The use of doubled haploids in barley breeding. I. Comparison of H1 and H2 generations

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Doubled haploid systems offer the quickest method of advancing heterozygous breeding lines to homozygosity. Currently the *Hordeum bulbosum* technique is the most efficient method of haploid production in barley. However, at present, the efficiency of this system is reduced by the suggested need for a multiplication phase following colchicine treatment of haploid plants prior to agronomic assessment. In this paper it is demonstrated that the rankings of the H1 (the first selfed generation after colchicine treatment) and H2 (second generation) generations remain unaltered. Furthermore it is shown that the correspondence between parents and progeny or H1 and H2 is determined by the relative magnitude of the additive genetic variation to the total phenotypic variation. The production of variation following a tissue culture phase (somaclonal variation) has been reported in barley. In this study the *H. bulbosum* doubled haploid system did not show any evidence of generating such variation.

INTRODUCTION

Spontaneously doubled microspore derived lines of barley produced from the cultivar Sabarlis have been shown to display considerable phenotypic variation (Powell, Hayter, Wood, Dunwell and Huang, 1984). It is therefore important to establish if such variation is generated when doubled haploid lines are produced from highly inbred cultivars by the *Hordeum bulbosum* technique (Huang, Dunwell, Powell, Hayter and Wood, 1984). Furthermore the current practice, when using the *bulbosum* technique, is to undertake a generation of multiplication following colchicine treatment. This policy was formulated following the work of Walsh, Reinbergs and Kasha (1973), who recommended the selfing of seed one generation after chromosome doubling to avoid seed source effects related to colchicine treated plants. Thus a plant breeder embarking on a scheme of doubled haploid production should know firstly the extent to which an *in vitro* tissue culture phase is likely to generate variation and secondly whether

colchicine treatment is inducing changes in the double haploid progeny.

MATERIALS AND METHODS

Experimental material

Three crosses between various “two-row” spring barley cultivars were used in this study and were, in fact, three of the five studied by Tapsell (1984).

Golden Promise × Mazurka
Golden Promise × Ark Royal
BH4/143/2 × Ark Royal

The first named cultivar was used as the female parent and with the exception of BH4/143/2 the cultivars have been widely grown commercially in the UK during the last decade. BH4/143/2 is a breeding line derived from the cross Akka × Midas.

Doubled haploid production

Twenty doubled haploid lines were produced from the F1 of the 3 crosses by the *H. bulbosum* technique (for details see Huang, *et al.*, 1984). In addition 5 doubled haploids were produced from Golden Promise, Mazurka, BH4/143/2 and a fourth cultivar Clipper. Clipper was included since

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it forms part of a larger series of experiments and is of particular interest since it differs from the other cultivars in the nature of its response to daylength (Thomson and Matthews, 1981). The seed harvested from colchicine treated plants was designated (H1), a portion of this seed was grown and allowed to self pollinate to produce the corresponding (H2) seed and the remaining portion retained.

Field Experiment

Twenty doubled haploid lines (each unique family being represented by the H1 and H2 generation) derived from each of the F1 of the 3 crosses, were grown together with the 4 parents. In addition the 5 doubled haploids produced from the cultivars described in the previous section were also included.

The experiment was sown with the N.I.A.E. walking plot seeder in April 1983 at the Murrays Farm, East Lothian. The experimental design was a randomised complete block of two replicates. Within each block each family was represented by a row of up to ten seeds, sown at 5 cm spacing, with a wheat guard at each end of each row. Rows were spaced 22.5 cm apart. During the growing season the following characters were scored on a row basis:

- Awn emergence, days from the 1st June until awns emerged from the flag leaf sheath of the main stem (AE).
- Maturity scored on a 1 to 9 scale with 1 = early, 9 = late (Mat).

After harvest, sheaves were returned to the laboratory and 5 randomly chosen plants from each row were scored for the following characters:

- Final height, measured from the base of the plant to the collar in cm. (Ht).
- The number of fertile tillers per plant (TN).

- The number of grains per ear (GN).
- The yield of grain on the main stem (MSW).
- The length of the ear (EL).
- Thousand grain weight (TGW) calculated from e and f.
- Grain yield of the whole plant, single plant yield (SPY).

