

## ***In vitro* ASSESSMENT OF DROUGHT TOLERANCE IN SUMMER SQUASH (*Cucurbita pepo* L.) CULTIVARS**

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### **ABSTRACT**

In this study, the growth performance of three different summer squash cultivars was evaluated under *in vitro* conditions using Murashige and Skoog (MS) nutrient media supplemented with varying concentrations of polyethylene glycol (PEG) at 0%, 2%, 4%, and 6%. In the study, parameters including germination rate (%), stem fresh and dry weight (g), root fresh and dry weight (g), shoot and root length (mm) were investigated. In addition, antioxidant capacity and total phenolic content in plant shoots were determined using the DPPH method. According to the findings, the highest stem fresh weight was recorded as 0.72 g in cultivar Pelin grown in control medium (without PEG). In terms of root fresh weight, the highest mean value among the media was also observed in control with 0.43 g. The longest shoot length as 48.91 mm was also determined in Pelin. Increasing concentrations of PEG were found to have a negative impact on all growth parameters.

**Keywords:** growth parameters, PEG, antioxidant capacity, squash, abiotic stress

### **INTRODUCTION**

Plants are increasingly exposed to multifactorial environmental stressors as a result of global warming and climate change, which significantly affect their growth, metabolism, and productivity. Among these stress factors, drought stands out as a major abiotic constraint that disrupts intracellular water relations, impairs photosynthetic efficiency, and alters ion homeostasis. These physiological disruptions lead to the overproduction of reactive oxygen species (ROS), which induce oxidative stress and ultimately hinder plant growth and reduce yield. Recent studies indicate that climate change is intensifying both the frequency and severity of drought events across many regions of the world. This trend has emerged as a critical concern for crop productivity, plant ecophysiology,

and global food security [Ali et al. 2025]. Environmental stress factors such as drought pose a significant threat to plant growth and productivity on a global scale [Koua et al. 2021, Khan et al. 2024]. Global warming contributes to this by raising temperatures that accelerate evaporation, reduce surface water availability, and rapidly dehydrate soils. That conditions collectively intensify drought stress [Passioura and Angus 2010, Devinentis 2020]. Moreover, as a result of ongoing climate change and global warming, drought episodes are becoming more frequent and severe [Seleiman et al. 2021]. The expansion of drought-affected areas, and the subsequent loss of arable land increasingly threaten global food security. Consequently, there is an urgent need to develop drought-tolerant and high-yielding crop cultivars to ensure sustained agricultural productivity [Ali et al. 2025]. In recent years, shifts in climate models have had a substantial impact on agricultural zones, with particularly pronounced effects observed in arid, semi-arid, and coastal regions [Stringer et al. 2021, Garcia-Caparros et al. 2025]. Currently, nearly one-third of the world's cultivable land is classified as arid or semi-arid, and the intensity of drought continues to increase. Drought has emerged as one of the most critical abiotic stressors, exerting severe negative effects on crop performance and productivity worldwide [Seleiman et al. 2021, Dietz et al. 2021, Garcia-Caparros et al. 2025].

Vegetables account for approximately 12% of global agricultural production, with species from the Solanaceae and Cucurbitaceae families representing a major share of total vegetable output. However, climate change poses significant threats to both agricultural productivity and ecosystem sustainability. Drought reduces the ability of plants to absorb water, lowers photosynthetic efficiency, and disrupts key physiological processes, collectively resulting in substantial declines in crop yield [Ulas et al. 2025]. *Cucurbita pepo* L. is one of the 15 recognized species within the genus *Cucurbita*, which belongs to the Cucurbitaceae family. Its plant is characterized by large, showy, yellow to orange flowers that are insect-pollinated, as well as broad, lobed leaves typically covered with fine, bristly hairs. Although different species within the genus may vary morphologically, their fruits often exhibit similar characteristics, which has led to the interchangeable use of the terms “pumpkin” and “squash” in various contexts [Adnan et al. 2017]. *Cucurbita pepo*. is native to northern Mexico, as well as the southwestern and eastern regions of the United States. Wild forms of the species can also be found in parts of Europe and Asia. The plant is valued not only for its agricultural importance, but also for its nutritional and medicinal properties. The immature fruits are commonly consumed as vegetables, while mature fruits are used in the preparation of desserts, confections, and beverages, often roasted or cooked [Martha and Gutierrez 2016, Ratnam et al. 2017]. Furthermore, *C. pepo* holds a prominent place in traditional medicine systems due to its reported antidiabetic, antihypertensive, anticancer, immunomodulatory, antibacterial, hypocholesterolemic, antiparasitic, anti-inflammatory, and analgesic activities [Fu et al. 2006, Conti et al. 2015].

