Salinity stress alleviation by use of silicon in *Ocimum basilicum* L.: an approach based on enhancing antioxidant responses

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Abstract

Basil (*Ocimum basilicum* L.) is one of the valuable medicinal plants. Negative effects of NaCl stress on plants have been reported and silicon may alleviate these negative effects through promoting antioxidant system. This study was conducted to investigate the effects of silicon as an elicitor and NaCl as salinity stress on some morphological, biochemical, and antioxidant parameters in basil. The plants were pretreated with silicon (0, 0.5, and 1.50 mM) and submitted to NaCl stress (0, 50 and 100 mM). Results showed that NaCl stress decreased dry and fresh weight of shoots and roots and chlorophyll and carbohydrate contents, but carotenoid and hydrogen peroxide (H_2O_2) contents and also superoxide dismutase (SOD) and catalase (CAT) activities increased ($p<0.05$). Silicon also increased dry and fresh weights of shoots and roots and carotenoid, chlorophyll, and carbohydrate contents, an also CAT and SOD activities, but it decreased H_2O_2 contents ($p<0.05$). Based on these findings, silicon, especially 1.50 mM concentration, is recommended for protection of basil under NaCl stress.

Keywords: basil, H_2O_2 content, CAT activity, SOD activity, sodium chloride stress.

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Introduction

Plants are constantly exposed to various stresses and pathogens. Their survival under harmful conditions depends on their metabolic and biochemical adaptations to adverse conditions (Muscolo et al., 2015). Salinity stress is one of several factors that impair plant growth and development through osmosis and ion toxicity, causing irreparable damage to plants (Hajiboland et al., 2017). It is reported that about 20% of the world's irrigable lands are affected by salinity (Zhu et al., 2020). Salinity has negative effects on plant physiological characteristics and reduces their growth such as dry weight and leaf area, (Abbasi et al., 2016) and germination percentage and number of leaves (Isayenkov and Maathuis, 2019). On the other hand, salinity reduces water ion imbalance, ion toxicity, coding genes in the synthesis of nucleic acid and proteins, and photosynthetic activity (Yarsi et al., 2017). One of the reasons for plant damage in salinity stress is the production of reactive oxygen species (Guo et al., 2015), which is modified by enzymatic and non-enzymatic antioxidant mechanisms. Production of antioxidant compounds to improve

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plant resistance to stress depends on the intensity of stress, plant genotype, and plant species (Daoud et al., 2018). The damage due to salinity or osmotic effect and ionic toxicity is stimulated under the influence of Cl^- and Na^+. The two ions prevent the absorption of ca^{2+}, k^+, and other nutrients (Acosta-Motos et al., 2017) since k^+ is an essential element which plays a significant role in enzyme activity, turgor adjustment, cell expansion, adjustment of membrane electrical potential, and PH homeostasis (Ragel de la Torre et al., 2019). Therefore k^+ could be considered as an essential element in plants' physiological and developmental procedures (Hasanuzzaman et al., 2018).

Silicon is the second most abundant element after oxygen (Soundararajan et al., 2017) which is absorbed by plants in the form of $Si\ (OH)_4$ (Yavaş and Aydın, 2017). Studies have shown that in addition to plants in yellow-brown, gold and diatoms algae to the extent significant silicon is found (Tubana et al., 2016). According to the definition of essential elements, the necessity of silicon for researchers is a little questionable, although its absence causes damage to plants and reduces their growth and development (Mauad et al., 2016). Therefore, researchers classify this element in the category of semi-essential (Rios et al., 2017). Silicon plays a significant role in controlling biological and non-biological stresses (Delavar et al., 2016). Silicone improves water absorption by affecting the osmotic potential and promotes better growth and development of plants (Khorasaninejad et al., 2020). Silicon accumulates in the epidermal tissue to protect the plant from damage caused by fungi and insects (Shanan and El Sadek, 2017) and also from the adverse effects of stress conditions (Luyckx et al., 2017).

Ocimum basilicum L., commonly known as basil, is an annual herb of the Lamiaceae family. It is cultivated at high levels as one of the most popular plants in human food, which is also of interest for researchers in medicinal plants (Jahan et al., 2015). Salinization of agricultural water and soil due to environmental problems and uncontrolled use of water resources poses a big challenge for agricultural practices. Therefore, this study was an attempt to investigate the possibility of increasing the resistance of *Ocimum basilicum* L. to salinity by using silicon under hydroponic cultivation.

