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Field performance of lines derived from haploid and diploid tissues of *Hordeum vulgare*

W. Powell [1], P. D. S. Caligari [1] and J. M. Dunwell [2]

[1] Scottish Crop Research Institute, Pentlandfield, Roslin, Midlothian EH25 9RF, Scotland
[2] John Innes Institute, Colney Lane, Norwich NR4 7UH, UK

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Summary. Plant tissue culture technology is of increasing interest to plant breeders. As part of a continuing investigation into breeding methods with spring barley two studies were conducted to assess the field performance of the progenies of material regenerated in tissue culture. The first study involved two spring barley cultivars, 'Golden Promise' and 'Mazurka' and compared lines produced from immature embryo (IE) derived callus with those from embryos developed by the *Hordeum bulbosum* (Hb) technique of chromosome elimination. In general the mean values for the seven characters scored were lower for the IE than the Hb material. In the second study F_1 hybrid material ('Golden Promise' × 'Mazurka') was used and doubled haploid lines produced by the *H. bulbosum* and microspore culture (M) techniques were compared with single seed descent (SSD) material. Analysis of these F_∞ samples indicated that the mean values for the M lines were significantly lower than those of the Hb and SSD lines. Furthermore, data from the M lines showed significant evidence of variation created during the culture phase. The implications of these findings for barley breeding are discussed.

Key words: *Hordeum vulgare* – Barley – Tissue culture – Haploid – Somaclonal variation

Introduction

In cultivated barley (*Hordeum vulgare*) doubled haploids are used extensively in breeding programmes (Choo et al. 1985). Such haploids are usually produced by the technique of chromosome elimination following interspecific pollination with *H. bulbosum*. However, microspore-derived doubled haploids produced by anther culture have also been used in breeding, for example as a means of rapidly isolating lines resistant to barley yellow mosaic virus (Foroughi-Wehr and Friedt 1984). In order to assess the practical value of these tissue culture techniques in breeding it is necessary to take into account the possible problem of induced genetic variation. There is evidence for the presence of such variation from studies on many species (Larkin and Scowcroft 1983; Evans et al. 1984) including barley (Powell et al. 1984). The present study of field grown material was designed to complement this earlier one; its particular objectives were:

1. To compare lines produced from immature embryo (IE) derived callus with those derived from the *H. bulbosum* (Hb) technique. Conventional seed generated material served as control.
2. To compare F_∞ samples of inbred lines produced from F_1 hybrids either by anther culture or the *H. bulbosum* technique with conventionally derived material – F_7 single seed descent (SSD) (Brim 1966) lines produced without conscious selection.

Materials and methods

Two cultivars of *H. vulgare*, 'Golden Promise' and 'Mazurka' and the F_1 hybrid ('Golden Promise' × 'Mazurka') were used.

1 Tissue culture and plant regeneration methods

a) Anther culture. Two seeds of the F_1 hybrid were sown per pot in John Innes Compost and grown at 18 °C in a growth room with a 16 h photoperiod provided by mercury halide lights (340 $\mu E\ m^{-2}\ s^{-1}$). Methods of spike collection, pretreatment at 4 °C and anther culture were as described previously (Huang and Sunderland 1982). Microspore derived calli larger

than 1 mm were transferred to regeneration medium (Huang and Sunderland 1982) and regenerated plants were subsequently separated and transferred to compost. From 14 individual calli a total of 91 green plants were isolated. The progenies of these plants will be considered in this study.

b) Hordeum bulbosum technique. Plants of 'Golden Promise', 'Mazurka' and their F_1 hybrid were grown in a glasshouse with a minimum 16 h photoperiod at a temperature of 20°–30 °C. Methods of emasculation, pollination and embryo regeneration were as described previously (Huang et al. 1984). A total of 20 doubled haploid (DH) lines were produced from the F_1 hybrid and from 'Mazurka' and 33 from the cultivar 'Golden Promise'.

c) Immature embryo regeneration. The techniques for plant growth, embryo excision and culture, and plant regeneration were as described previously (Dunwell et al. 1985). The progenies from a random sample of 19 regenerated plants were included in the comparison.

