.nivara* (16150)	1219	585	48.0
Total	9,790	3,452	35.3

Germination ability of interspecific hybrid embryos

In some interspecific hybridization, the embryo fails to develop at any stage of cleavage division to maturity (Bouharmont, 1961). To obtain a maximum number of hybrid seedlings, embryo culture for embryo rescue should be done as early as possible. It is therefore important to know from what age of the embryo, a sufficient germination can be expected from embryo culture. Before embryo culture was started, it was observed that the seeds were imperfectly developed after hybridization. Some of them were opaque and shrivelled at the eighth day of pollination (Figure 1A). This negative phenomenon is very common in wide hybridization of rice as reported by Jena and Khush (1984) that embryo deterioration appeared at two weeks after pollination when three rice cultivars were crossed with three wild rice species, as early as 10 to 14 days from pollination in most of the intergenomic crosses between seven rice varieties and eight wild species (Sitch *et al.*, 1989) and earlier than 10 days after pollination in the experiment of Tao and Sripichitt (2000) when four rice cultivars were crossed with three wild species. In this experiment, the germination ability of embryos at the age of 8 to 14 days after pollination was compared in 6 crosses and only the normal embryos in group 1 and opaque embryos in group 2 (Figure 1A) were cultured.

Not all embryos of the same cross appeared to germinate simultaneously nor grew at the same rate in the initial culture. The young embryos germinated by the 5th or 6th day after inoculation whereas the more mature ones started to germinate a few days earlier (Figure 1B). The maximum germination was obtained on the 8th to 12th day after culture depending on the crosses.

The germination ability of hybrid embryos of all crosses varied from 36.2 to 61.5% with an average of 51.9% (Table 4). The hybrid embryos of the crosses between two rice cultivars and *O. nivara* germinated at the higher frequency (61.4 to 61.5%) than those of the crosses between two rice cultivars and *O. meridionalis* which germinated at the rate of 36.2 – 56.0%. In the crosses between rice cultivar RD23 and two wild species (*O. meridionalis* acc. no. w-1625 and w-1629 and *O.*

nivara acc. no. 16150), hybrid embryos germinated at the higher percentages (56.0, 54.8 and 61.5%) than those of the crosses between CN1 and both wild species which germinated at the frequencies of 36.2, 41.7 and 61.4%. It was shown that the germination ability of the hybrid embryos depended on the wild species and rice cultivars used for crossing which was in accordance with the crossability of interspecific hybridization described previously. Similar observation had been made by Sitch *et al.* (1989) when seven rice varieties were pollinated with eight wild species and by Brar *et al.* (1991) when the thirteen rice cultivars were crossed with the eight wild species.

Table 4 Germination ability of the interspecific hybrid embryos between cultivated rice and wild species after cultured on the medium.

Crosses	No. of embryos cultured	Germinated embryos	
		No.	%
RD23/*O.meridionalis* (w-1625)	420	235	56.0
CN1/*O.meridionalis* (w-1625)	420	152	36.2
RD23/*O.meridionalis* (w-1629)	420	230	54.8
CN1/*O.meridionalis* (w-1629)	420	175	41.7
RD23/*O.nivara* (16150)	420	261	61.5
CN1/*O.nivara* (16150)	420	258	61.4
Total	2,520	1,311	51.9

Figure 1 A. The F$_1$ interspecific hybrid embryos between cultivated and wild rice at 12 days old after pollination: 1 – a normal embryo, 2 – embryos with opaque appearance, 3 – embryos with opaque and shrivelled appearance.
B. Germinated hybrid embryos after 8 days of culture on the medium.

Germination percentage of the hybrid embryos at different ages were shown in Table 5. Germination rates of the hybrid embryos of the four crosses involving RD23/*O. meridionalis* (w-1625), CN1/*O. meridionalis* (w-1625), RD23/*O. meridionalis* (w-1629) and CN1/*O. meridionalis* (w-1629) increased when the age of embryos increased and reached the highest percentages (75.0, 66.7 and 70.0%) at the age of 11 days after pollination in the three crosses RD23/*O. meridionalis* (w-1625), CN1/*O. meridionalis*(w-1625) and CN1/*O. meridionalis* (w-1629) and attained the highest rate (81.7%) at the age of 12 days after pollination in the cross RD23/*O. meridionalis* (w-1629). Germination percentages decreased as the age of embryos increased to 14 days after pollination in all four crosses. Similarly, Sitch *et al.* (1989) found that embryos of the intergenomic crosses between seven rice cultivars and eight wild species began to deteriorate when the age of embryos were between 10 to 14 days after pollination. Germination frequencies of the hybrid embryos from the crosses RD23/*O. nivara* (16150) and CN1/*O. nivar*a (16150) increased when the age of embryos increased and reached the highest percentage at the age of 14 days old after pollination. It was noticed that the hybrid embryos of the crosses between cultivated rice and *O. meridionalis* failed to germinate at the earlier stage of seed development than those of the crosses between cultivated rice and *O. nivara*. This phenomenon might be resulted from the disharmony between the embryo and endosperm which occurred at any stage of seed development (Allard, 1960a; Roy, 2000). It made the embryos became weakly before they had been placed on the medium. However, the hybrid embryos of the crosses between the two cultivated varieties and *O. nivara* developed relatively normal because they could germinate at the high percentages (86.7 and 85.0%) when they were more mature (14 days after pollination). It is suggested that the hybrid embryos of the crosses between cultivated rice and *O. meridionalis* should be cultured at the age of 11 - 12 days after pollination while embryo rescue of the crosses between cultivated rice and *O. nivara* is done later when they are 14 days old.

Table 5 Germination percentage of interspecific hybrid embryos at different ages after cultured on the medium.

Age of embryos[1] (days)	Germination (%)					
	RD23/ *O.meridionalis* (w-1625)	CN1/ *O.meridionalis* (w-1625)	RD23/ *O.meridionalis* (w-1629)	CN1/ *O.meridionalis* (w-1629)	RD23/ *O.nivara* (16150)	CN1/ *O.nivara* (16150)
8	38.3 c[2]	20.0 c	26.7 d	23.3 c	40.0 c	38.3 f
9	46.7 bc	41.7 b	41.7 c	60.0 a	43.3 c	43.3 ef
10	60.0 ab	60.0 a	55.0 bc	65.0 a	50.0 c	55.0 de
11	75.0 a	66.7 a	56.7 b	70.0 a	65.0 b	60.0 cd
12	66.7 a	40.0 b	81.7 a	45.0 b	65.7 b	70.0 bc
13	60.0 ab	13.3 c	66.7 b	15.0 c	80.0 a	78.3 ab
14	45.0 bc	11.7 c	55.0 bc	13.3 c	86.7 a	85.0 a
Average	56.0	36.2	54.8	41.7	61.5	61.4
C.V.(%)	18.5	19.1	14.1	18.5	11.8	12.3

[1] Number of days after pollination.

[2] Means within a column followed by a common letter are not significantly different at 95% level of confidence by DMRT.

Morpho-agronomic characters of the F_1 interspecific hybrids

Production of interspecific hybrid is somewhat difficult, however, 1,108 F_1 hybrid seedlings were obtained from the six interspecific crosses in this experiment (Table 6). The 1,086 F_1 hybrid plants could survive until maturity. The cross RD23/*O. nivara* (16150) produced highest number of F_1 hybrids (230 plants) followed by the crosses CN1/*O. nivara* (16150) and RD23/*O. meridionalis* (w-1625) which generated the F_1 hybrids of 226 and 198 plants, respectively.

Morphological and agronomic characters of the F_1 hybrid plants were investigated (Table 7 and Figure 2). They were intermediate in some morphological and agronomic characters between their parents. Nevertheless, ligule length, awn formation and color and panicle threshability were closer to the wild parents whereas leaf width, plant height and number of panicles per plant were closer to cultivated parents. This result implied that some characters were dominated by wild parent while the others were influenced by cultivated parents. Similar result was recovered when four rice cultivars were hybridized with three wild species in the work of Tao and Sripichitt (2000). Flag leaf length and leaf length of the F_1 hybrid plants between cultivated varieties and *O. meridionalis* were relatively intermediate between their parents but those of the F_1 hybrid between cultivated rice and *O. nivara* were much longer than both parents which might be caused by overdominance of the hybrid. All of the F_1 hybrid plants headed much earlier than their parents. This might be resulted from the overdominance of earliness which was caused by a series of dominant *Ef* genes controlling short vegetative growth stage (Chang and Li, 1991). Pollen fertility of the F_1 hybrid plants between cultivated rice and *O. meridionalis* was very low (0 - 1%) (Figure 2E) which resulted in very few seed setting (0 - 0.3%) on the hybrid plants. On the contrary, favorable high percentage of seed fertility (56 - 60%) was recovered on the hybrid plants between cultivated rice and *O. nivara* which was caused by the considerably high pollen fertility (80 - 83%) (Figure 2F).

Table 6 The F_1 interspecific hybrids between cultivated rice and wild species produced after transplanting.

Crosses	F_1 hybrid seedlings[1/]		Matured F_1 hybrid plants
	No.	%[2/]	
RD23/*O.meridionalis* (w-1625)	203	86.4	198
CN1/*O.meridionalis* (w-1625)	133	87.5	129
RD23/*O.meridionalis* (w-1629)	162	70.4	154
CN1/*O.meridionalis* (w-1629)	154	88.0	149
RD23/*O.nivara* (16150)	230	88.1	230
CN1/*O.nivara* (16150)	226	87.6	226
Total	1,108	84.5	1,086

[1/] At three-leaf stage.
[2/] Based on germinated embryos.

Table 7 Morpho - agronomic characteristics of the F_1 interspecific hybrid plants grown under greenhouse conditions.

