RESEARCH ARTICLE
Journal of Biometry Studies (2024) 4(1): 42-48
DOI: 10.61326/jofbs.v4i1.04
www.biometrystudies.com

Journal of Biometry Studies

PRENSİP
www.prensip.gen.tr

Development of double haploid lines using anther culture method with different F2 combinations

Berrin DUMLU[1], Umran KUCUKOZDEMIR[1], Halit KARAGOZ[1,*]

[1]East Anatolia Agricultural Research Institute, Erzurum/TÜRKİYE

[*]Corresponding author: halit.karagoz@tarimorman.gov.tr

Received: 30/05/2024, Accepted: 21/06/2024

Abstract

Breeders are turning to rapid breeding methods in order to respond to the increasing population for the breeding of the wheat plant, which has a very important place in human nutrition. One of the biotechnological methods to shorten wheat breeding, which takes 15-20 years with classical breeding methods, is double haploid. The aim of this study is to obtain double haploid lines by taking wheat genotypes that have reached the F2 generation after hybridization into anther culture and thus shorten the breeding period. When looking at the response to anther culture of the 15 different F2 combinations used in the study, F2-7 and F2-10 genotypes stood out as the most successful genotypes. It was observed that 4 different groups were formed among 15 genotypes according to the success rate. It was concluded that the success of anther culture varies depending on the genotypes used, the nutrient media and the suitability of laboratory conditions.

Keywords: Wheat, Double haploid, Speed breeding, F2 combination

Please cite this article as follows:

Dumlu, B., Kucukozdemir, U., & Karagoz, H. (2024). Development of Double Haploid Lines Using Anther Culture Method with Different F2 Combinations. *Journal of Biometry Studies*, *4*(1), 42-48. https://doi.org/10.61326/jofbs.v4i1.04

1. Introduction

Wheat, which meets a significant portion of people's daily protein and calorie needs, is the most cultivated plant species in the world and is the most important species that meets the food needs in Turkey. (Kucukozdemir et al., 2020; Kucukozdemir et al., 2021; Kucukozdemir et al., 2023). As of 2022, the wheat cultivation area in our country is approximately 6.6 million hectares, the production amount is 19.7 million tons and the grain yield per unit area is around 298 kg/da (TUIK, 2022; Dumlu, 2023). Wheat yield was 254 kg/da in 2009 (TUIK, 2010). This increase is directly proportional to the registration of high-yield varieties and their introduction into production. As in other plants, the "double haplodization" method, which is the method of obtaining 100% homozygous pure lines in the shortest time by doubling the chromosome numbers of haploid plants, is very important in wheat breeding (Keles et al., 2015). Obtaining pure lines in the classical breeding method requires long processes. Pure lines can be obtained after 10-12 years in foreign pollinated plants and 6-7 years in self-pollinated plants. This period can be reduced to 6-12 months with haploidy methods (Salantur et al., 2011). However, one of the most important bottlenecks in the double haploid method is genotype selection and the unknown success rate of these genotypes. This study was conducted using 15 different F2 genotypes to determine the suitability of these genotypes for the double haploid method and to develop genotypes with superior properties using the double haploid method.

2. Material and Methods

In the study, 15 different hybrid combinations were created and cultured at the F2 stage. Hybrid information of these combinations is given in Table 1.

Table 1. Hybrid information of the F2 genotypes used in the study

F2-1	Unknown/Alparslan
F2-2	Alacris /Alparslan
F2-3	Genessi/Alparslan
F2-4	Savalan/Grk//Pyn/Bau/3/Alparslan
F2-5	Müfitbey/Alparslan
F2-6	Unknown/Plk70//Frtl/3/Alparslan
F2-7	Ayyıldız /Alparslan
F2-8	Esperia/Alparslan
F2-9	Doğu 88/Alparslan
F2-10	Palandöken 97/Alparslan
F2-11	Bezostaya/Alparslan
F2-12	Unknown/Alparslan
F2-13	Unknown/Plk70//Frtl/3/Alparslan*
F2-14	Yugtına/Kauz/3/Agrı/Bjy//Vee/4/Alparslan*
F2-15	F130-L-1-12/5/Lov26//Lfn/Sdy(Es84-24)/3/Serı/4/Serı/6/F6038w12-1/7/Alparslan*

