IN VITRO SELECTION OF DROUGHT TOLERANT REGENERANTS IN DURUM WHEAT (*TRITICUM DURUM* DESF.)

BENLIOGLU, B.* – OZGEN, M.

Department of Field Crops, Agriculture Faculty, Ankara University, 06110 Diskapi, Ankara, Turkey (phone: +90-312-596-1325; fax: +90-312-596-1395)

**Corresponding author*
e-mail: benlioglu@ankara.edu.tr; phone: +90-312-596-1881

(Received 30th Nov 2020; accepted 3rd Mar 2021)

Abstract. In this study, a total of 12 genotypes were used, including 1 hulled emmer and 11 registered ones which are important as genetic resources and for durum wheat cultivation. The responses of these genotypes to different drought stress levels were determined in vitro. In this research, to create drought stress, high molecular weight polyethylene glycol (PEG 6000) was used. In the trials, 5 different PEG 6000 doses were administered to induce drought stress with rates of 0 (control), -0.50 bar, -1.48 bar, -2.95 bar and -4.91 bar. During the experiments, after the callus was formed with the endosperm supported mature embryo culture, regeneration capacity and plantlets they formed were evaluated by applying different levels of drought stress to the callus. Using these parameters, stress tolerance index, stress sensitivity index and tolerance index values of durum wheat genotypes were calculated. According to the results, there was a significant decrease in all parameters examined with the increase of drought stress. While Artuklu and Sarıçanak-98 cultivars had the highest drought tolerance, Çakmak-79, Çeşit-1252, Eminbey and Kunduru-1149 cultivars were sensitive to drought and *Triticum dicoccum*, which is an important gene source, was also sensitive.
Keywords: *drought stress, polyethylene glycol, endosperm-supported embryo culture, Triticum durum, Triticum dicoccum*

Introduction

To increase plant production under conditions of global warming and accompanying climate change, it is necessary to use cultivated plants and plant gene resources much more effectively (Ozgen et al., 2015). Today, the severity of global warming and environmental stress factors (drought, salinity, high and low temperatures, heavy metals, etc.) are gradually increasing. Among the environmental stressors, drought stress is the most prominent stress factor in both cultivated plants and wild forms globally. Climate change is predicted to create increasingly severe and prolonged drought periods in the next 30-90 years, as weather temperatures increase, affecting more than a third of the world, including the world's top food-producing areas (Dai, 2011, 2013; Cook et al., 2014; Hasanuzzaman et al., 2017).

The world population is estimated to reach 9.74 billion in 2050 (Desa, 2019) and the nutritional needs of the growing population are also increasing. One big challenge is that the agricultural areas that reach their natural limits are shrinking for various reasons and the pressure of stress factors on plants increases due to climate changes. Under these conditions, it is necessary to obtain maximum product per unit area. For this, new varieties with high yield and tolerance to biotic and abiotic stress factors should be developed (Ozgen et al., 2017).

Wheat (*Triticum* sp.) is the most widely cultivated crop worldwide with 214 million ha of cultivation area. Wheat forms the basis of human nutrition and provides an average of 531 kcal of energy per individual per day (Anonymous, 2020). Bread wheat

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1903_18131825

is spread over a wider area than durum wheat. The tolerance of common wheat against environmental stress factors is higher than durum wheat. For high yield and quality product, durum wheat is selective in terms of climate and soil requirements. Durum wheat will be one of the types that the increasing environmental stress factors, especially the drought pressure, will affect the most among cereals.

To develop new varieties with high drought tolerance, it is important to determine the tolerance levels of existing genotypes. For this purpose, selection at the cell and tissue level under fast and highly controlled in vitro conditions can give more reliable results than studies under outdoor conditions (Mohamed et al., 2000; Rai et al., 2010).

Mannitol, sorbitol, NaCl and polyethylene glycol (PEG) are used to create drought stress in vitro. Polyethylene glycols with high molecular weight are the most widely used stress agents in tissue culture due to their water-soluble polymer structure, non-toxicity, non-metabolism and not being absorbed by plant cells (Hassan et al., 2004; Caruso et al., 2008; Chen et al., 2010).

The aim of this study is to determine rapidly and consistently the tolerance of the durum wheat cultivars which are grown widely in Turkey as well as using as a genitor in wheat breeding programs and an emmer which is grown locally as well as resistant to several stresses against drought stress under in vitro conditions.

Materials and methods

This research was carried out at the Ankara University, Faculty of Agriculture, Department of Field Crops Biotechnology Laboratory. In the research, 11 registered cultivars which are used extensively in durum wheat cultivation in Turkey and Local hulled wheat genotype emmer (*Triticum dicoccum*) obtained from Kars province of Turkey were used as material (*Table 1*).

Table 1. *Durum wheat genotypes used in the research*

Genotypes	Type	Breeding company
Altın-40/98	Cultivar	Field Crops Central Research Institute
Artuklu	Cultivar	GAP International Agricultural Research and Training Center
Çakmak-79	Cultivar	Field Crops Central Research Institute
Çeşit-1252	Cultivar	Field Crops Central Research Institute
Eminbey	Cultivar	Field Crops Central Research Institute
Kızıltan-91	Cultivar	Field Crops Central Research Institute
Kunduru-1149	Cultivar	Field Crops Central Research Institute
Meram-2002	Cultivar	Bahri Dagdas International Agricultural Research Institute
Mirzabey-2000	Cultivar	Field Crops Central Research Institute
Sarıçanak 98	Cultivar	GAP International Agricultural Research and Training Center
Selçuklu-97	Cultivar	Bahri Dagdas International Agricultural Research Institute
T. dicoccum (Emmer)	Landrace	Collected from Kars province -Turkey

High molecular weight polyethylene glycol (PEG 6000) was used in the experiments to create drought stress. For the severity of drought stress, at the levels indicated as appropriate by previous researchers; It is set to 0, -0.50 bar (5% w/v), -1.48 bar (10% w/v), -2.95 bar (15% w/v), and -4.91 bar (20% w/v) (Abdel-Haddy and El-Naggar,

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1903_18131825

2007; Soni et al., 2011; Farshadfar et al., 2012a; El-Rawy and Hassan, 2014; Kacem et al., 2017).

