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The Effect of Ascorbic Acid Foliar Application on the Ecophysiological Characteristics of *Catharanthus roseus* L. Under Water Deficit Stress

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Abstract

Water is one of the most important environmental factors that regulate plant growth and development, and water deficit is considered the most important restricted factor for plant products, in that several chemical materials have been used to reduce the harmful effects of water deficit. One of these compounds is ascorbic acid and it has antioxidant effects in plants. For this purpose, a factorial pot experiment in the form of a completely randomized design with three replications was conducted to investigate the effect of water deficit on the morphological and physiological characteristics of *Catharanthus roseus* (Cape periwinkle) under ascorbic acid foliar spraying in 2019. The test factors include: Drought stress (I) at 4 levels based on field capacity (FC): 1) Control (no stress) (I1) Irrigation at 100% FC, 2) Mild stress (I2) Irrigation at 75% FC; 3) Medium stress (I3) Irrigation at 50% FC; and 4) Severe stress (I4) Irrigation at 25% FC; foliar application of ascorbic acid was at 4 levels: 0, 25, 50, 100 mM. The seeds of *C. roseus* were sown in a 50% field soil +30% peat + 20% perlite substrate. The obtained results showed that the concentration of chlorophyll a and carotenoids, leaf soluble sugar, proline amount and catalase enzyme activity increased with increasing severity of water deficit, but the morphological characteristics of root and shoot dry weight decreased. Application levels of ascorbic acid led to the improvement of the measured traits, and in most of the traits, the 50 mM level was significantly superior to other levels. According to the obtained results, it is recommended to use ascorbic acid to improve the growth of plants such as *C. roseus* under drought stress.

Keywords: Ascorbic acid, Catalase, Drought stress, Dry weight, Ornamental and medicinal plants.

INTRODUCTION

Catharanthus roseus (L.) G. Don. (Apocynaceae) derives its economic importance from highly valued leaf anticancer alkaloids vincristine and vinblastine and antihypertensive root alkaloid ajmalicine (Srivastava and Srivastava, 2007; Yang *et al*., 2018). All parts of the plant are rich in alkaloids, with maximum concentrations found in the root bark, particularly during flowering. An infusion of the leaves is used to treat menorrhagia (Dhyani *et al*., 2022). The juice of the leaves is applied externally to relieve wasp stings. All parts of the plant are credited with hypoglycaemic properties and are used to treat diabetes (Rizvi *et al*., 2016; Kaur *et al*., 2021).

Drought stress is one of the environmental stresses that, in addition to reducing vegetative growth and anatomical changes of the plant, causes changes in the synthesis pathways of compounds and secondary metabolites through the creation of secondary stress such as oxidative stress (Sharma *et al.*, 2012; Rajaeian *et al*., 2015).

Many studies have been reported on the increased accumulation of reactive oxygen species (ROS) during drought stress. Plants reduce the generated reactive oxygen species through enzymatic and non-enzymatic antioxidant mechanisms (Hasanuzzaman *et al.*, 2013). Accumulation of reactive oxygen species in the cell causes damage to membrane lipids, proteins and nucleic acid. During photosynthesis in the state of dehydration, a lot of electron leakage occurs towards O_2 and produces different types of reactive oxygen species (ROS) such as superoxide radicals (O_2•), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH•) and singlet oxygen radical ($^1O_2$) (Yang *et al*., 2021).

Plants have enzymatic and non-enzymatic antioxidant mechanisms to deal with oxidative stress caused by ROS. The superoxide radical may be converted to H_2O_2 by the superoxide dismutase enzyme and then to water by ascorbate peroxidase (APX) in the chloroplast; Also, the H_2O_2 released to the outer part of the chloroplast is cleaned by the enzyme catalase (CAT) in the leaf cells (Muhie, 2022). In the dry state, photorespiration increases due to the limitation of CO_2 absorption and fixation and the increase of RubisCO enzyme oxygenase activity, which results in the increase of H_2O_2 production (Miller *et al.*, 2010).

Various correlations have been reported between water deficit stress and the amount of antioxidants soluble in intracellular water. One of the ways to increase resistance is to raise the level of antioxidant enzyme substrates and intracellular antioxidant substances such as ascorbic acid (Smirnoff, 2013). Ascorbic acid is a small water-soluble molecule that has high antioxidant properties and plays a role as primary substrates in cyclic pathways for detoxification and neutralization of superoxide and single oxygen radicals (Hemmati *et al*., 2018). It also plays a role as a secondary antioxidant in the opening of α-tocopherol and other lipophilic antioxidants. This antioxidant molecule, along with other components of the antioxidant system, protects plant cells against oxidative damage caused by aerobic metabolism, photosynthesis and respiration, and even pollution (Elstner, 1991; Seminario *et al.*, 2017).

