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ENHANCING DROUGHT STRESS WITH POLYETHYLENE GLYCOL TOLERANCE IN SAFFLOWER (CARTHAMUS TINCTORIUS L.) WITH ASCORBIC ACID TREATMENT

Sufan Qamar Mohammad, Ph.D
Department of Field Crops, Faculty of Agriculture, Tishreen University, Lattakia, Syria
E-mail: 123qamar456@gmail.com

ABSTRACT
Drought is one of the most important environmental problems we are currently facing, so we must work on using some techniques to mitigate the harmful effects of drought on plants. This study was conducted to evaluate the effects of foliar spraying with ascorbic acid on some characteristics of safflower plants under drought stress in Burj Islam village - Latakia, Syria 2024 according to the randomized complete design (R.C.D) with three replications. The treatments consisted of three levels of polyethylene glycol PEG (15, 30 and 45%) and three concentrations of ascorbic acid (50, 150 and 200 $mg.l^{-1}$). Traits such as plant height, number of branches, (chlorophyll, proline and MDA) content in safflower leaves and seeds number were measured. The results of the study showed that the studied traits decreased significantly under the influence of drought stress except proline and MDA content in leaves. The lowest value for plant height (5 cm), number of branches (51 branch/plant) and seeds number (246 seed/plant) was at P_3 45%. Meanwhile, ascorbic acid spraying increased the plant height of safflower plants, especially at 150 $mg.l^{-1}$ concentrations AS_2. The ascorbic acid and PEG treatment significantly increased the plant height (14 cm), number of branches (95 branch/plant) and seeds number (642 seed/plant) at AS_2P_1 treatment. Hence, a 150 $mg.l^{-1}$ concentration of foliar ascorbic acid could be used in drought stress for better growth of safflower plants.

KEY WORDS
Plant height, foliar spraying, MDA, chlorophyll, proline, seeds number.

Safflower is the oldest cultivated crop, typically grown at a small-scale level. Each part of this crop has multiple uses ranging from oil, foods, flavors, coloring agents, dyes, and medicinal benefits [1]. Safflowers are high in Phyto-compounds and flavonoids, which have a wide spectrum of pharmacological activities. Flavonoids and Phyto-compounds from safflower have been used to prevent and treat a variety of disorders, including central nervous system abnormalities and cardiac vascular, reproductive, gastrointestinal, and metabolic disorders, as well as preventive medicines with antioxidant, anticoagulant, hypolipidemic, and many other pharmacological effects [2].

Safflower (*Carthamus tinctorius* L.) is an oil crop belonging to the Asteraceae family. Among the genera that belong to this family we find the safflower genus Carthamus. This family includes about 32,913 species within 13 families and 1,911 genera [3].

exists in various environments without recognizing borders and no clear warning thereby hampering plant biomass production, quality, and energy. It is the key important environmental stress that occurs due to temperature dynamics, light intensity, and low rainfall. Despite this, its cumulative, not obvious impact and multidimensional nature severely affects the plant morphological, physiological, biochemical and molecular attributes with adverse impact on photosynthetic capacity [4].

There is a need to obtain plant forms that combine resistance to various stresses with satisfactory consumer qualities. The needs of the current moment determine the directions of scientific research and stimulate the development of new research methods. Currently, new biotechnological methods that use micro and even nanotechnology are being actively created. At the same time, the ideology of environmental safety is becoming a priority, both for the approach itself and for the product it [5].

Drought poses a significant challenge, restricting the productivity of plants. The strain induced by drought can impede vital processes like respiration and photosynthesis, affecting various aspects of plants' growth and metabolism [6]. Drought has become a major abiotic stress that severely affects wheat production globally. Changing rainfall patterns, increased atmospheric CO_2 levels, rises in atmospheric temperature and hot and dry winds are the major causes of drought stress. It has morphological, physiological, and biochemical consequences such as reduced yield performance, yield attributing parameters, germination, and seed vigor, early leaf senescence, early maturity, decreased chlorophyll content, decreased Rubisco activity, decreased photosynthesis, and decreased starch accumulation [7].

More importantly, hydrogen peroxide, superoxide, peroxide, and hydroxyl radicals are produced during the photorespiratory reaction, which can effectively damage the biological membranes and cellular biomolecules [8]. Many enzymatic and non-enzymatic antioxidants accumulate to a considerable level in stressed plants to counteract ROS [9].