2 The field experiment

Progenies from the spontaneously doubled haploids produced by anther culture, together with the Hb and IE derived lines and 20 SSD lines from randomly produced F_2 seed were grown with the corresponding seed derived control material in a field experiment at the Murrays Farm, East Lothian in 1984.

The design was a randomised complete block with two replicates. Within a block each genotype was represented by a row of up to 20 seeds sown at 5 cm spacings with a wheat guard plant at each end of the row. Rows were spaced 22.5 cm apart and the whole experiment was netted to prevent bird damage. After harvest sheaves were returned to the laboratory and on 5 randomly chosen plants the following measurements were made: final plant height (cm) – Ht; ear length (cm) – EL; number of fertile tillers – TN; number of grains on the main stem – GN; grain yield (g) of main stem – MSW; thousand grain weight (g) – TGW; single plant yield (g) – SPY.

Results

Analyses of variance of the different populations are given in Table 1 which show significant differences between the 242 genotypes for the 7 characters scored. The genotypes may be partitioned into those originating from highly homozygous starting material (cultivars) and those from a heterozygous source (F_1 hybrids). These two types will be considered separately.

1 'Golden Promise'

There was no significant genetic variation between lines within the IE, Hb or seed derived populations for any of the characters scored. However, the means of these populations differed significantly for TN, GN, MSW, TGW and SPY. In particular, the IE, Hb and seed derived material differed significantly for EL, TN, MSW, TGW and SPY. The IE and Hb material differed significantly for EL, GN, MSW, TGW and SPY. The mean values for the different samples examined are shown in Table 2.

Table 1. Analyses of variance for the samples grown

Item	df	Character						
		Mean squares						
		Ht	EL	TN	GN	MSW (10^2)	TGW	SPY
1. Between reps	1	19,081.98	66.87	2.32	2.32	4.18	10.93	19.61
2. Between genotypes	241 (1)[a]	883.99***	8.54***	36.45***	75.43**	37.27***	183.83***	65.08***
a GP (C v IE v Hb)	2^{def}	31.96	1.67	33.89***	38.05***	19.76***	80.07***	37.13***
b C v (IE v Hb)	1	12.11	1.04	63.77***	0.87	3.51*	91.97***	33.33***
c IE v Hb	1	51.81	3.06***	4.01	75.23***	36.01***	68.17***	40.93***
d Between C lines	19	18.81	0.35	1.71	1.58	0.61	6.18	2.46
e Between IE lines	18***	26.64	0.27	2.29	1.86	0.89	6.11	2.68
f Between Hb lines	33	25.95	0.31	6.55	2.55	1.08	4.92	9.03
g Maz (C v Hb)	1^{hi}	40.56	0.73	27.57*	2.92	0.24	1.02	33.39
h Between C lines	19	26.87	0.17	6.89	1.76	0.80	3.71	11.60
i Between Hb lines	18	31.26	0.58	5.51	4.88	2.03	4.82	9.61
j GP × Maz F_1 (M v Hb v SSD)	2^{mno}	16.70	4.89	93.96***	119.81***	67.20***	137.13***	232.23***
k Mv Hb	1	15.70	6.37*	83.01***	136.96***	74.06***	138.08**	235.78***
l (M+Hb) v SSD	1	17.70	3.41	104.91***	102.66***	60.34***	136.18***	228.68***
m Between M lines	90	231.84*	1.64	7.90	24.02*	6.17	32.06***	12.55
n Between Hb lines	19	138.79*	1.48	2.99	3.85	8.32*	78.68*	11.90
o Between SSD lines	19	143.25*	1.93	4.68	5.28	5.91	63.59*	7.78
3. Reps × genotypes	236 (6)[a]	117.11***	1.78***	24.92***	19.03***	7.56***	29.55***	33.76***
4. Residual	1,884 (60)[a]	17.96	0.62	12.67	9.44	3.85	17.47	17.54

Superscripts indicate MS value used in VR test

[a] Value in backets denotes number of missing values.

