Fast tracking barley varieties using anther culture

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Breeder are constantly trying to develop plant varieties with increased yields, resistance to disease and improved product quality. The process begins with evaluation of introduced and local varieties to identify elite genetic material. Promising lines are then crossed or hybridised to produce crossbreds that combine the desirable aspects of both parents.

Following crossing, at least four generations are required to 'fix' the genetic make-up in self-fertilising crops such as barley, wheat, oats, lupins and peas. Using anther culture, true-breeding lines can be produced rapidly from hybrid material. This may take as little as eight months, developing lines that can enter commercial production about four years earlier than normal.
Western Australia produces about 800,000 tonnes of barley each year.

Anthers are the male sexual organs of flowering plants. They contain pollen cells, which in barley contain seven chromosomes, half the genetic complement of a fertile barley plant. At fertilisation these chromosomes combine with the seven chromosomes in the ovule, restoring the full complement required for fertile barley plants.

Anther culture involves the generation of plants from immature pollen cells, bypassing fertilisation and growth of the seed. Pollen cells and the plants derived from them are described as haploid as they contain only half the chromosomes of the parent. Haploid plants are of little use as they are sterile and will not set seed. However, if the chromosomes of a haploid plant are duplicated, the normal diploid chromosome number is restored and the plant will be fully fertile. In barley, spontaneous chromosome doubling occurs in about 70 per cent of plants generated from pollen cells. Such plants are called doubled haploids.

If doubled haploids are produced from early generation hybrids (the F, or F₁, generation), the genetic constitution of the lines is fixed by the chromosome doubling process. The new lines are then 100 per cent true-breeding from one generation to the next and there is no delay in identifying lines that could enter commercial production.

Limitations
Varieties differ markedly in their response to anther culture. Some varieties such as Chebec and Stirling respond extremely well, producing about 120 and 40 green plants for every 100 anthers cultured. However, it is difficult to generate green plants from other parents such as Franklin and Harrington so anther culture is not attempted in such crosses. By contrast, crosses such as Stirling x Harrington could be expected to perform reasonably well.

The production of white or albino plants is a further problem. These plants lack the special cell structures (chloroplasts) which enable plants to use light energy to produce carbohydrates. They survive in laboratory culture because all the requirements for growth and development are provided by the tissue culture medium. In some crosses the number of albinos may outnumber the number of green plants by up to 100 fold.

Western Australian results
Following the establishment of the Western Australian Barley Anther Culture Laboratory in April 1993, we selected four crosses for developing doubled haploid populations. They are expected to produce progeny with good malting quality adapted to large areas of Western Australia’s cropping zone.
Shoots are transferred to a root induction medium to encourage root formation.

Barley anthers are plated onto an induction medium (top left). After four weeks embryoids and callus develop from pollen cells (top right). Regenerant plants grow from these embryoids (bottom).

Technical Officer, Penny Priest, checks on the progress of donor plants in growth rooms where temperature and light can be carefully controlled.

All crosses responded well and large numbers of green plants have been generated (see Table 1). The number of lines generated compares very favourably with results from laboratories in Australia and overseas conducting anther culture on barley.

Current work is focused on the next eight crosses (see Table 2), while lines generated from the first crosses are being evaluated.

### Doubled haploids in plant breeding

Plant breeders have been interested in the potential of doubled haploids for many years but the biggest limitation has been production of sufficiently large numbers. Although doubled haploids can be produced using several methods, a successful anther culture system has the potential to produce the greatest number of doubled haploid plants because each anther contains between 2000 and 3000 pollen cells from which plants can theoretically be regenerated.

Anther culture is currently being used to produce doubled haploids in a number of species including canola, maize, potato, rice, sugarbeet, tobacco and wheat. Success varies considerably between species. It is particularly successful in canola and tobacco, and generally less successful in cereals.

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### Table 1. Plants produced from anther culture for four barley crosses

<table>
<thead>
<tr>
<th>Cross</th>
<th>Green plants/100 anthers</th>
<th>Total no. of green plants</th>
<th>Loss through deaths</th>
<th>Haploids</th>
<th>Final no. of doubled haploid lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stirling/Harrington//Skiff</td>
<td>53.6</td>
<td>790</td>
<td>20%</td>
<td>17%</td>
<td>462</td>
</tr>
<tr>
<td>Stirling/Harrington//O’Connor</td>
<td>49.9</td>
<td>845</td>
<td>11%</td>
<td>20%</td>
<td>557</td>
</tr>
<tr>
<td>Stirling/Harrington//Yagan</td>
<td>24.7</td>
<td>403</td>
<td>19%</td>
<td>27%</td>
<td>212</td>
</tr>
<tr>
<td>Stirling/Franklin//Skiff1</td>
<td>9.2</td>
<td>376</td>
<td>10%</td>
<td>10%</td>
<td>92</td>
</tr>
</tbody>
</table>

