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RESEARCH ARTICLE

Effects of Proline Applications on Plant Growth and Enzyme Activities in Forage Pea (*Pisum sativum* ssp. *arvense* L.) under Different Water Limit Conditions

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ABSTRACT

This study was conducted in 2024 in the greenhouses of Atatürk University plant production center in order to determine the effects of proline applications during the seedling period on plant development and some physiological and biochemical properties in forage pea grown under drought stress. The research was conducted in the form of a pot experiment with 3 irrigation levels [full irrigation (%100) (d0), 70% of field capacity (d1) and 40% of field capacity (d2)] and four proline applications (0, 5, 10, 20 mM) in 3 replications according to the completely randomized design. At the end of the experimental period, plant development parameters and some physiological and biochemical measurements and analyses were made in forage pea plants and the differences between the applications were evaluated. According to the research findings, significant differences emerged between the applications and levels. The effect of proline applications on plant development (plant height, stem diameter, fresh, dry weight, etc.) and some plant physiological and biochemical parameters [tissue electrical conductivity (mp), tissue relative water content (rwc), hydrogen peroxide (H_2O_2), malondialdehyde (mda), proline] was significant. At the end of the study, it was determined that drought conditions negatively affected plant development and decreased rwc and stomatal conductance. However, proline application improved plant development in forage pea under drought conditions and decreased rwc content compared to the control. As a result; it can be said that proline application affected the plant more positively in non-drought conditions.

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1. Introduction

Peas (*Pisum sativum* L.) are an important agricultural crop with a history dating back approximately 9,000 years, alongside wheat and barley (McPhee, 2003). They were first cultivated in Western Asia and have been widely grown in Europe for thousands of years. The subspecies *Pisum sativum* ssp. *sativum* is used for culinary purposes, while *Pisum sativum* ssp. *arvense* is used as forage. In Türkiye, forage pea (*Pisum arvense* L.) is grown as a spring crop in cold regions and as a winter crop in temperate regions, serving as both roughage and grain feed. In the Eastern Anatolia Region, it is primarily cultivated for seed and used as grain feed. The crude protein content of forage pea

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hay ranges between 16-18%, with a dry hay yield of 700-800 kg/ha (Tan et al., 2013).

Forage pea is a significant crop for both roughage and grain feed production due to its non-toxic nature, high fodder and seed yield, adaptability to various climate and soil conditions, and nitrogen-fixing ability that enriches the soil for subsequent crops. However, in places with a vegetation period such as Erzurum, grain feed crops that meet the energy needs of animals are largely limited to barley and vetch. Despite these advantages, the most significant factors negatively affecting the yield of forage pea are abiotic stresses such as drought, salinity and cold.

Abiotic stresses (drought, salinity, cold) are known to cause up to 50% yield losses in agricultural products globally. Approximately 26% of arable land is adversely affected by drought stress, 20% by mineral stress, and 15% by cold-frost stress (Blum & Jordan, 1985; Erdoğan Bayram, 2018). Drought is one of the most critical factors limiting plant production on a large portion of global agricultural land. Increasing population and global warming have rapidly depleted surface and groundwater resources. The lack of sufficient quality water has led to the use of low-quality water in agriculture, affecting soil structure negatively and causing issues such as salinization (Kutlar Yaylalı & Çiftçi, 2008).

Plants can be exposed to drought at different periods from germination to harvest. However, plants growing in arid environments throughout the entire development period are quite small in volume compared to plants growing in environments where water is not limited (F. Liu & Stützel, 2004; Tiryaki, 2016). The effects of drought stress on plants are classified at physiological, biochemical and molecular levels (Blum & Jordan, 1985). The first response of plants to water deficiency is to slow down cell growth (Taiz et al., 2015). The decrease in turgor pressure disrupts the water balance between plant tissues and negatively affects the amount of chlorophyll due to damage to photosynthetic pigments (Levitt, 1980). In addition, it has been reported that plant root structure elongates and top organs do not develop in water deficiency (Özel et al., 2016). In recent years, researchers have turned to various exogenous applications to alleviate the negative effects of drought on plants and to develop drought-resistant varieties (He et al., 2009). One of these applications is the use of amino acids. In addition to nourishing plants, amino acids improve biochemical processes and act as phytoregulators. This dual role makes them particularly valuable in stress reduction strategies. When plants encounter osmotic stresses such as water deficit, salinity, extreme temperatures, or heavy metal exposure, they exhibit initial physiological responses such as proline accumulation in cell vacuoles. Increased proline levels act as an osmoprotectant, stabilizing cellular structures and protecting enzymes under stress conditions. This increase in proline concentration within the cell is a critical indicator of how well the plant can tolerate stress and triggers a series of metabolic reactions that initiate the plant's defense mechanisms (Ünal, 2019). Numerous studies have shown that exogenous proline application significantly promotes plant growth under drought stress. Semida et al. (2020) reported that proline application significantly increased the growth and physiological performance of plants under water deficit conditions. Similarly, Kayak et al. (2022) observed that foliar application of proline improved drought tolerance by increasing osmotic regulation and reducing oxidative damage. Yamada et al. (2005) and Moustakas et al. (2011) also emphasized that proline supplementation provided better stress adaptation and recovery, and increased plant resistance. In addition, Ghaffari et al. (2019) found that proline application alleviated the negative effects of drought by increasing water holding capacity and maintaining higher photosynthesis rates. The aim of this study was to evaluate the effect of foliar application of proline on forage pea (*Pisum sativum*) grown under water-deficit conditions in greenhouse conditions. The study aimed to reveal the potential benefits of proline application in increasing drought tolerance in forage pea by evaluating various growth parameters, physiological traits and biochemical responses.

