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Araştırma
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Determination of Tolerance to Drought Stress of Two American Grapevine Rootstocks by PEG Application

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Abstract

Objective: This study was conducted to establish the in vivo protocol for the use of polyethylene glycol (PEG-6000) in 5BB (*V. berlandieri* x *V. riparia*) and 1103P (V. berlandieri x V. rupestris) American grapevine rootstocks, as well as to determine the plants' resilience to artificially induced drought stress.

Materials and Methods: The experimental design of this study was planned as a randomized complete plot design with 3 replications, each consisting of 10 plants. Polyethylene glycol (PEG-6000) was administered to the plants in each irrigation at doses of 0%, 2%, 4%, 8%, and 16%, based on the percentage of irrigation water. The application lasted for a total of 3 weeks. The study investigated the responses of plants to drought in terms of shoot development parameters (shoot fresh weight, shoot dry weight, shoot length, node and leaf number, leaf area, shoot tolerance ratio), root development parameters (root fresh weight, root number, rooting rate, root tolerance ratio, root length), and physiological development parameters (plant vitality, damage degree, leaf turgor weight, chlorophyll content, ion flux, and cell membrane damage rate).

Results: When examining the findings of the study, it was observed that polyethylene glycol material retained water, reducing the plant's water uptake and consequently creating artificial drought stress. The impact of drought induced by polyethylene glycol revealed that the 1103P rootstock exhibited higher resilience in shoot development parameters compared to the 5BB rootstock. On the contrary, the 5BB rootstock outperformed the 1103P rootstock in root development parameters. Concerning physiological development parameters, the severity of drought led to a significant decrease in plant vitality, chlorophyll content, and leaf turgor weight, while ion flux, cell membrane damage rate, and damage degree increased significantly to critical levels.

Conclusion: As a result of the research, the 1103P rootstock was found to be more successful in terms of shoot and physiological development under drought conditions, while the 5BB rootstock was found to be more successful in terms of root development parameters. Compared to other cultivation environments (in vitro, hydroponics), it was determined that polyethylene glycol (PEG) had a less pronounced effect at lower doses due to the difficulty of binding PEG in the soil. However, when compared to control plants, statistically significant differences were observed in the examined traits. Regarding the parameters investigated in this study, the 16% PEG concentration used was identified as the most effective dose in triggering drought stress.

Keywords: Abiotic stress, grapevine, plant growth parameters, polyethylene glycol

İki Amerikan Asma Anacının Kuraklık Stresine Toleransının PEG Uygulaması ile Belirlenmesi

Öz

Amaç: Bu çalışma 5BB (*V. berlandieri* x *V. riparia*) ve 1103P (*V.berlandieri* x *V.rupestris*) Amerikan asma anaçlarında hem polietilen glikolün (PEG-6000) *in vivo* şartlardaki kullanım protokolünü oluşturmak hem de bitkilerin yapay olarak oluşturulan kuraklık stresine olan dayanımlarını belirlemek için yürütülmüştür.

Materyal ve Yöntem: Çalışmadaki deneme deseni tesadüf parsellerine göre 3 tekerrürlü ve her tekerrürde 10 bitki olacak şekilde planlanmıştır.

Sulama suyunun yüzdesi olacak şekilde %0, 2, 4, 8 ve 16 dozlarında PEG-6000 her sulamada tarla kapasitesine göre bitkilere verilmiştir. Uygulama toplam 3 hafta sürmüştür. Çalışmada bitkilerin kuraklığa verdiği tepkiler sürgün gelişim parametreleri (sürgün yaş ağırlığı, sürgün kuru ağırlığı, sürgün uzunluğu, boğum ve yaprak sayısı, yaprak alanı, sürgün tolerans oranı), kök gelişim parametreleri (kök yaş ağırlığı, kök sayısı, köklenme oranı, kök tolerans oranı, kök uzunluğu) ve fizyolojik gelişim parametreleri (bitki canlılığı, zarar derecesi, yaprak turgor ağırlığı, klorofil miktarı, iyon akışı ve hücre zarı zararlanma) açısından ele alınmıştır.

