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The agronomic performance of wheat doubled haploid lines derived from wheat × maize crosses

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Summary. The agronomic performance of 9 doubled haploid (DH) lines of Chinese Spring, 6 DH lines of Hope, 14 DH lines of the single chromosome substitution line Chinese Spring (Hope 5 A) and their respective parents was analyzed under field conditions. Seventeen Chinese Spring DH lines derived from wheat × *Hordeum bulbosum* crosses were also included for comparison. No significant variation was detected in either population of Chinese Spring DH lines and neither DH population differed from its parent. The Hope DH lines differed significantly for tiller biomass, spikelet number per ear, ear grain weight and 50-grain weight. However, all the variation could be attributed to the poor performance of only one line. Chinese Spring (Hope 5 A) DH lines showed significant variation for ear emergence time, but this was probably due to genetic heterogeneity in the parental stock. Overall, the results suggest that most DH lines produced by the wheat × maize method resemble their wheat parent, and that the variation induced in DH production is likely to be similar to that found in DHs from wheat × *Hordeum bulbosum* crosses.

Key words: Wheat – Maize – *Hordeum bulbosum* – Doubled haploids – Gametoclonal variation

Introduction

Considerable work has been done on the use of *Hordeum bulbosum* for the production of wheat haploids via chromosome elimination (Sitch and Snape 1986, 1987 a, b, c; Snape et al. 1988). Chromosome elimination in early embryo development also leads to the production of haploid wheat embryos in wheat × maize crosses (Laurie and Bennett 1987, 1988 a, 1989). This system is of interest because maize appears to be insensitive to the action of dominant alleles at the wheat crossability (*Kr*) loci, which are present in most of the world's wheat varieties and which greatly reduce the frequency of fertilization in wheat × *H. bulbosum* crosses (Snape et al. 1979; Falk and Kasha 1981, 1983). For example, crosses between the wheat genotype Highbury (*Kr1, Kr2*) and the maize genotype Seneca 60 gave fertilization in up to 59% of florets (Laurie 1989) compared to about 0.5% in Highbury × *H. bulbosum* crosses (Sitch and Snape 1987 a).

Haploid plants have been recovered from wheat × maize crosses by spikelet culture (Laurie and Bennett 1988 b), ovary culture (Comeau et al. 1988), treating spikes with 2,4-D (Laurie et al. 1990) and injecting 2,4-D into the upper internode (Suenaga and Nakajima 1989). Fertile doubled haploid (DH) lines can subsequently be produced by colchicine treatment of the seedlings, but no assessment of the agronomic performance of DH lines from wheat × maize crosses has previously been reported.

Gametoclonal variation, which is usually detrimental to yield performance, has been found in DH lines from wheat × *H. bulbosum* crosses (Snape et al. 1988) or from anther culture (Baenziger et al. 1983, 1989; De Buyser et al. 1985; Winzeler et al. 1987; Carver and Johnson 1989; Marburger and Jauhar 1989). It is important to assess the extent of such variation in DH lines from wheat × maize crosses, since this obviously has important implications for the usefulness of this system of haploid production. In the present work, the agronomic performance of 29 DH lines from wheat × maize crosses was studied and, in the case of Chinese Spring, compared to an equivalent population of DH lines derived from crosses with *H. bulbosum*.

Materials and methods

Development of DH lines

A total of 29 DH lines was tested which were derived from the crosses between three hexaploid wheat (*Triticum aestivum* L.) genotypes and the maize (*Zea mays* L.) genotype Seneca 60 described in Laurie and Bennett (1988 b). The 29 DH lines comprised 9 DH lines of Chinese Spring (CS) (*kr1, kr2*), 14 DH lines of Chinese Spring (Hope 5 A) (CSH5A) (*kr1, Kr2*), a single chromosome substitution line developed by E.R. Sears (University of Missouri, Columbia, USA and 6 DH lines of Hope (*Kr1, Kr2*).

