Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi
Journal of Agricultural Faculty of Gaziosmanpasa University
http://ziraatdergi.gop.edu.tr/

Araştırma Makalesi/*Research Article*

JAFAG
ISSN: 1300-2910
E-ISSN: 2147-8848
(2019) 36 (2), 145-152
doi:10.13002/jafag4633

Drought Tolerance of Some Wine Grape Cultivars Under *In Vitro* Conditions

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Alındığı tarih (Received): 09.04.2019 Kabul tarihi (Accepted): 16.05.2019
Online Baskı tarihi (Printed Online): 30.08.2019 Yazılı baskı tarihi (Printed): 31.08.2019

Abstract: This study was conducted to determine the drought tolerance of 6 different economically important wine grape cultivars ('Sultani Seedless', 'Çalkarası', 'Emir', 'Boğazkere', 'Öküzgözü', 'Narince') of Turkey under *in vitro* conditions. Drought stress was induced on *in vitro*-grown explants by 3 different PEG (8000) (poly ethylene glycol) doses (2, 4 and 6 %). Plants were subjected to drought stress for 6 weeks and plant fresh weight, dry weight, shoot length, number of shoots, number of leaves, electrolyte leakage, relative water content, proline content and lipid peroxidation (MDA) were determined. Being more distinctive at higher doses, PEG treatments yielded significant decreases in fresh weight, dry weight, shoot length, number of shoots and number of leaves. As compared to the control, PEG treatments also yielded greater electrolyte leakage in all cultivars. All three PEG concentrations decreased relative water content of all cultivars. Proline content of explants increased with increasing PEG doses. While plant response to PEG treatments varied with the cultivars in 2 % PEG treatments, significant increases were observed in MDA content of all cultivars at higher doses (4 and 6 %).

Keywords: PEG, *in vitro* Screening, Proline, MDA

In Vitro Koşulları Altında Bazı Şaraplık Üzüm Çeşitlerinin Kuraklığa Toleransı

Öz: Bu araştırmada, Türkiye'de yetiştirilen bazı önemli şaraplık üzüm çeşitlerinin ('Sultani Çekirdeksiz', 'Narince', 'Öküzgözü', 'Boğazkere', 'Emir' ve 'Çalkarası') kuraklık stresine toleranslarını belirlemek amaçlanmıştır. Araştırmada çeşitlere ait *in vitro* şartlarda yetiştirilen eksplantlara kuraklık stresi 3 farklı PEG (8000) dozu (% 2,4 ve 6) olacak şekilde uygulanmıştır. Altı hafta süreyle strese maruz bırakılan bitkilerde yaş ağırlık, kuru ağırlık, sürgün uzunluğu, sürgün sayısı, yaprak sayısı, iyon sızıntısı, oransal su kapsamı, prolin miktarı ve lipid peroksidasyonu (MDA) belirlenmiştir. PEG uygulamaları, yüksek dozlarda daha belirgin olacak şekilde, yaş ağırlık, kuru ağırlık, sürgün uzunluğu, sürgün sayısı ve yaprak sayısında belirgin azalmalara neden olmuştur. İyon sızıntısı bütün asma çeşitlerinde kontrole kıyasla PEG uygulamalarında daha yüksek olarak ölçülmüştür. PEG'nin üç konsantrasyonu da bütün çeşitlerin eksplantlarının oransal su kapsamında azalmaya neden olmuştur. Explantlardaki prolin miktarı aratan PEG uygulama dozuna bağlı olarak artış göstermiştir. MDA içeriği açısından % 2'lik PEG uygulamasına verilen tepki çeşitlere bağlı olarak değişirken, yüksek konsantrasyonda (% 4 veya % 6) PEG uygulaması bütün çeşitlerin MDA içeriğinde önemli artışlara neden olmuştur.

Anahtar Kelimeler: PEG, *in vitro* Screening, Prolin, MDA

1. Introduction

Climate largely dominates geographical formations and human life (IPCC(Intergovernmental Panel On Climate Change) 2007). Anthropogenic activities, especially agricultural activities are also largely dependent on weather conditions and climate. Climate parameters have significant impacts on yield and quality of agricultural products (Adams, 2001; Sivakumar, 2006).

Together with current global warming and climate change, plants are exposed to various abiotic stress factors (drought alone or in combination with salinity, extreme temperatures, excessive CO_2 accumulation).

Climate change-induced abiotic stressors then exert significant threats on agricultural

sustainability, biodiversity, plant genetic sources and global food safety (Ahuja et al., 2010). Viticulture is a significant branch of agriculture worldwide and thus expected to be influenced significantly from the prospective global warming trends and resultant drought stress. Therefore, significant changes are expected in both physiological activities and grape yield and quality of grapevines (Carbonneau et al., 2007; Kunter et al., 2017).