RESULTS

The means and variances for the seed and H1 generations produced from Golden Promise, Mazurka, BH4/143/2 and Clipper are presented in table 1. The error variances for the two generations were tested for homogeneity by Bartlett's test, which indicated that the variances were not significantly heterogeneous ($P > 0.05$) and hence could be pooled. In the analyses of variance the doubled haploid families produced from the parental cultivars were regarded as being random effects but generations (H1 vs. seed) were considered fixed.

Results from the analyses of variance are given in table 2. There were significant differences between the 20 lines for: height, ear length, grain number, awn emergence, tiller number, thousand grain weight, main stem weight and single plant yield. However, most of these differences could be attributed to differences between the parental cultivars. There were no detectable differences within the Golden Promise and Mazurka lines for any of the characters scored and only single plant yield differed significantly at the 5 per cent level in the case of BH4/143/2. Clipper behaved differently to the other three cultivars and displayed significant variation for: grain number, tiller number and single plant yield. It was therefore examined further and the variance ratios for the between lines mean square (M.S.) against the within line M.S. for the seed and H1 generations are listed in

Table 1 Means and variances for the seed and H₁ generations produced from Golden Promise, Mazurka, BH₄/143/2 and Clipper

		MSW		SPY		Ht		EL		Mat		AE		TN		GN		TGW	
G. Promise	Seed	144.4	443.7	8.6	4.6	45.7	20.8	10.0	1.4	4.4	0.7	10.9	2.7	8.9	1.9	30.0	9.4	47.8	6.9
	H ₁	143.4	350.6	9.8	1.4	46.1	9.0	10.2	0.3	3.2	1.2	10.9	1.3	8.9	4.2	31.7	4.6	45.2	9.5
Mazurka	Seed	172.9	28.2	9.3	6.1	59.9	45.2	11.6	0.1	5.4	0.1	12.4	0.7	8.0	0.7	32.0	0.6	53.1	4.1
	H ₁	160.5	316.5	8.8	8.5	56.5	10.8	11.3	0.1	5.0	3.4	11.1	2.0	7.2	4.0	31.6	4.5	49.6	9.4
BH ₄ /143/2	Seed	143.0	54.3	8.4	4.8	44.0	3.5	10.3	0.2	4.5	2.0	11.9	3.5	8.2	3.9	29.2	1.4	46.9	23.3
	H ₁	151.3	110.8	8.8	24.0	46.6	10.7	10.9	0.1	2.5	0.9	15.8	0.9	7.2	7.0	32.5	0.6	46.4	4.5
Clipper	Seed	86.5	541.9	7.0	23.5	48.4	2.2	6.2	0.4	3.1	6.7	1.1	0.6	8.9	24.2	16.2	10.1	56.9	31.5
	H ₁	103.0	281.4	6.0	16.5	50.9	46.0	6.7	0.2	2.8	1.2	1.3	0.5	10.3	10.5	17.7	6.5	56.3	0.3

Means are in roman and variances in italics.

Table 2. Mean squares from the analyses of variance of the seed and H_1 generations from the parental cultivars