Ascorbic acid has a central role in photosynthesis and is found in high concentrations in chloroplast. Ascorbic acid plays a role in biochemical reactions in plants in three ways (El-Khamissi *et al*., 2018). First, it acts as an antioxidant directly in eliminating hydrogen peroxide produced by photo-reduction of oxygen in photosystem I (PSI). Second, mono-dehydro ascorbate produced by ascorbate peroxidase is a direct electron acceptor in photosystem PSI. Third, ascorbic acid is a cofactor for the viola-xanthin cycle, which protects plants against photo-oxidative damage (Foyer and Hanke, 2022). In addition, it has been found that ascorbic acid plays a series of roles in plant growth, such as cell division and elongation, cell wall

development, and other developmental processes (Vahdati *et al*., 2010; Malik and Ashraf, 2012; Latif *et al*., 2016).

Many works have already been carried out on this plant's medicinal and ornamental importance and growth regulator effects but the drought effects on this plant attracted a little attention (Aruna *et al*., 2015; Kaushik *et al*., 2017; Alhaithloul *et al*., 2019) The *C. roseus* is highly likely to experience prolonged water deficit, this investigation explores the effect of ascorbic acid foliar application on its morphological and physiological traits when grown under water deficit.

MATERIALS AND METHODS

The present study was carried out in the greenhouse located in the Kishestan greenhouse-town of Guilan province in the form of pots in 2019. This experiment was conducted as a factorial in the form of a completely randomized design (CRBD) with three replications. Experimental factors include: Drought stress (I) at 4 levels based on field capacity (FC): 1) Control (no stress) (I1) Irrigation at 100% FC, 2) Mild stress (I2) Irrigation at 75% FC; 3) Medium stress (I3) Irrigation at 50% FC; and 4) Severe stress (I4) Irrigation at 25% FC.

Foliar application of ascorbic acid (A) at 4 levels: 0, 25, 50, 100 mM, which are shown as A1, A2, A3, and A4, respectively.

In February 2019, F1 sterilized seeds of *C. roseus* were sown in plastic pots (19 cm in diameter ×17 cm in depth) filled with a 50% field soil +30% peat + 20% perlite substrate. After germination, the seedlings were well-watered until the age of 50 days. Thereafter, plants were subjected drought stress. Pots were kept in a greenhouse maintained at 65% humidity and a 12 h photoperiod under normal (17–22 ◦C) temperature.

The first foliar application of ascorbic acid took place 45 days after plant emergence, and the second application took place 60 days after plant emergence.

Traits measurement

Morphological traits

The flower number plant^{-1} was averaged in four plants per plot from the beginning of flowering until 50% of the flowers have wilted. Root length, number of nodes, internode length, and plant length were measured on four plants per plot at the end of the experiment. At the end of the experiment, plant, shoot and root fresh weights (FW) were measured using a 0.01-precision digital scale on four plants per plot. Plants were then oven-dried at 70 °C for 48 hours to calculate the dry weights.

Physiological traits

Before the end of the experiment, samples were taken to measure physiological traits.

Chlorophyll and carotenoid

The procedure of Arnon (1949) was used to measure chlorophyll a, b and total chlorophyll contents. To this end, the fresh leaves were extracted using 80 % acetone and it was read at 660 and 643 nm with a spectrophotometer (Apel-PD-303 UV). The following equations were employed to determine chlorophyll a, b and total chlorophyll in mg g^{-1} FW:

$$\text{Chlorophyll a} = 9.93(A_{660}) - 0.777(A_{643})$$
$$\text{Chlorophyll b} = 17.6(A_{643}) - 2.81(A_{660})$$
$$\text{Total chlorophyll} = 7.12(A_{660}) + 16.8(A_{643})$$
$$\text{Carotenoid} = 4.69\ (A_{440}) - 0.268\ (20.2)\ A_{645} + 8.02\ (A_{663})$$

Proline content of leaf

Proline content was measured using the method of Bates *et al*. (1973). An extract of 0.5 g of dry matter was prepared with 10 ml of 3% sulfo-salicylic acid and centrifuged at 6500 rpm for 20 minutes. 2 ml of centrifuged extract were added to 10 ml distilled water, 2 ml ninhydrin acid and 2 ml glacial acetic acid, and the samples placed in a 100 °C hot bath for 90 minutes. After cooling, 4 ml toluene was added to each sample and the samples were shaken for 30 minutes. The clear surface layer of the samples was read at 520 nm with a spectrophotometer and the amount of proline was obtained in mg g^{-1} FW with the aid of a standard curve.

Soluble carbohydrates content of leaf

Soluble carbohydrates or sugar in water were measured with anthrone reagent. After preparing the samples, the amount of soluble sugars was measured at a wavelength of 630 nm by a spectrophotometer. By using standard solutions and establishing a regression relationship between the concentration of the solution and the absorbance value at 630 nm, the concentration of water-soluble carbohydrates was determined in terms of ppm for each sample and then converted to mg g^{-1} DW (Yimm and Willis, 1954).

