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The production of haploid wheat plants from wheat × maize crosses

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Summary. Hybrid embryos from hexaploid wheat × maize crosses rapidly lose the maize chromosomes to produce haploid wheat embryos. Such embryos almost always aborted when left to develop on the plant, and only 1 was recovered from 2440 florets (0.17% of the expected number). Embryos had greater viability in spikelet culture, 47 (26.5% of the expected number) being recovered from 706 ovaries. Thirty-two of these embryos germinated to give green plants, 31 of which were haploid (21 wheat chromosomes) and 1 of which was euploid (42 wheat chromosomes). Spikelet culture enabled 17.1% of the expected number of embryos to be recovered as haploid plants, a 100-fold improvement on allowing embryos to develop in vivo. Ten haploid plants of 'Chinese Spring' (*kr1, kr2*), 13 plants of 'Chinese Spring (Hope 5A)' (*kr1, Kr2*), and 8 of 'Hope' (*Kr1, Kr2*) were recovered. The potential of wheat × maize crosses for wheat haploid production and for gene transfer from maize to wheat is discussed.

Key words: Wheat – Maize – Wide-crosses – Embryo culture – Haploids

Introduction

An embryo, an endosperm, or both are commonly found in 20%–30% of florets when hexaploid wheat is pollinated with maize. Zygotes contain one complete haploid chromosome set from each parent, confirming the hybrid origin of the embryos, but maize chromosomes have poorly defined centromeres and appear to have little affinity for spindle microtubules in zygotes and young embryos. As a result, they are lost during the first few cell division cycles to produce embryos whose cells contain a haploid complement of wheat chromosomes. Endosperm is either absent or highly abnormal (Laurie and Bennett 1986, 1988).

The above results include crosses where the wheat parent carried dominant alleles of the crossability loci *Kr1* and/or *Kr2*. Such alleles usually reduce seed set when wheat is pollinated by alien species, but 'Chinese Spring (Hope 5B)' (*Kr1, kr2*) × 'Seneca 60' maize crosses gave fertilization at a very similar frequency to that observed in 'Chinese Spring' (*kr1, kr2*) × 'Seneca 60' crosses (30.7% and 29.2% of florets, respectively). 'Highbury' (*Kr1, Kr2*) × 'Seneca 60' crosses resulted in fertilization in 14.4% of florets (Laurie and Bennett 1987). The relative insensitivity of maize to the action of *Kr1* and *Kr2* suggests that wheat × maize crosses could be a valuable alternative to wheat × *H. bulbosum* crosses (Barclay 1975; Snape et al. 1979; Sitch and Snape 1987) for the production of wheat haploids via chromosome elimination.

Wheat × maize crosses might also be used to transfer maize DNA to wheat, but to be useful for either purpose a reliable method is needed for recovering embryos, and ultimately green plants, from florets with little or no endosperm. This paper describes the use of a spikelet culture method (Mathias and Boyd 1988) that fulfills these criteria and compares results from this method with results from caryopses allowed to develop on plants.

Materials and methods

Plant material

Five genotypes of hexaploid wheat (*Triticum aestivum* L. $2n=42$) were used as female parents in crosses with maize, namely: (1) 'Chinese Spring' (*kr1, kr2*); (2) 'Hope' (*Kr1, Kr2*); (3) 'Highbury' (*Kr1, Kr2*); (4) 'Chinese Spring (Hope 5A)' (*kr1, Kr2*); and (5) 'Chinese Spring (Hope 5B)' (*Kr1, kr2*). The last two

are chromosome substitution lines produced by E. R. Sears (University of Missouri) in which the 'Chinese Spring' 5A and 5B chromosomes were replaced by the 'Hope' 5A (carrying *Kr2*) and the 'Hope' 5B (carrying *Kr1*) chromosomes respectively.

Three diploid maize (*Zea mays* L. $2n=20$) genotypes were used as male parents, namely: (1) 'Seneca 60', a single-cross F_1 hybrid sweetcorn; (2) 'Ac', a stock carrying the transposable element Activator (*Ac*); and (3) 'Zapalote Chico' (Oaxaca 57), an accession from southern Mexico.

Spikelet culture

In order to determine if the presence of a normally developing seed aided embryo development in cross-pollinated florets, two crossing procedures were used.

