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Priming the seeds of two mung bean (*Vigna radiata* L.) varieties with ascorbic acid under drought stress

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Abstract

Objective: Drought stress has posed a challenge to agriculture worldwide. For this reason, researchers are striving to improve agricultural production using various methods. In the present study, seed priming of Turkish and Iraqi mung bean (*Vigna radiata* L.) varieties with treatments of 50 and 100 mg/l of ascorbic acid was conducted to improve the biochemical performance and growth of this plant's seedlings under drought-stress conditions.

Methods: Seeds of both varieties were treated with ascorbic acid for 24 hours and dried after 48 hours. Drought stress was induced using polyethylene glycol 6000 (PEG 6000) at 0, -2, -4, and -8 bars. Fifteen seeds were placed in five replicates in a Petri dish and kept in a clean room for 10 days. The germination percentage, fresh weight, hypocotyl length, radicle length, leaf area, relative leaf water content, photosynthetic pigments, proline, total phenols and flavonoids, total sugars, total proteins, and antioxidant capacity were measured.

Results: The results showed that with the increase in drought stress levels, the fresh weight, hypocotyl length, radicle length, leaf area, germination percentage, and relative water content of the leaves decreased. In contrast, the levels of proline, phenols, flavonoids, sugars, proteins, and antioxidant capacity increased. The ascorbic acid treatment could not improve the growth characteristics and RWC compared to the control as the drought level increased. However, at the PEG of -2 bar, the 50 mg/l ascorbic acid did not significantly differ from the control for the hypocotyl length in both varieties. Also, in the Iraqi variety, both ascorbic acid concentrations had significantly higher seedling weight than the control at the -2 bar PEG. Furthermore, ascorbic acid alleviated the effect of drought stress in some cases concerning plant pigments. In addition, 100 mg/l of ascorbic acid was better than the 50 mg/l concentration concerning plant pigments, total sugars, total proteins, proline, total phenols and flavonoids, and antioxidant capacity in most cases. The Turkish variety performed better than the Iraqi genotype in most instances.

Conclusion: Drought stress decreased the growth characteristics and relative water content of the leaves of the Iraqi and Turkish mung bean varieties but, increased their biochemical compounds and antioxidant capacity. Except for some cases, applying the ascorbic acid treatment was not effective in alleviating the adverse effects of drought stress concerning growth characteristics and RWC.

However, in terms of plant pigments. Ascorbic acid reduced the negative effects of drought stress in some cases. Also, 100 mg/l ascorbic acid outperformed the 50 mg/l dosage concerning plant pigments, biochemical compounds, and antioxidant capacity of the mung bean seedlings.

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Introduction

Legumes are the most extensive and third-largest flowering plants in the world, with 650 genera and 20,000 different species. The mung bean (*Vigna radiata* L.) is one of the most widely distributed types of legumes in the world, consumed as part of the human food chain The mung bean plant can significantly help alleviate hunger because it has a very high nutritional value. This plant is rich in beneficial chemical compounds, including polyphenols, amino acids, oligosaccharides, vitamin C, vitamin B6, vitamin K, riboflavin, thiamine, niacin, magnesium, phosphorus, iron, and fiber. Mung beans have a high antioxidant capacity and can be used as medicine to treat heatstroke, heart conditions, digestive issues, cancer, skin problems, and Alzheimer's disease (Uppalwar *et al.* 2021).

Climate and weather changes in the world, have affected various ecosystems and led to widespread droughts (Naumann *et al.* 2018). Drought can reduce seed production by affecting the germination stage. Additionally, changes in enzymatic activity, reduced water absorption, and disruption in nutrient transport negatively affect cell division, leading to reduced seedling growth. Drought stress reduces the growth period of plants because it accelerates the transition from the vegetative to the reproductive stage. Therefore, seedlings that have grown in a shorter period have poorer performance (Farooq *et al.* 2012). Also, the reduction of water levels around the roots can lead to a decrease in the rate of nutrient absorption and distribution by the roots into the cells because the water potential around the roots and soil-water potential are diminished (He and Dijkstra 2014). During drought stress, the synthesis of free radicals increases, and the performance of photosynthetic systems decreases because free radicals have a high ability to bind to photosynthetic enzymes, causing significant disruptions in the functioning of photosynthetic systems. In addition, these radicals are highly toxic to cells and affect the plant's metabolism by binding to proteins and other plant compounds (Farooq *et al.* 2012). Plants can undergo extensive changes at the morphological and biochemical levels to cope with the stress effects (Aghajanzadeh *et al.* 2021). Some plants enter a natural dormancy during drought and resume their growth after the stress conditions pass. Also, some species complete their life cycle and endure stressful conditions before the onset of the dry seasons (Farooq *et al.* 2009). Plants can prevent water wastage by reducing biomass. In addition, making extensive changes to the root structure, such as root formation and root elongation, increases the rate of water absorption from the soil. One of the other important mechanisms for plants to increase resistance and survival in dry conditions is altering the plant's leaf system, as smaller leaves can survive with less water. Also, species exposed to drought stress can prevent water loss through transpiration by changing the orientation of leaves, altering the stomata, and changing the number of stomata (Yang *et al.* 2021).

Plants, with the help of a series of compatible salts such as mineral salts, organic compounds, plant hormones, soluble sugars, proteins, and secondary metabolites, maintain turgor pressure for stomatal activity and, by reducing water potential, lead to osmotic regulation and increased water absorption by the roots. Additionally, plants combat free radicals by increasing the synthesis of antioxidant compounds and protecting themselves from stress conditions (Taiz and Zeiger 2010).

It has been reported that ascorbic acid plays a crucial role in eliminating oxidative stress and free radicals in plants and can protect the plant's photosynthesis system from damage through its antioxidant activities (Shafiq *et al.* 2014). Vitamin C plays many roles in the biosynthesis of protective plant pigments such as xanthophylls. Vitamin C can play a role in the synthesis of various hormones such as ethylene and gibberellin and is used as a substrate for the enzymes involved in the synthesis of these hormones (Davey *et al.* 2000). One of the most important roles of vitamin C in plants is its high ability to react with reactive oxygen species (ROS) and free radicals. This compound can eliminate cell toxicity by forming chemical bonds with free radicals and ROS (Shafiq *et al.* 2014).