Araştırma Bulguları: Çalışmanın bulguları incelendiğinde polietilen glikolün suyu tutarak bitkinin su alımını azalttığı ve yapay kuraklık stresi oluşturabildiği gözlemlenmiştir. Polietilen glikolün neden olduğu kuraklığın etkisiyle beraber 1103P anacının, 5BB anacına göre sürgün gelişim parametreleri bakımından daha dayanıklı olduğu belirlenmiştir. 5BB anacı ise kök gelişim parametrelerinde 1103P anacına göre daha başarılı bulunmuştur. Fizyolojik gelişim parametrelerinde ise kuraklığın şiddeti her iki anaçta da bitkilerde canlılık, klorofil miktarı, yaprak turgor ağırlığı önemli oranda azalırken, iyon akışı, hücre zarı zararlanma oranı ve zarar derecesi kritik düzeyde yükselmiştir.

Sonuç: Araştırma sonucunda 1103P anacı kurak şartlarda sürgün gelişimi ve fizyolojik gelişim açısından daha başarılı bulunurken, 5BB anacı kök gelişim parametreleri açısından daha başarılı bulunmuştur. Diğer yetiştirme ortamlarına (*in vitro*, hidroponik) kıyasla toprakta polietilen glikolün bağlanmasının zorluğu nedeniyle, düşük dozlarda daha az etkiye sahip olduğu belirlenmiştir. Ancak kontrol bitkileri ile incelenen özellikler kıyaslandığında, istatistiksel olarak anlamlı farklılıklar gözlemlenmiştir. Bu çalışmada incelenen parametreler açısından, kullanılan %16'lık PEG konsantrasyonu kuraklık stresini tetiklemede en etkili doz olarak belirlenmiştir.

Anahtar kelimeler: Abiyotik Stres, asma, bitki büyüme parametreleri, polietilen glikol

Introduction

If plants cannot adapt well to their environment, they face a stress situation (Buyuk et al., 2012). Stress is a condition that prevents the growth and development of the plant and disturbs the metabolism. The level of tolerance of plants in this situation is called stress resistance (Levitt, 1980). It is known that plants encounter more than one stress condition throughout their lives, and these stress factors are known to occur mostly at the same time. Stress is classified into two different groups biotic and abiotic. Abiotic stress consists of external factors such as high salt, low and high temperature, high radiation, some chemicals, pesticides, heavy metals, floods, ozone, wind, and soil deprivation of nutrients, while biotic stress consists of pathogens, animals, and different anthropogenic activities (Mahajan and Tuteja 2005).

The contribution of the agricultural sector cannot be ignored for future generations to have a healthier and more balanced diet. Drought stress, which has already begun to be seen as a result of climate change and is expected to increase in severity over time, is predicted to cause serious problems for the nutrition of the current world population as well as causing changes in the entire population (Kusvuran and Dasgan 2019). In addition to the increase in both arid and semi-arid areas, global climate change may cause desertification, soil salinity, and soil erosion (Turkes, 1994). Today, the possibility of expanding agricultural lands in order to increase production is very low, and it is reported that the yield decrease in currently used agricultural lands has reached half due to abiotic stresses (Mahajan and Tuteja 2005). Latent reactions that do not reveal the negative effects of stress on plants can cause irreversible damage and permanent diseases later on (Ozcan et al., 2004).

Decreased germination is one of the first and most important effects of drought stress (Harris et al., 2002). The lack of water, which causes a decrease in the numbers and size of a leaves of the plants, also reduces photosynthesis and prevents leaf expansion (Rucker et al., 1995). The closure of stomata, one of the first reactions to drought, starts with the decrease in turgor pressure and causes water imbalance in the tissues as the plant experiences water loss through transpiration. Because of this imbalance, the deterioration of the metabolic and enzyme structures causes drying in plants (Levitt, 1980). At the same time, drought causes negative effects on development by reducing cell growth. However, different negative results can be encountered when drought stress continues, up to plant death (Bohnert and Jensen 1996). Plants take measures to combat stress, such as reducing the transpiration surface, taking water from the soil, increasing the water transmission capacity, and storing water (Kusvuran, 2010). It is known that water deficiency increases ABA concentrations in the

