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ORIGINAL PAPER

# Protective Role of Silicon (Si) Against Combined Stress of Salinity and Boron (B) Toxicity by Improving Antioxidant Enzymes Activity in Rice

**Muhammad Ansar Farooq**[1,2] · **Zulfiqar A. Saqib**[2] · **Javaid Akhtar**[2] ·
**Hafiz Faiq Bakhat**[3] · **Ratna-Kumar Pasala**[4] · **Karl-Josef Dietz**[1]

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**Abstract** The beneficial element silicon (Si) is known to enhance plant tolerance against various kinds of biotic and abiotic stresses. However, little is known about its protective role for plants facing multiple stresses such as salinity and boron (B) toxicity. Therefore, the current study was planned in pots to evaluate the beneficial role of exogenous applied Si (150 mg kg$^{-1}$) nutrition against salinity stress (10 dS m$^{-1}$), and B toxicity (2.5 mg kg$^{-1}$) alone or in combinations. Results showed that both salinity and B toxicity reduced plant growth and biomass of rice, with maximum damage under their combined stress due to increased uptake of toxic ions such as sodium (Na$^{+}$) and B. Contrarily, Si application helped the plants to overcome negative effects of these toxic ions by increasing silica and K$^{+}$ uptake and decreasing Na$^{+}$ and B entry in plants that ultimately lead to improvement in plant biomass. High silica uptake ability of rice significantly improved the efficiency of antioxidant mechanism, as indicated by reduced catalase (CAT) activity and improvement in guaiacol peroxidase (GPX) and ascorbate peroxidase (APX) activity by Si application under stress, resulting in reduced oxidative damage. From this study, we conclude that Si fertilization can enhance crop production in salt affected soils by helping plant defenses against salts as well as associated B toxicities; however, field trials should be carried out before setting any recommendations for farmers.

**Keywords** Boron · Salinity · Silicon · Rice

## 1 Introduction

Boron (B) toxicity is a significant constraint to cereal production in regions worldwide [1]. This problem is more severe in arid or semi-arid regions where irrigation water having a high level of B is used [2]. Due to low rainfall environments under such conditions, B is not sufficiently leached from the rhizosphere and consequently may accumulate to toxic levels [1, 2]. Natural weathering of B-containing rocks is also a major source of B compounds in water [2]. The conditions become even more detrimental for plant growth when salinity and toxicity of B occur concurrently particularly in saline-sodic soils. Salinity increases B toxicity symptoms in plants by increasing soluble B levels in inter and intracellular compartments in leaves, making the conditions more hostile for plant growth. Boron and salinity combined effects have significantly reduced biomass production and components of yield in wheat [3], tomato and spinach [4], and cucumber and tomato [5]. However, up till now, no unique reaction of plants to the combination of B toxicity and salinity has been identified and results of such studies are conflicting. Many reports on different plant species revealed no additive effects of salinity and high B, pointing to an independency of the combined effect [6,

✉ Muhammad Ansar Farooq
muhammad_ansar.farooq@uni-bielefeld.de

[1] Department of Biochemistry and Physiology of Plants, W5,
University of Bielefeld, University Str. 25, D-33501,
Bielefeld, Germany

[2] Intstitute of Soil & Environmental Sciences, University
of Agriculture, Faisalabad, Pakistan

[3] Department of Environmental Sciences, COMSATS, Institue
of Information Technology, Vehari Campus, 22060, Pakistan

[4] ICAR National Institute of Abiotic Stress Management,
Malegaon, Baramati-413115, Pune, Maharastra, India

7]. Other studies suggested the effect of both stresses was additive [3, 8]; even antagonistic effects were also reported [9].

