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The relationship between in vitro performance of haploid embryos and the agronomic performance of the derived doubled haploid lines in barley

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Summary. The relationship between in vitro performance of haploid embryos and the agronomic performance of the derived doubled haploid (DH) lines during the stages of field evaluation was investigated. The results showed a positive correlation between coleoptile height of haploid plantlets in culture and final plant height of their DH progeny lines in the field. These results illustrate that in vitro selection for plant height and other linked quantitative or pleiotropic characters can be carried out at the initial stages of a DH breeding programme.

Key words: Barley – *Hordeum bulbosum* – In vitro selection – Haploid

Introduction

The production of haploids at high frequencies is of great value to breeders and geneticists. It is the quickest way of advancing selected breeding lines to complete homozygosity and of increasing the efficiency of selection (Snape and Simpson 1981). The *Hordeum bulbosum* system has proved to be an efficient method of large-scale haploid production in barley and has made significant contributions to barley breeding programmes (Kasha and Kao 1970; Kasha and Reinbergs 1980; Shugar 1989). The efficiency of a DH breeding programme could be significantly increased if a direct relationship between in vitro performance during the initial stages of haploid development and the agronomic performance of the derived DH lines at the evaluation stages of the DH breeding programme could be established. This is because early selection of superior lines could be carried out and potentially inferior lines discarded. The objective of this investigation was to study variation in in vitro performance of haploid embryos and the agronomic performance of the derived DH lines, and to analyse the degree of relationship between them with a view of developing an in vitro selection procedure for agronomic traits in barley.

Materials and methods

Parental materials

F_1 hybrids from the crosses F_8 901-29 x 'Digger' (TAD), F_8 910-8 x 'Digger' (FHD), and F_8 904-3 x 'Digger' (HTD) were the parental materials. F_8 901-29, F_8 910-8, and F_8 904-3 were advanced selections from the winter barley breeding programme of Plant Breeding International (PBI), Cambridge. 'Digger', a commercial spring variety, was the male parent in all crosses. The F_1 plants were crossed with diploid *Hordeum bulbosum* clone PB1.

Procedures for developing the DH lines

a) *Emasculation, pollination, embryo excision, and culture.* These were as described by Simpson and Snape (1981). During the excision and culture periods the following charcteristics of the embryos were recorded; embryo size: length of the embryo in mm $\times 10^{-2}$ at the time of excision; germination period: the period recorded in days from excising the embryos to their germination, scored when coleoptiles were approximately 1 cm in height; coleoptile height: measured in cm 5 days after germination.

b) *Colchicine treatment.* This was as described by Simpson and Snape (1981).

Assessment of the field performance of the derived DH lines

a) *Design of experiments.* The field trial comprised five randomized blocks in which each genotype was represented by a single microplot. Each microplot consisted of a single row of 11 plants spaced 10 cm apart. Rows were spaced 30 cm apart. Eleven DH lines from the HTD cross, 14 DH lines from the FHD cross, and 16 DH lines from the TAD cross were used for the experiment.

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b) *Measurements of quantitative characters.* During growth, at maturity, and after harvest the following characters were measured on individual plots or single sample tillers. (1) Juvenile growth habit (GH) was scored on a scale of 1–5, where 1 was an extremely prostrate habit and 5 an extremely erect habit. (2) Ear emergence time (EET) was scored when the majority of plants in a plot had their awns emerged about 1 inch from the flag leaf. This was standardized to a convenient starting date, June 1st. (3) Average height (Hgt) of the plants in a plot (cm). (4) Plant tiller number (PTN), the total number of tillers/plot divided by number of plants in each plot. (5) Plot biomass: each plot was cut at ground level using a scythe, bound, and labelled individually. The weight of each plot was then measured (g). (6) Plot yield: harvested plots were threshed using a standing plot combine. To standardize moisture content, the grains from all harvested plots were dried to approximately 14% moisture content and then weighed (g).

Prior to harvest five leading tillers were taken from each plot for laboratory analysis, and the following characters measured; tiller biomass: the weight of five tillers in $g \times 10^2$; tiller yield: the ears were threshed by hand, and the grain yield was recorded in $g \times 10^2$; tiller grain number: total number of grains of five tillers.

