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## **New Cereal Doubled Haploid Facilities in Western Australia and the status of the Barley Doubled Haploid Program**

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# **Introduction**

The Department of Agriculture has invested in a new and expanded cereal doubled haploid facility for the Western Australian breeding programs. The establishment of the facility at South Perth is a \$1.2 million investment for the Department and provides the state with the capacity to maximise the benefits of this exciting technology to the barley and wheat breeding programs to the benefit of the Western Australian grains industry.

Doubled haploid technology has been used in the Department's barley and wheat breeding programs for several years with the most advanced doubled haploid lines now in advanced stages of the Crop Variety Testing Program. Two doubled haploid barley lines are scheduled for pilot brewing in 2001.

Doubled haploids form a significant part of the barley program, comprising approximately 25% of the total program and over 40% of the Stage 2 material (re-selected, fixed lines). Marker-assisted selection is well integrated into the program with disease and quality markers utilised in screening both pre- and post-tissue culture.

# **New Facilities in Western Australia**

The new facilities comprise four large controlled environment rooms as well as new laboratories, offices, plant handling areas and a new polycarbonate greenhouse. The expanded facilities will allow significant increases in doubled haploid production which is expected to treble in the next three years.

A significant part of the investment was the construction of four large controlled environment rooms (6 x 3 m) by Phoenix Research. Temperature, humidity, light

intensity and daylength can be carefully controlled and the rooms are used for plants both pre- and post tissue culture and are vital for the successful year round production of doubled haploids.

A large polycarbonate greenhouse was constructed by Advanced Environmental Structures. Rolling benches were used to maximise space and capillary matting was fitted to the benches with an automatic watering system.

# Barley Doubled Haploid Production in Western Australia

Crosses scheduled for doubled haploid production in 2001 are listed in Table 1. Green plant numbers, to date, are indicated. Thirty-seven crosses will be processed, with the aim of producing about 4,000 doubled haploid lines.

Selected crosses were prescreened for the *mlo* allele (resistance to powdery mildew) or the sd3 beta-amylase allele (improved b-amylase thermostability) using SNP markers (Paris *et al.*, 2001 a & b). Doubled haploid material segregating for these alleles, as well as Yd2 (resistance to barley yellow dwarf virus), will also be post-screened.

The doubled haploid program was relocated from the University of Western Australia's Field Station, Shenton Park, in September, 2000. Our first year in the new facilities has been somewhat experimental as we learn how the plants respond to their new growth conditions and cope with different pest and disease pressures.

**Table 1. Crosses in the Western Australian Barley Doubled Haploid Program (2001)**

<b>Pedigree</b>	<b>No. DHS Req'd</b>	<b>Method<sup>1</sup></b>	<b>Blended for IMC<sup>2</sup></b>	<b>Marker Prescreened</b>	<b>No. Green Plants</b>
TR251/2*Gairdner	200	AC			638
TR251/Gairdner//WABAR2080	200	AC/IMC	A, F		645
Alexis/Gairdner//WABAR2080				discarded, no mlo	
WABAR2080/Alexis//WABAR2080				discarded,	

				no mlo	
Kaputar/Alexis//Fitzgerald	100	AC/IMC	A, F	mlo	136
Kaputar/Alexis//Gairdner	100	AC/IMC	A, F, S	mlo	253
Kaputar/Alexis//WABAR2080	100	AC/IMC	A, F, S	mlo	154
WABAR2080/Skiff//Gairdner	100	AC			65
WABAR2080/Skiff//WABAR2080	100	AC			7
Optic/WABAR2080	100	AC			125
WB220/WABAR2080	100	AC			15
Chebec/WABAR 2110	100	AC			1
Chebec/WABAR2080	100	AC			189
Keel/WABAR 2110	100	AC/IMC	A, F		52
WABAR 2096/WABAR2080	100	AC/IMC	A, F, S		12
WABAR 2104/Keel	100	AC/IMC	A, F, S		662
B x 98A: 057/85	200	AC/IMC	S		97
WABAR2080/Keel	200	AC/IMC	S		536
WABAR 2104/WABAR2080	100	IMC	S		125
WI-3075/WABAR2080	100	IMC	S		41
Scarlett//WABAR2080	100	IMC	S		144
WB220/Gairdner//WABAR2080	100	IMC	S		201
Shyri/Gairdner	100	IMC	S		
Shyri/WABAR2080	100	IMC	S		
WABAR 2110/WABAR2080	100	IMC	S		
WABAR 2094//WABAR2080	100	IMC	S		
WABAR 2096/Keel	100	IMC	S		
TR250/Dekardo//WABAR2080	100	IMC	S		
TR250/Optic//WABAR2080	100	IMC	S		
TR250/Optic//47-31	100	IMC	S		
TR250/Kinu Nijo//WABAR2080	100	IMC	S		
WADH10307/WA5040	100	AC/IMC			
WADH10307/WA6370	100	AC/IMC			
WADH10307/Harrington	100	AC/IMC			
AB75//Fitzgerald	100	IMC		thermostable B-amy	
AB75//Gairdner	100	IMC		thermostable B-amy	
Magnif104/Prosa	100	AC/IMC			
Onslow/Gairdner	100	AC			
WABAR2104/WABAR 2110	100	AC			

<b>Total</b>	<b>4100</b>				
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<sup>1</sup> Method - AC anther culture; IMC isolated microspore culture

<sup>2</sup> Blended - A anthers, F florets, S whole spikes

Methods used for barley doubled haploid production include anther culture and isolated microspore culture (IMC).

