TÜBİTAK

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2015) 39: 408-419

doi:10.3906/bot-1406-84

Foliar spray of phyto-extracts supplemented with silicon: an efficacious strategy to alleviate the salinity-induced deleterious effects in pea (*Pisum sativum* L.)

Muhammad Adnan SHAHID[1,*], Rashad Mukhtar BALAL[1], Muhammad Aslam PERVEZ[2], Tahira ABBAS[1], Muhammad Anjum AQEEL[3], Muhammad Mansoor JAVAID[4], Francisco GARCIA-SANCHEZ[5]

[1]Department of Horticulture, University College of Agriculture, University of Sargodha, Punjab, Pakistan
[2]Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Punjab, Pakistan
[3]Department of Entomology, University College of Agriculture, University of Sargodha, Punjab, Pakistan
[4]Department of Agronomy, University College of Agriculture, University of Sargodha, Punjab, Pakistan
[5]Department of Plant Nutrition, Center for Soil Science and Applied Biology of Segura, Spanish National Research Council, Espinardo, Murcia, Spain

Received: 29.06.2014 • **Accepted:** 03.11.2014 • **Published Online:** 04.05.2015 • **Printed:** 29.05.2015

Abstract: A pot culture study was conducted to assess the ameliorative effect of silicon, *Melia azadirachta* leaf extract, and sugar beet root extract, each applied individually or in different combinations, on salinity-induced detrimental effects in pea (*Pisum sativum* L.). Salinity markedly inhibited the growth, various gas exchange attributes, total phenol contents, membrane stability index, and productivity. On the other hand, lipid peroxidation, electrolyte leakage, H_2O_2 content, antioxidant activities, and leaf free proline and glycinebetaine contents were significantly enhanced by salinity. However, exogenously applied Si and phyto-extracts markedly alleviated the salinity-induced drastic effects on growth, gas exchange attributes, and productivity. Both phyto-extracts supplemented with silicon gave highly salinity mitigating effects by markedly improving growth, gas exchange attributes, enzymatic activities, osmolytes, and yield. The phyto-extracts and Si suppressed lipid peroxidation, electrolyte leakage, and H_2O_2 content by strengthening the enzymatic and nonenzymatic (proline and glycinebetaine) antioxidant defense system. The phyto-extracts and Si application also checked the root/leaf sodium and chloride contents, but improved the silicon contents. Thus, it can be concluded that exogenous application of silicon in combination with phyto-extracts of *M. azadirachta* and sugar beet is a highly effective ameliorative approach to alleviate salinity-induced hazardous effects in plants, especially in pea, grown under a saline regime.

Key words: Salinity, silicon, *Melia azadirachta indica*, sugar beet, gas exchange, antioxidants

1. Introduction

Abiotic stresses drastically affect crops by minimizing yield. Plants face many distinctive abiotic stresses at different stages of plant growth and development. Among these stresses, salinity is prejudicious, limiting plant growth and productivity. Salt stress causes various deleterious effects on morphological, physiological, biochemical, and nutritional attributes. The formation of reactive oxygen species (ROS) is the significant consequence of salt stress. The major ROS comprise hydroxyl ($\cdot OH$), superoxide ($O_2^{\cdot-}$), and hydrogen peroxide (H_2O_2). The high ratios of ROS are responsible for lipid peroxidation (LPO) of tissues and cause deterioration of proteins, green pigments, and DNA (Schutzendubel and Polle, 2002). However, nature has equipped all vegetations with a defensive antioxidant system to counter the oxidative damage caused by ROS (Apel and Hirt, 2004). Plant growth under saline conditions is highly associated with its antioxidant activities, i.e. superoxide peroxidase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and glutathione reductase (GTR) activities. High antioxidant activities will inhibit LPO by eliminating the ROS.

Silicon (Si) is an excellent growth promoting agent. Silicon is reported to increase plant growth and stimulate productivity in various crop plants. Si application strengthened the plant biomass, height, and productivity under different stressed conditions (Ahmad et al., 2007). It also triggers growth by providing strength and extensibility to plant cells. It strengthens the endoderm of the mature basal cells and improves the extensibility of apical cells of the roots; thus Si contributes to a strong, extensive, and deep root system. However, it is also a vital element that has an excellent tolerance enhancing potential against abiotic

* Correspondence: dr.mas@uos.edu.pk

stresses, i.e. salinity, cold, drought, heavy metals, and disease (Mateos-Naranjo et al., 2013). It contributes highly in efficient water utilization of plants by improving the leaf water potential, transpirational rate, and photosynthesis under abiotic stressed conditions (Shen et al., 2010). It is also highly associated with osmotic adjustment and inhibition of ROS, because it accelerates the accumulation of various organic and inorganic osmolytes like proline and glycinebetaine (GB), and antioxidant activities in plants subjected to stressed environments (Ahmad and Haddad, 2011).

Plants are the natural source of various bioactive compounds and secondary metabolites like nitriles, flavonoids, alkaloids, and phenolic compounds that act as antifungal, insecticidal, herbicidal, and antioxidant agents (Ahmed et al., 2012). *Melia azedarchta* (MA) (L.) comprises various bioactives of agriculture use (Rao et al., 2012). MA is a plant of high medicinal importance due to high antioxidant (Nahak and Sahu, 2010), insecticidal (Rachokarn et al., 2008), and antifungal properties (Neycee et al., 2012). Its extracts have been successfully applied against various fungal diseases, insect pests, and ROS in animals but no work has been done regarding its role against plant abiotic stresses. Similarly, sugar beet (*Beta vulgaris* L.) roots contain high amounts of sucrose and are enriched with various compounds like GB and ferulic acid (Abbas et al., 2010), which can improve agricultural production. GB is a naturally occurring amino acid, present in many microorganisms, plants, and animals (Zwart et al., 2003; Makela, 2004). Physiologically, GB is involved in osmoregulation and maintains the performance of photosystem II protein complexes by protecting regulatory extrinsic proteins against denaturation. It protects these macromolecules during abiotic stresses, thus attaining the name of osmoprotectants. Plants subjected to abiotic stresses like salinity and drought accumulate high GB contents in their chloroplasts, which is the indication of osmotic adjustment potential, and resultantly improved plant growth under stressed conditions. Synthetic pure GB has been extensively used to alleviate the drastic effects of abiotic stresses (Khan et al., 2012). However, there are few reports in which direct plant-extract-based (natural source) GB has been used. Likewise, ferulic acid present in sugar beet has high antioxidant action.

The phyto-extracts of various plant species including MA and sugar beet contain various nutrients and organic and inorganic compounds such as potassium, calcium, sulfur, magnesium, phosphorus, vitamins, and amino acids. Therefore, it is thought that phyto-extracts can give more efficient effects as compared to the synthetically manufactured chemicals. Generation of ROS is the main consequence of salt stress; therefore, in this study we evaluated the ameliorating role of Si in combination with MA and sugar beet extract against ROS. MA leaf-extract (MAE) and sugar beet root-extract (SBE) have highly antioxidant and osmotic adjustment potential and so we hypothesized that exogenous application of Si in combination with phyto-extracts of MA leaves and sugar beet roots could effectively mitigate salinity-induced detrimental effects on growth and productivity of *Pisum sativum* by altering antioxidant activities. Thus, the two main objective of this investigation were to examine: (i) whether or not foliar application of three additives (Si, MAE, SBE) alone or in any combination with the other two additives could improve salinity tolerance ability of *Pisum sativum*, and (ii) whether exogenous application of phyto-extracts supplemented with a potential nutrient, Si, can better improve growth and the principal physiological mechanism associated with salt-tolerance than their application without Si supplementation.

