UDC 575.633.11
https://doi.org/10.2298/GENSR1902447K
Original scientific paper

LINE × TESTER ANALYSES FOR ANTHER CULTURE RESPONSE OF BREAD WHEAT (*Triticum aestivum* L)

Imren KUTLU[1*], Zeynep SIREL[2], Ozcan YORGANCILAR[2], Aysel YORGANCILAR[2]

[1]Eskisehir Osmangazi University, Faculty of Agriculture, Department of Biosystem Engineering, 26160 Eskisehir/Turkey
[2]Transitional Zone Agricultural Research Institute, 26160 Eskisehir/Turkey

Kutlu I., Z. Sirel, O. Yorgancilar, A. Yorgancilar (2019): *Line × tester analyses for anther culture response of bread wheat (Triticum aestivum L*.- Genetika, Vol 51, No.2, 447-461.

The aim of this study was to investigate the gene effects that play a role in the inheritance of the anther culture response of the F_1 and F_2 populations obtained from the hybridization between the seven doubled haploid (DH) wheat line and four bread wheat cultivar using line × tester method as well as, to determine suitable parents and promising hybrids. Eleven parents and their twenty-eight hybrid combinations were examined for androgenic properties related to anther culture response such as the number of callus, green, albino, haploid, spontaneous DH and fertile plants. The obtained data were evaluated by the combining ability analysis calculated using the line × tester method and by estimating the heritability degrees. According to the results, for all traits examined, the non-additive gene effects were found as significant and narrow sense heritability at a low level. Significant combining ability effects have been identified for some parents and hybrids regarding traits examined. DH19, DH22, and Harmankaya-99 have been identified as suitable parents to develop the androgenic traits, while crosses formed between them have been determined as promising. A total of 444 plants (142 F_1, 302 F_2) were transferred to the greenhouse from the plants obtained from the anther culture, and 205 (68 in F_1, 137 in F_2) were fertile. Ninety-three of the fertile plants were obtained from

Corresponding author: Imren Kutlu, Eskisehir Osmangazi University, Faculty of Agriculture, Department of Biosystem Engineering, 26160 Eskisehir/Turkey, Email: ikutlu@ogu.edu.tr, Phone: +90 222 3242990, Fax: +90 222 3242991

the parents. The seeds of 112 DH plants obtained from hybrids were replicated to be used in breeding programs.

Keywords: Androgenic Traits**,** Combining Ability, Doubled Haploid, Fertile Plants, Heritability.

INTRODUCTION

Wheat is one of the most planted and produced crops and breeding studies for improving wheat continue without decreasing. Desired characteristics can be gained to wheat with classical breeding methods. It was known that technologies are needed that enable gene transfer without affecting the length of the breeding period, hybridization difficulty, infertility, incompatibility, and unwanted gene transitions in addition to classical breeding methods for long years (TESTER and LANGRIDGE, 2010). Doubled haploid (DH) plant production accelerates and facilitates genetic and breeding studies. Anther culture (AC) is widely used in obtaining DH plants, and the routine applicability of the AC technique in wheat is a desire for researchers (ISLAM, 2010). The reason is that while the selection is applied for five to ten generations in order to obtain the homozygous genotypes in the classical plant breeding, this process can be reduced to a minimum by means of the AC technique and the homozygous genotypes can be produced in a single generation (PAUK *et al.,* 2003). The DH lines obtained by the AC technique are used for genetic studies such as quantitative trait inheritance, QTL mapping, genomics, gene identification, whole-genome mapping and production of transgenic plants as well as shortening breeding period (HUSSAIN *et al*., 2012).

To effectively use androgenetic haploids in breeding programs linked to AC in wheat, numerous genotypes and a sufficient number of DH plants must be economically produced (TRIGIANO and GREY, 2016). Economically produce haploid plants, it is essential to increase callus formation and green plant production, which demonstrate the success of the AC technique. However, low callus formation and green plant regeneration in many wheat varieties limit the use of AC in wheat breeding programs. A lot of researchers mentioned that it is an important problem since long years that some breeding materials with good agronomic performance are stubborn about producing green plants, even for hybrids of well-responded parents (ORLOV *et al.*, 1993; TYANKOVA and ZAGORSKA, 2008; PATIAL *et al.*, 2019). Among other parameters of androgenic response, green plant formation has low inheritance and is influenced by non-genetic factors (REDHA and TALAAT, 2008). The response to AC depends largely on the genotypic structure of the donor plants. For this reason, obtaining a high level of androgenic response should be optimized culture conditions for each genotype, and in the AC, should be used the hybrids between low responsive genotypes with highly responsive genotypes (YORGANCILAR *et al*., 2017). Obtaining more information about the combining ability of breeding material for AC response would be extremely beneficial for improving AC efficacy (DAGUSTU, 2008). In the studies on the inheritance of response to AC, callus formation and green plant development have been reported as inherited traits and general and specific combining abilities have been found as important (MARCINIAK *et al.*, 2003; DAGUSTU, 2008; YILDIRIM *et al*., 2008; AL-ASHKAR, 2014). MARCINIAK *et al.* (2003) reported that hybrids were generally more responsive than their parents. CHAUDHARY *et al*. (2003), GRAUDA *et al.* (2016) and DAGUSTU (2008) found that respectively, additive, dominant, and epistatic gene effects play a role in the inheritance of androgenetic traits. Heterosis for anther response is changed from genotype to genotype (AL-ASHKAR, 2014). It has been noted in some of the results that the anthers of the highly responsive genotypes were giving more responses (LANTOS *et al*., 2013). In some studies, the anther response is mentioned as a character that had a simple inheritance controlled by dominant genes (EL-HENNAWY *et al*., 2011). In that case, this character can be transferred from the highly responsive genotypes to the low responsive genotype with rapid genetic gain. The use of a parent as a male or female parent may cause changes in green plant production from F_1 hybrids and may reduce the utilization of breeding material (YILDIRIM *et al*., 2008). The importance of cytoplasmic genes in androgenetic features suggests that the use of genotypes known for good anther response as a female parent will increase the success.

In the study, the key components of the anther response in the AC such as the callus formation, regeneration ability, green and albino plant development, haploid and spontaneous DH plant numbers were evaluated regarding gene action and inheritance pattern in the F_1 and F_2 generations of line × tester wheat population. At the same time, suitable parents and promising hybrids have been identified, by determining the general and specific combining ability effects in the examined traits and DH lines obtained from this study were also appraised for fertility.