F_1 hybrid/ parents	Total no. of plants	Ligule length (mm)	Leaf width[1/] (cm)	Leaf length[1/] (cm)	Flag leaf length (cm)	Days to 50% flowering[2/]	Pollen fertility (%)	Seed fertility (%)	Plant height (cm)	No. of panicles per plant	Awn color	Panicle thresh-ability
RD23/ *O.meridionalis* (w-1625)	198	26	1.45	43.5	43.0	65	1	0.03	127	23	Straw	Easy
CN1/ *O.meridionalis* (w-1625)	129	27	1.50	46.2	46.0	65	0	0	122	20	Straw	Easy
RD23/ *O.meridionalis* (w-1629)	154	24	1.40	43.8	43.0	65	0.5	0.01	130	23	Straw	Easy
CN1/ *O.meridionalis* (w-1629)	149	26	1.45	46.4	46.0	65	0	0	120	20	Straw	Easy
RD23/ *O.nivara* (16150)	230	23	1.40	42.0	41.5	70	83	60	108	19	Straw	Easy
CN1/*O. nivara* (16150)	226	24	1.45	43.4	43.0	70	80	56	103	17	Straw	Easy
RD23	5	19	1.70	32.0	31.4	85	99	95	125	20	Awnless	Difficult
CN1	5	22	1.80	38.0	37.2	93	97	91	115	18	Awnless	Difficult
O. meridionalis (w-1625)	5	32	1.20	54.8	54.4	95	86	80	129	26	Straw	Easy
O. meridionalis (w-1629)	5	30	1.10	55.0	54.4	100	83	77	135	27.2	Straw	Easy
O. nivara (16150)	5	26	1.10	33.0	30.0	85	88	85	91	19	Red	Easy

[1/] The leaf below flag leaf

[2/] From date of culture to 50% flowering

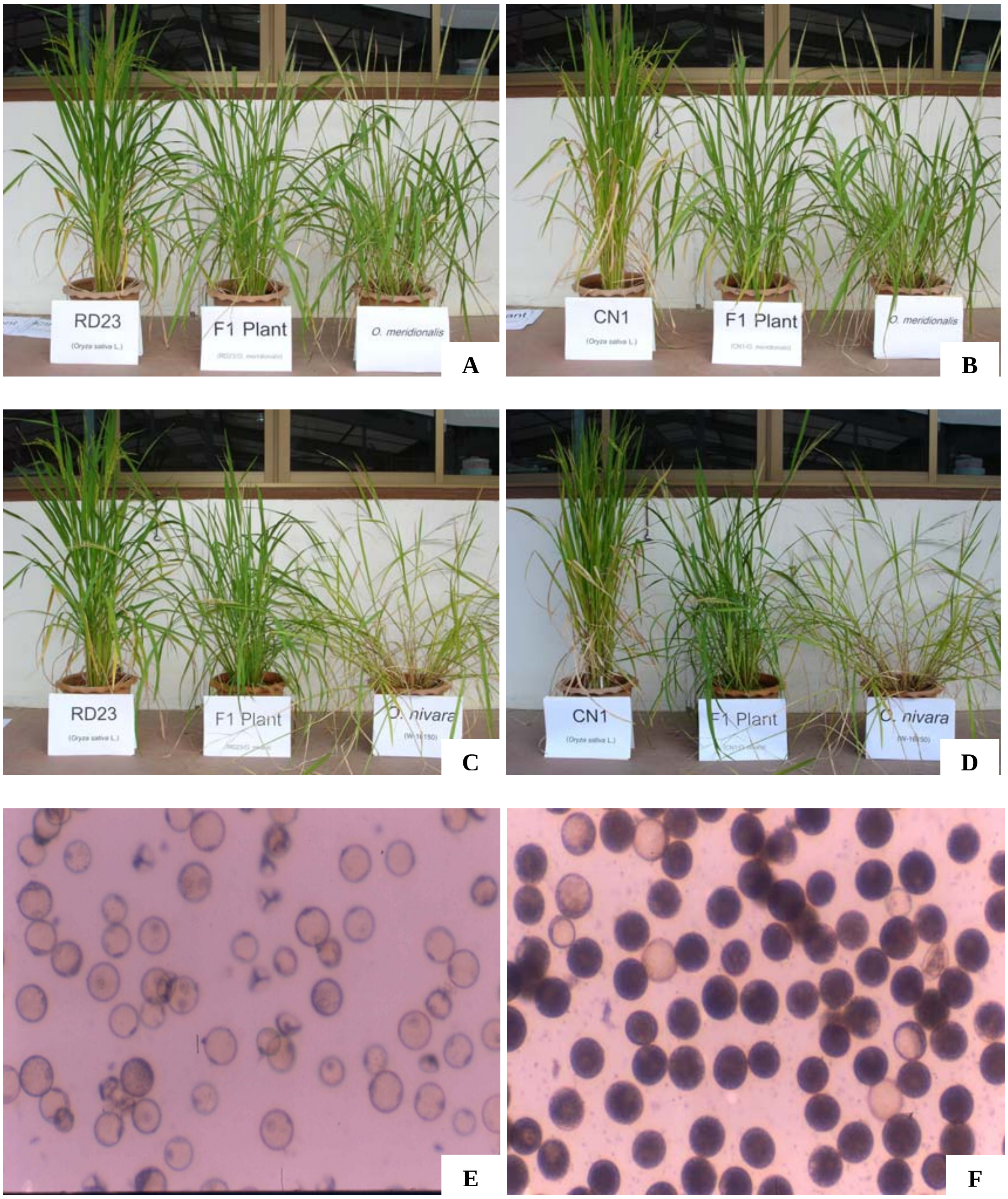

Figure 2 A. The F$_1$ hybrid plant (middle) between cultivated rice variety RD23 (left) and *O. meridionalis* (w - 1625) (right).
B. The F$_1$ hybrid plant (middle) between cultivated rice variety CN1 (left) and *O. meridionalis* (w - 1625) (right).
C. The F$_1$ hybrid plant (middle) between cultivated rice variety RD23 (left) and *O. nivara* (16150) (right).
D. The F$_1$ hybrid plant (middle) between cultivated rice variety CN1 (left) and *O. nivara* (16150) (right).
E. Completely sterile pollens of the F$_1$ hybrid (CN1/*O. meridionalis*, w-1625).
F. Partially sterile pollens of the F$_1$ hybrid (CN1/*O. nivara*, 16150).

Production of the backcross progenies

The F_1 hybrids between cultivated rice and wild species were backcrossed to the cultivated varieties to restore pollen fertility and good agronomic performance of the cultivated varieties. In all backcrosses, 5,730 spikelets of the F_1 hybrids were pollinated with the cultivated varieties and 1,023 seeds were produced (Table 8). Seed set extended from 10.5 - 35.0% with an average of 17.9% for all backcrosses. The F_1 hybrids between two cultivated varieties and *O. nivara* when backcrossed to cultivated varieties showed seed setting from 31.0 - 35.0% which was higher than the hybrids between two cultivars and *O. meridionalis* when introgressed to cultivars that presented the crossability from 10.5 – 17.2%. Especially, the F_1 hybrids between rice variety RD23 and two wild species (*O. meridionalis* acc. no. w-1625 and w-1629 and *O. nivara* acc. no. 16150) when backcrossed to RD23 exhibited the seed set rates of 17.2, 14.0 and 35.0% which were higher than the hybrids of CN1 and two wild species when introgressed to CN1 which manifested the crossability of 10.5, 11.0 and 31.0%, respectively.

The embryos derived from backcrossing were rescued by culturing on the medium (Table 9). The germiability of the embryos ranged from 30.0 to 45.0% with an average of 38.4% in all backcrosses. Both seed setting and germination percentage of the embryos were found higher in the F_1 interspecific hybridization than backcrossing. Jena and Khush (1986) also reported that crossability (12%) between three breeding rice lines and wild species *O. officinalis* was higher than that (1.3%) of the backcrossing of the F_1 interspecific hybrid to breeding lines, but the germination percentage (63.4%) of the F_1 interspecific hybrid embryos was lower than that (68%) of the backcrossed embryos.

The embryos derived from F_1 hybrids between two cultivated varieties and *O. nivara* backcrossing to cultivated varieties germinated at the frequency ranging from 43.0 - 45.0% which was higher than the embryos of hybrids between two cultivars and *O. meridionalis* introgressing into cultivars which showed germination rate of 30.0 – 36.7%. Prominently, the embryos of the hybrids between rice cultivar RD23 and *O. meridionalis* backcrossing to RD23 could germinate at the higher percentage (35.1 – 36.7%) than the embryos of the hybrids between CN1 and *O. meridionalis* introgressing to CN1 which germinated at the rate of 30.0 – 31.0%. However, germination percentage (43%) of the embryos derived from hybrid between RD23 and *O. nivara* backcrossing to RD23 was slightly lower than that (45.0%) of the embryos obtained from the hybrid between CN1 and *O. nivara* introgressing to CN1.

It was manifested that seed set in backcrossing and germination ability of the embryos derived from backcrosses still depended on the wild species and rice cultivars used in F_1 hybrid crosses before backcrossing which was relevant to the crossability and germinability of the embryos produced in the F_1 interspecific crosses. This observation is in agreement with the work of Jena and Khush (1986) when the F_1 interspecific hybrids between three breeding lines and *O. officinalis* were backcrossed to breeding lines.

The total 947 embryos derived from all backcrosses were cultured on the medium of which 364 embryos could germinate and survive to be 299 BC_1F_1 plants. Only 235 BC_1F_1 plant could produce BC_1F_2 seeds (Table 9) which were utilized for drought resistance screening.

