External application of salicylic acid on physiological changes and yield of sweet pepper (*capsicum annuum*) under drought stress

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Abstract

Drought is a global crisis that poses a serious threat to agriculture, which reduces growth as well as yield of plants. Salicylic Acid (SA) is a phenolic growth regulator that has a protective role against stress conditions in the plants. The purpose of the present research was to investigate the impact of foliar spray of SA on the resistance of sweet pepper plants to drought. This experiment was performed as factorial in a completely randomized design, and treatments consisted of three levels of salicylic acid (0, 0.5 and 1mM) and three levels of drought stress (irrigation at 100, 60, and 30% of field capacity). Findings suggested that drought reduced plant growth factors such as number and weight of pepper fruit, yield, plant height, photosynthetic pigments (chlorophyll a, b, and total) and carotenoids. It also increased activity rate of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), soluble sugars, proline, anthocyanins, malondialdehyde (MDA), and H_2O_2. SA treatment improved plant growth parameters, enhancing CAT, SOD, POD, chlorophyll a and b, proline contents, total chlorophyll, carotenoids, soluble carbohydrates, anthocyanins while decreasing the content of MDA and H_2O_2. The study concludes that application of SA enhances the activity of the enzymes and decreases the harmful impacts of drought.

Keywords: antioxidant enzymes, *capsicum annuum*, malondialdehyde, proline, salicylic acid

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Introduction

Bell Pepper (*Capsicum annuum* L.) is a thermophilic vegetable highly sensitive to drought stress, which can reduce crop yield and quality. Attention to irrigation is crucial during all growth stages to avoid smaller fruits and decreased yield (Yang et al., 2022).

Drought reduces the growth and development of plants, especially in arid and semi-arid areas of the world (Xie et al., 2019). Water-deficit prevents cell proliferation, slows down the growth and development, and leads to a decrease in the plant yield (Anupama et al., 2019). Drought increases production of reactive oxygen species (ROS) contents of superoxide (O_{-2}) and hydrogen peroxide (H_2O_2). ROS have potential to react with many cellular constituents and distort vital cellular macromolecules and other important macromolecules. In plant cells, antioxidant enzymes protect them from oxidative damage (Sun et al., 2015). One of the physiological mechanisms in plants is osmotic regulation by

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aggregation of solutes in cells; it can adapt to survive plants under drought stress (Kim et al., 2019). The most important compatible solutes include proline and soluble sugars that play an influential role in osmotic regulation in plants (Parmoon et al., 2019). A study conducted on wheat showed a significant positive correlation between proline accumulation and increased antioxidant activity as well as decreased ROS levels in the plant (Anupama et al., 2019). La et al. (2019)observed that leaf spray of SA enhanced proline metabolism as well as photosynthetic pigments. This can be considered as a reason to reduce the harmful impacts of drought on *B. juncea* L. and sunflower plants as result of positive impacts of salicylic acid.

Salicylic acid, a natural growth regulator, plays a role in plant physiological processes during biotic and abiotic stress. Research on wheat, tomato, and bean has demonstrated that SA can influence physiological and biochemical changes under drought conditions (Galviz et al., 2021). The present research has investigated the effect of SA on physiological and biochemical factors of sweet pepper seedlings under drought conditions.

Materials and Methods

Experimental details

This research was conducted as a factorial experiment in a completely randomized design in a pot in the Greenhouse of the Faculty of Agriculture, University of Mohaghegh Ardabili. The temperature of the greenhouse during the growing season was 18 °C and 25 °C night/day, and the relative humidity was 65%. Drought stress (control, soil moisture at 60% of field capacity, and soil moisture at 30% of field capacity) and salicylic acid treatment as foliar spray (0, 0.5 mM, and 1 mM) were applied with three replications. Each replication consisted three pots of 20 and 23 cm in diameter and height, respectively.

