Progress in Biological Sciences
Vol. 7, Number 2, Autumn/Winter 2017/147-157 - DOI: 10.22059/pbs.2019.288345.1336

Positive effects of salicylic acid on some biochemical and physiological parameters of *Aloysia citrodora* under drought stress

Behnam Mohammadi[1], Maryam Rezayian[1], Hassan Ebrahimzadeh[1,*], Javad Hadian[2] and Masoud Mirmasoumi[1]

[1] *Department of Plant Biology, School of Biology, College of Science, University of Tehran, 1417466191, Tehran, Iran*

[2] *Medicinal Plants and Drug Research Institute, Shahid Beheshti University, G.C., Evin, 1483963113 Tehran, Iran*

Received: September 02, 2019; Accepted: Deceber 30, 2019

ABSTRACT

Aloysia citrodora Palau is a valuable medicinal plant of the family Verbenaceae**.** This study was conducted to evaluate potential role of salicylic acid (SA) in reducing the harmful effects of drought stress on *A. citrodora* plants. SA (0.5 and 1 mM) was used to *A. citrodora* plants grown under stressed (5, 10, 15 and 20% PEG) and unstressed conditions. Fresh weight (FW) and relative water content (RWC) significantly decreased under water deficit stress. Increase in hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) content indicated drought-induced oxidative stress in *A. citrodora*. Water deficit stress significantly increased the protein content, proline content and antioxidative enzymes activities. The exogenous application of SA to drought-stressed plants reduced the content of MDA and H_2O_2 and increased superoxide dismutase (SOD), peroxidase (POX) and chlorophyll content. It is suggested that SA minimizes the negative effects of drought stress and could be used for amelioration of drought stress in *A. citrodora*.

Keywords: *Aloysia citrodora*; Salicylic acid; Antioxidative enzymes; Drought stress; MDA

Introduction

Aloysia citrodora Palau (syn. *Lippia citrodora* (Palau) Kunth.), the lemon verbena, a medicinal plant of the family Verbenaceae, is a perennial shrub or subshrub reaching to 2 m high. This species is native to South America, but was introduced into Europe at the end of the 17th century (1). Lemon verbena leaves are used as spice due to their lemon flavor. They also are used to make herbal teas. The major constituents in lemon verbena oil are citral, nerol and geraniol (2). Recently, cultivation area of *A. citrodora* in Iran has been increased to supply the demand of local markets and pharmaceutical industries. It is grown in the green-houses in temperate parts and in the field in subtropical areas of southern parts of Iran.

Drought stress affects the growth and productivity of plants by disturbing osmotic balance. Osmotic stress

* **Corresponding author:** ebizadeh@ut.ac.ir

affects several physiological and biochemical parameters both at the cellular and tissue levels (3). In order to decrease osmotic potential, osmolyte compounds are accumulated under drought stress. Three important groups of these components include nitrogenous compounds, carbohydrates and organic acids (4). The increase of reactive oxygen species (ROS) levels is one of the major effects of drought stress. Free radicals including superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH) cause plant damage through oxidizing photosynthetic pigments, membrane lipids, proteins, and nucleic acids (5, 6). To counteract the damaging effects of ROS, plant cells possess an antioxidant system consisting of low-molecular-weight antioxidants, as well as antioxidant enzymes such as SOD (Super-oxide dismutase), CAT (Catalase), and POD (per-oxidase) (7).

Salicylic acid (SA) or *ortho*-hydroxy benzoic acid is a modulator molecule in plant response to environmental stress (8). Several studies have indicated the role of SA in resistance to salinity, osmotic and heavy metal stress in plant species (9-13). Exogenous SA could regulate the activities of antioxidant enzymes and increase plant tolerance to abiotic stresses (14, 15).

There is a little information available so far about the effects of SA and water stress in Verbenaceae. Lemon verbena is an important medicinal member of this family. The leaves are reported to possess digestive, antispasmodic, antipyretic, sedative and stomachic properties. It has traditionally been used in infusions for the treatment of asthma, cold, fever, flatulence, colic, diarrhoea and indigestion (16). The aim of this study was to investigate the effect of water deficit on the biochemical and physiological parameters of *A. citrodora* and the possible ameliorating effect of SA on drought resistance.

Materials and methods

Scions of *A. citrodora* were obtained during autumn 2011 from Khoraman Pharmaceutical Company in Lorestan province of Iran. In order to promote rooting, scions were implanted in sand in a greenhouse with 16 hr light/8 hr dark period per 24 hr and day/night temperatures of 25/18°C for 45 days. Then, the seedlings were transferred to plastic pots (18×25 cm) filled with perlite and maintained under greenhouse condition and were nourished in half-strength Hoagland for two months. Water deficit was applied using polyethylene glycol (PEG) 6000 in different concentrations of 0%, 5.0%, 10.0% and 20.0% (equal to 0, −0.07, −0.13 and −0.49 MPa, respectively) without and with SA (0.5 and 1 mM). A foliar spray of SA was applied uniformly to the plants using an atomizer. The final harvest was performed after 30 days of treatment and leaves were collected.

Growth parameters and RWC

The plants were evaluated 4 weeks after treatment with SA and PEG in terms of fresh weight (FW). Relative water content (RWC) of leaves was estimated according to Wheatherley (17) and based on following formula:

$$RWC\ (\%) = [(FW\text{-}DW) / (SW\text{-}DW)] \times 100$$

Saturated weight (SW) of the plants was determined by keeping them in de-ionized water at 4°C in the dark for 24 h, and DW was obtained after oven drying at 45°C for 72 h.

