Pak J Sci Ind Res 2000 43 (4) 258-261

PRODUCTION OF HAPLOID PLANTS IN CROSSES BETWEEN F_1-GENERATION OF WHEAT WITH MAIZE

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(Received 18 June 1995; accepted 14 February 2000)

Eight crosses in F_1-generation of bread wheat were crossed with maize variety C-17. Fertilization frequency ranged from 10-21.9% of pollinated florets. A total of 50 embryos were recovered from 492 florets and 15 embryos germinated to have haploid seedlings.

Key words: Wheat, Maize, Haploid plants.

Introduction

Interspecific and intergeneric crosses between crop plants or between crop plants and their wild relatives are commonly known as "wide crosses". Until recently closely related species were used as parents in wide crossing. However recent work on crossing crop plants with wild relatives have shown encouraging results. Wheat-maize crossing i.e., crossing two sub-families of Gramineae, the Pooideae and Panicoideae (Hutchinson 1959) has become an excellent example in wide hybridization. It has provided a new system for wheat haploid production and also offers interesting possibilities for gene transfer. Fertilization and early seed development in wheat-maize crosses has been reported (Laurie and Bennett 1987, 1989, 1990). Observations on zygote confirm the hybrid origin of embryos, but these hybrids are cytologically highly unstable. Maize chromosomes get deleted from developing embryos and endosperm as they fail to move to the spindle poles during cell division (Barclay 1975; Sitch 1984). Loss of maize chromosomes during early cell division gives haploid embryos of wheat and haploid plantlets can be recovered through embryo rescue techniques (Inagaki and Tahir 1990; Riera-Lizarazy and Mujeeb 1990). This system has certain advantages over other methods because maize is a prolific pollen producer and is relatively insensitive to the action of dominant alleles at the wheat crossability loci Kr_1 and Kr_2 (Laurie and Bennett 1987). Thus it has a potential to produce haploids from many wheat cultivars which carry Kr_1 and/or Kr_2. Keeping in view the advantages of wheat-maize crossing system, crosses were made between F_1 hybrids of wheat and maize. Previous work on wheat-maize crosses has been carried out in controlled environment but the present experiment was performed in natural conditions to study the variations in fertilization frequency and production of haploid seedlings of wheat.

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Materials and Methods

Plant material and crossing procedure. The material used in wheat-maize crossing system consisted of six F_1's of bread wheat, *viz.*, Oasis/HD-2285, Ananda/V-87094, Ananda / Oasis, HD2570 // LU26 / HD2179, BL1020 // RL6043 / 4NAC and BL1022/Chakwal86. These hybrids were sown on November 22, 1990 at the experimental field of Wheat Research Institute, Faisalabad. Open pollinated maize variety C-17 was used as the male parent. The spikes of wheat plants were emasculated one to three days before anthesis and central florets from each basal and upper spikelets were removed, keeping only central, healthy spikelets for pollination (Fig 1). Florets were pollinated with freshly collected maize pollen when ovaries were in bifurcated position.

Cytological analysis. For determining fertilization frequencies, two spikes from each cross were analyzed. Two days after pollination, spikes were removed from plants and ovaries were fixed in 3:1 ethanol/acetic acid solution. Light microscopy of each embryo sac content was done according to Laurie and Bennett (1987). Each embryo sac was scored for the presence or absence of an embryo or endosperm.

Embryo rescue technique. Three spikes from each cross were used for embryo rescue study. They were pollinated with freshly collected maize pollen and just after pollination, 100 ppm 2, 4-D was injected into the upper internode of tiller. An apical spray of 75 ppm of gibberalic acid was applied twenty-four hours after pollination. The spikes were then rebagged and left to develop for two to three weeks. Later, "seeds" were removed and the surface was sterilized by dipping in 70% ethanol and then immersing in a 2% sodium hypochlorite solution for 5 minutes. They were then rinsed in sterile distilled water and dissected on sterile filter paper with the aid of stereoscope. Immature embryos (Fig 2) were then recovered and transferred to Gamborg's B5 medium supplemented with $20g1^{-1}$ of sucrose and $9g1^{-1}$ of Difco bacto-

agar. Embryos were incubated in dark at 18-20°C until they germinated and produced a coleoptile 1 to 2 cm in length. Thereafter, they were transferred to incubator at the same temperature under light condition. When seedlings have grown to top of culture tubes, they were transferred to soil. Chromosome number of seedlings were confirmed from root-tips as described by Laurie and Bennett (1988a).