MATERIAL AND METHODS

Seven DH wheat line with different the anther response was used as female (line), and four registered varieties (Altay-2000, Bezostaja-1, Harmankaya-99, Kate A-1) as male (tester) and they crossed according to line × tester matting design. The pedigrees of female parents were: 33IBSWN-S-244/Mufitbey for DH6, DH18, DH19, DH20, DH21, and DH22; Tosunbey/ Mufitbey for DH16. The DH lines used as a female parent were obtained in the F_2 generation of hybrids which pedigrees are given and had different genotypic properties. The experimental soil contained 1.2% organic matter and 9.6% lime; it was salt-free, clayey and slightly alkaline (pH 7.9-8.3). The total precipitation during the experiment year was 337.9 mm, the average temperature was 8.47°C and the average humidity was 73.3%. The 39 genotypes belong to F_1 and F_2 generations of line × tester wheat crosses were grown in randomized complete block design with three replications at Eskisehir Osmangazi University Agriculture Faculty research area in 2016-2017 growing season. The plots consisted of four rows 1 m long with 20 plants m^{-1} and 30 cm between rows. Standard agronomic practices (fertilization, irrigation, etc.) used in breeding programs were applied.

While in the early uninucleate stage, ten spikes from each combination were firstly exposed to the pre-cold application at 13°C for 13-14 days. The spikes were then removed from the stem and leaves, and rinsed with 2% sodium hypochlorite with 1-2 drops of Tween-20 for 20 minutes avoiding physical damage to the spikes and the spikes were washed with sterile water 4-5 times. After sterilization, the anthers were removed from spikes with a sterile forceps under the sterile cabin and planted in the previously prepared culture media. In the study, the MN6 culture media that its components given in Table 1, were used as the induction media (OUYANG *et al*., 1986). The liquid medium was prepared in Petri dishes of 60 mm diameter as 15 ml and four Petri dishes were used as replication from each genotype. One hundred anthers were planted in Petri dishes prepared for each wheat genotype in four replications. To prevent contamination in Petri dishes, the dishes were covered with parafilm and left in a dark incubator at 28°C. After a while, callus formation was observed. The resulting calluses were transferred to the 190-II-Cu nutrient media for green plant regeneration (ZHUANG and JIA, 1980). Petri dishes were wrapped 2-3 times with parafilm to prevent contamination and placed in plant growth cabinets for regeneration at 25°C, 16 h light and 8 h dark. If the regeneration started after 30 days, appropriate developmental calluses were transferred to test tubes with the 190-II Cu rooting medium (Table 1). The albino plants were counted and separated. Then, plantlets showing proper stem and shoot development were maintained in plant growth chambers (8°C/16 h in light; 4°C/8 h in dark) for 6 weeks. Plants showing sufficient root and shoot development in this condition were transferred to pots, and they were acclimated for two weeks at 16°C, 16 h in the light and 8 h in dark. The chromosome counts were determined from the root tips of the plants (BRUMMER *et al*., 1999). Spontaneous DH plants were transferred directly to the greenhouse, and haploid plants were transferred to the greenhouse by chromosome folding with 0.2% colchicine, 2% DMSO application. The numbers of callus, albino and green plant number, spontaneous DH and haploid plants were recorded for each replication while all these operations were carried out. In addition, in the plants transferred to the greenhouse, the plants forming the spikes and those non-forming were identified. Among the spiked plants, fertile ones were identified and the seeds they formed were counted.

Table 1. The components of induction (MN6) and regeneration (190-II Cu) media

MN6		190-II Cu	
Component	Amount (mg/l)	Component	Amount (mg/l)
KNO_3	1150	KNO_3	100
$/NH_4/2SO_4 \times 2H_2O$	100	$/NH_4/2SO_4$	200
$Ca/\ NO_3/2 \times 4H_2O$	100	$Ca/\ NO_3/2\times 4H_2O$	100
$(NH_4)_2\ SO_4$	80	KH_2PO_4	300
$MgSO_4 \times 7H_2O$	125	$MgSO_4 \times 7\ H_2O$	200
KH_2PO_4	200	KCl	40
KCl	35	Fe-Na-EDTA	20
2.4-D	1.5	$MnSO_4 \times 4H_2O$	8
Kinetin	0.5	$ZnSO_4 \times 7H_2O$	3
Ficoll	100.000	H_3BO_3	3
		Kl	0.5
Component	Amount (ml/l)	Glicine	2
Fe-Na-EDTA	5	Thiamin-HCl	1
Thiamin-HCl	1	Pyridoksine HCl	0.5
Maltose	100	Nicotinik acid	0.5
		Meso-inositol	100
		Sucrose	0.5
		NAA	0.5
		Kinetin	0.5
		$CuSO_4 \times 5H_2O$	5.7

*The pH was adjusted as 5.8 in both media.

Line × tester analyze was performed by the TarPopGen Statistical Package Program developed by OZCAN and ACIKGOZ (1999). The analyses of variance, general combining ability (GCA), specific combining ability (SCA), the variance of additive and dominant gene effects based on variances of GCA and SCA, as well as, broad and narrow sense heritability were calculated according to FALCONER and MACKAY (1996).

RESULTS AND DISCUSSION

The successful application of AC in wheat breeding programs is dependent on the plant regeneration ability of the genotypes. The callus numbers of the parents varied between 4 (Altay-2000) and 289 (DH22). The highest number of green plants was obtained from DH19 and DH22 lines as 34 and 28 plants, respectively, while the number of albino plants ranged from 0 to 128 (DH22). The formation of albino plants is one of the important factors limits the success of AC. DH19 and DH22 lines with high regeneration ability are also seen to have higher albino plant numbers. Of the green plants growing from callus, the number of haploids was highest in DH19 and DH22 lines, while spontaneous DH plant numbers ranged from 0 to 18 (Table 2).

