Volume 59(1):25-33, 2015
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

ARTICLE

Response of cucumber (*Cucumis sativus* L.) seedlings to exogenous silicon and salicylic acid under osmotic stress

Seyyed Reza Jafari[1]*, Seyyed Mohammad Javad Arvin[2], Khosrow Manoochehri Kalantari[1]

[1]Department of Biology, Shahid Bahonar University of Kerman, Kerman, Iran,
[2]Department of Horticulture, Shahid Bahonar University of Kerman, Kerman, Iran

ABSTRACT This study investigates the role of 1.5 mM silicon (Si) and 10 µM salicylic acid (SA) singly or in combination, in inducing cucumber seedlings tolerance to osmotic stress (15% PEG). Osmotic stress reduced shoot fresh and dry mass (SFM, SDM), shoot K^+ uptake and leaf area (LA) and increased malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and ion leakage (IL). Under osmotic stress, Si, SA and Si+SA, increased LA, SFM, SDM, relative water content, total phenolic compounds, anthocyanins, flavonoids, shoot K^+ and phenylalanine ammonia lyase (PAL) activity. In all cases the effect of Si+SA was more pronounced. Moreover, Si, SA and Si+SA reduced MDA, H_2O_2, ion leakage, proline and other aldehydes, under osmotic stress. Meanwhile, under osmotic stress, Si or SA improved seedling performance by enhancing antioxidant enzymes activity, but the better performance of the seedlings under osmotic stress treated with Si+SA was not associated with further enhancement of antioxidant enzymes activity. However, Si+SA treatment significantly increased non-enzymatic antioxidants, total phenolic compounds, anthocyanins, flavonoids, and Si, K^+, Ca^{2+} content in shoot and also PAL activity that might have contributed to higher tolerance of seedling to osmotic stress. **Acta Biol Szeged 59(1):25-33 (2015)**

KEY WORDS

antioxidant enzyme
cucumber
osmotic stress
silicon
salicylic acid

Introduction

The water deficit caused by drought adversely affects growth, membrane integrity, pigment content, osmotic adjustment, water relations, and photosynthetic activity. Also, this stress is responsible for an average yield loss of more than 50% in the major crops (Praba et al. 2009).

In plants, drought stress alters many morphological, physiological and metabolic processes such as reduced growth, stomatal closure and photosynthesis inhibition (Mooussa 2011), among others peanut (Celikkool et al. 2010) and chickpea (Sohrabi et al. 2012). Moreover, stomatal closure may induce over-reduction of photosynthetic electron chain and increases production of reactive oxygen species (ROS) such as superoxide anion (O^{2-}) which lead to the formation of H_2O_2, OH^- and other ROS (Iannone et al. 2009). Plant performance under stress conditions depends on the balance between the harmful effect of stress and a wide variety of protective and repair processes (Caldwell et al. 2007). In the absence of effective mechanisms which remove or scavenge free radicals, they can seriously damage plant by lipid peroxidation, protein degradation, breaking of DNA, and cell death (Tian and Li 2006). Plants protect cellular and sub-cellular system from the cytotoxic effects of active oxygen radicals with antioxidative enzymes such as superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), ascorbate-dependent peroxidase (APX, EC 1.11.1.11), and guaiacol-dependent peroxidase (GPX, EC 1.11.1.7) as well as non-enzymatic compounds, ascorbate, glutathione, phenolics, anthocyanins and flavones compounds, which normally maintain ROS balance within the cell (Ashraf et al. 2010).The application of chemicals such as silicon (Si) (Al-Aghabary et al. 2004; Zhu et al. 2004; Liang et al. 2006) and salicylic acid (SA) (Daneshmand et al. 2009; Hayat et al. 2010) can ameliorate the impairing effects of osmotic stress.

Si is recognized as a quasi-essential for the growth of higher plants (Epstein and Bloom 2003). Plants typically absorb bio-available silicon as a silicate –generally known as monosilicic or orthosilicic acid. Silicon is deposited as silica in the plant cell walls, improving cell wall structural rigidity and strength, plant architecture and leaf erectness. Si fertilization may improve yield, disease resistance, and tolerance to stresses such as cold, drought, and toxic metals (Romero-Aranda et al. 2006; Balakhnina and Borkowska 2013; Ma and Yamaji 2008; Zhu et al. 2004). It has been reported that Si applied by external foliar treatments or hydroponic supplementation has beneficial effects on plant growth and plays an important role in tolerance of plants to environmental stresses (Ma and Yamaji 2008). Gunes et al. (2007) reported that Si alleviated sodium and boron toxicity in spinach (*Spinacia*

Submitted Sept 30, 2014; Accepted Febr 28, 2015
*Corresponding author. E-mail: Seyydrezajfr13@gmail.com

oleracea L.) and tomato (*Solanum lycopersicum* L.) plants by reducing oxidative membrane damage. In sorghum (*Sorghum bicolor* Moench), plants grown in pots applied with silicon had higher relative water content and dry materials (Hattori et al. 2005). Reduced oxidative damage due to the addition of Si under saline conditions was also reported in barley (Liang et al. 2003, 2005) and cucumber (Zhu et al. 2004). Si increases the activity of antioxidative enzymes, thus mitigating the stress and improving the plants growth (Al-Aghabary et al. 2004), and/or improving plasma membrane H^+-ATPase activity in plants treated with NaCl (Liang et al. 2006). It is suggested that Si may contribute to affecting membrane lipid composition and maintaining optimal membrane fluidity of plants stressed. Moreover, Si maintains the integrity, stability and functioning of plasma membranes in salt-stressed barley (Liang et al. 2006). In addition, in *Sorghum* the Si application can affect stomatal conductance through modification of plant water status but not through any physical changes (Hattori et al. 2005). The beneficial effects are also attributed to Si deposition in cell walls of leaves, stems and hulls that protect plants against multiple abiotic and biotic stresses (Epstein 2009).