Seventeen CS DH lines derived from one CS DH line (Snape et al. 1988) were also grown. This DH/DH material provides an accurate measure of the variation produced by the wheat × *H. bulbosum* system, since it eliminates the problem of variation between DH lines arising from genetic heterogeneity in the parent. These lines therefore provide a useful comparison to the DH lines derived from wheat × maize crosses.

Field experiments

Plants of each genotype were grown in row plots in each of five randomized blocks, each of which contained one plot of each DH line and two plots of each parent. Rows, which were spaced 30 cm apart, were each sown with 11 seeds spaced 10 cm apart. Ear emergence time, final plant height, number of fertile tillers, plant yield (weight of grain per plant), tiller biomass, spikelet number per ear, ear grain weight and 50-grain weight were recorded. The last four characters listed were estimated from samples of four leading tillers taken at random from each plot.

Results

Chinese Spring DH lines

Analysis of variance (Table 1) showed no significant difference between DH lines derived from CS × maize crosses or between the DH population mean and the parental mean. A similar result was found for the DH/DH lines derived from CS × *H. bulbosum* crosses, which is in agreement with a previous analysis of these lines (Snape et al. 1988). Comparison of the maize-derived DH population with the *H. bulbosum*-derived DH/DH population showed significant variation for final plant height ($p < 0.01$), plant yield ($p < 0.001$) and 50-grain weight ($p < 0.05$). This was partly attributable to genetic variation between the parental CS lines. They differed significantly for 50-grain weight ($p < 0.05$) and this, together with a nonsignificant difference in tiller number, probably accounted for the significant difference in plant yield (Table 2). The reason for the difference in height between the DH populations is not known since the parents did not differ significantly for this character. This may indicated the presence of some gametoclonal variation in the maize-derived DH lines.

Chinese Spring (Hope 5 A) DH lines

Significant variation for ear emergence time (EET) was found between DH lines and between the DH population and the parent (Table 1). Only two DH lines had EETs similar to that of the parent (0.1 days earlier), while in the remaining 12 the EET was 1.2–2.6 (mean 1.85) days later than the parent (Table 2). The latest DH lines had an EET similar to that of CS. Since most DH lines had a later EET than the parent and since no other character showed significant variation, it is unlikely that variation in EET was due to gametoclonal variation. It is more likely that the parent(s) from which the later EET lines were derived was not of the correct genotype, perhaps because it did not have the Hope *Vrn1* allele on the 5A chromosome. Nevertheless, all CSH5A DH lines had the expected Hope 5 A band(s) of the isozyme systems β-amylase (Sharp et al. 1988), iodine binding factor (*Ibf*) (Liu and Gale 1989 a) and malate dehydrogenase (*Mdh*-3) (Liu and Gale 1989 b). It is possible that variation was due to one or more genes in the background, that the parent had a recombinant 5A chromosome that was only partly derived from Hope, or that the parent had a mutated *Vrn1* allele.

Hope DH lines

Significant variation was found for tiller biomass, spikelet number, ear grain weight and 50-grain weight (Table 1). This was due to lower values for these characters in one of the six DH lines (Table 2). The other five lines resembled the parent and no significant variation was detected when the aberrant line was excluded from the analysis. It is likely that the aberrant line was a gametoclonal variant.

Discussion

Maize is potentially superior to *H. bulbosum* for haploid wheat production because of its insensitivity to the action of the wheat crossability (*Kr*) loci (Laurie and Bennett 1987, 1988 a; Laurie 1989). However, this advantage would be of little value if the DH lines produced did not accurately represent the genotype of the wheat parent. The results from this pilot study were therefore encouraging in that they showed that the wheat × maize system was able to produce DH lines that were equivalent to their wheat parent in agronomic performance. Furthermore, CS DH lines from crosses with maize appeared to be equivalent to CS DH/DH lines from crosses with *H. bulbosum*, although there was possibly some gametoclonal variation in the former population, which tended to increase the mean height relative to the parental line (Table 2).

