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Silicon-mediated oxidative stress tolerance and genetic variability in rice (*Oryza sativa* L.) grown under combined stress of salinity and boron toxicity

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Abstract: The benefits of silicon (Si) in improving crop fitness by biotic and abiotic stress resistance are widely reported. However, investigations about its protective mechanisms for plants facing multiple stresses are very limited. Two contrasting rice cultivars, KS-282 (salt-tolerant) and IRRI-6 (salt-sensitive), were grown in a pot experiment to study the interrelation between Si supplementation (0 and 150 mg kg^{-1}) and boron (B) toxicity (0 and 2.5 mg kg^{-1}) under salinity stress with emphasis on growth response, mineral contents, physiology, and enzymatic antioxidant system response. The results revealed that adverse growth conditions, particularly the combined stress of salinity and B toxicity, severely affected the physiological attributes of rice. It reduced plant biomass by damaging the membrane, reducing special products analysis division values and photosynthetic efficiency, but Si application counteracted the adverse effects of stress by reducing the uptake of toxic ions such as sodium (Na^+) and B, lowering transpiration rate. Increased relative water contents and photosynthetic efficiency due to a higher Si and K^+ uptake ultimately led to better growth performance. Si significantly affected activities of enzymatic antioxidants in both genotypes, with increased ascorbate peroxidase, increased guaiacol peroxidase, and reduced catalase activity suggesting relieved stress by reduced oxidative damage. The response to stress and Si differed genotypically, with maximum damage to the salt-sensitive genotype (IRRI-6), particularly under the combined stress of salinity and B toxicity. In contrast, supplied Si improved the growth of the salt-tolerant genotype (KS-282) better than the IRRI-6 (salt-sensitive) genotype. These results support the protective role of Si in the regulation of salinity and/or B toxicity stress by improving growth, K^+/Na^+ ratio, physiology, and antioxidant capacity, suggesting it as a potential candidate for crops grown under such deteriorated soil conditions.

Key words: Antioxidant enzymes, boron, *Oryza sativa* (rice), oxidative stress, physiology, salinity, silicon

1. Introduction

Salinization is widespread in irrigated agriculture as about 20% of the irrigated lands of the world are affected by salt, particularly in countries of Asia and Africa, but also with large proportions of the irrigated land affected in Argentina, Egypt, Iran, Pakistan, and the USA (Ghassemi et al., 1995). The impact of salinity is most serious in countries where all or most of the agricultural production is based on irrigation, such as in Egypt and Pakistan, and where agriculture is a substantial part of the national economy (Rengasamy, 2001). The demands on soil and water resources for food and fiber have been increasing tremendously in such countries including Pakistan due to a building population pressure. Furthermore, most of the available good land is already under cultivation so it is very hard to increase the area under crop production. Rather, we have to increase crop yield per unit area and degraded land will have to be pushed into service manipulating all feasible options or solutions to get a sustainable crop yield on these degraded lands (Epstein, 2001).

Boron (B) is an essential micronutrient for plant growth and is needed in small amounts. Plants vary considerably in their requirements for boron. However, the range of soil solution B concentrations between deficient and toxic for plants is minute. Boron exists mainly as boric acid [$B(OH)_3$] in soil solution, which can be leached easily under heavy rainfall conditions, especially from sandy soils, and this may lead to B deficiencies in plants that grow in these areas (e.g., many regions in the USA, Brazil, China, and Japan) (Shorrocks, 1997; Yan et al., 2006). On the other hand, during low rainfall conditions, B cannot be leached sufficiently and consequently may accumulate to degrees that become very toxic for plant growth (Reid, 2007). This is very common in arid and/or semiarid regions, where groundwater can have varying amounts of B that accumulates in the soil when ground water is pumped for irrigation. Thus, the boron can reach toxic levels that

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result in a reduction in crop yields (Rashid, 2005). High concentrations of B are also observed in association with saline–sodic soils. Salinity increases the B toxicity symptoms of plants and the soluble B concentration in the inter- and intracellular compartments of leaves. In many cases, salinity and high B occur together, making the conditions more hostile for the plants. In addition to the direct affects, salinity also strongly interferes with the uptake and metabolism of mineral nutrients and B is an important nutrient affected by salinity (Saqib et al., 2009). Therefore, increased salt and B tolerance of crops is required to enhance food production and sustain agriculture in Pakistan to feed the increasing population.

Several chemical, physical (engineering), and biological approaches have been developed for crop production on such soils. An integrated use of these approaches is imperative in most situations due to economic and environmental limitations. Of all of the above approaches, exogenous application of some mineral nutrients has gained considerable ground as a shotgun approach to ameliorate the adverse effects of salt toxicity (Raza et al., 2006). For example, the adverse effects of salt were ameliorated with an exogenous application of K^+ on wheat (Zheng et al., 2008) and maize (Yousra et al., 2013), and by the application of Ca^{2+} on bean (Awada et al., 1995). Similarly, the use of many organic compounds like glycinebetaine (Ashraf et al., 2008) and proline (Misra and Gupta, 2005; Hayat et al., 2012) as well as growth hormones like abscisic acid (ABA) and indole acetic acid (IAA) were found to be tremendously important to counteract the adverse effects of salt stress (Gomez-Cardenas et al., 2003; Khadri et al., 2006). Among the mineral nutrients, there is an increasing body of literature describing that the silicon (Si) found abundantly in the earth's crust (ranked second after oxygen) is beneficial for plant growth, particularly under various biotic and abiotic stresses including salinity stress (Gunes et al., 2007a). The beneficial effects of Si are ascribed to its accumulation in the cell walls of the leaves, roots, and stems, thereby reducing intake and translocation of toxic ions such as Na^+ from roots to shoots (Ma et al., 2006). Si benefits in salt tolerance were also found to relate to improved activity of antioxidant enzymes during oxidative stress (Ma and Yamaji, 2008). These benefits of silica uptake are crop-specific as plants differ widely in their mechanisms of uptake and accumulation. Plant silica contents generally range from as low as 0.1%–0.5% (dicots such as members of the orders Poales and Aricales) to as high as 10%–15% (monocots such as rice, wheat, and maize) (Ma and Yamaji, 2008). This percent uptake is sometime higher than for the macronutrients. Among monocots, rice (a typical siliciphilous plant) has the power to absorb and accumulate Si metabolically while many upland crop plants seem to lack this ability (Mitani and Ma, 2005). Rice is also a staple food; it has been estimated that half of the world's population survives completely or partly on rice and more than 90% is currently grown in Asia, where it is the main item of the diet. In Pakistan, the region renowned for the production of rice is named "Kalar Tract". This region has low to moderate sodicity and salinity with high pH, partial water logging, and deteriorated soil structure. Growing rice on such deteriorated soils reduces growth, yield, and yield components up to 68%, resulting in a huge annual economic loss of approximately 1 million USD (Aslam et al., 2003).