Droughts and high temperatures are the primary outcomes of global climate change. Drought or continuous water deficits significantly influence plant growth and development, plant life and yield (Ahuja et al., 2010). Just depending on duration and severity, droughts generally result in stomal closure, reduce photosynthetic activity, influence the elasticity of cell membrane, generate some toxic metabolites and ultimately end up with total die outs (Rampino et al., 2006).

There are various measures to be taken against potential negative impacts of drought stress on plants. Drought stress-tolerant genotypes constitute a significant solution to cope with such stressors. Before the initiation of breeding programs to be designed for such purposes, initially tolerance status of available cultivars should be determined. Several studies were carried out to determine the effects of drought stress on physiological activities of various plant species (grapevines, apples, pears, peas, wheat and so on) (Dami and Hughes 1995; Šircelj et al., 2007; Parida et al., 2007; Sánchez et al., 2004; Alvarez et al., 2008; Carmo-Silva et al., 2009; Winning et al., 2009). Besides in vivo researches on recent drought stress in grapevines (Babalık 2012; Sabır 2016; Sucu et al., 2018; Tangolar et al., 2016; Canturk et al., 2018), there are some other studies carried out under in vitro conditions (Dami and Hughes, 1995; Babalık, 2015; Cui et al., 2016). *In vitro* studies are performed in small areas and shorter periods. It is also possible to control or observe plant behaviors efficiently. Therefore, such studies are generally used as complementary part of studies carried out under field conditions (Jain, 2001; Keskin and Kunter 2007, 2009; Manoj et al., 2011).

PEG is used to stimulate water stress in plants. It is a non-ionic osmoticum with high molecular weight. PEG is up taken by the plants without any toxic impacts and reduces water potential of nutrient medium. It was observed in previous studies that PEG increased water stress in *in vitro* culture (Sivritepe et al., 2008) of cherries and mulberries (Tewary et al., 2000).

This study was conducted to determine drought tolerance of 6 different economically important wine grape cultivars (‘Sultani Seedless’, ‘Çalkarası’, ‘Emir’, ‘Boğazkere’, ‘Öküzgözü’, ‘Narince’) of Turkey under *in vitro* conditions.

2. Material and Method

2.1. Plant material

Explants to be sued in this study were taken from ‘Sultani Seedless’ (K7 clone), ‘Çalkarası’, ‘Emir’, ‘Boğazkere’, ‘Öküzgözü’, ‘Narince’ grape cultivars. Initially, single-bud cuttings were taken from the vines of these grape cultivars and they were planted into perlite media in growth chambers at 24-25 ^{o}C for shooting. Before planting, cuttings were subjected to hot water treatments at 50 ^{o}C for 30 minutes and they were then surface sterilized through dipping into 1 % sodium hypochlorite (NaOCI) solution for 5 minutes. Again, before planting, the perlite medium to be used was saturated with distilled water and sterilized in an autoclave. Planted cuttings were irrigated with ½ Hoagland solution until the new shoots had 5-6 nodes.

2.2. In vitro propagation and stress treatments

About 0.4 – 0.5 mm single-bud (active bud) explants taken from newly developed shoots were cultured in MS (Murashige , Skoog 1962) growth medium supplemented with 0.5 mg L^{-1} BA (N^{6}- Benzyladenine), 3 % sucrose and 0.7 % bacto agar and a pH of 5.7- 5.8. Following the culture of explants in MS medium for 4 weeks, micro cuttings (2 cm long) were cultured in 300 ml glass jars including 50 ml MS

medium with different PEG supplementation levels (0, 2, 4, 6 %). Following measurements were performed after 6 weeks of stress treaments:

Growth measurements

Following 6 weeks of stress treatments, plant fresh and dry weights (mg), shoot lengths (mm) and number of leaves per shoot were determined.

Electrolyte Leakage

They were separated into 0.3 g equal pieces, placed into 25 mm × 150 mm glass tubes and supplemented with 15 ml distilled water. Samples were then shaken at 100 rpm for 24 hours. Following the incubation, electrical conductivity of the solution (EC1) was measured with an EC meter (Hach brand HQ 40d Model portative EC meter). Then the same samples were autoclaved at 115 ºC for 10 minutes. They were at room temperature for 24 hours and electrical conductivity of the samples (EC2) was measured again. Leaf electrolyte leakage was calculated as EL = EC1 / EC2 ×100 and expressed as percent (Özden et al., 2009).

