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Mitigation of Saline Stress Adverse Effects in Lettuce Plant Using Selenium and Silicon

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ABSTRACT

Two field experiments were conducted in the two successive seasons of 2012-2013 and 2013-2014 to investigate the effects of selenium (Se) and silicon (Si) application on two lettuce (*lactuca sativa* L.) cultivars Great lakes and Balady grown under salt stress (3.22 dSm^{-1}) in irrigation water. Two levels of Se (16 and 32 µM) as sodium selenate and Si (1 and 2 mM) as potassium silicate were used to adverse the destructive effect of salinity. Application of Se and Si improved growth parameters e.g., plant height, root length, number of leaves per plant, plant fresh weight, plant dry weight, chlorophyll a, b and carotenoids content. Si at 2 mM gave the highest significant increase of leaf relative water content (LRWC), accumulation of proline, total soluble sugars (TSS). Treatments also affected the activity of super oxide dismutase (SOD), peroxidase (POD), and the content of K and Ca. However, same treatments decreased lipid peroxidation (MDA), membrane permeability (MP) and Na content.

Keywords: Sodium selenate, potassium silicate, salinity stress, lettuce, enzyme activity.

Introduction

Lettuce regarded as one of the most important salad vegetable, which contains phytochemicals, including vitamins, carotenoids, polyphenols, as well as the fibers content and other antioxidants Pérez-López *et al.* (2013), Nicolle *et al*. (2004) and Serafini *et al*. (2002). Lettuce is determined to be moderately salt sensitive, with a threshold EC 1.3 dS m^{-1} (Ayers *et al.,* 1951). Researchers found that some lettuce types were significantly more salt tolerant than others and that salt tolerance increased with age in lettuce, as is sensitive during the early seedling stages and at flowering (Shannon *et al*., 1983).

Salinity is one of the major widespread abiotic stress factors that severely restricts crop productivity Hasegawa *et al.* (2000) and zuh (2001). It influences about 110 million ha in arid and semiarid regions. According to FAO, an estimated 20–30 million ha seriously deteriorated by salinity Leyva *et al.* (2011). In addition to natural salinity, a great amount of recently cultivated agricultural area has become saline because of land clearing or irrigation, both of which led water tables to rise and concentrate the salts in soil Munns & Tester (2008).

In general, the harmful effects of salinity on plant growth summarized as the reduction in the osmotic potential of the edaphic solution, diminishing water availability for the plant, causing specific toxicity of Na^+ and Cl^-, and consequently provoking a nutritional imbalance due to the competitive uptake of nutrients Tejera *et al.* (2006).

The initial and primary effect of salinity, especially at low to moderate concentrations, is due to its osmotic effects (Munns and Termaat, 1986; Jacoby, 1994, Amira Hegazi *et al* 2015). Roots are also reduced in length and mass but may become thinner or thicker. Maturity rate may be delayed or advanced depending on species. The degree to which growth is reduced by salinity differs greatly with species and to a lesser extent with varieties within a species. The severity of salinity response also mediated by environmental interactions such as relative humidity, temperature, radiation and air pollution (Shannon *et al.,* 1994).

Selenium, which is also an essential microelement for animals, positively affects growth and development of some plants by enhancing the activities of antioxidant enzyme (Daio *et al.,* 2014). The growth rate, photosynthetic pigment and proline content of cucumber increased by exogenous selenium treatment under salt stress, this application have protected the cell membrane and enhanced the salt tolerance of the cucumber and tomato (Hawrylak-Nowak, 2009). The growth and the photosynthesis of tomato seedlings induced by selenium application under salt stress condition (Daio *et al.,* 2014). Although both silicon and selenium applications reduced the toxic effects of salinity on various tomato genoytpes, silicone is more effective for improving salt tolerance (Avcu *et al.,* 2013). The role of silicon, which is the second most abundant element on the earth, has been poorly understood in plant biology (Zhu *et al.,* 2004);

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(Gong *et al.,* 2005). However, it is one of the most important elements, plays a critical role in tolerance against some environmental stress including salt stress on plants. (Zhu *et al.,* 2004), (Liang *et al.,* 2003). Silicone promotes the antioxidative defense mechanisms and reduces the lipid peroxydation under salinity stress (Zhu *et al.,* 2004). Antioxidant enzyme activities enhanced by exogenous silicon application in barley (Liang *et al.,* 2003) and cucumber (Zhu *et al.,* 2004) under salt stress.

The objective of this work was to determine the possibility of using Selenium and Silicon to mitigate the negative effect of salinity on growth, development, yield and chemical composition of lettuce plants grown using salty water of irrigation.

Materials and Methods

Plant materials and cultivation:

Seeds of two lettuce (*Lactuca sativa* L.) cultivars Balady and Great lakes were sown on 15th September in 2012-2013 and 2013-2014 seasons, at Ismailia Governorate using foam trays (209 cells) filled with a mixture of peat: vermiculite (1:1, v: v). Average day/night temperatures were 28/20˚C, respectively. Plants were transplanted into field 30 days after sowing and irrigated with saline water 3.22 dSm^{-1} using drip irrigation system. Fertilizers were added as recommended by the Ministry of Agriculture. A half dose of P_2O_5 (45 kg/fed) as single super phosphate (15% P_2O_5) and K_2O (50 kg/fed) as potassium sulfate (50% K_2O) with half dose of N (60 kg N/fed) in the form of ammonium nitrate were applied during soil preparation, while the remaining dose of N, K and P was applied 30 days after transplanting. Regular Standard agricultural practices common in the area were followed. Irrigation was regularly carried out each other day when the water level reach about 75% of the field capacity. Crop was harvested when heads attained the proper size.

Chemical analysis of cultivated sandy soil was as follows: ECe (ds m^{-1}) = 1.31 mmhos/cm, pH 8.91, Soluble anions (meq L^{-1}): HCO_3^- 1.0 meq L^{-1}, Cl^- 1.3 meq L^{-1}, SO_4^{--} 2.0 meq L^{-1}, Soluble cations (meq L^{-1}): Ca^{++} 2.0 meq L^{-1}, Mg^{++} 1.0 meq L^{-1}, Na^+ 2.5 meq L^{-1} while water EC was

EC_w (ds m^{-1}) = 3.22 mmhos/cm.

Treatments:

Two weeks after transplanting, foliar applications of Se and Si solutions were carried out and repeated twenty-one day intervals, the total number of foliar application reached 3 times. The concentrations of treatments were; 16, 32 µM selenium (Se) and 1, 2 mM silicon (Si) in addition to distilled water as a control. Two samples were taken at 35, 75 days after planting (DAP) for growth measurements, determination of leaf relative water content, electrolyte leakage and chemical analyses.

Plant growth measurements:

Three plants from each replicate were harvested, and data of plant growth parameters, i.e. plant height, root length, plant fresh weight, plant dry weight, number of leaves, head weight and head size. Dry weight was determined according to A.O.A.C. (2007). A known weight of plant was dried in a ventilated oven at 70°C for reaching the constant weight. Plant dry weight was determined and expressed as g.