In the past, several researchers reported the benefits of Si nutrition under salt stress or B toxicity in different crops such as growth improvement of tomato, spinach, wheat, and barley by decreasing the uptake of Na^+, Cl^-, and B (Alpaslan and Gunes, 2001; Gunes et al., 2007a), alleviating oxidative damage (Gunes et al., 2007b), increasing the activity of nonenzymatic antioxidants (Khadri et al., 2006), improving water status, and enhancing the net photosynthesis rate (Al-aghabary et al., 2004). However, investigations on the high silica uptake ability of rice crop and its benefits under salinity coupled with B toxicity problems are very limited. Taking all of this into consideration, a pot experiment was planned to evaluate the effect of Si application on various physiological and biochemical parameters of rice grown under salinity and/or B toxicity.

2. Materials and methods

2.1. Plant material

The seeds of two rice genotypes differing in salt tolerance, KS-282 (salt-tolerant) and IRRI-6 (salt-sensitive), were obtained from the Saline Agriculture Research Center (SARC) of the University of Agriculture in Faisalabad, Pakistan. They were treated with a 5% sodium hypochlorite solution (v/v) for surface sterilization, followed by thorough rinsing with deionized water and soaked between two layers of filter paper for 48 h in darkness. Later, they were sown in polyethylene-coated iron trays with a 5-cm-deep layer of washed sand. The moisture contents were optimized for seed germination and seedling establishment with deionized water and 10-day-old (2-leaf stage) nursery plants were shifted to glazed pots and placed in the wire house under natural conditions at the SARC Institute of Soil and Environmental Sciences. These pots were irrigated weekly with half strength Hoagland's solution (Hoagland and Arnon, 1938) as necessary.

2.2. Soil treatment and growth conditions

The soil used for the experiment was collected from the Research Farm of the University of Agriculture in Faisalabad. Before pot filling, the soil was air dried, thoroughly mixed, sieved (2 mm mesh size), and analyzed. The following physicochemical characteristics

were determined: EC_e of 1.96 dS m^{-1}, pH_s of 7.64, sodium adsorption ratio (SAR) of 5.8 ((mmolc $kg^{-1})^{1/2}$, available P (phosphorous) of 2.9 mg kg^{-1}, total N (nitrogen) of 0.03%, organic matter of 0.62%, citric acid extractable Si of 27 mg kg^{-1}, diethylenetriamene pentaacetic acid (DTPA) extractable Zn of 1.1 mg kg^{-1}, NH_4OAc and NaOAc extractable Na, K, and B of 11.5, 2.2 (cmol kg^{-1}), and 0.32 mg kg^{-1} respectively, and the textural class was sandy clay loam (60% sand, 17.5% silt, and 22.5% clay) (Page et al., 2009). Based on the mentioned results, this soil was fit to use as a control (nonsaline) and to develop salinity artificially (10 dS m^{-1}); a calculated amount of NaCl was mixed with a mechanical shaker. Each pot (27 cm in diameter) was filled with 12 kg of air-dried soil. At the age of 45 days, both varieties were tested (three homogeneous plants of each variety per treatment) against two levels of each of NaCl (0 and 10 dS m^{-1}), Si as Na_2SiO_3 (0 and 150 mg kg^{-1}), and B in the form of H_3BO_3 (0 and 2.5 mg kg^{-1}), alone and in combination. To compensate for the amount of Na added with Si-treated plants, an equivalent amount of Na (as NaCl) was added and subtracted from the Si-nontreated and saline-stressed plants, respectively. The experiment was arranged according to completely randomized design (CRD) with four replications.

The recommended doses of N, P, and K were used at the rates of 100, 67, and 67 kg ha^{-1}, respectively. Urea, SSP (single super phosphate) and SOP (sulfate of potash) were applied as the sources of N, P, and K respectively. A half dose of the N and full doses of the P and K were applied at the time of transplanting while the remaining half dose of the N was added 40 days after sowing. Zinc in the form of zinc sulfate was applied at the rate of 20 kg ha^{-1} at the time of sowing.

The plants were harvested at the booting stage and shoot fresh biomass was measured. Leaf samples were immediately frozen in liquid nitrogen and stored at –80 °C for further biochemical analyses, or dried in an oven at 65 ± 5 °C until reaching a constant dry weight for dry biomass and elemental analyses.

2.3. Elemental analyses

The elemental compositions of both genotypes for their shoot Na^+, K^+, B, and Si contents were determined. The dried plant material was ground to powder form and 1 g was used for digestion in a diacid mixture of nitric and perchloric acids (3:1) at 60 °C for 2 h followed by determination of Na^+ and K^+ contents by flame photometer (Jenway PFP-7) as described by Standford and English (1949).

