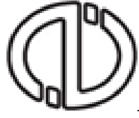ANADOLU ÜNİVERSİTESİ

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ARAŞTIRMA MAKALESİ / **RESEARCH ARTICLE**

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UTILIZATION OF ANTHER CULTURE FOR SCREENING OF DOUBLED HAPLOID LINES IN SOME TURKISH BREAD WHEAT HYBRIDS

ABSTRACT

In the current research, it is aimed to investigate the doubled haploid (DH) productivity of 30 F_2 derived wheat crosses constructed after hybridization of two Turkish bread wheat donors with different bread wheat genotypes, Sonmez01 and Es26. Especially, donor parents carrying out high yield performance and disease resistance selected from new developed bread wheat genotypes. Overall plant material sowed to the Transitional Zone Agricultural Research Institute field on 2010-2011 season, and an average of 40 spikes at suitable development stage harvested from each group. Under anther culture conditions, MN6 used as initial culture media and modified 190-II choosed as regeneration media. Callus obtained from two thirds of F_2 hybrids and they generated 16 albino plantlets. Extended ratios of callus production ranged between 3.25% and 50.5%. However green plantlet regeneration percentage calculated as 6.25% only in one hybrid, seven hybrid wheat samples generated only 0.25% green plantlet. According to overall results, five new DH wheat formed from different F_2 hybrids derived from Sonmez01, while there were any dihaploid plants observed in F_2 hybrids derived from Es26. In the sum, genome and phenome based evaluation of these F_2 derived five doubled haploid bread wheat hybrids can help to describe an extra population source for next generation breeding platforms that they designed to extract new resistant crops against to environmental stresses. Also, modified anther culture method used in this study might be employed for screening of other bread wheat cultivars, lines and registered genotypes in the future DH wheat population construction.

Keywords: Anther culture, Albino, Bread wheat, Callus, Doubled haploid, Hybrid.

BAZI TÜRK EKMEKLİK BUĞDAY MELEZLERİNDE KATLANMIŞ HAPLOİD HATLARIN TARANMASI İÇİN ANTER KÜLTÜRÜNÜN KULLANIMI

ÖZ

Mevcut çalışmada, iki Türk ekmeklik buğday çeşidinin (Sönmez01 ve Es26) farklı ekmeklik buğdaylarla melezlenmesi sonrası oluşturulmuş 30 F_2 kökenli buğday çaprazının katlanmış haploid (DH) verimliliğini araştırmak amaçlanmıştır. Özellikle verici ebeveynler yüksek verimli ve hastalıklara dirençli yeni geliştirilmiş ekmeklik buğday genotiplerinden seçilmiştir. Tüm bitki materyali 2010-2011 sezonunda Geçit Kuşağı Tarımsal Araştırma Enstitüsü arazisine ekilmiş ve her gruptan gelişimin uygun

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evresinde ortalama 40 başak hasat edilmiştir. Anter kültürü koşulları altında, MN6 başlangıç besiyeri olarak kullanılmış, 190-II rejenerasyon besiyeri olarak seçilmiştir. F_2 melezlerinin üçte ikisinden kallus elde edilmiş ve bunlar 16 albino bitkicik üretmişlerdir. Kallus üretiminin genişletilmiş oranları %3.25 ile %50.5 arasında değişmiştir. Yedi melez buğday örneği sadece %0.25 yeşil bitkicik üretmiş, ancak sadece bir melezde yeşil bitkicik rejenerasyon yüzdesi %6.25 olarak hesaplanmıştır. Es26'dan kökenlenmiş F_2 melezlerinde hiç katlanmış haploid bitki gözlenmemişken, tüm sonuçlara göre, beş yeni katlanmış haploid (DH) buğday Sönmez01'den kökenlenmiş farklı F_2 melezlerinden oluşmuştur. Özetle, F_2 kökenli bu beş katlanmış haploid ekmeklik buğday melezinin genom ve fenom temelli değerlendirilmesi, çevresel streslere karşı dayanıklı yeni bitki seçmede yeni nesil ıslah platformları için dizayn edilmiş ilave bir populasyon kaynağı tanımlamaya yardımcı olabilir. Ayrıca, bu çalışmada kullanılmış modifiye anter kültürü metodu, gelecekte diğer ekmeklik buğday çeşitleri, hatları ve tescilli genotipler için katlanmış haploid (DH) ekmeklik buğday populasyonu oluşturulmasında kullanılabilir.