Germination and seedling establishment are the first steps in the plants' development processes, and various studies are always striving to enhance germination speed and improve seedling performance by strengthening seeds in the early stages of germination. This set of processes, which are carried out in the early stages of germination and induce germination in seeds, is called priming, which includes hydro-priming, osmo-priming, halo-priming, chemical-priming, and bio-priming. Priming improves seedling establishment, germination speed, resistance, crop yield, and food security under unstable conditions (Zulfiqar 2021).

A laboratory study was conducted to investigate the priming of mung bean seeds with carrot root extract, garlic, and ascorbic acid under drought conditions, which resulted in a decrease in sugar content and an increase in protein content in the seedlings. Additionally, the examination of seeds treated with carrot root extract, garlic, and ascorbic acid showed that these compounds reduced the effects of drought in mung bean seedlings (Kasim *et al.* 2019). Also, the effects of seed priming of oat plants with ascorbic acid and alpha-tocopherol under drought conditions were investigated by El-Beltagi *et al.* (2022). The results showed that drought stress caused a decrease in the levels of sugars, proline, photosynthetic pigments, and proteins and an increase in antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase, and oxidase in the leaves.

Considering the climate changes and the increase in drought around the world, scientists are striving to find solutions to improve agricultural plants' yields. Therefore, given the effects of ascorbic acid in reducing oxidative damage in plants, the present study aimed to investigate the effects of ascorbic acid treatment on different varieties of mung beans during the germination stage.

Materials and Methods

Treatments

Seeds of Iraqi and Turkish mung bean varieties were obtained from Baghdad City, Iraq, and placed in a sodium hypochlorite solution for 5 min to eliminate pathogens. To eliminate the sterile seeds, the mung bean seeds were immersed in distilled water, and the seeds that settled at the bottom of the container were considered fertile, while the seeds floating on the water surface were considered sterile. For priming, 50 and 100 mg/l concentrations of ascorbic acid were prepared, and the seeds were placed in the solutions for 24 hours and then dried at 25 ^{o}C for 48 hours. Drought stress was induced using polyethylene glycol 6000 (PEG 6000), and different levels of drought stress, including 0, -2, -4, and -8 bars, were generated from the following relationship (Michel and Kaufmann 1973):

$$\Psi s = -(1.18 \times 10^{-2}) \times C - (1.18 \times 10^{-4}) \times C2 + (2.67 \times 10^{-4}) \times CT + (8.39 \times 10^{-7}) \times C2T$$

Where, Ψs = Osmotic potential of PEG 6000, C = The amount of polyethylene glycol in 1 liter of water, T = The temperature in degrees Celsius.

Finally, 15 seeds primed with ascorbic acid were added to Petri dishes containing 5 ml of PEG solution in 5 replicates, and the samples were kept in a clean room at 25 ^{o}C with a 16-hour light and 8-hour dark cycle for 10 days. The fresh weight of the seedlings was measured using a digital scale, and the radicle length, aerial parts, and leaf area were measured using the Digimizer software. Also, the germinated seeds were counted daily and then the germination percentage was calculated. Finally, the samples were transferred to the refrigerator for the measurement of metabolic characteristics (Figure 1).

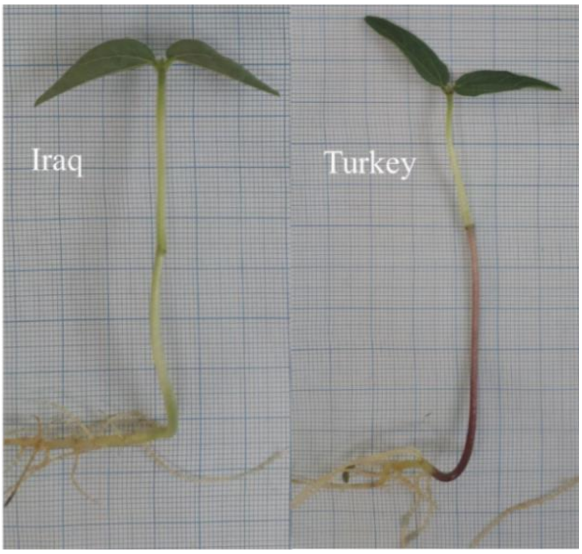


Figure 1. Mung bean seedlings of Iraqi and Turkish cultivars.

Relative water content

To examine the relative water content (RWC), the fresh weight of the plant tissue was measured immediately after harvesting. The plant tissues were also immersed in distilled water at 4 ^{o}C for 24 hours, and their weight was measured again. Additionally, the plant tissues were placed in an oven to dry, and their weight was measured. Finally, RWC was calculated using the following equation (Ritchie *et al.* 1990):

$$\mathrm{RWC} = \frac{\mathrm{Wf} - \mathrm{Wd}}{\mathrm{Wt} - \mathrm{Wd}} \times 100$$

Where, Wf = Fresh weight, Wt = Turgid weight, Wd = Dry weight.

Proline

To measure the proline in the aerial parts of the mung bean seedlings, they were weighed and ground with 3% sulfosalicylic acid in a mortar. The extracted and centrifuged extract, along with the ninhydrin reagent and pure acetic acid, was added to the supernatant and placed in a water bath for one hour. Finally, by adding toluene, the upper solution was separated and the absorbance was read at a wavelength of 520 nm. In the end, the amount of proline was expressed in terms of μmol^{-1} FW using the obtained standard curve (Bates 1973).

Photosynthetic pigments

To extract photosynthetic pigments, 0.05 gr of the aerial parts were ground with 3 ml of 80% methanol in a mortar. The extract was then filtered with filter paper and its absorbance was read with a spectrophotometer at wavelengths of 470, 652, and 665 nm. In the end, the amounts of chlorophyll a, chlorophyll b, and carotenoids were calculated using the following formulae in mg.g^{-1}FW (Arnon 1949):

$$\text{Chla} = ((16.82 \times \text{A665}) - (9.28 \times \text{A652})) \times (\text{V} \times 1000\text{W})$$

$$\text{Chlb} = ((36.92 \times \text{A652}) - (16.54 \times \text{A665})) \times (\text{V} \times 1000\text{W})$$

$$\text{CAR} = ((1000 \times \text{A470}) - (1.91 \times \text{Chla})\ (95.15 \times \text{Chlb}))/225 \times (\text{V} \times 1000\text{W})$$

Where, A= Absorbance, V= Extract volume, W= Leaf weight.