Colorimetric determination of B by azomethine-H was determined by dry ashing (Bingham, 1982). For dry ashing, 1 g of dried and ground plant material was put in porcelain crucibles. The crucibles were placed in a muffle furnace and the temperature was slowly raised to 550 °C. Ashing continued for 6 h after attaining 550 °C. The ash was made wet with 5 drops of deionized water, and then 10 mL 0.36 N sulfuric acid was added to the crucibles. After stirring with a plastic rod, the aliquot was filtered through Whatman No. 1 filter paper into a 50-mL polypropylene volumetric flask and then brought to volume with deionized water. The filtrate was used for B determination using a colorimetric procedure with azomethine-H.

For Si determination, 0.2 g of ground plant samples was digested in 2 mL of 50% H_2O_2 and 6 mL of 50% NaOH for 4 h at 150 °C (Elliot & Snyder, 1991). Si was measured in the digest samples using the amino-molybdate blue method using a UV visible spectrophotometer at a 650 nm wavelength (Shimadzu, Spectronic 100, Japan).

2.4. Physiological measurements

Leaf chlorophyll content special products analysis division (SPAD) values were measured with the help of a handheld SPAD-502 (Minolta, Osaka, Japan). For ease in handling and measurement, 2–3 fully mature young leaves from each treatment were selected and each leaf was measured several times. Other physiological parameters such as transpiration rate (E), photosynthetic efficiency (A), and stomatal conductance (gs) were measured using a portable infrared gas analyzer (IRGA LCA-4). As for the SPAD values, these measurements were also taken from 2–3 fully mature young leaves in the morning (0800–1000) at a steady state photosynthetic photon flux density of 1200–1400 μmol m^{-2} s^{-1} (Ben-Asher et al., 2006). Leaf relative water content (RWC) was calculated in accordance with the formula described by (Weatherley, 1950), as follows:

Relative water content (RWC) = [(Fresh wt. – Dry wt.)/(Turgid wt. – Dry wt.)] × 100

The plant leaves were kept at 100% humidity at 4 °C in the dark for 48 h in order to fully calculate turgid weight.

Membrane stability index (MSI) was recorded in accordance with the method of Premechandra et al. (1989), with modifications suggested by Sairam and Tayagi (2004).

2.5. Antioxidant enzyme assays

For the determination of antioxidant enzyme activity (catalase, ascorbate peroxidase, and guaiacol peroxidase), frozen plant leaves (0.1 g fresh weight) were ground to powder form in liquid nitrogen and extracted with appropriate buffers.

Catalase (CAT) activity was estimated according to Cakmak and Marschner (1992). The reaction mixture in a total volume of 2 mL contained 25 mM sodium phosphate buffer (pH 7.0) and 10 mM H_2O_2. The reaction was initiated by the addition of 100 μL of enzyme extract and activity was determined by measuring the initial rate of disappearance of H_2O_2 at 240 nm for 30 s.

Ascorbate peroxidase (APX) activity was determined according to Nakano and Asada (1981); the reaction mixture in a total volume of 2 mL consisted of 25 mM

(pH 7.0) sodium phosphate buffer, 0.1 mM EDTA, 0.25 mM ascorbate, 1.0 mM H_2O_2, and 100 µL enzyme extract. H_2O_2-dependent oxidation of ascorbate was followed by a decrease in the absorbance at 290 nm.

Guaiacol peroxidase (GPX) activity was measured using modification of the procedure of Egley et al. (1983); the reaction mixture in a total volume of 2 mL contained 25 mM (pH 7.0) sodium phosphate buffer, 0.1 mM EDTA, 0.05% guaiacol (2-ethoxyphenol), 1.0 mM H_2O_2, and 100 µL of enzyme extract. The increase in absorbance due to the oxidation of guaiacol was measured at 470 nm.

2.6. Statistical analysis

The data were subjected to statistical analysis by using analysis of variance (ANOVA) according to CRD. Treatments were compared by calculating means with standard error at 5% probability using Genstat Discovery edition.

3. Results

3.1. Growth response

Two contrasting rice varieties, KS-282 (salt-tolerant) and IRRI-6 (salt-sensitive), were grown with adequate Si (150 mg kg^{-1}) or without Si (0 mg kg^{-1}) in a pot experiment, in the absence or presence of 2.5 mg kg^{-1} B under control and saline conditions. Growth responses such as shoot fresh weight (SFW) and shoot dry weight (SDW) were recorded initially. The results showed that under salinity stress (10 dS m^{-1}), both varieties reduced their SFW but the effect was more pronounced and significant in genotype IRRI-6 (35%) than KS-282 (27%) with respect to the control (Figure 1). However, there was no significant change in the SDW of either genotype under salinity stress. B application alone caused a slight and nonsignificant decrease in plant biomass, but when applied together with salt stress B further exaggerated the problem with a reduction in the biomass