2. Materials and Methods

The study was conducted in pots in the greenhouses of Atatürk University Plant Production Application and Research Center. Taşkent forage pea variety was used as plant material in the study.

2.1. Establishment of the Experiment

The seedling study was conducted in a temperature-controlled greenhouse (45-50% humidity and 25±2°C). The seeds were planted in 2-liter pots filled with garden soil, peat, and sand mixture. Five seeds were sown in each pot at a depth of 1-1.5 cm. Standard fertilization was performed during planting with 3-5 kg/da N and 6-12 kg P2O5 kg/da (Tan, 2018). While calculating the fertilizer, it was assumed that there was 250 tons of soil in one decare area, the amount of fertilizer per pot (2 liters) was determined and applied by dissolving it in water (Bayhan et al., 2022). After seedling formation, thinning was performed so that 4 plants with a homogeneous appearance remained in each pot. Proline and water restriction applications were started 11 and 16 days after seed planting (three-leaf seedling period), respectively.

Throughout the experiment, the ambient temperature was kept constant at 25-30°C and irrigation was applied according to the amount of evaporation in the environment. In the experiment, three different irrigation subjects (completing the amount of evaporated water obtained from the evaporation pan (mm) to 100% (control), 70% and 40% of the usable water holding capacity depending on the relationship with the pot volume) were created. Before the experiment, the pot capacity (field capacity) and the usable water holding capacity (WWC) of each pot were determined accordingly (Çamoğlu, 2013). The pots were brought to pot capacity with planting. In the later stages of the pots that were brought to field capacity, water consumption was calculated according to evaporation and the study was carried out by restricting 100% water to the control group and 70-40% to the other pot groups. The water to be given in the study was Atatürk University drinking water and it was stated that it was suitable for irrigation. The trial was carried out with 4 proline applications (0 (P0), 5 (P1), 10 (P2), 20 mM (P3)), 3 water restriction applications (100% (D0), 70% (D1), 40% (D2)), 3 replications and 3 pots from each replication, in total 108 pots (4*3*3*3=108) (Table 1).

Table 1. Trial groups created in the study.

Total number of pots	Proline applications	Water restriction applications	Groups
108 pots	0 mM proline (P0)	100% (D0)	P0D0 (9 pots)
		70% (D1)	P0D1 (9 pots)
		40% (D2)	P0D2 (9 pots)
	5 mM proline (P1)	100% (D0)	P1D0 (9 pots)
		70% (D1)	P1D1 (9 pots)
		40% (D2)	P1D2 (9 pots)
	10 mM proline (P2)	100% (D0)	P2D0 (9 pots)
		70% (D1)	P2D1 (9 pots)
		40% (D2)	P2D2 (9 pots)
	20 mM proline (P3)	100% (D0)	P3D0 (9 pots)
		70% (D1)	P3D1 (9 pots)
		40% (D2)	P3D2 (9 pots)

Approximately 30 days after the trial was established, various parameters were examined from the seedlings in the greenhouse and in the laboratory. Measurements of each parameter were performed on 5 plants.

Seedling length (cm): In cm with a ruler. stem diameter (mm): In mm with a caliper. Plant fresh weight (g/plant): In grams (g) on a precision scale. Plant dry weight (g/plant): In grams (g) by drying in an oven at 68 0C until it reaches a constant weight. Root fresh weight (g/plant): In grams (g) on a precision scale. Root dry weight (g/plant): In grams by drying in an oven at 68 °C until it reaches a constant weight. Number of leaves (number/plant): Counted as pieces. Stomatal conductance (m^2s/mol): Stomatal conductance in the leaf was measured with a leaf porometer device at 10:00-11:00 hours while the plants were in pots and determined as m^2s/mol. Measurements were taken 3 days after the plants were irrigated in three different periods.

Leaf area (cm^2/plant): Leaf areas of the plants in each application were determined using a leaf area meter (LICOR, Model: LI-3100, Lincoln, NE, USA).

Chlorophyll content (SPAD): Chlorophyll content in plant leaves was measured with a chlorophyll meter (SPAD–502, Konica Minolta Sensing, Inc., Japan).