Pigments content

For measurement of pigments content including chlorophylls (Chl*a* and Chl*b*) and carotenoids, fresh leaves (0.2 g) were homogenized in 90% acetone using a pestle and mortar, then absorbance was measured by spectro-photometer (18).

Malondialdehyde content

Malondialdehyde (MDA) content was measured according to Heath and Packer method (19) as lipid peroxidation indicator. Fresh leaves (0.5 g) were homogenized in 2 ml of 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 10,000×g for 10 min in 25ºC and then 2 ml of 0.5% thiobarbituric acid (TBA) in 20% TCA was added to 1 ml supernatant. After getting prepared, this mixture was heated at 95ºC for 30 min and centrifuged at 10,000×g for 10 min. The absorbance of the supernatant was recorded at 532 nm and 600 nm. The MDA contents were measured by 155 $mM^{-1}\ cm^{-1}$ as extinction coefficient.

Proline content

Proline content was measured according Bates et al. (20). Fresh material (0.5 g) was homogenized in 5 ml sulfosalicylic acid (3%) and then was centrifuged at

13000 rpm for 20 min. Two ml of supernatant was mixed with acid ninhydrin (2 ml) and glacial acetic acid (2 ml) and then was boiled at 100°C for one hour. The reaction mixture was extracted with 4 ml toluene and the absorbance was recorded at 520 nm.

H_2O_2 content

H_2O_2 levels were measured according to Velikova et al. (21). 0.2 g of fresh leaves was homogenized in ice bath by 5 ml 0.1% (w/v) TCA, centrifuged at 12,000×g for 15 min, and 0.5 ml of the supernatant was added to 0.5 ml of 10 ml potassium phosphate buffer (pH 7.0) and 1 ml of 1 M KI. The absorbance of the supernatant was measured at 390 nm. The content of H_2O_2 was measured by a standard curve.

Antioxidant enzyme activity

For estimation of total protein content and enzyme activity, leaf material was homogenized at 4ºC with 1 M Tris-HCl (pH 7.5). The homogenate was centrifuged at 1324 ×g for 30 min at 4ºC. Supernatant was kept at –70ºC and used for protein determination and enzyme assays. The protein content was determined according to Bradford's method (22) using bovine serum albumin as a standard.

SOD (E.C. 1.15.1.1)

SOD activity was measured according to method described by Giannopolitis and Ries (23). The reaction solution contained 13 mM methionine, 75 µM NBT, 75 µM riboflavin, 50 mM sodium phosphate buffer (pH 7.5), 0.1 mM EDTA, and 100 µl of enzyme extract. The reaction mixture was irradiated for 16 min, and absorbance was read at 560 nm against the non-irradiated blank. One unit of SOD activity was defined as the amount of enzyme that is required for 50% inhibition of NBT reduction as monitored at 560 nm, and the activity were expressed as unit per milligram of protein.

POX (E.C. 1.11.1.7)

POX activity was measured according to the method of Abeles and Biles (24), in a reaction mixture consisting of 0.4 ml H_2O_2 (3%), 0.2 ml 20 mM benzidine, 4 ml of 0.2 M acetate buffer (pH= 4.8), and 50 µl enzyme extract. The increase of absorbance was recorded at 530 nm. The POX activity was defined as 1 µmol of benzidine oxidized per minute per milligram protein (U mg^{-1}).

Polyphenol oxidase (PPO; E.C. 1.14.18.1)

PPO activity was determined at 40ºC by the method described in Raymond et al. (25). The reaction solution contained 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.8), 0.2 ml pyrogallol 20 mM, and 100 µl enzyme extract. The activity of this enzyme was recorded at 430 nm. The PPO activity was defined as µmol of pyrogallol oxidized per minute per milligram protein (U mg^{-1} [protein]).

Statistical analysis

Each experiment was repeated three times and the data were analyzed by using either one- or two-way analysis of variance (ANOVA) using SPSS (version 20). Means were compared by Duncan's test at 0.05 level of confidence.

Results

Water deficit stress significantly reduced FW of *A. citrodora* plants when compared with control ($P < 0.05$). Under 10% and 20% PEG, FW showed 28.95% and 35.29% decrease as comparing to control, respectively. Treatment of 1mM SA increased this parameter in both stressed and unstressed plants, while concentration of 0.5 mM SA increased FW only in control plants (Fig. 1a). The improvement in growth under SA treatment was higher (49.16%) at 5% PEG than other concentration. Drought stress significantly decreased RWC and SA treatment increased this parameter, especially in 1 mM SA (Fig. 1b). There was a significant interaction among drought and SA, for FW and RWC (Table 1).

Content of chlorophyll *a*, *b*, total chlorophyll, and carotenoids increased in all concentrations of PEG when compared with control (Fig. 2). The concentration of 1 mM SA remarkably increased these parameters in both stressed and unstressed plants. SA (1 mM) application enhanced 37.66%, 81.31% and 48.64% total chlorophyll in three levels of drought as compared without SA, respectively. The improvement effect of SA treatment on carotenoid was prominent at 10% (3.93 fold) with comparison to other concentrations of drought.

Table 1. Results of two-way analysis of variance (ANOVA) in *A. citrodora*.

