RESEARCH NOTE

THE IMPORTANCE OF EMBRYO CULTURE TECHNIQUE IN PRODUCTION OF *ORYZA SATIVA* X *O. MINUTA* HYBRIDS

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A primary objective in plant breeding programs is to increase the genetic variability available for exploitation in germplasm collections and then to incorporate desirable traits into useful commercial cultivars. Hybridization between related species or genera is one approach to increase variability. Nevertheless the production of such interspecific or intergeneric hybrids is always limited by several incompatibility barriers such as low seed set, hybrid inviability and hybrid sterility.

Degeneration of the embryo at early stages of embryo development is the common phenomenon encountered in wide hybridization. Frequently this is the result of no endosperm or abnormal endosperm development which causes embryo starvation and abortion of the developing fruit. The technique of embryo culture has been extremely useful in the recovery of crosses where endosperm breakdown is the cause of incompatibility (Raghavan, 1985). It is presumed that tissue culture medium provides the necessary nutrients normally supply by endosperm tissues.

This paper reports the result from crosses between cultivated rice *Oryza sativa* and its wild relative *O. minuta* and the importance of embryo culture technique in obtaining viable F_1 and backcross progenies from such crosses.

Four varieties of rice, *O. sativa* L.; Mahsuri, Setanjung, MR84 and MR103, and one accession of wild rice *O. minuta* J.S. Presl. (IRRI's Acc. No. 101141) were used in the experiments. This *O. minuta*, originated from Philippines, was reported to be among the wild rice species which have a high level of resistance against diseases such as blast and bacterial blight (Sitch et al., 1989). *O. minuta* is a tetraploid (2n=48) species and has a genome composition of BBCC, distinct from *O. sativa* which is diploid (2n=24) and has a genome composition of AA.

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Crosses were made between cultivated rice and the wild species which served as the male parent. The original F_1 hybrids and the colchicine-treated F_1 were backcrossed to the respective variety of *O. sativa* as recurrent parents to produce backcross-1 (BC_1). Successive backcrosses to the recurrent parents was made to produce BC_2 progenies.

Overall, 14.8% seed set was obtained from crosses *Oryza sativa* x *O. minuta*. For the four varieties used, the seed set ranged from 9.5 to 25.1%. In the genus *Oryza*, the low seed set is a common phenomenon found in wide hybridization. According to Sitch et al. (1989), interspecific hybridization, especially those involving intergenomic crosses is normally characterized by low seed set (0-26.5%) and commonly less than 10%. Pre-fertilization incompatibility, which frequently occurs during the pollination process, was found to cause the low seed set.

In BC_1 production, seed set was lower, 0.11%, compared to the seed set in F_1 production. Extremely low seed set, 0.03%, was observed when the original triploid F_1 were backcrossed to *O. sativa* parents. A slightly higher seed set, 0.88%, was obtained when the colchicine-treated F_1 plants were backcrossed to *O. sativa*. In the MR84 x *O. minuta* crosses, no BC_1 plant was obtained even after pollinating 13,894 spikelets.

The F_1 and BC seeds obtained generally looked flat and small. They had imperfectly developed endosperm which subsequently results in the abortion of the embryo approximately two weeks after pollination. Among these seeds, there are some which were able to reach maturity on the mother plant regardless of the abnormality of the endosperm. However, their ability to survive was very poor although they could germinate well in the petri dish. This probably indicates that the degenerated endosperm affects the development of the hybrid seedlings to some extent. Thus, to overcome this embryo breakdown problem, rescuing the immature embryos on nutrient medium was an important procedure in ensuring viable interspecific derivatives.

Degeneration of hybrid embryos is a common post-fertilization barrier of many interspecific or intergeneric crosses. The abortion of the embryo is attributable to the failure of normal endosperm development. Embryo abortion can occur at different stages of development, depending upon the genomic relationships of two parental species. In cereals such as wheat and rice, hybrid embryos are rescued and cultured usually 10-14 days after pollination (Khush & Brar, 1992). In crosses between *O. sativa* and several of its wild species including *O. officinalis* (Jena & Khush, 1990), *O. australiensis* (Multani et al., 1994), *O. alta* and *O. ridleyi* (Brar et al., 1991), viable interspecific hybrids have been successfully rescued from 10-14 day old seeds. In contrast, 14 day old embryos of *O. sativa* x *O. brachyantha* hybrids showed poor viabilty and embryos were successfully rescued from 8 to 10 day old seeds (Sitch et al., 1989). In this study, upon rescuing 10-14 day old F_1 embryos on one-quarter strength of MS (Murashige 1962) medium, 93.50% of the embryo cultured have been germinated and of these, 414 (84.15%) F_1 plants have been successfully raised to maturity.

For the cultured BC_1 and BC_2 embryos, the germination percentage was slightly low, 79.07% and 72.73% respectively. Some of these BC embryos showed abnormal germination that produced rootless coleoptile and died off after 5-10 days germination. These embryos were considered not germinated and this indirectly reduced the germination percentage. Furthermore, the ability of the BC seedlings to grow either *in vitro* or in the soil is also varied. Some of the seedlings grew rapidly and some others grew abnormally and were stunted. Following hardening of the seedlings in Yoshida *et al.* (1976) nutrient solution prior to transplantation to soil, only 17 (39.53%) of BC_1 and 18 (16.36%) of BC_2 were grown until maturity. According to Khush and Brar (1992), the probable causes of the abnormality or lethality in the BC progenies include unfavorable nuclear-cytoplasmic interactions, differences in gene content between the parents and high chromosome numbers.

All the F_1 and BC_1 plants were perennial, vigorous and tillered profusely. Most of their morphological characters appeared like the wild parent. Amongst the BC_2 plants, there was variation in morphological characters. One of the plants , which was derived from the cross MR1 x *O. minuta*, closely resembled the parent *O. sativa* phenotype with no apparent morphological traits inherited from *O. minuta*. The plant was partially fertile with 58.8% pollen fertility and 12.47% spikelet fertility whereas the other plants were male sterile. The plant is of special interest and is being studied for the presence of wild genetic material.

In conclusion, the technique of embryo culture is particularly useful and necessary to circumvent natural barrier to rice interspecific survivability. With the procedure, it is possible to utilize distant relatives of crop plants in breeding programme.

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