(1) Spikes of glasshouse grown wheat plants of all five genotypes were prepared for pollination by removing the upper and basal spikelets and all but the primary and secondary florets of the remaining spikelets. All anthers were removed from one of the two remaining florets of the remaining spikelets and the bag used to prevent accidental outcrossing was folded tightly over the spike and fastened with paper clips to prevent the glumes gaping and shedding pollen onto the emasculated floret. The half-emasculated spikes were allowed to anthese and 2 days later the bags were removed and the emasculated side checked for enlarged ovaries or ovaries with shrivelled stigmas, indicative of stray self-pollination. Such ovaries were removed and the remaining unfertilized ovaries were pollinated with freshly collected 'Seneca 60' maize pollen to give what are subsequently referred to as SP/CP (self-pollinated/cross-pollinated) spikelets. Spikelets without seed set in the self-pollinated half were discarded.

(2) Spikes were prepared as described above but anthers were removed from both florets of the remaining spikelets and all florets were pollinated with 'Seneca 60' pollen at what was estimated to be an equivalent stage of maturity. This gave what are subsequently referred to as CP/CP (cross-pollinated/cross-pollinated) spikelets.

Two days after pollination spikes were removed, surface sterilized and washed four times in sterile distilled water. Spikes were blotted dry on sterile filter paper and the rachis was cut into individual spikelets. These were placed upright on MS medium (Murashige and Skoog 1962) containing 60 g l^{-1} sucrose and 0.1 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) at a density of 20 spikelets per 90 mm Petri-dish. Dishes were sealed with parafilm and incubated for 3 weeks in continuous light at 20 ± 1 °C.

Ovaries were then dissected and all embryos were transferred to tubes containing Difco Orchid agar medium (27.5 g l^{-1} supplemented with 8 g l^{-1} sucrose. Cultured embryos were kept in the dark at 20 ± 1 °C until they germinated, then transferred to continous light. Seedlings were transferred to pots in a controlled environment cabinet at 20 ± 1 °C with a 16 h day length.

Caryopses allowed to develop on plants

Plants of three wheat genotypes, 'Chinese Spring', 'Chinese Spring (Hope 5B)' and 'Highbury', were grown in a controlled environment cabinet at 20 ± 1 °C with a 16 h day length to determine the frequency of fertilization after pollination with 'Seneca 60' maize. Embryos were found in 28.0, 27.0 and 14.4% of florets, respectively (Laurie and Bennett 1987). A total of 107 further spikes, of which 47 were sprayed with a 75 mg l^{-1} solution of gibberellic acid 1 day after pollination with either 'Seneca 60', 'Ac' or 'Zapalote Chico', were left on plants for 10–14 days, removed, surface sterilized and dissected. All ovaries were harvested at this time rather than at 3 weeks since the majority were already shrivelled and necrotic and it was thought unlikely that embryos would survive longer. Only one embryo was found and this was cultured as described above.

Results

Caryopses allowed to develop on plants

A total of 2,440 florets were dissected 10–14 days after pollination (Table 1). From data on the frequency of egg-cell fertilization in ovaries fixed 48 h after pollination (Laurie and Bennett 1988), it was estimated that 592

Table 1. Number of embryos and haploid seedlings recovered from caryopses allowed to develop on plants

Hybrid combination and treatment	No. of spikes	No. of cross-pollinated florets	No. of embryos expected[a]	No. of embryos found		No. of haploid seedlings	
				n	%[b]	*n*	%[b]
CS × S60 no GA	11	266	71 (26.8% 343)	0	0	0	0
CS × S60 + GA	18	404	108 (26.8% 343)	0	0	0	0
CS × Ac no GA	39	954	239 (25.0% 40)	0	0	0	0
CS × ZC no GA	5	91	18 (19.4% 175)	1	5.5	1	5.5
CS (H5B) × S60 no GA	3	56	15 (27.0% 189)	0	0	0	0
CS (H5B × S60 + GA	19	364	98 (27.0% 189)	0	0	0	0
Highbury × S60 + GA	7	163	23 (14.4% 194)	0	0	0	0
Highbury × Ac no GA	5	142	20[c]	0	0	0	0
Overall total	107	2,440	592	1	0.17	1	0.17

