

# Influence of Exogenous Application of Silicon on Physiological Response of Salt-stressed Maize (*Zea mays* L.)

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## ABSTRACT

The influence of silicon (Si, 3 mM), sodium chloride (NaCl, 135 mM), and Si, 3 mM + NaCl, 135 mM supply on chlorophyll content, photosynthetic activity ( $^{14}\text{CO}_2$  -fixation), the concentration of malondialdehyde (MDA) and  $\text{H}_2\text{O}_2$ , activities of superoxide dismutase (SOD), catalase (CAT) enzymes, free proline and protein contents were studied in maize seedlings leaves after two month of treatments. The results indicated that silicon partially offset the negative impacts and increased tolerance of maize to NaCl stress by enhancing SOD and CAT activities, chlorophyll content and photosynthetic activity. Salt stress although decreased SOD, CAT activities and total soluble protein content, addition of silicon (3 mM) to the nutrient solution enhanced SOD and CAT activities and total protein. In contrast, salt stress considerably increased  $\text{H}_2\text{O}_2$ , free proline level and MDA concentration and Si addition significantly reduced  $\text{H}_2\text{O}_2$ , free proline level and MDA concentration in stressed maize leaves. Enhanced activities of SOD and CAT by Si addition may protect the plant tissues from salt induced oxidative damage, thus mitigating salt toxicity and improving the maize growth. These results suggest that the scavenging system forms the primary defense line in protecting oxidative damage under salt stress in crop plants.

**Key Words:** Maize (*Zea mays* L.); Antioxidant enzyme; Silicon; Salinity; Photosynthesis

## INTRODUCTION

Corn, (*Zea mays* L.) is one of the most important cereal crops growing in the Arab Republic of Egypt. It is used as a food for human consumption as well as food grain for animals (Moussa, 2001). Excessive soil salinity, resulting from natural processes or from crop irrigation with saline water, occurs in many semi-arid to arid regions of the world where it inhibits plant growth and yield (Lauchli & Epstein, 1990). Overcoming salt stress effects is main issue in order to ensure agricultural sustainability in food production. Attempts to improve tolerance to salinity through physiological selection criteria, has increased substantially to improve the probability of success by making empirical selection more efficient (Noble & Rogers, 1992).

It is now well known that salinity exerts oxidative stress due to the production of variety of active oxygen species (AOS) such as superoxide anion ( $\text{O}_2^{\cdot-}$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and hydroxyl ( $\text{OH}^{\cdot}$ ) radicals (McCord, 2000). To scavenge these toxic species, plants develop antioxidant enzymes, such as superoxide dismutase (SOD, EC. 1.15.1.1) is a major scavenger of  $\text{O}_2^{\cdot-}$  and enzymatic action results in the formation of  $\text{H}_2\text{O}_2$ . Catalase (CAT, EC. 1.11.1.6) scavenges hydrogen peroxide, the two electrons reduced from of oxygen ( $2\text{H}_2\text{O}_2$  to  $2\text{H}_2\text{O}$  &  $\text{O}_2$ ). Their activities and transcripts are altered when plants are subjected to different stressors including salinity (Hasegawa *et al.*, 2000; Hernandez *et al.*, 2000). Generally, it is assumed that salt-induced damage to membranes is negatively correlated with the capacity for increasing activities of enzymes in plants.  $\text{H}_2\text{O}_2$  is the first stable

compound among AOS produced in the plant cell under normal or stressful condition. Hence, it is the most probable candidate for AOS-mediated signal transduction. This compound is relatively stable, is able to penetrate the plasma membrane as an un-charged molecule, and therefore can be transported to the site of action (Foyer *et al.*, 1997). Previous findings showed that added silicon to salt treated barley significantly increased superoxide dismutase, catalase activity and decreased malondialdehyde (MDA) concentration in plant leaves (Liang, 1999).

Silicon, the mineral substrate is the most abundant element in the soils for most of world's plants (Epstein, 1994). Improvement of plant growth by addition of Si is beneficial for increased tissue tolerance of bean to high manganese concentration (Horst & Marschner, 1978) and in rice (Horiguchi, 1988), to alleviate both biotic and abiotic stresses in plants (Epstein, 1994) to enhance the salt tolerance of mesquite (Bradbury & Ahmad, 1990). Ahmad *et al.* (1992) reported that addition of small amount of soluble silicon enhanced salt tolerance of wheat. Si enhanced the growth of salt-treated barley, by improving the chlorophyll content and photosynthetic activity of leaf cell organelles of barley (Bradbury & Ahmad, 1990; Ahmad *et al.*, 1992; Liang, 1998).

