Efficiency of selenium and salicylic acid protection against salinity in soybean

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Abstract

This study was carried out in order to minimize risks associated with salinity. Soybean seedlings were treated with salicylic acid (SA) of 0 and 0.5 mM, selenium (Se) at 0, 25, and 50 mgl^{-1}, and/or NaCl, 0 and 100 mM. The supplements promoted shoot fresh mass and markedly relieved toxicity signs of salinity. Se and/or SA increased calcium and potassium contents in different organs and mitigated the inhibiting effects of salinity on the nutritional status. Simultaneous applications of these elicitors were the most effective strategy to induce antioxidant enzymes (peroxidase, polyphenol oxidase, and catalase). Also, these compounds increased proline, phenolics, and soluble carbohydrates in leaves and roots. Activities of phenylalanine ammonia lyase in roots and leaves were induced in the Se and/or SA-supplemented plants. In addition, the elicitors triggered accumulations of non-protein thiols. Therefore, the simultaneous application of Se and SA may be considered as a promising way to relieve the toxicity signs of salinity.

Keywords: elicitor; heavy metal; nutrition; salt stress

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Introduction

Selenium (Se) is an essential element suitable for human health. Se, at adequate levels, relieves symptoms of some physiological conditions, including cancer and cardiovascular disease (Safari et al., 2018; Babajani et al., 2019). The Food and Agriculture Organization of the United Nations suggest that human is allowed to consume daily up to 42 µg Se/day (Bitterli et al., 2010). While Se has antioxidant properties, in higher concentrations it produces superoxide which can be harmful to the cells (Seppänen et al., 2003). There are two kinds of selenium in soils, an inorganic type including selenide, elemental selenium, selenite, and selenate, mainly depending on pH and redox potential. The second type is organic form such as the volatile form dimethyl-selenide, selenoamino acids, and selenoproteins (Bitterli et al., 2010). Se is not considered as an essential nutrient for plants, but in lower concentration, it possesses some beneficial effects. Plants can absorb different types of Se because of its resemblance to Sulphur (S) and have restricted ability to differentiate between Se and S. Selenate, therefore, goes into active root cells by sulfate transporters. After that, it is reduced using Sulphur assimilation pathway in the chloroplast (Bitterli et al., 2010). However, most of the soils in the world contain low Se concentrations. Thus, plant bio fortification with Se is highly recommended to prevent Se deficiency

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in the human diet (Liu et al., 2017; Safari et al., 2018; Babajani et al., 2019). Some researchers showed that low concentration of Se increases the plant growth. The growth-promoting effects of Se, which mainly depend on its form, concentration, and species, have been recorded in various plants, including basil (Ardebili et al., 2015), garlic (Cheng et al., 2016), parsley (Ardebili and Moradi, 2016), wheat (Safari et al., 2018), and peppermint (Nazerieh et al., 2018). Also, the improving effects of Se supplementation on activating different resistance mechanisms have been reported in various plants counteracting different stress conditions, including salinity (Ardebili et al., 2015), UV (Golob et al., 2017), and heavy metal stress (Sun et al., 2016).

Many stressors such as salinity, cold, and drought can produce reactive oxygen species (ROS) in plants. The increased generation of ROS can pose a threat to plants. However, they also act as signals for stress response activation. Se can improve plant defense responses to counter environmental stress, mainly via regulation of antioxidants (Feng et al., 2013). Salinity or high electrical conductivity (EC) condition, known as a worldwide common issue and limiting factor, adversely affects plant growth, metabolism, development, and yield by oxidative stress, ion toxicity (especially sodium), nutrient, water deficiency, changed hormonal balance, and triggered changes in metabolism (Nazar et al., 2011). Soil salinity has been introduced as a worldwide key limiting factor in the productivity of legumes.

Salicylic acid (SA) is a secondary phenolic metabolite present in a wide range of organisms, including plants, which involves in a broad range of physiological processes (An and Mou, 2011). SA is considered as a signal triggering local and systemic plant defense-related reactions against the biotic agents (An and Mou, 2011). In addition, current opinion is that SA contributes to the plant response to various physicochemical stress conditions via triggering some critical signaling pathways and regulating different biochemical processes (Loutfy et al., 2012).

There are many efforts and interest to find suitable eco-friendly and economic supplements to improve plant growth and productivity as well as to minimize the detrimental impacts of different physicochemical stresses on plant growth, development, and metabolism (Asgari-Targhi et al., 2018). On the basis of our knowledge, there are limited scientific reports on elucidating short and long-term physiological responses triggered by Se and SA as well as comparing the efficiencies of individual and mixed applications of these signaling agents. With regard to the highlighted significance of the three factors addressed in this section (Se, SA, and salt stress), the current research was carried out to evaluate the effects of foliar supplementations of these chemicals on the physiology of soybean plants grown under two electrical conductivity (EC) conditions and to clarify the involved mechanisms. We aimed to provide a basis for developing methods to decrease toxicity associated with salinity and maintaining sustainable agriculture.