In order to determine the drought responses of genotypes by in vitro methods, the most realistic environment was tried to be prepared. For this:

- embryos of mature seeds were used as an explant source,
- drought stress applied to the developed calluses, which can represent a complete plant model and
- growth regulator was used only during callus formation phase, not during the stress phase.

For surface sterilization, mature seeds were treated with 70% (v/v) ethanol for 5 min, washed 2-3 times with sterile distilled water, sterilized for 25 min with Sodium Hypochlorite (NaClO), and washed several times with sterile distilled water. The seeds were then soaked in sterile distilled water for 2 h at 33 °C.

Endosperm supported mature embryo culture was applied to create callus from mature seeds (Ozgen et al., 1998). The embryos were gently separated without disconnecting the endosperm, allowing them to form callus in darkness for 11 days in medium containing only 8 mg^{-1} 2,4-D (Merck, Germany). The calli were then grown in MS (Murashige and Skoog, 1962) nutrient medium containing sucrose (20 g^{-1}), glycine (2 mg^{-1}) and Agar (7 g^{-1}) in darkness for 21 days. The obtained calli were transferred onto regeneration medium containing MS mineral salts, sucrose (20 g^{-1}), Agar (7 g^{-1}) and different doses of PEG-6000 (0, -0.50 bar (5% w/v), -1.48 bar (10% w/v), -2.95 bar (%15 w/v) and -4.91 bar (20% w/v)) in the plates. The transferred calli were incubated at 25 °C for 5-6 weeks under 16 h/8 h (light/dark) photoperiod at 25 °C.

Calli with green spots on them were considered to be regenerated, and the "regeneration capacity" was determined by proportioning the regenerated calli to the callus formed (Ozgen et al., 2017). Regenerants that did not remain in the form of shoot primordial and were graded at least 30-40 mm were accepted as plantlets and the "plantlet formation capacity" was determined by number of plantlets proportioning by the total number of calli (Kacem et al., 2017).

Stress tolerance index (STI) (Fernandez, 1992), stress sensitive index (SSI) (Fischer and Maurer, 1978) and tolerance index (TOL) (Rosielle and Hamblin, 1981) to better understand the responses of genotypes grown under stress conditions to these stresses calculated. Stress tolerance index is used to determine the varieties that show high value in terms of the properties examined both under stressful conditions and under normal conditions. Stress sensitive index, on the other hand, is used to identify varieties that have low value in terms of the trait examined but show high value under stress.

- Stress tolerance index (STI) $= \frac{(Ypi \times Ysi)}{Yp^2}$
- Stress sensitive index (SSI) $= \frac{1-(Ysi\ /\ Ypi)}{SI}$, where $SI = 1 - \frac{Ys}{Yp}$
- Tolerance index (TOL) $= \frac{(Ypi + Ysi)}{2}$

Ypi = The trait value of each type under stress-free conditions (control)
Ysi = The value of the traits of each type under stress
Yp = The average of the traits examined of the cultivars under stress-free conditions (control)
Ys = The average of the traits examined of the cultivars under stressed conditions (control)

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1903_18131825

Statistical analysis

A completely randomized design with three replications per species and per stress level was used. Petri dishes containing 25 seeds were considered the units of replication in callus induction and callus development stage.

Statistical analysis of the data obtained was made with MSTAT-C (Russel, 1994) and JMP-12 (SAS, Institute Inc., 2015). The effects of genotype and stress on culture responses were determined by analysis of variance and Duncan tests (Steel et al., 1980).

Results

First of all, we determined to the responses of durum wheat genotypes used in the experiments to tissue culture parameters with endosperm supported mature embryo culture. With the analysis of variance, it determined that the difference among the genotypes was statistically significant at $p < 0.01$ for all parameters examined (*Table 2*). The means of the genotypes in the parameters examined were shown in *Table 3*.

Table 2. *Analysis of variance for parameters obtained by endosperm-supported embryo culture in durum wheat genotypes*

V.R.	dF	Mean square			
		Callus induction	Callus weight	Regeneration capacity	Plantlet formation capacity
Genotype	11	137.2**	0.62**	205.2*	1039.1**
Error	24	15.7	0.08	17.9	42.2
Total	35	53.2	0.25	75.9	362.8

**Significantly different from zero at 0.01 probability

Table 3. *Response of durum wheat genotypes to tissue culture parameters*

Genotypes	Callus induction (%)	Callus weight (g)	Regeneration capacity (%)	Plantlet formation capacity (%)
Altın-40/98	89.3 a-c	3.132 ab	100.0 a	26.7 cd
Artuklu	96.0 a	2.595 b-d	100.0 a	30.0 bc
Çakmak-79	84.0 b-d	2.347 c-e	100.0 a	6.7 g
Çeşit-1252	90.7 ab	2.831 a-c	73.3 d	10.0 fg
Eminbey	94.7 a	3.279 a	83.3 c	40.0 b
Kızıltan-91	82.7 cd	2.687 b-d	100.0 a	13.3 ef
Kunduru-1149	77.3 d	2.623 b-d	93.3 ab	10.0 f
Meram-2002	82.7 cd	1.998 ef	96.7 ab	33.3 bc
Mirzabey-2000	77.3 d	1.687 f	93.3 ab	23.3 c-e
Sarıçanak 98	94.7 a	2.633 b-d	100.0 a	56.7 a
Selçuklu-97	80.0 d	2.140 d-f	96.7 ab	53.3 a
T. dicoccum	89.3 a-c	2.740 bc	90.0 bc	16.7 d-f
Mean	86.6	2.558	93.9	26.7

Means followed by the different letters are significantly different at the 0.05 probability

Among the durum wheat genotypes, the variety with the highest callus induction was Artuklu with 96%. This was followed by Eminbey and Sarıçanak-98. Kunduru-1149 has

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1903_18131825

the least callus induction. The varieties with the highest callus weight were Eminbey and Altın 40/98 respectively. All callus of Altın-40/98, Artuklu, Çakmak-79, Kızıltan-91 and Sarıçanak-98 cultivars regenerated and their regeneration capacity was calculated as 100%. In terms of the plantlet formation capacity obtained by counting the explants that had shoot elongation of at least 30-40 mm from the regenerated calli, Sarıçanak-98, Selçuklu-97 and Eminbey had the highest values, respectively (*Table 3*).

