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GROWTH, ION CONTENTS AND PHOTOSYNTHESIS OF SALT-SENSITIVE AND LESS SALT-SENSITIVE CUCURBITS TREATED WITH SILICON

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ABSTRACT

A study was carried out to evaluate the role of silicon in alleviating NaCl salinity effects on both salt-sensitive (cucumber) and less salt-sensitive (bitter gourd) cucurbit. The species were subjected to two levels of NaCl (0, 50 mM) and three silicon concentrations (0, 50, 100 mg L^{-1} sodium silicate) and data on growth, Na and Cl concentration of leaves, net photosynthesis (Pn) and stomatal conductance (g_s) were collected. When treated with 100 mg L^{-1} silicon, growth of salt-stressed and non-salt stressed plants for both species was significantly improved, with more beneficial effects recorded on the salt-stressed plants and salt-sensitive species. Plants treated with 50 mg L^{-1} silicon had 11.11% significantly lower Na^+ ion in leaf compared to 0 mg L^{-1} silicon, regardless of species and salinity condition. Treatment of silicon at 100 mg L^{-1} also significantly increased net photosynthesis and stomatal conductance by 12.13 and 30.14%, respectively. However, no significant beneficial effect of silicon in reducing Cl concentration was recorded. In conclusion, application of silicon can alleviate salinity stress in both salt-sensitive species (cucumber) and less salt-sensitive species (bitter gourd) by reducing sodium toxicity and increasing photosynthetic activity which evident in improvement of growth parameters.

Key words: Salt stress, silicon, *Cucurbitaceae*

INTRODUCTION

Among abiotic stresses, salt stress is one of the most prevalent factors that inhibit plant growth and yield worldwide (Fahad *et al*., 2015). Rise in salt concentration in the root zone area results in high osmotic potential of soil which leads to water deficit within plants. Most importantly, increase in Na and Cl ions beyond the threshold level causes nutritional imbalances and impaired the important biochemical processes in plants (Liang *et al*., 2015). Silicon (Si), the second most abundant mineral element in the soil after oxygen which comprises 31% of the earth's crust (Liang *et al*., 2007) has been reported to be beneficial in mitigating both biotic stresses and abiotic stresses (Sahebi *et al*., 2015). Mitigation of salinity stress by silicon is associated with the ability of silicon to reduce ion toxicity especially Na ions (Wang *et al*., 2015). Najib *et al*., (2017) reported that among four species of Cucurbit studied – cucumber, bitter gourd, bottle gourd and squash, cucumber was reported to be most salt-sensitive while bitter gourd was the least salt-sensitive. The objective of this study was to investigate the effects of varying concentrations of NaCl salinity (0, 50 mM) and silicon application (0, 50, 100 mg L^{-1}) on growth, ion contents and photosynthesis in both susceptible and tolerant species of Cucurbits.

MATERIALS AND METHODS

Plant materials and growth condition

The plant materials used in this study were cucumber (*Cucumis sativa*) and bitter gourd (*Momordica charantia*). Cucumber seeds were provided by MARDI (variety MT 1) while bitter gourd seeds were bought from a local seed supplier, Sin Seng Huat Seeds Sdn. Bhd. Seeds were sown in

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plug trays filled with peat at University's Agriculture Park nursery of Universiti Putra Malaysia. The seedlings were then transplanted into 1.5 litre pot. The growing media used was cocopeat with 6 g/L a complete compound fertilizer (15%N, 15% P_2O_5, 15% K_2O), 2 g/L ground magnesium limestone and 1 g/L mixture of micronutrients. The plants were grown under 50% shade on 1.2 meter bench.

Silicon treatments

Sodium silicate solution (extra pure) was bought from MERCK, Germany. This sodium silicate solution (Na_2SiO_3; Na_2O=7.5%, SiO_2=25.5%) was diluted to different concentration needed for this experiment. Plants were treated with 3 levels of sodium silicate (0, 50, 100 mg L^{-1}) by manually drenched approximately 50 mL of the solution to every pot. Plants were first treated with silicon after 1 day of transplanting, and every 3 days thereafter until the harvest date, 24 days after transplanting.