Ascorbic acid is commonly known as vitamin C and it is well known to regulate stress tolerance in plants as reported in several studies, e.g., pea [10], tomato [11] and maize [12]. Ascorbic acid activates a complex biological defence process and acts as an antioxidant [13].

Additionally, AsA serves as the first line of defence for plants to combat oxidative stress by removing many types of free radicals, primarily as a substrate of APX, a vital enzyme in the ascorbate glutathione pathway [14]. Furthermore, it acts as a co-factor for enzymes that work in various metabolic pathways [15].

The aim of this study was to: (1) clarify the level of drought tolerance of safflower plants; (2) Study the effect of foliar application strategy of ascorbic on some characteristics of safflower plants under drought stress.

MATERIALS AND METHODS OF RESEARCH

Foliar spraying with ascorbic acid (AS): the initial treatment was carried out by spraying ascorbic acid on the plants leaves at a rate of two sprays during the first month of transplanting, ascorbic acid concentrations were 150, 300 and 450 mg.l^{-1}: AS_0= 0 mg/L (Con), AS_1=150 mg.l^{-1}, AS_2= 300 mg.l^{-1}, AS_3= 450 mg.l^{-1}.

Drought stress treatments: artificial drought stress was induced by PEG-6000, PEG concentrations were 15, 30 and 45%: P_0= 0%(Con), P_1= 15%, P_2= 30%, P_3= 45%.

The research was carried out in both the scientific research laboratory-agricultural engineering - Tishreen University, and a farm in the village of Burj Islam – Lattakia, during 2024.

Safflower seeds Obtained from the local market from the Ghouta al-Sham Barakat Production Company- Syria. Seedlings were planted in plastic bags with a capacity of approximately 5-6 kg of dry soil (15 x 30 cm), Soil containing a mixture of sand and clay in a ratio of (2/1).

Chlorophyll content determination Pigments were extracted from the leaves. The extraction of leaf pigments was performed with 80% acetone, and the absorbance at 663 and 645 nm was measured with an Amersham spectrophotometer (Amersham Biosciences, Piscataway, NJ, USA). The total chlorophyll quantities were calculated according to the method of [16].

The proline content was determined according to [17]. Frozen leaf tissue (0.5g) was homogenized with 10 mL of 3% sulfosalicylic acid at 4°C. The extract was filtered with Whatman No. 2 filter paper. In a test tube, 2 mL of filtrate, 2 mL of acid-ninhydrin, and 2 mL of glacial acetic acid were mixed and incubated at 100°C for 1h. Th e reaction was terminated on ice, and the reaction mixture was then extracted with 4 mL of toluene. The chromophore-containing toluene was separated from the hydrated phase. The absorbance at 520 nm was spectrophotometrically determined with toluene as the blank. The proline concentration was calculated based on a standard curve and was expressed as μmol proline g^{-1} FW.

The concentration of MDA was determined using the extinction coefficient of 155 $mM^{-1}cm^{-1}$ according to the technique described by [18] 0.2 g frozen leaves were homogenized in an aqueous solution of trichloroacetic acid (5% w:v). Then, the homogenate was centrifuged at 14 000 rpm for 20 min and the supernatant were heated in 0.25% thiobarbituric acid. The amount of MDA was measured using a spectrophotometer at 532 nm. The value for non-specific absorption at 600 nm was subtracted.

Statistical analysis of the results from experiments with three or more mean values was used, a one-way analysis of variance (ANOVA) as dictated by the number of main effects, and this was followed by Tukey's test. The difference was statistically significant when $P<0.05$.

RESULTS AND DISCUSSION

The differences were highly significant between the treatments for all characteristics (plant height, number of branches, chlorophyll content in leaves, proline content in leaves, malondialdehyde content in leaves and Seeds number).

Plant height decreased significantly under the influence of drought stress (Fig.1). The lowest value for plant height was at P_3 45% (5 cm) as compared to control AS_0P_0 (11 cm). Meanwhile, ascorbic acid spraying increased the plant height of safflower plants, especially at 150 $mg.l^{-1}$ concentrations AS_2 (14 cm) as compared to control. The ascorbic acid and PEG treatment significantly increased the plant height at AS_2P_1 treatment (14 cm).