2.2. Tissue Electrical Conductivity (MP)

An indication of the damage caused by stress in the leaf tissue and especially in the cell membranes is the electrical conductivity measurements made on the fresh leaf tissues. For this purpose, the disks (1 cm in diameter) taken from the last developed real leaves of 2 randomly selected plants from each replication were placed in glass bottles containing 20 ml of distilled water and shaken in a shaker for 24 hours, and then the electrical conductivity of the soaking water was measured according to the method specified in Kaya et al. (2003) and the permeability (damage rate) of the cell membranes was determined (EC1). The samples were kept in an autoclave at 121°C for 20 minutes to ensure complete lysis of the cells and tissues, and then the second measurement was made (EC2). The ratio between EC1/EC2 and the relative electrical conductivity values were calculated.

2.3. Tissue Relative Water Content (RWC)

Leaf discs (1 cm in diameter) taken from 2 plants randomly selected among the surviving plants were weighed immediately and their fresh weights were determined (FW). After weighing, the discs were placed in petri dishes containing some distilled water and kept for 5 hours, then the excess water on the discs was wiped off with the help of blotting paper and weighed again and their turgor weights were determined (TW). Then, these discs were placed in petri dishes and dried in an oven set at 72°C for 48 hours and weighed again and their dry weights were determined (DW). Tissue water content was calculated according to the following formula stated in Kaya et al. (2003):

$$RWC = [(FW – DW) / (TW – DW)] \times 100 \quad (1)$$

Hydrogen Peroxide (H_2O_2): It was determined based on the method given in Özden et al. (2009). Lipid Peroxidation (Malondialdehyde-MDA): It was determined based on the method given in S. Liu et al. (2014). Catalase (CAT - EC:

1.11.1.6), Peroxidase (POD - EC: 1.11.1.7), Superoxide dismutase (SOD – EC: 1.15.1.1) enzyme activities were determined based on the method given in S. Liu et al. (2014).

2.4. Statistical Analysis

In the experiment, a completely randomized design was used. The data were subjected to analysis of variance (ANOVA) using the SPSS 20 software and means were separated by Duncan's multiple comparison test.

3. Results and Discussion

In this study, which was conducted to observe the effects of proline applications against drought stress in forage pea, the differences in plant height (cm), stem diameter (mm), plant fresh and dry weight (g), root fresh and dry weight (g) are presented in Table 2. Plant heights decreased with increased water restriction levels in all applications. In each application, the control group irrigation (D0) had the highest plant height. Proline applications generally affected plant height positively. P2 application gave better results than other applications even under water deficit conditions (D1, D2). The effects of the applications on stem diameter showed statistical differences. While stem diameters of control applications varied between 0.88-0.99 mm, proline applications varied between 0.77-1.29 mm. The highest stem diameter was obtained from P3D0 application. When the aboveground fresh mass results in forage pea were examined, it was seen that the highest values were obtained from full irrigation applications (D0). It is known that yield decreases under water deficit conditions. Accordingly, it is estimated that water scarcity is responsible for 17-70% of production losses (Ahmad et al., 2022). Among proline applications, P2D0 had the highest fresh mass value (4.16 g). In D1 applications, P1 and P2 applications reached higher fresh weight than the control (P0D1). In D2 values, which is the highest water restriction application (40%), all proline applications (P1, P2, P3) had higher fresh mass compared to the control applications. Butt et al. (2016) applied proline externally to plants under abiotic stress in their study (0.4 mM, 0.6 mM, 0.8 mM, 1 mM and 1.2 mM) and found that 0.8 mM proline concentration had the best effect and provided biomass increase. In fact, in our study, the best results were obtained from the proline application at a dose of 10 mM (P2). Plant dry weight decreased in each application depending on the decrease in water content. The highest plant dry matter weights were obtained from P2 applications among proline applications. While root fresh weights varied between 1.40-2.15 g in control applications, they varied between 0.70-1.44 g in proline applications. The first parts affected by water restriction in the plant are fresh and dry weights (Shao et al., 2008). When a significant water loss occurs from plant cells, the decrease in turgor pressure, which is the driving force for growth, and the negative effects on transpiration cause a decrease in mineral uptake, a decrease in photosynthesis and a decrease in growth rate (Capell et al., 2004; Eriş, 1990; McKersie & Leshem, 1994). With drought, the uptake of nutrients (Garg, 2003), mineralization (Bloem et al., 1992), transportation and the availability of nutrients on the root surface decrease. In addition, photosynthesis slows down in dry conditions and as a result, shoot development is weakened and root development is accelerated (Öztürk & Seçmen, 1992). In our study, it can be said that the root development of the control group was generally better compared to proline applications (Table 2). Studies have shown that in unstressed Arabidopsis seedlings, external proline supplementation at micromolar concentrations induced root elongation and branching, but when external proline was given at millimolar concentrations, root growth was inhibited with symptoms resembling cell death (Hellmann et al., 2000; Mattioli et al., 2009).

Table 2. Effect of applications on plant development in forage pea (*Pisum sativum* ssp. *arvense* L.).

