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PROGRESS IN WHEAT AND BARLEY HAPLOID INDUCTION USING ANTHER CULTURE

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ABSTRACT

Anther culture procedures in wheat and barley have been improved by: the use of liquid media BAC 1 and its replenishment; studies on concentrations of auxins, sucrose, glucose, inositol, Ficoll and agar (when used); anther innoculation density; culture temperatures; and pectolyase degradation of anther walls. A time schedule, technical procedures and hours required for the production of seed from doubled haploids in winter wheat are presented. Green haploid plant production is sufficient for the incorporation of these procedures into wheat breeding programmes. However, the currently used Bulbosum Method is more efficient for haploid use in barley breeding. Problems remaining are discussed.

KEYWORDS

Bulbosum Method, albinism, anther culture, wheat, barley.

INTRODUCTION

Doubled-haploid plants derived from anther culture have been used effectively for varietal development (Hu Han, 1984; Kasha and Seguin-Swartz, 1983) and, inevitably, this technology will be used more extensively in innovative programmes of winter cereal breeding. This paper outlines current research in anther culture of wheat and barley at the University of Guelph and indicates the directions of future research. Our goal is to reduce dramatically the time scale involved in the evaluation of crosses and the production of cultivars. An evaluation of the level of success recently achieved in haploid plant production from winter wheat hybrids and a schedule for the use of anther culture in an integrated breeding programme are presented. The Bulbosum Method of haploid production in barley is described by Falk and Guerrero (1986).

RECENT PROGRESS

The goal of current research has been to find a simple and reliable method of anther culture which permits microspore induction, cell growth, and embryogenesis in a range of genotypes. Research has recently centered on the use of liquid culture media (in contrast to semi-solid media with gelling agents such as agar and agarose) because media factors can be rapidly altered without transferring anthers. We found that the induction and development of embryoids within the anther appears to occur at similar frequencies in either liquid or agar medium. The use of liquid media also avoids the problem of dessication of emerging embryoids which may occur when using gelled media.

In the anther culture technique used for wheat and barley, anthers are cultured on BAC 1 induction medium containing high levels of auxin (8 mg/litre 2,4-D), sucrose (9%), and inositol (2000 mg/litre) for 10 days (Marsolais and Kasha, 1985). The medium is replenished at 10-day intervals with a medium containing low levels of IAA (1 mg/litre), 3% sucrose, and no inositol to promote embryogenesis. At the end of the incubation phase both the pH and water potential of the medium decreased (J. Lettre, pers. comm.); the replenishment medium apparently adjusts these conditions. Other factors which have been found to improve anther response and embryoid production are high inoculation density (20-80 anthers per ml), culture temperature (28 °C for barley, 30 °C for wheat), and Ficoll concentration (100-200 g/litre depending on the genotype). Other polysaccharides such as Dextran and soluble starch can be used at appropriate concentrations to replace the Ficoll component.

Preliminary studies with wheat indicate that anther response and embryoid production increase when the anther walls are partially degraded by 0.1% pectolyase for two hours before culture. The treated anthers enlarge in size relative to untreated anthers. Enzyme pretreatment may hasten equilibration and allow more efficient exchange between the medium and the microspores within the wheat anthers (which have relatively thick locule walls in comparison to barley). Other studies have indicated that moderate levels (17.5 g/litre) of glucose in the medium is generally best for embryoid induction but higher levels (30 g/litre) may improve the rates of green plant regeneration in some genotypes (S. Kelly, pers. comm.).

Research is being directed towards the characterisation of the conditioning factor, a tissue-produced factor which promotes early cell divisions within the microspores of cultured anthers. Inositol interacts synergistically with the conditioning factor (Xu and Sunderland, 1981). Isolation

Table 1. Evaluation of plant production from five winter wheat hybrids using anther culture.

Cross	Number F1 plants	Number spikes used	Number anthers plated	Number and % responsive anthers		Number and % embryoids produced		Number green plants
82-17 x Frederick	10	33	1876	305	16.3	853	45.5	210
Frederick x 82-17	9	32	1615	251	15.5	674	41.7	108
Harus x 82-17	10	30	2208	308	13.9	708	32.1	123
Houser x 81-5	10	23	1703	36	2.1	61	3.6	6
Houser x Harus	10	24	1184	44	3.7	83	7.0	3
Total	49	142	8586	944		2379		450
Mean (%)					11.0		27.7	

of the conditioning factor should greatly improve anther response and embryoid production in different genotypes in both wheat and barley and reduce the number of donor plants required.

