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Evidence for Enhancement of Salinity Induced Oxidative Damages by Salicylic Acid in Radish (*Raphanus sativus* L.)

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Abstract
In recent years some research results have appeared that exogenous salicylic acid provided significant mitigation effect against salinity induced injuries in plants. An experiment was conducted to investigate the effects of salt stress and salicylic acid on radish (*Raphanus sativus* L.) plants. After appearing first leaves, plants were treated by salicylic acid (0.2 mM) for 3 days and NaCl (180 mM) applied 48 hours after first treatment of salicylic acid. Results showed that salt stress decreased fresh weight of shoots and roots, chlorophyll *a*, chlorophyll *b*, anthocyanins and flavonoids contents of leaves. In opposite, this stress increased amount of leaves carotenoids, and both leaves and roots phenolic compounds, hydrogen peroxide, proline and lipid peroxidation content. Salicylic acid had similar effects on these factors. Applying salicylic acid in the saline condition decreased fresh weight of shoot and root, chlorophyll *a*, chlorophyll *b*, flavonoids, membrane stability index and increased amount of anthocyanins, carotenoids of leaves, phenolic compounds, proline, hydrogen peroxide, lipid peroxidation of leaves and root. We concluded that benefit of exogenous salicylic acid can be dose dependent and salinity plus salicylic acid, at concentrations applied in this study, were established potent secondary oxidative stress in plants.

Keywords: Growth; Oxidative stress; Radish (*Raphanus sativus* L.); Salicylic acid; Salinity

Introduction

Salinity conditions in soil or water is one of the major stresses especially in arid and semi-arid regions worldwide (Parvaiz and Satyawati 2008), which substantially affects almost all stages of plant growth and development. Thus, salinity is one of the most important stresses that hamper crop productivity worldwide. During salinity stress conditions, plants are subjected to both osmotic stress and ion toxicity, leading to secondary oxidative stress by activated oxygen species (AOS) production (Chaparzadeh *et al.* 2004). Plants can show adaptive biochemical and physiological mechanisms at the molecular, cellular and whole-plant levels, under various environmental stress conditions such as salinity (Shahbaz *et al.* 2012). Hence, changes in leaf water relations, the accumulation of various inorganic ions and organic metabolites including soluble sugars, proline and amines are the most important. AOS can induce cellular damage by chlorophyll, proteins and nucleic acids degradation, membrane lipid peroxidation, enzymes inactivation, gene expression alterations, and interfere in various pathways of metabolic processes (Ashraf 2004). The ability of higher plants to scavenge the toxic AOS seems to be a very important determinant of their tolerance to environmental stresses (Chaparzadeh *et al.* 2014). To scavenge the AOS, plants possess antioxidant enzymes and compounds such as reduced glutathione, ascorbic acid, tocopherols, carotenoids, flavonoids and phenolic compounds (Saed-Moucheshi *et al.* 2014). Phenolic compounds are naturally occurring substances in plants; many of those are thought to play

physiological roles such as antibacterial, antiviral, anticancer activities and scavenge of most types of oxidizing molecules. Under biotic and abiotic stresses such as pathogen attack, physical wounding and UV-light exposure, plants can induce a defense response and increase secondary metabolite levels**.** Anthocyanins are water-soluble pigments found in all plant tissues throughout the plant kingdom. Their subsequent production and localization allow the plant to develop resistance to a number of environmental stresses (Chalker-Scott 1999).

Salicylic acid (SA), a phenolic compound, is an endogenous growth regulator and signal molecule that is associated with stress tolerance in plants. Previous studies are showing that exogenously applied SA can induce tolerance against different types of stresses (Stevens *et al.* 2006; Ghorbani *et al.* 2011; Naser Alavi *et al.* 2014). Also, its exogenous application may influence a range of diverse processes in plants including growth, stomatal closure, ion uptake and transport, ethylene synthesis, seed germination, fruit yield and the inhibitory effect of abscisic acid (Horvath *et al.* 2007). Although increasing evidence indicates that SA may cause oxidative stress to plants through the accumulation of H_2O_2 (Chao *et al.* 2010), the application of exogenous SA with low concentrations may have stress induced adaptation effect, causing enhanced tolerance toward most kinds of abiotic stresses due primarily to enhanced antioxidative capacity (Horvath *et al.* 2007; Belkadhi *et al*. 2014).

