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Tests for the presence of gametoclonal variation in barley and wheat doubled haploids produced using the *Hordeum bulbosum* system

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Summary. To investigate whether the *Hordeum bulbosum* system of doubled haploid production generates gametoclonal variation, populations of second generation doubled haploid lines were developed from first generation doubled haploid lines of two barley varieties and three wheat genotypes. In barley, no variation between doubled haploids from doubled haploids was detected for a range of quantitative characters, suggesting the absence of any gametoclonal effects. However, the original selfed-seed stocks were shown to contain cryptic allelic variation for some of the characters investigated. In wheat, gametoclonal variation was detected for ear emergence time, plant height and yield, and its components for two out of the three genotypes investigated. The type and range of variation was similar to that reported from studies of somaclonal variation from immature embryos and gametoclonal variation from anther culture. Generally, the effects appeared to reduce the yield performance of individual lines. The difference in response between the two species and the consequences for the use of the doubled haploid system in breeding programmes are discussed.

Key words: Gametoclonal variation – Barley doubled haploids – Wheat doubled haploids – *Hordeum bulbosum*

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Introduction

In recent years great progress has been made in the development of doubled haploid (DH) systems in cereals (Snape 1982). In wheat and barley, two alternative systems are now available, namely anther culture and the "*Hordeum bulbosum* method". Snape et al. (1986b) have suggested that three criteria are important in the choice of a breeding program system: (1) the frequency of production of doubled haploids, (2) the logistical requirements of the alternative systems, and (3) the type and range of genetical variation produced. Information is now available on the first two criteria – for example, the work of Friedt et al. (1987) in barley and Snape et al. (1986a) in wheat. However, more information is required on the third criterion, and this could be critical in the choice of system to be used, since current evidence suggests that the efficiency and cost effectiveness of DH production by either systems is similar and also compares favourably to conventional breeding methods.

One factor which will affect the variation exhibited by DH lines derived from heterozygous parents is genetical changes induced by the production system. Both anther culture and the *H. bulbosum* system involve in vitro culture and, commonly, colchicine treatment for doubling the chromosome number. In recombinant progenies these treatments may induce variation over and above that derived from recombination and segregation of the parental genomes. Results from evaluation of the phenotypic performance of anther culture-derived DH lines in barley (Powell et al. 1984) and in wheat (Baenziger et al. 1983) suggests that anther culture does induce 'gametoclonal' variation. Such variation usually appears to be detrimental to

performance for yield characters and, as yet, does not appear to be useful for breeding purposes. It is important to establish whether the *H. bulbosum* system likewise induces genetical variation in derived DH progenies. The experiments described in this paper were designed to provide such information.

Materials and methods

Development of the experimental material

One criticism of earlier studies designed to detect gametoclonal variation in cereals has been the questionable purity of the starting material. Differences in DHs derived from selfed-seed stocks of inbred lines or varieties may only reflect the presence of residual heterozygosity in the parental material rather than induced mutational changes. The experimental system used in the present investigation was designed to circumvent this problem by deriving DH lines from previously developed DH lines. Consequently, any variation observed between the doubled haploids from doubled haploids (DH/DH) must relate to induced changes during culture and chromosome doubling, or to heterozygosity in the DH parent induced by the previous round of culture. This assumes, of course, that the frequency of spontaneous mutations will be very rare.

For the studies in barley two varieties were chosen for investigation; 'Sultan' and 'Julia'. Initially plants of these cultivars were germinated from selfed-seed stocks maintained at the Plant Breeding Institute and grown in a glasshouse. At heading time these plants were crossed with diploid *H. bulbosum* genotype PB1, and DH progenies were produced using the techniques described by Simpson and Snape (1981). This procedure produced at least one DH line from five different parental plants of each variety. DH seed was then taken at random from one of these lines for each variety and used for a second round of crossing with *H. bulbosum*. From the second generation, five DH/DH lines were developed from both 'Sultan' and 'Julia'.