2. Materials and methods

The seeds of pea (*Pisum sativum* L. cv. Olympia) were obtained from the Vegetable Research Section, Ayyub Agriculture Research Institute, Faisalabad, Pakistan. Healthy and vigorous seeds of uniform size were disinfected with sodium hypochlorite solution, and rinsed five times with double distilled water. The sterilized seeds were sown in plastic pots having fine sand as growth medium. The sand had pH of 6.0–6.5, with field capacity 7.09%, and incipient wilting at 1.22% (volume basis). There were five pots in each replication (total 25 pots per treatment) and 12 seeds were sown in each pot, but after the emergence of the first true leaves (15 days after germination), the number of plants per pot was adjusted to seven, by thinning out weak and less vigorous ones. Salinity treatments were applied at 30 days after germination (DAG). The plants were supplied with half-strength Hoagland solution as nutritional source. Overall, approximately 400 mL of distilled water having Hoagland solution (30 mL per liter of distilled water) was applied to each pot on alternate days. Si, MA, SB, and ethanol extract were used in various combinations (Si, MA, SBE, Si + MA, Si + SBE, MA + SBE, Si + MA + SBE) for foliar spray. First, an optimization experiment was carried out in plastic pots, and the best doses of Si, MA, and SB extract were identified on root length, shoot length, and plant fresh and dry biomass basis under saline conditions (data not shown). Potassium silicate (M. wt. = 154.28) of Sigma-Aldrich, Japan, was used as Si source. The optimized doses of Si (150 mg L^{-1}), MA (6.5 g L^{-1}), and sugar beet (3.5 g L^{-1}) were applied as foliar spray (each as alone, combination of two, and combination of three) at 5 and 10 days after salinity induction. Regarding the NaCl (salinity) level, concentrations above 8 dS m^{-1} proved highly drastic at the true leaf-stage; therefore the concentration (6 dS m^{-1}) below the lethal level was used in the study. The

pots were placed in a growth room adjusted to 26/16 °C day/night, relative humidity 85%, light intensity 62,200 lux from fluorescent tubes. Physiological and antioxidant aspects were evaluated 15 days after foliar spray, while morphological, plant biomass, and yield related attributes were measured at the end of the experiment (90 DAG).

2.1. Extraction

Fresh mature leaves of MA were harvested from a 10-year-old tree. The leaves were washed with distilled water and dried under shade. The dried leaves were powdered with an electric grinder (Moulinex, AR1044, Tokyo, Japan) and powdery material was extracted in ethanol through a Soxhlet apparatus (Sigma-Aldrich, Aldrich-Z556203, Tokyo, Japan) at 58 °C for 18 h. The ethanol extract thus obtained was kept in vacuum desiccators for complete separation of solvent, and to get extract in solid form. Similarly, pulp of locally procured sugar beet roots was prepared by an electric grinder (Moulinex, AR1044, Tokyo, Japan), and ethanol extract was taken by a Soxhlet apparatus.

2.2. Plant growth and yield attributes

The plant growth and yield related attributes, i.e. plant fresh and dry biomass, leaf area, number of pods per plant, number of seeds per pod, and 1000 seed weight, were recorded at the end of the experiment (90 DAG). Leaf area was evaluated by using a leaf area meter (Ll-3100; LICOR, Inc., Lincoln, NE, USA). Two plants per pot were selected for leaf area measurement and nine leaves from the two plants (3 + 3 + 3 from the top, middle, and basal portions, respectively) were used. However, for the estimation of fresh plant biomass (root and shoot fresh weight), two plants per pot (excluding pods) were harvested, washed, dried with filter paper, and then cut into separate root and shoot portions. The fresh weight of roots and shoots was measured individually by a digital balance. After that, both roots and shoots were placed in an oven (Memmert-110, Schawabach, Germany) at 65 °C for 1 week to record the dry biomass. The remaining 15 plants per replication (three from each pot) were used to evaluate yield attributes.

2.3. Photosynthesis, chlorophyll, and gas exchange attributes

For the measurement of photosynthetic activity (Pn), stomatal conductance (gs), transpiration rate (E), and water use efficiency (WUE), three young, fully developed, and healthy leaves per plant (three plants from each pot) were selected and placed individually in the chamber of a portable Infrared Gas Analyzer (IRGA) (Analytical Development Company, Hoddesdon, UK). All measurements were taken at times between 1000 to 1200 with the following conditions: molar air flow per unit leaf area 375.1 mmol m^{-2} s^{-1}; atmospheric pressure 95.2 kPa; water vapor pressure in the chamber ranging from 6.1 to 7.9 mbar; photosynthetic active radiation (PAR) at the leaf surface maximum 1587 μmol m^{-2} s^{-1}; leaf temperature ranging from 26.3 to 29.4 °C; ambient temperature ranging from 20.9 to 25.7 °C; ambient CO_2 concentration 380 ppm. However, the numbers of stomata was counted by separating a very thin abaxial layer (2 × 2 mm) from the lower epidermis of the leaf. This dry film was carefully separated, placed on a microscope slide under a coverslip, adjusted on the stage of a Nikon EFD-3 microscope (F-601, Type-104, Tokyo, Japan) and observed at various magnifications. The numbers of stomata were counted under a magnification of 40 × 10 on the full screen. The lengths and widths of stomata were measured in microns (Moya et al., 2003). The chlorophyll contents were estimated by the protocol of Arnon (1949).

2.4. Lipid peroxidation, membrane stability index, electrolyte leakage, total phenolic content, and H_2O_2

LPO was estimated by measuring the concentration of malondialdehyde (MDA) and thiobarbituric acid (TBA) as described by Heath and Packer (1968). Total phenolics were determined by the protocol of Julkenen-Titto (1985). For the estimation of membrane stability index (MSI), plant material (leaf 250 mg per tube) was taken in test tubes filled with 10 cm^3 of double distilled water. One set of these test tubes was heated at 40 °C for 30 min in a water bath, and the electrical conductivity (Ec) of the solution was recorded on a conductivity bridge (C1) (Yellow Springs, YSI-31, OH, USA). The second set of test tubes was heated at 100 °C on a boiling water bath for 10 min, and conductivity was measured on a conductivity bridge (C2). Finally, MSI was calculated by the formula

$$MSI = [1-(C1/C2)] \times 100$$

The total inorganic ions leaked out in the leaves were estimated by the method of Sullivan and Ross (1979). Twenty leaf discs (four from each per pot) were put in a test tube containing boiling deionized water (10 mL) and electrical conductivity was measured (EcA). After that, the material was subjected to temperatures of 45 °C and 55 °C for 30 min each in a water bath and electrical conductivity (EcB) was recorded. The material within the test tubes was again boiled at 100 °C for 10 min and electrical conductivity was noted (EcC). The electrolyte leakage was measured by using the formula

$$\text{Electrolyte leakage (\%)} = \frac{\text{EcB-EcA}}{\text{EcC}} \times 100$$

Estimation of H_2O_2 content was performed according to Liu et al. (2010).

2.5. Antioxidant enzymatic activities

For estimating antioxidant enzyme activities, fresh leaves (0.5 g) were ground in an ice-cooled tissue grinder in 5 mL of 50 mM cooled phosphate buffer (pH 7.8). The

homogeneous mixture was centrifuged at 15,000 × *g* for 20 min at 4 °C. The supernatant was used to determine the activities of the following enzymes. SOD activity was analyzed by calculating its potential to hinder the photoreduction of nitrobluetetrazolium (NBT), as described by Giannopolitis and Ries (1977). CAT and POD activities were measured by the method of Chance and Maehly (1955) with some modification as follows. The CAT reaction solution (3 mL) was composed of 50 mM phosphate buffer (pH 7.0), 5.9 mM H_2O_2, and 0.1 mL of enzyme extract. Changes in the absorbance of the reaction solution at 240 nm were recorded every 20 s. One unit of CAT activity was defined as an absorbance change of 0.01 units min^{-1}. The POD reaction solution (3 mL) was composed of 50 mM phosphate buffer (pH 5.0), 20 mM guaiacol, 40 mM H_2O_2, and 0.1 mL of enzyme extract. Changes in the absorbance of the reaction solution at 470 nm were recorded every 20 s. One unit of POD activity was defined as an absorbance change of 0.01 units per min. The activity of each enzyme was expressed on the basis of protein content. APX activity was estimated by the method of Nakano and Asada (1981). GPX activity was estimated by the method of Urbanek et al. (1991). GTR was measured by the procedure described by Smith et al. (1988).