[a] Calculated from the frequency of egg-cell fertilization found in florets fixed 48 h after pollination (the percentage fertilization is given in brackets together with the number of florets studied)

[b] Percentage of the expected number of embryos

[c] This cross was not studied 48 h after pollination. The expected number of embryos was calculated assuming that the frequency of egg-cell fertilization was the same as in 'Highbury' × 'Seneca 60' crosses

Table 2. Number of embryos and haploid seedlings recovered from spikelet culture

Hybrid combination and protocol		No. of cross pollinated florets	No. of embryos expected[a]	No. of embryos found		No. of embryos which did not germinate	No. that produced a shoot and root but died	No. that produced only a root and died	No. of haploid seedlings		No. of euploid seedlings	
				n	%[b]				*n*	%[b]	*n*	%[b]
CS × S60	SP/CP	63	17 (26.8% 343)	14	82.4	1	2	1	10	58.8	0	0
CS × S60	CP/CP	216	58 (26.8% 343)	0	0	0	0	0	0		0	0
CS (H5A) × S60	SP/CP	27	9 (31.8% 22)	4	44.4	1	1	0	1	11.1	1	11.1
CS (H5A) × S60	CP/CP	98	31 (31.8% 22)	14[c]	41.9[d]	2	0	0	12[c]	35.5[d]	0	0
CS (H5B) × S60	CP/CP	102	28 (27.0% 189)	0	0	0	0	0	0	0	0	0
Hope × S60	SP/CP	36	5 (12.5% 32)	8[c]	140.4[d]	1	2	0	5[c]	80.0[d]	0	0
Hope × S60	CP/CP	94	12 (12.5% 32)	7	58.3	2	1	1	3	25.0	0	0
Highbury × S60	CP/CP	70	10 (14.4% 194)	0	0	0	0	0	0	0	0	0
Overall total		706	170	47	26.5[d]	7	6	2	31	17.1[d]	1	0.6

[a] Calculated from the frequency of egg-cell fertilization found in florets fixed 48 h after pollination (the percentage fertilization is given in brackets together with the number of florets studied)
[b] Percentage of the expected number of embryos
[c] One floret contained two embryos, both of which developed into a haploid seedling
[d] The percentage is calculated taking each pair of twins as 1 embryo or plant on the assumption that they have arisen from the splitting of a single embryo

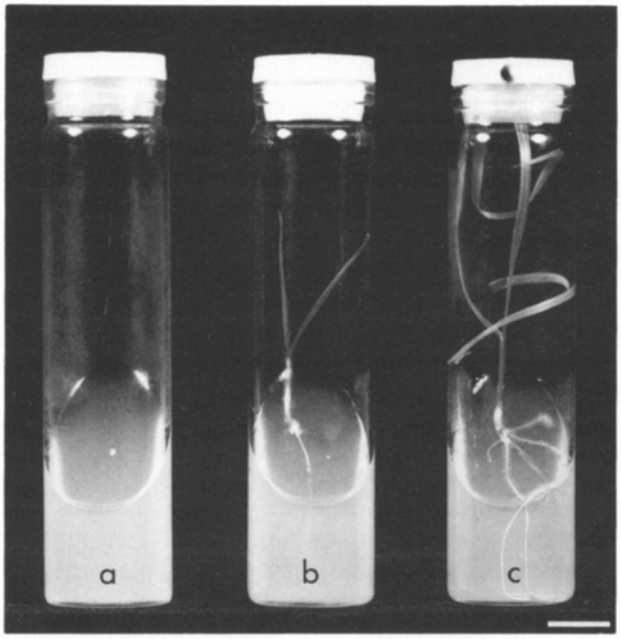

Fig. 1 a–c. Culture tubes containing embryos from 'Chinese Spring' × 'Seneca 60' crosses excised after 3 weeks in spikelet culture. **a** A freshly excised embryo. **b** A germinating embryo. **c** A seedling ready to be transplanted to soil. *Bar* represents 10 mm

embryos would be expected in a sample of this size; only 1 was found (0.17% of the expected number). This embryo, from a 'Chinese Spring' × 'Zapalote Chico' cross, was not accompanied by an endosperm and developed into a haploid wheat seedling. Embryo development did not appear to be improved by the application of gibberellic acid, and the single embryo produced was from an untreated spike. The low frequency of embryos found in dissected florets clearly suggested that the vast majority aborted early in development.