Results and Discussion

Fertilization frequencies. Wheat-maize crosses have previously been made in controlled environment of glass houses. This experiment was performed in field conditions to determine fertilization frequency and production of haploid seedlings for practical utilization of this technique to wheat breeding programme. Fertilization, early embryo and endosperm development in wheat was studied in ovaries fixed after 48 h of pollination with maize. Fertilization frequency of florets ranged from 10-21.9% with mean value of 14.9% (Table 1). Laurie and Bennett (1990) observed 29.7-30% of fertilization frequency in an experiment with wheat x maize crosses but in the present experiment, the reduction may be due to fluctuation in ambient temperature, sunshine and humidity (Sitch 1984). Out of 352 ovaries, only 27 (7.7%) had an embryo (Fig 3a) and 9 (2.6%) had only endosperm while 16 (4.5%) had both an embryo and an endosperm (Fig 3b). Thus the frequency of embryo development was higher than the frequency of endosperm development.

All the embryos were examined and their hybrid origin was confirmed from cytological study of zygotes at metaphase stage. Embryos with micronuclei indicated elimination of maize chromosomes (Laurie and Bennett 1986, 1988a). The poor embryo survival may be due to absence or abnormal development of endosperm which is the characteristic of all hexaploid wheat-maize crosses (Laurie and Bennett 1990).

The mechanism of elimination is same as in cytologically unstable interspecific hybrids (Finch and Bennett 1983). Endosperm development was highly abnormal and the observations suggest that this endosperm is the result of fusion of maize sperm nucleus with wheat polar nuclei (Table 1). The present results were similar to the previous studies of wheat-maize crosses (Laurie and Bennett 1988b, 1989).

Production of haploid wheat plants. Three spikes of each F_1 hybrid were treated with 100ppm 2,4-D solution and 75 ppm GA3 solution after pollination with maize. Florets were dissected out after 20-25 days of pollination and it was observed that ovaries had swollen to about half to three quarter size of normal seed of comparable age (Fig 4). 2,4-D enabled the embryos to survive and total of 50 embryos were recovered from 492 florets examined (Table 2). For each F_1-hybrid of wheat the number of embryos obtained, 20-25 days after pollination, were compared to an expected value calculated from the frequency of embryos present 48 h after pollination (Table 1). The data in Table 2 suggested that improvement in embryo rescue technique would increase the recovery of the expected number of embryos. Out of 50 embryos obtained only 15 germinated to produce healthy seedlings (Table 2) and rest of them failed to establish themselves as seedlings or produced roots only. Seedlings were obtained from all genotypes and their root-tip analysis confirmed that all seedlings were haploid (Fig 5). Recovery of haploid wheat plant from each genotype suggested that such crosses may be an attractive alternative to wheat-bulbosum crosses because maize is a prolific pollen producer and is relatively insensitive to dominant alleles at the wheat crossability loci Kr_1 and Kr_2 (Finch and Bennett 1983; Kisana 1991). Thus the present study shows that the results are encouraging and the process has the potential to produce haploid plants. There is a need to improve protocol by manipulating medium composition and hormone treatment so that number of embryos