For example, deposition of Si in the culms, leaves and hulls of rice enhances the strength and rigidity of cell walls and decreases transpiration, increasing the resistance to lodging, low and high temperature and drought stresses (Ma and Feng 2006).

Salicylic acid (SA, 2-hydroxybenzoic acid) is a phytohormone which influences a range of diverse processes in plants, including seed germination, ion uptake and transport, membrane permeability, and photosynthesis (Simaeia et al. 2011). Increasing evidence has shown that exogenous SA improves plant adaptation to stresses by various mechanisms such as improved photosynthetic capacity (Arfan et al. 2007), maintaining the stability of membranes and thereby improving the growth of salinity stressed barley plants (El-Tayeb 2005). Effects of SA on plants are concentration dependent, treatment duration, plant species, time of treatment and plant organ examined used for pretreatment (Shi et al. 2009). SA is an important signal involved in the activation of plant defense responses against abiotic and biotic stress and plays a crucial role for modifying plant responses to environmental stressors (El-Tayeb et al. 2005). Application of SA at lower concentration enhanced chlorophyll content and photosynthetic rate (Fariduddin et al. 2003) whereas, at higher concentrations, SA caused a high level of stress in plants (Hayat et al. 2010). It has been reported that SA is able to induce the expression of a gene encoding a novel proline-rich protein which is able to polymerize orthosilicic acid to insoluble silica (Kauss et al. 2003).

Regarding the separate roles of Si and SA in reducing osmotic stress and the role of SA in producing prolin-rich proteins, which polymerize Si to silica, the present study aims at investigating the separate and combined effects of Si and SA on growth parameters, antioxidative defense systems and ion accumulation and the mechanisms involved in cucumber seedlings grown under osmotic stress.

Materials and Methods

Plant material and treatments

Cucumber (*Cucumis sativus* L.) seeds (cv. Sina) were rinsed thoroughly with distilled water, soaked for 4 h in water and then germinated on moist filter paper in an incubator (Memmert Incubator ICP400) at 28 ºC. All experiments were conducts in a greenhouse at 25/18 ºC (day/night), 16 h light/8 h dark photoperiod (30 mol/m^2/s photon flux density-PFD) and 50% relative humidity. Three uniform seedlings were selected and grown hydroponically in 50 ml falcon tubes filled with half-strength Hoagland nutrient solution which had the following composition: 3 mM KNO_3, 0.5 mM $NH_4H_2PO_4$, 1 mM $MgSO_4 \cdot 7H_2O$, 2.5 mM $CaCl_2 \cdot 2H_2O$, 26.2 µM H_3BO_3, 9.15 µM $MnCl_2 \cdot 4H_2O$, 0.73 µM $ZnSO_4$ $7H_2O$, 0.32 µM $CuSO_4 \cdot 5H_2O$, 0.33 µM $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ and 0.0288 µM FeNaEDTA. The nutrient solution was turned over daily. After six days, the seedlings of uniform size were planted into hydroponics plastic pots (one seedling per pot) filled with 700 ml of half-strength Hoagland nutrient solution that was aerated continuously with an air pump. Meanwhile, the solution was also replaced with fresh one every 2 days to prevent nutrient deficiency and the pH of the nutrient solution was adjusted to 6.0 daily using 0.01 mol/l KOH and/or HCl. The seedlings were allowed to grow for 2 weeks. Pretreatment was initiated by adding Si. Sodium metasilicate (Na_2SiO_3) was used as the source of silicon. After 72 hours of pretreatment, the pots were divided into two groups and exposed to osmotic stress. Two factors including chemicals (control, silicon 1.5 mM (Si), salicylic acid 10 µM (SA) and salicylic acid 10 µM + silicon 1.5 mM (Si+SA)) and osmotic stress (0 and 15% PEG) were factorially arranged in randomized complete designs (RCBD) with three replicates. The applied levels of SA and Si used in experiments were based on previous preliminary tests.

All the seedlings were harvested 6 days after treatments with PEG and separated into leaves, stems and roots. The growth parameters (shoot fresh and dry mass and root dry mass) were assessed; the seedlings materials were washed by distilled water, immediately preserved in liquid N_2 and stored at -80 ºC prior to biochemical assays.

Measurement of the growth parameters

Following harvest, the seedlings were divided into shoots and roots. Total leaf area (LA) was measured by a leaf area meter

(portable area meter LI/3000A, LI-COR). Roots and shoots were washed 3 times in distilled water and dried at 70 ºC for 72 h, and their dry weights were determined.

Photosynthetic pigments

The amounts of photosynthetic pigments (chlorophyll a, b, total and carotenoids) were determined according to the method of Lichtenthaler (1987). Fresh leaves (0.25 g) were homogenized in acetone 80%, centrifuged at 10 000 g for 5 min and absorbance was recorded at wavelengths of 646.8 and 663.2 nm for chlorophyll assay and 470 nm for carotenoids assay by a UV/Vis spectrophotometer (Cary 50, Varian, Germany).