Catalase (CAT) activity

To determine the activity of CAT enzyme in the enzyme extract, the method (Chance and Maehly, 1955) was used. In this way, a mixture of 680 microliters of 100 mM H_2O_2 and 60 microliters of enzyme extract was taken and the volume of the solution was brought to 2 mg using 50 mM phosphate buffer solution (pH=7). Then, the resulting mixture was subjected to spectrometry at a wavelength of 240 nm, once every 5 seconds for 5 minutes. Then, the specific activity of the enzyme is converted into absorbance changes in unit/mg Pro min.

Statistical analysis

All data were subjected to combined analysis of variance (ANOVA). Significance of the effect of treatments was determined by the magnitude of the F-value ($P < 0.05$). In case F-test was significant for interactions, separation of the means were done using the LSMEANS procedure with the least significant difference (LSD) adjustment at $P < 0.05$. Statistical analysis of the results was performed using general linear model (GLM) in SAS software version 9.2.

RESULTS

Leaf chlorophyll content

The results of analysis of variance showed that the interaction effect of I × A on the concentration of chlorophyll a and b were significant (Table 1). The mean comparison of the interaction effect of I × A on the concentration of chlorophyll a showed that in 100 mM ascorbic acid under water deficit levels I1 and I4 showed the highest concentration of chlorophyll a, but in levels I2 and I3, between the levels of 100 and 25 mM ascorbic acid to statistically there was no significant difference (Fig. 1). Also, the highest amount of chlorophyll b was observed in severe water deficiency (I4) and the application level of 25 mM ascorbic acid (4.753 mg g^{-1} FW) was superior to other treatments (Fig. 2).

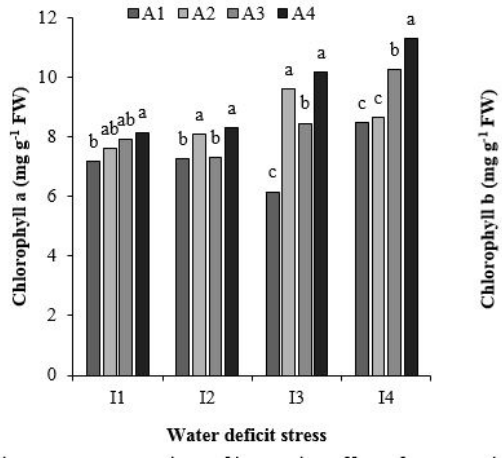

Fig. 1. Mean comparison of interaction effect of I × A on chlorophyll a.

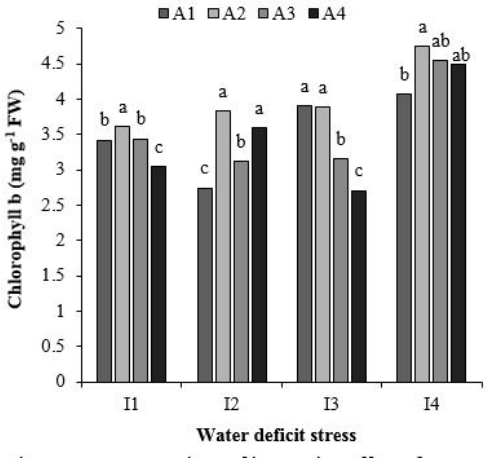

Fig. 2. Mean comparison of interaction effect of I × A on chlorophyll b.

Table 1. Analysis of variance the effect of ascorbic acid levels and water deficit stress on chlorophyll a, chlorophyll b, total chlorophyll and leaf carotenoids.

SoV	df	MS			
		Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoid
		mg g^{-1} FW			
I (Irrigation)	3	2.65*	0.319^{ns}	1.66^{ns}	3.69**
A (Ascorbic acid)	3	1.25^{ns}	0.539*	3.21^{ns}	7.42**
I × A	9	4.07**	0.896**	2.03^{ns}	2.275**
Error	32	0.898	0.195	1.65	0.535
CV (%)	-	11.8	12.4	16.09	6.23

*, ** and ^{ns}: Significant at P < 0.05, P < 0.01 and insignificant based on the LSD test, respectively.

Leaf carotenoid content

The results of analysis of variance showed that the interaction effect of I × A on leaf carotenoid concentration was significant at the level of 1% probability (Table 1). The mean comparison of the interaction effect of I × A on leaf carotenoid concentration showed that at each level of water deficit stress, there was a different reaction to the application of ascorbic acid, so that in level I1, between zero and 25 mM levels, they were superior to the other two levels. but there was no significant difference, while in levels I2, I3 and I4 showed the highest carotenoid concentration was obtained at 25, 50 and 100 mM levels of ascorbic acid respectively, and the highest carotenoid concentration from 100 mM level + I4 level of ascorbic acid (with an average of 13.35 mg g^{-1} FW) (Fig. 3).

