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High Frequencies of Fertilization and Haploid Seedling Production in Crosses Between Commercial Hexaploid Wheat Varieties and Maize

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With 3 tables

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Abstract

Nineteen commercial hexaploid wheat varieties were crossed with the maize F_1 hybrid 'Seneca 60'. Fertilization frequencies ranged from 32.1 % to 47.5 % of pollinated florets (mean 39.5 %) in the 14 winter wheat varieties and from 40.7 % to 51.4 % (mean 47.8 %) in the five spring wheat varieties. In some cases only an endosperm was formed and the frequencies of embryo formation were therefore slightly lower, being 28.2 % to 45.9 % (mean 36.4 %) for winter wheats and 39.8 % to 48.6 % (mean 45.1 %) for spring wheats. Mean values were significantly higher in the spring wheats but no significant variation was found between varieties within the spring or winter categories. In the five spring wheats the mean yield of embryos, and hence the potential yield of haploid plants, was 3.4-fold higher than with the tetraploid *Hordeum bulbosum* clone PB179. For the 14 winter wheats the figure was 10.9-fold higher. These differences were highly significant ($p < 0.001$) in all varieties. A single 2,4-D treatment given to spikes one day after pollination with maize enabled embryos to be recovered from all 19 varieties. A total of 311 embryos were recovered from 950 florets (an average of 7.3 embryos per spike) of which 191 germinated, giving an average yield of one haploid plant for every 5.0 florets pollinated (4.4 haploid plants per spike).

Key words: *Triticum aestivum* — *Zea mays* — wide-crosses — fertilization — embryo rescue — haploids

In hexaploid wheat × maize crosses the maize chromosomes are eliminated from the developing embryo during the first few division cycles (LAURIE and BENNETT 1986, 1988a, 1989). Several techniques can be used to produce plants from the resulting haploid wheat embryos (LAURIE and BENNETT 1988b, COMEAU et al. 1988, LAURIE et al. 1990, SUENAGA and NAKAJIMA 1989) and fertile doubled haploid plants can subsequently be produced by colchicine treatment of the seedlings. Preliminary studies indicate that the agronomic performance of the resultant doubled haploid lines is equivalent to that of the parental genotypes in most cases (LAURIE and SNAPE 1990).

Wheat × maize crosses are of interest for wheat haploid production because maize appears to be insensitive to the action of dominant alleles at wheat crossability (*Kr*) loci (LAURIE and BENNETT 1987, 1988a, LAURIE 1989a) which drastically reduce the frequency of fertilization in crosses between hexaploid wheat and *H. bulbosum* (SNAPE et al. 1979, FALK and KASHA 1981, 1983, SITCH and SNAPE 1987a). This is illustrated by results from crosses involving the wheat variety Highbury (*Kr1, Kr2*). Pollination with three tetraploid *H. bulbosum* clones resulted in an average seed set of 0.5 % (SITCH and SNAPE 1987a) whereas pollination with the maize variety 'Seneca 60' gave fertilization in up to 59 % of florets (LAURIE 1989a).

Previous work on wheat × maize crosses in this laboratory has concentrated on controlled environment experiments using the wheat vari-

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eties 'Chinese Spring' (*kr1*, *kr2*) and 'Highbury' (*Kr1*, *Kr2*), which have also been extensively studied in crosses with *H. bulbosum* (SITCH and SNAPE 1987a, b, c). To be useful for haploid production, however, pollination with maize must be effective in a wide range of genotypes. The present work compares the crossabilities of 19 current wheat varieties with maize and *H. bulbosum* under glasshouse conditions and describes a simple method for recovering plants from wheat × maize crosses.