Determination of membrane permeability (MP):

Twenty leaf discs (10 mm in diameter) obtained from three plants per replicate from the fully expanded leaves were placed in 50 ml glass vials, rinsed with distilled water to remove electrolytes released during leaf disc excision. Vials were then filled with 30 ml of distilled water and allowed to stand in the dark for 24 h at room temperature. Electrical conductivity (EC1) of the bathing solution was determined at the end of incubation period using an electrical conductivity meter (HANNA H199301). Vials were heated in a temperature-controlled water bath at 95◦C for 20 min, and then cooled to room temperature and the electrical conductivity (EC2) was measured. Electrolyte leakage was calculated as percentage of EC1/EC2 (Shi *et al.,* 2006).

Determination of leaf relative water content (LRWC):

Samples were taken from three plants per replicate. Individual leaves first detached from the stem and then weighed to determine fresh weight (FW). In order to determine turgid weight (TW), leaves were floated in distilled water. Leaf samples were weighed periodically, after gently wiping the water from the leaf surface with the tissue paper until a steady state achieved. At the end of imbibitions period, leaf samples were placed in a pre-heated oven at 80◦C for 48 h, in order to determine dry weight (DW). Values of FW, TW and DW were used to calculate LRWC using the equation below (Kaya *et al*., 2003):

$$LRWC\ (\%) = [(FW - DW) / (TW - DW)] \times 100$$

Biochemical analyses:

All biochemical analyses were determined in the fully expanded fourth leaf of three plants per replicate.

Determination of chlorophylls and carotenoids:

Chlorophyll a, b and total carotenoids contents were determined in representative fresh leaves samples according to Moran (1982**)**, as follows: Ten ml N, N-Dimethylformamide was added to 0.2 g of fresh leaves in tubes then they were placed overnight in dark. The obtained extracts from previous materials were measured by spectrophotometer at the wavelength of 664 nm for chlorophyll-a, 647 nm for chlorophyll-b and 470 nm for carotenoids, using N, N-Dimethylformamide as a blank.

Determination of proline:

Proline concentration was determined according to the method of Troll and Lindsley (1955) modified by Petters *et al*. (1997). Fresh leaf samples (0.5 g) were ground and homogenized with one volume of 100 mM sodium phosphate buffer (pH 6.0). The samples were centrifuged for 10 min at 11.000 x *g*. The reaction mixture contained 200 µl of the supernatant and 1 ml of ninhydrin solution (2.5 g dissolved in 100 ml of ortho-phosphoric acid, acetic acid, and water 15: 60: 25. V: V: V). The reaction proceeded for 1 h in boiling water bath and the developed dye was extracted with 1 ml of toluene and measured by the spectrophotometer at 515 nm by using UV–vis spectrophotometer (CT 200 spectrophotometer).

Determination of malondialdehyde:

The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) concentration by thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). One gram of fresh leaves tissue was homogenized in 5 ml of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 10000 x *g* for 5 min then 4 ml of thiobarbituric acid solution (0.5 g TBA / 100 ml TCA 20%) was added to 1 ml of the supernatant, the mixture was heated to 95 ºC for 30 min and then quickly cooled in ice bath. The contents were centrifuged at 10000 x *g* for 15 min and the absorbance of suspension was measured at 532 nm in spectrophotometer (CT 200 spectrophotometer). The concentration of MDA was calculated using an extinction coefficient of 155 $mM^{-1}cm^{-1}$and was expressed as µmol g^{-1} FW.

Determination of total soluble sugars:

Extraction of total soluble sugars (TSS):

For total soluble sugars extraction (A.O.A.C., 2007), added 0.5 g fresh sample + 5 ml heated ethanol (80%) in closed test tube, kept in water bath at 95°C for 10 min, centrifuged at 2500 rpm for 5 min. Take the supernatant and added 5ml heated ethanol (80%) to solid phase (starch), kept in water bath (95ºC) for 10 min and centrifuged at 2500 rpm for 5 min. Added 5ml heated ethanol (80%) to supernatant, kept in water bath (95ºC) for 10 min and centrifuged at 2500 rpm for 5 min. The supernatants were collected.

When the extract sample contained color pigments, added 0.5 g activated charcoal to remove the pigments, then filtrated before measurements. Take 1ml of supernatant (soluble sugars) +1 ml phenol 4%

+2 ml sulphoric acid 96%, and then add 9 ml distilled water (Dubois, 1956). The concentration of TSS was determined by reading the absorption at 490 nm, using UV– vis spectrophotometer (CT 200 spectrophotometer). The total soluble sugars concentration was determined by the standard curve of glucose (10-100 µg) and expressed as mg g^{-1} Fw.

Determination of antioxidant enzymes activity:

Preparation of enzymes extract:

Leaf tissues were homogenized in 100 mM chilled sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% polyvinyl pyrrolidone (PVP) (w/v) at 4 ºC. The extraction ratio was 4 ml buffer for each one gram of plant material. The homogenate was centrifuged at 11.000 x *g* for 15 min at 4 ºC. Supernatant was used to measure the activities peroxidase (POD) and superoxide dismutase (SOD). Protein concentration was determined according to the method of Bradford (1976). All enzymes activity was calculated per milligram of Protein per minute. The proteins concentration were calculated by using the standard curve of bovine serum albumin (BSA).

Determination of peroxidase activity:

The activity of peroxidase (POD; EC1.11.1.7) was assayed by the method of Hammerschmidt *et al*. (1982). The reaction mixture (2.9 ml) consisted of 0.25 % (v/v) guaiacol in 10 mM sodium phosphate buffer (pH 6 containing 10 mM hydrogen peroxide H_2O_2). Volume of 100 µl of the crude enzyme extract was added to initiate the reaction which was measured spectrophotometrically (CT 200 spectrophotometer) at 470 nm per min. One international (IU) of enzyme activity was expressed as Δ OD = 0.01 POX activity expressed as unit $min^{-1}mg^{-1}$ protein.

Determination of superoxide dismutase activity:

The activity of superoxide dismutase (SOD; EC 1.15.1.1) was assayed by the method of Beauchamp and Fridovich (1971) by measuring its ability of enzyme to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). A reaction mixture (3 ml) containing 40 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 µM NBT, 2 µM riboflavin, 0.1 mM EDTA and 100 µl of the crude enzyme extract was shaken and placed 30 cm below light source consisting of 15 W fluorescent lamp. The absorbance was recorded at 560 nm. One unit of SOD activity is the amount of protein required to inhibit 50% initial reduction of NBT under light. The activity of SOD was expressed at unit min^{-1} mg^{-1} protein.

Determination of the Na^+, K^+ and Ca^{++}:

The extraction and determination of the Na^+, K^+ and Ca concentrations were conducted according to Xu *et al.* (2006). Na^+, K^+ and Ca^{++} concentrations were measured using an atomic absorption spectrophotometer (Varian spectra AA 220, Varian, Palo Alto, CA, USA).