Dependent variable	Independent variable		
	Drought	SA	Drought×SA
Fresh weight	23.188***	7.175**	2.213*
RWC	8.033***	25.611***	3.750**
Protein content	6.537***	12.417***	2.672*
Proline content	42.827***	33.103***	4.003**
H_20_2 content	79.744***	54.237***	25.070***
MDA content	18.534***	10.624***	1.571^{ns}
SOD activity	3.954*	5.922*	1.196^{ns}
POX activity	10.580***	12.656***	6.817***
PPO activity	18.090***	10.520***	11.357***
chlorophyll a	104.193***	156.914***	81.875***
chlorophyll b	124.510***	329.421***	122.498***
Total chlorophyll	224.324***	283.156***	162.652***
Carotenoid	314.315***	300.159***	166.440***

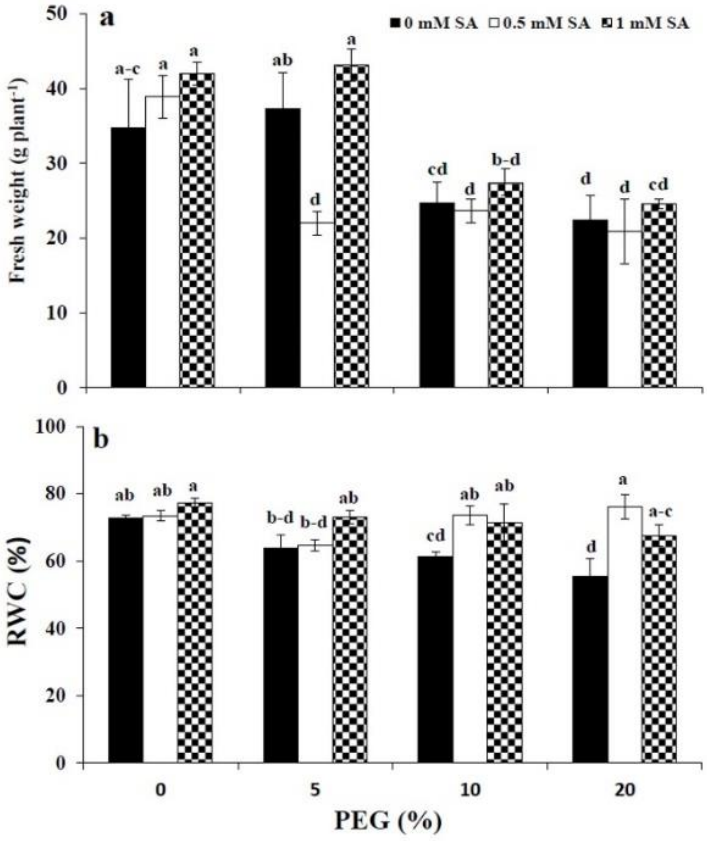

Figure 1. Effect of drought and SA treatment on fresh weight (a) and RWC (b) in *A. citrodora* plants. Different letters above columns indicate a significant difference at P < 0.05 using Duncan multiple range test.

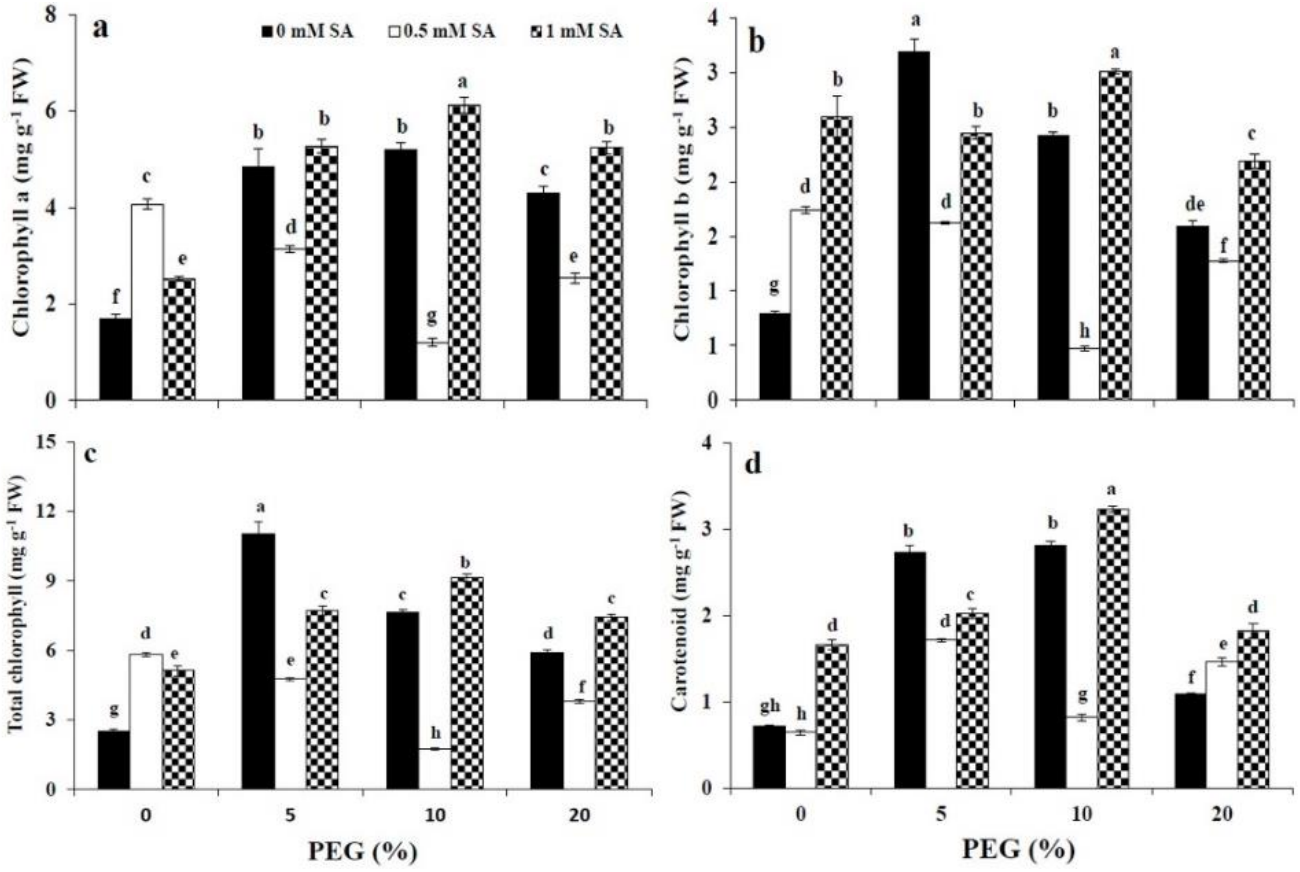

Figure 2. Change in content of chlorophyll and carotenoid in *A. citrodora* under drought stress and SA treatment. Different letters above columns indicate a significant difference at P < 0.05 using Duncan multiple range test.

