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Comparison of Orchard-grass and Sweet Maize for Doubled Haploid Plant Production via Wide Hybridization in Bread Wheat

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A R T I C L E I N F O	A B S T R A C T
	In this study, the potential of haploid regeneration was investigated in hybridization of six bread wheat F_1 hybrids known response to another culture with orchard-grass (*Dactylis glomerata* L.) and sweet maize varieties (Baron, Challenger and Merit). A total of 150 wheat spikes were pollinated with orchard grass and sweet maize and 2730 pseudo-seeds were produced. Although the high rate of developed pseudo-seeds was developed from bread wheat F_1 hybrids × orchard-grass, no embryos were produced. Developed pseudo-seeds (2057 number) of bread wheat × sweet maize produced 53 haploid embryos and only 8 of them were regenerated. Developed green plantlets were vernalized and applied colchicine and only four of them produced fertile seeds. The highest rate (5.9) of haploid embryo formation within wheat genotypes was determined in DH20 × Kate A-1. Although the highest haploid embryo formation was observed in Challenger with 3.5% among sweet maize genotypes, it had no effect on plant regeneration. Also, the mixture of pollen of sweet maize varieties increased haploid plant regeneration. It has been observed that some F_1 hybrids such as DH20 × Kate A-1 and DH6 × Altay 2000 with low anther response gave better results in terms of haploid embryo formation and regeneration. The means of fertile spike percentages and number of seeds per fertile spike were 26.75% and 9.83, respectively in developed green plants. As a result, bread wheat × sweet maize hybridization will be a good alternative to obtain a homozygous line in a short time in bread wheat genotypes with low anther response.

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Introduction

In wheat, which is vital for human nutrition, the limit of genetic variability has been approached to solve yield and quality problems. Accelerated breeding technologies are required for establishing wide variability in a short time, improve the efficiency of the selection, and quicken the occurring of new varieties. Double haploid technology is widely used for these aims. Producing numerous genotypes, economically, is related to use haploids in a wheat breeding program effectively. The most important advantage of using haploids is to offer the possibility of obtaining complete homozygosity.

Haploid plants in wheat can be obtained in artificial nutrient media by using anther and microspore cultures and wide hybridization of wheat with maize, cogongrass, etc. (Dang et al., 2011; Prasanna et al., 2012). Although anther and microspore culture are more widely used because they are easily applied methods, the root system

of the regenerant plant produced with a wide hybridization is healthier, and no albino plant formation is observed, which makes this method advantageous (Niu et al., 2014). Wheat × maize wide hybridization requires intensive workload; however, many researchers suggest that it is the most reliable and practical method in wheat breeding (Campell et al., 2000; Garcia-Llamas et al., 2004).

Growth conditions, pollen sources, plant growing methods, and wheat genotypes are important factors for producing doubled haploid plants, as well as the types and concentrations of the chemicals used (Knox et al., 2000). In previous studies, the effects of wheat genotype (Ltifi et al., 2019), pollen sources (Ding et al., 2019), emasculation methods (Patial et al., 2019) and hormone applications (Warchoł et al., 2016) on haploid plant regeneration of wheat were investigated.

In addition to maize, *Pennisetum glaucum* L. (Inagaki and Mujeeb-Kazi, 1995), sorghum (Maluszynski et al., 2003) and *Imperata cylindrica* (L.) Raeusch. (Chaudhary et al., 2005) species have been used to obtain haploid plants by wide hybridization with wheat. Zenkteler and Nitzsche (1984) reported that wheat, barley and rye were tested for haploid embryo formation with 15 species of Poaceae and Panicoideae and embryo formation can be obtained with *Agropyron repens* (L.) P. Beauv., *Alopecurus agrestis* L., *Dactylis glomerata* L., *Pennisetum americanum* L. and *Zea mays* L. However, it is important to investigate undiscovered pollen sources that will provide high haploid regeneration in different wheat genotypes and hybrids.