xylem sap and leaves of vines (Soar et al., 2004). Under drought stress, fruit ripening is delayed. In addition, blackening and shedding of old leaves, wilting of leaves, discoloration of shoots, sunburn in berries, dulling of colors, and slowing of growth are observed (Kocamaz, 1983). It is critical to maintain high yields even under drought stress conditions to meet the nutritional needs of the world's growing population, and it is critical to conduct studies to increase the plant's resistance to stress (Tuberosa and Salvi 2006). The use of plant species and varieties that are resistant to drought stress should also be part of a forward breeding strategy. For this, it is important to determine the drought resistance of the existing grapevine species and grape varieties (Safi, 2013). For this purpose, using polyethylene glycol (PEG), a series of polymers ranging from sticky liquids to waxy solids, in artificially inducing water stress has gained importance (Larher et al*.,* 1993). It was reported that PEG-induced osmotic stress decreases cell water potential (Govindaraj et al*.,* 2010).

Few studies have examined the effects of drought stress *in vivo* using PEG-6000 in viticulture (Min et al*.,* 2019). Therefore, in this study, it was aimed to determine the effectiveness of different polyethylene glycol doses that enable the artificial drought stress in grapevines and to determine the drought stress tolerance of American grapevine rootstocks included in the experiment under controlled conditions. This study was planned to propose the most appropriate PEG doses that can be used in physiological studies for drought stress on grapevine rootstocks.

Materials and Methods

In the experiment, grape cuttings of *Vitis vinifera L.* from 5BB and 1103P rootstocks obtained during the dormant period were used as plant material. The planting medium in the study was a 1:1 mixture of peat and perlite. Two-bud cuttings were blunted from the basal buds while dormant. They were planted in 13-liter pots, each containing 10 cuttings. Irrigation was applied according to field capacity until the cuttings reached the 4 to 5 leaf stage, as stated by Lorenz et al*.* (1995). Polyethylene glycol, dissolved in pure water, was administered to plants in the form of irrigation water. After the shoots had 4-5 leaves, 6 different concentrations of PEG (Merck-PEG 6000) (0%, 2%, 4%, 8%, or 16%) were added to the medium, thus drought stress was induced artificially. After the treatment, the top of the medium was covered with black polyethylene bags to prevent evaporation. Plants that were planted were kept in a room with white spiral LED bulbs with an average temperature of 26 °C, an average humidity of 63–65%, and a photoperiod of 16 hours of light and 8 hours of darkness. In order to determine the effectiveness of PEG applications in the experiment, the viability of the plants (%) was obtained by dividing the number of live plants by the total number of plants and multiplying by 100. Shoot and root lengths were determined with the help of a ruler. With an accuracy of 0.001 g, the fresh and dry weights of shoots, roots, and leaves were measured on a balance (Radwag WTB200). Then, the dry weights of the shoots were measured on the balance after drying in an oven (Memmert UN55) at 65°C for 72 hours. Rooting rate (%) was determined by dividing the number of root-forming plants by the total number of plants in drought-treated plants and multiplying by 100. Shoot and root tolerance rates were determined according to the formula specified for each PEG dose (Turhan et al*.,* 2005) Accordingly: STR and RTR (Tolerance rate) Tx/To,Tx: shoot and root dry weights (g) of plants treated with PEG at a certain concentration; To: shoot and root dry weights (g). The degree of damage to plants was evaluated according to the scale created by Sivritepe (2008). A plant with no signs of drought damage is considered to be at "1 degree," a plant with burns and dryness on the shoot tips and leaf margins is considered to be at "2 degrees," a plant with necrosis in some areas of the entire leaf and stem is considered to be at "3 degrees," and a plant that completely dries out and dies is considered to be at "4 degrees". Equal portions of 0.3 g of plant leaves were separated and placed in glass tubes measuring 25 mm by 150 mm. 15 ml of distilled water was then added, and the tubes were shaken in a shaker at 100 revolutions per minute for one day. The EC value obtained using the EC meter (HANNA HI 99300) after the shaking was finished was marked as EC_1. The EC_2 value was calculated after the same samples were autoclaved at 12^0C for 15 minutes and came to room temperature. In this manner, the formula; Ion Flux: $EC_1/EC_2 \times 100$ was used to calculate the ion flux in the leaves (Ozden et al*.,* 2009) The amount of chlorophyll in leaves was measured using a chlorophyll meter (SPAD-502) (Khan et al*.,* 2004). According to the experimental design, random plots were prepared with three replications per treatment, 10 plants were used in each interaction, and the LSD test was run using the JMP 13.2.0 package program at the 5% significant level to determine the existence of significant differences.