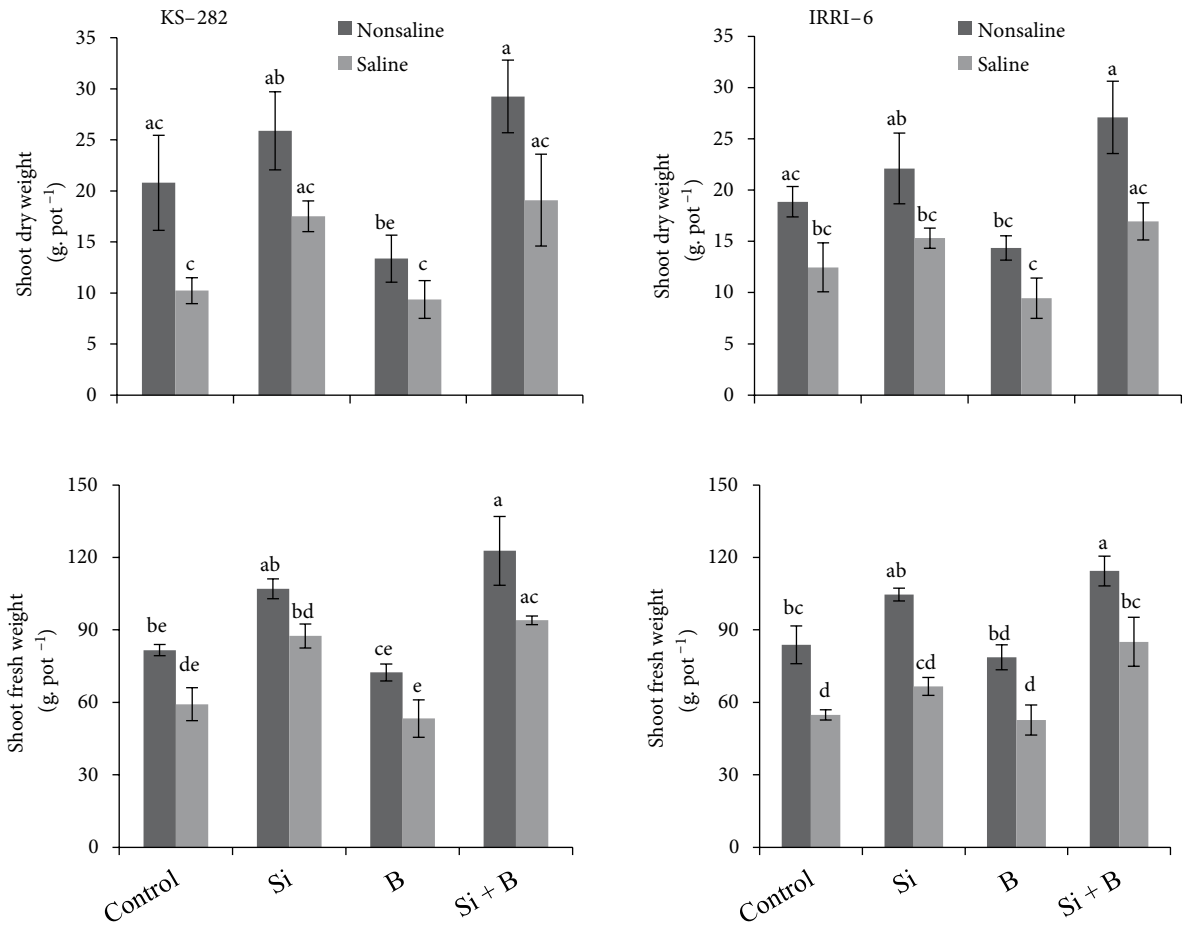

Figure 1. The effect of Si and B treatments on shoot fresh and dry weights of rice varieties KS-282 (left) and IRRI-6 (right) grown under nonsaline and saline conditions. Values are mean ± SE, n = 4. Different letters indicate means that are significantly different from each other at P = 0.05.

yields of both varieties up to 35%–38%, although the effect was nonsignificant. With the Si application under saline conditions, there was a 48% and 22% increase in the SFW of KS-282 and IRRI-6, respectively. However, compared to the control, the maximum improvement in SFW from the Si application was noted only under the combined stress of salinity and B toxicity, where up to 55%–59% inhibition in growth of both genotypes was overcome. A very similar trend of increase with Si supply under combined stress was recorded in the SDW of the KS-282 and IRRI-6 genotypes by a factor of 1.9 and 1.4, respectively. On plant biomass yield basis, genotypic variations existed and genotype KS-282 performed better.

3.2. Elemental analyses

Elemental analyses at the end of the experiment showed a slight but nonsignificant decrease in shoot Na^+ concentration in both genotypes by the application of either Si or B under nonsaline conditions (Table 1). However, the combined application of Si and B caused the shoot Na^+ content of the control plants to significantly increase by 18% and 11% in the KS-282 and IRRI-6 genotypes, respectively. In a converse way, there was significant reduction in Na^+ uptake from the application of both Si and B under saline conditions. This effect was more pronounced with the Si application, which caused 25%–28% less Na^+ concentration in both genotypes. Under salinity stress, a decrease in Na^+ uptake based on Si and B supply was correlated with increased plant uptake. Upon the addition of Si, shoots of the salt-affected KS-282 and IRRI-6 genotypes contained 37% and 29% more Si, respectively, as compared to the control plants. This uptake ability of silica under control conditions was also highest for genotype KS-282, where up to 55% more Si was recorded as compared to 46% in case of genotype IRRI-6. Antagonism between B and Na^+ under salinity stress resulted in a more drastic response due to the high B uptake (up to 37%) causing its toxicity, which was significantly reduced (up to 30%) by the Si supply in both genotypes.

The ability of the rice plants to maintain a high K^+/Na^+ ratio was also achieved significantly in both cultivars by the application of either Si or B under control conditions. While under saline stress, only genotype KS-282 showed a significant response to the Si and B application by

Table 1. Elemental composition of KS-282 (salt-tolerant) and IRRI-6 (salt-sensitive) shoots affected by Si and B treatments under nonsaline and saline conditions.

