Environmental Science and Pollution Research (2018) 25:26625–26642
https://doi.org/10.1007/s11356-018-2659-x

RESEARCH ARTICLE

Exogenous silicon alters ascorbate-glutathione cycle in two salt-stressed indica rice cultivars (MTU 1010 and Nonabokra)

Prabal Das[1] · Indrani Manna[2] · Asok K. Biswas[1] · Maumita Bandyopadhyay[2]

Received: 20 March 2018 / Accepted: 25 June 2018 / Published online: 12 July 2018

Abstract

Silicon is widely available in soil and is known to mitigate both biotic and abiotic stress in plants. Very low doses of silicon are becoming increasingly essential in rice for biofortification and preventing water loss. Soil salinity is a matter of grave concern in various parts of the world, and silicon is a suitable candidate to mitigate salinity-induced stress of important plants in affected areas. The present study investigates the protective capability of exogenously applied silicon in ameliorating NaCl-induced toxicity in two rice (*Oryza sativa* L.) cultivars, the salt-sensitive MTU 1010, and salt-tolerant Nonabokra. Rice seedlings were treated with three doses of NaCl (25, 50, and 100 mM), initially alone and subsequently in combination with 2 mM sodium silicate (Na_2SiO_3, $9H_2O$). After 21 days, these plants were examined to determine levels of reduced glutathione, ascorbic acid, cysteine, and activities of different enzymes involved in the ascorbate-glutathione cycle, viz., glutathione reductase (GR), ascorbate peroxidase (APX), glutathione peroxidase (GPx), and glutathione S-transferase (GST). Though ROS levels increased in both the cultivars with increasing NaCl concentrations, cv. MTU 1010 accumulated comparatively higher amounts. A differential response of NaCl-induced toxicity on the two cultivars was observed with respect to the various enzymatic and non-enzymatic antioxidants. APX and GST activities, as well as, cysteine contents, increased concomitantly with salt concentrations, whereas GR activity declined at increasing salt concentrations, in both cultivars. Activity of GPx increased in cv. Nonabokra but declined in cv. MTU 1010, under similar NaCl concentrations. Reduced glutathione (GSH) contents decreased in both cultivars, whereas ascorbate contents declined in only the sensitive cultivar. Application of silicon, along with NaCl, in the test seedlings of both the cultivars, reduced ROS accumulation and boosted antioxidant defense mechanism, through enhancing ascorbate and GSH levels, and activities of ascorbate-glutathione cycle enzymes as well. However, amelioration of salt-induced damages in the sensitive cv. MTU 1010 was more pronounced upon silicon administration, than the tolerant cv. Nonabokra. Thus, cv. MTU 1010 was found to be more responsive to applied silicon. Hence, this study was instrumental in realizing a successful strategy in silicon-mediated amelioration of salinity stress in plants.

Keywords ROS · Antioxidant · Ascorbate · Glutathione · Salinity · Silicon

Responsible editor: Philippe Garrigues

Prabal Dasb and Indrani Manna contributed equally.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11356-018-2659-x) contains supplementary material, which is available to authorized users.

✉ Maumita Bandyopadhyay
mbbot@caluniv.ac.in

[1] Plant Physiology and Biochemistry Laboratory, Centre of Advanced Study, Department Of Botany, Ballygunge Science College, University of Calcutta, 35, Ballygunge Circular Road, Kolkata 700019, India

[2] Plant Molecular Cytogenetics Laboratory, Centre of Advanced Study, Department Of Botany, Ballygunge Science College, University of Calcutta, 35, Ballygunge Circular Road, Kolkata 700019, India

Introduction

Salinity is an important abiotic stress that typically affects crop and forage production in the arid and semi-arid regions of the world (Munns and Gilliham 2015). According to a recent report 6% (approx.) of total land on earth (Tang et al., 2015), tantamounting to 20% of the arable regions, is affected by salinity (Rasool et al. 2013). In face of the specter of declining crop productivity and rapid urbanization, it has now become imperative to reclaim salinity affected areas, especially the coastal areas, for agriculture (Karan and Subudhi 2012). Plants growing in saline conditions accumulate high amounts of Na^+ in their tissues and exhibit characteristic symptoms of Na^+ toxicity that diminishes nutrient absorption and induces

oxidative damage, thus affecting plant physiology and fertility (Zhu et al. 2017).