Calli obtained by endosperm supported mature embryo culture of durum wheat genotypes (*Fig. 1*); regeneration capabilities determined by transferring MS media containing different severity of drought stress (0, -0.50, -1.48, -2.95 and -4.91 bar) (*Fig. 2*). As a result of the analysis of variance, it was seen that the difference between genotypes, stress levels and genotype x stress level interaction $p < 0.01$ level was statistically significant (*Table 4*). The "regeneration capacity" and the "plantlet formation capacity" of the genotypes, measured under different drought severity, are shown in *Table 5*.

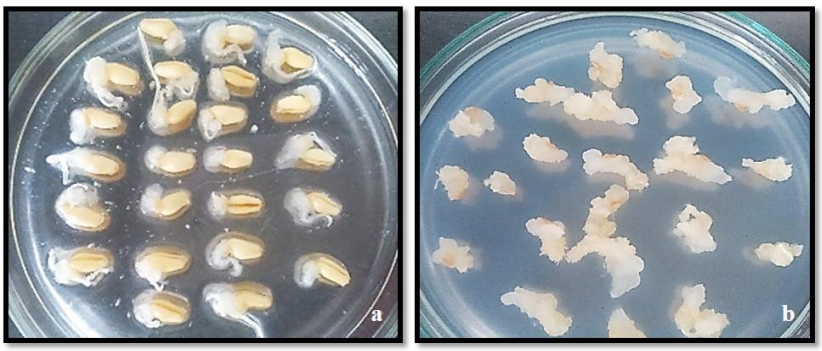

Figure 1. *Callus induction (a) and callus development stage (b)*

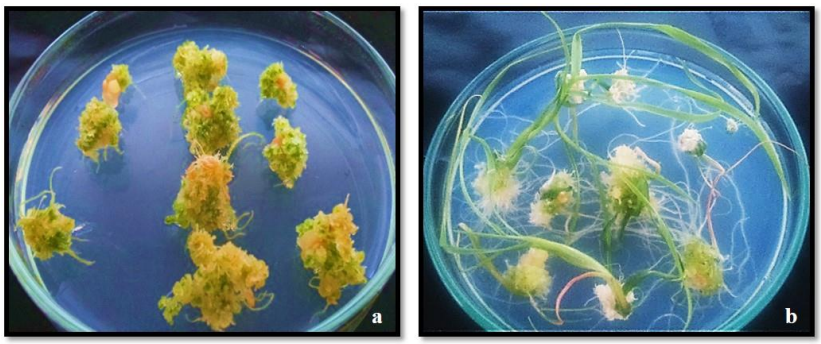

Figure 2. *Regeneration phase. (a) Callus remaining in the form of shoot primordian; (b) callus forming plantlets*

The regeneration capacity of Artuklu, Kızıltan-91, Kunduru-1149 and Sarıçanak-98 was measured 100% at the level of -0.50 bar drought stress. The genotype with the lowest regeneration capacity at this stress level was Selçuklu-97 (76.7%). The -0.50 bar stress level did not have a great effect on the regeneration capacity of the genotypes, and there was no stress effect in Artuklu, Eminbey, Kızıltan-91, Sarıçanak-98 and *T. dicoccum*. The varieties that created the highest plantlets at this stress level were Sarıçanak-98 (43.3%) and Artuklu (36.7%), respectively. Kunduru-1149 did not form

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1903_18131825

plantlets at this stress level. Mirzabey-2000 and *T. dicoccum* were the genotypes that produced the least plantlets with 6.7%. The varieties most affected by this drought level were Kunduru-1149 and Mirzabey-2000, with 100% and 71.2% stress-strain, respectively (*Table 5*).

Table 4. *Analysis of variance for the regeneration capacity and the plantlet formation capacity of durum wheat genotypes under different drought stress*

V.R.	dF	Mean square	
		Regeneration capacity	**Plantlet formation capacity**
Genotype (G)	11	5.201**	1.867**
Stress level (S)	4	10.486 **	2.504**
G x S	44	583**	180**
Eror	120	60	54
Total	179	737	251

**Significantly different from zero at 0.01 probability

Regeneration capacity at -1.48 bar drought stress level it was measured 100% in Artuklu, Kızıltan-91 and Sarıçanak-98 varieties. Genotypes with the lowest regeneration capacity at -1.48 bar; *T. dicoccum* (36.7%) and Çeşit-1252 (46.7%). When the changes in the regeneration capacity of the genotypes between the control group and the drought level of -1.48 bar were examined, the most stress-strain occurred in *T. dicoccum* (59.2%) and Selçuklu-97 (48.3%). The regeneration capacity of Artuklu, Kızıltan-91 and Sarıçanak-98 did not change according to the control group. At this stress level, Sarıçanak-98 obtained the highest plantlet formation capacity with 36.7%. Artuklu ranked second with 26.7% plantlets. Çakmak-79 and Eminbey were determined as the lowest (0%) genotypes of the plantlet formation capacity at -1.48 bar stress level. At this drought level, the stress-strain was at least Artuklu (11%) and Kızıltan-91 (24.8%) (*Table 5*).

