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Ameliorating Effect of Silicon on Growth Vigor, Physiological and biochemical Traits of Salinized Canola Seedlings (*Brassica napus* L.)

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ABSTRACT

In the present study the ameliorating effects of Si nutrition supplied as 1 mmol L^{-1} sodium silicate were proved on the seedling growth of canola (*Brassica napus L.*) seedlings under salinity stress (i.e. 0, 30, 60, 90 and 120 mmol L^{-1}sodium chloride). Salinity obviously disrupted cellular homeostasis, something was evident in the decreased percent of scavenging HO^- free radical and Metal chelating %, and hence reduction in photosynthetic pigments contents (chl.a and carotenoids). Silicon nutrition, however, enabled canola cells to balance between the steady-state levels of different ROS through improving the detoxification of the excess ROS. This was prevalent in higher scavenging percent of HO^- and metal chelating. In addition, Si maintained membranes integrity through improved levels of lipid peroxidation inhibition %. Si buffered oxidative stress through sustaining the enzymatic and non-enzymatic scavenging capacity of reactive oxygen species in salt-stressed plants. Enzymatic antioxidants such as (CAT, SOD, APX, and POS) activities as well as non-enzymatic antioxidants such as carotenoids contents phenolics contents increased at 120 mmol L^{-1} sodium chloride. Thus, silicon nutrition alleviated the deleterious effects of salinity on the growth of canola plants through increased reactive oxygen species scavenging capacity (enzymatically and non-enzymatically), maintaining the membrane integrity of seedling cells as evidenced by raising the reducing power contents, sustained higher levels of chlorophyll. Consequently, enhance seedlings growth observations.

Keywords: salinity, silicon, antioxidant enzymes, lipid peroxidation inhibition%, HO^- radical scavenging %, metal chelating %.

INTRODUCTION

One of the major agricultural concerns is salinity as it is very deleterious abiotic stresses. The detrimental effects of salinity hinder plant growth and productivity. Soil salinity causes substantially or partially unproductive lands (Medellín-Azuara *et al*., 2014). Salt affected cultivated land is over 800 million hectares worldwide (Munns and Tester, 2008). The problems of saline soil and water have serious implications in irrigated agricultural systems, and soil degradation caused by salinity is major challenge globally (Qadir *et al*., 2008). Soil salinity occurs naturally or through agricultural practices. Among soluble salts, NaCl is the most soluble and dominant salt with adverse effects on various morphological, physiological and microbiological and molecular aspects at whole plant level (Pessarakli and Szabolcs, 2010). High salt concentrations of soil are often associated with ion imbalances and hyperosmotic pressure, which eventually lead to oxidative stress conditions for plants (Abideen *et al.*, 2014). Purty *et al.*, 2008 indicated that tolerance against salinity stress is a complex trait that is governed by interconnected mechanisms at elevated levels, cellular, tissue and organ. Numerous agronomic practices have been made to improve the salinity tolerance of a variety of crops in addition to physiological and genetical methods, but no satisfactory commercial success has been reached so far.

Canola (*Brassica napus* L.) is oil seed crop, which is increasingly used as a source of edible vegetable oil (Iniguez-Luy and Federico, 2011). Besides its uses in food applications, canola is mainly used to produce clean-burning biofuel (Zapata *et al.*, 2012). Although maximum yields of canola are obtained under normal soil and environmental conditions, the quantity and quality of seed yields are affected by environmental stress (Singh *et al.*, 2014). Improving the tolerance of canola to stressful conditions would lead to increased yields of higher quality oil. Canola is categorized as moderate salinity-tolerant crop (Ashraf and McNeilly, 2004).

Timely silicon (Si) is usually classified under the beneficial element category. It has proven effects in enhancing abiotic stress tolerance (Eraslan *et al*., 2008; Haghighi and Pessarakli, 2013), improving pest (Han *et al.*, 2015) and increasing photosynthetic capacity and yield (Guntzer *et al.*, 2011) in various crops. According to its abundance in the lithosphere, silicon is classified as second most abundant element (Broadley *et al*., 2002). However, silicon is still not recognized as an essential element for plant growth and development. Over the last few years, several reports have highlighted the positive effects of silicon (Si) fertilization in agriculture (Liang *et al.*, 2015). Incidentally, the International Plant Nutrition Institute (IPNI) has recently added Si to its list of beneficial nutrients (Ouellette *et al*., 2017). Despite high abundance of silicon, it is never present in a free form in soil and is usually taken up by the plant as monosilicic acid ($Si(OH)_4$) (Ma and Yamaji, 2006). Exact mechanism of Si involvement in metabolic or physiological process of plants has not yet been elucidated (Guntzer *et al*., 2011). More explored, however, have been the influences of Si in alleviating deleterious effects of salinity and its suggested mechanisms of action in some plant species including soybean (Lee *et al*., 2010), strawberry (Wang and Galletta, 1998) and tomato (Haghighi and Pessarakli, 2013). However, there is still a shortage of information available regarding the role of supplied Si in ameliorating salinity in terms of antioxidant defense system and cell damage of canola.

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The present work assigned to study the influence of salinity in the presence or absence of sodium silicate on vegetative growth attributes, chlorophylls content, lipid peroxidation inhibition %, LOX enzyme, metal chelating %, reactive oxygen species scavenging % (H_2O_2 and OH^-), antioxidant enzymes (peroxidase, catalase, ascorbate peroxidase and superoxide dismutase) activities and some other physiological parameters (proteins, phenolics, flavonoids, reducing power and total antioxidants) in Sarw 4 canola cultivar.

MATERIALS AND METHODS

The seeds of canola (*Brassica napus* L.) cultivar Sarw 4, commonly used in Egypt, were kindly supplied by the Agricultural Research Center, Giza, Egypt. Seeds sterilized with NaClO 5% for 15 minutes and washed several times with distilled water and then spread on Petri dish (100 mm x 15 mm); lined with wet filter sheets. Canola seedlings were subjected to elevated NaCl levels (0, 30, 60, 90 and 120 mM). Silicon treatment was 1 mM Na_2SiO_3 combined with NaCl levels as: 0 + Si, 30 + Si, 60 + Si, 90 + Si, and 120 + Si. Each Petri dish represented as an experimental unit with 25 seed. All experiments were performed in six replications. The seeds were covered in the dark at 24˚C. The roots length and shoots height were measured over the course of 8 days and compared to the untreated control. After measurement, whole seedlings were washed twice with distilled water, dried gently with filter paper. The seedlings were quickly weighted for fresh weight determination, then oven-dried at 70°C for 48 hours in order to determine dry weight.