Over recent years, tissue culture-based approaches have become widely used in plant stress physiology research. Their main strength lies in enabling stress treatments to be applied under tightly controlled conditions, while also allowing rapid screening of large numbers of genotypes under uniform and reproducible exposure regimes [Vives-Peris et al. 2017, Pérez-Jiménez and Pérez-Tornero 2020]. The *in vitro* technique provides a valuable platform for studying plant development, genetic manipulation, stress responses, and mass propagation under *in vitro* conditions. Recent studies have shown that polyethylene glycol (PEG) serves as an effective osmotic agent for establishing controlled drought-like conditions *in vitro* [Sahu et al. 2023, Akram et al. 2024]. Consequently, PEG-induced osmotic stress has become a widely accepted approach for screening plant materials for drought tolerance [Zhang et al. 2018].

In this study, the drought tolerance of different squash varieties was evaluated *in vitro* under polyethylene glycol (PEG)-induced osmotic stress. Implementing such assessments within tissue culture systems provides a controlled platform that allows for the preliminary characterization and comparative evaluation of drought tolerance among the tested varieties.

## MATERIALS AND METHODS

Seeds of three summer squash cultivars, namely Pelin, Nazlı, and Şebnem were used as the plant material. Masuda and Skoog [1962] nutrient medium was used supplemented with polyethylene glycol (PEG 6000; Merck/Sigma-Aldrich) at four different concentrations: 0%, 2%, 4%, and 6%, in order to simulate drought conditions. The squash seeds used in the experiment were surface sterilized by immersion in a 30% sodium hypochlorite solution for 20 minutes. Following sterilization, the seeds were rinsed 4–5 times with sterile distilled water to ensure complete removal of any residual disinfectant, and to achieve full sterility. The seeds were aseptically transferred

into the pre-prepared culture media under a laminar flow. Sowing was performed in sterile 60 mm Petri dishes, with four seeds per dish. Seeds were positioned evenly and without contact to ensure uniform exposure to the culture medium. A total of 100 seeds per treatment were used (25 Petri dishes  $\times$  4 seeds). Following sowing, the Petri dishes were incubated in a plant growth chamber at  $25 \pm 2$  °C under a 16 h light/8 h dark photoperiod, with an illumination intensity of 3,000 lux. The cultures were maintained for 21 days after sowing, and measurements were performed at the end of the culture period.

Germination was assessed by counting the number of germinated seeds, and the germination rate was expressed as a percentage (%). For biomass determination, plants were randomly selected from each group, and stems and roots were separated. Fresh stem and root weights were measured immediately using an analytical balance and recorded in grams (g). The same samples were then dried in a laboratory oven at 55 °C until constant weight and reweighed to obtain dry stem and root weights (g). In addition, stem and root lengths were measured using a digital caliper and recorded in millimetres (mm) [Adem et al. 2025].

### Determination of antioxidant capacity

For antioxidant analysis, 0.25 g of powdered stem squash sample was placed into a sterile falcon tube and extracted with 20 mL of 80% methanol by shaking in a water bath at ambient temperature for 2.5 hours. The mixture was centrifuged at 7000 rpm for 15 minutes, and the resulting supernatant was filtered using blue band filter paper [Adem et al. 2025]. The clarified extracts were stored at +4 °C until further use. Antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay based on the method of Masuda et al. [1999], with slight procedural adjustments. In this method, 100  $\mu$ L of each extract was mixed with 1900  $\mu$ L of DPPH solution and incubated in the dark at room temperature for 30 minutes. After incubation, 200  $\mu$ L aliquots were transferred into microplate wells, and absorbance was recorded at 517 nm using a microplate reader (Tecan Infinite 200 Pro, Austria). The antioxidant capacity was quantified and expressed as  $\mu$ mol Trolox equivalents per gram of dry weight ( $\mu$ mol TE  $g^{-1}$  DW).

### Determination of total phenolic content

For the analysis of total phenolic content, the same extracts prepared for the DPPH assay were utilized. According to the procedure, 100  $\mu$ L of extract was mixed with 200  $\mu$ L of 10% Folin–Ciocalteu reagent and 800  $\mu$ L of 20% sodium carbonate solution. The mixtures were incubated in the dark at room temperature for 2 hours within a microplate. Following incubation, 200  $\mu$ L of each sample was transferred into new vials, and absorbance was measured at 765 nm using a microplate reader. Results were expressed as milligrams of gallic acid equivalent per 100 grams of dry weight (mg GAE 100  $g^{-1}$  DW) in accordance with the method described by Ainsworth and Gillespie [2007].

