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EXPLORING THE EFFICACY OF VARIOUS ASCORBIC ACID DOSAGES AND APPLICATION METHODS IN ALLEVIATING DROUGHT STRESS IN SUNFLOWER CULTIVATION

**BUSHRA SAEED[1], MUHAMMAD ZAFAR-UL-HYE[1*], NATASHA MALIK[2],
SAJID MASOOD[1] AND YASIR IFTIKHAR[3]**

*[1]Department of Soil Science, Faculty of Agricultural Sciences and Technology,
Bahauddin Zakariya University, Multan, 60000, Pakistan
[2]Department of Soil Science, Faculty of Agriculture and Environment, The Islamia university of Bahawalpur, Pakistan
[3]Department of Plant Pathology, College of Agriculture, University of Sargodha Pakistan
[*]Corresponding author's Email; zafarulhyegondal@yahoo.com*

Abstract

Drought stress is one of the devastating limitations for crop production in dry land regions. Application of exogenous molecules such as ascorbic acid (AsA) can mitigate drought stress on crop plants. However, optimum dose and proper application method of AsA have not been properly investigated so far. Apot experiment in the greenhouse was conducted to investigate the efficacy of seed soaking and foliar spray methods of ascorbic acid (AsA) on sunflower under normal irrigation and drought stress. The treatments included control, 0.5 mM, 1.0 mM, and 1.5 mM AsA by foliar spray, respectively, and 0.5mM, 1.0mM, and 1.5mM AsA by seed soaking, respectively. Treatments 1.5 mM AsA by foliar sprayhad the highest percentage increase in shoot fresh weight (104.5%), root fresh weight (138.72%), seed yield/plant (128.8%), nitrogen concentration (57.7%) and chlorophyll contents (28.3%) compared to control. Overall, the study suggests that the foliar application of AsA at a concentration of 1.5mM is a promising approach to enhance sunflower growth and yield attributes under both normal and drought stress conditions. This finding may have significant implications for farmers and agricultural practices, as it provides a potential solution to mitigate the negative effects of water stress on crop productivity.

Key words: Ascorbic acid, Biochemical attributes, Drought stress, Nutrient; Yield.

Introduction

Climate change and human activities have exacerbated the occurrence and intensity of drought stress in many parts of the world (Kuromori *et al*., 2022). Now a days drought has become a global problem and a major threat to food security, particularly in arid and semi-arid regions (More *et al*., 2023). Drought stress is caused by a combination of natural and human-induced factors that affect water availability and uptake by plants (Al-Qubatee *et al*., 2022). Lack of rainfall, high temperatures, soil properties, land use changes, water management practices, and climate change are some of the most common factors responsible for drought stress. These factors can lead to decreased soil moisture and water availability for plants, resulting in reduced yields, loss of biodiversity, and degradation of natural resources (Al-Qubatee *et al*., 2022).

The impact of drought stress on plants is multifaceted and can vary depending on the plant species, the severity, duration, and timing of drought stress (Kasim *et al*., 2017). One of the most visible effects of drought stress on plants is a reduction in their growth rate. Under drought conditions, plants tend to reduce their leaf area, root growth, stem elongation, and biomass accumulation (Marcos *et al*., 2018). This reduction in growth is often due to the plant's inability to absorb sufficient water and nutrients from the soil, leading to a decrease in cell expansion, division, and differentiation. In addition to growth reduction, drought stress can also lead to a decrease in plant yield (Xu *et al*., 2015). This reduction in yield is primarily due to a decrease in the number and size of reproductive organs, such as flowers, fruits, and seeds (Bijalwan *et al*., 2022). Under drought stress, plants allocate more resources towards survival and maintenance than reproduction, resulting in a lower yield. Moreover, drought stress can affect the quality of the yield, resulting in a decrease in nutritional value (Fathi & Tari, 2016).

Furthermore, drought stress can impact the photosynthetic activity of plants, which is critical for their growth and productivity(Bijalwan *et al*., 2022). Photosynthesis is the process by which plants convert light energy into chemical energy, and chlorophyll is the primary pigment responsible for this process (Baccari *et al*., 2020). It can also decrease chlorophyll content, reducing photosynthesis and, consequently, decreasing plant growth and yield(Baccari *et al*., 2020). Moreover, drought stress can also lead to a decrease in stomatal conductance, which is responsible for regulating water loss through transpiration (Al-Huqail *et al*., 2023). This reduction in stomatal conductance can cause a decrease in the availability of carbon dioxide for photosynthesis, leading to a decrease in plant growth and yield (Al-Huqail *et al*., 2023). Drought stress can also lead to increased reactive oxygen species (ROS) production in plants, which can cause cellular damage and oxidative stress (Jiang *et al*., 2013; Bodner *et al*., 2015; Hidangmayum *et al*., 2019).Therefore, understanding the underlying mechanisms of drought stress and identifying strategies to mitigate its effects on plants are critical for sustaining agricultural productivity and ensuring food security (Jiang *et al*., 2013; Bodner *et al*., 2015; Hidangmayum *et al*., 2019).