Preparation of the extract

Another fraction of fresh seedlings was immediately weighted and ground in a chilled mortar and pestle with 5 ml buffer solution containing Tris HCl pH 7, 50mM, containing 1 mM sodium (EDTA) and 3 mM $MgCl_2$. The extract was centrifuged at 4°C for 10 min at 5000 rpm. The resultant supernatant was used for the enzymatic and non-enzymatic antioxidants determinations in addition to antioxidant potential determinations.

Photosynthetic pigment Determination

The photosynthetic pigments were extracted from fresh plumule samples by suspending it in 5 ml of 95 % C_2H_5OH at 60˚C, until colorless. Then the total volume completed into 10 ml with 95% C_2H_5OH and absorbance readings were determined at 663, 644 and 452 nm spectrophotometerically. Chlorophylls and carotenoids concentrations were calculated as cited by Lichtenthaler (1987) as mg g^{-1} FW.

Determination of soluble proteins

Protein contents were determined in the plant extract by Folin reagent according to Lowry *et al.* (1951). A calibration curve was constructed using bovine serum albumin (BSA) and the data were expressed as mg BSA g^{-1} fresh matter.

Enzymatic antioxidants

Superoxide dismutase assay

SOD activity Determination carried out according to of Beauchamp and Fedovich (1976) method. The amount of enzyme causing the reduction of NBT by 50% was expressed as SOD Unit. The expression of specific activity was in terms of units per mg of protein.

Catalase assay

CAT activity determination carried according to Aebi, (1984). The decrease in H_2O_2 absorbance at A_{240} nm was used to calculate the activity.

Guaiacol peroxidase assay

GPX activity determination carried out following the method of Tatiana *et al.* (1999). The increase in absorbance at A_{470} nm due to the formation of tetraguaiacol was measured.

Assay of Ascorbate peroxidase

APX activity was assayed following the method of Jiang and Zhang (2002). The decrease in A_{290} following the oxidation rate of ascorbic acid was measured.

Assay of Lipoxygenase

The method of Minguez-Mosquera *et al*. (1993) was modified and used to assay lipoxygenase activity. The substrate was prepared by solubilizing 0.5 g linoleic acid with 0.5 g Tween 20 in deionized water and the final volume brought to 25 ml. Turbidity was cleared with a few drops of 2 N NaOH. The plant extract was reacted with the substrate in a spectrophotometer cuvette containing 3ml phosphate buffer 0.2 M, at pH 6.5 and the absorbance measured at 234 nm at 20s intervals for 1 min using a recording spectrophotometer. The rate of formation of conjugated diene reaction products, measured as an increase in A_{234} nm.

Non enzymatic antioxidants determinations

Total phenolics determination

Total phenolic contents were assessed according to Singleton and Rossi (1965). Folin-Ciocalteau reagent method was used. The measurements carried out at A_{765} nm. Gallic acid equivalents were used to express the data as µg g^{-1} FW using Molar Coefficient of 120 $\mu g^{-1} cm^{-1} ml^{-1}$.

Total Flavonoids determination

Content of total flavonoid was measured according to Moreno *et al.* (2000). Quercetin equivalents were used to express the absorbance at A_{415} nm as mg g^{-1} FW.

Activity of total antioxidant

The contents of total antioxidant were measured according to Prieto *et al*. (1999). The absorbance was measured at A_{695} nm.

Reducing power assay

The method of Oyaizu (1986) was used to detect reducing power of the plant samples. Ascorbic acid equivalents were used to express the absorbance at A_{700} nm as µg g^{-1} FW.

Hydroxyl radical (OH^-) scavenging assay

OH^- radical scavenging assay carried out according to Kunchandy and Rao, (1990). Absorbance of plant

was measured against a blank containing deoxyribose and buffer at A_{532} nm, and degradation inhibition of deoxyribose was used to calculate the inhibition in percent (I) was calculated by the formula

I= (Abs control – Abs sample) / Abs control X 100

Hydrogen peroxide (H_2O_2) scavenging

H_2O_2 radical scavenging assay carried out according to Long *et al*. (1999). Sodium pyruvate was used as the reference compound. The absorbance of the ferric-xylenol orange complex was measured at A_{560} nm.

Lipid peroxide formation inhibition

lipid peroxidation inhibition % carried out according to Janero (1990). The absorbance of the upper organic layer was measured at A_{532} nm. The inhibition in percent (I) was calculated by the formula

I= (Abs control – Abs sample) / Abs control X 100

Metal chelating assay

Metal chelating ability carried out according to Decker and Barbara (1990). The absorbance of the solution was measured at A_{562} nm. EDTA was used as a positive control.

Statistical analysis

The experiments were simple in complete Randomized (CR) design. Each treatment replicated in six Petri dishes. One-way ANOVA was performed on the data from two independent experiments with three replicates. The expressions of data as (mean ± SE). Analysis performed using the SPSS statistical 11.0 package. Comparing of means for significant differences Duncan's multiple range tests at ($p < 0.05$) were used. All the assessed attributes subjected to cluster analysis using a Correlations similarity distance with the software PAST version 2.11 for Windows (Hammer *et al.*, 2001). The matrix was then analyzed with Principle Component Analysis (PCA) variance regression ordination, using the Sørensen coefficient as the distance measure, to check the magnitude of change in attributes along the NaCl and NaCl + Si gradients by the same software.

RESULTS

Ameliorative effect of Si on canola seedling growth under NaCl stress

Salinity led to a significant reduction in fresh mass, while, exogenous Si treatment had a positive effect on fresh mass of NaCl-stressed seedlings (Table 1). Generally, the canola growth was completely stopped under 120 mM NaCl, while Si treatment sustained the survival of the seedlings under this concentration. Dry matter showed different trend towards elevated concentrations of salinity with or without Si application. Generally significant raise in dry matter was observed at elevated concentration of salinity, except for 120mM NaCl+Si where a significant reduction occurred.

As shown in table (1), salinity stress significantly decreased the shoot and root length, which was more prevelant in roots. In contrast, Si external application significantly enhanced the growth of shoots and roots.

The inhibitory effect of salinity can be observed in photosynthetic pigments as represented in table (1). Precisely, Ch.a and carotenoids contents decreased gradually with the rise of NaCl concentrations, while Ch.b content fluctuated under the elevated NaCl concentrations. On the other hand 1 mM Si promoted significant higher content of Ch.a, Ch.b, and carotenoids under different salinity levels as compared to NaCl levels without Si.

