

Comparative Response of Salt Tolerant and Salt Sensitive Maize (*Zea mays L.*) Cultivars to Silicon

¹Helal Ragab Moussa and ²Mohamed Abd El-Rahman EL-Galad

¹Radioisotope Department, Atomic Energy Authority, Malaeb El-Gamaa St., P.O. 12311, Dokki, Giza, Egypt. E-mail: helal_moussa@hotmail.com

²Agriculture Research Center, Soils, Water and Environment Research Institute, Giza

Abstract: Salinity stress is one of the most devastating stressful environments for plant growth and production. Two *Zea mays* cultivars, salt sensitive (Trihybrid 321) and salt tolerant (Giza 2), were grown in a complete nutrient solution in a growth room, to investigate the effect of silicon treatment, as Na₂SiO₃ (2.5 mM) on plants grown at two levels of salinity, 0.0 (Control), 150 mM of NaCl (S) which are equivalent to 15.5 dS m⁻¹, and supplied to the nutrient solution. Maize plants (Giza 2 and Trihybrid 321) grown at 15.5 dS m⁻¹ show a decreased in the chlorophyll (a+b), carotenoids, total protein contents, transpiration rate, relative water content (RWC), enzyme activity of ribulose-1,5-bisphosphate-carboxylase/oxygenase (RuBPCase), photosynthetic activity (¹⁴CO₂-assimilation) and macronutrient level (N, P, and K) as compared to the control plants. The results indicated that silicon (Si) treatment at 2.5 mM partially offset the negative impacts and increased tolerance of maize (Giza 2 and Trihybrid 321) to salinity stress by enhancing the above parameters. In contrast, salt stressed maize plants considerably increased proline content, Na⁺ and Cl⁻ levels, H₂O₂, malondialdehyde (MDA), and electrolyte leakage (EL). Application of silicon (2.5 mM) decreased significantly these parameters as compared to salt stressed maize plants. Salt tolerant (Giza 2), treated with silicon at 2.5 mM showed better performance than salt sensitive (Trihybrid 321). Salt tolerant (Giza 2), exhibited lower accumulation of Na⁺ and Cl⁻ levels, H₂O₂, MDA, and EL more than salt sensitive (Trihybrid 321). Overall, exogenously applied Si was found beneficial for improving salt tolerance of maize plants.

Key words: Chlorophyll, salinity stress, photosynthetic activity, ¹⁴CO₂-fixation.

1. Introduction

Salinity, a major abiotic stress at present [1] and the most devastating stressful environments for plant growth and production [2,3]. The response of plants to excessive salinity is multifaceted and involves changes in plant's physiology and metabolism [4], ultimately diminishing growth and yield [5] through osmotic effects, nutritional imbalances and specific ion toxicities [6, 7]. 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 [4]. One viable strategy of overcoming the salt-induced injurious effect on plant growth is the exogenous application of osmoprotectants and inorganic nutrients [8], Silicon (Si) is the second most abundant element on the surface of the earth, yet its role in plant biology has been poorly understood and the attempts to associate Si with metabolic or physiological activities have been inconclusive [9]. Addition of Si has been considered beneficial for improving crop tolerance to both biotic and abiotic stresses [9,10, 6]. A number of possible mechanisms are reported through which Si may increase salinity tolerance in plants [11] including increased plant water status [12], enhanced photosynthetic activity [13,4], immobilization of toxic Na⁺ ion [14] due to its complexation with Si [15], reduced Na⁺ uptake in plants and enhanced K⁺ uptake [16,17,18], improve photochemical efficiency of PSII by increased chlorophyll content, and limiting the transpiration rate [19, 20].

Corresponding Author: Helal Ragab Moussa

The current study was accomplished with the objectives to study the variation in salinity tolerance of two contrasting maize cultivars (salt sensitive, Trihybrid 321 and salt tolerant, Giza 2), and the physiological and biochemical insight of maize in response to Si applied under salinity stress.

2. Materials and methods

2.1. Plant materials and growth conditions

A homogenous lot of maize seeds (*Zea mays L.*) caryopses of Giza 2 (salt tolerant) and Trihybrid 321 (salt sensitive) were obtained from the Crop Institute, Agricultural Research Center, Giza, Egypt. The caryopses of both cultivars were kept at 4°C. They were surface sterilized in HgCl₂ solution (0.1%) for 10 min, and after rinsing the seeds with distilled water they were placed in sterile Petri plates containing two sheets of sterile filter paper moistened with distilled H₂O and allowed to germinate in the dark at 24 °C for five days, selected seedlings of equal size and vigor were transplanted to black polyethylene pots (40 cm high × 35 cm diameter) containing 14 Kg sand soil previously washed with HCl, the pots were irrigated with full-strength nutrient solution [21]. Five seedlings were planted in each pot. The plants were grown in a controlled growth chamber under the following growth conditions: 15-h photoperiod; 65-75% relative humidity; day and night temperature of 22°C and 20°C, respectively. The photosynthetic photon flux density at maximum plant height was about 440 μmol m⁻²s⁻¹. Cultural practices, such as weed control and irrigation, were performed as needed. After 15 days of seedling emergence salt stress treatments were