Material and Methods

Preparation, experimental design, and treatments

A pot experiment was conducted on the basis of a complete randomized design. Soybean seeds (*Glycine max* var. L17) were planted in pots (4 Kg), containing soil with a loam sandy texture (EC: 3.1 ds m^{-1}; pH: 8; P: 11.8 mg kg^{-1}; N: 0.06 %; K: 259 mg Kg^{-1}). The seeds were grown under natural condition, with the temperature about 25-27 °C at night and 31-35 °C during the day. 21-day-old seedlings were treated with three concentrations of Na_2SeO_4 (Sigma Chemical Co.) (0, 25, and 50 mgL^{-1}), two concentrations of SA (Sigma-Aldrich) (0 and 0.5 mM), and/or two concentrations of NaCl (Merck) (0 and 100 mM). NaCl 100 mM was added every 48 hours to the final EC of 7.1 dsm^{-1}. The foliar applications of Se and SA were done three times with one-week interval (tween was added to the prepared solutions). EC was measured by using a digital conductivity meter (WTW- Inolab Cond 720 Benchtop Conductivity Meter) to estimate the final concentration of NaCl. Plants were divided into twelve treatment groups. Eventually, they were harvested one week after the last treatment for the biochemical analysis.

Plants were grouped as follows: C, control, SA, 0.5 mM salicylic acid, Se25, 25 mgL^{-1} selenium, Se25-SA, simultaneous treatments of 25 mgL^{-1} selenium and salicylic acid, Se 50, 50 mgL^{-1}

selenium, Se50-SA, combined treatments of 50 mgL^{-1} selenium and salicylic acid, salinity, NaCl, SA-salinity, salicylic acid and NaCl, Se 25- salinity, 25 mgl^{-1} selenium and NaCl, Se25-SA-salinity, simultaneous treatments of 25 mgL^{-1} selenium, salicylic acid and NaCl, Se 50-salinity, 50 mgL^{-1} selenium and NaCl, Se 50-SA-salinity, simultaneous treatments of mgL^{-1} selenium, salicylic acid and NaCl.

Determination of minerals

Ash solution was used for elemental analysis. Potassium was analyzed by a flame photometer (Sherwood Model 410 Flame photometer, UK). Calcium was determined using an atomic absorption spectrophotometer (Varian Atomic Absorption Spectrometers, SpectrAA.200, USA). Samples were expressed against standard solutions that were determined concurrently.

Enzymes extraction

Enzymes were extracted at 4 °C in a mortar and pestle using phosphate buffer, 0.1 M pH 7.5 containing Na_2-EDTA 0.5 mM, and ascorbic acid 0.5 mM as an extraction buffer. The homogenates were centrifuged for fifteen minutes at 4 °C and supernatants were applied as enzyme extracts (Asgari-Targhi et al., 2018).

Determination of peroxidase (POX) activities

Peroxidase activity was measured based on the method described by Hemeda and Klein (1990). Change in absorbance at 470 nm in reaction mixture containing guaiacol and H_2O_2 was monitored and finally enzyme activity was expressed in $\Delta Amin^{-1}g^{-1}fw$.

Catalase (CAT) activity

Catalase activity was determined as the decrease in absorbance at 240 nm (Pereira et al., 2002). Enzyme extract was added to reaction mixture, including 0.1 M phosphate buffer (pH 7) and 240 mM H_2O_2. Differences in absorbance at 240 nm were recorded for 3 minutes. Finally, the enzyme activity was expressed in $\Delta Amin^{-1}g^{-1}fw$.

Poly phenol oxidase activity (PPO)

PPO activity was determined using pyrogallol (Raymond et al., 1993). An assay mixture containing 2.5 ml sodium acetate buffer (0.2 M, pH 7.6) and 200 µl pyrogallol (0.02 M) was used. The absorbance change was recorded at 430 nm. PPO activity was expressed as $\Delta Amin^{-1}g^{-1}fw$.

Quantification of proline and protein contents

Proline was extracted by sulfosalicylic acid 3% (w/v) and its content was quantified according to the previously described method of Bates et al. (1973). Proline contents were calculated based on a standard curve and expressed in $\mu gg^{-1}fw$. Protein content was measured using Bradford reagent (Bradford et al., 1976). BSA was used as standard.

Determination of phenolic compound

Total soluble phenols were extracted by methanol 50 % (v/v) at 70 °C. Reaction mixture contained 1 ml plant extract, 1 ml folin reagent (50 %), and 2 ml Sodium carbonate (21 %). The absorbance was recorded at 760 nm. Tannic acid was applied as a standard compound (Iranbakhsh et al., 2018b).