All statistical analyses were carried out using the computer programme, GENSTAT version 5 (GENSTAT 5 – Committee 1987). Initially, calculations were carried out on the raw data from the above parameters to obtain values standardized to a single plant or single tiller basis. Consequently, the following parameters were created: biomass per plant (Bio/P), yield per plant (Yld/P), biomass per tiller (Bio/T), yield per tiller (Yld/T), plant harvest index (HI/P), tiller harvest index (HI/T), tiller grain number (GN/T), and 50-grain weight (50-GW). Overall, 12 characters were available for analysis.

Results

Analyses of variance (not shown) revealed genetical variation between DH lines within and between crosses for all field-measured quantitative characters. The relationship between the three in vitro characters embryo size, germination period, and coleoptile height and the agronomic characters were then investigated using correlation analyses. Single linear correlation analyses based on the mean values of individual DH lines and the phenotypic value of the haploid parent were carried out.

Table 1 presents the correlation coefficients between the three in vitro characters and the 12 agronomic traits for the three crosses HTD, FHD, and TAD. In the HTD cross, based on 11 derived DH lines, embryo size was positively correlated with yield per tiller and plant harvest index. Germination period was negatively correlated with tiller yield, plant harvest index, tiller harvest index, and 50-grain weight. Coleoptile height was positively correlated with final plant height ($P<0.05$). In the FHD cross, based on 14 derived DH lines, again a positive correlation ($P=0.054$) was found between coleoptile height and plant height. In the TAD cross, based on 16 derived DH lines, there was also a strong positive correlation ($P<0.01$) between coleoptile height and plant height.

Among all the correlations mentioned above only the correlation between coleoptile height and final plant height appeared to be consistent over the three crosses. Figure 1 shows this relationship in the three crosses. Homogeneity tests of correlation coefficients were carried out and homogenous correlations, from Table 1, were pooled over the three crosses using Fisher's Z transformation (Snedecor and Cochran 1980). These are presented in Table 2. A highly significant positive correlation ($P<0.001$) was detected between coleoptile height and plant height. Coleoptile height also had a significant positive correlation ($P<0.05$) with growth habit and a positive and potentially useful correlation ($P=0.075$) with plant yield. Overall, these results suggest that haploid embryos with taller coleoptiles had taller DH progenies and were likely to have a erect growth habit and a higher yield.

Table 3 shows correlation coefficients between final plant height and biomass, and yield traits in DH lines of the three winter crosses. Final plant height showed positive correlations with yield and biomass of single plants

Table 1. Correlation coefficients between in vitro performance of haploid barley embryos and the agronomic performance of the derived DH lines of the three winter crosses

	GH	EET	Hgt	PTN	Bio/P	Yld/P	Bio/T	Yld/T	HI/P	HI/T	GN/T	50-GW
HTD (*df*=9)												
Embryo size	0.297	−0.237	0.072	−0.152	0.126	0.426	0.454	0.707*	0.715*	0.421	0.324	0.551
Germination period	−0.426	0.356	0.108	−0.125	−0.316	−0.574	−0.265	−0.714*	−0.650*	−0.748**	−0.147	−0.731*
Coleoptile height	0.506	−0.284	0.643*	−0.292	0.069	0.207	0.571	0.525	0.276	−0.097	0.285	0.354
FHD (*df*=12)												
Embryo size	0.165	−0.280	0.100	−0.183	−0.228	−0.451	−0.279	−0.375	−0.251	−0.243	−0.407	−0.251
Germination period	−0.118	0.140	−0.221	−0.297	−0.107	−0.124	−0.227	−0.166	0.038	0.164	−0.217	−0.018
Coleoptile height	0.493	−0.324	0.524[1]	0.462	0.214	0.248	0.030	−0.084	−0.286	−0.265	−0.051	−0.101
TAD (*df*=14)												
Embryo size	−0.147	0.134	−0.068	−0.129	−0.252	−0.153	−0.383	−0.218	0.061	0.151	−0.163	−0.212
Germination period	−0.160	0.093	−0.162	0.060	0.066	0.003	0.345	0.325	−0.094	0.091	0.481	−0.104
Coleoptile height	0.101	0.253	0.631**	0.336	0.407	0.388	0.042	0.095	0.182	0.071	0.060	0.076

Probability levels: [1] $P<0.054$; * $P<0.05$; ** $P<0.01$

Table 2. Correlation coefficients between in vitro performance of haploid barley embryos and the agronomic performance of the derived DH lines pooled over the three crosses

