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Comparative responses of tetraploid wheats pollinated with *Zea mays* L. and *Hordeum bulbosum* L.

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Abstract. Ten different tetraploid wheat (*Triticum turgidum*) genotypes were pollinated with maize (*Zea mays*). Fertilization was achieved in all ten genotypes and no significant difference in fertilization frequency between the tetraploid wheat genotypes was detected. A mean of 41.1% of pollinated ovaries contained an embryo. All these crosses were characterized by the elimination of the maize chromosomes, and the resulting embryos were haploids. Six of the tetraploid wheat genotypes were also pollinated with *Hordeum bulbosum*. Fertilization frequencies with *H. bulbosum* were much lower (mean = 13.4%), and significant differences between the tetraploid wheat genotypes were detected. Observation of pollen tube growth revealed that part of the incompatibility reaction between tetraploid wheats and *H. bulbosum* was due to an effect similar to that of the *Kr* genes, namely pollen tube growth inhibition. These results indicate that pollinations with maize may have potential as a broad spectrum haploid production system for tetraploid wheats.

Key words: *Triticum turgidum* ssp. *durum* – *Durum* wheat × maize – *Durum* wheat × *H. bulbosum* – Pollen tubes – Haploids

Introduction

Haploids and doubled haploids of self-pollinated crop plants are characterized by complete homozygosity. Consequently, doubled haploids can potentially be maintained and replicated through selfing. This makes them extremely useful as tools for both practical plant breeding applications (Snape 1982, 1989) and genetic analyses (Snape and Simpson 1981). Haploid production methods are available for bread wheat and barley, but until now, these methods have been of limited success in tetraploid macaroni or *durum* wheats.

The main methods used to produce haploids of bread wheat include (1) anther culture, (2) pollinations with *Hordeum bulbosum*, and more recently (3) pollinations with maize. Anther culture of tetraploid wheats has resulted in poor regeneration (Sharma et al. 1982) and albinism (Zhu et al. 1980; Hadwiger and Heberle Bors 1986). Fertilization with *Hordeum bulbosum* with the subsequent elimination of the *bulbosum* chromosomes has been used successfully with some bread wheats. However, this method has been restricted to only some genotypes carrying the recessive alleles at the crossability loci *Kr1* and *Kr2* located on chromosomes 5B (Riley and Chapman 1967) and 5A (Sitch et al. 1985), respectively. The dominant alleles at the *Kr* loci inhibit or reduce the crossability of bread wheat with *H. bulbosum* (Snape et al. 1979; Falk and Kasha 1981, 1983; Sitch et al. 1985), rye (Riley and Chapman 1967; Falk and Kasha 1981, 1983; and other Triticeae species (Thomas et al. 1981). This inhibition acts by arresting pollen tube growth at the base of the style and in the transmitting tissue of the ovary wall (Lange and Wojciechowska 1976; Sitch and Snape 1987a). The *Kr* gene constitution of tetraploid wheats is unknown, but when Krolow (1970) pollinated *durum* wheat genotypes with rye, variable seed set was obtained (mean = 11.09%), and much of the seed was shriveled and did not germinate. When pollinated with *H. bulbosum*, tetraploid wheats have also shown

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variation in seed set, indicating the presence of different crossability alleles (J. W. Snape, personal communication).

More recently a similar method involving wide hybridization with maize followed by the elimination of the maize chromosomes has been successfully developed to produce haploids of bread wheat (Laurie and Bennett 1988a; Comeau et al. 1988; Suenaga and Nakajima 1989; Riera-Lizarazu and Mujeeb-Kazi 1990; Inagaki and Tahir 1990). Furthermore, Laurie and Bennett (1987) showed that fertilizations by maize were largely insensitive to the *Kr* gene constitution of bread wheats. O'Donoughue and Bennett (1988) obtained 26.9% fertilization from the cross *T. turgidum* ssp. *durum* cv 'Kubanka' × *Z. mays* cv 'Seneca 60'. This was comparable to the fertilization frequencies originally obtained by Laurie and Bennett (1988b) in bread wheat × maize (about 27%).

The objectives of this study were to (1) determine if other tetraploid wheat genotypes could be successfully pollinated with maize, (2) compare fertilization frequencies obtained in crosses of tetraploid wheats with maize and *H. bulbosum* and (3) determine if these crosses were characterized by effects similar to that of the *Kr* genes in restricting pollen tube growth.

Materials and methods

Plant Material

The male parents used in this study were *Zea mays* L. cv 'Seneca 60' (2n = 2x = 20), an F_1 hybrid sweetcorn, and the two tetraploid (2n = 4x = 28) *Hordeum bulbosum* L. genotypes PB168 and PB179. The maize line was originally provided by Dr. D. Greyson, University of Western Ontario, Ontario, Canada, and the *H. bulbosum* genotypes were accessions from the IPSR Cambridge Laboratory collection.