Table (1): Growth attributes of canola (*Brassica napus L*. cultivar Sarw 4) as influenced by NaCl (mM) stress and silicon (1 mM Na_2SiO_3), different letters are significantly different at $p<0.05$, (mean ± SE; n = 3).

NaCl Conc. mM		Fresh Wt. g plant^{-1}	Dry Wt. g plant^{-1}	Plant height Cm	Root length Cm	Ch.a mg g^{-1}FWt.	Ch.b mg g^{-1}FWt.	Carot. mg g^{-1}FWt.
Control	**Zero**	0.039 ± 0.0029^{e}	0.0020 ± 0.0001^{b}	2.97 ± 0.15^{c}	1.50 ± 0.09^{b}	86.3 ± 2.0^{f}	5.2 ± 0.3^{d}	41.6 ± 0.9^{f}
	30	0.028 ± 0.0014^{d}	0.0022 ± 0.0002^{c}	2.07 ± 0.10^{b}	0.37 ± 0.09^{a}	62.1 ± 2.1^{d}	6.1 ± 0.4^{e}	32.5 ± 1.2^{d}
Without Si	**60**	0.022 ± 0.0013^{c}	0.0027 ± 0.0001^{g}	1.13 ± 0.07^{a}	0.53 ± 0.07^{a}	48.4 ± 3.2^{c}	3.0 ± 0.3^{c}	27.9 ± 2.0^{c}
	90	0.014 ± 0.0013^{b}	0.0023 ± 0.0001^{d}	1.03 ± 0.10^{a}	0.20 ± 0.07a	4.2 ± 1.0^{a}	6.9 ± 0.2^{f}	7.0 ± 0.7^{a}
	Zero^{+Si}	0.039 ± 0.0014^{e}	0.0028 ± 0.0001^{h}	3.40 ± 0.11^{c}	4.57 ± 0.17^{d}	97.7 ± 3.0^{g}	9.6 ± 0.5^{g}	46.5 ± 0.6^{g}
	30^{+Si}	0.039 ± 0.0011^{e}	0.0024 ± 0.0001^{e}	3.10 ± 0.15^{c}	4.53 ± 0.09^{d}	113.6 ± 3.4^{h}	14.3 ± 0.3^{i}	54.8 ± 0.7^{i}
With Si	**60^{+Si}**	0.059 ± 0.0024^{g}	0.0025 ± 0.0001^{f}	4.23 ± 0.10^{d}	2.07 ± 0.07^{c}	122.9 ± 2.0^{i}	11.8 ± 0.3^{h}	58.1 ± 1.0^{h}
	90^{+Si}	0.048 ± 0.0015^{f}	0.0031 ± 0.0001^{i}	2.00 ± 0.11^{b}	2.03 ± 0.11^{c}	62.3 ± 1.3^{e}	2.5 ± 0.2^{b}	34.5 ± 1.2^{e}
	120^{+Si}	0.011 ± 0.0010^{a}	0.0015 ± 0.0001^{a}	0.93 ± 0.07^{a}	0.27 ±0.06^{a}	17.8 ± 1.0^{b}	0.6 ± 0.1^{a}	9.1 ± 0.5^{b}

As shown in table (2), low salinity level (30 mM) exhibited a significant reduction in phenolics content with or without silicon, compared to control. Higher sodium chloride (60, 90, and 120 mM) concentrations plus or minus Si resulted in a significant rise in phenolics content. Salinity stress caused a significant decrease in the concentration of reducing power in canola seedlings (Table 2). Si treatment at 30 and 60 mM NaCl gave a similar response to that given by the applied salinity treatment individually.

While the content of reducing power doubled at 90 mM NaCl and even get higher at 120 mM NaCl. Application of Si at 0mM NaCl caused induction in the content of total antioxidants in canola seedlings more than absolute control by about 2.34 fold as represented in table (2).

There has been an increase in the content of the total antioxidants at elevated salinity levels, whether or not adding silicon, but cannot deny the obvious improvement in the content of total antioxidants by adding silicon except for 60 mM NaCl, which showed higher total antioxidants without Si application.

Flavonoids content were fluctuated under different salinity treatments with or without Si (Table 2). The

lowest concentration (30 mM NaCl) hindered the production of flavonoids when compared to control, while elevated concentrations of NaCl (60 and 90 mM) resulted in a significant induction in the flavonoids content. The external application of Si gave the same response, a reduction observed at (30 and 60 mM) NaCl. The production of flavonoids enhanced significantly with the addition of Si at 90 and 120 mM NaCl.

Lipid peroxidation inhibition % (Table 2) showed a significant induction under 30 and 60 mM NaCl without Si, while, a significant reduction can be observed at 90 mM NaCl. External application of Si improved significantly the inhibition percent of lipid peroxidation, when compared to control, or the corresponding salinity level without Si, while the significant reduction was observed at 120 mM NaCl with Si treatment.

A significant increase was observed in the values of the hydroxyl radicle scavenging % under the salinity effect, which is more apparent when applying the silicon treatment (Table 2).While amendment with Si at 120 mM NaCl could not improve the significant retardation in the scavenging percent of hydroxyl radicle.

Table (2): Antioxidants and Free Radical Scavenging Activities of canola (*Brassica napus L*. cultivar Sarw 4) as influenced by NaCl (mM) stress and silicon (1 mM Na_2SiO_3), different letters are significantly different at $p<0.05$, (mean ± SE; n=3).