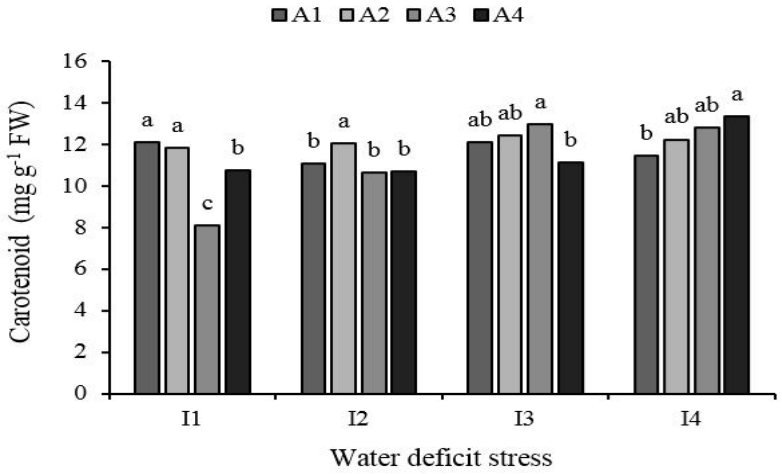

Fig. 3. Mean comparison of interaction effect of I × A on leaf carotenoid.

Root dry weight

The results of analysis of variance showed that the interaction effect of I × A on root dry weight was significant (Table 2). The average comparison showed that the dry weight of the root decreased in all treatments with the increase in the severity of water deficit. And there was a significant difference between the levels of ascorbic acid in all water stress levels. In most water deficit levels, 50 (A3) and 100 (A4) M levels were superior to A1 and A2 levels, although there was no significant difference between them in some stress levels (Fig. 4).

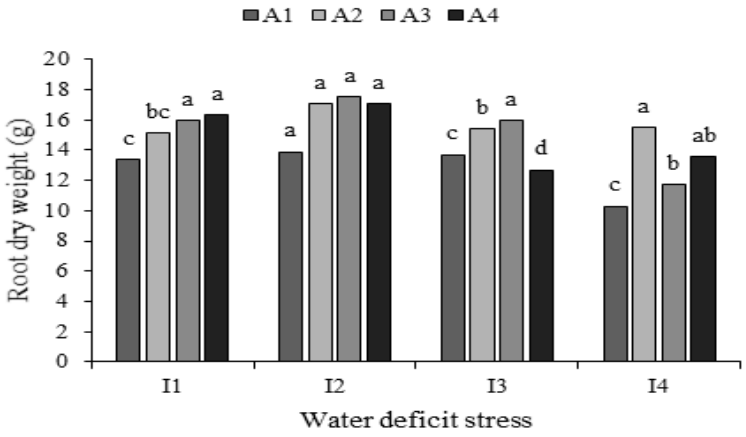

Fig. 4. Mean comparison of interaction effect of I × A on root dry weight.

Plant biomass

The results of analysis of variance showed that the interaction effect of I × A on total plant biomass was significant (Table 2). The average comparison showed that with the increase in the severity of water deficit, the biomass of the whole plant decreased in all treatments. Except for the I2 stress level, in all water deficit levels, the application of ascorbic acid significantly increased the biomass of the whole plant compared to the level of no ascorbic application (A0). In total, the highest biomass of the whole plant was obtained from A4 + I1 (Fig. 5).

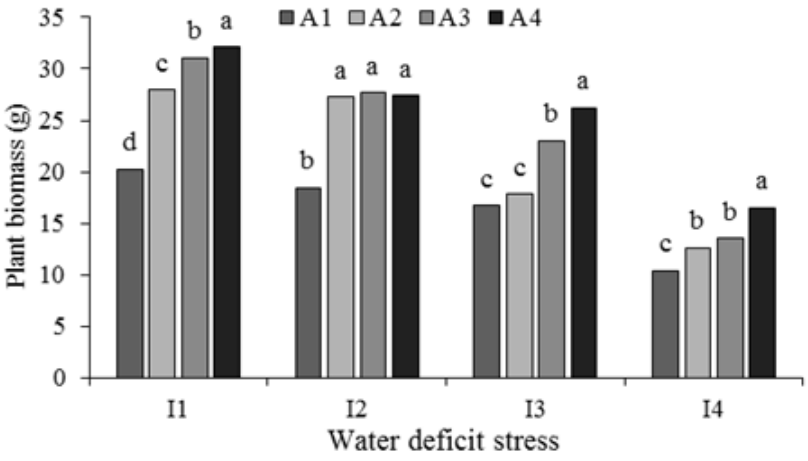

Fig. 5. Mean comparison of interaction effect of I × A on plant biomass.