Materials and Methods

Plant materials: 1. Hexaploid wheat (*Triticum aestivum*). Five spring wheat varieties ('Alexandria', 'Axona', 'Minaret', 'Tonic' and 'Wembley') and 14 winter wheat varieties ('Apollo', 'Avalon', 'Brimstone', 'Brock', 'Galahad', 'Hornet', 'Longbow', 'Mercia', 'Norman', 'Parade', 'Pastiche', 'Rendezvous', 'Riband' and 'Slejpner') were used as female parents. These comprise the list of wheat varieties fully or provisionally recommended for farmers in England and Wales in 1989 by the National Institute of Agricultural Botany (Farmer's leaflet No. 8). Two other spring wheats ('Chinese Spring' and the Japanese variety 'Soujuakage'), which have high crossability with tetraploid *H. bulbosum*, were used as controls.
2. Maize (*Zea mays*). The F_1 hybrid sweetcorn variety 'Seneca 60' was used as the the male parent.
3. *Hordeum bulbosum*. Plants of one tetraploid clone (PB179) were used as a male parent. This clone was previously used in studies of crossability with wheat (SITCH and SNAPE 1987a, b, c).

Crossing procedure and cytological analysis: Wheat spikes were emasculated one to four days before anthesis. Crosses with maize were made with freshly collected pollen when most of the florets in the spike were estimated to be at a developmental age equivalent to the day of anthesis (as described in LAURIE 1989a). Fertilization frequencies were analyzed in five spikes of each variety (or four spikes in the cases of 'Soujuakage' and 'Avalon'). A further one to three spikes of each European commercial variety were used for embryo rescue studies.

An additional five spikes of each variety were pollinated with *H. bulbosum* clone PB179. Crosses were made at a developmental age equivalent to two to four days before anthesis and were sprayed with a 75 mg l^{-1} solution of gibberellic acid 5 to 10 min after pollination (as described in SITCH and SNAPE 1987b).

Fertilization frequencies were determined from ovaries fixed in 3 : 1 ethanol/acetic acid four to eight days after pollination and prepared as described in LAURIE and BENNETT (1987). Each embryo sac was scored for the presence or absence of an embryo or endosperm. Karyotypes of seedlings were determined from root-tips as described in LAURIE and BENNETT (1988b).

Embryo rescue: One day after pollination with maize, the cavity of the upper internode of the tiller was filled with a 10 mg l^{-1} solution of 2,4-dichlorophenoxyacetic acid (2,4-D) using a syringe fitted with a fine (25G) hypodermic needle. The holes were covered with petroleum jelly to prevent leakage and a few drops of the 2,4-D solution were then placed in each floret. This protocol was derived from a combination of the methods of LAURIE et al. (1990) and SUENAGA and NAKAJIMA (1989). The spike was covered with a glassine bag and left for 14–21 days in the glasshouse, after which embryos were excised under sterile conditions and cultured on Difco Orchid Agar (27.5 g l^{-1} supplemented with 4 g l^{-1} sucrose) at 20 °C.

Results

Fertilization frequencies in wheat × maize crosses

Pollination with the maize variety 'Seneca 60' resulted in fertilization in all of the 21 varieties tested (Table 1). Fertilization frequencies in the 19 European wheat varieties ranged from 32.1 % to 47.5 % of florets (mean 39.5 %) in the 14 winter wheats and from 40.7 % to 51.4 % (mean 47.8 %) in the five spring wheats. The lowest fertilization frequencies found were in the two control varieties 'Chinese Spring' (17.3 %) and 'Soujuakage' (10.7 %). Overall, 80.8 % of florets in which fertilization had occurred contained only an embryo, 7.2 % had only an endosperm and 12 % had an embryo and an endosperm. Thus the frequencies of embryo formation were slightly lower than the total fertilization frequencies, being 28.2 % to 45.9 % (mean 36.4 %) for the 14 winter wheats, 39.8 % to 48.6 % (mean 45.1 %) for the five spring wheats, 15.4 % for 'Chinese Spring' and 8.0 % for 'Soujuakage'.