Determining the phenylalanine ammonia lyase (PAL) activity

The reaction mixture for PAL activity (EC 4.3.1.5) consisted of 6 µM phenylalanine, Tris-HCl buffer (0.5 M, pH 8), and 200µl enzyme extract. PAL activities were recorded based on the rate of conversion of L-phenylalanine to trans-cinnamic acid. PAL activity was expressed in $\mu gCin\ min^{-1}g^{-1}fw$) (Beaudoin-Eagan and Thorpe, 1985).

Determination of soluble carbohydrate

Briefly, sugars were extracted using 70 % (v/v) ethanol. Copper sulfate solution was added to each ethanolic extract. Then, the prepared samples were heated in a water bath for 10 minutes. After that, the tubes were cooled, and

Table 1
Effects of the Se and SA supplementations on different minerals and shoot fresh weight in *Glycine max* exposed to two levels of EC.

Treatments			Shoot fresh weight (g plant^{-1})	Leaf Ca^{2+} (mgg^{-1}dw)	Leaf K^+ (mgg^{-1}dw)	Root Ca^{2+} (mgg^{-1}dw)	Root K^+ (mgg^{-1}dw)
Control	-SA	-Se	6.63±0.12^{c}	13.99±0.33^{de*}	14.35±0.19^{d}	18.65±0.13^{a}	17.93±0.17^{bc}
		Se25	7.76±0.12^{a}	14.33±0.25^{cd}	16.39±0.19^{c}	18.16±0.22^{abc}	18.74±0.17^{b}
		Se50	6.46±0.15^{cd}	15.61±0.11^{b}	16.02±0.12^{c}	17.9±0.19^{c}	19.00±0.1^{b}
	+SA	-Se	6.2±0.15^{d}	17.013±0.26^{a}	17.55±0.33^{b}	18.43±0.13^{abc}	20.87±0.49^{a}
		Se25	7.28±0.22^{b}	17.506±0.31^{a}	17.84±0.26^{b}	18.076±0.09^{bc}	21.40±0.54^{a}
		Se50	7.53±0.11^{ab}	17.016±0.2^{a}	18.27±0.15^{a}	18.45±0.086^{ab}	22.03±0.11^{a}
Salinity	-SA	-Se	3.33±0.117^{h}	11.713±0.49^{f}	11.67±0.25^{f}	12.97±0.217^{f}	10.02±0.96^{f}
		Se25	4.7±0.04^{ef}	13.206±0.17^{e}	13.41±0.12^{e}	16.56±0.23^{e}	15.72±0.23^{de}
		Se50	3.93±0.09^{g}	14.63±0.23^{cd}	13.26±0.32^{e}	16.68±0.13^{e}	15.27±0.17^{e}
	+SA	-Se	4.66±0.05^{ef}	14.533±0.25^{cd}	14.06±0.13^{d}	16.35±0.086^{e}	16.07±0.32^{de}
		Se25	4.95±0.044^{e}	14.99±0.24^{bc}	14.286±0.1^{d}	16.63±0.28^{e}	16.34±0.1^{de}
		Se50	4.45±0.092^{f}	15.58±0.21^{b}	14.72±0.1^{d}	17.35±0.08^{d}	16.87±0.23^{cd}

*Significant differences in the mean value of each treatment group are represented by different lower case letters based on the Duncan test ($p \leq 0.05$, $n = 3$).

phosphomolybdic acid solution was added. The absorbance was recorded at 600 nm. The reducing sugar concentrations were quantified based on the standard curve of glucose

Determination of non-protein thiols

The total content of non-protein thiols in plants was determined as follows (Del Longo et al., 1993). Briefly, a plant sample (0.5 g) was homogenized in 0.3 ml of 5% (w/v) sulphosalicylic acid. The reaction mixture contained 0.1 ml of supernatant, 0.5 ml reaction buffer (0.1M phosphate buffer (pH 7), 0.5 mM EDTA), and 0.5 ml of 1 mM DTNB. The assay reaction was incubated for 10 min and absorbance was recorded at 412 nm.

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) using SPSS software. The mean values of each treatment group were submitted to variance analysis by the Duncan test at p≤5%.

Results

Growth characteristic and nutritional status

Shoot fresh mass significantly improved by 17% for the Se 25 group while it decreased by 49.7% in the salinity group and in the individual applications of SA or Se50 over the untreated control (Table 1). This characteristic increased by 13% and 9.8% for the Se50SA and Se25SA treatment groups, respectively compared with the control (Table 1). Interestingly, the reducing impact of salinity on the shoot fresh weight (49.7 %), was mitigated by the foliarly-applied compounds and reached to 29.7 %, 25.4 %, and 40.72 %, respectively for the SA-salinity, Se25-salinity, and Se50-salinity groups (Table 1). The applications of Se of 25 or 50 mgl^{-1} in combination with SA significantly alleviated the toxicity signs of salinity where the inhibiting rates on the shoot fresh mass declined to 25.3 % and 32.88 % for the Se25-SA-salinity and Se50-SA-salinity treatments, respectively (Table 1). Leaf calcium concentrations significantly increased by 21.5 % and 11.5 % for the individual treatments of SA and Se50, respectively, whereas it reduced by 16.3 % in the salinity control in comparison with the untreated control (Table 1). It was significantly enhanced by 25% for the Se50SA, Se25SA groups while the recorded differences between the SA-salinity, Se25-salinity, and Se50-salinity groups were not significant (Table 1). Interestingly, the leaf calcium in Se25-SA-salinity and Se50-SA-salinity was significantly higher than the salinity control by 28.11% and

