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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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ABSTRACT

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 62.1% with an average of 52.0%. 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 grown to be BC_1F_3 lines for further evaluation on drought resistance, yield and agronomic performance.

Key words: rice, wild rice, drought resistance, backcross, embryo culture

INTRODUCTION

Rice is the primary food source for more than a third of the world's population and the world will need more rice than what is produced today to feed the extra billions who will rely on it in the future. It is planted on almost 150 million ha annually or 11% of the world's cultivated land. More than 90% of rice is 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

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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. 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). Drought is an abiotic stress to be continuing threat to rice production. 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. Genetic variability of resistance for drought stress is limited in the cultivated germplasm while wild species offer useful sources of genes for drought avoidance such as *O. australiensis*, *O. breviligulata*, *O. glaberrima*, *O. longistaminata*, *O. meridionalis*, *O. nivara* and *O. rhizomatis* (Khush, 1997; Eizenga and Rutger, 2003). One of the exciting developments in biotechnology is to transfer useful genes from wild into cultivated rice across crossability barriers through embryo rescue and backcrossing. This method was applied to transfer bacterial blight, blast and brown planthopper resistance from wild species into cultivated rice (Jena and Khush, 1990; Amante-Bordeos, 1992). Little or no work has been done on the transfer of genes for tolerance to abiotic stresses from wild species to cultivated rice. The objective of this study was to transfer drought resistant character from wild species (*O. meridionalis* and *O. nivara*) to cultivated rice (*O. sativa* L.) by backcrossing in combination with immature embryo culture.

METERIALS AND METHODS

Plant materials

Two cultivated rice varieties susceptible to drought 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). Two wild species having drought avoidance traits (Eizenga and Rutger, 2003) to be used as donors were *O. meridionalis* Ng (acc. no. w-1625 and w-1629) and *O. nivara* (acc. no. 16150).

Hybridization

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

Embryo rescue

Spikelets dated at pollination time were harvested at different ages of 8-14 days old. The husks were removed from the seeds. Whole seeds were immersed in 70% ethanol for 5 minutes and then sterilized with 10% Chlorox 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 medium (Murashige and Skoog, 1962) supplemented with 3% sucrose and 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 16hrs 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.

Pollen fertility

Five spikelets were collected from individual F_1 hybrid plants before anthesis and fixed in 70% ethanol. 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).

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 the grain maturing stage.

Backcrossing

The F_1 hybrids between cultivated rice and wild species were backcrossed to cultivated varieties as the recurrent male parents. The BC_1F_1 seeds obtained were also cultured on half-strength MS medium for germination and the BC_1F_1 plants were selfed to produce BC_1F_2 seeds for drought screening.

Determination of morphological and agronomic characters

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

Agronomic characters of the F_1 hybrids were determined such as days to 50% flowering, plant height, panicle number, panicle threshability and seed fertility.

Drought screening

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 progeny 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 the resistant varieties had tightly rolled leaves at midday (10.00 am – 15.30 pm) to cause marked differences in drought sensitivity of the progeny and sprinkler irrigation was reused. The leaf drying and plant recovery scores were taken after 10 days following soaking in water.

The experiments were conducted from June 2004 to November 2005 in the laboratory and greenhouse at the Department of Agronomy, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand.

RESULTS AND DISCUSSION

Crossability of interspecific hybridization

In all cross combinations, 9,790 spikelets of cultivated rice (RD23 and CN1) were pollinated with the pollens of wild species (*O. meridionalis* and *O. nivara*) and 3,452 seeds (embryos) were obtained (Table 1). Seed sets 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.*

Table 1 Crossability and germinability of embryos derived from interspecific hybridization between cultivated and wild rice.