Statistical analysis:

Growth Parameters and biochemical analysis were determined by analysis of variance using the General Linear Models procedure of Statistical Analysis system. Significance between means was tested by Tukey's studentized range test at the 5% probability level (CoStat software, Version 6.4, 2008).

Results

Growth parameters Data in (Table, 1) showed that foliar application of (Se and Si) significantly improved all growth parameters (plant height, root length, number of leaves, fresh weight and dry weight) of lettuce plants (the two studied cultivars) under salt stress conditions compared with control plants in both seasons. The highest significant increase in growth parameters were recorded at the higher concentration of Si (2 mM) in both cultivars and seasons. Se at (32 µM) treatments gave the lowest values in all parameters compared with other treatments in both cultivars and seasons.

Table 1: Effect of selenium (Se) and silicon (Si) on growth parameters of two lettuce cultivars Great leaks (G) and Balady (B) under salinity stress after 75 days from planting during the two seasons.

Treatments		Plant height (cm)		Root length (cm)		No. of leaves/plant		FW (g)		DW (g)	
		G	B	G	B	G	B	G	B	G	B
1st season	Cont.	13.90	13.70	11.33	12.07	12.33	11.00	65.00	58.33	13.07	10.50
	Se1	17.87	16.90	14.00	14.03	20.67	20.00	143.67	183.33	26.13	24.73
	Se2	17.00	15.93	13.17	13.57	18.67	18.00	129.33	148.50	23.33	22.43
	Si1	17.83	17.87	14.47	14.03	20.67	20.00	163.33	194.60	28.20	25.77
	Si2	19.53	18.30	15.23	14.77	24.67	21.00	195.00	231.00	34.07	30.07
MSD		1.13	1.61	0.63	0.63	1.65	2.97	14.29	23.95	2.12	1.99
MSD C.		0.26		0.07		0.43		3.18		0.3	
2nd season	Cont.	14.30	14.56	11.23	11.07	13.33	10.33	66.67	52.67	10.93	10.22
	Se1	17.37	17.20	15.13	14.13	19.33	19.66	135.00	181.67	23.23	23.53
	Se2	15.73	15.70	14.27	13.90	18.33	18.00	120.67	160.00	20.03	20.27
	Si1	18.03	17.70	15.16	14.93	20.67	20.33	140.00	188.03	23.47	29.07
	Si2	19.10	18.17	15.90	15.20	22.67	23.00	181.67	216.33	25.90	31.97
MSD		1.31	0.94	0.86	1.14	1.65	2.52	13.94	11.1	1.69	1.55
MSD C.		0.23		0.12		0.42		2.4		0.34	

Leaf relative water content:

Data in (Figure, 1) illustrated that leaf relative water content (LRWC) was significantly affected by all treatments (Se and Si) positively compared with the control plants at the first date (35 DAP) in both cultivars and seasons. The maximum LRWC values observed with Si applications at the highest concentration 2 mM, followed by Si at 1 mM in both cultivars and seasons but there are no significant differences between treatments. However, Data in Figure, 1 showed that after 75 DAP, all treatments (Se and Si) were significantly increased LRWC compared with control plants. The maximum LRWC values were observed with plants treated with Si at higher concentration 2 mM, followed by Si at 1 mM in both cultivars and seasons. While, the lowest value of LRWC was observed in plants treated with Se at 32 µM in both cultivar and seasons compared with other treatments.

Membrane permeability:

Regarding the effect of different treatments on the cell membrane permeability (MP), data in (Figure, 1) showed that, at first plant sample (35 DAP), MP significantly decreased by all treatments (Se and Si) compared with untreated plants in both seasons. However, all treatments significantly reduced the negative effect of salinity on plasma membrane permeability of the two plant cultivars in both seasons. Plants treated with Si at the higher concentration (2 mM), gave the highest significant reduction of MP of two lettuce cultivars in both seasons, followed by the lower concentration of Si (1 mM) respectively, then Se at 16 µM. MP significantly decreased by all treatments (Se and Si) compared with untreated plants in both seasons 75 days from planting (Figure, 1) However, all treatments significantly reduced the negative effect of salinity on plasma membrane permeability of the two studied cultivars plants in both seasons. Si treatments at the higher concentration (2 mM) gave the highest significant reduction of MP of two cultivar of lettuce in both seasons followed by the lower concentration of Si (1 mM) then Se at 16 µM.

Yield:

Data in (Table, 2) illustrated that lettuce head weights and size were significantly affected by treatments compared with untreated plants. Thus, Si at 2 mM gave the highest significant head weight and head size in the two tested cultivars in both seasons followed by Si at 1 mM. While, the mean yield weight significantly increased by all treatments (Se and Si) compared with untreated plants in the two studied cultivars and seasons. However, foliar treatment with Si at 2 mM improved yield quality followed by Si at 1 mM in both seasons. Se at 32 µM treatment gave the lowest yield characters in both cultivars and seasons compared with other treatments.