Table 8 Crossability of the 1st backcrossing between F_1 hybrids and cultivated rice.

Crosses	No. of spikelets pollinated	Set seeds	
		No.	%
RD23/*O.meridionalis* (w-1625)//RD23	767	132	17.2
CN1/*O.meridionalis* (w-1625)//CN1	1,352	142	10.5
RD23/*O.meridionalis* (w-1629)//RD23	893	125	14.0
CN1/*O.meridionalis* (w-1629)//CN1	1,236	136	11.0
RD23/*O.nivara* (16150)//RD23	714	250	35.0
CN1/*O.nivara* (16150)//CN1	768	238	31.0
Total	5,730	1,023	17.9

Table 9 The number of cultured embryos, germinated embryos and viable BC_1F_1 plants obtained from six backcrosses.

Backcrosses	No. of embryos cultured	Germinated embryos		Viable BC_1F_1 plants*	BC_1F_1 plants producing seeds
		No.	%		
RD23/*O.meridionalis* (w-1625)//RD23	120	44	36.7	32	21
CN1/*O.meridionalis* (w-1625)//CN1	129	40	31.0	29	13
RD23/*O.meridionalis* (w-1629)//RD23	111	39	35.1	30	19
CN1/*O.meridionalis* (w-1629)//CN1	123	37	30.0	31	12
RD23/*O.nivara* (16150)//RD23	235	101	43.0	87	85
CN1/*O.nivara* (16150)//CN1	229	103	45.0	90	85
Total	947	364	38.4	299	235

* At three-leaf stage.

Variation in morpho-agronomic characters of the BC_1F_1 plants.

The 292 BC_1F_1 plants of six backcrosses were investigated for some morphological and agronomic characters. Variations in morphological and agronomic characters were found among the BC_1F_1 plants of each backcross (Table 10 and Figure 3). Totally, the BC_1F_1 plants of all six backcrosses showed variations in ligule length from 20.0 – 25.0 mm, leaf width from 1.2 – 1.7 cm, leaf length from 38.5 – 46.5 cm, flag leaf length from 37.5 – 45.5 cm, number of days to 50% flowering from 68 – 100 days, plant height from 92 – 135 cm, number of panicles per plant from 4 – 11 panicles, number of seeds per panicle from 0 – 95 seeds, awn formation from absent – present, awn length from short – long and panicle threshability from easy to difficult. The variations in morpho-agronomic characters of the BC_1F_1 plants might be due to genotypic segregation which was caused by the backcrossing of heterozygous F_1 interspecific hybrids to the homozygous cultivated rice. It was noticed that some BC_1F_1 could restore the good morpho-agronomic characters of the cultivated rice such as high number of seeds per panicle, awnless seeds and difficulty in panicle threshability. Particularly, the BC_1F_1 plants of the backcrosses RD23/*O. nivara* (16150)//RD23 and CN1/*O. nivara* (16150)//CN1 could set seeds in all plants. The high seed setting of the BC_1F_1 plants of these two backcrosses was inherited from their F_1 interspecific hybrid before backcrossing to cultivated rice (see Table 7).

Table 10 Variation in morpho - agronomic characteristics of the BC$_1$F$_1$ plants of six backcrosses.

Backcrosses	No. of plants observed	Ligule length (mm)	Leaf[1/] width (cm)	Leaf[1/] length (cm)	Flag leaf length (cm)	Days to 50% flowering[2/]	Plant height (cm)	No. of panicles per plant	No. of seeds/ panicle	Awning	Panicle thresh-ability
RD23/*O. meridionalis*	32	22.5-23.5	1.2-1.6	39.5-45.0	39.0-44.5	68-90	100-130	4-9	0-91	Absent-present	Easy - difficult
(w-1625)//RD23		(23)	(1.50)	(42.2)	(41.9)	(76)	(122)	(6.5)	(48.7)	short-long	
C.V. (%)		0.13	0.02	2.44	2.05	34.66	66.53	2.00	911.76		
CN1/*O. meridionalis*	29	23.5-25.0	1.3-1.7	40.0-46.5	39.5-45.5	69-95	98-130	4-10	0-87	Absent-present	Easy - difficult
(w-1625)//CN1		(24)	(1.52)	(43.1)	(42.5)	(78)	(120)	(6.2)	(47.5)	short-long	
C.V. (%)		0.70	0.01	3.51	2.91	56.65	91.07	3.54	396.94		
RD23/*O. meridionalis*	30	21.5-23.5	1.3-1.6	38.5-43.5	38.0-42.5	70-100	99-135	4-10	0-95	Absent-present	Easy - difficult
(w-1629)//RD23		(22)	(1.41)	(40.5)	(39.2)	(79)	(125)	(6.9)	(56.5)	short-long	
C.V. (%)		0.25	0.01	0.24	1.21	71.17	114.07	2.62	1007.1		
CN1/*O. meridionalis*	31	21.0-24.5	1. 4-1.7	39.5-45.5	39.0-44.0	70-100	98-131	4-11	0-90	Absent-present	Easy - difficult
(w-1629)//CN1		(23)	(1.46)	(43.4)	(42.6)	(82)	(118)	(6.7)	(44.2)	short-long	
C.V. (%)		0.80	0.01	3.09	2.27	99.58	111.77	3.24	679.14		
RD23/*O. nivara*	85	20.0-22.5	1.3-1.6	38.5-42.5	37.5-41.0	75-95	95-124	4-9	35-84	Absent-present	Easy - difficult
(16150)//RD23		(21)	(1.44)	(41.0)	(39.0)	(84)	(103)	(6.0)	(49.8)	short-long	
C.V. (%)		0.60	0.01	1.27	1.14	24.34	72.28	1.16	140.02		
CN1/*O. nivara*	85	22.0-24.0	1.3-1.7	38.5-43.0	38.0-42.0	75-98	92-124	4-8	30-83	Absent-present	Easy - difficult
(16150)//CN1		(23)	(1.46)	(40.6)	(39.1)	(85)	(102)	(5.7)	(43.1)	short-long	
C.V. (%)		0.56	0.01	1.50	1.02	28.67	62.22	0.80	136.89		
Total	292	20.0-25.0	1.2-1.7	38.5-46.5	37.5-45.5	68-100	92-135	4-11	0-95	Absent-present	Easy - difficult
		(22)	(1.46)	(41.4)	(40.1)	(82)	(110)	(6.2)	(47.6)	short-long	
C.V. (%)		1.53	0.01	3.06	3.80	52.89	168.05	1.91	412.43		

[1/] Leaf below flag leaf. [2/] From date of culture to 50% flowering. In parentheses are mean values.

Figure 3 A. The BC$_1$F$_1$ plants of RD23/*O. meridionalis*, w-1625//RD23 showing variations in morpho-agronomic characters.
B. The BC$_1$F$_1$ plants of CN1/*O. meridionalis*, w-1625//CN1 showing variations in morpho-agronomic characters.

Screening BC_1F_2 progenies for drought resistance

Visual drought scoring by an experienced researcher is apparently quite effective in discriminating drought avoidance in rice (O'Toole and Moya, 1978). Visual scoring has been used widely as the basis for drought resistance screening at vegetative growth stage (Loresto *et al.*, 1976; O'Toole and Cruz, 1980; O'Toole and Maguling, 1981; O'Toole and Moya, 1981; Theodore *et al.*, 1984; Malabuyoc *et al.*, 1985; Turner *et al.*, 1986; Singh and Mackill, 1991; Mitchell *et al.*, 1998; Singh *et al.*, 2004).

The BC_1F_2 progenies were also screened for drought resistance (Figure 4A) at the vegetative growth stage by visual scoring. At the 21st day after subjecting to drought stress, the susceptible check variety Taichung Native 1 (TN1) appeared its rolled leaves first followed by RD23, CN1, RD19, some BC_1F_2 plants of the six backcrosses and Salumpikit, respectively. Visual scores of leaf rolling (Figure 4B) were taken when the resistant check variety RD19 showed tightly rolled leaves at the 35th day of drought stress. Leaf drying and plant recovery were scored after rewatering for 10 days. Frequent distributions for leaf rolling, leaf drying and plant recovery scores of 452 BC_1F_2 progenies from six backcrosses, three susceptible and two resistant check varieties were shown in Table 11. High frequencies of plants having high scores of leaf rolling (7.5 – 8.5), low scores of leaf drying (0.4 – 0.8) and intermediate scores (1.2 – 3.6) of plant recovery were observed in the BC_1F_2 progenies of all backcrosses. In comparison, high frequencies of plants with high scores of leaf rolling (8.5 – 9.0) were found in three susceptible varieties RD23, CN1 and TN1 whereas high number of plants possessing low scores of leaf drying (0 - 0.7) and recovery (1.1 – 1.9) were manifested in two resistant varieties RD19 and Salumpikit.

The 162 BC_1F_2 progenies presenting as low scores of leaf drying and recovery as the resistant check variety RD19 were identified. These progenies were evaluated to be drought resistance because they showed less leaf drying when suffering from water stress and high capability to recover after reirrigation. Leaf rolling followed by leaf drying seems to be a reliable index of turgor loss caused by drought stress in rice. Turgor loss is induced by either a reduction in leaf water potential (O'Toole and Cruz, 1980) or turgor potential (Turner *et al.*, 1986). However, leaf rolling and leaf death can be reduced by osmotic adjustment which is an effective component of drought resistance (Hsiao *et al.*, 1984). Solutes such as sugars, organic acid, amino acid and sugar alcohols accumulated for cellular osmotic adjustment during water stress may be partially used for regrowth upon recovery after rewatering (McCree *et al.*, 1984). The drought resistant trait of the 162 BC_1F_2 progenies might be inherited from the donor wild species *O. nivara* and *O. meridionalis* which were claimed to be drought avoidance (Eizenga and Rutger, 2003).