Genotypes with the highest regeneration capacity at -2.95 bar drought stress level, were Sarıçanak 98 (100%), Altın-40/98 (96.7%), Artuklu (96.7%) and Çakmak-79 (96.7%), respectively. *T. dicoccum* showed the lowest regeneration capacity at this stress level (10%). Between the control group and -2.95 bar stress level of genotypes, the highest stress-strain in terms of regeneration capacity occurred at 88.9% and 55.2% in *T. dicoccum* and Selçuklu-97, respectively. At this drought level, Sarıçanak-98 (43.3%) and Artuklu (23.3%) achieved the highest plantlet formation capacity however, Çakmak-79, Eminbey, Kunduru-1149 and Selçuklu-97 could not form plantlets. At this stress level, in terms of regeneration capacity and plantlet formation capacity, the stress-strain was the least in Sarıçanak-98 and Artuklu.

The varieties with the highest regeneration capacity at -4.91 bar stress level, which is the maximum drought severity in the study, were Sarıçanak-98 (100%) and Kızıltan-91 (90%). *T. dicoccum* could not regenerate at this stress level. Eminbey regeneration capacity was calculated as 6.7% and stress-strain as 92%. At this drought level, the highest plantlet formation capacity was also formed by Sarıçanak-98 (26.7%) and Artuklu (13.3%). The varieties with the least stress-strain in terms of regeneration capacity and plantlet formation capacity were Sarıçanak-98 and Artuklu.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1903_18131825

Table 5. *Regeneration capacity and plantlet formation capacity of durum wheat genotypes under different drought stress levels*

Genotypes	Different drought stress levels (bar)					Mean
	0	**-0.50**	**-1.48**	**-2.95**	**-4.91**	
Regeneration capacity (%)						
Altın-40/98	100.0 a	93.3 a-c	93.3 a-c	96.7 ab	60.0 h-j	88.7 C
Artuklu	100.0 a	100.0 a	100.0 a	96.7 ab	86.7 b-e	96.7 AB
Çakmak-79	100.0 a	96.7 ab	93.3 a-c	96.7 ab	80.0 d-f	93.3 BC
Çeşit-1252	70.0 f-h	83.3 c-e	46.7 k-m	46.7 k-m	33.3 n	56.0 F
Eminbey	83.3 c-e	83.3 c-e	66.7 g-i	56.7 i-k	6.7 o	59.3 F
Kızıltan-91	100.0 a	100.0 a	100.0 a	93.3 a-c	90.0 a-d	96.7 AB
Kunduru-1149	93.3 a-c	100.0 a	80.0 d-f	70.0 f-h	53.3 j-l	79.3 D
Meram-2002	96.7 ab	93.3 a-c	83.3 c-e	60.0 h-j	53.3 j-l	77.3 D
Mirzabey 2000	93.3 a-c	86.7 b-e	76.7 e-g	56.7 i-k	33.3 n	69.3 E
Sarıçanak 98	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 A
Selçuklu-97	96.7 ab	76.7 e-g	50.0 j-l	43.3 l-n	33.3 n	60.0 F
T. dicoccum	90.0 a-d	90.0 a-d	36.7 mn	10.0 o	0.0 o	45.0 G
Mean	93.6 A	91.9 A	77.2 B	68.9 C	52.5 D	76.8
Plantlet formation capacity (%)						
Altın-40/98	26.7 e-h	20.0 g-j	6.7 k-m	10.0 j-m	3.3 lm	13.3 CD
Artuklu	30.0 d-g	36.7 c-e	26.7 e-h	23.3 f-i	13.3 i-l	26.0 B
Çakmak-79	6.7 k-m	10.0 j-m	0.0 m	0.0 m	0.0 m	3.3 G
Çeşit-1252	10.0 j-m	10.0 j-m	6.7 k-m	3.3 lm	3.3 lm	6.7 FG
Eminbey	40.0 cd	16.7 h-k	0.0 m	0.0 m	0.0 m	11.3 CDE
Kızıltan-91	13.3 i-l	13.3 i-l	10.0 j-m	6.7 k-m	0.0 m	8.7 EF
Kunduru-1149	10.0 j-m	0.0 m	6.7 k-m	0.0 m	0.0 m	3.3 G
Meram-2002	33.3 c-f	26.7 e-g	13.3 i-l	3.3 lm	0.0 m	15.3 C
Mirzabey 2000	23.3 f-i	6.7 k-m	10.0 j-m	10.0 j-m	0.0 m	10.0 DEF
Sarıçanak 98	56.7 a	43.3 bc	36.7 c-e	43.3 bc	26.7 e-h	41.3 A
Selçuklu-97	53.3 ab	16.7 h-k	6.7 k-m	0.0 m	0.0 m	15.3 C
T. dicoccum	16.7 h-k	6.7 k-m	3.3 lm	3.3 lm	0.0 m	5.9 FG
Mean	26.7 A	17.2 B	10.6 C	8.6 C	3.9 D	13.4

Means followed by the different letters are significantly different at the 0.05 probability

Regeneration capacity and plantlet formation capacity averages of genotypes at all drought stress levels were calculated as 76.8% and 13.3%, respectively (*Table 5*). Among the genotypes used in our study, the regeneration capacity and the plantlet formation capacity the average values at different stress levels of Artuklu and Sarıçanak-98 cultivars were found to be higher than these values (*Figs*. *3* and *4*).

Data belonging to stress tolerance, stress sensitive and tolerance indexes made with the data obtained from the parameters examined are shown in *Table 6*. In terms of stress tolerance index; Sarıçanak-98 formed the best scores in all parameters examined in all stress levels. Artuklu had the second-best scores. According to the stress-sensitive index, the most sensitive genotypes were determined as Eminbey, Selçuklu-97, Kunduru-1149 and *T. dicoccum*. The genotype with the highest tolerance index value was Sarıçanak-98 (*Table 6*).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1903_18131825

