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SADAF- A POTENTIAL DONOR FOR ENHANCING FREQUENCY OF DOUBLED HAPLOIDS IN WHEAT × MAIZE CROSSING SYSTEM

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To identify the genotypic effects of parents for wheat × maize crossing system, five F_1 wheat genotypes were crossed with five maize cultivars. The data for haploid seed and embryo production in each cross was recorded and analyzed. The data revealed that maize genotypes have significant variable effects on haploid wheat seed and embryo production. The line × tester analysis proved that wheat and maize genotypes along with their interactions, affect haploid seed and embryo production in wheat × maize crossing system. In this experiment a maize variety named Sadaf showed best performance on the basis of high GCA value as compared to others. It was also discovered that high seed production phenomenon could not be associated with high haploid embryo formation in a wheat × maize crossing system. It was concluded that maize genotypes having great potential for wheat haploid production should be identified and utilized in this system to improve its efficiency as in this study Sadaf is recommended for wheat × maize crossing system.
Keywords: Haploid wheat, wheat × maize, genotypic effect, gene interaction, homozygosity

INTRODUCTION

Doubled haploids (DH) are used for genetic studies like gene interaction, QTL mapping and cytogenetics. DH in plant breeding is the fastest method to attain complete homozygosity in a single generation from a heterogenous population (Mujeeb-Kazi, 2006ab; Akhtar *et al*., 2012). Production of haploid plants is the most important step for the development of DH. In wheat haploid plants can be produced through *in vitro* anther culture and wide hybridization (Khan *et al.,* 2012). But high genotype specificity, production of albino wheat plants and poor response of anthers to tissue culture media are main constraints in this technique, resulting in adoption of wide hybridization method. Haploids in wheat mediated through crosses between wheat × *hordeum bulbosum* were reported by Barclay (1975). But the presence of dominant Kr genes in most wheat genotypes causes incompatibility and hence reduces the haploid embryo formation. The wheat × maize crossing system is the most successful method of viable haploid production. The maize pollen hybridizes with wheat egg cell and produces hybrid zygote. The maize chromosomes are preferentially eliminated from hybrid zygote resulting in haploid (1n) wheat embryos after few mitotic divisions. First haploid embryo production in hexaploid wheat via wheat × maize crossing system was reported by Zenkteler and Nitzsche (1984). Their results were further confirmed by Laurie and Bennett (1986), through a systematic study.

The parental genotypic effect on haploid embryo production in wheat × maize cross system is still controversial phenomenon in literature. Studies indicate that haploid production is influenced by wheat and maize genotypes (Verma, 1999). Suenaga and Nakajima (1989) reported that maize genotypes influence the success of wheat × maize crossing system. But Suenaga (1994) found that wheat genotypes significantly affect the crossability. Laurie and Reymondie (1991) observed that in crosses of winter and spring wheat with F_1 hybrid of maize, significant differences for haploid embryo production were present between groups but absent within groups. Suenaga and Nakajima (1993) studied the effects of maize pollen on haploid wheat embryo production and observed differences in haploid embryo production by different maize genotypes on single wheat genotype. The genotypic specificity of the wheat × maize-mediated haploid production system is not up to the magnitude, as reported for other haploid production systems including anther culture (Kisana *et al.,* 1993; Bitsch *et al*., 1998) and wheat × *H. bulbosum* (Sitch and Snape, 1986). The genotypic specificity may reduce the efficiency of these systems to a level where they are not applicable to a breeding program. Ovary development and plant formation is affected by wheat genotype and embryo production influenced by maize genotypes. It is suggested that more responsive maize genotypes could be identified to ensure maximum production of haploid plants in this system. In the present study influence of both wheat and maize genotypes on haploid embryo production was studied by crossing 5 F_1wheat and maize genotypes in all possible combinations.

MATERIAL AND METHODS

This research was conducted at Agricultural Biotechnology Research Institute, AARI, Faisalabad and Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. Wheat lines were sown in the month of November in fields. The maize plants were grown in greenhouse under controlled conditions. When spikes partially emerged from flag leaf, the tillers were cut from the base and emasculated in lab. The premature florets at the base and top of the spikes were removed. After emasculation the detached tillers were kept in containers, half filled with distilled water. The spikes were covered with plastic bag to conserve moisture and avoid contamination and kept in growth room. Fresh maize pollens from each genotype were collected in separate perti dishes and dusted on receptive wheat stigma of emasculated tillers for pollination. After pollination tillers were kept in detached tiller culture media, containing 8 ml/L H_2SO_3, 30g/L sucrose and 100mg/L 2,4-D. After three days tillers were transferred to 2,4-D free media. The tillers were kept under 21°C temperature and 10,000 Lux light intensity for seed development. The culture solution was replaced with fresh solution regularly after three days to ensure continuous supply of nutrients for seed development. Generally 12-16 days after pollination the florets were detached from spike and collected in petri dishes. Crossed seeds were whitish in color, smaller in size and endosperm was absent. The seeds were sterilized with washing solution having 10-20ml/L sodium hypochlorite and 1-2 drops of tween twenty. Then seeds were washed 3-4 times with sterilized water. The seeds were dissected and immature haploid wheat embryos were placed in test tubes containing 10 mL ½ MS media at 21°C under 24 hours light regime (Mujeeb-Kazi, 2006b). In this media the embryos were developed into plantlets. When plantlets gained height to the opening of the tube approximately in 30-45 days, they were transferred to steam-sterilized soil mix of commercial potting soil/perlite (3:1). When plants reached 3-5 tiller stage they were uprooted and washed with tap water. The plants roots and crown were dipped in 1% colchicine solution with 8ml/L Dimethyl Sulfoxide (DMSO) and continuous air supplied with air pump. The Analysis of Line × Tester was performed using Minitab ® 16 version.