The purpose of this study was to provide additional information on exogenous application of Si and its ability to counteract salt inhibitory effects in maize. The hypothesis was whether the increased salt resistance by Si is mediated via the antioxidative system of the key enzymes involved in oxidation stress defense, such as SOD and CAT in stressed maize. Effect of Si on seedling dry weight, chlorophyll content, photosynthetic activity ( $^{14}\text{CO}_2$  -fixation) and  $\text{H}_2\text{O}_2$

accumulation and any protective role on soluble protein content, the level of proline and lipid peroxidation were determined.

## MATERIALS AND METHODS

**Plant material.** A homogenous lot of maize seeds (*Zea mays* L.) var. Giza 311, were obtained from the Crop Institute, Agricultural Research Center, Giza, Egypt. They were surface sterilized in 0.1% (w/v) sodium dodecyl sulphate (SOD) solution and then thoroughly rinsed with sterile deionized water. The seeds were placed in sterile Petri plates containing two sheets of sterile filter paper moistened with distilled H<sub>2</sub>O and allowed to germinate in the dark at 24°C for five days, selected seedlings of equal size and vigor were transplanted to plastic pots contained 3000 mL continuously aerated full-strength Hoagland's nutrient solution (Hoagland & Arnon, 1950) contained the control and the predetermined concentrations of three treatments [3 mM Si as Na<sub>2</sub>SiO<sub>3</sub>, sodium chloride (NaCl, 135 mM equivalent to an osmotic potential of 0.66 MPa) and Si, 3 mM + NaCl, 135 mM]. The pots were incubated at 25 ± 1°C and light intensity 3000 Lux 12 h throughout the experiment. The pH was monitored two times a week and maintained between 6 and 6.5 with H<sub>2</sub>SO<sub>4</sub>. Solution was replaced every 7 days. The experimental design was randomized complete block design with three replicates. One treatment has three replications, four plants in each plastic pot solution fitted with insulated covers. All plants were harvested two month after treatments and separated into leaves, stem and root. For antioxidant enzyme assays, lipid peroxidation and free proline level determination, leaves were stored at -20°C prior to analyses.

**Enzyme activity, lipid peroxidation and H<sub>2</sub>O<sub>2</sub> content.** The activity of catalase (CAT, EC. 1.11.1.6), was determined as described by Aebi (1984) and superoxide dismutase (SOD, EC. 1.15.1.1), was assayed as given by (Giannopotitis & Ries, 1977). Lipid peroxidation level was determined as the content of malondialdehyde (MDA) using the thiobarbituric acid reaction as described by Madhava-Rao and Sresty (2000). The contents of hydrogen peroxide in maize leaves were measured according to Patterson *et al.* (1984).

**Growth parameters and chlorophyll contents.** For dry weight determination, samples were oven dried at 70°C for 72 h and then weighed. Chlorophyll content was quantified according to Porra *et al.* (1989).

**Photosynthetic activity (<sup>14</sup>CO<sub>2</sub> -fixation).** Photosynthetic activity (<sup>14</sup>CO<sub>2</sub> -fixation) was measured in the Atomic Energy Authority Radioisotope Department, Cairo Egypt, with the method of Moussa (2001). One pot from each treatment was placed under a Bell jar, which was used as a photosynthetic chamber. Radioactive <sup>14</sup>CO<sub>2</sub> was generated inside the chamber by a reaction between 10% HCl and 50 μCi (1.87 × 10<sup>6</sup> Bq) NaH<sup>14</sup>CO<sub>3</sub> + 100 mg Na<sub>2</sub>CO<sub>3</sub> as carrier. Then the samples were illuminated with a tungsten lamp. After 30 min exposure time, the leaves were quickly

detached from the stem, weighed and frozen for 5 min to stop the biochemical reactions, then subjected to extraction by 80% hot ethanol. The <sup>14</sup>C was assayed from the ethanolic extracts in soluble compounds using a Bray Cocktail (Bray, 1960) and a Liquid Scintillation Counter (LSC2 -Scaler Ratemeter SR7, Nuclear Enterprises).