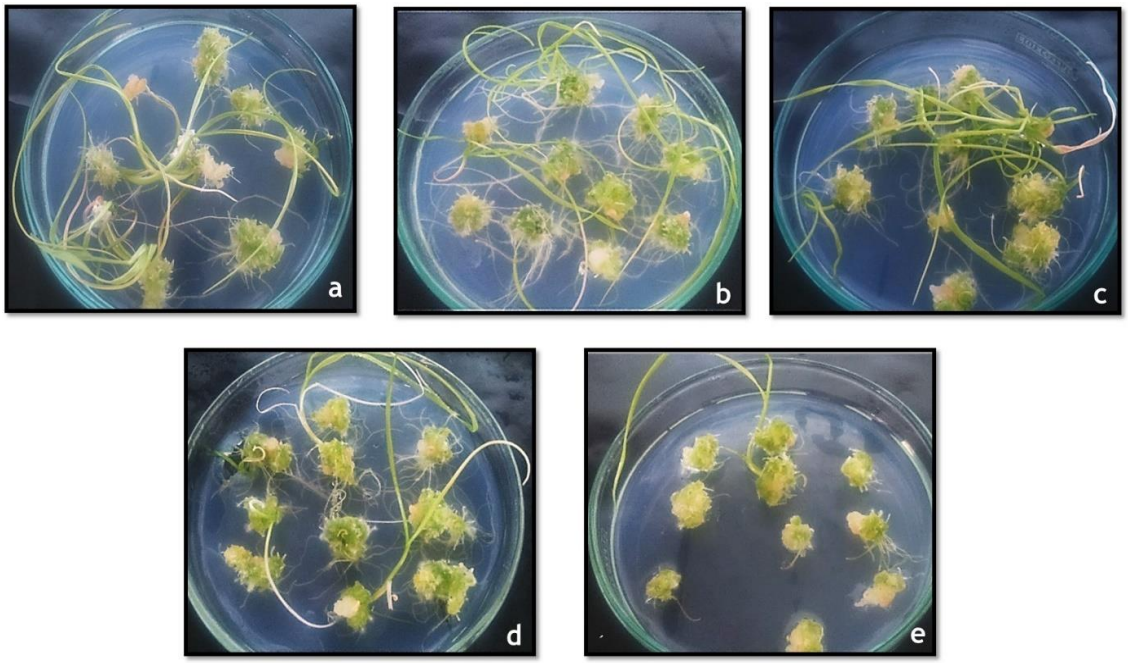

Figure 3. *Regeneration of Artuklu at different drought stress levels (a: control, b: -0.50 bar, c: -1.48 bar, d: -2.95 bar and e: -4.91 bar)*

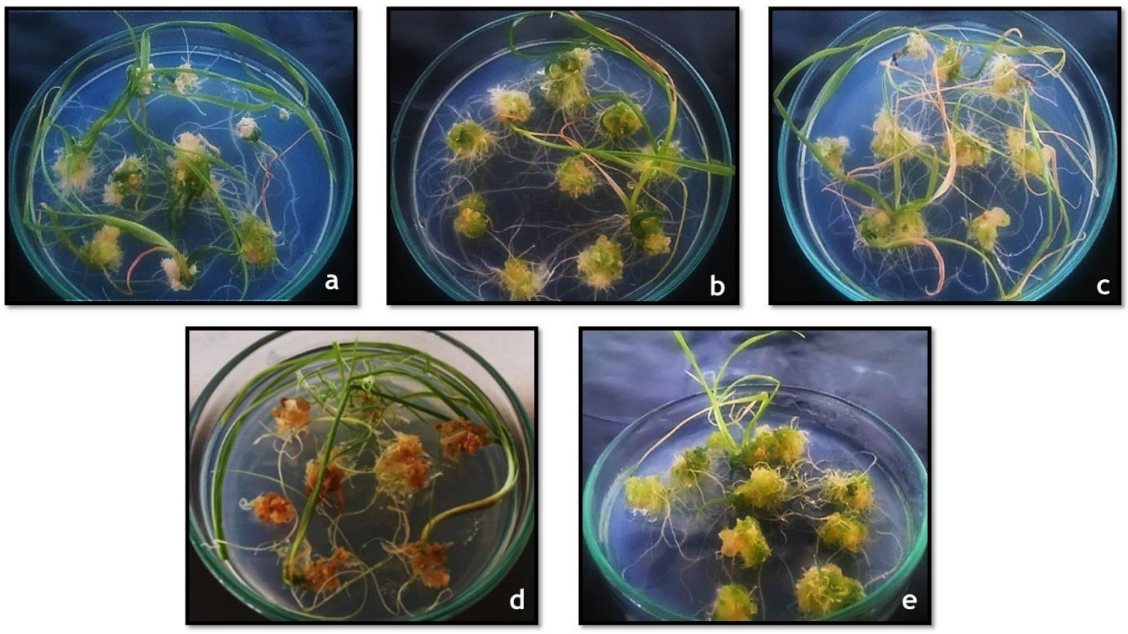

Figure 4*. Regeneration of Sarıçanak-98 at different drought stress levels (a: control, b: -0.50 bar, c: -1.48 bar, d: -2.95 bar and e: -4.91 bar)*

Discussion

The responses of durum wheat genotypes to tissue culture parameters differed significantly according to genetic structure. It has been clearly stated in previous studies that the genotype affects tissue culture parameters in wheat (Ozgen et al., 1998;

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1903_18131825

Pellegrineschi et al., 2004; Zale et al., 2004; Grigoryeva and Shletser, 2006; Farshadfar et al., 2012a, b; Ozgen et al., 2017; Kacem et al., 2017: Miroshnichenko et al., 2019; Jasdeep et al., 2019).