RESULTS

The frequencies of seed setting and haploid embryo production in crosses of 5 female wheat and 5 male maize genotypes is presented in Table 1. Statistical analysis displayed that both wheat and maize genotypes had significant effect on seed setting (Table 2). Moreover, the male parents showed significant differences for embryo development. The highest seed setting percentage in the wheat × maize crosses was observed by Neelum followed by Agate-85, Agate-2000, Sultan and Sadaf. But the male parent Sadaf gave better performance for haploid embryos production than other parents followed by Neelum, Sultan, Agate-2000 and Agate-85 (Table 1). The results also indicated that in all crosses the average seed production by Neelum was greatest and in case of Sadaf it was least. However, Sadaf produced maximum embryos in all crosses. It could be concluded that there was no direct relation in seed formation and haploid embryo production in wheat × maize crosses.

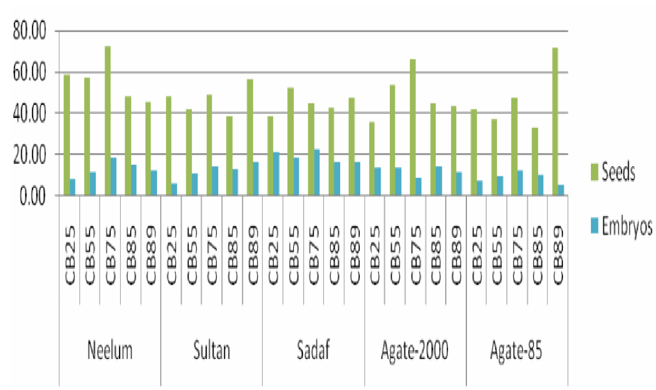

Figure 1. Frequency of seed and haploid embryo production by various wheat and maize parents.

The data showed a wheat genotype CB75 was positively significant for seed and embryo production with highest GCA value 7.24 and 2.188, respectively (Table 3).

DISCUSSION

In other haploid wheat embryo production techniques like anther culture and *bulbosum* technique genotype specificity is the main hindrance for successful haploid embryo production (Khan and Ahmad, 2011). The current study clearly indicated that maize genotypes have significant effects on haploid seed and embryo production in wheat × maize crosses. Although different experiments revealed that doubled haploid production with wheat × maize system is influenced by wheat genotypes. A substantial report also indicated that both wheat and maize genotypes posed striking differences on polyhaploid embryo production to plantlet regeneration in this crossing system. Suenaga *et al.* (1994) produced embryos by using wheat genotypes originated from diverse geographical regions and different maize varieties/lines. They observed that the efficiency of embryo formation varied between 0.9 to 35.8%. But this specificity does not limit the production of haploid wheat embryos as compared to other techniques (Bitsch *et al.*,

Table 1. Seed setting and embryo formation in crosses of wheat × maize

Male Parent		Seed Set %	Embryo Set %	Female Parent	No. of crosses	No. of seeds produced	% of seeds produced	No. of embryo formation	% of embryo formation (embryo/seed) embryo
Neelum				CN 25	228	134	58.77	11	8.21
				CN 55	191	109	57.07	13	11.93
	Maximum	72.58	18.33	CN 75	248	180	72.58	33	18.33
	Minimum	45.64	8.21	CN 85	275	132	48.00	20	15.15
	Average	56.41	13.20	CN 89	195	89	45.64	11	12.36
Sultan				CN 25	260	126	48.46	8	6.35
				CN 55	236	99	41.95	11	11.11
	Maximum	56.67	16.34	CN 75	231	113	48.92	16	14.16
	Minimum	38.89	6.35	CN 85	234	91	38.89	12	13.19
	Average	46.98	12.23	CN 89	270	153	56.67	25	16.34
Sadaf				CN 25	243	94	38.68	20	21.28
				CN 55	282	147	52.13	27	18.37
	Maximum	52.13	22.94	CN 75	244	109	44.67	25	22.94
	Minimum	38.68	16.42	CN 85	311	134	43.09	22	16.42
	Average	45.17	19.13	CN 89	203	96	47.29	16	16.67
Agate-2000				CN 25	242	86	35.54	12	13.95
				CN 55	199	107	53.77	15	14.02
	Maximum	65.90	14.56	CN 75	261	172	65.90	16	9.30
	Minimum	35.54	9.30	CN 85	229	103	44.98	15	14.56
	Average	48.69	12.73	CN 89	215	93	43.26	11	11.83
Agate-85				CN 25	223	94	42.15	7	7.45
				CN 55	171	64	37.43	6	9.38
	Maximum	71.86	12.62	CN 75	216	103	47.69	13	12.62
	Minimum	32.95	5.59	CN 85	173	57	32.95	6	10.53
	Average	46.41	9.11	CN 89	199	143	71.86	8	5.59

