III.4 Breeding New Rice Strains Through Anther Culture

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1 Introduction

Anther culture as a method of creating haploid strains has become more efficient in recent years (see Bajaj 1990). Because of their great usefulness in genetic studies and practical breeding, haploid strains have attracted much attention from geneticists and plant breeders.

The main advantage of doubled haploid breeding is that it can rapidly produce homozygous lines, so that doubled haploids can be used very effectively in selection programs. One useful area of plant selection where doubled haploids may have a major impact will be the selection of mutants from anther cultures, selection at the haploid level allowing direct selection for both dominant and recessive traits. After chromosome doubling, both dominant and recessive genes are expressed in the fertile plant. Mutagenesis at the haploid level should also be more efficient than at the diploid level when recessive traits involved. However, concerning the application of anther culture technique to crop breeding, there are different opinions. Some consider that in haploid breeding there is only one recombination in meiosis. As many of the agronomic characteristics, for example, yield and quality, are controlled by many genes, one recombination alone is not enough. Thus some authors doubt the usefulness of haploid breeding practice. However, in China, several rice and wheat varieties produced by this technique (Anonymous 1976; Li Y et al. 1985) are already being cultivated. The objective of this chapter is to report on several new rice strains by anther culture.

2 Anther Donor

It is well known that the differences in rice species have a marked effect on both callus formation and plantlet regeneration, and in the population of five species, they were in the order Oryza sativa var. > japonica cultivar > indica × japonica hybridization > hybrid rice > indica cultivar. The physiological and developmental state of the donor also influences the outcome of the culture. The anthers were excised at the late uninucleate stage and planted on medium. Through numerous trials and observation for the choice of anther, some empirical criteria

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have been established: total hull in light-white; anther in light-greenish blue-colored; total length of anther equal to half of hull. Cold pretreatment of anther is a well-tried technique for stimulating the production of calli from pollen.

3 Media

We tested the suitability of five media, and of these N6 medium was better than the MS, Blaydes, White’s or B5 for inducing callus, but MS medium was found to be better than other media for regeneration of plantlets. We observed the beneficial effect of combining an auxin with kinetin in the culture medium. If the medium was supplemented with 2,4-D 2 mg/l with a further addition of 0.5 mg/l of kinetin, callus induction increased significantly. Various combinations of auxins and cytokinins were tried; a combination of 6BA with 2,4-D, IAA, or NAA had no effect. High frequency and good callus was formed on N6 medium supplemented with 2,4-D (2 mg/l) + kinetin at 0.5 to 1 mg/l. This callus was hard, dense, crystalline lens, smooth, light greenish blue colored, and could be regenerated. Different types and concentrations of cytokinins have been tested to optimize green plantlet regeneration from anther callus of rice. Kinetin compared to 2-ip or 6BA in the range 2 to 4 mg/l had the best regeneration capacity and the highest survival and growth rates in the presence of 4 mg/l kinetin in MS medium. There is direct regeneration of plantlets on the anther, if the anthers are cultured on N6 medium supplemented with a low concentration of auxin and a high concentration of cytokinin.

4 Rooting of Plantlets and Transfer to Soil

Rooting of plantlet can be accomplished either by supplementing the medium with a low concentration of auxin (NAA 0.2–0.5 mg/l) and with high cytokinins (2–4 mg/l), or by inducing treatment with high cytokinins without auxin. Transfer of plantlets to a medium with auxin but without cytokinins also induced roots. According to our experience, all plantlets have the capability to form roots. Before transfer, the plants should be rinsed to remove traces of agar. In our experience, rice plantlets obtained from excised anther were sensitive to dehydration as compared to seedlings, so plantlets must be acclimated gradually to withstand the high humidity and weaker light intensities existing outside the test tube. When transplanted to soil (plantlets in small pots containing paddy soil), the plantlets have to be covered with a plastic sheet in the greenhouse or outdoors under humid conditions.