ISSN : 1302-7050

Namık Kemal Üniversitesi
Tekirdağ Ziraat Fakültesi Dergisi
Journal of Tekirdag Agricultural Faculty

An International Journal of all Subjects of Agriculture

Cilt** / Volume: **12 ***Sayı** / Number: **2*** ***Yıl** / Year: **2015***

Sahibi / Owner

Namık Kemal Üniversitesi Ziraat Fakültesi Adına
On Behalf of Namık Kemal University Agricultural Faculty

Prof.Dr. Ahmet İSTANBULLUOĞLU
Dekan / Dean

İndeksler / Indexing and abstracting

CABI tarafından full-text olarak indekslenmektedir/ Included in **CABI**

DOAJ tarafından full-text olarak indekslenmektedir / Included in **DOAJ**

EBSCO tarafından full-text olarak indekslenmektedir / Included in **EBSCO**

FAO AGRIS Veri Tabanında İndekslenmektedir / Indexed by **FAO AGRIS Database**

INDEX COPERNICUS tarafından full-text olarak indekslenmektedir / Included in **INDEX COPERNICUS**

TUBİTAK-ULAKBİM Tarım, Veteriner ve Biyoloji Bilimleri Veri Tabanı (TVBBVT) Tarafından taranmaktadır / Indexed by **TUBİTAK-ULAKBİM** Agriculture, Veterinary and Biological Sciences Database

Yazışma Adresi / Corresponding Address
Tekirdağ Ziraat Fakültesi Dergisi NKÜ Ziraat Fakültesi 59030 TEKİRDAĞ
E-mail: ziraatdergi@nku.edu.tr
Web adresi: http://jotaf.nku.edu.tr
Tel: +90 282 250 20 00

ISSN: 1302–7050

Tekirdag Ziraat Fakültesi Dergisi / Journal of Tekirdag Agricultural Faculty 2015 12(2)

İ Ç İ N D E K İ L E R / C O N T E N T S

Anther Culture Response to Different Media in F_2 Progenies of Bread Wheat (*Triticum aestivum* L.)

O. Yorgancilar[1] I. Kutlu[2*] A. Yorgancilar[1] P. Uzun[1]

[1]Transitional Zone Agricultural Research Institute, Biotechnology Division, Karabayir Bagları 6th km. Eskisehir-Turkey
[2]Eskisehir Osmangazi University, Faculty of Agriculture, Ali Numan Kirac Campus, Eskisehir-Turkey,imrenkutlu@hotmail.com

The anther culture is one of the most important methods for producing doubled haploid plants and the efficiency of this method is influenced by several mentioned factors such as genotype and induction media. In this study, it was investigated that anther response of different F_2 progenies of bread wheat (*Triticum aestivum* L.) hybrids using MN6 and P2 induction media. The results indicated that callus production, regeneration, green and albino plant numbers are higher on MN6 media than on P2 media for all genotypes. In addition to, all investigated parameters varied between genotypes. It can be said that the response of anthers depends mainly both the genotype and media, and the most suitable induction media for obtaining doubled haploid from our wheat hybrids was MN6 medium. However, it may be needed to develop other culture conditions for this population to utilize it in an actual breeding program.

Key Words: anther response, bread wheat (*Triticum aestivum* L.), callus production, induction media, regeneration.

Ekmeklik Buğdayın (*Triticum aestivum* L.) F_2 Döllerinin Farklı Ortamlarda Anter Kültürüne Tepkisi

Anter kültürü, double haploid bitki elde etmek için kullanılan en önemli yöntemlerden birisidir ve bu yöntemin etkinliği, genotip ve kültür ortamı gibi pek çok etmenden etkilenir. Bu çalışmada, MN6 ve P2 ortamları kullanılarak, farklı ekmeklik buğday melezi F_2 döllerinin anter kültürüne tepkisi araştırılmıştır. Sonuçlar, bütün genotiplerde kallus üretimi, rejenerasyon, yeşil ve albino sayısının, P2'ye göre MN6 ortamında daha yüksek olduğunu göstermiştir. Buna ek olarak, incelenen tüm özellikler bakımından genotipler arasında farklılıklar vardır ve anter kültürüne tepkinin büyük oranda hem genotipten hem de kültür ortamından etkilendiğini ve kullanılan melezler için en uygun kültür ortamının MN6 ortamı olduğu söylenebilir. Ancak, etkin bir ıslah programında bu populasyonun kullanabilmesi diğer kültür koşullarının da geliştirilmesiyle olasıdır.

Anahtar Kelimeler: anter tepkisi, ekmeklik buğday (*Triticum aestivum* L.) kallus üretimi, kültür ortamı, rejenerasyon

Introduction

Wheat, which has vital importance for human nutrition, is first as acreage and production in Turkey and world because it has wide adaptability and ease storage etc. Global demand for wheat has been growing day by day because of its features. Wheat production is so far away to meet increasing demand to wheat in Turkey and should be increased in. The production of wheat can be increased either by greater area under cultivation or by increasing per hectare yield. However, it is not feasible to increase area under wheat cultivation. Therefore, the only alternate left is to increase per hectare yield by developing high yielding varieties that are better adapted to wide range of environments and stresses (Ozgen, 1991). However, genetic variability which is required to solve yield and quality problems can be gained by traditional breeding methods. They, to evolve high yielding and quality wheat varieties should include a system of instant homozygosity of important characters after gene pyramiding to gain more variation (Inagaki, 1997). Many generation breeding cycles are needed to achieve uniformity in different agronomic traits which is time consuming (Hussain *et al*., 2012).

To speed up the breeding process, plant tissue culture methods could be used effectively. Among them, the technique of double haploid (DH) plants by anther culture is an important method, which enables significant shortening the breeding process (Kasha and Maluszynski, 2003; Belchev *et al*., 2004). As known, doubled haploid lines are homozygous of hundred percent, and they allow evaluating or screening the material in a very rather short time. Again, according to this method, it is possible to obtain new varieties even in 5–7 years, while conventional breeding usually takes 10–15 years (Grauda *et al.*, 2010). Doubled haploid production is widely used not only for the plant breeding (De Buyser *et al*., 1987; Pauk *et al*., 1995), but also for basic research (Orshinsky *et al.,* 1990), such as genomic mapping, haploid transformation and artificial seed production, etc (Tuvesson *et al*., 2003).

