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Research Article

Impact of Salicylic Acid Application on Growth, Photosynthetic Pigments and Organic Osmolytes Response in *Mentha arvensis* under Drought Stress

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Abstract

Background and Objective: The drought stress restricts the normal growth and development of plants. Salicylic acid (SA) is considered from the essential chemical compounds used to reduce the dangerous impacts of drought stress on plants. The objective of this study was to evaluate the growth attributes, some biochemical parameters of drought-stressed peppermint plants to salicylic acid application. **Materials and Methods:** A pot experiment was carried out to investigate the response of the morphological parameters (plant height, root length fresh and dry biomass), photosynthetic pigments (chlorophyll a and b, carotenoids and total pigments) and some organic osmolytes (proline, total soluble sugar and total soluble nitrogen) of peppermint (*Mentha arvensis* L.) grain pre-soaked in SA (0.05 M) and grown under drought conditions. **Results:** The data obtained in this study showed that drought stress significantly caused a massive reduction in the growth parameters and photosynthetic pigments but caused a remarkable increase in the root length and the organic osmolytes. Furthermore, the application of SA neutralized the reverse impacts of drought stress on the peppermint plant. The highest values of all the measured parameters were observed with droughted and nondroughted plants treated with SA. **Conclusion:** In general, results showed that water stress caused negative impacts on peppermint plant growth which was mitigated by the salicylic acid pre-soaking which was accompanying with some significant modification in some physiological processes. Moreover, these findings indicate that salicylic acid can be used to enhance the drought-tolerance in peppermint plant.

Key words: *Mentha arvensis*, salicylic acid, drought, pigments, organic osmolytes

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Peppermint (*Mentha* spp.), which belongs to the Labiatae family, stands out amongst the most prevalent essential oil crops and are broadly appropriated and developed. Among the different types of peppermint, *Mentha arvensis*, *M. piperita*, *M. citrata* and *M. cardiaca* are the primary types of the class which are grown in the calm, Mediterranean and subtropical areas of the world for volatile oil production¹.

Drought stress stands out amongst the most prohibitive factors in plant development and yield in numerous regions of the world². Under drought stress, water possibilities in the root zone become negative, bringing about a decrease in the water accessibility and thus influencing the plant's development and improvement³. Drought causes modification in the plant's morphology and metabolism such as stomatal regulation and photosynthesis⁴. It decreases the plant biomass in wheat⁵, *Mentha piperita*⁶ and 6 Lamiaceae species⁴. The growth parameters such as fresh and dry weights are greatly influenced by the dry seasons⁷. The growth parameters of peppermint had significantly reduced due to drought stress in many studies such as Hassanpour *et al.*⁸ with *Mentha pulegium* L. and Khorasaninejad *et al.*⁹ with *Mentha piperita* L.

Plants have many mechanisms including accumulation of compatible organic solutes (e.g., proline, soluble sugars and nitrogen) in order to adapt stress conditions. Under water stress, the accumulation of proline may be caused by synthesis stimulation, proline oxidation inhibition and/or proteins synthesis alteration¹⁰. Proline has a preventive role in supporting osmotic regulation through membranes stabilization, cellular structures protection and cellular redox potential regulation¹¹.

Salicylic acid (SA) is phenolic compound and it controls different physiological procedures in plants². It is involved in the regulation of important plant physiological processes such as nitrogen metabolism, photosynthesis, proline metabolism, antioxidant defines system and plant-water relations under various stress conditions and so it supplies plants protection against environmental stresses¹². Many investigations had indicated that SA enhanced the resistance of plants such as soybean to fungal disease¹³, pea to salinity¹⁴ and Shara to drought¹⁵.

This investigation was carried out to understand the impact of seed pre-soaking in salicylic acid on the growth parameters (growth vigour of shoot and root), pigments content (chlorophyll a and b, carotenoids and total pigments) and some organic osmolytes responses (total soluble sugar total, soluble nitrogen and proline) of peppermint plants (*Mentha arvensis* L.) grown under drought stress conditions.

