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Genetical analysis of microspore derived plants of barley (*Hordeum vulgare*)

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Summary. From an F_1 hybrid between the two barley (*Hordeum vulgare* L.) cultivars 'Golden Promise' and 'Mazurka' a series of doubled haploid (DH) lines were generated both from microspores by anther culture and from immature zygotic embryos after hybridization with *H. bulbosum*. The DH lines from both sources were used to monitor the segregation of the five major genes, rachilla hair length, DDT susceptibility, height, C hordein polymorphism and mildew resistance. Whereas the microspore-derived samples showed significant departures from the expected 1 : 1 ratio for three of the five genes, the *H. bulbosum* lines showed deviation for only one gene. Analysis of linkage data also showed differences between the two series of DH lines. Cytogenetic analysis revealed a mean chiasma frequency in the *H. bulbosum* lines which was very similar to the F_1 hybrid. In contrast, four of the ten microspore derived lines examined showed a reduced chiasma frequency. One showed evidence of translocation heterozygosity.

Key words: Barley – Doubled haploids – Somaclonal variation – Breeding

Introduction

Haploidisation and subsequent chromosome doubling to produce doubled haploids allows the development of completely homozygous lines from heterozygous parents in a single generation. Hence, the production of doubled haploids in large numbers is of great value to plant breeders and geneticists.

In cultivated barley, *Hordeum vulgare,* there are four principal methods of isolating haploids: the culture of microspores (Dunwell 1985), the culture of embryos following the interspecific hybridisation of *H. vulgare* with *H. bulbosum,* ovule culture, and the use of the haploid initiator gene (Kasha and Reinbergs 1982). However, only the *bulbosum* technique has been used in cultivar production (Choo et al. 1985). Although anther culture has been used in programmes to produce genotypes resistant to barley yellow mosaic virus (Foroughi-Wehr and Friedt 1984) its use in cultivar production is limited by a number of factors. These include the low frequency of regenerated plants and the high proportion of albinos amongst the regenerants (Day and Ellis 1985). However, the recent increases in efficiency achieved as a result of improvements in precultural and cultural methods (Huang and Sunderland 1982; Shannon et al. 1985; Lyne et al. 1986) suggest that this technique may be sufficiently advanced to be tested in barley breeding programmes.

The culture of barley anthers involves the induction of microspore derived callus, followed by the regeneration of plants, many of which are diploid. These non-haploids will be homozygous if they are derived from a single haploid cell but plants produced from unreduced gametes have been reported in many species including rye and potato (Wenzel et al. 1976). Furthermore, the anther is a complex structure consisting of both sporophytic and gametophytic tissues and the source of regenerating structures must be verified. Isozyme analysis (Corduan 1975; Zamir et al. 1981) is one method used for this purpose. It is also desirable that doubled haploids produced by anther culture represent a random sample of the parental gametes. Two studies (Chen et al. 1982, 1983), using a number of heterozygous lines of rice with either linked or unlinked markers, showed that the segregation ratios were as expected. However, there is evidence from barley (Kao et al. 1983; Foroughi-Wehr and Friedt 1984) for non random segregation and analogous data exist in *Brassica* (Orton and Browers 1985).

The present study on barley was conducted to examine the segregation ratios in microspore derived lines from an F_1 hybrid heterozygous for a number of major gene loci. These ratios were also examined in a similar series of lines produced by the *H. bulbosum* technique.

Materials and methods

Plant material

The F_1 hybrid of the cross 'Golden Promise' × 'Mazurka' was used in this study. The genetic markers segregating from this cross, their chromosomal locations and symbols used to represent them are given in Table 1.

Tissue culture methods

The methods of anther culture and the *H. bulbosum* techniques were those described previously (Huang et al. 1984; Powell et al. 1986). If the original doubled haploid plants produced from each technique are designated as generation 1, the material used for the analyses below were taken from generation 4.

DDT testing

Lines were tested for their reaction to DDT by spraying, at the two leaf stage, 16 plants from each family with a 0.2% aqueous emulsion of DDT (Hayes and Rana 1966). One week later, the plants were scored as being either resistant or susceptible to DDT.