Table 2. The number of total callus, green, albino, haploid and spontaneous DH plants obtained from wheat hybrids and parents by AC method

	Callus		Green plant		Albino plant		Haploid plant		Spontaneous DH plant	
Genotypes	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
DH-6	49	49	13	13	3	3	4	3	9	10
DH-6×Altay-2000	22	39	2	4	2	5	1	2	1	2
DH-6×Bezostaja-1	13	49	0	4	1	21	0	2	0	2
DH-6×Harmankaya-99	35	55	6	31	1	0	1	13	5	18
DH-6×Kate A-1	23	19	1	10	3	1	0	5	1	5
Altay-2000	4	4	0	0	0	0	0	0	0	0
DH-16	20	21	3	4	4	4	3	1	0	3
DH-16×Altay-2000	33	36	0	14	4	12	0	8	0	6
DH-16×Bezostaja-1	60	19	2	6	18	9	0	3	2	3
DH-16×Harmankaya-99	94	24	4	4	12	1	0	2	4	2
DH-16×Kate A-1	24	28	1	8	3	4	1	3	0	5
Bezostaja-1	19	19	0	0	0	2	0	0	0	0
DH-18	95	93	5	9	32	32	4	5	1	4
DH-18×Altay-2000	75	31	0	2	12	0	0	2	0	0
DH-18×Bezostaja-1	114	51	3	0	30	14	2	0	1	0
DH-18×Harmankaya-99	59	52	0	7	17	15	0	1	0	6
DH-18×Kate A-1	111	56	3	1	26	18	0	0	3	1
Harmankaya-99	31	31	10	17	0	0	4	7	6	10
DH-19	240	239	32	30	41	33	14	14	18	16
DH-19×Altay-2000	152	16	17	2	13	4	7	0	10	2
DH-19×Bezostaja-1	18	31	2	4	0	6	0	1	2	3
DH-19×Harmankaya-99	17	93	0	19	0	20	0	8	0	11
DH-19×Kate A-1	153	95	10	8	21	13	6	2	4	6
Kate A-1	17	17	1	0	4	0	1	0	0	0
DH-20	31	30	4	2	13	11	3	1	1	1
DH-20×Altay-2000	31	31	1	1	4	5	0	0	1	1
DH-20×Bezostaja-1	117	63	0	2	33	27	0	2	0	0
DH-20×Harmankaya-99	13	62	2	6	0	19	1	1	1	5
DH-20×Kate A-1	13	45	0	1	3	4	0	1	0	0

Table 2. The number of total callus, green, albino, haploid and spontaneous DH plants obtained from wheat hybrids and parents by AC method, cont.

DH-21	71	72	7	8	6	13	6	4	1	4
DH-21×Altay-2000	71	149	3	29	11	52	2	12	1	17
DH-21×Bezostaja-1	22	35	0	1	1	4	0	1	0	0
DH-21×Harmankaya-99	48	103	1	28	13	21	1	14	0	14
DH-21×Kate A-1	119	79	16	3	5	32	5	1	11	2
DH-22	224	289	19	28	78	128	10	15	9	13
DH-22×Altay-2000	68	43	5	16	0	14	0	4	1	12
DH-22×Bezostaja-1	64	42	0	0	0	6	0	0	0	0
DH-22×Harmankaya-99	94	39	17	4	6	7	7	1	10	3
DH-22×Kate A-1	163	24	6	3	0	6	3	1	3	2
Total	*2627*	*2273*	*196*	*329*	*420*	*566*	*86*	*140*	*106*	*189*

* Pink rows indicate female parents (line), blue rows indicate male parents (tester)

When the androgenic traits of the hybrid combinations are considered, it is seen that the number of calluses and plants obtained in the F_2 generation is higher (Table 2). Many researchers reported that the anther response was generally higher in the F_2 generation (PAUK *et al*., 2003; YORGANCILAR *et al*., 2015; BASER *et al*., 2016). The highest values for total callus, green, albino, haploid and spontaneous DH plant numbers of the hybrids in the F_1 generation were; 163 (DH22 × Kate A-1), 17 (DH19 × Altay-2000 and DH22 × Harmankaya-99), 33 (DH20 × Bezostaja-1), 7 (DH19 × Altay-2000 and DH22 × Harmankaya-99) and 11 (DH21 × Kate A-1), respectively. In the F_2 generation, the number of callus varied between 19 (DH6 × Kate A-1) and 149 (DH21 × Altay-2000), green plant numbers between 0 (DH18 × Bezostaja-1 and DH22 × Bezostaja-1) and 31 (DH6 × Harmankaya-99). The combinations with the lowest number of albino plants were DH6 × Harmankaya-99 (0), DH6 × Kate A-1 (1), DH16 × Harmankaya-99 (1), DH18 × Altay-2000 (0) hybrids. Total haploid plant numbers were the lowest in the DH18 hybrid series, while the highest in the DH21 hybrid series. The highest haploid plant was obtained from DH21 × Harmankaya-99 as 14. The number of spontaneous DH plants was also determined to be 14, and the highest spontaneous DH plant was detected in DH6 × Harmankaya-99 and DH21 × Altay-2000 hybrids (Table 2). In the F_1 generation, 142 genotypes in total were transferred to the greenhouse, while 302 plants were in F_2 generations. Among the 198 plants obtained in the F_1 generation, four plants were killed in the culture medium and die of 54 plants were after colchicine application. The number of plants that died in the F_2 generation is 27. Induced chromosome doubling by chemical agents, such as colchicine, results in high mortality rates, which decreases the process efficiency, therefore spontaneous doubling of chromosomes in AC is highly desirable (CASTILLO *et al*., 2009).

Altay-2000, Bezostaja-1 and Kate A-1 in the testers were not formed any plants, while Harmankaya-99 was formed 26 plants in total. The genotypes with the highest number of plants in the lines were detected as DH19 and DH22. The number of plants obtained from the hybrids was higher than that of their parents. However, the DH18 and DH20 hybrid series failed to form plants. Among these plants transferred to the greenhouse, spiked and unspiked were identified. The number of fertile plants and the number of seeds were also determined. In the first stage of DH plant production, obtaining a high number of fertile plants and seeds is important for the reproduction and evaluation of the obtaining lines. Obtaining yield DH and fertile plants depend on duplication (spontaneous or induced) of chromosomes. Achievement of chromosome doubling can be verified by checking fertile spikes. The DH19 genotype had the most number of fertile plants in the F_1 generation, followed by the DH22 × Harmankaya-99 hybrid with six plants. While the DH22 × Altay-2000 hybrid was the least seed-producing genotype, the DH21 × Kate A-1 hybrid produced 458 seeds in total. The number of seeds obtained from a total of 35 plants obtained from F_1 hybrids ranged from 1 to 271 per plant. The number of fertile plants in the F_2 generation was more. Some hybrids formed seeds in the F_1 generation but not F_2 generations. However, for some, the case was the opposite. There are 77 plants to be reproduced as cultivar candidates in the F_2 generation and their number of seeds per plant varies between 1 and 478 (Table 3).