For the studies in wheat three genotypes were used: 'Chinese Spring' (CS) and the single chromosome substitution lines of chromosome 5B of 'Chinese Spring' into the spring wheat varieties 'Highbury' and 'Sicco'. The latter lines, designated 'Highbury (CS 5B)' and 'Sicco (CS 5B)', are crossable with *H. bulbosum* due to the introduction of the crossability allele *kr1* from 'Chinese Spring' (Snape et al. 1986a).

Plants of these parental genotypes were grown in a glasshouse and, at heading, hybridized using pollen from a mixture of tetraploid *H. bulbosum* genotypes. DH lines were then produced using the techniques described by Sitch and Snape (1986) and Snape et al. (1986a). At least one DH line of each genotype was created. For the second generation of DH production, seeds of one of these DH lines of each genotype were germinated and the procedures repeated to produce the DH/DH lines. In all, 18 DH/DH lines of 'Chinese Spring', 26 lines of 'Sicco (CS 5B)', and 11 of 'Highbury (CS 5B)' were produced.

Seeds were taken from each DH/DH line, their original DH lines, and the initial parents and multiplied for one generation to produce sufficient seed for field experiments. Throughout these procedures all ears of the colchicine-treated haploids and their subsequent progenies were covered with cellophane bags to avoid contamination with extraneous pollen.

Table 1. Numbers of parental, doubled haploid (DH) and doubled haploid from doubled haploid (DH/DH) lines grown

Genotype	Parents	DH lines	DH/DH lines
Sultan	1	5	5
Julia	1	5	5
Chinese Spring	1	1	18
Highbury (CS5B)	1	1	11
Sicco (CS5B)	1	1	26

Field experiments

The DH and DH/DH genotypes were grown in field experiments sown in spring 1984. A separate experiment was carried out for each species to minimize effects of interspecific competition.

The experiment with barley included 22 genotypes consisting of one line from selfed-seed of each of the varieties, five DH lines each derived from a separate, randomly germinated plant of each variety, and five DH/DH's derived from a single parental DH of each variety (Table 1). The experiment with wheat contained 61 genotypes including selfed-seed of each original line, one first generation DH of each of these, and all DH/DH lines developed (Table 1).

The two experiments were sown adjacently with five replicate blocks of each. Within each replication, each genotype was represented by a single row of 11 plants, spaced 10 cm apart within rows and rows spaced 30 cm apart. During growth and at maturity a range of characters was measured on each plot, including ear emergence time and plant height, and scored as days from June 1st. At harvest, four random leading tillers were taken from each plot and used for the evaluation of single tiller yield components. The remainder of each plot was harvested, threshed, and plot yields measured. All these data were converted to values per plant or per tiller for analysis.

Results

Barley

The analysis of variance of differences between the eleven genotypes for each of the varieties 'Sultan' and 'Julia', for seven representative characters, is shown in Table 2. The variation between lines was partitioned into comparisons between the means of the three groups (parent, DH and DH/DH), and between DH lines within the DH and DH/DH groups.

Significant differences between all lines were exhibited for ear emergence time in both crosses, and this variation originates from two sources. First, there is a significant difference between the means of the three groups which, in both crosses, is accounted for by a comparison of the two DH group means with the parental variety mean. It appears that the parental seed sources used in this experiment, which were derived from selfing sib plants of those used for DH production, gave plants that were significantly earlier to flower than the derived DH lines.

Consequently, the original seed source for each variety must have been segregating for genes con-

Table 2. Analysis of variance of differences between doubled haploid lines of barley

Item	Mean squares							
	df	Ear emergence time (day)	Plant height (cm)	Yield/ plant (g)	biomass/ tiller (g)	Grain wt./ lear (g)	Grain no./ ear (g)	100 grain wt. (g)
Between Sultan lines	10	1.2145*	25.90	25.58	0.1385	0.0317	3.398	0.1954
Between groups[a]	2	2.3125*	7.78	50.30*	0.2263	0.0729*	10.982*	0.2698
Between DH lines	4	1.5400*	23.60	20.99	0.0873	0.0164	1.282	0.1117
Between DH/DH lines	4	0.3400	37.26	17.81	0.1458	0.0264	1.722	0.2419
Between Julia lines	10	1.6836*	17.04	14.91	0.0535	0.0154	2.519	0.0767
Between groups[a]	2	3.6180**	37.88	10.03	0.0995	0.0294	2.651	0.1131
Between DH lines	4	1.4600*	19.80	5.44	0.0177	0.0150	2.584	0.1296
Between DH/DH lines	4	0.9400	3.86	26.82	0.0613	0.0088	2.388	0.0056
Error	84	0.5623	16.94	14.24	0.0778	0.0209	2.102	0.1328