Table 6. *Stress tolerance index (STI), stress sensitive index (SSI) and tolerance index values of durum wheat genotypes*

	Genotypes	Stress tolerance index				Stress sensitivity index				Tolerance index			
		-0.50	-1.48	-2.95	-4.91	-0.50	-1.48	-2.95	-4.91	-0.50	-1.48	-2.95	-4.91
Regeneration capacity	Altın-40/98	1.06	1.06	1.10	0.68	3.69	0.38	0.13	0.91	96.7	96.7	98.4	80.0
	Artuklu	1.14	1.14	1.10	0.99	0.00	0.00	0.13	0.30	100.0	100.0	98.4	93.4
	Çakmak-79	1.10	1.06	1.10	0.91	1.82	0.38	0.13	0.46	98.4	96.7	98.4	90.0
	Çeşit-1252	0.67	0.37	0.37	0.27	-10.46	1.90	1.26	1.19	76.7	58.4	58.4	51.7
	Eminbey	0.79	0.63	0.54	0.06	0.00	1.14	1.21	2.09	83.3	75.0	70.0	45.0
	Kızıltan-91	1.14	1.14	1.06	1.03	0.00	0.00	0.25	0.23	100.0	100.0	96.7	95.0
	Kunduru-1149	1.06	0.85	0.75	0.57	-3.95	0.81	0.95	0.98	96.7	86.7	81.7	73.3
	Meram-2002	1.03	0.92	0.66	0.59	1.94	0.79	1.44	1.02	95.0	90.0	78.4	75.0
	Mirzabey 2000	0.92	0.82	0.60	0.35	3.89	1.02	1.49	1.46	90.0	85.0	75.0	63.3
	Sarıçanak 98	1.14	1.14	1.14	1.14	0.00	0.00	0.00	0.00	100.0	100.0	100.0	100.0
	Selçuklu-97	0.85	0.55	0.48	0.37	11.39	2.76	2.09	1.49	86.7	73.4	70.0	65.0
	T. dicoccum	0.92	0.38	0.10	0.00	0.00	3.38	3.37	2.28	90.0	63.4	50.0	45.0
Plantlet formation capacity	Altın-40/98	0.75	0.25	0.37	0.12	0.71	1.24	0.92	1.03	23.4	16.7	18.4	15.0
	Artuklu	1.54	1.12	0.98	0.56	-0.63	0.18	0.33	0.65	33.4	28.4	26.7	21.7
	Çakmak-79	0.09	0.00	0.00	0.00	-1.38	1.66	1.48	1.17	8.4	3.4	3.4	3.4
	Çeşit-1252	0.14	0.09	0.05	0.05	0.00	0.55	0.99	0.78	10.0	8.4	6.7	6.7
	Eminbey	0.94	0.00	0.00	0.00	1.64	1.66	1.48	1.17	28.4	20.0	20.0	20.0
	Kızıltan-91	0.25	0.19	0.12	0.00	0.00	0.41	0.73	1.17	13.3	11.7	10.0	6.7
	Kunduru-1149	0.00	0.09	0.00	0.00	2.81	0.55	1.48	1.17	5.0	8.4	5.0	5.0
	Meram-2002	1.25	0.62	0.15	0.00	0.56	1.00	1.33	1.17	30.0	23.3	18.3	16.7
	Mirzabey 2000	0.22	0.33	0.33	0.00	2.00	0.95	0.84	1.17	15.0	16.7	16.7	11.7
	Sarıçanak-98	3.44	2.92	3.44	2.12	0.66	0.58	0.35	0.62	50.0	46.7	50.0	41.7
	Selçuklu-97	1.25	0.50	0.00	0.00	1.93	1.45	1.48	1.17	35.0	30.0	26.7	26.7
	T. dicoccum	0.16	0.08	0.08	0.00	1.68	1.33	1.18	1.17	11.7	10.0	10.0	8.4

In previous studies to determine the responses of genotypes to drought stress in wheat in vitro, generally immature embryos were used as an explant source (Galovic et al., 2005; Abdel-Haddy and El-Naggar, 2007; Bouiamrine and Diouri, 2012; Farshadfar et al., 2012a; Mahmood et al., 2012; Mahmoud et al., 2012). On the other hand, we used endosperm supported mature embryo culture method in our study (Ozgen et al., 1998); Thus, it was ensured that the calli benefit from the nutrients of the endosperm and develop. In most of the previous studies, drought stress was applied during callus formation or callus development stages (Hsissou and Bouharmont, 1994; Almansouri et al., 2001; Biswas et al., 2001; Bouiamrine and Diouri, 2012; Farshadfar et al., 2012a, b). The callus formed in our study were expected to reach sufficient maturity and the full totipotency feature was allowed to occur. In our study, to determine the true potentials of genotypes, no growth regulator used during drought stress and only endosperm used as a nutrient for callus induction.

Our findings show that the regeneration capacity of genotypes decreases with increasing PEG 6000 doses. PEG is used as a drought stress agent; our results are similar to the results of the studies conducted on paddy (Biswas et al., 2002; Wani et al., 2010), durum wheat (Bajji et al., 2000; Almansouri et al., 2001; Lutts et al., 2004; Abdel-Haddy and El-Naggar,

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1903_18131825

2007; Bouiamrine and Diori, 2012; Razmjoo et al., 2015; Kacem et al., 2017), common bread wheat (Farshadfar et al., 2012a, b; Mahmood et al., 2012) and sorghum (Tsago et al., 2014).

In our study, Stress x Genotype interaction was found to be statistically significant in all parameters examined. In previous studies in which drought was induced by applying PEG in vitro conditions, durum wheat (Bouiamrine and Diouri, 2012; Razmjoo et al., 2015), common wheat (Farshadfar et al., 2012a, b; Mahmoud et al., 2012) and potato (Gopal and Iwama, 2007) were determined as the interaction of genotype x stress was statistically significant.

Abdel-Haddy and El-Naggar (2007); applied drought stresses by using different doses of PEG 6000 on callus they obtained from durum wheat. They stated that the regeneration capacity of the genotypes did not show a significant change at -0.50 bar stress level, but significant decreases occurred with -1.48 bar stress level. Most of the durum wheat genotypes we used in our study tolerated -1.48 bar of drought stress level. Besides, regeneration occurred at -4.91 bar stress level. Most of the durum wheat genotypes we used in our study tolerated -1.48 bar of drought stress level. Besides, regeneration occurred at -4.91 bar stress level. This difference is thought to be due to the genetic structures of genotypes. In addition, Abdel-Haddy and El-Naggar (2007); stated that among the varieties they used in their experiments, the most sensitive to drought stress was a local variety. In our study, we determined that emmer, which is a local wheat variety, is also sensitive to drought stress.

