II.1 Embryo Culture for Wide Hybridization in Rice

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1 Introduction

Interspecific hybridization is a tool of prime importance in the breeding programs of many crops, more particularly of some cereals, like wheat and oats. Nevertheless, the situation is very different in rice, where the introduction of wild genes in cultivated varieties has been very 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 1976). The broad genetic diversity of this species allowed an adaptation to very different growing conditions and a 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, applications of wide hybridization can be expected in the future in rice as well as for other crop plants.

2 The Genus *Oryza*

The number of species in the genus is a little higher than 20, but the classification is not stabilized, some species being periodically clumped or divided. This is the case for various spontaneous and adventitious forms related to *O. sativa* and for the old species *O. perennis*, which includes genotypes distributed on three continents. According to Chang (1976), *O. sativa*, the annual forms of *O. nivara* (*O. fatua*) and the asiatic perennial *O. rufipogon* are characterized by the same genome (AA). The genome of the second domesticated species (*O. glaberrima*) and its African wild and adventitious relatives (*O. barthii* and *O. stapfii*) is represented by A^eA^e. The perennial African species *O. longistaminata* (*O. perennis* subsp. *barthii*) can be represented by A^bA^b; the genome of *O. glumaepatula* (*O. perennis* subsp. *cubensis*)
is rather different (A\textsuperscript{eu}A\textsuperscript{cu}). Hybridization between all these species generally gives rise to viable seeds and plants, but the rate of successful crossing is very variable, and embryo culture has sometimes been used (Nowick 1986).

In some cases, when \textit{O. sativa} is pollinated by \textit{O. longistaminata}, the F\textsubscript{1} embryos and endosperms begin to deteriorate about 6 days after fertilization, and when \textit{O. longistaminata} is used as maternal parent, hybrid embryos fail after 3 days. According to Chu and Oka (1970), these observations can be explained by the presence of a barrier controlled by a set of complementary dominant lethal genes.

The development of the hybrid embryo generally fails when species with A, A\textsuperscript{g}, A\textsuperscript{b} and A\textsuperscript{eu} are crossed with the other ones, which are morphologically very different from the cultivated rice; some of them have a narrow geographic distribution. After observation of the chromosome pairing in these hybrids, five different genomes have been identified (B, C, D, E, F). Some species are diploid: \textit{O. officinalis} and \textit{O. eichingeri} (CC), \textit{O. australiensis} (EE), \textit{O. brachyantha} (FF). Others are allotetraploid: \textit{O. alta}, \textit{O. grandiglumis} and \textit{O. latifolia} (CCDD), \textit{O. minuta} and \textit{O. punctata} (BBCC). Several species include probably diploid and tetraploid cytotypes (\textit{O. eichingeri}, \textit{O. punctata}). Some genomes are not yet identified.

Interspecific hybrids combining genomes of the A group are generally highly sterile, but their chromosomes are nevertheless homologous, introgression is possible by backcrossing, fertility can be restored, and some genes have been introduced into cultivated varieties. On the contrary, there is almost no pairing between A chromosomes and other genomes, hybrids are completely sterile, allopolyploids have not been obtained, and these wild genomes have not been used in rice breeding up to now. Nevertheless, 12 monosomic addition lines combining the \textit{sativa} genome and the different chromosomes of \textit{O. officinalis} have been isolated after a second backcross of the triploid hybrid AAC by \textit{O. sativa} (Shin and Katayama 1979; Jena and Khush 1986). These lines could be used for the transfer of wild genes by translocation.

According to Shao and Jiang (1980), hybrids have been obtained between rice and sorghum: sorghum pollen tubes go through the rice stigmata and can fertilize the embryo sac, but the embryo and endosperm development stops after 5 and 7 days respectively: thus embryo culture seems useful. The hybrid nature of the recovered plants seems doubtful: Zu et al. (1985) claimed the presence of an enzymatic marker of sorghum, but the F\textsubscript{1} plants had a seed setting as high as 16.69\%, they displayed a phenotype of rice, meiotic anomalies and character segregation could be compared to the behavior of intraspecific hybrids.

### 3 Seed and Embryo Differentiation

In rice, as well as in other members of the grass family, embryo sac fertilization and growth of the embryo are rapid. The early differentiation of the embryonic organs is a useful feature for the application of embryo culture and for the rescue of hybrid progenies in the case of incomplete seed development.

The pollen tube reaches the embryo sac a few hours after pollination (2–12 h according to the conditions and authors). Egg and central cells are fertilized and the