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Short Communication

Evaluation of rapid crossing techniques for haploid production in wheat by wheat × maize crosses

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The wheat × maize system of producing wheat haploids has distinct advantages over other systems (anther culture, bulbosum system) - such as low genotypic specificity, negligible gametoclonal variation and absence of albinism. Currently, one of the most labour-intensive procedures in wheat × maize system involves the emasculation and pollination steps. In the generally followed method for these crosses, glumes are kept intact to ensure better seed development in contrast to the conventional wheat × wheat crosses where the glumes are clipped to expose the ovaries [1]. Intact glumes need to be forced open for emasculation as well as pollination. Moreover, the pollination process has to be carried out floret by floret rather than for the whole ear in one step. In this paper we have evaluated different modifications in manual crossing procedure in order to cut down the time and labour involved.

The experiment was conducted at Punjab Agricultural University's Research Station at Keylong (Dist. Lahaul-Spiti, H.P.) during the wheat off-season of 2001. Three wheat F_1s *viz.*, PBW 343 × Sasia (G1), PBW 343 × UP 2441 (G2) and PBW 445 × Cettia (G3) were used in crosses with 'Udaipur Local' maize. The wheat F_1s were sown in three replications in split plot design with genotype as main plot (six rows, 1m each) and crossing techniques as sub plot (2 rows, 1m each). The following emasculation-pollination (crossing) techniques were evaluated for conducting wheat x maize crosses:

In all the three techniques only primary florets of the spike were used for crosses. In case of CT-1, the pollinations were conducted at least a day prior to expected date of anthesis. The emasculation/clipping was performed 2-3 days before the anticipated date of anthesis. The crossed tillers in case of all the crossing techniques were administered injections of 2,4-D (100ppm) at 48hr, 72hr and 96 hrs after pollination. The 2,4-D was injected in the hollow of the culm of the uppermost internode. Spikes were harvested 18-20 days after pollination. Selfed seeds were sorted out on the basis the of presence of endosperm and embryo-carrying seeds were identified using the method of Bains *et al.* [2]. Embryos were cultured on Murashige and Skoog (MS) [3] based regeneration medium having MS basal salts + BAP (0.25mg/1) + Kinetin (0.25 mg/1) + Sucrose (3%) + Gelrite (0.2%). The observations recorded were: i) Crossed seed formation frequency (Percentage of florets carrying crossed seeds). ii) Embryo formation frequency (Percentage of crossed seeds carrying haploid embryos). iii) Plant regeneration frequency (Percentage of haploid embryos showing plant regeneration).

The analysis of variance revealed that the genotypes and crossing techniques were significant sources of variation for crossed seed formation frequency (Table 1). However, they did not contribute significantly in case of other efficacy parameters *viz.*, embryo formation frequency and plant regeneration frequency. Absence of significant variation due to crossing technique for the two critical efficiency parameters indicates the scope of rapid crossing techniques. Absence of significant interaction variance indicated that effectiveness of crossing techniques was not genotype specific.

The crossing techniques taken up in this study represent different levels of ease of crossing with CT-3, the standard technique, being most cumbersome. CT-1 represents the most rapid technique, which bypasses emasculation as well as the lengthy pollination procedure. It exploits the small time lag between ovary receptivity and anthesis in wheat. It also has as its basis the unambiguous distinction that can be made between crossed and selfed seeds in wheat × maize crosses.

Crossing techniques	Clipping of glumes	Emasculation	Pollination
Unemasculated - clipped florets (CT-1)	Done	Not done	Pollen broadcast on the spike
Emasculated-clipped florets (CT-2)	Done	Done	Pollen broadcast on the spike
Emasculated-unclipped florets (CT-3)	Not done	Done	Individual florets pollinated

Table 1. Analysis of variance with respect to different efficacy parameters for wheat × maize crosses

Source	df	Mean Squares		
		Cross-seed formation frequency (%)	Embryo formation frequency (%)	Plant regeneration frequency (%)
Replication	2	63.27	12.23	52.75*
Genotype	2	511.54*	63.62	6.76
Error (a)	4	41.98	11.54	3.11
Crossing techniques	2	441.02*	13.34	57.93
Interaction	4	45.49	7.19	8.92
Error (b)	12	89.14	3.84	18.67

Number of florets pollinated for various crossing techniques and genotypes, and the frequencies of crossed seed formation, embryo formation and plant regeneration in individual treatments is given in Table 2. With regard to crossed seed formation, the frequency obtained in case of CT-1 was significantly lower than the other two crossing techniques. This was probably due to higher percentage of setting in CT-1 as compared to CT-2 and CT-3 (in which emasculation prevents selfing). The slightly lower crossed seed formation frequency can, however, be more than compensated on account of a much larger number of spikes that can be handled by this method. In terms of embryo formation and plant regeneration frequency the three techniques were statistically at par, thereby indicating no impairment of efficacy on account of rapid techniques. Further, the difference in timing of pollination in CT-1 and CT-2 did not result in any significant variation in embryo formation frequency.

Regarding the estimation of saving in time in case of rapid methods, the number of spikes (per man-hour) that can be handled for clipping, emasculation and pollination with different techniques were recorded. Time taken for various crossing technique steps was estimated on the basis of three repeats of one hour each with each procedure (Table 3). The rapid technique (CT-1) was seen to be approximately four times faster than the standard technique, CT-3. The use of unemasculated spikes for wheat × maize crosses had also been recommended by Matzk and Mahn [4]. With respect to second modification i.e., use of clipped florets, our own experience in the main season at Ludhiana has shown its adverse effects on crossed seed size as well as embryo formation. The absence of detrimental effects of clipping at Keylong may be due to more conducive grain filling conditions at that location.

Table 2. Number of wheat florets pollinated and frequency of crossed seed formation, embryo formation and plant regeneration with different crossing techniques and genotypes

Crossing techniques	Genotype	Florets pollinated	Cross-seed formation frequency (%)	Embryo formation frequency (%)	Plant formation frequency (%)
Un-emasculated clipped florets (CT-1)	G1	1436	60.53	12.47	17.58
	G2	1014	57.53	7.19	21.43
	G3	1096	74.69	12.48	22.69
Mean			**64.25**	**10.71**	**20.57**
Emasculated clipped florets (CT-2)	G1	836	67.88	15.70	18.06
	G2	934	72.64	10.02	17.17
	G3	1022	87.21	10.33	17.02
Mean			**75.91**	**11.81**	**17.42**
Emasculated unclipped florets (CT-3)	G1	1114	75.62	12.42	16.06
	G2	1010	74.26	7.32	14.06
	G3	1480	81.16	8.87	17.31
Mean			**77.01**	**9.54**	**15.81**
			CD = 9.7	CD = N.S.	CD = N.S.

Table 3. Average time taken per spike to perform various crossing steps *viz.* clipping, emasculation and pollination

Crossing techniques	Clipping (min.)	Emascula-tion (min.)	Pollination (min.)	Total (min.)
CT-1	1.54	NIL	0.48	2.02
CT-2	1.49	3.43	0.52	5.44
CT-3	NIL	4.00	4.21	8.21

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