First, the seeds were sown in the chassis, and they were transferred to pots containing field soil, wind-blown sand, and leaf soil (6 kg of soil mixture) at 3-4 leaf stage. To determine the soil characteristics, the soil mixture was submitted to laboratory analysis (Table 1). Then, the pots were irrigated at an equal rate and at field capacity. At this stage, salicylic acid treatment was carried out as a foliar spray, and Tween 20 was used as a surfactant in the preparation of each concentration of salicylic acid. Irrigation treatments were carried out 72 hours after the application of salicylic acid until sampling, with daily weighing of the pots and addition of water consumed due to evaporation and transpiration. Young leaves were used to measure the desired factors. To determine their physicochemical parameters, leaf samples were collected from pepper seedlings at the green stage, characterized by 80% maturity. Subsequently, the samples were immediately immersed in liquid nitrogen and stored at a temperature of -80 °C.

Table 1
Physiochemical properties of the experimental soil

Characteristics	Units	Values
Moisture content	(%)	32
Phosphorus (P)	(ppm)	3.47
Potassium (K)	(ppm)	33.63
pH		7.3
Sand	(%)	22
Clay	(%)	11
Silt	(%)	67
Soil texture		Silty loam
Electronic Content (EC)	(ds/m)	0.7
Organic carbon	(%)	0.42
Total Nitrogen	(%)	0.04

Measurement of fruit yield

In conditions such as lack of humidity, high temperature, and sensitivity of the plant cultivar to drought, growth and economic performance are reduced, so it is not possible to keep the fruit on the plant in the greenhouse. As a result, the peppers were harvested four times. At the time of harvest, they were green and fully grown. Finally, some parameters such as plant weight, number of plants and total weight of fruit per plant were measured and total yield was evaluated.

Measurement of malondialdehyde

Malondialdehyde was measured following Ohkawa et al. (1979). About, 0.2 g of the leaf sample was homogenized with a homogenizer in 2 ml of 5% ice-cold trichloroacetic acid. After centrifugation at 12,000 rpm for 15 min the supernatant were removed. Then, 0.5 ml of the solution was mixed with 0.5 ml of 20% thiobarbituric and trichloroacetic acid solutions and incubated at 96 °C for 25 min, and then centrifuged at 10,000 rpm for 5 min in cold conditions. The supernatant adsorption was measured at 532, 450, 600 nm. Thiobarbituric 20% trichloroacetic acid solution was used as control.

Estimation of soluble carbohydrates

The amount of carbohydrates was determined using the method of Irigoyen et al. (1992). About 0.5 g of leaf sample was added to a porcelain mortar containing 95% ethanol and crushed before 70% ethanol was added to the supernatant and centrifuged. Following this, 3 ml of Entron reagent was added to 100 µl of the supernatant solution and placed in a boiling water bath, and finally the adsorption of the solution was recorded at a wavelength of 625 nm.

Estimation of proline contents

Bates et al. (1973) were followed to measure proline. For this purpose, we crushed 0.5 g of fresh plant leaves in a mortar containing 10 ml of 3% sulfosalicylic acid, then put the solution in a test tube, leaving it at room temperature for 10 min. The solution was than homogenized using a 15,000 g centrifuge for 10 min at 4 °C. After homogenization, 2 ml of the supernatant was added to the test tube containing 2 ml of ninhydrin and vortexed. The samples were heated at 100 °C for 1 hour. After cooling, 4 ml of toluene was added to the cold solution and vortexed again. The absorbance was read at 520 nm.

Measurement of chlorophyll and carotenoids

These parameters were calculated using the method of Lichthentaler (1987). In this method, about, 0.2 g of fresh leaves was completely crushed with 5 ml of distilled water in a Chinese mortar. The mixture was transferred to a test tube and increased to 12.5 ml, then 0.5 ml of the extract was mixed with 4.5 ml of 80% acetone and the solution was centrifuged at 3000 g for 10 min. Absorption was read at 663, 646 and 470 nm.

Determination of anthocyanins

Anthocyanin content was determined based on Wagner method (Wagner, 1979). About, 0.1 g of fresh leaf tissue was crushed in a mortar containing 10 ml of acidic methanol (methanol and hydrochloric acid, 1:99). The extract was transferred to a test tube and kept in the dark at 25 °C for 24 hours, then centrifuged at 4,000 g for 10 min and the absorbance was read at 550 nm.

Determination of hydrogen peroxide

Velikova et al. (2000)method was used to estimate the amount of hydrogen peroxide. Following this, 0.5 g of leaf tissue was crushed in a mortar with 30 ml of 0.1% trichloroacetic acid. The extract was centrifuged at 10,000 g for 15 min at 4 °C. Then, 0.5 ml of the supernatant was added to 0.5 ml of 10 mM potassium phosphate buffer and 1 mL of 1 M potassium iodide, and the absorbance was read at 390 nm.