More convincing evidence for gametoclonal variation was found in one of the six Hope DH lines (1/29 DH lines in all, assuming that variation in EET in the CSH5A DH lines was due to genetic heterogenity in the parent), but

Table 1. Analysis of variance of differences between DH lines

Item	*df*	Mean squares							
		Ear emergence time (days)	Plant height (cm)	Fertile tillers/ plant	Yield/ plant (g)	Biomass/ tiller (g)	Spikelet no./ear	Grain wt./ear (g)	50-grain wt.(g)
CS × maize									
Between CS lines	9	0.499	43.07	0.521	0.750	0.211	1.636	0.340	0.278
Between groups[a]	1	0.833	76.87	0.611	0.730	0.001	2.309	0.228	0.602
Between CS DH lines	8	0.176	40.41	0.562	0.767	0.244	1.753	0.351	0.113
CS × *H. bulbosum*									
Between CS lines	17	0.573	20.77	0.799	1.585	0.188	1.300	0.634	0.197
Between groups[b]	1	2.268	6.01	1.154	0.105	0.156	0.500	0.235	0.067
Between CS DH/DH lines	16	0.447	22.93	0.784	1.714	0.192	1.258	0.686	0.215
CSH5A × maize									
Between CSH5A lines	14	4.730***	29.65	1.076	1.495	0.088	0.977	0.289	0.269
Between groups[a]	1	21.667***	22.84	3.040	2.006	0.123	0.029	0.344	0.039
Between CSH5A DH lines	13	3.786***	32.19	1.007	1.320	0.092	1.093	0.306	0.267
Hope × maize									
Between Hope lines	6	0.563	47.14	1.140	1.465	0.282*	2.221*	0.919	0.672**
Between groups[a]	1	0.002	4.60	0.423	0.650	0.058	0.042	0.616	0.234
Between Hope DH lines	5	0.288	52.57	1.509	2.023	0.377*	3.096*	1.050*	0.862***
Error	189	1.332	24.13	1.048	1.179	0.131	1.066	0.455	0.194

[a] Comparisons of parental means with DH population means

[b] Comparison of parental DH means with DH/DH population means

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

Table 2. Means of parental lines and DH populations and ranges of DH means for those characters showing significant variation

	Ear emergence time (days)[a]	Plant height (cm)	Fertile tillers/ plant	Yield/ plant (g)	Biomass/ tiller (g)	Spikelet no./ear	Grain wt./ear (g)	50-grain wt.(g)
CS × maize								
Parent mean	11.80	83.10	5.90	5.51	3.76	18.03	1.79	1.63
DH lines mean	12.03	87.22	6.24	5.97	3.69	18.41	1.71	1.52
DH lines range	–	83.00–90.34	–	5.42–6.57	–	–	–	1.44–1.58
CS × *H. bulbosum*								
Parent DH mean	11.70	85.10	5.68	5.18	3.48	18.18	1.61	1.42
DH/DH lines mean	12.23	84.33	6.03	5.11	3.61	18.40	1.66	1.45
DH/DH lines range	–	81.20–88.09	–	4.12–6.62	–	–	–	1.34–1.56
CSH5A × maize								
Parent mean	9.49	79.37	5.56	4.75	3.37	16.41	1.57	1.60
DH lines mean	11.06	80.99	6.15	5.23	3.49	16.47	1.64	1.62
DH lines range	9.40–12.08	–	–	–	–	–	–	–
Hope × maize								
Parent mean	11.90	93.10	9.22	3.88	3.07	16.45	1.24	1.82
DH lines mean	11.88	92.32	8.98	3.98	2.98	16.38	1.22	1.76
DH lines range[b]	–	–	–	–	2.51–3.24	15.30–17.05	0.93–1.33	1.54–1.90

[a] Days from 1st June

[b] In all cases the lowest value is from the same DH line

the present experiment was too small to determine if this accurately reflects the frequency of induced mutations in DH lines from wheat × maize crosses. Overall, the results clearly indicate that it would be valuable to analyze a larger sample of DHs, preferably derived from commercial wheat varieties and breeders lines.

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