In order to harvest better crop yields from such deteriorated soils, there is a need of in-depth understanding for this limited and sporadic information. Several chemical, physical (engineering) and biological approaches could be used to counteract crop yield losses from such soils. Among them, exogenous application of some mineral nutrients like silicon (Si) has gained considerable ground as a shotgun approach to ameliorate the adverse effects of various biotic and abiotic stresses. Accumulation of Si as silicic acid in cell walls of leaves, roots and stems imparts several benefits to plants such as reducing ion toxicities by improving the activity of antioxidant enzymes during oxidative stress. This uptake and accumulation of Si in gramineous plants is generally higher than for other species [10]. Among cereals, rice is a typical siliciphilous plant capable of accumulating silica up to 10 % of its dry weight [10]. The region renowned for production of rice in Pakistan is named the "Kalar tract" which has low to moderate sodicity and salinity with high pH, causing significant yield losses of up to 68 % [11]. To our best knowledge, there is currently no information available about the possible beneficial effects of Si on stress tolerance of rice grown under the combined stress of salinity and B toxicity. Therefore, a pot experiment was conducted to investigate the protective role of Si in the growth performance of rice under such adverse soil conditions. We hope that this study will provide a basis for developing strategies to reduce the risks associated with the combined stress of salinity and B toxicity and maintaining sustainable rice production.

## 2 Materials and Methods

### 2.1 Plant Material and Growth Conditions

Seeds of rice *cv.* IR29 were obtained from the Saline Agriculture Research Center (SARC), University of Agriculture, Faisalabad-Pakistan and surface sterilized with 2 % sodium hypochlorite solution, followed by thorough rinsing with de-ionized water. Later, treated seeds were sown in trays having sand for germination. After ten days of germination, nursery plants were shifted to 12 kg glazed pots. The soil filled in pots was air dried, mixed and analyzed for the following physico-chemical characteristics: EC (electrical conductivity) = 1.90 dS $m^{-1}$, pH = 7.61, SAR (sodium adsorption ratio) = 5.6 $(\text{mmolc kg}^{-1})^{1/2}$, OM (organic matter) = 0.56 %, Si = 24 mg $kg^{-1}$, B = 0.31 mg $kg^{-1}$ and the textural class was sandy clay loam. Salinity treatment was applied by adding and mixing NaCl salt to develop EC = 10 dS $m^{-1}$ in half of the pots. Silicon was added as $Na_2SiO_3$ (150 mg $kg^{-1}$) and B in the form of $H_3BO_3$ (2.5 mg $kg^{-1}$) alone and in combinations in both saline and non-saline pots. The experiment was arranged according to completely randomized design with three replications. After transplantation, the pots were placed in a climate controlled glass house. These pots were irrigated weekly with half strength Hoagland's solution as necessary [12].

### 2.2 Measurements

At the booting stage, the photosynthetic efficiency of plants was measured by an IRGA (infra-red gas analyzer; LCA-4, Hoddesdon, UK) and plants were harvested. The shoot fresh biomass was recorded and fresh leaf samples were immediately frozen in liquid nitrogen and stored at -80 °C for enzyme assays. Later, plant shoots were kept in an oven at 80 °C until constant dry weight. The elemental composition of rice shoots for $Na^+$, $K^+$, B and Si content was determined after grinding dried plant material. The elemental analyses for shoot $Na^+$and $K^+$contents was measured with a Flame-photometer (Sherwood, 410, Cambridge, UK) as described by [13]. Boron and silicon were analyzed on a spectrophotometer by the Azomethine-H method [14] and [15] respectively. For antioxidant enzymes assay (catalase, ascorbate peroxidase and guaiacol peroxidase), frozen plant leaves were ground and extracted with appropriate buffers. The activity of catalase (CAT) was determined by a spectrophotometer at 240 nm [16], ascorbate peroxidase (APX) at 290 nm [17] and guaiacol peroxidase (GPX) at 470 nm according to the method of [18].

## 3 Statistical Analysis

The data were subjected to statistical analysis by using t-tests and treatments were compared by calculating means with standard deviation at $p \leq 0.05$.