Anahtar Kelimeler: Anther kültürü, Albino, Ekmeklik buğday, Kallus, Katlanmış haploid, Hibrid

1. INTRODUCTION

Enhancement of agricultural production is possible by increasing both productive lands and crop yield in the field per unit. Recently, it is not fully probable to increase agricultural areas in the world. According to the FAO (2004) reports, lands, reserved for agricultural activities, showed a decrease about 500.000 hectar between the years of 1990-2000. From this point of view, enhancement of productivity will only be possible by increasing the yield derived from per field unit. In Turkey, wheat production and yield showed differences from year to year as a result of several environmental changes. Also, decreasing wheat sowing areas that were measured as 67.58 million decare in the year of 2011 and 63.39 million decare in 2012 can be one of the evidences for this limitation by drawing a slope for wheat productivity (TUIK 2012). In addition, traits related to wheat quality are strictly fluctuated by genotype X environment interactions (Altay 2012; Mohammed 2009). Availability of diverse plant breeding technics promoted a set of tools for producing high quality and homozygous crops having adaptive traits with an accelarated pattern. Thus, these efforts will be the steps for sustainable feeding of future generations (Redden 2013).

Doubled haploidy is a key method for sufficiently obtaining homozygous populations without needing to multiple crosses between subsequent progenies. On the other hand, elimination of meiotic crossing-over in haploid microspore cells, results with a production of homozygous plant in anther culture. Thus, each plant in the regenerated population will carry the fixed gene in a recessive or dominant form that helps to select the more suitable one (Murovec and Bohanec 2012). In addition, microspore cells have totipotent nature that is very important to generate whole plant. Before doubled haploid plant selection, regeneration of immature haploid microspore cells is required (Ferrie and Caswell 2011). In a second approach, crossing with wild species like *Hordeum bulbosum* in barley or pollinating wheat with maize have been approved as two useful models for haploid crop production. Then, colchicine is routinely applied for doubling the chromosome number in regenerated haploid genome (Islam 2010). This basic pipeline has been used for enabling true breeding application in several plant species from apple to wheat (De Witte and Keulemans 1994; Chauhan and Khurana 2011). Recently, the efficiency of doubled haploid plant production has been started to test with molecular biology technics such as enzymatic mismatch cleaveage (Hofinger et al. 2013). With these features, double haploid crops are significant modulators for agriculture and they can be effectively used in the studies of gene mapping, marker/trait association research, finding dense QTL locations and genomics.

Since 1970s, intensive studies have been completed in the field of anther culture and its applications in wheat (Henry and De Buysert 1985; Hatipoglu et al. 1998; Sorrells et al. 2011; Lantos et al. 2013: Yorgancilar et al. 2013). However, anther culture has been accepted as a well established method, it is still complex procedure due to the limited access of doubled haploid plants. To see how double haploidy is used in agricultural practice, delivering some registered doubled haploid wheat genotypes can be accepted as the proofs of these efforts. For example, one wheat variety with a name tag "Florin" was developed in France. Also, two different wheat varieties commercially released from China with the names of "Jinghua No1" (Hu et al. 1983) and "764" (Hu et al. 1988) respectively. After a short period of time, Pauk et al. (1995) announced cultivar "GK Delibab" that

was developed by anther culture method and it has been started to use in active farming by Hungarian farmers. So, double haploidy, either it is performed by anther culture or using maize pollinators (Laurie and Bennett 1988), has been combined as an alternative approach to make homozygous lines in germplasm breeding. In addition, anther culture has been provided to save time at least 4-5 years as compared to classical breeding methods in crops. In self pollinating plants, cultivar breeding time can be shortened as 3-4 years. Clapham (1973) extensively accommodated anther culture as a method of obtaining haploid crops at one generation. Barloy et al. (1989) observed some high and low level of responses to the anther culture in dihaploid hybrid lines. Also, Hatipoglu et al. (1994) found that genotype derived effects shaped the callus production, green plantlet development in bread wheat under anther culture conditions. This was also approved by the study that has conducted to understand the genotype and nutrient media effects on anther culture in bread wheat (Baser et al. 1999). Under dynamic plant breeding environments and changing climatic conditions, we have still to need to investigate the responses of development of new crops under anther culture.