Drought stress caused a significant increase in protein content and the application of SA declined this content (Fig. 3a). Proline content was remarkably increased by 77.48%, 336.79% and 724.24% in the plants under drought as compared to control. A signi-ficant increase of proline content was observed in SA-treated plants. SA-induced proline content was much higher at 20% (26.68%) than other concentrate-ions of PEG (Fig. 3b).

It was found that drought causes a significant rise of MDA production in *A. citrodora* (Fig. 3c). Exo genous application of SA showed a decrease in MDA content in stressed plants but there was no significant difference between 0.5 and 1 mM SA treatments. H_2O_2 content considerably increased with increasing stress and SA application significantly reduced H_2O_2 level under water deficit stress (Fig. 3d). SA treatment caused 3-fold decrease H_2O_2 content at 20% PEG.

The activities of antioxidant enzymes significantly heightened in response to drought stress. Maximum activity of POX was noticed at low level of drought (5%) but high activity of SOD and PPO were at high level of drought (20%). SA application had an additive effect on the activities of antioxidant enzymes in unstressed and stressed plants (Fig. 4).

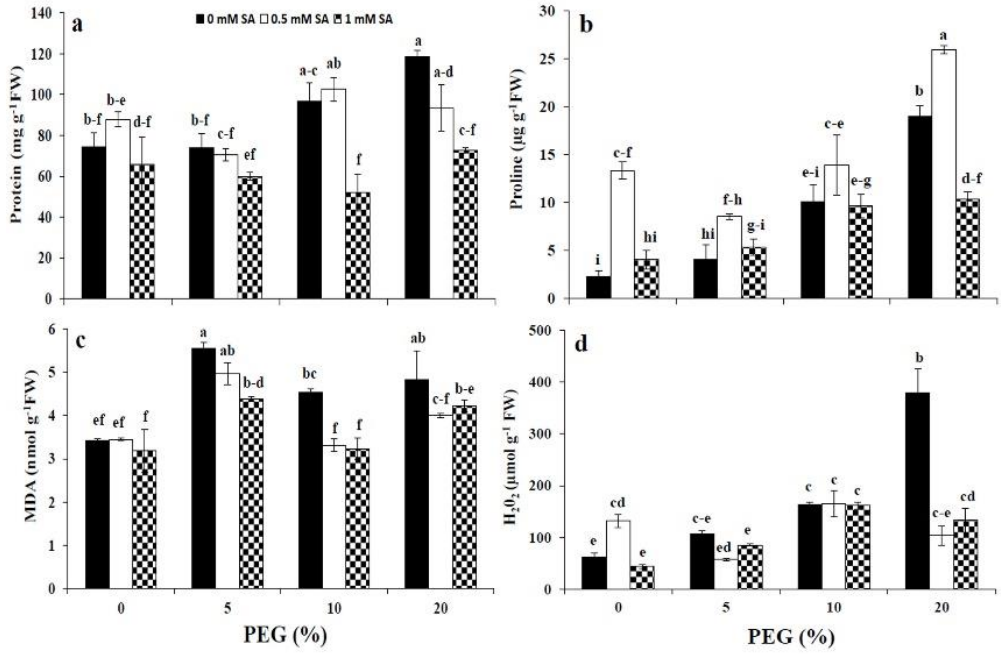

Figure 3. Effect of SA application on protein (a), proline (b), MDA (c) and H_2O_2 (d) content in *A. citrodora* under drought stress. Different letters above columns indicate a significant difference at P < 0.05 using Duncan multiple range test.

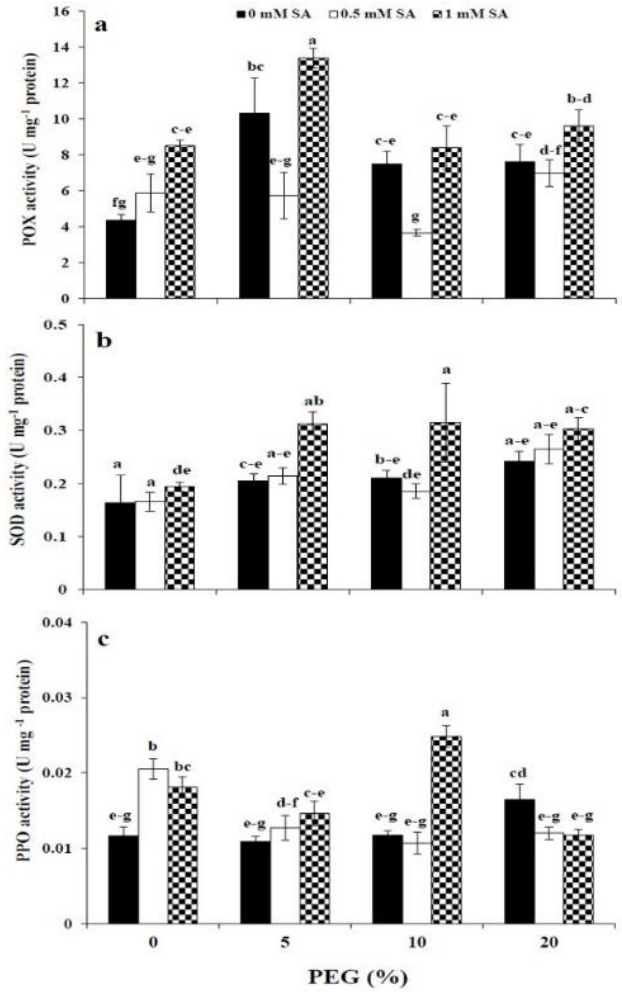

Figure 4. POX activity (a), SOD activity (b), and PPO activity (c) under drought and SA treatment in *A. citrodora*. Different letters above columns indicate a significant difference at P < 0.05 using Duncan multiple range test.