Total phenols and flavonoids

To extract total phenols and total flavonoids, 0.05 gr of the aerial parts were ground with 3 ml of 80% methanol in a mortar. Then, it was placed in the ultrasonic device for 10 min. At the end, the samples were placed in a centrifuge at a speed of 4000 RPM for 5 min, and then the supernatant was separated. The amount of total phenols was measured using the Folin-Ciocalteu colorimetric method. A 200 µl of extract was mixed with 1000 µl of 10% Folin reagent, and after five min, 800 µl of 7.5% sodium carbonate was added to it. After a 1 hour stop in the dark, the absorbance of the samples at a wavelength of 765 nm was read on the spectrophotometer. The total phenol content was calculated using the standard curve prepared from different concentrations of gallic acid and expressed as mg^{-1} FW (Ainsworth and Gillespie 2007). To measure the total flavonoids, the aluminum chloride colorimetric method was used. A 200 µl of the extract was mixed with 40 µl of 10% aluminum chloride, 40 µl of one molar potassium acetate, 600 µl of 80% methanol, and 1120 µl of distilled water, and after 30 min, the absorbance was read at a wavelength of 415 nm. Finally, using the standard curve obtained from different concentrations of rutin, the total flavonoid content was expressed in mg.g^{-1} FW (Akkol *et al.* 2008).

Total sugars

To extract the sugars, 0.05 gr of aerial parts were mixed with 3 ml of 100 mM potassium phosphate buffer (pH 7) and centrifuged at 4000 RPM for 5 min. The measurement of sugars was performed using the phenol-sulfuric acid method. The extract obtained was mixed with 200 µl of total phenols at 5%, and then 1000 µl of concentrated sulfuric acid was added to it. The samples were then mixed with a vortex device and incubated for 30 min at 38 ^{o}C. The absorbance of the samples at a wavelength of 485 nm was measured using a spectrophotometer. Finally, the sugar content was expressed as glucose concentration in mg.g^{-1}FW using the standard curve obtained from different glucose concentrations (Dubois *et al.* 1956).

Total proteins

A 0.05 gr of fresh leaves was ground with 3 ml of 50 mM potassium phosphate buffer at pH 7 and centrifuged at 12,000 RPM for 10 min at 4 ^{o}C. To measure the proteins, 100 µl of the extract was mixed with 1000 µl of Bradford solution, and after 10 min, its absorbance was measured at a wavelength of 595 nm. In the end, the total protein content was calculated using the standard curve obtained from different concentrations of bovine serum albumin and expressed as mg.g^{-1} FW (Bradford 1976).

Antioxidant capacity

A 0.05 gr of aerial parts was ground with 3 ml of 80% methanol in a mortar and placed in an ultrasonic device for 30 min. Then, the samples were centrifuged at a speed of 4000 RPM for five min, and the supernatant was separated. The antioxidant capacity was investigated using the ABTS cation radical scavenging method. A 40 mM potassium persulfate with a volume of 88 µl was added to 5 ml of a 7 mM ABTS solution ((3-ethylbenzothiazoline-6-sulfonic acid)2,2'-azino-bis)) and left in the dark in the laboratory for 16 hours. To determine the antioxidant capacity, 200 µl of the extract was added to the diluted ABTS solution, and after 10 min, their absorbance was measured at a wavelength of 734 nm. After calculating the percentage of inhibition, the ascorbic acid equivalent antioxidant capacity (AEAC) was expressed as mg.g^{-1} FW, using the standard curve obtained from different concentrations of ascorbic acid (Re *et al.* 1999; Matsui *et al.* 2011).

$$\mathrm{I} = \frac{(\mathrm{A0} - \mathrm{As})}{\mathrm{A0}} \times 100$$

Where, I= Percentage of inhibition, A= Control absorbance rate, AS= Sample absorption rate.

Statistical analysis

The results were statistically analyzed at a 5% significance level using a one-way analysis of variance and Duncan's multi-range test with SPSS 25 software. The charts were drawn using Excel 2013 software.

Results and Discussion

Growth characteristics

The results showed that increasing the level of drought stress led to a reduction in the germination percentage, length of hypocotyl and radicle, fresh weight, and leaf area in Turkish and Iraqi varieties of mung bean seedlings. The growth characteristics of the Turkish variety were higher compared to the Iraqi variety (Table 1). The adverse effect of drought on growth characteristics in this study was consistent with the research of others (Razaji *et al.* 2014; De Lima Nunes *et al.* 2020). Water stress is one of the environmental factors affecting the growth and performance of plants worldwide. For this reason, improving crop yields under stress conditions has always been of great importance (Akkuzu *et al.* 2013).

Germination is the most important part of the plant life cycle because it plays a role in the establishment of seedlings in the soil. During seed germination, the seeds absorb sufficient water and, through biochemical activities, expand the root system, preparing the seedlings for the vegetative and reproductive stages (Foschi *et al.* 2023). Drought stress reduces the water content of the plant and cell turgor and slows the plant's growth rate. In addition, the formation of free radicals during drought stress can affect cell divisions in plants and cause the cessation of cell elongation and leaf surface divisions. Drought reduces carbohydrate synthesis by damaging photosynthetic systems, leading to decreased plant growth. Additionally, drought stress disrupts the activity of enzymes and damages the plant membranes (Taiz and Zeiger 2010). The reduction in growth during drought stress is a natural occurrence, and plants reduce their water needs by decreasing weight, leaf area, and cell divisions, thereby coping with the stress conditions (Yang *et al.* 2021).

Ascorbic acid treatment could not improve the hypocotyl length, radicle length, leaf area, germination percentage, and fresh weight of mung bean seedlings compared to the control by increasing the drought level. However, at -2 bar PEG, there was no significant difference between the 50 mg/l ascorbic acid and the control for the hypocotyl length in Iraqi and Turkish varieties. Also, at -2 bar PEG, both ascorbic acid concentrations had significantly higher seedling weight than the control in the Iraqi variety. Furthermore, the treatment with 100 mg/l of ascorbic acid showed statistically better performance compared to the treatment with 50 mg/l in terms of radicle length in the Turkish variety, and for hypocotyl length, leaf area, and seedling weight in both varieties at some PEG levels (Table 1).