Materials and Methods

Plant material and treatment conditions

Seeds of *O. basilicum* were prepared form Pakan-Bazr Company, Isfahan, Iran. The seeds were then cultivated on a bed of perlite and irrigated with distilled water for 6 days. After emerging, the seedlings were freighted with 50% Long-Ashton solution (pH 6.5) (Hewitt, 1966). The seedlings were then transferred to hydroponic condition with 16 h light/8 h darkness and fed with Long-Ashton solution. The temperature program was 16 ± 2 °C and 24 ± 2 °C for night and day, respectively. Seedlings with 3 leaves were transferred to hydroponic media (Long Ashton solution) in 1.5 L plastic pots with 2 plants per pot, aerated by an air pump. Hydroponic media was changed every 5 days. The plants were treated with different concentrations of silicon in the form of $Na_2SiO_3.5H_2O$, (0, 0.5, and 1.50 mM) in hydroponic media (pH 6.5) (Hewitt, 1966) 30 days after planting and for 90 days. After that the plants were treated with NaCl (0, 50, and 100 mM) for 6 days. NaCl and silicon were prepared from Merk Company (Munich-Germany).

The plants were harvested and weighed to record fresh weight (FW). To assess enzyme activity, the plants were fixed in liquid azote and kept in -20 °C.

Assessment of morphological parameters

Fresh weights of roots and stems were measured with a digital scale with 0.001 accuracy. For measuring dry weights, roots and stems were wrapped in foil and placed in an oven at 72 °C for 48 hours. After drying, the dry weights of the samples were measured with the same digital scale.

Assessment of biochemical parameters

The amount of chlorophyll and carotenoids were measured and expressed in mg g^{-1} fresh weight (Porra et al., 1989). Carbohydrate contents were evaluated and expressed in mg g^{-1} fresh weight (Fales, 1951).

Assessment of enzyme activities and antioxidant parameters in shoots

Hydrogen peroxide (H_2O_2) content was assayed and expressed in nM g^{-1} weight (Sagisaka, 1976). Also, superoxide dismutase (SOD) activity was evaluated and expressed in Um g^{-1} protein (Giannopolitis and Ries, 1977) by using p-nitro blue tetrazolium. Catalase (CAT) activities was assayed and expressed in U mg^{-1} protein (Kar and Mishra, 1976)

Data Analysis

The present study was conducted as a factorial arrangement, consisting of silicon (0, 0.5, and 1.50 mM) and NaCl (0, 50, and 100 mM) treatments with 3 replications based on a completely randomized design. The data were normally distributed according to the Kolmogorov-Smirnov test. Analysis of variance and Duncan's test were used in SPSS software.

Result

Physiological parameters

Findings showed that shoot fresh weights (Fig I. A) significantly decreased in the plants treated with NaCl (50 and 100 mM) compared to the plants grown under non-stress condition ($p<0.05$). Application of silicon significantly increased shoot fresh weight ($p<0.05$) in plants treated with 1.50 mM silicon and NaCl (50 and 100 mM). Moreover, application of silicon with 0.5 mM increased shoot fresh weight under 100 mM NaCl, but it did not have a significant effect on plants treated with 50 mM NaCl ($p<0.05$).

Shoot dry weight (Fig I. B) significantly decreased in all levels of salinity compared to control plants ($p<0.05$). In the combined treatments of silicon (0.5 and 1.50 mM) and NaCl (50 and 100 mM), silicon caused a significant increase in shoot dry weight compared to non-silicon plants ($p<0.05$).