Mildew testing

Plants were grown in a spore proof greenhouse to the 3–4 leaf stage. Two segments, 35 mm in length, were cut from the middle of the youngest, fully expanded leaf on each plant and placed adaxial side uppermost on 0.5% agar containing 100 mg/l benzimidazole. The duplicate leaf segments from each line were arranged at random in a series of polystyrene boxes. The segments were inoculated in a settling tower with spores blown from an infected leaf segment. The culture CC/35 (virulent on *Mlg* and *Mlk* only) was used to score the segregation of the resistance gene *Mla7* among the F_1 doubled haploid derivatives. Following inoculation, leaf segments were incubated for 8 days at 17 °C with 12 h illumination per day. At the end of this period, the number of mildew colonies per leaf was counted and each family recorded as resistant or susceptible (resistant lines being identified by the absence of powdery mildew colonies).

Cytology

Seed of the microspore-derived lines and control plants were germinated on filter paper and primary roots sampled. Roots were pretreated in cold water (4 °C) 16 h before fixation in ethanol : glacial acetic acid (3 : 1), hydrolysis in 1 N HCl at 60 °C for 10 min, and staining in feulgen. Meiosis was examined in pollen mother cells, using inflorescences fixed, hydrolysed and stained as above. Estimates of chiasma frequency were made by scoring the number of arms bound by chiasmata at metaphase I.

Hordein analysis

a) Sample preparation. The embryo and a small part of the endosperm were dissected from the seed and retained for germination. The remainder of the endosperm was finely ground using a pestle and mortar and the resulting meal suspended in 0.2 ml of 55% v/v propan-2-ol containing 1% w/v dithiothreitol. The suspension was then placed in a heated water bath at 60 °C and periodically shaken. After 30 min the mixture was centrifuged at 5×10^3 rpm for 10 min and an aliquot of the supernatant removed and subsequently analysed.

b) Protein separation. The B and C hordeins were separated by high pressure liquid chromatography using a Gilson dual pump gradient chromatograph fitted with a 6.0 mm × 4.1 mm precolumn packed with Synchoprep RP-P (30 µm diameter, Anachem Ltd) and a 250 mm × 4.1 mm column packed with Synchropak RP-P [reversed phase (C_{18}) support 6.5 µm particle diameter, 300 Å pore diameter, Anachem Ltd.]. Column temperature was maintained at 25 °C and the elutant monitored at 210 nm (AUFS 0.5). Peak areas were integrated using a Shimadzu C-R3A chromatapac programmed for area normalisation.

The elution profile used for the separation of the proteins was as outlined by Marchylo and Kruger (1984) and consisted of a two-solvent system: A, 15% v/v acetonitrile containing 0.1% v/v trifluoroacetic acid; B, 80% v/v acetonitrile containing 0.1% v/v trifluoroacetic acid. The concentration of solvent B was increased from 25% to 60% over a period of 105 min and reduced gradually back to 25%. A regeneration time of 15 min was allowed between sample loading (20 µl) and the column regenerated daily by employing a steep linear gradient from 25% to 80% over a period of 10 min followed by a reduction to 25% over a further 10 min. All solvents used were of HPLC grade and degassed prior to use. Flow rate was maintained at 1.0 ml/min.

Ninety-six microspore-derived lines were screened for the five major gene markers. Since these progenies originated from 14 distinct calli, analyses for segregation at a single heterozygous locus and for the detection of linkage were based on these 14 values.

Results

Doubled haploids (DH) produced from the F_1 will segregate for rachilla hair type, reaction to DDT, growth habit, polymorphism at the C hordein locus (the B locus pattern did not differ in the parents) and mildew reaction. Variation between these DH lines is equivalent to that expected from a backcross of the F_1 to the double recessive parents. In the absence of

Table 1. Summary of genetic markers, their chromosomal location and gene symbol designations used in the linkage tests

Character pair	Gene		Symbol	Chromosome location (ref)
1. Long vs Short haired rachilla	*S*	v	*s*	7 (Nilan 1964)
2. DDT susceptibility vs resistance	*Ddt*	v	*ddt*	7 (Hayes and Rana 1966)
3. Tall vs dwarf	*Ert*	v	*ert*	7 (Thomas et al. 1984)
4. C hordein polymorphism	*A*	v	*B*	5 (Shewry et al. 1980)
5. Mildew resistant v susceptible	Mla_7	v	*O*	5 (Wolfe and Schwarzbach 1978)