**Free proline and protein content.** Free proline content was quantified according to the method of Bates *et al.* (1973). Protein content was measured according to Bradford (1976). **Statistical analysis.** Analyses of variance (ANOVA) for all the variables were carried out using SAS analysis. Treatment means were compared using the protected least significant difference (LSD) test at p<0.05 levels.

## RESULTS AND DISCUSSION

**Dry weight.** The data in (Table I) shows that added Si increased dry matter accumulation in all parts of maize plants under salt stress and the increase in leaf and total plant was significant (p<0.05). NaCl applied in nutrient solution resulted in considerable decrease in dry matter accumulation. Added Si did not affect dry matter accumulation under no salt stress, indicating that Si in nutrient solution alleviated the growth inhibition induced by added NaCl. These results are in corroborations with the findings of Liang (1998).

**Chlorophyll content.** Salt stress significantly decreased both chl a and b content, however, Si supplementation increased them under salt stress after two month of treatments (Table II). These results are in accordance with that of Al-aghabary *et al.* (2004).

**Photosynthetic activity (<sup>14</sup>CO<sub>2</sub> -fixation).** It is apparent that, application of salinity at 135 mM NaCl to maize plants induced remarkable inhibition (43.2%) in their photosynthetic activities as compared with the control samples (Table II). It has been noticed that NaCl stress decreased the rate of CO<sub>2</sub> assimilation and the leaf chlorophyll of barley (Popova *et al.*, 1995) and bean (Sibole *et al.*, 1998). Significant acceleration in <sup>14</sup>CO<sub>2</sub> -fixation rate was observed upon treatment with Si + NaCl by 42.3% as compared with stressed maize plants. Silicon treatment increased the photosynthetic efficiency by 22.2% as compared with the control plants. These results are in accordance with that of Liang (1998) and Al-aghabary *et al.* (2004), who found that added Si improved the chlorophyll content and photosynthetic activity of salt-treated barley and tomato. Added Si improved plant defense system to detoxify AOS induced under salt stress, which in turn helped in increased chlorophyll content and photosynthetic activity (Liang, 1998). These results confirm that the scavenging system forms the primary defense line in protecting oxidative damage under stress in crop plants.

**Enzymes.** Salt stress significantly decreased the SOD and CAT however, added Si improved the activity of CAT and SOD under salt stress. As salinity stress can cause membrane damage, reduced uptake of CO<sub>2</sub> as a result of stomatal closure, decreased hydrolytic enzyme activity and

**Table I. Changes in dry weight of leaf, stem, root and total plant in maize seedlings after two month of treatments with (0.0, 3 mM Si, 135 mM NaCl and 3 mM Si + 135 mM NaCl).**

Treatments	Leaf	Stem	Root (g plant <sup>-1</sup> )	Total Dry Weight
0.0	15.6±0.8 <sup>b</sup>	24.6±1.9 <sup>a</sup>	6.3±0.3 <sup>a</sup>	46.5±3.7 <sup>a</sup>
Si	17.8±0.9 <sup>a</sup>	22.5±1.8 <sup>a</sup>	7.1±0.6 <sup>a</sup>	47.4±3.6 <sup>a</sup>
NaCl	4.9±0.4 <sup>d</sup>	8.9±0.4 <sup>b</sup>	4.8±0.4 <sup>a</sup>	18.6±1.6 <sup>c</sup>
Si+NaCl	7.4±0.5 <sup>c</sup>	13.2±1.2 <sup>b</sup>	5.5±0.5 <sup>a</sup>	26.1±1.3 <sup>b</sup>
LSD 0.05	1.2	5.3	2.5	5.1

Data are means±SE (n=3). Means followed by the same letter are not significant different between the treatments at *p*<0.05 level of LSD.