Table 3. The number of total plants transferred to the greenhouse, spiked, unspiked, fertile, sterile plants and total seed obtained from wheat hybrids and parents by AC method

	Plants transferred to the greenhouse		Spiked plants		Non spiked plants		Fertile plants		Sterile plants		Total seed number	
Genotipler	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
DH-6	10	12	6	10	4	2	5	8	1	2	355	697
DH-6×Altay-2000	1	4	0	1	1	3	0	1	0	0	0	18
DH-6×Bezostaja-1	0	3	0	1	0	2	0	1	0	0	0	182
DH-6×Harmankaya	5	30	5	20	0	2	5	17	0	3	272	786
DH-6×Kate A-1	1	9	0	4	1	5	0	2	0	2	0	32
Altay-2000	0	0	0	0	0	0	0	0	0	0	0	0
DH-16	1	4	0	4	1	0	0	4	0	0	0	91
DH-16×Altay-2000	0	12	0	7	0	4	0	7	0	0	0	521
DH-16×Bezostaja-1	2	6	2	2	0	4	2	0	0	2	35	0
DH-16×Harmankaya	4	3	4	1	0	2	3	0	1	1	227	0
DH-16×Kate A-1	0	8	0	2	0	6	0	2	0	0	0	173
Bezostaja-1	0	0	0	0	0	0	0	0	0	0	0	0
DH-18	4	9	1	5	3	4	1	4	0	1	20	44
DH-18×Altay-2000	0	2	0	1	0	1	0	0	0	1	0	0
DH-18×Bezostaja-1	1	0	1	0	0	0	1	0	0	0	56	0
DH-18×Harmankaya	0	6	0	5	0	1	0	5	0	0	0	367
DH-18×Kate A-1	3	1	3	0	0	1	3	0	0	0	205	0
Harmankaya-99	9	17	2	17	7	0	0	14	0	3	0	1895
DH-19	30	21	30	16	0	5	23	12	7	4	2099	2501
DH-19×Altay-2000	11	2	11	2	0	0	5	2	6	0	351	232
DH-19×Bezostaja-1	2	4	1	2	1	2	1	2	0	0	57	249
DH-19×Harmankaya	0	18	0	8	0	10	0	6	0	2	0	644
DH-19×Kate A-1	5	7	0	4	5	3	0	3	0	1	0	145
Kate A-1	0	0	0	0	0	0	0	0	0	0	0	0
DH-20	1	2	1	0	0	2	1	0	0	0	151	0

Table 3. The number of total plants transferred to the greenhouse, spiked, unspiked, fertile, sterile plants and total seed obtained from wheat hybrids and parents by AC method, cont.

DH-20×Altay-2000	1	1	0	1	1	0	0	1	0	0	0	265
DH-20×Bezostaja-1	0	1	0	0	0	1	0	0	0	0	0	0
DH-20×Harmankaya	1	6	1	6	0	0	1	5	0	1	98	409
DH-20×Kate A-1	0	1	0	0	0	1	0	0	0	0	0	0
DH-21	3	8	0	4	3	4	0	3	0	1	0	309
DH-21×Altay-2000	3	29	0	10	3	19	0	6	0	4	0	139
DH-21×Bezostaja-1	0	1	0	0	0	1	0	0	0	0	0	0
DH-21×Harmankaya-99	0	27	0	13	0	14	0	10	0	3	0	595
DH-21×Kate A-1	11	2	4	1	7	1	4	1	0	0	458	194
DH-22	17	26	3	18	14	8	3	15	0	3	102	440
DH-22×Altay-2000	1	14	1	6	0	8	1	4	0	2	4	185
DH-22×Bezostaja-1	0	0	0	0	0	0	0	0	0	0	0	0
DH-22×Harmankaya	12	4	6	2	6	2	6	2	0	0	432	34
DH-22×Kate A-1	3	2	3	2	0	0	3	0	0	2	340	0

* Pink rows indicate female parents (line), blue rows indicate male parents (tester)

When the averages of the squares calculated by the line × tester analysis of androgenic traits were examined, all genotypes (treatments), parents, hybrids, and line × tester interaction were statistically significant at 1% for all traits in both F_1 and F_2 generations (Table 4). When the estimates of genetic variance of the androgenic traits are examined, the ratio of σ^2GCA / σ^2SCA to all traits in both generations was smaller than one, indicating that non-additive gene effects play a role in the heritability of these traits (Table 5). Dominance variance was greater than the additive variance for all the traits. These values show that there is a superior dominance in non-additive gene effects. CHAUDHARY *et al*. (2015) reported that the non-additive gene effects were predominant in green plant regeneration, while the additive gene effects were a presence in callus formation. EL-HENNAWY *et al*. (2018) informed that additive gene effects on variability observed in the androgenic process contributed more than non-additive gene effects. The features associated with androgenesis have a quantitative inheritance, and therefore gene expression of these properties may be different in various environments. The broad-sense heritability degrees of the androgenic traits were found high although the narrow-sense heritability was very low. This indicates that the genetic structure is higher than the environmental effects in the appearance of these traits, but the contribution of additive gene effects is less to heritability.

When parental GCA values are examined in terms of the number of callus, it is seen that four of the lines in the F_1 generation have positive GCA values, the effect of the three is significant and that the DH22 line has the highest GCA effect. The GCA effect on this line was negative in the F_2 generation. However, the GCA effect of the DH21 line was negligible in the F_1 generation, while it had the highest GCA effect value in the F_2 generation. The testers Kate A-1 and Harmankaya-99 had the highest GCA effects in F_1 and F_2 generations respectively. When the SCA effects of the hybrids were examined, 9 of the 18 hybrids in which significant SCA effect was detected in F_1 were positive. In the F_2 generation, the hybrids DH19 × Harmankaya-99, DH19 × Kate A-1, DH20 × Bezostaja-1 and DH × Altay-2000 showed positive and significant SCA effects. Combinations with positive effects in both generations with these hybrids may be followed as promising to increased callus formation in AC (Table 5).

Table 4. Squares averages and degrees of freedom calculated by line × tester analysis for androgenetic properties in bread wheat hybrids.