Analysis of variance after angular transformation of the percentage fertilization per spike showed that the two control varieties ('Chinese Spring' and 'Soujuakage') had significantly lower frequencies of embryo formation than the remaining 19 varieties and that the five European spring wheats had higher frequen-

Table 1. Numbers of embryos and mean fertilization frequencies in wheat varieties pollinated with *Zea mays* cv. 'Seneca 60' and *H. bulbosum* PB179

Female parent	a) × *Zea mays* 'Seneca 60'								b) × *H. bulbosum* PB179			
	No. of ovaries	Embryo only	Endo-sperm only	Embryo and endosperm	Total fertilization frequency n	%	Total no. of embryos n	%	No. of ovaries	Total no. of embryos[1] n	%	c) $\chi^2_{(1\ df)}$ [2]
Spring wheats												
Chinese Spring	104	16	2	0	18	17.3	16	15.4	128	45	35.2	12.0***
Soujuakage	75	5	2	1	8	10.7	6	8.0	109	85	78.0	93.3***
Alexandria	114	50	3	5	58	50.9	55	48.2	108	26	24.1	14.2***
Axona	107	47	3	5	55	51.4	52	48.6	118	9	7.6	51.1***
Minaret	99	40	2	6	48	48.5	46	46.5	117	21	18.0	20.6***
Tonic	99	30	5	12	47	47.5	42	42.4	105	7	6.7	38.5***
Wembley	113	40	1	5	46	40.7	45	39.8	111	12	10.8	26.1***
Winter wheats												
Apollo[3]	101	24	4	6	34	33.7	30	29.7	118	1	0.8	44.2***
Avalon	106	42	3	1	46	43.4	43	40.6	130	1	0.8	72.2***
Brimstone	87	17	5	9	31	35.6	26	29.9	117	2	1.7	36.8***
Brock	122	51	2	5	58	47.5	56	45.9	143	2	1.4	89.1***
Galahad	80	23	2	8	33	41.3	31	38.8	134	2	1.5	56.4***
Hornet[3]	113	34	3	5	42	37.2	39	34.5	113	3	2.7	42.0***
Longbow	117	39	2	2	43	36.8	41	35.0	128	8	6.3	33.8***
Mercia	102	34	3	6	43	42.2	40	39.2	134	2	1.5	63.6***
Norman	78	21	3	1	25	32.1	22	28.2	112	1	0.9	36.0***
Parade	105	44	4	1	49	46.7	45	42.9	102	5	4.9	45.6***
Pastiche	75	23	2	6	31	41.3	29	38.7	135	2	1.5	54.9***
Rendezvous	95	27	2	2	31	32.6	29	30.5	102	11	10.8	12.2***
Riband	80	26	4	4	34	42.5	30	37.5	122	12	9.8	22.2***
Slejpner[3]	97	29	2	8	39	40.2	37	38.1	130	3	2.3	53.9***

[1] Equal to the total fertilization frequency in all varieties except 'Chinese Spring' where one ovary had only an endosperm (giving a total fertilization frequency of 35.9 %).
[2] Results of 2 × 2 contingency χ^2 tests comparing the frequency of embryo formation in crosses with maize or *H. bulbosum*.
[3] Varieties with a 1 delete space 1BS.1RS translocation.

cies than the 14 winter wheats (Table 2). However, no significant variation was detected between varieties within these categories.

All embryos that were examined showed micronuclei, indicating the elimination of maize chromosomes. Endosperm was always highly abnormal, consisting of a small number of nuclei which were usually aberrant. Thus, early embryo and endosperm development appeared to be similar to that observed in previous studies of hexaploid wheat × maize crosses (LAURIE and BENNETT 1987, 1988a, 1989, LAURIE 1989a).