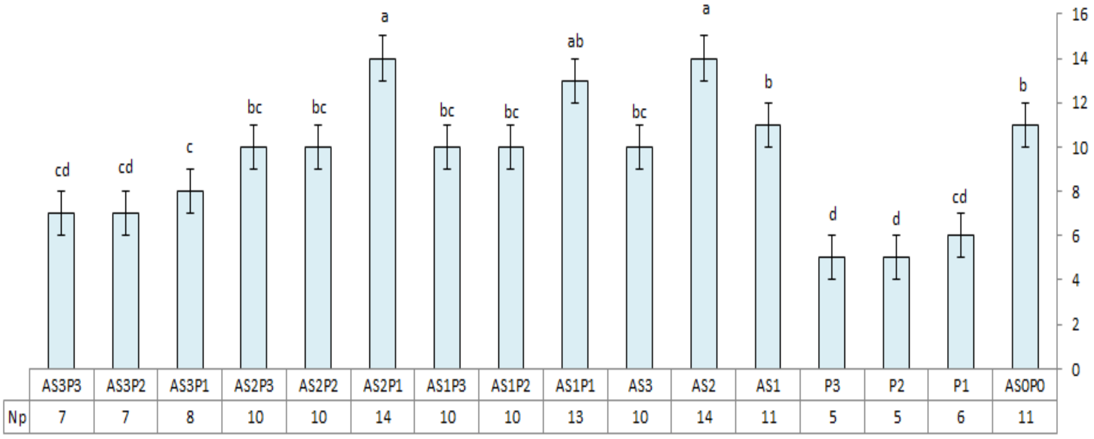


Figure 1 – Effect of ascorbic acid on the Safflower height plants under drought.

Drought stress reduced the plant height of safflower. It appears that under drought stress conditions, lower synthesis of photosynthates due to the limited access of plants to water, and CO_2 results in the lower allocation of photosynthates to plants' growing parts; therefore, compared to a situation with sufficient water, the plant's growth potential decreases significantly. In other words, decreases in turgor and cell growth, and so lower leaf area, stomatal closure, and limits in photosynthesis under water deficiency condition are the main effective reasons for the reduction in plant height and other morphological characteristics [19]. Reduction in morphological growth traits might be attributed to a reduction in cell expansion and cellular growth that resulted in declined plant height under drought stress [20].

Number of branches decreased significantly under the influence of drought stress (Fig.2). The lowest value for number of branches was at P_3 45% (51 branch/plant) as compared to control AS_0P_0 (80 branch/plant). Meanwhile, ascorbic acid spraying increased the number of branches of safflower plants, especially at 150 $mg.l^{-1}$ concentrations AS_2 (91 branch/plant) as compared to control. The ascorbic acid and PEG treatment significantly increased the number of branches at AS_2P_1 treatment (95 branch/plant).

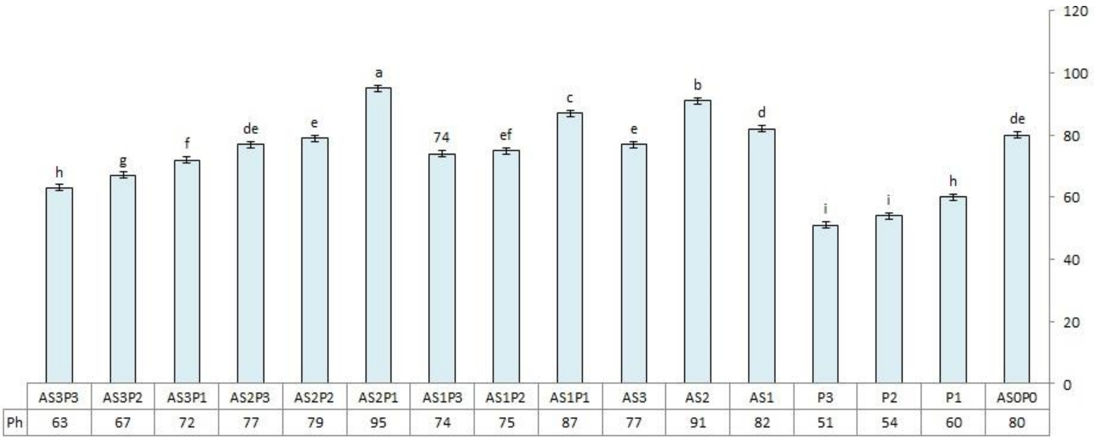


Figure 2 – Effect of ascorbic acid on the Safflower number of branches under drought.

Drought stress reduces productivity, synthesis of photosynthates, the components of yield and finally seed yield according to [21], [22] pointed out that, ascorbic acid (AS) is the most abundant antioxidant which protect cell, ascorbic acid Is currently considered to be a regulator on plant development and growth owing to its Impact on cell division and differentiation which reflected in Plant branches plant. However, [23] suggested that ascorbic acid could be a promising material utilized to decrease the harmful effect of water stress on the growth parameters and yield components of maize plants.