Androgenesis is a common methodology to develop haploids, and doubled haploids, in major grain crops. The formation of androgenetic structures and regenerated plants depends on the genotype of the donor plant, its growth environment, culture media and their interactions (Lazar *et al*., 1985; Redha and Talaat, 2008; Kondic-Spika *et al*., 2011).

It is not easy to find an anther culture medium, which gives good respond to many plant species (Ellialtioglu, 1999). Because the anther response changes even among the different genotypes of same species, common nutrient media is not recommended (Ellialtioglu, 1999). Several defined induction media, such as N6 (Chu, 1978), WI4 (Ouyang *et al*., 1989), Chu90 (Chu *et al*., 1990) and C17 (Pauk et al., 1991), and also less-defined media, such as the potato extract-media P2 (Chuang *et al*., 1978) and P4 (Ouyang *et al*., 1983), have been established for wheat anther culture (Puolimatka and Pauk, 2000).

It was reported that due to plants are not selected in F_1 generation, F_2 and F_3 progenies must be used as a material in anther culture (Pauk *et al*., 2003). Because genetic segregation in F_2 progenies are more evident than that of F_1 population and genetic variation reduction during doubled haploid plant production might be partially eliminated (Yorgancilar *et al*., 2013). Hence, in this research, F_2 population was used as a material.

In this study, it was investigated that anther response of different F_2 hybrids originated from Turkish winter bread wheat (*Triticum aestivum* L.) cultivars using MN6 and P2 induction media. Because there was no information about which components in these media affected callus induction and plant regeneration in these F_2 progenies.

Material and Methods

Twelve F_2 wheat hybrids created at the Transitional Zone Agricultural Research Institute were used in the research (Table 1).

Donor plants were grown under field conditions. Spikes were collected in the early uni- to mid-uninucleate and covered with plastic bags preserved at 4°C during two weeks. Then the developmental stage of microspores was determined by squashing in the acetic carmine on a glass slide (Jacquard *et al*., 2003). After pre-cold treatment, leaves and other parts thrown on spike and they were surface sterilized in 2% Sodium Hypochloride solution including Tween 80 for 20 minutes by continuous shaking to remove to surface contaminants (fungi, bacteria) and then they were rinsed in sterilized distilled water for four times (Pauk *et al.,* 2003).

Table 1. Pedigree of 12 bread wheat crosses

CROSS NUMBER	PEDIGREE
1	SIVAS111-33/ESER
2	AK702/CETINEL2000
3	KARAHAN99/ALPU01
4	KRC/BEZ1/3/TT-50-18/P101/TT-50-18/VG DWF/4/NACIBEY
5	PI-178383/YILDIZ98
6	1D13.1/MLT//TOSUNBEY
7	MV8/NACİBEY
8	ATILLA12/2*MUFITBEY
9	ONEARLY_S-248/2*KIRAC66
10	K431494/2*MUFITBEY
11	HARMANKAYA99/2*ONEARLY_S-148
12	CHINESEE SPRING × HYSTAR

Two different induction media, MN6 (Quyang *et al.,* 1987) and P2 (Chuang *et al.,* 1978) were used in this study and the media components showed Table 2. The media components were sterilized and autoclaved at 121˚C for 15 minutes. The induction media were poured into 90 mm diameter Petri dishes, 25 ml of medium in each one. 1000 anthers per cultivar and medium were placed Petri dishes at a density of 100 anthers per dish and ten replicates were used. Anthers were isolated under aseptic conditions and put on induction medium. From all F_2 combination total 12.000 anthers were isolated; isolated anthers of each spike were cultivated on both P2 medium and MN6 medium. Petri dishes where anthers incubated at 28˚C and 80% humidity in the dark for 4-5 weeks. When cultivation obtained callus are about 2 cm, they were transferred on the regeneration medium which its components showed Table 3 (Zhuang and Jia, 1980).

The cultures were maintained at 25˚C at a light intensity of 50 µmol s^{-1} m^{-2}, with photoperiod of 16/8 h. Green and albino plantlets were indentified and recorded. When the plantlets are approximately 1,0-1,5 cm, they were transferred to test tubes containing the same nutrient medium. Shooting plants with roots were transferred pots and acclimatization by covered nylon bags to provide a moist environment during 4-5 days.

Then, ploidy level is determined by examining the size of stomata under microscope (Olympus BH-2). Haploid plants were treated with 0.2% Colchicines, 2% Dimethyl Sulfoxide (DMSO), then transferred greenhouse, while spontaneous doubled haploid plants were directly transferred greenhouse.

A random design involving ten replications per variant (genotype × media) was used. Each petri dishes containing 100 anther was considered as an experimental unit. Data were collected on a per petri dishes basis. Statistical analyses were done using SPSS 17.0 statistical software.

Table 2. Component of induction media.

P2		MN6	
Component	Amount (mg/l)	Component	Amount (mg/l)
KNO_3	1000	KNO_3	1150
$Ca(NO_3)_2.4H_2O$	100	$/NH_4/2SO_4$ x $2H_2O$	100
$MgSO_4.7H_2O$	125	Ca/ NO_3/2 x $4H_2O$	100
$(NH_4)_2$ SO_4	100	$(NH_4)_2$ SO_4	80
KH_2PO_4	200	$MgSO_4$ x $7H_2O$	125
KCl	35	KH_2PO_4	200
$FeSO_4.7H_2O$	27.9	KCl	35
Na_2.EDTA	37.3	2,4-D	1,5
Sucrose	90.000	Kinetin	0,5
Agar	6000	Ficoll	100.000
Glutamin	500		
Thiamin.hcl	1		
2,4-D	1.5		
Kinetin	0.5		
Component	Amount (ml/l)	Component	Amount (ml/l)
Potato extract	100	Fe-Na-EDTA	5
		Thiamin-HCl	1
		Maltose	100

* pH 5.8 for both media.