Leaf relative water content

Leaf relative water content (LRWC) was calculated in accordance with Yamasaki, Dillenburg (1999). Leaf fresh weight (FW), turgor weight after keeping in distilled water for 6 hours (TW) and dry weight after drying at 80 ºC for 24 hours (DW) were used to calculate leaf relative water content (%) with the aid of the following equation;

LRWC = [(FW-DW) ÷ (TW-DW) x 100]

Proline content

About 0.5 g fresh leaf sample was homogenized with 3% sulphosalicylic acid and filtered through filter papers. Then, 1 ml of the filtrate was taken and supplemented with 1 ml acetic acid and 1 ml ninhydrine solution. Samples were placed into tubes, incubated at 100 ºC for 1 hour and the reaction was terminated on ice. Cooled samples were supplemented with 2 ml toluene, vortexed and reading was performed in a spectrophotometer at 520 nm. A graph was generated with proline standards (Sigma- Aldrich, Germany) and sample proline content was calculated with the aid of this graph (Bates et al., 1973).

MDA (Lipid peroxidation)

About 0.4 g fresh leaf sample was homogenized in 1 % TCA. The homogenate was centrifuged at 15000 rpm for 15 minutes. About 0.5 ml sample was taken from the resultant supernatant and supplemented with 20 % TCA including 1 ml 0.5 % (w/v) TBA. Samples were then incubated in a water bath at 95 ºC for 30 minutes, instantly cooled on ice, centrifuged at 10000 rpm for 15 minutes. Supernatant readings were performed at 532 nm. Resultant readings were subtracted from the readings made at non-specific 600 nm. Following the error corrections, MDA content of the samples was calculated by using an "extinction coefficient" of 155 mM^{-1} cm^{-1} (Heath and Packer 1968).

2.3. Statistical analysis

The experiment was arranged in completely randomized design with 5 replicates, each consisting of three plants. Thus, there were 30 plants in each treatment and a total of 240 plants in the experiment. Descriptive statistics wrew expressed as mean and standard deviation or (Standard error of mean). One-way ANOVA (Analysis of Variance) were used to analyze the data. Following the ANOVA, Duncan's multiple range test was performed to determine different groups. Statistically significant lewel was considered as 5% and SAS software was used all statistical computations.

3. Results and Discussion

PEG treatments yielded significant differences in fresh weight, dry weight, shoot length, number of shoots and number of leaves of all grape cultivars at 5 % level.

While higher PEG doses (4 and 6 %) yielded distinctive decreases in fresh weight of all cultivars, effects of lower doses (2 %) varied with the cultivars. As compared to the control, 2 % PEG treatments decreased fresh weight of Narince, Boğazkere, Emir and Çalkarası cultivars, however did not yield significant changes in fresh weight of Sultani Seedless and Öküzgözü cultivars. A similar case was also

observed in dry weights. The 4 and 6 % PEG treatments yielded significant decreases in dry weight of all cultivars. While 2 % PEG treatments did not result in significant changes in dry weight of Narince cultivar, they decreased the dry weight of the other cultivars. Similar with the present findings, PEG-induced drought stress inhibited growth and development of various other plants under *in-vitro* conditions (Sivritepe et al., 2008, Babalık et al., 2015, Dami and Hughes 1995, Al-Khayri and Al-Bahrany 2004). Such a case was mostly attributed to inhibition of plant water intake by PEG through reducing the osmotic potential of growth medium (Kaufmann and Eckard 1971; Bressan et al., 1982; Dami and Hudges, 1995; Sawwan et al., 2000, Al-Khayri and Al-Bahrany 2004, Chai et al., 2005). Except for 4 % PEG treatment in Emir cultivar, PEG treatments yielded significant decreases in shoot length of all cultivars. PEG treatments also resulted in significant decreases in number of leaves of all cultivars (Table 1).

Table 1. The effects of different PEG doses applied to varieties on growth and development parameters

Çizelge1. *Çeşitlere uygulanan farklı PEG dozlarının büyüme ve gelişme parametrelerine etkileri*