Salinity treatments

Treatment of sodium chloride (NaCl) commenced at the third time of silicon treatment to ensure the silicon is readily available in the plants, and continued every day until harvest. Two different levels of NaCl concentrations; 0 and 50 mM were given by manually drench approximately 300 mL of the solutions once per day for the first 5 days of NaCl treatment and twice per day for the following days as the plants grew. Electrical conductivity (EC) of the media extract determined using the pour-through method (Cavins *et al*., 2000) were 2.43 and 6.47 mS.cm^{-1} for 0 and 50 mM NaCl, respectively. The EC of solutions were checked by the EC meter (5061 Pen SHSX).

Vegetative growth

At 17th day after the first NaCl application, the whole plants were separated into leaves and stems. Leaf areas were measured and recorded as total leaf area per plant using automatic leaf area meter (MODEL LI-300, LI-COR). The dry weight of leaves and stem was determined after 72 hours drying at 75°C in a drying oven.

Sodium and Chloride concentration of leaves

At 20th day of treatment, the top fully expanded leaf samples were harvested and washed with deionized water prior to drying at 70°C for 72 h. Briefly 0.25 g of the dry sample was transferred to a 100 mL digestion flask and 5 mL concentrated H_2SO_4 was added. The flasks were then heated for 7 minutes at 450°C and 10 mL of 50% H_2O_2 was added to complete the process. The flasks were then removed from the digestion plate, cooled to room temperature and then made up to 100 mL with distilled water. Concentration of sodium (Na) was quantified using an atomic absorption spectrophotometer (Perkin Elmer, Model 3110, USA) while Chloride (Cl) was extracted from the dried samples using silver ion titration method (Richards, 1954).

Net photosynthesis and stomatal conductance

At 17 days of salinity treatment, net photosynthesis (Pn) and stomatal conductance (g_s) were measured on the third fully expanded leaves by using a portable close photosynthesis measurement system (Infra-Red Gas Analyzer, Li 6400, Licor, Lincoln, Nebraska, USA). The measurements were taken around 11:00 am using five measurements for each replication with an irradiance setting of 1000 µmol m^{-2} s^{-1}. Irradiance was provided by an LED RGB (Red Green Blue) light source (LI-6400-02B, Li-Cor Inc.).

Experimental design and data analysis

This study was conducted in a split-split plot design with species as main plot and NaCl concentration as sub-plot and silicon levels as sub-sub plot with three replications; 6 plants per replication. The data obtained was subjected to ANOVA using SAS (Version 9, SAS Institute Inc. Cary, North Carolina, USA) and differences between treatment means were compared using Duncan Multiple Range Test (DMRT) at $P \leq 0.05\%$. Pearson correlation coefficient (r) was determined between the variables in each species at $P \leq 0.05\%$.

RESULTS AND DISCUSSION

Leaf area, leaf and stem dry weight

The leaf areas of cucumber and bitter gourd were significantly different ($P<0.01$) and were significantly affected by salinity ($P<0.01$) and silicon ($P<0.05$), with significant interaction ($P<0.01$) recorded between salinity and silicon. Silicon effects on leaf area are highly dependable on the NaCl status of the plants. Supplementing silicon to non-salt stress plants had no significant increase in leaf area whereas addition of 50 mg L^{-1} silicon in salt-stress plants led to significant increase in leaf area by 58.01% (Table 1). Besides being beneficial in salinity conditions, alleviation effects of silicon was more beneficial in salt-sensitive species (cucumber) compared to less salt-sensitive species (bitter gourd). Salt-stressed cucumber treated with 100 mg L^{-1} silicon had 2.56 times higher leaf dry weight compared to those with 0 mg L^{-1} silicon (Figure 1) whereas in non-salt stressed cucumber, the rate was only 1.17 times. In bitter gourd, 100 mg L^{-1} silicon given to salt-stressed plants increased the leaf dry weight by 1.90 times compared to 0 mg L^{-1} silicon whereas the increase in leaf dry weight