NaCl Conc. mM		Phenolics µg g^{-1}F.Wt.	Reducing power µg g^{-1}F.Wt.	Total antioxidants Abs. (at 695 nm ml^{-1})	Flavonoids mg g^{-1}F.Wt.	Lipid peroxidation Inhibition %	OH^- radical scavenging %	H_2O_2 radical scavenging %	Metal Chelating %
Control	Zero	0.49 ± 0.02^{c}	0.27 ± 0.015^{g}	14.32 ± 0.7^{a}	0.33 ± 0.021^{d}	87.38 ± 2.2^{c}	92.98 ± 4.1^{b}	90.10 ± 1.9^{b}	92.29 ± 4.2^{c}
Without Si	30	0.39 ± 0.013^{b}	0.14 ± 0.013^{c}	14.31 ± 0.6^{a}	0.27 ± 0.022^{c}	94.74 ± 5.1^{h}	96.03 ± 2.2^{g}	96.02 ± 1.4^{c}	94.17 ± 3.5^{g}
	60	0.92 ± 0.02^{i}	0.21 ± 0.012^{d}	45.10 ± 0.7^{h}	0.46 ± 0.011^{g}	88.72 ± 1.1^{e}	94.50 ± 1.4^{e}	98.94 ± 2.5^{i}	92.57 ± 3.4^{d}
	90	0.82 ± 0.03^{g}	0.23 ± 0.011^{e}	39.08 ± 0.9^{f}	0.39 ± 0.010^{e}	86.87 ± 2.4^{b}	93.05 ± 0.9^{c}	98.74 ± 3.1^{h}	91.93 ± 1.5^{b}
With Si	$Zero^{+Si}$	0.77 ± 0.03^{f}	0.26 ± 0.014^{f}	33.62 ± 0.6^{e}	0.47 ± 0.014^{h}	88.64 ± 3.1^{d}	93.09 ± 1.8^{d}	96.59 ± 1.4^{d}	92.81 ± 4.2^{f}
	30^{+Si}	0.38 ± 0.021^{a}	0.09 ± 0.011^{a}	20.62 ± 1.0^{b}	0.24 ± 0.008^{b}	94.86 ± 1.1^{i}	97.63 ± 0.99^{i}	98.63 ± 3.4^{g}	96.16 ± 1.3^{i}
	60^{+Si}	0.61 ± 0.01^{d}	0.11 ± 0.012^{b}	30.24 ± 1.5^{d}	0.27 ± 0.011^{c}	94.21 ± 2.1^{g}	97.15 ± 3.1^{h}	98.23 ± 2.1^{f}	95.44 ± 0.9^{h}
	90^{+Si}	$0.84 \pm 0.0.02^{h}$	0.58 ± 0.01^{h}	41.89 ± 1.1^{g}	0.43 ± 0.013^{f}	89.15 ± 3.2^{f}	95.06 ± 2.9^{f}	97.50 ± 1.1^{e}	92.58 ± 2.1^{e}
	120^{+Si}	0.70 ± 0.01^{e}	1.65 ± 0.01^{i}	25.93 ± 1.9^{c}	0.09 ± 0.009^{a}	60.60 ± 4.3^{a}	84.24 ± 3.2^{ga}	87.39 ± 1.2^{a}	83.34 ± 3.1^{a}

Under elevated concentration of NaCl (30, 60 and 90 mM) canola seedlings showed significant raise in H_2O_2 radical scavenging % as compared to control (Table 2). Silicon application showed similar trend at 30 and 60 mM NaCl, while higher level of salinity (90 mM) exhibited lower scavenging percent of H_2O_2 radical as compared to corresponding salinity level without silicon. Another reduction in H_2O_2 radical scavenging % observed at 120 mM NaCl with Si.

At sodium chloride concentrations (30 and 60 mM) a significant induction appeared at the chelating Percent of metals, however, higher concentration of NaCl (90 mM) reduced significantly the metal chelating % (Table 2). On the other hand, external application of Si improved significantly the chelating percent of metals of canola seedling at (30, 60 and 90 mM) NaCl as compared to control or corresponding salinity concentration without Si. While a significant reduction at metal chelating % observed at 120 mM NaCl with Si.

Salinity resulted in reducing the activity of Lypoxygenase enzyme (LOX). Also individual application of Si resulted in a significant reduction in LOX activity without NaCl as compared to absolute control, then significant gradual increase in LOX activity was observed at 30, 60, 90 and 120 mM NaCl with Si (Table 3).

Table 3 is representing the change in soluble proteins. As compared to control, a significant reduction was observed under 30 mM NaCl, while high levels (60 and 90 mM) exhibited a significant increase in soluble proteins. In contrast, external amendment with Si caused significant reduction in soluble proteins content at almost all salinity levels. While the individual significant induction in soluble proteins observed with silicon treatment was at 120 mM NaCl, as compared to control.

It was recorded a decrease in enzymatic activity SOD and POD whether we added salinity individually or with silicon (Table 3). On the other hand, a significant increase of SOD activity at (60 and 90 mM NaCl) with silicon amendment could be observed as compared to corresponding salinity levels without Si. Another significant induction could be observed of SOD and POD at 120 mM NaCl with Si. It was also observed that the application of silicon without salinity had a positive effect on the activity of both enzymes if compared to the control. Catalase enzyme (CAT) showed slight induction at (30 mM NaCl) or showed no change at (90 mM NaCl), except at 60 mM NaCl a significant reduction was observed (Table 3). Exogenous Si application reduced the activity of CAT under 0 and 30 mM NaCl, while significant increases in CAT activity were observed under 60 and 90 when compared to corresponding salinity levels. Si external application induced greatly CAT 120 mM NaCl. Ascorbate peroxidase enzyme (APX) activity is shown in Table 3. General reduction in the APX activity was observed at different concentration of NaCl with or without Si application, but obvious significant rise was occurred at 120 mM NaCl with Si.

DISCUSSION

Soil salinity influences negatively growth and productivity of crop plants. In the Middle East 20×10^6 ha area is affected by increased groundwater and soil salinity, reasons being irrigation practices, high evaporation rates, growth of sabkhas (salt scalds), and increase in groundwater salinity. In Egypt 1×10^6 ha cultivable land along the Nile is salt-affected (Shahid, 2013). Canola is very promising oil crop cultivated in Egypt.

Table (3): Soluble proteins content,lipoxygenase (LOX) and some enzymatic antioxidants of canola (*Brassica napus L*. cultivar Sarw 4) as influenced by NaCl (mM) stress and silicon (1 mM Na_2SiO_3), different letters are significantly different at $p<0.05$, (mean ± SE; n=3).