	Treatm.	*Narince*	*Sultani Seedless*	*Öküzgözü*	*Boğazkere*	*Emir*	*Çalkarası*	*Mean*
Fresh weight	Control	C 413.50 a	A 518.20 a	A 548.50 a	A 530.50 a	BC 435.00 a	AB 492.00 a	489.61a
	%2 PEG	B 266.83 b	AB 452.50 a	A 588.33 a	A 266.00 b	B 237.17 b	AB 373.50b	364.06 b
	%4 PEG	B 180.50 bc	AB 220.70b	AB 216.67b	A 281.00 b	B 197.83 b	B 187.50 c	214.03 c
	%6 PEG	B 109.50 c	B 109.50 b	A 193.33 b	B 124.00 c	A 175.83 b	B 107.83 d	136.67 d
	Mean	242.58 C	358.78 A	376.44 A	324.22 AB	322.22 B	274.78 B	
Dry weight (mg)	Control	AB 83.00 a	A 106.83 a	A 102.00 a	A 99.16 a	C 80.33 a	B 89.33 a	93.44 a
	%2 PEG	AB 68.16 a	AB 55.17 b	A 73.00 b	B 39.33 b	AB 52.50 b	AB 52. 66 b	56.80 b
	%4 PEG	A 23.83 b	A 44.17 bc	A 37.83 c	A 45.66 b	A 46.67 b	A 28.16 c	37.72 c
	%6 PEG	A 20.00 b	A 20.00 c	A 28.33 c	A 17.16 c	A 30.00 b	A 24.66 c	23.36 d
	Mean	48.75 B	56.54 AB	60.29 A	50.33 B	52.37 AB	48.70 B	
Shoot lenght	Control	A 26.93 a	A 29.32 a	A 29.15 a	A 29.31 a	A 27.50 a	A 28.80 a	28.80 a
	%2 PEG	A 19.36 b	A 23.87 b	A 23.48 b	A 22.44 b	A 21.99 b	A 22.61 b	22.29 b
	%4 PEG	B 18.18 b	B 17.81 c	B 19.01 b	B 17.77 c	A 24.87 ab	B 18.96 bc	19.43 c
	%6 PEG	B 14.33 c	B 14.33 c	A 19.01 b	AB 15.05 c	B 14.07 c	AB 16.02 c	15.47 d
	Mean	19.70 B	21.33 AB	22.66 A	21.14 AB	22.11 A	21.60 AB	
Number of leaves/shoo	Control	A 8.61 a	A 8.70 a	A 9.51 a	A 8.70 a	A 8.02 a	A 9.23 a	8.80a
	%2 PEG	A 5.33 b	A 5.66 b	A 7.00 ab	A 6.75 ab	A 5.50 b	A 6.91 b	6.19 b
	%4 PEG	A 5.16 b	A 4.52 bc	A 5.33 b	A 5.33 b	A 4.66 b	A 4.66 b	4.88 c
	%6 PEG	A 4.16 b	A 4.16 c	A 5.16 b	A 5.00 b	A 4.50 b	A 4.30 b	4.61 c
	Mean	5.81 BC	5.76 BC	6.75 A	6.44 AB	5.67 C	6.28 ABC	

A,B,C : Different capital letter on on the sam eline represent statistically significant differences among the cultivars
a,b,c : Diffrent lowercase letter on the same coloumn represent statistically significant differences among the PEG doses ($p<0.05$).

Decrease in number of leaves was considered as a defense mechanism of plants against drought stress. Such a case probably resulted in less light absorption and reduced transpiration surface area (Molassiotis et al., 2006 and Sivritepe et al., 2008). Similar findings were also reported in several previous studies (Taiz and Ziger 1998; Pellegrino et al., 2005, Ghaderi et al.,, 2011; Babalık, 2012; Sabır, 2016).

PEG treatments had significant effects on leaf relative water content, proline and MDA values of all cultivars at 5 % level.

Cell membrane is the most critical component of the plants influenced by stress conditions. Electrolyte leakage is an important physiological parameter used in identification of plants with greater tolerance to drought and high temperatures (Leopold et al., 1981; Stevanovic et al., 1997; Bajji et al., 2001). Present drought treatments yielded distinctive increases in electrolyte leakage of all cultivars. As compared to the control, 4 and 6 % PEG treatments yielded almost 4 times greater electrolyte leakages. While all three PEG treatments had similar effects on electrolyte leakage of

Çalkarası cultivar, 4 and 6 % PEG treatments yielded significantly greater electrolyte leakage values than 2% treatments in the other cultivars. Similar findings were also reported for drought stress in kiwifruits (Savee et al., 1990) and figs (Karimi et al., 2012). Teulate et al. (1997) pointed out relative water content as a significant indicator for plant water condition. Akbarpour et al. (2017) investigated the drought response of almond cultivars under *in vitro* conditions and reported greater relative water content values for resistant cultivars as compared to sensitive ones. Rasouli (2000) indicated that the grapevine cultivars able to sustain higher relative water contents under drought stress were more resistant to droughts. In present study, PEG treatments significantly reduced explant relative water content of all cultivars. While all PEG concentrations had similar effects on relative water content of Boğazkere and Emir cultivars, greater concentrations were found to be more effective in the other cultivars. The relative water content of around 80 % measured in all cultivars decreased to 30 % levels with 6 % PEG treatments.