Pressure of increasing world population, and on the other hand, incessantly increasing of world saline lands, needs to bring new crops to saline agriculture. Plant responses to salinity and signal molecules are very complex, and species or genotype dependent, thus studies on salinity tolerance with similar conditions may result different conclusions. Radish has economic and nutritional value and is a good species to study (Curtis 2003). This plant was reported showing a strong tolerance to salinity (Sugimoto 2009). Despite the large body of the literature on salt stress, to our knowledge, there is no report about salinity and SA interactive effects on radish. Therefore, present experiment was designed to investigate the effect of SA on changes in growth and some physiological markers, in radish (*Raphanus sativus* L.) plants subjected to salinity conditions.

Materials and methods

Radish seeds were sterilized with sodium hypochlorite solution (1%) for five minutes and washed thoroughly with distilled water before use. At first, plants were irrigated by modified Hoagland nutrient solution. After appearing of first leaves, 0.2 mM solution of SA was applied for 3 days. The salt (NaCl 180 mM) treatment was applied 48 hours after first treatment of salicylic acid for 6 days. Seedlings were kept at 27±2/20±2 °C day/night, 16 h photoperiod, 250 µmol m-2s-1 photon fluence and relative humidity of 40%. All determinations were carried out 24 hours after last NaCl treatment on twenty-day-old plants.

Plants were harvested 24 hours after last treatment for measurement of shoot and root fresh weights by 8 replications.

For extraction of photosynthetic pigments, aliquots of fresh leaves were grinded with acetone

and rewashed another time with acetone. The homogenates were then centrifuged. The pellets were rewashed by acetone and centrifuged again. The supernatant absorbance was determined in 470, 646 and 663 nm by spectrophotometer and concentration of pigments was accounted (Lichtenthaler 1987). Results were expressed in mg g^{-1} FW.

For measuring the hydrogen peroxide content, aliquots of fresh leaves and roots were homogenized in 50 mM potassium phosphate buffer (pH, 6.5) and centrifuged. The solution was mixed with 1% titanium chloride (in concentrated HCl). Samples centrifuged and absorbance of supernatant was determined at 410 nm. The amount of H_2O_2 was calculated using the extinction coefficient of 0.28 $\mu M^{-1}cm^{-1}$ and the results expressed as μmol g^{-1} FW (Jana and Chaudhuri 1981).

Lipid peroxidation level was measured as the content of malondialdehyde (MDA) using thiobarbituric acid reacting substances **(**TBARS) method. Aliquots of fresh leaves and roots were homogenized in 20% trichloroacetic acid containing 0.5% thiobarbituric acid and incubated at 95◦C in water bath for 30 min and then quickly cooled in an ice bath. After centrifugation, the absorbance of the supernatant was recorded at 532 nm and corrected for nonspecific absorbance at 600 nm. The amount of MDA was calculated using the extinction coefficient of 155 $mM^{-1}cm^{-1}$ and the results expressed as μmol g^{-1} FW (Chaparzadeh *et al.* 2004).

Electrolyte leakage was used to assess membrane stability**.** Discs of leaves from the young fully expanded leaves were rinsed with distilled water to remove electrolyte released during excision. Then, leaf discs were submerged into double distilled water and incubated on a rotatory shaker for 24 h at room temperature. Electrical conductivity of bathing solution (EC_1) was determined at the end of incubation period. Samples were frozen (and thawed) and electrical conductivity of solution (EC_2) was measured again. The cell membrane stability (MSI) was calculated as: $MSI= [1 - (EC_1/EC_2)] \times 100$ (Chaparzadeh and Ghodrati Chagharlou 2013)

To determine levels of anthocyanins, aliquots of fresh leaves were homogenized with methanol containing 1% HCl and incubated for 24 hours. After centrifugation, the absorbance of supernatant was measured at 550 nm and data expressed as $Abs_{550\ nm}$ g^{-1} FW (Chaparzadeh and Ghodrati-Chagharlou 2013).