Discussion

Drought stress declined growth in *A. citrodora*. A similar decrease in growth has been previously observed under drought stress in *Populus kangdingensis* (26) and wheat (27). The SA treatment ameliorated adverse effect of water deficit on growth and improved growth in this plant. Based on our results, SA treatment had more effect on stress tolerance at low stress conditions. Our result is consistent with the findings in wheat (28) that stated decreased biomass under drought stress compensated by SA. Hussain et al. (29) also found that exogenous SA application was very effective in reducing adverse effects of drought stress in sunflower. The increase of growth by SA treatment in drought-stressed plants could be due to the protective role of SA in membrane integrity and regulation of ion uptake (30). RWC was reduced under drought stress in agreement with results obtained in barley (31), wheat (32), rice (33) and *Nigella sativa* (34). SA treatment caused in-creased RWC under drought which was similar to Farooq et al. (35) observations. SA caused increased accumulation of compatible osmolytes in plants and after that increase in water uptake.

According to our results, SA treatment enhanced chlorophyll content in *A. citrodora*. Positive effect of SA on this parameter indicates the involvement of SA in photosynthesis. Thus, these results explain that SA likely caused resistance of this plant to stress conditions by induction of photosynthetic rate. In other studies, application of SA increased pigments content in soybean (36), maize (37), wheat (38) and *Allium* spp. (39) grown under stress conditions. Similarly, Shi et al. (40) reported that photosynthetic pigments content had been reduced significantly under drought stress, and SA could alleviate this effect by moving the intracellular nitrate resources or increase of pigment biosynthesis. Very severe drought conditions cause limited photosynthesis due to a decline in Rubisco activity and reduced gas exchange (41). Similarly, Idrees et al. (42) reported that SA protected photosynthesis and enhanced Rubisco activity in treated wheat under water stress.

With increasing PEG concentration, the protein content increased, while SA treatment decreases this parameter in drought-stressed plants. Increased protein content under water deficit stress could be related to with over-expression of genes involved in the primarily metabolism, osmoregulation, trans-formers, protein catalyzers, detoxification and LEA protein (43). Singh and Usha (44) reported that the protein content decreased in wheat seedlings under SA treatment.
Among the important amino acids that accumulate in plant cells under drought stress, proline is the most important ones. Under drought stress plant could maintain water absorption by accumulation of compatible solutes like proline (45). Proline content was increased significantly under drought stress and also by SA (especially at 0.5 mM) that is in agreement with results obtained in *Torreya grandis* (46). Proline accumulation in response to abiotic stresses is widely reported and may play a role in stress adaptation of plants (47, 48). Proline is also considered to be involved in protection of enzymes (49), cellular structures (50), and to act as a free radical scavenger. Based on data obtained, the accumulation of this substance aids to osmotic adjustment in *A. citrodora* plants under stress.

In addition to their energetic role, lipids are important components involved in membrane structure (51). MDA and other aldehydes contents have been used for measurement of free radical damage to cell membranes under drought stress (52). The MDA content increased also in our plants under drought stress (Fig. 3c), which is in agreement with the findings of Niknam et al. (53). MDA content is more applicable to the plants, which are sensitive to stress than the resistant plants (54). SA alleviated this effect significantly; such effect has been reported also by Gunes et al. (30). H_2O_2 as a regulatory factor plays an important role in the genes activation which is involved in the oxidative stress response. In this study, H_2O_2 content was increased significantly under drought stress which is in agreement with Agarwal et al. (55) and Niknam et al. (53) (Fig. 3d). In addition, under SA treatment this content decreased significantly which is in contrary with Rao et al (56) and Chen et al. (57). Water deficit caused close of stomata and decrease in CO_2 assimilation (58); subsequently, there was an accumulation of NADPH, which resulted in the formation of ROS, causing damage to the membrane system and photosynthetic complexes. Overall, the positive effect of SA on soybean plant growth may be due to maintaining a balance between production and consumption of ROS.

Plants are able to protect their tissues from harmful effects of drought-accumulated ROS using enzymes such as SOD, CAT and APX (59). Drought tolerance is often related to a more efficient antioxidative system (60). Increased activities of POX, SOD and PPO suggested better tolerance to drought stress under SA treatment in *A. citrodora* plants. Furthermore, lower level of MDA content observed in SA-treated *A. citrodora* could be correlated with the increased activities of antioxid-ative enzymes. Our result is consistent with the findings in wheat (55) and maize (61). POX activity was increased under drought stress and SA treatment (1 mM). Increase in POX activity was also reported by Zhang et al. (62). Senaratna et al. (8) stated that in pea and tomato, SA caused an induction tolerance by activating antioxidant mecha-nism. Similar findings were presented in other plants such as SA-treated drought stressed *Lycopersicon esculentum* (63). POX plays a key role in decreasing H_2O_2 content, eliminating MDA and maintaining cell membrane integrity (64). SOD activity was increased significantly under drought stress and SA treatment (Fig. 4b). Hassanpour et al. (65) Found that SOD activity was increased significantly in root and shoot of *Mentha pulegium* under drought stress. Singh and Usha (44) stated that 1 and 2 mM SA increased SOD activity, while 3 mM SA decreased its activity.