Table 2. The effect of different PEG doses applied to the varieties on ion leakage, relative water content, proline accumulation and MDA content

Çizelge 2. *Çeşitlere uygulanan farklı PEG dozlarının iyon sızıntısı, nispi su miktarı, prolin miktarı ve MDA miktarı üzerine etkisi*

	Treatm.	*Narince*	*Sultani Seedless*	*Öküzgözü*	*Boğazkere*	*Emir*	*Çalkarası*	Mean
Electrolyte Leakage (%)	Control	A 17. 40 c	AB 16.12 c	AB 15.09 d	B 13.54 c	B 14.58 c	AB 15.59 b	15.39 c
	%2 PEG	B 33.51 b	B 31.29 b	AB 37.39 c	AB 34.83 b	AB 37.33 b	A 46.21 a	36.76 b
	%4 PEG	A 61.29 a	A 56.45 a	A 57.38 b	A 57.14 a	A 52.54 a	A 52.39 a	56.68 a
	%6 PEG	AB 57.68 a	A 57.68 a	B 68.52 a	B 60.02 a	B 58.20 a	B 54.33 a	58.93 a
	Mean	42.47 A	40.39 A	44.59 A	41.38 A	40.67 A	42.13 A	
Rel. water content (%)	Control	A 84.02 a	AB 83.22 a	AB 82.92 a	AB 80.51 a	B 77.07 a	A 83.67 a	81.90 a
	%2 PEG	A 47.90 b	A 49.07 b	A 55.16 b	A 38.47 b	A 42.39 b	A 47.63 b	46.77 b
	%4 PEG	AB 39.19 bc	C 33.72 c	C 33.21 c	BC 35.85 b	C 33.65 b	A 40.42 bc	36.01 c
	%6 PEG	A 32.14 c	A 32.14 c	A 33.32 c	A 32.50 b	A 33.34 b	A 33.72 c	34.86 c
	Mean	50.81 AB	49.45 ABC	51.15 A	46.83 BC	46.61 C	51.36 A	
Proline (*mg.g-*1FW)	Control	A 0.330 d	C 0.199 d	BA 0.297 d	BA 0.292 d	BA 0.270d	BC 0.236 c	0.272 d
	%2 PEG	B 0.530 c	A 0.620 c	A 0.630 c	CB 0.516 c	C 0.465 c	D 0.236 c	0.501 c
	%4 PEG	C 0.740 b	BC 0.828 b	C 0.755 b	AB 0.880 b	C 0.755 b	A 0.919 b	0.814 b
	%6 PEG	A 1.170 a	BC 0.979 a	BC 1.018 a	AB 1.107 a	C 0.910 a	AB 1.080 a	1.045 a
	Mean	0.699 A	0.657 BC	0.675 AB	0.699 A	0.601 D	0.618 CD	
MDA (nmol)	Control	C 49.98 c	C 50.63c	B 55.93b	AB 60.52 c	D 34.79c	A 62.08 b	52.32 d
	%2 PEG	AB 68.99 b	C 55.35c	B 63.05b	AB 67.51 c	D 47.78b	A 70.55 b	62.20 c
	%4 PEG	BC 77.47 b	CD 62.33c	AB 89.95a	A 107.47 b	D 54.38b	B 84.32 a	79.32 b
	%6 PEG	A 123.70 a	B 78.44a	B 89.11a	A 125.58 a	AB101.52a	B 80.18 a	99.75 a
	Mean	80.04 B	61.69 C	74.51 B	90.27 A	59.62 C	74.28 B	

A,B,C : Different capital letter on on the sam eline represent statistically significant differences among the cultivars
a,b,c : Diffrent lowercase letter on the same coloumn represent statistically significant differences among the PEG doses ($p<0.05$

Plants generates tolerance to stress factors through osmoticums (Hoekstra et al., 2001, Yokota et al., 2006; Ben Ahmed et al., 2008) and such osmatic regulations are realized by accumulation of osmatic compounds (Patakas and Noitsakis 2001). Proline accumulation increases with decreasing water potentials (Ragab and Moustafa 2008). Such an increase then aids in reducing cell water potential and preventing cell water loss and ultimately provide plant resistance to high evaporations without losing cell turgor (Mahajan and Tuteja 2005; Ben Ahmed et al., 2008). Similar findings supporting the above literatures were also observed in present study carried out with 6 different grapevine cultivars. PEG treatments yielded significant increases in proline content of all cultivars. Increasing proline contents were observed with increasing PEG doses. For instance in Narince cultivar, proline content of 0.33 $mg.g^{-1}$FW in control treatment increased to 1.17 $mg.g^{-1}$FW with 6 % PEG treatments. Similar cases were also observed in the other cultivars. MDA (malondialdehyde, a product of

