ACTA SCIENTIARUM POLONORUM

Acta Sci. Pol. Hortorum Cultus, 18(2) 2019, 159–169

www.acta.media.pl ISSN 1644-0692 e-ISSN 2545-1405 DOI: 10.24326/asphc.2019.2.15

ORIGINAL PAPER Accepted: 22.09.2018

EFFECT OF SALICYLIC ACID FOLIAR APPLICATION ON *Vitis vinifera* L. cv. 'SULTANA' UNDER SALINITY STRESS

Mehdi Oraei[1], Gholamreza Gohari[2]✉, Sima Panahirad[3], Elnaz Zareei[3], Fariborz Zaare-Nahandi[3]

[1] Department of Horticultural Sciences, Faculty of Agriculture, Miyaneh Branch, Islamic Azad University, Miyaneh, Iran

[2] Department of Horticultural Sciences, Faculty of Agriculture, University of Maragheh, Maragheh, Iran

[3] Department of Horticultural Sciences, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

ABSTRACT

The current survey aimed to study the effect of exogenous salicylic acid (SA) application on salinity stress of grapevine cv. 'Sultana'. The leaves of hydroponically cultivated grapes that were under 0, 75 and 150 mM salinity conditions treated with 0, 0.5, 1 and 1.5 mM SA and after two weeks, the factors such as Na^+, K^+, proline and MDA contents, leaf electrolyte leakage and enzymatic activities were measured. The results showed that all SA treatments were significantly effective at tolerance enhancement by reduction in Na^+/K^+ ratio, leaf electrolyte leakage, MDA and H_2O_2 values and promotion in proline content and the enzymatic activities (POD, APX, CAT and SOD) of grapes. These results indicated that SA application at salinity condition could be applied as a promising method for increasing the salinity tolerance of 'Sultana' grapes.

Key words: antioxidant activity, hydroponic, grape, salicylic acid, salinity

INTRODUCTION

Grape (*Vitis vinifera* L.), as the most widely cultivated perennial plant in the world, is exposed to adverse environmental factors such as high salinity that negatively affects the plant growth [Yildirim et al. 2008]. The reduction in plant growth in saline environments could be due to either adverse water relations or the toxic effects of Na^+ and Cl^- ions on metabolism [Munns and James 2003]. Elucidation of the plant biochemical and physiological mechanisms in response to abiotic stresses is very critical point for improvement of environmental stress tolerance in plants. Thus, salinity (NaCl), as an abiotic stress, is among most limiting factors of the plant growth, development and productivity [Yildirim et al. 2008]. Several studies have shown that salt stress induces the oxidative stress [Sairam and Tyagi 2004, Munns and Tester 2008, Zhang and Shi 2013] by the mitochondria and chloroplast that are important intracellular generators of reactive oxygen species (ROS) such as superoxide radical, peroxide radical, hydrogen peroxide and hydroxyl radical [Abd Elgawad et al. 2016]. Therefore, salinity as one of the most significant abiotic factors limiting the crop productivity and development of methods and strategies to improve the toxic effects of salt stress on plants, has received great attention [Yildirim et al. 2008].

Salicylic acid is an endogenous plant growth regulator and a derivative of phenolic compounds [Hayat and Ahmad 2007] that plays an important role in responses to biotic and abiotic stresses and regulation of plant growth and development [Yildirim et al. 2008, Wen et al. 2008, Hayat et al. 2010]. Salicylic acid has been recognized as an endogenous regulatory signal that

✉ gholamreza.gohari@gmail.com

plays several roles in the plant response to adverse environment conditions, such as salt and osmotic stresses [Wang and Li 2006, Yildirim et al. 2008]. Moreover, SA may be linked to oxidative stress playing an essential role in preventing the oxidative damage [Wang and Li 2006]. The roles of SA in inducing the stress tolerance depend on species, developmental phase of plants, concentration of SA applied and also experimental conditions [Arfan et al. 2007]. Therefore, low concentration of SA exhibited growth promoting and high concentration of SA exhibited growth inhibiting properties in grape (*Vitis vinifera* cv. 'Sultana') by increasing H_2O_2 concentration as a ROS in tissues [Szepesi et al. 2005]. The ROS content in plant cell is dependent on their producing systems and scavenging mechanism, both enzymatic and non-enzymatic ones [Apel and Hirt 2004]. Moderate doses of SA may activate the antioxidant mechanisms [Szepesi et al. 2005].

Several studies have reported positive effects of SA on the regulation of physiological processes. Additionally, activation of antioxidant enzymes such as catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) was enhanced by SA treatment [Wallace and Fry 1999]. Salicylic acid might affect the acclimation process that allows plants to adapt to subsequent stress condition and high levels of exogenous SA have adverse effects on plant growth and development including extensive oxidative damage. Thus, some studies reported positive effects of SA application on stress tolerance [Shakirova and Bezrukova 1997, Senaratna et al. 2000, Tari et al. 2002, Khodary 2004, Wang and Li 2006, Yordanova and Popova 2007, Wen et al. 2008, Yildirim et al. 2008].

At present, considerable interest has been provoked by the ability of SA to produce protective effects on plants under different abiotic stress. Therefore, the main aim of this paper was to study the effects of exogenous SA application on some plant physiological parameters and antioxidant properties under the salt stress in grape plant.

MATERIAL AND METHODS

Plant materials and experimental treatments

The grape stem cuttings (cv. 'Sultana') were placed in plastic pots under hydroponic conditions, which were irrigated by Hoagland nutritional solution. Two months after planting, considering plant deposition, salinity (NaCl) stress of 0, 75 and 150 mM was applied. After one month, foliar application of salicylic acid solution at four levels (0, 0.5, 1 and 1.5 mM) was applied onto the leaves. Two weeks after treatment, plant samplings were done to measure the enzymatic activity and other parameters. This experiment was carried out in a research greenhouse at University of Tabriz at ambient temperature, humidity and light intensity of 15–30°C, 40–50% and 500 µmol m^{-2} s^{-1}, respectively. The growing beds were regularly watered with tap water in 10 days intervals to prevent the salinity accumulation in growing media.