33%, respectively (Table 1). Collectively, the toxic impacts of the salinity treatment on the leaf calcium were mitigated by the applied supplements (about 21%). Similarly, the plant supplementation with Se or SA led to significant increases in the leaf potassium concentrations by 22.3 %, 11.5 %, and 9.68 % respectively for the SA, Se50, and Se25 treatment groups in contrast with the salinity treatment (19 %) (Table 1). Simultaneous applications of Se and SA were the most effective treatments to raise the leaf potassium (24.3 % and 27.3 %, respectively for the Se25SA and Se50SA treatment groups), compared to the control. The reduction rate in leaf K due to the salinity treatment was declined and reached to 6.6%, 7.5%, and 2% for the Se25- salinity, Se50– salinity, and SA-salinity groups while this effect was significantly mitigated with the applied supplements (Table 1). Under the high EC condition, the recorded changes in the leaf K between the SA-salinity, Se25-SA-salinity, and Se50-SA-salinity groups were not found to be significant (Table 1). The individual utilization of Se of 50 mgl^{-1} resulted in the slight decreases in the root calcium concentration when compared to the untreated control (Table 1). The dramatic reduction (31 %) in the root calcium was found in the salinity group while it reached 11.25 %, 10.5 %, 11 %, and 7 % for the Se25-salinity, Se50-salinity, SA-salinity, Se25-SA-salinity, and Se50-SA-salinity groups (Table 1). The individual applications of Se25, Se50, or SA led to increases in the root K by 4.5 %, 6%, and 16.4%, respectively, among which only the SA effect was significant in comparison with the control. The combined treatments of Se and SA were a more effective way to improve the root K (19.35% and 22.87% for the Se25-SA and Se50-SA groups, respectively) than the individual applications (Table 1). The salinity treatment resulted in a significant reduction (44%) in the root K concentration while the applied supplements significantly relieved this toxicity sign of salinity by 31.67 %, 29 %, 35 %, and 38 % for the Se25-salinity, Se50-salinity, Se25-SA-salinity, and Se50-SA-salinity groups, respectively (Table 1).

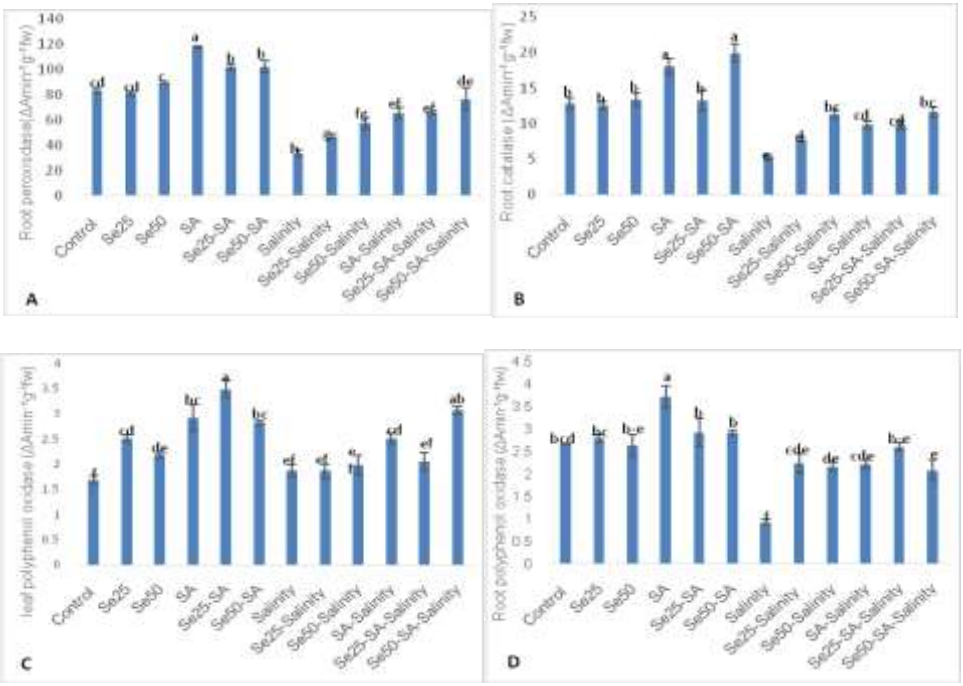

Fig. I. Induced-differences in the activities of antioxidant enzymes caused by the application of Se and SA in *Glycine max* grown under to two different EC conditions