2.6. Determination of proline, glycinebetaine, Na, Cl, and Si

The free proline contents of leaves were estimated by the method of Bates et al. (1973). The glycinebetaine content was determined by the method of Grieve and Grattan (1983). For the determination of sodium, the dried root and leaf samples were digested. Then these digested samples were diluted up to 50 mL in a volumetric flask and filtered. Sodium was determined from this filtrate by using a flame photometer (Jenway PFP-7, Tokyo, Japan). Chloride was calculated by adding the dried ground root/leaf samples in a test tube containing 10 mL of distilled water, and then incubated overnight at 25 °C. The test tubes were heated (80 °C) in a digestion block until the volume of water in them was half of the original volume. The test tubes were cooled and the volume was made up to 10 mL again by adding distilled water. Chloride concentration was measured from this water by chloride analyzer (Nelson-Jameson-926, WI, USA). Si in root and leaf tissues was measured by the method of Dai et al. (2005).

2.7. Statistical analysis

The experiment was laid out in a completely randomized design (CRD) with five replications and there were five pots (7 plants per pot = 35 plants per replication) in each replication. In this way there were in total 175 (35 × 5) plants per treatment. The data were analyzed by standard statistical procedures as described by Gomez and Gomez (1984). Least significance difference (LSD) was used to evaluate the significance of differences between the treatments at $P \leq 0.05$ (n = 5).

3. Results

Salt stress given through rooting medium significantly inhibited growth (plant fresh and dry biomass and leaf area) in pea plants (Figure 1). However, the foliar application of Si, MA leaf extract (MAE), and SBE individually or in various combinations with each other was very effective in elevating growth under saline conditions (Figure 1). Among all the treatments applied under saline conditions, the foliar application of MAE + SBE supplemented with Si showed an excellent response in mitigating deleterious effects of salinity by elevating the shoot fresh weight (95%), shoot dry weight (150%), root fresh weight (58%), root dry weight (118%), and leaf area (89%) with respect to the plants grown under saline conditions with foliar spray of distilled water (DW) (control). The combination of the three (Si + MAE + SBE) presented significant differences from that of the saline control (salinity + foliar spray with DW). However, in the case of root/shoot fresh and dry weights, the treatments, i.e. Si + MAE, Si + SBE and MAE + SBE, showed no significant variations, but showed variations regarding leaf area (Figure 1).

The gas exchange attributes, i.e. Pn, gs, *E*, WUE, and stomatal size, exhibited marked decreases in response to salt stress, compared to the nonsaline control (no salinity + foliar spray DW) (Figure 2). However, the exogenous application of phyto-extracts (MAE and SBE) along with Si, when applied individually or in combination, neutralized the toxicity and caused a marked improvement in the above-mentioned gas exchange attributes, compared to the plants exposed to salinity stress that were not sprayed with any phyto-extract or Si (Figure 2). Of the various treatments applied to plants under salt-stressed conditions, the foliar spray with the mixture containing MAE, SBE, and Si proved to be highly effective by significantly mitigating the detrimental effects generated by salinity. The plants subjected to a saline environment that were sprayed with the combination of MAE + SBE + Si exhibited increases in Pn of 93%, gs of 96%, E of 114%, WUE of 55%, and stomatal size of 108% with respect to the stressed plants treated with only distilled water (control). According to the statistical analysis, all the treatments tested under NaCl-stressed conditions significantly differed from the saline control (+NaCl and DW spray). However, in the case of WUE all treatments significantly differed from the control (–NaCl + DW), but did not show significant differences from each other. In this study, salt stress did not affect the number of stomata. Likewise, foliar application of the three additives also did not show any effect on the number of stomata (Figure 2).

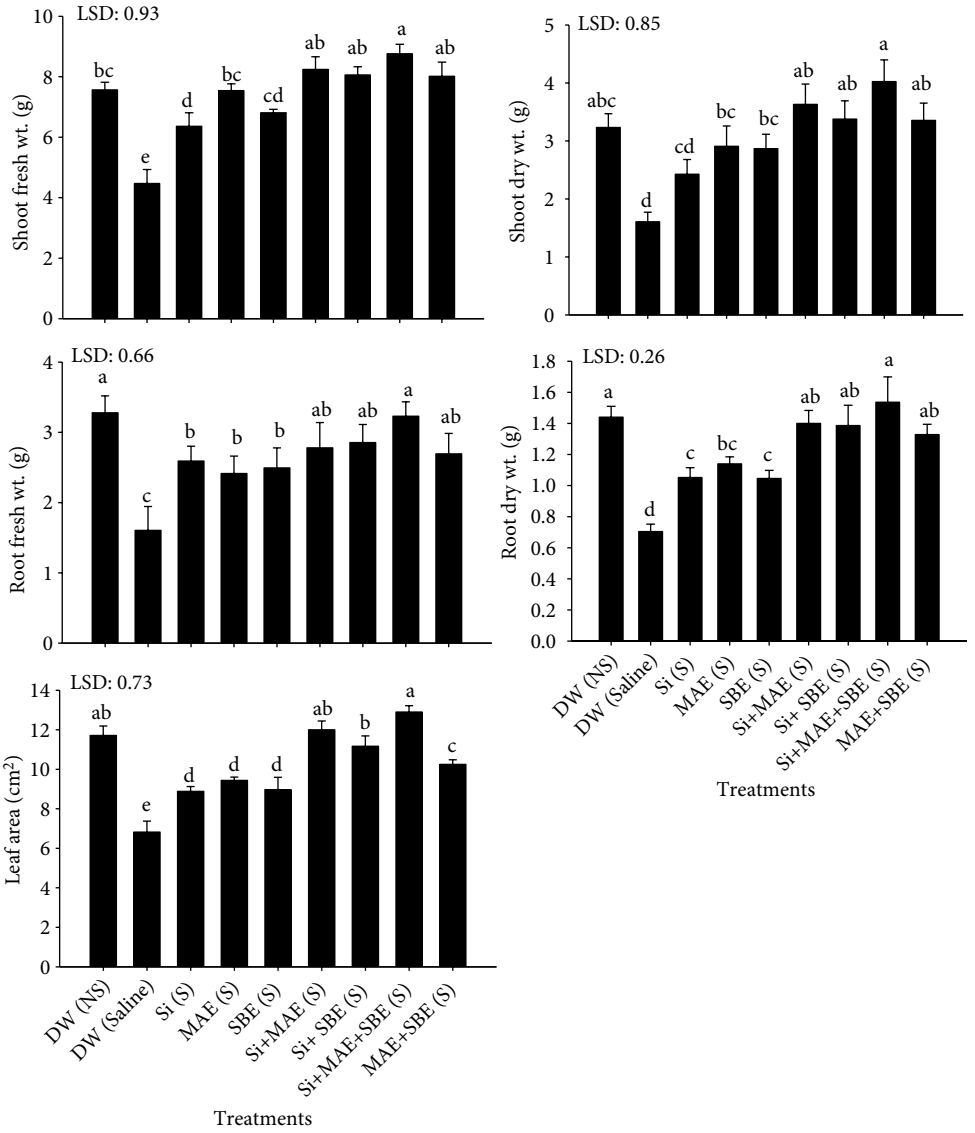

Figure 1. Effect of phyto-extracts (MAE and SBE) and Si on shoot/root fresh and dry weights and leaf area of salt-stressed pea plants. Each value represents the mean of 5 replicates ± standard error (SE) of the mean. Letters represent mean separation comparison utilizing least significant difference (LSD) test (P ≥ 0.05) (Si: silicon, MAE: *Melia azadarchta* leaf extract, SBE: sugar beet root extract).