Table 1. Main and interaction effects of two cucurbit species, salinity and silicon level on leaf area, leaf dry weight and stem dry weight

	Leaf area (cm^2)	Leaf dry weight (g)	Stem dry weight (g)
Species (Sp)			
Cucumber	916.70a	2.18a	1.07a
Bitter gourd	707.39a	1.69a	0.87a
Salinity (NaCl)			
0 mM	1008.88a	2.26a	1.18a
50 mM	615.21b	1.60b	0.76b
Silicon (Si)			
0 mg L^{-1}	742.77b	1.51b	0.75b
50 mg L^{-1}	783.25b	1.91b	0.93b
100 mg L^{-1}	910.11a	2.37a	1.23a
Sp	**	**	*
NaCl	**	**	**
Si *	**	**	*
Sp*NaCl	ns	ns	ns
Sp*Si	ns	ns	ns
NaCl*Si	**	ns	ns
Sp*NaCl*Si	ns	ns	ns

**Significant at 1% probability level, *Significant at 5% probability level, ns: Not significant. Means in each column with the different letters within each factor indicate significant differences at P≤0.05% level according to DMRT.

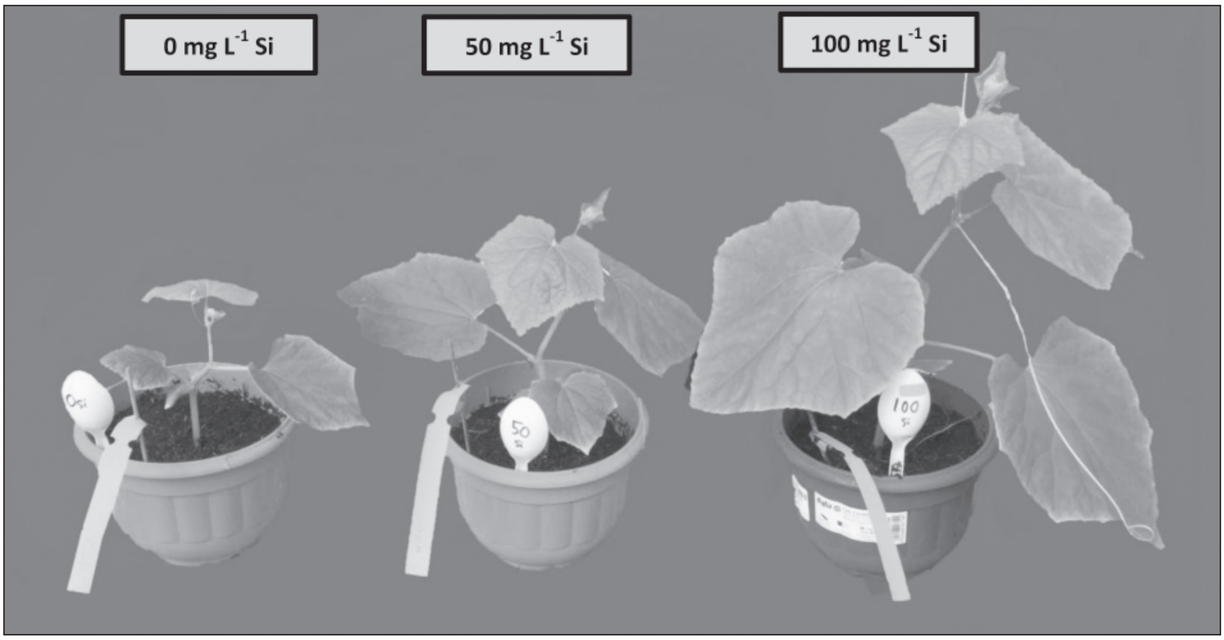

Fig. 1. Growth of salt-stressed cucumber at 8 days of NaCl treatment as affected by different silicon concentrations.