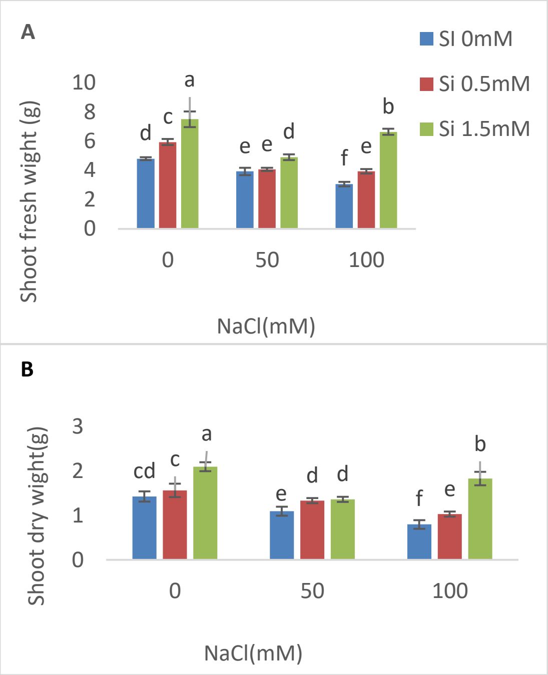

Fig. I. Effects of silicon on shoot fresh weight (A) and shoot dry weight (B), in basil grown under NaCl stress; the data are shown as means ± SE. Superscripts (a-f) show significant differences among groups ($p<0.05$).

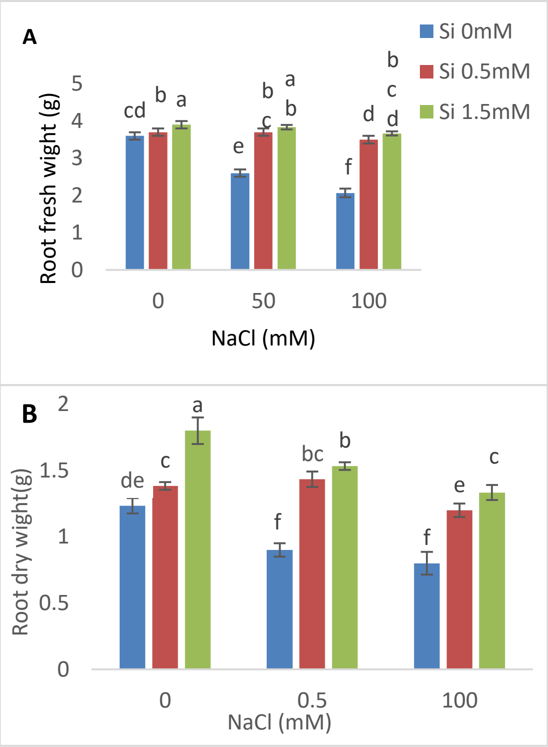

Fig. II. Effects of silicon on root fresh weight (A) and root dry weight (B) in basil grown under NaCl stress; the data are shown as means ± SE. Superscripts (a-f) show significant differences among groups ($p<0.05$).

Root fresh weight (Fig II. A) significantly decreased in all levels of salinity compared to control plants ($p<0.05$). Application of silicon significantly increased this parameter. In plants treated with silicon (0.5 and 1.50 mM) and salinity (50 and 100

Table 1
Chlorophyll and carbohydrate content in basil treated with silicon and NaCl

Treatment	Ch a (mg g^{-1} FW)	Ch b (mg g^{-1} FW)	T Ch (mg g^{-1} FW)	Root Carbohydrate (mg g^{-1} FW)	Shoot Carbohydrate (mg g^{-1} FW)
Control	2.59±0.137 b	0.81±0.167 c	3.40±0.300 b	7.76±0.35a-b-c	9.70±0.556 a-b
NaCl 1	1.15±0.165 e	0.99±0.133 c	2.14±0.040 d	6.68±0.401 c-d	8.72±0.541 b-c
NaCl 2	0.66±0.041 g	0.86±0.818 c	1.52±0.110 e	5.07±1.004 e	6.93±1.066 d
Si 1	2.90±0.040 a	1.76±0.172 a	4.66±0.208 a	8.13±0.251 a-b	9.76±0.907a-b
Si 2	2.95±0.037 a	1.98±0.187 a	4.93±0.152 a	8.70±0.400 a	10.80±0.400 a
<u>NaCl × Silicon</u>					
NaCl 1× Si 1	1.64±0.030 d	1.046±0.360 b	3.10±0.450 b-c	7.23±0.351 b-c	9.33±0.503 b
NaCl 1× Si 2	1.82±0.015 c	1.39f±0.642 b	2.83±0.075 c	7.70±0.75a-b-c	9.63±0.642 a-b
NaCl 2 × Si 1	0.93±0.020 f	1.90±0.055 a	3.22±0.077 b-c	5.76±1.05 d-e	7.73±1.040 c-d
NaCl 2 × Si 2	1.02±0.015 e-f	1.94±0.068 a	3.30±0.52 b	6.59±0.613 c-d	8.600±0.624 b-c

Si 1 (silicon 0.5 mM), Si 2 (silicon 1.5 mM), NaCl 1 (NaCl 50 mM), NaCl 2 (NaCL 100 mM); small letters in each column show the significance of mean comparison by Duncan multiple range test (P≤0.05).

