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High frequncies of fertilization and embryo formation in hexaploid wheat × *Tripsacum dactyloides* crosses

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Abstract The Hexaploid wheat variety Fukuho was crossed with *Tripsacum dactyloides* (2n = 4x = 72). The total fertilization frequencies for the egg cell, polar nuclei, and both, were 58.3%, 26.8% and 58.9% of the 168 ovaries examined. However, the fertilization frequency of single polar nuclei was much lower at only 0.6%. The total frequency of fertilization was higher than that in wheat × maize crosses. A total of 49 hexaploid wheat varieties, including Hope carrying the dominant genes *Kr1* and *Kr2*, were crossed with *T. dactyloides*, and most gave embryos. The embryo-formation frequencies ranged from 0.5% to 59.0%. A higher frequency of 32.0% embryo formation was obtained following pollination of the variety Hope. In comparison with embryo formation in wheat × maize crosses the difference of embryo-formation frequencies between the two crosses was significant. The results of high frequencies of fertilization and embryo formation in wheat × *T. dactyloides* crosses indicated that the *Kr* genes are as inactive in wheat × *T. dactyloides*, as they are in wheat × maize crosses, and also that the efficiency of fertilization and embryo formation is higher in wheat × *T. dactyloides* than in what × maize crosses. The potential of wheat × *T. dactyloides* crosses for wheat haploid production and wheat improvement is discussed.

Key words Wheat · *Tripsacum dactyloides* · Wide crosses · Fertilization · Embryo formation

Introduction

It has been demonstrated that the crossability between hexaploid wheat (*Triticum aestivum*) and rye (*Secale cereale*) is controlled by two genes, *Kr1* and *Kr2* (Lein 1943). Dominant alleles of either gene will cause poor crossability though *Kr1* has a stronger effect (Lange and Riley 1973; Lange and Wojciechowska 1976; Jalani and Moss 1980). Therefore, only a very few hexaploid wheat varieties or lines, including Chinese Spring which carries the recessive alleles *kr1* and *kr2*, have higher crossability with alien species. However, both *Kr1* and *Kr2* genes, which inhibit the crossability of wheat when pollinated with *Secale cereale* or *Hordeum bulbosum*, have little or no effect on the crossability of wheat with maize (Zenkteler and Nitzsche 1984; Laurie and Bennett 1986, 1987). In crosses between hexaploid wheat and maize, high frequencies of fertilization and embryo formation are found, and zygotes contain a complete haploid chromosome set from each parent. However, the maize chromosomes are eliminated from the developing embryo during the initial cycles of cell division, and haploid embryos are produced (Laurie and Bennett 1986, 1988).

In the present study we report and discuss the high frequencies of fertiliztion and embryo formation obtained in hexaploid wheat × *Tripsacum dactyloides* crosses.

Materials and methods

Determination of fertilization frequency

Hexaploid wheat (*T. aestivum*, 2n = 42) variety Fukuho was crossed as a female with *T. dactyloides* (2n = 72) in the field. One genotype of *T. dactyloides* was grown in the greenhouse. Spikes of the female parent were emasculated 1 or 2 days prior to anthesis, each with 20 florets remaining, and then enclosed in parchment bags. One or two days later, emasculated spikes were pollinated with freshly collected *T. dactyloides* pollen and then covered again. After pollination with *T. dactyloides* a solution of about 0.5 ml of 100 ppm 2.4-D was needle-injected into the uppermost internode of each pollinated spike. Ovaries were removed at 1, 2, 4, 8, 12, 24, 72, 96 and 120 h after pollination and fixed immediately in 3:1 ethanol/acetic acid for 24 h, then stored in 70% ethyl alcohol at 4 °C. For examination of fertilization frequency, whole ovaries were stained with Ehrliech's hematoxylin, dehydrated in a ethyl alcohol series and then embedded in paraffin. Sections were prepared at 10–12 μm thickness mostly longitudinally

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in the dorsi-ventral plane. The preparations were mounted and stained with Eosin. The rates of single and double as well as overall fertilization which occurred in 168 ovaries were examined using light microscopy.

Determination of embryo-formation frequency

This study was carried out from 1992 to 1994. A total of 49 hexaploid wheat varieties and lines, including the variety Hope carrying the *Kr1* and *Kr2* dominant genes, were used as female parents in crosses with one genotype of *T. dactyloides*. The 58 hexaploid wheat genotypes were pollinated with a mixture of pollen from two to three maize ($2n = 20$) genotypes, to be used as a comparison. All the plant stocks of wheat and *T. dactyloides* were grown in the field, while maize plant stocks were grown in the greenhouse. The crossing procedure is the same as that mentioned above. After being left on the plant for 14 days the pollinated spikes were collected. Embryos were excised from ovaries that grew over two thirds of the glum length. The number of embryos was counted according to the cross combinations designed. Furthermore, the embyo-formation frequencies (EFF) were calculated based on the number of embryos obtained from 100 pollinated florets.