Crosses	No. of spikelets pollinated	Set seeds		No. of embryos cultured	Germinated embryos	
		No.	%		No.	%
RD23/*O.meridionalis* (w-1625)	1,647	570	34.6	420	235	56.0
CN1/*O.meridionalis* (w-1625)	1,775	543	30.6	420	152	36.2
RD23/*O.meridionalis* (w-1629)	1,363	608	44.6	420	230	54.8
CN1/*O.meridionalis* (w-1629)	2,594	550	21.2	420	175	41.7
RD23/*O.nivara* (16150)	1,192	596	50.0	420	261	61.5
CN1/*O.nivara* (16150)	1,219	585	48.0	420	258	61.4
Total	9,790	3,452	35.3	2,520	1,311	51.9

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 3) 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.

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, we had the seeds were observed to be imperfectly developed after hybridization. Some of them were opaque and shrivelled at the eighth day of pollination (Figure 1A). This negative phenomenon was 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 were 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

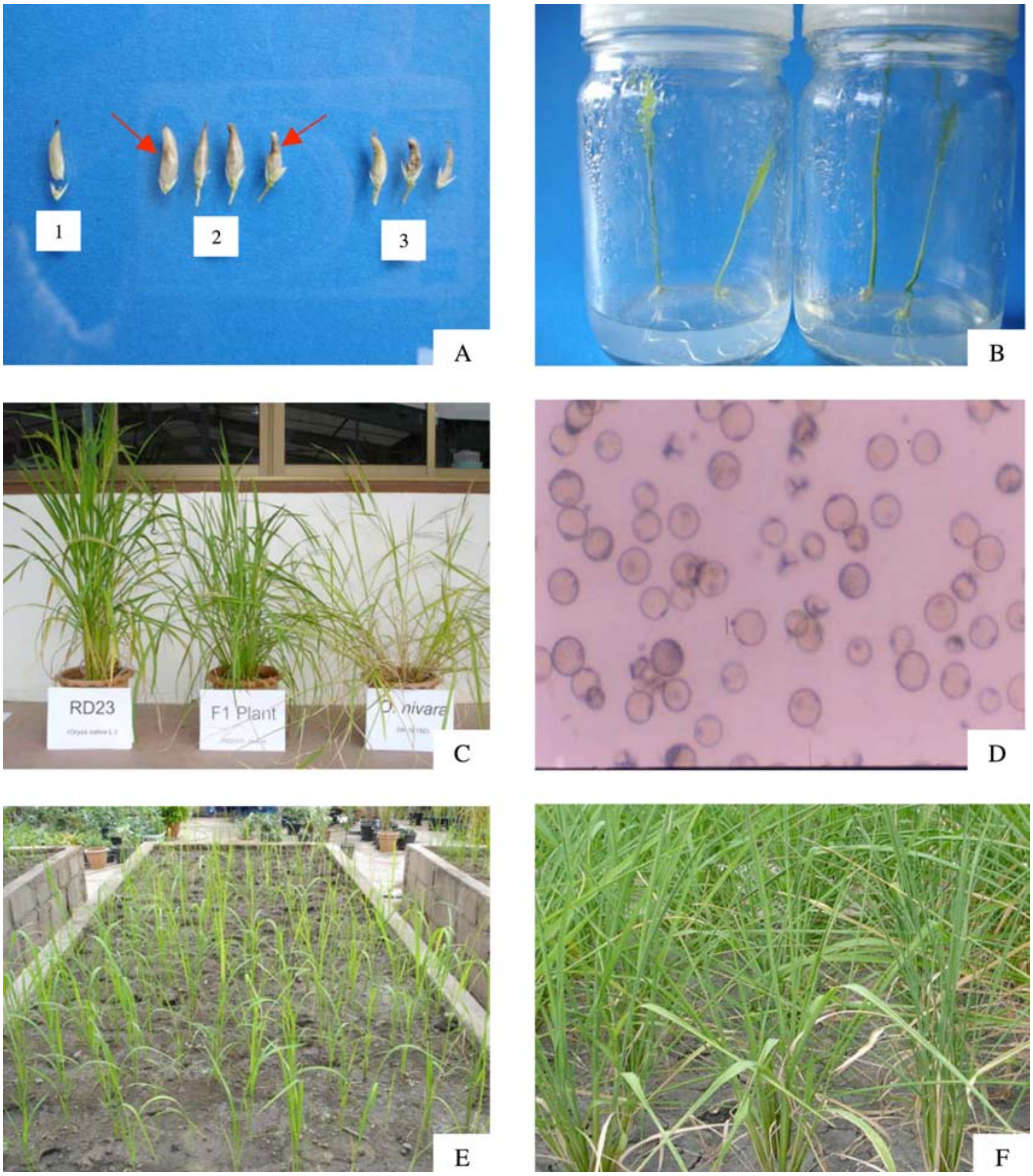

Figure 1 A. Embryos of the F_1 interspecific hybrids between cultivated and wild rice at 12 days old after pollination: 1 – a normal embryo, 2 – embryos with opaque appearance, 3 – embryos with opaque and shrivelled appearance.

B. Germinated embryos after 8 days of culture on the medium.

C. The F_1 hybrid plant (middle) between cultivated rice variety RD23 (left) and *O. nivara* (16150) (right).

D. Highly sterile pollens of the F_1 hybrid (CN1/*O. meridionalis*, w-1625).

E. The BC_1F_2 progenies screened for drought resistance.

F. The BC_1F_2 plants showed leaf rolling at the 35^{th} day of water stress.

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 (data not shown).

The germination ability of hybrid embryos of all crosses varied from 36.2 to 61.5% with an average of 51.9% (Table 1). The hybrid embryos of the crosses between two rice cultivars and *O. nivara* germinated at the higher frequency (61.4 - 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 was 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 cultivated rice varieties were crossed with the eight wild species.

Germination percentages of the hybrid embryos at different ages are shown in Table 2. 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

Table 2 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