In the greenhouse, from each F2 combination; The pollen found inside the anthers on the spikes of wheat genotypes was taken from 30 spikes in the early-mid univalent (single-nucleated) period (by examining the pollen under binocular). The spikes taken from the greenhouse were placed in a erlenmeyer flask containing water and covered with a nylon bag. These spikes were kept at 4 °C for 14-15 days. After the preliminary cold application, the stems and leaves of the spikes were cleaned, then they were placed in an erlenenmayer containing 1/1 sterile water and 0.5% sodium hypochlorite and shaken for 20 minutes in a way that would not cause physical damage to the spikes. After these procedures, the spikes were washed 3-4 times with sterile water under a sterile cabinet. After the sterilization process, the upper and lower spikelets on the spikes were removed. 90 anthers from the middle spikelets were taken with sterile forceps. Anthers were transferred to previously prepared petri dishes containing MN6 liquid medium. In this study, MN6 liquid medium was used for somatic embryo formation (Chu & Hill, 1988). 90 anthers were placed in each petri dish in 4 replicates. To prevent contamination in the Petri dishes, the dishes were covered with paraffin and left in a dark incubator at 29 °C for 40 days. The formed haploid embryos were transferred to 190-II (Xingzhi & Han, 1984) medium for plant regeneration. Developing plantlets were transferred to test tubes containing 190-II nutrient medium and rooting was ensured. The media and chemical components used in the experiment are given in Table 2. Rooted haploid plantlets were transferred to pots. The roots of the plantlets that developed roots in the pot were washed under water to remove soil. Roots were treated with 0.5% Colchicine for 1 hour.

Table 2. Media and chemical components used in the study

MN6		190-II Cu			
KNO3	1150 mg	KNO_3	100	Pyridoxine HCl	0,5
/NH4/2SO4	100 mg	$/NH_4/_2SO_4$	200	Nicotinik acid	0,5
Ca/ NO3/2 x 4 H2O	100 mg	Ca/ NO_3/2x 4 H_2O	100	Meso-inositol	100
MgSO4 x 7 H2O	125 mg	KH_2PO_4	300	Sakkarose	30 g
KH2PO4	200 mg	$MgSO_4$ x 7 H_2O	200	NAA	0,5
KCl	35 mg	KCl	40	Kinetin	0,5
Fe-Na-EDTA	5 ml	Fe-Na-EDTA	20	$CuSO_4$ x 5 H_2O	0,5
Thiamin-HCl	1 ml	$MnSO_4$ x 4 H_2O	8	Gelrite	3 g
Maltose	80 g	$ZnSO_4$ x 7 H_2O	3	pH	5,7
2,4-D	1,5 mg	H_3BO_3	3		
Kinetin	0,5 mg	Kl	0,5		
Ficoll	100 g	Glycine	2		
pH	5,8	Thiamin-HCl	1		

Parameters examined in the study: *Number of calluses (NC)* refers to the number of calluses formed from anthers taken from each genotype in the study. *Number of green plants (NGP)* refers to the number of green plants obtained from calluses. *Callus formation rate from anther (CFA)* is the percentage expression of the callus consisting of 90 anthers taken per petri dish. *Double haploid index (DHI)* is the percentage expression of green plantlets obtained from calluses. *Success rate (SR)* is the percentage expression of the number of natural and colchicine-grown plants from callus. (Salantur et al., 2011).

The study was designed according to the randomized block trial design with 4 replications. The results of the study were made using the Jump 17 program for variation analysis and were subjected to the LSD multiple comparison test. Heatmapper graphics were made with the heatmapper online program, PCA and Venn graphics were made with the tbtools program, and Dendogram graphics were made with the Srplot online program.

3. Results and Discussion

According to the results of the variation analysis, it was determined that there were statistically significant (p<0.01) differences between the genotypes in the NC, NGP, CFA, DHI and SR parameters (Table 3). In terms of NC, the F2-10 (15.5) genotype received the highest value, while the lowest NC value was determined in the F2-1 (4.5) genotype. The average NC value of the genotypes was 12.03. When we look at the NGP numbers, the highest NGP number was determined in the F2-10 genotype and the lowest NGP number was determined in the F2-12 and F2-13 genotypes. The average NGP numbers of the genotypes were 1.65. While the average CFA of the genotypes was 13.37%, the highest CFA was in the F2-10 (17.22%) genotype and the lowest CFA was in the F2-1 (5%) genotype. The highest DHI value was measured in F2-5 (50.63) and the lowest DHI value was measured in F2-12 (0) and F2-13 (0) genotypes. The average DHI value of the genotypes was 14.37. The highest SR value in the study was determined in the F2-5 (6.67) genotype, and the lowest SR value in the F2-12 (0) and F2-13 (0) genotypes, while the average SR value of the genotypes was 1.83 (Table 3).