To determine the amount of flavonoids, aliquots of fresh leaves were homogenized with 90% ethanol. The same volume of petroleum ether was added to supernatant. After shaking the absorbance of flavonoids containing phase was determined at 330 nm and data expressed as Abs 330 nm g^{-1} FW (Krizek *et al.* 1998).

For evaluation of total phenolic compounds, aliquots of fresh leaves and roots were homogenized with methanol. The total phenolic compounds content was determined using Folin-Ciocalteu reagents with gallic acid as the standard. After centrifuging, 0.1 ml of supernatant or standard solution was added to 0.9 ml distilled water and Folin-Ciocalteu reagent. After 5 minutes, 2 ml sodium carbonate was added to the mixture. The solution heated in a 45 ^oC oven for 15 min. After incubation for 60 min at room

temperature and dark, the absorbance at 750 nm was measured and calculated from a standard curve for gallic acid and expressed as mg g^{-1} FW (Chaparzadeh and Ghodrati-Chagharlou 2013).

For estimation of proline, aliquots of fresh leaves and roots were homogenized and kept in 80% ethanol in tubes. Then samples were heated to 60^{o}C for 30 min and homogenate was centrifuged. The supernatant was used for the estimation of proline content. The reaction mixture, consisted of 2 ml acid ninhydrin and 2 ml of glacial acetic acid, was boiled at 95^{o}C for 30 min. After termination of reaction in ice bath, the reaction mixture was extracted with 4 ml of toluene and absorbance was read at 520 nm. Proline contents were expressed as μmol g^{-1} FW (Raymond and Smirnoff 2002).

The results presented in this study were the means (± SE) of eight replications for growth characters and four replications for others. The means were compared by the Duncan's multiple range test ($p \leq 0.05$) using the SPSS software.

Results

Both salt and SA treatments led to decreasing of fresh weight of shoots without significant effect on roots fresh weight. However, applying SA in the saline condition had negative effect on fresh weight of both shoots and roots (Table 1).

Data show that both salt stress and SA treatments decreased chlorophyll *a*, chlorophyll *b* and total chlorophyll contents. Also applying SA in the saline condition decreased chlorophyll *a*, *b* and total chlorophyll (Table 2). In leaves, salinity, SA and salinity plus SA treatments showed a significant increase of carotenoids content compared with the control (Table 2). SA treatment enhanced the carotenoids content more than salinity plus SA. Hydrogen peroxide content of leaves and roots were increased in the salinity and SA treatments in comparison with the control plants. Applying SA in the saline condition led to maximum increasing of hydrogen peroxide content in leaves and roots (Table 1).

Pattern of lipid peroxidation of leaves was slightly different from roots. In leaves, under salinity plus SA pretreatment, lipid peroxidation was significantly more than the control plants. In roots, the exposure of salt stress greatly increased lipid peroxidation in both the presence and absence of SA (Table 1).

Salinity and SA per se didn't change membrane stability of leaf cells. When the SA pretreated plants were subjected to salinity condition, membrane stability was significantly less than the control plants (Table 2).

The anthocyanins content of SA-treated leaves were significantly decreased as compared with the control plants (Table 1). There was no significant increase of anthocyanins in the salinity plus SA treatment compared to the salinity alone (Table 2).

Salt stress didn't have significant effect on flavonoids content. SA application alone decreased flavonoids content. SA pretreatment in the saline condition decreased the flavonoids contents more than SA treated plants, however the difference was not significant (Table 2).

Phenolic compounds of leaves and roots were significantly increased in the SA treated and salt stressed plants compared to the control plants.