lipid peroxidation) is generated through peroxidation of membrane lipids and commonly used as an indicator of oxidative damage under stress conditions (Zhang and Kirkham 1996). Yağmur (2008) investigated drought resistance of different grapevine rootstock-cultivar combinations and indicated that drought stress yielded different rate of increases in MDA contents depending on the rootstocks and cultivars. Similar findings were also observed in present study. While lower PEG concentrations (2 %) increased MDA content of Narince and Emir cultivars, they did not result in significant changes in MDA content of the other cultivars. On the other hand, greater PEG concentrations (4 and 6 %) yielded distinctive increases in MDA content of all cultivars (Table 2). Zhong et al., (2012) reported increasing MDA content of grapevines with different PEG concentrations under *in vitro* conditions.

4. Conclusions

Present findings revealed that PEG supplement to *in vitro* growth medium yielded significant changes in investigated parameters of all grape cultivars. Such findings indicated that *in vitro* PEG treatments could efficiently be used in investigation of the effects of drought stress on grapevines. Considering the changes generated in fresh weights by 2 % PEG treatments, Öküzgözü and Sultani Seedless cultivars seemed to be more resistant to drought; but considering the other parameters, it is better to indicate that there were not significant differences in drought tolerance of the cultivars.

Acknowledgment

This Research Was Supported By The Scientific Research Projects Committee Of The Tokat Gaziosmanpaşa University (Project Number: BAP-2015/52)

References

Adams RM, Chen CC, Mc Carl BA, Schimmelpfennig, DE (2001). Climate variability and climate change: implications for agriculture. *Adv Econ Environ Resources* 3: 95-113.

Ahuja I, De Vos RCH, Bones AM, Hall RD (2010). Plant molecular stress responses face climate change. *Trends in Plant Science* 15(12):664–674

Akbarpour E, Imani A, Yeganeh SF (2017). Physiological and morphological responses of almond cultivars under ın vitro drought stress. *Journal of Nuts* 8 (1):61-72.

Al-Khayri JM, Al-Bahrany AM (2004). Growth, water content and proline accumulation in drought-stressed callus of date palm. *Biologia Plantarum* 48 (1): 105–108

Alvarez S, Marsh EL, Schroeder SG, Schachtman DP (2008). Metabolomic and proteomic changes in the xylem sap of maize under drought. *Plant Cell Environ.* 31: 325–340.

Babalık Z (2012). Effects of salt and water stress on some physiological and biochemical characteristics of grapevines. S.D.Ü. Institute of Science Department of Hortıculture. Ph. D. Thesis.249s., Turkey

Babalık Z, Türk FH, Baydar NG (2015). Determınatıon on some physiologıcal and biochemical characteristics of Kober 5 BB rootstcks ın vıtro conditions under water stress. *Selçuk Tarım ve Gıda Bilimleri Dergisi-A* 27: 552-561

Bajji M, Kinet JM, Lutts S (2001). The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. *Plant Growth Regulation* 00: 1–10

Bates LS, Waldren RP, Teare ID (1973). Rapid determination of free proline for water stress studies. *Plant Soil*. 39: 205–207

Ben Ahmed C, Ben Rouina B, Boukhris M (2008). Changes in water relations, photosynthetic activity and proline accumulation in one-year-old olive trees (O*lea europaea* l. cv. chemlali) in response to NaCI salinity. *Acta Physiologiae Plantarum* 30 (4): 553–560

Bressan RA, Handa AK, Handa S, Hasegawa PM (1982). Growth and water relation of cultured tomato cells after adjustment to low external water potentials. *Plant Physiol.* 701: 303–1309

Carbonneau A, Deloire A, Jaillard B (2007). The Grapevine: Physiology, Terroir, Growing. Dunod. 442. Paris; France

Carmo-Silva AE, Keys AJ, Beale MH, Ward JL, Baker JM, Hawkins ND, Arrabaça MC, Parry MAJ (2009). Drought stress increases the production of 5-hydroxynorvaline in two C4 grasses. *Phytochemistry* 70: 664–67

Chai TT, Fadzillah NM, Kusnan , Mahmood M (2005). Water stress-induced oxidative damage and antioxidant responses in micropropagated banana plantlets. *Biologia Plantarum* Volume 49 (1): 153–156

