ÇOMÜ Zir. Fak. Derg. (COMU J. Agric. Fac.)
2025: 13 (1): 151-164
ISSN: 2147–8384 / e-ISSN: 2564–6826
doi: 10.33202/comuagri.1505987

Research Article

In Vitro Investigation of Cornerstone and GF-677 Rootstocks Tolerance to Drought Stress Conditions

Rıdvan Parlar[1] Deniz Eroğul[1*] Nevzat Sevgin[2]

[1]Ege University Graduate School of Natural and Applied Sciences, Department of Seed Science and Technology, İzmir, Türkiye
[2]Şırnak University, Faculty of Agriculture Deparment of Horticulture, Şırnak, Türkiye
*Corresponding author: deniz.erogul@ege.edu.tr

Received Date:27.06.2024 Accepted Date: 21.02.2025

Abstract

With global warming, the use of drought-resistant rootstocks becomes mandatory. GF-677 (*Prunus amygdalus x Prunus persica*) rootstock shows a high tolerance to arid conditions, while it is not recommended for lands with heavy groundwater. It has high graft compatibility with peach, nectarine and almond varieties. Cornerstone (*Prunus dulcis x Prunus persica*) rootstock is more drought-resistant than Nemaguard (*P. persica x P. Davidiana*) and tolerates heavier soils. It is reported to be compatible with peach, nectarine, almond and even apricot varieties.This study evaluated the drought stress response of Cornerstone rootstock under *in vitro* conditions and compared it with GF-677 rootstock. For this purpose, artificial drought stress was created by adding PEG 8000 at different concentrations to the MS nutrient medium under *in vitro* conditions, and following the PEG 8000 application, characteristics such as shoot number per explant, shoot length, shoot fresh weight, shoot dry weight, chlorophyll content, leaf turgor weight, relative water content, and proline amount were examined in the rootstocks. As the concentration of PEG 8000 increased, the shoot number and shoot length decreased in both rootstocks. At higher PEG 8000 concentrations, Cornerstone rootstock showed a smaller decrease in chlorophyll content, while both rootstocks exhibited increased proline content. Cornerstone rootstock accumulated higher proline levels than GF-677 at all PEG 8000 concentrations. Fewer shoots, reduced shoot length, controlled increase in fresh and dry weights, stable dry weight under severe drought, higher proline levels, and minimal chlorophyll decrease indicate the Cornerstone rootstock's drought resistance. The study shows that both rootstocks exhibit different levels of drought resistance under varying conditions. Cornerstone, a new rootstock for Türkiye, is recommended for fruit growing in drought-prone areas.

Keywords: Cornerstone, drought, GF-677, rootstock, tissue culture

Cornerstone ve GF-677 Anaçlarının Kuraklık Stresi Toleranslarının *In vitro* Koşullarda Araştırılması

Öz

Küresel ısınma ile kuraklığa dayanıklı anaçların kullanımı zorunlu hale gelmektedir. GF-677 (*Prunus amygdalus x Prunus persica*) anacı, kurak koşullara yüksek tolerans gösterirken, ağır taban suyu bulunan araziler için önerilmez. Şeftali, nektarin ve badem çeşitleriyle yüksek aşı uyumuna sahiptir. Cornerstone (*Prunus dulcis x Prunus persica*) anacı, Nemaguard'a (*P. persica x P. Davidiana*) göre kuraklığa daha dayanıklıdır ve daha ağır toprakları tolere eder. Şeftali, nektarin, badem hatta kayısı çeşitleriyle uyumlu olduğu bildirilmektedir. Bu çalışmada Cornerstone anacının kuraklık stresine tepkisi *in vitro* koşullarda değerlendirilmiş ve GF-677 anacı ile karşılaştırılmıştır. Bu amaçla, *in vitro* koşullarda MS besin ortamına farklı konsantrasyonlarda Polietilen Glikol 8000 (PEG 8000) eklenerek yapay kuraklık stresi oluşturulmuş ve uygulamayı takiben, anaçlarda eksplant başına sürgün sayısı, sürgün uzunluğu, sürgün taze ağırlığı, sürgün kuru ağırlığı, klorofil içeriği, yaprak turgor ağırlığı, nispi su içeriği ve prolin miktarı gibi özellikler incelenmiştir. PEG 8000 konsantrasyonu arttıkça, her iki anaçta sürgün sayısı ve sürgün uzunluğunda azalma yaşanmıştır. Artan PEG 8000 konsantrasyonlarında klorofil içeriğindeki azalma Cornerstone anacında daha az olurken, her iki anaçta da prolin içeriğinde artış kaydedilmiştir. Cornerstone anacı tüm PEG 8000 konsantrasyonlarında GF-677 anacından daha yüksek prolin seviyeleri biriktirmiştir. Düşük sürgün sayısı, stres altında sürgün uzunluğunda azalma, hem taze hem de kuru ağırlıklarda kontrollü artış, şiddetli kuraklık stresi altında kontrol bitkilerine eşdeğer kuru ağırlık, yüksek prolin seviyeleri ve

klorofil içeriğinde önemsiz azalma, Cornerstone anacının kuraklık stresi koşullarına dirençli olduğunu göstermektedir. Çalışmanın sonucu, her iki anacın da farklı koşullar altında değişen seviyelerde kuraklığa direnç gösterdiğini ortaya koymaktadır. Türkiye için yeni bir seçenek olan Cornerstone anacı, kuraklığa eğilimli ortamlarda meyve yetiştiriciliği için uygun bir anaç olarak önerilmektedir.
Anahtar Kelimeler: Anaç, Cornerstone, doku kültürü, GF-677, kuraklık

Introduction

Global warming has various environmental impacts, including climate changes, with one significant consequence being the increase in drought events (Dolaş and Kılıç, 2008). Recent studies have focused on the physiological responses of plants to environmental stresses and especially on reducing water shortage and osmotic stress due to global warming (Moustakas et al., 2011). There is an immediate necessity to develop drought-tolerant rootstocks capable of adapting to changes in water use (Bielsa et al., 2018). Due to the impact of water shortages on numerous plant processes at molecular and morphological levels, the prioritized objectives in *Prunus* rootstock breeding programs include the development of drought-tolerant rootstocks and cultivars (Arismendi et al., 2015).