Table 1. The effect of salinity and SA on fresh weight, phenolic compounds, proline, hydrogen peroxide and thiobarbituric acid reacting substances contents of leaves and roots

Characters		Control	NaCl	SA	NaCl + SA
Fresh weight ($g\ plant^{-1}$)	Leaf	0.66 ± 0.13^{a}	0.55 ± 0.12^{b}	0.49 ± 0.08^{b}	0.39 ± 0.03^{c}
	Root	0.11 ± 0.01^{a}	0.10 ± 0.02^{a}	0.10 ± 0.01^{a}	0.09 ± 0.01^{b}
Phenolic compounds ($mg\ g^{-1}$ FW)	Leaf	1.23 ± 0.36^{c}	1.99 ± 0.36^{b}	2.32 ± 0.39^{b}	2.98 ± 0.66^{a}
	Root	1.25 ± 0.14^{d}	2.12 ± 0.34^{c}	2.89 ± 0.33^{b}	4.00 ± 0.46^{a}
Proline ($\mu mol\ g^{-1}$ FW)	Leaf	7.9 ± 0.84^{d}	9.22 ± 0.81^{c}	15.35 ± 0.8^{a}	12.77 ± 0.65^{b}
	Root	7.45 ± 0.25^{d}	8.5 ± 0.72^{c}	15.00 ± 0.19^{a}	12.35 ± 0.54^{b}
Hydrogen peroxide ($\mu mol\ g^{-1}$ FW)	Leaf	0.017 ± 0.003^{c}	0.030 ± 0.007^{b}	0.024 ± 0.004^{b}	0.039 ± 0.005^{a}
	Root	0.011 ± 0.001^{c}	0.023 ± 0.004^{b}	0.029 ± 0.001^{b}	0.036 ± 0.011^{a}
TBARS ($\mu mol\ g^{-1}$ FW)	Leaf	0.024 ± 0.004^{b}	0.029 ± 0.003^{ab}	0.026 ± 0.001^{ab}	0.030 ± 0.006^{a}
	Root	0.035 ± 0.013^{b}	0.049 ± 0.011^{a}	0.037 ± 0.010^{b}	0.058 ± 0.002^{a}

Note: Data are the mean of four replicates ± SE. Means followed by different letters in rows are significantly different at $P \leq 0.05$.

Furthermore, when the SA pretreated plants were grown in the saline condition, phenolic compounds of leaves and roots were higher than those of other conditions (Table 1).

Salt stress caused a significant increase in proline content of leaves and roots in radish. SA treatment induced the highest proline accumulation as compared to the control and salt stressed plants. Salinity exposure with SA pretreatment also increased proline content in leaves and roots of radish (Table 1).

Discussion

Growth evaluation is important indicator in plant physiology. Growth process is the result of lipids, proteins, DNA and RNA synthesis. Plants growth and distribution are limited by the environment conditions. Therefore, it is important to understand the environmental aspects that affect plant growth. According to our results, salt stress had negative effects on fresh weight of shoots and roots. Similar results were reported for other plants such as *Triticum aestivum* (Chaparzadeh *et al.* 2014) and *Calendula officinalis* (Chaparzadeh *et al.* 2004). The negative effects of salinity may due to low osmotic potential of soil solution (water stress), nutritional imbalance, specific ion effect (salt stress) or a combination of these factors (Parvaiz and Satyawati 2008). Cell division (meristem activity), enlargement and differentiation are affected by salt stress. Leaves are more sensitive than roots to salinity, simply because, toxic ions accumulate to higher levels in the shoot than in the root. Phytohormones are known to play a key role in the regulation of plant growth. Positive effects of exogenous SA on growth of different plants have been reported under water (Singh and Usha 2003; Idrees *et al.* 2010), salinity (Hussein *et al*. 2007) and heavy metals (Zhou *et al.* 2009) stresses. In the present study, applying SA led to decreasing of fresh weight of shoots but didn't have significant effect on roots. SA have dual functions as potent oxidant or effective antioxidant and its effect mostly depends on the concentrations and also the status of the environments with respect to each plant species (Hayat *et al.* 2009). When applied at appropriate low concentrations, it is able to reduce