In the Heatmapper chart, the colors of the genotypes gradually increase from red to green, and as they approach black, they get closer to the average value. Accordingly, in terms of all applications, F2-10, F2-7 and F2-8 genotypes stood out with higher values than the average. It was observed that while F2-3, F2-14, F2-13, F2-12, F2-15 and F2-11 genotypes had above average values in terms of NC and CFA, they had below average values in terms of NGP, SR and DHI parameters. F2-5 genotype had values close to the average in terms of NC and CFA parameters and was the genotype with the highest values in terms of NGP, SR and DHI (Figure 1).

According to PCA analysis, it was seen that the genotypes were divided into 4 different groups. F2-5 genotype in Group 1, F2-2, F2-3, F2-6, F2-7, F2-8, F2-10 and F2-14 genotypes in Group 2, F2-1, F2-4 in Group 3, and F2-9 genotypes, and F2-11, F2-12, F2-13 and F2-15 genotypes were included in Group 4 (Figure 2).

In the dendogram analysis, similar to the PCA analysis, it was seen that the genotypes were divided into 4 different groups. F2-5 genotype in Group 1, F2-2, F2-3, F2-6, F2-7, F2-8, F2-10 and F2-14 genotypes in Group 2, F2-1, F2-4 in Group 3, and F2-9 genotypes, and F2-11, F2-12, F2-13 and F2-15 genotypes were included in Group 4 (Figure 3).

Venn chart is a type of graph in which the common intersection set of genotypes that stand out in terms of applications is expressed. According to the Venn graph in this study, it is seen that there are two genotypes that stand out in terms of all parameters. These genotypes are F2-7 and F2-10 genotypes (Figure 4).

Table 3. Parameters examined in the study

	NC*	NGP*	CFA*	DHI*	SR*
F2-1	4.50^{f}	0.50^{ef}	5.00^{f}	15.63^{b-e}	0.56^{ef}
F2-2	11.50^{c-e}	1.50^{d-e}	12.78^{c-e}	13.13^{b-e}	1.67^{d-e}
F2-3	14.00^{a-c}	2.00^{b-d}	15.56^{a-c}	13.95^{b-e}	2.22^{b-d}
F2-4	8.50^{de}	0.50^{ef}	9.44^{de}	5.00^{d-f}	0.56^{ef}
F2-5	12.00^{bc}	6.00^{a}	13.33^{bc}	50.63^{a}	6.67^{a}
F2-6	11.75^{b-d}	1.75^{cd}	13.06^{b-d}	16,39^{b-d}	1.94^{cd}
F2-7	14.25^{a-c}	3.00^{b}	15.83^{a-c}	21.40^{bc}	3.33^{b}
F2-8	12.50^{a-c}	2.75^{bc}	13,89^{a-c}	22.74^{b}	3.06^{bc}
F2-9	8.25^{e}	1.50^{d-e}	9.17^{e}	19.58^{bc}	1.67^{d-e}
F2-10	15.50^{a}	3.00^{b}	17.22^{a}	19.81^{bc}	3.33^{b}
F2-11	13.25^{a-c}	0.50^{ef}	14.72^{a-c}	3.57^{ef}	0.56^{ef}
F2-12	15.00^{ab}	0.00^{f}	16.67^{ab}	0.00^{f}	0.00^{f}
F2-13	13.50^{a-c}	0.00^{f}	15.00^{a-c}	0.00^{f}	0.00^{f}
F2-14	12,75^{a-c}	1.25^{d-e}	14.17^{a-c}	9.83^{c-f}	1.39^{d-e}
F2-15	13.25^{a-c}	0.50^{ef}	14.72^{a-c}	3.71^{ef}	0.56^{ef}
Mean	12.03	1.65	13.37	14.37	1.83

*Means shown with different letters are statistically significantly different from each other according to the p<0.01 significance level in the LSD test.