Values are significant, $P=0.001$***, $P=0.01$**, $P=0.05$* level

Table 2. Means and ranges [high (H), low (L)] for the samples grown

Line			Character						
			Ht	EL	TN	GN	MSW (10^2)	TGW	SPY
'Golden Promise'		C	63.56	8.49	8.21	26.13	119.90	45.84	9.89
		IE	61.99	8.14	9.82	24.79	108.20	42.99	8.91
		Hb	63.44	8.52	9.84	26.94	120.70	44.66	10.21
'Mazurka'		C	77.81	10.24	8.37	29.88	152.80	51.12	10.57
		Hb	79.35	10.07	9.44	29.32	148.20	51.35	11.88
'Golden Promise'		H	86.40	11.31	11.50	30.60	164.01	58.75	14.71
×'Mazurka'	M	$\bar{x}$	71.36	9.11	7.72	25.25	120.50	46.76	8.20
		L	30.19	6.84	2.10	16.10	67.09	36.16	2.05
		H	82.00	9.31	12.20	30.10	161.77	56.31	15.37
	Hb	$\bar{x}$	71.40	8.66	9.33	27.88	136.40	48.82	10.88
		L	57.20	7.04	6.70	25.60	104.05	39.27	6.91
		H	85.00	11.28	13.90	30.50	172.72	55.96	15.42
	SSD	$\bar{x}$	71.25	9.34	9.78	27.94	137.50	49.14	11.28
		L	59.20	7.64	7.40	23.90	111.46	36.59	8.42

2 'Mazurka'

There was no significant difference between lines within the seed produced or the IE material. The means of these two populations did, however, differ significantly for TN, with the Hb mean being greater than that of the seed derived material (Table 2).

3 'Golden Promise'× 'Mazurka' F_1

The microspore-derived lines differed significantly for Ht, GN and TGW, whereas the Hb material differed significantly for Ht, MSW and TGW. The SSD material differed only for Ht and TGW. Comparison of these F_∞ samples indicated that the means of these populations differed significantly for TN, GN, MSW, TGW and SPY. There were significant differences between the M and Hb lines for all characters except height. Similarly, the means of the tissue culture (M + Hb) and SSD samples differed significantly for TN, GN, MSW, TGW and SPY.

Inspection of Table 2 reveals two important features. First, by deriving inbred material by M, Hb or SSD methods it is possible to transgress the parental range for characters of importance to barley breeders. Secondly, the means of the M sample are lower than the other two samples for TN, GN, MSW, TGW and SPY – characters involved with fertility. This feature will be further examined below. It is important to note that there was no difference between the means produced from 91 separate values or from 14 values (one from each callus "family").

Random samples of inbred lines derived from a cross by doubled haploidy (i.e. M or Hb) are expected to have the same phenotypic distribution about the same mean as the original parents of the cross. Differences between SSD and DH generations can be explained by linkage disequilibrium involving epistatic genes (Jinks and Pooni 1981). However, discrepancies between M and Hb samples cannot adequately be explained in this way. The observed ranking of the means for the M, Hb and mid-parent values are given in Table 3. It can be seen that only for the character Ht are the means equal. For four of the remaining six characters the Hb mean is not significantly different from the mid-parent value while the corresponding mean is significantly lower. For the character EL, the M mean equals the mid-parent value but is significantly greater than the Hb mean. For SPY, the Hb value is above the mid-parent value which in turn is higher than that of the M material. Thus, for five of the seven characters the Hb mean equals the mid-parent value and therefore provides confirmatory evidence that differential selection does not play a large role during the production of Hb doubled haploids. It is also notable that there is a strong correlation between the occurrence of sig-