Cui ZH, Bi WL, Hao XY, Xu Y, Li PM, Walker MA, Wang QC (2016). Responses of in vitro-grown plantlets (*Vitis vinifera*) to grapevine leafroll-associated virus-3 and peg-ınduced drought stress. *Front. Physiol.*, | Http://Dx.Doi.Org/10.3389/Fphys.2016.00203

Dami I, Hughes H (1995). Leaf anatomy and water loss of in vitro peg-treated 'Valiant' grape. *Plant Cell Tissue Organ Cult*. 42: 179-184

Ghaderi N, Talaie AR, Ebadi A, Lessani L (2011). The physiological response of three ıranian grape

cultivars to progressive drought stress. *J. Agr. Sci. Tech*. 13: 601-610

Heath RL, Packer L (1968). Photoperoxidation in isolated chloroplasts. ı kinetics and stoichiometry of fatty acid penoxidation, arch. *Biochem. Biophys*. 125: 189-198

Hoekstra FA, Golovina EA, Buitink J (2001). Mechanisms of Plant Desiccation Tolerance. *Trends in Plant Science* 6(9): 431–438

IPCC (Intergovernmental Panel On Climate Change) (2007). Workinggroup II report "ımpacts, adaptation and vulnerability". M.L. Parry, O.F. Canziani, J.P. Palutikof, P.J. Van Der Linden And C.E. Hanson(Eds). Cambridge University Press, Cambridge, United Kingdom And New York, NY, USA. 976 Pp.

Jain M (2001). Tissue culture-derived variation in crop improvement. *Euphytica* 118: 153–166

Karimi S, Hojati S, Eshghi S, Moghaddam RN, Jandoust S (2012). Magnetic exposure improves tolerance of fig 'Sabz' explants to drought stress induced *in vitro. Scientia Horticulturae*. Volume 137: 95–99

Kaufmann MR, Eckard AN (1971). Evaluation of water stress control with polyethylene glycols by analysis of guttation. *Plant Physiol*. 47: 453-456.

Keskin N, Kunter B (2007). Induction of resveratrol via UV irradiation effect in Erciş callus culture. Journal of Agricultural Science, 13(4): 379-384

Keskin N, Kunter B (2009). The effcets of callus age UV irradiation and incubation time on trans-resveratrol production in grapevine callus culture. Journal of Agricultural sciences, 15(1): 9-13

Kunter B, Cantür, S, Keskin N, Çetiner H (2017). Evaluation of viticultural performance of Ankara in relation to effective heat sum-vine phenology observations. 5th International Participation soil and water resources congress, I, 545-552

Leopold AC, Musgrave M E, Williams K M (1981). Solute leakage resulting from leaf desiccation. *Plant Physiol*. 68: 1222–1225

Mahajan S, Tuteja N (2005). Cold, salinity and drought stresses: an overview. *Archives of Biochemistry and Biophysics* 444(2): 139–158

Manoj KR, Rajwant KK, Rohtas S, Manu PG, Dhawan AK (2011). Developing stress tolerant plants through in vitro selection—an overview of the recent progress. *Environmental and Experimental Botany* 71: 89–98

Molassiotis AN, Sotiropoulos T, Tanou G, Kofidis G, Diamantidis ,Therios I (2006). Antioxidant and anatomical responses in shoot culture of the apple rootstock MM 106 treated with NaCl, KCl, mannitol or sorbitol. *Biol. Plant*. 50: 61-68

Murashige T, Skoog F (1962) A Revised Medium for Rapid Growth and Bioassay with Tobacco Tissue Cultures. *Physiology and Plants* 15: 472-497

Özden M, Demirel U, Kahraman A (2009). Effects of proline on antioxidant system in leaves of grapevine (*Vitis vinifera* l.) exposed to oxidative stress by H_2O_2. *Scientia Horticulturae* 119: 163–168

Parida AK, Dagaonkar VS, Phalak MS, Umalkar GV, Aurangabadkar LP (2007). Alterations in photosynthetic pigments, protein and osmotic components in cotton genotypes subjected to short-term drought stress followed by recovery. Plant *Biotechnol. Rep*. 1: 37–48

Patakas A, Noitsakis B (2001). Leaf age affect on solute accumulation in water-stressed grapevine. *Journal of Plant Physiology* 158: 63-69

Pellegrino A, Lebon E, Simmonneau T, Wery J (2005). Towards a simple ındicator of water stress in grapevine (*Vitis vinifera* l.) based on the differential sensitivities of vegetative growth component. *Austral. J. Grape Wine Res*. 11: 306-315

Ragab MH, Moustafa AS (2008). Comparative response of drought tolerant and drought sensitive maize genotypes to water stress. *Australian Journal of Crop Science Southern Cross Journals©* 1(1):31-36