### Statistical analysis

The experiment was conducted as a two-factor (cultivar and medium) factorial experimental design in randomized plots. The data obtained from the study were subjected to two-way analysis of variance (ANOVA) using JMP statistical software, version 13.0.1 (SAS Institute Inc., Cary, NC, USA), and when statistically significant differences were detected, means were separated using the least significant difference (LSD) test.

## RESULTS AND DISCUSSION

Measurements and evaluations of different squash cultivars grown under *in vitro* conditions, in nutrient media supplemented with various concentrations of PEG, were performed. The results revealed that, although germination observed in cultivars Nazlı and Şebnem on media containing 6% PEG, further plant development was not achieved under these stress conditions.

Table 1 presents the mean germination percentages of different squash cultivars grown on media containing various concentrations of PEG. Statistical analysis revealed that cultivar, medium, and the cultivar  $\times$  medium interaction were significant. Regarding cultivar, the highest average germination percentage was obtained from Pelin (63.00%), followed by Nazlı (49.17%) and Şebnem (31.81%). Among the media tested, the highest average germination rate was observed in the control medium with the average value of 60.74%, while the lowest was recorded in the 6% PEG medium with 26.14%. Germination of squash seeds Pelin at the highest PEG concentration decreased by approximately 1.7-fold, Nazlı 2.6-fold, and Şebnem as much as 5.5-fold compared to the control.

Brdar-Jokanović and Zdravković [2015] assessed the impact of PEG-induced drought stress on 15 tomato populations and found that increasing PEG levels significantly reduced both germination percentage and energy. Akte et al. [2016] examined five rice cultivars under PEG-simulated drought stress, and reported the highest germination (100%) in Binadhan-10 at 1% PEG. Similarly, Zayova et al. [2017] studied eggplant cultivars under PEG 8000 stress, and observed a decline in plant survival rates with rising PEG concentrations. In a study by Adem et al. [2025], different tomato cultivars were cultured *in vitro* on MS media containing various concentrations of PEG (0%, 1%, 2%, 4%, and 6%) to assess their growth responses. The highest average germination rate (70.00%) was recorded in the 0% medium without PEG (overall, the results indicated that increasing PEG concentrations led to a noticeable reduction in plant growth).

These findings clearly indicate that PEG-induced osmotic stress has a strong inhibitory effect on germination, and the magnitude of this reduction differs markedly among cultivars, demonstrating the cultivar dependent nature of drought tolerance.

**Table 1.** Germination percentages (%) of different squash cultivars in PEG supplemented media

PEG (%)	Cultivar			Medium avarege
	Pelin	Nazlı	Şebnem	
0	75.00b	63.00c	44.23e	60.74A
2	79.00a	62.00c	38.00f	59.67A
4	53.00d	46.25e	37.00f	45.42B
6	45.00e	25.42g	8.00h	26.14C
Cultivar average	63.00A	49.17B	31.81C	×

LSD<sub>cultivar</sub> = 1.446\*\*\* LSD<sub>medium</sub> = 1.669 \*\*\* LSD<sub>cultivarxmedium</sub> = 2.892\*\*\*

<sup>1</sup> statistical differences between the averages shown in separate letters in the same column were found to be significant

<sup>2</sup>\*\*\* $p \leq 0.001$

**Table 2.** Fresh stem weight (g) of different squash cultivars in PEG supplemented media

PEG (%)	Cultivar			Medium avarege
	Pelin	Nazlı	Şebnem	
0	0.72a	0.65b	0.47d	0.61A
2	0.56c	0.46d	0.29e	0.44B
4	0.30e	0.32e	0.20f	0.27C
6	0.21f	0	0	0.07D
Cultivar average	0.45A	0.36B	0.24C	×

LSD<sub>cultivar</sub> = 0.02\*\*\* LSD<sub>medium</sub> = 0.02 \*\*\* LSD<sub>cultivarxmedium</sub> = 0.04\*\*\*

<sup>1</sup> statistical differences between the averages shown in separate letters in the same column were found to be significant

<sup>2</sup>\*\*\* $p \leq 0.001$

The stem fresh weight values of different squash cultivars grown in media containing varying concentrations of PEG are presented in Table 2. The highest average stem fresh weight of 0.45 g was recorded in Pelin, regarding media, and cultivar × media interaction, the highest average stem fresh weight (0.61 g) was observed for Pelin in the medium without PEG. In contrast, the lowest interaction values were identified as 0.21 g for Pelin in 6% PEG medium and 0.20 g for Şebnem in 4% PEG medium. At the highest PEG concentration, the fresh shoot weight of Pelin decreased 3.5-fold compared to the control, while plants of the other cultivars died (Table 2).