Table 3. Rankings of the means for the microspore culture (M), *Hordeum bulbosum* (Hb) derived and mid parent (MP) values

Character	Ranking				
	1		2		3
Ht	Hb	=	M	=	MP
EL	M	=	MP	>	Hb
TN	Hb	=	MP	>	M
GN	MP	=	Hb	>	M
MSW	Hb	=	MP	>	M
TGW	Hb	=	MP	>	M
SPY	Hb	>	MP	>	M

Table 4. Model fitting (weighted least squares) for the micropore culture (M) and *Hordeum bulbosum* (Hb) derived lines

Line	Value	Character					
		Ht	EL	GN	MSW	TGW	SPY
M	m	70.69±0.169	9.36±0.018	28.00±0.046	136.26±0.030	48.47±0.078	8.20±0.487
	[*d*]	7.13±0.169	0.88±0.018	1.87±0.046	16.44±0.030	2.64±0.079	0.95±0.094
	[*i*]	–	–	–	–	–	(1.42±0.496)
	χ^2	0.0633	1.4299	13.5750***	1,667.05***	3.6919	8.1872**
Hb	m	70.74±0.161	8.66±0.061	27.98±0.042	136.35±0.029	48.49±0.077	10.88±0.173
	[*d*]	7.14±0.169	0.88±0.018	1.87±0.046	16.45±0.030	2.64±0.079	0.95±0.094
	[*i*]	–	(0.71±0.064)	–	–	–	(–1.26±0.196
	χ^2	1.3614	123.3313***	1.2785	0.1153	0.5698	41.1962***

, * For levels of significance, see Table 1

nificant differences between the means of the M and Hb lines and between the M means and mid-parent values. In other words, the lower than expected M means for fertility characters may have a common cause.

This feature of the data may be examined further by use of biometrical models (Mather and Jinks 1982). Where there are genetic differences between the two parents of a cross, their means may be represented as follows:

$\bar{P}_1 = m + [d] + [i]$

$\bar{P}_2 = m - [d] + [i]$

where m is the overall mean, [d] is the contribution of additive genetic effects and [i] is the contribution of additive × additive interactions. Doubled haploids produced at random from heterozygous F_1 hybrids will segregate 1 : 1 for alleles at each locus and hence the additive contributions of each allele pair to the DH progeny mean will cancel. In the absence of linkage, all possible genotypes will be produced in equal frequency and the interaction between alleles will also cancel. Hence the M and Hb progeny are expected to have a mean of m.

The genetic parameters m, [d] and [i] have been estimated from the means of the parents and Hb derived doubled haploids by weighted least squares model fitting procedures. The results of this model fitting are shown in Table 4. It can be seen that a simple additive genetic model is sufficient to explain the differences between the means for Ht, GN, MSW and TGW. For EL and SPY significant [i] type epistasis was detected. The test for non-allelic interactions is, however, confounded with selection during the production of F_∞ samples (Jinks 1983). Nevertheless, if non genetical reasons are ignored, the means of the DH generation are expected to differ from the mid-parent-value only in the presence of non allelic interactions. Results from the weighted least squares model fitting procedures for the M lines are also given in Table 4. The additive genetic model now fails for the characters GN and MSW. Furthermore, the χ^2 values for TGW approaches borderline significance. In addition there is no evidence of epistasis in the control of EL when using the M mean whereas there is significant positive [i] type epistasis for SPY. There is therefore overwhelming evidence that the two F_∞ samples, Hb and M, are different when subjected to model fitting analyses. Although the presence of epistasis is confounded with differential survival there is no adequate genetic model to explain the differences between the M and Hb means. Given that for 5 of the 7 characters, the M mean is less than the mid-parent value, it is reasonable to conclude that differential survival and/or genetic changes occur during the production of M lines.