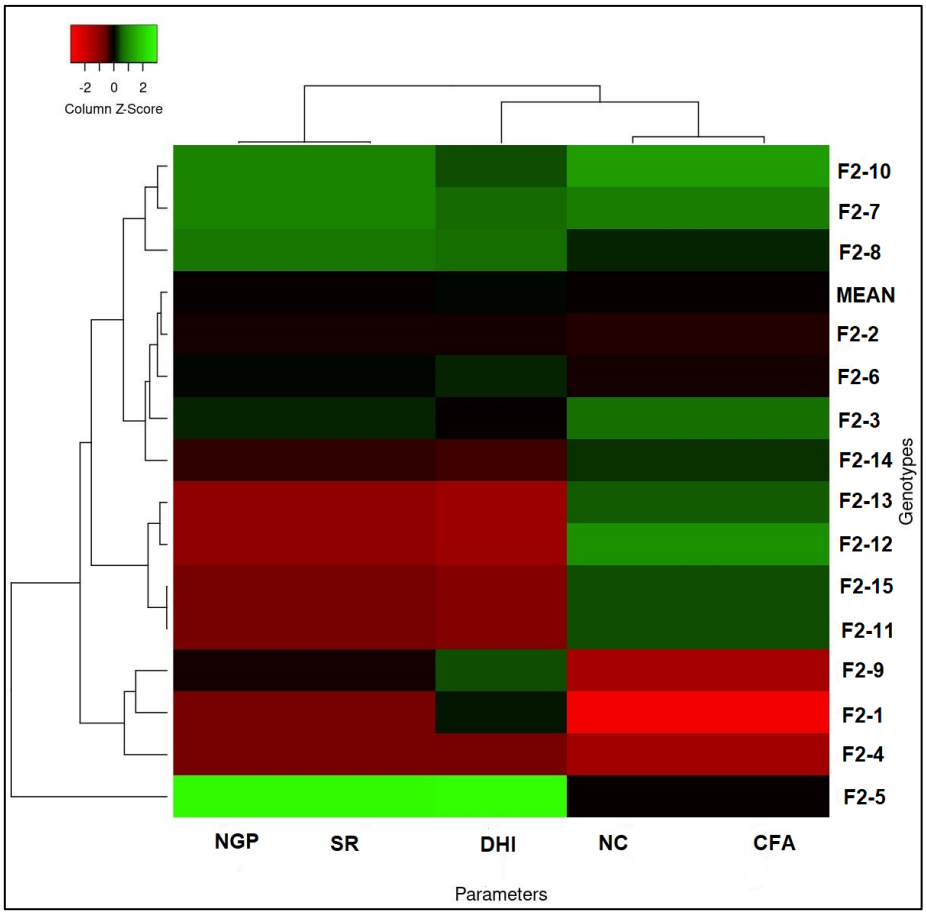

Figure 1. Heatmapper chart

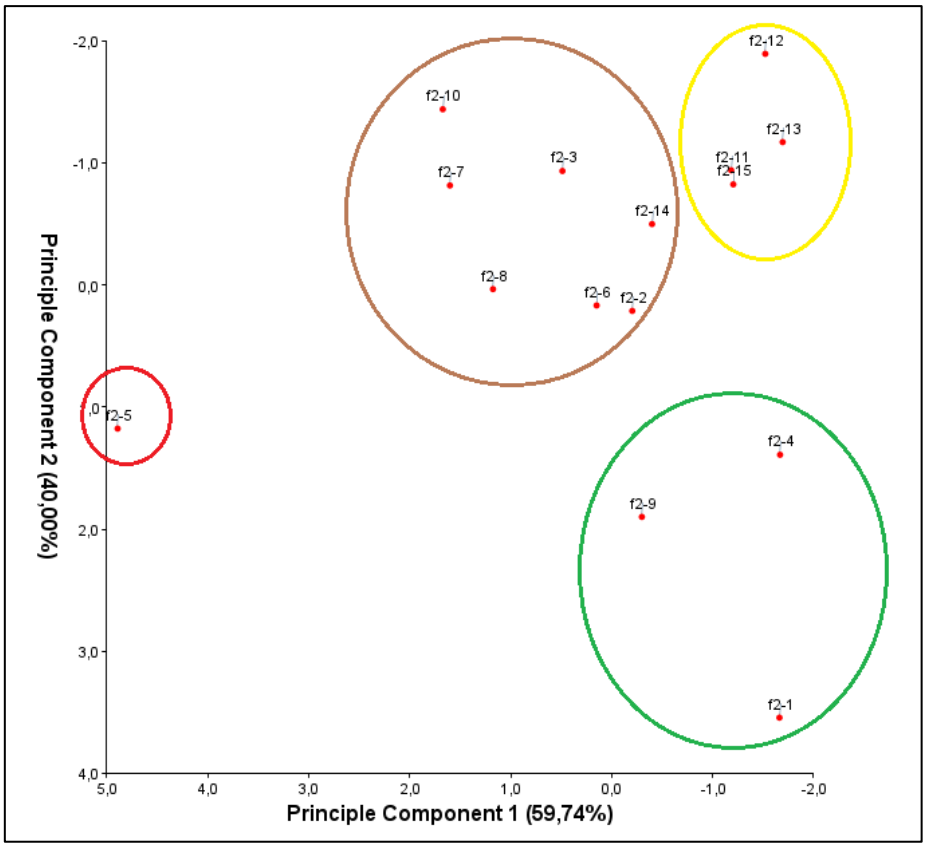

Figure 2. Principal component analysis

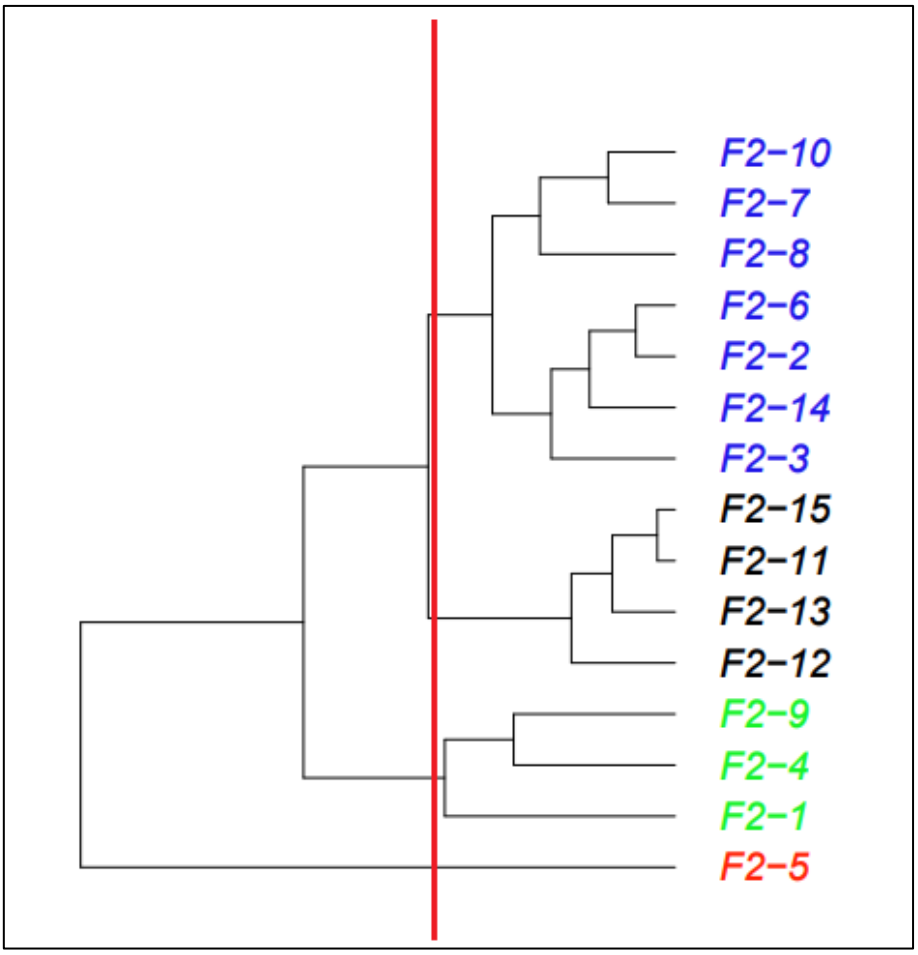

Figure 3. Dendrogram graph of 15 different genotypes

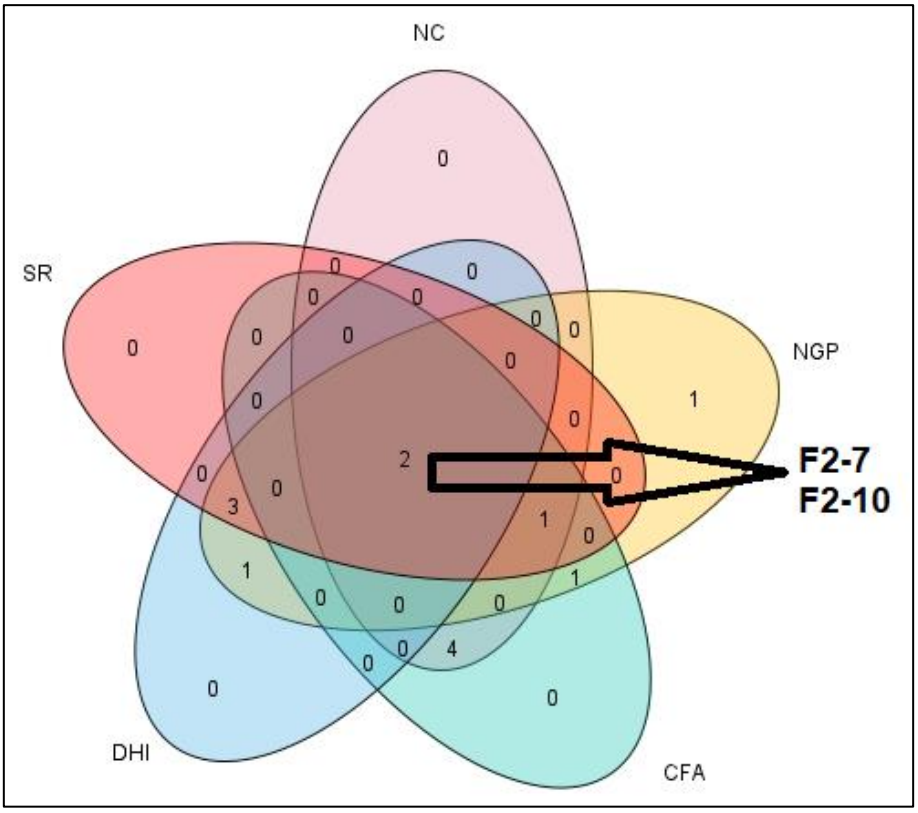

Figure 4. Venn chart of 15 different genotypes

4. Conclusion

In this study, the success rate obtained from anther culture varied depending on the genotypes. F2-7 and F2-10 genotypes in the study stood out as the most successful genotypes. Table 1, Figure 1 and 4). As a matter of fact, Szakacs et al. (1989) stated that despiegh the success of anther culture varies depending on the genotype; They reported that factors such as callus formation, plant regeneration, and the amount of double haploid plants success. In a study, it was stated that the rate of double haploid plants varies depending on not only the genotype but also the induction environment. (Orshinsky & Sadasivaiah, 1994). In another study, it