NaClConc. mM		LOX min mg^{-1} proteins	SOD unit mg^{-1} proteins	POD min mg^{-1} proteins	CAT min mg^{-1} proteins	APX min mg^{-1} proteins	Soluble Proteins mg g^{-1} F
Control	Zero	2.44 ± 0.10^{f}	16.0 ± 0.5^{h}	0.10 ± 0.004^{h}	0.10 ± 0.004^{d}	0.10 ± 0.002^{e}	30.0 ± 0.5^{e}
Without Si	30	1.02 ± 0.11^{b}	10.9 ± 0.9^{g}	0.09 ± 0.001^{g}	0.11 ± 0.006^{e}	0.07 ± 0.003^{d}	18.5 ± 1.1^{c}
	60	1.31 ± 0.12^{d}	7.2 ± 0.8^{a}	0.06 ± 0.001^{d}	0.06 ± 0.003^{a}	0.04 ± 0.002^{a}	38.8 ± 0.7^{i}
	90	0.99 ± 0.11^{a}	8.3 ± 0.2^{c}	0.08 ± 0.005^{f}	0.10 ± 0.009^{d}	0.10 ± 0.006^{e}	34.5 ± 2.0^{f}
With Si	$Zero^{+Si}$	1.17 ± 0.10^{c}	10.1 ± 0.3^{f}	0.06 ± 0.003^{c}	0.08 ± 0.004^{c}	0.05 ± 0.003^{b}	38.4 ± 1.0^{h}
	30^{+Si}	2.29 ± 0.15^{e}	7.9 ± 0.2^{b}	0.07 ± 0.003^{e}	0.07 ± 0.003^{b}	0.06 ± 0.002^{c}	14.0 ± 0.9^{a}
	60^{+Si}	2.54 ± 0.09^{g}	8.8 ± 0.2^{d}	0.05 ± 0.003^{a}	0.12 ± 0.006^{f}	0.06 ± 0.004^{c}	15.9 ± 1.1^{b}
	90^{+Si}	4.63 ± 0.10^{i}	9.9 ± 0.3^{e}	0.06 ± 0.003^{b}	0.08 ± 0.002^{c}	0.05 ± 0.001^{b}	26.1 ± 1.3^{d}
	120^{+Si}	4.29 ± 0.11^{h}	28.1 ± 0.4^{i}	0.14 ± 0.005^{i}	0.22 ± 0.002^{g}	0.14 ± 0.007^{f}	34.6 ± 1.6^{g}

One of the approaches to improve growth and productivity of crop plants under soil salinity is the fertilizer treatments, which are capable to withstand unfavorable environmental conditions. To resist or avoid stress conditions, plants have evolved complex mechanisms to counter NaCl toxicity in soil caused by salinity (Munns and Tester, 2008). Recently, it was reported that exogenous application of Si contributed to the growth of strawberry plants by modulating ion homeostasis and antioxidant defense system (Ouellette *et al*., 2017, Yaghubi *et al*., 2016). Our study clearly showed that exogenous Si application improved salt tolerance in canola seedlings. Something was obvious as external application of silicon sustained the survival of canola seedling under 120 mM NaCl. Seed germination and seedling establishment under high salt concentrations is very desirable response. The physiological responses implied that Si could modify the pace of physiological metabolisms and modulate the complex pathways of regulation under salt stress conditions. In this experiment, it has been shown that salt stress in canola caused very significant reductions in fresh weight. Our biomass data are in agreement with the work of Bar-Tal *et al.* (1991) for corn, and Kaya *et al.* (2001) for tomato. Inhibitory effect of salinity on plant growth may either be due to high ions (Na^+ and Cl^-) accumulation in plant tissues or to osmotic reduction in water availability (Gunes *et al*., 1995).

In the present study gradual raise in dry matter observed at elevated levels of salinity stress even at 90 mM NaCl. According to Abdul Qados (2011) the application of salinity at 60 and 120 mM NaCl in bean plant *(Vicia faba* L.) enhanced dry weight. Also, findings by Dantus *et al.* (2005) on cowpea (*Vigna unguiculata* L.), and Nedjimi *et al*. (2006), on (*Atriplex halimus* L.) agreed with the results of our study, they reported that with increasing concentrations of sodium chloride, dry weights of their seedlings increased.

On the other hand, there are findings, as well, representing the negative effect of salinity on dry matter. Such as a study on radish plants *Raphanus sativus* L by Jamil *et al*. (2007), another study on *Bruguiera gymnorrhiza* L., by Rui *et al*. (2009).

Salinity significantly suppressed shoot height and root length of canola, however root tissues received more stress than shoots (Table 1). Similar results were also reported on sugarcane, wheat and purslane (Kafi and Rahimi 2011; Ashraf et al. 2010; Tahir et al. 2010). It might be due to the direct contact of root tips with stress. Plant growth requires both proliferation and elongation of cells; so, growth reduction due to salinity stress may be attributed to osmotic stress, ion imbalance and ion toxicity (Marschner, 1995; Tahir et al., 2006; Yazici et al., 2007), that resulted in loosing the turgor and DNA synthesis for cell growth.

Supplementary silicon greatly improved plant growth and enhanced significantly the dry matter, Plant height, and root length of plants grown under salinity conditions. Similar results were observed in rice, cucumber, tomato and barley (Bonilla and Tsuchiya, 1998; Miyake, 1992; Liang, 1999). Ample evidences suggesting that Si plays very favorable role in plant growth under biotic and abiotic stresses. For instance, Tahir *et al.* (2010) found that silicon application significantly increased salt-treated plant biomass. External application of Si to salinity stressed plants has ameliorative effect through enhancing K^+/Na^+ ratio (Kafi and Rahimi, 2011), improving quenching capacity of ROS (Hasanuzzaman *et al*., 2013) and protecting the cell membrane against lipid peroxidation. Liang *et al*. (1996) reported that external application of silicon accompanied with high salinity levels enhanced the growth of barley. He attributed this response to reduced electrolyte leakage in the leaves. Zhu *et al.* (2004) suggested that Si may decrease the plasma membranes permeability and retarded the peroxidation of membrane lipids besides maintaining the membrane integrity and functions of salt-stressed cucumber, thus mitigating against salt toxicity and improving the growth of plants. Si supplementation helps the formation of secondary and tertiary cells of the endodermis, thus enabling higher root resistance and a stronger growth of roots (Munns and Tester, 2008).

Crop growth could be related to rate of photosynthesis which is directly proportional to chlorophyll contents in leaves. The change in Chlorophylls content is a sensitive indicator of the cellular metabolic state especially under stresses; the reduction in its content is a commonly reported phenomenon under salinity (Chutipaijit *et al*., 2011). According to our results, salinity reduced Chl a contents; however, external application of Si to these plants buffered and improved the adverse

effect of salinity on chl. a pigments. In the contrast, no mitigation impact of Si was found on chl.b (Table 3). Inhibition of chlorophyll biosynthesis (Haghighi and Pessarakli, 2013), acceleration of its degradation (Jamali *et al*., 2015) and oxidative damage induced by salinity (Munns and Tester, 2008) could be considered as main reasons for the declining the chlorophylls content. The present data of chlorophyll are also in agreement with the work of Yeo *et al*. (1990) on rice and that of Belkhodja *et al*. (1994) on barley, both showed the adverse effect of high NaCl on leaf chlorophyll concentrations.