A decreasing trend of response was observed when chlorophyll contents, MSI, and total phenolics were investigated in plants under stressed medium (Figures 3 and 4). However, the follow up treatment of the plants under salinity stress with Si/phyto-extracts elevated the chlorophyll contents, MSI, and total phenolic content more than those of the control (stressed plants with no Si or phyto-extracts). The plants treated with the blend of Si + MAE + SBE or only plant extracts (MAE + SBE) exhibited a better response than those treated with individual solutions of Si, MAE, and SBE. The stressed plants sprayed with the blend of the three (Si + MAE + SBE) showed maximum stress alleviating effect by increasing the chlorophyll a (49%), chlorophyll b (61%), total chlorophyll content (52%), MSI (50%), and total phenolic (56%). However, LPO, EL, and H_2O_2 contents were enhanced when plants were exposed to root applied salt stress (Figure 4). The application of Si and plant extracts individually or in combinations lowered the levels of LPO, EL, and H_2O_2. However, the plants with foliar application of Si supplemented with MAE and SBE exhibited high effectiveness in ameliorating the NaCl-induced toxicity by lowering the values of LPO by 184 %, EL by 59%, and H_2O_2 by 172% in comparison to those grown under NaCl stress but treated with DW only

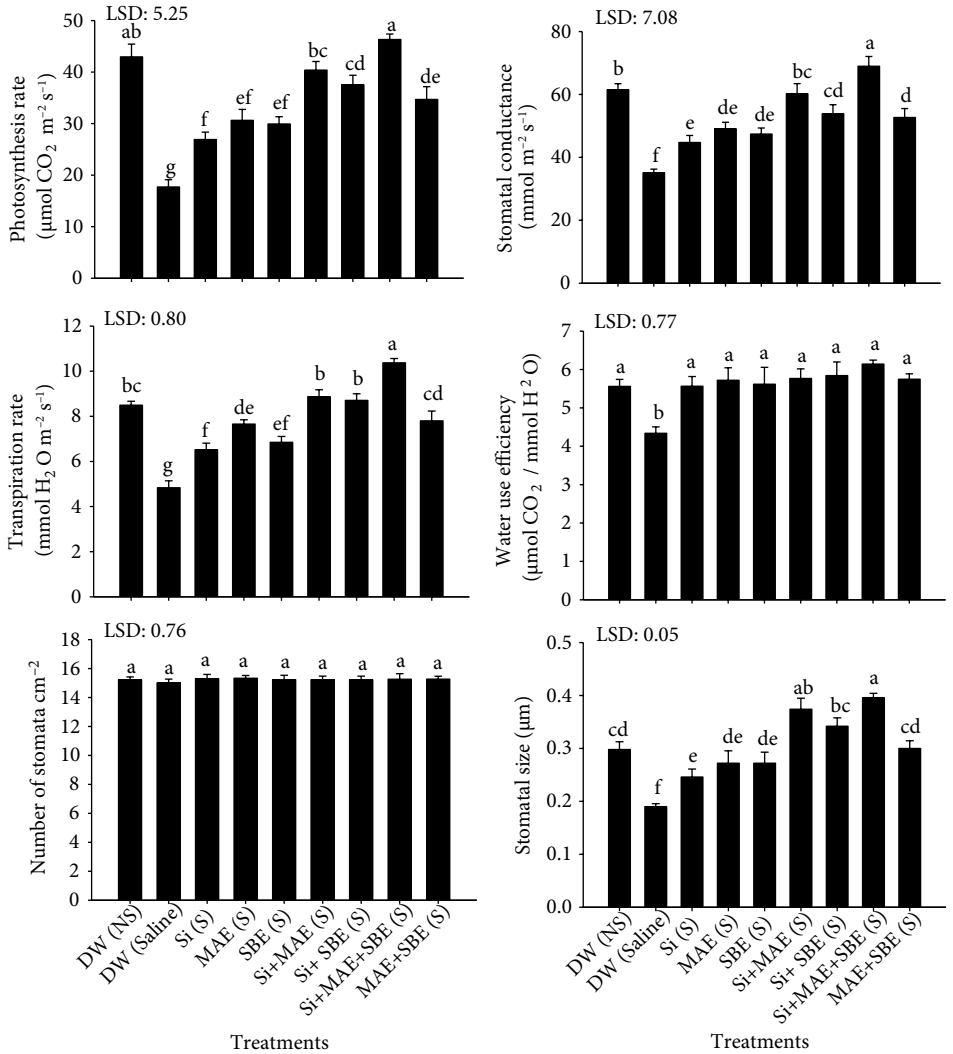

Figure 2. Effect of phyto-extracts (MAE and SBE) and Si on photosynthesis, stomatal conductance, transpiration rate, water use efficiency, number of stomata, and stomatal size of salt-stressed pea plants. Each value represents the mean of 5 replicates ± standard error (SE) of the mean. Letters represent mean separation comparison utilizing least significant difference (LSD) test ($P \geq 0.05$) (Si: silicon, MAE: *Melia azadarchta* leaf extract, SBE: sugar beet root extract).

(control). The spray of Si + MAE also induced a significant stress mitigating effect by elevating the chlorophyll a (46%), chlorophyll b (55%), total chlorophyll contents (48%), MSI (43%), and total phenolic content (52%) and lowering the LPO (133%), EL (82%), and H_2O_2 (117%) with respect to the DW-treated plants under saline conditions.

The level of different enzymatic activities, i.e. SOD, POD, CAT, APX, GPX, and GTR, showed an augmentation in response to salinity stress (Figure 5). All treatments applied under saline conditions overcame the NaCl toxicity by further accelerating the enzymatic activities of the above-mentioned enzymes but maximum acceleration was noted in the case of Si + MAE + SBE and Si + MAE. The exogenous application of these two mixtures (Si + MAE + SBE and Si + MAE) strengthened the stressed plants by increasing the SOD (by 45% and 41%), POD (by 44% and 41%), CAT (by 53% and 49%), APX (by 30% and 27%), GPX (by 41% and 38%), and GT (by 42% and 39%), respectively.

Leaf proline and GB contents were increased under salinity stress (Table), but exogenous application of the three additives (Si, MAE, SBE) alone and in combinations markedly improved the leaf free proline and GB contents. The application of additives in combinations of two or three gave better results as compared to their individual applications. Maximum increases in proline and GB contents were recorded in plants treated with a mixture of Si + MAE + SBE with respect to the control (DW + S).

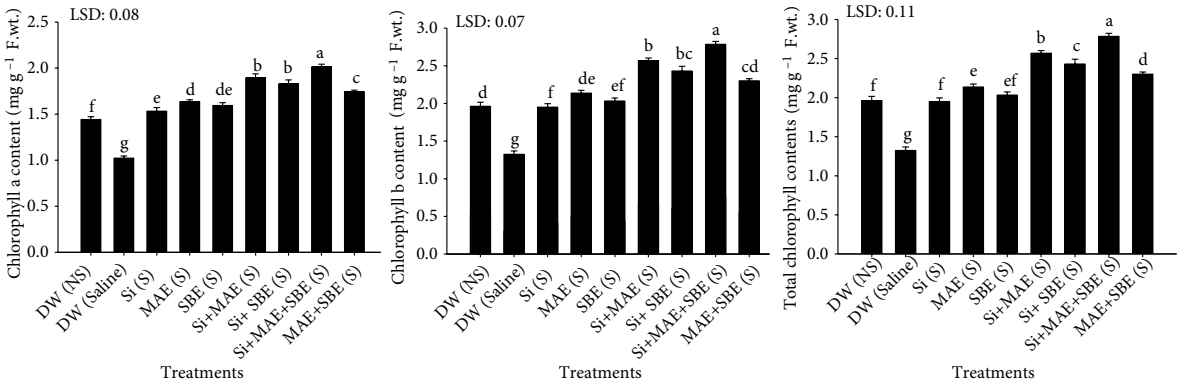

Figure 3. Effect of phyto-extracts (MAE and SBE) and Si on chlorophyll contents of salt-stressed pea plants. Each value represents the mean of 5 replicates ± standard error (SE) of the mean. Letters represent mean separation comparison utilizing least significant difference (LSD) test (P ≥ 0.05) (Si: silicon, MAE: *Melia azadarchta* leaf extract, SBE: sugar beet root extract).