Assay of physiological properties

Sodium and potassium concentration of the leaves. Plant samples were collected randomly from each treatment, washed, air dried and then dried in hot-air oven at 60°C for 18 h. The samples were ground in Willey mill. The powered plant samples were stored for nutrient analysis. The triacid digestion extract was used for estimation of potassium (K^+) and sodium (Na^+) by flame photometry as outlined by Tandon [1998] and expressed in mmol kg^{-1}. Thereby Na^+/K^+ ratio was determined.

Leaf electrolyte leakage. Leaf electrolyte leakage was measured as described by Lutts et al. [1996]. For this purpose, an electrical conductivity-meter Jenway Model 4330 was used. Leaves were excised and washed with deionized water. After drying with filter paper, 1 g fresh weight of leaves was cut into small pieces (about 1 cm^2) and then immersed in 20 mL deionized water and incubated at 25°C. After 24 h, electrical conductivity (EC_1) and after 48 h (EC_2) of the bathing solution was recorded of same samples. The results expressed as uS mg^{-1}.

Proline content. Proline content was calculated according to Bates et al. [1973]. Proline concentration was determined using calibration curve and expressed as umol/g FW (fresh weight). Plant material (0.5 g) was homogenized in 10 ml of 3% sulfosalicylic acid and the homogenate was filtered. The filtrate (2 mL) was treated with 2 mL ninhydrin reagent (1.25 mg ninhydrin in 30 mL of glacial acetic acid

and 20 mL 6 M H_3PO_4) and incubated at 95°C for 1 h. The reaction was terminated by placing in an ice bath. The reaction mixture was vigorously mixed with 4 mL toluene. After warming at 25°C, absorbance of the colored solutions was read at 520 nm. L-proline was used as a standard.

Protein extraction. For biochemical study, grape leaves were immediately frozen in liquid nitrogen and transferred to laboratory, where they were lyophilized and stored at –80°C until analysis. Superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POD) and catalase (CAT) specific activities were determined in crude protein extracts prepared from 0.1 g of plant material stored and frozen at −80°C. Each sample was ground in a mortar in the presence of liquid nitrogen and proteins were extracted with 2 mL of extraction buffer [20 mM Hepes (pH 7.5), 50 mM KCl, 1 mM EDTA, 0.1% (v/v) Triton X-100, 0.2% (w/v) polyvinylpyrrolidone, 0.2% (w/v) polyvinylpolypyrrolidone and 5% (v/v) glycerol], followed by addition of 0.2 mL of 'high salt buffer' (225 mM Hepes, pH 7.5, 1.5 M KCl and 22.5 mM $MgCl_2$). Homogenates were centrifuged and after removing precipitated material, the final protein extracts were stored at −80°C until used for enzymatic assays. The protein concentration in the extracts was determined by the method of Bradford [1976] using the Bio-Rad reagent and bovine serum albumin as a standard.

Antioxidant enzymes assay

Measurements of superoxide dismutase (SOD) activity. Total SOD activity was determined according to Beyer and Fridovich [1987] by monitoring the inhibition of nitroblue tetrazolium (NBT) photoreduction using the following colorimetric assay: the reaction mixtures (1 mL) containing 50 mM potassium phosphate buffer (pH 7.8), 9.9 mM methionine, 58 µM NBT, 0.025% (v/v) Triton X-100, 2.4 µM riboflavin (as the source of superoxide radicals) and the protein extract. After adding riboflavin, the reaction mixtures were irradiated (300 µmol m^{-2} s^{-1}, provided by three 23 W Osram DULUX PRO compact fluorescent lamps) for 10 min at 25°C, and the absorbance at 560 nm was measured, using a non-irradiated reaction mixture as a blank. One SOD unit was defined as the amount of enzyme that causes 50% inhibition of NBT photo-reduction under assay conditions. The results were expressed as units/min/mg protein.

Measurements of ascorbate peroxidase (APX) activity. The activity of APX was determined by measurement of the absorbance diminution of oxidized ascorbate at 290 nm (using quartz cuvette) according to Nakano and Asada [1981]. The 2 mL reaction mixture contained ascorbate (0.5 mM), EDTA (0.1 mM), H_2O_2 (1.2 mM) and protein extract. Reactions were initiated by adding H_2O_2 and the decrease in OD was monitored until it became stable. Control reactions with no protein extract were incubated in parallel to correct for non-enzymatic H_2O_2 reduction. Activity was calculated using the extinction coefficient (2.8 mM^{-1} cm^{-1} at 290 nm) for ascorbate and results were expressed as µmol ascorbate/min/mg protein.

Measurements of peroxidase (POD) activity. The peroxidase (POD) activity was determined using 4-methylcatechol as a substrate. The increase in the absorption caused by oxidation of 4-methylcatechol by H_2O_2 was measured at 420 nm spectrophotometrically. The reaction mixture contained 100 mM sodium phosphate buffer (pH 7.0), 5 mM 4-methylcatechol, 5 mM H_2O_2 and 500 µL of crude extract in a total volume of 3.0 mL at room temperature. One unit of enzyme activity was defined as 0.001 change in absorbance per min under assay conditions and results were expressed as unit/mg protein [Ghamsari et al. 2007].

Measurements of catalase (CAT) activity. CAT activity was determined by monitoring the disappearance of H_2O_2 at 240 nm according to the method of Aebi [1984]. The reaction mixture contained 50 mM K-phosphate buffer (pH 7.0), 33 mM H_2O_2 and enzyme extract. The results were expressed as µmol H_2O_2 min^{-1} mg^{-1} protein.