Table 5. Estimates of the additive genetic variance (D) obtained from the microspore culture (M) and *Hordeum bulbosum* (Hb) derived lines

Line	Character						
	Ht	EL	TN	GN	MSW	TGW	SPY
M	101.31	0.49	1.91	9.19	2.24	12.50	3.46
Hb	64.79	0.58	–	0.83	3.80	37.71	4.42
χ^2	1.04	0.13	3.15	7.56**	2.01	12.75***	0.22

Significantly different at $P = 0.001$*** and $P = 0.01$** level

If it is assumed that the inbred lines produced by the M and Hb methods are homozygous it is possible to estimate the additive genetic [D] component of variance from the expected mean squares appropriate to the experiment. Estimates of D from the two samples are given in Table 5. Whether or not the estimates of D from the different sources differ significantly can be tested by standard maximum likelihood model fitting procedures (Pooni et al. 1980; Powell et al. 1985). The test involves fitting a model to the between lines and within lines mean squares, assuming a single value of D but allowing the two within family mean squares to take their own values. Thus, three components are fitted to the 4 mean squares and there is one degree of freedom for testing the goodness of fit of the model. The model cannot fail unless the estimates of D from the two sources differ significantly. χ^2 values for the

Table 6. Analysis of variance for the 91 microspore derived lines produced from 14 calli

Item	df	Character						
		Mean squares						
		Ht	EL	TN	GN	MSW	TGW	SPY
1. Between reps	1 (3)[a]	1,454.45	4.55	0.22	1.55	0.87	12.64	1.14
2. Between calli	13 (3)	1,405.18***	8.85***	32.12***	109.18***	26.97***	155.85***	46.55***
3. Between lines within calli	77 (4)	33.75	0.22	3.81	9.64**	3.61**	11.19**	6.81
4. Error	90	29.22	0.67	4.09	5.65	1.70	7.06	5.63

, * For levels of significance, see Table 1

[a] Value in brackets denotes the MS used in the VR test

comparisons of the two samples are also given in Table 5 and indicate that there are significant differences between estimates of D for TGW and GN. It is samples differ significantly for a number of agronomic characters.

As mentioned above, the 91 M lines originated from 14 distinct calli, with a number of different plants produced from the same callus. Hence a hierarchical family structure can be obtained. Results from the analysis of variance for the 7 characters scored are given in Table 6. There are significant differences between calli for all the characters. This item is the highest hierarchy in the analysis and reflects the consequences of recombination in the F_1 hybrid. There are significant differences between lines within calli for GN, MSW and TGW. This variation between plants derived from the same microspore has been termed somaclonal (Larkin and Scowcroft 1983) or gametoclonal (Evans et al. 1984) and is associated with mutation during a prolonged callus phase. There are, therefore, at least two sources of genetic variation available for manipulation by the barley breeder. First, there is variation between calli (rank 1 variance) and secondly there is induced variation.

In order to compare further the variation between calli (σ_c^2) and the variation between lines within calli (σ_{lc}^2) the expected mean squares were calculated. Since the number of plants produced from a given callus was not constant (i.e. sample size in each family varied) it was necessary to use the harmonic mean (Snedecor and Cochran 1980) to calculate the appropriate coefficients. The expected mean squares together with their coefficients are given below:

Item	Df	Expected mean squares
1. Between reps.	1	$\sigma_e^2+2\sigma_{lc}^2+108.36\,\sigma_r^2$
2. Between calli	13	$\sigma_e^2+2\sigma_{lc}^2+15.48\,\sigma_c^2$
3. Between lines within calli	77	$\sigma_e^2+2\sigma_{lc}^2$
4. Error	90	σ_e^2

The components of variance calculated from the expected mean squares are given in Table 7 and have been scaled to sum to 100 in order to facilitate comparison. The main point of interest concerns the difference between σ_c^2 and σ_{lc}^2: in every case the σ_c^2 component is greater than the σ_{lc}^2 component. Thus, as a means of generating fixable additive genetic variation for breeding purposes, the generation of M lines from F_1 hybrids is to be preferred. However, the significant levels of somaclonal variation detected may indicate that the application of in vitro tissue culture techniques to adapted barley cultivars in conjunction with appropriate screening may offer a means of generating useful variability for specific traits.

