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Preliminary Report on Transfer Traits of Vegetative Propagation from Wild Rice Species to *Oryza sativa* via Distant Hybridization and Embryo Rescue

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ABSTRACT

There are diversified patterns of vegetative propagation in *Oryza* spp. If *Oryza sativa* is changed from annual type to perennial type via vegetative propagation, the perennial cultivar would be environmental sound and economical viable. There would be a great potential to increase rice harvest area via ratoon cropping or stubble cropping and some hope to break yield plateau via fixing heterosis by vegetative propagation. Another advantage is that it would shorten the time interval from hybridization to form fixed lines. A possible donor of the trait for ratoon or stubble cropping is *O. rufipogon*. The other species possessing rhizome formation ability for breeding of perennial rice are *O. longistaminata*, *O. officinalis*, *O. rhizomatis* and *O. australiensis*. In this study perennial trait was transferred from wild species *O. longistaminata*, *O. rhizomatis* and *O. officinalis* to cultivated rice *(O. sativa*) through distant hybridization. Genotypes of *O. sativa* and wild species, pollen fertility of male species and environmental factors could contribute to crossability or germination rate of the resulting embryos. Finally "false" hybrid problem and research on utilization of vegetative propagation in wild rice species were discussed.

Key words : vegetative propagation, wild rice, distant hybridization, embryo rescue, crossability

INTRODUCTION

For Asian cultivated rice *Oryza sativa*, seed is the predominant way of propagation. Among 23 species of genus *Oryza*, there are different patterns of vegetative propagation (Vaughan, 1994), which could be used to : 1) fix hybrid vigor (Xiu, 1995) 2) breed for perennial rice (Schmit, 1996) 3) culture ratooning rice (Krishnamurthy, 1988) and 4) multiply breeding lines and genetic stocks clonally (Mahadevappa *et al*., 1989).

Tillering is a common trait of vegetative propagation of all species within genus *Oryza*, which could be perennial (*O. rufipogon*, *O. glumaepatula*, *O. eichingeri*, *O. latifolia*, *O. alta*, *O. grandiglumis*, *O. longiglumis*, *O. meyeriana*, *O. granulata*) or annual (*O. sativa*, *O. nivara*, *O. meridionalis*, *O. glaberrima*, *O. barthii*, *O. punctata*). Under certain environment, perennial species *O. glumaepatula* has tillering ability as a mean of vegetative propagation (Oka and Morishima, 1967), which could be used as a pattern of vegetative propagation for all species of the genus *Oryza*.

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Stem regeneration as indicated by ratooning, stubble planting (Mahadevappa *et al*., 1989) or stoloniferous is also the common phenomenon among all *Oryza* spp. especially perennial species of AA genome (Oka and Morishima, 1967) which could also be used as a mean of vegetative propagation for all species of the genus *Oryza*. *O. australiensis*, *O. longistaminata*, *O. officinalis* and *O. rhizomatis* have a common trait of rhizome (Vaughan, 1994), which usually could make the species perennial and adapt to temporal drought. Thus it is the breeder's ideal choice of breeding for perennial ability in irrigated, upland or deepwater rice.

Apomixis is the most promising way to fix hybrid vigor (Hanna, 1995). However, the hope to find obligate apomixis in *Oryza* spp. is dim. The logical strategy is to transfer apomixis from other remote species or to induce genetic mutation in *Oryza* spp. *In vitro* propagation, especially artificial seed, is another way to multiply rice or fix F1 heterosis (Juliano *et al*., 1993; Yoshida and Kata, 1996).

From the viewpoint of breeding, strong regeneration ability of stem is useful for breeding of rice ratooning. Rhizome is the most logical pattern of propagation for breeding of perennial rice especially upland rice (Schmit, 1996). Apomixis and *in vitro* propagation are the most promising ways to fix heterosis of F1 hybrid.

