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Short Communication

Pollen tube behaviour and haploid wheat production via embryo rescue in wheat × maize crosses

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In wheat, anther culture has been used to a limited extent due to the occurrence of somaclonal variation, aneuploids, albinism and genotype specificity while the crossability loci (Kr_1 Kr_2 Kr_3) are the major limiting factors while crossing with *H. bulbosum* for haploid production in wheat [1]. Production of haploid wheat plants through wheat × maize crosses was first reported by Laurie and Bennett [2]. Since then, it has emerged as a system of choice for haploid production in wheat. Since maize pollen tube growth and fertilization appear to be insensitive to the crossability alleles of wheat, poly-haploids can be recovered across different genotypes. Wheat × maize system has been shown to be more efficient in haploid production when compared with anther culture [3] or *bulbosum* technique [4]. The growth of pollen tubes, including abnormalities if any, should be equally emphasized for complete understanding of all the pre-zygotic gametophytic competition in flowering plants [5]. The interaction between pollen and pistil and various pre-fertilization processes affect crossability. Therefore, in this study, an attempt has been made to produce haploids through wheat × maize crosses and to find out the correlation between various pre-fertilization factors *viz.* pollen germination, pollen tube growth and abnormal pollen tubes with frequency of embryo formation and haploid production.

Six genotypes of wheat were used as female parents (Table 2). The first five in the table are *Triticum aestivum* and the sixth (Synthetic 56) has *T. turgidum* and *T. tauschii* as its parents. The plants were grown in outdoor conditions in pots. A composite maize variety Kanchan was used as the pollen donor. Emasculation of wheat spikes was carried out by hand using cut glume method. The spikes were pollinated two days after emasculation when the stigmas of most of the emasculated florets were feathery and receptive.

Experiment 1: The spikes were sprayed with 75 ppm solution of 2, 4-D 1 day and 2 days after pollination and GA_3 @ 300 ppm on the third day. Two weeks after pollination the embryos were excised and cultured on half strength MS medium supplemented with 146 mg/L glutamine and 150 mg/L L-Asparagine and maintained at 4°C in the dark. After 5-6 days, cultures were transferred to 25°C and 16/8 hours light/dark cycle. When germinated embryos grew to become plantlets of 5-10 cm height, they were potted in soil. The chromosome number of the root tips of the haploid plantlets was determined using acetocarmine squash method. Influence of the genotype of wheat on embryo formation and haploid production was tested using $\sqrt{X}$ + 0.5 and $\sqrt{X}$ transformations, respectively in completely randomized design with unequal number of replications.

Pollination with maize pollen on wheat stigma followed by 2, 4-D and GA_3 treatment resulted in the formation of seed like structures lacking endosperm which were smaller than that of the normal selfed seeds of wheat. Out of 2981 florets pollinated, 2615 (87.7%) produced a seed like structure, of which only 171 (6.5%) contained embryos which were cultured on half strength MS medium. Out of 171 embryos only 80 (46.18%) germinated and produced 22 haploid plants. All the genotypes of wheat tested produced embryos with a frequency ranging from 2.2 to 9.5% (Table 1). This embryo formation rate was comparable to several

Table 1. Response of six wheat genotypes for embryo formation and haploid plantlets recovery

Genotypes	Florets pollinated	Seeds formed	Embryos obtained	Embryos germinated	Plants recovered
UP 2003	557	486 (87.2)	24 (4.9)	10 (41.7)	2 (8.3)
PBW 373	409	317 (77.5)	7 (2.2)	2 (28.6)	1 (14. 3)
PBW 343	624	562 (90.6)	46 (8.2)	22 (47.8)	6 (13.0)
PBW 175	663	619 (93.4)	59 (9.5)	32 (54.3)	9 (15.2)
PBW 65	396	351 (88.6)	22 (6.3)	10 (45.5)	3 (13.6)
Synthetic 56	332	280 (84.3)	13 (4.6)	4 (30.8)	1 (7.7)
	2981	2615 (87.7)	171 (6.5)	80 (46.8)	22 (12.9)

Values in parentheses indicate percentage of seeds formed, embryos obtained, embryos germinated and plants recovered.

Table 2. Pollen germination, pollen tube length (μm), pollen abnormalities and embryo formation (%) in selfed wheat and in wheat × maize crosses.

Cross	Mean pollen germination %	Mean pollen tube length	Mean abnormal pollen tube %	% embryo formed
UP 2003 (Selfed)	61.1 ± 5.15	88.4 ± 12.68	3.3 ± 0.82	
UP 2003 × Kanchan	33.1 ± 6.25	76.3 ± 37.18	12.5 ± 1.84	4.93
PBW 373 (Selfed)	52.6 ± 5.06	74.5 ± 12.65	6.4 ± 1.24	
PBW 373 × Kanchan	28.7 ± 7.06	70.3 ± 17.12	15.4 ± 3.09	2.20
PBW 343 (Selfed)	65.7 ± 6.04	95.9 ± 13.3	1.3 ± 0.60	
PBW 343 × Kanchan	41.3 ± 8.66	93.3 ± 18.96	9.3 ± 1.30	8.18
PBW 175 (Selfed)	64.4 ± 5.28	98.7 ± 14.13	1.3 ± 0.48	
PBW 175 × Kanchan	48.9 ± 7.64	97.9 ± 18.87	10.5 ± 2.08	9.53
PBW 65 (Selfed)	50.8 ± 4.67	92.3 ± 14.36	4.1 ± 0.83	
PBW 65 × Kanchan	41.4 ± 7.10	85.9 ± 18.72	12.5 ± 2.78	6.26
Synthetic 56 (Selfed)	60.5 ± 5.48	87.2 ± 11.93	1.6 ± 0.45	
Synthetic 56 × Kanchan	47.1 ± 7.14	83.0 ± 8.62	15.0 ± 2.58	4.64

other reports [3, 6] but it was much smaller than some of the other reports [6]. Since in this study, all the crosses were performed in the late season in outdoor conditions, so, perhaps due to temperature factor, a low percentage of embryos and haploid plants were recovered as compared to the other reports. The crucial role of temperature and light intensity during pollination, fertilization and embryo development has been reported and emphasized by Campbell [7].

Experiment 2: Pre-fertilization studies: In all the six wheat × maize crosses, crossed spikes were collected at 5 min, 15 min, 30 min and 2 hrs after pollination and fixed immediately in 1:3 aceto-alcohol. After 24 hrs, they were preserved in 70% ethyl alcohol till further use. Different prefertilization factors like pollen germination, pollen tube growth and abnormal pollen tubes were studied with a light microscope using 1% aniline blue as a dye for staining.

Maize pollen showed no germination on the wheat pistils at 5 min after pollination but a continuous increase in germination was observed from 15 min to 2hrs after pollination. Mean pollen germination showed no direct correlation with embryo formation and haploid recovery rate. Genotype with longest mean pollen tube length showed highest frequency of embryo and haploid recovery while the one with the least showed lowest embryo and haploid formation frequency (Table 2). Therefore, it can be assumed that in wheat × maize system also there is some mechanism of crossability (other than Kr_1, Kr_2 alleles) which makes some wheat genotype respond poorly with regard to pollen tube growth and embryo formation frequency as compared to other genotypes which lack this mechanism. Abnormal behavior of pollen tubes was studied in terms of coiling, twisting of pollen tubes, swelling of pollen tube tips, bursting of pollen tubes and growth in the wrong direction. A higher percentage of aberrations were recorded in crosses as compared to selfings. Number of embryos and haploids obtained showed a direct negative correlation with abnormal pollen tubes.

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