In a study by Razaji *et al.* (2014), priming of oilseed rape seeds with ascorbic acid under four levels of drought stress (0, -4, -6, -8, and -12 bar PEG 6000) showed that increasing in stress level caused a decrease in germination percentage, seedling fresh and dry weight, hypocotyl and radicle length and vigor index, but ascorbic acid treatments improved seedling growth. Also, two genotypes of *Vigna unguiculata* L. were treated with ascorbic acid under drought stress and results showed that 0.5 and 0.75 mM ascorbic acid enabled vigorous seedling development in both genotypes (De lima Nunes *et al.* 2020). Kumar *et al.* (2011) studied the effect of 50 µM ascorbic acid concentration on mung bean (*Vigna radiata* L.) under temperature stress in a hydroponic culture. They observed that

Table 1. The effect of ascorbic acid on the germination and seedling traits of Iraqi and Turkish mung bean cultivars under drought stress.

PEG (bars)	AA (mg/l)	Gr (%)	Hypocotyl length (mm)	Radicle length (mm)	Leaf area (mm^2)	Seedling fresh weight (g)
Iraqi						
0	0	92±2.9^{a}	73.69±2.2^{c}	44.08±4.2^{e}	152±6.8^{b}	0.28±0.016^{c}
-2	50	72±10.9^{b}	73.02±3.1^{c}	33.18±2.7^{fg}	89.88±4.7^{e}	0.30±0.001^{b}
	100	75±8.6^{b}	38.49±2.1^{e}	37.06±3.3^{f}	129.25±5.5^{c}	0.33±0.018^{a}
-4	50	51±7.6^{c}	38.30±2.5^{e}	25.64±2.3^{h}	79.3±1.6^{f}	0.21±0.009^{e}
	100	55±2.9^{c}	39.23±1.8^{e}	29.74±1.7^{gh}	86.19±5.4^{ef}	0.27±0.013^{c}
-8	50	25±5.5^{d}	13.23±0.5^{g}	19.86±1.6^{i}	24.3±1.3^{i}	0.10±0.005^{h}
	100	28±5.5^{d}	15.25±2.0^{g}	20.46±2.0^{i}	33.36±3.9^{h}	0.11±0.010^{h}
Turkish						
0	0 mg/l	96±3.6^{a}	107.15±5.0^{a}	84.37±6.5^{a}	159.92±4.9^{a}	0.33±0.007^{a}
-2	50 mg/l	76±7.6^{b}	107.64±0.9^{a}	63.70±4.4^{c}	131.33±1.4^{c}	0.30±0.005^{b}
	100 mg/l	76±5.9^{b}	107.88±1.4^{a}	70.45±4.0^{b}	136.37±5.1^{c}	0.30±0.008^{b}
-4	50 mg/l	53±4.7^{c}	88.23±0.3^{b}	58.22±1.2^{d}	91.84±5.2^{de}	0.26±0.007^{d}
	100 mg/l	56±3.6^{c}	85.96±1.4^{b}	67.94±5.7^{bc}	98.42±4.9^{d}	0.27±0.009^{c}
-8	50 mg/l	27±6.6^{d}	34.32±4.4^{f}	44.44±2.7^{e}	33.75±3.8^{h}	0.14±0.011^{g}
	100 mg/l	30±5.4^{d}	50.72±3.6^{d}	48.84±2.5^{e}	48.97±1.6^{g}	0.17±0.011^{f}

Means with different letter(s) in each column are significantly different at $p \leq 0.05$ based on Duncan's multiple range test; AA: Ascorbic acid, Gr: Germination.

the application of ascorbic acid reduced the damage caused by the temperature stress on germination and seedling growth. Ascorbic acid plays an important role in enhancing cell division, developing cells, and combating free radicals (Saedipour 2016). Ascorbic acid directly reacts with hydroxyl radicals, superoxide, and singlet oxygen. Therefore, the improvement in seedling traits can be attributed to the antioxidant properties of ascorbic acid (Shafiq *et al.* 2014; Davey *et al.* 2000; Smirnoff 2000).

Plant pigments

Analyzing the data of plant pigments in the seedlings of Turkish and Iraqi mung bean varieties showed that increasing levels of drought stress led to a decrease in the amounts of chlorophylls a, b, and carotenoids. However, Turkish varieties showed better performance compared to Iraqi genotypes (Figure 2).

The adverse effects of drought stress on photosynthesis pigments have also been shown in other crops (Farooq *et al.* 2013; Hussein and Khursheed 2014; Kasim *et al.* 2019; MacDonald *et al.* 2022; Gharred *et al.* 2023). Photosynthetic pigments in plants are of great importance, and chlorophylls are the most important organic molecules on Earth because they drive photosynthesis. Carotenoids, as photosynthetic pigments, can ensure the survival of plants by protecting the photosynthetic systems (Davies 2018). Drought stress reduces water in the cell, causes the loss of stability in chlorophyll, protein, and lipid complexes, and decreases the formation of plastids and chlorophylls. In addition,