Applications	Seedling height (cm)	Stem diameter (mm)	Plant fresh weight (g)	Plant dry weight (g)	Root fresh weight (g)	Root dry weight (g)
Proline (P)						
P0	31.67 b	0.94 a	2.49 a	0.36 a	1.60 a	0.51 a
P1	34.56 ab	0.96 a	2.62 a	0.34 a	1.24 b	0.31 b
P2	40.33 a	0.96 a	2.94 a	0.38 a	1.22 b	0.31 b
P3	35.00 ab	1.02 a	2.48 a	0.30 a	0.93 b	0.22 b
Water restriction (D)						
D0	40.92 a	1.12 a	3.58 a	0.45 a	1.50 a	0.41 a
D1	37.00 b	0.96 b	2.68 b	0.35 b	1.08 b	0.25 b
D2	28.25 c	0.83 c	1.64 c	0.25 c	1.16 b	0.35 ab
P x D						
P0D0	35.67 c	0.99 cd	3.43 abc	0.45 ab	2.15 a	0.72 a
P0D1	35.67 c	0.95 cde	2.55 cd	0.39 b	1.25 bc	0.29 cd

Table 2. (continued)

Applications	Seedling height (cm)	Stem diameter (mm)	Plant fresh weight (g)	Plant dry weight (g)	Root fresh weight (g)	Root dry weight (g)
P0D2	23.67 e	0.88 cdef	1.50 e	0.24 d	1.40 b	0.51 b
P1D0	38.67 bc	1.04 bc	3.06 bc	0.38 bc	1.17 bc	0.28 cd
P1D1	35.33 c	1.00 cd	2.99 bc	0.37 bc	1.11 bcd	0.25 cd
P1D2	29.67 d	0.85 def	1.80 de	0.28 cd	1.44 b	0.40 bc
P2D0	49.33 a	1.18 ab	4.16 a	0.52 a	1.40 b	0.36 bcd
P2D1	41.67 b	0.90 cdef	3.13 bc	0.39 b	1.16 bc	0.28 cd
P2D2	30.00 d	0.81 ef	1.53 e	0.23 d	1.10 bcd	0.30 cd
P3D0	40.00 bc	1.29 a	3.66 ab	0.43 ab	1.28 b	0.29 cd
P3D1	35.33 c	1.00 cd	2.07 de	0.25 d	0.82 cd	0.18 d
P3D2	29.67 d	0.77 f	1.72 de	0.24 d	0.70 d	0.19 cd

Means marked with different letters are statistically different.

The number of leaves has an average of 18.25 pcs/plant in control applications. It was determined as 17.62 pcs/plant in P1 applications, 20.03 pcs/plant in P2 applications and 16.47 pcs/plant in P3 applications (Table 3). As in plant fresh weight, the number of leaves in P2 application was more effective than other applications. Chlorophyll content is an important indicator of the growth status of a plant (Pavlović et al., 2014). All applications except POD2, PID0, P2D2 were statistically in the same group. In general, the chlorophyll content increased as water restriction increased in all applications. The researchers attributed this increase to the decrease in the unit area of the leaf and the increase in leaf thickness (Küçükkömürcü, 2011). Under control conditions, the leaf area value was determined as 178.25-205.20 cm2/plant (P0D2-P0D0) (Table 3). In the irrigation regimes where proline applications affected the leaf area, the highest value was calculated as 211.37 cm^2/plant in P3D0, while the lowest value was calculated as 189.48 cm2/plant in P1D2 level. It is numerically seen that the average value of control plants (P0D0, P0D1, P0D2) is 191.97 cm^2/plant, and the average value of proline application is 200.90 cm^2/plant with high leaf area. It is seen that proline applications generally increase the leaf area compared to control and have a positive effect. The best effect was obtained from P3 applications with the highest proline dose. RWC measurement was performed to determine the effect of proline application on water status in the plant against drought stress (Table 3). Drought stress caused a statistical increase in RWC values in the plant. It was determined that P2 application among proline applications regulates plant water status better than other groups. The fact that RWC values are consistent in P2 applications, as in plant root fresh and dry weights, shows that proline regulates the development of response to drought stress at a certain concentration (10 mM). This shows that the healing effect of proline varies according to the concentration. Electrical conductivity values increased with increasing stress in all applications (Table 3). Membrane permeability, which is considered as an indicator of damage occurring in plant cells under drought conditions, is measured as EC and is expressed as an ion imbalance that develops due to intracellular and extracellular osmotic incompatibility, especially in plants under salt and water stress (Kuşvuran, 2010). Ors et al. (2016) found that water restriction applied during the seedling period in squash increased EC in the plant. Stomatal conductance decreased with increasing stress in the control group. An increase was observed in the proline group. Studies have reported that stomatal conductance decreased under stress conditions (Yıldız, 2017). Stomatal conductance in proline applications increased with increased application dose. This can be attributed to the effect of proline in reducing plant stress.

Table 3. Effect of applications on leaf number, chlorophyll value (SPAD), leaf area, tissue proportional water content, electrical conductivity, stomatal conductivity in forage pea (*Pisum sativum* ssp. *arvense* L.).