In the present study silicon nutrition can recover the chlorophyll a content of canola plants under salinity. Previous studies have pointed to the positive effect of silicon to improve the chlorophyll content of canola and barley under salt stress (Liang *et al*., 1996; Kafi and Rahimi, 2010). This was interpreted as possible influence of silicon on the biosynthesis of new chlorophylls and the protection of existing chlorophylls against salinity-induced oxidative stress (Shekari *et al.*, 2015). Our data reviled negative impact of salinity stress on carotenoids particularly at elevated concentration of salinity (Table 1). Mane *et al*. (2011) reported that, carotenoids can preserve its function as accessory pigments at low levels of salt concentration, but these molecules are inhibited and unable to protect chloroplast from photo-oxidative damage at higher levels. The inhibitory effect of Salinity over carotenoid content has been previously reported in maize and wheat genotypes (Singh *et al.*, 2008; Sairam *et al.*, 2002). The data obtained in this study, showed clearly a pronounced increase in the carotenoid content when Si incorporated with salt treatments. Mane *et al.* (2011) also reported that carotenoids can protect plants against oxidative stress. They are one of the non-enzymatic antioxidants along with vitamin C, vitamin E and lipoic acid.

In the current study, the determination of lipid peroxidation inhibition %, hydroxyl radical scavenging %, H_2O_2 radical scavenging % and metal chelating % were used to evaluate the ability of the antioxidant system of the stressed cell to protect its different compartments and membranous system from peroxidation and deterioration under salinity stress, in addition to evaluate the protective action of silicon. Our data showed that incorporation of Si into salt-treatment increased lipid peroxidation inhibition % compared with the treatment of salt alone (Table 2). In previous study, it was reported that Si enhanced the stability of lipids in cell membranes of rice plants exposed to drought and heat stresses, suggesting that Si prevented the structural and functional deterioration of cell membranes when rice plants were exposed to environmental stress (Agarie *et al*., 1998).

Hostile environments such as salinity impairs cellular electron transport within the different subcellular compartments and leads to generation of reactive oxygen species (ROS) such as hydrogen peroxide, superoxide, hydroxyl radical and singlet oxygen (Lee *et al*., 2001), which triggers phytotoxic reactions such as lipid peroxidation and membrane damage (Parihar *et al.*, 2015). Results presented in table (2) showed that external application of silicon obviously enhanced the ability of canola cells to scavenge the hydroxyl free radical and metal chelating as compared with seedling subjected to salinity without silicon.

Loading plot of different studied attributes correlations to the first two Principle component analysis (PCA) axes (Fig. 1) and Cluster analysis (Fig. 2) emphasized a strong positive correlation among the scavenging percent of hydroxyl, metal chelating % and lipid peroxidation inhibition. This findings implying to the protective effect of silicon against ROS generation under salinity stress which had a strong stabilizing effect on membranes function and integrity, thus our data suggests that Si application could maintain the permeability of plasma membranes and sustain the membrane integrity and functions of canola plant under salinity, mitigating against oxidative burst and salt toxicity. Something reflected in the final improvement of seedling growth.

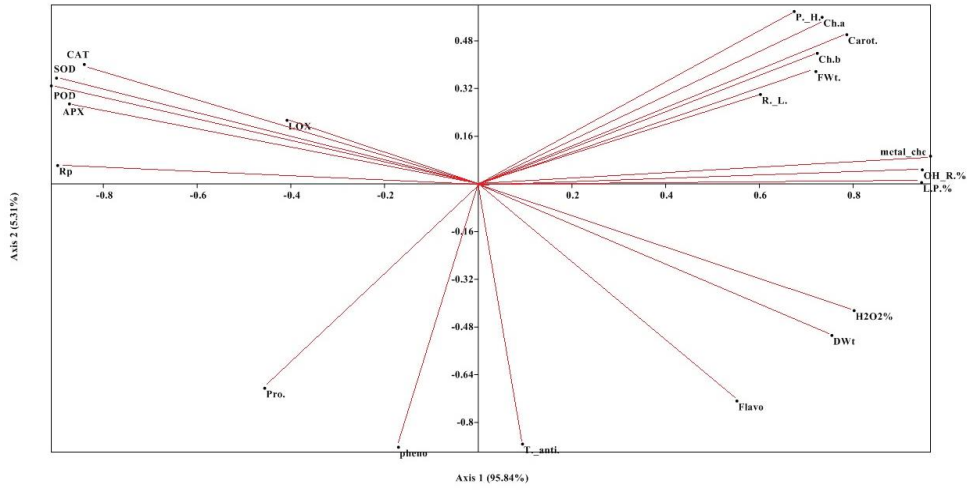

Figure (1): Loading plot of different studied attributes correlations to the first two Principle component analysis (PCA) axes. Abbreviations: Pheno = Total Phenolics, T-anti = Total Antioxidants, Pro = Proteins, Flavo. = Total Flavonoids, Rp = Reducing Power, LOX = Lipoxygenase, POD = Peroxidase, SOD = Superoxide Dismutase, CAT = Catalase, APX = Ascorbate peroxidase, Ch.a = Chlorophyll a, Ch.b = Chlorophyll b, Carot. = Carotenoids, LP% = Lipid peroxidation inhibition %, OH-R = Hydroxyl radical scavenging %, MC = Metal chelating %, H_2O_2 %= Hydrogen peroxide scavenging %, R.L= Root length, P.H. = Shoot height, DWt = Dry weight, FWt = Fresh weight.

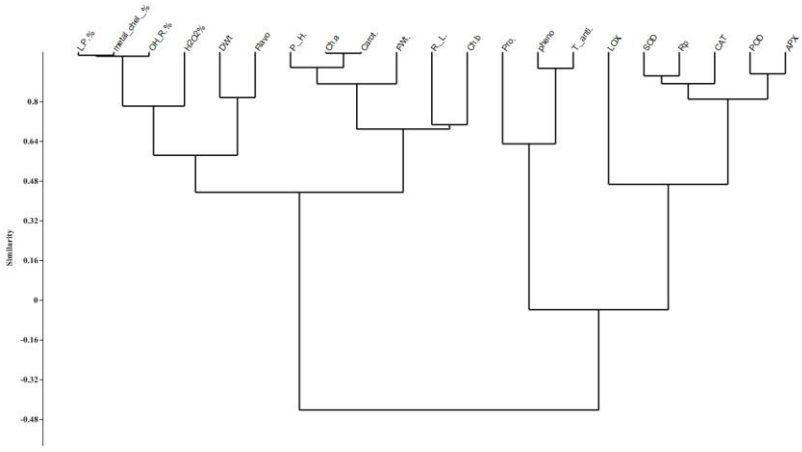

Figure (2): Cluster analysis of measured attributes show significance.