**Table II. Changes in chlorophyll a & b and photosynthetic activity in maize leaves after two month of treatments with (0.0, 3 mM Si, 135 mM NaCl and 3 mM Si + 135 mM NaCl).**

Treatments	Chl a (mg g <sup>-1</sup> FW)	Chl b (mg g <sup>-1</sup> FW)	Chl a+b (mg g <sup>-1</sup> FW)	Photosynthetic Activity ( <sup>14</sup> C <sup>14</sup> mg <sup>-1</sup> FW)
0.0	2.18±0.29 <sup>a</sup>	0.77±0.07 <sup>a</sup>	2.95±0.21 <sup>a</sup>	12.643 <sup>a</sup>
Si	1.87±0.05 <sup>b</sup>	0.66±0.03 <sup>b</sup>	2.53±0.12 <sup>b</sup>	15.459 <sup>b</sup>
NaCl	1.45±0.05 <sup>c</sup>	0.52±0.03 <sup>c</sup>	1.97±0.12 <sup>c</sup>	7.178 <sup>c</sup>
Si+NaCl	1.68±0.04 <sup>c</sup>	0.59±0.04 <sup>bc</sup>	2.27±0.15 <sup>bc</sup>	10.214 <sup>d</sup>
LSD 0.05	0.27	0.085	0.29	1.094

<sup>a</sup> Kilobecquerel (10<sup>3</sup>Bq).

Data are means±SE (n=3). Means followed by the same letter are not significant different between the treatments at *p*<0.05 level of LSD

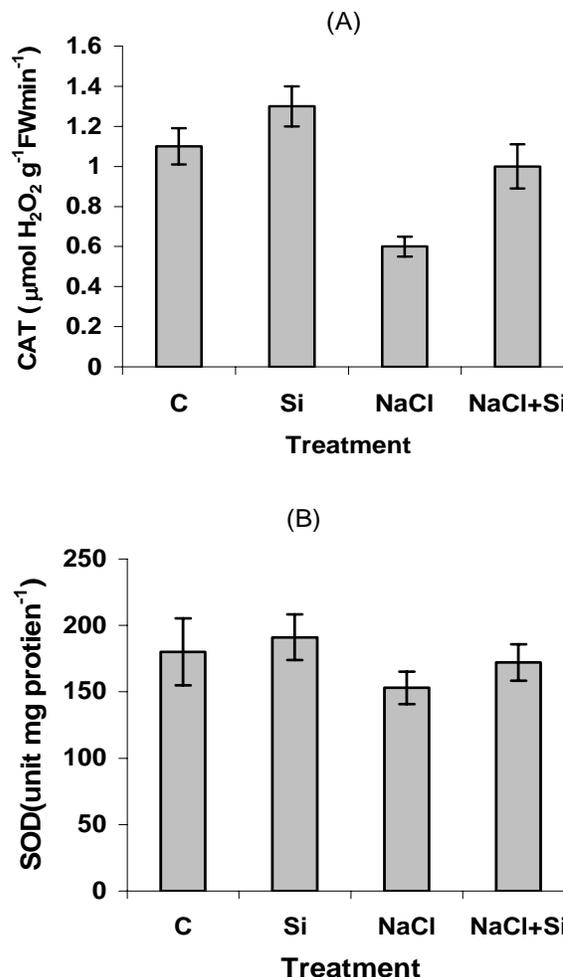
increased lipid peroxidation level, it may stimulate formation of AOS, such as H<sub>2</sub>O<sub>2</sub>, O<sub>2</sub><sup>•-</sup> and OH<sup>•</sup> radicals. Among AOS, superoxide is dissimulated by SOD enzyme into H<sub>2</sub>O<sub>2</sub> and is further scavenged by CAT and various peroxidases. Bor *et al.* (2003) reported a better protection from oxidative damage caused by salt treatment by increasing activities of SOD and CAT in beet leaves. According to Scandalios (1993), SOD and CAT are the most effective antioxidant enzymes in preventing cellular damage. A lower lipid peroxidation resulting from elevated activities of antioxidants under salt stress was also reported on salt-tolerant wild tomato species (Shalata & Tal, 1998), wheat (Dionisio-Sese & Tobita, 1999), and cotton (Gossett & Millhollon, 1994). These results suggest that salt stress led to significant changes in the activities of SOD and CAT enzymes involved in oxygen metabolism. This may be the result of changes in the lipid peroxidation mediated by free oxygen radicals. SOD is thought to be one of the most important defense systems, which detoxifies superoxide anion free radicals, by the formation of H<sub>2</sub>O<sub>2</sub> (Fridovich, 1986). In the present study, added Si significantly increased the activity of SOD and CAT (Fig. 1a & b) in leaves of salt stressed maize. These results are in accordance with that of Liang (1998) and Al-aghabary *et al.* (2004), suggesting that the damage due to increased production of AOS under salt stress was alleviated by Si addition by virtue of increased activities of SOD and CAT and the decrease content of MDA and H<sub>2</sub>O<sub>2</sub>. These results are in good agreement with the results of Liang (1998), who found that under salt stress,