Determination of Hydrogen peroxide (H_2O_2) concentration. To quantify H_2O_2 concentration, 0.2 g fresh leaves were ground and extracted with 1.5 mL cold acetone. After centrifugation (12,000 g, 15 min), the supernatant was used to determine H_2O_2 concentration according to the method of Patterson et al. [1984].

Statistical analysis

The experiment was conducted as factorial in a randomized complete block design with four replications. Experiment treatments included salinity of sodium chloride (NaCl) at three levels (0, 75 and 150 mM) and SA foliar application at four concentrations (0, 0.5, 1 and 1 mM). Data analysis was performed by SAS software and the mean values were processed by Duncan's multi-domain test at a probability level of $P \leq 0.05$.

Results and discussion

Sodium and potassium contents. Sodium contents of leaves significantly increased as the salinity increased (Fig. 1A), but decreased in the leaves at high concentrations of SA (1 and 1.5 mM). However, 0.5 mM SA showed no significant change as compared to controls (Fig. 1B). Unlike sodium, the potassium concentrations of leaves decreased when salinity stress increased ($P \leq 0.05$) at all concentrations (Fig. 2A). At the highest concentration of SA, potassium values significantly increased (Fig. 2B). The interaction of salinity and SA demonstrated that all SA treatments significantly affected the Na^+/K^+ ratio and the best result was obtained at 1.5 mM of SA (Fig. 3). Potassium plays an important role in balancing the membrane potential and turgor, activating enzymes and regulating the osmotic pressure, stomatal movement and tropisms. Potassium leakage normally happens as a result of NaCl induced membrane depolarization under saline conditions [Shabala 2003]. Given that intracellular Na^+/K^+ homeostasis is essential for cell metabolism and is considered a key component of salinity tolerance in plants, the interaction of salinity stress and salicylic acid on sodium ratio was important. Salinity has been shown to increase the uptake of Na^+ and decrease the uptake of K^+. Reducing Na^+ content after SA treatments results in low membranes injury [Yildirim et al. 2008]. These results are in agreement with Gunes et al. [2005], who found that exogenously applied SA decreased Na^+ and raised K^+ contents compared to those of non-treated ones under salt stress. According to the results of present study, potassium content of grape leaves increased significantly, which was previously reported by Kaya et al. [2003] and different levels of salicylic acid reduced the Na^+/K^+ ratio of leaves compared to the control and caused tolerance enhancement of 'Sultana' cultivar during salinity stress. Also, the highest effect was observed at the highest (1.5 mM) level of salicylic acid. The results were also in agreement with Szepsi et al. [2005].

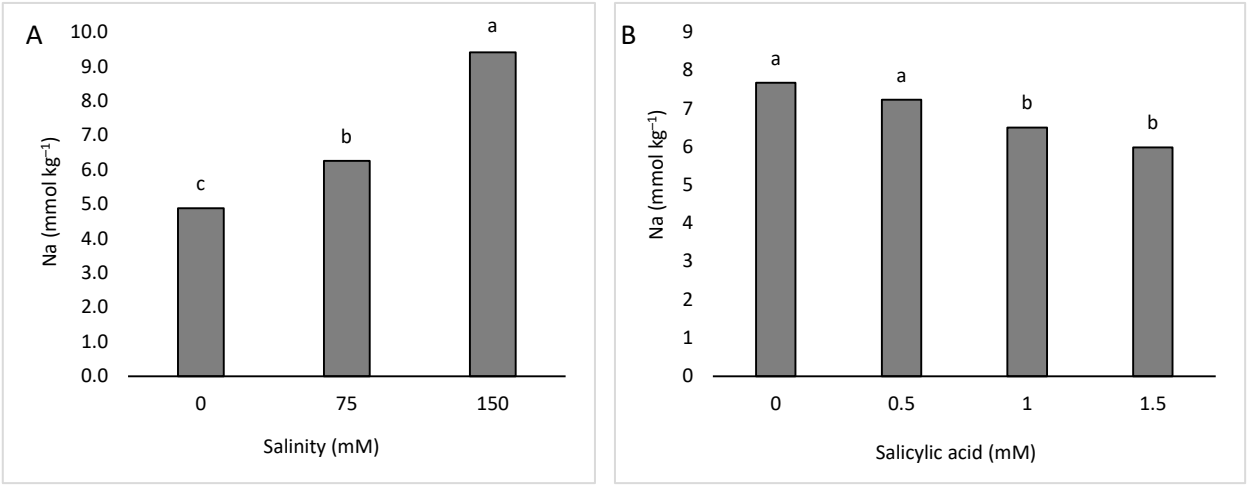

Fig. 1. Effects of salinity (A) and salicylic acid (B) on sodium (Na) concentration in grape leaves. Values marked with different letters are significantly different at $P \leq 0.05$