Enzyme extraction method

Leaf samples (0.5 g) were homogenized in 100 mM potassium phosphate buffer (pH: 7.8), 5% polyvinylpyrrolidone, and 1 mM EDTA before centrifuging at 10,000 g for 30 min. The supernatant was used to assay antioxidant enzymes (Korkmaz et al., 2013).

Measurement of catalase enzyme

Catalase was measured using Dhindsa et al. (1981)method. Catalase activity was measured by calculating the reduction of hydrogen peroxide at 240 nm. The reaction mixture contained 3 ml of 15 mM phosphate buffer (pH: 7) and 15 mM hydrogen peroxide. To start the reaction, 0.1 ml of enzymatic extract was added to the mixture.

Measurement of superoxide dismutase (SOD) enzyme activity

Superoxide dismutase enzyme was assayed following Dhindsa et al. (1981). To this end, 3 ml of the reaction mixture including 50 mM potassium phosphate (pH 7.8), 13 mM methionine, 75 µM nitroblutrazolium chloride, 0.1 mM ethylene diamine tetra acetic acid (EDTA), 360 µM of riboflavin, and 30 µl of enzymatic extract were added to the test tubes, which were placed under fluorescent light (40 µmol m^{-2} s^{-1}) at 10 min. Then, the absorbance of the reaction mixture was read at 560 nm.

Duncan test was used to compare the means at 5% probability level.

Table 2
Comparison of mean effects of salicylic acid and drought stress on morphological parameters of pepper plants.

Treatments	Plant height (cm)	Fruit number	Fruit weight (g)	Total yield (kg)
Salicylic acid				
0 mM (control: Distilled water)	67.11^{c}	4^{c}	2.34^{b}	1.032^{c}
0.5 mM	89.50^{b}	6^{b}	2.60^{b}	1. 60^{b}
1 mM	100.185^{a}	8.44^{a}	5.57^{a}	5.60^{a}
Drought stress				
0 (control: Full irrigation)	100.34^{a}	4^{a}	4.21^{a}	4.49^{a}
60%	66.61^{b}	2.73^{c}	3.66^{c}	1.69c
30%	73.83^{b}	3.31^{b}	4.08^{b}	2.06^{b}

The same letters in each column indicate no significant difference at 5% probability level according to the Duncan test.