MATERIALS AND METHODS

Experimental site and time: A pot experiment was conducted in the Department of Biology, Faculty of Science and Humanitarian Studies, Sattam Bin Abdulaziz University, KSA (24.07°N, 47.58°E), from January-March, 2019. This area has a desert climate and the temperature varies from 10-44°C.

Research procedure: The seeds were supplied by the Ministry of Agriculture of Saudi Arabia. The seeds were sterilized through soaking for 3 min in 0.01% HgCl₂ and subsequently rinsed several times by sterile H₂O. The sterilized seeds were separated to two sets: the seeds of the 1st and the 2nd set were separately soaked in distilled H₂O or SA (0.05 M) respectively. On 15 January, 2019, the seeds of each set were planted in plastic pots (5 seeds/pot, 15 cm width × 20 cm height) filled with 3 kg mixed soil (clay and sand = 2:1, v/v). The pots were kept in a greenhouse and the plants were subjected to natural day/night conditions (minimum/maximum air temperature and relative humidity were 8/19°C and 10/45%, respectively). At the soil water content became below 60% of its initial value, irrigation to the field's capacity was executed.

Experimental design: After 15 days from planting, the 1st set was separated to 2 groups (12 pots/group): control and drought. Following this, the 2nd set was also separated to 2 groups (12 pots/group): salicylic acid and salicylic acid+drought. Water stress was induced by withholding water twice at the vegetative stage for 30 days. Each treated pot acquired 200 mL of water when the period ends. During the drought period, the nondroughted plants were irrigated to the field capacity.

Data collection: Three replicates were taken from each treatments of the peppermint plants that were exposed and unexposed to the drought stress twice at 4 and 6 weeks after planting (4 and 6 WAP) and were used to calculate the mean of all parameters measured (growth parameters, photosynthetic pigments, total soluble sugars, total soluble nitrogen and free proline).

Parameters measured

Growth parameters: Measurements were taken of the plant height and root system length, shoot and root system fresh weights and shoot and root system dry weights. To obtain the dry weight, the shoots and roots were taken immediately, put in sacks and left to dry in a drying oven at 80°C until the weight was stable¹⁶.

Determination of photosynthetic pigments: Leaves (0.1 g) of the peppermint plants were grounded in 80% cold acetone and centrifuged at 5000 g for 10 min. The absorbance of the purified chlorophyll samples was measured at 470, 646 and 663 nm (Shimadzu, Japan). Chlorophylls and carotenoid contents were calculated according to Lichtenthaler and Wellburn¹⁷.

Determination of the total soluble sugars: The total soluble sugars were determined using a modified version of the Riazi *et al.*¹⁸ method as modified by Ibrahim¹⁹. The TSS were analyzed by causing 0.1 mL of alcoholic extract to react with 3.0 mL of freshly prepared anthrone in a boiling water bath for 10 min and reading the cooled samples at 625 nm in a Spectronic 21D spectrophotometer.

Determination of total soluble nitrogen: The total soluble nitrogen was extracted and determined by the conventional semimicro modification of the Kjeldahl method²⁰. An aliquot of the extract was taken into a digestion flask and heated for at least 8 h with 0.5 g catalyst (K_2SO_4 80 g, $CuSO_4 \cdot 5H_2O$, 20 g and SeO_2 0.3 g), 2 mL of ammonia-free concentrated H_2SO_4 and 1 cm^3 of distilled water. The solution was treated with 15 mL of 40% NaOH and steam distilled in the conventional manner into 5 mL of 0.05 N- H_2SO_4 . The distillate was made up to volume and was used to determine total soluble nitrogen by estimating ammonia.