The use of rootstocks that provide efficiency in water use and resistance to abiotic stresses will be of great importance in fruit cultivation in the future (Webster, 2002). In contemporary fruit cultivation, the resistance level of both the rootstock and the variety against biotic and abiotic stress conditions plays a crucial role in achieving economically and environmentally sustainable cultivation. Particularly in addressing abiotic challenges, the selection of rootstock may hold greater significance than that of the variety (Webster, 2002). In fruit cultivation, rootstocks have been shown to mitigate the adverse impacts of various abiotic stressors (Bolat ve İkinci, 2019). Therefore, the selection of rootstocks and propagation conditions are crucial for improving drought tolerance (Dutra de Souza et al., 2017).

In fruit production, establishing orchards with varieties grafted onto drought-tolerant rootstocks has become increasingly important. Nowadays, with declining irrigation options, the significance of rootstocks that facilitate fruit cultivation with reduced water usage has increased. In Türkiye, almond and peach are intensively cultivated, especially in the Aegean and Mediterranean regions. One of the commonly used rootstocks in the cultivation is GF-677 (*Prunus amygdalus x Prunus persica*). The GF-677 rootstock is a hybrid of almond (*P. amygdalus*) and peach (*P. persica*). The GF-677 rootstock shows high graft compatibility with peach, nectarine, and almond varieties, contributing positively to the fruit yield and quality of the grafted plants. It is known for its high tolerance to calcareous soils and drought conditions (Stylinanides et al., 1989; Küden, 2000).

However, there is a need for different rootstocks for cultivation under drought conditions. The Cornerstone (*Prunus dulcis x Prunus persica*) rootstock, new for our country, has been reported to be suitable for salty and drought conditions (Burchell Nursery Inc, 2023). Cornerstone rootstock is a hybrid of almond (*Prunus. dulcis*) and peach (*Prunus persica*). It is reported to be compatible with peach, nectarine, almond and even apricot varieties (Slaughter et al., 2010). It is tolerant to high pH calcareous soils, salt and iron chlorosis (Sandhu et al., 2020; Burchell Nursery Inc., 2023). According to reports, high-yield and sweeter fruits are obtained from the varieties grafted on this rootstock (O'Connell and Stefanell, 2018). Although some studies have been conducted on its suitability for salty conditions (Sandhu et al., 2020), research on its adaptation to drought conditions is limited.

In *in vitro* studies, drought stress is usually induced using polyethylene glycol (PEG) (Osmolovskaya et al., 2018). PEG-induced drought conditions offer a convenient, cost-effective, simple, and rapid method for evaluating drought stress tolerance, enabling the screening of numerous genotypes (Manoj and Uday, 2007; Esan et al., 2018; Carvalho et al., 2019). Gisela 5 cherry rootstock, cherry rootstocks, GF-677 rootstock, Garnem rootstock, Myrobolan 29C, citrus rootstocks, almond cultivars, apple cultivars and cherry cultivars were evaluated for their tolerance to drought stress using PEG (Sivritepe et al., 2008; Karimi et al., 2012; İpek, 2015; Şimşek et al., 2018; Hamayat et al., 2020).

The aim of this study was to assess the suitability of GF-677 rootstock, which is commonly used in establishing almond and peach orchards in drought-prone regions of our country, and to evaluate the potential of Cornerstone rootstock as an alternative, particularly in areas with limited irrigation facilities, by comparing it to GF-677. Hence, this tissue culture study was conducted to assess the drought resistance level of GF-677 and Cornerstone rootstocks, to compare them, and to evaluate the potential of Cornerstone as an alternative rootstock.

Materials and Methods

Plant Materials

The GF-677 rootstock used in the study wasthe sourced from the controlled greenhouse facilities of Bademli Biotechnology Industry and Trade Limited Company (İzmir, Türkiye), while the Cornerstone rootstock was obtained from Parlar Fidancilik Company (İzmir, Türkiye).

Method

The nutrient medium described by Murashige and Skoog (1962) was used for *in vitro* tissue culture propagation of rootstocks. 30 g sucrose per litre, 7 g agar per litre, plant growth regulator BAP 1 mg/ml and NAA 0.01 mg/ml were added to the medium.

Approximately 0.5 cm long 'eyes' or bud explants were obtained from mother plants. For surface sterilization, explants were washed under tap water for one hour, followed by treatment with 70% ethyl alcohol for 30 seconds. After washing, explants were soaked in 15% commercial bleach solution for 15 min and rinsed thrice with sterile distilled water.

After sterilization, explants were placed on MS medium in culture tubes and allowed to grow in a climate chamber with a temperature of 23 ± 2 °C. At the end of the four-week growth period, healthy explants free of microbial contamination were subcultured. Before starting the experiments, subculturing was carried out every three weeks in order to obtain a sufficient number of micro-shoots; after the desired number of micro-shoots was reached, drought stress experiments were started.

The nutrient media to be used in drought stress experiments were prepared by adding PEG 8000 at different concentrations, adjusted to pH 5.8 using 1 N NaOH or 1 N HCl and then sterilized in an autoclave at 121 °C for 15 minutes at a pressure of 1.4 atmospheres. The cultured plants were grown in climate chambers at 23 ± 2 °C under a light intensity of 3000 lux, 16 h light and 8 h dark photoperiod. A total of 80 explants were used in each experiment for each rootstock and all experiments were carried out with 4 replicates and 5 explants.

In order to determine the effects of drought stress on micro-shoots, Cornerstone and GF-677 rootstocks were subjected to artificial drought stress with polyethylene glycol 8000 (PEG-8000) *in vitro*. Drought stress experiments were carried out at 2%, 4% and 6% PEG-8000 concentrations.

A total of 3 *in vitro* experiments were conducted, where each lasted 4 weeks. At the end of the fourth week *in vitro*, the number of shoots formed per explant and the mean shoot lengths of the explants of GF-677 and Cornerstone rootstocks exposed to drought stress were measured with the help of a millimetric ruler. At the end of the stress, the fresh weights of the leaf samples taken from the tissue culture plants were calculated to determine the relative water content, then the leaves were kept in pure water for 4 hours and their turgor weights were measured at the end of this period. The leaf samples whose weights were determined were dried in an oven at 65°C for 48 hours and then their dry weights were calculated. Tissue water content was calculated using the following formula described by Kaya et al (2003).

$$LRWC=(FW-DW)/(TuW-DW)x100 \quad (1)$$

LRWC: Leaf Relative Water Content, FW: Fresh Weight, DW: Dry Weight, TuW: Turgor Weight

After 4 weeks of drought stress treatments, the fresh weights of the selected plants were weighed in milligrams using a precision balance and the dry weights of the same samples were determined in mg after drying in a 65ºC oven for 48 hours according to Sanchez et al (2004).

