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Accelerating variety release with double haploids

Sue Broughton
sue.broughton@agric.wa.gov.au

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ACCELERATING VARIETY RELEASE WITH DOUBLED HAPLOIDS

The use of plant tissue culture to produce special lines called doubled haploids is reducing the time taken to breed new varieties of cereal crops by up to three years. Sue Broughton outlines what doubled haploids are, how they are produced, and why they have been able to short-circuit the usual lengthy breeding process.

Plant breeding is traditionally a slow process, with the release of new cereal varieties taking up to 12 years.

The process begins with the evaluation of introduced and local breeding lines to identify elite genetic material. Promising lines are then crossed or hybridised to produce crossbreds that combine the desirable aspects of both parents.

After crossing, at least four generations (usually four years) are required to obtain true breeding or homozygous lines, with minimal genetic variation, in self-fertilising crops such as wheat, barley, lupins, oats and peas.

However, when tissue culture techniques are used to generate true breeding lines, this time can be reduced to less than a year. These tissue culture derived lines are called doubled haploids.

Doubled haploids are plants generated directly from pollen or egg cells. These cells are termed haploid as they contain only half the chromosome complement of the parent plant. Under normal conditions, the full chromosome complement—diploid—is restored after fertilisation and the union of pollen and egg cells.

The production of doubled haploids, however, bypasses this process. Haploid plants are generated from pollen or egg cells and subsequently require chromosome doubling to restore fertility and the normal diploid chromosome number. Chromosome doubling may either occur spontaneously or be induced by the application of certain chemicals. The term, doubled haploid, is used to differentiate these plants from conventionally bred lines.

To accelerate the breeding process, doubled haploids are produced from early generation hybrids (F₁ or F₂). An F₁ or hybrid plant contains genetic material from both parents and the genetic recombination that occurs during the formation of pollen and egg cells (a process
Where doubled haploids fit into the breeding program

Methods of producing doubled haploids in barley and wheat

Two similar methods of producing doubled haploids are anther and microspore culture. Anthers contain immature pollen cells, called microspores, which may be cultured whole (anther culture) or macerated first to release microspores (microspore culture).

Microspore culture is technically a more difficult method although, if successful, can yield better results as microspores are freed from the restrictive competitive confines of the anthers.

Both techniques divert the microspores away from their normal developmental pathway, which is to become a mature pollen cell. Instead, the microspores develop directly into haploid plants. In barley, spontaneous chromosome doubling occurs in about 70 per cent of plants generated from microspores and these plants become fertile doubled haploids. Haploid plants are sterile and will not set seed.

While anther and microspore culture methods work well in barley, they are not very successful in wheat. Therefore, an alternative method, called wide-crossing, is used.

Wide-crossing involves the pollination of wheat with maize. Before pollination, all wheat anthers are removed to prevent self-fertilisation. Fresh maize pollen is then applied to wheat flowers, and because maize and wheat are distant "relatives", a brief partial fertilisation occurs.
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Because the cross is so wide, all maize chromosomes are rapidly eliminated from the developing embryo. The result is a haploid embryo, containing only wheat chromosomes. No endosperm is formed so the embryos must be dissected from the developing seed in a process called embryo rescue.

The embryos are then germinated on a plant tissue culture medium and become haploid plants. The plants are treated with a chemical, such as colchicine, which induces chromosome doubling to produce fertile doubled haploid plants.

Doubled haploids and plant breeding

Plant breeders have been interested in the potential of doubled haploids for many years and, in addition to barley and wheat, they are produced in crops such as canola, maize, potato, rice, sugarbeet, and tobacco.

Doubled haploids are also extremely useful in gene mapping and the development of molecular markers due to their total homozygosity. They are easier to work with than conventional lines, where there is often some residual genetic variability or heterozygosity.

Wheat embryos must be rescued about two weeks after pollination because no endosperm has formed. Seeds are surface sterilised and haploid wheat embryos are dissected from the seed and placed on a nutrient medium. Embryos germinate and develop roots and shoots in about three to four weeks.

