

# INTERSPECIFIC HYBRID PLANTS RECOVERED FROM IN VITRO EMBRYO RESCUE IN RICE

R.K. Niroula\*, L.P. Subedi\*\*, R.C. Sharma\*\* and M.P. Upadhyay\*\*\*

\*Scientist, Biotechnology Unit, Nepal Agriculture Research Council, Khumaltar, Lalitpur, Nepal

\*\*Professors, Institute of Agriculture and Animal Science, Rampur, Chitwan, Nepal

\*\*\*Senior scientist, Nepal Agriculture Research Council, Khumaltar, Lalitpur, Nepal

**Abstract:** Embryo rescue is an efficient plant breeding tool to accomplish wide hybridization in the genus *Oryza*. Following this technique several intra and intergenomic hybrids have been successfully produced in wide arrays of crop plants. The present study was carried out taking different cultivars of *O. sativa* as female parent and *O. officinalis* and *O. granulata* as male parent to assess the crossability affinity between cultivated and wild species. All the F<sub>1</sub> embryos prior to abortion (7-10 day old) were aseptically excised and cultured *in vitro* during 2001-2002. Culture was maintained at 25 ± 1°C under dark until germination and then continuous light (~ 110 foot candle). The germination of embryo among hybrids varied from 0-66.67%. Interspecific hybrids were successfully produced only from *O. sativa* x *O. officinalis*. The crossability between *O. sativa* and *O. officinalis* ranged from 0-2.44%, depending upon the cultivars of *O. sativa* used, whereas it was almost zero for *O. sativa* x *O. granulata* suggests that the existence of strong crossability barrier between these species. The present results showed that embryo rescue is a potential technique to overcome post fertilization barriers; however, it is no longer effective where abortion of embryo occurs at early stages of development. Therefore, efforts should be concentrated to optimize the conditions for the rescue of hybrid embryo aborting at very early stages of development.

**Key words:** Embryo; Genepool; *Oryza*, hybrid; Embryo abortion.

## INTRODUCTION

Wide hybridization in cereals is a significant plant breeding tool for the incorporation of desirable genes from wild into cultivated species. However, several reproductive isolation mechanisms restrict the production of intergenomic hybrids, and cause barriers to gene flow between cultivated and wild species (Stebbins, 1958; Hadley and Openshaw, 1980). Based on these restriction and ease of gene transfer, the gene pools in the genus *Oryza* can be grouped into primary (*sativa* complex), secondary (*officinalis* complex) and tertiary (other complex) (Khush, 2000). These pools are excellent and largely untapped source against the unpredictable future genetic vulnerability (Chang, 1976; Chang and Vaughan, 1991).

Niles (1951) was the first who initiated *in vitro* germination in rice. Since then a number of reports on rice embryo culture have been published either for the production of interspecific hybrids or standardization of *in vitro* culture conditions (Bouharmont, 1991, 1961; Ko *et al.*, 1983; de Guzman, 1983; Yie and Liaw, 1975; Iyer and Govila, 1964; Li *et al.*, 1961; Nakajima and Morishima, 1958; and Amemiya *et al.*, 1956). Jena and Khush (1984) have demonstrated the potential role of embryo rescue as a handy tool for the production of

intergenomic hybrids in the genus *Oryza*. They produced several interspecific hybrids by culturing 10-14 days old embryos on ¼ MS medium. Following this technique, a number of researchers have successfully produced a series of interspecific hybrids across the crossability barriers (Abdullah and Somantri, 1995; Brar *et al.*, 1991; and Sitch *et al.*, 1989b).

Among various kinds of pre and post reproductive barriers exist in wide hybridization in cereals; the earlier abortion of hybrid embryo at different developmental stage is the characteristic feature in rice (Brar and Khush, 1995). Subsequent progress in the technique of embryo culture now facilitated the transfer of several alien genes of economic importance from distantly related wild taxa to cultivated rice (Brar and Khush, 1997). To facilitate the gene transfer from these pools, particularly secondary and tertiary, into cultivated species, embryo rescue is essential (Brar and Khush, 1995). Several workers have already transferred BPH and RTSV resistance genes from *O. officinalis* into elite lines of cultivated rice (Jena and Khush, 1990; Kobayashi *et al.*, 1992). Therefore, the present study was undertaken to produce the intergenomic hybrids so as to examine the crossability between various cultivars of cultivated rice and *O. officinalis*, and *O. granulata* of Nepalese origin.

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Author for Correspondence: R.K.Niroula, Scientist, Biotechnology Unit, Nepal Agriculture Research Council, Khumaltar, Lalitpur, Nepal. E-mail: rkn27st@yahoo.com.