Lipid peroxidation

Lipid peroxidation was determined using the thiobarbituric acid (TBA) reaction followed by measurement of malondialdehyde (MDA) content (Heath and Packer 1968). One gram of fresh tissue was ground in 10 ml of 0.5% TBA in 20% (w/v) trichloroacetic acid (TCA). The mixture was incubated at 95 °C in a water bath for 30 min and quickly cooled in an ice bath. Then, the homogenate was centrifuged at 10 000 g for 15 min. The absorbance of the supernatant was read at 532 nm. After substracting the non-specific absorbance at 600 nm, the MDA concentration was calculated using an extinction coefficient of 155/mM/cm.

Hydrogen peroxide (H_2O_2) content

H_2O_2 content was determined using the method given by Velikova et al. (2000). Shoot samples (0.2 g) were extracted with 5 ml of 0.1% TCA and centrifuged at 12 000 g for 15 min. Then 0.5 ml of supernatant was mixed with 0.5 ml of 10 mM phosphate buffer (pH 7.0) and 1 ml of 1 M potassium iodide and the absorbance was determined at 390 nm. The amount of H_2O_2 was calculated using the extinction coefficient 0.28/ mM/cm and expressed as μmol/g FW (fresh weight).

Electrolyte leakage

The electrolyte leakage (EL) was determined as described by Ben Hamed et al. (2007). The shoot samples (0.2 g) were placed in test tubes containing 10 ml of double distilled water. The tubes were incubated in a water bath at 32 ºC for 2 h and the initial electrical conductivity of the medium (EC1) was measured by an EC meter (Metrohm, Switzerland).The samples were autoclaved at 121 ºC for 20 min to release all the electrolytes, cooled at 25 ºC and the final electrical conductivity (EC2) measured. The electrolyte leakage was calculated by using the formula: EL = (EC1/ EC2) ×100.

Enzyme extraction and activity determination

Leaves (500 mg) were homogenized in 50 mM potassium phosphate buffer (pH 7.0) containing 1% soluble PVP, 1 mM ethylenediaminetetraacetic acid (EDTA) and 1 mM phenylmethanesulfonyl fluoride (PMSF) with the addition of 10 mM ascorbic acid in the case of the APX assay. The homogenate was centrifuged at 20 000 g for 20 min and the supernatant was used for determination of enzyme activity. All procedures were performed at a temperature of 4 °C. The supernatant was used for measurement of total soluble protein according to Bradford (1976) and expressed as mg/g FW. Bovine serum albumin was used as standard.

Superoxide dismutase (SOD) (EC 1.15.1.1) activity was determined by method of Giannopolitis and Ries (1977). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM Na-EDTA, 75 μM riboflavin, 13 mM methionine and 0.05 ml the enzyme extract. One unit of SOD was defined as the amount of enzyme required to cause 50% inhibition of the reduction of nitroblue tetrazolium (NBT) as monitored at 560 nm.

Ascorbate peroxidase activity (APX, EC 1.11.1.11) was measured in the presence of 0.5 mM ascorbic acid, 0.15 mM H_2O_2, 0.1 mM EDTA and 50 μl enzyme extract by monitoring the decrease in absorption at 290 nm (Nakano and Asada 1981). Nanomole ascorbate consumed per min was defined as one unit of APX.

Catalase activity (CAT, EC 1.11.1.6) was assayed by following the decomposition of H_2O_2 at 240 nm (Dhindsa et al. 1981). The unit (U) of CAT activity was defined as the amount of enzyme that decomposed 1 mM H_2O_2 per minute per mg protein in 100 μl enzyme extract are given in U/mg of protein.

Total phenolic compounds

Total phenolic content was determined using the Folin-Ciocalteau method Singleton and Rossi (1965) as modified by Velioglu and coworkers (1998). Samples (100 mg) were extracted with 80% methanol containing 1% hydrochloric acid (5 ml) at room temperature on a shaker for 2 hours. The mixture was centrifuged at 3000 g for 10 min. The supernatant was used to determine total phenolics. One hundred microliter of extract was mixed with 0.75 ml of Folin-Ciocalteu reagent (previously diluted tenfold with distilled water) and allowed to stand at 22 °C for 5 min; 0.75 ml of sodium bicarbonate (60 g/l) solution was added to the mixture. After 90 min at 22 °C, absorbance was measured at 725 nm. Gallic acid was used for constructing the standard curve. Results were expressed as mg gallic acid (GA) per gram of the fresh weight.

Table 1. Individual and combined effects of silicon (1.5 mM), salicylic acid (10 µM) and PEG (15%) on leaf area, shoot fresh mass, shoot dry mass, root dry mass and RWC.

	Leaf area (cm^2)		Shoot fresh mass (g/ seedling)		Shoot dry mass (g/ seedling)		Root dry mass (g/ seedling)		RWC (%)	
Treatment	- PEG	+ PEG	- PEG	+ PEG	- PEG	+ PEG	- PEG	+ PEG	- PEG	+ PEG
Control	429 c	229 f	24.93 ab	14.07 d	2.21 bc	1.55 e	0.44 c	0.61 a	88.7 b	71.1 f
Si	459 b	314 e	25.26 ab	17.50 c	2.44 a	2.05 d	0.45 c	0.52 b	89.7 a	76.8 e
SA	481 a	323 e	26.60 a	19.54 c	2.52 a	2.09 cd	0.48 c	0.55 b	86.4 b	81.3 c
Si+SA	475 a	341 d	26.40 a	22.74 b	2.57 a	2.28 b	0.45 c	0.51 b	87.1 b	86.3 b

For each parameter, means with similar letter(s) are not significantly different P<0.05 according to Duncan's multiple range tests.