The female parents, listed in Table 1, are all tetraploids (2n = 4x = 28). The hexaploid, *T. aestivum* (L.) Thell. cv 'Chinese Spring' (2n = 6x = 42), was used as a control in the pollinations with *H. bulbosum*. The last four cultivars listed in Table 1 were recommended by the National Institute of Agricultural Botany, Cambridge, UK for 1989 and were pollinated only with maize and in a different season.

Pollination methods

The plants were maintained in a glasshouse at a daylength of about 16 h. Wheat plants were transferred to a growth cabinet at 20 °C with a 16 h day about a week before anthesis. Emasculations and pollinations with maize were as described in Laurie and Bennett (1986). Pollinations with *H. bulbosum* were performed in a similar way, except that the spikes were sprayed 1 day after pollination with 75 ppm gibberellic acid plus a drop of polyethylene sorbitan monolaurate (Tween 20) as a surfactant (Sitch and Snape 1987b).

Light microscopy of embryo sacs

In order to determine fertilization frequencies, ovaries from each of four spikes per cross combination were removed 3–4 days after pollination, fixed in absolute ethanol/glacial acetic acid (3:1) and stored at 4 °C. For observations, the ovaries were Feulgen stained, and the embryo sacs were dissected out as described in Laurie and Bennett (1987). Fertilization of the egg cell and/or polar nuclei, presence/absence of maize chromosomes and/or presence/absence of micronuclei were noted.

Observation of pollen tube growth

For all crosses with *H. bulbosum* and some crosses with maize, the ovaries from one side of each spike were fixed 3–4 days after pollination for estimation of fertilization frequencies as described above. The ovaries from the other side of the spike axis were fixed 1 day after pollination to study pollen tube growth.

The latter ovaries were stained using the aniline blue method modified from Sitch and Snape (1987a). Thus, the ovaries were washed and rehydrated in distilled water for about 30 min, placed in 70% lactic acid in a boiling water bath for 1 min, cooled at room temperature, washed in distilled water for 1h and left in 0.1 *M* K_3PO_4 buffer overnight. They were then stained in decolorized aniline blue (0.2% w/v in 0.1 *M* K_2HPO_4 buffer, pH 11) for at least 15 min.

Each ovary was then cut in half with a razor blade between the two stigmas and through the micropyle. They were mounted under a coverslip in aniline blue and examined using a Zeiss epifluorescence miroscope. The yellow fluorescence emitted by the stained callose plugs and lining of the pollen tubes was visualized after excitation with ultra-violet light (near 365 nm, G365). The emitted fluorescence was filtered by barrier LD418 (Zeiss filter set 02, chromatic splitter FT420).

The number of germinated pollen grains out of 20 random grains on the stigmas of each of five ovaries in four different spikes per cross combination (i.e. 400 pollen grains per cross combination) were recorded . For each ovary, the number of pollen tubes at the base of the style, the top of the ovule and beyond the top of the ovule were scored. In this paper the expression "beyond the top of the ovule" refers to pollen tubes that have grown beyond the transmitting tract of the ovary wall and can be observed between the ovule and the ovary wall towards the micropyle (Fig. 2b).

Results

Fertilization frequencies

Table 1 lists fertilization frequencies obtained 3–4 days after pollination of tetraploid wheat cultivars with *Z. mays* cv 'Seneca 60' and both *H. bulbosum* genotypes. These were recorded 3–4 days after pollination as it had been determined from previous timing studies that all potential fertilization events would be achieved at that time (O'Donoughue 1990). For pollination with maize both the total fertilization frequency and the frequency of embryos are listed. Unlike in pollinations with *H. bulbosum* where double fertilization is usually the rule, pollination with the *Triticum* spp. can lead to fertilization of only the egg cell or the polar nuclei. On average, in this study, the frequency of embryos was 11% lower than the total fertilization frequency with maize (Table 1). Double fertilization in the tetraploid wheat × maize crosses ranged from 33.4% of the fer-

Table 1. Fertilization frequencies (%) and embryo frequencies (%) resulting from pollination of tetraploid wheats with *Z. mays* and *H. bulbosum*. Average of 4 spikes scored 3–4 days after pollination