Recently, ascorbic acid, also known as Vitamin C, has been identified as a potential antioxidant for mitigating drought stress in plants (Seminario *et al*., 2017). Ascorbic acid can scavenge ROS and protect plants from oxidative damage, enhancing their drought stress tolerance (Wang & Huang, 2019). Several studies have investigated the use of ascorbic acid as a mitigation strategy for drought stress in plants. For example, one study showed that the exogenous application of ascorbic acid increased the relative water content, proline content, and photosynthetic pigments in drought-stressed wheat plants (El-Beltagi *et al*., 2020). As a result, the wheat plants exhibited increased growth and improved water-use efficiency compared to the non-treated plants (El-Beltagi *et al*., 2020).

Sunflower (*Helianthus annuus* L.) is an important oilseed crop cultivated worldwide for oil purposes (Kosar *et al*., 2021).It is a valuable protein, oil, and fiber source and is widely used in the food and feed industries. However, like most crops, sunflower is susceptible to environmental stresses such as drought (Kosar *et al*., 2021). The importance of sunflower as a valuable crop, combined with its susceptibility to drought stress, highlights the need for research on strategies to mitigate the effects of drought on sunflower production (Kosar *et al*., 2021).

Although research on the use of ascorbic acid (AsA) for mitigating drought stress in plants has shown promising results, there is a need for research on the effectiveness of foliar application and seed soaking methods in enhancing plant tolerance to drought stress. As there is limited research comparing the effectiveness of different application methods in mitigating drought stress, there is a knowledge gap in the optimal application method and dosage of ascorbic acid for achieving maximum potential benefits under drought stress. This work aimed to investigate the effects of the exogenous application of AsA on physiological, nutritional uptake, and biochemical response of sunflower plants under normal irrigation practices and drought conditions. The investigation was done on the yield and yield related characteristics, chlorophyll SPAD, proline accumulation, electrolyte leakage and oil content in sunflower plants under normal and drought stress with foliar and seed soaking application of ascorbic acid application. It was assumed that AsA could prevent cell damage and enhance crop production.

Material and Methods

Apot experiment was conducted in a greenhouse at the Department of Soil Science, Bahauddin Zakariya University, Multan. A completely randomized design (CRD) was employed with three replications of each treatment. Seven treatment plans were devised for ascorbic acid application, including T_1 (control), T_2 (0.5mM AsA by foliar spray), T_3 (1.0 mM AsA by foliar spray), T_4 (1.5 mM AsA by foliar spray), T_5 (0.5mM AsA by seed soaking), T_6 (1.0mM AsA by seed soaking), and T_7 (1.5mM AsA by seed soaking). Two moisture levels were applied, including 75% water holding capacity (WHC) under normal irrigation and 40% WHC under drought stress. Both moisture conditions were maintained using the weight-based method (Table 1).

Seed sowing and pot preparation: To ensure controlled conditions for the growth and development of sunflower (Hysun 33), the seeds were obtained from Ayyub Agricultural Research Center, Faisalabad. Prior to sowing, the sunflower seeds were sterilized using a 1% sodium hypochlorite (NaOCl) solution for 10 minutes and then washed with tap water for 1 minute. The seeds were then sown in pots filled with 10 kg 2 mm sieved soil. The sowing season for the seeds was in January 2020. To ensure adequate germination, five sunflower seeds were planted in each pot. After full germination, the number of plants was reduced to one healthy plant per pot to avoid competition for resources.

Irrigation and fertilizer application: The recommended amounts of nitrogen, phosphorus, and potassium (200:80:80 kg ha^{-1}) were applied to each treatment, including T1 (control), to maintain optimal soil fertility. Prior to initiating the drought stress treatment, the plants were irrigated daily until the field capacity was reached. The drought stress treatment was imposed after seed germination. Their moisture content was monitored regularly to maintain the desired moisture levels in the drought-stressed pots. This was achieved by weighing each pot daily and adding normal irrigation water as required to ensure that the weight of the pot was equivalent to the weight calculated for 50% WHC.

Ascorbic acid application: The experiment consists of three concentrations of ascorbic acid (0.5 mM, 1.0 mM, and 1.5 mM) through foliar spraying and seed soaking methods. The control group was treated with distilled water only. For the seed soaking method, the seeds were first sterilized and then subjected to the respective ascorbic acid solutions before being sown. Foliar application of the ascorbic acid solutions was carried out at the plant's vegetative stage. The concentrations of the ascorbic acid solutions used were consistent for both the seed soaking and foliar spray methods.

Table 1. Soil properties of experiment site.