Table 2. Individual and combined effects of silicon (1.5 mM), salicylic acid (10 µM) and PEG (15%) on lipid peroxidation (MDA), hydrogen peroxide (H_2O_2), proline, ion leakage and other aldehydes.

	Lipid peroxidation (MDA) (nmol/g Fw)		H_2O_2 (µmol/g Fw)		Proline (mg/g Fw)		Ion leakage (%)		Other aldehydes content (nmol/g Fw)	
Treatment	- PEG	+ PEG	- PEG	+ PEG	- PEG	+ PEG	- PEG	+ PEG	- PEG	+ PEG
Control	2.47 d	20.2 a	2.13 e	10.8 a	0.22 e	1.75 a	12.40 d	61.4 a	2.41 c	21.48 a
Si	3.30 d	12.4 b	2.34 e	7.60 b	0.38 de	1.24 b	13.20 fd	42.4 b	2.47 c	8.35 b
SA	3.61 d	11.2 b	3.26 d	7.33 b	0.52 d	0.95 c	13.80 d	37.8 b	2.89 c	9.11 b
Si+SA	3.31 d	7.1 c	2.89 de	6.10 c	0.54 d	0.77 c	13.00 fd	27.4 c	1.92 c	8.25 b

For each parameter, means with similar letter(s) are not significantly different P<0.05 according to Duncan's multiple range tests.

Total flavonoids

One hundred milligrams of samples were extracted with 10 ml 80% aqueous methanol. The mixture was centrifuged at 2000 g for 10 min. Supernatants were used for subsequent analysis. The flavonoid content was measured employing the colorimetric assay described by Zhishen and coworkers (1999). Aliquots of extracts (0.5 ml) were added to 10 ml volumetric flask containing 4.5 ml distilled water. After 5 min, 0.3 ml 5% sodium nitrite and then 0.6 ml of 10% aluminum chloride was added to each aliquot. After 6 min, 2 ml of 1 M sodium hydroxide was added to the mixture following by the addition of 2.1 ml distilled water. Absorbance was recorded at 510 nm, and flavonoid content was expressed as mg of rutin equivalent (RE) per 100 g of fresh weight.

Anthocyanins

Determination of anthocyanin contents was carried out using the method of Wagner (1979). Samples (0.1 g) were soaked in 10 ml acidified methanol (methanol/HCl, 99:1, v/v). The tissues were crushed and kept in the dark at 25 ºC for 24 h. The extracts were then centrifuged at 4 000 g for 5 min at room temperature. The absorption rate of the supernatant was read by spectrophotometer at 550 nm. To calculate the amount of anthocyanins, the extinction coefficient 33,000/ mol/cm was used and anthocyanin content were expressed as µmol/g FW.

Element analysis by inductively coupled plasma atomic emission spectroscopy (ICP-OES)

Samples of root and shoot were oven dried at 70 °C for 72 h and after determination of dry biomass, 0.5 g samples dissolved in 10 ml 65% (w/v) nitric acid (supra pure, Merck). After digestion, the volume of each sample was adjusted to 50 ml using double deionized water. Total concentration of K^+, Na^+ and Si was determined by ICP-OES (720-ES, Varian). The stability of the device was evaluated after determination of every ten samples by examining the internal standard. Reagent blanks were also prepared to detect potential contamination during the digestion and analytical procedure. The samples were analyzed in triplicates. Also, we used standards solutions with a final K^+, Na^+ and Si concentrations in range of seedlings in the analyzed solution for quality control.

Statistical analysis

Data were subjected to two-way ANOVA using the SPSS (Statistical Package for Social Sciences) software. Significant differences between treatment means were determined by

Table 3. Individual and combined effects of silicon (1.5 mM), salicylic acid (10 µM) and PEG (15%) on superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), guaiacol-dependent peroxidase (GPX) and protein content.

	SOD (Unit/mg protein)		APX (Unit/mg protein)		CAT (Unit/mg protein)		GPX (Unit/mg protein)		Protein (mg/g Fw)	
Treatment	- PEG	+ PEG	- PEG	+ PEG	- PEG	+ PEG	- PEG	+ PEG	- PEG	+ PEG
Control	7.70 c	12.67 b	2.10 d	3.02 bc	1.51 cd	1.96 bc	1.88 d	9.20 a	12.6 a	9.96 c
Si	9.20 bc	17.56 a	2.16 d	3.77 ab	1.65 cd	2.25 b	2.07 d	5.47 c	12.9 a	10.53 bc
SA	10.90 bc	17.09 a	2.32 cd	4.28 a	1.33 d	3.06 a	2.43 d	6.91 b	13.1 a	11.05 b
Si+SA	11.73 b	17.44 a	2.24 cd	4.07 a	1.64 cd	3.26 a	1.97 d	4.43 c	13.2 a	11.03 b

For each parameter, means with similar letter(s) are not significantly different P<0.05 according to Duncan's multiple range test.

Table 4. Individual and combined effects of silicon (1.5 mM), salicylic acid (10 µM) and PEG (15%) on K^+, Ca^{2+} and Si concentrations in shoot and root.

