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ORIGINAL PAPER

Stimulating the Anti-Oxidative Role and Wheat Growth Improvement Through Silicon Under Salt Stress

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Abstract
Purpose Salt stress poses a threat to wheat performance that can be managed by silicon application. Therefore, the current short term experiment was aimed to learn the effect of silicon on the defense system, ionic composition and growth of wheat in the presence and absence of salt stress.
Methods In the current experiment, the influence of silicon (6 mM) on anti-oxidative potential and dry matter yield of wheat genotypes (salt sensitive; Fareed-06 and salt tolerant; Inqlab-91) was studied under saline (10 dS/m) and control (2 dS/m) hydroponic solutions. The whole Si was applied to the pots of the Si+ treatment (6 mM) using calcium silicate solution. $CaCl_2$ solution was applied to the pots of the Si-deficient treatment to balance the same total of Ca as in the Si+ treatment to obtain the sole effect of Si. Various ionic, biochemical and growth parameters of wheat plants were recorded after 34 days of transplanting according to standard procedures.
Results Silicon addition to the hydroponic solution increased wheat seedling weight, K:Na with limited Na and enhanced K uptake. Moreover, Si increased chlorophyll *chl* a/b ratio and promoted the actions of superoxide dismutase (SOD) and catalase (CAT).
Conclusions It can be concluded that silicon supplementation improved the wheat growth and plant defense suppressed by the salt applied. Further, salt tolerant variety gave better performance than the salt sensitive variety.

Keywords Silicon · Salt tolerance · SOD · CAT · Potassium · Sodium · NaCl

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Abbreviations:

Abbreviation	Explanation
Si	Silicon
SOD	Superoxide dismutase
CAT	Catalase
Chl	Chlorophyll

1 Introduction

Salinity causes not only the osmotic and ion effects on plants, but it also induces oxidative stress [1]. The toxic effect of salinity accelerates the production of reactive oxygen species such as singlet oxygen, superoxide hydrogen peroxide and hydroxyl radical [2] which destroy lipids, nucleic acids and proteins within the plant body [3] and reduces chlorophyll content [4]. Membrane lipids are deteriorated by ROS known as lipid peroxidation that disrupts the cell structure [27]. All these para-normal activities reduce the crop growth and the yield [28, 29] because several bio-chemical, biological and physiological processes occurring in the plant body are severely affected by the presence of excessive salts in the root zone [5].

In response to salt stress, the intrinsic defensive mechanism in plants enables them to alleviate the toxic salt by producing different enzymatic (SOD, CAT, APX) and non-enzymatic (ascorbate, glutathione and a-tocopherol) antioxidants [6]. This basic defense is still not enough to save the plant, instead it needs additional support. Many approaches have been tested against salt stress and the silicon (Si) amendment is one of them. Under salt stress the Si application significantly decreases the production of ROS like H_2O_2, MDA and ELP by stimulating the activities of antioxidants such as SOD, CAT, APX, GPX, GR and H^+ATPase [7, 8].

Sufficient information regarding the anti-oxidative role of Si in local wheat germplasm is lacking. Different plant species and even crop varieties have different response and tolerance mechanisms. Therefore, the current short experiment was done to recertify the effectiveness of Si in improving the defense system and growth of wheat in the presence of NaCl with changes in added Si and wheat genotypes compared with previous experiments [28].

2 Materials and Methods

2.1 Plant Material and Experimental Conditions

The current experiment was executed under saline (EC=10 dS m^{-1}) and control (EC=2 dS m^{-1}) hydroponics culture in the wheat cvs (salt sensitive; Fareed-06 and salt tolerant; Inqlab-91) in response to Si application (Si+ = 6 mM) against control (Si− = 0 mM). The whole Si was applied to the pots as calcium silicate solution. Initially, nursery plants were grown for two weeks and uniform size seedlings were transferred to Hoagland nutrient solution tubs. Completely randomized design with three replicates in factorial arrangement was followed. Required EC was developed by adding NaCl. Two months old wheat seedlings were subjected to analytical and growth parameters according to standard procedures as given below.