Table 2. Data from F_∞ samples from M and Hb doubled haploids showing segregation ratios for genetic markers located on chromosome 5 and 7

Sample	Marker and χ^2 value														
	ert	*Ert*	χ^2	*S*	*s*	χ^2	*Ddt*	*ddt*	χ^2	*A*	*B*	χ^2	Mla_7	*0*	χ^2
M	4	10	2.57	3	11	4.57*	11	3	4.57*	11	3	4.57*	8	6	0.29
Hb	49	53	0.16	41	61	3.92*	47	38	0.95	6	5	0.09	18	16	0.12
Total	53	63	2.73	44	72	8.49*	53	41	5.52	17	8	4.66	26	22	0.41

* $P<0.05$

Table 3. Linkage tests for gene loci on chromosomes 5 and 7

a Chromosome 7

Sample

	ert/S	*ert/s*	*Ert/S*	*Ert/s*	*ert/ddt*	*ert/Ddt*	*Ert/ddt*	*Ert/Ddt*
M	1	3	1	9	2	2	1	9
Hb	29	20	12	41	31	14	7	33
Total	30	23	13	50	33	16	8	42

	Seg *ert/Ert*	Seg *S/s*	Linkage	Seg *ert/Ert*	Seg *Ddt/ddt*	Linkage
M	2.57	4.57*	2.57	2.57	4.57*	4.57*
Hb	0.16	3.92*	14.16***	0.29	0.95	21.75***
Total	2.73	8.49**	16.73***	2.86	5.52	26.32

b Chromosome 5

Sample

	A/O	A/Mla_7	*B/o*	B/mla_7
M	6	5	0	3

	Seg *A/B*	Seg Mla_7/O	Linkage
M	4.57*	0.29	1.14

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

linkage, equal frequencies of the phenotypic classes are expected (Mather 1938). The individual segregation ratios for the 5 loci studied are given in Table 2 for both the microspore derived and *Hordeum bulbosum* (Hb) samples. Individual segregation ratios are significantly different from 1 : 1 at three of the five loci in the microspore derived sample. In the Hb sample, only one locus, that for rachilla hair, differs significantly from the expected ratio.

The results of the linkage tests for rachilla hair type, DDT reaction and the *ert* locus are given in Table 3. Also given are segregation data for the hordein loci and mildew reaction which are located on chromosome 5. Considering data from the microspore derived sample it can be seen that there is a significant departure from the 1 : 1 : 1 : 1 ratio for the *ert* gene and rachilla hair ($\chi^2_{(3)}=9.71$ $P=0.05$–0.01). Linkage was not detected and the deviation from the 1 : 1 : 1 : 1 ratio was therefore due to abnormal segregation at the rachilla hair locus. Similarly, there was a significant departure from the expected ratio in the case of DDT reaction ($\chi^2_{(3)}=11.7$, $P=0.01$–0.001). Linkage and aberrant segregation at the DDT reaction locus were responsible for this departure. Hb-derived doubled haploid lines have been used previously in combination with F_2 and translocation stocks to locate the 'Golden Promise' *ert* locus on chromosome 7 (Thomas et al. 1984). The relevant data from that experiment have been tabulated (Table 3) and it is of interest to compare segregation ratios from the Hb and microspore-derived samples. The Hb data clearly indicate linkage of the *ert* locus to the rachilla hair and DDT reaction loci. Linkage between the *ert* locus and rachilla hair was not detected in the microspore derived sample, whereas