Table 2
Carotenoid and H_2O_2 contents and enzyme activities in basil treated with silicon and NaCl

Treatment	Carotenoid (mg g^{-1} F.W)	H_2O_2 (nMl g^{-1}F.W)	Catalase (Umg $^{-1}$ protein)	Superoxide dismutase (Umg $^{-1}$ protein)
control	0.406±0.015 g	58.56±0.305 d	0.963±0.152 d	120.40±0.916 i
NaCl 1	0.443±0.020 e-f	63.73±0.152 c	.0826±0.020 f	136.76±0.152 d
NaCl 2	0.460±0.010 d-e	75.76±0.152 a	0.630±0.010 g	149.66±0.471 b
Si 1	0.433±0.015 f	49.20±0.264 g	1.076±0.015 b	123.86±0.251 h
Si 2	0.440±0.010 e-f	47.86±0.251 h	1.483±0.025 a	125.40±0.200 g
<u>NaCl × Silicon</u>				
NaCl 1× Si 1	0.470±0.010 c-d	58.96±0.513 d	0.966±0.020 d	134.70±0.173 e
NaCl 1× Si 2	0.520±0.010 b	57.43±0.152 e	1.040±0.020 c	132.36±0.152 f
NaCl 2 × Si 1	0.486±0.015 c	70.20±0.264 b	0.890±0.020 e	144.33±0.152 c
NaCl 2 × Si 2	0.580±0.010 a	54.40±0.100 f	0.910±0.222 e	150.50±0.360 a

Si 1 (silicon 0.5 mM), Si 2 (silicon 1.5 mM), NaCl 1 (NaCl 50 mM), NaCl 2 (NaCL 100 mM); small letters in each column show the significance of mean comparison by Duncan multiple range test (P≤0.05).

mM) root fresh weight significantly increased compared to non-silicon plants ($p<0.05$).

Root dray weights (Fig II. B) significantly decreased in the plants treated with NaCl (50 and 100 mM) compared to the plants grown under non-stress condition ($p<0.05$). In plants treated with silicon (0.5 and 1.50 mM) and salt (50 and 100 mM), root dry weights significantly increased compared to non-silicon plants ($p<0.05$).

Biochemical parameters

Analysis of the obtained data revealed that the concentration of chlorophyll a (Table 1) significantly decreased in the plants treated with NaCl (50 and 100 mM) compared to those grown under non-stress condition ($p<0.05$). Treatment with silicon (0.5 and 1.50 mM) significantly increased chlorophyll a contents in plants under 50 and 100 mM salinity compared to non-silicon plants ($p<0.05$).

Content of chlorophyll b (Table 1) did not show any significant change in salt stress group compared to control plants ($p<0.05$). In plants treated with silicon (0.5 and 1.50 mM) and salt (50 and 100 mM) chlorophyll b content showed a significant increase compared to non-silicon plants ($p<0.05$).

Content of total chlorophyll (Table 1) showed significant decreases under all levels of salinity compared to control plants ($p<0.05$). In addition, in plants treated with silicon (0.5 and 1.50 mM) and salt (50 and 100 mM) total chlorophyll showed a significant increase compared to non-silicon plants ($p<0.05$).

Significantly decrease ($p<0.05$) was observed in root carbohydrate contents of the plants treated with NaCl (100 mM) compared to those grown under non-stress condition (Table 1). Application of silicon at the highest concentration (1.50 mM) increased root carbohydrate in plants treated with 100 mM NaCl ($p<0.05$).

Concentration of shoot carbohydrate (Table 1) showed a significant decrease under salt stress treatment (100 mM) compared to control condition ($p<0.05$). In plants treated with silicon (1.5 Mm) and NaCl (100 mM) shoot carbohydrate contents significantly increased compared to non-silicon plants. On the other hand, application of silicon with 0.5 mM concentration did not result in any significant change on carbohydrates contents of shoots ($p<0.05$).