In this study, it is aimed to investigate the doubled haploid productivity of 30 F_2 derived wheat crosses constructed after hybridisation of two Turkish bread wheat donors, Sonmez01 and Es26. Hence, doubled haploid wheat availability and anther culture responses will be tested at variety and line based level in F_2 wheat samples.

2. MATERIAL and METHODS

2.1. Plant Material

In the present work, different 30 F_2 derived hybrid combinations were constructed by using Sonmez01 and ES26 genotypes as donor parental lines. Pedigree of these crosses listed in Table 1. Sonmez and ES26 genotypes were originally registered Turkish bread wheat cultivars.

2.2.Harvest and Preparation of Hybrid Wheat Spikes

In the early-to-mid-uninucleate period, nearly 40-50 spikes were collected from every wheat combination and examined according to their microspore structure under microscope. Spikes ensuring the above mentioned criteria collected from field, and placed in water filled flasks and deposited in polyethylene bags for cold pretreatment at 4 °C over 14 days. Then, spikes were separated from leaves and stalks, then these spikes were put into 250 ml flasks containing sterile water and 2% sodium hypochloride. Spikes were shaked by hand and waited for 20 minutes to make surface sterilization and washed 4-5 times with sterile water under laminar airflow workstation (Thermo Scientific). After sterilization, both top and down spikelets of spikes were removed.

2.3. Anther Culture Conditions

Anthers derived from spikelets in the middle position of spikes were transferred to the steril petri dishes (60x10 mm) containing MN6 nutrient media (Han and Hongyuan 1986) Table 2. Approximately, 100 anthers were placed in each petri dish and all groups were replicated at four times. Petri dishes were covered with parafilm to prevent contamination and they were incubated at 28°C in dark period. Callus were transferred into 190-II nutrient media (Zhuang and Jia 1983) for regeneration of green plantlets (Table 2). Petri dishes were put into plant growth chambers (BINDER KBW-400 and NUVE TK-252) for obtaining the vegetative plant parts at 25°C in 16 h light and 8 h dark period and observations performed daily to follow the plant development. After 30 days, root and and shoot tissue emerged samples were transferred into the test tubes contained 190-II Cu (rooting medium) regeneration media. Albino plantlets were determined and seperated. Samples showing proper root and shoot development were incubated in plant growth chambers at 8°C/16 h day and at 4°C/8 h dark over 6 weeks for vernalization. Seedlings were transferred to soil filled pots (Fig. 1) and incubated at 16°C in 16 h light and 8 h dark period over 2 weeks for adaptation. Ploidy level has been determined by examining the size of stomata under the microscope. While spontaneous diploid seedlings were directly transferred to greenhouse, the haploid plants were subjected to chromosome doubling via colchicine (2%) and DMSO (2%) application. These plants were transferred to greenhouse like diploid plants until seed maturation (Fig. 2).

Table 1. List of bread wheat samples used in the field trials TZARI (Transitional Zone Agricultural Research Institute)