The cereal, rice (*Oryza sativa* L.), is the primary source of calorie for approximately three billion people globally (Ullah et al. 2017). Countries of SE Asia are traditionally the largest producers, as well as consumers of rice (Cuong et al. 2017). India has the largest rice growing area in the world (44.0 Mha) and is the second largest producer after China (Anonymous 2016). A glycophyte, rice, is very sensitive to salinity, particularly at seedling stage (Lutts et al. 1995). In fact, salinity is one of the primary problems which affect rice production along the coastal regions of India (Gupta and Abrol 1990).

Rice grown in saline soil is plagued by inhibition of germination, malformed leaves, reduction in dry mass, and infertility (Folkard and Marco, 2001). These negative effects of salinity can be minimized with reclamation, watering, and drainage, but the cost of management and technology driving such endeavors is prohibitive (Murtaza et al. 2009). Declining profitability in agriculture and limited usage of advanced agricultural equipment by farmers has refocused global attention on the necessity of enhancing rice production per unit area with reduced toxic effect to the soil and environment.

Increased production of ROS at molecular level characterizes biochemical perturbations in plants during stress. In fact, enhanced ROS is one of the most common consequences in plants subjected to salinity and varies widely with the duration and degree of stress imposition, and the types of crop as well (Hasanuzzaman et al. 2011). To encounter the destructive effect of ROS, plants build up multiple anti-oxidant-based defense systems, one of which is the ascorbate-glutathione (AsA-GSH) cycle, composed of enzymatic [glutathione reductase (GR), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), and dehydroascorbate reductase (DHAR)] and non-enzymatic components (휙-tocopherol, non-protein amino acids, ascorbic acid (AsA), glutathione (GSH), alkaloids, and phenolic compounds), as well as other enzymes, like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione S-transferase (GST) (Fig. 1). All these components act in a harmonious symphony to detoxify ROS and confer protection against oxidative damage (Hasanuzzaman et al. 2011, 2012). Glutathione is a well-known low molecular weight thiol, which under unstressed conditions is present in its reduced form (GSH) and a small amount of its oxidized form (GSSG) (Noctor et al. 2002). Cysteine-rich GSH plays the role of storehouse of reduced sulfur in the cell and also act as substrate for glutathione S-transferases (GST) that removes various toxic compounds, interacting with ROS on one hand and the stable oxidized form of ascorbate on the other, which it reduces to ascorbate (Noctor et al. 2002). GSH in plant cells is synthesized via a two-step process: L-glutamate reacting with L-cysteine to produce γ-glutamylcysteine in the presence of the enzyme γ-glutamylcysteine synthetase, followed by glycine being added by help of the enzyme glutathione synthetase. Glutathione reductase (GR) converts GSSG to its reduced form GSH and thereby helps in maintaining the reduced state of GSH (Murgia et al. 2004), while GST, APX, and GPX link peroxide reduction to GSH oxidation. Increased availability of H_2O_2 and cellular glutathione status are thus closely related, synchronized with the ascorbate content too. The ascorbate-glutathione cycle thus connects H_2O_2 reduction to NAD(P)H oxidation via ascorbate and glutathione pools, and analysis of key components of this pathway can be used as reliable stress markers in plants (Noctor et al., 2012, b).