Flower number plant^{-1}

The results of analysis of variance showed that the interaction effect of I × A on Flower number plant^{-1} was significant at the level of 1% probability (Table 2). A comparison of the means for all treatments shows that the that in 50 and 100 mM ascorbic acid under water deficit levels I1 showed the highest flower number (28.6 and 30.8 flowers plant^{-1}), but in levels I4 between the levels of 25 and 50 mM ascorbic acid to statistically there was no significant difference and even level 100 mM ascorbic acid showed a lower number of flowers (Fig. 6).

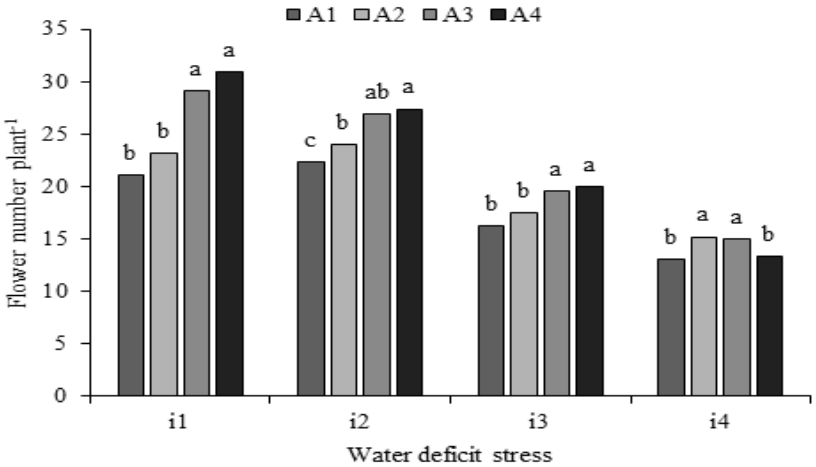

Fig. 6. Mean comparison of interaction effect of I × A on flower number plant^{-1}.

Ion leakage content

The results of analysis of variance showed that the ion leakage percentage was only affected by water deficit (Table 3). The comparison of the main effect of water deficiency on the percentage of ion leakage showed that as the intensity of water deficiency stress increased, the percentage of ion leakage increased so that the maximum ion leakage was obtained from the I4 level (Fig. 7).

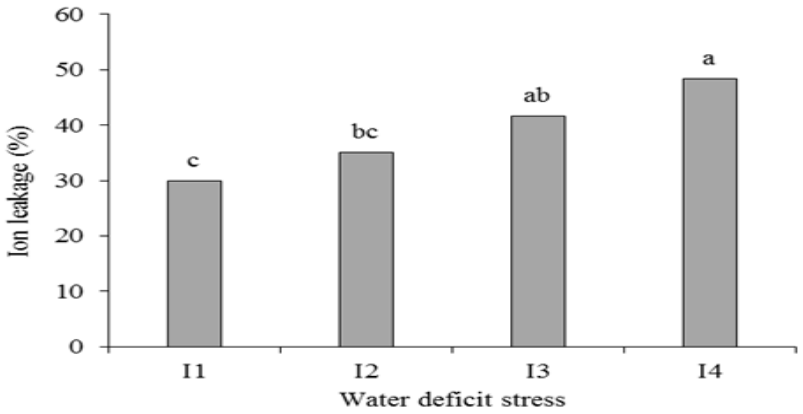

Fig. 7. Mean comparison of main effect of water deficit on ion leakage.

Table 2. Analysis of variance the effect of ascorbic acid levels and water deficit stress on root weight, total biomass, flower number and plant height.

		MS			
SoV	df	Root weight	Total biomass	Flower number plant^{-1}	Plant height (cm)
		g plant^{-1}			
I (Irrigation)	3	33.96**	112.7**	19.1*	8.20^{ns}
A (Ascorbic)	3	17.4**	55.3**	188.6**	1.23^{ns}
I × A	9	8.279*	47.9**	98.5**	2.48^{ns}
Error	32	3.22	8.85	4.28	3.37
CV (%)	-	7.81	10.62	11.09	26.37

*, ** and ^{ns}: Significant at P < 0.05, P < 0.01 and insignificant based on the LSD test, respectively.

Table 3. Analysis of variance the effect of ascorbic acid levels and water deficit stress on ion leakage, prolin, soluble carbohydrates and catalase.

		MS			
SoV	df	Ion leakage (%)	Proline	Soluble carbohydrates	Catalase (unit mg^{-1} Pro min)
			mg g^{-1} DW		
I (Irrigation)	3	294*	0.319^{ns}	1789**	0.0128**
A (Ascorbic)	3	47.5^{ns}	0.539*	71.1*	0.00182^{ns}
I × A	9	26.9^{ns}	0.896**	492**	0.0038^{ns}
Error	32	77.5	0.195	24.5	0.0017
CV (%)	-	32.8	12.4	16.9	33.9

*, ** and ^{ns}: Significant at P < 0.05, P < 0.01 and insignificant based on the LSD test, respectively.