Spikelet culture

In contrast to the above results, 47 embryos and 31 haploid wheat plants (Fig. 1) were recovered from 706 cross-pollinated florets grown in spikelet culture (Table 2). When 'Chinese Spring' (*kr1, kr2*) was used as the female parent, 14 embryos were recovered from 63 self-pollinated/cross-pollinated (SP/CP) spikelets, which was 82.4% of the expected number. The 10 embryos that germinated (58.8% of the expected number of embryos) all gave rise to haploid seedlings. These embryos probably arose from hybrid zygotes, since 69 unpollinated florets from self-pollinated/unpollinated (SP/UP) 'Chinese Spring' spikelets gave no embryos. Interestingly, em-

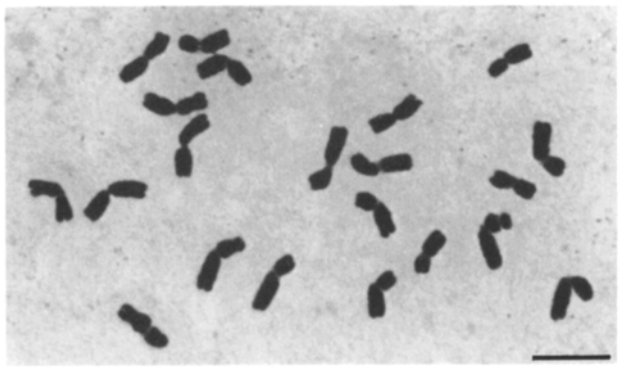

Fig. 2. Root-tip squash from a haploid 'Hope' seedling showing 21 chromosomes. *Bar* represents 10 μm

bryos also failed to develop in 216 florets from cross-pollinated/cross-pollinated (CP/CP) spikelets, where 58 were expected.

Using 'Chinese Spring (Hope 5A)' (*kr1, Kr2*), 4 embryos were recovered from SP/CP spikelets (44.4% of the expected number), one of which developed into a haploid seedling (11.1% of the expected number of embryos) and 1 into a euploid seedling with 42 wheat chromosomes. The latter may have been a spontaneously doubled haploid since it was not accompanied by an endosperm and is therefore unlikely to have been a contaminant arising from a stray wheat pollen grain. In contrast to the results from 'Chinese Spring', embryos were also recovered from CP/CP spikelets of 'Chinese Spring (Hope 5A)'. Fourteeen, including 2 from 1 floret, were found in 98 florets (41.9% of the expected number). The transformed percentage recovery of embryos per cross-pollinated floret appeared to be the same in SP/CP and CP/CP spikelets ($F_{1,3}$ 0.2 n.s.), although the sample consisted of only two and three dishes respectively. Twelve embryos from CP/CP spikelets germinated and all developed into haploid seedlings.

Using 'Hope' (*Kr1, Kr2*), 8 embryos, again including 2 from 1 floret, were recovered from 36 SP/CP spikelets. This was greater than the number expected (140.0%) even though the twin embryos were classed as 1, perhaps because of the small sample size (32 florets) on which the expected number of embryos was based (Table 2). Five of the embryos, including the twin pair, developed into haploid seedlings (Fig. 2). Seven embryos were also recovered from 94 CP/CP spikelets (58.3% of the expected number), of which 3 developed into haploid seedlings. The transformed percentage recovery of embryos was significantly higher in the SP/CP florets ($F_{1,3}$ 11.2, $p<0.05$) in the small sample of 2 SP/CP and 3 CP/CP dishes compared.

All haploid seedlings were colchicine treated and doubled haploid seed was recovered from 9 'Chinese Spring', 13 'Chinese Spring (Hope 5A)' and 7 'Hope' plants. Crosses involving 'Chinese Spring (Hope 5B)' (*Kr1, kr2*) or 'Highbury' (*Kr1, Kr2*) produced no embryos, even though both genotypes are known to hybridize with maize (Laurie and Bennett 1987, 1988). However, these genotypes were only tested as CP/CP spikelets, which also failed to yield embryos with 'Chinese Spring'.