Jezdinsky et al. [2012] investigated the effects of drought stress on leek by evaluating various morphological parameters. Their findings revealed that drought conditions led to significant reductions in total fresh weight, leaf area, stem length, and stem diameter. Similarly, Mehmandar et al. [2023] assessed the responses of three Iranian

melon genotypes Girke, Ghobadloo, and Toghermezi to sorbitol (0.1, 0.2, and 0.4 M) and PEG (0.009, 0.012, and 0.015 M) treatments *in vitro* conditions. The genotype Girke has been determined for its drought tolerance in Iran. The study reported that both PEG and sorbitol stress increased the accumulation of proline and malondialdehyde (MDA), while reduced coleoptile length, fresh plant weight, and photosynthetic pigment contents. In another study, Kara et al. [2024a] examined the effect of drought stress on leek using PEG-induced drought *in vitro* conditions. PEG was added to the medium at concentrations of 0%, 1%, 2%, 4%, and 6% to simulate varying drought levels. The highest stem fresh weight (0.0219 g) was recorded in the control (0% PEG), whereas the lowest (0.0104 g) was observed in the 6% PEG treatment, indicating that increased PEG concentrations negatively affected stem development in leek seedlings.

Findings of the present study clearly demonstrate that increasing PEG concentrations exert a strong inhibitory effect on stem biomass accumulation, consistent with the general pattern observed in previous drought-stress studies. The marked reduction in stem fresh weight at 6% PEG, particularly in Nazlı and Şebnem where no measurable biomass was produced, suggests that severe osmotic stress limits cell expansion and water uptake to a degree that prevents normal stem development. The superior performance of Pelin across all PEG levels indicates a comparatively greater capacity for osmotic adjustment and maintenance of cellular turgor under stress. Overall, the cultivar-dependent variation observed in stem biomass under PEG-induced drought supports the conclusion that early-stage growth parameters such as stem fresh weight can serve as reliable indicators for distinguishing drought-tolerant and drought-sensitive squash cultivars *in vitro*.

Statistical analysis showed that cultivar, media, and the cultivar × media interaction had significant effect on stem dry weight (Table 3). Among the media, the highest average stem dry weight was observed in the control medium (0.08 g), while the lowest was recorded in the 6% PEG medium (0.01 g). Regarding cultivar, Pelin and Nazlı exhibited the highest average values, both at 0.06 g. The cultivar × medium interaction was statistically highly significant. The cultivars responded differently to the PEG doses. The highest individual values, belonging to the same statistical group, were obtained from Nazlı at the 2% dose (0.09 g), Nazlı at the 0% dose (0.08 g), and Pelin at the 0% dose (0.08 g).

**Table 3.** Dry stem weight (g) of different squash cultivars in PEG supplemented media

PEG (%)	Cultivar			Medium average
	Pelin	Nazlı	Şebnem	
0	0.08a	0.08a	0.07b	0.08A
2	0.07cd	0.09a	0.07bc	0.07B
4	0.06d	0.07bc	0.06e	0.06C
6	0.04f	0	0	0.01D
Cultivar average	0.06A	0.06A	0.05B	×

LSD<sub>cultivar</sub> = 0.003\*\*\* LSD<sub>medium</sub> = 0.003\*\*\* LSD<sub>cultivarxmedium</sub> = 0.007\*\*\*

<sup>1</sup> statistical differences between the averages shown in separate letters in the same column were found to be significant

<sup>2</sup>\*\*\**p* ≤ 0.001

Kara et al. [2024b] tested the development of white cabbage seeds in the MS media supplemented with varying concentrations of PEG (0%, 1%, 2%, 4%, 6%) to determine drought tolerance levels. The highest stem dry weight was observed in the control as 0.0110 g, indicating a negative effect of drought stress on biomass accumulation. In a related study, Rehman et al. [2024] reported that drought conditions significantly suppressed plant growth, with cabbage showing notable reductions in both fresh and dry weights of shoots and roots. Similarly, Torun and Sarı [2025] subjected 192 melon genotypes from the Cucurbitaceae genetic resources collection at Çukurova University to drought stress induced by 5% PEG 6000 for two months. They assessed parameters such as stem length, stem and root dry weights, leaf number, and a drought tolerance score (0–5 scale). Most parameters showed a decrease under stress, except root dry weight with 3% increase. Kaya [2025] investigated the effects of increasing concentrations of PEG-6000 on the morpho-physiological parameters of melon seedlings (*Cucumis*