Leaf soluble sugar content

The results of analysis of variance showed that the triple interaction effect, I × A × C, on leaf soluble sugar content was significant (Table 3). The average comparison showed that in both fenugreek cultivars in the conditions of no water shortage and irrigation at 25% of the field capacity, the levels of 50 and 100 mM ascorbic acid showed the highest concentration of soluble sugar, but in the conditions of irrigation at 35 and 50% of moisture depletion, the levels 50 and 100 mM compared to zero and 25 mM levels of ascorbic acid showed lower soluble sugar. In total, the highest concentration of soluble sugar was obtained in untreated plants with ascorbic acid (A1) + irrigation at 50% FC (I4), with an average of 3.24 mg g^{-1} DW (Fig. 8).

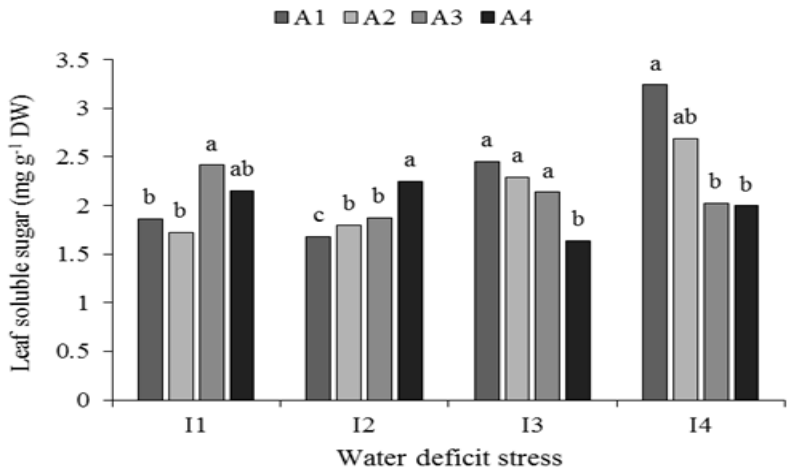

Fig. 8. Mean comparison of interaction effect of I × A on leaf soluble sugar.

Leaf proline content

The results of analysis of variance showed that the double interaction effect, I × A, on leaf proline concentration was significant (Table 3). Comparison of the average interaction effect, I × A on leaf proline content showed that in the condition of no stress (I1) there was no significant difference between the levels of ascorbic acid, but with the increase in the severity of water deficit, the difference between the levels of ascorbic acid in terms of proline increased and the application levels increased ascorbic acid (A1) did not use ascorbic acid, increased the proline concentration of the plant, so the levels of ascorbic application compared to A1 increased the concentration of proline in levels of water deficit stresses I2, I3, and I4, respectively, 10.1 - 19.4%, 12.3 - 35.5, and 24.6 - 49.3. In total, the highest concentration of proline was obtained from plants treated with 100 mM under 35% moisture depletion with an average of 45.08 mg/g fresh weight (Fig. 9).

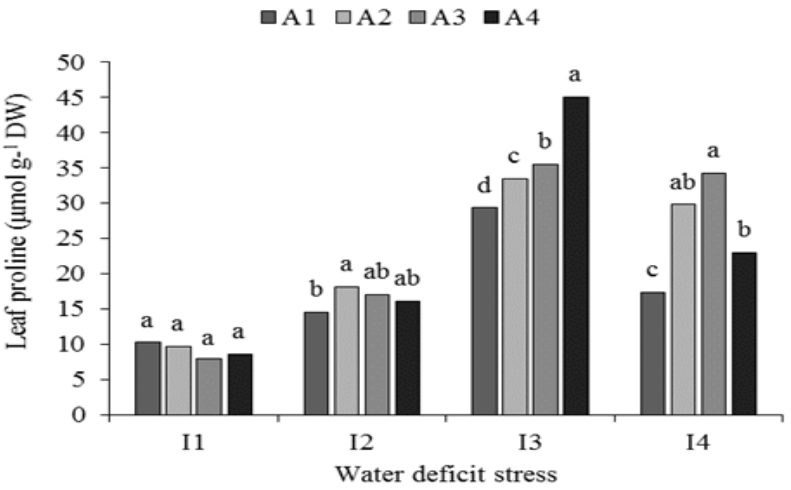

Fig. 9. Mean comparison of interaction effect of I × A on leaf proline content.

Catalase activity

The results of analysis of variance showed that the main effect of water deficiency and ascorbic acid on catalase (CAT) activity was significant (Table 3). Comparison of the main effect of water deficit on catalase enzyme activity showed that with the increase of water deficit intensity, catalase enzyme activity increased so that the maximum activity of catalase enzyme was obtained from I4 level (Fig. 10). The comparison of the main effect of ascorbic acid on catalase enzyme activity showed that by increasing the use of ascorbic acid up to A3 level, catalase enzyme activity increased significantly, but at A4 level, its activity decreased. Application (A1) showed significant superiority (Fig. 11).