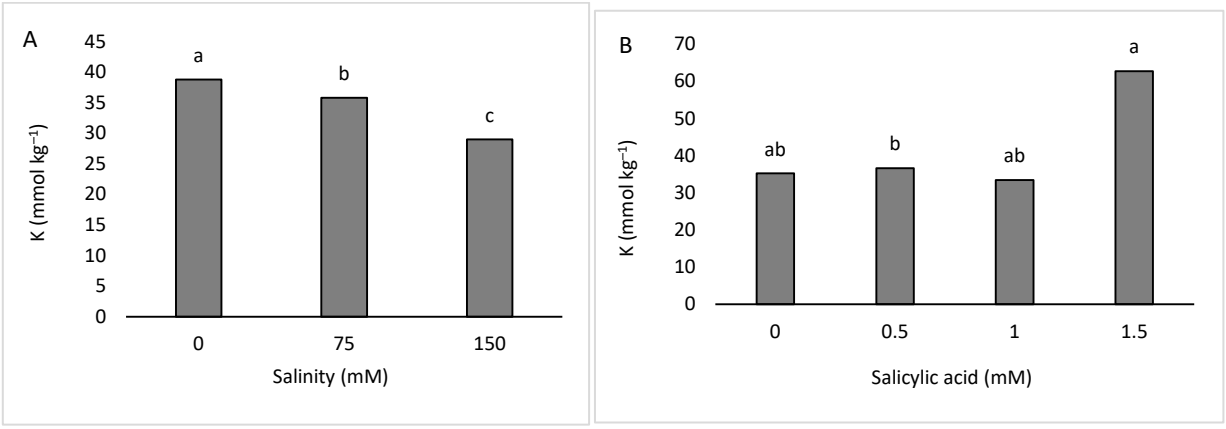

Fig. 2. Effects of salinity (A) and salicylic acid (B) on potassium (K) concentration in grape leaves. Values marked with different letters are significantly different at $P \leq 0.05$

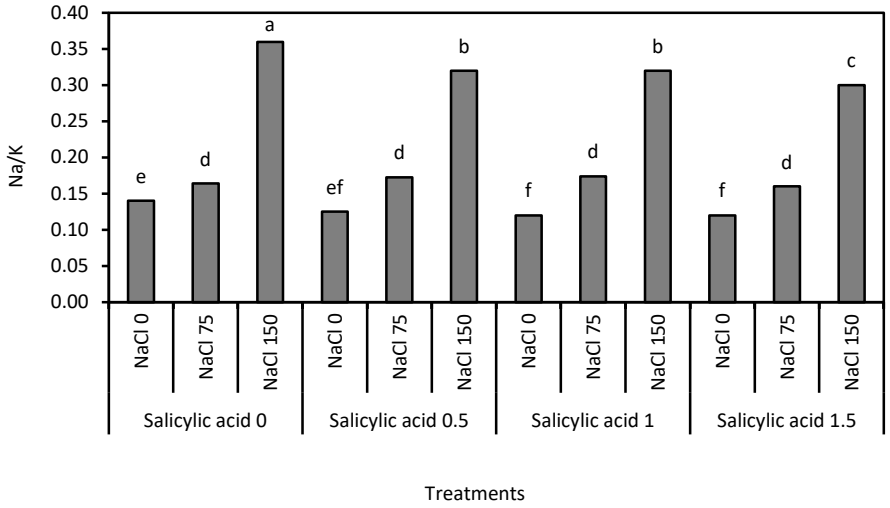

Fig. 3. Effects of salinity and salicylic acid on sodium to potassium ratio (Na/K) in grape leaves. Values marked with different letters are significantly different at $P \leq 0.05$

Leaf electrolyte leakage. This parameter was included in order to get more information on membrane stability and thereby on the relative ion content in the apoplastic space. Salinity impaired membrane permeability caused the increase in electrolyte leakage. Enhancement at salinity levels caused electrolyte leakage of 'Sultana' cultivar leaves, but based on the results, up to 75 mM, did not show significant difference and just when increasing salinity to 150 mM, the significant increase was observed in comparison with control. In other words, salinity stress up to 75 mM had not undesirable effects on the cell membrane properties on grape leaves (Fig. 4A). Application of SA on grape leaves caused significant decrease in electrolyte leakage and significant decrease was observed at 1.5 mM SA compared to the others (Fig. 4B). Electrolyte leakage causes cell membrane injury when plants are subject to salinity stress [Stevens et al. 2006]. Furthermore, application of salicylic acid (SA) partly maintained membrane permeability as SA application could influence membrane permeability [Yildirim et al. 2008]. Positive effect of SA

application on reduction of electrolyte leakage was previously reported by Shi et al. [2006].

Proline content of the leaves. The plants treated with salt had higher amount of proline as compared to the controls. At salinity conditions, the increase at concentrations of SA caused the increase in proline content (Fig. 5). It was showed that 1.5 mM SA and 150 mM NaCl caused increase in proline content by supporting the defense mechanism of the plant. Some studies reported that this compound could have a defense mechanism supporting the role for the plants under stress. Proline, as compatible solute, accumulates in plant cells under salinity condition and acts as a free radical scavenger and enzyme protectant to be involved in stress resistance [Hare and Cress 1997]. Proline indirectly increased the metabolic activation by providing osmoregulation of plants under stress [Pesserakli et al. 1989]. Based on the results of current study, all concentrations of SA increased in proline content significantly, which improved the regulation of osmotic potential of the cell. It could be concluded that SA application might activate the metabolic consumption of soluble sugars to form new cell constitutes as a mechanism to stimulate growth and leading to the increased proline accumulation to reduce the injurious effects of salinity on the salt stress of plants [Szepsi et al. 2005].

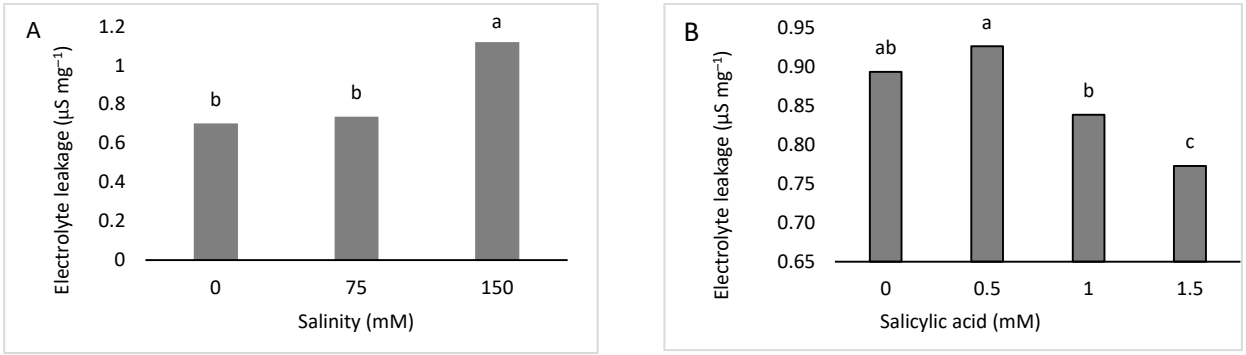

Fig. 4. Effects of salinity (A) and salicylic acid (B) on electrolyte leakage in grape leaves. Values marked with different letters are significantly different at $P \leq 0.05$