*melo* Kirkağaç 589). Seedlings with two true leaves were grown for 30 days in a peat:perlite:vermiculite (6:1:1) substrate containing 5%, 10%, 15%, and 20% PEG-6000. Measurements included plant height, stem diameter, fresh/dry weights, dry matter content, leaf area, leaf temperature, chlorophyll content (SPAD), relative water content, turgor loss, and electrolyte leakage. The findings indicated that increasing drought severity led to significant reductions in plant height, biomass, and relative water content. Our results clearly demonstrate that increasing PEG concentrations markedly restricted dry matter accumulation, indicating that stem dry weight is highly sensitive to osmotic stress. In our experiment, the superior performance of the cultivars Pelin and Nazlı, particularly at lower PEG levels, suggests a greater capacity to maintain tissue hydration and carbon allocation under drought-like conditions. Overall, the cultivar-dependent variation observed in this study reinforces the value of stem dry weight as a reliable indicator of drought tolerance in squash seedlings.

Table 4 summarizes the root fresh weight responses of squash cultivars exposed to increasing PEG concentrations *in vitro*. Root fresh weight differed significantly among treatments and cultivars. The PEG-free control produced the highest mean root fresh weight (0.43 g). When averaged across media, Pelin had the greatest root fresh weight (0.35 g), whereas Nazlı and Şebnem showed markedly lower values (0.12 g and 0.08 g, respectively). In a related study, Yıldırım et al. [2020] examined the effect of drought stress on growth, nutrient content, and various physiological and biochemical traits in bean under irrigation levels at the 100%, 80%, and 60% of field capacity. Results indicated that drought stress adversely affected leaf area as well as fresh and dry weights of leaves, stems, and roots. Similarly, Tajaragh et al. [2022] assessed the physiological and biochemical responses of three Iranian local *Cucurbita* cultivars (Tanbal Ajili – *C. maxima*, Ajili Razan – *C. pepo*, Balghabakhi – *C. moschata*) under osmotic stress induced by PEG 6000 and mannitol *in vitro*. Seedlings were transferred to MS media supplemented with varying concentrations of PEG (0.009–0.015 M) and mannitol (0.1–0.4 M). The treatments significantly increased shoot and root dry weights, malondialdehyde (MDA), proline, and various antioxidant components, while reducing coleoptile length, shoot and root fresh weights, and photosynthetic pigment contents especially under higher stress levels. Kara et al. [2024c] evaluated onion growth performance under *in vitro* drought conditions induced by PEG concentrations (0%, 1%, 2%, 4%, 6%) added to MS medium. The highest root fresh and dry weights were recorded in the control (0.383 g and 0.031 g, respectively), while the lowest were observed in 6% PEG (0.02 g and 0.001 g, respectively). These findings clearly demonstrate that increasing PEG concentrations negatively affect root biomass and indicate increasing sensitivity to drought stress. Our results clearly indicate that root fresh weight is highly sensitive to PEG-induced osmotic stress, with marked reductions occurring even at moderate stress levels. The superior performance of Pelin suggests a stronger root system capable of maintaining water uptake under declining water availability, highlighting its potential drought-tolerance advantage.

**Table 4.** Fresh root weight (g) of different squash cultivars in PEG supplemented media

PEG (%)	Cultivar			Medium average
	Pelin	Nazlı	Şebnem	
0	0.78a	0.26bc	0.24c	0.43A
2	0.32b	0.12d	0.06def	0.17B
4	0.20c	0.09de	0.004ef	0.11C
6	0.11d	–	–	0.03D
Cultivar average	0.35A	0.12B	0.08B	×

LSD<sub>cultivar</sub> = 0.036\*\*\* LSD<sub>medium</sub> = 0.042\*\*\* LSD<sub>cultivarxmedium</sub> = 0.072\*\*\*

<sup>1</sup> statistical differences between the averages shown in separate letters in the same column were found to be significant

<sup>2</sup>\*\*\* $p \leq 0.001$

The highest root dry weight in term of media was recorded in the medium without PEG (Table 5). In the control medium, the cultivar Pelin exhibited the highest root dry weight (0.043 g), followed by the cultivars Şebnem (0.032 g) and Nazlı (0.029 g).