1. Results to June 1994

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### Table 2. Current crosses in the Barley Anther Culture Laboratory

#### Crosses for high rainfall cropping zones
- Franklin/Chariot
- Franklin/Waveney
- Franklin/Europa/Stirling/Europa

#### Crosses for medium and low rainfall cropping zones
- 83S:514/Harrington
- 83SM:522/Harrington
- 83SM:522/TG121-l
- 83SM:522/Natasha
- Yagan/TG121-l

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Table 2. Current crosses in the Barley Anther Culture Laboratory
The anther culture procedure

The procedure used in Western Australia is based on a system developed in Germany and adopted by anther culture laboratories at the University of Adelaide and the South Australian Research and Development Institute.

The production of healthy stress-free donor plants is critical to the success of the technique. The plants are raised in growth rooms where the temperature, light intensity and daylength can be carefully controlled. Young barley ears, still within the tillers of the plant, are harvested to obtain anthers for culturing. A few anthers from each ear are squashed and examined under the microscope to determine the stage of pollen development. Pollen cells must be at a particular stage before they can be induced to regenerate into new plants. If they are too old or too young the ear is discarded.

The excised barley ears are then sealed into petri dishes and given a cold treatment involving three or four weeks storage at 4°C. Cold treatment is used commonly in barley anther culture and helps to increase the number of regenerated plants obtained from each anther.

After cold treatment, 30 to 40 anthers are dissected from each ear and plated on an induction medium which contains nutrients, a carbohydrate source (maltose), vitamins, amino acids and plant growth hormones. During a four-week incubation a small proportion of pollen cells within the anthers may develop into embryoids or callus. Embryoids are similar to a developing embryo in a seed while callus is a mass of cells without any defined structure.

The embryoids and callus are transferred to a regeneration medium to encourage shoot development, then to another medium to encourage root growth. The resulting plantlets are transferred to sterile soil mix and covered with plastic lids which maintain humidity and prevent the young plants from drying out. The covers are removed as the plants harden off. After about four to six weeks the plants are transferred to larger pots where they are grown through to maturity and seed is harvested. Plants that set seed will be doubled haploids, and this seed will represent true-breeding lines for evaluation in the Department of Agriculture's barley breeding program.

Research Officer, Sue Broughton, dissects barley anthers from an ear with the aid of a microscope.

Young barley ears are dissected out to remove the important pollen cells.

Plantlets are transferred to seedling trays. Plastic covers help maintain humidity until the plants harden off.

The procedure used in Western Australia is based on a system developed in Germany and adopted by anther culture laboratories at the University of Adelaide and the South Australian Research and Development Institute.
Self-fertilising plants

Barley (Hordeum vulgare), like wheat, is a self-fertilising plant. This means that the pollen fertilises the ovule of the same plant. Thus, the plant in the next generation is solely the product of one parent. Because the parent is true-breeding, there is no genetic variation. In a commercial barley crop all plants are genetically similar and their characteristics are reproduced exactly from one cropping season to the next.

Barley genetics
Barley is a diploid species which means that all the cells of the plant, except the reproductive cells, carry pairs of chromosomes (called homologous pairs). Barley contains seven pairs of homologous chromosomes resulting in a diploid number of 14. The reproductive cells (pollen and egg) are haploid and carry only one member of each pair of homologous chromosomes. At fertilisation, the union of pollen and egg cell restores the chromosome number of the embryo to diploid.

At least 24 doubled haploid barley varieties have been released since 1980, although these were not produced from anther culture. To date, three wheat varieties and more than 100 strains and varieties of rice have been released using anther culture. The first successful plant regeneration from barley pollen was reported in the early 1970s, although regeneration rates were extremely low. Research throughout the 1980s led to significant improvements by altering the carbohydrate source of the media from sucrose to maltose, and by changes in the chemical that solidifies the culture media (gelling agent). Today, barley anther culture laboratories are servicing breeding programs in Canada, China, Europe, Japan, UK, USA and Australia.

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Special thanks also to Dr Sue Logue (University of Adelaide) and Dr Phil Davies (South Australian Research and Development Institute) who greatly assisted in the establishment of the laboratory.

Above right: Rate at which genes become fixed in the generations following hybridisation.

Below right: Green plant regeneration from anther culture for some barley varieties.

* Results courtesy of Dr P. Davies (SARDI) and Dr S. Logue (University of Adelaide).