Treatment		KS-282 (salt-tolerant)				IRRI-6 (salt-sensitive)			
		Na^+	K^+	B	Si	Na^+	K^+	B	Si
Control	nonsaline	28.08bd	240.14d	3.23f	201.50d	29.11bd	232.74c	3.42f	211.75d
	saline	34.88ab	224.47e	3.75d	181.00e	36.14ab	222.22c	3.86d	175.50e
Si	nonsaline	21.87d	277.26bc	2.98f	312.00a	20.65d	270.46b	3.12f	309.50a
	saline	25.9cd	241.89d	3.49e	75.25b	26.26cd	233.86c	3.61e	272.75b
B	nonsaline	22.13d	267.12c	4.91b	49.75f	22.57d	264.37b	5.13b	146.75f
	saline	27.13cd	240.86d	5.14a	3.50g	29.92bd	237.07c	5.25a	126.00g
Si + B	nonsaline	33.16ac	303.82a	4.58c	27.00c	32.40ac	290.21a	4.83c	238.25c
	saline	38.42a	282.51b	4.86b	185.00e	39.46a	277.60ab	5.06b	178.75e
Si	nonsaline	22.12 ↓	15.46 ↑	7.74 ↓	54.83 ↑	29.06 ↓	16.21 ↑	8.77 ↓	46.16 ↑
	saline	25.75 ↓	7.65 ↑	6.93 ↓	52.07 ↑	27.33 ↓	5.24 ↑	6.48 ↓	55.41 ↑
B	nonsaline	21.19 ↓	11.24 ↑	52.01 ↑	25.68 ↓	22.47 ↓	13.59 ↑	50 ↑	30.69 ↓
	saline	22.22 ↓	7.30 ↑	37.07 ↑	26.24 ↓	17.21 ↓	6.68 ↑	36.01 ↑	28.20 ↓
Si + B	nonsaline	18.09 ↑	26.52 ↑	41.79 ↑	12.66 ↑	11.30 ↑	24.69 ↑	41.23 ↑	12.51 ↑
	saline	10.15 ↑	25.86 ↑	29.6 ↑	2.21 ↑	9.19 ↑	24.92 ↑	31.09 ↑	1.85 ↑

The upper values are means (n = 4) of elemental contents (mM) on a dry weight basis. The lower part depicts percent changes with an upward arrow (increase) or downward arrow (decrease) with respect to the control. Values showing different letters are significantly different from each other at P = 0.05.

increasing tissue K^+ contents up to 8%. For genotype IRRI-6, the response was the same but nonsignificant. However, comparing the effectiveness of Si and B in increasing shoot K^+ content for the two genotypes resulted in a more beneficial response to Si. However, the maximum uptake of K^+ (24%– 26%) by both genotypes was observed by a combined Si and B application, irrespective of salinity stress or not.

3.3. Physiological response

The physiological response of the two contrasting rice genotypes grown under differential experimental conditions was determined by measuring total chlorophyll contents (SPAD value), IRGA parameters (such as photosynthetic rate, stomatal conductance, and transpiration rate), and relative water content (RWC). The data showed that salinity stress significantly decreased the total chlorophyll content (TCC) of both genotypes and application of B exaggerated this damage to the plants, irrespective of salinity stress (Table 2). However, together with salt stress, the application of B resulted in a maximum reduction of TCC by 38% and 44% in genotypes KS-282 and IRRI-6, respectively, as compared to the control conditions. Furthermore, for the Si application under control as well as stress (salinity and/or B toxicity) conditions, there was an increase in the SPAD chlorophyll value of both genotypes. However, only genotype KS-282 showed significant improvement (42% more TCC) with Si under salinity stress compared to stress conditions without Si. Similarly, a significant increase of 80% and 92% in TCC by Si addition was recorded in the leaves of KS-282 and IRRI-6, respectively, growing under the combined stress of salinity and B toxicity.

The data regarding photosynthetic rate revealed that photosynthetic efficiency of both genotypes was also reduced by all type of stresses (salinity and/or B toxicity) but there were no significant differences between the stress conditions and the respective controls (Table 2). The extent of the damage to the photosynthetic system of both cultivars was at its highest (up to 65%) under the combined stress of salinity and B toxicity as compared to normal growth conditions. On the other hand, application of Si under both control and saline conditions significantly improved the photosynthetic rate of genotype KS-282. The response to Si was similar but nonsignificant for genotype IRRI-6. Si also improved photosynthetic performance of both genotypes under both B stress alone and salinity coupled with B toxicity stress, with an improvement in the range of 2– 3.6 factors as compared to the corresponding stressed plants without Si.

RWC in the leaves of the rice genotypes grown under both type of stresses improved with Si application (Table 3). Compared to the control, the lowest RWC (27% and 38% lower in the leaves of KS-282 and IRRI-6, respectively) was observed under the combined stress of salinity and B toxicity and was increased significantly by Si supplementation as compared to the stressed plants receiving no Si. The improvement in RWC by Si addition was also noted under the control and saline conditions; however, the response was nonsignificant as compared to the respective controls except for the IRRI-6 plants grown under nonsaline conditions. Similarly, the transpiration rate data revealed that plants of both genotypes grown under stress conditions (salinity and/or B toxicity) were under the severe influence of a water potential gradient due to a greater salt content in the rooting medium (Table 3). This resulted in reduced upward movement of water inside the plant body, thereby reducing the transpiration rate under any type of applied stress. However, compared to the control, the greatest significant reduction in transpiration rate (25% and 40% in the KS-282 and IRRI-6 genotypes, respectively) was noted due to the combined stress of salinity and B toxicity. The application

Table 2. The effect of Si and B treatments on SPAD chlorophyll value and photosynthetic rate (μmol m^{-2} S^{-1}) of rice varieties KS-282 (salt-tolerant) and IRRI-6 (salt-sensitive) grown under nonsaline and saline conditions.

Treatment	SPAD chlorophyll value				Photosynthetic rate (μmol m^{-2} S^{-1})			
	KS-282 (salt-tolerant)		IRRI-6 (salt-sensitive)		KS-282 (salt-tolerant)		IRRI-6 (salt-sensitive)	
	nonsaline	saline	nonsaline	saline	nonsaline	saline	nonsaline	saline
Control	38.90ac	32.50bd	36.50ac	29.25bd	4.89bd	3.12cd	4.39ac	2.95bc
Si	48.55a	45.95a	44.19a	40.38ab	11.35a	8.23ab	8.96a	6.95ab
B	30.25cd	24.32d	27.55cd	20.45d	2.85cd	1.75d	1.58c	1.50c
Si + B	46.90a	43.90ab	45.70a	39.28ab	8.65ab	6.36bc	7.25ab	5.25ac

Values are means of 16–24 measurements from 4 different experiments. Values showing different letters are significantly different from each other at P = 0.05.