Property	Unit	Value	References
pH*s*	-	8.37	(Mclean, 2015)
Electrical conductivity (EC)	dSm^{-1}	2.02	(Rhoades, 1996)
Organic matter	%	0.73	(Nelson & Sommers, 1982)
Nitrogen	%	0.0023	(Bremner & Mulvaney, 1982)
Available phosphorus	µg/g	5.43	(Kuo, 1996)
Extractable potassium	µg/g	109	(Pratt, 2016)
Sand	%	10	(Gee & Bauder, 1986)
Silt	%	70	
Clay	%	20	
Texture	-	Silt loam	

Plant analysis: A total 21 sunflower plants were harvested when they reached 85% maturity. The leaf samples were collected in three replicates, with each replicate obtained from three pots. Therefore, each treatment group consisted of three plants in total. The fresh samples were taken to the laboratory and analyzed for various biochemical characteristics using standard procedures. Additionally, data for agronomic characteristics, such as root and shoot fresh weights, plant height, etc. were recorded, and the fresh samples were preserved for further analysis. After that, the plants were dried in an oven at 60°C for three days, and the dry weights of roots and shoots, number of achenes, and thousand achenes weight were calculated.

Electrolyte leakage: The method for estimating plant electrolyte leakage (EL) involved incubating leaves in distilled water for 24 hours in the dark at a temperature of 23°C. Following incubation, the samples were vortexed, and the initial electrical conductivity was measured using a conductivity meter. After recording the initial conductivity, the samples were autoclaved for 15 minutes at a temperature of 60°C. Once the process was complete, the samples were allowed to cool to room temperature, and the final conductivity was measured using a conductivity meter. The electrolyte leakage was then calculated using the following equation (Lutts *et al*., 1996).

$$\text{EL } (\%) = [(C1 - C0) / (Ct - C0)] \times 100$$

where EL represents electrolyte leakage as a percentage, C1 represents the conductivity of the sample after autoclaving, C0 represents the conductivity of the distilled water used for incubation, and Ct represents the conductivity of the sample before autoclaving.

Estimation of proline content: To estimate the proline content, fresh leaves weighing 0.1 g were extracted using 5 mL of sulfosalicylic acid (3%) and then centrifuged at 10,000 g for 15 minutes. A 1 mL aliquot of the supernatant was taken and mixed with 1 mL of glacial acetic acid and 1 mL of acidic ninhydrin mixture in a test tube. The mixture was then boiled for 10 minutes at 100°C and immediately cooled in an ice bath. After cooling, the mixture was vortexed for 20 seconds and allowed to cool to room temperature. The absorbance of the resulting solution was measured at a wavelength of 520 nm using a spectrophotometer. This process was repeated for each sample to determine the proline content of each sample (Bates *et al*., 1973).

Proline concentration (μmol/g FW) = [(Absorbance at 520 nm – 0.0575)/0.0456] x (V_f/V_i) x (1/w)

where V_f is the final volume of the reaction mixture, V_i is the volume of the extract taken, and W is the fresh weight of the sample.

Statistical analysis

The standard statistical procedure was followed for the statistical analysis of data (Steel *et al*., 1997). The two-factorial analysis of variance (ANOVA) was used to analyze the effects of independent variables (factors) on a dependent variable. Statistix@ 8.1 was used to perform the ANOVA. After the ANOVA was performed, the fishers' least significant difference (LSD) test was applied to evaluate the significant difference among the treatments. The LSD test is a post-hoc test used to determine which treatment means differ significantly.

Results

Under normal irrigation, the plant height progressively increased from T1 to T4 and slightly decreased from T4 to T7. T1 had the lowest plant height at 71.70 cm, while T4 had the highest at 133.18 cm. The percentage increase in plant height compared to T1 was 4.4%, 53.9%, and 85.5% for T2, T3, and T4, respectively. T5, T6, and T7 had a smaller percentage increase compared to T1 at 8.1%, 14.5%, and 26.4%, respectively. Under drought stress, the plant height decreased compared to normal irrigation for all treatments. The percentage decrease in plant height compared to T1 was 18.7%, 4.6%, 65.2%, 14.4%, 16.5%, and 23.7% for T2, T3, T4, T5, T6, and T7, respectively. Although T4 had the highest plant height under normal irrigation, it had the highest percentage decrease under drought stress (Table 2).

Main effects were significant but interactive effect was non-significant for head diameter. The results indicate that head diameter varied significantly among the different treatments. Treatment T4 had the largest head diameter with a mean value of 15.54 cm, followed by T2, T3, T6, and T7, which had mean values of 12.42 cm, 12.52 cm, 12.72 cm, and 12.64 cm, respectively. In contrast, T1 had the smallest head diameter with a mean value of 9.75 cm. In comparison to the control treatment (T1), the percentage increase in head diameter was highest in T4, with a 59.08% increase, followed by T2 (27.08%), T3 (28.51%), T6 (30.77%), and T7 (29.44%). However, T5 had a negligible increase in head diameter (3.28%) compared to T1. On the other hand, the head diameter under drought conditions decreased by 8.96% compared to normal irrigation (Table 2).