The data presented indicate that the first and second degree statistics estimated from the M and Hb samples differ for a number of agronomic traits. In the context of a barley breeding programme, it is also important to determine the proportion of F_∞ lines generated by both these methods which transgress given standards. The proportion of lines transgressing P_1 and P_2 (the highest and lowest scoring parents, respectively) for the M and Hb samples are given in Table 8. Also provided are the contingency χ^2 values used to test whether the four proportions calculated are significantly different. Analysis shows that five of the seven characters are significantly different ($P < 0.001$) with Ht and EL being non significant. Furthermore, for most characters the barley breeder will be concerned with transgressing P_1 and hence the Hb sample provides a greater proportion of inbred lines falling into this category.

Another important aspect of the results is the variation between plants within a family (i.e. the between plant variance). Previous results have indicated significant variation between genotypes but it is also important to establish whether there is significant variation between plants within a row. The between plant variances for the M and Hb lines, together with the parental cultivars, are given in Table 9. Also given

Table 7. Components of variance for the microspore derived lines (scaled to sum to 100)

Variance σ^2		Character						
		Ht	EL	TN	GN	MSW	TGW	SPY
Between reps	σ^2_r	9.84	3.10	0.00	0.00	0.00	0.07	0.00
calli	σ^2_c	66.52	43.64	31.48	45.68	36.24	50.56	29.24
lines within calli	σ^2_{lc}	1.70	0.00	0.00	14.17	22.94	11.17	6.70
environment	σ^2_e	21.94	53.26	68.52	40.14	40.83	38.20	64.05

Table 8. Proportion of microspore culture (M) and *Hordeum bulbosum* (Hb) derived lines transgressing the P_1 and P_2 values. Values for the M lines are provided for 91 separate lines and also for 14 separate callus "families"

Line	Character											
	Ht		EL		GN		MSW		TGW		SPY	
	$>P_1$	$<P_2$	$>P_1$	$<P_2$	$>P_1$	$<P_2$	$>P_1$	$<P_2$	$>P_1$	$<P_2$	$>P_1$	$<P_2$
M_{91}	0.31	0.19	0.10	0.26	0.02	0.39	0.02	0.46	0.10	0.32	0.12	0.57
M_{14}	0.07	0.29	0.07	0.21	0.00	0.57	0.00	0.57	0.07	0.29	0.00	0.93
Hb	0.35	0.25	0.05	0.35	0.00	0.10	0.35	0.35	0.50	0.35	0.45	0.20
M_{91} v M_{14} (χ^2)	5.56*		0.12		3.12		2.68		0.26		17.79***	
M_{91} v HB (χ^2)	0.19		2.90		16.81***		34.81		13.83***		36.29***	

*, *** For levels of significance, see Table 1

Table 9. Between plant variances for the microspore culture (M), *Hordeum bulbosum* (Hb) derived and parental genotypes, 'Golden Promise' (GP) and 'Mazurka' (Maz)

Line	Character						
	Ht	EL	TN	GN	MSW	TGW	SPY
M	17.726	1.607	10.816	12.765	0.058	19.947	14.279
Hb	16.483	0.430	13.878	7.415	0.053	15.906	24.445
GP	21.283	0.747	10.595	10.680	0.038	19.583	13.887
Maz	24.935	0.626	12.700	8.370	0.034	15.144	17.524
$\chi^2_{(3)}$	10.72*	139.68***	5.63 NS	24.40***	24.38***	6.91*	23.08***

*, *** For levels of significance, see Table 1
NS = not significant

in Table 9 are the χ^2 values for the Bartlett test for homogeneity of variance. These tests indicate that the variances are heterogeneous for six of the seven characters scored. Furthermore, the variance for the M sample is larger than the remaining three variances in the case of EL, GN, MSW and TGW.