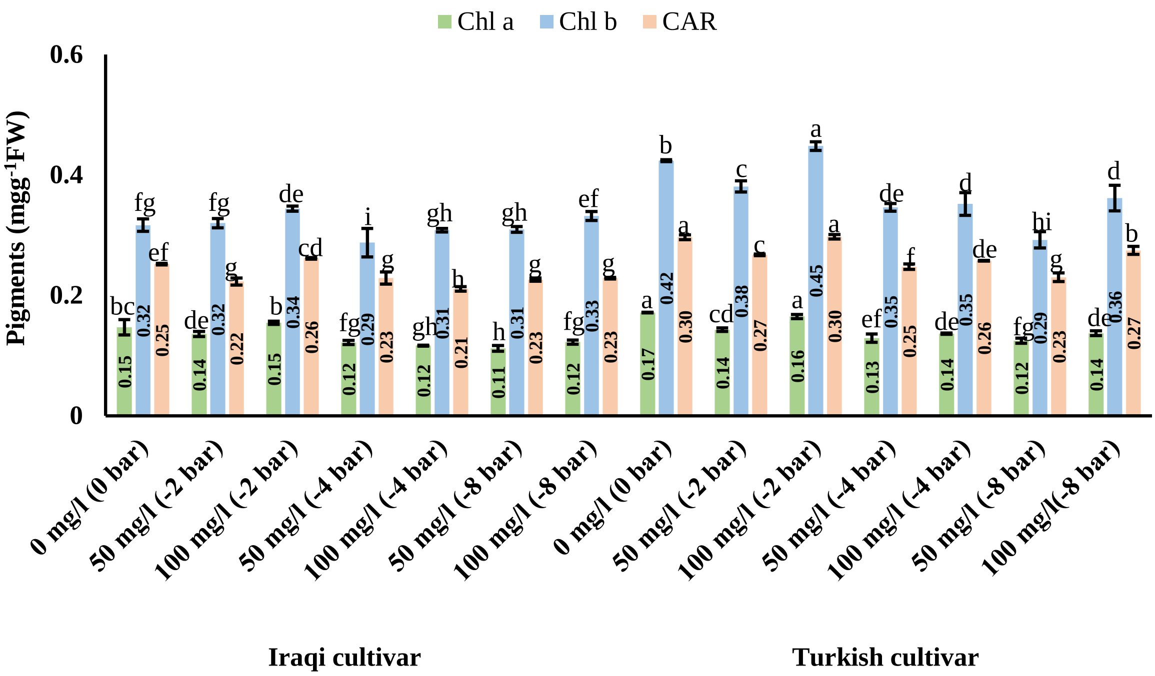


Figure 2. Pigments in Iraqi and Turkish cultivars of mung bean as affected by the treatment with ascorbic acid and drought stress; Chl: Chlorophyll, CAR: Carotenoid, Iraq: 0 PEG + 0 ascorbic acid treatment on the Iraqi cultivar, Turk: 0 PEG + 0 ascorbic acid treatment on the Turkish cultivar; Means with different letter(s) in each trait are significantly different at $p \leq 0.05$ based on Duncan's multiple range test.

drought stress damages photosynthetic systems by forming free radicals and ROS, reducing photosynthetic performance (Farooq *et al.* 2012). Research has shown that drought stress can increase the expression of genes encoding chlorophyllase enzymes (Yang *et al.* 2016). It has also been determined that the decrease in carotenoid levels in plants is due to the degradation of beta-carotene and the increased synthesis of zeaxanthins (Sultana *et al.* 1999).

Ascorbic acid treatments were able to alleviate the negative effects of drought stress in some cases concerning plant pigments. Also, the treatment with 100 mg/l of ascorbic acid showed better performance compared to the treatment with 50 mg/l of ascorbic acid, leading to a significant increase in the levels of photosynthetic pigments at different drought levels in most cases (Figure 2).

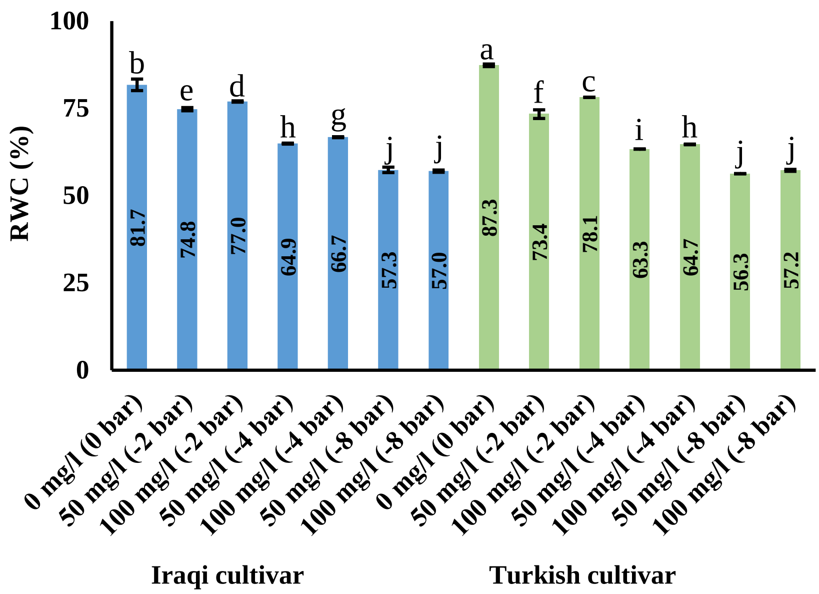
In a study on mung bean, the use of 20 mM ascorbic acid was beneficial under water stress conditions and improved the amount of photosynthetic pigments (Jahanbakhshi *et al.* 2024). According to MacDonald *et al.* (2022), the priming of *Brassica oleracea* seeds with ascorbic acid (0, 1, and 10 ppm) increased chlorophyll content under drought stress. In a study on wheat, seeds of two cultivars were primed for 10 hours with two methods of hydro-priming and osmo-priming by ascorbic acid and sown in pots with 70 and 35% of water-holding capacity. The results showed that drought stress decreased chlorophyll contents while osmo-priming with ascorbic acid increased chlorophyll contents more than hydro-priming (Farooq *et al.* 2013). Hussein and Khursheed (2014) also reported the alleviation of drought-stress effects in wheat seedlings by spraying with 200 ppm ascorbic acid. Ascorbic acid can enhance the stability of the enzymes involved in chlorophyll metabolism, and has a very high antioxidant property to eliminate free radicals and prevent the degradation of chlorophyll and carotenoids (Shafiq *et al.* 2014; Davey *et al.* 2000; Smirnoff 2000).

Relative water content and proline

Increasing the level of drought stress led to a decrease in RWC in the leaves of Turkish and Iraqi mung bean seedlings. The highest RWC in the leaves was observed in the control treatment seedlings (Figure 3). The results of the present study were consistent with the other research on *Brassica oleracea* (MacDonald *et al.* 2022) and *Medicago polymorpha* (Gharred *et al.* 2023). High relative water content maintains cell turgor and improves growth. The reduction of water content in plant tissue is due to the decrease in water potential in the rhizosphere, which prevents sufficient water from entering the plant (Rao and Mendham 1991).

The ascorbic acid treatments could not improve RWC, however, the Turkish cultivar showed better performance compared to the Iraqi cultivar (Figure 3). On the other hand, some studies have shown the beneficial effects of seed priming with ascorbic acid under drought stress and salinity stresses in *Medicago polymorpha* L. (Gharred *et al.* 2023) and under drought stress in *Brassica oleracea* (MacDonald *et al.* 2022). Ascorbic acid can influence metabolic pathways, the biosynthesis of primary and secondary compounds, and the regeneration of these compounds, and consequently, improves water absorption (Shafiq *et al.* 2014; Davey *et al.* 2000; Smirnoff 2000). However, some studies have shown that ascorbic acid alone cannot improve the growth of plants under drought stress, and the plant requires an increase in the antioxidant defense system's capacity with the help of other biochemical compounds (Zehra *et al.* 2013).