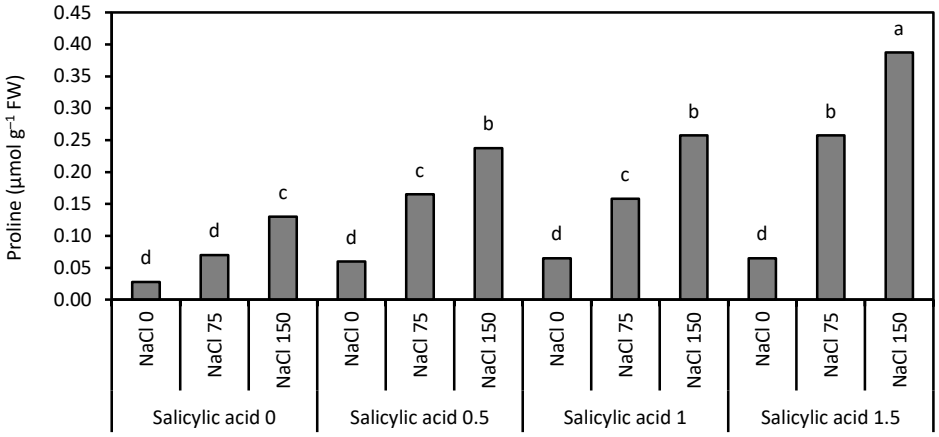

Fig. 5. Effects of salinity and salicylic acid on proline content in grape leaves. Values marked with different letters are significantly different at $P \leq 0.05$

Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA). According to the results of present study, the significant increase was observed in H_2O_2 levels (Fig. 6A) due to salinity stress, which indicates oxidative stress. Salicilic acid application at concentration of 1 mM caused significant decrease in H_2O_2 values ($P \leq 0.05$), but other concentrations showed no significant different in this regard (Fig. 6B). High concentrations of H_2O_2 have toxicity effect and cause the cell death, however accumulation of H_2O_2 to a certain degree in plants may indicate a signaling role during acclimation to abiotic stress [Wang and Li 2006]. Thus, sharp increase in H_2O_2 values at high salinity might be hazardous and reduction in its amount after SA application might cause tolerance to salt stress.

According to the results (Fig. 7), the MDA content, as a trait of oxidative stress, showed decreasing trend as salicylic acid levels increased. The highest decrease was observed at highest concentration of SA at the highest level of salinity stress. Also, application of SA (1 mM) was significantly effective at decreasing the MDA content ($P \leq 0.05$). Therefore, it could be concluded that salicylic acid reduces the oxidative stress caused by salinity stress and induces the tolerance to it.

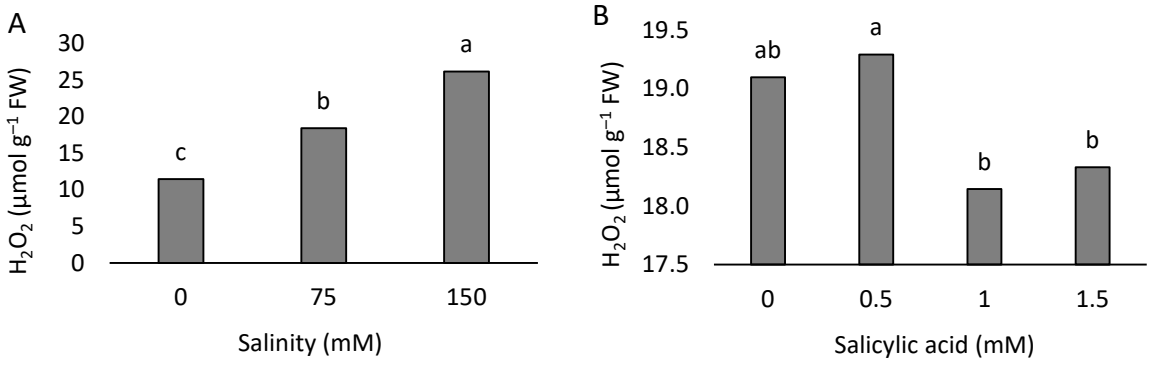

Fig. 6. Effects of salinity (A) and salicylic acid (B) on H_2O_2 content in grape leaves. Values marked with different letters are significantly different at $P \leq 0.05$

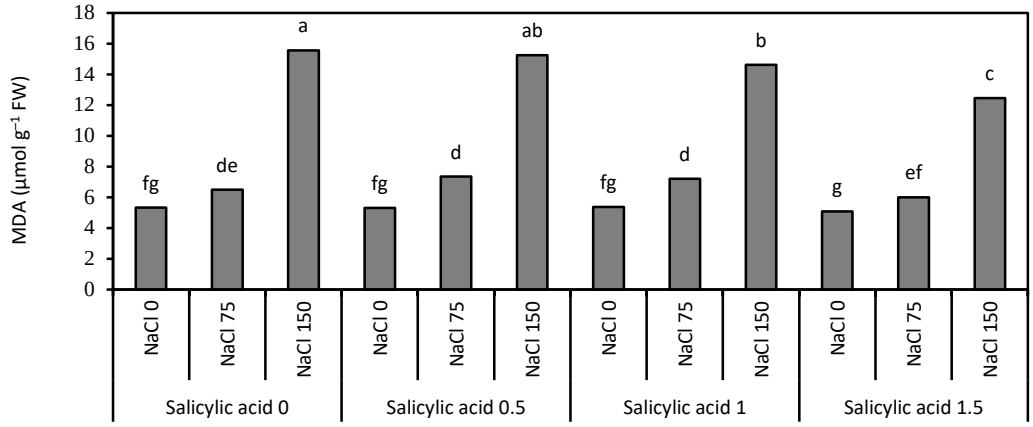

Fig. 7. Effects of salinity and salicylic acid on MDA content in grape leaves. Values marked with different letters are significantly different at $P \leq 0.05$

