Cost effective doubled haploids for accelerated wheat and oat breeding

Summary

The project successfully regenerated wheat double haploids (DHs) using isolated microspore culture (IMC) technology at levels similar to those for commercial barley breeding. This is the first time such high levels of regeneration have been achieved using IMC in Australia, and at 55% of the cost for using DHs in the existing wheat x maize method.

The project also successfully regenerated oat DHs at levels far greater than achieved previously. A key improvement was the incorporation of 1% activated charcoal in the medium. A surprising finding was the high (80%) level of spontaneous chromosome doubling in regenerant plants, eliminating the need for colchicine treatment if IMC was used for breeding.

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Conclusions

Wheat
The IMC method was successfully applied to Australian wheat germplasm, providing levels of plant regeneration similar to the barley IMC method. One disadvantage with wheat is that there is a low level of spontaneous chromosome doubling (20%) compared to barley (more than 70%) so that colchicine treatment of regenerant wheat plants is necessary. This incurs an additional cost of production. Even with this additional cost, the IMC method reduces the cost per DH plant by almost half (55%) compared to the wheat x maize method previously used. Because of the increased costs of labour, energy and infrastructure, the overall costs of DH production for wheat and barley have exceeded the economic threshold required by commercial breeding companies, which are now seeking alternative methods for accelerated breeding.

Oats
The IMC method for oats, developed at SARDI, has been improved significantly to produce DHs at approx. 20% of the efficiency of barley and wheat. Although this is a significant achievement from a scientific viewpoint, it is not of sufficient magnitude to allow the technique to be adopted by the National Oat Breeding Program.

General comments
The economics of DH implementation for breeding are no longer favourable. Since 1997, seven barley and 11 wheat varieties have been released by Australian doubled haploid (DH) laboratories and breeding programs to deliver significant gains to the grains industry by the early adoption of significant yield traits. The project contributed to big improvements in wheat and oats DH, but these benefits cannot be realised in the existing economic climate.

Recommendations

Recommendations:
1) That methods developed in this project be published in scientific journals so they can be implemented if the economic climate becomes favourable for their adoption.
2) That scientists and technicians with expertise in the field of plant cell culture and doubled haploid (DH) production be employed in other roles related to cereal breeding so their expertise can be called on if required.

Outcomes

Objectives
This project aimed to develop techniques to deliver more efficient and commercially viable production of doubled haploids
(DHs) for wheat and oats.

**Background**

DH technology bypasses the natural sexual reproductive processes to create genetic efficiencies in plant breeding. It allows cereal varieties to be released three to five years earlier than conventional methods and has resulted in the development of several Australian wheat and barley varieties. But the use of wheat DHs in breeding has declined significantly because of the relatively high technology costs. For breeding oats, it is not yet efficient enough for commercial use.

**Research**

This project adapted the isolated microspore culture (IMC) method, used successfully for barley DH production, to produce DHs for wheat and oats. A method was developed for wheat that produces plants at a similar frequency to the barley method. Two IMC derived populations have been provided to breeding companies. For oats, the level of plant regeneration has improved, but it is still only about 20% of the level for wheat and barley.

**Outcomes**

A useable wheat IMC protocol has been developed, which is available to breeders.

**Economic:**

Doubled haploids (DH) plants are homozygous and true-breeding, and when incorporated into cereal breeding programs can decrease the time to produce new varieties by three to five years with significant economic gains for the grains industry. But the costs to produce DHs is increasing with costs of technical labour and rising energy costs. This project succeeded in producing wheat DHs at about half the cost of methods available before this project, which is a significant achievement. Whether this will be sufficient to allow its long-term adoption for commercial breeding is yet to be tested.

**Environmental:**

Environmental outcomes from breeding relate primarily to producing disease resistant varieties that require fewer fungicide applications, more weed-competitive varieties that need less herbicide and varieties less dependent on fertiliser inputs because of efficient nutrient use. All these outcomes can be accelerated through the use of DH technology.

**Achievements/Benefits**

The aim of this project was to develop methods for doubled haploid (DH) production in wheat and oat using the isolated microspore culture (IMC) technique. For wheat, the specific aim was to develop a method that was efficient as the IMC method used commercially for barley breeding. For oats, the specific aim was to develop a method efficient enough to deliver DHs to the National Oat Breeding Program.

**Wheat**

During the course of this project, more than 4,000 wheat spikes were cultured in experiments designed to achieve maximum microspore growth and plant regeneration.

An isolated microspore culture (IMC) method for doubled haploid (DH) production in wheat has been successfully developed and two breeding populations have been provided to breeding companies.

Critical findings for success of the method were:

- Culture temperature of 28°C is far more successful than 22°C for the development of microspore-derived colonies.
- W14 basal medium produces more microspore-derived colonies than many other media, including 190-2 (which is the second best basal medium) and the standard barley medium, KFWC.
- 0.3M mannitol pre-treatment of wheat spikes at 4°C produces more colonies and regenerant plants than spikes pre-treated at 4°C in water.
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Genotype has an impact on capacity to regenerate, but in general wheat has similar levels of regeneration to the best barley IMC protocol.

- Levels of spontaneous chromosome doubling in wheat (20%) are much less than in barley (approx. 70%) so chemical doubling using colchicine is essential.
- Attempts to double the chromosome number \textit{in vitro} in this project were unsuccessful.
- Costs of wheat DH production using IMC were compared with the wheat x maize method and the barley IMC method. The cost of a wheat DH plant produced using IMC is about 45% of the cost of a wheat DH produced by the wheat x maize method. The cost of a wheat DH produced using IMC is 15% higher than the cost of barley because of the additional chromosome doubling step required.

Oats

Throughout this project, 4,000 oat panicles were cultured in experiments designed to achieve maximum growth and plant regeneration.

Excellent microspore growth and improved levels of plant regeneration have been achieved from oat IMC.

Critical findings were:

- Barley ovaries significantly increase the proportion of oat microspores that will grow to form colonies.
- 2, 4-D is an essential growth regulator for the induction of microspore development and plant regeneration.
- The growth regulators in the induction medium are more critical for green plant regeneration than growth regulators in the regeneration medium.
- Cold pre-treatment (4°C) of panicles in water is more effective than cold pre-treatment in 0.3M mannitol.
- 190-2 is a more effective basal medium than W14.
- Consistent levels of plant regeneration are not possible without the addition of 1% activated charcoal to the solid regeneration medium.
- There is a high level (85%) of spontaneous doubling in regenerant plants.

Additional information

Publications

Papers on isolated microspore cultures (IMCs) are being prepared for peer-reviewed scientific journals.