The balance in the steady-state level of superoxide radicals and hydrogen peroxide, together with sequestering of metal ions, is thought to be important to prevent the formation of the highly toxic hydroxyl radical via the metal-dependent Haber-Weiss or the Fenton reactions (Asada and Takahashi, 1987). Timely induction of cellular antioxidant machinery is considered as a vital approach for protection against various abiotic stresses via scavenging or detoxifying the ROS generated therein (Khare *et al.*, 2015).

In the present study, salinity induced the content of phenolics in seedlings of canola significantly with or without Si. Navarro *et al.* 2006 showed similar results, he reported an enhancement in total phenolic contents at moderate saline levels in red peppers (*Capsicum annuum*). Phenolic compounds can act as antioxidant to scavenge ROS in plants under stresses (Solecka, 1997). The present data indicated that moderate and high salinity concentrations enhanced flavonoid without Si (Table 2). This may be due to enzymatic activity inductions occurring under salinity condition and resulting in synthesis of different flavonoid compounds (Haghighi *et al.*, 2012). It has been found that there is considerable increase in flavonoid levels following biotic and abiotic stresses, such as wounding, drought, metal toxicity and nutrient deprivation (Winkel-Shirley, 2002). Flavonoids and other phenolics, these secondary metabolites play multiple roles in plants, including scavenging of ROS induced under different stress, they promote roles in plant protection against damaging effects (Sonar *et al*., 2011).

The measurement of reducing power of a compound may serve as a significant indicator of its potential antioxidant activity. An obvious increase in reducing power assay was observed when Si incorporated at (90 and 120 mM NaCl), while a significant induction was observed when salinity applied alone at (30 and 60 mM NaCl). The results showed that Si application can improve the redox status of canola under high salinity levels.

On the other hand, salt stress incorporated with Si significantly increased the total antioxidants content of canola seedlings, which increased with increasing the salinity levels. In the present work, PCA and cluster analysis showed positive correlation between total antioxidants and phenol contents. Statistical evaluation by pearson correlation between total antioxidants and total phenolic contents (Table 4) was found to be highly significant (r= 935**). On the other hand, correlation between the total antioxidants and reducing power was found to be non-significant (r= 0.023). Another non-significant correlation was observed between reducing power content and the total phenolic contents (r= 0.23).This indicates that phenolic compounds might be a major contributor to the antioxidant capacities under salinity stress.

It has been reported by many researchers the positive correlation between the total phenolic content and antioxidant activity (Chew *et al.*, 2008 and Wang *et al*., 2009). Djeridane *et al.* (2006) reported synergistic interactions in a mixture of phenolic and interactions between the antioxidants. It has been proven that the antioxidant activity of plant extracts is mainly ascribable to concentration of phenolic compounds in plant (Heim *et al*., 2002). Based on these results (Gressel and Galun, 1994) showed that adaptation to ionic and osmotic stresses caused by salinity need complex mechanisms evolved by plants. The capacity of the antioxidant defense system to increase under stress conditions may be associated with salt tolerance (Abogadallah, 2010). Increased LOX activity, presented in this work under salinity stress with Si compared to salinity without Si treatments suggests higher lipolytic activity of the membranes and oxidation of membrane-bound fatty acids, which propagates higher lipid peroxidation (Tavallali *et al*., 2010). On the other hands, the previous data of lipid peroxidation inhibition % shows higher level of membranes protection under Si treatment. This could be attributed to the biosynthesis of enzymatic and non-enzymatic antioxidants to protect membranens. SOD, CAT, APX and POS are the major antioxidant enzymes associated with scavenging the ROS (Marschner, 1995). In this study, SOD and POD activities were decreased by adding the NaCl to nutrient medium (Table 3). On the other hand, external application of Si improves the activity of SOD at 60 and 90 mM NaCl.

Among ROSs, H_2O_2 is a functional element, which is produced by activity of superoxide dismutase (Eraslan *et al.*, 2008). High ratios of hydrogen peroxide as well as malondealdehyde under salinity are responsible for membrane deterioration (Gupta and Huang, 2014). Mitigation role of Si on salt-induced cell damage is well documented (Eraslan *et al*., 2008; Haghighi and Pessarakli, 2013). Shekari *et al*., (2015) revealed that application of Si to salt-subjected plants decreased significantly the content of MDA and H_2O_2. Our results give supporting information that cellular damage induced by NaCl is alleviated by Si, as represented by higher lipid peroxidation inhibition % as well as higher hydroxyl radicle scavenging %.

Silicon application enhanced the activity of CAT and APX at 60 mM NaCl as compared to corresponding salinity level without Si. In addition to these findings, work showed that CAT and APX activities even got higher at 120 mM NaCl. Gossett *et al*. (1994) reported that in cotton, NaCl decreases the activity of APX. Results further highlighted that external application of Si with increasing salt concentrations, resulted in escalation in CAT and APX activities, which indicating their crucial role in scavenging O_2^- during salt stress.

The present study showed higher protein concent-rations in salt stressed seedling without Si than in salt stress seedling with Si (Table 3). To survive under stress, plants accumulate proteins that protect cells from stress effects (Wang *et al.*, 2003). Protein contents in *Vignia unguiculata* (L) were not affected compared to controls, while it significantly increased in the stems of plants grown with 100 mM of sodium chloride (Franco *et al*., 1999). This could be a consequence of salinity stress on protein synthesis as previously reported by (Omar *et al*., 1993). Salinity stress which frequently leads to oxidative stress may cause denaturing of structural and functional proteins (Shanker *et al*., 2004; Mandhania *et al*., 2006). The diverse environmental stresses often promote similar cell signaling pathways (Foyer *et al*., 1994) and cellular responses, such as the production of stress proteins and up regulation of antioxidants (Zhu *et al*., 1997).

Table 4: Correlation coefficient values (r2) among different parameters of canola (*Brassica napus L*. cultivar Sarw 4) as influenced by NaCl (mM) stress and silicon (1 mM Na_2SiO_3).