In this study, the potential of haploid plant regeneration was investigated by crossing six bread wheat F_1 hybrids, response to another culture are known, with sweet maize (*Z. mays* var. *saccharata*) and orchard-grass (*D. glomerata*).

Materials and Methods

In the study, six bread wheat F_1 hybrids known anther culture response were used and their features were shown in Table 1. As an inducer, the population of orchardgrass (*D. glomerata*) which is distributed in Campus of Agriculture Faculty, Eskişehir Osmangazi University, and sweet maize (*Z. mays* var *saccharata*) varieties [(Baron (B), Challenger (C) and Merit (M)] and mixture pollen of maize (MP) were used.

After wheat seedlings were vernalized at 4°C for 5 weeks, they were grown in pots containing soil, peat and vermiculite (3:2:1). Maize seeds were sown periodically every week at four times, and the flowering period of the wheat plant was provided to coincide with maize. Wheat spikes that slightly emerged from flag leaves were emasculated, and then these spikes were covered with isolation envelopes. Emasculated spikes were pollinated with inducer plants within 1-3 days. 2.4-D solution (213.05 mg/l, pH = 10.36) was applied within 24-48 hours following hybridization to support healthy embryo formation as described by Niu et al. (2014).

Spikes were collected at 16-19 days after fertilization and the pseudo-seeds were removed from the spikes. Picked pseudo-seeds were sterilized in 70% alcohol for 1 minute. Then, they were kept in 20% bleach (Domestos, 4.26% sodium hypochlorite) for 15 minutes and rinsed with sterile distilled water at least 3 times. The haploid embryos were carefully removed from pseudo-seeds under a stereo microscope and cultured on MS medium (Murashige and Skoog, 1962) containing 5% sugar and, 0.7% agar and were kept in the dark condition at 22±0.5°C for 1-2 weeks. Germinated embryos were

transferred in ½ MS media containing 3% sugar and 0.7% agar and kept for 2 weeks under the photoperiod of 16/8 (light/dark) at 22±0.5°C. When plants growing from haploid embryos reached to 5-6 cm, they were transferred to pots containing peat and vermiculite (3:1). Haploid plants with 2-3 tillers were removed without damaging their roots and colchicine doubling solution [colchicine (0.45 g/l) + DMSO (20 ml/l) + GA_3 (100 mg/l) + Tween 80 (0.3 ml/l), pH = 5.5] was applied (Niu et al., 2014). These treated plants were rinsed under tap water overnight, and then they were planted to pots again and transferred to the climate cabinet at 18 ±0.5°C and 75% humidity.

Chromosome observations were performed by modifying the method as described by Arslan et al. (2012). First, root tips of haploid plants were kept in alpha-monobromonaftalin solution for 3 hours and then fixed in glacial acetic acid for 30 minutes at room temperature. The fixed root tips were hydrolysed in 1N HCL for 12 minutes at 60°C. Then, root tips were left to be stained for 1 - 1.5 hours in 1 % aceto-orsein solution.

The data of individual factors were given with standard error and interaction between wheat and maize genotypes was measured by the chi-squared test ($\chi2$) for developed pseudo-seeds, embryo, and plantlets.

Results and Discussion

The successful application of wide hybridization in wheat breeding programs is dependent on ability to form pseudo-seeds, embryo and plant regeneration of the genotypes. In this study, a total of 2730 pseudo-seeds were obtained from pollinated 150 wheat spikes, and 53 of them (1.94%) generated haploid embryos. Only 8 (0.29%) of the haploid embryos were regenerated, and 4 (0.14%) of them were improved seed set.

The effect of wheat F_1 hybrids on developed pseudo-seeds, embryo formation, and plant regeneration indicated differences (Table 2). The highest number of developed pseudo-seeds, embryo formation and plant regeneration were recorded in DH6 × Altay 2000, DH20 × Kate A-1 and DH21 × Kate A-1, respectively. High numbers of developed pseudo-seeds and embryo-forming wheat F_1 hybrids did not produce a high number of green plants. It is still a controversial issue of whether haploid embryo formation was affected by wheat and maize genotypes. Some researchers observed embryo percentage affected only by maize cultivars, not wheat genotypes, whereas numerous other studies proved wheat genotypes distinctly affect the production of embryos per florets (Filomena Martins-Lopes et al., 2001; Niroula and Thapa, 2009, Xynias et al., 2014).