Applications	Number of leaves (pcs/plant)	Chlorophyll SPAD	Leaf area (cm^2/plant)	RWC (%)	MP	Stoma
Proline (P)						
P0	18.25 a	35.10 a	191.98 b	79.18 a	38.52 a	21.52 a
P1	17.62 a	32.63 a	193.60 b	68.95 a	47.51 a	19.92 b
P2	20.03 a	34.22 a	201.53 ab	71.60 a	42.63 a	22.30 b
P3	16.48 a	34.65 a	207.59 a	69.87 a	38.97 a	27.69 b
Water restriction (D)						
D0	19.47 a	32.76 b	203.75 a	0.51 a	31.97 b	21.82 a
D1	20.38 a	34.21 ab	201.30 a	0.65 b	38.47 b	23.16 a
D2	14.44 b	35.48 a	190.97 b	0.67 c	55.27 a	23.60 a
P x D						
P0D0	19.55 abcd	33.66 ab	205.20 abc	86.37 a	32.45 cd	22.50 cd
P0D1	22.44 ab	34.73 ab	192.48 bcd	81.05 ab	36.44 cd	21.86 cd
P0D2	12.77 e	36.09 a	178.25 abcd	70.12 bcdef	46.66 abc	20.22 cd
P1D0	20.00 abcd	31.50 b	191.43 bcd	76.55 abcd	40.06 bcd	18.71 d
P1D1	17.77 abcde	33.40 ab	199.88 abc	67.17 cdef	43.19 abcd	20.14 cd
P1D2	15.11 cde	33.01 ab	189.48 cd	63.13 def	59.27 a	20.91 cd
P2D0	20.55 abc	32.13 ab	207.02 ab	83.34 ab	29.29 cd	21.42 cd
P2D1	23.77 a	33.57 ab	202.57 abc	70.80 bcdef	42.22 abcd	22.16 cd
P2D2	15.77 cde	36.97 a	195.02 abc	60.67 ef	56.38 ab	23.34 cd
P3D0	17.77 abcde	33.76 ab	211.37 a	77.83 abc	26.10 d	24.64 bc
P3D1	17.55 bcde	35.14 ab	210.28 a	73.90 abcde	32.04 cd	28.49 ab

Means marked with different letters are statistically different.

Proline has a role that can be naturally synthesized in the plant and its amount can change according to the stress experienced by the plant. In the scope of the study, 3 different doses (5 mM, 10 mM and 20 mM) were selected in order to determine the best proline dose to be applied to the leaves. The selected doses were applied to the leaves in the form of external pulverization in forage peas grown under full and restricted irrigation conditions. At the end of the seedling development period, Catalase (CAT), Superoxide dismutase (SOD), peroxidase (POD), H_2O_2 (mmol/kg), MDA (nmol/g) values in the plants were measured and the obtained data are given in Table 4. Restricted irrigation and proline applications did not play a decisive role in CAT antioxidant enzyme activity. All applications except P2D1, P3D2 applications were statistically included in the same group. When the POD values were examined, it was seen that the control applications had the lowest values among all applications. The highest POD values were determined in P3 applications. SOD antioxidant enzyme was positively affected by external applications and decreased in all proline applications compared to the control under water limited conditions (D1, D2). All proline D1 applications (P1D1, P2D1, P3D1) were found to be lower than the control D1 applications (P0D1). This situation is valid for D2 and D3 applications. Anjum et al. (2012) applied a total of 4 different water stress subjects as 80%, 60%, 40% and 20% in their study to determine the physiological responses of two pepper (*Capsicum annuum* L.) varieties under drought stress. It has been reported that with the onset of drought conditions, the antioxidant enzyme activities of catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) increased, then decreased with the severity of drought and reached lower levels than the relevant control levels. However, they stated that the growth, development and yield values of the variety with high activities of SOD, POD and CAT were higher than the other variety. As a result of the study, they stated that due to the presence of high antioxidant enzymes, reduced lipid peroxidation, better osmolyte accumulation and preservation of tissue water content in the plant, better growth and yield were recorded and drought resistance was increased. In many studies, it has been reported that the working principle of enzyme activity depends on a combination of parameters such as the type of stress conditions, its function in the plant and the plant species (Bhaduri & Fulekar, 2012; Malecka et al., 2001; Shah et al., 2001). In our study, H_2O_2 level in the control group decreased in D1 application and increased again with increasing drought at D2 level. This is the highest value among all applications (16.84 mmol/kg). In proline applications, it increased or remained the same in D1 applications from D0 process and decreased in D2 applications. However, all applications except P0D2, P3D2 applications were statistically in the same group and proline applications did not have a determining effect. When the study findings are examined, it was observed that MDA levels were the lowest in the control group. In proline applications, an increase was observed in MDA values due to the increase in water stress and proline dose. Similar studies have shown that MDA accumulation increases due to stress. It has been reported that drought stress increases the amount of MDA in wheat (Naveed et al., 2014).