Bouiamrine and Diori (2012); applied different drought stresses with PEG to calli obtained from immature embryos of durum wheat genotypes. The researchers stated that the mean regeneration capacity was 88.73% in the control group and 26.91% at -4.91 bar stress level. In our study, the mean regeneration capacity of the genotypes was 93.6% in the control group and 52.5% at -4.91 bar stress level. While the regeneration capacity values in the control group are close to each other, there is a significant difference between mean of the regeneration capacity at -4.91 bar stress level. It is thought that this difference may be due to the source of the explant and the method used, as well as the genetic structure.

Farshadfar et al. (2012b); under in vitro conditions, by applying different drought stress to mature embryos of 20 bread wheat genotypes, they examined the genotypes callus induction and callus development. In their study, they stated that callus induction depends on the genotype, not the stress factor. In our study, drought stress was applied to mature calli and their regeneration ability was determined under stress conditions. It is thought that the application of the stress agent in the regeneration phase may be more determinant in measuring tolerance to drought stress.

Conclusion

The results confirmed a significant variation for plant regeneration ability in durum wheat genotypes under drought stress condition that can be used in durum wheat breeding programmes. We propose that this protocol to in vitro selection for drought tolerance would be a suitable and rapid way to characterize parental lines and to develop drought-tolerant lines in durum wheat.

According to the results, the genotypes with the most drought stress tolerance were determined as Sarıçanak-98 and Artuklu. On the other hand, Çakmak-79, Çeşit-2002, Eminbey, Kunduru-1149 and *Triticum dicoccum* (emmer) were determined as genotypes sensitive to drought stress.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1903_18131825

REFERENCES

[1] Abdel-Hady, M. S., El-Naggar, H. M. (2007): Wheat genotypic variation and protein markers in relation with in vitro selection for drought tolerance. – Journal of Applied Sciences Research 3(10): 926-934.
[2] Almansouri, M., Kinet, J. M., Lutts, S. (2001): Effect of salt and osmotic stresses on germination in durum wheat (Triticum durum Desf.). – Plant and Soil 231(2): 243-254.
[3] Anonymous (2020): Plant Production Statistics. – FAO, Rome. http://wwwfaoorg/faostat/en/#data/QC.
[4] Bajji, M., Lutts, S., Kinet, J. M. (2000): Physiological changes after exposure to and recovery from polyethylene glycol-induced water deficit in callus cultures issued from durum wheat (Triticum durum Desf.) cultivars differing in drought resistance. – Journal of Plant Physiology 156(1): 75-83.
[5] Biswas, J., Chowdhury, B., Bhattacharya, A., Mandal, A. B. (2002): In vitro screening for increased drought tolerance in rice. – In Vitro Cellular and Developmental Biology-Plant 38(5): 525-530.
[6] Bouiamrine, H., Diouri, M. (2012): Response of durum wheat (Triticum durum Desf.) callus culture to osmotic-induced drought stress caused by PEG. – Annals of Biological Research 3(9): 4555 4563.
[7] Caruso, A., Chefdor, F., Carpin, S., Depierreux, C., Delmotte, F. M., Kahlem, G., Morabito, D. (2008): Physiological characterization and identification of genes differentially expressed in response to drought induced by PEG 6000 in Populus canadensis leaves. – Journal of Plant Physiology 165(9): 932-41.
[8] Chen, J., Wu, W., Zheng, Y., Hou, K., Xu, Y., Zai, J. (2010): Drought resistance of Angelica dahurica during seedling stage under polyethylene glycol (PEG-6000)-simulated drought stress. – China Journal of Chinese Materia Medica 35(2): 149-53.
[9] Cook, B. I., Smerdon, J. E., Seager, R., Coats, S. (2014): Global warming and 21st century drying. – Climate Dynamics 43: 2607-2627.
[10] Dai, A. (2011): Drought under global warming: a review. – Wiley Interdisciplinary Reviews: Climate Change 2: 45-65.
[11] Dai, A. (2013): Increasing drought under global warming in observations and models. – Nature Climate Change 3: 52-58.
[12] Desa, U. N. (2019): World Population Prospects 2019: Highlights. – United Nations Department for Economic and Social Affairs, New York.
[13] El-Rawy, M. A., Hassan, M. I. (2014): A diallel analysis of drought tolerance indices at seedling stage in bread wheat (Triticum aestivum L.). – Plant Breeding and Biotechnology 2(3): 276-288.
[14] Farshadfar, E., Jamshidi, B., Cheghamirza, K., Hashemzadah, H. (2012a): Evaluation of drought tolerance in breat wheat (Triticum aestivum L.) using immature embryo culture. – Annals of Biological Research 3(1): 330-338.
[15] Farshadfar, E., Jamshidi, B., Cheghamirza, K., Teixeira da Silva, J. A. (2012b): Evaluation of drought tolerance in bread wheat (Triticum aestivum L.) using in vivo and in vitro techniques. – Annals of Biological Research 3(1): 465-476.
[16] Fernandez, G. C. J. (1992): Effective selection criteria for assessing plant stress tolerance. – Proceedings of Symposium, Taiwan, 13-16 Aug. 1991, pp. 257-270.
[17] Fischer, R. A., Maurer, R. (1978): Drought resistance in spring wheat cultivars: I. Grain yield responses. – Australian Journal of Agricultural Research 29: 897-912.
[18] Galovic, V. O., Kotaranin, Z., Denčić, S. S. (2005): In vitro assessment of wheat tolerance to drought. – Genetika 37(2): 165-171.
[19] Gopal, J., Iwama, K. (2007): In vitro screening of potato against water-stress mediated through sorbitol and polyethylene glycol. – Plant Cell Reports 26(5): 693-700.
[20] Grigoryeva, L. P., Shletser, I. A. (2006): Screening wheat cultivars for morphogenesis ability in immature embryo culture in vitro. – Biologia 3: 64-66.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1903_18131825