One of the cost-effective ways being explored is chemical modification of the saline soil by the use of silicon (Si). It is reported that silicon-enriched fertilizers not only sustain rice production but also decrease emission of the greenhouse gas methane during rice cultivation (Ali 2013). Silicon, though second most abundant element, is termed as quasi-essential for plants (Epstein and Bloom 2005; Coskun et al. 2016). Though not absolutely essential for plant survival, Si highly improves metabolic, biochemical, and physiological functioning of plants. Beneficial role of silicon has been exploited in many experimentally and economically important plants as well like rice, tomato, sorghum, cucumber, wheat, among others (Coskun et al. 2016; Li et al. 2015; Amani et al. 2017; Wang et al. 2015; Ibrahim 2016). Role of silicon application in salinity amelioration is well documented (Liang et al. 2007). Silicon helps a salt-stressed plant in a cascade of events starting from cessation of bypass flow of Na^+ uptake by creating a mechanistic barrier, blockage of transpiration by depositing on cell walls (Gong et al. 2005; Wang et al. 2015), increase in total leaf water content to check hyper-osmotic changes (Romero-Aranda et al. 2006), increase in antioxidant enzyme activities (major enzymes namely SOD, GPX, APX, CAT), all of which are responsible for warding off increase in salinity-related ROS quantity (Zhu et al. 2004) or H^+-ATPase activity accretion in the cell cytosol (Wang et al. 2015). It has recently come to light that silicon alleviates the hyperosmotic response thus controlling osmotic stress (Munns and Tester 2008; Romero-Aranda et al. 2006), mainly by controlling stomatal movements and aquaporin-mediated root conductance (Liu et al. 2015) and by accumulating osmolytes like polyamines (Wang et al. 2015). Aquaporins, which belong to the ancient family of major intrinsic proteins (MIP) (Maurel et al. 2015), are primarily responsible for specialized water transport (Zhang et al. 2016). They are the chief intra-cellular mineral transporters (Maurel et al. 2009; Rios et al. 2017). Silicon uptake too is highly regularized by aquaporins, while they transpass from the apoplastic to the symplastic network (Rios et al. 2017). Due to its non-corrosive and non-dissociative nature, silicon over-accumulation does not damage plants and silicate fertilizers can contribute in developing ecologically sustainable green agriculture. Both silicate and nano-silicate additives have positive results against salinity-

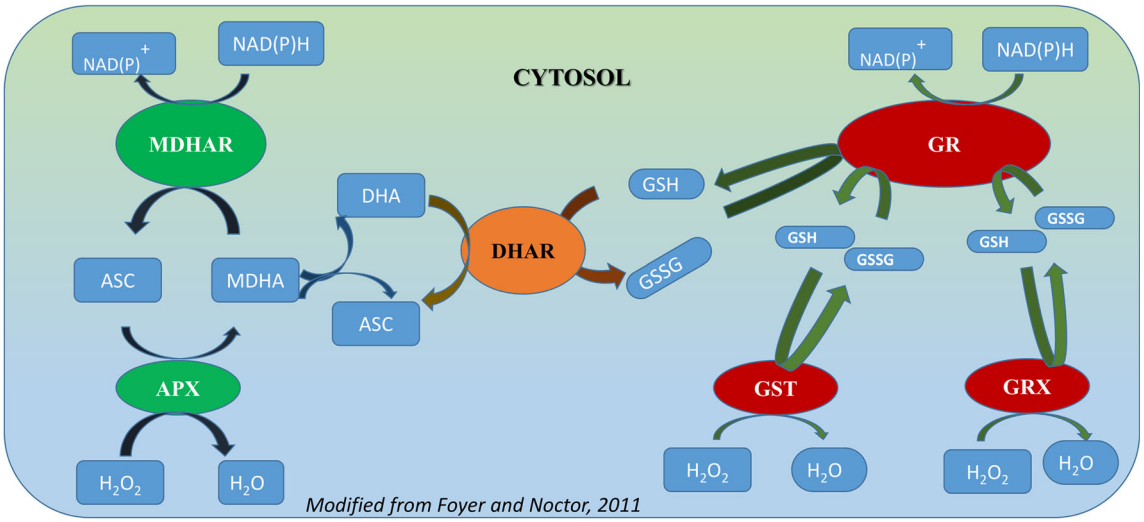

Fig. 1 Diagrammatic representation of ascorbate-glutathione cycle

induced drought in many plant species (Haghighi and Pessarakli 2013); however, here, sodium silicate was chosen because of its wide availability and cheap price. Recent studies have confirmed the role of silicon in ameliorating harmful effects of salt stress in many economically important crops, and not only that, silicon has been reported to mitigate heavy metal toxicity in various plants as well (Singh et al. 2011; Tripathi et al., 2015a, b, Tripathi et al., 2016a, b).