The results showed that all treatments significantly affected shoot fresh weight compared to T1 ($p<0.05$). Under normal irrigation conditions, T4 had the highest shoot fresh weight (12.82 g) followed by T3 (11.78 g), while T1 had the lowest (8.41 g). T2, T5, T6, and T7 had intermediate values with mean shoot fresh weights of 9.72 g, 7.83 g, 9.16 g, and 9.84 g, respectively. In contrast, under drought stress, all treatments had lower shoot fresh weights compared to their corresponding treatments under normal irrigation. T4 still had the highest shoot fresh weight (10.87 g) followed by T3 (8.84 g), while T1 had the lowest (5.32 g). T2, T5, T6, and T7 had mean shoot fresh weights of 7.60 g, 6.81 g, 7.93 g, and 8.77 g, respectively. Under normal irrigation, T4 had the highest percentage increase in shoot fresh weight compared to T1 (52.7%), followed by T3 (40.2%), T7 (17.2%), T2 (15.5%), T6 (8.9%), T5 (-6.9%). Under drought stress, T4 had the highest percentage increase compared to T1 (104.5%), followed by T3 (66.7%), T7 (64.3%), T2 (42.1%), T6 (49.1%), T5 (28.6%) (Table 2).

The percentage increase was calculated to compare the shoot dry weight results for each treatment with the control treatment T1. The shoot dry weight of T1 under normal irrigation was 0.4017 g, while under drought stress it was 0.295 g. Compared to T1, T4 had the highest percentage increase in shoot dry weight, with an increase of 146.4% under normal irrigation and an increase of 148.8% under drought stress. T3 also showed a considerable increase of 77.1% and 132.2% under normal irrigation and drought stress, respectively. T2, T6, and T7 also had increased shoot dry weight compared to T1 under both normal irrigation and drought stress, although to a lesser extent. T5 showed a higher shoot dry weight than T1 under normal irrigation, but a lower value under drought stress (Table 2).

The control group (T1) had a root fresh weight of 1.51fg in the normal irrigation treatment and 1.3533g in the drought treatment. Comparing the treatments to the control group, it was found that T2 had a percentage increase of 53.37% in the normal irrigation treatment and 71.47% in the drought treatment. Similarly, T3 had a percentage increase of 82.21% in the normal irrigation treatment and 104.29% in the drought treatment compared to the control group. In contrast, T4 had the highest percentage increase of 171.85% in the normal irrigation treatment and 138.72% in the drought treatment compared to the control group. T5 had a percentage increase of 34.56% in the normal irrigation treatment and 24.98% in the drought treatment, while T6 had a percentage increase of 54.28% in the normal irrigation treatment and 46.61% in the drought treatment. Finally, T7 had a percentage increase of 76.27% in the normal irrigation treatment and 134.87% in the drought treatment compared to the control group (Table 2).

When comparing the treatments for root dry weight to the control group, it was found that T2 had a percentage increase of 18.69% in the normal irrigation treatment and 6.67% in the drought treatment. Similarly, T3 had a percentage increase of 28.15% in the normal irrigation treatment and 10.86% in the drought treatment compared to the control group. In contrast, T4 decreased 29.61% in the normal irrigation treatment and 15.86% in the drought treatment compared to the control group. T5 had a percentage decrease of 33.33% in the normal irrigation treatment and 22.79% in the drought treatment, while T6 had a percentage decrease of 49.49% in the normal irrigation treatment and 53.53% in the drought treatment. Finally, T7 had the highest percentage decrease of 59.39% in the normal irrigation treatment and 59.82% in the drought treatment compared to the control group (Table 2).

When comparing the treatments for number of achenes to the control group, it was observed that T2 showed a percentage increase of 6.89% in the normal irrigation treatment and 7.96% in the drought treatment. Similarly, T3 exhibited a percentage increase of 28.77% in the normal irrigation treatment and 13.17% in the drought treatment as compared to the control group. Furthermore, T4 displayed the highest percentage increase of 39.12% in the normal irrigation treatment and 28.81% in the drought treatment compared to the control group. In contrast, T5 showed a slight percentage increase of 2.5% in the normal irrigation treatment and 0.16% in the drought treatment, while T6 had a modest percentage increase of 1.87% in the normal irrigation treatment and 8.14% in the drought treatment. Finally, T7 showed a percentage increase of 6.34% in the normal irrigation treatment and 8.17% in the drought treatment compared to the control group (Table 3).

The Thousand Achene Weight (TAW) results showed that the control group (T1) had a TAW of 26.465l in the normal irrigation treatment and 27.763k in the drought treatment. When compared to the control group, it was found that T2 had a significant increase in TAW by 74.94% in the normal irrigation treatment and 52.44% in the drought treatment. Similarly, T3 also showed a significant increase in TAW by 75.14% in the normal irrigation treatment and 56.18% in the drought treatment compared to the control group. Furthermore, T4 had the highest TAW in both normal irrigation (54.337a) and drought (49.315b) treatments, with a significant percentage increase of 105.18% and 77.94%, respectively, compared to the control group. In contrast, T5 had a lower TAW with only a 27.07% increase in the normal irrigation treatment and 14.54% increase in the drought treatment compared to the control group. Similarly, T6 had a relatively lower TAW with only a 42.17% increase in the normal irrigation treatment and 17.36% increase in the drought treatment compared to the control group. Finally, T7 had a TAW of 39.213f in the normal irrigation treatment and 34.113h in the drought treatment, with a significant percentage increase of 48.11% and 22.89%, respectively, compared to the control group (Table 3).