Table 4. Phenotypic correlations for the M (n = 14) and Hb (n = 20) samples

Character		Correlation coefficient					
		Single plant yield	Ear length	Grain no.	Height	Main stem wt	Thousand grain wt
Ear length	M	0.0818					
	Hb	0.0832					
Grain no.	M	0.7747	−0.4227				
	Hb	0.4089	0.1767				
Height	M	0.8769	0.3325	0.6069			
	Hb	0.7697	0.4871	0.1426			
Main stem wt	M	0.8347	−0.1778	0.8682	0.6887		
	Hb	0.8171	0.2315	0.6049	0.7181		
Tiller no.	M	0.8623	0.2856	0.5215	0.7964	0.4669	
	Hb	0.7802	−0.0308	0.0373	0.5403	0.3502	
Thousand grain wt	M	−0.2237	−0.5216	−0.1411	−0.3214	−0.1793	−0.3046
	Hb	−0.1197	−0.0782	0.1014	−0.2425	−0.3524	0.1322

linkage was detected between the DDT reaction and the *ert* locus. Furthermore, the *M1a* locus is known to be linked to the hordein loci on chromosome 5 (Shewry et al. 1980). However, a significant departure from a 1 : 1 : 1 : 1 ratio was not observed for alleles at these two loci in the microspore derived sample (Table 3, $\chi^2_{(3)} = 6.0$, $P = 0.3–0.1$) and hence caution should be exercised when attempting to use anther culture for linkage and gene mapping purposes.

The phenotypic correlations between all pairwise combinations of characters were obtained for the M and Hb samples and are given in Table 4. A correlation greater than 0.4973 for the M sample or 0.4227 for the Hb sample is significant ($P < 0.05$). In the absence of differential survival or selection, the phenotypic distributions of inbred populations derived by anther culture and the *H. bulbosum* technique should be identical. Similarly, the relationship between quantitatively varying characters should not differ significantly in these two samples. However, inspection of Table 4 indicates that the phenotypic correlations between: GN and EL, Ht and GN and TN and GN, although not formally significant, differ in the two samples. These differences are associated with the character grain number which is a fertility character. Indeed, a previous study (Powell et al. 1986) has shown that the means of the M sample were significantly lower than the mid-parental values for fertility charcters. These lower mean scores may be associated with cytological abnormalities.

Genetic markers have been used to determine whether regenerants from anther culture are of sporophytic or gametophytic origin. The 96 M lines used in this study may be traced back to 14 distinct calluses (see "Materials and methods"); all the regenerants examined were homozygous for the gene loci studied and are therefore of gametophytic origin.

A preliminary chromosome count allowed the classification of families according to their ploidy level. With the exception of one tetraploid family derived from callus number 2 all the other progenies were diploid.

Meiosis was examined in progenies derived from 10 of the 14 calluses. Pairing in the tetraploids (from callus 2) was largely a mixture of bivalent and quadrivalent configurations. Translocation heterozygosity was found amongst families from callus no. seven (Fig. 1). Progeny from the remaining eight calluses formed seven bivalents.

Mean chiasma frequency in the Hb progeny (13.90) was very similar to that of the F_1 hybrid (13.92). Therefore, there is no reason to suspect that there has been any reduction of chiasma frequency induced by the Hb technique. If this Hb chiasma frequency is used as a control and compared with the values obtained for M derivatives (Table 5), it can be seen that lines from callus numbers 3, 6 and 9 have a reduced chiasma frequency. However, chiasma frequency in callus lines 7, 12 and 13 are quite similar to that of the control.

Genetical analyses of the hordeins (alcohol soluble storage proteins) have indicated that the two main groups of hordein polypeptides are controlled by single loci (*hor 1* controlling the C polypeptides and *hor 2* the B hordein polypeptides) (Shewry et al. 1980). The segregation of these major gene loci may be monitored by unidirectional SDS polyacrylamide gel electrophoresis (PAGE). More recently, a reversed phase HPLC system has been used to separate the hordeins (Marchylo and Kruger 1984) and this system was applied to