	GH	EET	Hgt	PTN	Bio/P	Yld/P	Bio/T	Yld/T	HI/P	HI/T	GN/T	50-GW
Winter crosses (*df*=35)												
Embryo size	0.070	−0.089	0.025	−0.159	−0.149	−0.119	−0.139	–	–	0.089	−0.130	−0.020
Germination period	−0.217	0.178	−0.119	−0.110	−0.100	−0.207	−0.009	−0.148	–	–	0.100	−0.270
Coleoptile height	0.354*	−0.080	0.600**	0.245	0.264	0.300[1]	0.188	0.158	0.040	−0.089	0.080	0.089

Probability levels: [1] $P=0.075$; * $P<0.05$; ** $P<0.001$

Table 3. Correlation coefficients between final plant height and biomass and yield traits of DH lines of the three winter crosses

Cross	Bio/P	Yld/P	Bio/T	Yld/T
HTD (*df*=9)	0.173	0.262	0.672*	0.397
FHD (*df*=14)	0.547*	0.407	0.451	0.336
TAD (*df*=14)	0.465	0.362	0.405	0.207
Overall[a] (*df*=35)	0.430**	0.350*	0.500**	0.300[1]

Probability levels: [1] $P=0.075$, * $P<0.05$; ** $P<0.001$
[a] Correlation coefficients pooled over the three winter crosses after homogeneity test

as well as single tillers in all three crosses, with varying degrees of magnitude. Overall, plant height had stronger correlations ($P<0.01$) with biomass than with yield. This is probably because taller plants tended to have a greater biomass, which in turn contributed towards higher grain yield because of a source and sink relationship. Although these results do not provide sufficient evidence on the kind and degree of genetical association between plant height and these characters, they nevertheless indicate that selection for increased height would affect yield and biomass in a positive direction.

The present results suggest that coleoptile height in culture and plant height in the field are likely to be under a common genetical control and that selection for plant height and other agronomically related characters can be effectively carried out by selecting haploid plantlets in culture during the initial stages of production. Based on this information an in vitro selection procedure for agronomic traits could be developed. This was examined on the present data by evaluating haploid coleoptile height as a selection criterion. The DH lines were divided into two groups based on the performance of their haploid parents at the culture stage. The first group (H_1) were those whose haploid parents had a coleoptile height of less than 3 cm measured 5 days after germination, and the second group (H_2) were those with a coleoptile height of more than 3 cm measured 5 days after germination.

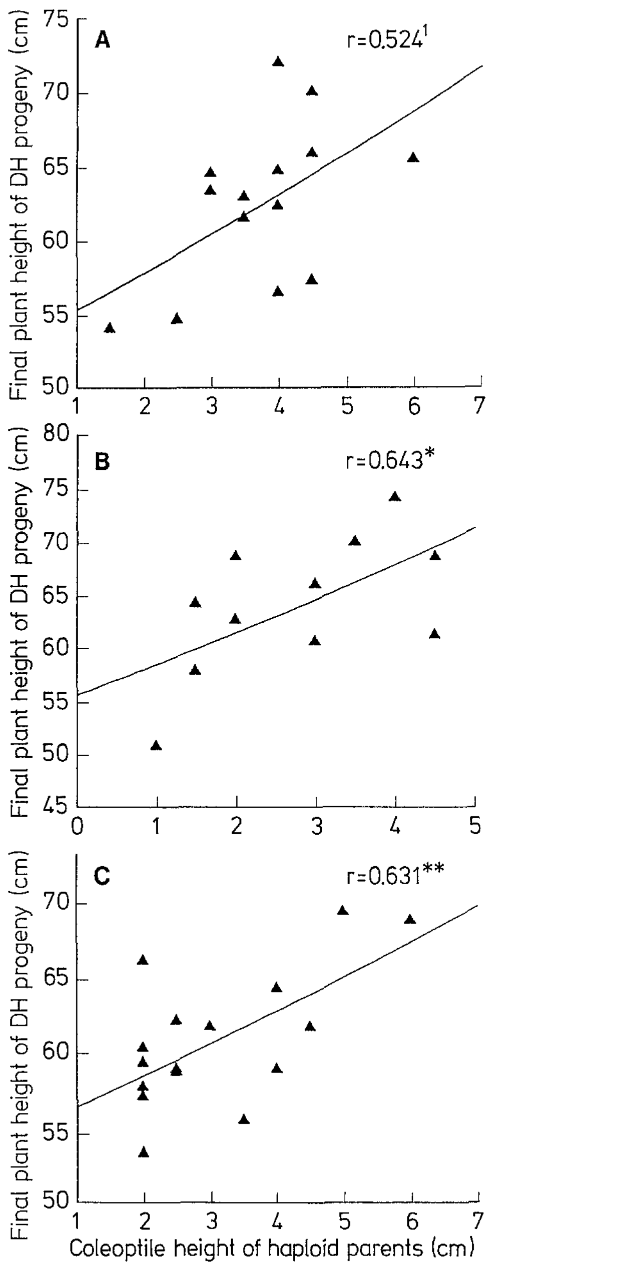

Fig. 1A–C. Relationship between coleoptile height and final plant height for the three crosses **A** FHD, **B** HTD, **C** TAD. Probability levels: *1*=0.054, * $P<0.05$, ** $P<0.01$