## Anther Culture

Our anther culture results (green plants per spike) have been slightly lower than previous years, with an average of about 4 green plants per spike over 15 crosses. Some crosses have performed well, yielding about 11 green plants per spike, eg. TR251/2\*Gairdner and TR251/Gairdner/WABAR 2080. Other crosses have proved particularly recalcitrant, yielding less than 1 green plant per spike, eg. Chebec/WABAR 2110, WABAR2080/Skiff/WABAR2080, WABAR 2096/WABAR2080 and WB220/WABAR2080. Plant numbers for these crosses are very low (Table 1).

## IMC

We commenced the 2001 program testing a homogeniser-style blender (Ystral D-79282) which has proved beneficial for wheat microspore culture (Patel, 1997). Compared with the Waring blender, there were no advantages when blending barley anthers, and disadvantages when blending whole spikes or florets. Using the Ystral blender, it took longer to achieve the same results obtained with the Waring blender, possibly damaging microspores. Additionally, it was more difficult to clean spike debris from the Ystral blender.

After returning to the Waring blender, we concentrated on whole spike blending and made improvements by increasing the spike number per blend, from six to ten. Our current IMC protocol is based on that of Davies and Morton (1998) with some modifications from Kasha *et al.* (2001).

Key points in pretreatment and isolation include:

- 1 Selecting spikes with microspores at an optimum stage for IMC,
- 2 Mannitol or cold pretreatment,
- 3 Blending whole spikes (10 per blend) using a Waring blender with container 8580,
- 4 Additional wash after first centrifugation,
- 5 Isolating viable microspores on a maltose/mannitol gradient,

6 Suspension of microspores at  $1 \times 10^5$  -  $4 \times 10^4$  per ml in liquid drops of liquid KFWC medium,

7 Keeping microspores cold ( $4^{\circ}\text{C}$ ) during isolation by using chilled equipment and chilled liquid media. Cells kept on ice during rest phases.

The most exciting aspect of the IMC protocol outlined above is that we are now routinely blending whole spikes. The alternative, anther removal prior to blending, is a time-consuming process. Previous attempts at whole spike blending, in our laboratory, have been largely unsuccessful but a critical factor appears to be the use of refrigerated equipment and solutions during microspore isolation (Kasha *et al.*, 2001).

Inconsistency in response between blends means that we are still processing six to eight blends per cross, to ensure we obtain the desired numbers of doubled haploid lines. However, we were obtaining similar inconsistencies when blending anthers, so blending whole spikes is an attractive speedier option. Often one successful blend can produce sufficient regenerant plants (ie. 200 + plants) for an entire cross. The challenge is to increase consistency and reduce the number of blends so that we can process more crosses.

Mannitol pretreatment is our preferred pretreatment at this time. It involves pretreating whole spikes in liquid 0.3 M mannitol for 3-5 days at  $4^{\circ}\text{C}$  (Kasha *et al.*, 2001). Although data are not yet finalised, the yield of viable microspores appears consistently greater, culture response appears better and from results to date, green plant numbers are also higher.

Genotype variation continues to be a problem and generally crosses that respond poorly to anther culture will also respond poorly to IMC. In our laboratory, recalcitrant crosses generally perform slightly better with anther culture than with IMC. Important factors that we can control include donor plant health and microspore stage. These factors are vital to the success of IMC and anther culture and should not be underestimated.

The lighting configuration in our new controlled environment rooms dictates the use of either metal halides or sodium vapour lamps. At present, metal halide lamps are installed. However, anecdotal evidence from the South Australian barley doubled haploid program suggests that sodium vapour lamps may provide a superior light source for donor plants destined for microspore culture. We are considering changing the lamps in one controlled environment room to sodium vapour for the 2002 program.

## Conclusion

It appears that whole spike blending can be used successfully in routine barley doubled haploid production. Certain factors, such as chilling equipment and solutions during isolation, appear important. Factors such as microspore stage and donor plant growth conditions are also crucial. The challenge for our program is to improve consistency and green plant response with whole spike blending and IMC.

# Acknowledgements

Funding was provided by the GRDC, the Grain Pool of WA, Joe White Maltings Pty Ltd, Kirin Australia Pty Ltd, The Matilda Bay Brewery and The Swan Brewery Company Ltd. Technical support from Melissa Beeck and Ian Watson is gratefully acknowledged.

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