For plant breeders, one of the biggest limitations can be the production of large enough numbers for practical plant breeding purposes.

A major problem, especially with anther and microspore culture, is that varieties differ markedly in the way they respond to these methods, with some varieties responding very poorly or not at all. In Western Australia, barley varieties such as Stirling responded well to anther culture, while other varieties such as Franklin, Chariot, and Mundah responded poorly.

The numbers of plants produced (per 100 anthers cultured) by different varieties are outlined in Table 1. Crosses involving at least one responsive parent (e.g. Stirling x Franklin) will generally respond to anther culture, but it is more difficult to produce large numbers of doubled haploids from crosses where both parents are recalcitrant to anther culture (e.g. Franklin x Chariot).
The number of doubled haploid plants produced when using the wheat and maize wide-crossing method is less dependent on the variety used. However, differences in production are still found between crosses, with variations occurring in the number of embryos obtained and the numbers of embryos that successfully germinate.

**Stress pretreatments**

Experiments carried out in the Western Australian program in 1997 identified improvements in green plant regeneration by altering the stress pretreatment.

Stress pretreatments are frequently used in anther culture to induce microspore embryogenesis. Cold pretreatment, where excised spikes (barley ears) are stored at 4°C for 14 to 28 days, is widely used in many programs.

An alternative is mannitol pretreatment, where anthers are pretreated on a medium containing mannitol for 3 to 5 days. Pretreatment of barley anthers with high concentrations of mannitol significantly improved green plant regeneration compared with cold pretreatment.

The mannitol pretreatment resulted in improvements for varieties that were both responsive (Stirling) and non-responsive (Franklin). Further improvements were made with Franklin by combining the mannitol pretreatment with increased carbohydrate levels in the induction medium.

The best treatment resulted in 120 and 54 green plants per 100 anthers for Stirling and Franklin, respectively.

The mannitol pretreatment was compared with cold pretreatment in a number of crosses in the Western Australian program, resulting in improved green plant regeneration in all crosses. The results are outlined in Table 2.

At present, anther culture with mannitol pretreatment is the main method used to...
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Table 3: Number of doubled haploid barley lines produced and evaluated in field trials in Western Australia.

<table>
<thead>
<tr>
<th>Year</th>
<th>No. Crosses</th>
<th>No. DH lines produced</th>
<th>Seed bulking &amp; preliminary evaluation</th>
<th>Breeding program stage 2 trials</th>
<th>Crop variety testing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BS2/B2D</td>
<td>B2.1</td>
</tr>
<tr>
<td>1993</td>
<td>3</td>
<td>1,060</td>
<td></td>
<td>1,060</td>
<td></td>
</tr>
<tr>
<td>1994</td>
<td>11</td>
<td>2,101</td>
<td></td>
<td>1,958</td>
<td></td>
</tr>
<tr>
<td>1995</td>
<td>8</td>
<td>1,559</td>
<td></td>
<td>1,559</td>
<td>1,628</td>
</tr>
<tr>
<td>1996</td>
<td>12</td>
<td>2,068</td>
<td></td>
<td>1,704</td>
<td>1,075</td>
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<tr>
<td>1997</td>
<td>31</td>
<td>2,816</td>
<td></td>
<td>1,798</td>
<td>1,840</td>
</tr>
<tr>
<td>1998</td>
<td>32</td>
<td>2,474</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>97</td>
<td>12,078</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Due to improved seed production following DH production, some of these lines were progressed directly to breeding program stage 2 trials.
2. Refer Figure 1

Due to improved seed production following DH production, some of these lines were progressed directly to breeding program stage 2 trials.

Breeding program stage 2 trials:
- BS2/B2D
- B2.1
- B2.2
- Stage 3

The most advanced doubled haploid lines are currently in their first year of Crop Variety Testing with promising malting and yield results.

The wheat doubled haploid program is newer and began in 1997. To date, about 3500 wheat doubled haploid lines have been produced, and the first wheat doubled haploids entered field trials in 1998.

Acknowledgements

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