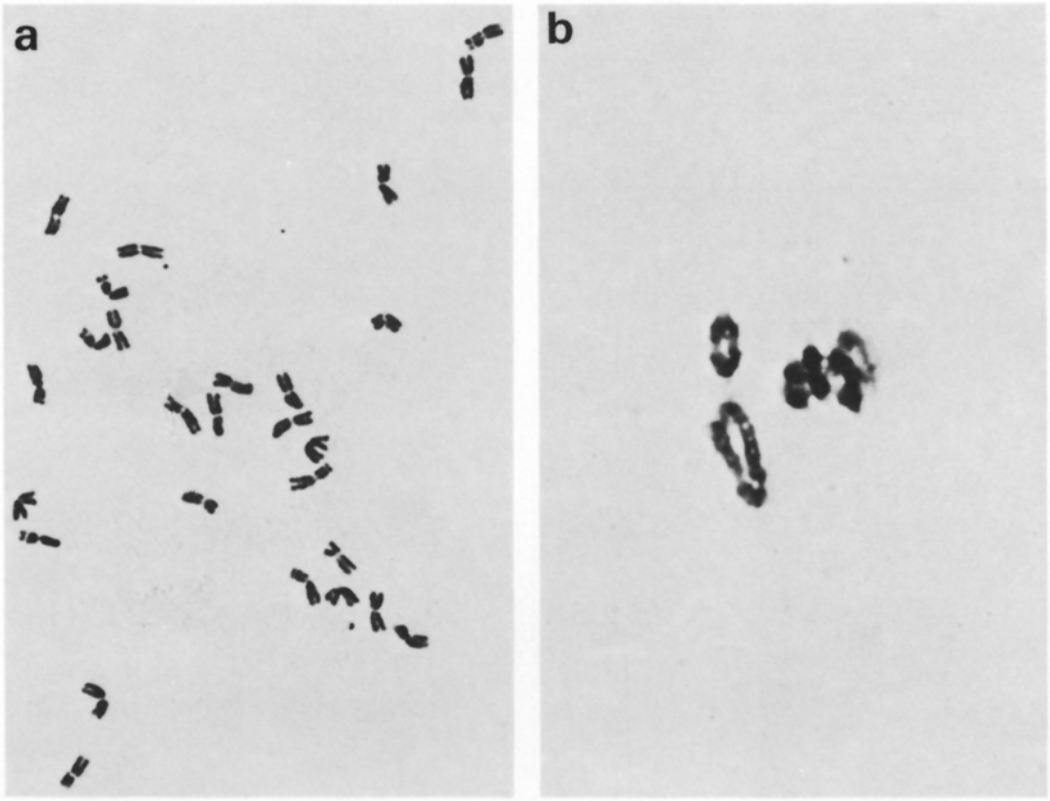

Fig. 1 a, b. Cytologically abnormal cells in the progeny of microspore-derived plants. **a** Mitosis in root tip cell of plant derived from callus 2 ($2n = 4\times - 1 = 27$); **b** Meiosis in a translocation heterozygote from callus 7, showing five ring bivalents, and one quadrivalent

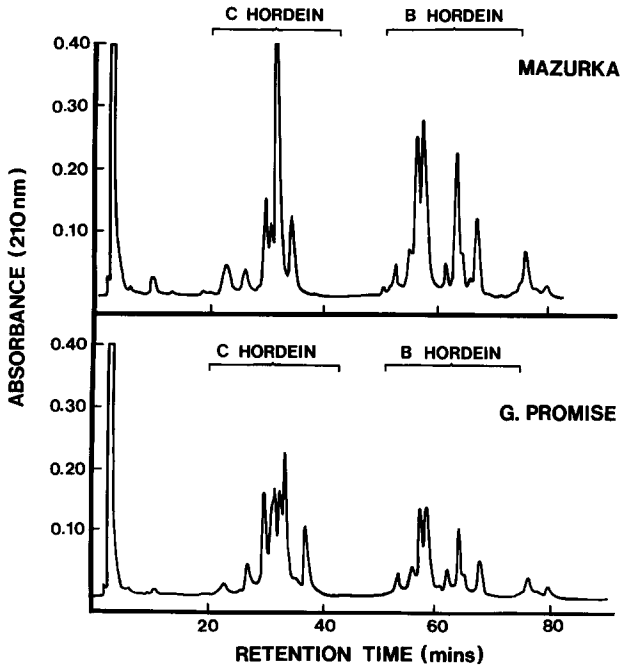

Fig. 2. HPLC elution profiles for B and C hordeins for 'Mazurka', 'Golden Promise'

the M progenies in an attempt to provide a qualitative and quantitative assessment of segregation at the hordein loci.