Plants exposed to drought stress for 4 weeks by adding different concentrations of PEG 8000 to the culture medium were homogenized using 80% acetone. Subsequently, the concentrations of chlorophyll a and b (measured in mg L^{-1}) were determined through spectrophotometric analysis at wavelengths of 663 nm and 645 nm, respectively, following the procedure outlined by Qados (2011). Additionally, the free proline content was assessed using the method described by Bates et al (1973).

Statistical analysis

The data collected from the experiments were analyzed using analysis of variance (ANOVA) with the JMP Pro 16 statistical software package. Duncan's multiple comparison test was used to determine the statistical significance of the differences between treatments.

Results and Discussion

When the effect of drought stress induced by different concentrations of PEG 8000 added to the medium under *in vitro* conditions on the number of shoots per explant of Cornerstone and GF-677 rootstocks was analyzed, the effect of rootstock factor ($p \leq 0.001$) and PEG 8000 ($p \leq 0.05$) were found

to be significant. However, the effect of (rootstock) x (PEG 8000) interaction was found to be insignificant.

Similarly, when the effect of drought stress induced by different concentrations of PEG 8000 added to the medium under *in vitro* conditions on the shoot length per explant of Cornerstone and GF-677 rootstocks was analyzed, the effect of PEG 8000 was found to be significant ($p \leq 0.005$). However, the effect of rootstock factor and (rootstock) x (PEG 8000) interaction was found to be insignificant ($p = 0$).

It was shown that PEG 8000 concentration (drought stress) and rootstock type (Cornerstone and GF-677) affected the number of shoots and these effects were statistically significant. However, the interaction between rootstock and PEG 8000 (e.g., the combination of a specific rootstock and PEG 8000 concentration) was found to have no significant effect. For shoot length, the effect of PEG 8000 was found to be significant, whereas the effects of rootstock type and the (rootstock) x (PEG 8000) interaction were found to be insignificant.

Table1. Analysis of variance of rootstock, PEG 8000 concentration and (rootstock) x (PEG 8000) concentration interaction on shoot number and shoot length *in vitro*.

	Sources Of Variance	DF	MS	SS	F
Shoot number	Rootstock	1	308.16	308.16	107.18*
	PEG 8000	3	49.83	149.49	5.78**
	Rootstock X PEG 8000	3	13.83	41.49	1.60
Shoot length	Rootstock	1	0.534	0.534	0.347
	PEG 8000	3	17.605	52.81	3.809*
	Rootstock X PEG 8000	3	5.373	16.12	1.162

*,** The difference between mean values is significant at $p \leq 0.05$, $p \leq 0.001$ level, respectively
DF: Degree of Freedom MS:Mean Square SS:Sum of Square

The GF-677 had approximately the same and the highest number of shoots between the control group of 0% PEG 8000 and 2% PEG 8000, while a 35% decrease was observed when the PEG 8000 concentration was 6%. This indicates that the capacity of GF-677 rootstock to produce shoots decreased in response to increasing drought stress. In Cornerstone rootstock, the number of shoots showed approximately the same results at 0% PEG 8000, 2% PEG 8000, and 4% PEG 8000 levels, while a 27% decrease was observed at 6% PEG 8000. As the PEG 8000 concentration increased, a decrease in the mean number of shoots was observed. While a significant decline in the number of shoots was observed in GF-677 rootstock with increasing PEG 8000 concentration, this rate was less in Cornerstone rootstock (Figure 1).

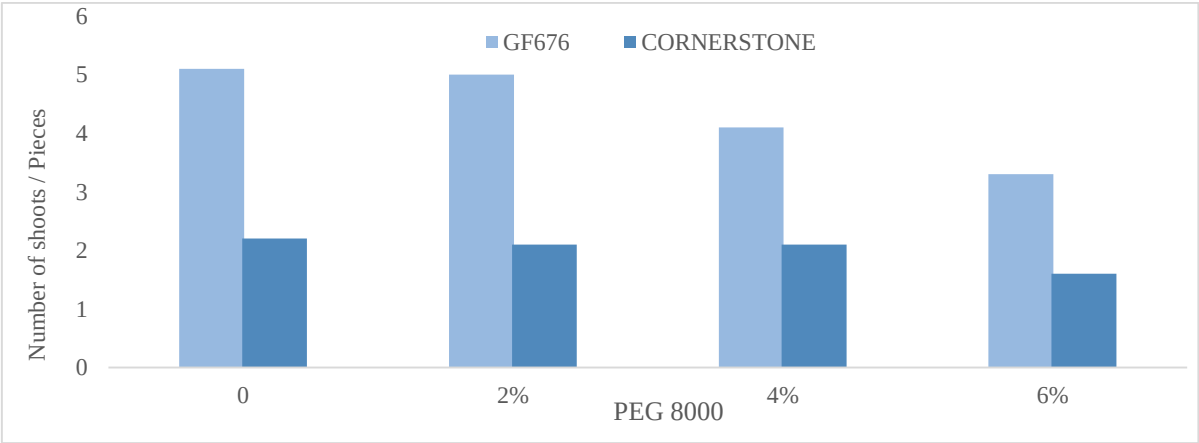

Figure 1. The mean number of shoots per explant in rootstocks at different PEG 8000 concentrations *in vitro*.

GF-677 rootstock yielded a mean number of 5.1 shoots per explant in the control group, whereas the control group of Cornerstone rootstock produced 2.2 shoots per explant (Figure 1).

Drought, one of the abiotic stress factors, negatively affects plants' growth and development and vegetative growth stage (He et al., 2018). In this study, drought stress negatively affected the

vegetative growth of the plants and reduced the number of shoots. This decrease was more pronounced in GF-677 rootstock (Figure 2; Figure 3; Figure 4; Figure 5).

Figure 2.Comparison of PEG 8000 Control group plants of Cornerstone and GF-677 rootstocks.

Figure 3.Comparison of PEG 8000 2% group plants of Cornerstone and GF-677 rootstocks.