	Shoot						Root					
	K^+ (mg/g DW)		Ca^{2+} (mg/g DW)		Si (mg/g DW)		K^+ (mg/g DW)		Ca^{2+} (mg/g DW)		Si (mg/g DW)	
Treatment	- PEG	+ PEG	- PEG	+ PEG	- PEG	+ PEG	- PEG	+ PEG	- PEG	+ PEG	- PEG	+ PEG
Control	40.8 abc	35.3 d	7.85 a	6.59 bc	0.5 e	0.22 e	20.63 c	23.47 bc	2.44 bc	2.94 abc	0.25 c	0.15 c
Si	42.3 abc	39.3 bc	6.56 d	7.14 b	11.2 a	4.07 d	22.56 bc	21.70 c	1.88 cd	3.71 a	7.14 a	4.65 b
SA	40.6 abc	38.3 cd	7.80 a	6.95 bc	0.2 e	0.17 e	20.56 c	22.20 bc	1.44 d	3.34 ab	0.30 c	0.09 c
Si+SA	44.3 a	43.4 ab	7.06 b	7.61 a	8.00 b	5.81 c	27.40 a	25.46 ab	2.74 abc	3.30 ab	4.30 b	3.73 b

For each parameter, means with similar letter(s) are not significantly different P<0.05 according to Duncan's multiple range test.

Duncan's multiple range test (DMRT, 5%) using MSTAT-C.

Results

Growth parameters, leaf relative water contents (RWC), shoot K^+ uptake and leaf protein contents were reduced under osmotic stress (Table 1). Meanwhile, MDA, H_2O_2, proline, ion leakage and other aldehydes (Table 2) and also the enzyme activity of SOD, APX and GPX were increased under osmotic stress but the leaf protein decreased and no significant changes were observed in the activity of CAT (Table 3).

Under *non-stress* conditions, Si and SA similarly improved growth parameters, leaf RWC, shoot K^+ uptake and leaf protein content and the effect of combined Si+SA was not superior to individuals effects (Table 1, 3 and 4). Moreover, no significant change was observed in enzyme activity (Table 3).

Under osmotic stress, Si and SA were highly effective in reducing negative effect of stress and the combined effect of Si+ SA was more pronounced. Relative to corresponding control, and under osmotic stress Si, SA and Si+SA, increased LA (37%, 41% and 48%), SFM (25%, 39% and 62%), SDM (32%, 34% and 47%), RWC (8%, 14% and 21%), total phenolic compounds (20%, 23% and 58%), anthocyanins (15%, 20% and 49%), flavonoids (12%, 10% and 30%), shoot K^+ (11%, 8% and 23%) and PAL activity (22%, 25% and 57%), respectively (Table 1, 2, 3 and 4). Moreover, Si, SA and Si+SA reduced MDA (50%, 43% and 61%), H_2O_2 (33%, 32% and 50%), LA (38%, 36% and 50%), proline (30%, 51% and 74%) and other aldehydes (44%, 35% and 60%) under osmotic stress (Table 2). However, under osmotic stress, Si or SA significantly increased the activity of antioxidant enzymes (Table 3) but the better seedling growth performance (LA, SFM, SDM and RWC) and reduced MDA, H_2O_2 and IL in seedlings treated with Si+SA under osmotic stress was not associated with further enhancement of the enzyme activity. However, shoot K^+ and Si, total phenolic compounds, anthocyanins, total flavonoids and PAL activity were significantly improved with Si+SA that may have contributed toward better performance.

Discussion

The present study indicated that the cucumber growth was significantly affected by osmotic stress. Similar drought-induced reduction in growth attributes have been reported in a number of crop plants including *Populus cathayana* L. (Yang et al. 2009) and *Olea europaea* L. (Ennajeh et al. 2009). Osmotic stress causes oxidative stress, because an imbalance between ROS and their removal makes macromolecules and

Table 5. Individual and combined effects of silicon (1.5 mM), salicylic acid (10 µM) and PEG (15%) on total phenolic, total flavonoid and anthocyanin contents of shoot.

	PAL (Unit/mg protein)		Total phenolic (mg GA/g fw)		Total flavonoid (mg Ru/100 g fw)		Anthocyanin (µmol/g)	
Treatment	- PEG	+ PEG	- PEG	+ PEG	- PEG	+ PEG	- PEG	+ PEG
Control	3.1 d	4.2 bc	1.20 de	1.14 cd	32 d	31 cd	7.23 c	7.65 c
Si	3.9 c	4.3 bc	1.31 cd	1.54 bc	37 cd	38 b	7.69 c	8.97 bc
SA	3.7 c	4.4 b	1.45 c	1.72 bc	36 d	44 bc	8.12 c	9.11 bc
Si+SA	4.1bc	5.8 a	1.38 cd	2.03 a	35 d	54 a	8.45 bc	11.7 a

For each parameter, means with similar letter(s) are not significantly different P<0.05 according to Duncan's multiple range test.

membranes damaged, thus lead to the reduction of plant growth grown under osmotic stress (Ennajeh et al. 2009).

Under *non-stress* conditions, Si, SA or Si+SA improved seedling performance rather similarly. Although Si has not been generally recognized as an essential nutrient for higher seedlings, its beneficial effects on growth and development have been demonstrated (Epstein 2009). Improved performance of plant growth with Si in soybean (Hamayun et al. 2010) and wheat (Zhu et al. 2004) under *non-stress* conditions has been reported. Meanwhile, it is notable to state that Si treatment in soybean plant increased GA_1 and GA_4 hormones that play a pivotal role in the growth and development of plants, when added to control or stressed plants (Hamayun et al. 2010). In present study, improved seedling performance under Si treatment was associated with increased RWC under *non-stress* conditions. Silicon increased extensibility of young elongating cell walls which might be brought about by a silicon-induced decrease in thickness of the primary cell wall itself (Hossain et al. 2002), the physiological mechanism of which is unknown. The better performance of seedling growth treated with SA under *non-stress* conditions in the present study was associated with increased LA and SDM which is in line with the previous findings in wheat (Shakirova et al. 2003), maize (Gunes et al. 2007), and chamomile (Kováčik et al. 2009). The better growth of wheat seedlings treated with SA was related to increases in indole acetic acid and cytokinine (Shakirova et al. 2003). The effect of exogenous SA on growth depends on the plant species, developmental stage, and the SA concentrations tested.