Table 2. The effect of SA and salinity on photosynthetic pigments, anthocyanins, flavonoids and cell membrane stability index of leaves

Characters	Control	NaCl	SA	NaCl + SA
Chlorophyll *a* (mg g^{-1} FW)	0.84 ± 0.11^{a}	0.57 ± 0.03^{b}	0.57 ± 0.09^{b}	0.54 ± 0.02^{b}
Chlorophyll *b* (mg g^{-1} FW)	0.26 ± 0.04^{a}	0.16 ± 0.01^{b}	0.20 ± 0.01^{c}	0.17 ± 0.01^{bc}
Total chlorophyll (mg g^{-1} FW)	1.10 ± 0.10^{a}	0.73 ± 0.04^{b}	0.77 ± 0.09^{b}	0.72 ± 0.02^{b}
Carotenoids (mg g^{-1} FW)	0.04 ± 0.01^{c}	0.10 ± 0.01^{ab}	0.12 ± 0.02^{a}	0.08 ± 0.00^{b}
Anthocyanins (Abs $_{550\ nm}$ g^{-1} FW)	2.08 ± 0.20^{ab}	2.06 ± 0.25^{ab}	1.71 ± 0.30^{b}	2.63 ± 0.54^{a}
Flavonoids (Abs $_{330\ nm}$ g^{-1} FW)	14.47 ± 1.66^{a}	13.34 ± 1.88^{ab}	12.16 ± 1.31^{b}	11.66 ± 1.85^{b}
Membrane stability index (%)	49.36 ± 7.66^{a}	50.39 ± 3.86^{a}	49.04 ± 5.97^{ab}	42.09 ± 5.32^{b}

Note: Data are the mean of four replicates ± SE. Means followed by different letters in rows are significantly different at $P \leq 0.05$.

the severity of stress factors (Horvath *et al*. 2007). The inhibitive effects found on leaves of radish suggest that SA may lead to a toxic effect which decreases growth characters.

Photosynthesis is a key process in plants that is influenced by salt stress (Parida and Das 2005). Oxidative effect of salt stress was reported to damage the photosynthetic machinery at multiple levels, such as pigments, stomatal functioning and gaseous exchange, structure and function of thylakoid membrane, electron transport and enzymes (Yusuf *et al.* 2008; Wu *et al.* 2010). Chlorophylls have central role in photosynthesis, therefore, any changes in their level can affect plant growth. Salinity stress decreased *a*, *b* and total chlorophylls in radish (Table 2). Similar results have been recorded in other plants such as *Thellungiella halophila* (Vera-Estrella *et al.* 2005), *Brassica juncea* (Yusuf *et al.* 2008). The decrease was due to instability of protein complexes and increased chlorophyllase activity. Furthermore, SA treatment decreased chlorophylls content in radish leaves (Table 2). In a research program, exogenous treatment with SA decreased chlorophyll content of *Brassica juncea* (Fariduddin *et al*. 2003), however, the effect of SA was opposite in *Triticum aestivum* (Naser *Alavi et al*. 2014). In *Cymbopogon flexu*osus, salicylic acid neutralized the effect of water stress on total chlorophyll and carotenoids content (Idrees *et al.* 2010). The concentration of applied SA may play an important role in plant responses. According to Fariduddin *et al.* (2003), the low concentration of SA (10^{-5} M) generated highest values for all the characteristics (dry mass, chlorophyll content, pod number, seed number, seed yield) at the 60-d-stage in *Brassica juncea* plants. However, the values decreased as the concentration of SA was increased and reached below that of the control at the highest concentration. Thus, SA has a bidirectional effect on plants. At low concentration improves photosynthetic machinery function but at high concentration can offer negative effects. Inhibition of chlorophyll biosynthesis and acceleration of destruction may be two main reasons for decreasing of chlorophyll by high concentration of SA.