	Source of variation	DF	Callus	Green plant	Albino plant	Haploid plant	Spontaneous DH plant
	Replication	3	5.66^{ns}	0.10^{ns}	0.89^{ns}	0.12^{ns}	0.16^{ns}
	Treatment	38	919.80^{**}	13.11^{**}	59.00^{**}	2.57^{**}	4.18^{**}
	Parents	10	1987.75^{**}	26.20^{**}	151.42^{**}	4.75^{**}	8.11^{**}
F_1	Interaction	1	274.85^{**}	55.43^{**}	127.18^{**}	17.19^{**}	10.05^{**}
	Hybrids	27	548.15^{**}	6.69^{**}	22.25^{**}	1.22^{**}	2.51^{**}
	Lines	6	705.89^{ns}	6.72^{ns}	34.70^{ns}	1.54^{ns}	1.87^{ns}
	Testers	3	499.46^{ns}	5.64^{ns}	11.74^{ns}	1.03^{ns}	1.89^{ns}
	Line × Tester	18	503.68^{**}	6.86^{**}	19.85^{**}	1.15^{**}	2.82^{**}
	Error	114	21.18	0.27	1.16	0.16	0.23
	Source of variation	DF	Callus	Green plant	Albino plant	Haploid plant	Spontaneous DH plant
	Replication	3	12.11^{ns}	2.26^{ns}	2.23^{ns}	3.03^{*}	1.68^{ns}
	Treatment	38	723.26^{**}	19.93^{**}	119.72^{**}	8.67^{**}	6.38^{**}
	Parents	10	1986.06^{**}	27.77^{**}	354.32^{**}	7.10^{**}	8.56^{**}
F_2	Interaction	1	1363.49^{**}	12.50^{*}	32.11^{**}	5.18^{**}	2.29^{ns}
	Hybrids	27	231.86^{**}	17.30^{**}	32.11^{**}	3.60^{**}	5.72^{**}
	Lines	6	453.81^{ns}	21.08^{ns}	49.60^{ns}	5.23^{ns}	5.99^{ns}
	Testers	3	73.34^{ns}	36.91^{ns}	1.26^{ns}	6.08^{ns}	13.17^{ns}
	Line × Tester	18	184.29^{**}	12.77^{**}	31.41^{**}	2.64^{**}	4.39^{**}
	Error	114	27.72	1.99	2.76	0.53	1.04

$^{*}P<0.05$, $^{**}P < 0.01$

The GCA effects calculated for the number of green plants varied between -0.67 (DH20) and -1.35 (DH18) and 0.95 (DH19) and 1.96 (DH21) in the F_1 and F_2 generations, respectively. The numbers of parents with positive GCA effects were five in F_1 and six in F_2. The DH6 and DH16 lines showing a negative GCA effect in F_1 showed a positive effect in F_2. The high frequency of green plants is an aim to be attained in AC studies. Parents with positive GCA should be included in breeding programs because of this trait enhancing properties. The SCA effect values of the hybrids were between -1.95 (DH19 × Harmankaya-99) with 2.44 (DH19 × Altay-2000) in F_1, while it changed between -2.48 (DH16 × Harmankaya-99) with 2. 86 (DH21 × Altay-2000) in F_2. Eighteen hybrids in the F_1 generation and ten hybrids in the F_2 generation showed significant SCA effect statistically and seven (F_1) and four (F_2) of them had positive values (Table 5).

Table 5. General combining ability (GCA) and specific combining ability (SCA) in bread wheat hybrids and their parents for androgenic traits

	Callus		Green plants		Albino plants		Haploid plants		S. DH plants	
Genotypes	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
DH-6	-9.54**	-2.40	-0.36**	0.71	-1.36**	-0.91*	-0.20*	0.41*	-0.15	0.30
DH-6×Altay-2000	-1.10	-0.17	0.00	-2.14**	0.21	-1.12	0.10	-0.91*	-0.10	-1.23*
DH-6×Bezostaja-1	-1.78	4.29	0.11	-0.32	-1.36*	3.05	0.13	-0.23	-0.03	-0.09
DH-6×Harmankaya	9.22**	1.08	0.61*	1.89*	1.29*	-0.30	0.10	0.77*	0.51*	1.12*
DH-6×Kate A-1	-6.35*	-5.20*	-0.71**	0.57	-0.14	-1.62	-0.33	0.37	-0.38	0.20
Altay-2000	0.16	-0.02	0.00	0.58*	-0.46	0.25	0.03	0.22	-0.03	0.36
DH-16	-4.61	-5.96**	-0.48**	0.09	-0.11	-1.41**	-0.27**	0.22	-0.21	-0.13
DH-16×Altay-2000	-3.29	2.64	-0.37	0.98	-0.54	1.12	-0.09	0.78*	-0.29	0.20
DH-16×Bezostaja-1	5.04*	0.36	0.73**	0.80	1.64**	0.55	0.20	0.20	0.54*	0.60
DH-16×Harmankaya	9.29**	-3.61	0.23	-2.48**	0.29	-1.30	-0.09	-1.04**	0.32	-1.44**
DH-16×Kate A-1	-11.04**	0.61	-0.59*	0.70	-1.39*	-0.37	-0.02	0.06	-0.57*	0.63
Bezostaja-1	-1.41	-1.98*	-0.61**	-1.24**	0.86**	0.07	-0.26**	-0.45**	-0.35**	-0.79**
DH-18	5.39**	-1.65	-0.48**	-1.35**	2.89**	-0.54	-0.20*	-0.59**	-0.28*	-0.76**
DH-18×Altay-2000	-2.79	-2.92	-0.37	-0.58	-1.54**	-2.75	-0.15	0.09	-0.22	-0.67
DH-18×Bezostaja-1	8.54**	4.04	0.98**	0.74	1.64**	0.93	0.63**	0.27	0.35	0.47
DH-18×Harmankaya	-6.46**	-4.42	-0.52*	-0.54	-1.46**	-0.43	-0.15	-0.48	-0.37	-0.06
DH-18×Kate A-1	0.71	3.29	-0.09	0.38	1.36*	2.25	-0.33	0.12	0.24	0.26
Harmankaya-99	-4.41**	1.98*	0.14	1.29**	-0.54**	-0.07	0.03	0.54**	0.12	0.75**
DH-19	5.27**	2.35	0.95**	0.21	0.02	-0.35	0.48**	-0.09	0.47**	0.30
DH-19×Altay-2000	16.59**	-10.67**	2.44**	-2.14**	1.59**	-1.94	0.91**	-0.91*	1.53**	-1.23*
DH-19×Bezostaja-1	-15.34**	-4.95	-0.70**	0.18	-2.98**	-1.26	-0.55**	0.02	-0.15	0.16
DH-19×Harmankaya	-12.59**	6.58*	-1.95**	1.39	-1.59**	2.38	-0.84**	0.77**	-1.12**	0.62
DH-19×Kate A-1	11.34**	9.04**	0.22	0.57	2.98**	0.81	0.48*	0.12	-0.26	0.45
Kate A-1	5.66**	0.02	0.46**	-0.63*	0.14	-0.25	0.20*	-0.31*	0.26**	-0.32
DH-20	-5.11**	0.22	-0.67**	-1.22**	0.39	0.40	-0.27**	-0.53**	-0.40**	-0.70**
DH-20×Altay-2000	-3.29	-4.79	0.06	-0.95	-1.04	-2.44	-0.09	-0.47	0.15	-0.48
DH-20×Bezostaja-1	19.79**	5.17*	0.42*	1.12	4.89**	3.24	0.20	0.70	0.22	0.41
DH-20×Harmankaya	-3.21	0.95	0.17	-0.42	-1.96**	1.38	0.16	-0.54	0.01	0.12
DH-20×Kate A-1	-13.29**	-1.33	-0.65**	0.26	-1.89**	-2.19	-0.27	0.31	-0.38	-0.05
DH-21	0.27	10.54**	0.39**	1.96**	-0.23	3.78**	0.17	0.97**	0.22	0.99**
DH-21×Altay-2000	1.34	14.39**	-0.50*	2.86**	1.34*	5.94**	-0.03	1.03**	-0.47*	1.83**
DH-21×Bezostaja-1	-9.34**	-12.14**	-0.64*	-2.32**	-2.48**	-5.88**	-0.24	-1.04**	-0.40	-1.28*
DH-21×Harmankaya	0.16	0.89	-1.14**	1.89*	1.91**	-1.49	-0.28	1.20**	-0.87**	0.69
DH-21×Kate A-1	7.84**	-3.14	2.29**	-2.43**	-0.77	1.44	0.54**	-1.19**	1.74**	-1.24*
DH-22	8.33**	-3.09*	0.64**	-0.41	-1.61**	-0.97*	0.29**	-0.40*	0.35**	-0.01
DH-22×Altay-2000	-7.47**	1.52	-1.25**	1.98**	-0.04	1.19	-0.65**	0.40	-0.60*	1.58**
DH-22×Bezostaja-1	-6.90**	3.23	-0.89**	-0.20	-1.36*	-0.63	-0.37	0.08	-0.53*	-0.28
DH-22×Harmankaya	3.60	-1.48	2.61**	-1.73*	1.54**	-0.24	1.10**	-0.67	1.51**	-1.06*
DH-22×Kate A-1	10.78**	-3.27	-0.46*	-0.05	-0.14	-0.31	-0.08	0.19	-0.38	-0.24