Table 3 Morpho - agronomic characteristics of the F1 interspecific hybrid plants grown under greenhouse conditions.

F1 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 threshability
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

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 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 (Roy, 2000). It made the embryos became weak 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 was 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* was done later when they were 14 days old.

Morpho-agronomic characters of the F_1 interspecific hybrids

Production of interspecific hybrid is somewhat difficult, however, a thousand of F_1 hybrid plants between two rice cultivars and two wild species were generated in this experiment. Morphological and agronomic characters of the F_1 hybrid plants were investigated (Table 3 and Figure 1C). 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 parent whereas leaf width, plant height and the 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 cultivars of rice were hybridized with three wild species in the experiment 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 over dominance 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 1D) 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%).

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 4). 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 (of three accessions) when backcrossed to RD23 exhibited the seed set

Table 4 Crossability, embryo germinability and viable BC_1F_1 plants obtained from the 1st backcrossing between F_1 hybrids and cultivated rice.

Backcrosses	No. of spikelets pollinated	Set seeds		No. of embryos cultured	Germinated embryos		Viable BC_1F_1 plants*	BC_1F_1 plants producing seeds
		No.	%		No.	%		
RD23/*O.meridionalis* (w-1625)//RD23	767	132	17.2	120	44	36.7	32	21
CN1/*O.meridionalis* (w-1625)//CN1	1,352	142	10.5	129	40	31.0	29	13
RD23/*O.meridionalis* (w-1629)//RD23	893	125	14.0	111	39	35.1	30	19
CN1/*O.meridionalis* (w-1629)//CN1	1,236	136	11.0	123	37	30.0	31	12
RD23/*O.nivara* (16150)//RD23	714	250	35.0	235	101	43.0	87	85
CN1/*O.nivara* (16150)//CN1	768	238	31.0	229	103	45.0	90	85
Total	5,730	1,023	17.9	947	364	38.4	299	235

* Plants at three - leaf stage.

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 manifested the crossability of 10.5, 11.0 and 31.0%, respectively.