Under osmotic stress, exogenous Si counteracted the PEG-induced oxidative stress in present study and improved performance of seedling growth and RWC. Similarly, increased seedling dry mass in maize (Kaya et al. 2006), wheat (Gong et al. 2003) and soybean (Hamayun et al. 2010) and improved water use efficiency in sorghum (Eneji et al. 2005), and tomato (Romero-Aranda et al. 2006) plants subjected to osmotic or salt stress has been reported when treated with silicon.

The improved performance of seedlings treated with Si under osmotic stress was associated with increased antioxidant activity (SOD, APX and CAT) which is in line with previous findings in wheat (Gong et al. 2003). Added Si decreased the permeability of plasma membrane of leaf cells (Liang et al. 2006), and significantly improved the ultrastructure of chloroplasts which were badly damaged by the added NaCl with double membranes disappearing and the grannae being disintegrated in the absence of Si (Liang 1998).

In present study, Si treatment prevented the increase of MDA and H_2O_2 contents and ion leakage, thereby alleviating the damage normally caused by osmotic stress. Moreover, Si increased K^+ uptake in shoot and root. Similar results were reported in tomato (Al-Aghabary et al. 2004) and sugarcane (Ashraf et al. 2009) treated with Si under salt stress. It has been reported that efficacy of Si in maintaining membrane integrity may be indirect and lie in part in its effect on increasing K^+ concentrations in tissues and cells of salt stressed barley plants (Liang et al. 2003) and also increased the activity of plasma membrane H^+-ATPase. Moreover, in present study Si increased phenolics, anthocyanins, flavones compounds and also PAL activity which is the first key enzyme in phenylpropanoid biosynthesis and known to be linked with enhanced phenylpropanoid biosynthesis (Gomez- Vasquez et al 2004).

Silicon application significantly increased phenolic components in rose (Shetty et al. 2011) and cucumber (Fawe et al. 1998) under biotic stress and in maize (Kidd et al. 2001) under Al toxicity. Furthermore, Si increased the level of phenolic components, flavonoids and anthocyanins which act, as stated by Winkel-Shirley (2002), as terminators of free radical chains and as cheaters of redox-active metal ions, capable of catalyzing lipid peroxidation, by terminating the Fenton reaction (Jung et al. 2003).

The present study showed that SA improved seedling performance under osmotic stress which was associated with enhanced levels of antioxidant enzymes activity leading to increased K^+ and Ca^{2+} uptake. Similar results were reported by Kaydan and coworkers (2007) in wheat.

Moreover, the present study revealed that the combined effect of Si+SA was more effective than the individual effects in improving seedling performance under osmotic stress.

However, the better performance of seedlings with Si+SA was not associated with further enhancing the activity of antioxidant enzymes, though significant higher shoot K^+ and Si contents were observed with Si+SA.

Nevertheless, Si+SA combination further increased non-enzymatic antioxidants including phenolics, anthocyanins and flavones compounds synthesized in the cytosol which might protect cells from oxidative damage. Chérif et al. (1994) reported that silicon had no effect on phenolic concentrations of plants in the absence of pathogen infection. In the other words, most of the research on SA has focused on its role in the local and systemic response against microbial pathogens, and on defining the transduction pathway leading to gene expression induced by SA. Probably, in the current study, application of SA amplified Si role in improved seedling performance under osmotic stress. Moreover, combined Si+SA may increase formation of silica in silicon low concentrations because it was reported that during the induction of SAR by SA in cucumber, the expression of a gene encoding a novel proline-rich protein was enhanced. The C-terminus of this protein contained a high density of lysine and arginine residues proposed to catalyse the localized deposition of silica at the site of vulnerability. This insoluble silica, in turn, reduces the damaging effects of osmotic stress (Kauss et al. 2003). In present study and under osmotic conditions, the shoot Si content was increased in seedlings treated with SA. This could explain the higher Si concentration observed on osmotic cucumber seedlings treated with SA and the better performance of seedlings treated with Si+SA. There are limited reports on the combined effects of Si+SA on plants. In spinach, combined effect of Si+SA was not more effective that individual effects (Eraslan et al. 2008). It is worth to mention that spinach is not a silica accumulator and was exposed to very mild osmotic stress. In maize (Mohsenzadeh et al. 2011) a silica accumulation species and in potato (Arvin et al. 2014), the combined effect of Si+SA was more effective in reducing the damaging effect of cadmium, compared with individual effects.

As a conclusion, the results of present study highlighted the role of Si and SA in regulating the osmotic responses by enhancing the activity of enzymatic and non-enzymatic antioxidants and the synergistic effect of combined Si+SA was partly because of the enhanced non-enzymatic antioxidants along with an increase accumulation of Si in shoot which strengthened cell wall structure.

References

Al-Aghabary K, Zhu Z, Shi Q (2004) Influence of silicon supply on chlorophyll content, chlorophyll fluorescence, and antioxidative enzyme activities in tomato plants under salt stress. J Plant Nutr 27(12):2101-2115.