**Table 5.** Dry root weight (g) of different squash cultivars in PEG supplemented media

PEG (%)	Cultivar			Medium avarege
	Pelin	Nazlı	Şebnem	
0	0.043a	0.029b	0.032b	0.030A
2	0.023c	0.014d	0.006e	0.010B
4	0.020c	0.009de	0.006e	0.010B
6	0.011de	–	–	0.004C
Cultivar average	0.02A	0.01B	0.01B	×

LSD<sub>cultivar</sub> = 0.002\*\*\* LSD<sub>medium</sub> = 0.002\*\*\* LSD<sub>cultivarxmedium</sub> = 0.005\*\*\*

<sup>1</sup> statistical differences between the averages shown in separate letters in the same column were found to be significant

<sup>2</sup>\*\*\*p ≤ 0.001

**Table 6.** Stem length (mm) of different squash cultivars in PEG supplemented media

PEG (%)	Cultivar			Medium avarege
	Pelin	Nazlı	Şebnem	
0	70.39a	51.05c	48.50c	56.65A
2	51.15b	42.95d	31.76e	43.30B
4	43.45d	32.86e	18.17g	31.49C
6	26.64f	–	–	8.90D
Cultivar average	48.91A	31.71B	24.61C	×

LSD<sub>cultivar</sub> = 1.958\*\*\* LSD<sub>medium</sub> = 2.261\*\*\* LSD<sub>cultivarxmedium</sub> = 3.915\*\*\*

<sup>1</sup> statistical differences between the averages shown in separate letters in the same column were found to be significant

<sup>2</sup>\*\*\*p ≤ 0.001

Turan and Samur [2024] examined the effects of pre-treatments with gibberellic acid (GA<sub>3</sub>) and boric acid (BA) on germination and seedling development of rapeseed (*Brassica napus* L.) under PEG-induced drought stress. Their findings indicated that the highest seedling dry weight (0.046 g) was obtained in treatments combining the control with GA<sub>3</sub> and BA at concentrations of 1.00, 1.5, and 2.00 mg L<sup>-1</sup>. Our results indicate that root dry weight is highly sensitive to increasing PEG concentrations, as reductions were evident even at moderate stress levels. The superior performance of Pelin suggests a more robust root system capable of maintaining structural integrity under osmotic stress, underscoring its potential drought tolerance.

Among the media, the highest stem length was observed in the 0% medium as 56.65 mm, while the lowest value was recorded in the 6% PEG medium as 8.90 mm, indicating a clear reduction in stem length with increasing PEG concentration (Table 6). Pelin showed the greatest average stem length (48.91 mm), whereas Şebnem had the shortest (24.61 mm). These findings highlight the significant influence of both cultivar and PEG concentration on stem development.

In support of these findings, Ayaz et al. [2015] induced artificial drought stress using five PEG-6000 concentrations in two tomato cultivars (Nagina and 17905) and reported that, especially under 8% PEG treatment, seed germination in Nagina dropped to 60%. Additionally, phenotypic traits such as shoot length, leaf length, leaf number, and leaf area decreased significantly along increasing PEG levels. Similarly, Dolgun and Çifci [2018] investigated the effects of drought stress on germination and early seedling development in wheat varieties using four levels of PEG-induced stress (2.5, 5.0, 7.5, and 10.0 bars) and distilled water as the control. Morphological parameters including germination rate, vigor index, root and seedling length, and biomass were significantly suppressed by increasing drought severity. Tran et al. [2020] studied the effects of drought stress induced by mannitol (35 g L<sup>-1</sup> in

1/2 MS medium) and heat pre-treatment (45 °C for 120 minutes) on *in vitro* shoot development in *Solanum lycopersicum* L. Their results revealed that shoot length, leaf number, and total leaf area were reduced by nearly 50% compared to the control, emphasizing the substantial limiting effects of osmotic stress on plant development. In our study, stem elongation was highly sensitive to PEG-induced osmotic stress, with substantial reductions observed even at moderate stress levels. Among the cultivars evaluated, Pelin maintained the greatest stem length, suggesting a higher capacity to sustain cell expansion under limited water availability and, consequently, comparatively greater drought tolerance.

The highest average root length was recorded in the control (62.37 mm), while the shortest was observed in the 6% PEG medium (10.75 mm); see Table 7. In terms of cultivar, Pelin exhibited the longest average root length (51.92 mm), whereas Şebnem showed the shortest (27.99 mm). These results indicate that root development was significantly influenced by both variety and PEG concentration.