Chlorophyll content decreased significantly under the influence of drought (Fig.3). The lowest value for chlorophyll content was at P_3 45% (1.75 µg/g) as compared to control AS_0P_0 (2.75 µg/g). Meanwhile, spraying with ascorbic acid increased the chlorophyll content of safflower plants, especially at AS_2 150 mg.l^{-1} (3.04 µg/g) as compared to control. The ascorbic acid and PEG treatment significantly increased the chlorophyll in leaves at AS_2P_1 treatment (3.24 µg/g).

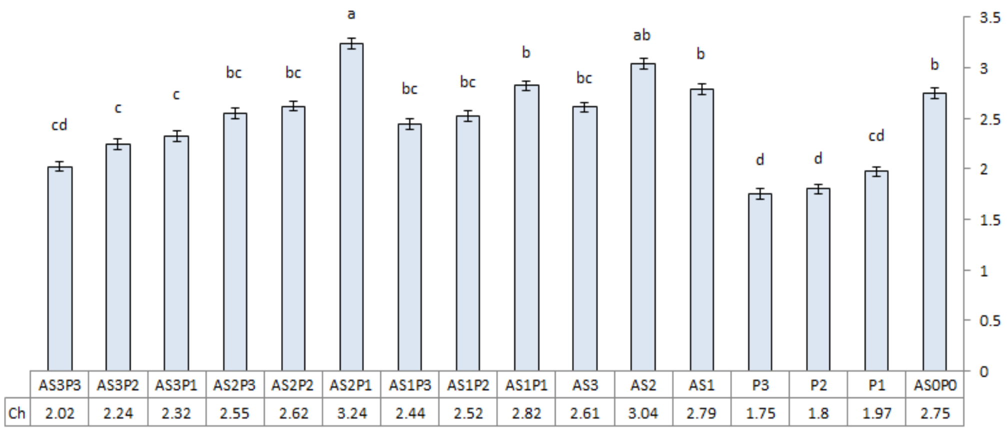


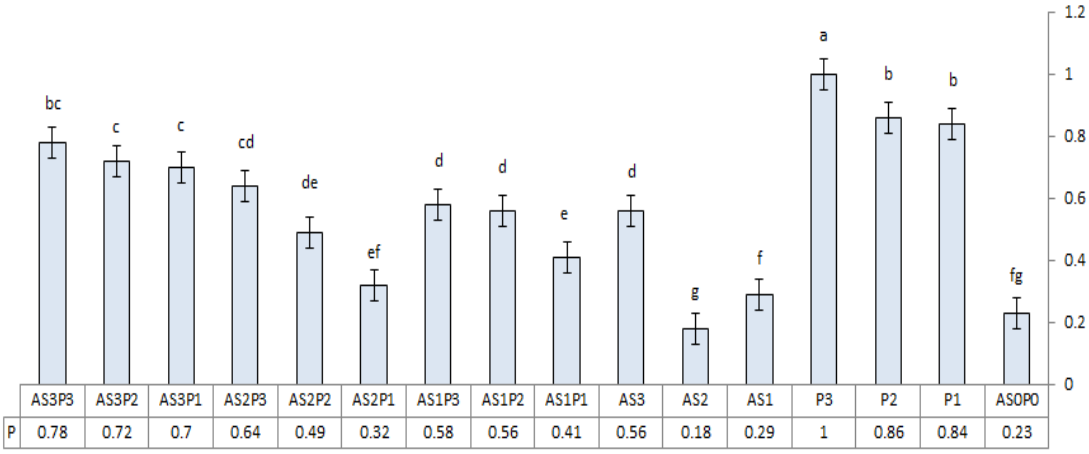
Figure 3 – Effect of ascorbic acid on the chlorophyll content under drought

It is well known that drought stress can considerably decrease the photosynthetic pigments which result in reduced plant growth and yield [24]. Drought stress reduces plant height and biomass, leaf chlorophyll content and photosynthesis rate, yield components, oil content and yield, and fatty acid composition of safflower [25].

Such reduction in chlorophyll pigments may have been due to the enhanced activities of chlorophyllase and peroxidase involved in the breakdown of chlorophyll under stress conditions [26]. The reduction of chlorophyll is common in drought stress conditions [27].