Estimation of free proline: Free proline was estimated using the procedure described by Bates *et al.*²¹. A 2% homogenate of the fresh peppermint leaf was prepared with 3% aqueous sulfosalicylic acid and centrifuged at 11500 rpm at 4°C for 15 min. After that 2 mL of glacial acetic acid and ninhydrin reagent was added to 2 mL of supernatant. The mixture was boiled in water bath for 60 min and then cooled on ice. Subsequently 4 mL of toluene was added and incubated at room temperature for 30 min. Tubes were then shaken for 15 sec and allowed to stand for 10 min for phase separation. The upper phase was separated and absorbance was measured using spectrophotometer and the concentration of free proline was calculated using proline standard.

Statistical analysis: The main effect of factors (drought stress, salicylic acid and growth stages) was evaluated using the Statistical Package for the Social Sciences (SPSS) version 11.0 computer software package²². A 2-tailed $p < 0.05$ was statistically significant. The paired sample t-test was used to

compare the means with and without the treatment of salicylic acid. A one-way analysis of variance (ANOVA) was used to find the significant differences in the peppermint plant growth parameters and some physiological responses among the different treatments with a level of significance of less than 5% ($p < 0.05$).

RESULTS

Response of the growth vigour of shoot: In general, the shoot biomass (fresh and dry) and plant height, of peppermint plants had increased in an orderly manner in the control and stressed plants during the experiment period (4 and 6 WAP) (Table 1). As compared to the control values, the drought caused a marked decrease ($p < 0.05$) in all growth vigor of shoot during the both growth stages. Moreover, SA application induced a significant excess ($p < 0.05$) in the shoot growth vigor of the treated and untreated peppermint plants. The maximum values of all measured parameters were recorded from the unstressed plants treated with SA at stages 4 and 6 WAP.

Response of the growth vigour of root: The root length and root biomass (fresh and dry) of peppermint plants had increased in the control and stressed plants during the experiment time (4 and 6 WAP) (Table 2). The drought stress caused a significant decrease ($p < 0.05$) in the root biomass as compared to the untreated plants but increased the root length at the both growth stages. Alternatively, the SA application promoted to cause an obvious excess ($p < 0.05$) in the root biomass as well as root length of droughted as well as nondroughted peppermint plants. The maximum values of root biomass were recorded from unstressed plants treated with SA at the both growth stages. Furthermore, the root length maximum values were obtained from stressed plants treated with SA at the both growth periods.

Effect of drought and SA on the photosynthetic pigments: In relation to the control, the untreated peppermint plant leaves had higher pigment contents (chlorophyll a and b, carotenoids and total pigments) at all the growth stages (Table 3). Drought conditions led to a noticeable reduction ($p < 0.05$) in all the pigment contents of the treated plants at 4 and 6 WAP but a remarkable reduction in all the measured parameters occurred at the 4 WAP growth stage. Further, the SA application caused an obvious increase ($p < 0.05$) in the photosynthetic pigments content in the stressed and unstressed peppermint plants. The highest values of all the

Table 1: Effects of salicylic acid on shoot growth vigor of droughted peppermint plants at various growth stages

| Parameters | Shoot growth vigor | | | | | |
|-------------------------|--------------------|-----------------|------------------------|-----------------|----------------------|-----------------|
| | Plant height (cm) | | Shoot fresh weight (g) | | Shoot dry weight (g) | |
| | 4 WAP | 6 WAP | 4 WAP | 6 WAP | 4 WAP | 6 WAP |
| Groups | Mean±SD | | | | | |
| Control | 13.12±2.31 | 18.50±2.50 | 5.27±1.41 | 8.64±1.63 | 1.26±0.25 | 2.44±0.35 |
| Drought | 10.53±1.60 | 14.24±2.23 | 3.80±0.72 | 4.96±0.34 | 0.89±0.09 | 1.31±0.24 |
| Salicylic acid | 13.32±2.13 | 18.83±1.72 | 6.41±1.54 | 9.34±1.56 | 1.45±0.45 | 2.56±0.31 |
| Salicylic acid+Drought | 12.40±1.50 | 16.32±2.64 | 4.62±1.31 | 6.89±0.91 | 1.09±0.34 | 2.13±0.53 |
| ANOVA-one way (p-value) | 5.55 (0.003)* | 18.24 (0.000)** | 6.15 (0.000)* | 15.24 (0.000)** | 6.15 (0.000)* | 15.24 (0.000)** |