Kumar et al. [2017] reported that increasing PEG concentration proportionally reduced root growth, highlighting the critical role of the root system in plant survival under drought stress. They suggested that changes in root development may reflect the level of drought tolerance, as drought-tolerant genotypes typically possess more extensive root systems. Similarly, Wickramasinghe and Seran [2019] assessed the effects of PEG at 30, 60, and 90 g L<sup>-1</sup> on germination and seedling development of the tomato cultivar KC-1 in *in vitro* conditions. Their results showed a significant negative correlation ( $p < 0.0001$ ) between PEG concentration and all morpho-physiological traits, including shoot and root length, fresh and dry weight, and chlorophyll a, b, and total chlorophyll content. In the control group, after 4 weeks of culture, shoot and root lengths were 2.88 and 1.67 cm, respectively, while fresh and dry weights were 60.37 and 8.93 mg, respectively, and total chlorophyll content was 1.86 mg g<sup>-1</sup>. Clearly, the 60 and 90 g L<sup>-1</sup> PEG treatments restricted germination and seedling development. Bouchyoua et al. [2024] aimed to identify drought-tolerant *Brassica napus* L. genotypes under *in vitro* conditions using PEG-6000 to simulate osmotic stress at three levels (-0.7, -0.9, -1.1 MPa). Parameters such as germination percentage (GP), germination rate (GR), mean germination time (MGT), root length (RL), shoot length (SL), root-shoot ratio (RSR), seedling vigor index (SVI), and elongation rates (SER, RER) were evaluated. Results indicated significant effects of genotype, drought level, and their interaction on all parameters. Under severe drought, only mean germination time and root-shoot ratio increased by 56% and 76%, respectively, while reductions of 53–96% in other measured parameters were recorded. These findings confirm that root elongation is highly sensitive to PEG-induced osmotic stress, with severe reductions occurring as water availability declines. The superior root length of cultivar 1 suggests a greater capacity for root system maintenance under drought-like conditions, indicating a potential advantage for soil moisture foraging and overall drought resilience.

**Table 7.** Root length (mm) of different squash cultivars in PEG supplemented media

PEG (%)	Cultivar			Medium average
	Pelin	Nazlı	Şebnem	
0	68.92a	64.22b	53.98c	62.37A
2	57.18c	40.98e	32.52f	43.55B
4	49.34d	33.93f	25.48g	36.25C
6	32.26f	–	–	10.75D
Cultivar average	51.92A	37.78B	27.99C	×

LSD<sub>cultivar</sub> = 2.115\*\*\* LSD<sub>medium</sub> = 2.442\*\*\* LSD<sub>cultivarxmedium</sub> = 4.229\*\*\*

<sup>1</sup> statistical differences between the averages shown in separate letters in the same column were found to be significant

<sup>2</sup> \*\*\* $p \leq 0.001$

DPPH based antioxidant capacity measured in stem tissues of *in vitro* grown squash cultivars exposed to PEG is summarized in Table 8. Both the main effects (cultivar and PEG level) and the cultivar × medium interaction were statistically significant, indicating that antioxidant capacity varied according to genotype and its response to osmotic stress. When averaged across treatments, Pelin showed the highest antioxidant capacity (2.23 µmol TE g<sup>-1</sup> DW), whereas Şebnem had the lowest (1.46 µmol TE g<sup>-1</sup> DW). Across media, the PEG-free control yielded the greatest mean antioxidant capacity (2.93 µmol TE g<sup>-1</sup> DW).

For example, Popović et al. [2016], reported that PEG 6000-induced water stress elicited marked genotype-specific variation in antioxidant capacity and polyphenol metabolism, with certain genotypes maintaining or enhancing DPPH radical-scavenging activity through increased activation of the phenylpropanoid pathway. Similarly, our significant cultivar × medium interaction suggests that the ability to maintain stem antioxidant capacity under PEG differs among squash cultivars, supporting the use of DPPH-based antioxidant capacity as a complementary indicator of drought-related stress tolerance in this study.