Carotenoids represent a diverse group of pigments widely distributed in nature (Lu and Li 2008). In the present study, a significant increase was occurred under treatments as compared with the control plants (Table 2). Increasing of carotenoids under SA treatment has been appeared in *Arabidopsis thaliana* (Rao *et al.* 1997). By down regulation of β-carotene hydroxylase and increase of β-carotene (and total carotenoids), the enhancement of salt stress tolerance in transgenic cultured cells of sweet potato was occurred (Kim *et al.* 2012). Carotenoids have a fateful role as antioxidant molecules for free radical scavenging, therefore, increasing of carotenoids in plants under salt stress and SA treatment could enhance their capacity to reduce the damage caused by AOS (Azooz 2009).

According to Table 1, salinity, SA and salinity plus SA treatments caused a significant accumulation of hydrogen peroxide both in leaves and roots. Salinity and SA didn't have significant effect on peroxidation and membrane stability (Tables 1 and 2). These oxidative indicators were affected by applying SA under saline condition. Increasing of peroxide hydrogen content and peroxidation process during salinity have been reported in many plants such as *Phaseolus vulgaris* (Nagesh Babu and Devaraj 2008), *Poncirus trifoliata* (Wu *et al.* 2010), *Arabidopsis* (Borsani *et al.* 2001), *Calendula officinalis* (Chaparzadeh *et al.* 2004) and *Triticum aestivum* (Chaparzadeh *et al.* 2014). Applying of SA in *Panax ginseng* (Babar *et al.* 2007) and *Arabidopsis* (Rao *et al.* 1997) led to increasing of hydrogen peroxide content. In *Linum usitatissimum*, seeds presoaking with SA for 8 hours led to an enhanced production of H_2O_2 in dose dependent manner of seedlings (Belkadhi *et al.* 2014). Under stress conditions, SA and H_2O_2 may probably use a common signaling mechanism to improve tolerance to oxidative stress (Belkadhi *et al.* 2014).

Lipids are the most effective source of storage energy and they play important roles as the structural constituents of the cellular membranes. The cellular membranes have vital roles in the tolerance of stressors at plants. Lipid oxidation is problematic as enzymes don't control many oxidative chemical reactions and some of the products of the attack are highly reactive species that modify proteins and DNA (Parida and Das 2005). When a radical reacts it always produces another free radical and this is called a chain reaction mechanism. Lipid peroxidation produces a variety of toxic aldehydes such as MDA. MDA is commonly measured as the end product of lipid peroxidation and is known to react with nucleophilic side chains of proteins. Results of the present study showed that salinity induced oxidative stress via increased amount of hydrogen peroxide, consequently increased lipid peroxidation of root membranes. Also, the results achieved from this study showed that salinity and SA had synergic effects on lipid peroxidation, and hence, on the reduction of membrane stability and integration of leaf cells. SA-increased peroxidation was, also, reported in *Arabidopsis thaliana* (Rao *et al.* 1997).

Anthocyanins are a large class of water-soluble flavonoid pigments found in all plant tissues throughout the plant kingdom (Chalker-Scott 1999). The anthocyanin biosynthetic pathway is controlled by environmental and internal factors (Horbowicz *et al.* 2008) and

anthocyanin synthesis is known to be inducible under stress. Species with high levels of foliar anthocyanin are more tolerant to drought conditions (Hughes *et al.* 2010). Anthocyanins have antioxidative properties arising from their capability as hydrogen or electron donors, their ability of the polyphenol-derived radicals to stabilize and delocalize the unpaired electron and their ability to chelate transition metal ions (Leao *et al*. 2014). In the present study, the toxic effects of salinity and SA on anthocyanin accumulation were not significant. However, salt stress plus SA treatment increased anthocyanin accumulation in leaves (Table 2). Under this condition, growth and amount of chlorophyll were decreased (Tables 1 and 2). Thus, this reduction could suggest that an anthocyanin overproduction of carbon structure may be related to harmful effects on the biosynthesis of nitrogen containing compounds.