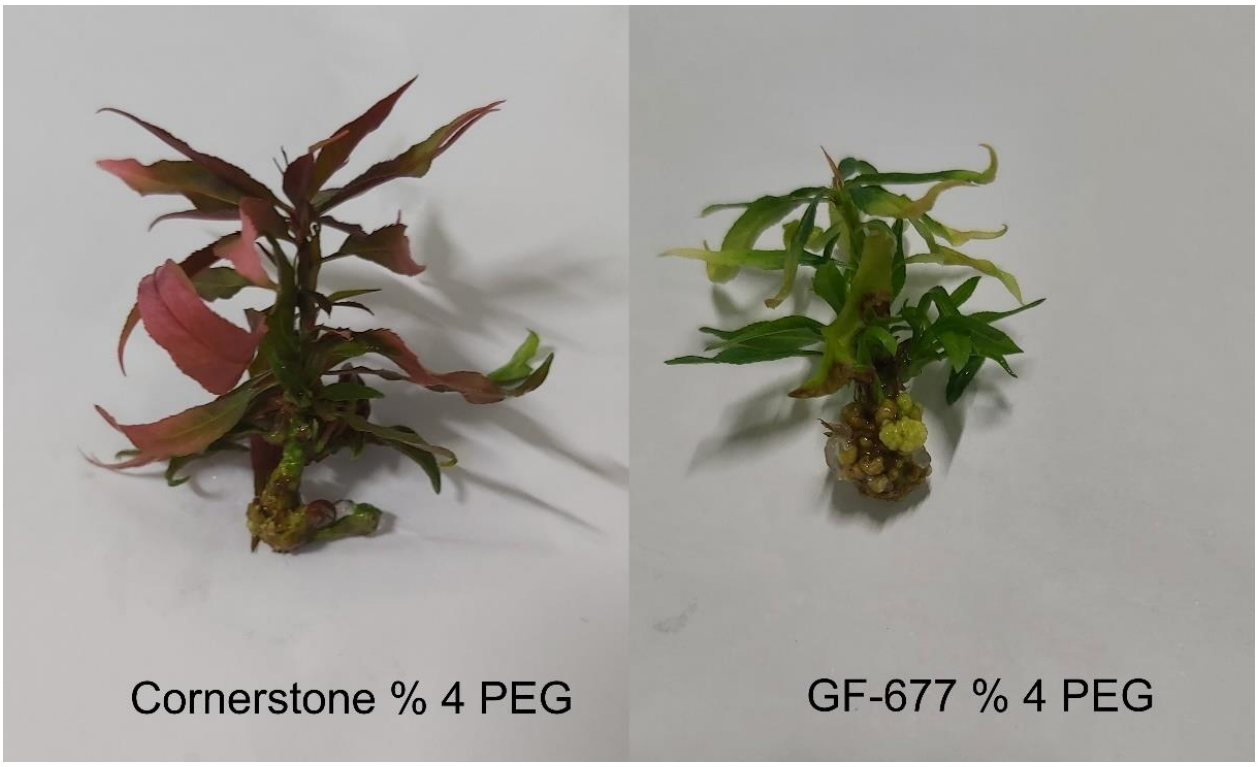

Figure 4.Comparison of PEG 8000 4% group plants of Cornerstone and GF-677 rootstocks.

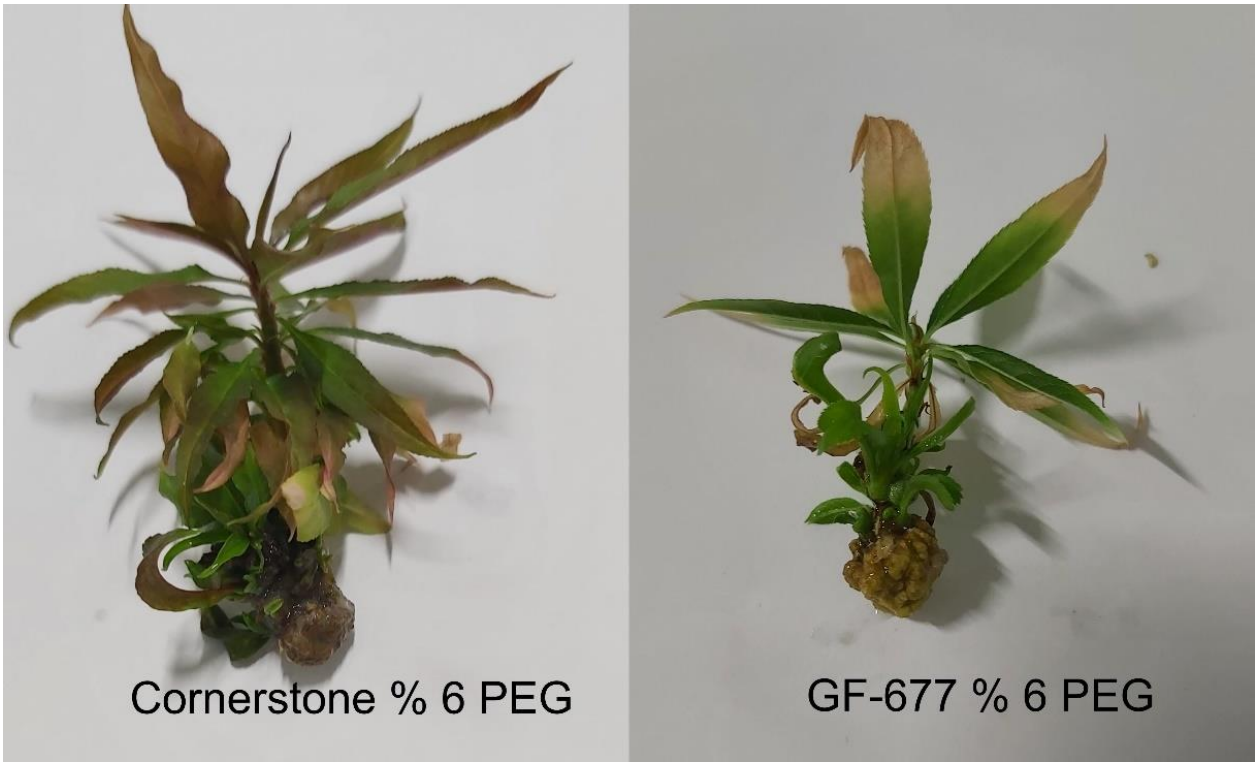

Figure 5.Comparison of PEG 8000 6% group plants of Cornerstone and GF-677 rootstocks.

The effect of PEG 8000 on shoot length was found to be significant. ($p \leq 0.005$). In the rootstock GF-677, a 19% reduction in shoot length was observed between 0% and 2% PEG 8000 concentration. Between 2% and 4% PEG 8000 concentration, the shoot length value was approximately maintained in GF-677 rootstock type. Between 0% and 6% PEG 8000 concentration, a 27% reduction in shoot length was recorded. In Cornerstone rootstock, a 21% reduction in shoot length was observed between 0% and 2% PEG 8000 concentration. In GF-677 rootstock, a 23% decrease occurred from PEG 8000 2% to 4%.