PPO is the major enzyme responsible for oxidation of phenolic compounds. Phenolic compounds act as an intermediary ROS acceptor in the vacuole. They are oxidized to phenoxyl radicals and reduced ascorbate into monodehydroascorbate, averting the cellular damage under unfavorable conditions such as drought stress (66). The decrease of PPO activity under water deficit stress agrees with the results reported on other plants such as bean (67) and *Mentha pulegium* (65) under drought. SA significantly increased PPO activity under low (5% PEG) and moderate (10% PEG) drought stress (Fig. 4c). Janda et al. (61), found that SA pre-treatment in maize increased antioxidant enzyme activity. The results of the present work suggest that the application of SA in combination with drought stress improved antioxidant systems like activities of POX, SOD and PPO to repair the damage caused by ROS. Increasing activities of these enzymes with the application of SA proved the role of this plant growth regulator in mitigating the adverse effects of water stress in *A. citrodora*.

In conclusion, exogenous SA treatment mitigated the drought-induced inhibition of the whole-plant growth. SA represents a promising candidate agent for use in crop enhancement and protection. The current results can provide new insights to better realizing the responsible mechanisms to regulate drought stress resistance in *A. citrodora*.

Acknowledgments

The financial support of this research was provided by University of Tehran. We are grateful to the Khoraman Pharmaceutical Company for providing the plants of *Aloysa citrodora*.

REFERENCES

1. Carnat, A., Carnat, A.P., Fraiss, D., and Lamaison, J.L. (1999) the aromatic and polyphenolic composition of lemon verbena tea. *Fitoterapia*, **70**, 44-49.
2. Argyropoulou, C., Daferera, D., Tarantilis, P.A., Fasseas, C., and Polissiou, M. (2007) Chemical composition of the essential oil from leaves of *Lippia citriodora* at two developmental stages. *Biochem. Syst. Ecol.*, **35**, 831-837.
3. Lutts, S., Kinet, J.M., and Bouharmont, J. (1996) NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *J. Ann. Bot.*, **78**, 389-398.
4. Sairam, R.K., and Tyagi, A. (2004) Physiology and molecular biology of salinity stress tolerance in plants. *Curr. Sci.*, **3**, 406-421.,
5. Reddy, A.R., Chaitanya, K.V., and Vivekanandan, M. (2004) Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *J. Plant Physiol.*, **161**, 1189-1202.
6. Bor, M., Ozdemir, F., and Turkan, I. (2003) The effect of salt stress on lipidperoxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L. and wild beet *Beta maritima* L. *J. Plant Sci.*, **164**, 77-84.
7. Aghaleh, M., Niknam, V., Ebrahimzadeh, H., and Razavi, K. (2011) Effect of salt stress on physiological and antioxidative responses in two species of Salicornia (*S. persica* and *S. europaea*). *J. Acta Physiol. Plant.*, **33**, 1261-1270.