Conclusions

1. There was no significant genetic variation between families produced from the IE and Hb derived material. However, there were significant differences between the means of the samples; invariably the mean of the IE derived material was lower than the corresponding seed and Hb material.
2. The F_∞ samples produced by microspore culture (M), *Hordeum bulbosum* (Hb) and SSD methods displayed significant genetic variation for a number of characters of agronomic importance.
3. With the exception of Ht and EL the mean of the M population was lower than the corresponding Hb mean. In most cases, the mean of the Hb sample equalled the mid-parent value, as expected from the genetical models derived by Jinks and Pooni (1981). It is important to appreciate that the M lines were produced from callus. Regeneration from callus is associated with a range of genetic changes, some of which are attribut-

able to chromosomal rearrangements (Karp and Bright 1985). Tetraploids were eliminated from the material used in this study and the results of cytological from the material used in this study and the results of cytological analyses will be presented in a future publication.

4. The biometrical models confirm that the differences between the means of the M and Hb samples cannot be adequately explained in simple genetical terms. Although there are significant differences between the means of the M and SSD samples for a number of characters, the Hb and SSD means only differ for EL. This difference can be explained in terms of linkage disequilibrium involving epistatic genes (Jinks and Pooni 1981). It is therefore reasonable to conclude that the poor performance of the M lines may be due to differential survival during the extraction of the doubled haploids and/or mutational events during the culture phase.

5. Standard maximum likelihood model fitting procedures were used to compare estimates of D from the Hb and M samples. This is the fixable portion of the additive genetic variation (Mather and Jinks 1982) and there were significant differences between estimates of D for GN and TGW. In a plant breeding context the most important statistic is the proportion of inbreds that fall outside the parental range ($> P_1 < P_2$). These proportions were presented in Table 8 and two important points emerge. First, there are significant differences between the proportions estimated from the M and Hb samples. Secondly, with the exception of Ht and EL, the Hb sample consistently produced the highest proportion of such inbreds when compared to the M sample.

6. The data indicated that the mean and variance estimated from the M sample was different to that obtained from the Hb sample. This M sample was analysed further by use of the expected mean squares. Using this procedure it was possible to calculate the percentage of the variation attributable to the between calluses (σ^2_c) and the between lines within callus (σ^2_{lc}) items. The σ^2_c component accounted for a far greater proportion of the variability than σ^2_{lc}. The σ^2_{lc} component is equivalent to the somaclonal variation described in other systems (Larkin and Scowcroft 1983).

7. Variation between plants within the parental and Hb lines is due to environmental causes. The general trend would indicate that there is more variation between lines within the M sample than the parental and Hb material. This variation may have arisen from unreduced gametes (Collins et al. 1974) (though these were never observed in the original material) or from changes during the callus phase. Previously, Kao et al. (1983) demonstrated that microspore regenerants from barley F_1 hybrids were not produced at random, for example the ratio of 6 row to 2 row plants deviated significantly from the 1:1 ratio expected by the random assortment of genes. Data from the segregation of major gene markers will be the subject of a later publication.

8. The final point to be discussed concerns the utilisation of the variation generated following the production of F_∞ samples. For example, if the breeder wishes to maximise the additive genetic variability available for selection, then it is necessary to sample gametes at random and hence the production of inbreds without a callus phase, by the *H. bulbosum* technique or SSD, is to be preferred. It should be noted, however, that recent technical improvements in anther culture (Lyne et al. 1985) show that direct microspore embryogenesis is possible in barley. If variation induced in vitro is deemed necessary then an intermediate callus phase should be encouraged. Although the presence of such somaclonal variation has been demonstrated previously in barley (Powell et al. 1984) it has yet to be proved that this variation is of value in breeding programmes.

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