Drought stress increased the proline levels in the leaves of Turkish and Iraqi mung bean seedlings (Figure 3). Others have also shown an increase in proline content under drought stress in wheat (Farooq *et al.* 2013; Hussein and Khursheed 2014), alfalfa (Salemi *et al.* 2019), oilseed rape (Razaji *et al.* 2014), and *Medicago polymorpha* L. (Gharred *et al.* 2023. Proline is part of a group of osmo-protective compounds called osmolytes that play a role in osmotic stress. During drought stress, osmolytes play a role in cell turgor by accumulating in plant cells (Omari Alzahrani 2021). During drought stress, proline accumulates in the cytosol and stabilizes the structure of proteins. Proline, by forming hydrogen bonds, helps maintain membrane integrity (Hossain *et al.* 2019). Proline increases



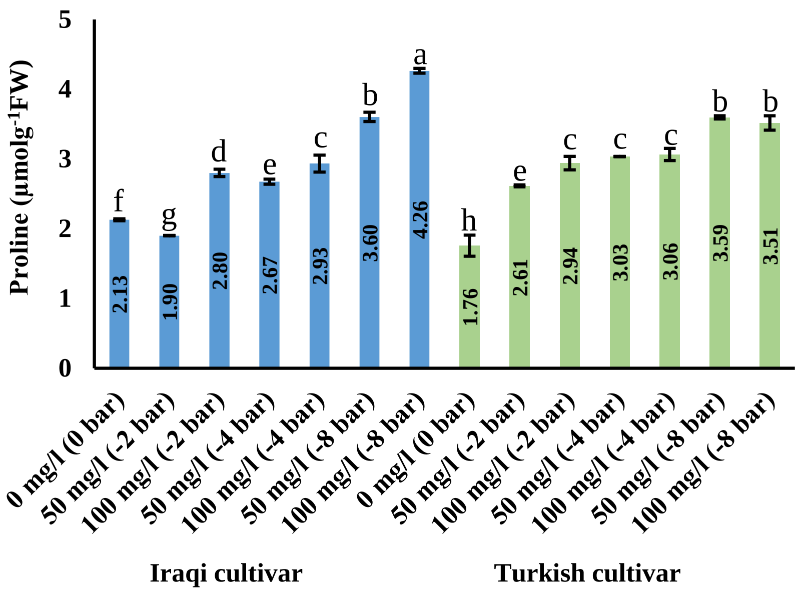


Figure 3. Relative water content (RWC) and proline in Iraqi and Turkish cultivars of mung bean as affected by the treatment with ascorbic acid and drought stress; Iraq: 0 PEG + 0 ascorbic acid treatment on the Iraqi cultivar, Turk: 0 PEG + 0 ascorbic acid treatment on the Turkish cultivar; Means with different letter(s) in each trait are significantly different at $p \leq 0.05$ based on Duncan's multiple range test.

the absorption potential and enhances water absorption in the cell. Additionally, proline is an antioxidant molecule that is effective in antioxidant defense against free radicals (Omari Alzahrani 2021).

The results showed that the treatment with 100 mg/l ascorbic acid performed better compared to the treatment with 50 mg/l ascorbic acid in most cases concerning the proline content (Figure 3). Ascorbic acid is a small but powerful molecule that plays an effective role in the enhancement of carbohydrates, proteins, and secondary metabolites such as proline in the cell (Shafiq *et al.* 2014; Davey *et al.* 2000; Smirnoff 2000).

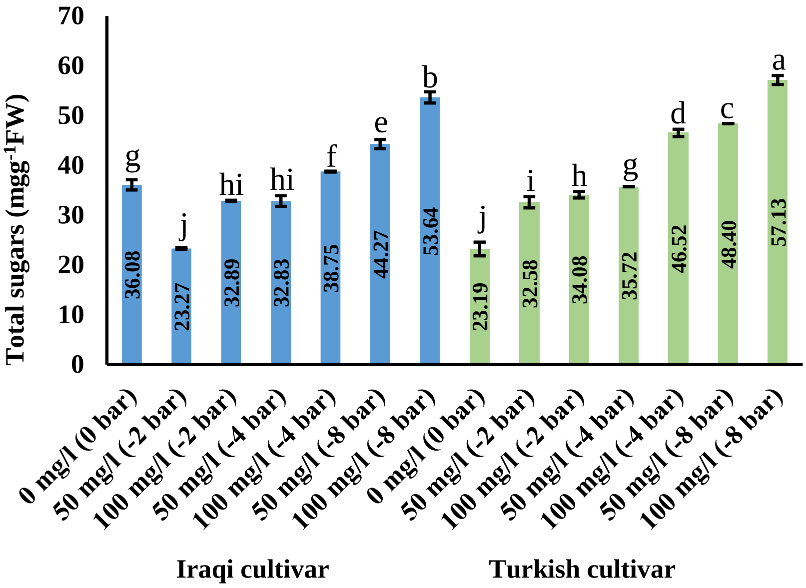
Total sugars and proteins

The results showed that increasing the drought stress level led to an increase in the total sugar and protein content in the seedlings of Turkish and Iraqi mung bean varieties. The highest levels of sugars and proteins were observed at the highest drought levels (Figure 4). Carbohydrates play a vital role in the synthesis of secondary metabolites in plants. Carbohydrates in plants act as energy sources and are used in the synthesis of starch, cellulose, amino acids, and fatty acids (Trouvelot *et al.* 2014). Plant proteins also play an effective role in signaling and increasing the resistance of plants to stress (Fatehi *et al.* 2012). Scientists believe that increasing the rate of photosynthetic electron transfer and stimulating pigment biosynthesis can enhance the synthesis of metabolites, carbohydrates, and proteins (Mutale-Joan *et al.* 2020).