added Si decreased the permeability of plasma membrane of leaf cells and significantly improved the ultrastructure of chloroplasts which were badly damaged by the added NaCl (Liang *et al.*, 1996). In accordance with the results in the present study, Dionisio-Sese and Tobita (1998) showed that, the activity of SOD a decrease upon exposure to salt stress in maize (Fig. 1).

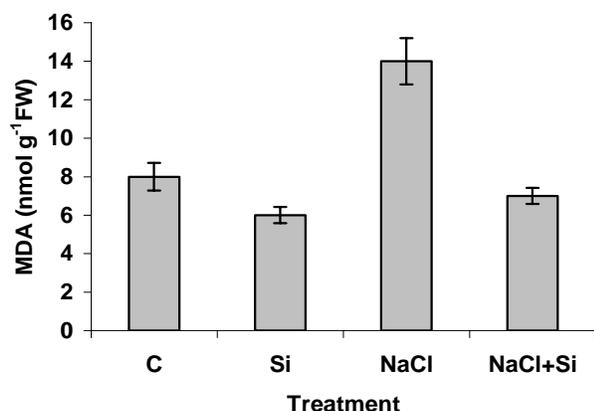
**H<sub>2</sub>O<sub>2</sub> content.** No matter salt was added, added Si decreased the H<sub>2</sub>O<sub>2</sub> level in the leaves (Fig. 5). Salt stress significantly increased the H<sub>2</sub>O<sub>2</sub> level and added Si decreased the H<sub>2</sub>O<sub>2</sub> level. These results are in accordance with that of Al-aghabary *et al.* (2004).

**Malondialdehyde (MDA).** Exogenously added Si considerably decreased MDA content in leaves under salt stress (Fig. 2). These results are in accordance with that of Liang (1999) and Al-aghabary *et al.* (2004). Added NaCl significantly increased the MDA content under absence of Si, however, MDA content was less increased by NaCl addition in presence of Si in nutrient solution. This study

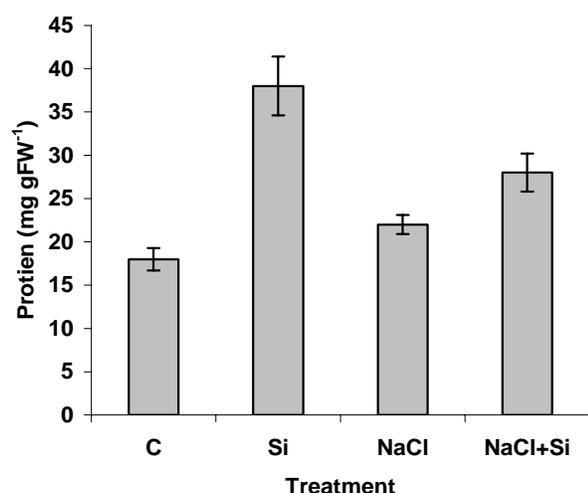
**Fig. 1. Changes in enzyme activities, (a) catalase (CAT) and (b) superoxide dismutase (SOD) in leaves of maize seedlings (with or without Si). C: control. Values are mean±SE based on three replicates (n = 3).**



**Fig. 2. Changes in the malondialdehyde content (MDA) in leaves of maize seedlings under salinity stress (with or without Si). C: control. Values are mean±SE based on three replicates (n = 3).**



**Fig. 3. Salt stress-induced changes in soluble protein content in leaves of maize seedlings under salinity stress (with or without Si).C: control. values are mean ± SE based on three replicates (n = 3).**