Results and Discussion

Effects of PEG on shoot growth

In Table 1, it was determined that the length of the shoots in the plants was negatively affected by increasing PEG doses. The highest shoot length was obtained at 0, 2, and 4% PEG concentrations. In turn, the lowest shoot length was obtained from a 16% PEG dose. According to the general average results for the rootstocks studied, it was determined that *1103P* rootstock formed longer shoots (24.96 cm) than *5BB* rootstocks (19.66 cm). In the rootstock × treatments interaction, the control group of *1103P* rootstock had the highest value (28.97 cm). With increasing PEG concentrations, the fresh weight of the shoots was negatively affected. When Table 2 was examined, it was determined that the highest number of nodes was 6.55 in the control group, whereas the lowest number of nodes was 3.81 in the 16% PEG treatment. Based on the average nodes for individual rootstocks, it was found that *1103P* formed more nodes than *5BB* rootstock. The interaction results (rootstock × treatments) indicated that the lowest number of nodes was found in *5BB* rootstocks treated with 16% PEG. Moreover, it was determined that the highest number of leaves was noted in the control plants, and the least number of leaves belonged to the 16% PEG treatment. Leaf area values decreased significantly, depending on the increase in PEG concentrations. When comparing leaf turgor weights, there was no statistically significant difference among the rootstocks used. However, as drought severity increased, reductions in turgor weight were observed. Accordingly, when comparing PEG concentrations, the lowest turgor weight was obtained from plants treated with 16% PEG (0.42 g). Upon analyzing the interactions, the lowest turgor weight was obtained from plants exposed to a 16% PEG concentration in both rootstocks. Conversely, the interaction resulting in the highest leaf turgor weight was identified as *5BB* x 4% PEG (1.24 g).

Table 1. The effects of PEG concentrations on shoot characteristics of *5BB* and *1103P* American grapevine rootstocks

PEG concentration (%)	Shoot length (cm)			Shoot fresh weight (g)			Shoot dry weight (g)		
	Rootstock			Rootstock			Rootstock		
	1103P	5BB	Aver.	1103P	5BB	Aver.	1103P	5BB	Aver.
0%	28.97 a	24.00 abc	26.48 A	4.94 a	5.10 a	5.02 A	0.90 a	0.99 a	0.95 A
2%	28.34 a	20.47 bc	24.41 A	5.07 a	4.33 a	4.70 A	0.95 a	0.71 a	0.83 A
4%	26.88 ab	23.43 abc	25.15 A	5.12 a	4.84 a	4.98 A	0.89 a	0.93 a	0.91 A
8%	26.81 ab	19.21 cd	23.01 B	4.55 a	3.83 a	4.19 A	0.84 a	0.74 a	0.79 A
16%	13.80 de	11.21 e	12.50 B	1.27 b	1.43 b	1.35 B	0.27 b	0.25 b	0.26 B
Aver.	24.96 A	19.66 B		4.19	3.91		0.77	0.73	
$R_{LSD\ 5\%}$			2.88			N. S			N. S
$PEG_{LSD\ 5\%}$			4.56			1.15			0.21
$R \times PEG_{LSD\ 5\%}$			6.45			1.63			0.3

LSD least significant difference, within column, means followed by a different letter differ significantly at p<0.05 by LSD. N.S: Not significant, R: Rootstock, R × PEG: Rootstock × PEG, Aver: Average

Table 2. The effects of PEG treatments on shoot and leaf characteristics of *5BB* and *1103P* American grapevine rootstocks