## 4 Results

Results (Table 1) of the growth parameters revealed that under all adverse conditions i.e. salinity and/or B toxicity, plant growth was negatively affected as indicated by a decrease in shoot fresh and dry weight. However, the extent of damage was maximum under salinity coupled with B toxicity stress. When compared to the control, approximately 40 % less biomass yield was recorded under this aggravated stress. Addition of Si to growth medium improved the growth of plants under both control and stress conditions. The maximum significant response by Si application was for combined stress, where shoot fresh and dry biomass increased by 60 % and 40 %, respectively. On

**Table 1** Effect of Si, B, and NaCl treatments on growth, antioxidants activity and elemental contents of rice grown under normal and saline soil conditions

| Determinants | Normal soil | | | | Saline soil | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | Si | B | Si+B | Control | Si | B | Si+B |
| Growth | | | | | | | | |
| Shoot fresh wt. (g/plant) | 28.1bc | 34.9ab | 26.2bd | 38.2a | 18.3d | 22.2cd | 17.6d | 28.4bc |
| Shoot dry wt. (g/plant) | 6.3ac | 7.4ab | 4.8bc | 9.1a | 4.2bc | 5.1bc | 3.2c | 5.7ac |
| Photosynthesis (µmol $m^{-2}s^{-1}$) | 4.4ac | 8.9a | 1.6c | 7.3ab | 2.9bc | 6.9ab | 1.5c | 5.3ac |
| Antioxidant activity (mmol $min^{-1}$ $g^{-1}$) | | | | | | | | |
| Ascorbate peroxidase | 13.1cd | 16.8a | 10.1e | 14.4bc | 11.9d | 15.8ab | 7.8f | 12.1b |
| Guaiacol peroxidase | 9.5h | 15.5d | 13.3f | 18.2b | 11.8g | 16.8c | 14.9e | 19.4a |
| Catalase | 2.0c | 1.4d | 2.8 b | 1.9c | 3.2b | 2.2c | 4.1a | 2.9b |
| Elemental contents (%) | | | | | | | | |
| Boron | 100 | 91 | 150 | 141 | 100 | 106 | 154 | 69 |
| Potassium | 100 | 116 | 113 | 131 | 100 | 106 | 107 | 75 |
| Sodium | 100 | 72 | 78 | 112 | 100 | 73 | 83 | 91 |
| Silicon | 100 | 146 | 64 | 113 | 100 | 129 | 60 | 98 |

Values represent means from 3 independent experiments (n = 6). Different letters indicate means that are significantly different from each other at p = 0.05

the other hand, the photosynthetic yield of control plants was not significantly different as compared to salinity or B toxicity stress alone. Contrarily, application of B toxicity stress together with salinity decreased the photosynthetic value by 65 % which was improved by Si application under all growth conditions, however, the response was non-significant.

Similarly, results of the shoot elemental composition (Table 1) showed that B, $Na^+$ and Si concentrations were all highest under their respective treatments. However, the interactive pattern among them under different experimental conditions was quite variable. Under normal soil, application of either B or Si reduced entry of $Na^+$ in plants which was increased by their combined application. Due to the highest uptake rate of Si and B under stress conditions, $Na^+$ uptake rate was reduced in their presence. As compared to non-stressed plants, there was a 30 % increase in shoot silica contents due to Si application under saline conditions. Similarly, under combined stress, there was a 37 % increase in B uptake due to its application; thereby making conditions more hostile for plant growth due to B toxicity. However, application of Si under such conditions relieved the stress by reducing its uptake by 30 % as compared to stressed plants missing Si supply. Likewise, due to the decrease in $Na^+$ uptake by Si or B application, the uptake trend for $K^+$ in their presence always increased. Comparing the effectiveness of B and Si in enhancing shoot $K^+$, contents of rice plants showed maximum uptake in the presence of Si supply. However for both normal and saline soils, a general increase of up to 26 % in $K^+$ uptake by their combined application caused an additive effect on $K^+$ uptake.

An antioxidant network consisting of ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and catalase (CAT) plays a significant role in the detoxification of hydrogen peroxide ($H_2O_2$) i.e. a toxic byproduct of oxygenic photosynthesis under stress. Therefore in the present study, these antioxidants were measured in rice leaves as a marker of stress and defense response (Table 1). Results revealed that due to either stress i.e. salinity and/or B toxicity, APX activity dropped substantially. However, the maximum significant decrease by 34 % occurred under combined stress. Contrastingly, application of Si (150 mg $kg^{-1}$) improved its activity by up to 32 % under salt stress alone, but no difference was observed under combined stress. Whereas, GPX and CAT showed a distinct but contrasting pattern of response under stress and Si application. Their activities were significantly enhanced upon salinity and/or B toxicity stress exposure of control plants. As for APX activity response, maximum damage occurred under combined stress where CAT activity was enhanced by a factor of 2 as compared to 1.5 times under salt stress alone. Similarly GPX activity was also more pronounced under combined stress which was further enhanced up to 30 % by Si application as compared to stressed plants missing Si supply. But in contrast, CAT activity was lowered due to Si application under both control and stress conditions (salinity and/or B toxicity).