Table 4. Effects of treatments on antioxidant enzyme activities (Prolin, CAT, POD, SOD, H_2O_2, MDA) in forage pea (*Pisum sativum* ssp. *arvense* L.).

Applications	**CAT-(EU/Gta)**	**POD-(EU/gTA)**	**SOD-(EU/Gta)**	**H_2O_2-(mmol/kg)**	**MDA- (nmol/g)**
Proline (P)					
P0	0.01 a	17.87 c	82.13 a	14.60 ab	0.55 a
P1	0.01 a	23.03 b	78.54 ab	15.86 a	0.58 a
P2	0.04 a	22.61 b	72.11 ab	14.11 ab	0.64 a
P3	-0.0356 a	30.52 a	70.45 b	11.02 b	0.67 a
Water restriction (D)					
D0	0.004 a	23.25 a	75.81 a	14.29 a	0.51 b
D1	0.04 a	21.21 a	77.50 a	14.17 a	0.65 a
D2	-0.02 a	26.06 a	74.11 a	13.23 a	0.67 a
P x D					
P0D0	0.013 ab	17.58 d	76.53 ab	14.37 ab	0.43 c
P0D1	0.016 ab	18.41 d	85.24 a	12.59 ab	0.67 abc
P0D2	0.003 ab	17.62 d	84.61 a	16.84 a	0.56 abc
P1D0	-0.020 ab	22.19 bcd	76.08 ab	16.08 ab	0.45 bc
P1D1	0.006 ab	22.04 cd	81.40 a	16.10 ab	0.62 abc
P1D2	0.046 ab	24.85 bc	78.14 ab	15.42 ab	0.67 abc
P2D0	0.023 ab	25.04 bc	78.33 ab	14.83 ab	0.58 abc
P2D1	0.110 a	17.59 d	77.42 ab	15.87 ab	0.63 abc
P2D2	0.006 ab	25.21 bc	60.59 b	11.65 ab	0.70 ab
P3D0	0.000 ab	28.21 b	72.29 ab	11.90 ab	0.57 abc
P3D1	0.030 ab	26.82 bc	65.96 ab	12.14 ab	0.68 abc
P3D2	0.136 b	36.54 a	73.10 ab	9.03 b	0.75a

Means marked with different letters are statistically different.

4. Conclusion

In the study investigating the effect of proline doses under different water constraint conditions, it was determined that the increase in drought negatively affected the plant in all applications. The positive effect of proline applications was detected more clearly in applications without water restriction (D0). Leaf number, leaf area, plant fresh weight are the parameters on which P2 doses are effective. In water-limited conditions, the best results were obtained from P2 dose and D1 combinations. According to the results obtained from our study, it can be said that proline applications can be an important strategy for plant growth and development in areas without water shortage. New studies are needed to test different water-limited conditions and proline doses.

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Conflict of Interest

The authors have no conflict of interest to declare.

References

Ahmad, H. M., Fiaz, S., Hafeez, S., Zahra, S., Shah, A.N., Gul, B., Aziz, O., Rahman, M. U., Fakhar, A., Rafique, M., Chen, Y., Yang, S. H., & Wang, X. (2022). Plant growth-promoting rhizobacteria eliminate the effect of drought stress in plants: A review. *Frontiers in Plant Science*, *13*, 875774. https://doi.org/10.3389/fpls.2022.875774

Anjum, S. A., Farooq, M., Xie, X. Y., Liu, X. J., & Ijaz, M. F. (2012). Antioxidant defense system and proline accumulation enables hot pepper to perform better under drought. *Scientia Horticulturae*, *140*, 66-73. https://doi.org/10.1016/j.scienta.2012.03.028

Bayhan, M., Özkan, R., Albayrak, Ö., Akıncı, C., & Yıldırım, M. (2022). Bazı arpa (*Hordeum vulgare* L.) genotipleri ile f1 melezlerinin kontrollü koşullarda değerlendirilmesi. *ISPEC Journal of Agricultural Sciences*, *6*(3), 563-572. https://doi.org/10.1007/BF00018060

Bhaduri, A. M., & Fulekar, M. H. (2012). Antioxidant enzyme responses of plants to heavy metal stress. *Reviews in Environmental Science and Bio-Technology*, *11*, 55-69. https://doi.org/10.1007/s11157-011-9251-x

Bloem, J., De Ruiter, P. C., Koopman, G. J., Lebbink, G., & Brussaard, L. (1992). Microbial numbers and activity in dried and rewetted arable soil under integrated and conventional management. *Soil Biology and Biochemistry*, *24*(7), 655-665. https://doi.org/10.1016/0038-0717(92)90044-X

Blum, A., & Jordan, W. R. (1985). Breeding crop varieties for stress environments. *Critical Reviews in Plant Sciences*, *2*(3), 199-238. https://doi.org/10.1080/07352688509382196