was stated that pre-cold application increased the amount of naturally double haploids. (Pauk et al., 2003). Additionally, Bajaj (1990) reported that many factors such as the growing conditions of donor plants in anther culture, genotype, pre-applications to flower buds, structure and composition of the nutrient medium, and incubation conditions affect the success rate in anther culture. However, most researchers have stated that the biggest problem for the production of double haploid plants is still genotype dependency. (Barbanas, 2003; Inagaki, 2003; Zamani et al., 2003; Özü, 2006; Ahmet & Adak, 2007). In our study, the formation of 4 different groups among 15 genotypes according to the success rate is a good indicator of how success depends on the characteristics of the genotype. It is thought that hybrids made from Palandöken 97 and Ayyıldız varieties respond better to anther culture and can be used as parents. As a result, in order to achieve success in the double haploid method, which shortens the process in wheat breeding, which requires many years and labor, and saves time and labor for breeders, it is necessary to conduct studies on more genotypes and determine properties of this genotypes.

Acknowledgement

This article was produced from the Eastern Anatolian Wheat Breeding Research Project. We thank the General Directorate of Agricultural Research and Policy for supporting this project.

Conflict of interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

References

Ahmet, H., & Adak, M. S. (2007). Callus Induction and Plant Regeneration in Some Iraqi Common Wheat Varieties. *Journal of Agricultural Sciences*, *13*(3), 285-292.

Bajaj, Y. P. S. (1990). *Haploids in crop improvement I*. Springer-Verlag.

Barbanas B. (2003). Protocol for producing doubled haploid plants from anther culture of wheat (*Triticum aestivum* L.). In M. Maluszynski, K. J. Kasha, B. P. Forster, & I. Szarejko (Eds.), *Doubled Haploid Production in Crop Plants* (pp. 65-70). Springer.