Results and discussion

Fertilization frequency

The pollen grains of *T. dactyloides* germinated normally on the stigmas of hexaploid wheat. The pollen tubes penetrated into the embryo sac 1 h after pollination and discharged their contents. Fertilization subsequently occurred. The male gametes may fuse with the egg cell and the polar nuclei only or else with both. Therefore ovaries containing embryos without endosperms, or with both embryo and endosperm, could be observed (Fig. 1).

In 168 ovaries examined a total of 58.3% of the egg cells and 26.8% of the polar nuclei were fertilized while the total frequency of fertilized ovaries was 58.9%. This is different from wheat × maize crosses where either single fertiliztion of or else double fertilization of polar nuclei had occurred with relatively lower frequencies (Laurie and Bennett 1987). In wheat × *T. dactyloides* crosses not only the egg cell but also both egg cell and polar nuclei were fertilized simultaneously with a higher frequency. In the present study, the total frequencies of fertiliztion for the single egg cell and for both the egg cell and the polar nuclei were 32.1% and 26.2% of the ovaries examined, respectively. Accordingly they were 54.5% and 44.4% of the fertilized ovaries. The total frequencies of the single fertilization of polar nuclei were 0.6% of the ovaries examined and 0.1% of the fertilized ovaries, respectively. That the polar nuclei were fertilized with a lower frequency is similar to that observed by Laurie and Bennett (1988). The fertilization frequency of 58.9% in the present study was higher than that in hexaploid wheat × maize crosses where fertilization frequencies of 51.2%, 28.6% (Wang et al. 1991); 30.7%, 29.2%, 14.4% (by Laurie and Bennett 1987); and 23.66% (by Sun et al. 1992) were obtained in crosses with different hexaploid wheat and maize genotypes. From these results it is clear that, although hexaploid

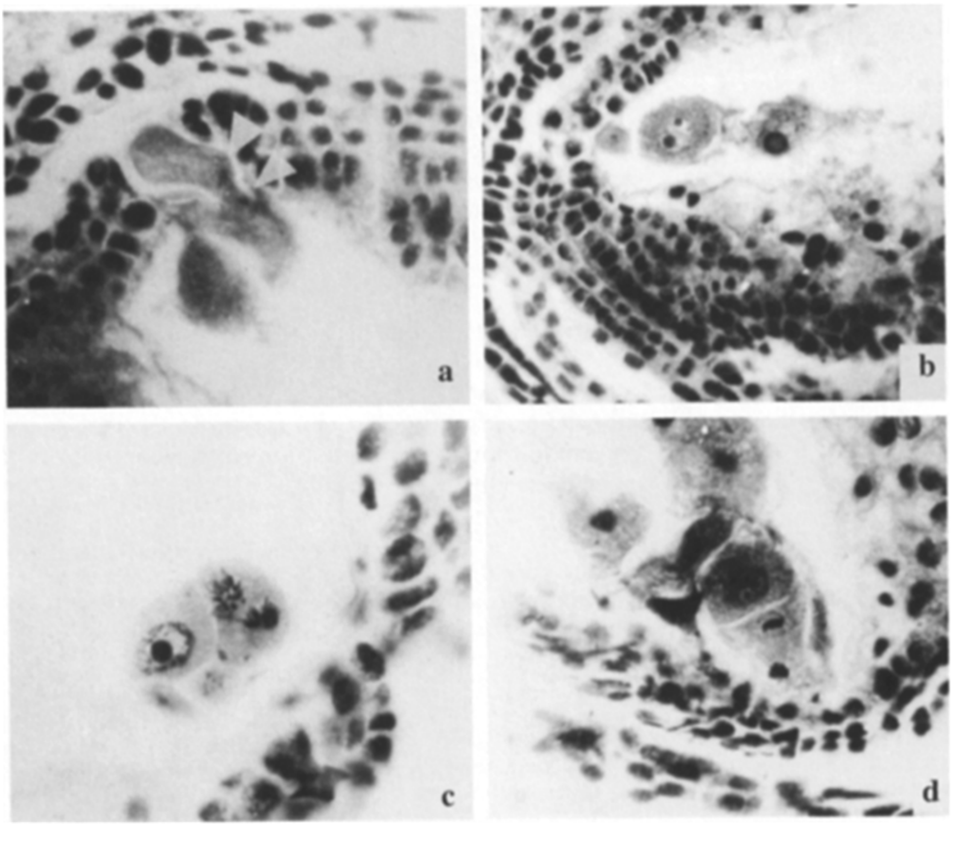

Fig. 1a Pollen tube penetration into the embryo sac 1 h after pollination. *Arrows* shows sperm. **b** Fertilized egg cell with two nuclei. The larger one is the egg cell nucleus, the smaller one the male nucleus. **c** A proembryo without endosperm in a ovary 72 h after pollination with *T. dactyloides*. **d** A proembryo with endosperm in a ovary 72 h after pollination with *T. dactyloides*