Compared to the control treatment T1, which received normal irrigation, the percentage increase in seed yield/plant was notable in some other treatments. T4 had the highest percentage increase of 186.9%, followed by T3 with an increase of 125.2%. The treatments T6 and T7 had a more modest increase of 45.1% and 57.4%, respectively. However, T2 and T5 had a lower seed yield/plant than the control treatment T1, with 4.4% and 23.2% percentage decreases, respectively. On the other hand, when considering the drought treatment, the results showed a different pattern. T4 still had the highest seed yield/plant, but the percentage increase compared to T1 was lower, with a value of 128.8%. T3 had an increase of 77.2%, while T6 and T7 showed increases of 26.7% and 30.3%, respectively. In this case, T5 had a percentage decrease of 11.8% when compared to T1, while T2 had a higher decrease of 17.3% (Table 3).

When comparing the nitrogen concentration of the plants under normal irrigation conditions, it is evident that the percentage increase in nitrogen concentration varied across the treatments. The highest percentage increase was observed in T4, with a value of 34.3% compared to T1. T3 had an increase of 11.4%, while T7 had a more modest increase of 10.1%. On the other hand, T2, T5, and T6 had lower nitrogen concentrations than T1, with percentage decreases of 4.2%, 1.7%, and 0.6%, respectively. Under drought conditions, the percentage increase in nitrogen concentration compared to T1 was lower for all treatments. The highest increase was again observed in T4, with a value of 57.7%. T3 had an increase of 29.8%, while T7 had an increase of 27.1%. The treatments T2, T5, and T6 had a lower nitrogen concentration than T1, with percentage decreases of 21.5%, 17.3%, and 5.5%, respectively (Table 4).

When comparing the percentage increase in P concentration compared to T1, it is evident that the percentage increase varied across the treatments. The highest percentage increase was observed in T4, with a value of 49.3% compared to T1. T3 had an increase of 39.6%, while T6 had an increase of 27.6%. On the other hand, T2, T5, and T7 had lower P concentrations than T1, with percentage decreases of 10.8%, 10.8%, and 15.7%, respectively (Table 4).

The results showed that T4 had the highest percentage increase in K concentration, with a value of 43.9%. T3 also had a considerable increase of 31.8%, while T6 had a moderate increase of 24.2%. In contrast, T2, T5, and T7 showed a reduction in K concentration compared to T1, with decreases of 9.9%, 8.2%, and 13.6%, respectively (Table 4).

When comparing the electrolyte leakage of the different treatments to T1, it is evident that the treatments had varying effects on the electrolyte leakage of the plants. T4 had the largest percentage decrease in electrolyte leakage compared to T1, with a value of 43.6%. T3 had a considerable decrease of 35.5%, while T7 had a moderate decrease of 11.7%. On the other hand, T2, T5, and T6 had higher electrolyte leakage values than T1, with percentage increases of 13.0%, 1.2%, and 4.1%, respectively (Table 5).

The highest percentage increase was observed in T4, with a value of 302.6% compared to T1. T3 had the second-highest increase of 194.5%, while T7 had the lowest increase of 201.2%. On the other hand, T1 had the lowest proline concentration value of 3.393µmol/g FW, while T5 had the second-lowest value of 8.03 µmol/g FW. Under drought conditions, the data also showed that all the treatments had a higher proline concentration than T1, suggesting that the treatments were effective in increasing proline accumulation and helping the plants to cope with water stress. T4 had the highest percentage increase in proline concentration compared to T1, with a value of 344.7%. T3 had the second-highest increase of 140.6%, while T7 had the lowest increase of 131.3%. In contrast, T1 had the lowest proline concentration value of 4.503 µmol/g FW, while T5 had the second-lowest value of 8.623µmol/g FW (Table 5).

Table 2. Effect of treatments on plant height, head diameter, shoot and root fresh and dry weight under normal irrigation and drought stress.

