Production of haploid wheat through intergeneric crosses

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Frequencies of haploid production from wheat (*Triticum aestivum* L.) genotypes were compared in the crosses with *Hordeum bulbosum* L. and with maize (*Zea mays* L.). All the wheat genotypes produced embryos when crossed with maize, only if followed by the application of 2,4-dichlorophenoxyacetic acid (2,4-D). All plants regenerated from embryos on the B5 medium were euhaploids having a complement of 21 chromosomes. Overall frequencies of wheat haploid production were 0.2 % from the *H. bulbosum* cross, and 9.5 % from the maize cross. Use of the doubled haploid lines produced from F_1 hybrid plants for genetic analyses allowed an assessment of the effects of the semi-dwarfing genes *Rht1* and *Rht2* on yield performance.

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The initial objective of wide hybridization in cereal improvement programs is to enlarge the germplasm variation, particularly by incorporating desirable traits from wild into cultivated species. In some crosses, contrary to expectation, hybrid zygotes result in the formation of haploid embryos following the preferential elimination of chromosomes of one parent (ZENKTELER and NITZSCHE 1984). Since haploid plants carry only one set of alleles at each locus, after chromosome doubling the doubled haploid (DH) lines are completely homozygous and homogeneous. The dihaploidization process can thus provide a rapid method of developing recombinant inbred lines from hybrid progenies.

Since the performance of inbred lines can be accurately assessed in replicated experiments, the use of a random sample of recombinant inbred lines is advantageous in analyzing the linkage of gene(s) for agronomic traits, as well as for selecting lines for breeding purposes. Therefore, efficient production of haploid plants is of great interest to cereal geneticists and breeders.

This report presents a further development of the methods for haploid production in wheat (*Triticum aestivum* L.) through intergeneric crosses since the publication of a previous report (INAGAKI 1989), and gives an example how to use the DH lines derived from F_1 hybrid plants in analyzing the effects of semidwarfing genes on yield performance.

Overcoming cross-incompatibility of wheat

Intergeneric hybridization of wheat with *Hordeum bulbosum* L. results in the production of haploid embryos of wheat (BARCLAY 1975). However, the success of fertilization depends on which wheat genotypes are used for the cross (SNAPE et al. 1980). Cross-incompatibility of wheat is genetically controlled by the dominant genes *Kr1* and *Kr2*, located on chromosomes 5B and 5A, respectively (SITCH et al. 1985). At present, the wheat genotypes used for haploid production are restricted to cross-compatible lines originating from Asia (SNAPE et al. 1979; FALK and KASHA 1981; INAGAKI 1986; LI and HU 1986).

In order to overcome the cross-incompatibility barrier of wheat, the use of alternative pollen from maize (*Zea mays* L.) and the application of a plant hormone were attempted here. Four check varieties of wheat, i.e., Norin 61, Chinese Spring, Mexipak 65, and Highbury were used as female parents. The two pollen sources were populations of *H. bulbosum* and maize. On each of two consecutive days after pollination, wheat culms with pollinated spikes were needle-injected with a 100 ppm solution of 2,4-dichlorophenoxyacetic acid (2,4-D). Two weeks after pollination, immature embryos were aseptically cut out and transferred onto B5 medium (GAMBORG et al. 1968) for plant regeneration.

Table 1. Effect of 2,4-D application on embryo formation (%) in four wheat varieties crossed with *H. bulbosum* and with maize

Pollen source	2,4-D application	Wheat variety			
		Norin 61	Chinese Spring	Mexipak 65	Highbury
None	—	0.0	0.0	0.0	0.0
None	+	0.0	0.0	0.0	0.0
H. bulbusom	—	23.6	16.9	0.0	0.0
H. bulbosum	+	38.5	25.0	0.0	0.0
Maize	—	0.0	0.0	0.0	0.0
Maize	+	17.5	21.1	18.9	8.3

Table 1 shows the frequencies of embryo formation in the four wheat varieties crossed with *H. bulbosum* and maize. In the cross with *H. bulbosum*, two wheat varieties, Norin 61 and Chinese Spring, produced embryos at frequencies of 23.6 % and 16.9 %, respectively. These frequencies increased when 2,4-D was applied. Without 2,4-D application, the crosses of wheat with maize did not produce any embryos, whereas with 2,4-D application, all wheat varieties produced embryos and the frequencies of embryo formation ranged from 8.3 % to 21.1 % among the wheat varieties. Immature embryos ca. 1.0 mm in size obtained from crosses with *H. bulbosum* and maize were regenerated to green plants, within three weeks of incubation. A total of 226 plants were obtained from 534 embryos cultured. The frequency of success was 43.1 %. All regenerated plants that were examined cytologically were euhaploids, having a complement of twenty-one wheat chromosomes.

Recent cytological evidence shows that crosses of wheat with maize, irrespective of the presence of *Kr* gene(s), resulted in the production of wheat haploid proembryos after elimination of maize chromosomes, apparently by a process similar to that in crosses with *H. bulbosum* (LAURIE and BENNETT 1986, 1987). These proembryos degenerated rapidly, but were capable of regenerating haploid plants through in vitro culture of pollinated wheat florets (LAURIE and BENNETT 1988). All the wheat varieties crossed with maize and treated with 2,4-D successfully developed embryos of sufficient size to regenerate haploid plants, suggesting that the application of 2,4-D enhanced the development of hybrid zygotes to haploid embryos (SUENAGA and NAKAJIMA 1989; INAGAKI and TAHIR 1990).