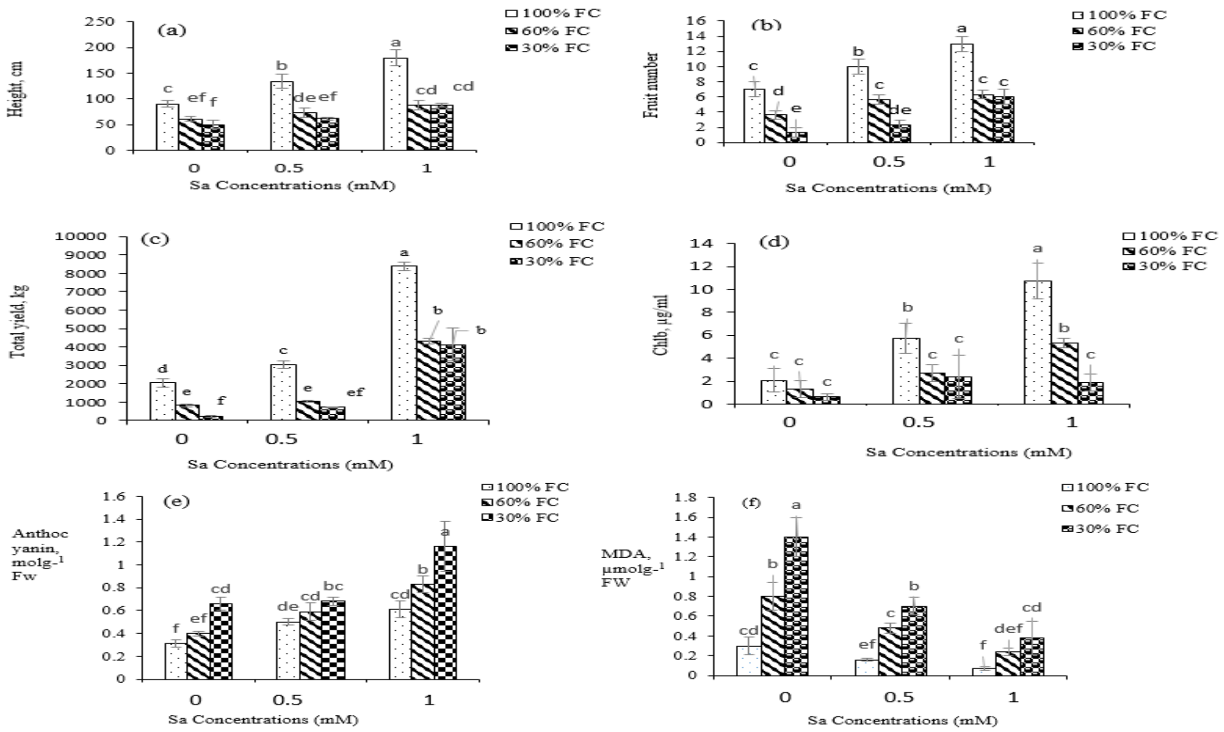

Fig. I. Interaction effects of drought stress and salicylic acid treatment on (a) plant height, (b) fruit number, (c) yield, (d) Chlorophyll b, (e) anthocyanin, and (f) MDA in pepper leaves under drought stress

Measurement of peroxidase enzyme activity

Peroxidase activity was estimated using the method of Kochba and Spiegel-Roy (1977). The reaction mixture consisted of 20 mM phosphate buffer (pH 6), 20 mM guaiacol, 40 mM hydrogen peroxide, and 40 ml of enzymatic extract. The absorption changes were read at 470 nm.

Statistical Analysis

SPSS software was used to analyze the data. EXCEL software was used to draw the graphs, and

Results

Plant height

Analysis of the obtained data (Tables 1 & 2) showed that drought stress decreased plant height. Increasing drought level decreased plant height significantly, whereas the foliar salicylic acid application enhanced plant height. The combined effect of both drought and salicylic acid treatments improved seedling height (Fig. I. a).

Fruit yield

Table 3
Mean comparison of salicylic acid and drought stress effects on Photosynthetic pigments of pepper plants

Treatments	Chlorophyll a (µg /ml)	Chlorophyll b (µg /ml)	Total Chlorophyll (µg /ml)	Carotenoids (µg /ml)	Anthocyanin (M g^{-1} FW)
Salicylic acid					
0mM (control)	6.91^{a}	1.36^{c}	8.28^{c}	2.98^{c}	0.45^{c}
0.5 mM	8.26^{a}	3.61^{b}	11.87^{b}	8.42^{b}	0.59^{b}
1 mM	8.92^{a}	5.99^{a}	14.92^{a}	16.71^{a}	0.87^{a}
Drought stress					
0(control)	10.36a	6.19a	15.53a	12a	0.47c
60%	6b	1.64c	7.75c	7.35b	0.84a
30%	7.73b	3.13b	11.78b	8.75b	0.61b

The same letters in each column indicate no significant difference at 5% probability level according to Duncan test.

Table 4
Means comparison of salicylic acid and drought stress effects on biochemistry and physiological parameters of pepper plants.

Treatments	Proline (µg g^{-1}FW)	H_2O_2 (µmolg^{-1} FW)	MDA (µmol g^{-1}FW)	Total soluble carbohydrates (mg g^{-1} DW)	SOD (Ug^{-1}FW)	POD (Ug^{-1}FW)	CAT (Ug^{-1}FW)
Salicylic acid							
0mM(control)	17.53c	0.11a	0.89a	0.15c	0.95c	1.01c	1.71c
0.5 mM	27.77b	0.02b	0.45ab	0.18b	2.28b	3.27b	4.69b
1 mM	43.09a	0.02b	0.23c	0.21a	4.15a	6.81a	10.05a
Drought stress							
0(control)	21.14c	0.03c	0.21c	0.12c	1.32c	2.60c	3.85c
60%	38.71a	0.06a	0.83a	0.24c	4.07a	5.23a	7.59a
30%	27.53b	0.05b	0.52b	0.17b	1.98b	3.26b	5.01b

The same letters in each column indicate no significant difference at the 5% probability level according to Duncan test.