Table 3. Components of regeneration and growth media.

After P2 Induction Media			After MN6 Induction Media (190-II Cu)	
Component	Amount for Regeneration (mg/l)	Amount for Growth (mg/l)	Component	Amount (mg/l)
KNO_3	950	1000	KNO_3	100
$Ca(NO_3)_2.4H_2O$	-	500	$/NH_4/2SO_4$	200
$MgSO_4.7H_2O$	-	71.5	$Ca/\ NO_3/2x\ 4H_2O$	100
$(NH_4)_2\ SO_4$	185	-	KH_2PO_4	300
KH_2PO_4	85	300	$MgSO_4$ x 7 H_2O	200
NH_4NO_3	825	1000	KCl	40
$CaCl_2.2H_2O$	220	-	Fe-Na-EDTA	20
$FeSO_4.7H_2O$	13.9	13.9	$MnSO_4$ x $4H_2O$	8
$Na_2.EDTA$	18.6	18.6	$ZnSO_4$ x $7H_2O$	3
$MnSO_4.4H_2O$	22.3	4.9	H_3BO_3	3
$ZnSO_4.7H_2O$	8.6	2.7	KI	0,5
H_3BO_3	6.2	1.6	Glicine	2
KI	0.83	0.75	Thiamin-HCl	1
$Na_2MoO_4.2H_2O$	0.25	-	Pyridoksine hcl	0,5
$CuSO_4.5H_2O$	0.025	-	Nikotinik asit	0,5
$CoCl_2.6H_2O$	0.025	-	Meso-inositol	100
Agar	20.000	20.000	Sucrose	0,5
Glutamin	6000	8000	NAA	0,5
Glycine	100	-	Kinetin	0,5
Pyridoxin.HCl	2	20	$CuSO_4$ X $5H_2O$	5,7
Thiamin.HCl	0.5	5		
Nikotinik asit	0.1	1		
2,4-D	0.5	5		
IAA	0.5	-		
Kinetin	-	1		
KNO_3	0.5	-		

* pH 5.8 for both media.

Results

The main effects of the genotype, media and their interaction were all significant at the $p < 0{,}01$ level for number of callus, genotype was significant at the $p < 0{,}01$ level for regeneration and albino plants and genotype was significant at the $p < 0{,}05$ level for green plant. However, media and genotype x media interactions were not significant for regeneration and green plant. Media and genotype x media interactions could not be analysed for albino plant because of any albino plant was not occurred in P2 media (Table 4).

Table 4. Variance analyses of genotype, media and their interactions for callus number, plant regeneration, green plant number and albino plant number.

Dependent Variable	Independent Variable		
	Genotype	Media	Genotype × Media
Callus	$3{,}99^{**}$	$36{,}86^{**}$	$2{,}50^{**}$
Regeneration	$3{,}26^{**}$	$3{,}62^{ns}$	$0{,}13^{ns}$
Green Plant	$2{,}08^{*}$	$0{,}41^{ns}$	$0{,}02^{ns}$
Albino Plant	$13{,}73^{**}$	na	na

** Significant at *P<0,01*, * Significant at *P<0,05*, ^{ns} not significant, na: not analysed

The effect of different induction media on anther response (callus induction) of F_2 progenies was presented in Figure 1. All genotypes of F_2 progenies analyzed provided of callus induction except for Cross 4 and Cross 5 on P2 media. Differences were observed between genotypes and culture media with respect to number of callus. The Cross 11 showed the highest total callus induction both on MN6 media (252) and on P2 media (57). The Cross 4 which had the lowest value (32) on MN6 media did not produce callus on P2 media. The best anther culture response was obtained on MN6 media.

Regeneration was higher on MN6 media depending on the number of obtained callus from initiation medium (Figure 2). It is clear from the results that genotype influences the regeneration as well as media. Cross 8 was the highest regeneration to callus cultivation on the MN6 media (33) and Cross 9 was the highest regeneration on the P2 media (4). Three crosses (Cross 8, Cross 9, and Cross 11) regenerated on P2 media, although all crosses regenerated on MN6 media.

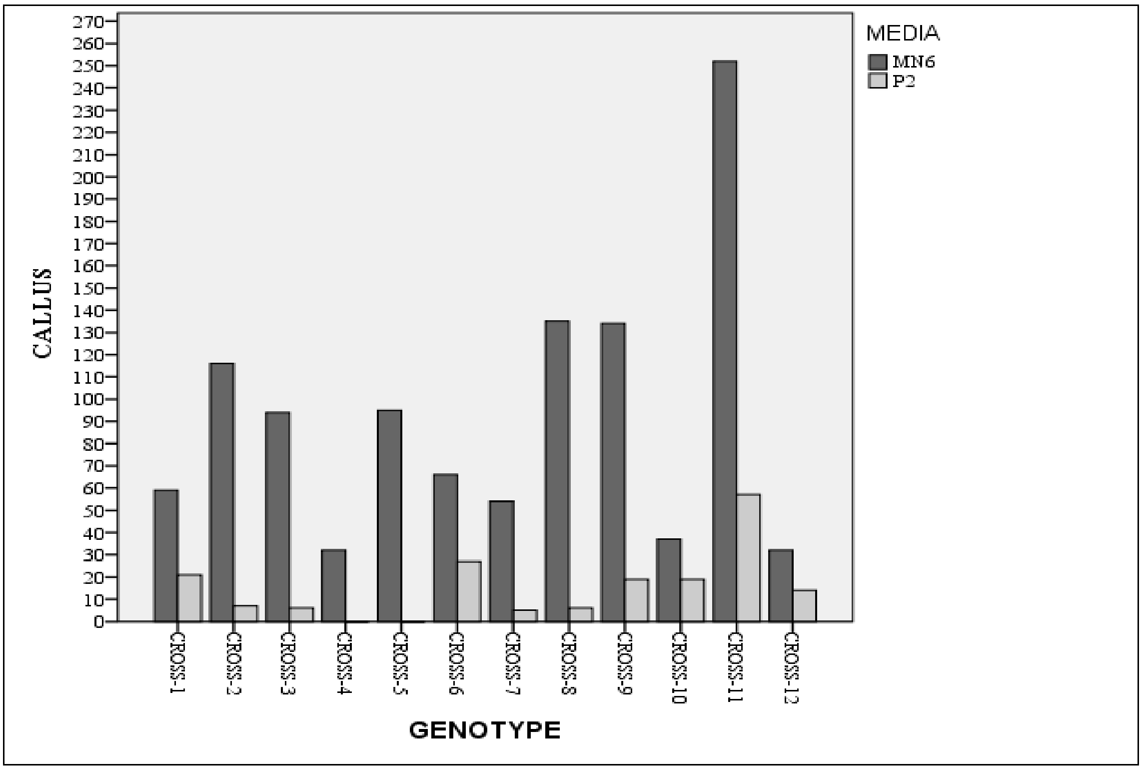

Figure 1. Sum callus induction for 12 genotypes on MN6 and P2 media.

