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Effects of Different Ploidy Levels of Wheat (Hexaploids and Tetraploids) on Seed Set, Embryo Formation and Haploid Production in Wheat × Maize Crosses

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Abstract: The results of crosses between the best pollen sources and various female wheat parents of different ploidy levels (hexaploid and tetraploid) indicated that hexaploid wheat perform better than tetraploid in crosses with maize. However, successful production of embryos at a relatively high frequency could be achieved in tetraploid wheats with FSH-399. The highest frequency of embryo formation (59.0%) was produced from a cross between the hexaploid wheat (F1 Cross-5) and maize hybrid FSH-399.

Key words: Haploids, wheat x maize crossing, ploidy levels, *Triticum aestivum*, *Triticum durum*

INTRODUCTION

The maize system provides an efficient tool for achieving homozygosity in wheat crosses and reducing the time for the development of new cultivars.

The first report on the crossing between relatives of bread wheat and maize was published by O'Donoughue and Bennett[1]. They conducted a wide hybridization programme involving pollination of 16 *Aegilops* and *Triticum* species of varying ploidy levels with maize to determine the frequency of embryo and endosperm development. The results indicated that the frequency of fertilization varied greatly between species. Various earlier scientists working with haplo-breeding in tetraploids reported poor response of *Triticum turgidum* (var. durum) to haploid production[2,3]. Another attempt at producing durum haploid plants from the maize system was made by Sarrafi *et al.*[4].

The objective of the present study was to determine if responsive female parents exist at both the tetraploid and hexaploid levels of wheat (4x and 6x).

MATERIALS AND METHODS

The studies were conducted in the fields and laboratories of Agricultural Biotechnology Research Institute, Faisalabad during, 2002-03. The materials comprising following twelve F_1 crosses, 8 of hexaploid wheats (*Triticum aestivum*, 2n = 6x = 42, AABBDD) and 4 of tetraploid wheats (*T. durum*, 2n = 4x = 28, AABB) were used in making crosses with three maize (*Zea mays* L.) genotypes namely, FSH-399, Composite-20 and Akbar.

Triticum aestivum

Cross-1:	Punjab-96/Karwan-2
Cross-2:	Punjab-96/Crow
Cross-3:	Inqilab-91/Kentana
Cross-4:	MH-97/Tanager
Cross-5:	Auqab-2000/Fasan
Cross-6:	V-87094/Vulture
Cross-7:	Kohistan-97/Oasis
Cross-8:	Inqilab-91/Chenab-2000

Triticum durum

Cross-19:	Altar-84/Chen
Cross-20:	Durum-97/Chen
Cross-21:	Altar-84/Cham-4
Cross-22:	Durum-97/Cham-4

Wheat tillers were detached from plants 2-3 days before expected anthesis, kept in distilled water, hand emasculated by clipping anthers with the help of fine pointed forceps and covered with butter paper bags. Maize pollen was collected by gently shaking the pollen producing tassel over a craft paper bag or petri dish. The collected pollen was transferred to the wheat stigmas within 10-15 min of pollen release, with the aid of a small camel hair brush when the ovaries were receptive. After pollination wheat tillers were transferred into a solution, containing, 8 ml L^{-1} sulfurous acid (6% SO_2), 40 g L^{-1} sucrose and 100 mg 2,4-D (for first 3 days only) following the method by Inagaki[5]. Tillers were kept at 21°C under lights. Fresh culture solution was added to the containers every 2-3 days. Twelve to sixteen days after pollination each floret was pealed off the spike, the palea and lemma removed and the intact caryopsis placed in a petri dish. Thecaryopsises were sterilized with a solution having 10-20 ml L^{-1} sodium hypochlorite (1-2%) and few

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drops L^{-1} of tween-20 for ten minutes followed by 3-4 rinsings with autoclaved distilled water. Embryos were aseptically excised and cultured on ½ MS nutrient solution supplemented with 30 g L^{-1} sucrose and 2 g L^{-1} Gel-gro. The immature embryos were incubated in a culture cabinet. The first phase of culturing for one to two weeks was done in the dark at 22±2°C. When properly differentiated, the embryos were transferred to a growth room at 25±2°C with a 16 h light regime. The regenerated plantlets were then moved to the controlled greenhouse.

RESULTS

The frequencies of seed set, embryo formation and haploid plant production resulting from crossing the 12 wheat F_1 crosses with different maize genotypes are presented in Table 1 and 2. Statistical analysis of the data indicated that both the male and female parents affected the seed set. Considerable differences ($p<0.01$) in embryo formation and haploid production were observed among the ploidy levels (Table 3). Maize genotype FSH-399 resulted in the highest percentage of embryo formation followed by Composite-20 and Akbar (Table 4).