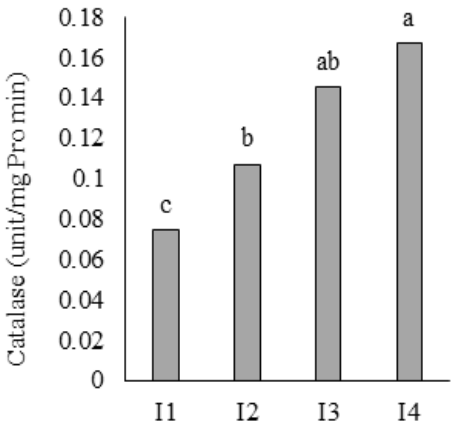

Fig. 10. Mean comparison of main effect of water deficit on catalase enzyme activity.

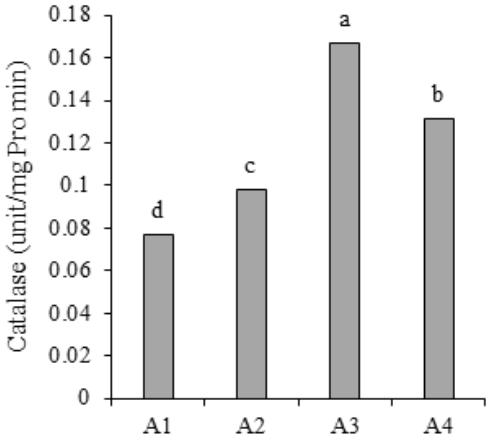

Fig. 11. Mean comparison of main effect of ascorbic acid on catalase enzyme activity.

DISCUSSION

The present study investigated the effect of different levels of ascorbic acid on increasing plant tolerance to water deficit conditions, and the results showed that water deficit has a negative effect on the morphological and physiological traits of *C. roseus*, but the application of ascorbic acid reduces the negative effect of drought stress. The examination of leaf chlorophyll content revealed that under water deficit I_1 and I_4, foliar application of 100 mM ascorbic acid showed the highest concentration of chlorophyll a, but at the level of I_2 and I_3, there was no statistically significant difference between the levels of 100 and 25 mM ascorbic acid. The highest amount of chlorophyll b was also obtained in severe water deficit (I_4) and application of 25 mM ascorbic acid. A decrease in chlorophyll a, b and total of *Catharanthus roseus* due to drought stress has been reported (Hashemabadi *et al.*, 2019; Ali *et al*., 2021). By examining drought stress on two species of *Populus cathayana* for 12 weeks in greenhouse conditions, they determined that the reason for the decrease of chlorophyll in severe drought stress is the slow rate of synthesis or rapid decomposition of chlorophyll (Joshi and Bains, 2021). Quantification of ascorbic acid, bioactive compounds and antioxidant activity in some unconventional leafy greens. (Arzhang *et al*., 2015). Other factors include the reduction of chlorophyll content when plants are exposed to drought stress, the production of reactive oxygen species, and subsequent lipid peroxidation and chlorophyll degradation. In general, water stress through the reduction of the leaf surface, the closing of the stomata, the reduction in the ability of the stomata to conduct, the reduction in water absorption of chloroplasts and other parts of the protoplasm (which somehow reduces the efficiency of photosynthesis), the reduction of protein and chlorophyll synthesis causes

a reduction The process of photosynthesis. Water stress can directly affect the biochemical processes related to photosynthesis and indirectly reduce the entry of carbon dioxide into the stomata that are closed due to dehydration conditions. Therefore, the transfer of photosynthetic materials is also affected by water stress and limits photosynthesis. It is obvious that by limiting photosynthetic products in water shortage conditions, plant growth and ultimately its performance suffer violations (Halopheoes *et al*., 2013).

Ascorbic acid, along with antioxidant enzymes, plays a role in neutralizing superoxide ions, which are produced by the Mehler reaction and photorespiration (Noctor and Foyer, 2016). Foliar application of ascorbic acid increases resistance to drought stress and reduces the harmful effect of oxidative stress (El-Beltagi *et al*., 2022). In response to the increase in the production of active oxygen species, the capacity of antioxidant defense and the activity of antioxidant enzymes increase (Noctor and Foyer, 1998).

It has been suggested that ascorbic acid has an effect on the plasma membrane and ATP-ase proton pumps (Carrasco-Luna *et al*., 1995) and according to the acid hypothesis it causes cell wall loosening factors. As a result, the development of the cell wall increases and the cell enlarges (Rayle and Clelend, 1992). There is also evidence of increasing the progress of cell division from G1 to S phase of onion root meristem by external application of ascorbic acid (Arrigoni, 2014).