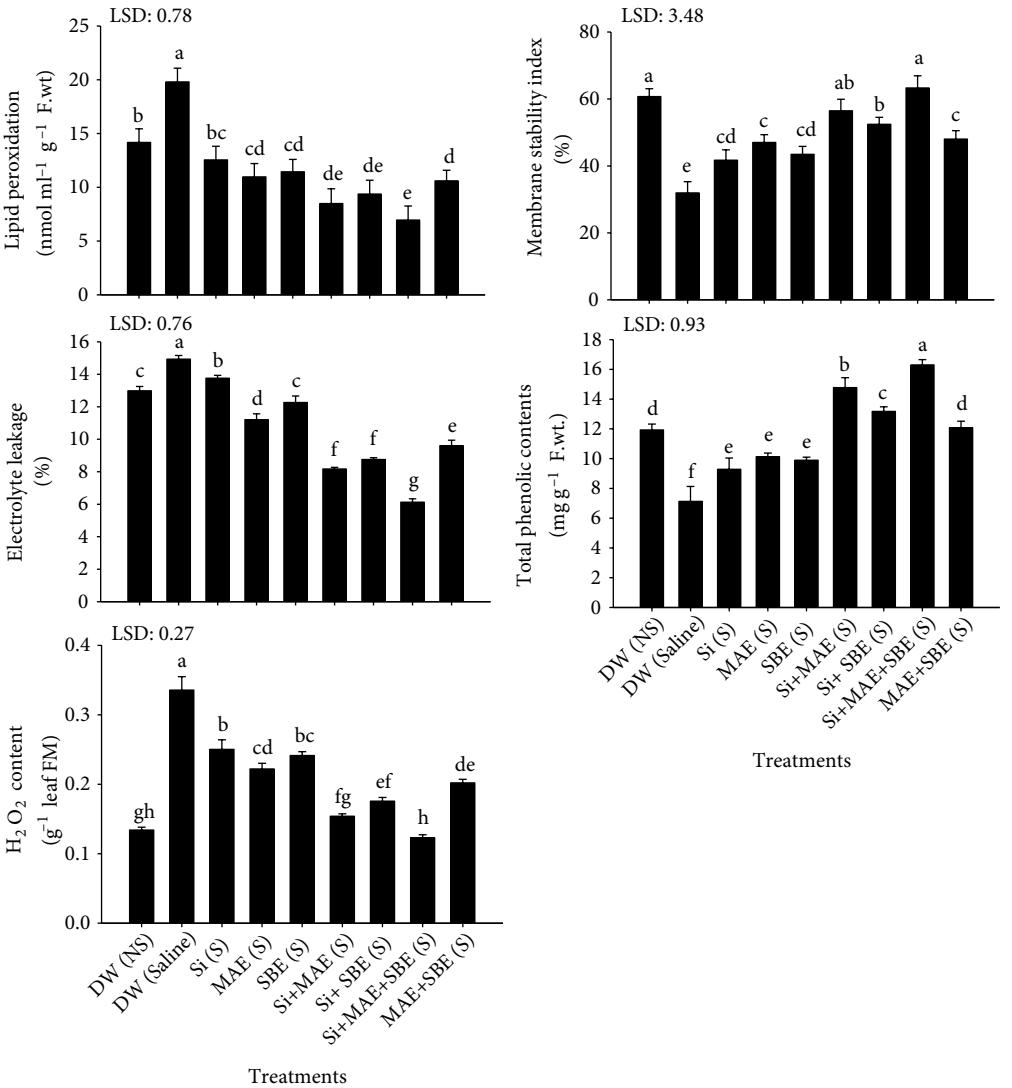

Figure 4. Effect of phyto-extracts (MAE and SBE) and Si on lipid peroxidation, membrane stability index, electrolyte leakage, total phenolic contents, and H_2O_2 content of salt-stressed pea plants. Each value represents the mean of 5 replicates ± standard error (SE) of the mean. Letters represent mean separation comparison utilizing least significant difference (LSD) test (P ≥ 0.05) (Si: silicon, MAE: *Melia azadarchta* leaf extract, SBE: sugar beet root extract).