On Cornerstone rootstock, a 64% reduction in shoot length was recorded between 0% and 6% PEG 8000 concentration. Both rootstocks showed a decrease in shoot length as PEG 8000 concentration increased, but Cornerstone rootstock showed a greater decrease than GF-677 at higher PEG 8000 concentrations (Figure 6). GF-677 rootstock showed a 27% reduction in shoot length at 6% PEG 8000 treatment, while Cornerstone showed a 63% reduction (Figure 6). Nevertheless, the interaction effect (rootstock) x (PEG 8000) was not significant (Table 1).

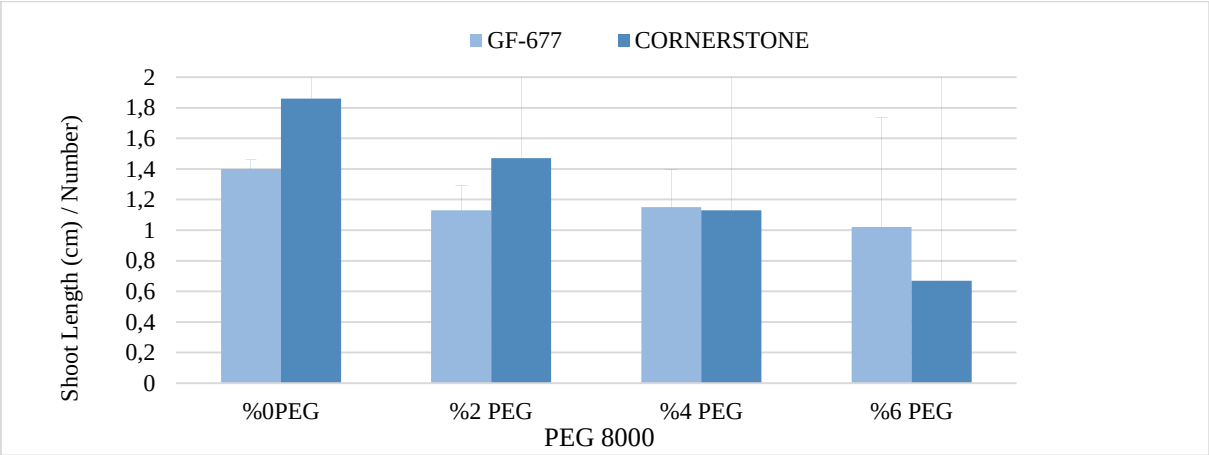

Figure 6. The mean length of shoots per explant in rootstocks at different PEG 8000 concentrations *in vitro*.

Kuşvuran and Daşgan (2017) noted a decrease in both the number and length of shoots as the severity of drought increased under drought stress conditions induced by a limited irrigation method and determined that it was affected by drought stress in proportion to increasing concentrations of PEG 8000. The damage to plants was also reported to increase with the increase in PEG dose. Govindaraj et al. (2010) reported that osmotic stress induced by PEG decreased cell water potential. Khodarahmpour (2011) reported that osmotic stress induced by PEG resulted in a reduction in shoot length across certain plant species.

Numerous studies have demonstrated that drought stress often leads to a slowdown or reduction in plant length. This effect was reported in different grapevine varieties (Yağmur, 2008; Çakır, 2011; Babalık et al., 2015; Bilir and İlhan, 2022), various almond species and GF-677 rootstock (Karimi et al., 2012), citrus rootstocks (Çerçi, 2012) and Myrobolan 29C and Garnem rootstocks (İpek, 2015). In this study, shoot number and length decreased with increasing drought stress and the results were similar to the results of the previous studies.

When the effect of different concentrations of drought (PEG 8000) stress on the fresh and dry weight of Cornerstone and GF-677 rootstocks under *in vitro* conditions was analyzed, the effect of rootstock, PEG 8000 and (rootstock) x (PEG 8000) interaction was found to be insignificant (Table 2).

Table 2. Analysis of variance of the effect of rootstock, PEG 8000 concentration and (rootstock) x (PEG 8000) concentration interaction on fresh and dry weight *in vitro*.

	Sources Of Variance	DF	MS	SS	F
Fresh Weight	Rootstock	1	10.67	10.67	4.25
	PEG 8000	3	17.00	51.00	2.26
	Rootstock X PEG 8000	3	6.64	19.92	0.88
Dry weight	Rootstock	1	0.03	0.03	1.81
	PEG 8000	3	0.11	0.33	2.20
	Rootstock X PEG 8000	3	0.02	0.06	0.45

* The difference between mean values is significant at 0 level in all cases
DF: Degree of Freedom MS:Mean Square SS:Sum of Square

With 2% and 4% PEG 8000 treatments, 41% and 119% increase in wet weight occurred in GF677 rootstock, respectively, while a 22% decrease occurred in 6% PEG 8000 treatment. In Cornerstone rootstock, in all three treatments (2%, 4%, and 6% PEG 8000), the wet weight increased by 68%, 69%, and 24% in PEG 8000 treatments compared to control plants (Figure 7).

In both rootstocks, an increase in dry weight was observed in 2% and 4% PEG 8000 treatments (Figure 8). This increase was determined as 12.5% in 2% PEG 8000 treatment in GF 677 rootstock and 62.5% in 4% PEG 8000 treatment. The 6% dose of PEG 8000 treatment resulted in a 25% decrease in the dry weight of GF 677 rootstock. Increasing doses of PEG 8000 at 2% and 4% caused a 20% and 40% increase in dry weight of Cornerstone rootstock, respectively. However, when the rate of PEG 8000 was increased from 4% to 6%, the dry weight value of Cornerstone rootstock was determined to be the same as the dry weight value of control plants (Figure 8.). When evaluating the dry weight parameter, Cornerstone rootstocks were less affected by drought stress compared to GF-677 rootstock. Under stress conditions, the increase in both wet and dry weight of Cornerstone rootstock was comparatively limited, with no observed decrease. This indicates that Cornerstone rootstock experienced minimal changes under stress conditions.