Fertilization frequencies in wheat × *H. bulbosum* crosses

Fertilization occurred in all 19 European varieties (Table 1) but the frequencies were always substantially lower than those obtained from crosses with maize. Values ranged from 6.7 % to 24.1 % in the European spring wheat varieties and from 0.8 % to 10.8 % in the winter wheats where only the varieties 'Longbow', 'Rendezvous' and 'Riband' gave frequencies over 5 %. As expected, mean fertilization frequencies were higher in the two control varieties (35.9 % in 'Chinese Spring' and 78.9 % in 'Soujuakage'). In contrast to wheat × maize crosses, most florets in which fertilization occurred had both an embryo and an endosperm, the only exception being one floret of 'Chinese Spring' which contained only an endosperm.

For each of the 21 wheat varieties, 2 × 2 contingency χ^2 tests were used to compare the frequencies of embryo formation, and hence the potential yields of haploid plants, after pollination with maize and *H. bulbosum*. In 'Chinese Spring' and 'Soujuakage' the frequency of embryo formation was significantly higher ($p < 0.001$) in crosses with *H. bulbosum* but in the remaining 19 commercial varieties the frequency of embryo formation was significantly higher ($p < 0.001$) in crosses with maize (Table 1). Overall, the yield of embryos from crosses with maize was 3.4-fold higher in the five European spring wheats and 10.9-fold higher in the winter wheats. It should be noted that although the control varieties had the highest fertilization frequencies with *H. bulbosum* and the lowest frequencies with maize this may not reflect a simple negative correlation between crossabilities since the European spring wheats had a higher mean fertilization frequency than the winter wheats with both pollinators. It would be valuable to investigate the relationship between crossabilities further using crosses between 'Soujuakage' and European varieties.

The fertilization frequencies in wheat × *H. bulbosum* crosses (Table 1) were in agreement with the data of SITCH and SNAPE (1986a) which showed that clone PB179 gave very low seed set in 16 winter wheat genotypes including five varieties ('Avalon', 'Brimstone', 'Mercia', 'Norman' and 'Rendezvous') used in the present experiment. The only discrepancy was in 'Rendezvous', where the seed set was zero (SITCH and SNAPE 1986a) but where the fertilization frequency was 10.8 % (Table 1). This may have been due to environmental effects on fertilization frequency or to poor seed survival in this cross.

Table 2. Analysis of variance of frequency of embryo formation

Item	df	Mean square
Between control and European varieties	1	3525.4***
Between controls	1	128.6
Within European varieties		
Between habit (spring vs. winter)	1	478.0**
Between spring varieties	4	22.7
Between winter varieties	13	55.7
Error	82	61.2
Total	103	

** $p < 0.01$
*** $p < 0.001$

Haploid seedling production from wheat × maize crosses

In addition to the spikes fixed for cytological analysis, one to three spikes of each of the 19 European varieties were treated with a 10 mg l^{-1} 2,4-D solution one day after pollination. Seeds were dissected 14 to 21 days after pollination at which time the ovaries had swollen to about half to three quarters of the size of normal seeds of comparable age. The 2,4-D treatment enabled embryos to survive in all 19 genotypes, a total of 311 being recovered from the 950 florets examined (an average of 7.6 embryos per spike) (Table 3).

For each genotype, the number of embryos present 14 to 21 days after pollination was compared to an expected value calculated from the frequency of embryos present 4 to 6 days after pollination (Table 1). This comparison needs to be treated with caution, since only one to three spikes were used for the embryo rescue treatment, but the figures indicate that, overall, 85.3 % of embryos had developed to a stage where they were recognizable at the time of dissection. The data in Table 3 suggest that the spring wheats tended to produce fewer embryos than expected, indicating that the embryo rescue method was less efficient for these varieties.