Arfan M, Athar H, Ashraf M (2007) Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress? J Plant Physiol 164:685-694.

Arvin MJ, Moslemi K, Sarchashmepur M, Karamat B, Olumi H, Daneshmand F (2014) Interactive effects of salicylic acid and silicon on growth, development and cadmium accumulation in tuber of potato plants under cadmium stress. JCPP 4(11):185-196.

Ashraf MA, Ashraf M, Ali Q (2010) Response of two generally diverse wheat cultivars to salt stress at different growth stages: leaf lipid peroxidation and phenolic contents. Pak J Bot 42(1):559-565.

Balakhnina T, Borkowska A (2013) Effects of silicon on plant resistance to environmental stresses: review. Int Agrophys 27:225-232.

Ben Hamed K, Castagna A, Elkahoui S, Ranieri A, Abdelly C (2007) Sea fennel (*Crithmum maritimum* L.) under salinity conditions: a comparison of leaf and root antioxidant responses. Plant Growth Regul 53:185-194.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantites of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248-254.

Caldwell M, Bornmam J, Ballare C, Fint S, Kulandavelu G (2007) Terrestrial ecosystems, increased solar ultraviolet radiation, and interactions with other climate changes factors. Photochem Photobiol 6:252-266.

Celikkol A, Ercan O, Kavas M, Yildiz L (2010) Drought-induced oxidative damage and antioxidant responses in peanut (*Arachis hypogaea* L.) seedlings. Plant Growth Regul 61:21-28.

Chérif M, Asselin A, Belanger RR (1994) Defense responses induced by soluble silicon in cucumber roots infected by *Pythium* spp. Phytopathology 84:236-242.

Daneshmand F, Arvin MJ, Kalantari KM (2009) Effect of acetylsalicylic acid (Aspirin) on salt and osmotic stress tolerance in *Solanum bulbocastanum* in vitro: enzymatic antioxidants. Am-Euras J Agric Environ Sci 6(1):92-99.

Dhinds RS, Dhindsa P, Thorpe AT (1981) Leaf senescence correlated with increased levels of membrane permeability and lipid peroxidation and decrease levels of superoxide dismutase and catalase. J Exp Bot 32:93-101.

El-Tayeb MA (2005) Response of barley grains to the interactive effect of salinity and salicylic acid. Plant Growth Regul 45:215-224.

Eneji E, Inanaga S, Muranaka S, Li J, An P, Hattori T (2005) Effect of calcium silicate on growth and dry matter yield of *Chloris gayana* and *Sorghum sudanense* under two soil water regimes. Grass Forage Sci 60:393-398.

Ennajeh M, Mohamed A, Khemira H (2009) Osmoregulation and osmoprotection in the leaf cells of two olive

cultivars subjected to severe water deficit. Acta Physiol Plant 31:711-721.

Epstein E (2009) Silicon: its manifold roles in plants. Ann Appl Biol 155:155-160.

Eraslan F, Inal A, Pilbeam DJ, Gunes A (2008) Interactive effects of salicylic acid and silicon on oxidative damage and antioxidant activity in spinach (*Spinacia oleracea* L. cv. Matador) grown under boron toxicity and salinity. Plant Growth Regul 55:207–219.

Fariduddin Q, Hayat S, Ahmad A (2003) Salicylic acid influences net photosynthetic rate, carboxylation efficiency, nitrate reductase activity and seed yield in *Brassica juncea*. Photosynthetica 41:281-284.

Fawe A, Abou-Zaid M, Menzies JG, Bélanger RR (1998) Silicon-mediated accumulation of flavonoid phytoalexins in cucumber. Biochem Cell Biol 88:396-401.

Giannopolitis C, Ries S (1977) Superoxide dismutase. I. Occurrence in higher plants. Plant Physiol 59:309-314.

Gomez-Vasquez R, Day R, Buschmann H, Randles S (2004) Phenylpropanoids, phenylalanine ammonia lyase and peroxidases in elicitor-challenged cassava (*Manihot esculenta*) suspension cells and leaves. Ann Bot 94:87-97.

Gong H, Chen K, Chen G, Wang S, Zhang C (2003) Effects of silicon on growth of wheat under drought. J Plant Nutr 26:1055-1063.

Gunes A, Inala A, Bagcia EG, Coban S, Pilbeam DJ (2007) Silicon mediates changes to some physiological and enzymatic parameters symptomatic for oxidative stress in spinach (*Spinacia oleracea* L.) grown under B toxicity. Sci Hortic 113:113-119.

Hamayun M, Sohan Sohan EY, Khan SA, Shinwari ZK (2010) Silicon alleviates the adverse effects of salinity and drought stress on growth and endogenous plant growth hormones of soybean (*Glycine max* L.). Pak J Bot 42(3):1713-1722.

Hattori T, Inanagaa S, Arakib H, Ana P, Moritac S, Luxova G (2005) Application of silicon enhanced drought tolerance in *Sorghum bicolor*. Physiol Plant 123:459-466.

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

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125:189-198.

Hossain M, Hossain MT, Mori R, Soga K, Wakabayashi K, Yamamoto R, Hoson T (2002) Silicon stimulates oat leaf growth by modifying cell wall properties. Proceedings of the Second Silicon in Agriculture Conference August 22–26, Tsuruoka, Yamagata, Japan 121-124.