Table 4. Means (±SE) for agronomic characters of the DH lines based on the initial growth of their parental haploid embryos in culture

Character	HTD			FHD			TAD		
	H_1 [a]	H_2 [a]	SD [b]	H_1	H_2	SD	H_1	H_2	SD
GH	1.80±0.26	2.80±0.43	§	1.90±0.10	2.87±0.40	*	3.02±0.30	3.31±0.47	n.s.
EET	38.64±1.24	35.27±2.10	n.s.	34.90±0.30	38.68±1.84	§	35.07±1.51	36.17±2.08	n.s.
Hgt	60.80±3.04	66.77±2.15	n.s.	54.30±0.30	63.78±1.29	***	59.38±1.17	63.03±1.92	n.s.
Bio/P	20.22±2.27	20.72±1.24	n.s.	16.62±3.98	20.85±1.07	n.s.	18.23±0.83	19.96±1.20	n.s.
Yld/P	8.84±0.87	9.55±0.53	n.s.	8.63±2.24	9.82±0.53	n.s.	8.79±0.51	9.97±0.88	n.s.
Yld/T	0.95±0.05	1.12±0.04	§	0.96±0.07	1.03±0.05	n.s.	0.98±0.05	0.98±0.06	n.s.
GNT	22.78±1.14	23.38±1.04	n.s.	21.76±2.24	22.20±0.68	n.s.	21.10±0.86	21.13±0.96	n.s.
50-GW	2.08±0.09	2.40±0.08	*	2.22±0.06	2.31±0.05	n.s.	2.32±0.04	2.31±0.08	n.s.

[a] Coleoptile height (cm) measured 5 days after germination where $H1<3$ and $H2\geq3$
[b] Significance of difference between DH groups tested by analyses of variance. Probability levels: n.s.$=P<0.1$; § $P<0.1$, * $P<0.05$, ***$P<0.001$

Table 4 shows comparisons between the two groups for 8 agronomic characters. Differences between means of the groups were tested in an analysis of variance against the variances within groups. H_2 plants tended to have an erect growth habit (GH), while H_1 plants had a prostrate growth habit in all three crosses. Thus, the genotype for this character can perhaps be classified in single embryos if a single gene is involved. For plant height the H_2 plants were taller than the H_1 plants in all three crosses. This difference was most distinct in the FHD cross with a highly significant ($P<0.001$) level, of difference between the two groups. The H_2 plants also had a higher plant biomass, plant yield, tiller biomass, tiller yield, tiller grain number and 50-grain weight in all the three crosses. Although the difference between the two groups was not statistically significant in all cases, nevertheless the direction of difference between the two groups was consistent for all the characters, except for ear emergence time, in all three crosses. This was perhaps because of the low number of lines in individual crosses involved in the comparisons.

Table 5 shows the comparisons between the two groups for all DHs combined together for the 8 agronomic characters. This shows that overall the H_2 plants were significantly taller and tended to be more erect, slightly later in maturity with a higher plant biomass, plant yield, tiller biomass, tiller grain number, and 50-grain weight. Thus, the overall results suggest that the H_2 plants tended to be superior to the H_1 plants for nearly all the agronomic traits studied.

The results above suggest that selection for final plan height in the field can be carried out during the initial stages of a DH breeding programme. However, the extent of success of a selection programme depends on the heritability of the character under study. Narrow sense heritability (h^2), based on mean performance of individual plots, of all the agronomic characters were estimated (not shown). Overall, most characters had high heritabilities with growth habit ($h^2=0.74$) and final plant height ($h^2=0.72$) having the highest while plant tiller number ($h^2=0.29$) and plant biomass ($h^2=0.29$) had the lowest. This is undoubtedly because these characters (PTN, Bio/P) are influenced by environmental factors such as population density to a much larger extent.