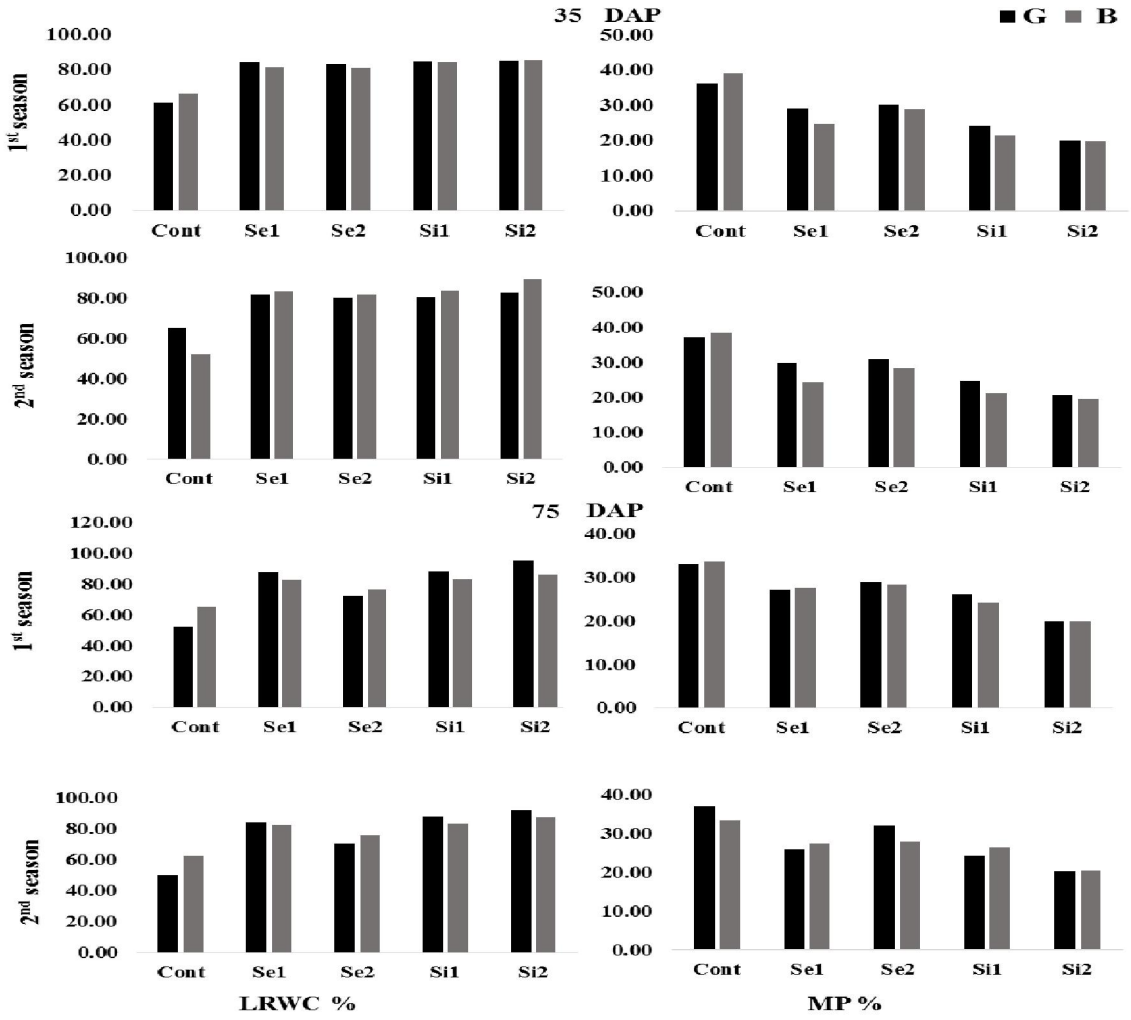

Fig. 1: Effect of Selenium (Se) and Silicon (Si) on Leaf relative water content (LRWC) and Membrane permeability (MP) of two lettuce cultivars Great leaks (G) and Balady (B) under salinity stress at 35 and 75 days after planting during two seasons.

Table 2: Effect of Selenium (Se) and Silicon (Si) on yield of two lettuce cultivars Great leaks (G) and Balady (B) under salinity stress after 90 days from planting during the two seasons.

Treatments		Head weight (g)		Head size (cm^3)		Yield ton /fed	
		G	B	G	B	G	B
1st season	Cont.	112.00	78.50	59.21	21.84	3.58	2.51
	Se1	181.00	206.50	96.98	47.39	5.79	6.61
	Se2	164.50	178.50	80.38	31.88	5.26	5.71
	Si1	230.00	221.50	102.35	64.08	7.36	7.09
	Si2	270.50	268.50	124.98	87.25	8.66	8.59
	MSD	21.05	15.85	21.61	10.17	0.67	0.3
	MSD C.	2.71		2.81		0.087	
2nd season	Cont.	119.33	76.50	49.72	24.22	76.50	2.45
	Se1	199.00	179.50	81.81	44.68	179.50	5.74
	Se2	163.00	153.50	59.10	35.79	153.50	4.91
	Si1	213.50	195.00	85.20	57.48	195.00	6.24
	Si2	286.00	230.00	102.12	75.05	230.00	7.36
	MSD	22.72	20.61	8.2	6.93	20.61	0.66
	MSD C.	3.28		1.5		0.11	

Biochemical constituents:

Plant pigments:

Chlorophyll a, b and Carotenoids concentration:

Data presented in (Figure, 2) showed that, after 35 DAP, treatments increased chlorophyll a concentration compared with untreated plants in both seasons. Se treatment at the lower concentration (16 µM) recorded the highest values of chlorophyll a concentration (2.68 and 1.99 mg g^{-1} FW) in both seasons followed by Si at the higher concentration (2.24 and 1.35 mg g^{-1} FW) when compared with untreated plants (1.25 and 0.70 mg g^{-1} FW) in Great leaks cultivar. Moreover, Si and Se treatment at (1 mM and 32 µM) gave the lowest values of chlorophyll a concentration in both seasons compared with other treatments. Balady cultivar plants treated with the higher concentration of Si (2 mM) recorded the greatest values of chlorophyll a (2.46 and 1.4 mg g^{-1} FW) followed by Si at 1 mM treatments (2.36 and 1.43) in both seasons respectively. Se treatment at (16 and 32 µM) gave the lowest values of chlorophyll a concentration in both seasons comparing with other treatments.