Table 5. General combining ability (GCA) and specific combining ability (SCA) in bread wheat hybrids and their parents for androgenic traits, cont.

SH(Line)	1.15	1.27	0.13	0.35	0.27	0.42	0.10	0.18	0.12	0.25
SH(Tester)	0.87	0.96	0.10	0.27	0.20	0.31	0.08	0.14	0.09	0.19
SH(SCA)	2.30	2.54	0.26	0.70	0.54	1.83	0.20	0.36	0.24	0.51
σ^2GCA/ σ^2SCA	0.01	0.02	-0.01	0.03	0.01	0.002	0.00	0.03	-0.01	0.03
σ^2A	1.48	1.59	-0.01	0.15	0.08	0.02	0.002	0.03	-0.01	0.04
σ^2D	120.62	39.64	1.65	2.70	4.67	7.16	0.25	0.53	0.65	0.84
H^2	0.97	0.94	0.96	0.89	0.97	0.95	0.88	0.82	0.90	0.83
h^2	0.01	0.03	-0.01	0.04	0.01	0.002	0.005	0.03	-0.01	0.03

$^{*}P<0.05$, $^{**}P < 0.01$; pink rows indicate female parents (line) , blue rows indicate male parents (tester)

The occurrence and frequency of albino plants remain a serious problem in the AC of cereal species. Researchers studying on albinism (LANTOS *et al.*, 2013; HASAN *et al.*, 2014; KRZEWSKA *et al.*, 2015) reported that albino production rate is a heritable feature, and that nuclear genomes dominate over controlling this phenomenon, even though the period collected anthers of donor plant or some pre-treatment also affects the albino number. The GCA effects of six and seven hybrids for albino plant numbers were found to be negative in the F_1 and F_2 generations, respectively (Table 5). DH6, DH16, and DH22 are potentially usable as parents in breeding programs to reduce the number of albinos in AC due to their effect on reducing the number of albino. Sixteen of the F_1 hybrids had negative SCA effects and ten of them were statistically significant. The negative SCA effect was detected in 15 hybrids in the F_2 generation, and only one of them (DH-21 × Bezostaja-1) is statistically significant. The hybrids of DH6 × Kate A-1, DH16 × Kate A-1, DH20 × Kate A-1, DH18 × Harmankaya-99, DH19 × Bezostaja-1 and DH20 × Altay-2000, with high and negative SCA effect, should also be pursued for this feature.

The GCA effects of hybrids for haploid plant numbers varied from -0.27 to 0.48 in F_1. Among the lines, the highest GCA effect was found on the DH19 line, and among the tester, Kate A-1 was the parent with the highest GCA effect. In the F_2 generation, the GCA effect of the DH21 line was the highest and the lowest GCA effect was obtained from the DH18 line. Within the testers, Harmankaya-99 was found to be the parent with the highest contribution. The SCA effects of the hybrids ranged from -0.84 (DH19 × Harmankaya-99) to 0.91 (DH19 × Altay-2000) in F_1 and from -1.19 (DH21 × Kate A-1) to 1.20 (DH21 × Harmankaya-99) in the F_2 generation (Table 5). Hybrids that have a high SCA effect in both generations should be monitored as promising hybrids for this feature.

Since spontaneous chromosome doubling is frequently observed in the cereal AC technique, determination of the level of ploidy is necessary for plants that are produced by AC. Haploid plants should be treated with colchicine to make them fertile. Since colchicine is toxic to humans, the ability to reproduce spontaneous DH plants is a tremendous advantage by avoiding manipulation. In androgenesis, chromosomal doubling occurs at very early stages of embryogenesis, and the resulting plants are then completely doubled and fertile. Compared to plants treated with colchicine, spontaneous DH plants produce much more seed even when first obtained and can rescue a generation for seed multiplication before phenotyping for quantitative traits (CHAUDHARY *et al*., 2015). When the combining ability effects calculated for the number of spontaneous DH plants are examined, it is seen that four parents in the F_1 generation and five parents in the F_2 generation have a positive GCA effect (Table 5). Since DH19, DH22 and Harmankaya- 99 of these parents are also significant statistically to GCA effects; they can be used as parents in breeding programs to increase the number of spontaneous DH plants. The SCA effects of the hybrids ranged from -1.12 (DH19 × Harmankaya-99) to 1.74 (DH21 × Kate A-1) in the F_1 generation, and -1.44 (DH16 × Harmankaya-99) to 1.83 (DH21 × Altay-2000) in the F_2 generation. In both generation, DH6 × Harmankaya-99, DH16 × Bezostaja-1, and DH18 × Bezostaja-1 hybrids, which have a positive SCA effect, should be followed with hope for spontaneous DH plant number (Table 5).