In order to transfer rhizome character from wild rice species to the cultivated species so as to fix heterosis of rice, it is a prerequisite condition to have diversified genetic resources. *O. longistaminata*, *O. rhizomatis*, *O. officinalis* and *O. australiensis* should be the donors of perennial character. This paper is to report preliminary results of transferring rhizome character from *O. longistaminata*, *O. officinalis*, *O. rhizomats* to cultivated rice so as to breed for perennial hybrid rice via distant cross and embryo rescue.

MATERIALS AND METHODS

Cultivated rice

KDML 105 (or Khao Dawk Mali 105, *indica* rice)

RD23 (*indica* rice)

CT6241-17-1-5-1 (*japonica* rice)

IR 42 (*indica* rice)

Wild species

O. longistaminata (accession no. unknown) was kindly supplied by Prof. Dr. Hiroshi Hyakutake which was derived from the Ministry of Agriculture and Forestry, Japan.

O. rhizomatis (accession no. 20133) was supplied by Pathumthani Rice Research Center, Thailand and *O. rhizomatis* (accession no. W 95018 and accession no. unknown) were introduced from International Rice Research Institute (IRRI) via Yunnan Academy of Agricultural Sciences (YAAS), China.

O. officinalis (accession no. W9502 and W9509) were introduced from IRRI via YAAS.

Planting method

All materials were planted in pots and grown in a greenhouse at the Department of Agronomy, Kasetsart University. Each accession comprised 5-7 plants.

Hybridization

Hybridization was done between cultivated rice and wild rice by emasculation of the female parents. Upper one third of the glume of each spikelet was cut off using scissors after 4 p.m.. Then the anthers were removed using forceps. The emasculated panicles were pollinated heavily from the male parents one day afterwards for the duration of 3 days.

Embryo rescue

After pollination, ovaries were excised and surface-sterilized by soaking in 75 % ethanol for 5 minutes followed by 25 % Chlorox contatining a few drops of wetting agent, Tween 20, for 20 minutes. After washing in sterilized distilled water 4 times, the lower part of the ovary was excised and cultured on $^1/_4$ MS (Murashige and Skoog, 1962) medium (3 % sucrose and 0.7 % agar, pH 5.8) in the dark at 25°C (Guzman Emerita, 1983; Jena and Khush, 1984). After germination, seedlings were kept in the light until they reached the three-leaf stage. For acclimatization, the cultured bottles were transferred to a room without direct sunlight for one week, then the seedlings were cultured in pipe water with little NPK compound fertilizer for another week before the plantlets were transplanted into soil in pots and grown in a greenhouse.

Pollen fertility

Six spikelets were sampled from one panicle of the wild species used as male parents before anthesis. Pollen grains were crushed out and stained with 2-KI solution. Dark, round and big pollen grains were considered as fertile ones, and about 1,000 pollen grains were counted for each material.

RESULTS

Observation on rhizome character of different species of wild rice

O. rhizomatis was collected from seasonally dry grassland in Sri Lanka. The extensive thick root system and rhizomes suggest its usefulness perhaps as a source of drought tolerance (Vaughan, 1990). It had vigorous tillers and the growth of rhizome did not overlap with the main cropping. When cutting the main cropping at maturation stage, all rhizome grew and had tillers vigorously within 10 days, and new ratooning tillers could head simultaneously. However, the plant height and panicle length of regenerated cropping were shorter than the main cropping (Table 1). The cropping of rhizome separating and planting was much better than that of *in situ* plant.

The perenniality of *O. longistaminata* was due to permanent rhizome formation. It was easy to form clonal population via rhizome propagation. However, tillering ability was weak and generation overlapping was obvious. Thus, it was not easy to induce panicle initiation.

Both accessions of *O. officinalis* had rhizome and strong tillering ability. Though they possessed

Table 1 Plant height and panicle length of the main cropping and ratoon cropping for different species of wild rice.