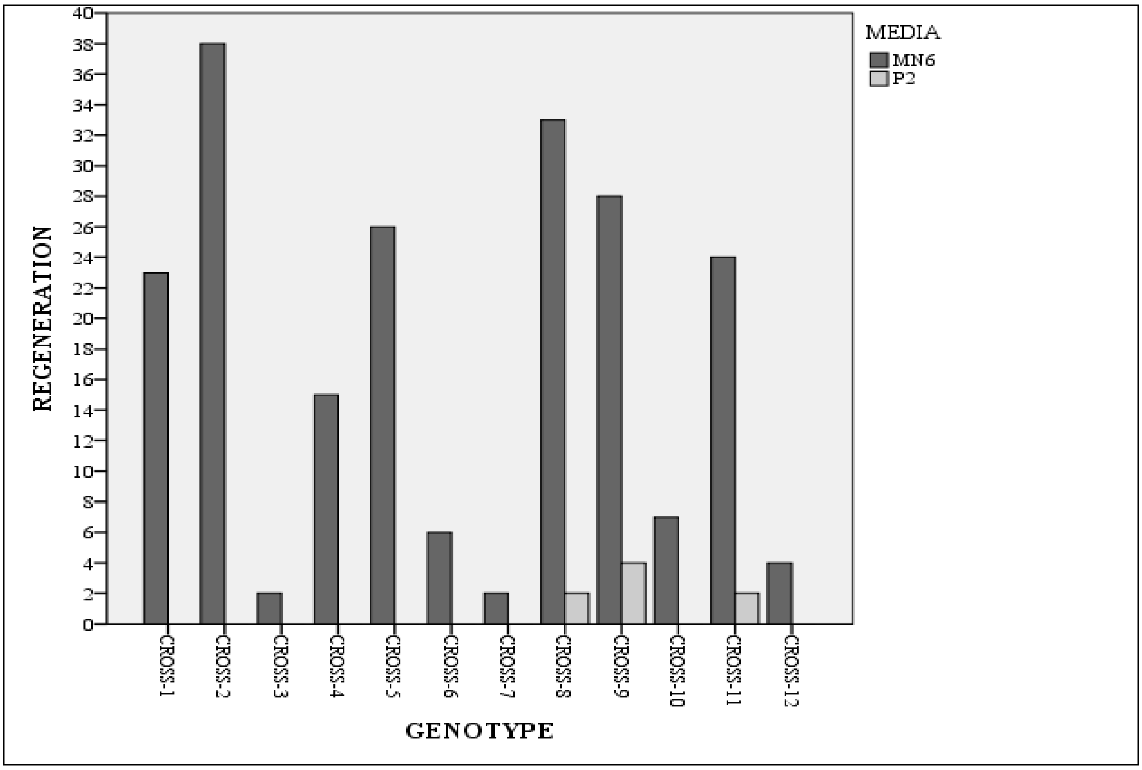

Figure 2. Sum regenerated plant number for 12 genotypes on MN6 and P2 media.

Likewise, all regenerated callus were produced green plants both on P2 media and on MN6 media (Figure 3). The highest green plant gained from Cross 8 (22), and this cross followed by Cross 9 (18), Cross 5 (12), Cross 2 (11) and Cross 11 (9) with higher values than other crosses. In total, 94 and 8 well-rooted green plantlets were regenerated from the 12 breeding crosses on MN6 media and P2 media, respectively. These plantlets were acclimatized to greenhouse conditions, and the rate of survival following acclimatization was approximate 63% on both media.

The albino plants total number was changed from 1 (Cross 3 and Cross 12) to 27 (Cross 2) on MN6 media. On P2 media, any cross was produced albino plant (Figure 4).