Table 1. Pedigree of F_1 hybrids used as donor plant and their anther culture response.

Pedigree	Callus induction	Plant regeneration	Fertile plants	Gained seeds
DH6 × Altay 2000	22	2	0	0
DH18 × Altay 2000	75	0	0	0
DH18 × Kate A-1	111	3	3	205
DH19 × Altay 2000	152	17	5	351
DH20 × Kate A-1	13	0	0	0
DH21 × Kate A-1	119	16	4	458

*The values shown in the table were obtained from the previous study (Kutlu et al., 2019)

Table 2. Effect of wheat F_1 hybrids on the yield of developed pseudo-seeds, embryos and plants

Wheat F1 hybrids	Developed pseudo-seeds (n)	Embryos	Plants
		%*	%
DH6 × Altay 2000	117	2.9	0.4
DH18 × Altay 2000	85	0.6	0.4
DH18 × Kate A-1	74	0.7	0.0
DH19 × Altay 2000	108	1.7	0.5
DH20 × Kate A-1	90	5.9	0.3
DH21 × Kate A-1	72	2.5	0.7
Standard error	4.3	1.4	0.4

*Percentage values were calculated using the following formula: (Embryo or Plant numbers/Developed pseudo seeds) × 100

Table 3. Effect of inducers on the yield of developed pseudo seeds, embryos and plants

Inducers	Developed pseudo seeds (n)	Embryos	Plants
		%*	%
Baron	97	2.1	0.2
Challenger	93	3.5	0
Merit	70	1.0	0.3
Pollen mixture of maize	83	3.4	1.2
Orchard-grass	112	-	-
Standard error	4.0	1.0	0.7

*Percentage values were calculated using the following formula: (Embryo or Plant numbers/Developed pseudo seeds) × 100

Table 4. The effect of bread wheat F_1 hybrids × inducers interaction on developed pseudo seeds (number)

Wheat F1 hybrids	Inducers				
	Baron	Challenger	Merit	Mixture of maize	Orchard-grass
DH6 × Altay 2000	134	113	53	122	161
DH18 × Altay 2000	78	81	47	86	132
DH18 × Kate A-1	75	89	48	71	88
DH19 × Altay 2000	108	76	106	100	150
DH20 × Kate A-1	106	114	96	59	76
DH21 × Kate A-1	82	86	67	60	66

Interaction is significant at P=0.01(df=20) since the calculated χ^2 =105.6> theoretical χ^2= 37.56.

Table 5. The effect of bread wheat F_1 hybrids × inducers interaction on embryo formation (%)

Wheat F1 hybrids	Inducer			
	Baron	Challenger	Merit	Pollen mixture of maize
DH6 × Altay 2000	2.2	3.5	0	5.7
DH18 × Altay 2000	1.2	0	0	1.2
DH18 × Kate A-1	0	1.1	2	0
DH19 × Altay 2000	0	3.9	0	4
DH20 × Kate A-1	7.5	7.9	0	6.8
DH21 × Kate A-1	0	3.5	4.5	1.7

Interaction is significant at P=0.01(df=15) since the calculated χ^2 =33.0> theoretical χ^2= 30.5.

In terms of the inducer effect on haploid plant regeneration, wheat F_1 hybrids × *D. glomerata* hybridization produced a high rate of developed pseudo-seeds (Table 3). However, none of them had haploid embryos after 16-19 days. Zenkteler and Nitzsche (1984) reported that a high rate of embryos was obtained from wheat × *D. glomerata* hybridization, but the embryos disappeared after ten days. In terms of embryo formation, Challenger and pollen mixtures of sweet maize showed similar results. However, the effect of the pollen mixture of sweet maize on plant regeneration was found to be higher. Zhang et al. (1996) observed that the effect of maize genotypes on embryo formation and plant regeneration was important, and the effect of wheat genotypes was insignificant.