Butt, M., Ayyub, C. M., Amjad, M., & Ahmad, R. (2016). Proline application enhances growth of chilli by improving physiological and biochemical attributes under salt stress. *Pakistan Journal of Agricultural Sciences*, *53*(1), 43-49. https://doi.org/10.21162/PAKJAS/16.4623

Çamoğlu, G. (2013). The effects of water stress on evapotranspiration and leaf temperatures of two olive (*Olea europaea* L.) cultivars. *Zemdirbyste-Agriculture*, *100*(1), 91-98. https://doi.org/10.13080/z-a.2013.100.012

Capell, T., Bassie, L., & Christou, P. (2004). Modulation of the polyamine biosynthetic pathway in transgenic rice confers tolerance to drought stress. *PNAS*, *101*(26), 9909-9914. https://doi.org/10.1073/pnas.0306974101

Erdoğan Bayram, S. (2018). Su stresi ve bitkilerde su stresine bağlı fizyolojik değişimler. *Tralleis Elektronik Dergisi*, *3*(2), 219-228. (In Turkish)

Eriş, A. (1990). *Bahçe bitkileri fizyolojisi*. Uludağ Üniversitesi Ziraat Fakültesi Yayınları. (In Turkish)

Garg, B. K. (2003). Nutrient uptake and management under drought: Nutrient-moisture interaction. *Current Agriculture*, *27*(1/2), 1-8.

Ghaffari, H., Tadayon, M. R., & Nadeem, M., Cheema, M., & Razmjoo, J. (2019). Proline-mediated changes in antioxidant enzymatic activities and the physiology of sugar beet under drought stress. *Acta Physiologiae Plantarum*, *41*, 23. https://doi.org/10.1007/s11738-019-2815-z

He, L., Gao, Z., & Li, L. (2009). Pretreatment of seed with H_2O_2 enhances drought tolerance of wheat (*Triticum aestivum* L.) seedling. *African Journal of Biotechnology*, *8*(22), 6151-6157. https://doi.org/10.5897/AJB09.490

Hellmann, H., Funck, D., Rentsch, D., & Frommer, W. B. (2000). Hypersensitivity of an Arabidopsis sugar signaling mutant toward exogenous proline application. *Plant Physiology*, *123*(2), 779-789. https://doi.org/10.1104/pp.123.2.779

Kaya, C., Higgs, D., Ince, F., Amador, B. M., Cakir, A., & Sakar, E. (2003). Ameliorative effects of potassium phosphate on salt-stressed pepper and cucumber. *Journal of Plant Nutrition*, *26*(4), 807-820. https://doi.org/10.1081/PLN-120018566

Kayak, N., Kal, Ü., Dal, Y., Yavuz, D., & Seymen, M. (2022). Do proline and glycine betaine mitigate the adverse effects of water stress in spinach? *Gesunde Pflanzen*, *75*(1), 97-113. https://doi.org/10.1007/s10343-022-00675-6

Küçükkömürcü, S. (2011). *Tuzluluk ve kuraklık streslerine tolerans bakımından bamya genotiplerinin taranması* (Master's thesis, Çukurova University). (In Turkish)

Kuşvuran, Ş. (2010). *Kavunlarda kuraklık ve tuzluluğa toleransın fizyolojik mekanizmaları arasındaki bağlantılar* (Doctoral dissertation, Çukurova University). (In Turkish)

Kutlar Yaylalı, İ., & Çiftçi, N. (2008). *Tuzlu suların tarımda kullanımı ve domates yetiştiriciliğinde verime etkisi*. Konya Kapalı Havzası Yeraltısuyu ve Kuraklık Konferansı Bildiriler Kitabı. (In Turkish)

Levitt, J. (1980). *Responses of plants to environmental stresses. Volume II. Water, radiation, salt, and other stresses*. Academic Press.

Liu, F., & Stützel, H. (2004). Biomass partitioning, specific leaf area, and water use efficiency of vegetable amaranth (*Amaranthus* spp.) in response to drought stress. *Scientia Horticulturae*, *102*(1), 15-27. https://doi.org/10.1016/j.scienta.2003.11.014

Liu, S., Dong, Y., Xu, L., & Kong, J. (2014). Effects of foliarapplications of nitric oxide and salicylic acid on saltınduced changes in photosynthesis and antioxidative metabolism of cotton seedlings. *Plant Growth Regulation*, *73*(1), 67-78. https://doi.org/10.1007/s10725-013-9868-6

Malecka, A., Jarmuszkiewicz, W., & Tomaszewska, B. (2001). Antioxidative defense to lead stress in subcellular compartments of pea root cells. *Acta Biochimica Polonica*, *48*(3), 687-698.

Mattioli, R., Falasca, G., Sabatini, S., Altamura, M. M., Costantino, P., & Trovato, M. (2009) The proline biosynthetic genes *P5CS1* and *P5CS2* play overlapping roles in *Arabidopsis* flower transition but not in embryo development. *Physiol Plantarum*, *137*(1), 72-85. https://doi.org/10.1111/j.1399-3054.2009.01261.x

McKersie, B. D., & Leshem, Y. (1994). *Stress and stress coping in cultivated plants*. Kluwer Academic Publishers.