Species	Accession no.	Plant height (cm)			Panicle length (cm)		
		Main	Ratoon	Difference	Main	Ratoon	Difference
O. officinalis	W 9502	171	180.3	-9.3	29.8	31	-1.2
	W 9509	150.2	174.3	-24.1	29.4	30.5	-1.1
O. rhizomatis	W 95018	171.6	122.4	49.2**	31.4	22.4	9*
	O. rhizomatis	170.4	119	51.4**	27.9	22.4	5.5**

* ** indicate significance at the 0.05 and 0.01 level of probability, respectively.

permanent rhizome formation and tillering, the plant usually showed synchronous flowers. When cutting the plant at maturation stage, rhizome growth and tiller formation were becoming vigorously within 10 days, and there was no obvious change in plant height, panicle length of main cropping and those of regenerated cropping. This type of vegetative propagation is promising for breeding of perennial ability.

Crossability and germination rate of different interspecific combinations

RD23/*O. longistaminata* could give the highest percentage of seed set (42.86 %) (Table 2) since both parents have the same genome (AA), followed by *O. sativa*/*O. rhizomatis* (0.26-19.3 %),

Table 2 Interspecific hybrids produced via embryo rescue.

Crosses	No. florets pollinated	Seed set		No. embryos cultured	Germination rate (%)	No. hybrid plants
		No.	%			
RD 23/*O. rhizomatis* (no. 20133)	172	14	8.14	8 (9 days old)	50	1
IR 42/*O. rhizomatis* (no. 20133)	696	58	8.33	22 (5 days old)	22.73	0
KDML 105/*O. rhizomatis* (no. 20133)	1,154	3	0.26	3 (8 days old)	33.33	0
RD 23/*O. rhizomatis*	490	83	16.94	45 (5-10 days old)	0	0
RD 23/*O. rhizomatis* (W 95018)	1,011	196	19.39	27 (6-10 days old)	0	0
RD 23/*O. officinalis* (W 9502)	479	8	1.67	3 (13 days old)	0	0
RD 23/*O. officinalis* (W 9509)	388	27	6.96	2 (13 days old)	0	0
CT 6241/*O. officinalis* (W 9502)	991	11	1.2	6 (15 days old)	82.33	3
RD 23/*O. longistaminata*	119	51	42.86	33 (5-10 days old)	3.03	1

and *O. sativa*/*O. officinalis* with the lowest seed set (1.2-6.96 %). *O. rhizomatis* (no. 20133) was crossed with three different cultivars of *O. sativa*. Among the three cultivars, KDML 105 gave the lowest seed set (0.26 %) while IR 42 exhibited the highest seed set (8.33 %). The rate of seed set varied from 8.14 to 19.39 % when the cultivated variety RD23 was used as a female parent and hybridized with different accessions of *O. rhizomatis*, whereas the percentage of seed set ranged from 1.67 to 6.96 when RD23 was crossed with two different accessions of *O. officinalis*. The results indicated that there was crossability difference within the species of *O. rhizomatis* and *O. officinalis*.

In order to obtain interspecific hybrids, embryo rescue is necessary. The embryos of *O. sativa*/*O. rhizomatis* began to degenerate about 8-10 days after pollination and the embryo older than 10 day old was not sucessfully rescued. The hybrid embryos of *O. sativa*/*O. longistaminata* began to degenerate 6 days after pollination. The 5-10 day-old embryos gave considerably low germination rate (3.03 %). Development of the seeds of CT6241-17-1-5-1/*O. officinalis* (no. W9502) was very poor, but the degeneration rate was rather slow. Fifteen-day-old embryos could germinate quite well (82.33 %).

Relationship between crossability and pollen fertility of male species

Partial pollen sterility is a common phenomenon for wild species of rice. The results of Table 3 indicated that there was some relationship between pollen fertility of male species and crossability. The higher fertility of pollen caused the higher percentage of seed set. To confirm the concordance observation, five plants of *O. rhizomatis* (no. 20133) were crossed with RD23 separately and pollen fertility of each plant was investigated. The results once again indicated that there was some relationship between pollen fertility of male species (or different plant within accession) and crossability (Table 4).