Figure 4 A. The BC_1F_2 progenies screened for drought resistance.
B. The BC_1F_2 plants showed leaf rolling at the 35th day of water stress.

Table 11 Frequency distribution for leaf rolling, leaf drying and recovery scores of BC$_1$F$_2$ progenies of six backcrosses, three drought susceptible varieties and two drought resistant varieties.

BC$_1$F$_2$/ Variety	No. of plants observed	Characters	Score frequency distribution						Average
			0	1	3	5	7	9	
RD23/ *O. meridionalis* (w-1625)//RD23	114	Leaf rolling[1/]	-	-	-	27	31	56	7.5
		Leaf drying[2/]	62	52	-	-	-	-	0.5
		Recovery[3/]	-	41*	26	24	15	8	3.6
CN1/ *O. meridionalis* (w-1625)//CN1	111	Leaf rolling	-	3	5	12	29	62	7.6
		Leaf drying	81	26	2	2	-	-	0.4
		Recovery	-	48*	38	18	4	3	1.2
RD23/ *O. meridionalis* (w-1629)//RD23	93	Leaf rolling	-	-	-	3	26	64	8.3
		Leaf drying	50	43	-	-	-	-	0.5
		Recovery	-	21*	38	20	9	5	3.2
CN1/ *O. meridionalis* (w-1629)//CN1	57	Leaf rolling	-	-	2	7	14	34	7.8
		Leaf drying	34	18	3	2	-	-	0.6
		Recovery	-	24*	17	9	5	2	3.0
RD23/*O. nivara* (16150)// RD23	46	Leaf rolling	-	-	-	5	12	29	8.0
		Leaf drying	28	15	3	-	-	-	0.5
		Recovery	-	17*	10	11	7	1	3.5
CN1/*O. nivara* (16150)// CN1	31	Leaf rolling	-	-	-	1	5	25	8.5
		Leaf drying	17	8	6	-	-	-	0.8
		Recovery	-	11*	9	8	3	-	3.2
RD23 (susceptible variety)	8	Leaf rolling	-	-	-	-	-	8	9.0
		Leaf drying	-	3	4	1	-	-	2.5
		Recovery	-	-	5	2	1	-	4.0
CN1 (susceptible variety)	14	Leaf rolling	-	-	-	-	2	12	8.7
		Leaf drying	4	8	2	-	-	-	1.0
		Recovery	-	4	9	1	-	-	2.6
TN1 (susceptible variety)	32	Leaf rolling	-	-	-	-	8	24	8.5
		Leaf drying	-	15	11	6	-	-	2.4
		Recovery	-	-	10	8	9	5	5.6
RD19 (resistant variety)	38	Leaf rolling	-	-	-	2	7	29	8.4
		Leaf drying	21	13	4	-	-	-	0.7
		Recovery	-	22	15	1	-	-	1.9
Salumpikit (resistant variety)	39	Leaf rolling	-	3	5	17	12	2	5.3
		Leaf drying	38	1	-	-	-	-	0.0
		Recovery	-	37	2	-	-	-	1.1

[1/] *Leaf rolling:* 0=leaves healthy, 1=leaves start to fold (shallow V-shape), 3=leaves folding (deep V-shape), 5=leaves fully cupped (U-shape), 7=leaf margins touching (O-shape) and 9=leaves tightly rolled.

[2/] *Leaf drying:* 0=no symptoms, 1=slightly tip drying, 3=tip drying extended up to ¼ length in most leaves, 5=one-fourth to ½ of all leaves fully dried, 7=more than 2/3 of all leaves fully dried and 9=all plants apparently dead.

[3/] *Recovery:* 1=90-100% plants recovered, 3=70-89% plants recovered, 5=40-69% plants recovered, 7=20-39% plants recovered and 9=0-19% plants recovered.

* Plants evaluated to be highly resistant to drought.

Variation in agronomic characteristics of the BC_1F_2 progenies

The 452 BC_1F_1 progenies of six backcrosses evaluated for drought resistance were allowed to grow in the cement blocks with normal irrigation until maturity. Some agronomic characters of the BC_1F_2 progenies were determined.

Variations in agronomic characters of the BC_1F_2 progenies were observed among the BC_1F_2 progenies of each backcross compared to the check varieties RD23 and CN1 (Table 12 and Figure 5 – 10). Overall, the BC_1F_2 progenies of all six backcrosses exhibited variations in the number of days to 50% flowering from 78 – 115 days, plant height from 71 – 135 cm, number of panicles per plant from 2 – 17 panicles, number of seeds per panicle from 5 – 140 seeds, 100 seed weight from 2.16 – 2.41 g, seed yield per plant from 4.6 – 27.0 g, awn formation from absent – present, awn length from short – long and panicle threshability from easy – difficult. Variations in the number of days to 50% flowering, plant height, the number of panicles per plant and number of seeds per panicle were wider in the BC_1F_2 progenies than the BC_1F_1 plants (Table 10). The variation in agronomic characters of the BC_1F_2 progenies might be contributed from the genetic segregation of the heterogeneous BC_1F_1 plants after selfing. It was clearly shown that the BC_1F_2 progenies could retain good agronomic characters of the recurrent parents especially the seed fertility. Although, they were subjected to drought stress at the vegetative growth stage, they could set seeds with a wide variation in the number. Particularly, some BC_1F_2 progenies produced higher number of seeds per panicle than the check varieties RD23 and CN1 although the average number of seeds per panicle of all backcross progenies (46.7 seeds/panicle) was lower than those of the check varieties RD23 (76.0 seeds/panicle) and CN1 (89.0 seeds/panicle). The variation in number of panicles per plant was wider in BC_1F_2 progenies (2- 17 panicles/plant) than the check varieties RD23 and CN1 (4 – 6 panicles/plant) resulting in the higher average number of panicles per plant of BC_1F_2 progenies (6.4 panicles/plant). The average seed yield per plant of the BC_1F_2 progenies (producing awnless seeds) of all six backcrosses (10.9, 11.1, 14.5, 16.4, 15.6 and 20.2 g/plant) were higher than those of the check varieties RD23 (8.9 g/plant) and CN1 (10.6 g/plant). The high average seed yield per plant of the BC_1F_2 progenies might be caused the high number of panicles per plant and high number of seeds per panicle which were contributed from some number of BC_1F_2 progenies (Figure 5 – 10 c, d). Fukai and Cooper (1995) also reported that grain yield of rice lines grown under different environments was more closely related to the grain number than to the grain weight because grain weight is relatively stable across environments.

Selection for drought resistance

Many of the BC_1F_2 progenies could restore favorable agronomic performance from cultivated rice after backcrossing which included medium plant height, high seed fertility, high seed weight and yield (per plant) and awnless seeds. Selection was made for 39 BC_1F_2 plants having high seed yield per plant (>10.6 g) and good agronomic performance as their cultivated parents and resistant to drought (recovery score 1) of which three were derived from RD23/*O. meridionalis* (w-1625)//RD23, ten from CN1/*O. meridionalis* (w-1625)//CN1, five from cross RD23/*O. meridionalis* (w-1629)//RD23, eight from cross CN1/*O. meridionalis* (w-1629)//CN1, six from cross RD23/*O. nivara* (no. 16150)//RD23 and seven from cross CN1/*O. nivara* (no. 16150)//CN1 (Appendix Table 1 – 6). They will be grown to be BC_1F_3 lines for further evaluation on drought resistance, yield and agronomic performance.

Table 12 Variation in agronomic characteristics of the BC$_1$F$_2$ progenies of six backcrosses.

Backcrosses	No. of plants observed	Days to 50% flowering	Plant height (cm)	No. of panicles/ plant	No. of seeds/ panicle	100 seed weight[*] (g)	Seed yield/ plant[*] (g)	Awning	Panicle thresh-ability
RD23/*O. meridionalis*	114	81–110	80–125	3–15	5–140	2.20–2.41	4.6–27.0	Absent-present short-long	Easy – difficult
(w-1625)//RD23		(94)	(107.1)	(6.2)	(45.9)	(2.29)	(10.9)		
C.V. (%)		30.37	147.29	6.40	1063.27	0.002	32.15		
CN1/*O. meridionalis*	111	80–112	75–125	2–13	7–138	2.21–2.36	4.6–23.8	Absent-present short-long	Easy – difficult
(w-1625)//CN1		(97)	(106.2)	(6.0)	(47.1)	(2.27)	(11.1)		
C.V. (%)		42.85	1128.61	6.20	755.47	0.002	34.74		
RD23/*O. meridionalis*	93	79–110	79–131	4–15	6–109	2.24–2.36	4.6–27.4	Absent-present short-long	Easy – difficult
(w-1629)//RD23		(94)	(108.2)	(7.1)	(43.1)	(2.29)	(14.5)		
C.V. (%)		48.94	193.06	6.28	692.56	0.002	73.23		
CN1/*O. meridionalis*	57	87–115	75–135	4–17	6–120	2.20–2.32	5.3–22.1	Absent-present short-long	Easy – difficult
(w-1629)//CN1		(99)	(107.2)	(6.9)	(55.8)	(2.26)	(16.4)		
C.V. (%)		64.26	182.45	12.33	1024.1	0.001	31.98		
RD23/*O. nivara*	46	78–100	71–120	3–13	10–97	2.16–2.34	10.6–21.0	Absent-present short-long	Easy – difficult
(16150)//RD23		(87)	(99.7)	(6.3)	(41.1)	(2.24)	(15.6)		
C.V. (%)		34.52	181.80	6.97	546.00	0.004	10.29		
CN1/*O. nivara*	31	80–105	75–115	2–12	9–110	2.20–2.24	15.1–25.8	Absent-present short-long	Easy – difficult
(16150)//CN1		(91)	(96.8)	(6.1)	(50.3)	(2.22)	(20.2)		
C.V. (%)		34.10	92.03	7.92	1221.25	0.0002	12.01		
Total	452	78-115	71-135	2-17	5-140	2.16-2.41	4.6-27	Absent-present short-long	Easy – difficult
		(94)	(105.7)	(6.4)	(46.7)	(2.27)	(13)		
C.V. (%)		52.90	167.54	7.41	882.05	0.002	43.81		
RD23	8	90–93	109–112	4–6	65–82	2.31–2.38	7.8–9.5	Absent	Difficult
		(92)	(110.0)	(5.0)	(76.0)	(2.34)	(8.9)		
C.V. (%)		1.50	1.00	0.75	27.5	0.0004	0.32		
CN1	14	96–100	103–106	4–6	69–94	2.34–3.46	8.9–11.4	Absent	Difficult
		(98)	(105.0)	(5.0)	(89.0)	(2.39)	(10.6)		
C.V. (%)		2.78	0.86	0.57	49.43	0.001	0.55		

[*] From the plant producing awnless seeds. In parentheses are mean values.