Treatments	Normal irrigation	Drought	Mean	Normal irrigation	Drought	Mean	Normal irrigation	Drought	Mean
	Plant height (cm)			**Head diameter (cm)**			**Shoot fresh weight (g)**		
T1	71.70fg	65.77g	68.74E	9.84g	9.67g	9.75C	8.41fg	5.32j	6.86F
T2	75.02ef	78.15def	76.59D	13.33c	11.50ef	12.42B	9.72d	7.60h	8.66D
T3	110.26b	85.19cd	97.72B	13.26cd	11.77def	12.52B	11.78b	8.84ef	10.31B
T4	133.18a	109.01b	121.10A	16.20a	14.87ab	15.54A	12.82a	10.87c	11.85A
T5	77.48def	75.03ef	76.25D	10.38fg	9.87g	10.12C	7.83h	6.81i	7.32E
T6	82.00cde	79.31def	80.65CD	13.42bc	12.02cde	12.72B	9.16e	7.93gh	8.54D
T7	90.51c	81.43de	86.00C	13.29c	11.99cde	12.64B	9.84d	8.77ef	9.30C
Mean	**91.46 A**	**81.98 B**		**12.82 A**	**11.67 B**		**9.94 A**	**8.02 B**	
	Shoot dry weight (g)			**Root fresh weight (g)**			**Root dry weight (g)**		
T1	0.40h	0.30i	0.35E	1.51fg	1.35g	1.43G	0.99a	0.73c	0.35E
T2	0.66de	0.62ef	0.64C	2.32d	2.32d	2.32D	0.81b	0.69cd	0.64C
T3	0.71cd	0.69cd	0.70B	2.76c	2.76c	2.76C	0.71cd	0.66de	0.70B
T4	0.99a	0.74c	0.86A	4.11a	3.2367b	3.67A	0.70cd	0.62ef	0.86A
T5	0.50g	0.34hi	0.42D	2.03e	1.69f	1.86F	0.66de	0.57f	0.42D
T6	0.70cd	0.57f	0.64C	2.33d	1.99e	2.16E	0.50g	0.34hi	0.64C
T7	0.81b	0.66de	0.73B	2.67c	3.18b	2.92B	0.407h	0.30i	0.73B
Mean	**0.68 A**	**0.56 B**		**2.53 A**	**2.36 B**		**0.68 A**	**0.56 B**	

Values are means of 3 replicates. Different letters (capital letters showing main effect and small letters showing interactive effect) showed significant difference at $p \leq 0.05$; Fisher's LSD. Values having no letters did not show any significant change at $p \leq 0.05$

Table 3. Effect of treatments on number of achenes per head, thousand achene weight and seed yield/ plant under normal irrigation and drought stress.

Treatments	Normal irrigation	Drought	Mean	Normal irrigation	Drought	Mean	Normal irrigation	Drought	Mean
	Number of achenes head^{-1}			**1000achene weight (g)**			**Achene yield (g plant^{-1})**		
T1	338.50g	314.50h	326.50D	26.47	27.76k	27.11G	8.95i	8.74i	8.85G
T2	361.83d	339.17g	350.50C	46.23c	42.31e	44.27C	16.73c	14.36e	15.54C
T3	435.83b	356.50def	396.17B	46.37c	43.45d	44.91B	20.21b	15.50d	17.86B
T4	471.83a	404.50c	438.17A	54.34a	49.32b	51.83A	25.64a	19.95b	22.80A
T5	347.17defg	315.50h	331.33D	33.63h	31.77j	32.70F	11.68g	10.03h	10.86F
T6	344.83efg	340.17g	342.50C	37.61g	32.55i	35.08E	12.98f	11.08g	12.03E
T7	360.17de	340.83fg	350.50C	39.21f	34.11h	36.66D	14.13e	11.64g	12.88D
Mean	**380.02 A**	**344.45 B**		**40.55 A**	**37.32 B**		**15.76 A**	**13.04 B**	

Values are means of 3 replicates. Different letters (capital letters showing main effect and small letters showing interactive effect) showed significant difference at $p \leq 0.05$; Fisher's LSD

Table 4. Effect of treatments on nitrogen, phosphorus and potassium concentration under normal irrigation and drought stress.

Treatments	Normal irrigation	Drought	Mean	Normal irrigation	Drought	Mean	Normal irrigation	Drought	Mean
	N (%)			P (%)			K (%)		
T1	2.76def	2.28g	2.53D	0.41g	0.37h	0.39F	0.46ef	0.42f	0.44E
T2	2.92bcd	2.67f	2.79C	0.44ef	0.42fg	0.43E	0.49de	0.50de	0.50D
T3	3.10b	2.98bc	3.039B	0.57ab	0.51c	0.54B	0.62a	0.54cd	0.58B
T4	3.73a	3.59a	3.66A	0.60a	0.56b	0.58A	0.65a	0.61ab	0.63A
T5	2.88cde	2.95bcd	2.91BC	0.46de	0.41g	0.43E	0.51cde	0.46ef	0.48DE
T6	2.97bcd	2.72ef	2.84C	0.51c	0.48d	0.50C	0.56bc	0.53cd	0.55BC
T7	3.059bc	2.92bcd	2.99B	0.48d	0.45ef	0.46D	0.53cd	0.50de	0.51CD
Mean	**3.06 A**	**2.87 B**		**0.50 A**	**0.46 B**		**0.54 A**	**0.51B**	

Values are means of 3 replicates. Different letters (capital letters showing main effect and small letters showing interactive effect) showed significant difference at $p \leq 0.05$; Fisher's LSD. Values with no letters showed no significant change at $p \leq 0.05$

Table 5. Effect of treatments on electrolyte leakage, proline, chlorophyll and oil content under normal irrigation and drought stress.