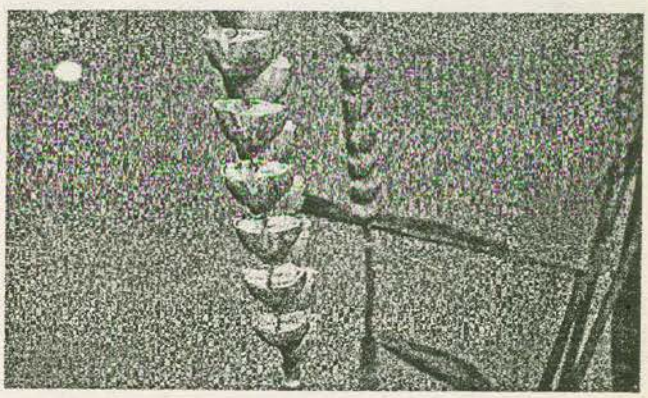

Fig 1. Hand emasculated wheat spike.

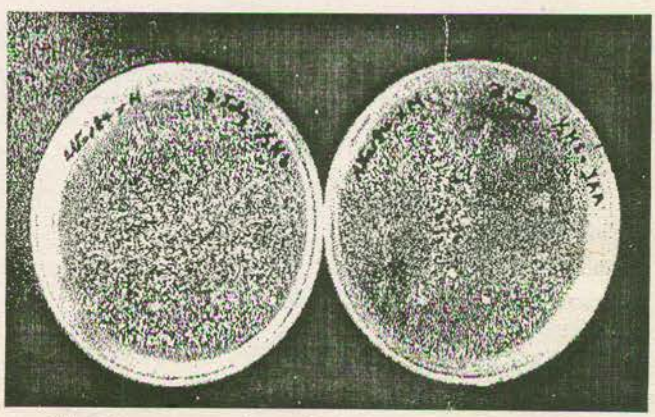

Fig 2. Immature embryo of wheat x maize cross.

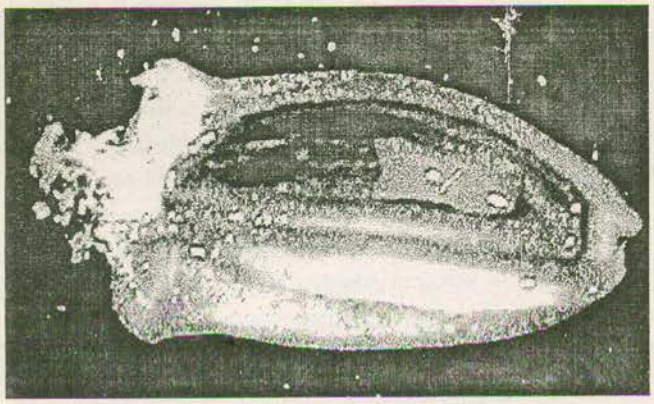

Fig 3a. Wheat x maize crossed seed with only embryo.

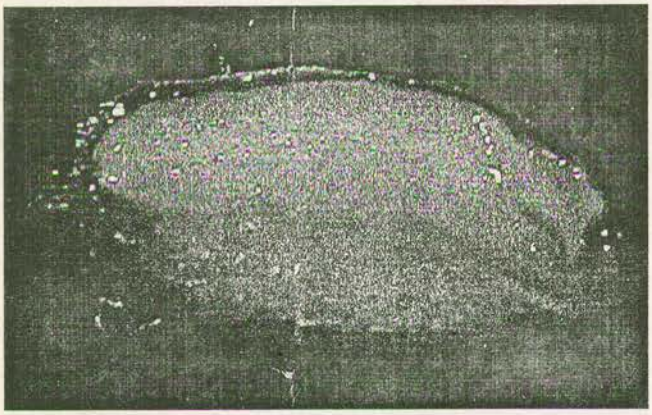

Fig 3b. Wheat x maize crossed seed with embryo and endosperm.