In this study, fruits yield significantly decreased by higher levels of drought stress in plants. Application of SA enhanced the yield of pepper plants under drought conditions (Table 2). Moreover, combined treatment of both drought and salicylic acid improved seedling height (Fig. I. c).

Fruit yield component

SA concentrations and different levels of drought showed significant changes in fruit weight and number. Drought stress caused a significant decrement in the weight and number of fruits. Leaf application of SA enhanced the number of pepper fruits compared with control plants. As a result of SA spraying, the weight and number of fruits of pepper were significantly exacerbated (Table 2). The effect of both treatments led to an increase in this factor (Fig. I. b).

Leaf chlorophyll and carotenoids contents

In this study, photosynthetic pigment contents of pepper were significantly different compared with control plants under drought. By higher levels of drought stress, the amount of Chl a, Chl b, Chl total, and carotenoids decreased; however, exogenous SA application increased all photosynthetic pigments of leaves (Table 3). The findings showed that by increasing levels of drought and SA concentration, the content of chlorophyll b decreased and increased, respectively (Fig. I. d).

Anthocyanin content

The content of anthocyanins in plants under drought stress significantly increased compared to control plants. Also, salicylic acid increased the content of anthocyanins in pepper plants (Table 3). The interaction of both drought and SA treatments increased the amount of anthocyanin (Fig. I. e).

H_2O_2 content

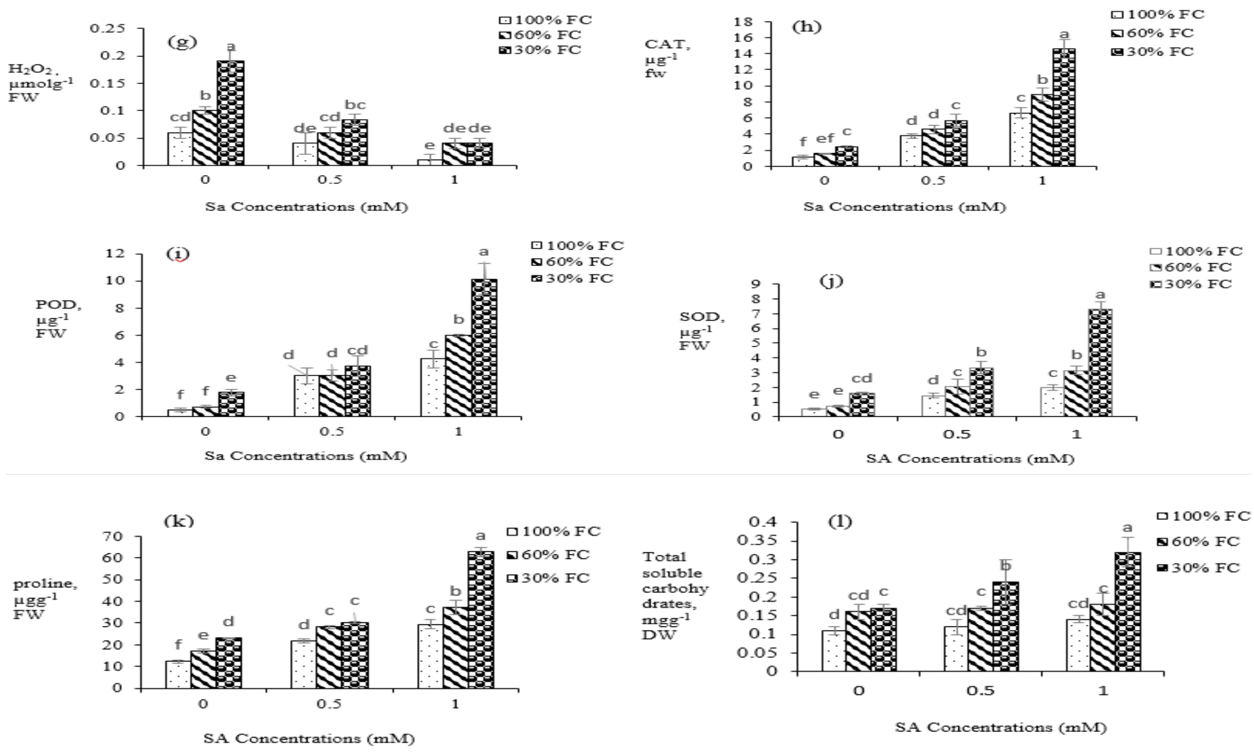

Fig. II. Interaction effects of drought stress and salicylic acid treatment on (g) H_2O_2, (h) CAT, (i) POD, (j) SOD, (k) Proline, and (l) Total soluble carbohydrates in pepper leaves under drought stress