The percentage of florets setting seed was high in hexaploid wheats compared to tetraploid wheats. The range of florets setting seed was 82.0-99.5% among the hexaploid wheats with a mean of 90.3% and from 60.3-78.1% in the tetraploid wheats with a mean of 71.4% (Table 1 and 2).

Significant differences ($p<0.01$) were found in embryo formation between hexaploid wheat × maize crosses as well as tetraploid wheats × maize crosses. All tetraploid wheats produced embryos (Table 2). The mean amount of embryo formation in hexaploid wheats was significantly higher than that in tetraploid wheats (Fig. 2 and Table 4). It ranged from 13.1-59.0% with a mean of 29.8% in crosses involving hexaploid wheats and from 3.5-25.0%

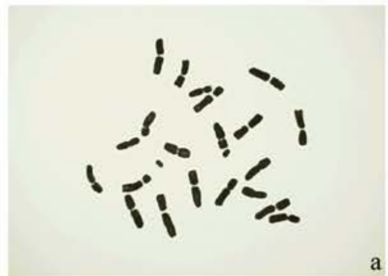

Fig. 1a and b: Chromosome counting of haploids of hexaploid (a) tetraploid (b) wheats

Table 1: Varietal differences for percentage of seed set, embryo formation and haploid production in hexaploid wheat × maize crosses

Maize genotypes	Hexaploid wheat	No. of pollinated florets	Seeds as % of pollinated florets	Embryos as % of pollinated florets	Embryos as % of seeds	Haploids as % of embryos
FSH-399	Cross-1	346	90.5	29.8	32.9	52.4
	Cross-2	408	91.4	35.8	39.1	56.2
	Cross-3	388	85.6	33.3	38.8	53.5
	Cross-4	432	99.5	47.9	48.1	58.9
	Cross-5	378	92.3	59.0	63.9	53.8
	Cross-6	520	90.6	24.2	26.8	55.6
	Cross-7	476	98.5	45.8	46.5	54.1
	Cross-8	422	89.3	19.2	21.5	55.6
Total/Average		3370	92.4	36.6	39.6	55.2
Composite-20	Cross-1	230	85.2	20.4	24.0	61.7
	Cross-2	212	90.6	25.5	28.1	59.3
	Cross-3	244	82.0	23.0	28.0	58.9
	Cross-4	208	95.7	27.9	29.2	62.1
	Cross-5	266	83.1	31.6	38.0	59.5
	Cross-6	314	89.2	19.8	22.1	58.1
	Cross-7	240	92.1	30.4	33.0	58.9
	Cross-8	290	86.9	15.9	18.3	56.5
Total/Average		2004	87.9	24.0	27.3	59.4
Akbar	Cross-1	212	89.2	20.8	23.3	59.1
	Cross-2	198	89.9	24.2	27.0	62.5
	Cross-3	184	84.8	24.5	28.9	55.6
	Cross-4	284	90.1	24.7	27.3	62.7
	Cross-5	208	89.4	29.8	33.3	59.7
	Cross-6	236	92.0	22.0	24.0	59.6
	Cross-7	242	90.1	26.7	29.8	61.5
	Cross-8	206	86.9	13.1	15.1	63.0
Total/Average		1770	89.2	23.3	26.2	60.5

Table 2: Varietal differences for percentage of seed set, embryo formation and haploid production in tetraploid wheat × maize crosses

Maize cultivars	Tetraploid wheat	No. of pollinated florets	Seeds as % of pollinated florets	Embryos as % of pollinated florets	Embryos as % of seeds	Haploids as % of embryos
FSH-399	Cross-19	468	75.0	25.0	33.3	37.6
	Cross-20	620	73.0	18.0	24.7	25.0
	Cross-21	542	73.0	13.6	18.6	21.6
	Cross-22	484	78.1	14.0	17.9	22.0
Total/Average		2114	74.6	17.5	23.5	27.8
Composite-20	Cross-19	242	71.9	9.5	13.2	30.4
	Cross-20	408	69.6	8.3	11.9	35.2
	Cross-21	194	60.3	6.7	11.1	23.0
	Cross-22	308	70.1	3.5	5.0	36.3
Total/Average		1152	68.7	7.0	10.2	32.1
Akbar	Cross-19	268	70.1	10.0	14.3	25.9
	Cross-20	210	68.1	7.6	11.1	31.2
	Cross-21	238	65.1	4.6	7.1	18.1
	Cross-22	244	66.8	4.5	6.7	18.1
Total/Average		960	67.6	6.8	10.0	24.6