A qualitative examination of the HPLC elution profiles of the B and C hordeins of 'Golden Promise' and 'Mazurka' (Fig. 2) revealed that both varieties had identical B hordein profiles, with six major peaks eluting between 50 and 70 min after injection. In contrast, major differences with regard to the C hordein profiles were observed. The 'Mazurka' profile is dominated by a major peak at 32 min. The C hordein profile of 'Golden Promise' consists of a less well defined group of peaks with broadly similar intensities spread over 29 to 33 min as well as an additional peak at 37 min after injection. No trace of a peak with a similar retention was observed in the 'Mazurka' C hordein profile. Single seeds from M plants were analysed and the results (Table 6) revealed that of the fourteen plants examined eleven had protein profiles identical to 'Golden Promise' and three identical to 'Mazurka'. The ratio of B hordein to C hordein was also determined and is shown in Table 6. The majority of the seed analysed had ratios similar to those found in 'Mazurka' and 'Golden Promise', although one line from callus num-

Table 5. Mean chromosome pairing at first metaphase of meiosis in the parents, their F_1 hybrid and Hb and M Lines

Genotype			Configurations					Mean chiasma frequency	No. of cells
			I	ring II	rod II	ring IV	chain IV		
'Golden Promise'				6.93	0.07			13.93	195
'Mazurka'				6.67	0.33			13.67	139
F_1				6.92	0.08			13.92	176
Hb				6.90	0.10			13.90	309
M lines									
Callus no.	Line no.	Ploidy							
3	1	(2x)		6.57	0.43			13.57	7
	2	(2x)		5.10	1.90			12.10	29
6	1	(2x)		6.27	0.73			13.27	200
	2	(2x)	0.02	5.57	1.42			12.55	183
9	1	(2x)		5.25	1.75			12.25	8
12	1	(2x)		6.85	0.15			13.85	65
	2	(2x)		6.84	0.16			13.84	113
	3	(2x)		6.66	0.34			13.66	241
13	1	(2x)		6.86	0.14			13.86	135
	2	(2x)		6.86	0.14			13.86	100
	3	(2x)		6.86	0.14			13.86	149
Lines with aberrant meiosis									
2	1	(4x)	0.13	7.20	1.27	2.33	0.40	26.19	15
	2	(4x)		7.25	1.25	2.75		26.75	4
7	1	(2x)[a]		4.94	0.06	0.87	0.13	13.81	148
	2	(2x)[a]		4.89	0.12	0.95	0.04	13.85	259

[a] Plants with a translocation

Table 6. The relative area of B and C hordein peaks in the M lines

Sample	Relative area		Ratio	Type
	C hordein	B hordein	C:B	
'Mazurka'	15.09	22.93	1:1.5	–
'G. Promise'	15.01	24.63	1:1.6	–
Callus line				
1	17.72	30.56	1:1.7	'Golden Promise' (A)
2	21.19	29.79	1:1.4	'Mazurka' (B)
3	21.01	28.93	1:1.4	'Golden Promise' (A)
4	20.81	32.58	1:1.6	'Golden Promise' (A)
5	11.47	17.19	1:1.5	'Golden Promise' (A)
6	15.65	35.51	1:2.3	'Golden Promise' (A)
7	10.51	16.82	1:1.6	'Golden Promise' (A)
8	27.87	38.95	1:1.4	'Golden Promise' (A)
9	16.95	30.79	1:1.8	'Golden Promise' (A)
10	17.38	26.90	1:1.5	'Golden Promise' (A)
11	17.18	17.11	1:1.0	'Golden Promise' (A)
12	29.30	41.53	1:1.4	'Golden Promise' (A)
13	25.36	28.80	1:1.1	'Mazurka' (B)
14	31.14	41.73	1:1.6	'Mazurka' (B)

ber 6 appeared to have a considerably increased ratio of 1:2.3 as compared with the average value of approximately 1:1.4.

Discussion

The 96 anther culture families examined in this study were homozygous for all five marker genes tested. Hence, the plants were of microspore origin and the haploid chromosome complement had doubled during in vitro development.

It is also of interest to determine whether random assortment and survival of gametophytes occurs during the anther culture process. Evidence from the homozygous diploids for deviations in the segregation of genes from the expected 1:1 ratio suggests non random survival during the production and/or differentiation of microspore derived plants. The gene markers used were located on chromosome 5 and 7.