imposed. The seedlings were irrigated with full Hoagland's solution containing two levels of salinity, 0.0 (Control), 150 mM of NaCl (S) which are equivalent to EC=15.5 dS m⁻¹ and 2.5 mM of Si as Na₂SiO₃. The pots were rinsed with 400 ml of distilled water once a week to avoid salt accumulation. The experimental design was randomized complete block design with three replicates. All determinations were carried after 40-day-old.

2.2 Chemical analysis

Hydrogen peroxide was measured according to methods of [22]. Transpiration rate was determined as described by [23]. Determination of protein content was done spectrophotometrically according to [24]. Ribulose-1,5-bisphosphate-carboxylase/oxygenase (RuBPCase, EC 4.1.1.39) was determined following [25]. Lipid peroxidation was measured in terms of malondialdehyde (MDA) content using the thiobarbituric acid reaction as described by [26]. Chlorophyll *a*, *b* and total carotenoids were determined according to [27]. Photosynthetic activity (¹⁴CO₂-fixation) was measured at the Atomic Energy Authority Radioisotope Department, Cairo, Egypt, with the method of [4]. Proline was determined according to the method described by [28]. Electrolyte leakage (EL) was calculated to appraise membrane permeability, this method is based on [29]. Relative water content (RWC) content was measured according to [30].

2.3 Elemental analysis

Determination of Na⁺, K⁺ and Cl⁻ were determined with a flame photometer (Jenway, model, PFP-7, Chelmsford, Essex, England). The method of [31] was used to estimate phosphorus. Total nitrogen was determined by using the Kjeldahl method [32].

2.4 Statistical Analysis

Data were analyzed using ANOVA (SPSS 10.0, SPSS Inc., Chicago, IL, USA). Means were separated with Duncan's Multiple Range Test.

3. Results and Discussion

Photosynthesis characteristics are the possible tool for salinity tolerance screening in crops genotypes [33]. Measurement of photosynthesis characteristics including net CO₂ uptake, photosynthetic pigments and transpiration rate for monitoring plant responses to salt stress [34, 4]. The present study showed that on overall basis the salinity level at EC=15.5 dS m⁻¹ decreased the photosynthetic pigment contents (chlorophyll *a+b* and carotenoids), the rate of CO₂ assimilation, the transpiration rate, RuBPCase activity of both cultivars of maize plants salt tolerant, Giza 2 and salt sensitive, Trihybrid 321 (Table 1). However, Si supplementation at 2.5 mM increased these parameters as compared to salt stressed maize plants which are more obvious in salt tolerant (Giza 2) than salt sensitive (Trihybrid 321).

Table 1. Effect of Si on chlorophyll *a+b*, carotenoids, photosynthetic activity, transpiration rate, and enzyme activity of RuBPCase for maize cultivars differing in salt tolerance.

Treatment	Giza 2 (Salt tolerant)			Trihybrid 321 (Salt sensitive)		
	C	S	S+Si	C	S	S+Si
Chlorophyll (<i>a+b</i>) (mg g ⁻¹ FW)	3.82 ^a	2.89 ^c	3.51 ^b	3.68 ^a	2.64 ^c	3.34 ^b
Carotenoids (mg g ⁻¹ FW)	1.75 ^a	1.08 ^c	1.62 ^b	1.62 ^a	0.93 ^c	1.47 ^b
Photosynthetic activity (*KBq mg ⁻¹ FW)	16.63 ^a	8.15 ^c	14.64 ^b	14.63 ^a	7.05 ^c	12.95 ^b
Transpiration rate (mMH ₂ O m ⁻² s ⁻¹)	5.27 ^a	3.97 ^c	4.99 ^b	5.08 ^a	3.62 ^c	4.69 ^b
RuBPCase (μmolCO ₂ min ⁻¹ mg ⁻¹ protein)	41.65 ^a	33.21 ^c	39.16 ^b	40.08 ^a	30.18 ^c	37.58 ^b

C = water only control; S = 150 mM NaCl and Si = 2.5 mM.

*Kilo Becquerel (10³ Bq).

Values within rows followed by the same letter are not significantly different, Duncan's Multiple Range Test, P<0.01.