PEG concentration (%)	Number of nodes (n)			Number of leaf (n)			Leaf area (cm^2)		
	Rootstock			Rootstock			Rootstock		
	1103P	5BB	Aver.	1103P	5BB	Aver.	1103P	5BB	Aver.
0%	7.73 a	5.36 b	6.55 A	8.00 a	6.00 b	7.00 A	24.68 a	22.53 ab	23.61 A
2%	7.87 a	4.37 bc	6.12 A	8.00 a	4.00 cd	6.00 A	20.47 bc	24.30 a	22.39 AB
4%	7.80 a	5.43 b	6.62 A	8.00 a	5.00 bcd	6.00 A	20.17 bcd	20.98 abc	20.58 B
8%	7.72 a	5.4 b	6.59 A	8.00 a	5.00 bc	6.00 A	17.87 cd	16.67 d	17.27 C
16%	4.10 bc	3.53 c	3.81 B	4.00 cd	3.00 d	4.00 B	12.14 e	12.33 e	12.23 D
Aver.	7.04 A	4.83 B		7.00 A	5.00 B		19.07	19.36	
$R_{LSD\ 5\%}$			0.63			0.62			N. S
$PEG_{LSD\ 5\%}$			0.99			0.98			2.65
$R \times PEG_{LSD\ 5\%}$			1.40			1.38			3.75

LSD least significant difference, within column, means followed by a different letter differ significantly at p<0.05 by LSD. N.S: Not significant, R: Rootstock, R × PEG: Rootstock × PEG, Aver: Average

Effects of PEG on root growth

Plants exposed to 16% PEG had the decreased root weight. The difference between rootstocks was statistically significant and the average root fresh weight of *5BB* rootstocks (1.31 g) was higher than that of *1103P* rootstock (0.44 g). Moreover, *1103P* rootstocks treated with 16% PEG had the lowest root fresh weight of 0.08 g in the rootstock × treatment

interactions, while *5BB* rootstocks in the control group had the highest root weight values (Table 3). The effect of PEG on root dry weight showed similar results to its effect on root fresh weight; as the PEG dose increased, the root dry weight decreased. The influence of PEG doses on root length was found to be significant in all treatments. The longest roots had the control group of *5BB* rootstocks, while at 16% PEG the *1103P* rootstocks had the shortest roots (Table 3). According to the rootstock averages, the *5BB* rootstocks formed more roots than *1103P*. Moreover, while the control group had the most roots there was a decrease in the number of roots when the PEG dose increased (Table 4). When the rootstock × treatment interaction was considered, it was found that at 16% PEG the *1103P* rootstock had the least number of roots. In terms of rooting rate, *5BB* rootstock showed better development than *1103P*. While the control group had the best rooting rate of 93.33% compared to the average obtained with individual doses of PEG, the lowest rooting rate was obtained at 16% PEG dose (61.66%). In both rootstocks, a decrease in rooting rate was noted with the increase of the PEG concentration applied.

Table 3. The effects of PEG treatments on root properties of *5BB* and *1103P* American grapevine rootstocks

PEG concentration (%)	Root fresh weight (g)			Root dry weight (g)			Root length (cm)		
	Rootstock			Rootstock			Rootstock		
	1103P	5BB	Aver.	1103P	5BB	Aver.	1103P	5BB	Aver.
0%	0.86 bc	1.56 a	1.21 A	0.86 bc	1.56 a	1.21A	8.94 c	10.04 a	9.49 A
2%	0.58 cd	1.86 a	1.22 A	0.58 cd	1.86 a	1.22 A	8.41 c	9.43 a	8.92 A
4%	0.41 cde	1.69 a	1.05 A	0.41 cde	1.69 a	1.05 A	7.20 c	9.73 ab	8.46 A
8%	0.26 de	1.07 b	0.66 B	0.26 de	1.07 b	0.66 B	5.74 c	9.27 bc	7.50 A
16%	0.08 e	0.38 e	0.23 C	0.08 e	0.38 cde	0.23 C	2.30 c	5.34 c	3.82 B
Aver.	0.44 B	1.31 A		0.06 B	0.13 A		6.52 B	8.76 A	
$R_{LSD\ 5\%}$			0.22			0.03			1.41
$PEG_{LSD\ 5\%}$			0.34			0.05			2.23
$R \times PEG_{LSD5\%}$			0.48			0.08			3.16

LSD least significant difference, within column, means followed by a different letter differ significantly at $p<0.05$ by LSD. N.S: Not significant, R: Rootstock, R × PEG: Rootstock × PEG, Aver: Average

Table 4. The effects of PEG treatments on root properties of *5BB* and *1103P* American grapevine rootstocks