wheat and *T. dactyloides* belong to two different subfamilies of the Gramineae, high frequencies of fertilization had occurred in the wheat × *T. dactyloides* crosses.

The frequency of embryo formation

Of forty nine hexaploid wheat varieties and lines all, except for very few, gave rise to embryos (Fig. 2). The frequencies of embryo formation ranged from 0.5% to 59.0% from 1992 to 1994. A total of 2242 embryos were obtained from 12 462 florets pollinated. Relatively high frequencies of embryo formation were found. Among the 49 wheat varieties and lines tested, 31 of them formed embryos at a rate over 5%, and 22 over 10%. They account for 63.27% and 44.90% of the total genotypes tested respectively (Table 1). It is to be noted that a frequency of embryo formation of 32.0% was obtained from the wheat variety Hope (*Kr1 Kr1 Kr2 Kr2*), which is cross-incompatible with rye and *H. bulbosum*. The result that high values of embryo formation were found in wheat × *T. dactyloides* crosses contrast sharply with the result that the crossabilities of most wheat genotypes were lower than 5% in wheat × rye crosses (Lein 1943; Lange and Wojciechowska 1976; Zeven 1987). The data indicated that wheat genotypes behave independently in wheat × *T. dactyloides* crosses.

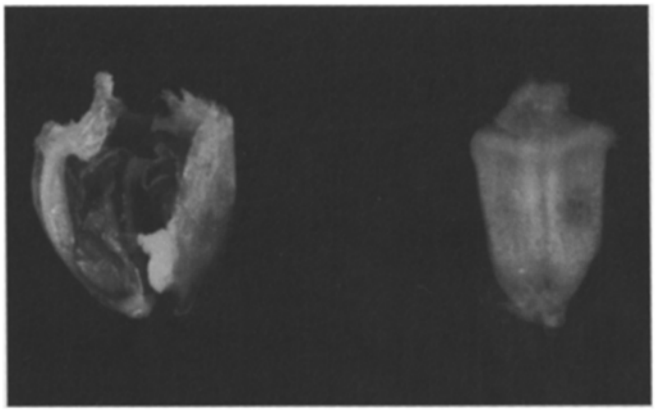

Fig. 2 A caryopsis of wheat 14 days after pollination with *T. dactyloides* (right) and an embryo in a dissected seed (left)

Table 2 shows a comparison of embryo-formation frequencies (EFF) in 58 wheat varieties and lines crossed with *T. dactyloides* and 49 crossed with maize during 1992–1994. For either, the highest or the average value of EFF, *T. dactyloides* showed better results than did maize when they were used as pollinators. The highest value of EFF pollinated with *T. dactyloides* was 59.0% while that pollinated with maize was 30.0%. In the three crossing seasons the average values of EFF were 5.22%, 3.40% (1992); 13.19%, 4.40% (1993); and 29.87%, 11.14% when *T. dactyloides* and maize were used as male parents, respectively. The overall average EFF values were 18.01% for *T. dactyloides* and 6.76% for

Table 1 Thirty one wheat varieties and lines with more than 5% embryo formation among 49 wheat genotypes crossed with *Tripsacum dactyloides*

Wheat genotype	No. of florets pollinated	No. of embryos	Embryo formation(%)	Year
As 375 10-A	80	7	8.75	1992
Spring 90-1	200	10	5.00	1993
90053	200	10	5.00	1993
Zhong 5	191	12	6.28	1993
Spring 90-2	200	14	7.00	1993
86966	200	17	8.50	1993
901701	200	17	8.50	1993
H2G-43	200	17	8.50	1993
Guinong 87-11/Youyimai	200	19	9.50	1993
Jia 84s-438/Guinong 87-6	180	19	10.56	1993
Guinong 87-11	200	23	11.50	1993
I-M (white)	200	24	12.00	1993
0105585	200	27	13.50	1993
I-M (red)	200	28	14.00	1993
Qingfeng 1	220	36	16.36	1993
Long 85-5216	200	34	17.00	1993
6535	194	33	17.01	1993
Long 85-5226	160	29	18.13	1993
Long 80-10881	520	102	19.62	1993
Fukuho	203	46	22.66	1993
Spring 90-3	200	59	29.50	1993
Fr 81-8	188	93	49.47	1993
Gansu 4	200	118	59.00	1993
Vee's' Scri 82	98	12	12.24	1994
Panjiang 1	197	26	13.20	1994
Chinese Spring	432	70	16.20	1994
Toluka F73	200	43	21.50	1994
Hope	286	93	32.50	1994
J11	226	86	38.05	1994
Alondra 's'/957 kabra3	99	39	39.39	1994
Qianhua	1383	579	41.87	1994