2.2 Parameters

Plants were harvested, and left for sun drying. After that samples were oven dried to constant weight and weighed with a spring balance and yield per plant was recorded. The oven dried and ground leaf material (0.1 g) was digested with a mixture of 2 mL of sulfuric acid and hydrogen peroxide according to the method of [10]. Potassium and sodium in the digested material were determined with a flame photometer (Jenway, PFP-7). Si concentration was measured using the calorimetric amino molybdate blue color method [30]. The chlorophylls a and b were determined according to the method of Arnon [31].

For the extraction of antioxidant enzymes, fresh leaves (0.5 g) were ground in a pestle and mortar using 50 mM cooled phosphate buffer (pH 7.8). After filtration through a cheese cloth, the homogenate was centrifuged at 15000 x *g* for 20 min at 4 °C and the supernatant was used for enzymes assays. The activity of SOD was assayed following the method of [13] by monitoring the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm. Activities of CAT were assayed following [14] with some modification.

3 Results

Dry matter yield (Fig. 1A) was badly affected in the presence of salt stress as against the control in both varieties. The seedling weight increased considerably, with the addition of silicon not only under saline but also under control environments in both varieties when compared with Si-deficient treatment. Comparing both the varieties, Inqlab-91 produced more weight than Fareed-06 both under saline and non-saline conditions

The data (Fig. 1B, C) showed that salt stress (10 dS/m) significantly reduced the flag leaf Si and K^+: Na^+in comparison to control (2 dS/m) conditions in both varieties. The addition of Si (6 mM) significantly (p <0.05) increased the Si content (Fig. 1C) and K^+: Na^+in wheat plants than those grown deprived of silicon both under control and saline conditions in both genotypes. Comparing varieties, better K^+: Na^+ratio and Si uptake were seen in Inqlab-91 (salt tolerant) in contrast to Fareed-06.

Antioxidants (CAT and SOD (Fig. 1D, E) in flag leaves were enhanced due to NaCl as compared to non-saline in both varieties. Silicon addition further increased CAT and SOD contents considerably under saline and non-saline conditions in both the varieties. Comparing both the varieties, Inqlab-91 showed improved defense activities than Fareed-06.

Salinity (Fig. 1F) significantly lowered the *Chl* a/b as against non-saline conditions in both varieties. Added Si enhanced the *Chl* a/b to a significant extent under saline and non-saline conditions in both varieties. Among varieties, Inqlab-91 exhibited higher values in comparison to Fareed-06 under control and saline environments.

4 Discussion

The current data indicate that owing to the addition of NaCl in the hydroponic nutrient medium, the Na^+content was significantly increased in wheat flag leaves. The presence of excessive Na^+disturbed the metabolic and growth activities including the production of ROS, loss of chlorophyll content, reduced K^+uptake and finally the dry matter production was significantly reduced. The production of