Treatments	Normal irrigation	Drought	Mean	Normal irrigation	Drought	Mean
	Electrolyte leakage (%)			Proline (µmol/g FW)		
T1	59.93	62.58	61.25A	3.39i	4.50h	3.95E
T2	50.12	56.66	53.39B	7.89g	9.84de	8.87C
T3	38.96	39.98	39.47C	9.99de	10.85c	10.42B
T4	31.10	37.98	34.54C	13.67b	15.50a	14.59A
T5	60.17	61.03	60.60A	8.03fg	8.62f	8.33D
T6	56.99	60.58	58.79AB	8.59f	9.51e	9.05C
T7	53.22	54.92	54.07B	10.23cd	10.42cd	10.33B
Mean	**50.07 B**	**53.39 A**		**8.83 B**	**9.89A**	
	Chlorophyll (SPAD)			Oil content (%)		
T1	28.47b	20.53fgh	24.50CD	30.33gh	28.78hij	29.56DE
T2	26.67bc	24.95cde	25.81BC	34.67de	32.45f	33.56C
T3	28.07b	26.29bcd	27.18B	36.72bc	35.78cd	36.25B
T4	33.44a	26.40bcd	29.92A	42.51a	37.81b	40.16A
T5	23.49def	18.64h	21.06E	29.60hi	28.14ij	28.87E
T6	22.90efg	20.36gh	21.63E	31.99fg	27.59j	29.79DE
T7	24.69cde	20.26gh	22.47DE	33.63ef	27.07j	30.35D
Mean	**26.82 A**	**22.49 B**		**34.21 A**	**31.09 B**	

Values are means of 3 replicates. Different letters showed significant difference at $p \leq 0.05$; Fisher's LSD. Values having no letters did not show any significant change at $p \leq 0.05$

Under normal irrigation, T4 showed the highest percentage increase in SPAD readings compared to T1, with an increase of 17.4%. T3 had an increase of 4.4%, while T7 had an increase of 0.9%. On the other hand, T5 and T6 had lower SPAD readings than T1, with percentage decreases of 17.3% and 19.6%, respectively. T2 also had a lower SPAD reading than T1, with a percentage decrease of 6.4%. Under drought stress, T4 again showed the highest percentage increase in SPAD readings compared to T1, with an increase of 28.3%. T3 had an increase of 16.2%, while T7 had an increase of 1.5%. On the other hand, T5 and T6 had lower SPAD readings than T1, with percentage decreases of 8.3% and 7.4%, respectively. T2 also had a lower SPAD reading than T1, with a percentage decrease of 27.9%. These results suggest that T4 had the most effective response to drought stress to maintain chlorophyll content, while T2 had the least effective response (Table 5).

When compared to control T1, it is evident that there were variations in the percentage increase across the treatments. T4 had the highest percentage increase in oil contents (%) under normal irrigation conditions, with a value of 40.2% compared to T1. T3 also had a substantial increase of 20.8%, while T2 and T7 had increases of 14.3% and 10.6%, respectively. On the other hand, T5 and T1 had lower oil contents (%) than T1, with percentage decreases of 6.8% and 5.1%, respectively. Under drought conditions, the percentage increase in oil contents (%) compared to T1 was lower across all treatments. T4 had the highest percentage increase, with a value of 31.2% compared to T1. T3 also had a notable increase of 24.3%, while T2 and T7 increased 13.0% and 6.1%, respectively. Similar to normal irrigation conditions, T5 and T1 had lower oil contents (%) than T1, with percentage decreases of 5.1% and 5.9%, respectively. Overall, the results suggest that drought conditions had a negative impact on the oil contents (%) of the plants, as there was a reduction in the percentage increase compared to T1 (Table 5).