Most embryos appeared well differentiated and 62 % (191/311) germinated to give healthy green seedlings (Table 3). A further 89 embryos also germinated but these either failed to establish themselves as seedlings or only produced roots. Seedlings were recovered from all varieties except 'Alexandria' and 'Axona' where the embryos were lost because of fungal contamination. However, these embryos were

Table 3. Frequencies of haploid seedling production from wheat × maize crosses following 2,4-D treatment

	No. of florets[1]	No. of embryos obtained	(expected[2])	No. of seedlings
Spring wheats				
Alexandria	42 (2)	7	(20)	0[3]
Axona	40 (2)	10	(19)	0[3]
Minaret	38 (2)	11	(18)	8
Tonic	48 (3)	12	(20)	11
Wembley	33 (2)	13	(13)	12
Winter wheats				
Apollo	74 (3)	19	(22)	12
Avalon	44 (2)	17	(18)	14
Brimstone	75 (3)	29	(22)	18
Brock	26 (1)	5	(12)	3
Galahad	58 (2)	21	(23)	7
Hornet	52 (2)	16	(18)	11
Longbow	52 (2)	19	(18)	13
Mercia	48 (2)	19	(19)	14
Norman	48 (2)	19	(14)	12
Parade	49 (2)	15	(23)	11
Pastiche	50 (2)	18	(19)	10
Rendezvous	45 (2)	18	(14)	6
Riband	74 (3)	29	(28)	22
Slejpner	54 (2)	14	(21)	7
Totals	950 (41)	311	(361)	191

[1] The number of spikes is given in brackets.
[2] Calculated from the frequencies of embryo formation given in Table 1.
[3] Embryos were lost because of fungal contamination.

very similar in appearance to those from other genotypes at the time of excision and it is likely that they would have been capable of germination. In the spring wheats (excluding 'Alexandria' and 'Axona') one seedling was recovered for every 3.8 florets pollinated (4.4 per spike) while in the winter wheats the yield was one for every 4.7 florets pollinated (5.3 per spike).

Two pieces of evidence suggested that all embryos were the result of hybridization with maize. Firstly, all embryos fixed four to six days after pollination that were examined in detail contained micronuclei, indicating elimination of maize chromosomes. Secondly, no embryos were found in two unpollinated spikes of each genotype (a total of 763 florets) dissected 14 to 21 days after 2,4-D treatment. Thus, there was no evidence for parthenogenic development of the egg-cell.

From previous work on wheat × maize crosses it was expected that all maize chromosomes would be lost during embryo development. Analysis of seedling root-tips confirmed that this was the case. However, seven of the 191 plants (3.6 %) had an abnormal wheat karyotype. Firstly, about half the cells in one root of one plant of 'Apollo' showed separation of a wheat chromosome into two telocentrics. Secondly, the three plants produced from Brock had only 20 chromosomes, suggesting that the wheat parent was a spontaneous monosomic. This interpretation was supported by examination of selfed progeny of the parent plant since eight out of 11 seedlings had 41 chromosomes (selfing a monosomic plant is expected to give approximately 75 % monosomic progeny, TSUNEWAKI 1964). Thirdly, three of the 11 plants of 'Parade' had 20 biarmed chromosomes plus a telocentric chromosome. A telocentric was also found in five out of nine selfed progeny of the parent plant. Thus six of the seven abnormal plants could be accounted for by aneuploidy in the parent.

Discussion

The results show that wheat × maize crosses are an effective means of producing wheat haploids in a range of commercial wheat genotypes. The yields of haploids (4.4 per spike for spring varieties and 5.3 per spike for winter varieties) compare very favourably with the 1.7 haploids per spike produced by the *H. bulbosum* system from two spring varieties which had been substituted with the 5B chromosome of 'Chinese Spring' in order to introduce the recessive *kr1* crossability allele (SNAPE et al. 1986).