Item	df.	MSW M.S.	SPY M.S.	Ht M.S.	EL M.S.	Mat M.S.	AE M.S.	TN M.S.	GN M.S.	TGW M.S.
1. Overall Reps	1	4.9	5.9	155.6†	0.7	6.3	2.6	14.4	0.5	0.1
2. Lines	19	3,500.3§	31.5†	135.6‡	15.4§	6.5	96.8§	20.0†	174.5§	79.8‡
(i) Bet. Cultivars	3	18,204.1§	43.5	688.1§	93.4§	16.6	587.0§	18.7	1,029.6§	288.8§
(ii) Bet. G.P.	4	804.7	3.2	11.5	1.8	3.2	1.7	2.7	12.9	15.6
(iii) Bet. Maz.	4	332.0	10.6	36.7	0.1	3.4	4.2	1.4	7.5	18.0
(iv) Bet. BH ₄	4	302.8	32.9†	19.7	0.2	4.5	5.6	17.0	3.8	19.9
(v) Bet. Clipper	4	1,498.5	70.3‡	60.1	1.1	7.2	0.6	60.1§	32.4†	33.7
3. Generations (H_1 v Seed)	1	157.2	1.8	9.7	0.8	16.0	3.6	0.1	48.5†	43.1
4. Lines × Gen.	18 (1)*	436.3	14.7	44.9	0.7	4.3	5.1	7.9	6.7	30.6
(i) Cultivar × Gen.	3	910.7	6.0	45.2	0.9	5.4	15.8	4.8	14.2	100.5†
(ii) GP × Gen.	4	749.3	8.6	47.9	1.7	0.6	6.2	9.5	15.7	16.9
(iii) Maz. × Gen.	4	356.2	18.5	75.2	0.2	3.6	1.4	8.0	2.6	30.5
(iv) BH ₄ × Gen.	4	37.4	33.1	11.5	0.6	2.9	4.3	6.4	0.3	47.5
(v) Clipper × Gen.	4	146.5	9.7	36.5	0.3	8.6	0.4	9.4	0.8	29.8
5. Replicate Error	31 (8)*	586.6	11.1	28.5	1.0	4.3	6.0	8.3	10.7	31.2

* = number of missing plot. † $P < 0.05$ ‡ $P < 0.01$ § $P < 0.001$

Table 3 The variance ratios for the seed and H_1 generations for the variety clipper

	MSW	SPY	Ht	EL	Mat	AE	TN	GN	TGW
Seed (4df/4df)	21.5§	56.9§	0.4	5.3	3,034.5§	1.0	115.3§	7.8†	7.5†
H_1 (4df/4df)	3.9	29.6‡	4.8	1.4	3.1	1.2	6.5†	3.5	0.5†

† $P < 0.05$ ‡ $P < 0.01$ § $P < 0.001$

table 3. There was significant variation between the seed produced lines for: main stem weight, single plant yield, maturity, tiller number, grain number and thousand grain weight. The H_1 population, on the other hand, only displayed significant variation for two of these characters, namely single plant yield and tiller number and would suggest that the haploidisation process in this case is not generating variability.

The main comparison of interest in the analyses of variance given in table 2 is that between the H_1 lines and the corresponding seed derived parents. Grain number was the only character which displayed border line significance ($P \approx 5$ per cent), in all other cases there was no significant difference between the mean of the H_1 and seed derived populations.

Indeed, many of these variance ratios were sub-significant, which, of course, requires some explanation. They, in fact, arise because soon after the seeds germinated, before the experiment was netted, the experiment suffered from attack by birds. They did not, however, randomly damage the experiment but did so from one end of both replicates. Thus the damage they produced created

a gradient in plant growth over the experiment which ran equally up both replicates. This systematic pattern of imposed variation would clearly lead to such sub-significance being shown and therefore these significance tests can only be viewed with suspicion. Such doubts are not, however, applicable to the test for the interaction with lines and these items are the ones of most interest in the present context. Significance of these items would indicate that the ranking of the lines changed between generations. Examination of table 2 revealed only two significant interactions and both of these were only significant at the 5 per cent probability level. Since 45 such tests have been carried out, it would be expected that 2 would appear significant at this level by chance and thus they could be attributed simply to this and provide no evidence of any change in the ranking of the lines between the seed and H_1 generation for any of the material.

The comparison between the H_1 and H_2 generations was carried on the 3 crosses separately. An initial Bartlett's test indicated that the H_1 and H_2 error variances were homogeneous and hence could be pooled.

Golden Promise × *Mazurka* (TT1)

The results from this analysis are given in table 4a and indicate that there was significant variation between the doubled haploid families for: ear length, main stem weight, single plant yield, thousand grain weight, height, grain number and awn emergence. There were no significant differences between the means of the H1 and H2 populations and no significant interactions between lines and generations were detected for any of the characters scored.

Golden Promise × *Ark Royal* (TT3)

The results of the analyses of variance for this cross are given in table 4b. Significant genetic variation existed between the doubled haploid lines for: ear length, maturity, thousand grain weight, height and awn emergence. There were no significant differences between the means of the populations and no significant lines × generations interaction.