WAP: Week after planting, *p<0.05, **p<0.01

Table 2: Effects of salicylic acid on root growth vigor of droughted peppermint plants at various growth stages

| Parameters | Root growth vigor | | | | | |
|-------------------------|-------------------|-----------------|-----------------------|-----------------|---------------------|-----------------|
| | Root length (cm) | | Root fresh weight (g) | | Root dry weight (g) | |
| | 4 WAP | 6 WAP | 4 WAP | 6 WAP | 4 WAP | 6 WAP |
| Groups | Mean±SD | | | | | |
| Control | 14.52±1.60 | 16.52±2.12 | 1.65±0.21 | 2.98±0.23 | 0.68±0.08 | 1.12±0.51 |
| Drought | 15.45±1.82 | 20.66±2.20 | 0.71±0.03 | 1.47±0.45 | 0.34±0.03 | 0.77±0.04 |
| Salicylic acid | 13.31±1.51 | 17.55±1.83 | 1.98±0.23 | 3.12±0.37 | 0.87±0.05 | 1.24±0.01 |
| Salicylic acid+Drought | 16.23±1.32 | 22.51±3.71 | 1.23±0.34 | 2.23±0.21 | 0.53±0.02 | 1.02±0.06 |
| ANOVA-one way (p-value) | 4.25 (0.000)* | 12.24 (0.000)** | 6.15 (0.000)* | 15.24 (0.000)** | 16.35 (0.000)** | 14.15 (0.000)** |

WAP: Week after planting, *p<0.05, **p<0.01

Table 3: Effects of salicylic acid on pigments content of droughted peppermint plants at various growth stages

| Parameters | Pigments content (mg g ⁻¹ d.wt.) | | | | | | | |
|-------------------------|---|---------------|-----------------|---------------|---------------|---------------|----------------|---------------|
| | Chl a | | Chl b | | Carotenoids | | Total pigments | |
| | 4 WAP | 6 WAP | 4 WAP | 6 WAP | 4 WAP | 6 WAP | 4 WAP | 6 WAP |
| Groups | Mean±SD | | | | | | | |
| Control | 4.08±1.31 | 4.58±1.24 | 1.45±0.32 | 1.78±0.67 | 1.23±0.42 | 1.38±0.32 | 6.76±1.25 | 7.74±1.34 |
| Drought | 3.56±1.09 | 3.76±1.35 | 0.86±0.12 | 1.34±0.25 | 0.80±0.31 | 1.16±0.24 | 5.22±1.34 | 6.62±1.23 |
| Salicylic acid | 4.34±1.24 | 4.78±1.10 | 1.49±0.18 | 1.70±0.23 | 1.27±0.14 | 1.45±0.15 | 6.10±1.41 | 7.93±1.57 |
| Salicylic acid+Drought | 3.78±1.05 | 4.13±0.57 | 1.14±0.15 | 1.54±0.41 | 1.09±0.13 | 1.25±0.09 | 6.01±1.37 | 6.92±1.48 |
| ANOVA-one way (p-value) | 4.46 (0.000)* | 6.46 (0.000)* | 17.56 (0.000)** | 3.53 (0.000)* | 6.53 (0.000)* | 6.46 (0.000)* | 5.52 (0.000)* | 3.98 (0.000)* |