PEG concentration (%)	Number of roots (n)			Rooting rate (%)		
	Rootstock			Rootstock		
	1103P	5BB	Aver.	1103P	5BB	Aver.
0%	8.00 ab	9.00 a	9.00 A	93.33 a	93.33 a	93.33 A
2%	6.00 bc	6.00 abc	6.00 B	83.33 c	86.66 b	84.99 B
4%	6.00 bc	7.00 abc	6.00 B	86.66 b	83.33 c	84.99 B
8%	4.00 cd	4.00 cd	4.00 B	63.33 e	73.33 d	68.33 C
16%	2.00 d	6.00 abc	4.00 B	40.00 f	83.33 c	61.66 D
Aver.	5.00 B	7.00 A		73.33 B	83.99 A	
$R_{LSD\ 5\%}$			1.48			3.40
$PEG_{LSD\ 5\%}$			2.34			5.36
$R \times PEG_{LSD\ 5\%}$			3.32			7.57

LSD least significant difference, within column, means followed by a different letter differ significantly at $p<0.05$ by LSD. N.S: Not significant, R: Rootstock, R × PEG: Rootstock × PEG, Aver: Average

Effects of PEG on selected physiological parameters

The difference between PEG doses and interactions was significant for the plant viability parameter. While the highest plant viability was obtained at the 0, 2 and 4 doses, the lowest plant viability was determined at the 16% PEG with a value of 82.33%. The viability of the plant decreased significantly, especially at the 8% (87.44%) and 16% (82.33%) PEG concentrations (Table 5). Plants treated with 8% or 16% PEG had the significantly lower chlorophyll content than control plants. The *5BB* rootstocks exposed to 8% PEG were found to have the lowest chlorophyll level of the combinations studied (Table 5). As drought stress increased, the degree of plant damage increased. The treatment group with the highest degree of damage was 16% PEG (Table 5). The shoot tolerance rate decreased as the PEG dose increased. Moreover, the *1103P* rootstock showed higher resistance to artificial drought conditions created by PEG, with a shoot tolerance value of 0.75, compared to *5BB* rootstocks (0.58) (Table 6). When the rootstock × treatments interaction was considered, the lowest shoot tolerance was obtained from 16% PEG dose in both rootstocks.

On the other hand, the effect of PEG exposition on root tolerance ratio showed that *1103P* rootstock was less tolerant (0.52) than that of *5BB* rootstock (0.62).

Furthermore, there was a decrease in root tolerance due to the increase in PEG dose compared to the general average for the treatment. Control plants had the highest root tolerance, with a value of 1, while plants treated with 16% PEG showed the lowest root tolerance, with a value of 0.12 (Table 6). The plants treated with PEG 16% were characterized by the highest ion flow rate (34.97%), while the control group plants with the lowest ion flow rate (20.35%). When the rootstock × treatments interaction was examined, the combination with the highest ion flow (43.35%) was 8% PEG treatment of *1103P* rootstock (Table 7). The cell membrane damage rate was found to be statistically significant in all combinations. According to the general average for the rootstock, the cell damage of *1103P* was higher (22.17%) than that of the *5BB* rootstocks (11.50%). Considering the treatments, cell membrane damage increased with the increase of PEG concentration. The highest value of this parameter was 31.9% at 16% PEG, while the least cell membrane damage was noted in the control group (Table 7).

In this study, artificial drought stress was induced by applying different doses of PEG, resulting in observed reductions in shoot and root development. It has been reported by several researchers that plant growth is adversely affected by increasing drought severity (Serra et al*.,* 2013; Min et al*.,* 2019). In terms of shoot length, the *1103P* rootstock reached higher values under both drought and control conditions. Additionally, the control plants showed insignificant differences in shoot length development when subjected to 2% and 4% PEG concentrations. Regarding shoot fresh and dry weight, the highest values were observed in plants grown under control conditions, while the lowest values were observed, particularly in plants subjected to 16% PEG concentration.