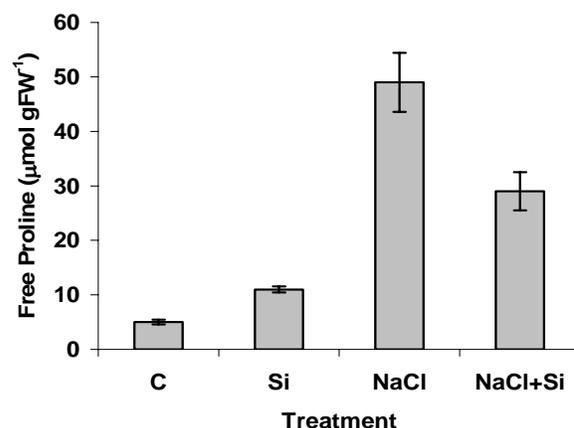


demonstrated that lipid peroxidation induced by NaCl was significantly lower in the Si-treated maize seedlings under salt stress than those under salt stress without Si treatment, which revealed protection of lipid membranes from AOS (McCord, 2000; Jain *et al.*, 2001), primarily by more effective scavenging of AOS by means of Si application. Since, Singha and Choudhuri (1990) proposed that H<sub>2</sub>O<sub>2</sub> accumulation in leaves of *Vigna* and *Oryza* seedlings under salt stress resulted from the decrease in the activity of CAT, H<sub>2</sub>O<sub>2</sub> level might have increased due to decreased activity of CAT in seedlings of maize under salt stress (Fig. 2). By considering that H<sub>2</sub>O<sub>2</sub> treatment to excised leaves of maize plants caused lipid peroxidation increment (Lin & Kao, 1998), elevated level of H<sub>2</sub>O<sub>2</sub> as a result of decreased CAT activity seemed due to an increase in MDA level of maize

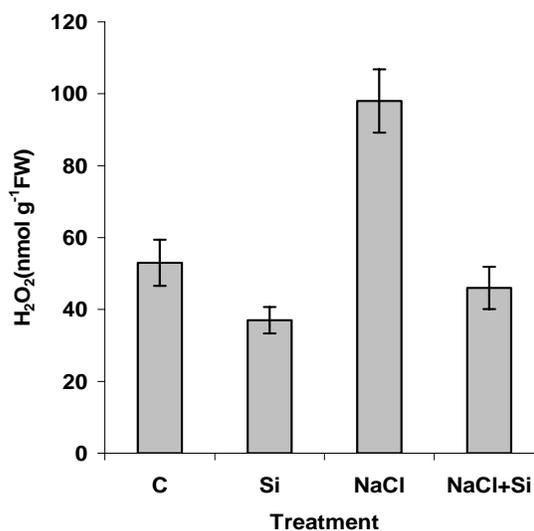
under salt stress.

**Free proline contents.** Free proline content showed no remarkable increase with Si application (Fig. 4). Salt stress caused a remarkable increase in free proline content. Nonetheless, Si treatment caused a significant decrease in free proline level salt stress compared to the seedlings treated with NaCl alone. Proline has been considered as a carbon and nitrogen source for rapid recovery from stress and growth, a stabilizer for membranes and some macromolecules and also a free radical scavenger (Jain *et al.*, 2001). Free proline content increased remarkably in maize seedlings with salinity but decreased with Si + NaCl treatment. It seems plausible that Si shows a protective role for maize seedlings to prevent them from being severely

**Fig. 4. Free proline content in leaves of maize seedlings under salinity stress (with or without Si). C: control. Values are mean±SE based on three replicates (n=3).**



**Fig. 5. Changes in H<sub>2</sub>O<sub>2</sub> content in leaves of maize seedlings under salinity stress (with or without Si). C: control. Values are mean±SE based on three replicates (n = 3).**



affected by salinity stress. Therefore, the level of proline accumulated in salt-stressed with Si treatment was not as high as it was in salt-stressed without Si treatment (Fig. 4). This is the first result showing the role of Si on proline accumulation under salt stress conditions.

**Protein content.** Soluble Protein content of maize leaves seedlings increased significantly in all other three groups in comparison with the control group (Fig. 3). In addition, exogenous application of Si significantly increased soluble protein content of maize seedlings in both Si and Si + NaCl treated groups compared with the control group. These results are in corroborations with the findings of Liang (1998) and Al-aghabary *et al.* (2004).

Finally, Si may act to alleviate salt stress in maize by decreasing the permeability of plasma membranes and maintenance of cell form and structure due to the increase of antioxidative enzymes SOD and CAT. Si partially offset the negative impacts of NaCl stress due to increased the tolerance of maize leaves to NaCl salinity by enhancement of chlorophyll content and photosynthetic activity.

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