## 5 Discussion

This study aimed to investigate the protective effects of Si on rice crops exposed to the combined stress of higher NaCl salt and toxic B concentrations. The results indicated that B toxicity in rice caused substantial damage to plant growth along with salinity stress and in most of the determinants the effect of B is additive with salinity. Without Si supplementation, the plant biomass and photosynthetic efficiency of rice were significantly affected under stress, particularly the combined stress of salinity and B toxicity. Higher uptake of toxic ions like $Na^+$ and B under such deteriorated soil conditions creates ionic imbalances such as decreased $K^+$ entry in plants. This results in oxidative stress in plants which severely hampers growth and yield of crop plants [19]. Similar results of poor growth response in cereals (e.g. maize and sorghum) and vegetables (e.g. cucumber and tomato) are also reported under the combined stress of salinity and B toxicity [5, 20]. However, Si application in such deteriorated soils significantly enhanced the growth of rice plants. Higher silica contents in rice shoots due to Si application may be correlated with decreased uptake of $Na^+$ and B under such conditions. Irreversible precipitation of silica in the cell wall of leaves and shoots results in the formation of boron-silicate complexes both in the plant and soil, hence lowered B availability and uptake [21]. B is taken up passively by plants via a transpiration stream, while Si is either taken up actively via transporters or passively via the transpiration stream; therefore Si application may also result in reduced B uptake due to antagonistic behavior between them [4]. Accumulation of Si in the cell walls and lumens of plants helps improved tolerance against various biotic and abiotic stresses [21]. From this report, improved tolerance to salinity and/or B toxicity by Si supplementation is correlated with lower uptake of toxic ions such as $Na^+$ and B and enhanced efficiency of antioxidant mechanism which ultimately causes reduced oxidative damage. Maintaining a proper $K^+/Na^+$ ratio in plants is also the key to various metabolic processes, as it maintains the turgor pressure in the cell and activates a number of metabolic enzymes [22]. Components of the antioxidant system are very essential in maintaining higher photosynthetic rates as well as the detoxification of toxic by-products of oxygenic photosynthesis i.e. reactive oxygen species (ROS). This antioxidant system is either activated or disabled under variable growth conditions in order to keep the concentration of ROS at a minimum level. It is reported that application of Si not only improves the photosynthetic efficiency of plants by increasing light interception due to more erectness in leaves and higher Rubisco activity [4, 21], but it also improves the efficiency of the antioxidant system, which is required for the detoxification of ROS. In this experiment, higher activities of CAT and GPX under salinity and/or B toxicity depict higher oxidative stress which was lowered by Si application as shown by lowered CAT activity. These results are in agreement with the reports of [23], who documented higher CAT activity under salinity or B toxicity in apple rootstocks. However, activities of APX and GPX in the presence of Si were significantly higher compared to stressed plants missing Si supply. These results suggest active involvement of APX and GPX in the detoxification of ROS as compared to CAT and such a coordinated response between them is thought to promote oxidative stress tolerance. Improvement in APX activity by Si application to cucumber and potato seedlings grown under salt stress is also reported by [24, 25].

In conclusion, results of this experiment demonstrate severe damage to rice growth under stress, particularly under the combined stress of salinity and B toxicity and indicate a protective role of Si in regulating the stress response of rice. From the results of the present study, we suggest that Si could be used as a potential candidate to improve crop performance under adverse soil conditions. The findings of this study suggest enhanced tolerance of rice against salinity and/or B toxicity by reduced oxidative damage due to improved activities of antioxidant enzymes (APX, GPX and CAT), however, further investigations are needed particularly under field conditions.

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