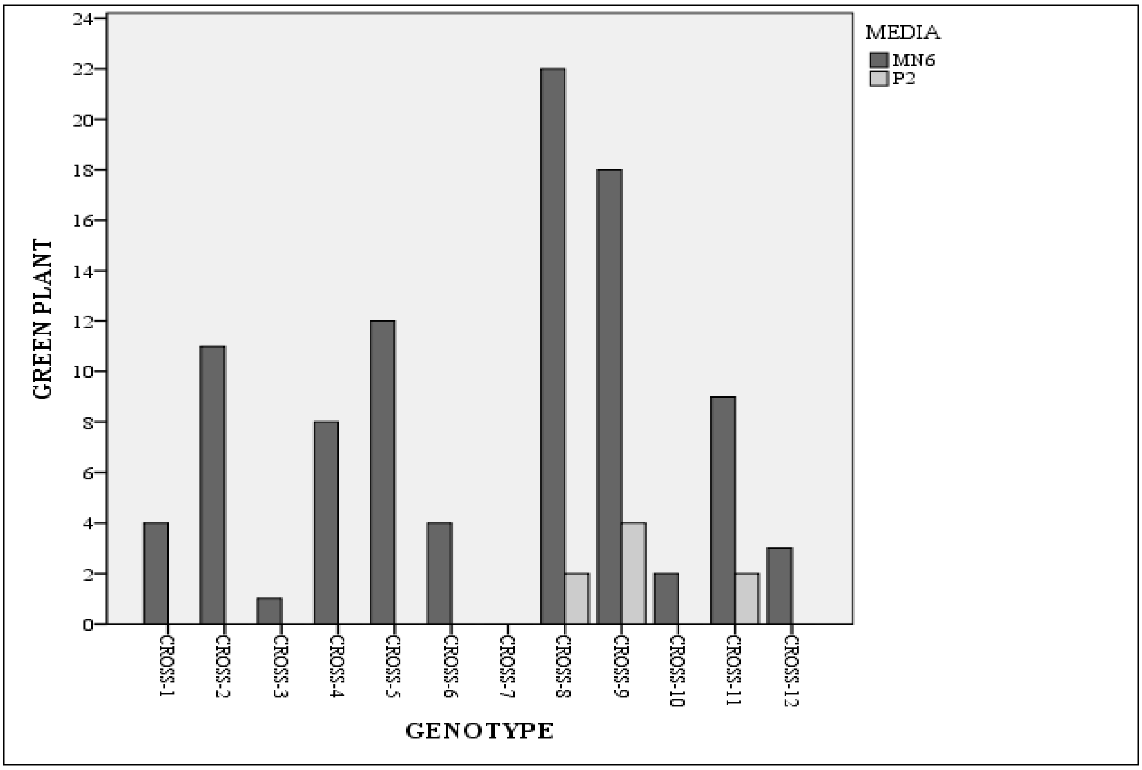

Figure 3. Sum green plant number for 12 genotypes on MN6 and P2 media.

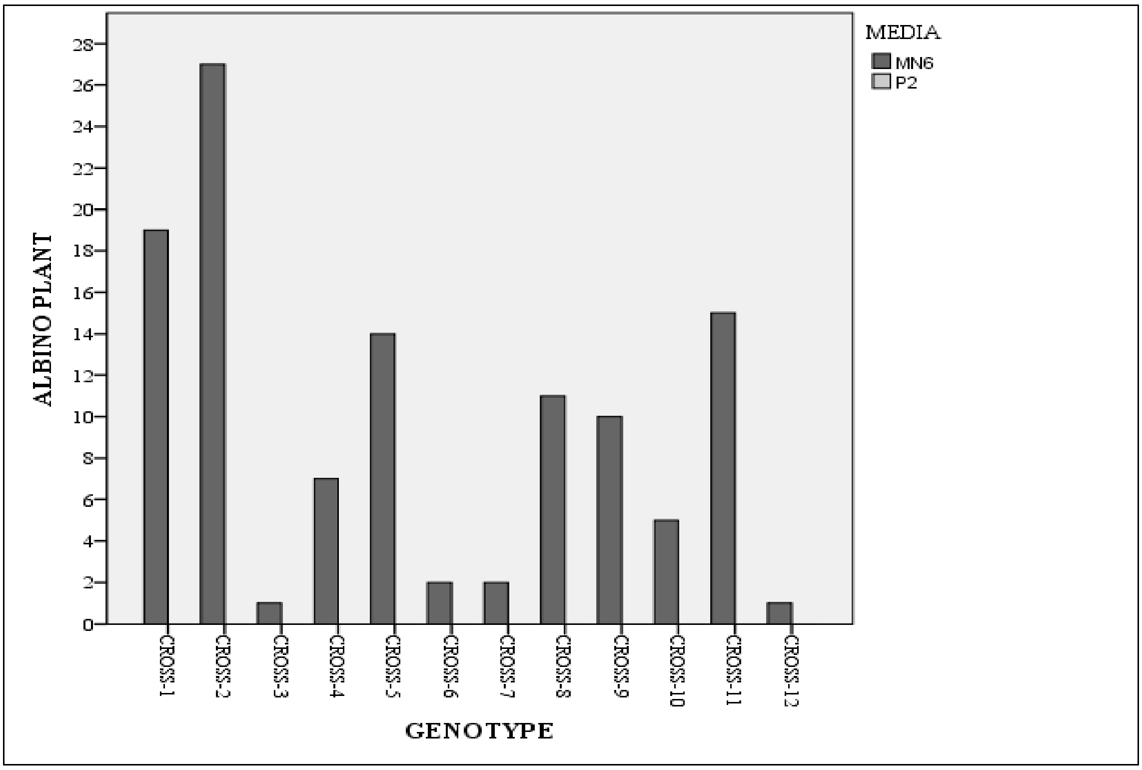

Figure 4. Sum albino plant number for 12 genotypes on MN6 and P2 media.

The number of haploid plants obtained from regenerated green plants were changed from 1 (Cross 4, Cross 6 and Cross 11) to 5 (Cross 8 and Cross 9) on MN6 media and only 2 haploid plants gained from Cross 9 on P2 media (Figure 5). On P2 media, the Cross 8 and Cross 11 produced spontaneous double haploid plants were their number 1 and 2 respectively (Figure 6). On MN6 media, all crosses produced spontaneous double haploid plants. The highest spontaneous double haploid plants gained from Cross 8 on MN6 media (8) and from Cross 11 on P2 media (2). In total, 19 haploid and 40 spontaneous double haploid plants were obtained from MN6 media, but only 2 haploid and 3 spontaneous double haploid plants were obtained from P2 media.

Discussion and Conclusion

Anther culture is one of the most important methods for producing doubled haploid plants and the efficiency of this method is influenced by several mentioned factors such as genotype and induction media. This study was carried out to evaluate twelve different F_2 progenies bread wheat hybrids capacity for callus production and plant regeneration by using two different media.

Total callus number changed from 32 to 252 on MN6 media, while it changed from 0 to 57 on P2 media. These results mainly indicated that media effect on anther response, even though anther response was greatly genotype dependent. Many researchers reported that the response of anthers depends mainly on the genotype used (Konieczny *et al*., 2003; Kim and Baenziger, 2005; Cistu´e *et al*., 2006; Kang *et al*., 2011; Yorgancilar *et al*., 2013). In addition to Redha and Talaat (2008) stated that manipulation of media components is of particular interest and has led to success in some cases. Also, Kondic-Spika *et al*. (2011) reported that the differences in the genotypes' reaction to the induction media indicate that formation of callus and plant regenerants (green and albino plants) depends on the genotype and culture conditions.