Chu, C. C. & Hill, R. D. (1988). An Improved Anther Culture Method for Obtaining Higher Frequency of Pollen Embryoids in *Triticum aestivum* L. *Plant Science*, *55*(2), 175-181. https://doi.org/10.1016/0168-9452(88)90174-4

Dumlu, B. (2023). *Evaluation of Kırik wheat populations in terms of cold resistance, some agricultural features and quality* [PhD dissertation, Atatürk University]. (in Turkish)

Inagaki, M. N. (2003). Doubled haploid production in wheat through wide hybridization. In M. Maluszynski, K. J. Kasha, B. P. Forster, & I. Szarejko (Eds.), *Doubled Haploid Production in Crop Plants* (pp. 53-58). Springer.

Keles, D., Pinar, H., Ata, A., Taskin, H., Yildiz, S. & Buyukalaca, S. (2015) Effect of Pepper Types on Obtaining Spontaneous Doubled Haploid Plants via Anther Culture. *HortScience*, *50*(11), 1671-1676. https://doi.org/10.21273/HORTSCI.50.11.1671

Kucukozdemir, U., Dumlu, B., Yalcin, Z., & Karagoz, H. (2020). Determination of the Yieldy and Cold Hardiness Characteristics of Some Bread Wheat (*Triticum aestivum* L.) Genotypes in Aqueous Conditions. *Anatolian Journal of Biology*, *1*(1), 12-15.

Kucukozdemir, U., Dumlu, B., Karagoz, H., & Yilmaz, O. (2021). Determination of Yield, and Cold Hardiness of Some Triticale (xTriticosecale Wittmack) Genotypes in Eastern Anatolia Region. *Journal of Agricultural Production*, *2*(1), 26-31. https://doi.org/10.29329/agripro.2021.344.4

Kucukozdemir, U., Dumlu, B., Yilmaz, O., Turkoglu, A., Karagoz, H., & Kizilgeci, F. (2023). A Study On the Determination of Cold Resistance of Barley Genotypes with Different Cold Acclimatization Different Times and Growing Nature. *Fresenius Environmental Bulletin*, *32*(6), 2452-2459.

Orshinsky, B. R., & Sadasivaiah, R. S. (1994). Effects of Media on Embryoid Induction and Plant Regeneration from Cultured Anthers of Soft White Spring Wheats (*Triticum aestivum* L.). *Plant Science*, *102*(1), 99-107. https://doi.org/10.1016/0168-9452(94)90023-X

Özü, M. E. (2006). *Effect of growing conditions of donor plants and different incubation temperatures on*

haploid plant regeneration from anthers of bread wheat [Master's thesis, Çukurova University]. (in Turkish)

Pauk, J., Mihaly, R. & Puolimatka, M. (2003). Protocol for wheat (*Triticum aestivum* L.) anther culture. In M. Maluszynski, K. J. Kasha, B. P. Forster, & I. Szarejko (Eds.), *Doubled Haploid Production in Crop Plants* (pp. 59-64). Springer.

Salantur, A., Yazar, S., Dönmez, E., & Akar, T. (2011). Determination of the Response of Winter Bread Wheat F2 Populations to Plant Regeneration in Anther Culture. *Journal of Field Crops Central Research Institute*, *20*(1), 15-21.

Szakacs, E., Kovacs, G., Pauk, J. & Barbanas, B. (1989). Substitution Analysis of Callus Induction and Plant Regeneration from Anter Culture in Wheat (*Triticum aestivum* L.). *Plant Cell Reports*, *7*, 218-222.

TUIK, (2010). Agricultural structure production, price, value. Turkish Statistical Institute, Ankara.

TUIK, (2022). Agricultural structure production, price, value. Turkish Statistical Institute, Ankara.

Xingzhi, W., & Han, H. (1984). The Effect of Potato II Medium for Triticale Anther Culture. *Plant Science Letters*, *36*(3), 237-239. https://doi.org/10.1016/0304-4211(84)90175-5

Zamani, I., Gouli-Vavdinoudi, E., Kovacs, G., Xynias, I., Roupakias, D., & Barbanas, B. (2003). Effect of Parental Genotypes and Colchicine Treatment on the Androgenic Response of Wheat F1 Hybrids. *Plant Breeding*, *122*, 314-317. https://doi.org/10.1046/j.1439-0523.2003.00866.x