**Table 8.** Determination of antioxidant capacity in shoots of different squash varieties grown in PEG containing nutrient media by the DPPH method ( $\mu\text{mol TE g}^{-1} \text{DW}$ )

PEG (%)	Cultivar			Medium avarege
	Pelin	Nazlı	Şebnem	
0	2.85b	2.72c	3.21a	2.93A
2	3.10b	2.76bc	1.38f	2.41B
4	1.64d	1.50e	1.23g	1.46C
6	1.33fg	–	–	0.44D
Cultivar average	2.23A	1.74B	1.46C	×
LSD <sub>cultivar</sub> = 0.056*** LSD <sub>medium</sub> = 0.065*** LSD <sub>cultivarxmedium</sub> = 0.112***				

<sup>1</sup> statistical differences between the averages shown in separate letters in the same column were found to be significant

<sup>2</sup>\*\*\* $p \leq 0.001$

Table 9 presents the total phenolic content measured in the stems of different squash cultivars grown in nutrient media containing various concentrations of PEG. Statistical evaluations showed that cultivar, media, and the cultivar × media interaction were all significant factors. Among media, the highest total phenolic content was recorded in the control medium (0%) as 1.27 mg GAE 100 g<sup>-1</sup> DW. Ahmad et al. [2020] reported that PEG-induced drought stress substantially increased phenolic compound accumulation in *Stevia rebaudiana*, with the highest total phenolic content occurring under the 4% PEG treatment. This observation suggests that *Stevia* enhances phenolic biosynthesis as a protective strategy against ROS generated under water-deficit conditions. In contrast, our findings revealed an opposite pattern in squash cultivars, where increasing PEG concentrations resulted in a gradual and marked reduction in total phenolic content. The highest TPC value was recorded in the control treatment (1.82 mg GAE 100 g<sup>-1</sup> DW in Pelin), while phenolic levels steadily declined with rising PEG concentrations, reaching their minimum under the 6% PEG treatment. Collectively, these contrasting outcomes indicate that phenolic compound production is strongly dependent on species-specific stress response mechanisms. Whereas PEG functions as an elicitor that stimulates phenolic accumulation in *Stevia*, the same osmotic stress appears to suppress phenolic biosynthesis in squash, underscoring fundamental physiological and metabolic differences in how plant species cope with drought-induced oxidative stress.

**Table 9.** Determination of total phenolic content in the shoots of different squash varieties grown in PEG containing nutrient media (mg GAE 100 g<sup>-1</sup> DW)

PEG (%)	Cultivar			Medium avarege
	Pelin	Nazlı	Şebnem	
0	1.82a	1.17b	0.81d	1.27A
2	1.13c	0.49g	0.42h	0.68B
4	0.55e	0.42h	0.28i	0.41C
6	0.51f	–	–	0.17D
Cultivar average	1.00A	0.52B	0.38C	×
LSD <sub>cultivar</sub> = 0.007*** LSD <sub>medium</sub> = 0.008*** LSD <sub>cultivarxmedium</sub> = 0.014***				

<sup>1</sup> statistical differences between the averages shown in separate letters in the same column were found to be significant

<sup>2</sup>\*\*\* $p \leq 0.001$

## CONCLUSION

It is an expected result that the highest values in all tested parameters were obtained in the PEG-free, (control) environment. This result confirms that the osmotic stress induced by PEG exerted a suppressive effect on both morphological and physiological traits. Especially, the reductions observed in shoot and root length, as well as in fresh and dry biomass with increasing PEG concentrations were directly proportional to the severity of the imposed stress. Among the cultivars evaluated, Pelin showed the best growth performance. These findings indicate that this cultivar can have a relatively higher level of adaptation and tolerance to drought stress. Its superior performance for both morphological parameters (shoot/root length, fresh and dry weight) and physiological parameters (antioxidant capacity, total phenolic content) highlights its potential as a promising candidate for future breeding studies aimed at enhancing drought resilience. The PEG treatments employed successfully simulated drought under *in vitro* conditions, thus enabling the effective assessment of drought tolerance among the tested genotypes. This supports the use of PEG-induced osmotic stress as a practical and reproducible method for evaluating plant responses to drought stress, particularly during early developmental stages. Transferring drought tolerance studies to tissue culture conditions to get preliminary idea will be advantageous in terms of time and cost.

## AUTHORSHIP CONTRIBUTION STATEMENT

B.E.: setting up experiments and performing all analyses, statistical analyses and writing; E.K.: data analysis-review, editing and writing; Ş.D.: all analyses, A.K.E.: antioxidant analyses, H.T.: data analysis-review, editing and writing; G.B.: designing of the study, controlling of all analysis and measurements, supervising and writing.

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## DATA AVAILABILITY

All data of this work are available in this paper.

## CONFLICT OF INTEREST

All authors have nothing to disclose.

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