% of seeds produced = (Seed/Crosses) * 100 % of embryo formation = (Embryos/Seeds) * 100

Table 2. Line × tester analysis for haploid seed and embryo formation in wheat × maize crosses

Sources	df	MS	
		Haploid seed formation	Embryo formation
Crosses	24	326.246**	57.717**
Wheat genotypes	4	518.953**	34.691**
Maize genotypes	4	307.287**	198.968**
Wheat × maize interaction	16	282.837	28.161
Error	48	10.476	0.574

*p ≤ o,05 and **p ≤ 0.01

1998). As in this case it varied from 5.59 to 22.94 %. The genotypes in this study were not too much diverse and selected from the local Pakistani origin as compared to Bitsch *et al.* (1998) who used maize genotypes from different geographical regions.

The investigations for effect of parental genotypes on haploid embryo have been conducted to assess the utility of this system. In most of the studies considerable varietal differences among wheat (Suenaga, 1994; Bitsch *et al.,* 1998) and maize genotypes (Suenaga *et al.,* 1991) were observed. Furthermore, the analysis also revealed that the genotypic interaction present between wheat and maize significantly influenced the recovery of haploid embryo formation and plant regeneration.

The results of the present study concordat well with the findings of Suenaga and Nakajima (1989) who reported a significant difference in the frequency of haploid embryo formation when one wheat genotype was pollinated with four maize genotypes. It appears that specific maize genotypes are more suitable than others for haploid wheat production (Suenaga and Nakajima*,* 1993). The influence of maize genotypes to crossing with various wheat genotypes was well visible when five F_1 wheat genotypes were crossed to five maize varieties. In this study the number of haploid wheat embryos varied from 5.59 to 22.94%. The genotypic influence of the maize was obvious in the case of the variety Agate-85. It not only yielded minimum number of seed setting and embryos in crosses with CN85 and CN 89, respectively but overall produced less number of haploid embryos in all crosses. Therefore, haploid embryo production rate in wheat via maize mediated system can be improved by selecting more responsive wheat or maize genotypes. Significantly higher number of haploid embryos in the combination CN75 × Sadaf and higher output of haploid embryos among all wheat genotypes were observed. With high GCA value 5.854, Sadaf strongly showed interactions existing between specific maize genotype and embryo production (Verma *et al.,* 1999).

Table 3. GCA effects of wheat and maize genotypes on frequencies of haploid seed formation and embryo production in wheat × maize crosses

Source d.f.		GCA	
	Wheat	**Seed**	**Embryo**
1	CB 25	-3.96	-1.833
2	CB 55	-0.29	-0.319
3	CB 75	7.24	2.188
4	CB 85	-7.22	0.688
5	CB 89	4.24	-0.724
	Maize		
1	Neelum	7.77	-0.085
2	Sultan	-1.76	-1.051
3	Sadaf	-3.63	5.854
4	Ag-2000	-0.09	-0.549
5	Ag-85	-2.29	-4.167

Wheat parent CN25 yielded high number of haploid embryos (21.28%) in cross with Sadaf. But the same female parent produced 6.35% embryos with Sultan. It clearly indicated that in case of wheat both the seed and embryo formation is influenced by female parent. Moreover, it was also observed that in case of male parent frequency of embryo formation was not related to seed formation. In specific cross male parent Neelum produced the highest number of seed (72.58%) in cross with CN75 but the maximum embryos were produced by Sadaf. Among male parents, Neelum showed maximum average seed production but Sadaf produced more haploid embryos as compared to other genotypes. Some maize genotypes can produce high number of caryopsis but it is not necessary to show high rate of embryo formation (Niroula and Thapa, 2009). These results indicate that response to haploid embryo formation in wheat depends on other factors than the way of production i.e., anther culture or interspecific cross. However, it is worthy to note that using interspecific crossing the variety Sadaf produced 110 embryos out of 1238 crosses. The high rate of green haploid plant production is one of the most important advantages of interspecific hybridization over the anther culture method in wheat. In barley, interspecific crossing gives superior results over anther culture especially in cases where genotype response to anther culture is poor (Kisana *et al.,* 1993). In short the most innovative finding of present study is that maize variety Sadaf proved superior over others for use in wheat × maize crossing system to make that doubled haploids with high frequency. As the variety Sadaf is well adapted in local environment, it is recommended to use Sadaf in doubled haploid programs being run in Pakistan.

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