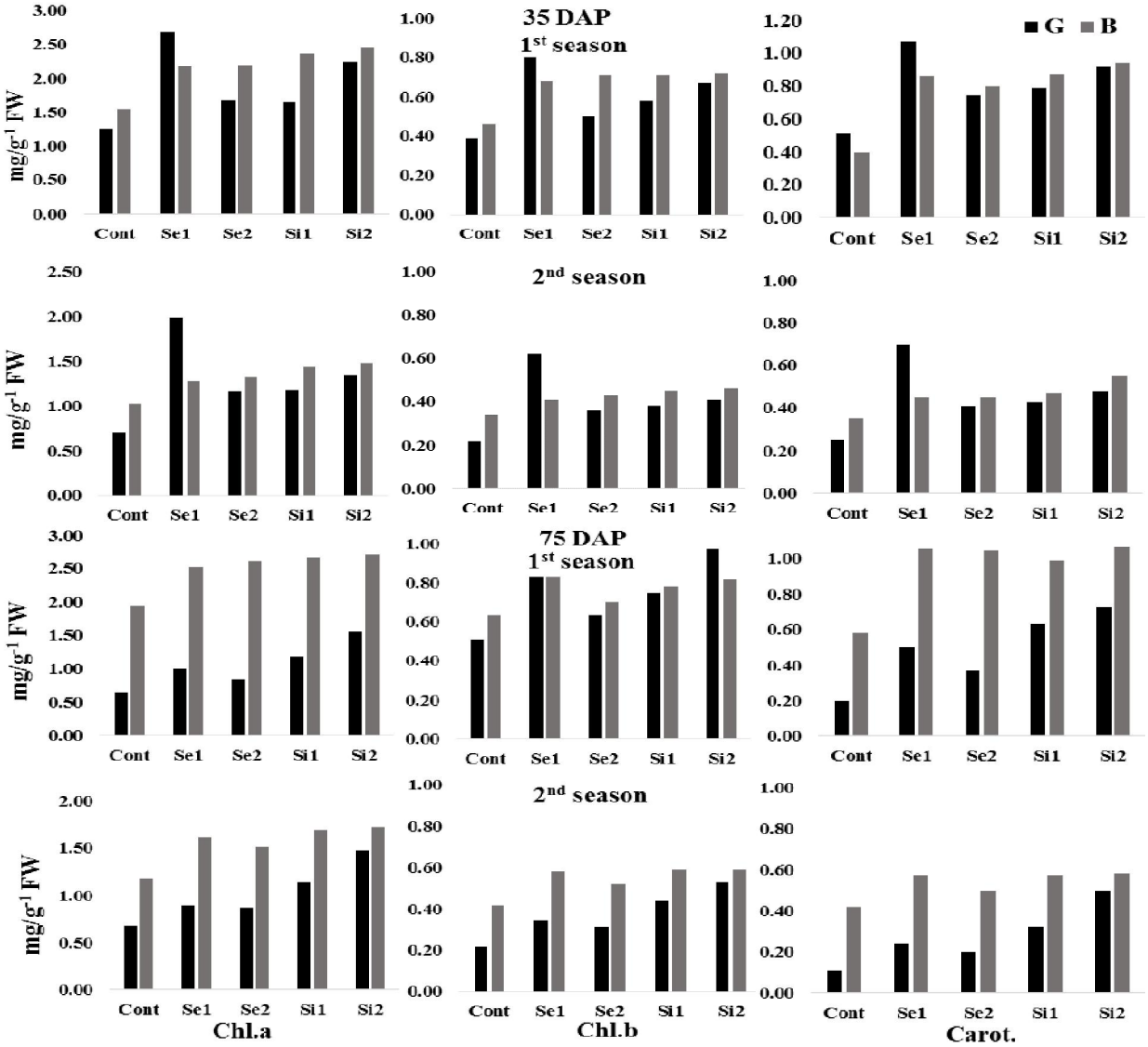

Fig. 2: Effect of Selenium (Se) and Silicon (Si) on chlorophylls (Chl.a, b) and carotenoids (Carot.) concentrations of two lettuce cultivars Great leaks (G) and Balady (B) under salinity stress at 35 and 75 days after planting during two seasons.

Chlorophyll b concentrations increased by various treatments compared with untreated plants in both seasons. Se at (16 µM) recorded the highest Chlorophyll b values followed by Si at the higher (2 mM)

concentration respectively comparing with untreated Great leaks cultivar in both seasons. Moreover, Se treatment at (32 µM) gave the lowest values of chlorophyll b concentration in both seasons comparing with other treatments. However, in Balady cultivar treatment Si at higher concentration (2 mM) recorded the highest values followed by Si at 1 Mm treatments in both seasons respectively.

(Figure, 2) illustrated that after 75 DAP, all treatments increased chlorophyll a and b concentration compared with untreated plants in both cultivars and seasons. Si at 2 mM gave the highest significant increase followed by Si at 1 mM then Se 16 µM compared with untreated plants in the two lettuce tested cultivars Great leaks and Balady. Se at 32 µM gave the lowest values of chlorophyll b concentration in both cultivar and seasons compared with other treatments.

In comparison to untreated plants, data in (Figure, 2) at the first sample (35 DAP) showed that, carotenoids significantly increased in treated plants. In the same manner, chlorophyll a and b Se (16 µM) recorded the highest values followed by Si at the higher concentrated comparing with untreated plants in both seasons respectively in Great leaks cultivar. Moreover, Se treatment at (32 µM) gave the lowest values of carotenoids concentration in both seasons comparing with other treatments. However, in Balady cultivar, Si treatment at the (2 and 1Mm) concentration recorded the highest values followed by se at (16 µM) treatments in both seasons respectively. Se treatment at (32 µM) gave the lowest values of carotenoids concentration in both seasons compared with other treatments.

At 75 DAP (Figure, 2) obtained results showed that carotenoids significantly increased by treatments compared with untreated plants in both cultivars and seasons. Si at 2 mM gave the highest significant increment followed by Si at 1 mM then Se 16 µM compared with untreated plants in both cultivars and seasons. Se treatment at (32 µM) gave the lowest values of carotenoids concentration in both cultivars and seasons compared with other treatments.

Proline:

Proline concentration was significantly increased in plants by Si and Se treatments compared to untreated ones at the first date (35 DAP) in both seasons under salt stress conditions (Figure 3). The highest significant values recorded by Si at 2 mM followed by Si at 1 mM then Se 16 µM compared with untreated plants in both season and both cultivar. The same results found in great leaks cultivar but the differences between treatments were not significant in both seasons.

After 75 days from planting Data in (Figure 3) illustrated that the highest proline content significantly detected by Si at 2 mM followed by Si at 1 mM then Se 16 µM ppm respectively comparing with untreated plants in both cultivar and both season. Se treatment at 32 µM gave the lowest values of proline in both cultivars and both seasons comparing with other treatments.

Malondialdehyde:

(Figure 3) also show that, all treatments significantly reduced MDA concentration in both seasons compared with untreated plants. The highest significant reduction recorded by Si at both concentrations compared to other treatments and untreated plants in both seasons. Se at the higher concentration 32 µM gave the lowest reduction values at first and second dates (35 and 75 DAP) in both cultivar and both seasons respectively.

At second sample (75 DAP) the lowest significant values were recorded by Si at 2 mM followed by Si 1 mM then Se 16 µM compared with untreated plants in both cultivars and seasons. Plants treated with 32 µM gave the highest values of Malondialdehyde concentration in both cultivars and seasons as compared with other treatments (Figure 3).

Total soluble sugars:

(Figure 4) revealed that, total soluble sugars significantly increased by all treatments (Se and Si) compared with untreated plants in both seasons at first sampling (35 DAP). The highest significant values recorded by Si at 2 mM followed by Si at 1 mM and Se 16 µM compared with untreated plants in both season respectively in Great leaks cultivar plants. The same results found in Balady cultivar plants.

After 75 days from planting, data in Table (14) illustrated that the highest significant values of TSS noted in plants treated with Si at 2 mM then Si at 1 mM then Se 16 µM compared with untreated plants in both seasons respectively. In Balady cultivar, the highest significant values recorded by Si at 2 mM followed by Si at 1 mM then Se 16 µM compared with untreated plants in both seasons.

At first and second sample (35, 75 DAP) (Figure 4) the total soluble sugars (TSS) significantly increased by all treatments Se and Si compared with untreated plants in both cultivar and seasons. The highest significant values were recorded by Si at 2 mM followed by Si at 1 mM then Se 16 µM comparing with untreated plants in both cultivar and season.