Enzyme activity. The results showed that salinity and SA increased the concentrations of all antioxidant enzymes tested (POD, APX, CAT and SOD). The POD enzyme activity at low (75 mM) and high (150 mM) salinity significantly ($P \leq 0.05$) increased as compared to the control (Fig. 8A). Moreover, POD activity at 1 and 1.5 mM increased significantly compared to 0.5 mM SA and control (Fig. 8B). The APX increased significantly as salinity increased, so that 75 and 150 mM salinity caused the same increase at APX activity compared to the control (Fig. 9A). Application of SA at 1 and 1.5 mM caused significant enhancement at APX activity, but 0.5 mM SA had no significant difference as compared with the control (Fig. 9B). Likewise, CAT enzyme activity increased whenever the salinity increased. The highest activity was observed at 150 mM NaCl (Fig. 10A). The SOD enzyme activity increased significantly ($P \leq 0.05$) at 150 mM salinity (Fig. 10B) and SA application increased the SOD activity at all concentration (Fig. 11).

Plants with high levels of antioxidants have greater resistance to oxidative damage. Salt stress leads to the formation of reactive oxygen species that disturb normal metabolism through oxidative damage. Prevention of oxidative damage to cells during stress has considered as one of the mechanisms of stress tolerance [Wang and Li 2006]. The improvement of stress tolerance is often related to enhancement of antioxidant activities in plants and it was reported that SA acts as an antioxidant [Agarwal et al. 2005]. Enhanced activity of certain antioxidant enzymes with SA applications under stress conditions including salinity has been observed [Shi et al. 2006, Stevens et al. 2006]. Different studies have reported the effect of SA application on antioxidant activity of plants. For example, SA application decreased the apoplastic catalase (CAT) enzyme activity, increased apoplastic peroxidase (POD) and polyphenol oxidase (PPO) activities [Tasgin et al. 2006]. SA application increased POD, CAT, SOD and APX enzyme activities [Agarwal et al. 2005]. Significant increase in POD, APX and SOD activities after SA application was also reported by Wang and Li [2006]. SA application might cause the induction of antioxidant response that increases the tolerance of plant to damage [Yildirim et al. 2008]. In conclusion, salicylic acid may mediate the acclimation of plants to environmental stress, and may interact with other cellular metabolites and environmental factors in the regulation of stress responses [Yordanova and Popova 2007]. Our results are in agreement with above mentioned studies and could be described by them.

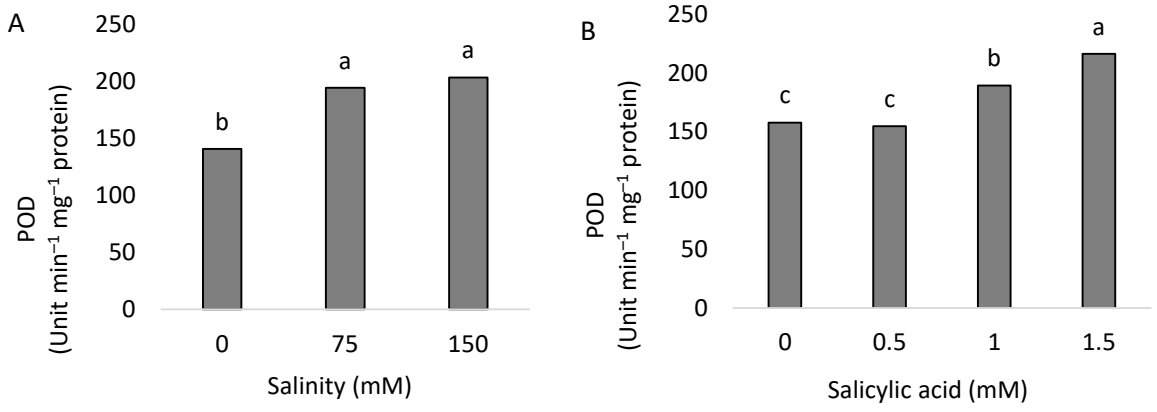

Fig. 8. Effects of salinity (A) and salicylic acid (B) on POD activity in grape leaves. Values marked with different letters are significantly different at $P \leq 0.05$

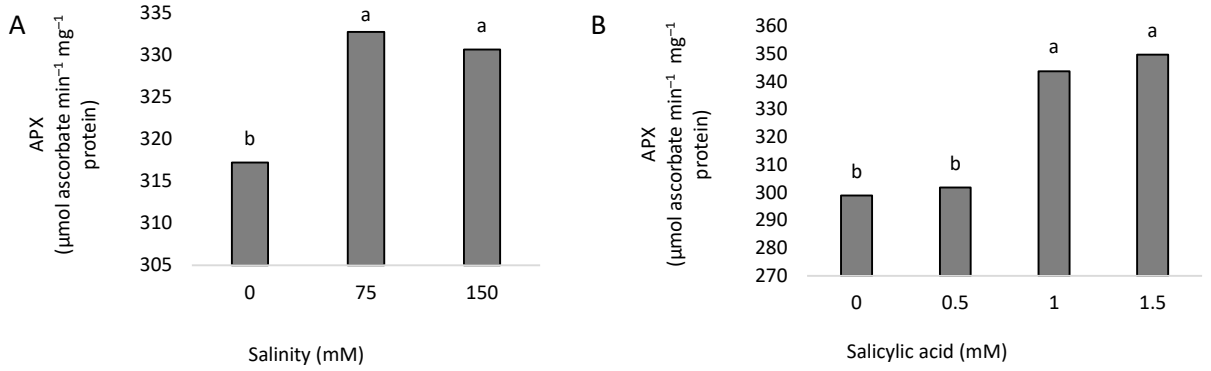

Fig. 9. Effects of salinity (A) and salicylic acid (B) on APX activity in grape leaves. Values marked with different letters are significantly different at $P \leq 0.05$