Previous linkage studies using *H. bulbosum* derived doubled haploids, translocation stocks and conventional breeding have provided precise map distances for these 5 loci (Thomas et al. 1984; Shewry et al. 1980). With the exception of rachilla hair type the doubled haploid sample produced by the *H. bulbosum* technique segregated in the expected 1:1 ratio and the linkage data obtained by this method were consistent with that obtained from F_2 samples (Thomas et al. 1984). Those gynogenetically produced haploids therefore reflect the random segregation products of meiosis. The microspore products in the present study displayed distorted segregation ratios and caution should therefore be exercised when using anther culture as an alternative to traditional genetical methods. There are at least two previous cases of distorted segregation ratios being observed in microspore derived lines of barley (Kao et al. 1983; Foroughi-Wehr and Friedt 1984). Both reports indicate an excess of 6 row progenies arising from 2 row×6 row crosses. Although a random assortment of gametes is essential for genetical investigations, such distortions for major genes may prove to be advantageous in breeding programmes. For example, in the study of Foroughi-Wehr and Friedt (1984) an excess of virus resistant genotypes was observed in the microspore progenies (79R: 53S, $\chi^2_{(1)} = 5.62$)*. In other words, differential survival or selection during the in vitro phase, resulting in an excess of desirable recombinants, would prove advantageous to the barley breeder. However, a recent study on a number of quantitative characters in barley (Powell et al. 1986) has indicated that the mean of the microspore sample was significantly lower than the corresponding control samples.

The present protocol for barley anther culture involves regeneration from callus (Sunderland and Huang 1985). Such regeneration is genetically determined and it is conceivable that the excess of certain phenotypes in M progenies may be due to linkage of these loci to genes responsible for regeneration capacity. Genetic changes occur in tissue culture and these may be associated with a prolonged callus phase (Larkin and Scowcroft 1981). This phenotypic variability may also be associated with chromosomal changes (Edallo et al. 1981). The cytological examination of the M progenies in this study revealed changes both in chromosome number and more subtle changes as revealed by lower chiasma frequencies in the M lines than in the controls. It is possible that disruption of the barley genome during callus culture may be useful in obtaining introgression of genetic material following wide crosses (Orton 1979). A previous study on tomato has shown increased frequencies of recombination in plants regenerated from cotyledons (Sibi et al. 1984).

In the present study M lines were monitored for B and C hordein content using reversed-phase HPLC, a system which as well as being more sensitive than SDS-PAGE also allowed a quantitative analysis. The results showed a non random distribution amongst the M derivatives with an excess of the 'Golden Promise' phenotype.

When considering the potential applications of anther culture in barley improvement it is necessary to be aware of possible non-random segregation of gametes and the induction of genetic variation. However, the production of such somaclonal variation does not necessarily mean that it will be of value in barley breeding. Lack of genetic variability is not usually the limiting factor in barley breeding; the chief constraint is the difficulty of identifying and isolating desirable recombinants. In order to meet this objective, elimination of the callus phase and the formation of diploid embryoids direct from haploid pollen (Lyne et al. 1986) would be advantageous. Such a system would shorten the culture procedure and reduce the dangers of chromosomal change and plastid mutation (Day and Ellis 1985).

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References

Chen CC, Chiu WL, Yu LJ, Ren SS, Yu WJ, Lin MH (1983) Genetic analysis of anther-derived plants of rice: independent assortment of unlinked genes. Can J Genet Cytol 25: 18–24

Chen CM, Chen CC, Lin MH (1982) Genetic analysis of anther-derived plants of rice. J Hered 73:49–52

Choo TM, Reinbergs E, Kasha KJ (1985) Use of haploids in breeding barley. Plant Breed Rev 3:219–252

Corduan G (1975) Isozyme variation as an indicator for the generative or somatic origin of anther derived plants of *Digitalis purpurea* L. Z Pflanzenzücht 76:47–55

Day A, Ellis THN (1985) Deleted forms of plastid DNA in albino plants from cereal anther culture. Curr Genet 9:671–678