Antioxidant enzyme activity

The individual application of Se (25 and 50 mgL^{-1}) did not provoke significant changes in the root peroxidase activities while the significantly higher activity of this enzyme was recorded in the SA-treated seedlings (about 41 % compared to the untreated control) (Fig. IA). It should be noted that the salinity treatments significantly inhibited the root peroxidase by 59 %. However, this percentage has declined to 56%, 30%, and 21.5% for the Se25-salinity, Se50-salinity, and SA-salinity groups, respectively (Fig. IA). The Se50-SA-salinity group had the highest activity of the root peroxidase under the high EC condition (about twofold more than the salinity control) (Fig. IA). Similarly, individual Se treatments did not have significant effects on the root catalase activity while the utilization of SA led to significant increases in this parameter by 40% over the control (Fig. IB). The highest activity of the catalase enzyme was recorded in the Se50-SA group (56% higher compared to the control) (Fig. IB). This characteristic decreased by 58% for the salinity group, which was mitigated by the Se and SA treatment and reached to 39%, 11.5%, 23%, 21.5%, and 9% for the Se25-salinity, Se50-salinity, SA-salinity, Se25-SA-salinity, and Se50-SA-salinity groups, respectively (Fig. IB). The polyphenol oxidase activities were affected by the applied supplements, where the Se or SA, especially the latter, significantly induced the enzyme activities in the leaves by 49 %, 29.7 %, 73 % for the Se25, Se50, and, SA groups, respectively (Fig. IC). The highest activity of the polyphenol oxidase in leaves was found in the Se25-SA group by 2 folds as compared with the control (Fig. IC) while the observed changes in the Se25-salinity and Se50-salinity groups were not significant in comparison with the salinity group (Fig. IC). Under the high EC condition, the simultaneous application of Se50 and SA (63 %) was found to amplify the inducing effects of SA (34%) on the polyphenol oxidase activities when compared to the salinity group (Fig. IC). Regarding the individual treatments of Se or SA in the non-saline condition, only the SA application made a significant alteration in the activity of the root polyphenol oxidase by 39% over the control (Fig. ID). Salinity significantly reduced the activity of the root polyphenol oxidase by 66%, where this percentage was mitigated by the applied supplements and reached to 16.5%, 18.7%, 2.2%, 16%, 2.2%, and 21.7% for the Se25-salinity, Se50-salinity, SA-salinity, Se25-SA-salinity, and Se50-SA-salinity groups, respectively in comparison with the control (Fig. D).

Table 2
Modifications in proline and phenol contents and PAL activity triggered by the applied levels of Se and SA in soy bean plants

Treatments			Leaf proline ($mgg^{-1}fw$)	Leaf phenol ($mgg^{-1}fw$)	Root phenol ($mgg^{-1}fw$)	Leaf PAL ($mgCin\ min^{-1}\cdot g^{-1}fw$)	Root PAL ($\mu gCin\ min^{-1}\ g^{-1}fw$)
Control	-SA	-Se	$0.231\pm0.009^{e*}$	1.37 ± 0.055^{fg}	1.04 ± 0.026^{cde}	0.074 ± 0.003^{d}	15.67 ± 0.52^{e}
		Se25	0.322 ± 0.018^{d}	1.73 ± 0.025^{cd}	1.11 ± 0.055^{bcd}	0.123 ± 0.008^{ab}	16.306 ± 0.71^{de}
		Se50	0.417 ± 0.008^{b}	1.64 ± 0.013^{cde}	0.98 ± 0.01^{de}	0.097 ± 0.008^{cd}	15.23 ± 0.76^{e}
	+SA	-Se	$0.39\pm0.011b^{c}$	1.95 ± 0.076^{ab}	1.09 ± 0.02^{bcd}	0.111 ± 0.008^{bc}	18.06 ± 0.51^{bcd}
		Se25	0.363 ± 0.003^{c}	2.02 ± 0.11^{a}	$1.27\pm.069^{a}$	0.136 ± 0.006^{a}	18.753 ± 0.60^{bc}
		Se50	0.394 ± 0.01^{bc}	1.98 ± 0.08^{ab}	1.02 ± 0.021^{cde}	0.128 ± 0.004^{ab}	17.36 ± 0.69^{cde}
Salinity	-SA	-Se	0.327 ± 0.014^{d}	1.35 ± 0.046^{fg}	0.68 ± 0.038^{f}	0.097 ± 0.001^{cd}	11.65 ± 0.35^{f}
		Se25	0.390 ± 0.006^{bc}	1.48 ± 0.066^{ef}	0.92 ± 0.04^{e}	0.083 ± 0.001^{d}	20.17 ± 0.71^{b}
		Se50	0.394 ± 0.004^{bc}	1.29 ± 0.047^{g}	0.95 ± 0.075^{e}	0.096 ± 0.004^{cd}	13.076 ± 0.95^{f}
	+SA	-Se	0.460 ± 0.012^{a}	1.63 ± 0.031^{de}	1.16 ± 0.018^{abc}	0.113 ± 0.004^{abc}	17.24 ± 0.67^{cde}
		Se25	0.412 ± 0.015^{b}	1.57 ± 0.02^{de}	1.22 ± 0.064^{ab}	0.109 ± 0.01^{bc}	21.12 ± 0.93^{a}
		Se50	0.415 ± 0.018^{b}	1.82 ± 0.023^{bc}	0.99 ± 0.011^{de}	0.096 ± 0.002^{cd}	19.8 ± 0.38^{ab}