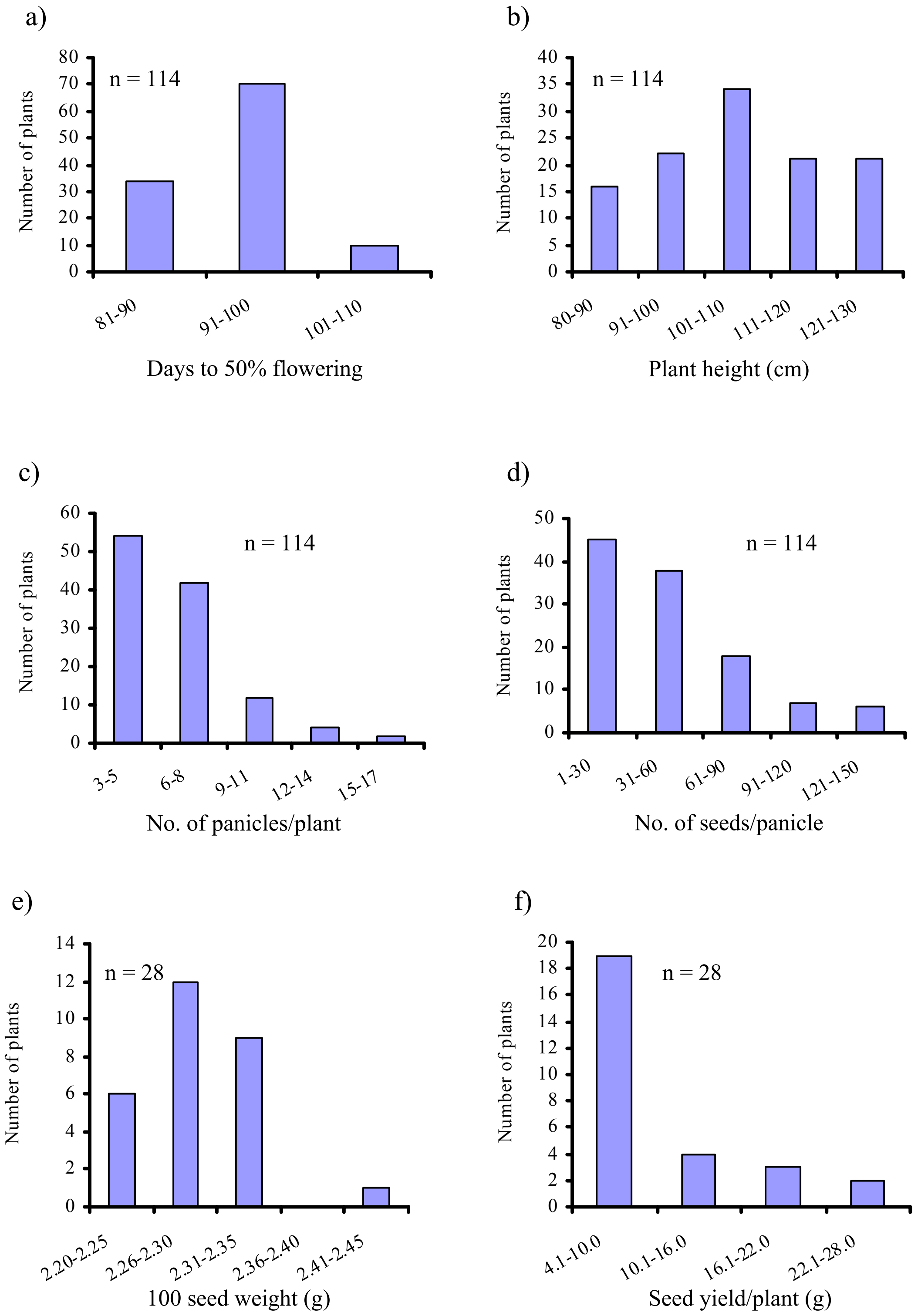

Figure 5 Distribution of the BC_1F_2 progenies of backcross RD23/*O. meridionalis* (w-1625)//RD23 for six agronomic characters: a) Days to 50% flowering, b) Plant height, c) Number of panicles per plant, d) Number of seeds per panicle, e) 100 seed weight, f) Seed yield per plant.

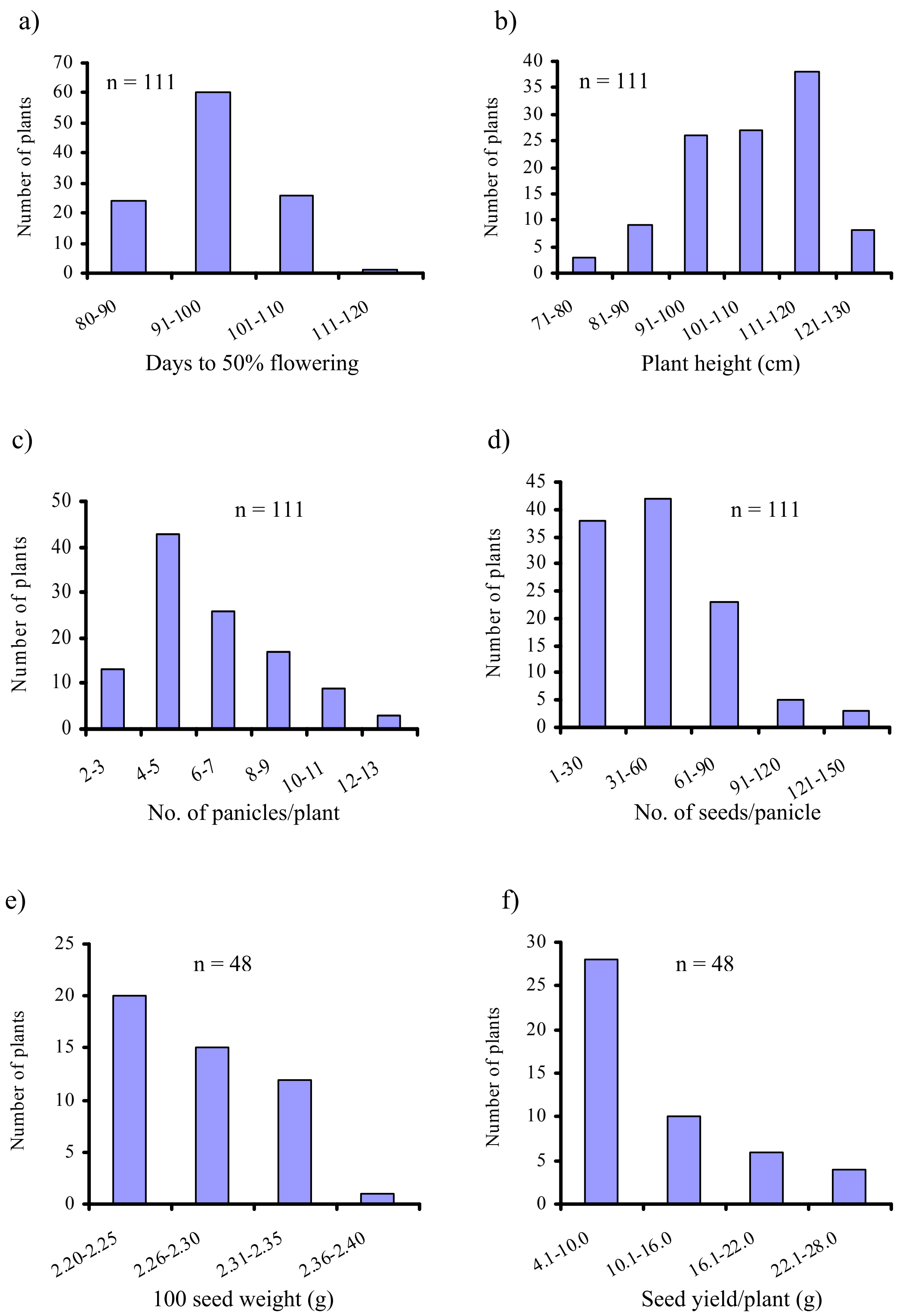

Figure 6 Distribution of the BC_1F_2 progenies of backcross CN1/*O. meridionalis* (w-1625)//CN1 for six agronomic characters: a) Days to 50% flowering, b) Plant height, c) Number of panicles per plant, d) Number of seeds per panicle, e) 100 seed weight, f) Seed yield per plant.