WAP: Week after planting, *p<0.05, **p<0.01

Table 4: Effect of salicylic acid on some organic osmolytes in leaves of droughted peppermint plants at various growth stages

| Parameters | Organic osmolytes (mg g ⁻¹ d.wt.) | | | | | |
|-------------------------|--|-----------------|---------------------|---------------|---------------|---------------|
| | Total soluble nitrogen | | Total soluble sugar | | Proline | |
| | 4 WAP | 6 WAP | 4 WAP | 6 WAP | 4 WAP | 6 WAP |
| Groups | Mean±SD | | | | | |
| Control | 2.15±0.84 | 3.25±0.74 | 17.13±2.24 | 22.34±3.15 | 0.32±0.06 | 0.45±0.24 |
| Drought | 4.24±1.14 | 5.46±1.24 | 21.14±2.17 | 28.25±2.43 | 0.41±0.13 | 0.68±0.16 |
| Salicylic acid | 2.56±0.93 | 3.67±1.14 | 18.12±1.35 | 24.46±3.24 | 0.71±0.16 | 1.24±0.45 |
| Salicylic acid+Drought | 5.12±1.24 | 6.41±1.20 | 26.35±3.17 | 30.21±2.68 | 1.17±0.43 | 2.48±0.86 |
| ANOVA-one way (p-value) | 5.24 (0.000)* | 12.34 (0.000)** | 3.15 (0.000)* | 7.56 (0.000)* | 4.51 (0.000)* | 3.68 (0.000)* |

WAP: Week after planting, *p<0.05, **p<0.01

measured pigment contents were obtained from the unstressed plants treated with SA.

Effect of drought and SA on the total soluble sugars: The total soluble sugars in the peppermint leaves had increased in

an ordinary manner in the control and droughted plants at the 2 growth stages(4 and 6 WAP) (Table 4). Drought stress led to a noticeable increase (p<0.05) in the TSS of the peppermint plant leaves at both growth periods as compared to control values. On the other hand, the SA treatment resulted in an

additional accumulation in the TSS of the peppermint plant. The maximum accumulations of TSS (26.35 and 30.21 mg g⁻¹dwt) were recorded with the stressed plants treated with SA at 4 and 6 WAP, respectively.

Effect of drought and SA on the total soluble nitrogen: As shown in Table 4, the stressed and unstressed plants had accumulated more TSS during the experiment time (4 and 6 WAP). Furthermore, drought led to a remarkable increase ($p < 0.05$) in the TSN content of the peppermint plants at the two growth stages regarding the control values. The external application of SA induced an additional accumulation ($p < 0.05$) in the TSN in the peppermint leaves in stressed and unstressed plants. The maximum accumulations of TSN (5.12 and 6.41 mg g⁻¹dwt) were recorded with stressed plants treated with SA at 4 and 6 WAP, respectively.

Effect of drought and SA on the proline content: In relation to the control values, water shortage induced a remarkable increase ($p < 0.05$) in the proline content in peppermint leaves during the experiment time (4 and 6 WAP) (Table 4). On the other hand, SA resulted in more accumulation in proline content. The highest values of proline (1.17 and 2.48 mg g⁻¹ dwt) were recorded with stressed plants treated with SA at 4 and 6 WAP, respectively.

DISCUSSION

Growth and development of plant are affected by various internal as well as external factors in addition to its genetic makeup, which are considered as significant tools for evaluating the yield in many plants. The results in Table 1 and 2 showed that drought conditions led to a noticeable reduction in the growth parameters of shoot and root of peppermint plants but caused a significant increase in the root length. Indeed the reduction in the plant height was reported in other studies by Garcia-Caparros *et al.*⁴, Hassanpour *et al.*⁸ and Khorasaninejad *et al.*⁹. In this regard, Manivannan *et al.*²³ stated that the insufficient plant height could be as a result of the decrease in the cell expansion due to more leaves senescence and low turgor pressure under dry conditions.