[a] Variation between means of parent, DH group mean and DH/DH group mean
Significance levels: * = 0.05–0.01; ** < 0.001

Table 3. Analysis of variance of differences between DH/DH lines of wheat

Item	Mean squares						
	df	Ear emergence time (days)	Plant height (cm)	Yield/ plant (g)	biomass/ tiller (g)	Grain wt./ ear (g)	50 grain wt. (g)
Between Chinese Spring DH/DH lines	17	0.1464	5.715	4.317	0.1775	0.06205	0.02401
Between Sicco (CS5B) DH/DH lines	25	0.3671**	4.352	10.028**	0.2148	0.11840*	0.02706
Between Highbury (CS5B) DH/DH lines	10	0.3345*	12.051*	3.808	0.2272	0.11098	0.02218
Error	248	0.1758	5.252	4.262	0.1808	0.06847	0.02117

Significance levels: * = 0.05–0.01; ** = 0.01–0.001

trolling this character. This is confirmed by the second source of variation, that of significant differences between the means of lines within the DH group, where each line originated from a different parent plant.

Differences between the means of the three groups were also exhibited for yield/plant, grain weight/ear, and grain number/ear by the 'Sultan' lines. This again demonstrates that the parental seed source for this variety was segregating for alleles controlling these traits.

Conequently, the seed sources of both these varieties are heterogeneous for alleles controlling these characters, although the magnitude of the phenotypic variation was small. For example, the difference in group means for flowering time, although statistically significant, was only one day. Such small differences are unlikely to be important during selection for varietal purity, and some genetical variation for these characters has obviously remained within the seed stocks over many generations of multiplication.

For all characters, however, there was no variation exhibited between the DH/DH lines within the population of either cross. Further, there were no significant differences between the means of each DH/DH line and the mean of their DH parent. Consequently, these results suggest that the *H. bulbosum* method of haploid production does not generate gametoclonal variation in barley, at least in these varieties.

Wheat

An examination of variation at major gene loci for glume color and awning in the Highbury (CS 5B) DH/DH population and for biochemical markers (glutenin and gliadin sub-unit composition) in all DH/DH populations showed that all DH/DH lines were identical to their DH parent in allelic constitution at these loci. However, this test only samples a small proportion of the genome for variation. Quantitative characters, being determined by several loci, are likely to provide a more sensitive test.

The analysis of variance between lines within each DH/DH population, for six representative quantitative characters, is shown in Table 3. Significant differences between genotypes were exhibited – for ear emergence

Table 4. Mean performance and range for parent DH and DH/DH lines of Highbury (CS5B) and Sicco (CS5B)

	Ear emergence time (days)	Plant height (cm)	Yield/plant (g)	Grain wt./ear (g)
Sicco (CS5B) parent DH mean	2.40	100.2	18.16	2.40
Sicco (CS5B) DH/DH mean	3.07	102.4	17.13	2.31
Sicco (CS5B) DH/DH range	2.6–3.6*	100.6–104.4	13.48–20.20	1.84*–2.49
Highbury (CS5B) parent DH mean	3.40	108.8	15.62	3.34
Highbury (CS5B) DH/DH mean	3.71	108.7	15.23	3.52
Highbury (CS5B) DH/DH range	3.4*–4.0*	106.0–111.0	13.20*–16.55	3.25–3.71

* Significantly different from parent DH mean at 5% level

time in the Sicco (CS 5B) and Highbury (CS 5B) populations, for plant height in the Highbury (CS 5B) population, and for grain weight/plant and grain weight/ear in the Sicco (CS 5B) population. Changes have occurred in the development of these lines which are expressed as genetical differences between the DH/DH progenies. In contrast, no variation was exhibited by the Chinese Spring lines.