Rampino P, Pataleo S, Gerardı C, Mıta G, Perrott C (2006). Drought stress response in wheat: physiological and molecular analysis of resistant and sensitive genotype. *Plant Cell Environ*. 29: 2143–2152

Sabır A (2016). Physıologıcal and morphologıcal responses of grapevıne (*V. vinifera* l. cv. 'Italıa') leaf to water defıcıt under dıfferent rootstock effects. Issn 1644-0692 www.acta.media.pl *Acta Sci. Pol. Hortorum Cultus* 15(1): 135-148

Sánchez FJ, De Andrés EF, Tenorio JL, Ayerbe L (2004). Growth of epicotyls, turgor maintenance and osmotic adjustment in pea plants (*Pisum sativum* l.) subjected to water stress. *Field Crops Res*. 86: 81–90

Savee R, Adillon J (1990) Comparision between plant water relations of in vitro plants and rooted cuttings of kiwifruit. *Acta Horticulturae* 282: 53-57

Sawwan J, Shibli RA, Swaidat I, Tahat M (2000) Phosphorus regulates osmotic potential and growth of african violet under in vitro- induced water deficit, *Journal of Plant Nutritio*n 23 (6): 759-771, DOI: 10.1080/01904160009382057

Šircelj H, Tausz M, Grill D, Batič F (2007). Detecting different levels of drought stress in apple trees (*Malus domestica* borkh.) with selected biochemical and physiological parameters. *Scientia Horticulturae* 113 (4): 362–369.

Sivakumar MVK (2006). Climate prediction and agriculture: current status and future challenges. *Climate Research* 33(1): 3-17

Sivritepe N, Erturk U, Yerlikaya C, Türkan I, Bor M, Özdemir F (2008). Response of the cherry rootstock to water stress induced in vitro. *Biol. Plant*. 52: 573-576

Stevanovic B, Sinzar J, Glisic O (1997). Electrolyte leakage differences between poikilohydrous and homoiohydrous species of gesneriaceae. *Biol. Plant.* 40: 299–303

Sucu S, Yağcı A, Yıldırım K (2018). Changes in Morphological, Physiological Traits And Enzyme Activity Of Grafted And Ungrafted Grapevine Rootstocks Under Drought Stress. Erwebs-Obstbau, Doi:10.1007/s10341-071-0345-7 (Yayın No: 3572292).

Taiz L, Zeiger E (1998). Plant Physiology 2nd Ed. Sinauer Associates, Inc., Sunderland, Mass

Tangolar SG, Tangolar S, Kelebek H, Topcu S (2016). Determination of phenolics, sugars, organic acids and antioxidants in the grape variety kalecik karasi under different bud loads and irrigation amounts. *Korean Journal of Horticultural Science and Technology* 34 (3): 495-509

Teulate B, Rekika D, Nachit MM, Monneveux P (1997). Comparative Osmotic adjustments in barley and tetraploid wheats. Plant Breeding 116, 519–523.

Tewary P, Ardhana S, Raghunath M, Sarkar A (2000) - In vitro response of promising mulberry (*Morus* sp.) genotypes for tolerance to salt and osmotic stresses. *Plant Growth Reg.* 30: 17-21

Winning H, Viereck N, Wollenweber B, Larsen FH, Jacobsen S, Søndergaard I, Engelsen SB (2009). Exploring abiotic stress on asynchronous protein metabolism in single kernels of wheat studied by nmr spectroscopy and chemometrics. *J. Exp. Bot* 60: 291–300

Yağmur Y (2008). İnvestigation of some physiological and Biochemical tolerance parameters against Drought stress of different grapevine (*Vitis vinifera L.*) Cultivars. E Ü., Institute of Science, M. Sc. Thesisi, Biology Department. Februrary. 124s, Turkey.

Yamasaki S, Dillenburg LR (1999). Measurements of leaf relative water content in Araucaria angustifolia. *Revista Brasilleira De Fisiologia Vegetal* 11(2): 69-75

Yokota A, Takahara K, Akashı K (2006). Water stress. Physiology and Molecular Biology of Stress Tolerance in Plants Pp 15-39

Zhang X, Luo Z, Tang J, Lu W , Yi Y (2004). Effect of high temperature and drought stress on free proline content and soluble sugar content of (*Taxiphyllum Taxirameum*). *Europe Pmc*. 24(6): 570-573

Zhong W, Pan X, Liu W, Zhou J (2012). Response of wild *Vitis quinquangularis* "huaxi-4" tube seedlings to peg stress in morphology and physiology. Journal of Northwest A , F University. *Natural Science Edition* 40 (6): 181-188.