Table 3. The effect of Si and B treatments on relative water content (%) and transpiration rate (mmol m^{-2} S^{-1}) of rice varieties KS-282 (salt-tolerant) and IRRI-6 (salt-sensitive) grown under nonsaline and saline conditions.

Treatment	Relative water contents (%)				Transpiration rate (mmol m^{-2} S^{-1})			
	KS-282 (salt-tolerant)		IRRI-6 (salt-sensitive)		KS-282 (salt-tolerant)		IRRI-6 (salt-sensitive)	
	nonsaline	saline	nonsaline	saline	nonsaline	saline	nonsaline	saline
Control	66.35abc	53.24ae	66.20a	46.92bc	1.73bc	1.62be	1.64bc	1.19ad
Si	84.38b	66.05ac	80.09de	53.59ab	1.51ce	1.20ac	1.65c	1.49ac
B	65.08a	48.41a	62.89acd	41.35b	1.42b	1.29ae	1.07ae	0.99e
Si + B	4.08ce	64.16c	66.16a	58.39ae	1.15ad	1.01ad	0.71e	0.65e

Values are means of 16–24 measurements from 4 different experiments. Values showing different letters are significantly different from each other at P = 0.05.

of Si under control and saline conditions also reduced the transpiration rate of both genotypes but the response was not significant as compared to their respective controls. However, the lowest significant decrease in transpiration rate of both genotypes was noted due to Si addition under combined stress of salinity and B toxicity, where 42% and 60% decreases in transpiration value were recorded for the KS-282 and IRRI-6 genotypes, respectively, as compared to the control conditions.

The data showed that both salinity stress and B toxicity damaged membranes and reduced the stomatal conductance of water in both genotypes (Table 4). However, the highest damage to both attributes occurred under the combined stress of salinity and B toxicity, although the impact was nonsignificant when compared to the respective controls. The membrane stability index (MSI) was reduced 44%–46% due to salinity coupled with B toxicity stress as compared to the control conditions. Similarly, as compared to the control conditions, the lowest stomatal conductance of water (60% and 67% less in KS-282 and IRRI-6, respectively) was noted under the combined stress. The application of Si under control and stressed conditions (salinity and/or B toxicity) overcame the damage to some extent but the trend was nonsignificant. The improvement in MSI by Si supplementation for plants facing combined stress was a factor of approximately 1.9 for both genotypes. Similarly, the stomatal conductance of water also doubled for both genotypes by the application of Si under combined stress as compared to those receiving no Si; however, the trend was nonsignificant. Taken all together, the physiological efficiency of both genotypes under different combinations of treatments indicates that genotype KS-282 (salt-tolerant) performed better than IRRI-6 (salt-sensitive).

3.4. Antioxidant system response

Enzymatic antioxidants such as CAT and APX are known for their role in detoxification of H_2O_2 in cytosols, peroxisomes, and chloroplasts. Due to their involvement in

Table 4. The effect of Si and B treatments on membrane stability index (%) and stomatal conductance (mmol m^{-2} S^{-1}) of rice varieties KS-282 (salt-tolerant) and IRRI-6 (salt-sensitive) grown under nonsaline and saline conditions.

Treatment	Membrane stability index (%)				Stomatal canductance (mmol m^{-2} S^{-1})			
	KS-282 (salt-tolerant)		IRRI-6 (salt-sensitive)		KS-282 (salt-tolerant)		IRRI-6 (salt-sensitive)	
	nonsaline	saline	nonsaline	saline	nonsaline	saline	nonsaline	saline
Control	17.45ab	15.25ab	15.75ac	12.50ac	0.05a	0.03a	0.06ab	0.04ab
Si	24.75a	21.25a	21.73a	19.75ab	0.06a	0.05a	0.08a	0.06ab
B	13.56ab	9.50b	10.25bc	8.75c	0.03a	0.02a	0.03b	0.02b
Si + B	23.25a	18.35ab	20.25ab	16.25ac	0.05a	0.04a	0.04ab	0.04ab

Values are means of 16–24 measurements from 4 different experiments. Values showing different letters are significantly different from each other at P = 0.05.

antioxidant metabolism, their contents in the leaves of the rice genotypes were measured as representative enzymes (Figures 2A and 2B). For both enzymes, the results showed a distinct but contrasting pattern of response to Si application and to salinity and/or B toxicity stress. When compared to the control, a significant increase in CAT activity but a decrease in APX activity was observed in the leaves of both genotypes under the stress of both B toxicity alone and salinity coupled with B toxicity. However, under salt stress alone, CAT activity significantly increased and, despite decreasing, APX activity was still in the range of the control for both genotypes. The highest CAT activity was recorded under the combined stress, where genotypes KS-282 and IRRI-6 showed significant increases by factors of 1.8 and 2, respectively, as compared to the control. In contrast, the APX activity dropped significantly to a lowest