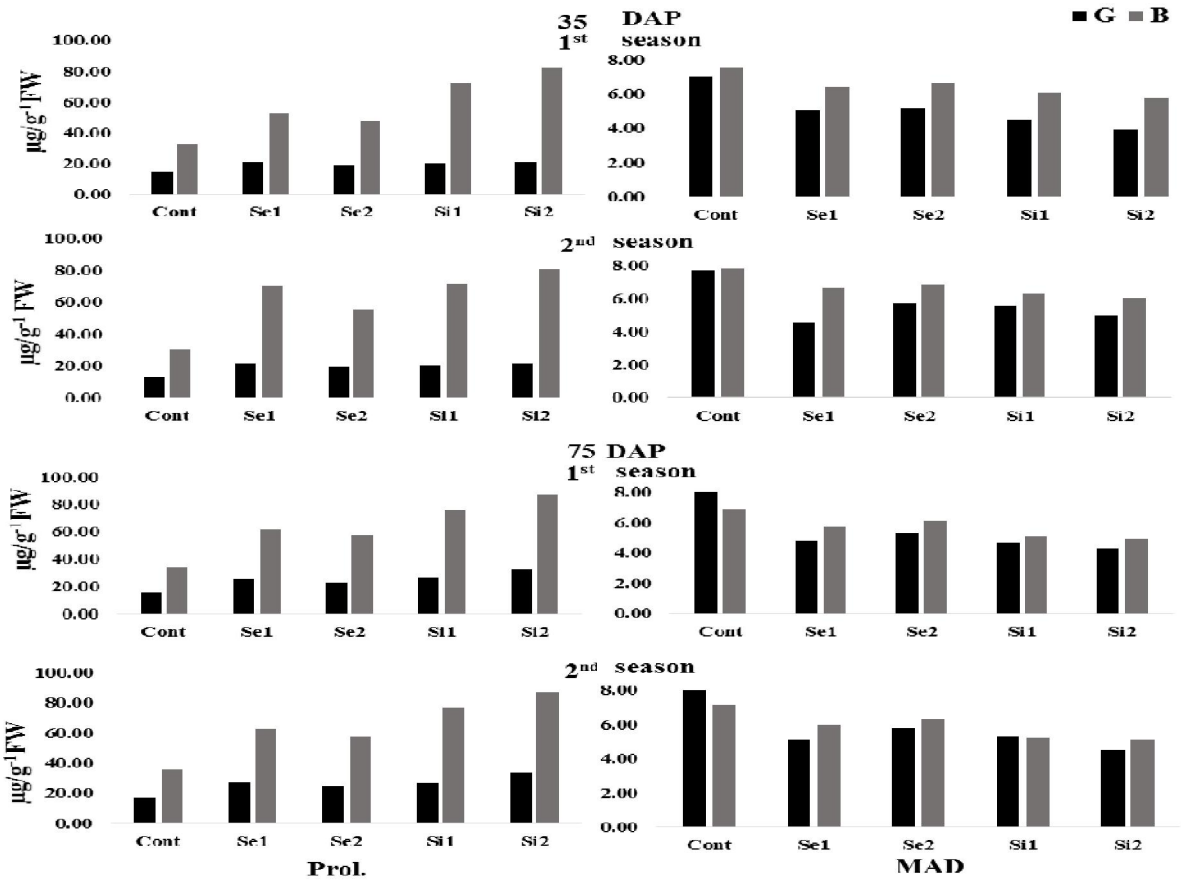

Fig. 3: Effect of Selenium (Se) and Silicon (Si) on proline (prol.) and malondialdehyde (MDA) concentrations of two lettuce cultivars Great leaks (G) and Balady (B) under salinity stress at 35 and 75 days from planting during two seasons.

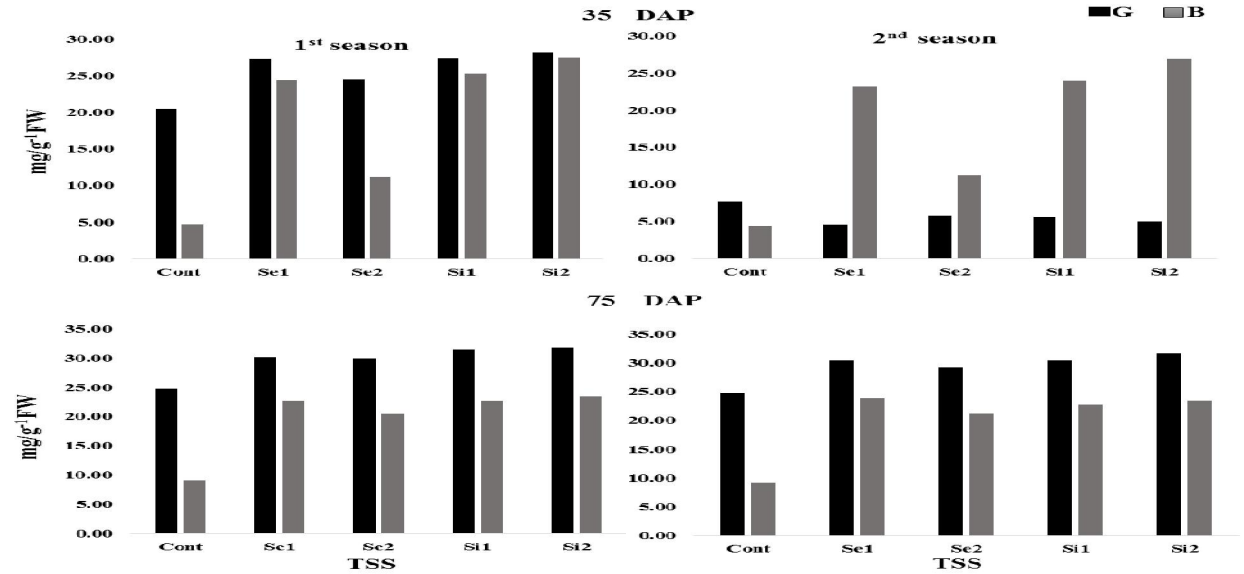

Fig. 4: Effect of Selenium (Se) and Silicon (Si) on total suable sugar (TSS) concentrations of two lettuce cultivars Great leaks (G) and Balady (B) under salinity stress at 35 and 75 days from planting during two seasons.

Antioxidant enzymes activity:

The results in Figure (5) revealed that treatments significantly increased POD and SOD activities at the two dates in both cultivars as compared to untreated plants under saline conditions. The highest significant improvement was recorded by Se at 16 µM compared to other treatments and control plants at two dates in both cultivar and seasons.

Plants treated with Se at 16 µM recorded the highest significant activity of POD (increased by88.16 – 83.76%) followed by Si at 2 mM (increased by 69.03 and 50.69% respectively) in Great leaks cultivar in both seasons. However, Se at 32 µM showed the lowest significant POD activity in both cultivar and both seasons. The same results were found in Balady cultivar.

In comparison with untreated plants Se at 16 µM recorded the highest significant activity of SOD (increased by %135.07 – 147.34%) followed by Si at 2 mM (increased by112.48 – 122.83%) in both seasons in Great leaks cultivar. The same result was found in Balady cultivar. However, Se at 32 µM showed the lowest significant SOD activity in both cultivar and both seasons.