	F.Wt.	D.Wt.	Shoot H.	Root L.	Ch.a	Ch.b	Carot	LOX	SOD	POD	CAT	APX	Pro	pheno	Rp	T-anti	Flavo.	LP %	OH˙R. %	$H_2O_{2\,\%}$
D.Wt.	0.547																			
shoot H.	.871**	0.274																		
Root L.	0.58	0.439	.704*																	
Ch.a	.864**	0.345	.947**	.756*																
Ch.b	0.521	0.224	.753*	.708*	.730*															
Carot	.881**	0.417	.929**	.749*	.995**	.725*														
LOX	0.193	-0.107	-0.079	-0.025	-0.047	-0.406	-0.064													
SOD	-0.421	-.785*	-0.318	-0.33	-0.372	-0.548	-0.446	0.556												
POD	-0.645	-.902**	-0.492	-0.47	-0.534	-0.524	-0.597	0.318	.91**											
CAT	-0.39	-.793*	-0.274	-0.431	-0.391	-0.401	-0.465	0.473	.90**	.81**										
APX	-0.572	-.898**	-0.403	-0.48	-0.546	-0.388	-0.614	0.301	.84**	.91**	.84**									
Pro	-0.566	-0.018	-0.539	-0.257	-0.584	-0.589	-0.592	-0.118	0.25	0.21	0.08	0.20								
pheno	-0.239	0.414	-0.46	-0.232	-0.49	-0.493	-0.46	0.101	-0.08	-0.23	-0.09	-0.15	.75*							
Rp	-0.468	-0.55	-0.5	-0.332	-0.523	-0.651	-0.576	.688*	.90**	.77*	.84**	.70*	0.35	0.232						
T-anti	-0.078	0.611	-0.343	-0.083	-0.329	-0.255	-0.28	0.054	-.35	-.48	-.29	-.38	0.51	.935**	0.023					
Flavo.	0.208	.816**	0.044	0.199	0.055	0.006	0.12	-0.401	-.69*	-.71*	-.80**	-.69*	0.42	0.561	-0.564	0.608				
LP%	0.606	0.646	0.549	0.384	0.598	0.637	0.659	-0.513	-.90**	-.81**	-.84**	-.78*	-0.51	-0.283	-.960**	-0.036	0.519			
OH˙R. %	0.649	0.65	0.551	0.395	0.629	0.657	.692*	-0.395	-.89**	-.82**	-.80**	-.79*	-0.61	-0.294	-.911**	-0.007	0.428	.980**		
$H_2O_{2\,\%}$	0.322	.778*	0.193	0.287	0.252	0.516	0.324	-0.461	-.95**	-.89**	-.77*	-.80**	-0.25	0.2	-.749*	0.498	0.597	.775*	.801**	
m. ch.%	0.65	0.614	0.623	0.473	.678*	.735*	.732*	-0.474	-.89**	-.81**	-.81**	-.77*	-0.57	-0.334	-.948**	-0.065	0.441	.987**	.986**	.771*

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

CONCLUSION

In conclusion, silicon had ameliorative effects on canola germination, growth, chlorophyll a, and antioxidant parameters under salinity. This can provide a basis for attempting new strategies for diminishing the salinity damages and establishing a functional link between silicon function, morphophysiological response and salt stress tolerance in canola plants. Our results showed that the higher reactive oxygen species scavenging capacities induced in canola seedlings by Si application and this ability was associated with the higher level of lipid peroxidation inhibition %, reducing power and carotenoids contents. These data suggest silicon can improve the redox status of canola cells. This agricultural practice is recommended for saline soils.

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التأثيرالمحسن لعنصر السليكون على صفات النمو والصفات الفسيولوجية والبيوكيميائية لبادرات الكانولا (*Brassica napus* L.) تحت الأجهاد الملحي

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الملخص العربي

تستهدف الدراسة الحالية التعرف علي الدور الذي يمكن أن يقوم به عنصر السيليكون (سيليكات الصوديوم بتركيز 1 ملل مول) في تحسين التأثير الضار للإجهاد الملحي (كلوريد الصوديوم بتركيزات 0، 30، 60، 90،120ملل مول) علي نمو بادرات نبات الكانولا (*Brassica napus L.*). أظهرت الدراسة التأثير السلبي للملوحة علي التوازن الخلوي والذي أتضح من خلال أنخفاض قيم النسب المئوية لكبح الشقوق الحرة وبالأخص النسب المئوية لكبح مجموعة الهيدروكسيل (HO^-) كأحد الشقوق الحرة وقيم النسب المئوية لإزالة المعادن (مخلبية المعادن) مما ترتب عليه توهين تخليق الخضوب النباتية (الكلورفيل أ والكاروتينات). مع ذلك،استطاعت التغذية بعنصر السيليكون أن تحسن من التوازن الخلوي في خلايا بادرات الكانولا عن طريق رفع قدرة الخلية علي التخلص من الشقوق الحرة الزائدة. والذي أتضح من رفع قدرة الخلية علي التخلص من الهيدروكسيل (HO^-) كشق حر وزيادة القدرة المخلبية للتخلص من المعادن. بالإضافة إلى ذلك، حافظ السيليكون على سلامة الأغشية منخل الرفع قدرة الخلية علي منع التأكسد للأحماض الدهنية في الاغشية البلازمية. ساعد عنصر السيليكون علي حماية الخلية من ضغوط الأكسدة تحت الاجهاد الملحي عن طريق تحسين النظام (الانزيمي واللاإنزيمي) المضاد للأكسدة. حيث حسن نشاط عدة أنزيمات المضادة للأكسدة (CAT, SOD, APX, POS). بالأضافة لزيادة نشاط مضادات الاكسدة اللاأنزيمية مثل (الكاروتينات والفينولات) تحت تركيز 120 ملل مول كلوريد صوديوم. وهكذا يتضح ان عنصر السيليكون لعب دورا هاما في مقاومة الاجهاد الملحي في بادرات نبات الكانولا من خلال عدة طرق أهمها زيادة قدرة الخلية علي كبح الشقوق الحرة والتخلص منها ومن ثم تقليل الإجهاد التأكسدي بالخلية. كما لعب السليكون دورا واضحا في حماية الاغشية البلازمية من الأكسدة. الامر الذي انعكس علي معدلات النمو لبادرات الكانولا من خلال رفع المحتوي من مكونات الأختضاب (الكلوروفيل).

الكلمات المفتاحية: الملوحة، السيليكون، الإنزيماتالمضادة للأكسدة، النسبة المئوية لتثبيط تأكسد الدهون في الأغشية، النسبة المئوية لكبح الشق الحر HO^-،النسبة المئوية لإزالة المعادن(مخلبية المعادن).