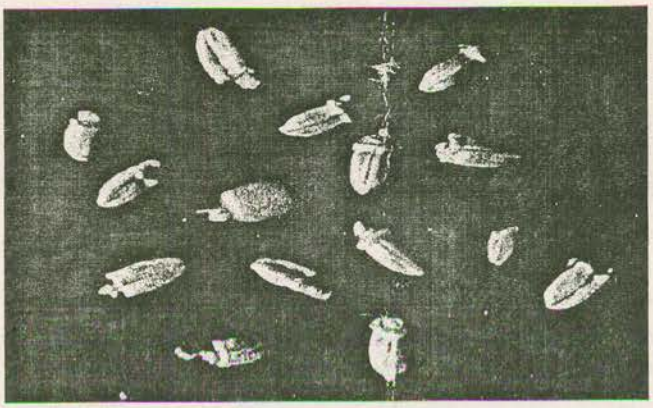

Fig 4. Comparison of normal and wheat x maize crossed seed (Central bold seed is normal while others are crossed.

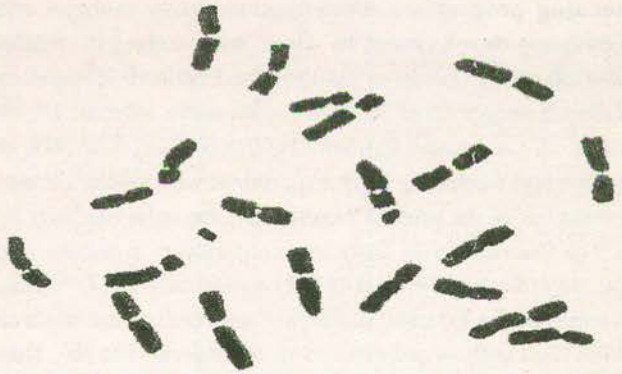

Fig 5. Haploid chromosome count in wheat x maize crossed embryo (In = 21).

might be increased for large number of seed setting in wheat-maize crossing system (Kisana 1991).

Potential application of wheat-maize crosses. Haploids are important to plant breeding programmes because ever increasing demand of food and geometric increase in population has put lot of stress on plant breeders to enhance yield at each production level; For this purpose plant breeders require the means to sort out best crosses as early as possible. This technique is directly applicable to breeding programme in the process of releasing new cultivars because it offers a mean for repidly advancing selected breeding lines to complete homozygosity and of increasing the efficiency of selection (Snape and Simpson 1981).

In addition, a smaller population size is required to obtain

Table 1

Fertilization frequencies in F_1 hybrids of wheat pollinated with maize

Female parents	No.of ovaries	Embryo only	Endosperm only	Embryo & endosperm	Total fertilization frequency		Mean %
					N	%	
Oasis/HD2285	40	2	0	2	4	10.00	
Ananda/V-87094	50	3	1	2	6	12.00	
Ananda/Oasis	75	6	2	3	11	14.70	
HD2570//LU26/HD2179	75	8	2	4	14	18.60	14.9
BL1020//RL6043/4*NAC	80	5	2	3	10	12.50	
BL1022/Chakwal-86	32	3	2	2	7	21.90	
Total	352	27	9	16	52	89.70	

Table 2
Production of haploid seedlings from wheat-maize crosses

No. of florets	No. of embryos obtained	No. of embryos expected**	No. of seedlings
65(3)*	3	7	1
63(3)	4	6	2
60(3)	5	7	1
60(3)	6	10	2
58(3)	10	6	4
66(3)	5	10	2
60(3) BTN89/Perwaz-94	6	—***	2
60(3) BTN89/V-88220	11	—	1
(Total) 492	50		15

* The number of spikes is given in parenthesis;
** Calculated from frequency of embryo formation is given in Table 1;
*** Not studied for fertilization frequency.

desirable genotypes in haploid breeding as compared to conventional segregating population (Nie 1963). Production of haploids is particularly attractive for genetic analysis of characters which can be assessed easily on single plant basis (Snape and Simpson 1981). However, these potential benefits can be successfully manipulated by increasing frequency of embryo formation since it is desirable to recover as many embryos as possible for developing into the plants.

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