Significant increases ($p<0.05$) were recorded in carotenoid contents of the basil plants under all levels of salinity compared to the control (Table 2). In plants treated with silicon (0.5 and 1.50 mM) and salt (50 and 100 mM) carotenoid contents significantly increased compared to non-silicon plants ($p<0.05$).

Enzyme activity and oxidative parameters

Results of the study showed that hydrogen peroxide content (Table 2) significantly increased in salt-stressed plant while application of silicon (0.5 and 1.5 mM) significantly decreased hydrogen peroxide contents ($p<0.05$)

Catalase activity (Table 2) showed a significant decreased in all levels of salinity compared to control plants ($p<0.05$). In basil plants treated with silicon (0.5 and 1.50 mM) and salt (50 and 100 mM) catalase activity showed a significant increase compared to non-silicon plants ($p<0.05$).

Moreover, a significant increase ($p<0.05$) was observed in activity of the plants under all levels of salinity compared to control plants (Table 2). Application of silicon in non-salinity conditions significantly increased this parameter. Silicon at a concentration of 0.5 mM resulted in a significant decrease in the activity of superoxide dismutase under all levels of salinity. Furthermore, interaction of salinity (100 mM) and silicon (1.50 mM) showed the highest increase in SOD activity ($p<0.05$).

Discussion

Plant growth and development are affected by various factors and environmental stresses (Farzamisepehr et al., 2021). Salt stress is one of the damaging factors affecting morphological, physiological, and biochemical traits in plants. The results of this study showed that salinity reduced fresh and dry weights of roots and shoots, which was similar to the results of a number of other studies (Heidarian and Roshandel, 2020; Hoffmann et al., 2020; Yarsi et al., 2017). Reduced fresh and dry weights of plants under salt stress seems to be due to osmotic effects, reduced water absorption, and reduced osmotic potential against salinity (Rezende et al., 2017). Accumulation of sodium ion in plants that are exposed to salinity causes osmotic changes and reduces the water potential in the root zone, which in turn reduces water absorption in roots and impedes the entry of water into the plant. Under salt stress, the relative water content, leaf water potential, water retention, and optimal water use capacity are reduced. Plants that are not able to adapt and regulate osmotic conditions cannot maintain the turgor pressure. Numerous studies have pointed out that the growth and development of plants depend primarily on the turgor pressure. When turgor pressure decreases, stomata are closed, photosynthesis is reduced, and cell divisions are reduced (Abbasi et al., 2016; Abdel-Latif and El-Demerdash, 2017). Studies have revealed that under saline conditions salt accumulates in old leaves and causes a decrease in carbohydrates and growth hormones in meristematic zone and consequently leads to growth decline. In other words, as a result of reduction in photosynthesis and nutrient uptake, the synthesis of specific metabolites decreases, eventually impeding plant growth (Acosta-Motos et al., 2017).

Application of silicon nanoparticles was found to improve the growth of strawberry under salinity stress (Avestan et al., 2019). Also, in their study on *Borago officinalis* (Torabi et al., 2015) reported the positive effects of silicon against salt stress. In another study on cucumber (Wang et al., 2015) it was shown that the use of silicon improved hydrolytic conductivity and root access to water. Also, in another study on *Oryza sativa* silicon was reported to cause lignin and suberin deposition cell wall, and then root growth and development was stimulated (Zhu et al., 2019). Lignification in sorghum species increases xylem resistance to water loss and also maintains leaf turgor pressure by increasing leaf surface cuticle (Abdel-Latif and El-Demerdash, 2017). A similar study showed that in wheat, the negative effects of salt stress on growth, tillering and nutritional parameters were reduced by silicon (Daoud et al., 2018).

In the present study, the amount of chlorophyll a, total chlorophyll, and soluble carbohydrates in salt stress treatments decreased. A similar result was reported in a study on *Physalis peruviana* exposed to 0.5% and %1 sodium chloride, where total chlorophyll content reduced(Rios-Lozano et al., 2023). This may be due to the decreased absorption of essential nutrients, increased chlorophyllase enzyme, decreased chlorophyll synthetase, production of active oxygen, and degradation of photosynthetic pigments, resulting in reduced soluble sugars (Rezende et al., 2017).