Frequencies of haploid production

Average frequencies of embryo formation in twenty wheat varieties from West Asia and North Africa crossed with *H. bulbosum* and maize are compared in Table 2. Overall frequencies of wheat haploid production were 0.2 % using the *H. bulbosum* cross, and 9.5 % using the maize cross, indicating that the maize cross was more efficient than the *H. bulbosum* cross. This high frequency of haploid production has been confirmed using F_1 hybrid plants as starting materials.

This study demonstrated that maize pollination with subsequent 2,4-D application onto wheat florets results in the production of wheat embryos capable of regenerating haploid plants, even for wheat genotypes not crossable with *H. bulbosum*. However, this technique is not always amenable to haploid production of durum wheat (*T. turgidum* var. *durum* L.), although the reasons are not understood (INAGAKI and TAHIR, unpublished) and further development is required for application to other related crops in the Triticeae.

Table 2. Average frequencies of haploid production from twenty wheat varieties in crosses with *H. bulbosum* and with maize

Pollen source (%)	No. of florets pollinated	No. of seeds set (%)	No. of embryos obtained (%)	No. of plants regenerated
H. bulbosum	2296	1354 (59.0)	7 (0.3)	5 (0.2)
Maize	1128	906 (80.3)	245 (21.7)	107 (9.5)

Use of DH lines for assessing linkage effects

Although the DH lines may display genetic variations during the process of dihaploidization, these variations are rare and negligible for use in genetical analysis (INAGAKI 1987; LAURIE and SNAPE 1990).

A random sample of thirty DH lines produced from an F_1 hybrid (Norin 50 × Fukuhokomugi) using the *H. bulbosum* cross (INAGAKI 1989) was used to assess the linkage effects of the semi-dwarfing genes *Rht1* and *Rht2* on yield performance. The two parental varieties, Norin 50 and Fukuhokomugi, carried *Rht2* and *Rht1*, respectively. The genotypes of the DH lines were identified in terms of segregation ratios for the gibberellin (GA) response of the F_2 populations derived from crosses with the parental varieties (GALE et al. 1981). Yield performance was examined in a randomized block with two replications. Each line within a block was represented by a one-row plot of twenty plants, 10 cm apart. Rows were spaced 60 cm apart.

Agronomic traits of the DH lines identified for the semi-dwarfing genes are listed in Table 3. The four classes of DH lines examined for the assortment of semi-dwarfing genes fitted well with the expected ratio due to two-gene segregation at P = 0.75–0.90. The mean number of spikes/plant did not differ significantly among the four genotypes. On the other hand, significant differences were found for plant height, grain yield/plant and 1000-grain weight. The plants were remarkably short in the lines with the *Rht1 · Rht2* genotype, intermediate in those with either the *Rht1 · rht2* or the *rht1 · Rht2* genotype, and tall in those with the *rht1 · rht2* genotype. The values of grain yield/plant and 1000-grain weight of the lines with the *Rht1 · Rht2* genotype were lower than those of the lines with the *Rht1 · rht2*, *rht1 · Rht2*, and *rht1 · rht2* genotypes. No significant differences

were found among these three genotypes. In the lines with the *Rht1 · Rht2* genotype, the value of grain yield/plant was more reduced than that of 1000-grain weight.

Random assortment of semi-dwarfing genes was ascertained in the wheat DH lines examined, so that the effects of these genes on agronomic traits could be assessed. The differences in mean plant height among the four genotypes indicated that the effects of *Rht1* and *Rht2* were additive for the reduction of plant height. However, the combined effect of *Rht1* and *Rht2* adversely affected grain yield. The low grain yield of the dwarf lines (carrying both *Rht1* and *Rht2*) may be attributed to the decrease in both grain weight and, probably, number of grains, which was not measured in this study. The semi-dwarf lines (carrying either *Rht1* or *Rht2*) produced the same grain yield as did tall lines (carrying neither *Rht1* nor *Rht2*), which were artificially supported to prevent lodging. Therefore, the semi-dwarfing genes are effective in reducing plant height independently without causing an adverse effect on grain yield (GALE and YOUSSEFIAN 1985). In addition, the recombinant genetic background of the semi-dwarfing genes modified both plant height and grain yield.

Conclusion

The cross-incompatibility barrier in wheat has been successfully overcome by using maize pollen. The maize technique is currently being improved as an alternative to the bulbosum technique for wheat haploid production.

Use of the doubled haploid lines in genetical analysis allowed an assessment of the linkage effects of semi-dwarfing genes on yield. As molecular technologies have recently developed DNA sequence polymorphisms and physical linkage maps,

Table 3. Means[1] for agronomic traits in 30 doubled haploid lines derived from wheat F_1 hybrid (Norin 50 × Fukuhokumugi)

Genotype	No. of DH lines	Plant height (cm)	No. of spikes/plant	1000-grain weight (g)	Grain yield/ plant (g)
rht1 · rht2	7	106.0[a]	17.1[a]	32.0[a]	31.1[a]
Rht1 · rht2	10	87.0[b]	18.3[a]	30.1[a]	30.5[a]
rht1 · Rht2	6	83.4[b]	17.3[a]	31.0[a]	29.5[a]
Rht1 · Rht2	7	53.7[c]	17.5[a]	23.6[b]	17.6[b]
Norin 50	–	85.7	22.7	27.8	35.7
Fukuhokomugi	–	82.9	18.7	31.6	31.6

[1] Numbers followed by the same letter are not significantly different at the 5 % probability level

the recombinant inbred lines produced through intergeneric crosses could be used as materials for the detection of the loci for quantitative traits of agronomic importance.

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