It is assumed that salinity reduced transpiration rates mainly by effects on stomatal opening [35]. The obtained results are in accordance with that of [4, 6]. It is generally known that reduced photosynthetic rate leads to reduced plant growth in most plants. This decrease in plant photosynthesis under salt stress takes place due to closing of stomata that result in a decrease in leaf transpiration rate and reduced leaf internal CO₂ concentration [36]. Salt stressed maize plants (Giza 2 and Trihybrid 321) considerably increased proline content, Na⁺ and Cl⁻ levels, H₂O₂, malondialdehyde (MDA), and electrolyte leakage (EL) as compared to the control plants (Table 2). However, silicon treatment (2.5 mM) significantly decreased these parameters which are more obvious in salt tolerant, Giza 2 than salt sensitive, Trihybrid 321 as compared to salt stressed plants. These results are in agreement with that of [1].

Electrolyte leakage (EL) was measured to identify the membrane stability or permeability. Added NaCl into the solution culture impaired the membrane stability and significantly increased the EL in both cultivars in comparison to the control. Inclusion of Si into the solution culture significantly decreased EL under saline conditions in both cultivars as compared to the control (Table 2). Among maize cultivars, Trihybrid 321 showed higher values of EL indicating more susceptibility as compared to Giza 2 both under salinity stress conditions (Table 2). Exogenous application of Si in saline growth medium reduces electrolyte by maintaining the integrity and functions of membrane, thus mitigating salt toxicity [14]. The present study showed that addition of Si into the solution culture decreased electrolyte leakage under salt stress showing a negative correlation between Si content and

electrolyte leakage. This ameliorative effect of Si may be due to its hydrophilic nature by maintaining plant water status and by protecting the plants from physiological drought [1].

Table 2. Effect of Si on MDA, proline, total protein, H₂O₂, electrolyte leakage (EL) and relative water content (RWC) of maize cultivars differing in salt tolerance.

Treatment	Giza 2 (Salt tolerant)			Trihybrid 321 (Salt sensitive)		
	C	S	S+Si	C	S	S+Si
Total Protein (mg g ⁻¹ DW)	75 ^a	58 ^c	69 ^b	70 ^a	51 ^c	67 ^b
Proline (μmol g ⁻¹ DW)	6.1 ^b	17.9 ^a	5.7 ^c	6.9 ^b	18.7 ^a	6.8 ^b
H ₂ O ₂ (μmol g ⁻¹ FW)	102 ^b	120 ^a	95 ^c	108 ^b	126 ^a	102 ^c
MDA (μmol g ⁻¹ FW)	419 ^b	443 ^a	410 ^c	426 ^b	458 ^a	418 ^c
EL (%)	18.1 ^b	25.2 ^a	16.9 ^c	18.6 ^b	26.8 ^a	17.8 ^c
RWC (%)	95.7 ^a	75.9 ^c	89.6 ^b	94.5 ^a	73.9 ^c	86.2 ^b

C = water only control; S = 150 mM NaCl and Si = 2.5 mM.

Values within rows followed by the same letter are not significantly different, Duncan's Multiple Range Test, P<0.01.

Table 3 . Effect of Si on ion contents, Na, Cl, N, P, and K (mg g⁻¹DW) of maize cultivars differing in salt tolerance.

Treatment	Giza 2 (Salt tolerant)					Trihybrid 321 (Salt sensitive)				
	Na	Cl	N	P	K	Na	Cl	N	P	K
C	45 ^b	53 ^b	309 ^a	198 ^a	184 ^a	57 ^{bb}	64 ^b	273 ^a	192 ^a	176 ^a
S	67 ^a	75 ^a	278 ^c	168 ^c	153 ^c	73 ^a	82 ^a	257 ^c	161 ^c	143 ^c
S+Si	41 ^c	50 ^b	293 ^b	187 ^b	177 ^b	52 ^c	61 ^c	268 ^b	185 ^b	169 ^b

C = water only control; S = 150 mM NaCl and Si = 2.5 mM.

Values within columns followed by the same letter are not significantly different, Duncan's Multiple Range Test, P<0.01

Salinity stress decreased significantly the protein and relative water contents (Table 2), which increased by exogenous application of Si. The obtained results are similar to that of [1, 37, 4]. Maximum contents of Na⁺ and Cl⁻ were observed at 150 mM of NaCl, while least contents of Na⁺ and Cl⁻ at same salinity level was obtained with exogenous application of Si as compared to the control plants (Table 3).

These results are in accordance with that of [6, 38] reported that Si-induced reduction in transpiration rate and partial blockage of the transpiration bypass flow. Therefore, Na concentration in the shoots of plant was decreased by reduction in transpiration rate. Silicon also inhibits Na⁺ uptake and its onward translocation to shoots partly by the inhibitory

effect of Si on the transpiration stream [16]. Application of Si increased concentration of N, P, and K in maize plants (Giza 2 and Trihybrid 321), as compared to salt stressed plants as shown in Table (3). These results are similar to that of [1].

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Author Profile



Dr. Helal Ragab Moussa

Professor in Plant Physiology and Nuclear Techniques Radioisotopes Department, Atomic Energy Authority, Malaeb El-Gamaa St., P.O. 12311, Dokki, Giza, Egypt