Female parent	Male parent						
T. turgidum ssp.	*Z. mays*			*H. bulbosum* PB168		*H. bulbosum* PB179	
	n	Total fertilization frequency (%)	Frequency of embryos (%)	*n*	Fertilization frequency (%)	*n*	Fertilization frequency (%)
durum cv Kubanka	93	64.4	54.6	61	5.0	54	1.6
durum cv Langdon	91	62.0	44.0	61	0.0	54	4.2
turgidum cv Rampton Rivet	102	48.8	41.3	58	11.8	56	11.5
durum cv Mexicali 75	72	44.6	30.0	28	23.0	30	21.3
durum cv Cappelli	107	42.6	35.2	46	30.3	57	16.0
durum cv Wakona	95	48.9	41.5	54	3.9	48	31.8
durum cv Ambral[a]	79	59.0	53.1				
durum cv Arcour[a]	90	57.0	52.4				
durum cv Cando[a]	90	66.6	62.4				
durum cv Flodur[a]	76	59.4	47.8				

n, Number of ovaries scored
[a] Crosses performed in a different season

Table 2. The nature of fertilization in tetraploid wheat genotypes pollinated with *Zea mays* cv 'Seneca 60'. Average of four spikes per cross combination. The numbers of ovaries scored is as in Table 1. The numbers are summed over spikes; the percentages in parentheses, are the mean of the percentages for each spike

Female parent ssp.	Number and percentage of fertilized ovaries containing		
	Endosperm only	Embryo only	Both
durum cv Cappelli	8(18.8)	22(47.8)	17(33.4)
durum cv Wakona	7(14.8)	17(34.0)	22(51.2)
durum cv Rampton Rivet	8(14.2)	11(20.5)	31(65.3)
durum cv Mexicali 75	10(42.5)	3(8.1)	19(49.4)
durum cv Kubanka	9(14.9)	8(14.1)	42(71.0)
durum cv Langdon	16(29.4)	8(15.2)	31(55.4)
durum cv Flodur[a]	9(19.7)	11(23.9)	25(56.3)
durum cv Ambral[a]	5(9.5)	26(54.7)	16(35.8)
durum cv Arcour[a]	4(9.8)	31(56.8)	17(33.4)
durum cv Cando[a]	4(5.9)	35(60.1)	21(34.0)

[a] Crosses performed in a different season

tilized ovaries in 'Cappelli' and 'Arcour' to 71% in 'Kubanka' (Table 2). All embryos and endosperms observed are believed to be the result of fertilization by the maize or *H. bulbosum* sperm nuclei rather than selfs because either the recognizably smaller maize chromosomes (Fig. 1a) or micronuclei (Fig. 1b) resulting from maize or *H. bulbosum* chromosome elimination were observed.

Statistical analysis revealed that the frequency of embryos obtained with *Z. mays* were significantly ($P<0.001$) higher than that obtained with either of the *H. bulbosum* genotypes. Embryos were seen in 30–54.6% (mean: 41.1%) of the ovaries pollinated with maize as opposed to 0–31.8% (mean: 13.4%) of those pollinated with *H. bulbosum* (Table 1). In addition, frequencies of fertilization were consistently high with maize as no significant differences in fertilization frequencies were detected between the *durum* wheat genotypes. Four more *durum* genotypes were pollinated with maize in a different season, and again, all 4 yielded egg cell fertilization frequencies ranging from 47.8 to 62.4% with a mean of 53.9% (Table 1).

Least significant differences (L.S.D.) comparisons revealed that the two *H. bulbosum* genotypes were not

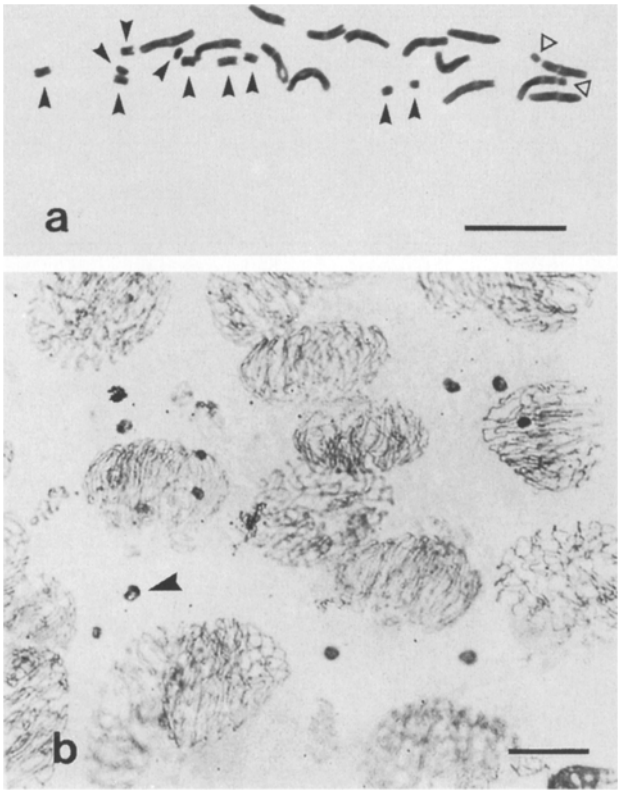

Fig. 1. a Zygote of *T. turgidum* ssp. *durum* cv 'Wakona' × *Z. mays* cv 'Seneca 60' showing the 10 smaller maize chromosomes (*arrowed*) and the 14 wheat chromosomes; the two wheat satellites are indicated by *arrowheads*. *Bar*: 20 μm. **b** Micronuclei resulting from *bulbosum* chromosome elimination in an endosperm with nuclei at interphase in a 3-day-old cross between *T. turgidum* ssp. *durum* cv 'Wakona' and *H. bulbosum* clone PB179. *Bar*: 30 μm