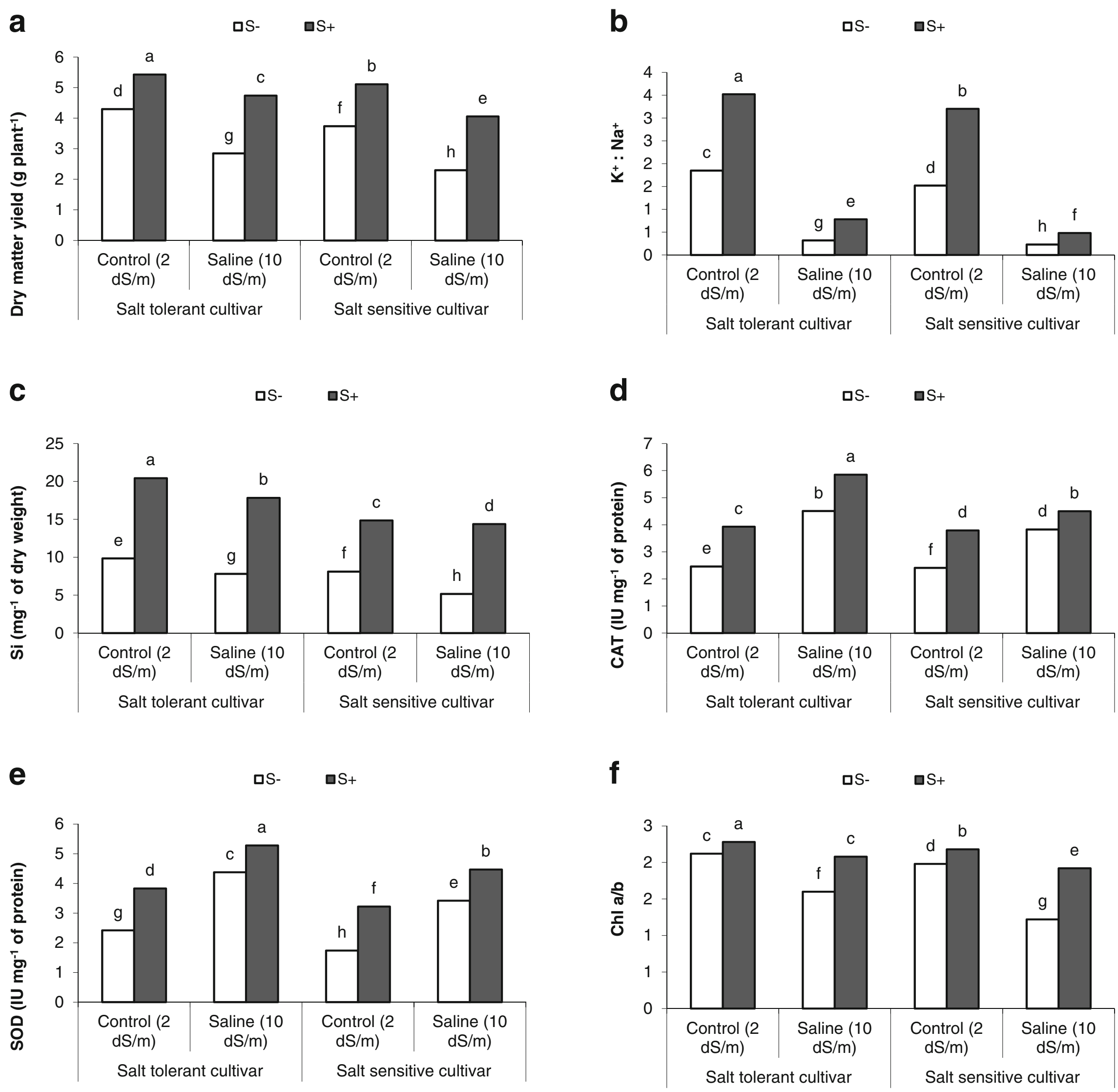

Fig. 1 Influence of Si on K^+: Na^+, catalase, peroxidase, superoxide dismutase and dry matter production of wheat cultivars both under saline and non-saline conditions. Si- and Si+ represent 0 and 6 mM of Si respectively. Values in columns sharing the different letters differ statistically at P ≤0.05. (The values are means of three replicates)

ROS like hydrogen peroxide, superoxide, singlet oxygen and hydroxyl radical suppressed the anti-oxidants making the plant defense system weak [1, 2]. Ionic imbalance also occurred in the cell due to excessive accumulation of Na^+ which reduced the uptake of K^+ [12] as depicted in the current data. Chlorophyll contents and their ratios were also considerably decreased in the presence of toxic NaCl in comparison to the control and the same has been reported by [18].

Any factor which can reduce the accumulation and translocation of Na^+ in a plant body enables the plant to tolerate the salt stress. Though Si is non-essential, its application in a saline environment is beneficial [4]. In current results, the Si supplementation significantly increased the Si contents in wheat plants that significantly reduced the Na^+ concentration in the leaves of both varieties under stressful and control environments. The presence of silicon produces a polymer gel in the root exodermis and endodermis and physically blocks the apoplastic transport of toxic Na^+ to aerial plant parts [22]. Owing to this feature, Si ameliorated the oxidative stress in both varieties by exciting the production of antioxidants (SOD and CAT) [23–25]. It also occurred in tomato, cucumber [8] and barley [25]. This detoxification of ROS resulted in a remarkable increase in *Chl a* as compared to *Chl b*. which in turn helped to cause the enhancement in photosynthesis and higher dry matter yield that was obtained where silicon was applied. Another factor in growth improvement was the increased uptake of

K^+ which in turn increased plant water status and protected the plant from salinity induced water stress [22, 26].

It was concluded from the current research that salt tolerance in Si-applied wheat is accredited to decreased uptake of Na^+ and enhanced uptake of K^+, improved chlorophyll ratio and enhanced antioxidant activity finally leading to increased wheat growth. Note, significantly, that our previous results were verified with the use of a lower dose of Si and different wheat varieties.

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