F_2 ID	***Pedigree***
1	CBR/5133//MT/3/KKC/4/LFN//ND/2*P101/5/N057/PEX/6/KREMANA/LOV/7/SONMEZ01
2	BEZ2B/CGN//VRZ/3/SONMEZ01
3	GALAHAD/*2OROVCHANKA LS172//SONMEZ01
4	MV17//KREMENA/LOV29/3/KATEA-1/4/SONMEZ01
5	OKTYABRINA70/SONMEZ01
6	TX73V203*3/AMIGO//SONMEZ01
7	TAST/PCH//BEZ2B/CGN/3/SONMEZ01
8	EKG15//TAST/SPRW/3/2*ID800994.W/VEE/4/SONMEZ01
9	TRK13 RESEL//TRAP#1/BOW/3/SONMEZ01
10	AGRI/BJY//VEE/6/SN64//SKE/2*ANE/3/SX/4/BEZ/5/SERI/7/F10S-1/8/SONMEZ01
11	58.182/DRC//SPN/3/KATIA/4/BJNC47/5/TSI/VEE//2*TRK13/6/SONMEZ01
12	15.99/SONMEZ01
13	YMH/HYS//HYS/TUR3055/3/DGA/4/VPM/MOS/5/5/TAM200/KAUZ/6/ SONMEZ01
14	F10S-1//STOZHER/KARL/3/SONMEZ01
15	ERYT1620.91 (OD120/YUBILEJNAYA75)//2*MV17/3/SONMEZ01
16	KONYA2002/SONMEZ01
17	BOW/NKT/7/WRM/4/FN/3*TH//K58/2*N/3/MY54/N10B//AN/5/PEL 72380/ATR71/6/KVZ/CGN//GLE/8/SONMEZ01
18	FILIN/SABRE//2*BEZ1/3/SONMEZ01
19	KOSAVA/BOKA//SONMEZ01
20	ZITNICA/GK KALASZ//SONMEZ01
21	F12.71/COC/KAUZ//ALP01/3/SONMEZ01
22	TX71A039-V1*3*/AMI/3/BEZ/NAD//KZM/4/KIRAC/5/SONMEZ01
23	SOM-6//CA8055/GRK/3/SONMEZ01
24	AYTIN98/3/AGRI/BJY//VEE/4/SONMEZ01
25	AGRI/NAC//MLT/3/SOM-6/4/SULTAN95/5/SONMEZ01
26	ANKARA093-44//BEZ1/KRC66/3/SONMEZ01
27	BEZOSTAYA1/3/AUS GS50AT34/SUNCO//CUNNINGHAM/4/SONMEZ01
28	BAYRAKTAR2000/MUFITBEY//ES26
29	TAST/PREW//ZAR/3/MUFITBEY/4/ES26
30	MUFITBEY/3/AUS GS50AT34/SUNCO//CUNNINGHAM/4/ES26

Table 2. Composition of MN6 and 190-II nutrient media.

MN6	**Amount**	**190-II Cu**	**Amount**
KNO_3	1150 mg/L	KNO_3	100 mg /L
$(NH_4)_2SO_4$	100 mg/L	$(NH_4)_2SO_4$	200 mg/L
$Ca(NO_3)_2.4H_2O$	100 mg/L	$Ca(NO_3)_2.4H_2O$	100 mg/L
$Ca(NO_3)_2.2\ H_2O$	80 mg/L	KH_2PO_4	300 mg/L
$MgSO_4.7\ H_2O$	125 mg/L	$MgSO_4.7H_2O$	200 mg/L
KH_2PO_4	200 mg/L	KCl	40 mg/L
KCl	35 mg/L	Fe.NaEDTA	20 mg/L
Fe.NaEDTA	5 ml/L	$MnSO_4.4H_2O$	8 mg/L
Thiamin-HCl	1 ml/L	$ZnSO_4.7H_2O$	3 mg/L
Maltose	80 g/L	H_3BO_3	3 mg/L
Potato extract	100 ml/L	Kl	0.5 mg/L
2,4-D	1.5 mg/L	Glycine	2 mg/L
Kinetin	0.5 mg/L	Thiamin-HCl	1 mg/L
Ficoll	100 g/L	Pyridoxine HCl	0.5 mg/L
Gelrite	-	Nicotinic acid	0.5 mg/L
pH	5.8	Meso-inositol	100 mg/L
		Saccarose	30 g /L
		NAA	0.5 mg/L
		Kinetin	0.5 mg/L
		$CuSO_4.5H_2O$	0.5 mg/L
		Gelrite	3 g/L
		pH	5.7