Table 5. Means (±SE) of agronomic characters of the DH progeny lines based on initial growth of their parental haploid embryos in culture pooled over the three crosses

Character	H_1 [a]	H_2 [a]	SD [b]
GH	2.50±0.24	2.98±0.25	n.s.
EET	36.16±1.00	37.16±1.17	n.s.
Hgt	59.19±1.20	64.29±0.97	**
Bio/P	18.65±0.93	20.57±0.66	§
Yld/P	8.79±0.43	9.80±0.36	§
Yld/T	0.97±0.03	1.04±0.03	n.s.
GN/T	21.69±0.64	22.18±0.50	n.s.
50-GW	2.24±0.04	2.33±0.03	§

[a] Coleoptile height (cm) measured 5 days after germination where $H1<3$ and $H2\geq3$
[b] Significance of difference between DH groups tested by analyses of variance. Probability levels: n.s.$=P<0.1$; § $P<0.1$, **$P<0.01$

Discussion

The important correlation found in this study was that between coleoptile height in culture and plant height in the field. It seems likely that these two characters are under the same genetical control. This would suggest that selection for plant height can be effectively carried out two generations in advance through selection of haploid plantlets in culture. Alternatively, selection for reduced height, an important component of lodging resistance, could be carried out in vitro when a DH system is being utilized. In addition, this can further contribute to a breeding programme when the character height is genetically linked or when the genes are pleiotropic with other quantitatively inherited characters. This will, of course, depend on the genetic architecture of the quantitative characters and their interrelationships in the particular crosses under investigation. In such cases in vitro selection for height can also be effective for the other characters and may be considered as an indirect selection for these characters. Although there were insufficient data to suggest involvement of linkage or pleiotropic association of plant height with other agronomic characters in this investigation, nevertheless, the present results suggest that selection for height would affect other agronomic characters in a positive direction.

The results reported by other workers also suggest a direct relationship between height and yield. For example, Vazquez and Sanchez-Monge (1987) showed a positive correlation between height and yield in barley in a 6×6 diallel cross. Baron and Kibite (1987) also reported a positive correlation between these characters in eight six-rowed barley lines. In contrast, Thomas et al. (1991) reported the association of *Denso* and *GP-ert* dwarfing genes with low grain weight and low plant yield. However, these are the effects of individual mutant genes and thus perhaps not surprising. In wheat, Law et al. (1978) showed a positive correlation between height and yield in a 4×4 diallel cross from four diverse varieties. However, as this relationship is contrary to the breeder's objectives of selecting for high yielding lines with short straws, they suggested that a way of utilizing this relationship would be to introduce independently acting dwarfing genes and then to select for tall, high-yielding plants. Such a strategy could also work in barley using the *Denso* and *GP-ert* dwarfing genes.

References

Baron VS, Kibite S (1987) Relationships of maturity, height and morphological traits with whole-plant yield and digestability of barley cultivars. Can J Plant Sci 67:1009–1017

Genstat 5-Committee (1987) Genstat 5 reference manual. Statistical department, Rothamsted Experimental Station. Clarendon Press, Oxford

Kasha KJ, Kao KN (1970) High frequency haploid production in barley (*Hordeum vulgare* L.). Nature 225:874–876

Kasha KJ, Reinbergs E (1980) Achievements with haploids in barley research and breeding. In: Davies DR (ed) The plant genome. The John Innes Charity, Norwich, pp 215–230

Law CN, Snape JW, Worland AJ (1978) The genetical relationship between height and yield wheat. Heredity 40:133–151

Shugar LP (1989) Commercialization of the *Bulbosum* method in barley (*Hordeum vulgare* L.). In: Maluszynski R (ed) Current options for cereal improvement. Kluwer Academic Publ, Dordrecht, pp 161–170

Simpson E, Snape JW (1981) The use of doubled haploids in a winter barley programme. In: Barley Genetics IV. Edinburgh University Press, Edinburgh, pp 716–720

Snape JW, Simpson E (1981) Uses of doubled haploid lines for genetical analysis in barley. In: Barley Genetics IV. Edinburgh University Press, Edinburgh, pp 704–709

Snedecor GW, Cochron WG (1980) Statistical methods, 7th ed. The Iowa State University Press, Ames, Iowa

Thomas WTB, Powell W, Swaminston JS (1991) The effects of major genes on quantitatively varying characters in barley. 4. The *GPert* and *denso* loci and quality characters. Heredity 66:381–389

Vazquez JF, Sanchez-Monge E (1987) Correlations, epistasis, and heterosis of plant height and internode length in barley. Genome 29:532–536