The present data also allow a comparison with previous work using *H. bulbosum* for haploid production in winter wheats. SITCH and SNAPE (1986a) obtained three seedlings from 7272 florets pollinated with *H. bulbosum* while in the present experiment 160 seedlings were obtained from 749 florets (Table 3). Thus, the number of florets pollinated per haploid was 2424 and 4.7 respectively. Since the amount of time needed per spike is equivalent in the maize and *H. bulbosum* systems, this indicates that the maize system is a least 500-times more efficient for winter wheat varieties. The yield of haploids from wheat × *H. bulbosum* crosses could probably be improved by using the 2,4-D injection technique to promote embryo survival but the low fertilization frequency found in crosses with *H. bulbosum* (Table 1) would still make the maize system superior. In addition, the results also suggest that successful haploid production by the wheat × maize system may be less genotype dependent than anther or microspore culture. This may offer a significant advantage, particularly for the development of doubled haploid populations for genetic mapping.

The advantages of the wheat × maize system discussed above assume that the level of induced (gametoclonal) variation in the resultant doubled haploid lines will be comparable to that found in other haploid production systems. Preliminary analysis by LAURIE and SNAPE (1990) of 29 doubled haploid lines produced from wheat × maize crosses by a spikelet culture method (LAURIE and BENNETT 1988b) suggest that this is a reasonable expectation, but further work is needed to clarify this point.

The present results may also be important for attempts to transfer maize DNA, particularly transposable elements, to wheat. If the conclusions from crosses with 'Seneca 60' apply to other maize genotypes, the number of embryos obtained, and hence the number of opportunities for gene transfer, will be greater if commercial varieties are used in preference to genotypes such as 'Chinese Spring' which are usually used for wide-hybridization with other species in the Triticeae (GALE and MILLER 1987). The applications of wheat × maize hybridization are likely to benefit from further improvements in the embryo rescue method and, in addition, higher frequencies of haploid production might be obtainable by using other maize genotypes, or other species such as sorghum (LAURIE and BENNETT 1988c) or pearl millet (LAURIE 1989b), as pollen parents.

Zusammenfassung

Hohe Befruchtungsraten und Erzeugung von hexaploiden Keimpflanzen nach Kreuzungen von haploiden Handelssorten des Weizens mit Mais

Es wurden 19 haploide Handelssorten des Weizens mit der Mais-F_1-Hybride 'Seneca 60' gekreuzt. Die Befruchtungsraten variierten bei den 14 Winterweizensorten von 32,1 % bis 47,5 % der bestäubten Blütchen (im Mittel 39,5 %); bei den 5 Sommerweizensorten reichten sie von 40,7 % bis zu 51,4 % (im Mittel 47,8 %). In einigen Fällen wurde nur Endosperm gebildet. Als Folge davon verringerten sich die Zahlen für die Embryobildung leicht auf 28,2 % bis 45,9 % für Winterweizen (im Mittel 36,4 %) und auf 39,8 % bis 48,6 % für Sommerweizen (im Mittel 45,1 %). Die Mittelwerte für den Sommerweizen waren signifikant höher als die für den Winterweizen. Es wurden jedoch keine signifikanten Unterschiede zwischen den einzelnen Winter- bzw. Sommerweizensorten festgestellt. Bei den 5 Sommerweizensorten war die mittlere Ausbeute an Embryonen und damit der mögliche Gewinn an haploiden Pflanzen 3,4mal höher als bei der Verwendung des tetraploiden *Hordeum bulbosum*-Klons PB179 als Bestäuber. Bei den 14 Winterweizensorten wurde eine diesbezügliche Steigerung um das 10,9fache festgestellt. Diese Unterschiede waren bei allen Sorten signifikant ($p < 0{,}001$). Eine einmalige Behandlung der Ähren mit 2,4-D einen Tag nach der Bestäubung mit Mais führte dazu, daß von allen 19 Sorten Embryonen erhalten werden konnten. Insgesamt wurden von 950 Blütchen 308 Embryonen gewonnen (im Durchschnitt 7,3 Embryonen je Ähre), von denen 191 zum Auskeimen gebracht werden konnten, so daß von jeweils 5 bestäubten Blütchen eine haploide Pflanze erhalten wurde (4,4 haploide Pflanzen je Ähre).

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