The use of silicon in the present study modulated the reduction of chlorophyll and thus modulated the reduction of soluble sugars under salinity stress. Salinity reduces photosynthesis by causing osmotic stress, and ion imbalance and toxicity. Silicon increases photosynthesis by improving water absorption and transport (Rios et al., 2017). Research showed that silicon increased total chlorophyll and carotenoids in *Zea mays* (Moussa and Galad, 2015). Also, chlorophyll content under salinity reduced by 0.5% in *Physalis peruviana*, and the use of silicon at a concentration of 0.5 (g l^{-1}) improved the reduction of chlorophyll a, b, and total (Rezende et al., 2017). Silicon is an element that prevents chlorophyll degradation by maintaining water balance (Shanan and El Sadek, 2017). Photosynthesis and especially the preservation of the structure of photosystem II was reported to improve and increase photosynthesis and thus increase soluble carbohydrates (Zhu et al., 2019). On the other hand, silicon has a positive effect on the pentose cycle of phosphate and Kelvin, thereby increasing the efficiency of photosynthesis and modulating the negative effects of stress (Soundararajan et al., 2017).

Carotenoids are a type of photosynthetic pigment that also play as an antioxidant against stress. In this study, carotenoids increased under salinity stress. There was a similar result in *Salvinia auriculate* (Pervaiz et al., 2023).Perhaps the reason for the increase in carotenoids can be attributed to their antioxidant effect and protection of its plasma membrane (Gomes et al., 2017; Soundararajan et al., 2017).

In this study, the amount of H_2O_2 as an indicator of antioxidant damage and the activity of catalase and superoxide dismutase enzymes were measured as it modulates antioxidant damage. Results showed that hydrogen peroxide increased in plants under salinity stress. Many studies indicate that salinity stress is a stimulus in the production of reactive oxygen species such as O_2^-, H_2O_2, and OH^-, and increasing these compounds causes cell damage such as peroxidation of lipids and damage to proteins, chloroplast membranes, mitochondria, and cells. Applying silicon reduces Na^+ contents in roots and stems and increases the K^+/Na^+ ratio (Zhu et al., 2020). One of the reasons for increasing K^+/Na^+ by silicon is plasma membrane H^+ ATPase, H^+ pyrophosphates plasma membrane, and tonoplast amplification. Silicon increases potassium and potassium is an essential element in the structure of 160 enzymes (Abdel-Latif and El-Demerdash, 2017). Also, silicon produces enzymatic antioxidants such as CAT and SOD to fight ROS and protects the plant against these damages (Abbasi et al., 2016).

Other studies have shown that the application of silicon in *Zea mays* under salinity stress increased the activities of CAT and SOD (Moussa and Galad, 2015) which is similar to the results obtained in the present study. Similar studies reported the positive effect of silicon on increasing antioxidant enzymes in rice, cucumber, and tomato plants under salinity stress (Hoffmann et al., 2020; Mauad et al., 2016; Zhu et al., 2020). On the other hand, the amount of H_2O_2 in *Rose hybrida* decreased under silicon treatment (Soundararajan et al., 2017), which is similar to the results obtained in the present study. SOD converts O_2 to H_2O_2 and then in a biochemical reaction H_2O_2 is converted through POD enzyme to O_2 and H_2O, and its toxic effect is reduced (Zhang et al., 2017). (Alves et al., 2020) showed that H_2O_2 levels in silicon-treated lettuce reduced, and this is similar to a study(Sattar et al., 2019) on *Triticum aestivum* treated with both silicon and drought. Also decreased H_2O_2 levels were reported in *Glycyrrhiza glabra* plants treated with silicon (Yazdani et al., 2021).

From this research it is concluded that salt stress reduces plant growth while the use of silicon mitigates the adverse effects of salinity. Silicon stimulates multiple responses such as nutrient uptake, production of antioxidants, synthesis of osmolytes, and production of phenolic compounds (Khan et al., 2019). In general, in this study, the positive effects of using silicon to ameliorate the negative effects of salinity was observed especially at 1.50 mM concentration, where the negative effects of salinity on photosynthesis pigment and antioxidant enzyme were mitigated. Applying silicon as an oscillator in this study moderated the effects of salinity stress by affecting photosynthetic pigments and antioxidant enzymes and improving the morphological and biochemical properties of basil under salinity stress. The optimal concentration of silicon in the study, i.e. 1.5 mM, enabled the basil plant to withstand the damage caused by salinity stress.

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