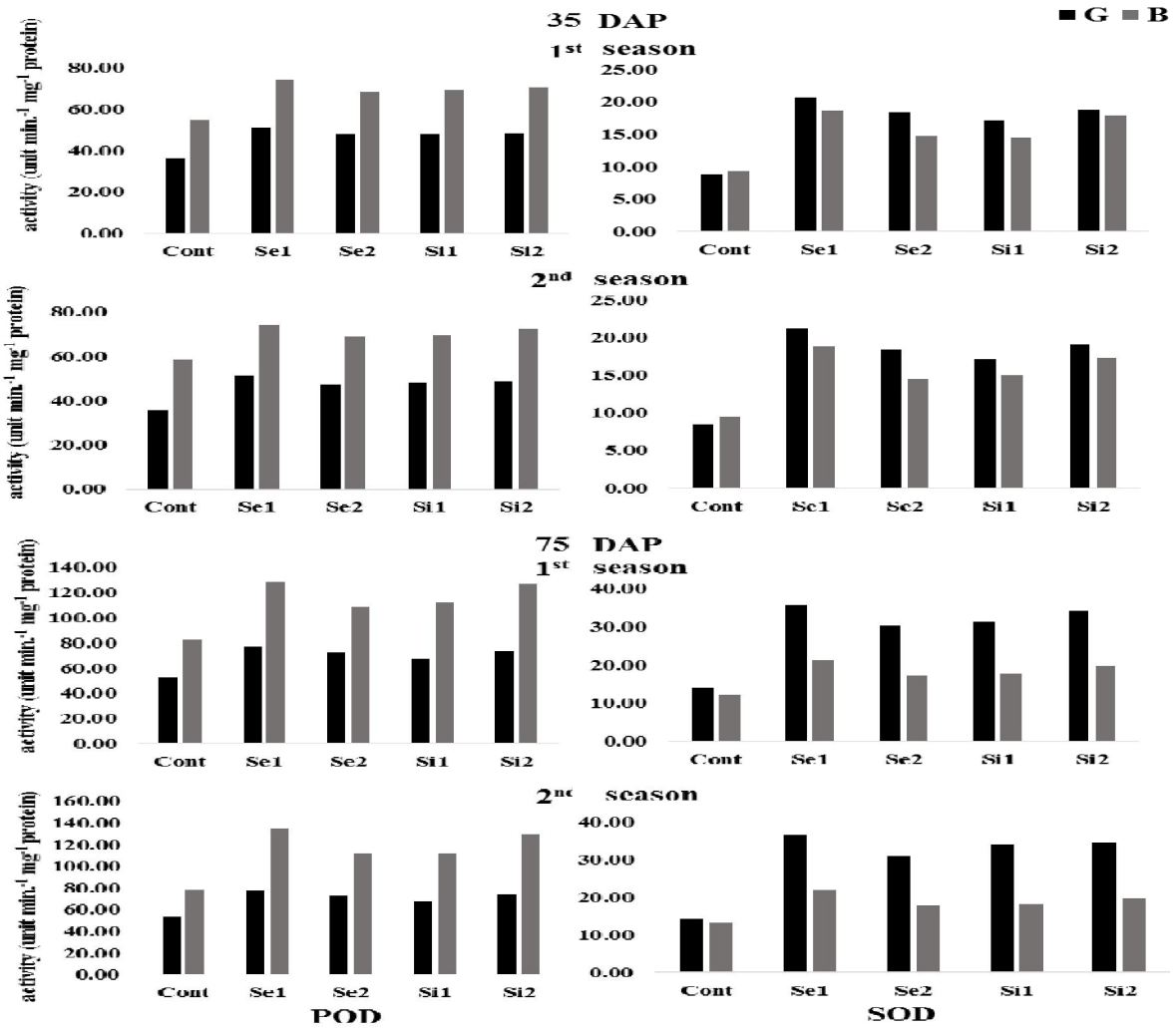

Fig. 5: Effect of Selenium (Se) and Silicon (Si) on peroxidase (POD) and superoxide dismutase (SOD) activities in two lettuce cultivars Great leaks (G) and Balady (B) under salinity stress at 35 and 75 days from planting during two seasons.

Mineral concentration:

Data in (Table 3) showed that, at 75 DAP, Si at 2 mM significantly decreased concentration of Na^+, followed by Si at 1 mM then Se at 16 µM in Great leaks cultivar in both seasons. Se at 32 µM showed the

lowest significant decreased concentration of Na^+ in both seasons. The same result was found in Balady cultivar.

Data in (Table 3) showed that, Si at 2 mM significantly increased concentration of K^+ and Ca^{++} followed by Si at 1 mM then Se at 16 µM in Great leaks cultivar in both seasons. Se at 32 µM showed the lowest significant increased concentration of K^+ and Ca^{++} in both seasons. The same result was found in Balady cultivar.

Table 3: Effect of Selenium (Se) and Silicon (Si) on Na^+, K^+ and Ca^{++} concentrations leaves of two lettuce cultivars (C) Great leaks (G) and Balady (B) under salinity stress during two seasons.

Treatments		Na%		K%		Ca%		K/Na	
		G	B	G	B	G	B	G	B
1st season	Cont.	5.25	4.04	0.50	0.50	0.75	0.71	0.10	0.12
	Se1	3.00	1.45	1.75	0.75	1.04	1.08	0.58	0.52
	Se2	3.25	1.52	1.50	0.63	0.96	0.96	0.46	0.41
	Si1	2.88	1.39	1.88	0.88	1.25	1.25	0.65	0.63
	Si2	2.00	1.14	2.63	2.13	1.54	1.54	1.32	1.87
	MSD	0.36	0.34	0.36	0.36	0.24	0.23		
	MSD C.	0.061		0.060		0.045			
2nd season	Cont.	7.51	5.58	0.57	0.75	0.68	0.69	0.08	0.13
	Se1	4.29	2.10	2.00	1.15	1.02	1.19	0.47	0.55
	Se2	4.65	2.12	1.81	1.00	0.89	0.94	0.39	0.47
	Si1	3.82	2.00	2.33	1.31	1.38	1.53	0.61	0.66
	Si2	2.72	1.57	2.99	3.08	1.70	1.88	1.10	1.96
	MSD	0.49	0.51	0.43	0.54	0.25	0.26		
	MSD C.	0.084		0.083		0.044			

Discussion

The present study showed that exogenous foliar application of selenium (Se) and silicon (Si) promoted the growth of lettuce plants under salinity stress; plants treated with Se and Si exhibited improvement of growth parameters, physiological functions and yield compared with untreated plants.

Stress factors such as salinity causes common reactions in plants, which lead to cellular damages mediated by reactive oxygen species (ROS). Oxygen radicals in different plants exposed to abiotic stress causes an inhibition of protein synthesis, inactivation of several chloroplast enzymes, impairment of electron transport, increased membrane permeability, and increased activity of the H_2O_2 scavenger system. Antioxidative enzymes such as superoxide dismutase (SOD) and catalase (CAT) play an important role against salinity stress.

Selenium (Se) and silicon (Si) are suggested as beneficial elements for some plants: they are not required by all plants but can promote plant growth and may be essential for particular species. These elements have been reported to enhance resistance to abiotic stresses such as salinity, drought, and nutrient toxicity or deficiency (Hasanuzzaman *et al*. 2010a, b; Hasanuzzaman and Fujita 2011b; Tahir *et al.* 2012).