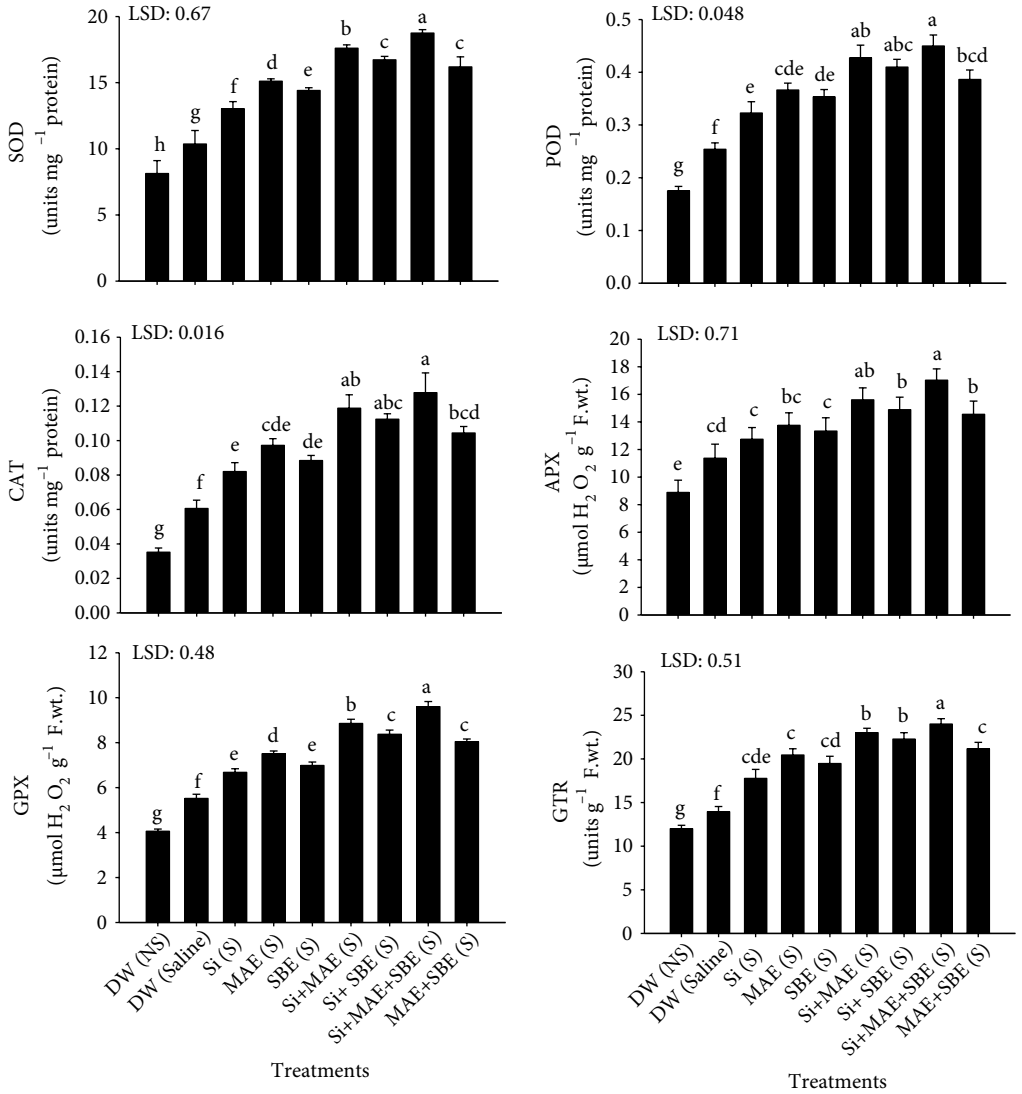

Figure 5. Effect of phyto-extracts (MAE and SBE) and Si on antioxidant activities of salt-stressed pea plants. Each value represents the mean of 5 replicates ± standard error (SE) of the mean. Letters represent mean separation comparison utilizing least significant difference (LSD) test (P ≥ 0.05) (Si: silicon, MAE: *Melia azadarchta* leaf extract, SBE: sugar beet root extract).

Sodium and chloride concentrations in leaves and roots were enhanced in response to salt stress, but silicon concentration was decreased (Table). However, foliar spray of Si and the two phyto-extracts, applied individually or in combinations, limited the Na and Cl contents in leaves and roots, but improved the Si contents in both plant parts. The stressed plants foliar-applied with a blend of MAE and SBE supplemented with Si had low root/leaf Na and Cl contents, whereas plants sprayed with Si + MAE + SBE showed the highest improvement in root/leaf silicon contents.

Salt stress significantly reduced the number of seeds per pod and 1000 seed weight but did not affect the number of pods per plant (Figure 6). The application of Si, MAE, and SBE in various combinations enhanced the number of seeds per pod and 1000 seed weight in stressed plants. Among all the treatments applied under saline environment, Si + MAE + SBE and Si + MAE gave extraordinary results by markedly elevating the values for number of seeds per pod and 1000 seed weight. The spray of Si + MAE + SBE and Si + MAE increased number of seeds per pod by 47% and 44% and 1000 seed weight by 58% and 52%, respectively.

4. Discussion

In the present investigation, salt stress significantly reduced growth (plant fresh weight, plant dry weight, and leaf area) and yield (number of seeds per pod and seed weight). These results are equivalent to what was reported earlier

Table. Effect of phyto-extracts of *Melia azedarchta* leaves and sugar beet roots supplemented with silicon on root/leaf sodium, chloride, silicon, leaf free proline, and leaf glycinebetaine contents of salt-stressed pea plants. Letters represent mean separation comparison utilizing least significant difference (LSD) test (P ≥ 0.05).

Treatments	Parameters							
	Sodium (Na) (mg g^{-1} DW)		Chloride (Cl) (mg g^{-1} DW)		Silicon (Si) (mg g^{-1} DW)		Free proline (µmol g^{-1} FW)	Glycinebetaine (µmol g^{-1} FW)
	Root	Leaf	Root	Leaf	Root	Leaf		
DW (NS)	0.79f	0.55h	1.30g	1.06g	0.09d	0.12e	0.43g	1.54g
DW (S)	1.52a	1.31a	3.44a	2.98a	0.06e	0.07f	0.57f	1.72fg
Si (S)	1.36b	1.24ab	3.10b	2.39b	0.13cd	0.26b	0.95e	1.81ef
MAE (S)	1.31bc	1.13cd	2.13d	1.78d	0.10cd	0.11e	1.12d	1.99de
SBE (S)	1.28bcd	1.19bc	2.74c	1.98c	0.12cd	0.16d	1.08d	2.03de
Si + MAE	1.18d	0.93f	1.60f	1.30f	0.14c	0.11e	1.46b	3.07b
Si + SBE	1.22cd	1.02ef	1.80e	1.47e	0.20b	0.20c	1.31c	2.67c
Si + MAE + SBE	1.07e	0.79g	1.42g	1.19f	0.25a	0.32a	1.62a	3.49a
MAE + SBE	1.22cd	1.09de	1.90e	1.47e	0.13cd	0.13de	1.28c	2.20d
LSD at (P ≤ 0.05)	0.10	0.09	0.16	0.12	0.037	0.034	0.08	0.22

DW: distilled water, NS: nonsaline, S: saline, SBE: sugar beet root extract, MAE: *Melia azedarach* leaf extract

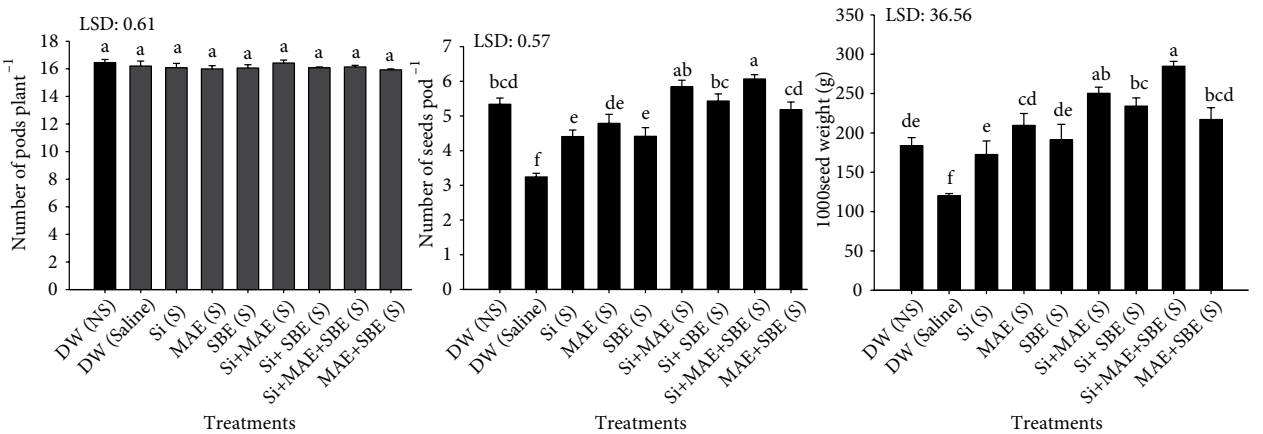

Figure 6. Effect of phyto-extracts (MAE and SBE) and Si on yield attributes of salt-stressed pea plants. Each value represents the mean of 5 replicates ± standard error (SE) of the mean. Letters represent mean separation comparison utilizing least significant difference (LSD) test (P ≥ 0.05) (Si: silicon, MAE: *Melia azadarchta* leaf extract, SBE: sugar beet root extract).

for pea (Shahid et al., 2012, 2013). However, exogenous application of Si and phyto-extracts (MAE and SBE) had a marked effect in improving pea growth under saline conditions. Reports indicate that Si stimulates growth and productivity under stressed conditions (Mateos-Naranjo et al., 2013), whereas MAE and SBE extracts are rich in various growth promoting compounds such as flavonoids, steroids, tocopherols, proline, GB, and many phenolic compounds (Mack et al., 2007; Suresh et al., 2008; Sultana et al., 2013). Therefore, the elevation in growth and yield attributes in NaCl-stressed plants could have been due to the alleviating effect of Si to reduce Na and Cl toxicity by decreasing oxidative stress and those of the growth promoting compounds present in MAE and SBE.