An effect of stress on plants is their H_2O_2 content. This factor indicates the severity of stress as affecting the plants (Table 4). Drought stress led to the increased H_2O_2 production in leaves. The H_2O_2 content in leaves significantly increased under drought stress conditions; however, foliar application of salicylic acid decreased H_2O_2 content in plants. The H_2O_2 content markedly increased parallel to enhancing levels of drought and decreased when foliar SA was applied, compared with control conditions.

MDA

In plants, lipid peroxidation is used to evaluate membrane damage response to drought stress. Results of statistical analyses showed that the MDA content of leaves increased in stressed plants compared to control plants. External application of salicylic acid reduced the amount of MDA in plants (Table 4). The interaction of drought and salicylic acid treatments revealed that increasing drought stress levels led to higher MDA contents. In contrast, when the concentration of SA increased, its content decreased (Fig. I. f).

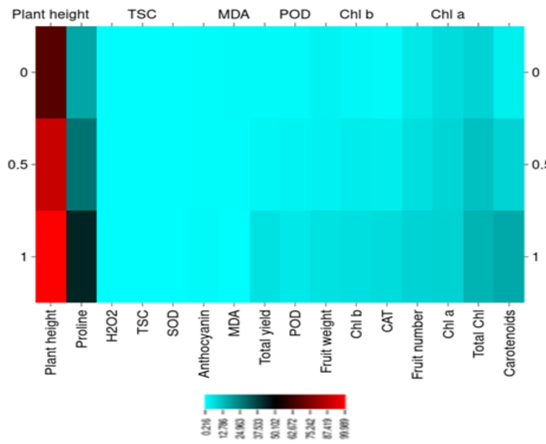

Fig. III. Heatmap showing the effects of salicylic acid treatment (0, 0.5 and 1 mM) on Plant height, Fruit number, Yield, Chlorophyll a and b, Anthocyanin, MDA, H_2O_2, CAT, POD, SOD, Proline, and Total soluble carbohydrates in pepper leaves

Soluble carbohydrates contents

In this experiment, leaf soluble carbohydrate content in pepper seedlings under different drought stress levels significantly increased. Treatment with salicylic acid spray accumulated the soluble carbohydrate content of plants (Table 4). The combined effect of both treatments showed that by higher levels of both treatments, the carbohydrate solution raised (Fig. II. i).

Table 5
Correlation coefficients (r) between pepper seedlings traits

	Plant height (cm)	Fruit number	Fruit weight (g)	Total yield (kg)	Chl. a (µg	Chl. b (µg /ml)	Chl. t (µg /ml)	Carotenoid (µg /ml)	Anthocyanin (mol g^{-1} FW)	MAD (µmol g^{-1}FW)	H_2O_2 (µmolg^{-1} FW)	CAT (ug^{-1}FW)	POD (ug^{-1}FW)	SOD (ug^{-1}FW)	Proline (µg g^{-1}FW)	Total soluble carbohydrates (mg g^{-1} DW)
Plant height	1															
Fruit number	0.94**	1														
Fruit weight	0.77**	0.77**	1													
Total yield	0.86**	0.83**	0.95**	1												
Chl. a	0.73**	0.73**	0.57**	0.56**	1											
Chl. b	0.88**	0.82**	0.76**	0.84**	0.66**	1										
Chl. t	0.79**	0.82**	0.73**	0.74**	0.82**	0.85**	1									
carotenoid	0.74**	0.74**	0.88**	0.90**	0.51**	0.78**	0.73**	1								
anthocyanin	-.06	-0.08	0.34	0.25	-0.23	-0.04	-0.06	0.43*	1							
MAD	-0.72**	-0.82**	-0.70**	-.71**	-.56**	-0.67**	-.72**	-0.77**	-0.05	1						
H_2O_2	-0.44*	-0.52**	-0.46*	-.49**	-0.32	-0.45*	-0.42*	-0.69**	-0.30	0.79**	1					
CAT	0.17	0.17	0.56**	0.48**	-0.01	0.16	0.14	0.66**	0.90**	-0.37	-0.56**	1				
POD	0.17	0.15	0.55**	0.46*	0.04	0.15	0.17	0.63**	0.86**	-0.37	-0.53**	0.96**	1			
SOD	-0.04	-0.06	0.31	0.24	-0.19	-0.08	-0.11	0.44*	0.93**	-0.17	-0.47*	0.94**	0.92**	1		
Proline	0.03	0.02	0.43*	0.35	-0.10	0.02	0.01	0.52**	0.93**	-0.22	-0.47*	0.97**	0.96**	0.96**	1	
Total soluble carbohydrates	-0.26	-0.35	0.02	-0.04	-0.38*	-0.28	-.41*	0.13	0.76**	0.11	0.29	0.73**	0.72**	0.86**	0. 78**	1