In addition, the biomass (fresh and dry) reduction of the roots and shoots of the treated peppermint plants could determine the reduction in the plant's growth and these results were in accordance with previous studies Hassanpour *et al.*⁸ and Khorasaninejad *et al.*⁹. In this regarding, Garcia-Caparros *et al.*⁴ stated that the total biomass of *Mentha piperita* had reduced under drought

stress conditions. In general, there is a severe dissimilarity between the roots and shoots in their drought response. This might be clarified by the various rates of osmotic adjustments of root and shoot cells or the different loosening capabilities of the leaves cell walls from the root cell walls²⁴. The loosening capability of a growing cell wall might be influenced by hormones like abscisic acid and auxins. The amount of internal abscisic acid had increased in the roots and leaves and an additional amount abscisic acid was delivered to the shoot from the root under drought stress². Moreover, drought stress stimulates the root growth that results in root length increase which has an adapted role that assists in soil-water absorption. These findings were in conformity with other results acquired by Aldesuquy *et al.*⁵ and Huang *et al.*²⁵.

On the other hand, the external application of SA appeared to ameliorate the influence of water stress on the growth vigor of shoots and roots of both the controlled and the droughted peppermint plants. Similar results were found in other investigations with some medicinal plants, for instance, in sunflower²⁶, *Cymbopogon flexuosus*²⁷, *Satureja hortensis*²⁸ and *Musa acuminata*²⁹. In addition, Alam *et al.*³⁰ found that foliar spray with 50 μ M SA ameliorated the short-term drought stress in mustard (*Brassica juncea* L.) seedlings by up regulation of the antioxidant defence and glyoxalase system. They reported an increase in the leaf relative water content, chlorophylls, ascorbate/ascorbic acid and reduced glutathione but a decrease in the oxidized glutathione content and maintained a higher ration of glutathione S-transferase/oxidized glutathione. Furthermore, the reduced need for water by the stressed peppermint plants due to the external application of SA could be the main purpose behind the considerable enhancing in the shoot fresh weight.

Photosynthesis is considered to be a vital plant metabolic pathway. The conservation of plant growth under drought stress requires maintenance the right photosynthetic rate³¹. The obtained results showed that drought led to a significant reduction in the photosynthetic pigment contents in the peppermint leaves (Table 3). It was observed that the leaves became yellow when they had minimum water potentials in a certain period. These results are in conformity with those obtained by Jalal *et al.*¹⁵ with *Plectranthus tenuiflorus* and Aldesuquy *et al.*⁵ with *Triticum aestivum* under drought stress. Furthermore, Fathi and Tari³² stated that drought stress affected photosynthesis by closing of stomata, transfer of CO₂ in chloroplasts and a decrease in cell water potential, which could result in a marked reduction in the plant's productivity. Moreover, the results in Table 3 indicated that the SA

pre-soaking caused significant increases in the photosynthetic pigment contents in droughted as well as nondroughted peppermint leaves. The enhancing impact of SA might be a result of the improvement in the immortality of leaves in water-stressed plants by maintaining their pigment content, thus prevent them from aging. Furthermore, in other investigations, the application of SA improved the pigment contents in maize³³, mustard³⁰ and wheat^{5,34} under stress conditions. Moreover, Kang *et al.*³⁵ stated that the application of SA over expressed the proteins associated with signal transduction, stress defence, photosynthesis, carbohydrate metabolism, protein metabolism and energy production and led to an improvement in the growth and drought tolerance of wheat seedlings. On the other hand, Aldesuquy *et al.*⁵ stated that the enhancing in the growth, production and drought-tolerance of wheat plants resulting from the application of SA was correlated with the increase in the photosynthetic rate.

Carbohydrates, which considered one of the main organic solutes in the plant, were influenced by drought conditions. It is appeared from Table 4 the TSS increased in the peppermint plants. Similar results were found by Aldesuquy *et al.*⁵ and El Tayeb and Ahmed³⁶ with wheat plants. Indeed, TSS play an important role as a compatible solute under drought stress³⁷. Moreover, Siringam *et al.*³⁸ reported that, the increased in the TSS decline the water potential, cell turgidity and osmotic adjustment by increasing the storage reserves for the normal function of plant cells under stress conditions.