A considerable decline in Pn was seen in salt-stressed plants in the present investigation. However, among all the treatments applied, foliar application of solution containing Si, MAE, and SBE significantly overcame the injurious effect of NaCl by improving the Pn, since in the current study plant biomass, number of seeds per pods, and seed weight of pea are positively associated with Pn, which shows that combined application of Si + MAE +

SBE increased growth and yield by accelerating Pn. Salt stress also markedly lowered the gs, *E*, and stomatal size, which is the reason for decreased Pn under salinity stress. The exogenous application of MAE + SBE supplemented with Si suppressed the deleterious effect of NaCl stress by enhancing gs and *E*. The elevations in gs and *E* due to spray of Si + MAE + SBE were found to be linked with improved stomatal size. Green pigments in the leaf play a significant role in photosynthetic activity, but salt stress causes substantial damage to chlorophyll contents (Shu et al., 2013). In the present study, stressed plants also exhibited a marked decline in chlorophyll a, chlorophyll b, and total chlorophyll contents, but exogenous application of Si, MAE, and SBE, individually or in various combinations, reduced the chlorophyll degradation and supported the photosynthetic apparatus by promoting the chlorophyll contents. However, the stressed plants with spray of Si + MAE + SBE had maximum improvement in chlorophyll contents. The stressed plants with high chlorophyll contents also showed excellent growth, which shows a strong correlation between growth and green pigments in the current investigation. The same kind of correlation has also been reported by Ayumi et al. (2004). It has been found that maximum green pigments show maximum production of chemical energy and plant metabolism, which improves plant growth. This fact may also be the reason for improved Pn, growth, and yield in Si + MAE + SBE treated plants subjected to a saline environment. The findings of this study also indicate that plants with higher Pn had high growth and productivity, which proves the existence of a correlation between Pn and growth. Various reports indicate the presence of a positive correlation between Pn and growth (Iqbal et al., 2012). The literature also indicates that application of Si (Mateos-Naranjo et al., 2013), proline (Kaur et al., 2011), and GB (Kanechi et al., 2013) accelerated the photosynthetic activity by enhancing the chlorophyll contents. The findings of our study are in accordance with these reports. It is already mentioned that MA and SB extracts are enriched with various compounds including proline (Suresh et al., 2008) and GB (Mack et al., 2007). Therefore, in the present investigation, stressed plants receiving spray of Si + MAE + SBE showed high chlorophyll contents and Pn, which might have been due to the combined action of Si, proline, and glycinebetaine present in this blend. In addition, the plants treated with Si + MAE + SBE had an increment in WUE, which may also be attributed to regulation in Pn and *E* under a saline environment.

In the present study, salt stress significantly reduced the MSI and enhanced the EL, which is highly linked with LPO and $H_2O_2^-$, but foliar application of Si and phyto-extracts strengthened the membranes by improving the MSI and lowering the EL. However, the plants treated with a blend of Si with MAE and SBE gave the maximum increase in MSI and a high decline in EL.

To overcome the oxidative damage under stressed conditions, especially salinity stress, plants develop an antioxidant defense system comprising various antioxidant enzymes such as APX, GPX, CAT, SOD, and POD (Apel and Hirt, 2004). This antioxidant system maintains the ROS at a less toxic level within the cell by converting them into water and oxygen. In this study the stressed plants showed enhanced values of antioxidant enzymes (APX, GPX, GTR, POD, SOD, and CAT), but foliar application of Si supplemented with MAE + SBE further accelerated the activities of antioxidant enzymes. Enhanced antioxidant activities in response to spray of Si + MAE + SBE in NaCl-stressed plants indicated that these plants are well adapted to the saline environment by eliminating the ROS. A strong relationship was established between antioxidant activities and growth in this investigation.

Salinity causes an ionic and osmotic effect that results in oxidative damage, because reduced supply of CO_2 under stressed conditions results in carbon reduction in the Calvin cycle and ultimately a reduction in electron acceptor ($NADP^+$) in photosynthesis. Extra reduction in ferrodoxin during electron transfer in Pn results in the generation of superoxide radicals due to the transfer of electrons to oxygen from PS-I by a mechanism referred to as the Mehler reaction (Hsu and Kao, 2003). It initiates a chain of reactions that produces ROS, which disturbs the metabolic processes within the cell by oxidative degradation of lipid, nucleic acids, and proteins (McCord, 2000). These ROS also disintegrate the membranes by peroxidation of lipids and constituent of membranes (Jain et al., 2001). In this study salt stress enhanced the LPO but spray of Si or plant extracts markedly inhibited it. The plants treated with combinations such as Si + MAE, Si + SBE, MAE + SBE, and Si + MAE + SBE showed maximum inhibition as compared to the individual application of Si, MAE, and SBE. Among the four blends, the mixture containing Si along with MAE and SBE exhibited the maximum decline in LPO. A strong negative correlation was observed with the rate of LPO and salt tolerance. Our results regarding the LPO are in accordance with the findings reported by Azuma et al. (2010), who observed a significant enhancement in the LPO of salinized pepper plants. It is suggested that the conspicuous increase in MSI and decrease in LPO and EL in Si + MAE + SBE treated plants are due to acceleration in the antioxidant activities of SOD, POD, CAT, APX, GPX, GTR, proline, and GB, which detoxified the ROS.

Under stressed conditions, plants suffer from osmotic stress; therefore they adopt a mechanism of osmotic adjustment by accumulating various osmolytes like proline, GB, and phenolic compounds. In the present study, plants sprayed with a mixture of Si + MAE + SBE exhibited better

growth and development, which could have been due to the efficient osmotic adjustment potential. It has already been discussed above that plant extract of MAE contained flavonoids, tocopherols, and phenolic compounds while SBE is enriched with GB. Similarly, Si itself has a role in osmotic adjustment but its mode of action is not yet elucidated. GB is mainly found in leaf chloroplasts and protects the thylakoid membranes from the drastic effect of salt stress by osmotic adjustment (Genard et al., 1991). Proline is another organic solute that mostly occurs in higher plants in higher ratios than amino acids. It promotes the deposition of useable nitrogen and enhances the membrane stability under salt stress. Therefore, it is suggested that a spray of a blend comprising three things, i.e. Si + MAE + SBE, indirectly provides proline and GB to the stressed plants, which might have fortified osmotic adjustment mechanisms and played a role in mitigating the drastic effects of salinity, by raising nutritional and moisture levels within plant tissues, resulting in better growth and productivity.

It is concluded that growth melioration in response to exogenous application of Si + MAE + SBE was attributed to improved MSI, green pigments (chlorophyll a, chlorophyll b, and total chlorophyll contents), Pn, gs, *E*, WUE, stomatal size, and osmotic adjustment potential due to enhanced activities of antioxidant enzymes (SOD, POD, CAT, APX, GPX, and GTR) and osmolytes (proline and GB). Si and natural extracts of MA and sugar beet in the form of a blend were highly effective in improving some major physiological processes in pea plants exposed to saline conditions, and so it can be a cheap and easily manageable remedy to tackle the deleterious effects of salt stress, and can be used to enhance pea productivity in marginal saline areas with salty underground water.

References

Abbas W, Ashraf M, Akram NA (2010). Alleviation of salt-induced adverse effects in eggplant (*Solanum melongena* L.) by glycinebetaine and sugarbeet extracts. Sci Hortic 125: 188–195.

Ahmad F, Rahmatullah, Aziz T, Maqsood MA, Tahir MA, Kanwal S (2007). Effect of silicon application on wheat (*Triticum aestivum* L.) growth under water deficiency stress. Emir J Food Agr 19: 1–7.

Ahmad ST, Haddad R (2011). Study of silicon effects on antioxidant enzyme activities and osmotic adjustment of wheat under drought stress. Czech J Genet Plant Breed 47: 17–27.

Ahmed MA, Rao AS, Ahemad SR, Ibrahim M (2012). Phytochemical studies and antioxidant activity of *Melia azedarach* leaves by dpph scavenging assay. Int J Pharm Appl 3: 271–276.

Apel K, Hirt H (2004). Reactive oxygen species: metabolism oxidatives and signal transduction. Ann Rev Plant Physiol Plant Mol Biol 55: 373–399.