significantly different from each other in effecting fertilization of *durum* wheat cultivars. However, unlike with maize, the *durum* wheat genotypes did vary in fertilization frequencies when pollinated with *H. bulbosum* ($0.001 < P < 0.01$). An L.S.D. test revealed no clear-cut groupings of the *durum* wheat genotypes. However, 'Kubanka' and 'Langdon', with fertilization frequencies at or below 5%, had low or negligible crossability, whereas the other genotypes, with fertilization frequencies between 11.5 and 31.8%, exhibited a higher level of crossability (Table 1). The three spikes of the crossable hexaploid wheat genotype 'Chinese Spring', which were included as a control, gave 58.3% fertilization frequency when pollinated with *H. bulbosum* PB179, a value consistent with the 65% fertilization obtained by Sitch and Snape (1987a) for the same cross combination.

Pollen tube growth

Pollinations with maize

Since there were no significant differences in fertilization frequencies between *durum* wheat genotypes when pollinated with maize, no differences in pollen tube behavior was expected. Therefore, maize pollen tube growth was observed only in the *durum* genotypes exhibiting the highest and lowest fertilization frequencies with maize, namely, 'Kubanka' and 'Cappelli', respectively. In both, the maize pollen germinated readily on the wheat stigmas and grew normally down the style (Fig. 2a). There was no apparent inhibition of pollen tube growth in the transmitting tissue of the ovary wall, and 3–5 pollen tubes were observed beyond the top of the ovules in all ovaries scored. However, in both genotypes the maize pollen tubes exhibited some aberrations, which took place mainly beyond the top of the ovule when they were no longer in the transmitting tissue of the ovary. These included swelling, branching of the tubes and erratic growth plants. Also the pollen tubes tended to accumulate and become intertwined at or near the micropyle (Fig. 2b).

Pollinations with *H. bulbosum*

The pollen of both *H. bulbosum* genotypes germinated readily on the stigmas of the *durum* wheats (Fig. 2c). There was no significant differences between the male parents or the female parents in pollen germination, which averaged 74% for clone PB 168 and 72% for clone PB179 (data not shown). All *durum* wheat genotypes showed some *H. bulbosum* pollen tube growth beyond the top of the ovule in some ovaries. However, evidence of growth inhibition was observed in some genotypes (Fig. 2d). The inhibition reduced both the number of ovaries in which some pollen tube growth proceeded normally (Table 3) and the number of pollen tubes in each ovary which did so (Table 4). 'Kubanka' was the only genotype with significantly fewer ovaries having any pollen tube growing towards the micropyle ($P < 0.001$) (Table 3). The data in Table 3 are expressed for various locations in the ovaries and show that the inhibition in 'Kubanka' started in the style with 20–25% of the ovaries having no pollen tube at the base of the style. A 30% reduction in the number of ovaries with pollen tubes occurred between the base of the style and the top of the ovule, and an additional 20% reduction was seen beyond the top of the ovule.

A more sensitive expression of pollen tube growth inhibition is obtained by dividing the number of pollen tubes observed at one point along their path toward the micropyle by the number of pollen tubes seen at a previous location. Table 4 gives the data for such a reduction in the number of pollen tubes. Again,