Proline

The proline content of pepper leaves increased significantly under drought compared to control plants (Table 4). Foliar spray of SA had a positive effect on proline content of leaves under stress. The combined effect of both treatments on plants showed that proline content increased after foliar spray of SA in plant leaves (Fig. II. k).

Antioxidant enzyme activities

Effects of both treatments on the activities of antioxidant enzymes (CAT, SOD, and POD) in pepper seedlings showed that both foliar application of SA and drought increased the activity of enzymes in sweet pepper seedlings (Table 4), improving the oxidative properties of pepper. The combined effect of both treatments on plants showed that the antioxidant enzyme activity increased significantly by higher levels of drought and ASA concentration (Fig. II. h, i, j).

Correlational analysis

Analysis of the obtained data (Table 5) showed a high correlation between plant height, number of fruits, fruit weight, and total yield. Also, it was found that chlorophyll a was positively correlated with total chlorophyll, CAT, and POD while negatively correlating with H_2O_2 and soluble carbohydrates. Moreover, there was a positive correlation between chlorophyll b and total chlorophyll, carotenoids, and CAT while a negative correlation was observed with H_2O_2. Total chlorophyll showed a positive and significant correlation with carotenoids CAT, POD, and SOD and also had significant negative correlation with H_2O_2 and soluble carbohydrates. furthermore, a significant positive correlation was observed between anthocyanin, CAT, POD, and SOD activities and proline while the significant correlation with H_2O_2 was negative (Table 5). Besides, correlations between anthocyanin and proline, soluble carbohydrates, and the activity of enzymes CAT, POD, and SOD were positive and significant. Significant negative correlations were observed between MDA and CAT, POD, and SOD activities as well as with H_2O_2 and proline under drought condition (Table 5). Also, there was a positive correlation between H_2O_2 level and CAT enzyme, and a significant negative correlation was observed between POD and SOD activities and proline content. Furthermore, the correlations between proline, carbohydrate, and CAT enzyme on the one hand and POD, SOD, soluble carbohydrates, and proline were significant. Also, SOD enzyme significantly correlated with soluble carbohydrates and proline. Finally, POD enzyme showed correlation with SOD, proline, and soluble carbohydrates.

Discussion

In the present study, drought stress decreased fruit morphological traits, fruit yield, plant height, and chlorophyll and carotenoids in leaves while increasing MDA, H_2O_2, soluble carbohydrates, proline, anthocyanins, and antioxidant enzymes. Drought is one of the abiotic stresses that impress various physiological and biochemical processes of plants clearly affecting their growth and development.

Plants have a number of enzymatic and non-enzymatic antioxidants. Increasing drought levels led to decrease in yield and its components (number and weight of fruit) in this study while SA application increased these attributes. This is in line with the findings of Bakundi and Yahaya (2017)who reported increased fruit water content and active cell division in sweet pepper due to water availability following SA treatment, which plays a role in the regulation of physiological processes and stress tolerance in plants. La VanHien, et al. (2019)reported that SA induces stress tolerance in plants through its effects on metabolic and physiological responses.