The embryos derived from backcrossing were rescued by culturing on the medium. 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 percentages 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 to 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 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 the 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 generated in the F_1 interspecific crosses. This observation was 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 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 plants produced BC_1F_2 seeds which were utilized for drought resistance screening.

Screening of 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 (O'Toole and Maguling, 1981; Malabuyoc *et al.*, 1985). The BC_1F_2 progenies were also screened for drought resistance at the vegetative growth stage (Figure 1E) 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 1F) 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. Frequency 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 are shown in Table 5. 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 CN1, RD23 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 varieties 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).

Many of the BC_1F_2 progenies could restore good 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 (data not shown). Selection was made for 39 BC_1F_2 plants having high seed yield per plant and promising 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* (16150)//RD23 and seven from cross CN1/*O. nivara* (16150)//CN1. They will be grown to be BC_1F_3 lines for further evaluation on drought resistance, yield and agronomic performance.

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.

Table 5 Frequency distribution for leaf rolling, leaf drying and recovery scores of BC_1F_2 progenies of six backcrosses, three drought susceptible varieties and two drought resistant varieties.

BC_1F_2/ Variety	No. of plants observed	Characters	Score frequency distribution						Average
			0	1	3	5	7	9	
RD23/	114	Leaf rolling[1/]	-	-	-	27	31	56	7.5
O. meridionalis		Leaf drying[2/]	62	52	-	-	-	-	0.5
(w-1625)//RD23		Recovery[3/]	-	41*	26	24	15	8	3.6
CN1/	111	Leaf rolling	-	3	5	12	29	62	7.6
O. meridionalis		Leaf drying	81	26	2	2	-	-	0.4
(w-1625)//CN1		Recovery	-	48*	38	18	4	3	1.2
RD23/	93	Leaf rolling	-	-	-	3	26	64	8.3
O. meridionalis		Leaf drying	50	43	-	-	-	-	0.5
(w-1629)//RD23		Recovery	-	21*	38	20	9	5	3.2
CN1/	57	Leaf rolling	-	-	2	7	14	34	7.8
O. meridionalis		Leaf drying	34	18	3	2	-	-	0.6
(w-1629)//CN1		Recovery	-	24*	17	9	5	2	3.0
RD23/	46	Leaf rolling	-	-	-	5	12	29	8.0
O. nivara		Leaf drying	28	15	3	-	-	-	0.5
(16150)// RD23		Recovery	-	17*	10	11	7	1	3.5
CN1/	31	Leaf rolling	-	-	-	1	5	25	8.5
O. nivara		Leaf drying	17	8	6	-	-	-	0.8
(16150)// CN1		Recovery	-	11*	9	8	3	-	3.2
RD23	8	Leaf rolling	-	-	-	-	-	8	9.0
(susceptible		Leaf drying	-	3	4	1	-	-	2.5
variety)		Recovery	-	-	5	2	1	-	4.0
CN1	14	Leaf rolling	-	-	-	-	2	12	8.7
(susceptible		Leaf drying	4	8	2	-	-	-	1.0
variety)		Recovery	-	4	9	1	-	-	2.6
TN1	32	Leaf rolling	-	-	-	-	8	24	8.5
(susceptible		Leaf drying	-	15	11	6	-	-	2.4
variety)		Recovery	-	-	10	8	9	5	5.6
RD19	38	Leaf rolling	-	-	-	2	7	29	8.4
(resistant variety)		Leaf drying	21	13	4	-	-	-	0.7
		Recovery	-	22	15	1	-	-	1.9
Salumpikit	39	Leaf rolling	-	3	5	17	12	2	5.3
(resistant variety)		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 $^1/_4$ length in most leaves, 5=one-fourth to $^1/_2$ 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.

2. The F_1 interspecific hybrid embryos obtained could be rescued by culturing on the half-strength MS medium with germinability of 52.0% 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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