On the other hand, the pre-soaking of SA led to additional increases in the TSS content in the leaves of both the stressed and unstressed plants (Table 4). This finding is consistent with the results obtained by Azooz and Youssef³⁴ and Kang *et al.*³⁵ with *T. Aestivum* and Yazdanpanah *et al.*²⁸ with *Satureja hortensis*. In addition, Habibi³⁹ stated that exogenous application of salicylic acid alleviates the oxidative damage of barley plants under drought stress by increasing the dry mass and the net CO₂ assimilation rate.

The drought stress led to a remarkable increase in the TSN content in peppermint leaves (Table 4). These results were compatible with those obtained in many studies with various medicinal plants, for instance in *Satureja hortensis*²⁸. In this regard, Aldesuquy *et al.*⁵ reported that drought stress led to an increase in the TSN content in the leaves of the droughted wheat plants which might be due to the suppression of transporting from root to shoot, the protein synthesis inhibition or the protease activity increase. Moreover, Idrees *et al.*²⁷ pointed out that the drought enhanced the

activities of nitrate reductase and carbonic anhydrase and increased the proline and free amino acid contents in *Cymbopogon flexuosus* plants under water stress conditions.

The pre-soaking of peppermint seeds with SA enhanced the TSN amount in the peppermint leaves (Table 4) and these finding were compatible with many investigations^{5,28,27,36}. Furthermore, Idrees *et al.*²⁷ stated that the foliar application of 10 µM SA moderated the activities of nitrate reductase, carbonic anhydrase and increased the proline and free amino acid contents which led to improvement in the growth parameters in *Cymbopogon flexuosus* plants. Moreover, SA induced genes encoding chaperone proteins, heat shock proteins, antioxidants and secondary metabolites of different plant types and was involved in a mitogen-activated protein kinase regulation and expression⁴⁰.

The higher accumulation of the proline content noticed in drought stressed plants indicates an effective mechanism for osmotic adjustment, stabilization of sub-cellular structures and cellular adaptation to drought conditions which are supported by other investigations^{2,27,41-43}. Thus, water stress led to a significant increase in the proline content in the peppermint leaves extract (Table 4). These findings suggested that the increase in proline levels could be from of the metabolic replies precipitated in the translocation passage which associate the recognition of various abiotic stresses conditions to the induction of physiological responses⁴⁴. Furthermore, proline acts as nitrogen reservoir in the restricted growth stages, hydrates the polymers⁴⁵. In this respect, Nadeem *et al.*⁴⁶ reported that proline acts as a signaling compound to regulate the function of mitochondria, has an impact on cell proliferation by means of activating particular genes, which are essential for stress recovery under drought conditions and the increase in proline content assists in preserving membrane integrity through reducing lipids oxidation by guarding cellular redox potential and scavenging free radicals.

It is clear from Table 4 that the SA application led to additional accumulation in the free proline concentration in the peppermint leaves and these finding were compatible with many investigations^{2,29,43,47}. In this regarding, Yazdanpanah *et al.*²⁸ reported that the pre-soaking in SA induced to a significant increase in protein, proline and sugar contents but reduced the lipid peroxidation in *Satureja hortensis* plant under drought stress. In addition, proline might be seemed as an significant compound in the spectra of SA-induced ABA-mediated preventive reactions of plants replay to water shortage, participating in the mitigation of the

drought harmful impacts and hastening of the recompensing operations after stress conditions, indicating the preservative effect of salicylic acid on plant growth².

CONCLUSION

In this study, water stress caused negative influence on peppermint plant growth which was mitigated by the salicylic acid pre-soaking treatment. This was accompanying with some physiological processes modification such as enhancing in photosynthetic pigments and organic solutes as TSS, TSN and proline content. Moreover, these findings indicate that salicylic acid can be used to improve the plant growth and development under drought conditions.

SIGNIFICANCE STATEMENT

This study discovers that salicylic acid can be applied to improve the growth, photosynthetic pigments and some organic osmolytes of peppermint plant grown under water stress conditions. This study will help the farmers to use the SA seed-pres soaking doses so as to increase the drought stress tolerance and the cultivation of peppermint plant in arid and semiarid soils.

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