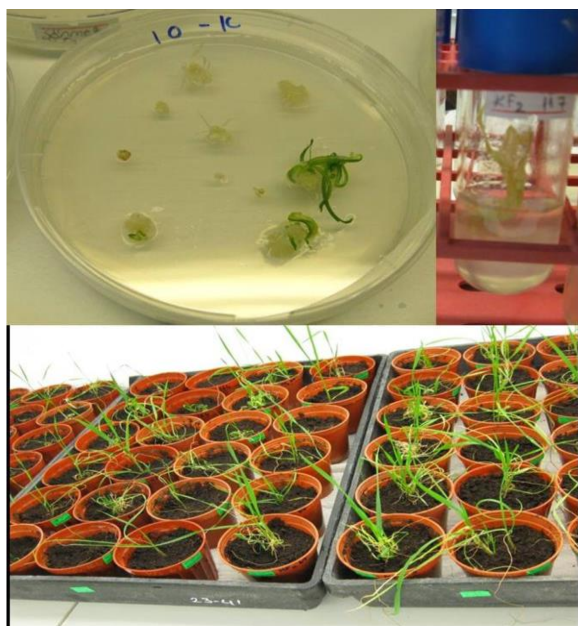

Figure 1. Anther culture pipeline from green plantlet to seedling stage of haploid plants in small pots.

Figure 2. Doubled haploid wheat spikes derived from Sonmez01 donor at maturation phase.

3. RESULTS and DISCUSSION

In this research, a total of 12000 anthers belonging to 30 different wheat hybrids were examined according to their anther culture responses, haploid and doubled haploid plant productivity. In Table 3, production of total plantlets ranged from 1 to 25 and the highest plantlet number noted in "ERYT1620.91(OD120/YUBILEJNAYA75)//2 *MV17/3/SONMEZ01" F_2 hybrid. While there were no calli produced in the following bread wheat hybrids that were indicated with the numbers of 3, 12, 13, 15, 18, 24, 26, 27, 29, 30. Rate of callus production changed between the range of 3.25-50.5% for the remaining F_2 hybrids. Ekiz and Konzak (1997) observed the effects of light and dark on callus production in spring wheat genotypes and defined the most effective light application time and intensity. Beside, some plant growth regulators such as 2,4 D and BAP have inductive effects on callus production in some wheat genotypes (Can et al. 2002; Mehmood et al. 2013). Also, environmental stresses such as salt and heat less affected callus production in wheat cultivar called as "Mahon-Demia" than other wheat "Hidhab" and there were differences observed for two wheat cultivars at high salt concentration (Benderradji et al. 2012). Different tissues has been used to produce callus in several studies (Haliloglu 2006; Turhan and Baser 2004: Tuvesson et al. 2008). Most of the studies tended to use anthers due to the high regeneration efficiency (Dogramaci et al. 2001; Cistué et al. 2009; Grauda et al. 2010).

According to our results, albino plantlets observed in 15 hybrids between the percentage of 0.25-4.25 and green plantlet regeneration calculated as 0.25-6.25% in 5 hybrids. In a recent research of Lantos et al. (2013), genotype could be able to effective on albino plantlet regeneration. One of the F_2 derived bread wheat hybrid, "TAST/PCH//BEZ2B/CGN/3/SONMEZ01" (F_2 ID7) produced four haploid plants from four green plantlets. On the other hand, F_2 hybrid KONYA2002/SONMEZ01 (F_2 ID16) resulted the most abundant green plantlet number that was counted as 25, and this hybrid also provided three haploids and one doubled haploid plant. The maximum haploid plant number (4) detected in F_2 ID7 wheat line and there were any doubled haploid plant derived from this hybrid. The highest number of callus detected as 202 in "ZITNICA/GK KALASZ//SONMEZ01" (F_2 ID20) hybrid combination and from this group, 12 albino, 16 green plantlets and 2 haploid plants were obtained. After colchicine treatment, one of these haploid plants converted into the form of doubled haploid wheat. Also, callus number of the following