"False" hybrids problem

False hybrid was an important problem for distant cross breeding (Chen *et al*., 1989). From the review of literatures, the average rate of false hybrid for intraspecific hybridization was below 5 %. The self-fertilization seed set of emasculated panicles in this experiment was below 1 %. However, the data in Table 2 shows that most plantlets of embryo rescue were false hybrids. It was surprising that a few normal developmental seeds were obtained when RD23 and IR42 were

Table 3 Pollen fertility of the male species and seed set of interspecific crosses.

	No. florets pollinated	Pollen fertility of male species (%)	Seed set	
			No.	%
RD 23/*O. rhizomatis* (no. 20133)	1,561	60.17	207	13.26
RD 23/*O. rhizomatis*	490	31.75	83	16.94
RD 23/*O. rhizomatis* (W 95018)	1,011	86.00	196	19.39
RD 23/*O. officinalis* (W 9502)	497	16.08	8	1.67
RD 23/*O. officinalis* (W 9509)	388	51.31	27	6.96
CT 6241/*O. officinalis* (W 9502)	991	16.08	11	1.11
RD 23/*O. Longistaminata*	199	64.49	51	42.86

hybridized with *O. rhizomatis*, while the seeds germinated normally. All plants of IR42/*O. rhizomatis* (no. 20133) and 3 out of 7 plants of RD 23/*O. rhizomatis* (no. 20133) died after 6-7 days of germination, the remaining were all false hybrids.

RD23 was crossed with 5 separated plants of *O. rhizomatis* (no. 20133). Each cross obtained could form very few normal seeds (Table 5). However, all plants of these progenies were apparently like the maternal parent in morphology. The rate of normal seed set was lower than 1 %, which could be regard as true false hybrids of self fertilization during the course of emasculation. The normal seeds could be obtained for RD23/*O. rhizomatis* (no. 20133-3) as high as 10.67 %, which could not explain the situation of self-fertilization.

Another possible explanation of false hybrid is the pollen of wild species could induce parthenogenesis, and the plant like the maternal parent is presumed to be resulted from haploid gametes stimulated by the pollen of wild species. This phenomenon is known as matromorphy (Farooq *et al*., 1996). If it is true, there would be another effective way to produce diploid plant of rice from female gametophyte. To confirm the hypothesis, CMS line V20A and Fl hybrid of

Table 4 Pollen fertility of the male plants of *O. rhizomatis* (no. 20133) and seed set of interspecific crosses.

	No. florets pollinated	Pollen fertility of male plants (%)	Seed set	
			No.	%
RD 23/No. 20133-1	246	82.01	74	30.08
RD 23/No. 20133-2	451	50.39	37	8.20
RD 23/No. 20133-3	300	54.46	72	24.00
RD 23/No. 20133-4	232	57.28	9	3.88
RD 23/No. 20133-5	332	39.55	15	4.52

Table 5 Normal seed set of certain interspecific hybrids.

	No. florets pollinated	Normal seeds obtained		Germination rate (%)
		No.	%	
IR 23/*O. rhizomatis* (no. 20133)	362	2	0.55	100
RD 23/*O. rhizomatis* (no. 20133)	136	7	5.15	100
RD 23/*O. rhizomatis* (no. 20133-1)	246	2	0.81	100
RD 23/*O. rhizomatis* (no. 20133-2)	451	9	1.96	100
RD 23/*O. rhizomatis* (no. 20133-3)	300	32	10.67	93.75
RD 23/*O. rhizomatis* (no. 20133-4)	232	2	0.86	100
RD 23/*O. rhizomatis* (no. 20133-5)	332	2	0.60	100

RD23/KDML 105 are suggested to be used as female parents to hybridize with *O. rhizomatis* (no. 20133 plant 3).