was only by 1.44 times (Figure 2). The same pattern of responses can also be observed in stem dry weight (Figure 3). Li *et al*. (2007) explained that beneficial effects of silicon are not remarkable in optimal conditions but rather evident when the plants are under stress conditions. Hashemi *et al*. (2010) demonstrated in canola plant that the dry weight improved significantly under saline conditions when silicon was introduced to the growing media. However, silicon alone (i.e. under non-saline conditions) did not increase the dry matter. The reason behind this is still ambiguous and research elucidating this response is still lacking in present time.

Sodium and Chloride concentration of leaves

The Sodium (Na) concentration in leaves of the two species were significantly different ($P<0.01$)

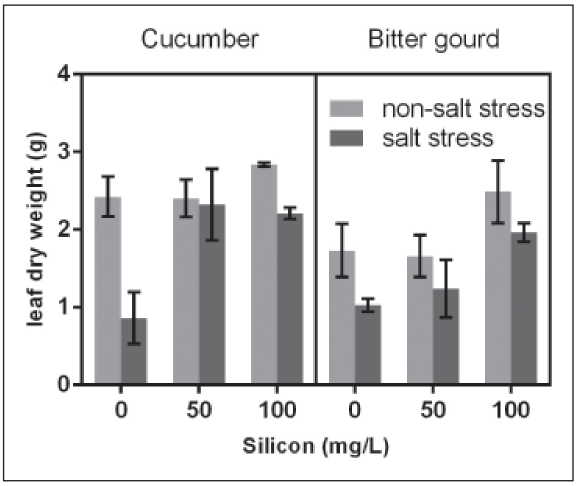

Fig. 2. Effects of silicon concentrations, salt stress conditions and species on leaf dry weight (Mean ± S.E; n=3).

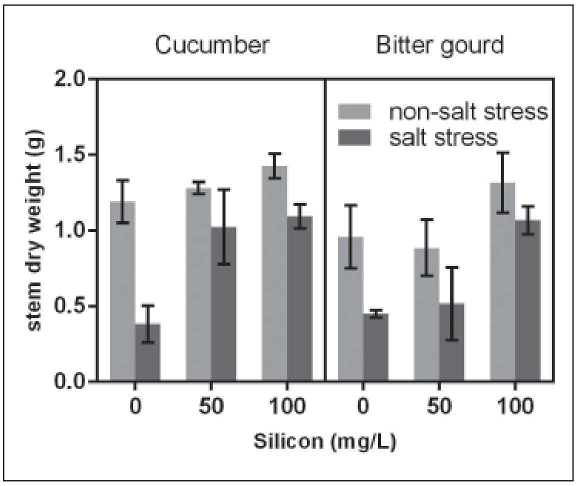

Fig. 3. Effects of silicon concentrations, salt stress conditions and species on stem dry weight (Mean ± S.E; n=3).