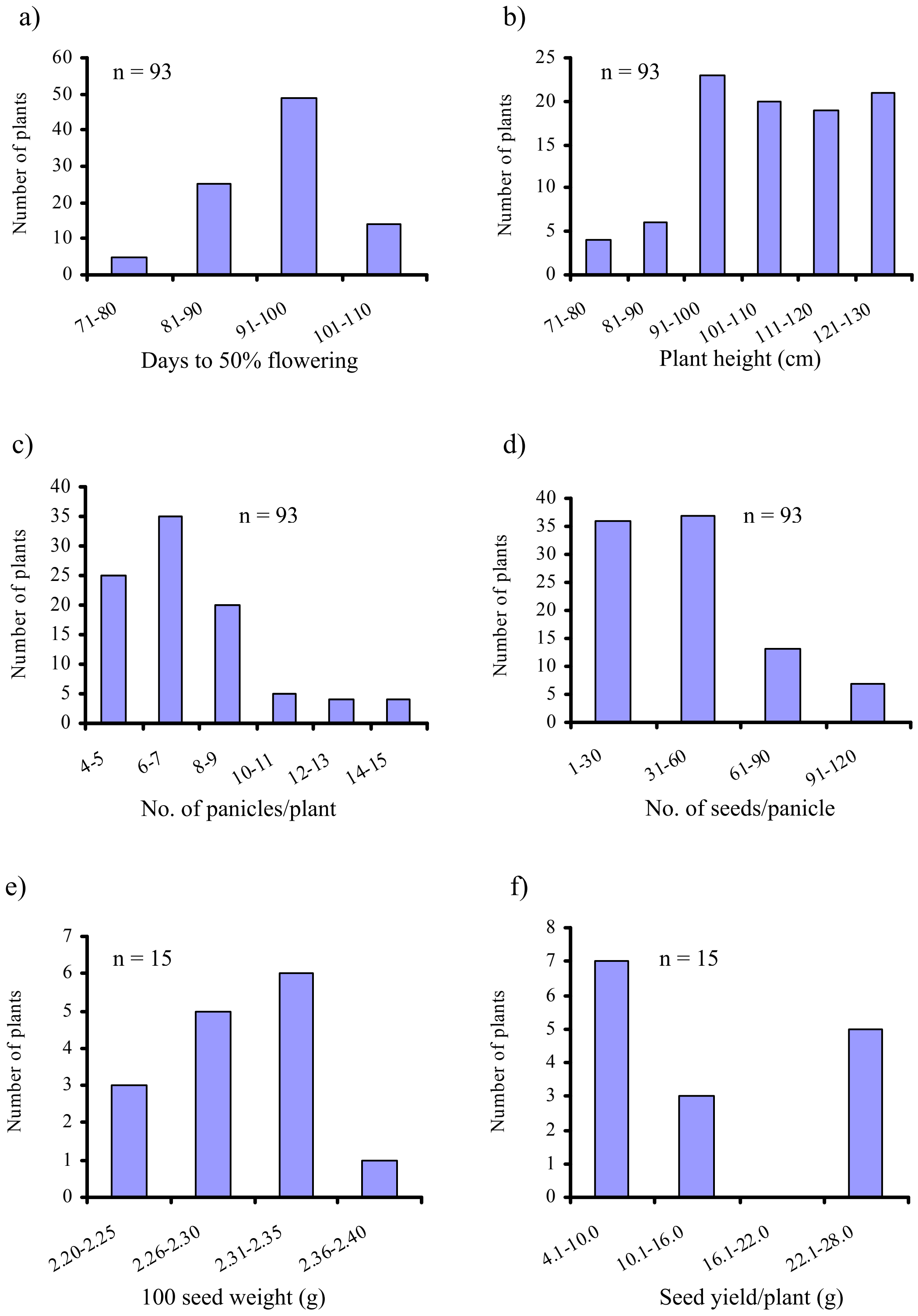

Figure 7 Distribution of the BC_1F_2 progenies of backcross RD23/*O. meridionalis* (w-1629)//RD23 for six agronomic characters: a) Days to 50% flowering, b) Plant height, c) Number of panicles per plant, d) Number of seeds per panicle, e) 100 seed weight, f) Seed yield per plant.

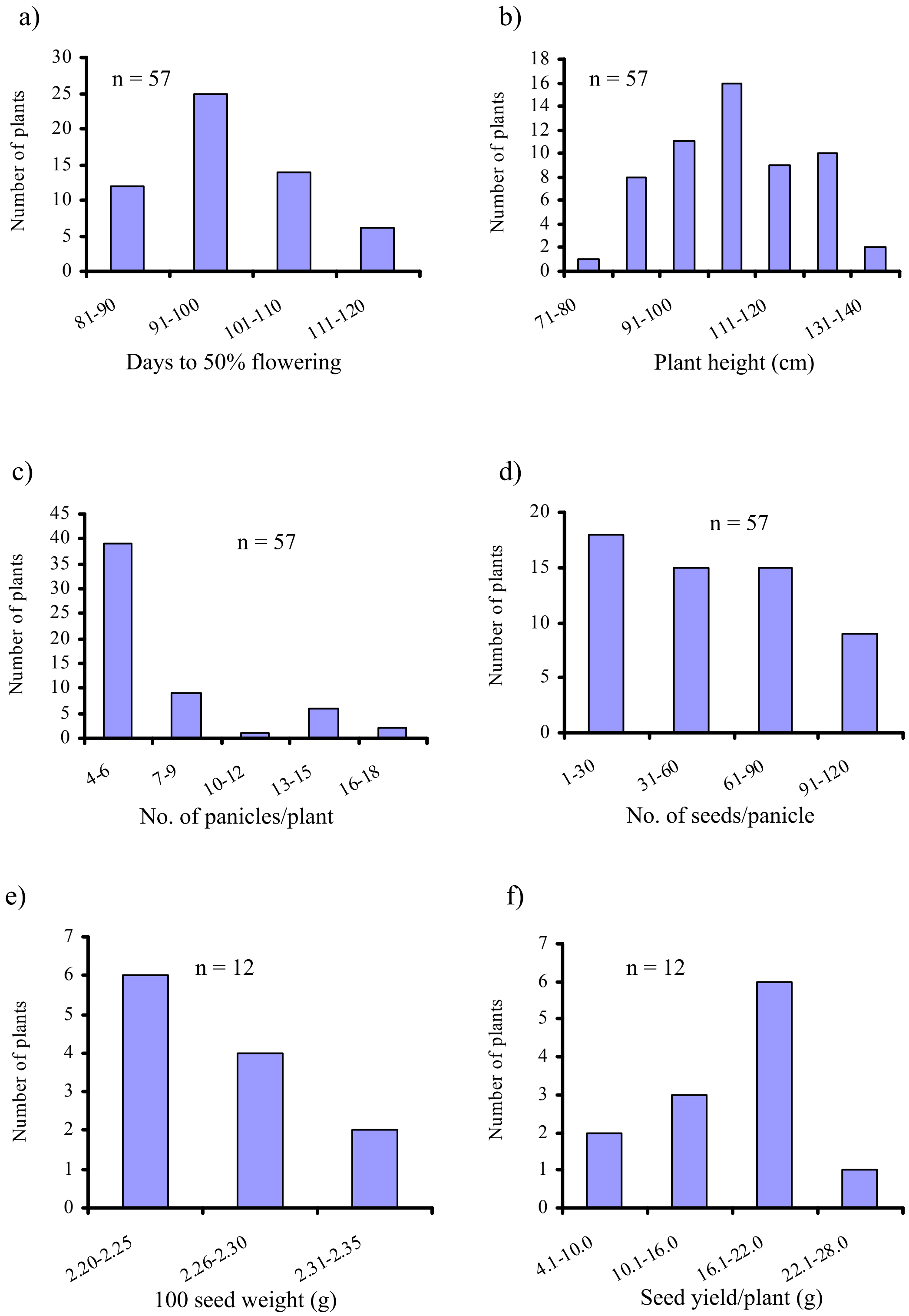

Figure 8 Distribution of the BC_1F_2 progenies of backcross CN1/*O. meridionalis* (w-1629)//CN1 for six agronomic characters: a) Days to 50% flowering, b) Plant height, c) Number of panicles per plant, d) Number of seeds per panicle, e) 100 seed weight, f) Seed yield per plant.