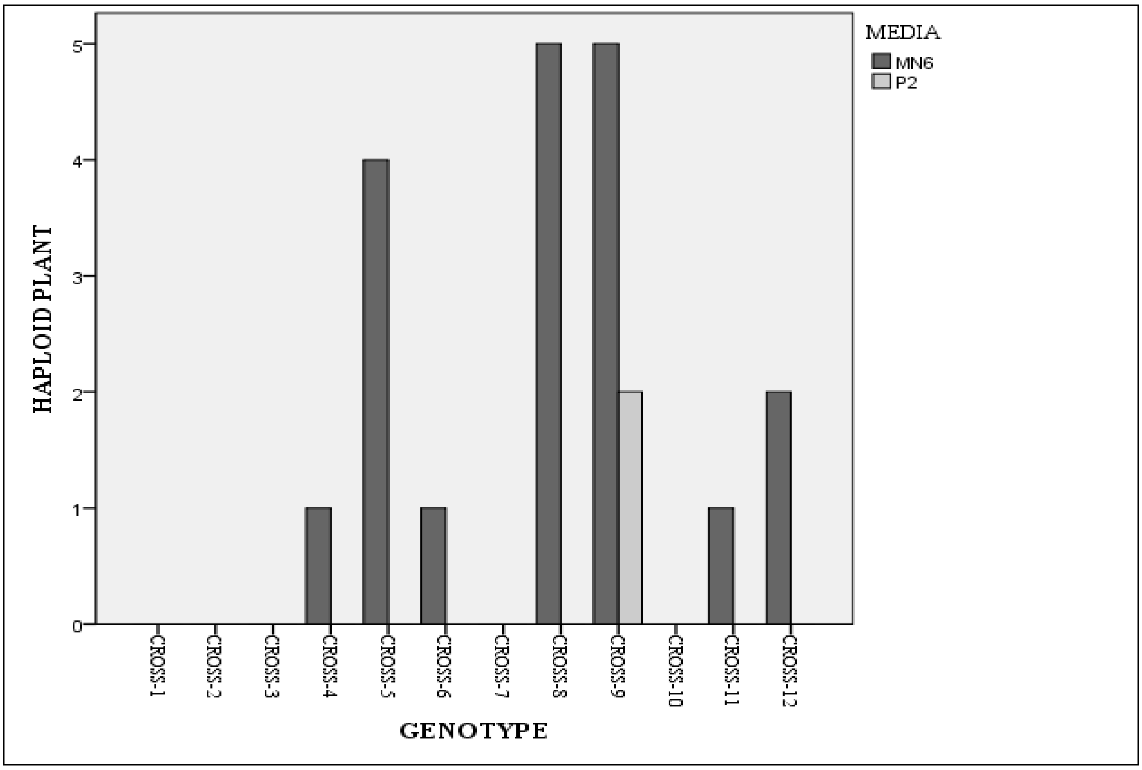

Figure 5. Sum haploid plant number for 12 genotypes on MN6 and P2 media.

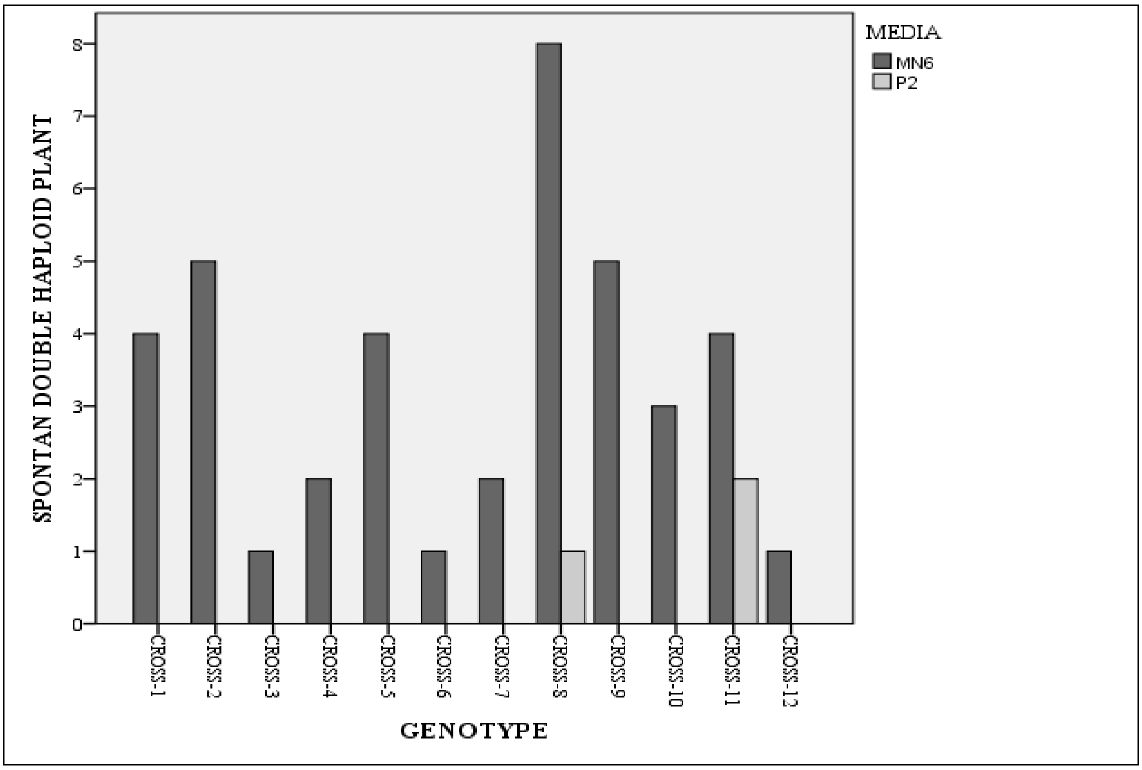

Figure 6. Sum spontanoeus double haploid plant number for 12 genotypes on MN6 and P2 media.

Regeneration, green and albino plant numbers are higher on MN6 than on P2 media. The reason for this may be high callus production on MN6 media. MN6 media including maltose could yield sufficient green plants from anther culture in these F_2 populations.