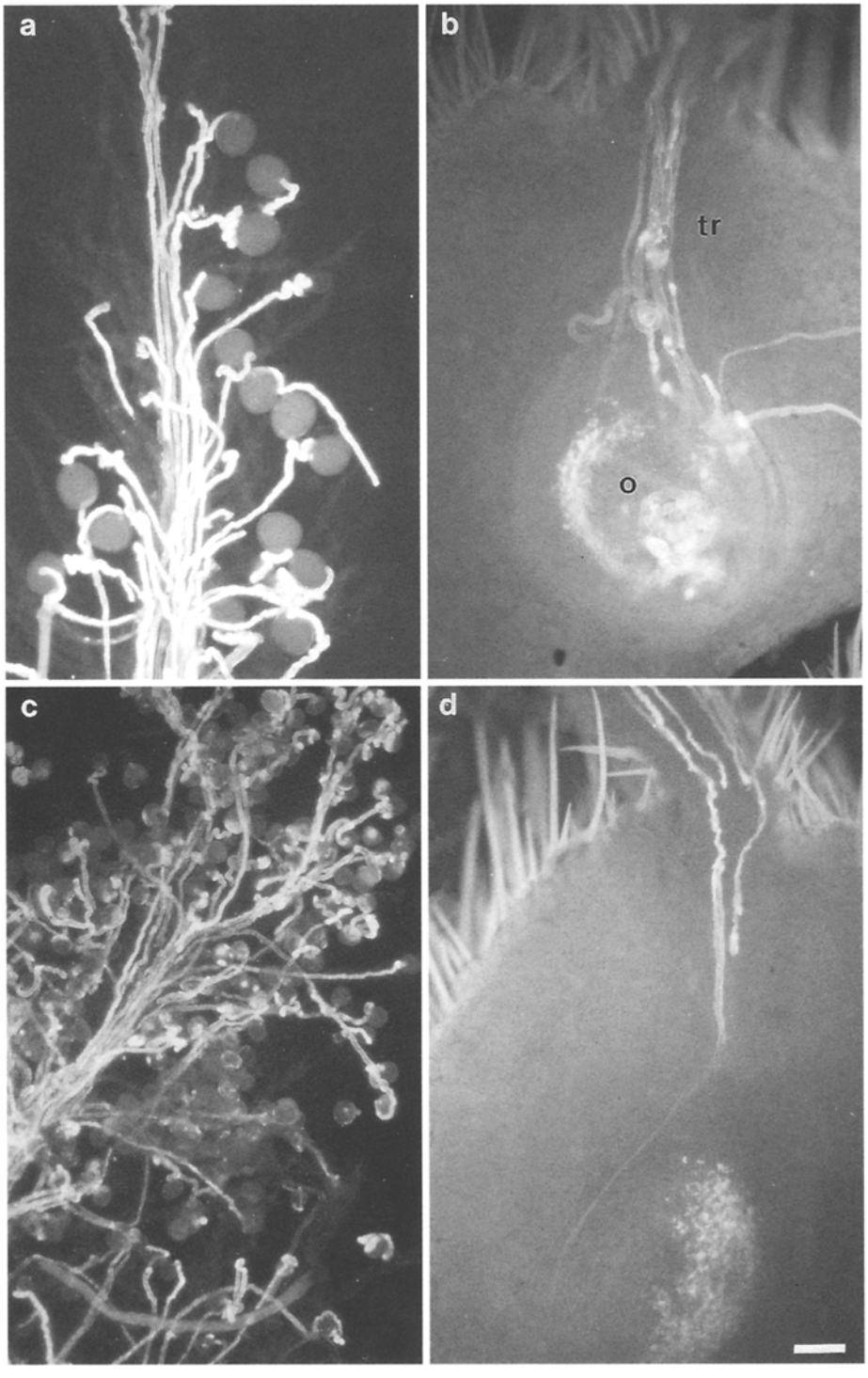

Fig. 2a–d. Maize and *H. bulbosum* pollen tube growth through tetraploid wheat ovaries **a** Maize pollen grains germinating on the stigma of *T. turgidum* ssp. *durum* cv 'Cappelli' with good pollen tube growth down the style; **b** maize pollen tubes growing through the transmitting tract (*tr*) and outside of the ovule (*o*) wall towards the micropyle in *T. turgidum* ssp. *durum* cv 'Kubanka', **c** pollen germination on the stigma and pollen tube growth down the style in the cross *T. turgidum* ssp. *durum* cv 'Langdon' × *H. bulbosum* clone PB168; **d** ovary from the cross *T. turgidum* ssp. *durum* cv 'Kubanka' × *H. bulbosum* clone PB168; showing inhibition of some pollen tubes at the base of the style and in the transmitting tissue of the ovary, as well as the continued growth of one pollen tube towards the micropyle. *Bar*: 100 μm

there were significant differences ($P < 0.001$) between the *durum* genotypes for all four variates listed in Table 4. 'Kubanka' and 'Langdon' both showed more inhibition than the other genotypes. Again, these data show that the inhibition in 'Kubanka' started in the style and extended throughout the ovary. For 'Langdon', the inhibition was initiated beyond the base of the style, with a 65% reduction in the number of pollen tubes occurring in the transmitting tract. Inhibition also occurred beyond the top of the ovule as only 35% of the

Table 3. The numbers and percentages (in parentheses) of ovaries with pollen tubes (p.t.) at various locations in the ovary, in crosses between tetraploid wheats and *H. bulbosum* clones PB168 and PB179