McPhee, K. (2003). Dry pea production and breeding. *Journal of Food Agriculture and Environment*, *1*(1), 64-69.

Moustakas, M., Sperdouli, I., Kouna, T., Antonopoulou, C. I., & Therios, I. (2011). Exogenous proline induces soluble sugar accumulation and alleviates drought stress effects on photosystem II functioning of *Arabidopsis thaliana* leaves. *Plant Growth Regulation*, *65*, 315-325. https://doi.org/10.1007/s10725-011-9604-z

Naveed, M., Hussain, M. B., Zahir, Z. A., Mitter, B., & Sessitsch, A. (2014). Drought stress amelioration in wheat through inoculation with *Burkholderia phytofirmans* strain PsJN. *Plant Growth Regulation*, *73*, 121-131. https://doi.org/10.1007/s10725-013-9874-8

Ors, S., Ekinci, M., Yildirim, E., & Sahin, U. (2016). Changes in gas exchange capacity and selected physiological properties of squash seedlings (*Cucurbita pepo* L.) under well-watered and drought stress conditions. *Archives of Agronomy and Soil Science*, *62*(12), 1700-1710. https://doi.org/10.1080/03650340.2016.1168517

Özden, M., Demirel, U., & Kahraman, A. (2009). Effects of proline on antioxidant system in leaves of grapevine (*Vitis vinifera* L.) exposed to oxidative stress by H_2O_2. *Scientia Horticulturae*, *119*(2), 163-168. https://doi.org/10.1016/j.scienta.2008.07.031

Özel, S. D., Gökkuş, A., & Alatürk, F. (2016). Farklı sulama seviyelerinin macar fiği (*Vicia pannonica* Crantz.) ve yem bezelyesinin (*Pisum arvense* L.) gelişimine etkileri. *Alinteri Journal of Agriculture Science*, *30*(1), 46-52. (In Turkish)

Öztürk, M. A., & Seçmen, Ö. (1992). *Bitki ekolojisi*. Ege Üniversitesi Fen Fakültesi Yayınları. (In Turkish)

Pavlovic, D., Nikolic, B., Djurović, S., Waisi, H., Anđelković, A., & Dragana, M. (2014). Chlorophyll as a measure of plant health: Agroecological aspects. *Pesticidi i Fitomedicina*, *29*(1), 21-34. https://doi.org/10.2298/PIF1401021P

Semida, W. M., Abdelkhalik, A., Rady, M. O., Marey, R. A., & Abd El-Mageed, T. A. (2020). Exogenously applied proline enhances growth and productivity of drought stressed onion by improving photosynthetic efficiency, water use efficiency and up-regulating osmoprotectants. *Scientia Horticulturae*, *272*, 109580. https://doi.org/10.1016/j.scienta.2020.109580

Shah, K., Kumar, R. G., Verma, S., & Dubey, R. S. (2001). Effect of cadmium on lipid peroxidation superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings. *Plant Science*, *161*(6), 1135-1144. https://doi.org/10.1016/S0168-9452(01)00517-9

Shao, H. B., Chu, L. Y., Jaleel, C. A., & Zhao, C. X. (2008). Water-defi cit stress-induced anatomical changes in higher plants. *Comptes Rendus Biologies*, *331*(3), 215-225. https://doi.org/10.1016/j.crvi.2008.01.002

Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2015). *Plant physiology and development*. Sinauer Associates.

Tan, M. (2018). *Baklagil ve buğdaygil yem bitkileri*. Atatürk Üniversitesi Ziraat Fakültesi Yayınları. (In Turkish)

Tan, M., Koç, A., Dumlu Gül, Z., Elkoca, E., & Gül, İ. (2013). Determination of dry matter yield and yield components of local forage pea (*Pisum sativum ssp. arvense* L.) ecotypes. *Journal of Agricultural Sciences*, *19*(4), 289-296. https://doi.org/10.1501/Tarimbil_0000001254

Tiryaki, İ. (2016). Yoncada (*Medicago sativa* L.) kuraklık stresi ve tolerantlık mekanizması. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, *19*(3), 296-304. (In Turkish)

Ünal, Z. (2019). *Bazı abiyotik streslerin prolinle desteklenen turuçgil anacı çöğürleri üzerine morfolojik, fizyolojik ve biyokimyasal etkileri* (Master's thesis, Akdeniz University). (In Turkish)

Yamada, M., Morishita, H., Urano, K., Shiozaki, N., Yamaguchi-Shinozaki, K., Shinozaki, K., & Yoshiba, Y. (2005). Effects of free proline accumulation in petunias under drought stress, *Journal of Experimental Botany*, *56*(417), 1975-1981, https://doi.org/10.1093/jxb/eri195

Yıldız, S. (2017). *Kuraklık ve tuz stresinin lahanada fide gelişimi ve bazı fizyolojik özellikler üzerine etkisi* (Master's thesis, Atatürk University). (In Turkish)