HAPLOID PRODUCTION IN WINTER WHEAT HYBRIDS

Recent work with five winter wheat hybrids demonstrated that anther culture techniques can produce sufficiently high numbers of green plants (Table 1) with relatively low technical labour requirements (Table 2) and growth room space. Two classes of winter wheat genotypes were used as parents for the hybrids in this study: (1) cultivars that were moderately good for anther culture (Harus, OAC 82-17); (2) cultivars that were poor for anther culture (Frederick, OAC 81-5, Houser). Crosses were chosen on the basis of their potential for plant breeding in Ontario. After vernalisation, nine or 10 plants of each F1 hybrid were used (two plants per 15 cm pot) requiring a total of 1.8 m^2 of growth room space. Tillers were taken from these plants and the section of the sheath containing the spike was swabbed in 75% ethanol before the spike was removed. The spike was not sterilised anymore. About 100-150 anthers bearing microspores at the mid-uninucleate stage were then cultured in 60 x 15 mm petri dishes on 3 ml of induction medium containing BAC 1 nutrients, 100 g/litre Ficoll, 8 mg/litre 2,4-D, 2000 mg/litre inositol, 60 g/litre sucrose and 17.5 g/litre glucose for 10 days at 30 °C within a dark incubator (Marsolais and Kasha, 1985). At 10-day intervals thereafter, 1 ml of medium was drawn off and replaced with 1 ml of replenishment medium containing BAC 1 nutrients, 100 g/litre Ficoll, 1 mg/litre IAA, 30 g/litre sucrose and 17.5 g/litre glucose. Embryoids were transferred when they were about 2 mm in diameter (about 35-40 days after initiating culture) onto MS medium containing 1 mg/litre IAA, 1 mg/litre kinetin, 146 mg/litre glutamine and 0.7% agar (Schaffer *et al.*, 1979) and incubated under low light conditions at 22-24 °C for plant regeneration.

Table 2. Time study of doubled-haploid seed production using anther culture for all five winter wheat hybrids in Table 1.

Activity	Technician hours	
1) Preparation of 2 batches of induction medium	6	
2) Preparation of 3 batches of replenishment medium	9	
3) Anther plating: about 800 anther/hour	10	
4) Preparation of 3 batches of regeneration medium	12	
5) Transfer of embryoids to regeneration medium	34	
6) Transfer of regenerated plants to flats	10	estimated
7) Colchicine doubling of haploid and repotting	30	estimated
8) Harvesting doubled-haploid seed	10	estimated
TOTAL	121	estimated

There was 11% anther response and 27.7 embryoids produced per 100 anthers plated when figures for the five crosses were averaged (Table 1). In total, 450 green plants were regenerated from the five crosses. The entire procedure from media preparation to doubled-haploid seed harvesting took an estimated 121 technician hours of labour during several months (Table 2). The costs to a breeding programme seem reasonable considering the overall time advantages which can be realised. Genotypic variation for anther culturability was observed but it is not necessarily restrictive. Although some crosses had a low response such limitations could be overcome by increasing the number of plants grown and increasing the number of anthers cultured to ensure adequate recovery of green plants. Additional effort could also be devoted to crosses of special interest by growing additional plants of each cross at 2-3 week intervals, thus enabling more time to be spent later on those crosses which were observed initially to have low response. While most crosses should give adequate numbers of doubled haploids a few will nevertheless either not respond to the present methods or will mostly produce albinos.

Doubled-haploid methods are employed to the best advantage when integrated into a conventional breeding

Table 3. Winter wheat doubled-haploid programme.