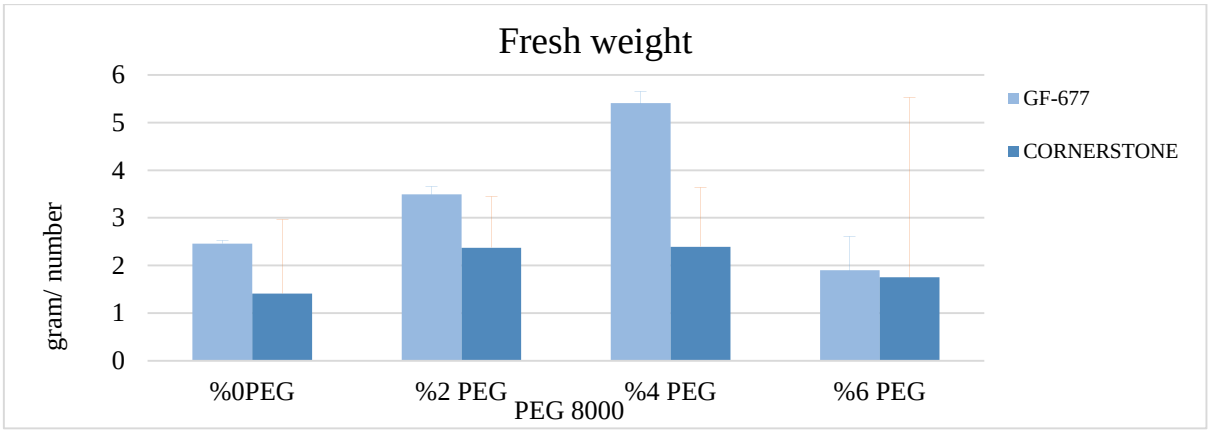

Figure 7. Mean wet weight of rootstocks at different PEG 8000 concentrations *in vitro*.

In citrus fruits, grapevine rootstocks, Myrobolan 29C, and Garnem rootstock, the fresh and dry weights were found to decrease with increasing PEG treatment doses (Simşek et al., 2018; Babalık et al., 2015; Ipek, 2015; Mese and Tangolar, 2019).

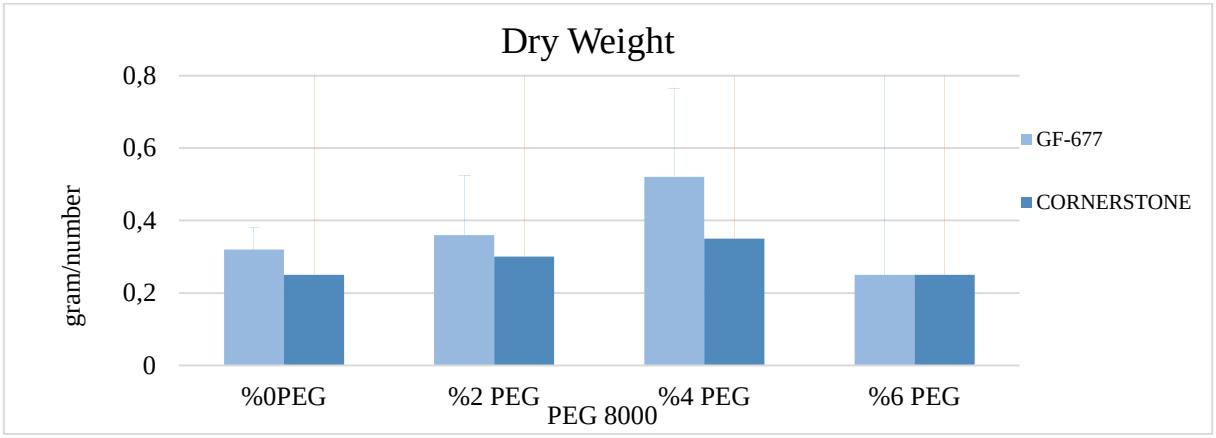

Figure 8. Mean dry weight of rootstocks at different PEG 8000 concentrations *in vitro*.

When the effect of different concentrations of drought stress (PEG 8000) on the chlorophyll amount of Cornerstone and GF-677 rootstocks *in vitro*, the effect of PEG 8000 was found to be significant ($p \leq 0.05$), but the effect of rootstock and (rootstock) x (PEG 8000) interaction was found to be insignificant (Table 3).

Table 3. Analysis of variance of the effect of rootstock, PEG 8000 concentration and (rootstock) x (PEG 8000) concentration interaction on chlorophyll amount *in vitro*.

Sources Of Variance	DF	MS	SS	F
Rootstock	1	1.23	1.23	0.7915
PEG 8000	3	14.95	44.95	3.2157*
Rootstock X PEG 8000	3	2.43	7.29	0.5233

* The difference between mean values is significant at p≤0.05 level
DF: Degree of Freedom MS:Mean Square SS:Sum of Square

In both rootstocks, chlorophyll a and b values were highest in the control group (0% PEG 8000), while a decrease was observed at 2%, 4% and 6% PEG 8000 concentrations. As the level of PEG 8000 increased in GF-677 rootstock, indicating more severe drought conditions, there was a decrease in both chlorophyll a and chlorophyll b amounts (Figure 9 and Figure 10). In such cases, the photosynthetic activity of the plant is expected to decrease.

A similar situation was observed for Cornerstone rootstock. However, the decrease in chlorophyll a and b levels was less when compared with GF-677 rootstock plants of the same treatment. This suggests that the decrease in photosynthetic activity in Cornerstone rootstock caused by drought stress was more limited. It was determined that Cornerstone rootstock was less affected by drought stress.

Metabolic disorders caused by drought stress may also lead to impaired chlorophyll synthesis. Reduced chlorophyll amount is a common symptom of altered plant morphology under drought stress (Mishra and Singh, 2011; Arbona et al., 2013). It was noted that the chlorophyll amount decreased with the increase in PEG concentration in grapevine (Mese and Tangolar, 2019; Bilir and Ilhan, 2022).

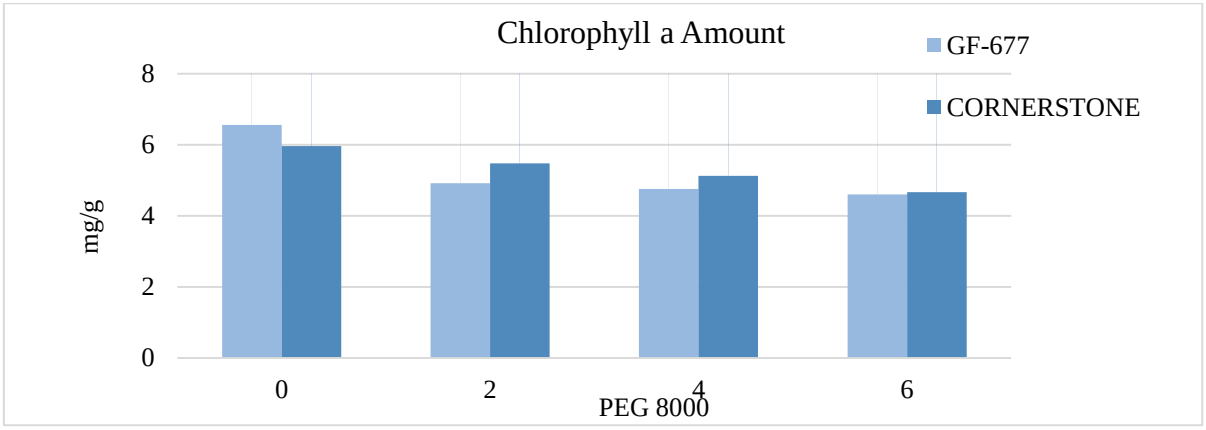

Figure 9. Chlorophyll a amount in rootstocks at different PEG 8000 concentrations *in vitro*.