Cross combination	Number of ovaries scored	Number of ovaries with p.t. at the base of the style[a]	Number of ovaries with p.t. at the top of the ovule[a]	Number of ovaries with p.t. beyond the top of the ovule[a]
Cappelli × PB168	43	43(100.0)	43(100.0)	39(91.1)
Cappelli × PB179	54	54(100.0)	53(98.3)	45(83.9)
Kubanka × PB168	56	46(81.0)	19(32.7)	11(18.9)
Kubanka × PB179	43	43(73.2)	18(30.1)	12(19.6)
Langdon × PB168	55	55(100.0)	46(83.1)	39(70.2)
Langdon × PB179	58	58(100.0)	51(87.7)	39(67.0)
Mexicali 75 × PB168	36	36(100.0)	35(97.5)	32(90.0)
Mexicali 75 × PB179	34	34(100.0)	33(97.5)	32(95.0)
Wakona × PB168	42	42(100.0)	41(97.9)	40(96.0)
Wakona × PB179	50	50(100.0)	48(95.8)	46(92.7)
Rampton Rivet × PB168	57	55(96.2)	46(88.0)	43(77.6)
Rampton Rivet × PB179	53	47(90.5)	46(89.0)	34(70.6)

[a] Mean of the percentages of each of four spikes

Table 4. The mean number of pollen tubes (p.t.) at the base of the style and the ratios expressed as percentages of the number of p.t. at various locations in the ovaries, of crosses between tetraploid wheats and *H. bulbosum* clones PB168 and PB179. Average of four spikes per cross combination

Cross combination	Number of ovaries scored	Number of p.t. at the base of the style	Number of p.t. at the top of the ovule/number of p.t. at the base of the style (%)	Number of p.t. beyond the top of the ovule/number of p.t. at the top of the ovule (%)	Number of p.t. beyond the top of the ovule/number of p.t. at the base of the style (%)
Cappelli × PB168	43	10.72	58	57	35
Cappelli × PB179	54	12.74	60	48	30
Kubanka × PB168	56	5.97	11	21	7
Kubanka × PB179	59	2.66	14	16	6
Langdon × PB168	55	9.34	35	34	17
Langdon × PB179	58	11.65	38	37	15
Mexicali 75 × PB168	36	15.84	50	49	26
Mexicali 75 × PB179	34	18.65	52	59	33
Wakona × PB168	42	12.91	49	68	37
Wakona × PB179	50	16.59	56	58	35
Rampton Rivet × PB168	57	7.68	45	40	21
Rampton Rivet × PB179	53	8.31	53	38	22

pollen tubes reaching the top of the ovule in 'Langdon' grew beyond that point. The overall reduction in the number of pollen tubes was 85%. The overall reduction in the other genotypes was 65–70%, being somewhat larger in 'Rampton Rivet' (ca. 80%). Good pollen tube growth was observed especially in cvs 'Cappelli' and 'Wakona' with up to ten pollen tubes observed at the micropyle in 'Wakona' × PB179. Linear regressions between the reduction in number of ovaries showing pollen tube growth and fertilization frequencies, and between the reduction in the number of pollen tube and fertilization frequency, both showed significant association of the two variables ($0.02 < P < 0.05$ and $0.01 < P < 0.02$, respectively).

Discussion

The tetraploid wheat genotypes used in the present study were varied and included two different sub-

species, *T. turgidum* ssp. *durum* and *T. turgidum* ssp. *turgidum*, an old *durum* landrace, 'Kubanka', older tall commercial cultivars such as 'Cappelli', 'Langdon' and 'Wakona' and more modern dwarf cultivars such as 'Ambral', 'Arcour', 'Cando' and 'Flodur'. Despite these differences in genetic backgrounds successful fertilization by *Z. mays* cv 'Seneca 60' was achieved in all of these genotypes, and an average of 41.1% (53.9% for the crosses performed in the summer months) of the pollinated ovaries contained an embryo (Table 1). Furthermore, no significant difference in the frequency of embryos was detected between the tetraploid wheat genotypes pollinated in the same season. Thus, it appears that, as in hexaploid wheat × maize crosses (Laurie and Bennett 1987), fertilization by maize is largely unaffected by the female parent genotype in tetraploid wheat × maize crosses. Also, rapid maize chromosome elimination characterized all these crosses (Fig. 1). These results imply that the method could be used to produce haploids of many if not all *durum* wheat genotypes. Interestingly, double fertilization was seen in more than 30% of the fertilized ovaries in all of the tetraploid wheat genotypes pollinated with maize (Table 2), whereas double fertilization occurred in only 12.0% of the fertilized ovaries in the cross between the hexaploid wheat 'Chinese Spring' and 'Seneca 60' maize performed by Laurie and Bennett (1988b). However, Laurie (1989) showed that the proportion of double fertilization in hexaploid wheat × maize crosses could be strongly influenced by the developmental age of the ovary at the time of pollination. Nevertheless, the consistently higher incidence of an endosperm associated with the embryo in these tetraploid wheat × maize crosses suggests that plant recovery may be facilitated.