*Significant differences in the mean value of each treatment group are represented by different lower case letters based on the Duncan test ($p\leq0.05$, n = 3).

Proline, phenolic contents, and PAL activity

The individual Se or SA supplementations, especially Se 50 mgl^{-1}, significantly increased the leaf proline by 38.5%, 80%, and 61%, respectively for the Se25, Se50, and SA when compared with the control (Table 2). Also, the individual salinity treatment enhanced this parameter by 41.5%. Similarly, the Se25-SA and Se50-SA groups had significantly higher amounts of the leaf proline by (57%) and (68%), respectively compared with the control (Table 2). The highest rises in the leaf proline were recorded in the SA-salinity group (about 2 fold), Se50-SA-salinity (81%), and Se25-SA-salinity (78%) (Table 2). In comparison with the control, Se or SA individually improved the leaf phenols by 26%, 19.7%, and 42% for the Se25, Se50, and SA groups, respectively (Table 2). However, the changes recorded between the control and the salinity group was found to be insignificant. The highest amounts of this parameter were recorded in the groups simultaneously treated with Se and SA (Se25SA and Se50SA) under the non-saline condition (Table 2). The observed differences in the leaf phenol concentrations between the Se25-salinity and Se50-salinity groups were not found significant, as compared with the control while the leaf phenols were significantly higher by 19%, 14.6%, and 32.8%, respectively for the SA-Salinity, Se25-SA-salinity, and Se50-SA-salinity groups compared to the salinity treatment (Table 2). The individual effects of the Se or SA treatments were not significant on the root phenols whereas the salinity treatment significantly reduced this characteristic by 34.6%, compared to the control (Table 2). The Se25-SA treatment significantly increased root phenols by 27% while the effect of Se50SA treatment on this characteristic was not found to be significant (Table 2). There was no