8. Senaratna, T., Touchell, D., Bunn, E., and Dixon, K. (2000) Acetyl salicylic acid and salicylic acid induce multiple stress tolerance in bean and tomato plants. *Plant Growth Regul.*, **30**, 157-161.
9. Shakirova, F.M., and Bezrukova, M.V. (1997) Induction of wheat resistance against environmental salinization by salicylic acid. *Biol. Bull.*, **24**, 109-112.
10. Mishra, A., and Choudhuri, M.A. (1999) Effects of salicylic acid on heavy metal-induced membrane deterioration mediated by lipoxygenase in rice. *Biol. Plant.*, **42**, 409-415.
11. Pal, M., Szalai, G., Horvath, E., Janda, T., and Paldi, E. (2002) Effect of salicylic acid during heavy metal stress. Proceedings of seventh Hungarian congress on plant physiology. *Acta Biol. Szegediensis*, **46**, 119-120.
12. Sakhabutdinova, A.R., Fatkhutdinova, D.R., Bezrukova, M.V., and Shakirova, F.M. (2003) Salicylic acid prevents damaging action of stress factors on wheat plants. *Bulg. J. Plant Physiol.*, 214-219.
13. Singh, B., and Usha, K. (2003) Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. *Plant Growth Regul.*, **39**, 137-141.
14. He, Y.L., Liu, Y.L., Chen, Q., and Bian, A.H. (2002) Thermotolerance related to antioxidation induced by salicylic acid and heat hardening in tall fescue seedlings. *J. Plant Physiol. Mol. Biol.*, **28**, 89-95.
15. Erasalan, F., Inal, A., Gunes, A., and Alpaslan, M. (2007) Impact of exogenous salicylic acid on the growth, antioxidant activity and physiology of carrot plants subjected to combined salinity and boron toxicity. *Sci. Hortic.*, **113**, 120-128.
16. Newall, C.A., Anderson, L.A., and Phillipson, J.D. (1996) Herbal medicines- A guide for health-care professionals. The Pharmaceutical Press. London, p. 179.
17. Wheatherley, P.E. (1950) Studies in the water relations of cotton plants. I. The field measurement of water deficit in leaves. *J. New Phytol.*, **49**, 81-87.
18. Lichtenthaler, H. (1987) Chlorophylls and caroteno ids, the pigments of the photosynthetic bio membranes. *Meth. Enzymol.*, **148**, 350-382.
19. Heath, R.L., and Packer, L. (1968) Photoperoxidatin in isolated chloroplasts. I. Kinetics and stochiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.*, **125**, 189-198.
20. Bates, L.S., and Waldren, R.P., and Teare, L.D. (1973) Rapid determination tolerance in cucumber plants. *Pak. J. Biol. Sci.*, **61**, 16-22.
21. Velikova, V., Yordanov, I., and Edreva, A. (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Protective role of exogenous polyamines. *Plant Sci.*, **151**, 59-66.
22. Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**, 248-254.
23. Giannopolitis, C.N., and Ries, S.K. (1997) Superoxide dismutase:II purification and quantitative relationship with water soluble protein in seedlings. *J. Plant Physiol.*, **59**, 315-318.
24. Abeles, F.B., and Biles, C.L. (1991) Characterization of peroxidase in lignifying peach fruit endocarp. *J. Plant Physiol.*, **95**, 269-273.
25. Raymond, J., Rakariyatham, N., and Azanza, J.L. (1993) Purification and some properties of polyphenoloxidase from sunflower seeds. *J. Phytochem.*, **34**, 927-931.
26. Yin, C., Peng, Y., Zang, R., Zhu, Y., and Li, C. (2005) Adaptive responses of *Populus kangdingensis* to drought stress. *J. Physiol. Plant.*, **123**, 445-451.
27. Rane, J., Maheshwari, M., and Nagarajan, S. (2001) Effect of pre anthesis water stress on growth, photosynthesis and yield of six wheat cultivars differing in drought tolerance. *Ind. J. Plant Physiol.*, **6**, 53-60.
28. Gomez, L., Blanca, L., and Antonio, C.S. (1993) Evidence of the beneficent action of the acetyl salicylic acid on wheat genotypes yield under restricted irrigation. In: Proc. scientific meeting on Forestry. Livestock and Agriculture Mexico., p. 112.
29. Hussain, M., Malik, M.K., Farooq, M., Ashraf, M.Y., and Cheema, M.A. (2008) Improving drought tolerance by exogenous application of glycinebetaine and salicylic acid in sunflower. *J. Agro. Crop Sci.*, **194**, 193-199.
30. Gunes, A., Inal, A., Alpaslan, M., Eraslan, F., Bagci, E.G., and Cicek, N. (2007) Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity. *J. Plant Physiol.*, **164**, 728-736.
31. Kocheva, K.V., Busheva, M.C., Goorgegiev, G.I., Lambrev, P.H., and Goltsev, V.N. (2005) Influence of short-term osmotic stress on the photosynthetic activity of barley seedlings. *Biol. Plant.*, **49**, 145-148.

32. Lei, Y., Yin, C., Ren, J., and Li, C. (2007) Effect of osmotic stress and sodium nitroprusside pretreatment on proline metabolism of wheat seedlings. *Biol. Plant.*, **51**, 386-390.
33. Hsu, S.Y., and Kao, C.H. (2003) The protective effect of free radical scavengers and metal chelators on polyethylene glycol-treated rice leaves. *Biol. Plant.*, **46**, 617-619.
34. Kabiri, R., Nasibi, F., and Farahbakhsh, H. (2014) Effect of exogenous salicylic acid on some physiological parameters and alleviation of drought stress in *Nigella sativa* plant under hydroponic culture. *Plant Protect. Sci.*, **50**, 43-51.
35. Farooq, M.W.A., Kobayashi, N., Fujita, D., and Basra, S.M. (2009) Plant drought stress: effects, mechanisms and management. *Argon. Sustain Dev.*, **29**, 185-212.
36. Zhao, H.J., Lin, X.W., Shi, H.Z., and Chang, S.M. (1995) The regulating effect of phenolic compounds on the physiological characteristics and yield of soybeans. *Acta Agron. Sin.*, **21**, 351-355.
37. Khodary, S.E.A. (2004) Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt-stressed maize plants. *Int. J. Agric. Biol.*, **6**, 5-8.
38. Arfan, M., Athar, H.R., and Ashraf, M. (2007) Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress. *J. Plant Physiol.*, **164**, 685-694.
39. Ahmad, A.M., Murali, P.V., and Marimuthu, G. (2014) Impact of salicylic acid on growth, photosynthesis and compatible solute accumulation in *Allium cepa* L. subjected to drought stress. *Int. J. Agric. Food Sci.*, **4**, 22-30.
40. Shi, Q., Bao, Z., Zhu, Z., Ying, Q., and Qian, Q. (2006) Effects of different treatments of salicylic acid on heat tolerance, chlorophyll fluorescence and antioxidant enzyme activity in seedlings *Cucumis sativa* L. *Plant Growth Regul.*, **48**, 127-135.
41. Bota, J., Flexas, J., and Medrano, H. (2004) Is photosynthesis limited by decreased Rubisco activity and RuBP content under progressive water stress. *New Phytol.*, **162**, 671-681.
42. Idrees, M., Khan, M.M.A., Aftab, T., Naeem, M., and Hashmi, N. (2010) Salicylic acid induced physiological and biochemical changes in lemongrass varieties under water stress. *J. Plant Int.*, **5**, 293-303.
43. Ingram, J., and Bartels, D. (1996) The molecular basis of dehydration tolerance in plant. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, **47**, 347-403.
44. Singh, B., and Usha, K. (2003) Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. *Plant Growth Regul.*, **39**, 137-141.
45. White, D.A., Turner, N.C., and Galbraith, J.H. (2000) Leaf water relations and stomatal behavior of four allopatric Eucalyptus species planted in Mediterranean southwestern Australia. *Tree Physiol.*, **20**, 1157-1165.
46. Shen, C., Hu, Y., Du, X., Li, T., Tang, H., and Wu, J. (2014) Salicylic acid induces physiological and biochemical changes in *Torreya grandis* cv. Merrillii seedlings under drought stress. *Trees*., **28**, 961-970.
47. Ashraf, M., and Foolad, M.A. (2007) Improving plant abiotic-stress resistance by exogenous application of osmoprotectants glycine betaine and proline. *Environ. Exp. Bot.*, **59**, 206-216.
48. Sperdouli, I., and Moustakas, M. (2012) Interaction of proline, sugars, and anthocyanins during photosynthetic acclimation of *Arabidopsis thaliana* to drought stress. *J. Plant Physiol.*, **169**, 577-585.
49. Solomon, A., Beer, S., Waisel, Y., Jones, G.P., and Poleg, L.G. (1994) Effect of NaCl on the carboxylating activities of Rubisco from *Tamarix jordanis* in the presence and absence of proline related compatible solutes. *Physiol. Plant.*, **90**, 189-204.
50. Van Resenburg, L., Kruger, G.H.J., and Kruger, H. (1993) Proline accumulation as drought tolerance selection criterion: Its relationship to membrane integrity and chloroplast ultrastructure in *Nicotiana tabacum* L. *J. Plant Physiol.*, **141**, 188-194.
51. Ashraf, M., and Harris, P.J.C. (2004) Potential biochemical indicators of salinity tolerance in plants. *Plant Sci.*, **166**, 3-16.
52. Halliwel, B., and Gutteridge, J.M. (1984) Oxygen toxicity, oxygen radicals, transition metal and disease. *Biochem. J.*, **219**, 1-14.
53. Niknam, V., and Zafari, S. (2012) Effect of phytoplasma infection on metabolite content and antioxidant enzyme activity in lime (*Citrus aurantifolia*). *Acta Physiol. Plant.*, **34**, 561-568.
54. Juan, M., Rivero, R.M., Romero, L., and Ruiz, J.M. (2005) Evaluation of some nutritional and biochemical indicators in selecting salt-resistant tomato cultivars. *Environ. Exp. Bot.*, **54**, 193-201.