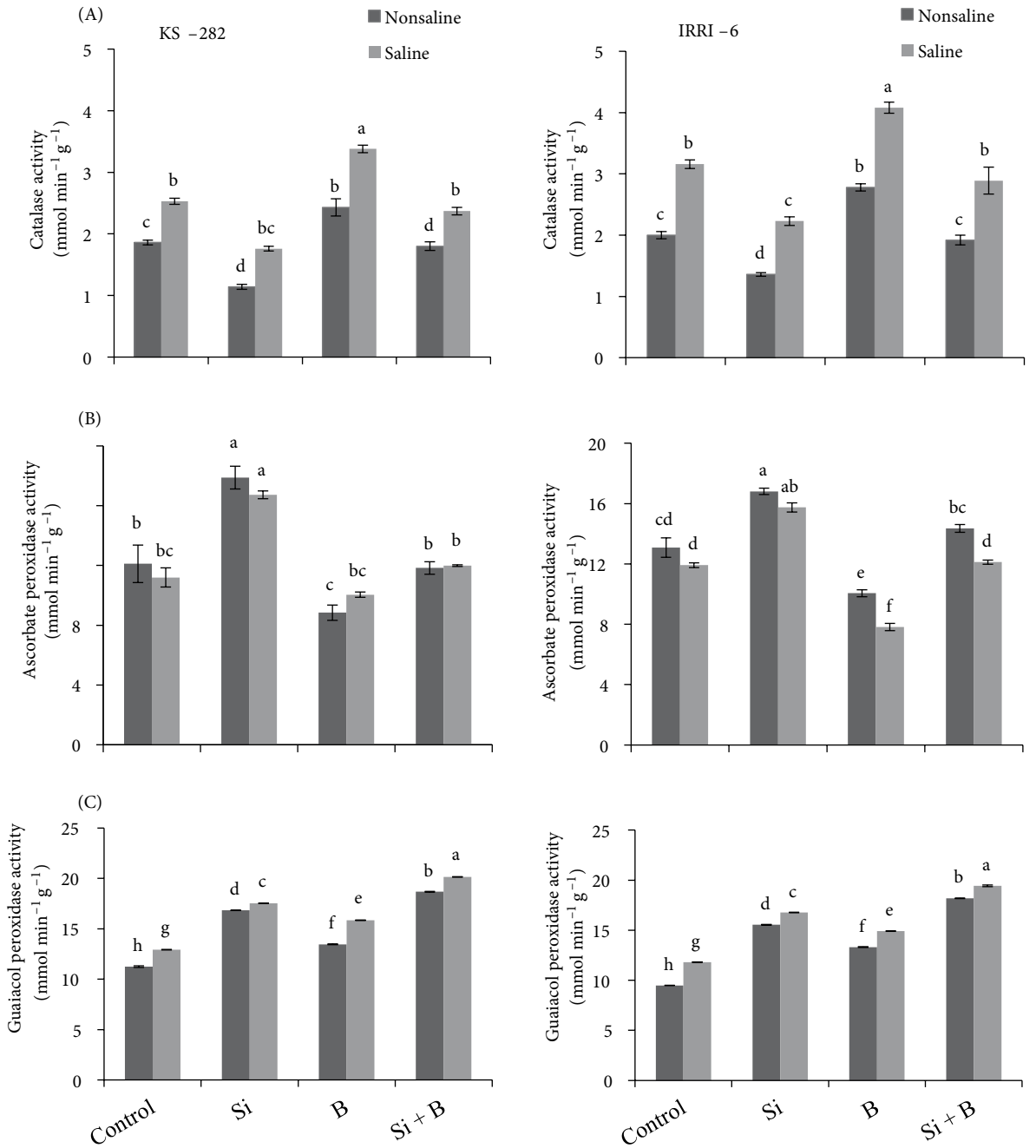

Figure 2. The activity of CAT (A), APX (B), and GPX (C) in the leaves of rice varieties KS-282 (left) and IRRI-6 (right) as affected by Si and B treatments under nonsaline and saline conditions. Values are mean ± SE, n = 4. Different letters indicate means that are significantly different from each other at P = 0.05.

value for IRRI-6 under the combined stress. Furthermore, Si application caused a significant decrease in CAT activity in the control for both genotypes and under saline stress for genotype IRRI-6 only as compared to the respective control. Both genotypes suffering from the stress of B toxicity alone as well as salinity coupled with B toxicity also responded significantly to the Si application by a decrease in CAT activity as compared to the Si-deprived respective stress conditions. In contrast, APX activity was significantly increased by the Si application under control and salinity stress conditions for both genotypes. The pattern of response was highest in genotype KS-282, where up to a 49% increase in APX activity was recorded under both control and saline conditions. When compared to B toxicity stress alone, both genotypes showed significant increase in APX activity upon the addition of Si. However, Si application under the combined stress significantly increased APX activity only in the leaves of genotype IRRI-6 as compared to the Si-deprived stress conditions.

As an indicator of stress and defense response, GPX activity was measured under differential experimental conditions (Figure 2C). For both genotypes, GPX activity was significantly increased upon salinity and/or B toxicity stress exposure for the control plants. However, the pattern of increase in GPX activity was more pronounced with Si addition under all growth conditions, particularly under the combined stress of salinity and B toxicity. With the Si application, there was a 27%–30% increase in GPX activity in both genotypes under the combined stress as compared to the stressed plants without a Si supply.

4. Discussion

Sustainable crop production in arid and semiarid climate regions including Pakistan is at great risk due to the presence of salt-affected soils and the associated nutrient toxicities such as B, which requires special attention. Increasing crop productivity by better management practices on such marginal lands is required to meet food demands. Therefore, this study aimed to investigate the beneficial effects of Si on rice crops exposed to toxic B concentrations associated with salt stress.