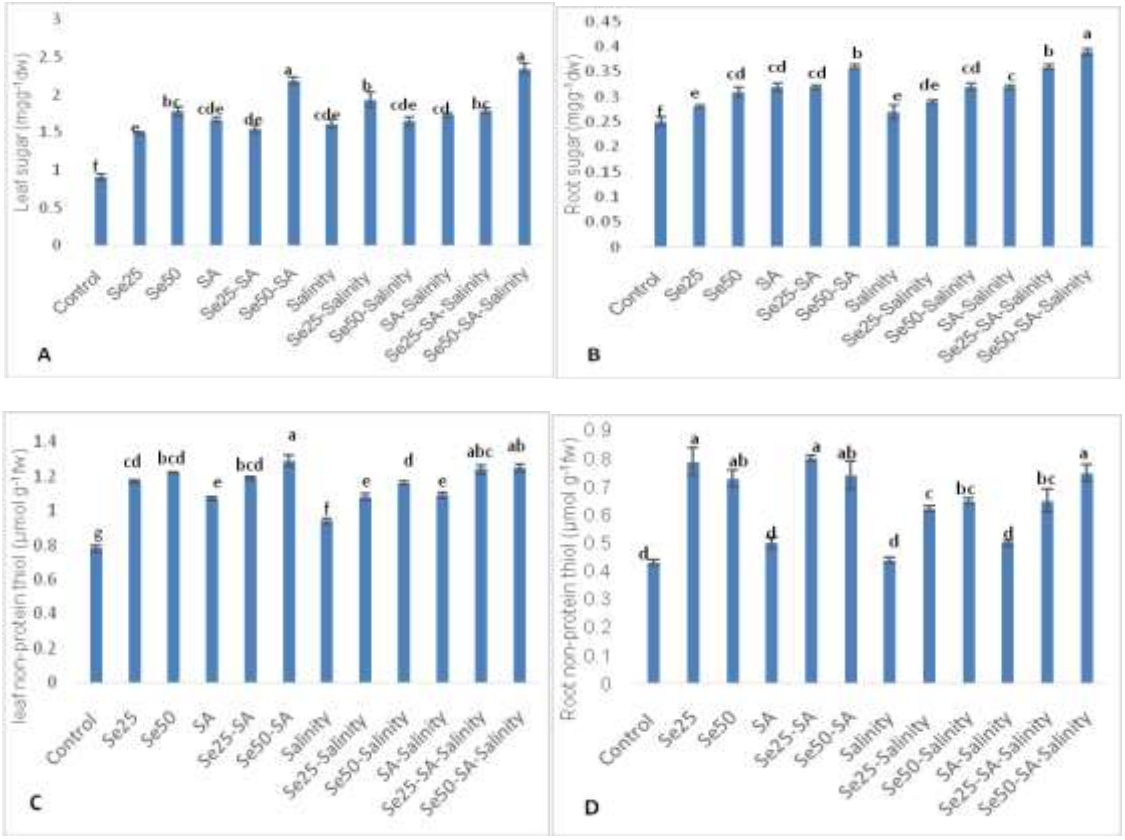

Fig. 2. Effects of the Se and/or SA supplementations on sugar and non-protein thiol in leaves and roots of soy beans grown under two different EC conditions

significant difference between the Se25-salinity or Se50-salinity and the control (Table 2). However, the root phenol concentrations in these groups were significantly higher than the salinity group by 35% and 39.7%, respectively (Table 2). Similarly, the root phenols increased by 70%, 17%, and 63%, respectively for the SA-salinity, Se25-SA-salinity, and Se50-SA-salinity groups, over the salinity treatment. The individual application of Se or SA led to inductions in leaf PAL activity by 66%, 31%, and 48.6%, compared with the control. Also, this parameter in the salinity group was significantly higher than the control (Table 2). The highest activities of PAL in leaves were observed in the groups simultaneously treated with Se or SA in the seedlings grown under the non-saline condition (Table 2). The achieved data indicated that the Se50 or SA treatments in the plants counteracted with the salinity stress improved the leaf PAL activity by 31% and 52.7% relative to the control whereas Se25 did not make a significant difference (Table 2). It should be noted that the PAL activities in the leaves of Se25-SA-salinity and Se50-SA-salinity groups were higher by 47% and 29%, respectively compared with the control (Table 2). SA individually induced PAL activity in roots by 15% when compared to the control (Table 2). However, the salinity treatment declined this parameter by 25% (Table 2). The simultaneous application of Se and SA led to the significant enhances in the root PAL activity by 19.5%, and 11%, respectively for the Se25SA and Se50SA groups, respectively (Table 2). Except for the Se50-salinity treatment, the PAL activity in the roots of seedlings exposed to salinity increased by 28.7%, 10%, 34.78%, and 26.3% for the Se25SA, Se25-SA-salinity, and Se50-SA-salinity groups (Table 2).

Soluble carbohydrates, non-protein thiols, and protein contents

Se, SA, or salinity individually increased leaf sugar concentration (Fig. 2A). Under the non-saline condition, the simultaneous application of Se or SA led to the dramatic rises in this parameter, when compared to the control (Fig. 2A). The exposure of the seedlings to the salinity condition dramatically enhanced the leaf sugars in the Se or SA-treated seedlings among which the Se50-SA, Se25-salinity, and Se25-SA-salinity groups, respectively by 2.5 folds, 2 folds, and 96% (over the control) had the highest amounts (Fig. 2A). Similarly, a significant rise in root sugar provoked by Se, SA, or salinity in the Se25, Se50, SA, and salinity groups (Fig. 2B). The increasing effect of salinity on the root sugar concentration was amplified by the foliar supplementation with Se and/or SA where the recorded amounts in Se50-salinity, SA-salinity, Se25 salinity, and Se50-SA-salinity were significantly higher by 18.5%, 19%, 33%, and 44%, respectively compared with the salinity group (Fig. 2B). The individual effect of Se, SA, or salinity on the leaf and root sugar concentrations was significant while the interactions between independent factors were not found to be significant (Table 6). Under the non-saline condition, the individual foliar applications of Se or SA led to the promotion in the leaf non-protein thiols, where the effectiveness of Se was much higher than that of the SA (Fig. 2C). Similarly, simultaneous applications of Se and SA significantly enhanced this parameter in the seedlings grown under low EC condition among which the highest amount was found in the Se50-SA group (Fig. 2C). Also, the significant increase in the leaf non-protein thiols was recorded for the salinity treatment; however, this amount was significantly fewer than the SA and/or Se-supplemented seedlings (Fig. 2C). In non-saline condition, only the effects of the Se treatment on the root non-protein thiols were significant while the SA effect was not found to be statistically significant (Fig. 2 D). While the difference between the salinity control and the untreated control groups was not statistically significant, Se-treated seedlings under High EC condition had the significantly higher concentrations of the non-protein thiols in roots (Fig. 2D). The highest values of the non-protein thiols were recorded in roots of the seedlings exposed to (Fig. 2D). Salinity condition was recorded in the Se50-SA groups (Fig. 2D). The simultaneous foliar applications of Se and SA slightly improved the effectiveness of the individual Se treatment (Fig. 2D).