Discussion

Drought stress is a significant environmental factor that has a negative impact on plant metabolism, growth, and yield (Sabetfar *et al*., 2013). Even mild drought stress can significantly reduce plant growth in many economically important crops (Arvin *et al*., 2012). This study aimed to investigate the ameliorative effects of ascorbic acid (AsA) on pot-grown sunflower plants under normal and drought moisture conditions. Our investigation found that water stress reduces physiological and biochemical processes, directly or indirectly affecting plant growth. These findings are consistent with previous research conducted by Jaleel *et al*., (2009). In addition, our study results are in line with those of Shafiq *et al*. (2014), who explored the role of AsA in modulating growth and different physio-biochemical attributes of canola plants under well-watered as well as water-deficit conditions. According to their results, drought stress imposed on 60% field capacity significantly decreased shoot and root fresh and dry weights, leaf chlorophyll contents, shoot and root P, root K, and catalyze enzyme (CAT) activity, while increasing chlorophyll a/b contents and proline in canola cultivars. Our study found that drought stress significantly ($p<0.05$) suppressed the root and shoot fresh and dry weights in sunflower. This finding is consistent with a number of studies that have reported the adverse effects of drought stress on the fresh and dry biomass of many crop plants, including maize (Jabeen *et al*., 2008), rice (Mostajeran & Rahimi-Eichi, 2009), sunflower (Hossain *et al*., 2010) due to alteration in different physio biochemical processes. It is important to note that the harmful effects of water stress on crop growth can vary depending on the specific stage of growth and the genetic nature of a crop/cultivar. Some crops may be more resilient to drought stress during certain growth stages compared to others, while different cultivars of the same crop may exhibit varying levels of tolerance to water stress. Therefore, it is crucial to consider these factors when studying the effects of water stress on crop growth and developing strategies to mitigate its impact (Arshad *et al*., 2008). Our findings revealed that the root and shoot fresh and dry weights were relatively higher under well-watered conditions than under drought conditions, indicating that water stress significantly impacts plant growth. This result is consistent with previous research that has shown that water stress can reduce plant growth, development, and productivity. Additionally, it has been observed that the efficiency of photosynthesis and photochemical reactions can also be hampered under stress conditions, such as drought stress. These processes are critical for plant growth and development, as they play a vital role in converting light energy into chemical energy, which is essential for plant metabolism. The reduction in plant growth and photosynthetic efficiency under water stress can be attributed to several factors. For instance, the plant's ability to take up water and nutrients from the soil is significantly reduced under drought conditions. This limitation can impede the plant's ability to carry out vital metabolic processes, leading to a decline in plant productivity (Taiz & Zeiger, 2006; Ashraf & Harris, 2013). However, ascorbic acid is believed to play a key role in photosynthesis and Protects plants against oxidative damage (Ashraf, 2009; Yazdanpanah *et al*., 2011).Studies have shown that Ascorbic acid (AsA) can potentially mitigate salinity's destructive effects on plant growth(Hamada and Al-Hakimi, 2009; Hassan *et al*., 2021). This may be achieved by increasing the endogenous levels of antioxidant enzymes and proline, which in turn can result in improved growth. In addition, the exogenous application of AsA has been found to positively impact plant growth. This was demonstrated by an increase in both shoot length and diameter in response to the application of AsA (Sofy *et al*., 2020). Other possible positive roles of proline under stress have been proposed, including stabilizing proteins and scavenging hydroxyl radicals (Smirnoff and Cumbes, 1989).In our study, we found that water-deficit stress increased leaf proline content, which may have helped the plant adjust osmotically and maintain turgor pressure, enabling it to adapt to limited water availability. Furthermore, the use of Ascorbic acid (AsA) helped scavenge reactive oxygen species (ROS) and prevent further proline biosynthesis. These findings suggest that AsA plays a critical role in alleviating the negative impacts of water-deficit stress on plant growth and development (Dolatabadian *et al*., 2009).Earlier, Reddy *et al*.(2003) have reported that the amount of proline in the drought stress time would increase, in that amino acid proline is a key in osmosis regulation. Similarly, the investigations by Sairam *et al*., (1998) reported that increasing the proline would lead to increased salt stress resistance. Ascorbic acid would affect the metabolism of plant reactions and would lead to many changes in them. These changes are sometimes accounted for as adaptabilities which increase the tolerance or resistance of plants against the environmental factors (Metwally *et al*., 2003). On the other hand, previous research reports that increasing proline will cause the preservation of the cellular inflammation and reduction of the membrane damage in plants, so the osmosis regulation is as an adaptability which increases the plant tolerance or resistance to drought stress (Inze & Van Montagu, 1995). It was noted that the concentration of proline has a direct and positive relationship with increasing the created resistance in the plants exposed to non-biological stress (Ramanjulu *et al*., 1998). The significant increase in the agronomic attributes and biochemical characteristics of sunflower plants following foliar spray of Ascorbic acid (AsA) in our study provides strong evidence of its effectiveness. Our results demonstrate that higher doses of AsA were particularly effective in enhancing plant growth and yield, even under both normal irrigation and drought stress conditions.

Conclusions

In conclusion, our study demonstrates that the foliar application of ascorbic acid positively impacts plant growth, physiological and yield characteristics. Among tested treatments, the higher dose of ascorbic acid at a concentration of 1.5 mM as a foliar spray was found to be the most effective in mitigating the adverse effects of water stress. This treatment improved growth parameters such as plant height, root and shoot dry and fresh weight, and an increase in the number of achenes and yield per plant. The osmolyte proline content was also enhanced, which is crucial for combating water stress under drought conditions. Overall, our findings suggest that the foliar application of ascorbic acid may be a promising strategy for improving plant growth and yield under water-stressed conditions. However, further investigations, especially field experimentation, are recommended to confirm the performance of these outcomes.

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