Chlorophyll content in leaves was significantly affected by drought stress and AS. The data demonstrated that drought stress significantly decreased the content of chlorophyll. The ascorbic acid treatment significantly increased the leaf chlorophyll content exposed to severe drought. The content of Chlorophyll in leaves decreased by increasing of drought intensity and increased with raising AS [28].

Proline content in leaves increased significantly under the influence of drought stress (Fig.4). The highest value for proline content in leaves was at P_3 45% (1 µg/g) as compared to control AS_0P_0 (0.23 µg/g). Meanwhile, ascorbic acid spraying decreased the proline content of safflower leaves, especially at 150 mg.l^{-1} concentrations AS_2 (0.18 µg/g) as compared to control. The ascorbic acid and PEG treatment significantly increased the proline content in leaves at AS_2P_1 treatment (0.32 µg/g).



	AS3P3	AS3P2	AS3P1	AS2P3	AS2P2	AS2P1	AS1P3	AS1P2	AS1P1	AS3	AS2	AS1	P3	P2	P1	AS0P0
P	0.78	0.72	0.7	0.64	0.49	0.32	0.58	0.56	0.41	0.56	0.18	0.29	1	0.86	0.84	0.23

Figure 4 – Effect of ascorbic acid on the proline content under drought

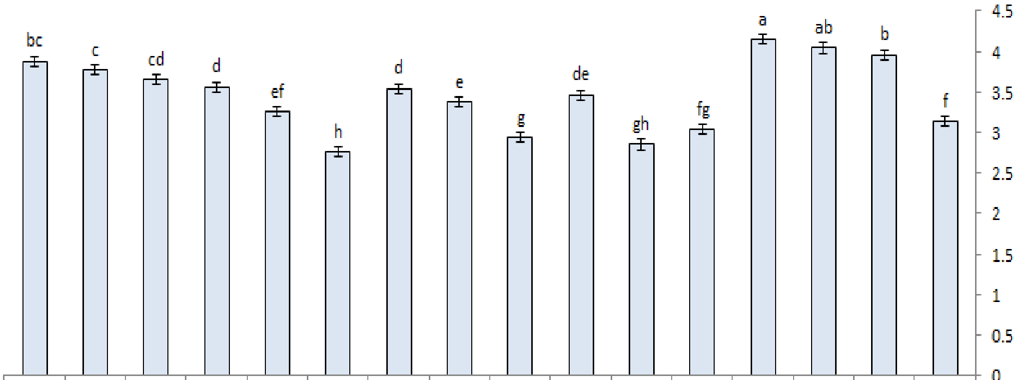
Proline is an amino acid produced by plants when facing abiotic stress in the form of drought. This is one of the adaptation mechanisms possessed by plants to survive in extreme environmental conditions [29]. This shows a link between proline accumulation and resistance to drought stress. This accumulation of proline is a crucial factor in determining plant tolerance to extreme environmental conditions, such as drought [30], safflower plants showed enhanced accumulation of proline under water stress [31]. The results indicated that proline content was affected by drought and AS foliar application. The content of proline in free leaves increased with increasing drought stress in pepper, and its content increased significantly after application of ascorbic acid in leaves [28].

Different levels of foliar applied AS, especially 150 mg/L^{-1}, significantly improved the proline contents safflower cultivars. [32] reported that AS might be essential for hydroxyproline synthesis, a non-essential amino acid derivative. Likewise, ascorbate is also essential for collagen synthesis, especially the hydroxylation of prolyl residues. [33] in a comprehensive review reported that ascorbate is involved in cell division and expansion of plants as a cofactor for prolyl hydroxylase that hydroxylates proline residues in cell wall hydroxyproline-rich glycoproteins.

MDA content in leaves increased significantly under the influence of drought stress (Fig.5). The highest value for MDA content in leaves was at P_3 45% (4.14 nmol/g) as compared to control AS_0P_0 (3.14 nmol/g). Meanwhile, ascorbic acid spraying decreased the MDA content of safflower leaves, especially at 150 mg.l^{-1} concentrations AS_2 (2.85 nmol/g) as compared to control. The ascorbic acid and PEG treatment significantly increased the MDA content in leaves at AS_2P_1 treatment (2.76 nmol/g).

This study showed that drought stress effectively increased the leaf MDA contents as compared with control plants. Exogenous application of ascorbic acid decreased the MDA content in plants exposed to medium and severe drought stressed conditions, and MDA content were raised with increasing of drought intensity and decreased with enhancing foliar spray of AS [28].