Table 5. The effects of PEG treatments on the physiological properties of *5BB* and *1103P* American grapevine rootstocks

PEG concentration (%)	Plant vitality (%)			Chlorophyll content (SPAD)			Degree of damage (1-4)		
	Rootstock			Rootstock			Rootstock		
	1103P	5BB	Aver.	1103P	5BB	Aver.	1103P	5BB	Aver.
0%	90.00 a	90.00 a	90.00 A	24.68 a	25.46 a	25.07 A	1.00 c	1.06 c	1.03 B
2%	90.00a	90.0 a	90.00 A	25.15 a	24.10 ab	24.63 A	1.21 c	1.06 c	1.14 B
4%	90.00 a	90.0 a	90.00 A	24.28 ab	24.81 a	24.55 A	1.21 c	1.15 c	1.18 B
8%	90.00 a	84.88 b	87.44 A	23.55 ab	22.08 b	22.81 B	1.28 c	1.29 c	1.28 B
16%	74.6 c	90.00 a	82.33 B	23.60 ab	23.81 ab	23.70 AB	2.86 a	2.00 b	2.43 A
Aver.	86.93	88.97		24.25	24.05		1.51 A	1.31 B	
$R_{LSD\ 5\%}$			N. S			N. S			0.17
$PEG_{LSD\ 5\%}$			3.37			1.58			0.27
$R \times PEG_{LSD\ 5\%}$			4.77			2.23			0.38

LSD least significant difference, within column, means followed by a different letter differ significantly at p<0.05 by LSD. N.S: Not significant, R: Rootstock, R × PEG: Rootstock × PEG, Aver: Average

Table 6. The effects of PEG treatments on the physiological properties of *5BB* and *1103P* American grapevine rootstocks

PEG concentration (%)	Leaf turgor weight (g)			Shoot tolerance ratio (STR)			Root tolerance ratio (RTR)		
	Rootstock			Rootstock			Rootstock		
	1103P	5BB	Aver.	1103P	5BB	Aver.	1103P	5BB	Aver.
0%	0.84 bc	0.83 bc	0.84 B	1.00 a	1.00 a	1.00 A	1.00 a	1.00 a	1.00 A
2%	1.04 ab	0.92 bc	0.98 AB	0.98 a	0.71 c	0.85 B	0.61 b	0.92 a	0.77 B
4%	0.92 bc	1.24 a	1.08 A	0.83 b	0.58 d	0.71C	0.54 bc	0.60 b	0.57 C
8%	0.88 bc	0.65 cd	0.77 B	0.73 c	0.40 e	0.56 D	0.35 cd	0.45 bc	0.40 D
16%	0.39 d	0.45 d	0.42 C	0.24 f	0.21 f	0.22 E	0.10 f	0.15 de	0.12 E
Aver.	0.81	0.82		0.75 A	0.58 B		0.52 B	0.62 A	
$R_{LSD\ 5\%}$			N. S			0.04			0.10
$PEG_{LSD\ 5\%}$			0.22			0.07			0.15
$R \times PEG_{LSD\ 5\%}$			0.30			0.10			0.22

LSD least significant difference, within column, means followed by a different letter differ significantly at p<0.05 by LSD. N.S: Not significant, R: Rootstock, R × PEG: Rootstock × PEG, Aver: Average

Table 7. The effects of PEG treatments on the physiological properties of *5BB* and *1103P* American grapevine rootstocks

PEG concentration (%)	Ion flow (%)			Cell membrane damage rate (%)		
	Rootstock			Rootstock		
	1103P	5BB	Aver.	1103P	5BB	Aver.
0%	18.02 c	22.68 bc	20.35 B	0.00 ı	1.00 ı	0.5 E
2%	23.80 c	18.01 c	20.90 B	15.89 e	10.64 f	13.26 D
4%	28.89 abc	17.00 c	22.94 B	24.65 d	5.75 h	15.19 C
8%	43.35 a	17.19 c	30.27 AB	40.28 a	6.29 g	23.15 B
16%	33.13 abc	36.82 ab	34.97 A	29.06 c	34.82 b	31.94 A
Aver.	29.43	22.34		22.17 A	11.44 B	
$R_{LSD\ 5\%}$			N. S			4.06
$PEG_{LSD\ 5\%}$			11.42			6.42
$R \times PEG_{LSD\ 5\%}$			16.15			9.28

LSD least significant difference, within column, means followed by a different letter differ significantly at $p<0.05$ by LSD. N.S: Not significant, R: Rootstock, R × PEG: Rootstock × PEG, Aver: Average