To bolster rice plants in saline conditions, it is important to understand the gamut of physio-biochemical changes that the exposed plants undergo, by comparing response of a salt-tolerant and a salt-sensitive cultivar. The authors have earlier reported that there are certain physiological consequences of salt-stress in MTU1010, a salt-sensitive cultivar of rice, and the possibility of salt stress modulation by silicon administration (Das et al. 2016). The present work was designed to elucidate upon the effect of salinity on the ascorbate-glutathione cycle, a very important mechanism for ROS detoxification, as a comparative study in two rice cultivars, the salt-sensitive MTU 1010 and salt-tolerant Nonabokra. We also intended to explore the efficacy of using silicon for ameliorating the adverse effects of salinity and understand the possible mechanisms involved in silicon-induced augmentation of salt tolerance. This strategy may be employed in mitigation of salt stress in rice plants growing in saline-prone regions.

Material and methods

Plant material, culture method, concentration of Si, and salinity treatment

Seeds of two low land rice (*Oryza sativa* L.) cultivars, MTU 1010 and Nonabokra, used in this study were obtained from the State Rice Research Station, Chinsurah, Hooghly, West Bengal, and were surface sterilized with 5% (*v*/*v*) sodium hypochlorite, and then washed thoroughly with water. About 50 seeds for each treatment were spread over in petri dishes (φ 10 cm) lined with filter papers. The seeds were kept in dark and humid conditions for 48 h in a germinator at 30 ± 2 °C. Then, the germinating seeds were exposed to 25, 50, and 100 mM concentration of sodium chloride (NaCl: Merck, India) solutions (*w*/*v*) with or without 2 mM sodium meta-silicate (Na_2O_3Si, $9H_2O$: Loba-chemie, India) solution (*w*/*v*) and exposed to 16 h photoperiod (260 μmol m^{-2} s^{-1} PFD) for 21 days in the presence of modified Hoagland solution (Rafi and Epstein, 1999, b), which was renewed every alternate day. Two millimolar concentration of Si was chosen for amelioration because this dose showed better performance under NaCl stress which was selected after multiple trial studies considering membrane lipid peroxidation reported previously (Das et al. 2016). Throughout this period, pH of the nutrient solution was adjusted in the range of 5.5–6.0 using 0.1 M HCl to reduce polymerization of silicates. The plants were harvested after 21 days of the treatment, washed with distilled water to remove any residues, stored separately into shoot and root at − 80 °C.

Detection of intracellular ROS with fluorescent dye

Salinity inspired intracellular ROS formation in root and shoot of control, and treated samples was studied qualitatively by 2′,7′-dichloroflourescein acetate. Tissue was treated with 0.25 μM DCFH-DA (Sigma-Aldrich) solution (Faisal et al. 2013) in PBS and inspected under Laser Scanning Confocal Microscope (LSCM-Olympus, 1X CLSM 81). Software version: Flouview FVV 1000, 495-530 filter.

Estimation of cysteine

Cysteine, an important alleviator of oxidative stress (Genisel et al. 2015), was estimated in 0.5 g of samples which were homogenized in 5% (*v*/*v*) chilled perchloric acid (PCA) and centrifuged at 10,000×*g* for 10 min at 4 °C. The absorbance of the supernatant was taken using acid-ninhydrin reagent at 560 nm following the protocol of Gaitonde (1967) with minute modifications.

Estimation of ascorbate content

Ascorbate contents were measured according to Mukherjee and Choudhuri 1983). Root and shoot samples were homogenized in 6.0 ml ice-cold 6% TCA and homogenates centrifuged at 11,500*g* for 15 min at 4 °C. Reaction mixture consisting 4 ml of supernatant, 2 ml of 0.2% DNPH (2′,4′-dinitrophenylhydrazine) in 0.5 N HCl, and 0.01 ml 10% thiourea in 70% ethanol was kept in boiling water bath for 15 min and cooled, and 5 ml concentrated H_2SO_4 was added. Absorbance of the mixture was monitored at 530 nm. A standard curve was prepared using known concentrations of ascorbic acid. Ascorbate contents were expressed as microgram ascorbate per gram FW.