While the highest developed pseudo-seeds were observed in the hybridization of DH6 × Altay 2000 with low anther response and *D. glomerata*, the lowest value was obtained from DH18 × Altay 2000 and Merit hybrid (Table 4). The highest value (7.9 %) for embryo formation occurred in the DH20 × Kate A-1 with low anther response and Challenger hybrid (Table 5). In terms of plant regeneration from haploid embryos, the effect of interaction was found insignificant (Table 6). Although Challenger induced the high rate of embryo formation in wheat F_1 hybrids, its effect was not observed in plant regeneration. Lefebvre and Devaux (1996) and Cherkaoui et al. (2000) reported that wheat × maize interaction was significant in haploid embryo formation and plant regeneration.

Table 6. The effect of bread wheat F_1 hybrids × inducers interaction on plant regeneration (%)

Wheat F1 genotypes	Inducer		
	Baron	Merit	Pollen mixture of maize
DH6 × Altay 2000	0	0	1.7
DH18 × Altay 2000	1.3	0	0
DH18 × Kate A-1	0	0	0
DH19 × Altay 2000	0	0	2
DH20 × Kate A-1	0	0	1.7
DH21 × Kate A-1	0	1.5	1.7

Interaction is not significant at P=0.01(df=10) since the calculated χ^2 =13.3< theoretical χ^2= 23.2.

Table 7. Some characters of regenerated plants in wheat × maize hybrids

Characters	Wheat × sweet maize hybrids				
	DH21×Kate A-1×MP	DH6×Altay 2000×MP-1	DH6×Altay 2000×MP-2	DH21×Kate A-1×M	Mean
Fertile spike (%)	19.3	9.7	40.0	38	26.75
Seed number per fertile tiller	13.5	1.3	10.5	14	9.83

MP and M indicate pollen mixture of sweet maize and cv. Merit, respectively.

Among the wheat genotypes, only plants obtained from DH6 × Altay 2000 with low anther response and DH21 × Kate A-1 with high anther response genotypes have produced seeds (Table 7). The number of fertile spikes ranged from 9.7% in DH6 × Altay 2000 × MP-1 to 40% in DH6 × Altay 2000 × MP-2, and the mean fertile spike was 26.75%. The mean seed number per fertile spike was 9.83 and the lowest value was obtained from DH6 × Altay 2000 × MP-1. In the previous study indicated in Table 1, no plants were obtained from the combination of DH6 × Altay 2000 among these genotypes by the anther culture method (Kutlu et al., 2019). It is a pleasing result to obtain high number of seeds from two plants obtained by the wheat × maize crossbreeding method.

Consequently, the efficiency of *D. glomerata* and sweet maize genotypes for haploid plant production in some bread wheat F_1 hybrids were determined. Even though there was a high rate of developed pseudo-seeds in the wheat × orchard-grass hybrids, no haploid embryo was found. Thus, effects of different hormone type and times of embryo rescue should be evaluated for haploid embryo production between wheat and orchard grass cross. In wheat × sweet maize hybrids, four green plants in two different wheat F_1 hybrids were obtained. Differences in haploid plant regeneration were observed according to the wheat and maize genotypes. Achieving for haploid embryo formation and regeneration from wheat genotypes with a low anther culture response suggest that wheat × maize wide hybridization will be a good alternative to obtain efficient doubled haploid plant regeneration in bread wheat genotypes with low anther response. In genotypes with good anther response and good maternal haploids, the haploid technique to be used is optional. However, the high rate of albino plants may make it preferable to wide hybridization instead of anther technique. Although the source of the pollen to be used depends on the compatibility with the wheat genotypes to be used as a female parent, the effective one on many genotypes can be chosen. However, as proved in this study, applying maize pollen by mixing will guarantee embryo production.

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