The culture media composition is reported to be a major factor for the anther culture response and induction of development of green and albino plants from microspores (Fadel and Wenzel, 1990; Hu, 1997; Kao, 1981). Dogramaci-Altuntepe *et al.* (2001) reported that a strong media effects on anther response and callus production. Our findings were similar and callus production and plant regeneration were better on MN6 than on P2 media. Whereas some researchers (Moieni and Sarrafi, 1995, 1996; Danci *et al*., 2010; Kondic-Spika *et al*., 2011) reported that the P2 media has been effectively used in anther culture of hexaploid wheat. In contrast to, the results of this study showed that P2 media is not effective enough in our genotypes. Shirdelmoghanloo *et al*. (2009) stated that P2 media is not suitable wheat isolated microspore culture, too. On the other hand, Rashid *et al*. (2002) reported that N6 medium as a recommended medium for callus induction.

One of the important results of this study, spontaneously doubled haploids accounted almost 68% of plants. Similar results found that Cistu´e *et al*., 2006. Spontaneous doubling is generally assumed to generate from endomitosis or by nuclear fusion (Sunderland *et al*., 1974). However, since both mechanisms are infrequent and inconsistent events ((Loh and Ingram 1983), the reason for the high spontaneous chromosome doubling among regenerated plants is unknown.

It was shown that the most suitable induction media for obtaining doubled haploid from wheat hybrids was MN6 medium. This medium was the suitable for callus production and to develop green plants. P2 media was not suitable for investigated wheat hybrids. Therefore, we may need to develop other culture conditions for this population to utilize it in an actual breeding program. Cross 8, Cross 9 and Cross 11 gave desirable regeneration answers, but Cross 8 exhibited better values for green and spontaneous double haploid plants and therefore may be worthy genotype to serve in the future as a promising donor material for breeding based on anther culture. More plants- regenerates contained spontaneous double haploid, but some of them had also haploid. In addition, the use of doubled haploid lines will be gained great possibilities and they will be bring novel genes at the especially biotechnologically level in the wheat breeding.

References

Belchev, I., M. Tchorbadjieva and I. Pantchev, 2004. Effect of azacytidine on callus induction and plant regeneration potential in anther culture of wheat *(Triticum aestivum* L.). Bulg. J. of Plant Physiol. 30 (1–2): 45–50.

Chu, C. C. 1978. The N6 medium and its applications to anther culture of cereal crops. In: Proceedings of Symposium on Plant Tissue Culture, Beijing, pp. 43-50.

Chu, C. C., R. D. Hill and A. L. Brule-Babel, 1990. High frequency of pollen embryoid formation and plant regeneration in *Triticum aestivum* L. on monosaccharide containing media. Plant Sci. 66: 255-262.

Chuang, C. C., T. W. Ouyang, H. Chia, S. M. Chou and C. K. Ching, 1978. A set of potato media for wheat anther culture. In: Proceedings of Symposium on Plant Tissue Culture, Scientific Press, Peking, China, pp. 51-56.

Cistu´e, L., M. Soriano, A. M. Castillo, M. P. Vall´es, J. M. Sanz and B. Ech´avarri, 2006. Production of doubled haploids in durum wheat (*Triticum turgidum* L.) through isolated microspore culture. Plant Cell Rep. Cell Biol. and Morphogen. 25: 257–264.

Danci, M., O. Danci, F. Berbentea, I. David, G. Bujanca, S. Alda, S. S. Chis, L. Alda and G. Carciu, 2010. Factors that influence wheat (*Triticum aestivum*) somaclones and gametoclones regeneration**.** Journal of Hortic. Forest. and Biotech. 14 (2): 243-249.

De Buyser, J., Y. Henry, P. Lonnet, R. Hertzog and A. Hespeb, 1987. 'Florin' a doubled haploid wheat variety developed by the anther culture method. Plant Breeding. 98: 53-56.

Dogramaci-Altuntepe, M., T. S. Peterson and P. P. Jauhar, 2001. Anther culture derived regenerants of durum wheat and their cytological characterization. The Am. Genet. Assoc. 92: 56-64.

Ellialtioglu, S. 1999. The factors effecting success on haploid plant regeneration via androgenesis. Journal of Biotechnol. (Kükem) 24 (1): 11-22.

Fadel, F. and G. Wenzel, 1990. Medium-genotype interaction on androgenetic haploid production in wheat. Plant Breeding. 105:278–282.

Grauda, D., N. Lepse, V. Strazdina, I. Kokina, L. Lapina, A. Mikelsone, L. Lubinskis and I. Rashal, 2010. Obtaining of doubled haploid lines by anther culture method for the latvian wheat breeding. Agron. Res. 8 (Special Issue III): 545–552.

Hu, H. 1997. In vitro induced haploids in wheat. In: In Vitro Haploid Production in Higher Plants. Vol 4, Cereals (Ed(s): Jain, S.M., S.K. Sopory, R.E Veilleux). Dordrecht, The Netherlands: Kluwer. Pp. 73–97.

Hussain, M., M. Niaz, M. Iqbal, T. Iftikhar and J. Ahmad, 2012. Emasculation techniques and detached tiller culture in wheat x maize crosses. J. Agr. Res. 50(1): 1-20.

Inagaki, M. 1997. Technical advances in wheat haploid production using ultra-wide crosses*.* JIRCAS 4: 51-62.

Jacquard, C., G. Wojnarowiez and C. Clément, 2003. Anther culture in barley. In: Doubled Haploid Production in Crop Plants (Ed(s): Maluszymski, M., K.J. Kasha, B.P. Forster and I. Szarejko). Kluwer Academic Publishers, Dordrecht. pp. 21–27.

Kang, M., Y. Hai, B. Huang, Y. Zhao, S. Wang, L. Miao and X. Zhang, 2011. Breeding of newly licensed wheat variety huapei 8 and improved breeding strategy by anther culture. Afr. J. of Biotech. 10(85): 19701-19706, Available online at http://www.academicjournals.org/AJB. (accessed 28 December, 2011).