and were significantly affected by salinity ($P<0.01$) and silicon ($P<0.05$), with significant interaction ($P<0.01$) recorded between species and salinity (Table 2). Significant interaction between species and salinity revealed that under salt-stressed condition, concentrations of leaf Na in both species were significantly higher compared to non-salt stressed condition. However, the degree of increment was different between the species. In cucumber, concentration of leaf Na in salt-stressed plants was 5.33 times higher compared to control whereas in bitter gourd, it was 5.11 higher compared to control (Figure 4). Alleviation of salt toxicity by silicon is normally closely attributed by the reduction of Na ions in salt stressed plants in order to maintain ion homeostasis (Yin *et al*., 2015). The underlying mechanism of how silicon reduce Na ion toxicity lies in the activities of plasma membrane in removing the Na ions or compartmentalizing the ions in vacuole, which is made possible by the Na^+/H^+ antiporter. This antiporter is driven by H^+-ATPase and H-PPase (Liang *et al.,* 2007). The activity of H^+-ATPase decreased under salt stress but when the plants were treated with silicon, the activities of H^+-ATPase increased significantly (Sahebi *et al.,* 2015). This may facilitate removal of Na ions from the cell and thus avoiding ion toxicity. This is in accordance with results obtained in this study where treatment of 50 mg L^{-1} silicon significantly reduced leaf Na concentration by 11.11% compared to non-treated plants, regardless of the species. Besides removing the Na ions or compartmentalizing the ions in vacuole, silicon also reduce Na ions uptake in the root through long chain of phytoliths that binds Na and restrict its translocation to the upper parts of plant (Khan *et al*., 2016). Apart from Na ions, accumulation of Chloride (Cl) ions is also deleterious in salt stressed plants. The Cl concentration in leaves of the two species were significantly different ($P<0.01$) and was significantly affected by salinity ($P<0.01$). Salinity significantly increased Cl ions by 88.80% in salt-stressed plants compared to non-stressed plants. However, silicon has no significant effect on the reduction of Cl in leaves (Table 2). This is interesting since it shows that even without the alleviative effects of silicon on leaf Cl, the plants can still thrive under high Cl environment. However, this is only true for bitter gourd only. There was significant negative correlation between vegetative growth parameters and leaf Cl in cucumber but it was not the case for bitter gourd. Ruling out the silicon factor, this suggests that in cucumber, the combination of Na and Cl ions toxicity is deleterious to its growth whereas in bitter gourd, growth inhibition is mainly due to Na ion toxicity.

Net photosynthesis and stomatal conductance

Generally, reduction in photosynthetic rate results in reduced plant growth in most plants. This is because under salt stress, stomata are signaled to close due to osmotic stress resulted in a decrease in leaf transpiration rate and reduced leaf internal CO_2 concentration (Parveen & Ashraf, 2010). Furthermore, salinity stress also reduces the content of ribulose-1,5-biphosphate carboxylase/oxygenase (RuBisCO), and other enzymes essential for photosynthesis and synthesis of chlorophyll (Chaves *et al*., 2009). Correlation analysis in both species revealed significant negative correlation between stomatal conductance and leaf Na, leaf Cl, which indicates that plants tend to have lower stomatal conductance as a result of Na^+ and Cl^- ions accumulations. Reduce in stomatal conductance subsequently leads to a reduction in photosynthetic

Table 2. Main and interaction effects of two cucurbit species, salinity and silicon level on Na and Cl concentration, net photosynthesis and stomatal conductance

	Na (%)	Cl (%)	Pn (μmol CO_2 m^{-2} s^{-1})	g_s (mol H_2O m^{-2} s^{-1})
Species (Sp)				
Cucumber	0.33b	1.61a	6.64a	0.326a
Bitter gourd	0.48a	0.96a	5.34b	0.141b
Salinity (NaCl)				
0 mM	0.13b	0.89b	6.47a	0.261a
50 mM	0.68a	1.68a	5.51b	0.205b
Silicon (Si)				
0 mg L^{-1}	0.45a	1.54a	5.69b	0.209c
50 mg L^{-1}	0.40b	1.21ab	5.90b	0.220b
100 mg L^{-1}	0.36b	1.10b	6.38a	0.272a
Sp	**	**	**	**
NaCl	**	**	**	**
Si *	ns	**	**	*
Sp*NaCl	**	ns	ns	ns
Sp*Si	ns	ns	ns	ns
NaCl*Si	ns	ns	ns	ns
Sp*NaCl*Si	ns	ns	ns	ns

**Significant at 1% probability level, *Significant at 5% probability level, ns: Not significant. Means in each column with the different letters within each factor indicate significant differences at P≤0.05% level according to DMRT.