Table 2 The comparison of embryo formation frequencies (EFF) between wheat × maize and wheat × *Tripsacum dactyloides* crosses

Year	Wheat × Maize					Wheat × *Tripsacum dactyloides*					*t* Value[a]
	No. of wheat genotype	No. of florets pollinated	No. of embryos	EFF(%) highest	EFF(%) average	No. of wheat genotype	No. of florets pollinated	No. of embryos	EFF(%) highest	EFF(%) average	
1992	21	3944	134	9.09	3.40	6	1380	72	8.75	5.22	3.00**
1993	9	2751	121	21.46	4.40	34	6824	900	59.00	13.19	12.57**
1994	28	4526	504	30.04	11.14	9	4258	1272	41.87	29.87	23.50**
Overall	58	11 221	759	30.04	6.76	49	12 462	2244	59.00	18.01	

[a] ** $t_{0.01} = 2.58$

maize. According to previous reports from other workers, the highest embyo-formation frequencies (by embryo excision) so far obtained were 25.1% (with the Fukuho wheat variety, Suenaga and Nakajima 1989), 34.5% (with Chinese Spring, Inagaki and Y. Tahir 1990), 28.7% (with Morocco, Riera-Lizarazu et al. 1990) and 30.29% (with Zheng zhou 721, Li et al. 1994) in wheat × maize crosses. These values were lower than the 59.0% obtained in the crosses with *T. dactyloides*, although different wheat varieties and crossing procedures were used. The difference of EFF between the crosses of wheat × *T. dactyloides* and wheat × maize were examined by a *t*-test; the *t* values for each of the three crosing years were significant.

The different embryo-formation levels of wheat genotypes probably show that in addition to both parent genotypes, there are other factors, such as temperature, humidity, pollen quality, the developmental level of the stigma and crossing technique that affect the level of embryo formation. In our study we found that the same wheat genotype gave different frequencies of embryo formation when workers or crossing seasons were changed. Moreover, the interspike-difference in the same wheat genotype when pollinated with alien pollen was also notable, even if the crossing was carried out by one person and in one crossing season. When the wheat variety Chinese Spring was crossed with *T. dactyloides* a different embryo-formation rate (7.35%, 9.44% and 16.20%) was obtained each year from 1992 to 1994. After examining ten spikes of the wheat variety Fukuho crossed with *T. dactyloides*, the highest embryo formation of an individual spike was 38.89% while the lowest was 10.0%. Therefore, we take the highest level of embryo formation obtained for a wheat genotype to represent its embryo forming capacity, although in practice its potentiality is probably much higher.

These results indicated that: (1) *Kr* genes which markedly reduce the crossability in wheat × rye and wheat × *H. bulbosum* crosses are inactive in wheat × *T. dactyloides* crosses, as they are in wheat × maize crosses; and (2) the efficiencies for fertilization and embryo formation are higher in wheat × *T. dactyloides* crosses than in wheat × maize crosses. High frequencies of fertilization and embryo formation in wheat × *T. dactyloides* crosses have great prospects in wheat breeding. Firstly, it is possible that wide-ranging wheat genotypes could be successfully used in wide crosses with *T. dactyloides.* Secondly, since *T. dactyloides* is noted for its wild genotype, more tillers, prolific pollen and longer florescence characteristics, it can offer a larger quantity of fresh pollen all through the crossing season. Thirdly, if the chromosomes of *T. dactyloides* are completely eliminated from the developing embryo, it would be possible to find a new way to efficiently produce wheat haploids. Finally, under the condition that the chromosomes of the male parent would be incompletely eliminated it is possible that some of the characters of *T. dactyloides* could be transferred to wheat. This would be of great value to wheat improvement. Somatic chromosome number was examined in root-tip cells of plantlets that were regenerated from wheat embryos crossed with *T. dactyloides*. The results (unpublished) indicated that the chromosomes of *T. dactyloides* may be either completely or incompletely eliminated, because plantlets were found with 21, or more than 21, chromosomes.

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