Flavonoids play important roles in protecting plants against different stressors. The antioxidant activity of flavonoids towards free radicals and AOS is well known (Zhao *et al.* 2009). During the present study flavonoids content decreased under SA and salt plus SA treatments. Increasing of flavonoids under salt stress in *Hordeum vulgare* was reported by Ali and Abbas (2003). However, decreasing of flavonoids in low concentration of salinity and its increasing in high salinity in *Thellugiella calli* have been recorded (Zhao *et al.* 2009). Application of SA in *Panax ginseng* increased flavonoids content (Babar *et al.* 2007) that was different from our results. Salt and high concentration of SA treatments reduce photosynthesis rate by decreasing photosynthetic pigments. Hence, necessary energy to the synthesis of flavonoids won't be available. Also, reducing of flavonoids may be due to channeling to anthocyanin overproduction.

According to Table 1, phenolic compounds were increased significantly in shoots and roots under salinity, SA and salinity plus SA treatments. Similar results were recorded in barely during salt stress (Ali and Abbas 2003). Also, SA treatment increased phenolic compounds of *Panax ginseng* (Babar *et al.* 2007). Increased phenylalanine ammonia-lyase (PAL) activity could be a response to the cellular damage provoked by higher NaCl concentrations (Gao *et al.* 2008). So enhancement of PAL activity could be related to the implication of enzyme in the plant response to stresses. It is believed that phenolic compounds, as antioxidants, can reduce the toxic effects of stressors and thus prevent physiological damages of plants, however, this is critically dependent on the salt sensitivity of plants (Yuan *et al.* 2010). Induced accumulation of phenolic compounds can control the production of H_2O_2, so, these compounds may play an important role in the oxidative stress tolerance of plants (Lu and Li 2008). SA is considered to be a plant signaling molecule that plays a key role in the plant growth, development and defense responses. SA, probably, can induce particular enzymes of the secondary metabolism to produce defense compounds such as phenolic compounds.

Proline is known to occur widely in higher plants and normally accumulates in response to environmental stress (Hayat *et al.* 2009). It is an important component of the defense system of the plants to counter stress. Proline, also acts as osmotic pressure regulation, an O_2 quencher, thereby, revealing its antioxidant capability (Gupta and Huang 2014), improve photosynthetic

activity and maintain water status (Ahmed *et al.* 2010), protects quaternary structures of proteins and stabilizes the cell membranes (Hayat *et al.* 2012). Proline accumulation can serve as a selection criterion for the tolerance of most species to stress conditions (Parida and Das 2005). Results from the present work showed that salinity, SA and salinity plus SA treatments caused a significant accumulation of proline both in leaves and roots (Table 1). The results from salinity stress are similar to the experiments on *Vigna radiata* (Manivannan *et al.* 2007), *Brassica juncea* (Yusuf *et al*. 2008), *Dioscorea rotundata* (Abdul Jaleel *et al.* 2008) and *Lycopersicum esculentum* (Dogan *et al.* 2010). Increasing of proline content by SA treatment has been recorded in *Panax ginseng* (Babar *et al.* 2007). SA application under drought stress failed to alter proline content of *Cymbopogon flexu*osus (Idrees *et al.* 2010). As mentioned above, salinity and SA reduced chlorophyll content. Since glutamate is common precursor of chlorophyll and proline biosynthesis, decreasing of chlorophyll content can give opportunity for proline production during stress conditions.

Conclusion

In conclusion, salinity and SA, with the concentrations used in this study, had remarkably negative effects on growth of radish plants. The results are opposite to many reports that state the stress induced effects in plants can partially overcome by SA application. Probably, salinity and SA can affect plants with concentration dependent manner. Our finding, clearly, are showing synergetic function of salinity and SA in generating AOS and establishment of secondary oxidative stress in plants.

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