The differences in fertilization frequencies obtained with *H. bulbosum* seem to result in part from an effect similar to the *Kr* gene effect known in hexaploid wheats pollinated with rye (Lange and Wojciechowska 1976) and *H. bulbosum* (Sitch and Snape 1987a, b), namely the inhibition of pollen tube growth in the ovary tissue. However, the inhibition observed in tetraploid wheats pollinated with *H. bulbosum* was not complete. This is unlike the situation in the non-crossable hexaploid wheat 'Highbury' (*Kr1*, *Kr2*) pollinated with *H. bulbosum* where complete arrest of pollen tube growth at the base of the style was observed in most ovaries (Sitch and Snape 1987a). Even in 'Kubanka', where the inhibition was strongest, some pollen tubes grew beyond the top of the ovule (Fig. 2d).

Pollen tube growth inhibition in tetraploid wheats could be due to the "weaker" *Kr2* (Snape et al. 1979; Falk and Kasha 1981, 1983; Sitch et al. 1985) gene, or perhaps weaker alleles might be present at the *Kr* gene loci. Allelic variations at both the *Kr1* and *Kr2* loci has been shown in hexaploid wheats using chromosome 5A and 5B substitution lines from different cultivars into 'Chinese Spring' (Falk and Kasha 1983; Sitch and Snape 1986). Furthermore, it is clear that pollen tube growth inhibition is not the only aspect of the incompatibility reaction between tetraploid wheats and *H. bulbosum*. 'Kubanka' was the only genotype where most of the fertilization failures could be explained by pollen tube growth inhibition. Though some pollen tube growth inhibition was observed in 'Langdon', 'Mexicali 75' and 'Rampton Rivet', in the worst case (Langdon × PB179), 67% of the ovaries had pollen tubes beyond the top of the ovule whereas the fertilization frequency was only 4%. Thus, the incompatibility must also have been interfering with the penetration of the micropyle by the pollen tube, and/or the release of the sperm cells and/or their migration and fusion to the egg cell and polar nuclei. Overall, the incompatibility reaction between tetraploid wheats and *H. bulbosum* is apparently more complex than that in hexaploid wheats, though part of it can be explained by an effect similar to that of the *Kr* genes.

Interestingly, though no significant difference in fertilization frequencies was noted between the tetraploid wheat genotypes when pollinated with maize, the two tetraploid wheat genotypes, 'Kubanka' and 'Langdon', which gave the lowest fertilization frequencies with *H. bulbosum* yielded the highest fertilization frequencies with maize. Linear regressions of the mean fertilization frequencies obtained with maize versus those obtained with both *H. bulbosum* clone PB168 and PB179 gave regression lines that were significantly different from 0 as well as significant correlation coefficients ($r_{x,y} = -0.81$ for PB168 versus maize and $r_{x,y} = -0.83$ for PB179 versus maize). The reason for this is unclear and does not appear to be due to an effect on pollen tube growth. Laurie and Reymondie (1991) found a similar loose negative correlation between the fertilization frequencies obtained with maize and *H. bulbosum* in hexaploid wheats.

In summary, the results of this study indicate that successful fertilization of tetraploid wheat genotypes with maize can be achieved at relatively high frequencies and that these crosses are characterized by the rapid elimination of the maize chromosomes and consequent formation of haploid embryos. Furthermore, fertilization by maize of tetraploid wheats is largely insensitive to genes limiting the crossability of these tetraploid wheats with *H. bulbosum*. Therefore, the method has potential as a broad spectrum haploid production system for tetraploid wheats. Though the rapid elimination of maize chromosomes is a disadvantage for potential gene transfer from maize to wheat, the high fertilization frequencies (mean = 51.9%) obtained in tetraploid wheats (Table 1) when compared to hexaploid wheat × maize crosses (mean = 39.1%) (Laurie and Reymondie 1991) may increase the chances of

achieving gene transfer by inducing translocations in very early seed development.

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References

Comeau A, Plourde A, St Pierre CA, Nadeau P (1988) Production of doubled haploid wheat lines by wheat × maize hybridization. Genome 30 [Supp1]:482 (abstr)

Falk DE, Kasha KJ (1981) Comparisons of the crossability of rye (*Secale cereale*) and *Hordeum bulbosum* onto wheat (*Triticum aestivum*). Can J Genet Cytol 23:81–88

Falk DE, Kasha KJ (1983) Genetic studies of the crossability of hexaploid wheat with rye and *Hordeum bulbosum*. Theor Appl Genet 64:303–307

Hadwiger MA, Heberle-Bros E (1986) Pollen plant production in *Triticum turgidum* ssp. *durum*. In: Horn W, Jensen CJ, Odenbach W, Schieder O (eds) Genetic manipulation in plant breeding. EUCARPIA 1985.) Watter de Gruyter, Berlin, pp 303–305

Inagaki M, Tahir M (1990) Comparison of haploid production frequencies in wheat varieties crossed with *Hordeum bulbosum* L. and maize. Jpn J Breed 40:209–216

Krolow KD (1970) Untersuchungen über die kreuzbarkeit zwischen weizen und roggen. Z Pflanzenzuecht 64:44–72