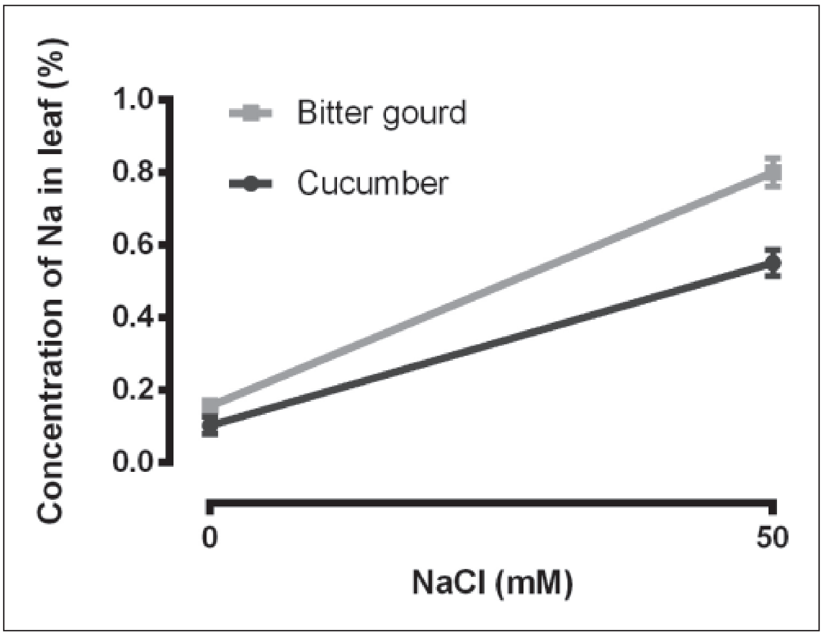

Fig. 4. Effects of Cucurbit species and salinity on Leaf Na concentration (Mean ± S.E; n=3). Means in each species with the different letters indicate significant differences at P≤0.05% level according to DMRT.

rate as indicated by the significant positive correlation between stomatal conductance and photosynthetic rate in cucumber (r=0.73, p≤0.01) and bitter gourd (r=0.87, p≤0.01). Silicon has been widely reported to be able to improve plant photosynthesis under salt stress (Shi *et al.*, 2013) and the same findings as well in this study. Net photosynthesis (Pn) and stomatal conductance (g_s) of cucumber and bitter gourd were significantly different (P<0.01). Main effects of salinity and silicon significantly affected (P<0.01) Pn and g_s, with no significant interactions recorded between the main effects (Table 2). Between the species, cucumber had significantly higher net photosynthesis and stomatal conductance compared to bitter gourd. Regardless of the species and silicon rate, net photosynthesis and stomatal conductance in salt stressed plants were reduced by 14.83 and 21.45%, respectively compared to non-salt stressed plants. Treatment of 100 mg L^{-1} silicon significantly increased net photosynthesis by 12.13% compared to 0 mg L^{-1} treatment while no significant increment was recorded for 50 mg L^{-1}. For stomatal conductance, treatment of silicon at every interval significantly increased the stomatal conductance. Plants supplemented with 50 and 100 mg L^{-1} silicon had respectively, 5.26 and 30.14% higher stomatal conductance, compared to plants supplemented with 0 mg L^{-1} silicon. This could be explained by the deposited silicon in the epidermal cells of leaf that act as a "window" which promotes efficiency of light-use by facilitating light transmission to the photosynthetic tissue, thus increasing the net photosynthesis (Klancnik *et al*., 2014). In addition, previous studies also demonstrated that silicon supplementation in salt-stressed plants could improve photochemical efficiency of PSII by increasing chlorophyll content, regulating transpiration rate and detoxifying ROS via the accumulation of silicon in leaves (Mohsenzadeh *et al*., 2011).

CONCLUSION

Alleviation effects of silicon on growth of salt-stressed plants was more beneficial on salt-sensitive species (cucumber) compared to less salt-sensitive species (bitter gourd). Regardless of the species, growth of salt-stressed plants treated with silicon was improved by alleviating salt-induced damages such as Na ion toxicity and impaired photosynthetic activity.

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