Discussion

Nutritional status and antioxidant enzymes

The obtained findings clearly indicated that the adverse impacts of the salinity on the

plant growth, the nutritional status, and antioxidant enzymes were considerably mitigated by the foliar applications of Se and SA. Interestingly, the positive effects of Se on the growth and plant resistance against salinity were amplified by the simultaneous application of Se and SA. Moreover, it should be noted that SA was the more effective agent than the Se treatment to induce the long-term reactions (systemic) in the root. These provoked changes by the Se and SA treatments may be due to the modifications in the source/sink relations, alterations in hormonal balances, and/or possible induced signal transduction. There is strong evidence confirming the involvement of Se on modifying some critical phyto-hormones, especially salicylic acid, jasmonic acid, and ethylene (Tamaoki et al., 2008), each may contribute to specific signaling pathways and activate especial defense mechanisms. Interestingly, the Se treatment delayed fruit ripening, mainly via stimulation of antioxidant system, and a down-regulation of ethylene biosynthesis-related genes (Zhu et al., 2017). Also, improvements in the plant nutritional status by the exogenous SA contributed as a key mechanism in mitigation of salinity symptoms in tomato (Wasti et al., 2012). Our findings are consistent with those reported by some other researchers representing the potential benefits of Se (Ardebili et al., 2015; Cheng et al., 2016; Yusuf et al., 2016) and SA (Ardebili et al., 2014) to ameliorate signs of stress in the exposed plants. Changes in the nutritional status, antioxidant system, specific gene expression, and triggering complex metabolic networks have been described as a key mechanism involved in ameliorating the adverse impacts of stress (Feng et al., 2013; Ardebili et al., 2014; Ardebili et al., 2015; Cheng et al., 2016; Sun et al., 2016; Wu et al., 2017; Iranbakhsh et al., 2018a, b; Safari et al., 2018; Babajani et al., 2019). It is interesting to note that Se nanoparticle changed the expression pattern of HSFA4A gene in wheat (Safari et al., 2018).

Proline, phenols, and PAL

The obtained findings revealed that the leaf proline concentrations were modified by foliar applications of Se and/or SA. This may be attributed to the modifications in the nitrogen assimilation which may act as a crucial mechanism, thereby counteracting destructive signs of salinity, triggering signaling pathways, and improving stress tolerance. Some protective functions of proline are as a protein stabilizer, an antioxidant agent, a source of carbon and nitrogen, and a cell membrane stabilizer (Celik and Unsal, 2013). Proline plays a vital role as a signaling agent and alters the expression pattern of specific genes, thereby improving plant resistance against various physicochemical stress conditions (Szabados and Savouré, 2010). It has been stated that Se may improve plant resistance against abiotic stress condition via the modifications in the nitrogen assimilation pathway (Sun et al., 2016; Safari et al., 2018) and proline metabolism (Yusuf et al., 2016). Moreover, the Se and/or SA induced the changes in the phenolic concentrations and activities of PAL, the main enzyme in phenylpropanoid metabolism. These results are in agreement with findings of Ježek et al. (2011) in Solanum tuberosum and Golob et al. (2017) in *Triticum aestivum*. Therefore, these responses may be regarded as critical signs of possible specific signaling, modification in the secondary metabolism, and inductions in the defense-related reactions by which Se and SA may improve plant resistance to salinity conditions.

Soluble carbohydrates and non-protein thiols

Improvements in the carbohydrates caused by Se, SA, and/or salinity could be attributed to the changes in photosynthesis rate, osmotic regulations, alteration in nutritional status (especially elements with cofactor roles), and/or the induced modifications in the activities of enzymes implicated in carbohydrates metabolism. This is supported by the findings of Feng et al. (2013), Ardebili et al. (2014), Ardebili et al. (2015), and Ardebili and Moradi (2016). Enhancement in the non-protein thiols may be regarded as a key protection mechanism to ease detrimental impacts of salt stress. There is evidence indicating that the non-protein thiols help the plant to counteract stress (Zagorchev et al., 2013). Exogenously applied Se may enhance the sulfur uptake which leads to synthesizing more glutathione (Ardebili et al., 2015; Wu et al., 2017). Interestingly, it has been stated that SA is

responsible for enhancing Cd tolerance by modulating glutathione metabolisms (Guo et al., 2016).

Conclusion

The current research provides valuable data and evidence for the various short (local) and long-distance (systemic) physiological responses triggered by Se and SA, thereby improving plant growth and resistance against salt stress. Collectively, it seems that the simultaneous foliar supplementations of Se and SA may be regarded as an effective and eco-friendly strategy for ameliorating the toxicity signs of stress via possible crucial mechanisms, including regulating the nutrient uptake, accumulating cellular protectant (like proline and phenols), activating the antioxidant system and secondary metabolism, and/or enhancing the non-protein thiols. It is obvious that more convincing molecular studies are required to clarify the exact implicated mechanisms in these kinds of interactions, regarding sustainable agriculture and environmental issues.

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