In this study, plant height significantly decreased under drought stress. On the other hand, SA could reduce the harmful effects of drought stress on plant height. Dianat et al. (2016), observed that plant height in *Lippia citriodora* L. significantly decreased under drought stress while SA foliar spray was able to diminish the harmful effects of drought stress on the plant, also similar to our findings in pepper seedlings.

Osmoprotectants such as proline and soluble sugars play an essential role in the adaptation and survival of plants exposed to drought and the amount of these solutions increases under stress in plants (Barnaby et al., 2019). According to the findings of this study, salicylic acid and drought stress treatments enhanced the concentration of proline and soluble sugars in both control and treated plants. This confirms the findings of the study by La et al. (2019), in *Brassica rapa* under drought. Accumulation of compatible solutions can cause osmotic stabilization of cells, the aggregation of carbon and nitrogen, resistance to water depletion, reduction of membrane damage, and improvement of plant growth under stress conditions (Abrantes et al., 2019). This is also in line with the findings of the present study.

As important component of chloroplast for photosynthesis is chlorophyll, and there is a positive relationship between chlorophyll content and photosynthesis rate. Therefore, reduced chlorophyll in drought conditions is a symptom of oxidative stress in plants. This can be a result of oxidation of photosynthetic pigments and degradation as well as reduction of chlorophyll content. Arivalagan and Somasundaram (2015)reported that photosynthetic pigment contents decreased under drought stress compared with control plants in sorghum. This is consistent with the findings of the present study.

In their study, Arivalagan and Somasundaram (2015)observed that carotenoids play a critical role via eliminating reactive oxygen species, stabilizing photosynthetic pigments, helping to conserve energy, and also reducing the side effects of drought stress on plants. Carotenoid content reduced under drought stress in sorghum plants that was in agreement with the results of the present study.

Salicylic acid-treated pepper seedlings in this study increased the amounts of photosynthetic pigments relative to control plants. Application of salicylic acid in plants such as *Sorghum bicolor* (L.) Moench improved chl. a, chl. b, and carotenoids compared with control plants (Arivalagan and Somasundaram, 2015), which is consistent with the results of the present study. Salicylic acid is suggested to enhance the synthesis of compounds such as carotenoid pigments and improve the antioxidant capacity of plants, and thereby reducing the effects of oxidative stress.

According to the statistical results of the present study anthocyanin content of pepper seedlings increased under drought stress while foliar application of salicylic acid enhanced its level. This is consistent with the findings of La et al. (2019) in *Brassica napus*. Anthocyanins play a role as osmotic regulators under stress conditions in plants that tolerate drought stress (Dabravolski and Isayenkov, 2023). The plants exposed to the highest concentration of PEG showed the maximum total antioxidant capacity; salicylic acid increased the antioxidant capacity of both control and PEG plants. It was observed that anthocyanins, through their hydrogen donor potential, can inactivate reactive oxygen species and eventually mitigate the damage caused by drought stress in plants.

In this study, the amount of MDA and H_2O_2 in plants under drought stress increased while salicylic acid treatment resulted in a significant decrease in their levels, similar to the findings of La et al. (2019) in wheat. Foliar application of salicylic acid reduced the harmful effects of stress by increasing the activity of antioxidant enzymes, thus improving the growth of pepper seedlings. Reactive oxygen species under drought stress play a role in peroxidation of cell membrane lipids, thereby altering the balance of plant cells and reducing cell membrane stabilization (Kim et al., 2019). MDA is used as an appropriate biomarker to determine the amount of lipid peroxidation induced by oxidative stress in cells (La et al, 2019). In sum, salicylic acid improves cell walls and effectively eliminates the reactive oxygen species produced during stress.

Drought stress increases the amount of reactive oxygen species. removing the ROS leads to more stress resistance in plants. Plants are able to cope with drought stress through their antioxidant system including CAT, POD, and SOD enzymes. For instance, SOD converts superoxide to H_2O_2, and H_2O_2 is further reduced to H_2O and O_2 by catalase and peroxidase enzymes (Kim et al., 2019).

Finally, in line with our study, some researchers (Lin et al., 2024)reported a significant positive correlation between changes in antioxidant enzyme activity, which could improve plant tolerance to stress. Lin et al. (2024) further found a significant positive correlation between proline and soluble carbohydrates in soybean plants under drought stress, also confirming the findings of the present study.

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