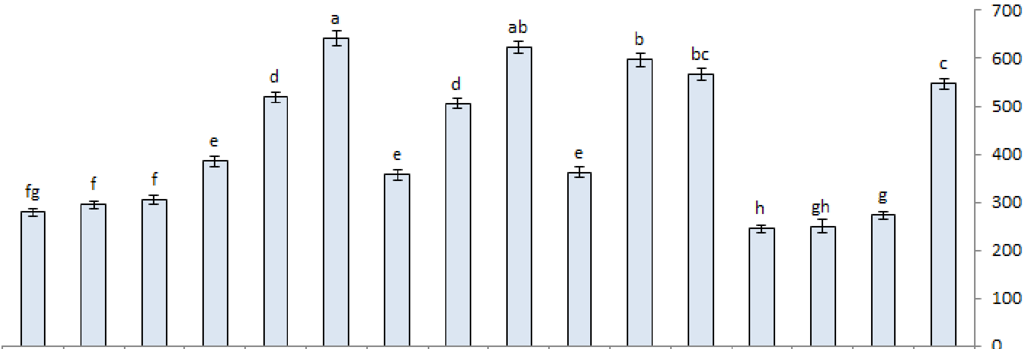
High lipid peroxidation results due to the elevated levels of ROS damaging the plant ultrastructures under water deficit conditions, high accumulation of MDA was observed in safflower plants subjected to water deficit conditions. On the other hand, exogenously applied AS has played a significant role in lowering the MDA content in leaves [31].



	AS3P3	AS3P2	AS3P1	AS2P3	AS2P2	AS2P1	AS1P3	AS1P2	AS1P1	AS3	AS2	AS1	P3	P2	P1	AS0P0
MDA	3.88	3.77	3.65	3.56	3.25	2.76	3.54	3.37	2.93	3.46	2.85	3.04	4.14	4.04	3.96	3.14

Figure 5 – Effect of ascorbic acid on the MDA content under drought

Seeds number decreased significantly under the influence of drought stress (Fig.6). The lowest value for seeds number was at P_3 45% (246 seed/plant) as compared to control AS_0P_0 (547 seed/plant). Meanwhile, ascorbic acid spraying increased the seeds number of safflower plants, especially at 150 mg.l^{-1} concentrations AS_2 (597 seed/plant) as compared to control. The ascorbic acid and PEG treatment significantly increased the seeds number at AS_2P_1 treatment (642 seed/plant).



	AS3P3	AS3P2	AS3P1	AS2P3	AS2P2	AS2P1	AS1P3	AS1P2	AS1P1	AS3	AS2	AS1	P3	P2	P1	AS0P0
Ns	280	295	305	385	520	642	358	505	623	362	597	568	246	250	273	547

Figure 6 – Effect of ascorbic acid on the Seeds number under drought

Drought stress through its impacts on the enzymes involved in photosynthesis, stomatal closure, and reducing photosynthesis, decreases LAI and accelerates leaf aging, which leads to lower plant economic yields [34]. A substantial yield reduction in various economically important crops has been reported due to water deficit stress [35].

Drought stress reduces crop yield significantly by decreasing plants' physiological and morphological processes [36]. These stresses cause nutritional and ionic imbalances, which have negative impacts on a variety of physiological and biochemical pathways involved in plant growth and development [37]. salinity had an additive effect on dry-matter accumulation; drought stress leads to alterations in metabolism during the growth phase of the vegetative and reproductive stages. Therefore, the metabolic changes in the plant resulted in lowering of water potential, iron deficiency and toxicity, stomatal closure, and reduced carbon dioxide (CO_2) assimilation [38].

Ascorbic acid is a non-enzymatic antioxidant which is believed to be beneficial for protecting plants under water deficit stress [13], and it is one of the easily available growth regulators that can be conveniently used by the farmers to Induce stress tolerance, including drought tolerance in plants. Ascorbic acid (AS) can improve plant growth and elevate yield through improvement of resistance to stress [39]. Previous study addressed that AS is involved in increasing yield and stress tolerance in plants [40].

CONCLUSION

Results of this study showed that PEG increased ROS levels and had negative effects on morphological and chemical traits, however, when drought stress was applied with AS, studied characteristics was improved and ROS levels decreased. Therefore, by increasing the activity of antioxidant enzymes, it can stimulate tolerance to drought stress.

Ascorbic acid foliar spraying at 150 mg.l^{-1} concentrations increased the plant height, branches number and seeds number of safflower plants. Further studies under field conditions are needed to optimize ascorbic acid concentrations to achieve adequate increase in seed yield.

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