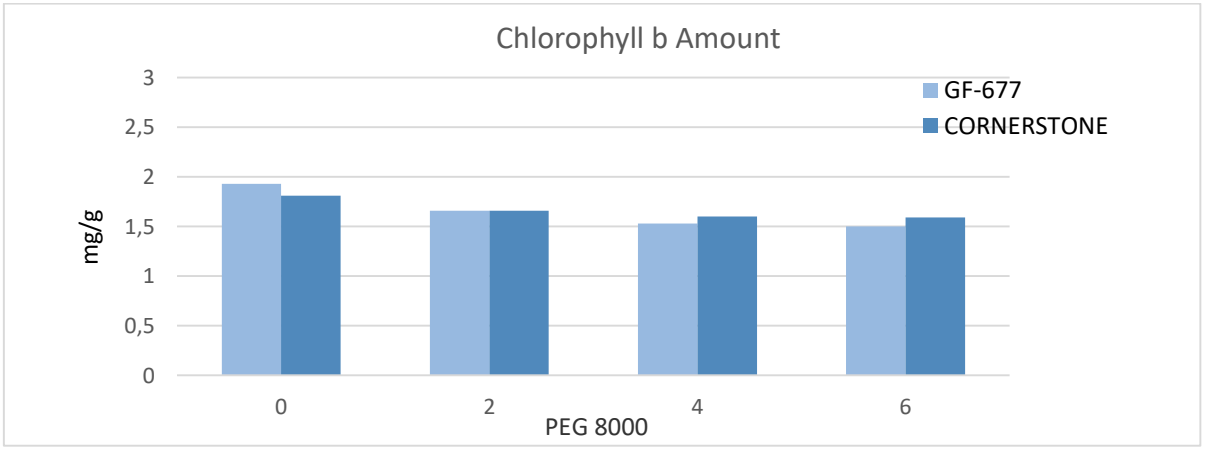

Figure 10. Chlorophyll B amount in rootstocks at different PEG 8000 concentrations *in vitro*.

When drought and salt stress intensify, the enzymes required for chlorophyll synthesis cannot function and this leads to the inability of chlorophyll biosynthesis, the lack of chlorophyll to provide the

necessary light energy for photosynthesis and the decrease in photosynthesis rate (Al Absi, 2005; Bertamini et al., 2006; Dajic, 2006).

In a study, it was noted that chlorophyll amount decreased with increasing PEG doses. However, plants in the 2% and 4% PEG groups showed similar chlorophyll amounts, belonging to the same statistical group in terms of this feature (Bilir and İlhan, 2022).

When the effect of different concentrations of drought stress (PEG 8000) on % Leaf Relative Water Content (%LRWC) and Proline of Cornerstone and GF-677 rootstock under *in vitro* conditions was analyzed, the effect of rootstock, PEG 8000 and (rootstock) X (PEG 8000) interaction was found insignificant in terms of LRWC. (0) (Table 4). However, the effect of rootstock and polyethylene glycol (PEG 8000) ($p \leq 0.001$) and the effect of (rootstock) X (PEG 8000) interaction was found to be significant ($p \leq 0.005$) (Table 4).

Table 4. Analysis of variance of rootstock, PEG 8000 concentration and (rootstock) x (PEG 8000) concentration interaction on %LRWC and Proline *in vitro*.

	Sources Of Variance	**DF**	**MS**	**SS**	**F**
% LRWC	Rootstock	1	2.30	2.30	0.01
	PEG 8000	3	142.78	428.34	0.29
	Rootstock X PEG 8000	3	198.68	596.04	0.40
Proline	Rootstock	1	319.06	319.06	127.224*
	PEG 8000	3	131.65	394.95	17.498*
	Rootstock X PEG 8000	3	53.72	161.16	7.140**

*,** The difference between mean values is significant at $p \leq 0.05$, $p \leq 0.001$ level, respectively

For GF-677 rootstock, PEG 8000 drought stress treatments applied at different amounts in the medium did not significantly affect the leaf relative water content of the plants and the leaf relative water content of the treated plants and control plants were similar. When the concentration of PEG 8000 was raised to 4% and 6%, the leaf relative water content of plants in these treatments closely resembled that of the control plants (Figure 11).

The findings suggested that at low PEG 8000 concentrations, Cornerstone rootstock exhibited improved water retention by increasing LRWC. However, its water retention capacity declined as the concentration of PEG 8000 increased. In contrast, GF-677 demonstrated a more balanced response in terms of LRWC (Figure 11).

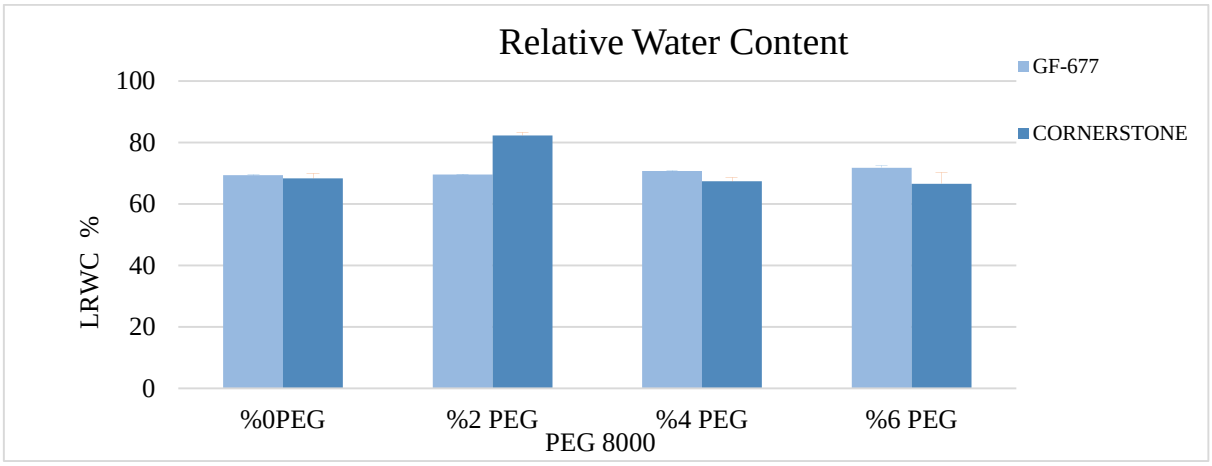

Figure 11. Relative Water Content of rootstocks at different PEG 8000 concentrations *in vitro*

Drought conditions are known to reduce the processes of division and growth in plant cells. This results in a reduction in the growth rate of plants, accompanied by decreases in the relative moisture content of leaves and leaf water potential (Lawlor and Cornic, 2002; Capell et al., 2004; Cakmakci, 2009).