The results of the present study showed that salinity and/or B toxicity stress significantly hampered plant growth, particularly when no Si was added to the growth medium. Increased nutritional imbalances and high cytosolic Na^+ concentrations associated with salt stress result in reduced K^+ uptake due to competition at the plasma membrane uptake site (Maathuis and Amtmann, 1999). The decreased K^+ uptake leads to secondary effects such as production of reactive oxygen species (ROS), causing membrane damage and disturbance in water uptake at the root medium leading to physiological consequences (Shabala et al., 2009) and poor plant growth. Reduced plant growth due to salinity coupled with B toxicity has also been reported in some vegetables (Alpaslan and Gunes, 2001) and cereals (Ismail, 2003). The effects were minimized by the exogenous application of Si as it improved K^+ and reduced Na^+ content in shoot tissues due to exclusion of Na^+ from the root or inclusion into the vacuole by maintaining plasma membrane H^+-ATPase activity (Liang et al., 2006b). Silicon deposition in the root endodermis also creates binding sites for Na^+ and reduces its translocation from the root to the shoot (Saqib et al., 2009). A higher K^+/Na^+ ratio in shoot tissues may also be due to reduced water transpiration (Serrano et al., 1999; Romero-Aranda et al., 2006) from the Si deposition in the epidermis (Liang, 1999). These higher cytosolic K^+ contents help improve plant growth by charge balancing in cytosols, activation of the number of enzymes involved in key metabolic processes, and maintenance of the turgor pressure in the cell. Therefore, maintaining a proper K^+/Na^+ ratio is a key component of salinity tolerance in plants (Hossain et al., 2002). Earlier studies also documented growth improvement by Si application under different abiotic stresses such as heavy metal stress in cucumber, rice, and maize (Yeo et al., 1999; Rogalla and Roemheld, 2002; Liang et al., 2003); drought stress in sorghum and wheat (Hattori et al., 2003); salt stress in barley, tomato, and cucumber (Yeo et al., 1999; Al-aghabary et al., 2004; Ben-Asher et al., 2006); B stress in wheat; and the combined stress of salinity and B toxicity in tomato and spinach (Gunes et al., 2006).

In addition, increased tissue silica contents upon Si application significantly reduced B concentration in both genotypes. The concentrations of Na^+ and B were higher in the salt-sensitive (IRRI-6) than in the salt-tolerant (KS-282) genotype. This positive effect of Si application in decreasing B uptake might be attributed to the formation of boron-silicate complexes in the soil. The greater silica uptake ability of KS-282 caused a greater reduction of Na^+ and B uptake as compared to IRRI-6, which is in contrast to previous results for rice by Yeo et al. (1999), who reported sensitive cultivars to be more Si-responsive than tolerant ones.

From these results it is evident that reduced Na^+ uptake by Si supplementation maintained the membrane integrity and can minimize the damage to cell organelles such as chloroplasts. Silicon application in the root medium increased the plant photosynthetic efficiency as well as total chlorophyll content. Moreover, the exogenous application of Si under no-stress conditions produced beneficial effects on rice growth. Si-treated leaves were erect and intercepted more light for photosynthesis (Miyake and Takahashi, 1983); furthermore, the Si increased plant photosynthetic efficiency by changing the rubisco concentration and its activity (Gunes et al., 2007b;

Epstein, 2009). Furthermore, Si increased plant growth by increasing the cell wall extensibility in the plant roots and shoots (Hossain et al., 2002; Hattori et al., 2003). Similar to the effects of salinity stress, B application also reduced plant growth and severely affected the photosynthetic efficiency, most probably by damaging the chloroplast membranes. Exogenous application of B in the root medium resulted in an increased concentration of B in the shoot tissue. Previous studies showed that high levels of B in plants results in membrane damage, necrosis, and chlorosis (Reid and Fitzpatrick, 2009). The reduced chlorophyll may be due an accumulation of starch and hexose sugars that decrease the chlorophyll formation and have a negative effect on photosynthetic enzymes synthesis (Cave et al., 1981; Schaffer et al., 1986). The application of Si completely overcomes the adverse effects of B on plant growth by increasing the chlorophyll content and ultimately increasing the photosynthetic efficiency (Gunes et al., 2007a).

The results suggested that plant defenses were activated against salt stress by increased activity of CAT and GPX, showing that the antioxidant system was activated to recover from the effects of salinity stress. However, salt stress was exaggerated when combined with B toxicity, causing higher oxidative damage and resultantly higher CAT activity. The application of Si alleviated the combined stressed and resulted in a decrease in CAT activity, which indicates a reduction in oxidative stress due to Si and consequently a lower activity of CAT antioxidant enzymes. Contrasting results have been reported by various authors about the response of plants to salinity stress in the context of CAT activity. Previous studies reported both a decrease (Zhu et al., 2004) and an increase in CAT activity (Furtana and Tıpırdamaz, 2010) when plants are under stress. However, a variation in this trend was observed in the two other antioxidant system enzymes APX and GPX. GPX exhibited a similarly increasing trend against salt stress to CAT, but behaved differently when B and Si were applied along with salt stress. GPX activity increased with the application of both B and Si; however, a higher rate of activity was observed when Si was applied alone or in combination with B in both varieties as previously reported by Inal et al. (2009). On the other hand, APX activity was reduced under salt stress and this reduction was more pronounced when salts were combined with B toxicity stress. The application of Si enhanced its activity under salt stress alone as well as when applied in combination with B toxicity, but interestingly, this trend was not observed in the salt-tolerant rice variety KS-282, which showed overall insignificant variation in APX activity when comparing saline and nonsaline conditions. The treatment effect was significant and Si increased the enzyme activity in instances where it was lowered by the application of B toxicity; however, it was improved when Si was added with B toxicity.

In conclusion, the results of this study show hampered plant growth due to salinity and B toxicity, with maximum damage under their combined stress. However, Si addition relieved the stress effects (particularly for salinity coupled with B toxicity) due to maintaining membrane integrity in relation to a high K^+/Na^+ ratio and reduced oxidative stress. Si supplementation reduced uptake and transport of B and Na^+ in rice shoots due to decreased permeability of the plasma membrane to these toxic ions in both genotypes. There was a net increase in total chlorophyll content, photosynthetic rate, and relative water content, as well as a reduction in transpiration rate due to Si supply, particularly under stress conditions, all of which ultimately improved biomass yield. Genotypically, the extent of damage from any type of stress, as indicated by reduction in biomass, photosynthetic efficiency, and other physiological attributes, was higher for the salt-sensitive (IRRI-6) than the salt-tolerant (KS-282) genotype. However, the high silica and K^+ uptake ability of the KS-282 genotype helped in greater exclusion of Na^+ and B, resulting in better response and adaptability than IRRI-6. From this report, we suggest Si to be considered in the list of elements essential for sustainable crop production, particularly under stress conditions. However, field trials should be carried out before setting any recommendations for farmers.

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