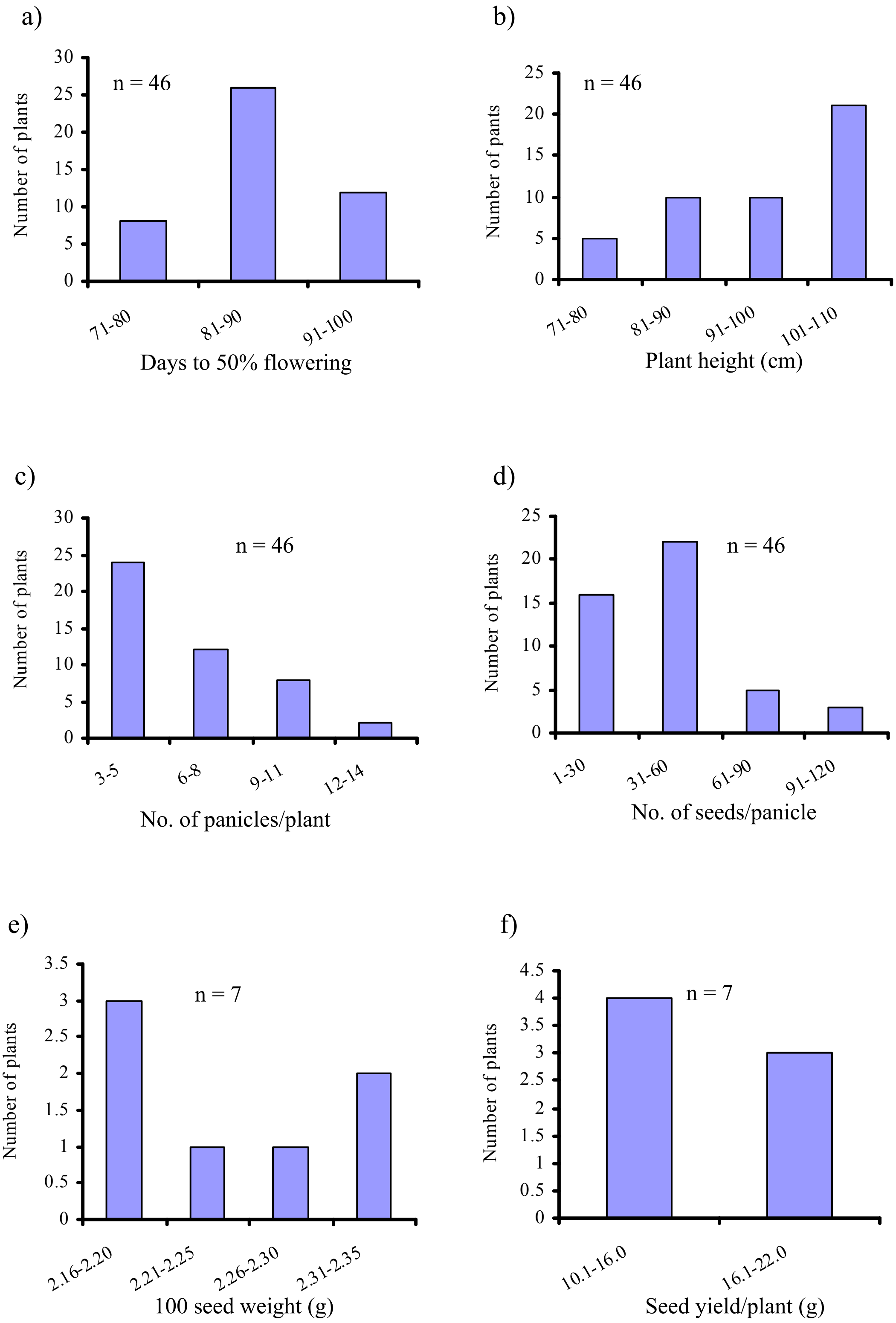

Figure 9 Distribution of the BC_1F_2 progenies of backcross RD23/*O. nivara* (16150)//RD23 for six agronomic characters: a) Days to 50% flowering, b) Plant height, c) Number of panicles per plant, d) Number of seeds per panicle, e) 100 seed weight, f) Seed yield per plant.

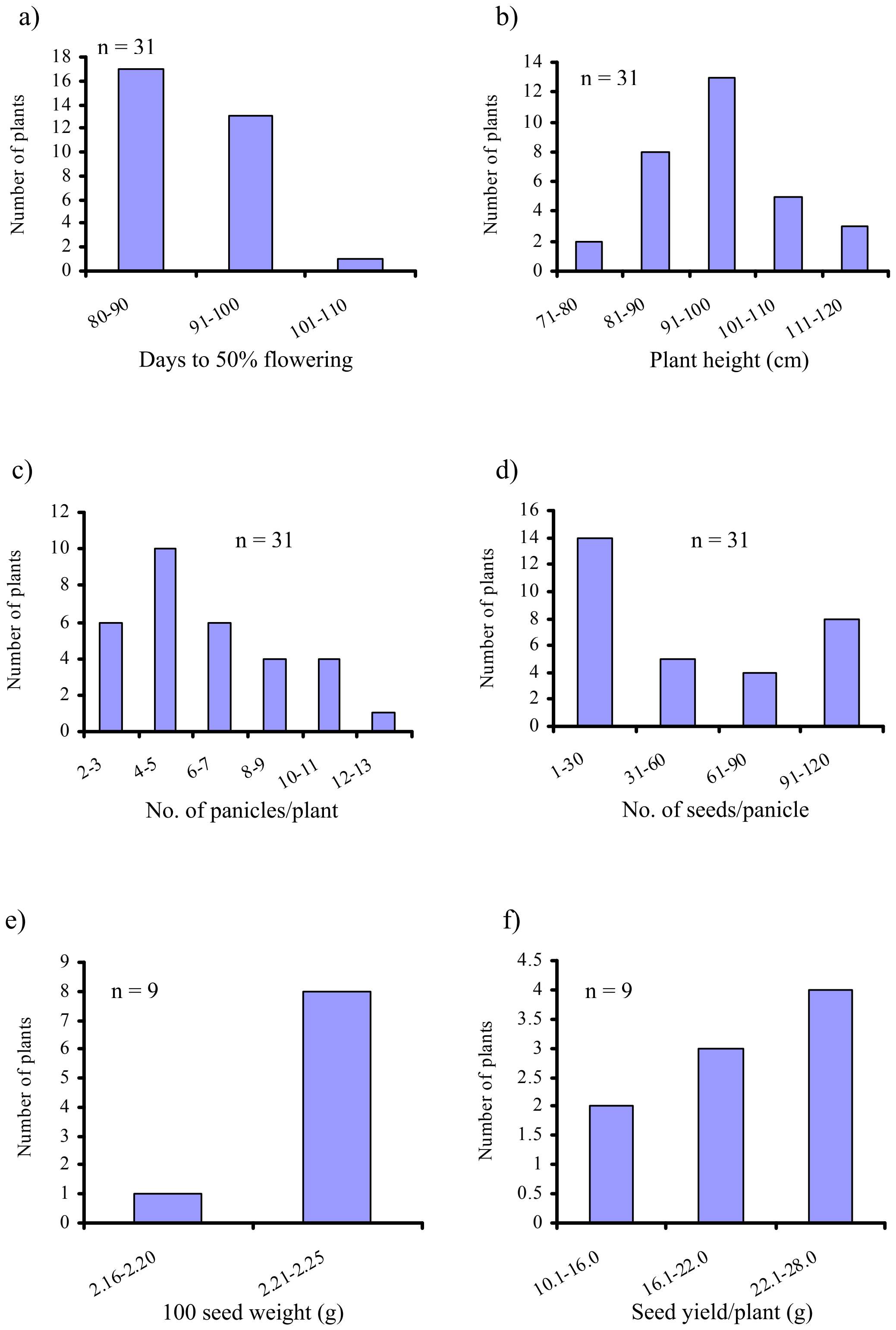

Figure 10 Distribution of the BC_1F_2 progenies of backcross CN1/*O. nivara* (16150)//CN1 for six agronomic characters: a) Days to 50% flowering, b) Plant height, c) Number of panicles per plant, d) Number of seeds per panicle, e) 100 seed weight, f) Seed yield per plant.

CONCLUSION

1. Interspecific hybridization between cultivated rice (*O. sativa*) and wild species (*O. meridionalis* and *O. nivara*) was achieved with crossability of 35.3% by average.

2. The F_1 interspecific hybrid embryos obtained could be rescued by culturing on the half-strength MS medium with germinability of 51.9% by average.

3. Rescue of hybrid embryos derived from the crosses between cultivated rice and *O. meridionalis* should be done at the age of 11 – 12 days after pollination and 14 days after pollination for embryos obtained from the crosses between cultivated rice and *O. nivara*.

4. Drought resistant character could be transferred from *O. meridionalis* and *O. nivara* to cultivated rice by backcrossing in combination with embryo culture.

5. Thirty nine BC_1F_2 progenies from six backcrosses having drought resistance and high seed yield per plant were selected for further study.

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APPENDICES

Appendix Table 1 Agronomic characteristics of the BC$_1$F$_2$ progenies of the backcross RD23/*O. meridionalis* (w-1625)//RD23.

Plant no.[1/]	Pedigree	Days to 50% flowering	Plant height (cm)	No. of panicles/ plant	No. of fertile seeds/ panicle	100 seed weight (g)	Seed yield/ plant (g)	Awning	Panicle thresh ability
1	4	95	88	5	67	2.26	7.6	Absent	Difficult
2	5	90	100	4	56	2.34	5.2	Absent	Difficult
3	13	92	112	7	75	2.28	12.0	Absent	Easy
4	24	98	115	11	35	2.41	9.3	Absent	Difficult
5	26	98	115	6	99	2.28	13.5	Absent	Difficult
6	29*	90	90	10	112	2.25	25.2	Absent	Difficult
7	36	95	125	15	80	2.25	27.0	Absent	Easy
8	38	90	99	6	124	2.21	16.4	Absent	Easy
9	39	95	110	4	68	2.22	6.0	Absent	Difficult
10	43	98	115	11	79	2.30	20.0	Absent	Easy
11	49	90	95	8	54	2.20	9.5	Absent	Difficult
12	62	88	85	7	45	2.33	7.3	Absent	Difficult
13	63	95	106	7	54	2.29	8.7	Absent	Difficult
14	66	95	98	5	76	2.26	8.6	Absent	Easy
15	69	95	105	7	46	2.29	7.4	Absent	Easy
16	70	95	89	5	52	2.31	6.0	Absent	Easy
17	74	95	120	12	65	2.34	18.3	Absent	Easy
18	76	95	90	3	98	2.25	6.6	Absent	Easy
19	78	95	110	4	107	2.29	9.8	Absent	Difficult
20	82	98	120	3	135	2.32	9.4	Absent	Easy
21	87	98	104	7	46	2.28	7.3	Absent	Easy
22	88	95	90	3	67	2.28	4.6	Absent	Difficult
23	92*	98	115	4	128	2.32	11.9	Absent	Difficult
24	96	92	98	8	54	2.32	10.0	Absent	Easy
25	99	95	110	4	95	2.30	8.7	Absent	Easy
26	101*	93	95	5	130	2.31	15.0	Absent	Difficult
27	105	95	106	7	51	2.29	8.2	Absent	Difficult
28	108	98	108	6	43	2.31	6.0	Absent	Difficult

[1/] Plants producing awnless seeds.

