Rice microspore culture: a fast-track to new varieties

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IN A RICE HULL

- Immature pollen grains or microspores (with one set of chromosomes, haploid) can be made to divide and grow into a complete haploid plant on a specialised culture medium
- The microspore-derived plants can then be induced to double their chromosome number to obtain doubled-haploid plants (with two identical set of chromosomes, thus homozygous), which is an attractive alternative to repeated cycles of in-breeding in self-pollinating crops like rice
- Establishment of a microspore culture system for rice at Yanco would mean breeders will have direct access to doubled-haploid plants, thus development of better varieties faster

The investigation and development of novel plant breeding techniques could potentially take years off the lead-up to producing new rice varieties. Previous articles in this edition have documented new techniques to make the selection of specific plant characteristics quicker and more precise. Another technique being investigated at the Yanco Agricultural Institute is microspore culture.

In flowering plants, like rice, the pollen grain (microspore or male gamete) normally fertilises the egg cell (female gamete) to produce a seed embryo (zygote) that germinates and grows into a fertile plant. Both male and female gametes contain only one set of chromosomes (haploid number), whereas the seed embryo (zygote) contains two sets of chromosomes (diploid number, one set coming from each parent).

In conventional breeding, two parental lines (one commercial variety as the recipient parent and the other donor parent with one or more desirable traits) are crossed to produce F1 hybrid plants. The two sets of chromosomes in the F1 plant segregate randomly for various agronomic traits in the subsequent generations. Breeders have to select for the desirable lines and then grow them (in-breeding) for at least 8–10 generations, with continuous selection, until the two sets of chromosomes within in each of their selected crossbred lines become identical (homozygous).

True breeding lines in 1–2 years

In anther and/or microspore culture technique, the immature pollen grains (with one set of chromosomes, thus haploid) are induced to divide and double their chromosome number so that the plants regenerated from them have two sets of chromosomes that are the mirror image of each other (doubled-haploid). All these steps in a doubled-haploid production can be achieved within one generation (10–18 months). Therefore, if the microspore culture step is performed with the F1 hybrid plants, it would result in homozygous plants within one generation thus reducing the time required in developing uniform (true-breeding) commercial varieties. In addition, due to the true-breeding nature of the doubled-haploid plants, they are an ideal material to study the genetic control and linkage of various agronomic traits.

The anther and/or microspore culture technique has been routinely used for a number of crop plants around the world. For rice, both intact anther and isolated microspore culture methods have been successful in generating doubled-haploid plants. However, the anther culture procedure, where intact anthers are cultured on a specialised solid medium, is not very efficient and can generate plants from diploid cells of the anther wall. In contrast, microspore culture technique is more efficient and ensures that all plantlets are derived from the haploid microspores.

At Yanco, rice breeders had been relying on a limited number of doubled-haploid plants generated from the anther culture program at Cobbitty. Unfortunately, due to quarantine restrictions for plant material entering the MIA, the access to these doubled-haploid populations is generally delayed. Therefore, attempts were made to set up a microspore culture system at Yanco and a protocol (previously established at Adelaide University) involving culture of spontaneously shed microspores from intact anthers was used. In this procedure, immature panicles (with anthers containing young pollen grains at the single nucleus stage) are harvested. The anthers are treated so that the microspores contained within are spontaneously shed into a medium and start dividing into cell clusters (forming a callus or embryos), while anther walls shrivel off and die. These cell clusters and/or embryos are then transferred onto a specialised regeneration medium, where whole plants are produced. The unique advantage of this method is that it eliminates the extra steps involved in mechanical isolation of microspores, thus preventing their possible mortality and reduced efficiency of doubled-haploid plant production.

Promising results at Yanco

At Yanco, results from a preliminary trial on the microspore culture technique are very promising. Immature pollen grains from plants of crosses made between Australian cultivars and salt tolerant lines (to incorporate the salt tolerance traits into the Australian cultivars) have been cultured. Young anthers isolated from immature panicles (Figure 1) of F1 plants of the salt-tolerant crossbred showed a good response (callus and embryo formation) to microspore culture (Figure 2). This was comparable (even better) to the response observed in a culture of a highly responsive variety Nipponbare (Figure 3). The microspore derived cell clusters from the crossbred line (Figure 4) are currently being tested on a range of tissue culture media for their regeneration into normal doubled-haploid plants.

In the next season, a number of F1 crossbreds derived from cold and salt tolerant lines will be put through this microspore culture technique, to generate doubled-haploid populations at Yanco. In addition, a range of parental lines important to breeders will be tested for their response to microspore culture and plant regeneration potential. It is anticipated that this in-house microspore culture system would allow rice breeders to have direct access to the doubled-haploid plant populations, overcoming the quarantine barriers encountered before, and will expedite their efforts in developing new varieties.

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Figure 1 *The microspore culture process starts with isolating young anthers from immature panicles*

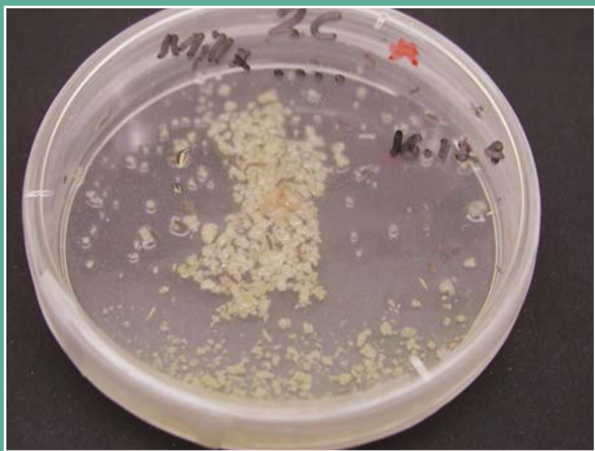

Figure 2 *Cell clusters from microspores of a cross of an Australian cultivar and a salt tolerant line formed, at eight weeks*

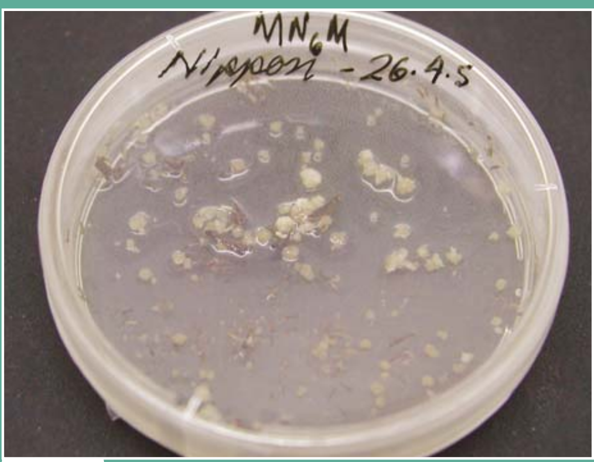

Figure 3 *Eight week cell clusters of Nipponbare, a variety known to respond well to microspore culture*

Figure 4 *Microspore derived cell clusters on a solid culture medium ready for plant regeneration*