BH4/143/2 × *Ark Royal* (TT4)

The results of the analyses of variance for the 9 variates scored are given in table 4c. There were significant differences between the lines for: main stem weight, thousand grain weight, height and awn emergence. Again no significant differences

between the means of the populations were detected and there were no significant interactions.

In the three crosses examined the means of the H1 and H2 generations did not differ significantly. Biased estimates of the between generations item may have resulted from the gradient in the field. However, this factor should not influence the interaction item which was found to be non-significant for all the characters scored. Hence the rankings of the H1 and H2 generations remained unaltered.

In addition to their use in varietal production doubled haploids produced from different crosses have been used to identify the potential frequency of transgressive segregants (Reinbergs, Park and Song, 1976, Snape and Simpson, 1981). To predict the distribution of inbred lines that can be obtained from a cross, accurate estimates of the mean and additive genetical variance of the population are needed (Jinks and Pooni, 1976). Estimates of the additive genetical variance (D or $D+I$) obtained from an analysis of variance of the H1 and H2 generations are presented in table 5. Whether or not the estimates of D obtained from the H1 and H2 generations differed significantly can be tested by standard maximum likelihood model fitting procedures (Pooni, Jinks and Pooni, 1980). By fitting a model to the between lines and within lines mean squares and assuming a single value of D but allowing the two within family mean squares to take their own values, the significance of difference between estimates of D can be tested. Since

Table 4a Analysis of variance of the 20 doubled haploid lines produced from the F_1 of the G.P. × Maz. (TT1) cross

		MSW	SPY	Ht	EL	Mat	AE	TN	GN	TGW
Item		M.S.	M.S.	M.S.	M.S.	M.S.	M.S.	M.S.	M.S.	M.S.
1. Overall Reps.	1	4.8	38.5	217.9§	0.3	0.3	0.1	43.4	2.2	1.6
2. Bet. DH lines	19	2,250.7§	48.6‡	105.2‡	5.6‡	9.5	21.4‡	27.4	32.2‡	96.1‡
3. $H_1 \times H_2$ Gen.	1	1.6	5.9	20.6	0.1	1.4	29.0	3.6	0.5	2.6
4. Lines × Gens.	19	737.1	20.5	24.8	1.3	3.5	4.4	15.3	10.2	20.2
5. Rep. Error	32 (7)*	525.1	19.0	29.9	0.8	3.4	5.1	11.9	6.9	26.9

4b Analysis of variance of the 20 doubled haploid lines produced from the F_1 of the G.P. × A.R. (TT3) cross

1. Overall Reps.	1	1.7	15.0	190.1§	1.1	7.1	9.4	27.5	1.3	2.4
2. Bet. DH lines	19	1,968.0	51.4	387.6§	6.4‡	15.9‡	46.6§	36.1	16.3	136.8‡
3. $H_1 \times H_2$ Gen.	1	75.7	109.4	14.4	2.5	0.0	28.8	38.4	12.4	0.8
4. Lines × Gens.	19	947.4	43.8	59.8	0.9	3.9	6.7	29.5	12.7	55.7
5. Rep. Error	36 (3)*	974.3	29.8	77.4	0.9	2.8	4.1	22.9	16.4	42.5

4c Analysis of variance of the 20 doubled haploid lines produced from the F_1 of the BH₄ × A.R. (TT4) cross

1. Overall Reps.	1	1.1	109.8‡	424.8§	1.8	4.7	4.4	31.3	0.2	17.6
2. Bet. DH lines	19	2,182.0	20.9	305.2§	2.6	10.4	25.4‡	12.1	17.4	128.7‡
3. $H_1 \times H_2$ Gen.	1	1,687.7	21.5	16.6	0.3	4.3	0.2	0.4	40.1	8.7
4. Lines × Gens.	19	1,152.1	58.8	60.0	1.6	4.0	4.1	26.1	16.8	30.1
5. Rep. Error	33 (6)*	997.7	37.1	33.7	1.7	4.8	3.7	20.6	13.9	44.3

* = number of missing plots. † $P < 0.05$ ‡ $P < 0.01$ § $P < 0.001$

three components have been estimated from 4 mean squares, there is one degree of freedom left for testing the goodness of fit of the model. The model will fail if the estimates of D from the two sources differ significantly. Chi-square (χ^2) values for the comparisons of the two sources are also given in table 5. A significant χ^2 value was only obtained for ear length in the TT1 cross.