Preliminary observation on hybrids

One plant of RD23/*O. rhizomatis,* three plants of CT6241-17-1-5-1/ *O. officinalis* (no. W9502) and one plant of RD23/*O. longistaminata* were morphological characterized. They were intermediate in morphological characters between the two parents, but much closer to their respective wild parents (Figure 1-6). They headed much earlier than their cultivated parents in winter. The spikelets were awned, small and easy shattering. Some discriminative traits such as ligule length of lower leaves, presence of rhizome, presence of whorl of branches at panicle base and spikelets inserted in the lower half of lower panicle branches were dominant or recessive traits in F1 generation according to different interspecific hybrids (Table 6).

Pollen fertility of RD23/*O. rhizomatis* (no. 20133) ranged from 0 to 1.54 %. No seed set was observed when the hybrid was backcrossed to RD23; even 2,886 florets were pollinated by RD23. RD23/*O. longistaminata* hybrid had rhizome and indehiscent anthers (Figure 7). Pollen fertility of the hybrid was as low as 32.53 % while the male parent *O. longistaminata* possessed 64.51 % pollen fertility before hybridization.

DISCUSSION

There are diversified pattern of vegetative propagation in *Oryza* spp. If *Oryza sativa* is changed from annual habit to perennial type via vegetative propagation, the perennial crop would have the potential to provide environmental sound and economically viable alternatives for the use on upland, irrigated, rainfed lowland and flood-prone ecosystem (Schmit, 1996; Wagoner, 1990). There might be an alternative to double or triple cropping of rice in tropical and subtropical area to expand rice harvest area via ratooning (Krishnamurthy, 1988). Another advantage is to fix heterosis via vegetative propagation so as to make hybrid rice become available to poor farmers and fragile ecosystems (upland, flood-prone and rainfed lowland ecosystems) (Xiu, 1995). Subsequently, rice yield per hectare could be increased on a large scale. For breeding strategies, perennial character could shorten the time interval from hybridization to form fixed lines. Thus, breeding rice for perennial or vegetative character has environmental, economical, and theoretical preference.

Interspecific crossability is a complex character which could be contributed by female variety of *O. sativa,* male species or accession of wild rice, pollen fertility of male species or accession, and environmental factors. The present results indicated that it is important to select accession or plant of high pollen fertility as male parent so as to get high seed set when interspecific cross is produced.

Nearly all hybrids between *O. sativa* and wild species of the genus *Oryza* have been successfully obtained (IRRI, 1993). Some useful traits have been successfully transferred to *O. sativa* from wild species of rice. However, very little attention was paid on research and utilization of propagation habit. There is a trend for food production toward intensive and fragile area. Research and utilization of diversified vegetative habit could partly meet today’s pressing global concerns on agriculture, availability of food, conservation of resources, and sustainability of the environment.

Finally, hybrid of RD23/*O. longistaminata* might be useful for transferring rhizome character from wild species to cultivated rice and to tag the gene (s) responsible to rhizome formation because it has rhizome in Fl generation and relatively high

Table 6 Morphological characters of certain interspecific hybrids.

Crosses or parents	Rescue date (m/d)	Transplanting date (m/d)	Heading date (m/d)	Plant height (cm)	Panicle length (cm)	Flag leaf length (cm)	Flag leaf width (cm)	Ligule length of flag leaf (mm)	Ligule length of the third leaf (mm)	Length of awn (cm)	Rhizome presence	Whorl of branch presence	Spikelets inserted in the lower half of lower panicle branches
RD 23[1]	10/29	11/4	1/27	96	24.5	46	1.8	11	29	0	no	no	yes
RD 23/*O. rhizomatis*[2]	10/16	11/10	12/17	80	21.5	33	1.6	7	6	1.0	no	no	yes
O. rhizomatis (no. 20133)[1]	10/29	11/4	1/20	65	15.5	23.5	1.1	2	5	0	yes	no	yes
CT 6241-17-1-5-1[1]	12/18	1/13	3/28	60	25	44	1.7	4	17	0	no	no	no
CT 6241/*O. officinalis*[2]	12/4	1/13	2/20	65	19	28	2.1	4	8	2.5	no	yes	no
O. officinalis (W 9502)[2]	12/18	1/13	3/17	120	31	35.5	2.5	3	4	2	yes	yes	no
RD 23[2]	12/28	1/27	4/9	102	25	27	1.7	7	20	0.4	no	no	yes
RD 23/*O. longistaminata*[2]	12/28	2/24	4/13	127	41	69	2.3	30	31	3.3	yes	no	yes
O. longistaminata[3]	-	2/24	-	-	-	-	-	-	-	-	yes	-	-