[21] Hasanuzzaman, M., Shabala, L., Brodribb, T. J., Zhou, M., Shabala, S. (2017): Assessing the suitability of various screening methods as a proxy for drought tolerance in barley. – Functional Plant Biology 44(2): 253-266.
[22] Hassan, N. S., Shaaban, L. D., El-Sayed, A. H., Seleem, E. E. (2004): In vitro selection for water stress tolerant callus line of Helianthus annus L. Cv Myak. – International Journal of Agriculture and Biology 1-13.
[23] Hsissou, D., Bouharmont, J. (1994): In vitro selection and characterization of drought-tolerant plants of durum wheat (Triticum durum Desf). – Agronomie 14(2): 65-70.
[24] Jasdeep, P., Avijit, T., Varsha, S., Harinder, V., Sanjay, S. (2019): Cultivar specific response of callus induction and plant regeneration from mature embryos in different elite Indian wheat. – Research Journal of Biotechnology 14(2): 1-8.
[25] Kacem, N. S., Delporte, F., Muhovski, Y., Djekoun, A., Watillon, B. (2017): In vitro screening of durum wheat against water-stress mediated through polyethylene glycol. – Journal of Genetic Engineering and Biotechnology 15(1): 239-247.
[26] Lutts, S., Almansouri, M., Kinet, J. M. (2004): Salinity and water stress have contrasting effects on the relationship between growth and cell viability during and after stress exposure in durum wheat callus. – Plant Science 167(1): 9-18.
[27] Mahmood, I., Razzaq, A., Hafiz, I. A., Kaleem, S., Khan, A. A., Qayyum, A., Ahmad, M. (2012): Interaction of callus selection media and stress duration for in vitro selection of drought tolerant callus of wheat. – African Journal of Biotechnology 11(17): 4000-4006.
[28] Mahmoud, S. N., Aljibouri, A. M., Abd, A. S., Al-Hassini, Z. A., Ismail, E., Majeed, D. M. (2012): Evaluation of bread wheat Triticum aestivum L. callus genotypes for water stress tolerance using Polyethylene Glycol (PEG). – Baghdad Science Journal 9(3): 391-396.
[29] Miroshnichenko, D. N., Klementyeva, A. A., Salina, E. A., Dolgov, S. V. (2019): Evaluation of in vitro plant regeneration efficiency in Siberian wheat cultivars. – Current Challenges in Plant Genetics, Genomics, Bioinformatics and Biotechnology 24: 126-128.
[30] Mohamed, M. A. H., Harris, P. J. C., Henderson, J. (2000): In vitro selection and characterisation of a drought tolerant clone of Tagetes minuta. – Plant Science 159: 213-222.
[31] Murashige, T., Skoog, F. (1962): A revised medium for rapid growth and bio assays with tobacco tissue cultures. – Physiologia Plantarum 15(3): 473-497.
[32] Ozgen, M., Turet, M., Altinok, S., Sancak, C. (1998): Efficient callus induction and plant regeneration from mature embryo culture of winter wheat (Triticum aestivum L.) genotypes. – Plant Cell Reports 18(3-4): 331-335.
[33] Ozgen, M., Adak, M. S., Ulukan, H., Benlioglu, B., Peskircioglu, M., Koyuncu, N., Yıldız, A., Tuna, D. E. (2015): Climate change and plant genetic resources. – Turkey Agricultural Engineering 8. Technical Conference, 12-16 January 2015 Ankara, pp. 184-211.
[34] Ozgen, M., Avci Birsin, M., Benlioglu, B. (2017): Biotechnological characterization of a diverse set of wheat progenitors (Aegilops sp. and Triticum sp.) using callus culture parameters. – Plant Genetic Resources: Characterization and Utilization 15(1): 45-50.
[35] Pellegrineschi, A., Brito, R. M., Mclean, S., Hoisington, D. (2004): Effect of 2.4-Dichlorophenoxyacetic acid and NaCl on the establishment of callus and plant regeneration in durum and bread wheat. – Plant Cell Tissue and Organ Culture 77: 245-250.
[36] Rai, R., Agrawal, M., Agrawal, S. B. (2010): Threat to food security under current levels of ground level ozone: a case study for Indian cultivars of rice. – Atmospheric Environment 44(34): 4272-4282.
[37] Razmjoo, M., Mohammadi, R., Shooshtari, L. (2015): In vitro evaluation of durum wheat genotypes for drought tolerance. – Journal on New Biological Reports 4(1): 33-40.
[38] Rosielle, A. A., Hamblin, J. (1981): Theoretical aspects of selection for yield in stress and nonstress environments. – Crop Science 21: 943-946.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1903_18131825

[39] Russell, O. F. (1994): MSTAT-C v. 2.1 (a computer-based data analysis software). – Crop and Soil Science Department, Michigan State University, USA.
[40] SAS Institute Inc. (2015): JMP® Statistical Discovery Software, version 12.0; – SAS Institute Inc., Cary, NC.
[41] Soni, P., Rizwan, M., Bhatt, K. V., Mohapatra, T., Singh, G. (2011): In-vitro response of Vigna aconitifolia to drought stress induced by Peg 6000. – Journal of Stress Physiology and Biochemistry 7(3): 108-121.
[42] Steel, R. G., Torrie, J. H., Dickey, D. A. (1980): Principles and Procedures of Statistics: A Biometrical Approach. – McGraw-Hill, New York.
[43] Tsago, Y., Andargie, M., Takele, A. (2014): In vitro selection of sorghum (Sorghum bicolor L. Moench) for polyethylene glycol (PEG) induced drought stress. – Plant Science Today 1(2): 62-68.
[44] Wani, S. H., Sofi, P. A., Gosal, S. S., Singh, N. B. (2010): In vitro screening of rice (Oryza sativa L.) callus for drought tolerance. – Communications in Biometry and Crop Science 5: 108-115.
[45] Zale, J. M., Wier, H. B., Kidwell, K. K., Steber, C. M. (2003): Callus induction and plant regeneration from mature embryos of a diverse set of wheat genotypes. – Plant Cell Tissue and Organ Culture 76: 277-281.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1903_18131825