55. Agarwal, S., Sairam, R.K., Srivastava, G.C., Tyagi, A., and Meena, R.C. (2005) Role of ABA, salicylic acid, calcium and hydrogen peroxide on antioxidant enzymes induction in wheat seedlings. *Plant Sci.*, **169**, 559-570.
56. Rao, M.V., Paliyath, G., Ormrod, D.P., Murr, D.P., and Watkins, C.B. (1997) Influence of salicylic acid on H_2O_2 production, oxidative stress, and H_2O_2-metabolizing enzymes. *Plant Physiol.*, **115**, 137-149.
57. Chen, Z., Ricigliano, J.W., and Klessig, D.F. (1993) Purification and characterization of a soluble salicylic acid-binding protein from tobacco. *Proc. Natl. Acad. Sci. USA.*, **90**, 9533-9537.
58. Ozkur, O., Ozdemir, F., Bor, M., and Turkan, I. (2009) Physiochemical and antioxidant responses of the perennial xerophyte *Capparis ovate* Desf to drought. *Environ. Exp. Bot.*, **66**, 487-492.
59. Verhagen, J., Put, M., Zaal, F., and van Keulen, H. (2004) Climate change and drought risks for agriculture. The impact of climate change on dry lands. Springer, Netherlands, pp. 49-59.
60. Tahi, H., Wahbi, S., Modafar, C.E., Aganchich, A., and Serraj, R. (2008) Change in antioxidant activity and phenol component in tomato plants subjected to partial root drying and regulated deficit irrigation. *Plant Biosyst.*, **142**, 550-562.
61. Janda, T., Szalai, G., Tari, I., and Paldi, E. (1999) Hydroponic treatment with salicylic acid decrease the effects of chilling injury in Maize (*Zea mays* L.) plants. *Planta*, **208**, 175-180.
62. Zhang, M., Duan, L., Tian, X., He, Z., Li, J., and Wang, B. (2007) Uniconazole induced tolerance of soybean to water deficit stress in relation to changes in photosynthesis, hormones and antioxidant system. *J. Plant Physiol.*, **164**, 709-717.
63. Hayat, S., Hasan, S.A., Fariduddin, Q., and Ahmad, A. (2008) Growth of tomato (*Lycopersicon esculentum*) in response to salicylic acid under water stress. *J. Plant Int.*, **3**, 297-304.
64. Jaleel, C.A., Gopi, R., Panneerselvam, R. (2008) Biochemical alterations in white yam (*Dioscorea rotundata* Poir.) under triazole fungicides: impacts on tuber quality. *Czech J. Food Sci.*, **26**, 298-307.
65. Hassanpour, H., Khavari-Nejad, R., Niknam, V., Najafi, F., Razavi, Kh. (2012) Effects of penconazole and water deficit stress on physiological and antioxidative responses in pennyroyal (*Mentha pulegium* L.). *Acta Physiol. Plant.*, **34**, 1537-1549.
66. Tekalign, T., Hammes, S., and Robbertse, J. (2005) Paclobutrazol induced leaf, stem and root anatomical modifications in potato. *J. Hort. Sci.*, **40**, 1343-1346.
67. Demir, Y., and Kocaliskan, I. (2001) Effects of NaCl and proline on polyphenol oxidase activity in bean seedlings. *Biol. Plant.*, **44**, 607-609.

Editorial Note

Volume 7, issue 2 of Progress in Biological Sciences was initialy scheduled to be published in December 31, 2017. However, some administrative changes leaded to a major delay in processing of the manuscripts. This issue is actually published in May 1, 2020. Editor-in-chief apologizes deeply for any inconvenience caused especially to the authors.