[1] Direct germination

[2] Embryo rescue

[3] Rhizome propagation.

Figure 1 Plants of RD 23 (left), RD 23/*O. rhizomatis* (middle) and *O. rhizomatis* (no. 20133, right).

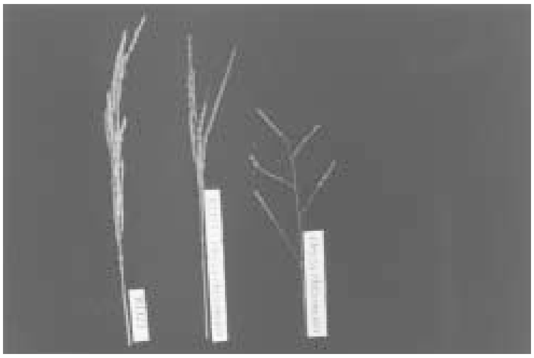

Figure 2 Panicles of RD 23 (left), RD 23/*O. rhizomatis* (middle) and *O. rhizomatis* (no. 20133, right).

Figure 3 Plants of CT 6241-17-1-5-1 (left), CT 6241/*O. officinalis* (middle) and *O. officinalis* (no. W 9502, right).

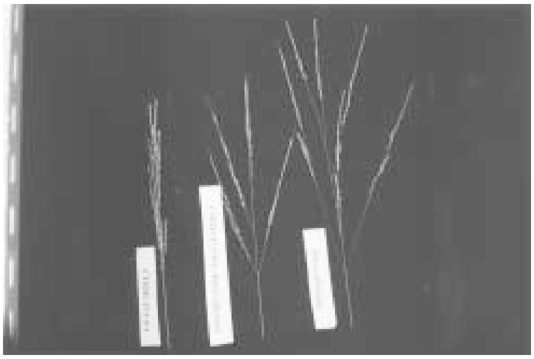

Figure 4 Panicles of CT 6241-17-1-5-1 (left), CT 6241/*O. officinalis* (middle) and *O. officinalis* (no. W 9502, right).

Figure 5 Plants of RD 23 (left), RD 23/*O. longistaminata* (middle) and *O. longistaminata* (right).

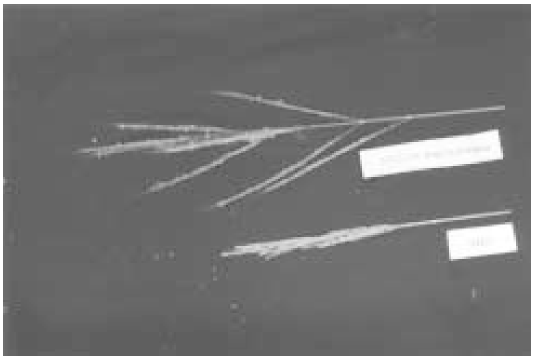

Figure 6 Panicles of RD 23(lower) and RD 23/*O. longistaminata* (upper).

Figure 7 Rhizome of RD 23/*O. longistaminata* (F1 generation).

pollen fertility. It was different from other hybrids reported at least on rhizome expression (Ghesquiere, 1991).

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