Arnon DI (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24: 1–15.

Ayumi T, Masumi H, Ryoichi T (2004). Chlorophyll metabolism and plant growth. Kagaku Seibutsu 42: 93–98.

Azuma R, Ito N, Nakayama N, Suwa R, Nguyen NT, Larrinaga-Mayoral JA, Esaka M, Fujiyama H, Saneoka H (2010). Fruits are more sensitive to salinity than leaves and stems in pepper plants (*Capsicum annuum* L.). Sci Hortic 125: 171–178.

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

Chance B, Maehly AC (1955). Assay of catalase and peroxidase. Method Enzymol 2: 764–775.

Dai W, Zhang KQ, Duan BW, Sun CX, Zheng KL, Cai R, Zhuang JY (2005). Rapid determination of silicon content in rice. Rice Sci 12: 145–147.

Genard H, Saos JL, Hillard J, Tremolieres A, Boucaud J (1991). Effect of salinity on lipid composition, glycinebetaine content and photosynthetic activity in chloroplasts of *Suaeda maritima*. Plant Physiol Bioch 29: 421–427.

Giannopolitis CN, Ries SK (1977). Superoxide dismutase I. Occurrence in higher plants. Plant Physiol 59: 309–314.

Gomez KA, Gomez AA (1984). Statistical Procedures for Agricultural Research, 2nd ed. New York, NY, USA: Wiley.

Grieve C, Grattan S (1983). Rapid assay for determination of water soluble quaternary ammonium compounds. Plant Soil 70: 303–307.

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

Hsu SY, Kao CH (2003). Differential effect of sorbitol and polyethylene glycol on antioxidant enzymes in rice leaves. Plant Growth Regul 39: 83–90.

Iqbal N, Nazar R, Khan MIR, Khan NA (2012). Variations in photosynthesis and growth of mustard cultivars: Role of ethylene sensitivity. Sci Hortic 135: 1–6.

Jain M, Mathur G, Koul S, Sarin NB (2001). Ameliorative effects of proline on salt induced lipid peroxidation in Cell lines of groundnut (*Arachis hypogea* L.) Plant Cell Rep 20: 483–468.

Julkenen-Titto R (1985). Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. Agr Food Chem 33: 213–217.

Kanechi M, Hikosaka Y, Uno Y (2013). Application of sugarbeet pure and crude extracts containing glycinebetaine affects root growth, yield, and photosynthesis of tomato grown during summer, Sci Hortic 152: 9–15.

Kaur G, Kumar S, Thakur P, Malik JA, Bhandhari K, Sharma KD, Nayyar H (2011). Involvement of proline in response of chickpea (*Cicer arietinum* L.) to chilling stress at reproductive stage. Sci Hortic 128: 174–181.

Khan MIR, Iqbal N, Masood A, Khan NA (2012). Variation in salt tolerance of wheat cultivars: role of glycinebetaine and ethylene. Pedosphere 22: 746–754.

Liu ZJ, Guo YK, Bai JG (2010). Exogenous hydrogen peroxide changes antioxidant enzyme activity and protects ultra-structure in leaves of two cucumber ecotypes under osmotic stress. J Plant Growth Regul 29: 171–183.

Mack G, Hoffmann CM, Marlander B (2007). Nitrogen compounds in organs of two sugar beet genotypes (*Beta vulgaris* L.) during the season. Field Crop Res 102: 210–218.

Makela P (2004). Agro-industrial uses of glycinebetaine. Sugar Technol 6: 207–212.

Mateos-Naranjo E, Andrades-Moreno L, Davy AJ (2013). Silicon alleviates deleterious effects of high salinity on the halophytic grass *Spartina densiflora*. Plant Physiol Bioch 63: 115–121.

McCord JM (2000). The evolution of free radicals and oxidative stress. Am J Med 108: 652–659.

Moya LJ, Gomez-Cadenas A, Primo-Millo E, Talon M (2003). Chloride absorption in salt-sensitive *Carrizo citrange* and salt-tolerant *Cleopatra mandarin* citrus rootstocks is linked to water use. J Exp Bot 54: 825–833.

Nahak G, Sahu RK (2010). In vitro antioxidative acitivity of *Azadirachta indica* and *Melia azedarach* Leaves by DPPH scavenging assay. J Am Sci 6: 123–128.

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

Neycee MA, Nematzadeh GHA, Dehestani A, Alavi M (2012). Assessment of antifungal effects of shoot extracts in chinaberry (*Melia azedarach*) against 5 phytopathogenic fungi. Int J Agr Crop Sci 4: 474–477.

Rachokarn S, Piyasaengthong N, Bullangpoti V (2008). Impact of botanical extracts derived from leaf extracts *Melia azedarach* L. (Meliaceae) and *Amaranthus viridis* L. (Amaranthaceae) on populations of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) and detoxification enzyme activities. Commun Agr Appl Biol Sci 73: 451–457.

Rao AS, Ahmed MF, Ibrahim M (2012). Hepatoprotective activity of *Melia azedarach* leaf extract against simvastatin induced hepatotoxicity in rats. J Appl Pharm Sci 2: 144–148.

Schutzendubel A, Polle A (2002). Plant responses to abiotic stresses: heavy metal induced oxidative stress and protection by mycorrhization. J Exp Bot 53: 1351–1365.

Shahid MA, Ashraf MY, Pervez MA, Ahmad R, Balal RM, Garcia-Sanchez F (2012). Impact of salt stress on concentrations of Na^+, Cl^- and organic solutes concentration in pea cultivars. Pakistan J Bot 45: 755–761.

Shahid MA, Balal RM, Pervez MA, Abbas T, Ashfaq M, Ghazanfar U, Afzal M, A. Rashid A, Garcia-Sanchez, Mattson NS (2012). Differential response of pea (*Pisum sativum* L.) genotypes to salt stress in relation to the growth, physiological attributes antioxidant activity and organic solutes. Aust J Crop Sci 6: 828–838.

Shen X, Zhou Y, Duan L, Li Z, Eneji AE, Li J (2010). Silicon effects on photosynthesis and antioxidant parameters of soybean seedlings under drought and ultraviolet-B radiation. J Plant Physiol 167: 1248–1252.

Shu S, Yuan L, Guo S, Sun J, Yuan Y (2013). Effects of exogenous spermine on chlorophyll fluorescence, antioxidant system and ultrastructure of chloroplasts in *Cucumis sativus* L. under salt stress. Plant Physiol Bioch 63: 209–216.

Smith IK, Vierheller TL, Thorne CA (1988). Assay of glutathione reductase in crude tissue homogenates using 5,5-dithiobis (2-nitrobenzoic acid). Ann Biochem 175: 408–413.

Sultana S, Akhtar N, Asif HM (2013). Phytochemical screening and antipyretic effects of hydro-methanol extract of *Melia azedarach* leaves in rabbits. Bangladesh J Pharmacol 8: 214–217.

Suresh K, Deepa P, Harisaranraj R, Achudhan V (2008). Antimicrobial and phytochemical investigation of the leaves of *Carica papaya* L., *Cynodon dactylon* (L.) Pers., *Euphorbia hirta* L., *Melia azedarach* L. and *Psidium guajava* L. Ethnobot Leaflets 12: 1184–1191.

Sullivan CY, Ross W (1979). Selecting for drought and heat resistance in grain sorghum. Stress Physiol Crop Plant 1: 263–281.

Urbanek H, Kuzniak-Gebarowska E, Herka K (1991). Elicitation of defense responses in bean leaves by *Botrytis cinerea* polygalacturonase. Acta Physiol Plant 13: 43–50.

Zwart FJ, Slow S, Payne RJ, Lever M, George PM, Gerrard JA, Chambers ST (2003). Glycinebetaine and glycinebetaine analogues in common foods. Food Chem 83: 197–204.