## MATERIALS AND METHODS

The study was carried out taking two Nepalese wild species viz. *O. officinalis* ( $2n = 24$ , CC), and *O. granulata* ( $2n=24$ , GG), and seven cultivated rice viz. IR 54, IR 72, Manshara, Jhinuwa, Kalanamak, Jethobudo and Pokhreli, respectively as male and female parents. Seed of *O. officinalis* was kindly provided by Agri-Botany Division, Nepal Agricultural Research Council, Khumaltar, Lalitpur. Live plants of *O. granulata* were collected from river side forest of Piple -6 Chitwan. Collected seeds of *O. officinalis* were germinated by keeping dehulled treated seeds in incubator at  $33^{\circ}\text{C}\pm 1^{\circ}\text{C}$  for 7-9 days following the technique of Vaughan (1994). Germinated seeds and collected live plants were planted into ten plastic buckets filled with sterilized soils. Stagger planting of female parent was made continuously at four day of intervals to synchronize the flowering time. Each female seeds were planted in two replications; three buckets/replication, and two seeds/bucket at a time. Usual agronomical practices were followed to raise the parental materials. Each female parent was crossed with *O. officinalis* and *O. granulata*. Hand pollination was done repeatedly for 2-3 days and pollinated spikelets were treated with  $\text{GA}_3$  and NAA @ 75 ppm (1:1) once a day regularly up to four days after pollination.

Immature 7-10 day old embryos, prior to abort, were excised following the method of Ko *et al.* (1983). The method consisted of removing the envelope from the cut made at the time of pollination. Excised ovaries were surface sterilized in freshly prepared solution of sodium hypochlorite (1%) for 18 minutes. Isolated ovaries were thoroughly washed thrice in sterile distilled water and the lower part of the ovary was cut and pressed out the embryo with the help of sterile forceps. Isolated embryos were inoculated with the aid of needle with loop on modified Whites medium (Bouharmont, 1991). The medium was gelled by 0.7% Agar, after adjusting the pH to 5.8.

Culture was maintained in a temperature-controlled chamber at  $25^{\circ}\text{C}\pm 1^{\circ}\text{C}$  under dark until germination and then continuous light (~110-foot candles). Regenerated seedlings were removed out from the culture tubes at three-leaf stage. The roots of the seedlings were thoroughly washed in tap water to remove agar and finally washed with sterilized distilled water. Hardening of seedlings was done following the technique of Niroula *et al.* (2003) to enhance rooting and make plant hardened in the external environment. The hardened seedlings were planted in plastic buckets filled with well-fertilized sterilized soil and grown in the glass house during winter season in 2001- 2002. Crossability of each species with cultivated rice was calculated following the method of Wu *et al.* (1963) after the establishment of hybrid plants in the pot. Pollen fertility of regenerated hybrid plants was determined by staining 1% IKI solution as described by Virmani *et al.* (1997).



**Figure 1:** 7 day old hybrid (*O. sativa* cv. Kalanamak/*O. officinalis*) embryo ready to germinate.

## RESULTS AND DISCUSSION

Time of embryo degeneration after fertilization was varied with remoteness of the wild species used. It was quite earlier in *O. sativa/O. granulata* (5-10 days) than in *O. sativa/O. officinalis* (9-17days) depending upon the female genotypes used. Though all interspecific hybrid embryos obtained from crosses were cultured *in vitro*, hybrid plants were only obtained from four of the seven cross combinations of *O. sativa/O. officinalis* (Table 1). The germination of hybrid embryo was varied from 0-66.67% (Figure 1). In some of the combinations the germination of *O. sativa/O. granulata* embryos was also found good. Only 21 hybrid embryos were germinated out of 83 cultured, but all were died after germination (Table 1). Therefore, hybrids between *O. sativa* and *O. granulata* were not obtained. Most of the caryopsis in this combination were without embryo or only filled with watery endosperm and thus very few embryos were obtained. Only one hybrid seedling was obtained, but that was also died during hardening process indicating that there should be strong pre and post crossability barriers, or mostly handicapped by post germination barrier.

Such pre and post-fertilization barriers were also commonly observed by Sitch *et al.* (1989a), Sitch and Romero (1990) in the genus *Oryza* and reported for other taxa like *O. brachyantha*, *O. minuta*, *O. ridleyi*, when crossed with *O. sativa*, they all showed strong pre-fertilization barriers. Other several researchers observed variations regarding the hybrid caryopsis formation and their *in vitro* germination (Brar *et al.*, 1991, Sitch *et al.*, 1989b; Jena and Khush, 1984; Iyer and Govilla, 1964). Jena and Khush (1984) obtained ranged of germination that were varied from 38.2-80% when embryos from three sets of hybrids between IRRIs lines and three wild species of rice (*O. officinalis*, *O. brachyantha* and *O. australiensis*) were cultured on  $\frac{1}{4}$  MS medium. On the other hand 16 *O. sativa/O. officinalis* hybrid plants were



**Figure 2:** *In vitro* regenerated hybrid plants, *O. sativa* cv. Manshara/*O. officinalis*.

recovered by culturing 133 embryos (Figure 2). Most of the F<sub>1</sub> hybrids had less than 4% fertile pollen and 100% spikelet sterility (data not shown). The morphology of F<sub>1</sub> plant was vigorous and showed intermediate characters in some respects, but there was preponderance of wild phenotype (Figure 3).

