12 Anther Culture for Doubled Haploid Production

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12.1 Introduction

Breeding programs typically require several years to develop a new variety. The process begins with a cross-pollination to combine desirable parental traits. The first generation of offspring, the F1, are heterozygous but genetically uniform. Segregation occurs during reproduction by the F1. Segregation is the separation of homologous chromosomes and genes from different parents at meiosis (Poehlman 1987), and produces genetic variability within the F2 population. With successive generations, self-pollinating crops such as rice become progressively more homozygous, because the heterozygosity decreases by 1/2 with each generation. By the F5 generation, plants are nearly 97% homozygous, which is sufficiently homozygous for advanced testing. However, a considerable portion of the time required to develop a new variety entails this reproduction of several generations to achieve a relatively homozygous condition following the initial cross-pollination.

Anther culture provides a method for the production of homozygous lines over the course of a few months, rather than the several generations required using conventional whole plant techniques (Choo et al. 1985; Morrison and Evans 1988; Snape 1989). The doubled haploid plants resulting from anther culture are homozygous and breed true. Also, because they harbor no hidden traits, the use of doubled haploids for breeding also improves the efficiency with which superior genotypes can be identified (Knapp 1991; Mitchell et al. 1992; Bjornstad et al. 1993).

Anthers contain pollen, and anther culture involves the culture of these structures in vitro. The immature pollen or microspores contained within the anther either gives rise directly to embryos, called androgenesis, or to callus tissue, which in turn is induced to regenerate plants under the influence of growth regulators added to the culture medium. Pollen is haploid, and the cells produced from pollen or microspores during culture are haploid as well. Other methods for the production of haploids include the culture of unfertilized ovules or ovaries, resulting in gynogenesis, or by the chromosome elimination method as used in the Hordeum bulbosum method (Bajaj 1983).

When plants are regenerated from haploid cells, a haploid plant is produced. Haploid plants are sterile and can produce no seed. However, a spontaneous duplication of chromosomes often occurs within anther culture-derived callus
cells, resulting in the production of fertile, doubled haploid plants. Because the two copies of genetic information within such plants are identical, the plants are fully homozygous and breed true. In rice anther culture, roughly half the plants are haploid, and the rest are diploid, along with an occasional plant of higher ploidy (Chu and Croughan 1989). With experience, haploid plants can be distinguished from diploid plants with about 90% certainty at the point at which plants are transferred to the greenhouse. Subtle differences in stature, color, leaf shape, tillering, and root development can be used to eliminate most haploids at this stage if greenhouse resources are limited.

Colchicine can be used to induce polyploidization and offers the possibility to increase the number of diploid plants produced, especially when direct androgenesis is involved (Bajaj 1983). Our experience in using colchicine in rice anther culture (Chu and Croughan 1989) has indicated that more diploid plants can be produced by expending the same effort on plating additional anthers, rather than utilizing colchicine treatments.

Several factors affect the success of anther culture, especially the genotype of the plant from which anthers are obtained (Bajaj 1983; Chu and Croughan 1989) and the composition of the nutrient media (Gaillard et al. 1991; Biddington et al. 1993; Hoekstra et al. 1993). Other factors include the condition of the donor plant, developmental stage of the pollen, and thermal shock pretreatment of the anthers (Bajaj 1983).

Rice anther culture was first successfully conducted by Niizeki and Oono (1968), and several new rice cultivars have been developed from anther culture (Zhang and Chu 1986). The practical application of rice anther culture entails the use of heterozygous breeding lines. The use of $F_1$ donor plants results in the greatest savings in time to produce new homozygous plants. The use of individual $F_2$ plants saves less time, but offers the advantage of concentrating the effort on superior individuals within the $F_2$ population. Also, using $F_2$ donor material provides an additional opportunity for useful genetic recombination to occur (Singsit et al. 1990). Another consideration is that $F_2$ plants might be better than the $F_1$ for anther culture if one of the parents in the original cross was a poor genotype for anther culture.

12.2 Objectives and Goals

- To provide technologies for the successful culture of rice anthers, and to apply the procedures for production of haploid and doubled haploid plants.
- To transfer the regenerated plants to greenhouse and field conditions, grow the plants to maturity, and harvest seeds from fertile doubled haploid plants derived from anther culture for use in planting progeny rows.