Iannone MF, Rosales EP, Groppa MD, Benavides MP (2009) Reactive oxygen species formation and cell death in catalase-deficient tobacco leaf disks exposed to cadmium. Protoplasma 10:20-28.

Kauss H, Seehaus K, Franke R, Gilbert S, Dietrich RA, Kro N (2003) Silica deposition by a strongly cationic proline-rich protein from systemically resistant cucumber plants. Plant J 33:87-95.

Kaya C, Tuna L, Higgs D (2006) Effect of silicon on plant growth and mineral nutrition of maize grown under water-stress conditions. J Plant Nutr 29:1469-1480.

Kaydan D, Ya mur M, Okut N (2007) Effects of salicylic acid on the growth and some physiological characters in salt stressed wheat (*Triticum aestivum* L) Tar Bil Derg 13 (2):114-119.

Kidd PS, Llugany M, Poschenrieder C, Gunse B, Barcelo J (2001) The role of root exudates in aluminum resistance and silicon-induced amelioration of aluminium toxicity in the three varieties of maize (*Zea mays* L.). J Exp Bot 52(359):1339-1352.

Kováčik J, Klejdus B, Ba kor M (2009) Nitric oxide signals ROS scavenger-mediated enhancement of PAL activity in nitrogen-deficient *Matricaria chamomilla* roots: side effects of scavengers. Free Rad Bio Med 46:1686-1693.

Liang Y (1998) Effect of silicon on leaves ultra-structure, chlorophyll content and photosynthetic activity of barley under salt stress. Pedosphere 8(4):289-296.

Liang Y, Chen Q, Liu Q, Zha W (2003) Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (*Hordeum vulgare* L.). J Plant Physiol 60:1157-1164.

Liang Y, Sun M, Zhu YG, Christie P (2007) Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: A review. Environ Pollut 147:422-428.

Liang Y, Zhang W, Chenc Q, Ding R (2005) Effects of silicon on H^+-ATPase and H^+-PPase activity, fatty acid composition and fluidity of tonoplast vesicles from roots of salt-stressed barley (*Hordeum vulgare* L.). Environ Exp Bot 53:29-37.

Liang Y, Zhang W, Qin C, Liu Y, Ding R (2006) Effect of exogenous silicon (Si) on H^+-ATPase activity, phospholipids and fluidity of plasma membrane in leaves of salt-stressed barley (*Hordeum vulgare* L.). Environ Exp Bot 57:212-219.

Lichtenthaler H (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol 148:350-382.

Ma JF, Feng J (2006) Silicon uptake and accumulation in higher plants. Trends Plant Sci 11(8):392-397.

Ma J, Yamaji N (2008) Functions and transport of silicon in plants. Cell Mol Life Sci 65:3049 - 3057.

Mohsenzadeh S, Shahrtash M, Mohabatkar H (2011) Interactive effects of salicylic acid and silicon on some physiological responses of cadmium-stressed maize seedlings. IJST A1:57-60.

Mooussa HR (2011) Low dose of gamma irradiation enhanced drought tolerance in soybean. Bulg J Agric Sci

17(1):63-72.
Nakano Y, Asada K (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplast. Plant Cell Physiol 22:867-880.
Praba M, Cairns J, Babu R, Lafitte H (2009) Identification of physiological traits underlying cultivar differences in drought tolerance in rice and wheat. J Agron Crop Sci 195:30-46.
Romero-Aranda MR, Jurado O, Cuartero J (2006) Silicon alleviates the deleterious salt effect on tomato plant growth by improving plant water status. J Plant Physiol 163:847-855.
Shakirova MF, Sakhabutdinova AR, Bezrukova MV, Fatkhutdinova RA, Fatkhutdinova DR (2003) Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. Plant Sci 164:317-322.
Shi GR, Cai QS, Liu QQ, Wu L (2009) Salicylic acid-mediated alleviation of cadmium toxicity in hemp plants in relation to cadmium uptake, photosynthesis, and antioxidant enzymes. Acta Physiol Plant 31:969-977.
Simaeia M, Khavarinejada RA, Saadatmanda S, Bernardb F, Fahimia H (2011) Interactive effects of salicylic acid and nitric oxide on soybean plants under NaCl salinity. Russ J Plant Physiol 58(5):783-790.
Sohrabi Y, Heidari G, Weisany W, Golezani K, Mohammadi K (2012) Some physiological responses of chickpea (*Cicer aritinum* L.) cultivars to arbuscular mycorrhiza under drought stress. Russ J Plant Physiol 59(6):708-716.
Tian X, Li Y (2006) Nitric oxide treatment alleviates drought stress in wheat seedlings. Biol Plant 50(4):775-778.
Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Plant Sci 151:59-66.
Velioglu YS, Mazza G, Gao L, Oomah BD (1998) Antioxidant activity and total phenolics in selected fruits, vegetables, and grainproducts. J Agric Food Chem 46:4113-4117.
Wagner GJ (1979) Content and vacuole/extra vacuole distribution of neutral sugars free amino acids, and anthocyanins in protoplast. Plant Physiol 64:88-93.
Winkel-Shirley B (2002) Biosynthesis of flavonoids and effects of stress. Curr Opin Plant Biol 5:218-223.
Yang F, Xu X, Xiao X, Li C (2009) Responses to drought stress in two poplar species originating from different altitudes. Bio Plant 53(3):511-516.
Zhishen J, Mengcheng T, Jianming W (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 64(5):555-559.
Zhu Z, Wei G, Li J, Qian Q, Yu J (2004) Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (*Cucumis sativus* L.). Plant Sci 167:527-533.