Table 3: Analysis of variance (MS) of seed set, embryo formation and regeneration of haploid plantlets in crosses of eight hexaploid and four tetraploid wheats with three maize pollen sources

		MS		
Source	df	seed set (%)	Embryos/ florets (%)	Haploids/ embryos (%)
Maize (M)	2	347.1 **	3632.8 **	4486.5 **
Ploidy (P)	1	15978.9 **	1796.2 **	14867.8 **
M × P	2	93.8 ns	236.3 **	389.4 **
Error	525	37.7	59.5	71.5
Total	530			

ns = Non-significant, ** = $p<0.01$

Table 4: Means and SE for seed set, embryo formation and haploid production in crosses of eight hexaploid and four tetraploid wheats with three maize pollen sources

	Seed set		Embryo formation		Haploid production	
	Mean	SE	Mean	SE	Mean	SE
Maize						
FSH399	79.7	0.67	27.2	0.67	48.8	0.66
Composite-20	80.9	0.75	17.8	0.80	55.4	0.82
Akbar	81.6	0.76	17.5	0.80	55.6	0.82
Ploidy						
Hexaploid	90.3	0.37	29.8	0.50	57.1	0.48
Tetraploid	71.4	0.53	12.2	0.62	28.1	0.74

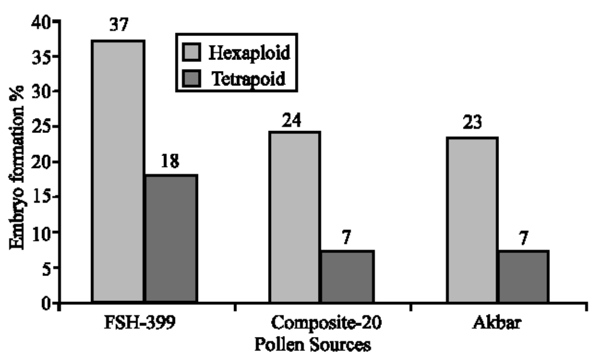

Fig. 2: Comparison of two ploidy levels of wheat parents on embryo formation as influenced by three maize hybrid pollen parents

with a mean of 12.2% in tetraploid wheats. The cross between FSH-399 × Cross-5 was the most successful with respect to embryo formation (59.0%). Among the tetraploids, Cross-19 produced the highest number of embryos when crossed with FSH-399 (25.0%).

Significant differences ($p<0.01$) in haploid production were found between the wheats of different ploidy levels. All the genotypes of both ploidy levels produced haploids (Fig. 1a and b). The haploid production was considerably higher among the crosses involving hexaploid wheat × maize than the crosses involving tetraploid wheat × maize (Table 4). This ranged from 52.4-63.0% for the hexaploid wheats and from 18.1-37.6% for the tetraploid wheats (Table 1 and 2).

DISCUSSION

In contrast to hexaploid wheat, little research has been conducted into the lower ploidy levels of wheat or its relatives for haploid production via the maize system. The investigations which had been conducted with tetraploid or diploid wheats in anther culture have shown that these species are recalcitrant because of the low frequency of embryo production and the high number of regenerated albino plants[6-8].

The results show an overall increase in the frequency of embryo formation with increasing ploidy levels. Hexaploid wheats produced significantly more embryos than tetraploid wheats. One of the main causes for this low frequency of embryo formation in tetraploid wheats is their low number of florets that produce seeds (Table 1 and 2).

Embryo viability was low in tetraploid wheat compared to hexaploid wheat because of the size of embryos. If the parents have different chromosome numbers, success is influenced by quantitative and qualitative factors. When the species to be crossed belongs to a different genus and has a completely different chromosome number and genome quality, the success of crossing depends only on qualitative factors[9].

The highest frequency of embryo formation (59.0%) was obtained in the cross Cross-5 × FSH-399. This is better than the frequencies obtained previously[10-14].

The frequency of haploid wheat embryos produced by the maize system is superior to the output from anther or microspore culture and the *bulbosum* method[1,15]. Successful production of embryos in tetraploid wheats with FSH-399 indicated that ploidy level is not a barrier to the production of haploid embryos using this technique. The results also indicated that the production of embryos in tetraploid wheats with maize can be achieved at relatively high frequencies compared to anther culture which was not successful because of the low frequency of embryoid formation and the high frequency of albino plant[6].

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