* Selected plants

Appendix Table 2 Agronomic characteristics of the BC$_1$F$_2$ progenies of the backcross CN1/*O. meridionalis* (w-1625)//CN1.

Plant no.[1/]	Pedigree	Days to 50% flowering	Plant height (cm)	No. of panicles/ plant	No. of fertile seeds/ panicle	100 seed weight (g)	Seed yield/ plant (g)	Awning	Panicle thresh ability
1	6	98	105	6	55	2.23	7.4	Absent	Easy
2	7	80	92	7	36	2.24	5.6	Absent	Difficult
3	11	98	90	6	61	2.21	8.1	Absent	Difficult
4	15	88	98	4	65	2.24	5.8	Absent	Difficult
5	16	90	95	5	45	2.26	5.1	Absent	Difficult
6	19	92	105	3	67	2.31	4.6	Absent	Difficult
7	20	95	100	6	54	2.23	7.2	Absent	Difficult
8	25	93	102	5	46	2.25	5.2	Absent	Easy
9	26*	100	116	10	95	2.29	21.8	Absent	Difficult
10	27*	90	105	9	95	2.32	19.8	Absent	Difficult
11	30	98	115	8	33	2.25	5.9	Absent	Difficult
12	34	90	95	4	60	2.31	5.5	Absent	Easy
13	35	92	103	5	65	2.23	7.2	Absent	Difficult
14	38	95	115	8	64	2.21	11.3	Absent	Easy
15	40	102	119	10	56	2.32	13.0	Absent	Easy
16	41*	95	115	9	90	2.35	19.0	Absent	Difficult
17	48	98	115	9	56	2.28	11.5	Absent	Easy
18	52	98	115	6	85	2.22	11.3	Absent	Easy
19	54*	98	112	10	89	2.27	20.2	Absent	Difficult
20	56*	95	115	8	82	2.32	15.2	Absent	Difficult
21	59	95	97	7	55	2.25	8.7	Absent	Difficult
22	60	98	102	6	47	2.26	6.4	Absent	Difficult
23	62	95	98	7	54	2.21	8.4	Absent	Easy
24	63	92	90	6	53	2.23	7.1	Absent	Easy
25	66	100	115	11	95	2.28	23.8	Absent	Easy
26	69	98	95	6	67	2.26	9.1	Absent	Easy
27	70	100	120	8	28	2.31	5.2	Absent	Difficult
28	71*	92	115	8	101	2.30	18.6	Absent	Difficult
29	72	95	120	11	42	2.32	10.7	Absent	Easy
30	75*	90	110	8	122	2.26	22.1	Absent	Difficult
31	77	100	115	11	47	2.31	11.9	Absent	Easy
32	78	98	115	12	85	2.26	23.1	Absent	Easy
33	80	92	97	8	50	2.24	9.0	Absent	Easy
34	83	95	103	5	56	2.31	6.5	Absent	Easy
35	84	95	89	6	54	2.32	7.5	Absent	Difficult
36	85	98	100	4	64	2.23	5.7	Absent	Difficult
37	87*	90	95	9	76	2.28	15.6	Absent	Difficult
38	89	90	98	5	64	2.23	7.1	Absent	Difficult
39	91	90	107	7	59	2.25	9.3	Absent	Easy
40	92*	102	112	11	84	2.30	21.3	Absent	Difficult

[1/] Plants producing awnless seeds.

* Selected plants

Appendix Table 2 Continued

Plant no.[1/]	Pedigree	Days to 50% flowering	Plant height (cm)	No. of panicles/ plant	No. of fertile seeds/ panicle	100 seed weight (g)	Seed yield/ plant (g)	Awning	Panicle thresh ability
41	97	90	100	11	45	2.30	11.4	Absent	Difficult
42	99	95	106	8	38	2.24	6.8	Absent	Difficult
43	100	98	110	7	46	2.36	7.6	Absent	Difficult
44	102*	88	85	7	95	2.29	15.2	Absent	Difficult
45	104	95	120	13	78	2.24	22.7	Absent	Easy
46	108	95	111	6	65	2.21	8.6	Absent	Easy
47	109	92	113	4	71	2.27	6.4	Absent	Easy
48	111	88	85	6	38	2.34	5.3	Absent	Difficult

[1/] Plants producing awnless seeds.

* Selected plants

Appendix Table 3 Agronomic characteristics of the BC_1F_2 progenies of the backcross RD23/*O. meridionalis* (w-1629)//RD23.

Plant no.[1/]	Pedigree	Days to 50% flowering	Plant height (cm)	No. of panicles/ plant	No. of fertile seeds/ panicle	100 seed weight (g)	Seed yield/ plant (g)	Awning	Panicle thresh ability
1	2*	93	95	15	75	2.24	25.2	Absent	Difficult
2	5	90	79	4	50	2.32	4.6	Absent	Difficult
3	17	95	98	8	42	2.34	7.9	Absent	Easy
4	25*	93	100	11	52	2.35	13.4	Absent	Difficult
5	26	90	89	9	67	2.36	14.2	Absent	Difficult
6	33	98	120	4	108	2.21	9.5	Absent	Difficult
7	36*	98	113	12	97	2.35	27.4	Absent	Difficult
8	39	95	110	6	64	2.24	8.6	Absent	Difficult
9	44	95	120	11	98	2.29	24.7	Absent	Easy
10	46	98	100	4	76	2.28	6.9	Absent	Difficult
11	55	90	79	6	45	2.31	6.2	Absent	Easy
12	58	95	115	6	40	2.26	5.4	Absent	Difficult
13	72	95	103	6	75	2.30	10.4	Absent	Easy
14	88*	95	111	14	84	2.31	27.2	Absent	Difficult
15	93*	90	80	14	81	2.26	25.6	Absent	Difficult
RD23		82	110	5	76	2.34	8.9	Absent	Difficult
CN1		88	105	5	89	2.39	10.6	Absent	Difficult
TN1		80	78	5	61	1.95	5.9	Absent	Difficult
RD19		145	65	6	69	2.60	10.8	Absent	Difficult
Salumpikit		75	125	6	76	2.38	10.9	Absent	Difficult

[1/] Plants producing awnless seeds.

* Selected plants

Appendix Table 4 Agronomic characteristics of the BC_1F_2 progenies of the backcross CN1/*O. meridionalis* (w-1629)//CN1.

Plant no.[1/]	Pedigree	Days to 50% flowering	Plant height (cm)	No. of panicles/ plant	No. of fertile seeds/ panicle	100 seed weight (g)	Seed yield/ plant (g)	Awning	Panicle thresh ability
1	2*	90	115	8	95	2.20	18.6	Absent	Difficult
2	3*	95	115	14	76	2.22	15.2	Absent	Difficult
3	4*	98	108	11	89	2.25	21.3	Absent	Difficult
4	7	95	92	6	54	2.26	11.4	Absent	Difficult
5	9	93	105	17	103	2.22	5.3	Absent	Easy
6	11	98	115	14	106	2.25	5.9	Absent	Easy
7	19*	95	110	15	108	2.28	21.8	Absent	Difficult
8	26	102	135	4	89	2.32	20.2	Absent	Difficult
9	36*	93	110	15	76	2.27	15.6	Absent	Difficult
10	37*	95	115	14	102	2.23	22.1	Absent	Difficult
11	42*	100	112	7	119	2.31	19.0	Absent	Difficult
12	49*	100	113	8	85	2.30	19.8	Absent	Difficult

[1/] Plants producing awnless seeds.
* Selected plants

Appendix Table 5 Agronomic characteristics of the BC_1F_2 progenies of the backcross RD23/*O. nivara* (16150)//RD23.

Plant no.[1/]	Pedigree	Days to 50% flowering	Plant height (cm)	No. of panicles/ plant	No. of fertile seeds/ panicle	100 seed weight (g)	Seed yield/ plant (g)	Awning	Panicle thresh ability
1	10*	95	105	13	56	2.31	16.8	Absent	Difficult
2	14*	90	89	11	76	2.16	18.1	Absent	Difficult
3	15*	95	90	6	97	2.26	13.2	Absent	Difficult
4	17*	90	85	9	75	2.34	15.8	Absent	Difficult
5	35	95	85	5	96	2.20	10.6	Absent	Difficult
6	45*	95	71	7	88	2.19	13.5	Absent	Difficult
7	46*	93	80	10	95	2.21	21.0	Absent	Difficult

[1/] Plants producing awnless seeds.
* Selected plants

Appendix Table 6 Agronomic characteristics of the BC_1F_2 progenies of the backcross CN1/*O. nivara* (16150)//CN1.

Plant no.[1/]	Pedigree	Days to 50% flowering	Plant height (cm)	No. of panicles/ plant	No. of fertile seeds/ panicle	100 seed weight (g)	Seed yield/ plant (g)	Awning	Panicle thresh ability
1	4*	98	95	11	95	2.23	23.3	Absent	Difficult
2	5*	95	115	11	96	2.21	23.3	Absent	Difficult
3	12*	95	105	10	102	2.20	22.4	Absent	Difficult
4	14*	93	90	8	98	2.24	17.6	Absent	Difficult
5	15*	95	90	9	96	2.24	19.4	Absent	Difficult
6	23*	95	95	12	97	2.22	25.8	Absent	Difficult
7	29	90	85	9	76	2.21	15.1	Absent	Easy
8	30	90	85	11	65	2.23	15.9	Absent	Easy
9	31*	95	80	9	96	2.23	19.3	Absent	Difficult

[1/] Plants producing awnless seeds.

* Selected plants