Regarding Se, the beneficial effects of low doses of Se have received little attention compared to toxic effects that typically occur at higher concentrations. Understanding of the effects of beneficial elements is important to improve crop productivity and enhance plant nutritional value for a growing world population.

The physiological roles of Se in plants have been studied by many researchers although Se has not been confirmed to be an essential micronutrient in higher plants. There are several evidences on its positive effect on plant growth and productivity at low concentrations (Turakainen *et al*. 2004; Hasanuzzaman *et al*. 2010a,b; Hasanuzzaman and Fujita 2012; Hasanuzzaman *et al*. 2012b). However, the specific physiological mechanisms underlying the beneficial role of Se in plants have not been clearly elucidated. It is already established that the plants supplemented with Se have shown enhanced resistance to certain abiotic stresses including salinity (Djanaguiraman *et al*. 2005; Filek *et al*. 2008; Hawrylak-Nowak 2009; Cartes *et al.* 2010; Chu *et al.* 2010; Djanaguiraman *et al.* 2010; Hasanuzzaman *et al.* 2010b; Yao *et al.* 2010a, b; Hasanuzzaman and Fujita 2011b; Hasanuzzaman *et al.* 2011b). One of the major effects of Se on abiotic stress tolerance is associated with its antioxidative capacity (Djanaguiraman *et al.* 2005; Hasanuzzaman *et al.* 2011b; Hasanuzzaman and Fujita 2011). A plenty of research results have shown the ability of Se to protect plants from salt stress-induced damages when applied at low concentration. The interaction of Se with soil salinity has been studied earlier by Terry *et al*. (2000). Kong *et al.* (2005) reported that at low concentrations (1–5 ㎜), Se tends to stimulate the growth, the activities of SOD and POD, as well as the accumulation of water- soluble sugar in leaves of sorrel (*R. patientia* × *R.*

tianshanicus) seedlings. However, at higher concentrations (10–30 mM), Se exerted diminished beneficial effects on growth and enzyme activities. Results revealed that SOD and POD activity of salt-stressed increased when exposed to Se. In *C. sativus* leaves, Se treatments at 5 and 10 mM significantly improved the growth rate and increased the photosynthetic pigments and proline contents when subjected to salt stress (Hawrylak-Nowak 2009). Additionally, Se enhanced the salt tolerance of *G. max* seedlings by protecting the cell membrane against lipid peroxidation, also Se-treated plants have increase proline content (Djanaguiraman *et al.* 2005), however, the mechanisms and the reasons for proline accumulation in Se-supplied plants have not been fully investigated. Walaa *et al.* (2010) observed that NaCl-induced lipid peroxidation which led to increase the percentage of electrolyte leakage, were effectively minimized when the cucumber seedlings were pretreated with Se. Se-supplemented seedlings also showed enhanced antioxidant activities and proline content. Concomitant increases in the levels of H_2O_2 and MDA were also measured. However, further investigation revealed that Se treatment led to a reduction in the levels of MDA in treated plants as compared to salt stress untreated plants.

Silicon (Si) is the second most abundant element on the earth crust after oxygen and it is accumulated in plants at a rate comparable to those of macronutrient elements like Ca, Mg and P (Epstein 1999). Although Si is a major constituent of plants, its essentiality has not been established completely yet. Its deficiency can cause various dysfunction in regards to plant growth, development and reproduction, so it may be considered a 'quasi essential' element for plants. Moreover, supplementation with Si extend a number of beneficial effects on growth and yield of several plant species (Richmond and Sussman 2003; Pilon-Smits *et al.* 2009). Numerous studies have been achieved to understand the possible mechanism for Si to enhance resistance and/or tolerance to abiotic stresses in higher plants. It is found to stimulate enzymatic and non-enzymatic antioxidant under stress condition (Liang *et al.* 2007). The possible mechanisms of Si-mediated protective effects under salt stress may include increased plant water status (Romero-Aranda *et al.* 2006), enhanced photosynthetic activity and maintenance of leaf organelles (Shu and Liu. 2001), Dismutation of ROS (Zhu *et al.* 2004), immobilization of toxic Na^+ (Liang *et al.* 2003), reduced Na^+ uptake in plants and enhanced K^+ uptake (Liang *et al.* 2005; Tahir *et al.* 2006) and higher K^+: Na^+ selectivity (Hasegawa *et al.* 2000). Si was recently confirmed to mitigate salinity stress by enhancing Na^+ exclusion and decreasing lipid membrane peroxidation through stimulation of enzymatic and non-enzymatic anti- oxidants (Saqib *et al.* 2008; Hasanuzzaman and Fujita 2011b). The protective effect of Si on salinity has been examined in many plants. In *B. napus*, exogenous Si mitigated the harmful effects of salinity on the growth by lowering tissue Na^+ contents, maintaining the membrane integrity of root cells by reduced lipid peroxidation and lignifications; and increased ROS scavenging capacity (Hashemi *et al.* 2010). While studying with *Z. mays*, Parveen and Ashraf (2010) reported that under saline condition exogenously applied Si significantly increased growth of plant, stomatal conductance and transpiration. Applying Si to *Medicago sativa* could modify the activity of antioxidative enzyme of one or several organs of plants to improve the salt tolerance (Wang *et al.* 2011b). The plants under NaCl salinity stress treated with Si significantly increased POD activity in shoots, but decreased the SOD activity in roots (Wang *et al.* 2011b). In addition, Ali *et al.* (2012) found that Si supplementation into the root medium improved significantly the K^+ and K^+: Na^+ ratio, leaf water potential and stomatal conductance, but reduced the Na^+ in *T. aestivum*. Lima *et al.* (2011) reported that Si application in the nutrient solution significantly increased growth parameters and decreased ion leakage in *Z. mays* seedlings, whereas this response was not observed in *Vigna unguiculata*. In *Glycine max*, an addition of Si to salt stressed plants substantially alleviated the adverse effects of NaCl on growth, as it enhanced endogenous GA_3, while reducing the levels of ABA and proline (Lee *et al.* 2010). The addition of Si also showed reduced levels of H_2O_2 and MDA in salt-stressed seedlings compared to salt stress alone. Our results suggested that the exogenous application of Si led the plants to be more tolerant to salt stress by enhancing their antioxidant defense (Hasanuzzaman and Fujita 2011b). Tahir *et al.* (2012) reported that application of Si increased shoot and root dry weight and plant water contents in both normal and saline conditions in wheat plants.

Conclusion

Regarding many researcher findings, it is clear that salt stress have destruction effects on the growth, development, physiology and yield of plants. The response to salinity greatly differs among various plant species and the levels of stress as well as the environmental condition. The exogenous treatment of Se and

Si under salt stress condition effectively alleviate salt- induced damages. However, Si treatments improved the tolerance of lettuce plants in general.

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