Decreased shoot development is associated with internode shortening and inhibition of shoot elongation (Schultz and Matthew, 1988; Hardie and Martin, 2000). In terms of bud and leaf number the *1103P* rootstock was found to be statistically more successful compared to the *5BB* rootstock. Significant growth reductions were observed in plants grown under 16% PEG concentration in these parameters. In contrast to shoot parameters, the *5BB* rootstock exhibited greater success in root development compared to the *1103P* rootstock. Consequently, in terms of shoot length, root fresh and dry weight, root number, and rooting rate, the *5BB* rootstock showed statistically more positive results. Despite being more sensitive to drought compared to the *1103P* rootstock, the success of *5BB* in rooting is based on the physiology of grapevines. Therefore, the decrease in root growth is less pronounced than shoot growth in the presence of drought symptoms in grapevines (Dry et al., 2000). Furthermore, in plants under drought stress, reductions in root growth rate occur due to the accumulation of abscisic acid in the root zone (Yamaguchi, 2010). To fully understand the response mechanisms of the rootstocks to drought, it is necessary to investigate the interaction of exogenous factors and genotypes with the environment (Serra, 2013). The width of plant leaves decreased with increasing drought severity. The lowest leaf area was observed in plants exposed to a 16% PEG concentration. There was no statistically significant difference in plant vitality and leaf chlorophyll content among the rootstocks. However, when examining the treatments, plants subjected to drought showed a significant decrease in plant vitality and chlorophyll content. Consequently, stomatal closure in grapevines and a decrease in photosynthesis due to reduced chlorophyll content are among the responses to drought (Chaves et al., 2003). The degree of damage to plants, which is an important factor in stress studies, increased with increasing drought severity. Additionally, the *5BB* rootstock was found to have a lower degree of damage compared to the *1103P* rootstock. Furthermore, statistically significant differences were found in tolerance parameters associated with stress response mechanisms. However, in both the *1103P* and *5BB* rootstocks, plants exposed to a 16% PEG concentration exhibited the lowest tolerance rate. Similarly, with increasing drought severity, both the shoot and root tolerance rates of plants decreased.

When plants are subjected to stress, damage first occurs at the cellular level. As a result, there is an increase in cell integrity, permeability, and electrolyte leakage (Collado et al., 2010). The extent of cell membrane damage can vary depending on the cultivation, plant growth, and leaf position (Premachandra and Shimada, 1987; Gavuzzi et al., 1997). Considering this information, it has been determined that ion flux reaches its highest levels in plants treated with a 16% PEG concentration in this study. Similarly, the cell membrane damage rate, which shows parallel results with the ion flux parameter, increased with the 16% PEG concentration in plants. Although no statistically significant difference was observed in ion flux among the rootstocks, a statistically significant difference was found in cell membrane damage. Reductions in shoot length, shoot fresh and dry weight, root length, root fresh and dry weight, root number, bud number, and leaf number have also been reported in grapevines in the presence of drought stress by various researchers (Gao et al., 2009; Mese and Tangolar, 2019; Gecene, 2020; Cochetel et al., 2020). Drought stress, which poses a serious threat to plants, adversely affects plant growth parameters not only in

grapevines but also in other species (Gopal and Iwama, 2007; George et al*.,* 2013; Ipek, 2016).

Conclusion

With increasing PEG concentrations, there were significant decreases in leaf area, plant vitality, root fresh and dry weight, shoot fresh and dry weight, chlorophyll content, shoot and root tolerance rates, root and shoot lengths, and leaf turgor weights. On the other hand, as the PEG dosage increased, the degree of damage, ion flux, and cell membrane damage in plants also increased. The drought-tolerant rootstock *1103P* exhibited higher values for shoot length, node number, leaf number, shoot tolerance rate, and cell membrane damage rate compared to the more drought-sensitive rootstock *5BB*. In *5BB* rootstock, higher values were found for root fresh weight, root dry weight, root length, root number, and rooting rate. Due to the difficulty of PEG binding in the soil compared to other cultivation mediums (*in vitro*, hydroponics), it had less impact at low doses. However, statistically significant differences were observed in most of the examined plant characteristics compared to the control group. In terms of the parameters studied in this study, the 16% PEG dosage was determined to be the most effective in inducing drought stress.

Conflicts of interest

There is no conflict of interest between the authors.

Authors statement of contribution

Mİ: Contributing to the establishment and execution of trials, laboratory analysis, statistics of data and writing of the article.

HBE: During the planning of the research, the establishment and conduct of the trials, the evaluation of the data and the writing of the article.

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