When all the androgenic traits are considered together, DH19, DH21, and DH22 lines appear to be suitable parents for breeding trials to improve the AC response, based on the total number of callus and plant numbers and the GCA effects calculated for them. Although anther response has been reported to be controlled mostly by nuclear genes, there are studies reporting that it is managed by both nuclear and cytoplasmic genes (CHAUDHARY *et al*., 2003; YILDIRIM *et al*., 2008, respectively). The combining ability of the lines was found to be higher than the testers. It can be said that this is due to the fact that the lines used as female parents are produced by AC technique and that they have the ability to transfer this high response characteristic to their offspring.

Among the hybrids, DH6 × Harmankaya-99, DH19 × Harmankaya-99, DH19 × Kate A-1, DH21 × Harmankaya-99, and DH22 × Altay-2000 were the best performers in terms of all androgenic traits. Despite the fact that DH6 × Bezostaja-1, DH18 × Bezostaja-1, and DH20 × Bezostaja-1 hybrids also have high callus formation and regeneration ability and SCA effects are high, the number of plants transferred to the greenhouse due to the high albino ratio of these hybrids is very low. These hybrids may be followed up promising with other hybrids if the defect of high albino formation is eliminated.

HASAN *et al*. (2014) studied on performance of AC response by comparing F_1 and F_2 generations, and they found that the F_2 generation had better performance due to the polygenic effect of F_2 and that this dominance may be due to the recombination of superior genes. CHAUDHARY *et al*. (2003) suggested that the use of non-additive components of genetic diversity is limited to hybrids only because it cannot be fixed in the segregating generations and that the low narrow-sense heritability level for callus induction refers to dominant gene effects on this trait. Therefore, they proposed that it would be true to usage of F_1 population in this variation component because the effect of dominance can disappear in later generations. In this study, for all the investigated androgenic traits, both the GCA effects of the parents and the SCA effects of the hybrids were found to be higher in the F_2 generation. At the same time, the performance of the hybrids was higher in the F_2 generation. The recombination of superior genes in the F_2 generation may be responsible for the better performance of the F_2 generation.

CONCLUSION

The DH19 and DH22 lines and the Harmankaya-99 tester have been identified as important parents in that they are able to transfer their high callus and plantlet formation abilities to their offspring. In the determination of hybrids had high anther response, both callus, green plant numbers and high plant numbers transferred to the greenhouse and positive SCA effect were taken into account. Some hybrids had a positive SCA effect in both generations, while some others were negative in F_1 and positive in F_2. Those with high numbers of plants from these hybrids were evaluated as promising. Although all combinations of the DH16 line formed a few calluses, the number of plants they produce was high. On the contrary, a high number of calluses were obtained in all combinations of the DH18 line, but the number of plants they produced was low. It is thought that promising combinations can be obtained and removed their incomplete aspects if these hybrids are resolved by crossing different genotypes or by back-crossing. Hybrids suitable for follow-up for androgenetic properties are DH6 × Harmankaya-99, DH19 × Altay-2000, DH19 × Harmankaya-99, DH19 × Kate A-1, DH20 × Harmankaya-99, DH21 × Altay-2000, DH21 × Harmankaya-99, DH21 × Kate A-1, DH22 × Altay-2000 and DH22 × Harmankaya-99. Totally 112 DH plants (35 from the F_1 generation, 77 from the F_2 generation) plants were produced for our winter wheat breeding program.

ACKNOWLEDGMENT

This research supported by the Scientific Research Projects Commission of Eskisehir Osmangazi University (project no: 201723009).

Received, August 18th, 2018
Accepted May 18th, 2019

REFERENCES

AL-ASHKAR, I.M. (2014): Genetic contribution of parental genotypes on anther culture response of bread wheat F_1 hybrids. Middle East Journal of Agriculture Research**,** *3(3):* 472-478.

BASER, I., K.Z., KORKUT, O., BILGIN, A., BALKAN (2016): Obtaining of doubled haploid plants from bread wheat (*Triticum aestivum* L.) hybrid populations by anther culture technique. J. Central Res. Institute for Field Crops, *25*(SI-1): 232-236.

BRUMMER, E.C., M., SLEDGE, J.H., BOUTON, G., KOCHERT (1999): Molecular marker analyses in alfalfa and related species. In: PHILLIPS, R. L., I. K. VASIL (eds), DNA-Based Markers in Plants. Springer, Dordrecht, p. 169-180.

CASTILLO, A.M., L., CISTUÉ, M.P., VALLÉS, M., SORIANO (2009): Chromosome doubling in monocots. In: Advances in Haploid Production in Higher Plants. Springer, Dordrecht, p. 329-338.

CHAUDHARY, H.K., I., DHALIWAL, S., SINGH, G.S., SETHI (2003): Genetics of androgenesis in winter and spring wheat genotypes. Euphytica**,** *132:* 311–319.

CHAUDHARY, H.K., A., BADIYALA, N.S., JAMWAL (2015): New frontiers in doubled haploidy breeding in wheat. Agric. Res. J., *52*(4): 1-12.

DAGUSTU, N. (2008): Diallel analysis of anther culture response in wheat (*Triticum aestivum* L.). Afr. J. Biotech.**,** *7(19):* 3419-3423.

EL-HENNAWY, M.A., A.F., ABDALLA, S.A., SHAFEY, I.M., AL-ASHKAR (2011): Production of doubled haploid wheat lines (*Triticum aestivum* L.) using anther culture technique. Ann. Agric. Sci., Ain Shams Univ., Cairo. *56*: 63–72.

EL-HENNAWY, M.A., E.I., ZAAZAA, I.M., AL-ASHKAR, E.A. EID (2018): Genetic analysis of anther culture response in some bread wheat crosses under drought stress conditions. J. Biol. Chem. Environ. Sci., *13*(2): 189-213.

FALCONER, D.S., T.F., C., MACKAY (1996): Introduction to quantitative genetics. Longman, 464 p.

GRAUDA, D., K., ŽAGATA, G., LANKA, V., STRAZDINA, V., FETERE, N., LISINA, N., KRASNEVSKA, O., FOKINA, A., MIKELSONE, R., ORNICANS, I., BELOGRUDOVA, I., RASHAL (2016): Genetic diversity of wheat (*Triticum aestivum* L.) plants-regenerants produced by anther culture. Genetics and Breeding, *20*(4): 537-544.