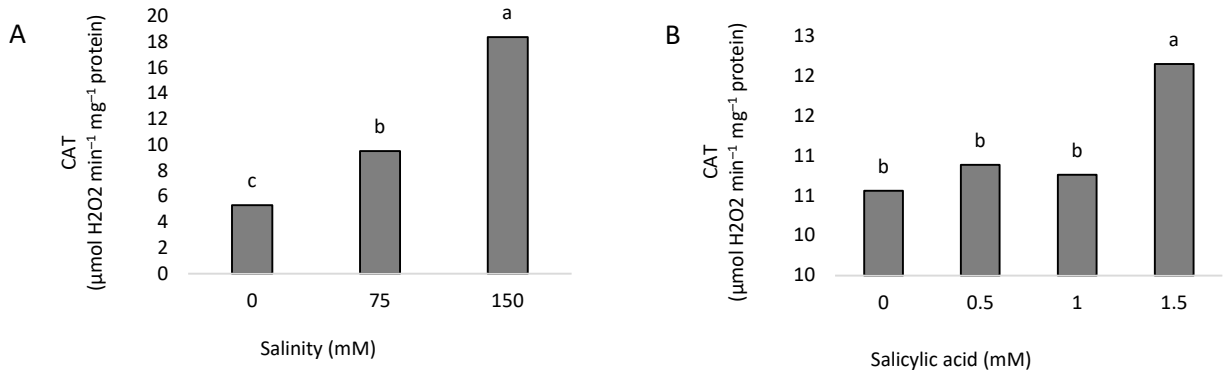

Fig. 10. Effects of salinity (A) and salicylic acid (B) on CAT activity in grape leaves. Values marked with different letters are significantly different at $P \leq 0.05$

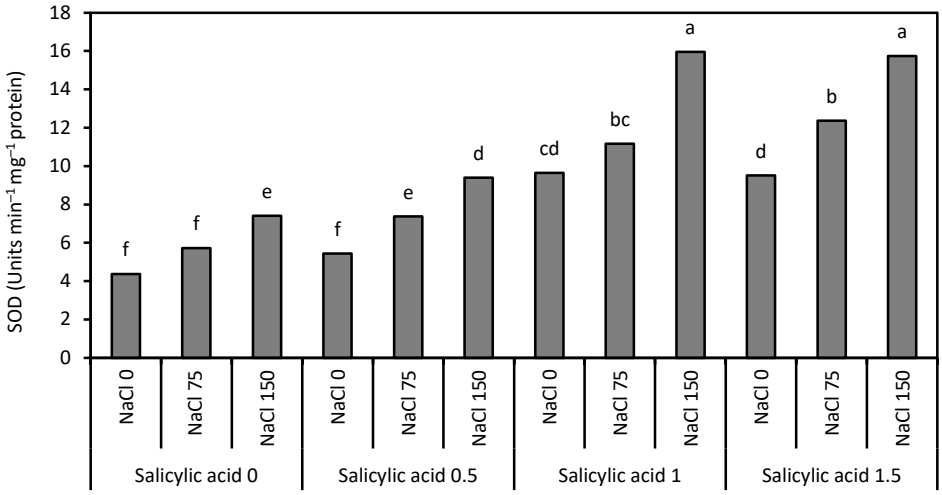

Fig. 11. Effects of salinity and salicylic acid on SOD activity in grape leaves. Values marked with different letters are significantly different at $P \leq 0.05$

CONCLUSIONS

Salicylic acid (SA), as a plant regulator with great effects on biotic and abiotic resistance of plants, might be applied as a helpful turn to maintain tolerance during salinity stress. Thus, its application on grapes in the course of saline condition could decrease the negative effects of salinity. In general, SA application on grapes under salinity stress improved antioxidant enzyme activities (POD, APX, CAT and SOD) and proline content as desirable characters and decreased Na^+/K^+ ratio, leaf electrolyte leakage, MDA and H_2O_2 values as undesirable ones. The results of this work conclude that SA application may be a satisfactory routine that could increase the salinity tolerance of grapes.

ACKNOWLEDGMENTS

This work was supported by a research grant from Research Deputy of the Islamic Azad University, Miyaneh Branch.

REFERENCES

AbdElgawad, H., Zinta, G., Hegab, M., Pandey, R., Asard, H., Abuelsoud, W. (2016). High salinity induces different oxidative stress and antioxidant responses in maize seedling organs. Front Plant Sci., 24, 134–141.

Aebi, H. (1984). Catalase *in vitro*. Methods Enzymol., 105, 121–126.

Agarwal, S., Sharma, S., Agrawal, V., Roy, N. (2005). Caloric restriction augments ROS defense in S. cerevisiae, by a Sir2p independent mechanism. Free Radical Res., 39, 55–62.

Arfan, M., Athar, H.R., Ashraf, M. (2007). Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthesis capacity in differently adapted spring wheat cultivars under salt stress? J. Plant Physiol., 6, 685–694.

Bates, L.S., Waldren, R.P., Teare, I.D. (1973). Rapid determination of free proline for water-stress studies. Plant Soil., 39, 205–207.