"F12.71/COC/KAUZ//ALP01/3/SONMEZ01" (F_2 ID21) hybrid group was counted as 182, and one doubled haploid plant also obtained from the same set after colchicine application. Two F_2 hybrids, "TAST/PCH//BEZ2B/CGN/3/SONMEZ01", "BAYRAKTAR2000/MUFITBEY//ES26", produced 13 callus that were also represented the lowest number of callus during anther culture application. Other doubled haploid plants observed in "AGRI/NAC//MLT/3/SOM6/4/SULTAN95/5/SONMEZ01" and "EKG15//TAST/SPRW/3/2*ID800994.W/VEE/4/SONMEZ01" hybrids. Producing doubled haploids ensures stable, homozygous plant material for map construction and marker identification. As an exception, non-haploids may arise from; somatic tissue of anther walls, fusion of nuclei, endomitosis within the pollen grain, irregular microspores (Sunderland and Dunwell 1977).

In this study, an average of 1.46 green plantlets calculated per 100 anther and doubled haploid index changed between 4-50%. Salantur et al. (2011) obtained 1.11 green plantlet per 100 anther and doubled haploid index as 33.3-70.6% in some winter bread wheat population. Hybrids having high regeneration rates for green plantlet production might be beneficial bread wheat sources. To extend the use of haploidy technic, obtaining green plantlets and increasing regeneration ability of plants is important for calli production. Our results concordant with Hatipoglu et al. (1994) and Korkut et al. (2001) and these findings also showed the importance of selection of beginning plant material that were placed as donors in anther culture studies.

Briefly, regenerated green plantlet percentage was calculated as 16.6% in overall hybrids and four of them were belong to the Sonmez01 derived F_2 wheat material, while there were only one ES26 derived F_2 plant (Table 3). A total of 17 haploid plants, that were originated nine different hybrids, generated five DH wheat plants that were also transferred into soil for following their growth till seed maturation (Fig. 2). There were any response from ten different hybrid groups that were containing 2 of ES26 and 8 of Sonmez01 derived F_2 hybrids. Significantly, callus regeneration observed in the remaining 20 F_2 hybrids. These results showed a wide variation in response to the anther culture among all F_2 derived wheat samples.

Table 3. Callus, albino, green plantlet and doubled haploid plant numbers of some Turkish bread wheat genotypes.

F_2 ID	**Number of ınoculated anthers**	**Regenarated callus number**	**Albino plantlet number**	**Green plantlet number**	**Haploid plant number**	**Diploid plant number**
1	400	60	5	1	-	-
2	400	36	-	1	-	-
4	400	104	1	3	1	-
5	400	15	-	7	-	-
6	400	45	4	1	-	-
7	400	13	-	4	4	-
8	400	93	2	7	1	1
9	400	63	2	2	-	-
10	400	67	7	3	-	-
11	400	29	1	1	1	-
14	400	45	8	1	-	-
16	400	82	6	25	3	1
17	400	28	-	1	-	-
19	400	48	2	3	-	-
20	400	202	12	16	2	1
21	400	182	17	22	1	1
22	400	82	7	4	-	-
23	400	111	4	12	2	-
25	400	40	3	2	2	1
28	400	13	-	1	-	-
29	400	No response				
30	400	No response				
3	400	No response				
12	400	No response				
13	400	No response				
15	400	No response				
18	400	No response				
24	400	No response				
26	400	No response				
27	400	No response				

4. CONCLUSION

It is obviously determined that both donor plant material should give positive effects to the anther culture, and reciprocal effects of genotypes under culture conditions should be tested to produce more feasible doubled haploid plant population for future research. So, using large population size and anther sources might reduce the genotype based fluctuations by enlighting the behaviour of each individual plant under anther culture. In this study, crosses including Sonmez01 as donor Turkish bread wheat cultivar, draw a distinct profile under the anther culture conditions when it was compared to the other wheat cultivar Es26. Under dynamic plant breeding environments and changing climatic conditions, we have still to need to investigate the responses of new developed crops under anther culture.

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