Kao, K. N. 1981. Plant formation from barley anther cultures with ficoll media. Z Pflanzen Physiol. 103:437–443.

Kasha, K.J. and M. Maluszynski, 2003. Production of doubled haploids in crop plants. In: Doubled Haploid Production in Crop Plants. (Ed(s): Maluszymski, M., K. J. Kasha, B. P. Forster and I. Szarejko) Kluwer Academic Publishers, Dordrecht. pp. 1–4.

Kim, K.-M., and P.S. Baenziger. 2005. A simple wheat haploid and doubled haploid production system using anther culture. In Vitro Cell. Dev. Biol. Plant 41:22–27.

Kondic-Spika, A., M. Ukosavljev, B. Kobiljski and N. Hristov, 2011. Relationships among androgenetic components in wheat and their responses to the environment. J. of Biol. Res-Thessaloniki. 16: 217 – 223.

Konieczny, R., A. Z. Czaplicki, H. Golczyk and L. Przywara, 2003. Two pathways of plant regeneration in wheat anther culture. Plant Cell Tiss. Org. 73: 177–187.

Lazar, M. D., G. W. Schaeffer and P. S. Baenziger, 1985. The physical environment in relation to high frequency callus and plantlet development in anther cultures of wheat (*Triticum aestivum* L.) cv. Chris. J. Plant Physiol. 121: 103-109.

Loh, C. S., and D. S. Ingram, 1983: The response of haploid secondary embryoids and secondary embryogenic tissue of winter oilseed rape to treatment with colchicine. New Phytol. 95: 359-366.

Moieni, A. and A. Sarrafi, 1995. Genetic analysis for haploid-regeneration responses of hexaploid-wheat anther cultures. Plant Breeding. 114: 247-249.

Moieni, A. and A. Sarrafi, 1996. Effects of donor plant genotype and media composition on androgenesis of iranian spring wheat genotypes and F_1 hybrids. J. Genet. Breeding. 50: 383-386.

Orshinsky, B. R., L. J. Mcgregor, G. I. E. Johnson, P. Huel and K. K. Kartha, 1990. Improved embryoid induction and green shoot regeneration from wheat anthers cultured in medium with maltose. Plant Cell Reports. 9: 365-369.

Ouyang, J. W., S. G. Jia, C. Zhang, X. D. Chen and G. H. Feng, 1989. A new synthetic medium (W14 medium) for wheat anther culture. Annual Report of the Institute of Genetics, Academia Sinica for 1987-1988, 91-92.

Ouyang, J. W., S. M. Zhou and S. E. Jia, 1983. The response of anther culture to culture temperature in *Triticum aestivum.* Theor. Appl. Genet. 66: 101-109.

Özgen, M., 1991. Yield stability of winter barley (*Hordeum Sp.*) cultivar and lines. Proc.6th Int. Barley Gen.Sym.22-27 July., Hensingborg, 407-409.

Pauk, J., Z. Kertesz, B. Beke, L. Bona, M. Csosz and J. Matuz, 1995. New winter wheat variety: 'GK Delibab' developed via combining conventional breeding and *in vitro* androgenesis. Cereal Res. Commun. 23: 251-256.

Pauk, J., O. Manninen, I. Ma'itila, Y. Salo and S. Pulli, 1991. Androgenesis in hexaploid wheat f_2 populations and their parents using a multiple-step regeneration system. Plant Breeding. 107: 18-27.

Pauk, J., R. Mihaly and M. Puolimatka, 2003. Protocol for wheat (*Triticum aestivum* L.) anther culture. In: Doubled Haploid Production in Crop Plants. (Ed(s): Maluszymski, M., K. J. Kasha, B. P. Forster and I. Szarejko). Kluwer Academic Publishers, Dordrecht, pp. 59-64.

Puolimatka, M. and J. Pauk, 2000. Effect of induction duration and medium composition on plant regeneration in wheat *(Triticum aestivum* L.) anther culture. J. Plant Physiol. 156: 197-203.

Quyang, J. W., D. G. He, G. H. Feng and S. E. Jia, 1987. The response of anther culture to culture temperature varies with growth conditions of anther-donor plants. Plant Sci. 49: 145-148.

Rashid, H., R. A. Ghani, Z. Chaudhry, S. M. S. Naqvi and A. Quarishi, 2002. Effect of media, growth regulators and genotypes on callus induction and regeneration in wheat (*Triticum aestivum*). Biotechnol. 1(1): 49-54.

Redha, A. and A. Talaat, 2008. Improvement of green plant regeneration by manipulation of anther culture induction medium of hexaploid wheat. Plant Cell Tiss. Org. 92: 141–146.

Salantur, A., S. Yazar, E. Donmez and T. Akar, 2011. Determination of Plant Regeneration Response of Winter Bread Wheat F_2 Population under Anther Culture. Journal of Field Crops Central Research Institute 20 (1): 15-21

Shirdelmoghanloo, H., A. Moieni and A. Mousavi, 2009. Effects of embryo induction media and pre-treatments in isolated microspore culture of hexaploid wheat (*Triticum aestivum* L. cv. Falat). Afr. J. Biotech. 8 (22): 6134-6140.

Sunderland, N., G. B. Collins, and J. M. Dunwell, 1974. The role of nuclear fusion in pollen embryogenesis of Datura innoxia Mill. Planta 117: 227-241.

Tuvesson, S., R. Von Post and A. Ljungberg, 2003. Wheat anther culture. In: Doubled Haploid Production in Crop Plants. (Ed(s): Maluszymski, M., K. J. Kasha, B. P.Forster and I. Szarejko). Kluwer Academic Publishers, Dordrecht, pp. 71-76.

Yorgancilar, O., A. Yorgancilar, O. Bilir and A. Yumurtaci, 2013. Impacts of F_2 derived winter bread wheat progenies on callus production and regeneration frequencies through anther culture**.** Res Plant Biol 3(3): 10-17.

Zhuang, J. J. and X. Jia, 1980. Studies on the differentiation of pollen calli of wheat. In: Annual Report of the Institute of Genetics, Academia Sinica, Beijing, pp. 70-72.