The treatment with 100 mg/l of ascorbic acid performed better compared to the 50 mg/l treatment and resulted in a significant increase in total sugars and proteins under drought stress (Figure 4). Jahanbakhshi *et al.* (2024), also reported the positive effect of ascorbic acid treatments on mung bean protein under drought stress conditions. In a study, ascorbic acid spraying (200 ppm) enhanced proteins and total sugars of wheat seedlings under different drought levels (Hussein *et al.* 2014). According to Salemi *et al.* 2019), drought stress decreased proteins and total sugars, while ascorbic acid ameliorated the detrimental impacts of water stress in alfalfa. The use of ascorbic acid can increase protein content because ascorbic acid prevents the degradation and oxidation of protein structures by removing free radicals (Alayafi 2020). Osmolytes play a special role in osmotic regulation and improve the plant's resistance to stress conditions (Omari Alzahrani 2021). Ascorbic acid is recognized as a powerful molecule in the synthesis of soluble sugars and improving plant growth (Shafiq *et al.* 2014; Davey *et al.* 2000; Smirnoff 2000).

Total phenols and flavonoids

By examining the results, it was determined that the increase in drought stress levels led to an increase in the amount of phenolic compounds in the seedlings of Turkish and Iraqi mung bean varieties in most cases. Additionally, the Turkish variety seedlings showed better performance in terms of phenolic compounds compared to the Iraqi variety. The highest level of phenolic compounds was observed at the highest level of drought stress (Figure 5). Other scientists have also reported an increase in phenolic compounds under salinity stress in mung bean (Nawaz *et al.* 2021), and under



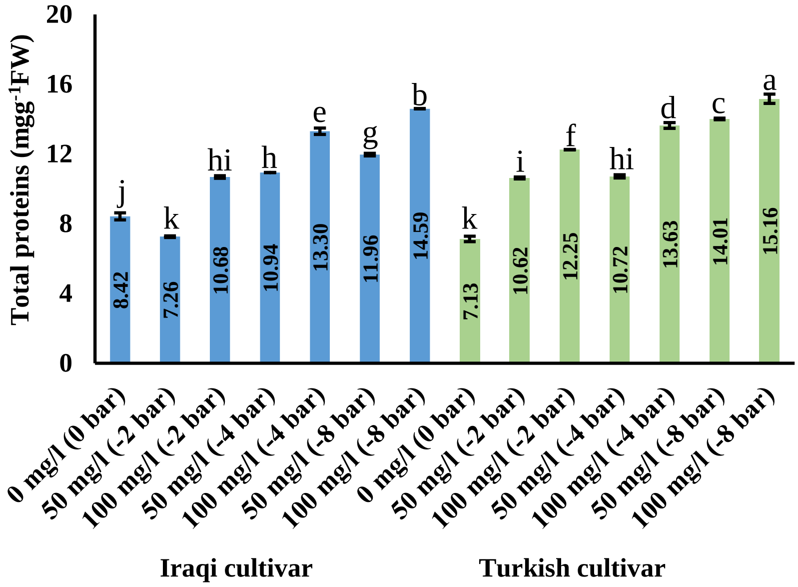


Figure 4. Total sugars and proteins in Iraqi and Turkish cultivars of mung bean as affected by the treatment with ascorbic acid and drought stress; Iraq: 0 PEG + 0 ascorbic acid treatment on the Iraqi cultivar, Turk: 0 PEG + 0 ascorbic acid treatment on the Turkish cultivar; Means with different letter(s) in each trait are significantly different at $p \leq 0.05$ based on Duncan's multiple range test.

drought stress in wheat (Farooq *et al.* 2013) and oat plants (El-Beltagi *et al.* 2022). Phenolic compounds have antioxidant properties and play an effective and preventive role under oxidative stress conditions in plants (Hemmaty *et al.* 2011; Alara *et al.* 2021Park *et al.* 2022).

The treatment with 100 mg/l ascorbic acid had higher phenolic compounds compared to the use of 50 mg/l ascorbic acid at different drought levels (Figure 5). Nawaz *et al.* (2021) showed the positive effects of salicylic acid and ascorbic acid under salinity stress in mung bean. In a study, the wheat