Year	Month	Activity
1	mid-August	Seed parents for crossing block; vernalise
	early-December	Make crosses in growth room/cabinet
	mid-December	Excise F1 embryos and culture to regenerate F1 plants
	early January	Vernalise F1 plants
	mid-February	Grow F1 plants in growth room/cabinet
	early April	Culture anthers
	early May	Regenerate plants from embryoids
	early June	Double haploids with colchicine, pot plants; vernalise
2	early October	Harvest doubled-haploid seed
	mid-October[3]	Seed hill plot preliminary yield trial and observation nursery
3	August[1]	Harvest hill plots
	September	Seed replicated yield trial
	August	Harvest yield trial
4	mid August[2]	Seed parents for crossing back

[1]Total time to re-cycle advanced lines = 24 months.
[2]Total time through cycle = 36 months.
[3]Except for regions with early winter conditions e.g. Western Canada, Northern USA.

system. For example, F1 hybrids from crosses between adapted lines having relatively few gene differences could be handled directly using anther culture, whereas crosses between adapted and exotic lines could be pre-selected at the F2 level and the population subsequently used for both anther culture and bulk population selection. The F2 seed available from the F1 donor plants used in anther culture, in particular those which prove difficult to culture, can be further used without jeopardising the breeding programme.

Valentine (1984) suggests that doubled-haploid methods do not compare favourably with accelerated pedigree methods. Table 3 outlines a doubled-haploid breeding programme for winter wheat employing both anther culture and embryo rescue techniques. This scheme could be used to produce, evaluate, and re-cycle advanced lines in two years. This compares to the three-year interval in the accelerated pedigree selection system. Another major advantage of the doubled-haploid programme is the ability to select among uniform homozygous lines. Also the growth room space requirements are not unusually large in contrast to the needs of the accelerated pedigree selection methods. Therefore, the use of doubled haploids derived from anther culture saves time and thereby increases the genetic gain per year, conserves growth room space, and simplifies selection. In addition, anther culture may be more readily integrated with *in vitro* selection and genetic engineering methods as these biotechnologies develop.

REMAINING PROBLEMS

While progress has been achieved in anther culture of wheat and barley some problems remain which, when overcome, would ensure more widespread use of this technology for breeding. For wheat in general, the problem occurs early in the culture process during the microspore induction phase: the frequency of microspores within responding anthers which generate viable embryoids is low. However, embryoid quality and green plant regeneration is acceptable in most genotypes. In barley, the induction phase has not been the limitation, it is the occurrence of albinism in many genotypes. The block which inhibits chloroplast development in barley may be caused by developmentally programmed deletions within the plastid genome of the microspore or by the lack of proper physiological signals during culture which prompt chloroplast development within the embryoids (Dunwell, 1985). Our observations of wheat suggest that the carbohydrate levels within the media might strongly influence the rate of recovery of green plants. This effect encourages us to speculate that altered media composition may also prove beneficial in barley.

Somaclonal variation may by induced by the culturing of anthers and may, depending upon the situation, be advantageous or disadvantageous. The literature contains contradictory evidence (mostly in tobacco) on its existence and importance. The contradictions are evidence that this variation may be controlled and further research on this topic is required.

CONCLUSIONS

Anther culture for doubled-haploid production in winter wheat has reached a level where it can now be incorporated with a conventional breeding programme. Efforts to isolate the conditioning factor which assists the induction process, and a better understanding of the physiological interactions during anther culture could improve anther response and embryoid production. In barley, albinism remains the limiting factor for the use of anther culture. Anther culture procedures are being used in

barley breeding (Freidt and Foroughi-Wehr, 1983) but its efficiency has not been comparable to the more widely used Bulbosum Method (Huang *et al.*, 1984). Thus, the Bulbosum Method (reviewed by Kasha and Reinbergs, 1981; and Kasha and Sequin-Swartz, 1983) remains the best method for obtaining homozygosity in one generation until anther culture limitations are overcome.

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SYMPOSIUM DISCUSSION

Prof. D. von Wettstein, Carlsberg Laboratory

It is said that the haploid technique would be useful in combining genes that are close together. Do you have a good example where this has been achieved in barley or wheat?

Kasha

No. There has been some theoretical work done looking at linkage and the suggestions are that where genes are linked, it is better to go to F_2 or perhaps F_3 to produce double haploid because with the cross linkage there would be further chance for combination in F_2.

Efficiency of recombination is not good enough to warrant taking it any further than F_3. In most of the studies where there are different groups involved, the haploids are a random sample of the gametes, so it should be possible to use a smaller population size of double haploids versus an F_2 population.