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.

Beyer, W.F., Fridovich, I. (1987). Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal. Biochem., 161, 559–566.

Ghamsari, L., Keyhani, E., Golkhoo, S. (2007). Kinetics properties of guaiacol peroxidase activity in *Crocus sativus* L. corn during rooting. Iran Biomed. J., 11, 137–146.

Gunes, A., Inal, A., Alpaslan, M., Cicek, N., Guneri, E., Eraslan, F., Guzelordu, T. (2005). Effects of exogenously applied salicylic acid on the induction of multiple stress tolerance and mineral nutrition in maize (*Zea mays* L.). Arch. Agro. Soil Sci., 51, 687–695.

Hare, P.D., Cress, W.A. (1997). Metabolic implications of stress-induced proline accumulation in plants. Plant Growth Regul., 21, 79–102.

Hayat, S., Ahmad, A. (2007). Salicylic acid: A plant hormone. Springer, Dordrecht, The Netherlands.

Hayat, Q., Hayat, S., Irfan, M., Ahmad, A. (2010). Effect of exogenous salicylic acid under changing environment: A review. Environ. Exp. Bot., 68, 14–25.

Kaya, C., Higgs, D., Ince, F., Amador, B.M., Cakir, A., Sakar, E. (2003). Ameliorative effects of potassium phosphate on salt-stressed pepper and cucumber. J. Plant Nut., 26, 807–820.

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.

Lutts, S., Kinet, J.M., Bouharmont, J. (1996). NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. Ann. Bot., 78, 389–398.

Munns, R., James, R.A. (2003). Screening methods for salinity tolerance: A case study with tetraploid wheat. Plant Soil. 253, 201–218.

Munns, R., Tester, M. (2008). Mechanisms of salinity tolerance. Annu. Rev. Plant Biol., 59, 651–681.

Nakano, Y., Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant Cell Physiol., 22, 867–880.

Pessarakli, M., Huber, T.J., Tucker, T.C. (1989). Dry matter yield, nitrogen absorption and water uptake by sweet corn under salt stress. J. Plant Nutr., 12, 279–290.

Sairam, R.K., Tyagi, A. (2004). Physiology and molecular biology of salinity stress tolerance in plants. Curr Sci., 86, 407–421.

Senaratna, T., Touchell, D., Bunn, E., Dixon, K. (2000). Acetyl salicylic acid (aspirin) and salicylic acid induce

multiple stress tolerance in bean and tomato plants. Plant Growth Regul., 30, 157–161.

Shabala, S., Cuin, T.A., Pang, J., Percey, W., Chen., Z., Conn, S., Eing, C., Wegner, L.H. (2003). Xylem ionic relations and salinity tolerance in barley. Plant J. 61, 839–853.

Shakirova, F.M., Bezrukova, M.V. (1997). Induction of wheat resistance against environmental salinization by salicylic acid. Biol Bull., 24, 109–112.

Shi, Q., Bao, Z., Zhu, Z., Ying, Q., Qian, Q. (2006). Effects of different treatments of salicylic acid on heat tolerance, chlorophyll fluorescence, and antioxidant enzyme activity in seedlings of *Cucumis sativa* L. Plant Growth Regul., 48, 127–135.

Stevens, J., Senaratna, T., Sivasithamparam, K. (2006). Salicylic acid induces salinity tolerance in tomato (*Lycopersicon esculentum* cv. 'Roma'): associated changes in gas exchange, water relations and membrane stabilisation. Plant Growth Regul., 49, 77–83.

Szepesi, A., Csiszar, J., Bajkan, S., Gemes, K., Horvath, F., Erdei, L., Deer, A.K., Simon, M.L., Tari, I. (2005). Role of salicylic acid pre-treatment on the acclimation of tomato plants to salt- and osmotic stress. Acta Biol. Szegediensis, 49, 123–125.

Tari, I., Csiszar, J., Szalai, G., Horvath, F., Pecsvaradi, A., Kiss, G., Szepesi, A., Szabo, M., Erdei, L. (2002). Acclimation of tomato plants to salinity stress after a salicylic acid pre-treatment. Acta Biol. Szegediensis, 6, 55–56.

Tandon, H.LS. (1998). Methods of analysis of soils, plants, water and fertilizers. Fertiliser Development and Consultation Organisation, New Delhi, India.

Patterson, B.D., Macrae, E.A., Ferguson, I.B. (1984). Estimation of hydrogen peroxide in plant extracts using titanium(IV). Anal. Biochem., 139, 487–492.

Wang, L.J., Li, S.H. (2006). Thermotolerance and related antioxidant enzyme activities induced by heat acclimation and salicylic acid in grape (*Vitis vinifera* L.) leaves. Plant Growth Regul., 48, 137–144.

Wen, P.F., Chen, J.Y., Wan, S.B., Kong, W.F., Zhang, P., Wang, W., Zhan, J.C., Pan, Q.H., Huang, W.D. (2008). Salicylic acid activates phenylalanine ammonia-lyase in grape berry in response to high temperature stress. Plant Growth Regul., 55, 1–10.

Yildirim, E., Turan, M., Guvenc, I. (2008). Effect of foliar salicylic acid applications on growth, chlorophyll, and mineral content of cucumber grown under salt stress. J. Plant Nutr., 31, 593–612.

Yordanova, R., Popova, L. (2007). Effect of exogenous treatment with salicylic on photosynthetic activity and antioxidant capacity of chilled wheat plants. General App. Plant Physiol., 33, 155–170.

Zhang, J.L., Shi, H. (2013). Physiological and molecular mechanism of plant salt tolerance. Photosynth. Res., 115, 1–22.

Wallace, G., Fry, S.C. (1999). Action of diverse peroxidases and laccases on six cell wall-related phenolic compounds. Phytochemistry, 52, 769–773.