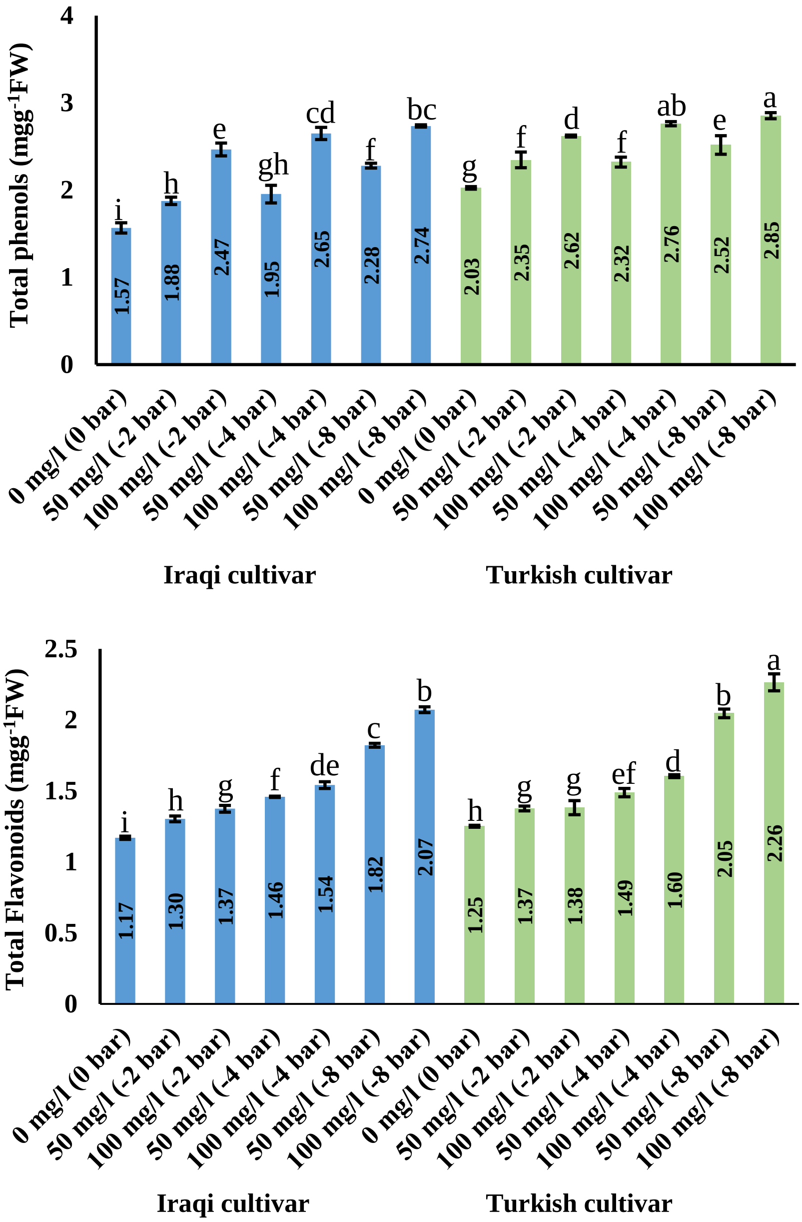


Figure 5. Total phenols and flavonoids in Iraqi and Turkish cultivars of mung bean as affected by the treatment with ascorbic acid and drought stress; Iraq: 0 PEG + 0 ascorbic acid treatment on the Iraqi cultivar, Turk: 0 PEG + 0 ascorbic acid treatment on the Turkish cultivar; Means with different letter(s) in each trait are significantly different at $p \leq 0.05$ based on Duncan's multiple range test.

seeds primed by ascorbic acid under drought stress helped the seedlings to improve their resistance to drought by increasing phenolic compounds and antioxidant properties (Farooq *et al.* 2013). In addition, treatments of α-tocopherol and ascorbic acid mitigated the adverse effects of drought stress on oats by improving the antioxidant defense system (El-Beltagi *et al.* 2022). Ascorbic acid improves the synthesis of antioxidant compounds and secondary metabolites by creating a stable environment and removing free radicals (Davey *et al.* 2000; Smirnoff 2000; Shafiq *et al.* 2014). Phenolic compounds may play a fundamental role in enhancing antioxidant properties. Plants activate a set of defense mechanisms to cope with the harmful effects of drought stress and free radicals, which leads to an increase in antioxidant properties and consequently the plant's resistance (Elmastaş *et al.* 2006).

Antioxidant capacity

The results showed that with the increase in drought stress levels, the antioxidant capacity in the Turkish and Iraqi varieties of mung bean increased. The antioxidant capacity in the Turkish cultivar seedlings showed a greater response compared to the Iraqi cultivar. Also, the highest level of antioxidant capacity was observed at the highest levels of drought stress. In addition, the treatment with 100 mg/l of ascorbic acid performed better in improving the antioxidant capacity in the seedlings of Iraqi and Turkish mung bean varieties compared to the treatment with 50 mg/l of ascorbic acid in most cases (Figure 6). According to Nawaz *et al.* (2021), ascorbic acid was able to partially increase plant resistance by accumulating metabolites and antioxidant compounds in mung bean. Also, El-Beltagi *et al.* (2022) treated oat plants with ascorbic acid and showed that ascorbic acid mitigated the adverse effects of drought stress in this species.

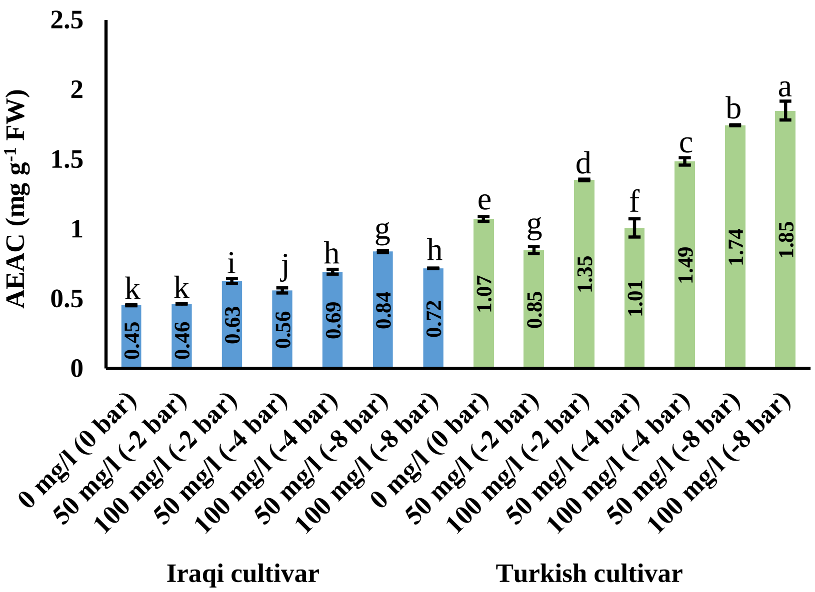


Figure 6. Antioxidant capacity in Iraqi and Turkish cultivars of mung bean as affected by the treatment with ascorbic acid and drought stress; Iraq: 0 PEG + 0 ascorbic acid treatment on the Iraqi cultivar, Turk: 0 PEG + 0 ascorbic acid treatment on the Turkish cultivar; AEAC: Ascorbic acid equivalent antioxidant capacity; Means with different letter(s) are significantly different at $p \leq 0.05$ based on Duncan's multiple range test.

Conclusion

The results from priming the seeds of Turkish and Iraqi mung bean varieties with different concentrations of ascorbic acid under drought stress that showed increasing the drought-stress level led to a decrease in seedling fresh weight, hypocotyl length, radicle length, leaf area, relative water content, amounts of chlorophylls a, b, and carotenoids, and increased proline, total sugars, total proteins, total phenols and flavonoids, and antioxidant capacity. However, Turkish varieties showed mostly better performance compared to the Iraqi genotypes.

The ascorbic acid treatment could not improve the growth characteristics and RWC compared to the control by increasing the drought level. However, at the PEG of -2 bar, the 50 mg/l ascorbic acid was not significantly different from the control for the hypocotyl length in both varieties. Also, at -2 bar PEG, both concentrations of ascorbic acid had significantly higher seedling weight than the control in the Iraqi variety. Alternatively, the treatment with ascorbic acid alleviated the effect of drought stress in some cases in terms of plant pigments. Also, 100 mg/l of ascorbic acid was better than the 50 mg/l concentration concerning plant pigments, total sugars, total proteins, proline, total phenols and flavonoids, and antioxidant capacity in most cases. More detailed experiment is needed to conclude about the effect of ascorbic acid on alleviating the adverse effects of drought stress in mung bean.

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Conflict of Interest

The authors declare that they do not have any relevant financial or non-financial competing interests.

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