HASAN, M., J., PAUK, Z., KERTESZ (2014): *In vitro* androgenesis of some wheat (*Triticum aestivum* L.) varieties and their F_1, F_2 progenies and combining ability of embryoid production. Progressive Agriculture, *18(2)*: 57-60.

HUSSAIN, B., M.A., KHAN, Q., ALI, S., SHAUKAT (2012): Double haploid production is the best method for genetic improvement and genetic studies of wheat. IJAVMS, *6(4)*: 216-228.

ISLAM, S.M.S. (2010). The role of drought stress on anther culture of wheat (*Triticum aestivum* L.). Plant Tissue Culture & Biotechnology, *20(1)*: 55-61.

KRZEWSKA, M., I., CZYCZYLO-MYSZA, E., DUBAS, G., GOLEBIOWSKA-PIKANIA, I., ZUR (2015): Identification of QTLs associated with albino plant formation and some new facts concerning green and albino ratio determinants in triticale (× *Triticosecale* Wittm.) anther culture. Euphytica, *206*: 263–278.

LANTOS, C., J., WEYEN, J.M., ORSINI, H., GNAD, B., SCHLIETER, V., LEIN, S., KONTOWSKI, A., JACOBI, R., MIHÁLY, S., BROUGHTON, J., PAUK (2013): Efficient application of in vitro anther culture for different European winter wheat (*Triticum aestivum* L.) breeding programs. Plant Breeding, *132*: 149–154.

MARCINIAK, K., Z., KACZMAREK, T., ADAMSKI, M., SURMA (2003): The anther-culture response of triticale line × tester progenies. Cellular & Molecular Biology Letters, *8:* 343-351.

OUYANG, J.W. (1986): Induction of pollen plants in *Triticum aestivum* L. In: HU, H. Y. YANG (eds) Haploids of higher plants *in vitro*. China Academic Publishers, Beijing, p. 26–41.

OZCAN, K., N., ACIKGOZ (1999): Development of a statistical package program for population genetics. In: Third Symposium on Computer Applications in Agriculture, Adana, Turkey.

PAUK, J., R., MIHALY, M., PUOLIMATKA (2003): Protocol for wheat (*Triticum aestivum* L.) anther culture. In: MALUSZYMSKI, M., K. J. KASHA, B. P. FORSTER, I. SZAREJKO (eds), Doubled haploid production in crop plants. Kluwer Academic Publishers, Dordrecht, p. 59-64.

REDHA, A., A., TALAAT (2008): Improvement of green plant regeneration by manipulation of anther culture induction medium of hexaploid wheat. Plant Cell Tissue and Organ Culture, *92:*141-146.

TESTER, M., P., LANGRIDGE (2010): Breeding technologies to increase crop production in a changing world. Science, *327(5967)*: 818-822.

TRIGIANO, R.N., D.J., GRAY (2016): Plant tissue culture, development, and biotechnology. CRC Press.

TYANKOVA, N., N., ZAGORSKA (2008): Factors affecting in vitro androgenesis in cereals. Int. J. Plant Dev. Biol., *2(1):* 59-78.

YILDIRIM, M., B., BAHAR, I., GENC, R., HATIPOGLU, S., ALTINTAS (2008): Reciprocal effects in anther cultures of wheat hybrids. Biologia Plantarum, *52 (4):* 779-782.

YORGANCILAR, O., I., KUTLU, A.,YORGANCILAR, P., UZUN (2015): Anther culture response to different media in F_2 progenies of bread wheat (*Triticum aestivum* L.). J. Tekirdag Agricultural Faculty, *12(2)*: 99-109.

YORGANCILAR, O., I., KUTLU, A., YORGANCILAR, Z., SIREL (2017): Optimization of factors affecting success in anther culture technique for wheat. In: ARAPGIRLIOGLU H., A. ATIK, R. ELLIOTT, E. TURGEON (eds), Researches on science and art in 21st century Turkey, Gece Publishing, Ankara/Turkey, p. 802-814. ISBN:978-605-180-771-3.

ZHUANG, J.J., X., JIA (1980): Studies on the differentiation of pollen calli of wheat. In: Annual report of the institute of genetics. Academia Sinica, Beijing, p. 70-72.

LINIJA × TESTER ANALIZA ODGOVORA KULTURE ANTERA HLEBNE PŠENICE (*Triticum aestivum* L)

Imren KUTLU[1*], Zeynep SIREL[2], Ozcan YORGANCILAR[2], Aysel YORGANCILAR[2]

[1]Eskisehir Osmangazi Univerzitet, Poljoprivredni fakultet, Departman za biosistemski inžinjering, 26160 Eskisehir, Turska
[2]Tranziciona zona poljoprivrednog istraživačkog instituta, 26160 Eskisehir, Turska

Izvod

Cilj ovog rada bio je ispitati efekte gena koji imaju ulogu u nasleđivanju odgovora kulture antera populacija F1 i F2 dobijenih hibridizacijom između sedam duplih haploidnih (DH) linija pšenice i četiri sorte hlebne pšenice korišćenjem linije × tester metoda, kao i da se odrede odgovarajući roditelji i obećavajući hibridi. Jedanaest roditelja i njihovih dvadeset i osam hibridnih kombinacija ispitivano je za androgena svojstva vezana za odgovor kulture antera, kao što je broj kalusa, zelenih, albino, haploidnih, spontanih DH i fertilnih biljaka. Dobijeni podaci su procijenjeni analizom kombinacione sposobnosti izračunatom metodom linije × tester i procenom stepena heritabilnosti. Prema rezultatima, za sva ispitivana svojstva, neaditivni efekti gena su nađeni kao značajni, a heritabilnost u užem smislu bila je na niskom nivou. Utvrđeni su značajni efekti kombinacione sposobnosti kod nekih roditelja i hibrida u pogledu ispitivanih osobina. DH19, DH22 i Harmankaia-99 su identifikovani kao pogodni roditelji za razvoj androgenih osobina, dok su ukrštanja formirana između njih ocenjena kao obećavajuća. Ukupno 444 biljke (142 F1, 302 F2) su prenesene u staklenik od biljaka dobijenih iz kulture antera, a 205 (68 u F1, 137 u F2) su bile fertilne. Od roditelja je dobijeno 90 fertilnih biljaka. Seme 112 DH biljaka dobijenih od hibrida umnoženo je za upotrebu u oplemenjivačkim programima.

Primljeno 18.VIII.2018.
Odobreno 18. V. 2019.