Estimates of the narrow sense heritability (h_n^2) were calculated for the 9 variates scored using the following formula:

$$h_n^2 = \frac{\text{additive genetic variance}}{\text{total phenotypic variance}}$$

These estimates, together with the regression slopes based on the family means for the 3 crosses, are given in table 6. Where there were no significant differences between lines the regression of H1 onto the H2 generations, slopes and heritabilities were not calculated. The narrow sense heritabilities estimate the proportion of the phenotypic variation that is additive in origin and will therefore appear in progeny generations in such a way as to cause similarities between the parental and off-spring generations. It is not surprising therefore that h_n^2 and the regression slopes calculated for each variate within each cross are very similar. Thus the regressions along with the h_n^2 identify those characters for which initial selection on the H1 generation could be practiced. Characters with high regression

slopes such as awn emergence, height and thousand grain weight are likely to exhibit the greatest repeatability between the H1 and H2 generations.

CONCLUSIONS

The main conclusions from this study are:

- (1) Doubled haploid populations produced from the cultivars Golden Promise, Mazurka, BH4/143/2 did not display any more phenotypic variation than was present in the corresponding seed derived lines. Doubled haploids produced from Clipper displayed phenotypic variability for single plant yield and tiller number but this variability was also expressed in the seed generation. Hence the haploidisation process does not appear to be generating random changes but sampling the variation present in the parental material.
- (2) The ranking of the H1 and H2 generations are unaltered and it would appear perfectly justifiable to carry out initial selection on the H1 population. This study has further highlighted that the correspondence between parents and progeny or H1 and H2 is determined by the relative magnitude of D or $D+I$ to the total phenotypic variation. Hence a low correlation between H1 and H2 is due mainly to the relatively low value of D rather than any real differences in H1 and H2.

Table 5 Estimates of the additive genetic variation (D or $D+I$) obtained from the expected mean squares for the H₁ and H₂ generations

		MSW	SPY	Ht	EL	AE	GR	TGW
Golden Promise × Mazurka (TT1)	H ₁	303.7	1.8	13.4	0.6	5.2	4.2	17.0
	H ₂	636.5	12.1	20.9	2.1	2.3	9.7	12.8
	$\chi^2_{(1)}$	0.8	1.1	0.2	3.6†	0.9	1.2	0.1
Golden Promise × Ark Royal (TT3)	H ₁	196.5		83.1	1.5	13.3		20.0
	H ₂	294.5		61.0	1.2	9.6		33.9
	$\chi^2_{(1)}$	0.1		0.2	0.1	0.4		0.3
Bh ₄ /143/2 × Ark Royal	H ₁	1,390.0		90.6		5.2		35.0
	H ₂	185.5		57.3		5.6		
	$\chi^2_{(1)}$	2.1		0.9		0.0		2.9

† $P < 0.05$

Table 6 Estimates of the narrow sense heritabilities (h_n^2) and regression slopes (b) for the three crosses

		MSW	SPY	Ht	EL	Mat	AE	GR	TGW
TT1	h_n^2	0.45	0.28	0.39	0.59		0.44	0.48	0.39
	b	0.40 ± 0.15	0.24 ± 0.13	0.48 ± 0.16	0.43 ± 0.11		0.76 ± 0.20	0.38 ± 0.13	0.61 ± 0.17
TT3	h_n^2	0.20		0.50	0.61	0.70	0.72		0.36
	b	0.37 ± 0.23		0.79 ± 0.17	0.88 ± 0.17	0.65 ± 0.20	0.88 ± 0.18		0.45 ± 0.23
TT4	h_n^2	0.23		0.67			0.59		0.32
	b	0.37 ± 0.23		0.79 ± 0.17			0.88 ± 0.18		0.45 ± 0.23

(3) All the estimates necessary for univariate cross prediction (m and D) can be obtained from the H1 generation and can be used in a programme to rank the value of various crosses.

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