The proline levels of GF-677 and Cornerstone rootstock showed differences along with the increased amount of PEG. In GF-677 rootstock, the proline level of control plants was measured at 1.33 µmol/g. Under drought stress treatments (2%, 4%, and 6% PEG 8000), proline amounts were determined

as 1.62 µmol/g, 2.17 µmol/g, and 3.81 µmol/g, respectively. In Cornerstone rootstock, the proline level of control plants was measured at 5.39 µmol/g. Under drought stress treatments (2%, 4%, and 6% PEG 8000), proline amounts were determined as 6.66 µmol/g, 12.31 µmol/g, and 14.21 µmol/g, respectively (Figure 12). Proline concentration was found to increase significantly in plants under drought stress (Choudhary et al., 2005; Mohammadkhani and Heidari, 2008; Ghorbanli et al., 2012; Bohalima, 2017; Buhroy et al., 2017; Celik et al., 2017; Hunt et al., 2017; Aghaie et al., 2018; Nahar and Ullah, 2018; Noori et al., 2018; Wang et al., 2018; Akgul, 2019).

Ben Ahmed et al. (2008) pointed out that the amount of proline accumulated in plants under stress conditions shows differences according to species. Drought resistance of different cultivars of the same species varies, and accordingly, the amount of proline they contain may be different (Arji and Arzani, 2000).

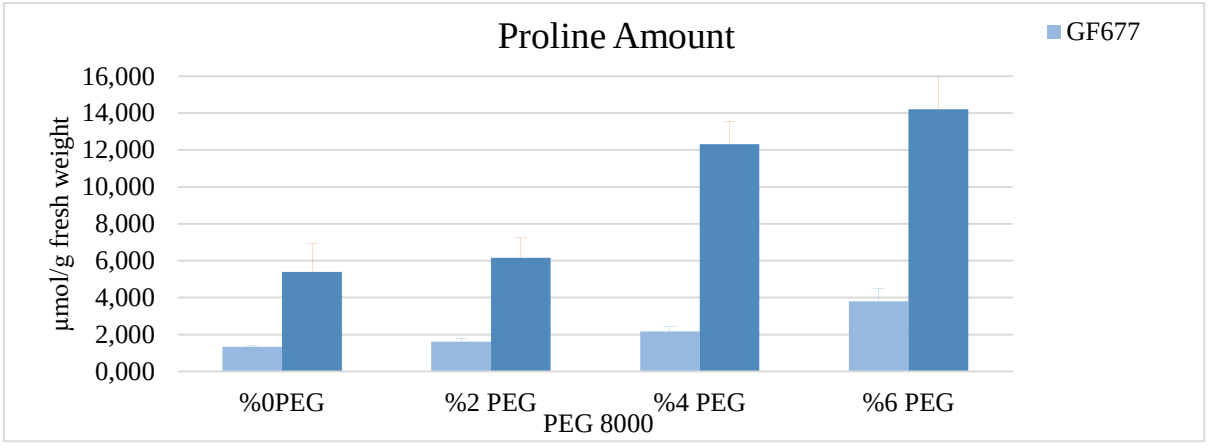

Figure 12. Amount of proline produced in rootstocks *in vitro* at different PEG 8000 concentrations (µmol/g fresh weight).

Proline levels vary widely among different rootstocks and cultivars (Jiménez et al., 2013). Cornerstone showed much higher proline levels than GF-677 rootstock at certain PEG 8000 concentrations. This suggests that Cornerstone rootstock may show better resilience to drought conditions and could serve as a more tolerant rootstock under water-limited conditions (Figure 12). The accumulation of proline in plants under stress conditions can vary significantly from species to species, and in certain cases, it can be up to 100-fold higher than in control plants (Hunt et al., 2017). In this study, an increase in proline levels was observed in both rootstocks under drought stress treatments. However, the proline amounts of Cornerstone rootstock, including control plants, were determined to be higher. Research indicates that stress-tolerant plant species typically show higher proline concentrations than species sensitive to stress conditions (Saed-Moucheshi et al., 2013). In this study, the initial proline levels in the Cornerstone rootstock were higher compared to those in GF-677. Furthermore, under artificial drought conditions induced by increasing PEG 8000 concentrations, proline levels increased significantly. A notable difference in proline levels was observed between the GF-677 and Cornerstone rootstocks.

Conclusion

Differences in the adaptability to drought conditions were observed between GF-677 and Cornerstone rootstocks. The GF-677 showed adaptation ability with an increase in shoot wet and dry weight and shoot number at low and medium PEG 8000 concentrations. Conversely, Cornerstone rootstock initially displayed a lower shoot number but demonstrated superior protection in terms of LRWC and chlorophyll levels as water stress intensified. The low number of shoots, reduction in shoot length with stress conditions, controlled increase in wet and dry weight, and the same dry weight as the control plants in the highest drought stress treatment, high proline amount and no high decrease in chlorophyll amount indicate that Cornerstone is a resistant rootstock under stress conditions. This indicates that Cornerstone may better adapt to drought stress under certain conditions than GF-677. However, both rootstocks experienced similar difficulties at high levels of drought stress and showed adaptation to drought stress through an increase in proline levels. Our results indicate that the

Cornerstone and the GF-677 rootstock, showed drought tolerance, with both rootstocks demonstrating varying levels of resistance under diverse conditions. Based on the findings of the study, it is recommended that Cornerstone rootstock, as a new one in Türkiye, be considered for cultivation in peach, nectarine, almond, and apricot, especially under drought conditions.

Acknowledements

This article contains the results and findings of Ege University Faculty of Agriculture Master's thesis titled 'Investigation of Drought Stress Tolerance of Cornerstone and GF-677 Rootstocks under *In vitro* Conditions'. We would like to thank Parlar Fidancılık and Bademli Biotechnology Industry and Trade Limited Companies for their contributions.

Researchers' Contribution Rate Declaration Summary

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Conflict of Interest Declaration

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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