Unlike many metabolites, the amount of soluble sugars in the leaves increases drastically with the increase of drought stress in the presence or absence of ascorbic acid. This increase in leaf soluble sugars is completely related to stress and may be due to the breakdown of starch and non-reducing sugars such as sucrose in drought stress and the production of reducing sugars. However, the decrease in the amount of starch and non-renewable leaf sugars such as sucrose in drought stress has also been reported in other plants, including soybean (Huber *et al*., 1984) and other species of the Fabaceae family (Keller and Ludlow, 1993), which may be due to a decrease in the speed Photosynthesis or increasing the speed of decomposition of sucrose and starch. Also, in the leaves of a type of bean, an increase in the activity of enzymes that decompose starch (such as amylase) and sucrose (such as acid invertase and sucrose synthase) has been observed, which can cause a decrease in starch and sucrose in the leaves and an increase in the accumulation of reducing sugars (Keller and Ludlow, 1993). A change in the starch concentration of leaves may indicate a change in the ratio of source to reservoir. In fact, in drought stress, due to the reduction of the source-reservoir ratio, carbon partitioning between sucrose and starch changes and sucrose export increases, and as a result, starch concentration in leaves decreases (Goldschmidt and Huber, 1992). Therefore, reducing the osmotic potential and ascorbic acid treatment probably by changing the source-to-reservoir ratio on the one hand and by increasing the activity of starch and sucrose degrading enzymes on the other hand, will cause a decrease in the amount of starch and sucrose in the leaf and an increase in reducing sugars.

In stress conditions, the concentration of proline amino acid increases compared to other amino acids. It seems that ascorbic acid reduces the damaging effect of stress by scavenging oxygen free radicals, reducing damage to fatty acids and proteins. Therefore, proline accumulation is reduced as one of the plant's responses to stress. Changes in metabolism and conversion of sugars in stress conditions have a decisive role in stress tolerance.

One of the mechanisms that plants use to deal with the harmful effects of water deficit is the synthesis of compatible solutions, including soluble sugars and proline, which are involved in osmotic regulation and preferably keep the membrane surface hydrated (Zhu *et al*.,

2018; Akram *et al*., 2020). But it seems that in this experiment, osmolytes also did not enable *Catharanthus roseus* plants to overcome the effects of drought stress, because the tissue water percentage decreased in these plants under drought stress.

Proline is an osmotic substance and osmotic protector in maintaining water balance, maintaining the stability of proteins, C and N storage source for growth after stress relief, reducing the risks of ROS production, scavenging hydroxyl radicals and quenching singlet oxygen (and as a result It plays a role in protecting cellular macromolecules such as proteins, lipids, and DNA from free radical damage, regulating cellular pH, and regulating the $NADP^+$/ NADPH ratio) (Verbruggen and Hermans, 2008). Accumulation of proline in plant tissues can result in:

a) Decrease in proline breakdown, b) Increase in proline biosynthesis, c) Decrease in protein synthesis or use of proline and d) Hydrolysis of proteins. Therefore, increasing the amount of proline in drought stress in plant cells can be explained in different ways (Saudy *et al*., 2021).

An increase in the concentration of sugars, in addition to causing the osmotic potential in the cytoplasm to become more negative, also plays a role in the osmotic protection of membranes and proteins, as well as the scavenging of free oxygen radicals (Hemmati *et al*., 2018), the most important of which is the protection of membranes and walls cellular. In this study, despite the decrease in the amount of photosynthetic pigments and the decrease in tissue water and growth indicators, the accumulation of soluble sugars in the leaves of the plant led to the improvement of the cell wall. There is a direct relationship between physiological processes such as: photosynthesis, transport, respiration, growth and changes in the amount of carbohydrates. The use of ascorbic acid increased photosynthetic pigments and tissue water content and improved growth indicators and reduced the accumulation of soluble sugars. Smirnoff (2013) believes that ascorbic acid, as a small molecule but with a high physiological power, can induce the processes of material production and especially the production of sugars in a direction that ultimately leads to plant growth.

CONCLUSION

The obtained results showed that the concentration of chlorophyll and carotenoid, leaf soluble sugar, proline amount, protein amount and catalase enzyme activity increased with increasing severity of water deficit, but the morphological traits of plant height, dry weight of root and shoot decreased. The application of ascorbic acid led to the improvement of the measured traits, and the results of the application of ascorbic acid were significantly superior to its non-use, and the level of 50 mM had a greater effect on most of the traits than other levels. As a non-enzymatic defense mechanism, ascorbic acid seems to have played a key role in the resistance of *C. roseus* to water deficit stress. In fact, it plays a role as a primary substrate in cyclic pathways, for detoxification and neutralization of superoxide and single oxygen radicals, and also as a secondary antioxidant in the α-tocopherol cycle and other lipophilic antioxidants. According to the obtained results, it is recommended to use ascorbic acid to improve the growth of plants such as *C. roseus* under drought stress.

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