Regardless of the other factors, based on the embryo rescue worked the crossability between *O. sativa*/*O. officinalis* was ranged from 0-2.44% with average pooled mean 1.35 % among all crosses with cultivars (Table 1). It was zero for cv. of *O. sativa* and *O. granulata*, although a large number of florets pollinated and a lot of *in vitro* efforts were carried out up to two years. However, the present result was comparable to the report of Jena and Khush (1986). They found that the crossability between three lines of *O. sativa* and *O. officinalis* varied from 1.0-2.3 % and pooled mean was 1.7%. Brar *et al* (1991), on the other hand, obtained quite low crossability and was ranged from 0-1.1% with pooled mean 0.15% in five varieties of *O. sativa* pollinated with *O. officinalis*.

The successful recovery of hybrid plants from *O. sativa* and *O. officinalis* suggests that there might be less crossability barriers than that found in *O. sativa*/*O. granulata*. They mostly showed post fertilization barrier and can be overcome by embryo culture. Several investigators have successfully overcome such barriers by adopting embryo rescue in different species hybrids (Philips *et al.*, 1992; Sitch *et al.*, 1989b; Williams *et al.*, 1987; Jena and Khush, 1984; Skirm, 1942; Laibach, 1929). Brar *et al.* (1991), however, obtained 3 hybrid plants from *O. sativa*/*O. granulata*, in one combination out of three following this usual technique. This suggests that degree of incompatibility is varying with genotypes of *O. sativa* and ecotype of *O. granulata* used in the crossing program. This indicates that there should be strong crossability barriers between cultivars of *O. sativa* and *O. granulata* used in this study. There is no evidence to such slight discrepancy obtained in this study and that results found by earlier investigators, except to say differences in strains used in crossing. This overall result suggests that production of hybrid plants from *O. sativa*/*O. granulata* cross is very difficult (Khush, 2000, Ghose *et al.*, 1960). Therefore, it is concluded that embryo rescue technique has great promise for wide hybridization and subsequent gene/s transfer in plant breeding. Moreover the crossability between species can be enhanced through the manipulation of various factors that affect the successful plant regeneration from early aborting intergenomic hybrid embryos.

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**Figure 3:** Morphology of *O. sativa* cv. Manshara (Left), F<sub>1</sub> hybrid (center), and *O. officinalis* (right)

**Table 1:** Results of embryo rescue and crossability among *O. sativa*, *O. officinalis*, and *O. granulata*

Parental combination	No. of florets pollinated	Seed set	Seeds without embryo	No. of embryos cultured	Germination %	Died after germination	No. of seedlings grown	Hardening	No. of F <sub>1</sub> plants obtained	Crossability %
Jhnuwa/ <i>O. granulata</i>	411	140	116	24	20.83	5	-	-	-	0
IR 72/ <i>O. granulata</i>	413	80	73	7	14.29	1	-	-	-	0
IR 64/ <i>O. granulata</i>	389	85	72	13	23.08	3	-	-	-	0
Manshara/ <i>O. granulata</i>	211	25	21	4	-	-	-	-	-	0
Pokhrelli/ <i>O. granulata</i>	221	36	26	10	30.00	3	-	-	-	0
Jethobudo/ <i>O. granulata</i>	341	75	60	15	53.33	7	1	1	-	0
Kalanamak/ <i>O. granulata</i>	147	23	13	10	23.07	2	-	-	-	0
Jhnuwa / <i>O. officinalis</i>	205	41	23	18	50.00	4	5	5	5	2.44
IR 72 / <i>O. officinalis</i>	115	36	21	15	20.00	3	-	-	-	0
IR 64/ <i>O. officinalis</i>	163	11	8	3	66.67	2	-	-	-	0
Manshara/ <i>O. officinalis</i>	203	44	24	20	55.00	6	5	5	4	1.97
Pokhrelli/ <i>O. officinalis</i>	174	26	13	13	61.54	4	4	4	3	1.72
Jethobudo/ <i>O. officinalis</i>	125	10	2	8	37.50	3	-	-	-	0
Kalanamak/ <i>O. officinalis</i>	201	60	42	18	33.33	2	4	4	4	1.99
Pooled crossability mean ( <i>O. sativa</i> / <i>O. officinalis</i> )										1.35

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