Discussion

Although wheat and maize are in separate sub-families of the Gramineae (Hutchinson 1959), fertilization of the egg-cell, the polar nuclei, or both, has been found in up to 40% of florets fixed 48 h after pollination (Laurie and Bennett 1988). This, together with the observation that hybrid embryos rapidly eliminate maize chromosomes (Laurie and Bennett 1986, 1987, 1988) suggests that wheat × maize crosses have the potential to produce haploid wheat plants in up to 33% of florets, provided that the embryos develop to a stage where they are capable of germinating into healthy plants.

Unfortunately, embryos had very poor viability when left to develop on plants and only 1 was recovered from 2,440 florets. Spikelet culture enabled 47 embryos unaccompanied by any detectable endosperm to be recovered from 706 cross-pollinated florets and 31 of these embryos developed into healthy haploid seedlings. Spikelet culture was estimated to be 156 times more efficient for recovering embryos (0.17% vs 26.5%) and 100 times more efficient for recovering haploid plants (0.17% vs 17.0%, Tables 1 and 2).

Results from the three wheat genotypes from which haploids were recovered showed an interesting difference between protocols. Embryos were recovered from 'Chinese Spring' only when using SP/CP spikelets and although 'Chinese Spring (Hope 5A)' and 'Hope' yielded embryos from both SP/CP and CP/CP spikelets, the percentage of recovery was higher in SP/CP spikelets in both cases (Table 2), the difference being significant ($P<0.05$) for 'Hope'. The above results are from small samples and must be interpreted with caution. However, they may suggest a "feeder effect" in which normally developing seeds in SP/CP spikelets produce a diffusable product, perhaps a hormone, which aids the development of the embryo in the cross-pollinated floret.

The results were very encouraging in that wheat haploids were produced not only from 'Chinese Spring' (*kr1, kr2*) and 'Chinese Spring (Hope 5A)' (*kr1, Kr2*) but also from 'Hope' (*Kr1, Kr2*), a variety that previously gave no seed set when pollinated with *H. bulbosum* (Snape et al. 1979). If the spikelet culture method can be developed to yield haploids from other wheat genotypes such as 'Chinese Spring (Hope 5B)' (*Kr1, kr2*) and 'High-

bury' (*Kr1, Kr2*), which are known to hybridize with maize (Laurie and Bennett 1987), wheat × maize crosses may have general applicability as an alternative to wheat × *H. bulbosum* crosses for the production of wheat haploids via chromosome elimination.

Furthermore, the ability of the maize sperm nucleus to fuse with the wheat egg-cell opens up exciting areas for experiments in gene transfer. No embryos were found in the unpollinated controls, and it is therefore very likely that the haploid plants arose from hybrid zygotes. If this is so it may be possible to transfer maize DNA, including active maize transposable elements, into wheat by sexual hybridization.

All hexaploid wheat × maize genotype combinations studied so far have been karyotypically unstable, rapidly losing maize chromosomes to give haploid wheat embryos (Laurie and Bennett 1988). This may be a general property of wheat × maize crosses and the most practicable way to transfer maize DNA into wheat may be by inducing intergenomic translocations before chromosome elimination. This might be done by, for example, exposing spikes containing hybrid zygotes or very young hybrid embryos to ionizing radiation.

The frequency of intergenomic translocations is likely to be low because of: (1) the rapid loss of the maize chromosomes; (2) the great disparity of genome sizes (85% of the DNA in a 'Chinese Spring' × 'Seneca 60' maize zygote would be from wheat); and (3) the likelihood of parental genome separation in hybrid nuclei, as has been found in other cereal wide hybrids (Finch et al. 1981; Bennett 1984; Schwarzacher-Robinson et al. 1987). Also, the amount of radiation-induced damage in the wheat genome must not be so great as to render the plants inviable. However, the major technical obstacle was the failure to recover plants from wheat × maize crosses (Zenkteler and Nitzsche 1984; Laurie and Bennett 1986, 1987) and the present data suggest that spikelet culture overcomes this problem. Provided that the haploid plants produced arose from hybrid zygotes, the transfer of maize DNA to wheat via sexual hybridization is now a realistic goal.

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