Lange W, Wojciechowska B (1976) The crossing of common wheat (*Triticum aestivum* L.) with cultivated rye (*Secale cereale* L.). 1. Crossability, pollen grain germination and pollen tube growth. Euphytica 25:609–620

Laurie DA (1989) Factors affecting fertilization frequency in crosses of *Triticum aestivum* cv 'Highbury' × *Zea mays* cv 'Seneca 60'. Plant Breed 103:133–140

Laurie DA, Bennett MD (1986) Wheat × maize hybridization. Can J Genet Cytol 28:313–316

Laurie DA, Bennett MD (1987) The effect of the crossability loci *Kr1* and *Kr2* on fertilization frequency in hexaploid wheat × maize crosses. Theor Appl Genet 73:403–409

Laurie DA, Bennett MD (1988a) The production of haploid wheat plants from wheat × maize crosses. Theor Appl Genet 70:100–105

Laurie DA, Bennett MD (1988b) Chromosome behaviour in wheat × maize, wheat × sorghum and barley × maize crosses. In: Brandham PE (ed) Kew Chromosome Conference III (Proc 3rd Chromosome Conf 1987). Her Majesty's Stationary Office, London, pp 167–177

Laurie DA, Reymondie S (1991) High frequencies of fertilization and haploid seedling production in crosses between commercial hexaploid wheat varieties and maize. Plant Breed 106: 182–189

O'Donoughue LS (1990) Chromosome behaviour and reproductive physiology in cereal wide crosses. PhD thesis, Cambridge University, UK

O'Donoughue LS, Bennett MD (1988) Wide hybridization between relatives of bread wheat and maize. In: Miller TE, Koebner RMD (eds) Proc 7th Int Wheat Genet Symp vol 1. Institute of Plant Science Research, Cambridge, pp 397–402

Riera-Lizarazu O, Mujeeb-Kazi A (1990) Maize (*Zea mays* L.) mediated wheat (*Triticum aestivum* L.) polyhaploid production using various crossing methods. Cereal Res Commun 18:339–345

Riley R, Chapman V (1967) The inheritance in wheat of crossability with rye. Genet Res 9:259–267

Sharma GC, Wang WC, Sapra VT (1982) Effect of genotype, media and temperature pretreatment on callus initiation in triticale, wheat and rye anther culture. Cereal Res Commun 10:143–150

Sitch LA, Snape JW (1986) Allelic variation at the crossability loci in wheat (*Triticum aestivum*). Wheat Inf Serv 63:11–15

Sitch LA, Snape JW (1987a) Factors affecting haploid production in wheat using the *Hordeum bulbosum* system. 1. Genotypic and environmental effects on pollen grain germination, pollen tube growth and frequency of fertilization. Euphytica 36:483–496

Sitch LA, Snape JW (1987b) Factors affecting haploid production in wheat using the *Hordeum bulbosum* system. 3. Post-fertilization effects on embryo survival. Euphytica 36:763–773

Sitch LA, Snape JW, Firman SJ (1985) Intrachromosomal mapping of crossability genes in wheat (*Triticum aestivum*). Theor Appl Genet 70:309–314

Snape JW (1982) The use of doubled haploids in plant breeding. In: Broertjes C (ed) Induced variability in plant breeding. Centre for Agricultural Publishing and Documentation, Wageningen, pp 52–58

Snape JW (1989) Doubled haploid breeding: theoretical basis and practical applications. In: Mujeeb-Kazi A, Sitch LA (eds) Review of advances in plant biotechnology 1985:88. (Second Int Symp Genet Manipulation Crops. CIMMYT and IRRI, Mexico and Manila, pp 19–31

Snape JW, Simpson E (1981) Uses of doubled haploid lines for genetical analysis. In: Asher et al. (eds) Barley genetics IV. (Proc 4th Int Barley Genetics Symp.) Edinburgh University Press, Edinburgh, pp 704–709

Snape JW, Chapman V, Moss J, Blanchard CE, Miller TE (1979) The crossabilities of wheat varieties with *Hordeum bulbosum*. Heredity 42:291–298

Suenaga K, Nakajima K (1989) Efficient production of haploid wheat (*Triticum aestivum*) through crosses between Japanese wheat and maize (*Zea mays*). Plant Cell Rep 8:263–266

Thomas JB, Kaltsikes PJ, Anderson RG (1981) Relation between wheat-rye crossability and seed set of common wheat after pollination with other species in the Hordeae. Euphytica 30:121–127

Zhu ZQ, Wang JJ, Sun JS (1980) The induction of albino pollen plants and preliminary observation of their ploidy in *Triticum durum* Desf. In: Davies DR, Hopwood DA (eds) The plant genome. (Fourth John Innes Symp and 2nd Int Haploid Conf.) The John Innes Charity, Norwich, pp 254–255