Dunwell JM (1985) Anther and ovary culture. In: Bright SWJ, Jones MGK (eds) Cereal tissue and cell culture. Nijhoff/Junk, Dordrecht, pp 1–44

Edallo S, Zucchianali C, Perenzin M, Salamini F (1981) Chromosomal variation and frequency of spontaneous mutation associated with in vitro culture and plant regeneration in maize. Maydica 26:39–56

Foroughi-Wehr B, Friedt W (1984) Rapid production of recombinant barley yellow mosaic virus resistant *Hordeum vulgare* lines by anther culture. Theor Appl Genet 67: 377–382

Hayes JD, Rana MS (1966) Genetic resistance to DDT in *Hordeum.* 1. Linkage studies in diploid barley. Heredity 21:581–593

Huang B, Sunderland N (1982) Temperature-stress pretreatment in barley anther culture. Ann Bot 49:77–88

Huang B, Dunwell JM, Powell W, Hayter AM, Wood W (1984) The relative efficiency of microspore culture and chromosome elimination as methods of haploid production in *Hordeum vulgare* L. Z Pflanzenzücht 92:22–29

Kao KN, Wetter LR, Dyck J, Horn D, Ye CM, Harvey BL (1983) Inheritance of certain genetic traits in pollen plants from F_1 hybrid barley. Genet Soc Can Bull 14:D7

Kasha KJ, Reinbergs E (1982) Recent developments in the production and utilization of haploids in barley. In: Proc 4th Int Barley Genet Symp. Edinburgh, pp 655–665

Larkin PJ, Scowcroft WR (1981) Somaclonal variation: a new source of variability from cell cultures for plant improvement. Theor Appl Genet 60:197–214

Lyne LR, Bennett RI, Hunter CP (1986) Embryoid and plant production from cultured barley anthers. In: Alderson PG, Withers LA (eds) Plant Tissue Culture and its Agricultural Applications. Butterworths, Guildford (in press)

Marchylo BA, Kruger JE (1984) Identification of Canadian barley cultivars by reversed-phase high performance liquid chromatography. Cereal Chem 61:295–301

Mather K (1938) The measurement of linkage in heredity. Methuen, London

Nilan RA (1964) The cytology and genetics of barley 1951–1962. Monograph Supplement No 3, Washington State University, Washington

Orton TJ (1979) Haploid barley regenerated from callus cultures of *Hordeum vulgare* × *H. jubatum.* J Hered 71: 780–782

Orton TJ, Browers MA (1985) Segregation of genetic markers among plants regenerated from cultured anthers of broccoli (*Brassica oleracea* var. 'italica'). Theor Appl Genet 69: 637–643

Powell W, Caligari PDS, Dunwell JM (1986) Field performance of lines derived from haploid and diploid tissues of *Hordeum vulgare.* Theor Appl Genet 72:458–465

Shannon PRM, Nicholson AE, Dunwell JM, Davies DR (1985) Effect of anther orientation on microspore-callus production in barley (*Hordeum vulgare* L.) Plant Cell Tissue Organ Cult 4:271–280

Shewry PR, Faulks AJ, Pickering RA, Jones IT, Finch RA, Miflin BJ (1980) The genetic analysis of barley storage proteins. Heredity 44:383–389

Sibi M, Biglary M, Demarly Y (1984) Increase in the rate of recombinants in tomato (*Lycopersicon esculentum* L.) after in vitro regeneration. Theor Appl Genet 68:317–321

Sunderland N, Huang B (1985) Barley anther culture – The switch of programme and albinism. Hereditas (Suppl) 3:27–40

Thomas WTB, Powell W, Wood W (1984) The chromosomal location of the dwarfing gene present in the spring barley variety 'Golden Promise'. Heredity 53:177–183

Wenzel G, Hoffman F, Thomas E (1976) Heterozygous microspore-derived plants in rye. Theor Appl Genet 48: 205–208

Wolfe MS, Schwarzbach E (1978) Patterns of race changes in powdery mildews. Annu Rev Phytopathol 16:159–180

Zamir D, Tanksley SD, Jones RA (1981) Genetic analysis of the origin of plants regenerated from anther tissues of *Lycopersicon esculentum* Min. Plant Sci Lett 21:223–227