The direction of the differences in the Highbury (CS 5B) and Sicco (CS 5B) DH/DH populations are shown in Table 4. In both populations, lines significantly later than the parental DH line for ear emergence time were apparent, indicating that mutational changes retarding development had occurred. In contrast, in the Highbury (CS 5B) population there was also one line which was flowering significantly earlier. As with the barley lines, however, the range of variation for this character, although statistically significant, was only just over one day, indicating the generation of small rather than large effects. Such changes were frequent in the Sicco population, where five out of the 26 lines were significantly later than the parental DH line. There was a directional effect for changes in plant height where all significantly deviant lines were taller. A similar directional pattern was shown for yield/plant and grain weight/ear, where all lines were significantly lower than the parent. This suggests the presence of mutations which reduce fitness. Overall, these results are similar to those reported for anther culture (Baenziger et al. 1983), where the occurrence of gametoclonal variation always appeared to be detrimental to yield performance.

There was no apparent correlation between the deviations of individual lines for different characters, suggesting that mutations were at random relative to different DH/DH lines and characters. Also, although individual DH/DH lines were different from the DH parents, overall population means were not significantly different, other than for ear emergence time in the Sicco (CS 5B) population. Generally, the magnitude of the changes for all characters was small.

Discussion

These results indicate that the *H. bulbosum* system of doubled haploid production generates gametoclonal variation in wheat but not in barley. This difference in response between the species probably reflects the difference in ploidy level, a characteristic shown to affect the response of these species to other systems, for example, pollen irradiation (Snape et al. 1983). Wheat (a hexaploid), is well known to tolerate aneuploidy, chromosomal rearrangements, and a wide range of mutations while still producing viable and fertile progeny (Law and Worland 1972). Barley (a diploid), on the other hand, is well known to react adversely to changes in its genome (Tsuchiya, personal communication). In the present study it is possible that changes were generated during culture in barley, but that no viable mutated progeny survived to produce fertile DH lines. These results contrast with the studies of Powell et al. (1984), where they indicated that gametoclonal variation for a similar range of quantitative characters was generated in DH progenies from anther culture of the barley variety 'Sabarlis'. However, their lines were developed from selfed seed stocks and they were unable, therefore, to unambiguously separate the effects of gametoclonal variation from seed source effects.

The direction and range of effects exhibited between the DH/DH lines of wheat shown here is similar to that shown in studies of the performance of somaclonal lines regenerated from immature embryos (Larkin and Scowcroft 1981; Larkin et al. 1984), and for DH lines derived from anther culture (Baenziger et al. 1983). However, it may be misleading to compare the present study with these two systems, since in the present study there is no callus phase, only the stimulation of germination and the development of immature but differentiated embryos. The mechanisms underlying these changes are unclear. It is possible that mutagenic effects of colchicine are responsible for the variation, rather than the processes of haploid plant production. Colchicine is known to have widespread effects on plant cell metabolism and

development (Hart and Dinkar 1976), and there is evidence of complex formation between colchicine and DNA (Ilan and Quastel 1966).

The detection of cryptic variation within the selfed-seed stocks of the barley varieties used vindicates the use of the experimental system of developing DH's from a previously developed DH parent. These results also add a note of caution to the interpretations of experiments used to detect somaclonal or gametoclonal variation in cereals. Even where studies have used selfed-seed stocks as controls, there is the possibility of erroneously concluding the presence of induced variation because of sampling differences between the selfed controls and the plants used for culture or haploid production.

The present results also have conequences for the choice of a DH system to be used in cereal breeding programs. It appears that, in wheat, both anther culture and the *H. bulbosum* system are generating variation over and above that which would be obtained from reassortment and recombination of genomes in an F_1 between two varieties. However, accumulated evidence suggests that this variation is not qualitatively different from that already available in the species, or from that obtained from induced mutation studies. The effects on yield and its component characters appear to be detrimental. Consequently, it is unlikely that this variation will improve the benefits of using doubled haploid systems. This is also likely to be true for anther culture in barley, although the *H. bulbosum* system appears to be exempt from this problem. Fortunately, it is likely that this variation will be relatively trivial when compared with that released by reassortment and recombination of the parental genomes, and its effects should not hinder genetic advancement.

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