Estimation of GSH content and enzyme activities

a. Total glutathione content from root and shoot samples of 21-day-old rice seedlings was extracted in 5% (*m*/*v*) sulfosalicylic acid (SSA) containing 1 mM ethylenediamine-tetraacetic acid disodium salt (Na_2EDTA) and centrifuged at 10,000*g* for 20 min. Total thiols were measured in a 100 mM phosphate buffer (pH 7.5) containing 1 mM Na_2EDTA, 6 U cm^{-3} GR, 10 mM 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), and 0.16 mg cm^{-3} NADPH. Absorbance was recorded at 412 nm (Sedlak and Lindsay 1968) using a spectrophotometer (Hitachi 2000, Tokyo, Japan).

b. Further, root and shoot samples were extracted using in 100 mM phosphate buffer (pH 7.5) containing 0.5 mM Na_2EDTA and centrifuged at 15,000*g* and 4 °C for 20 min. GR (EC 1.6.4.2) activity was assayed in a 100 mM phosphate buffer (pH 7.5) containing 0.5 mM Na_2EDTA, 0.75 mM DTNB, 0.1 mM NADPH, and 1 mM oxidized glutathione (Smith et al. 1988). The reaction mixture was incubated at 35 °C; meanwhile, absorbance at 412 nm was measured for up to 5 min. The activity was calculated using the coefficient of absorbance (ε) of 6.22 mM^{-1} cm^{-1}.

c. For GPx (EC 1.11.1.9) estimation, 100 mg tissue was extracted in 220 mM Tris-HCl buffer (pH 7.4) containing 250 mM sucrose, 50 mM potassium chloride, 1 mM magnesium chloride, 160 mM β-mercaptoethanol, and 0.57 mM phenylmethanesulfonyl fluoride. The GPx activity was assayed in a 20 mM sodium acetate buffer (pH 5) with 30 mM H_2O_2, and 2 mM guaiacol added just prior to estimation. Absorbance at 470 nm was recorded, and the activity was calculated using $\varepsilon = 26.6$ mM^{-1} cm^{-1} (Ranieri et al. 2001).

d. For GST assay, root and shoot samples were extracted in a 100 mM Tris-HCl buffer (pH 7.5) containing 2 mM Na_2EDTA, 14 mM β-mercaptoethanol, and 7.5% (*m*/*v*) polyvinylpyrrolidone (PVP). After centrifugation at 15,000*g* for 15 min, the activity of GST (EC 2.5.1.18) was measured in a 100 mM phosphate buffer (pH 6.5), 5 mM GSH, and 1 mM 1-chloro-2,4-dinitrobenzene mixture based on absorbance at 340 nm. The activity of GST was calculated using $\varepsilon = 9.6$ mM^{-1} cm^{-1} (Ando et al. 1988). Protein content was estimated from assay samples according to Lowry et al. 1951).

e. APX activity was assayed according to Nakano and Asada 1981). Root and shoot samples were homogenized in 0.1 M sodium phosphate buffer (pH 7.0) containing 10% PVP, centrifuged at 12,000*g* for 20 min, and supernatant was used to assay the enzyme activity. Reaction mixture consisted of 0.1 M sodium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H_2O, 0.1 mM EDTA, and enzyme extract. The change in absorbance at 290 nm was recorded for every 30 s for 180 s. APX activity was calculated using extinction coefficient of 2.8 mM^{-1} cm^{-1} for ascorbate oxidized at 290 nm and expressed as micromole ascorbic acid decomposed per microgram protein per minute.

f. Monochlorobimane is a dye that becomes fluorescent only on conjugation with lower group thiols especially reduced glutathione catalyzed by glutathione S-transferase forming a monochlorobimane-GSH adduct (Kamencic et al. 2000). Qualitative estimation of reduced glutathione was done by using the protocol of Muller et al. (1999) by immersing root tips in 0.25 μM solution of monochlorobimane in PBS for 30 min and documenting them under Laser Scanning Confocal Microscope (LSCM- Olympus, 1X CLSM 81). Software version: Flouview FVV 1000, 394-430 filter.

Statistical analysis

The experiments were carried out in a completely randomized design (CRD) with three replicates, each replica comprising a single petri dish containing an average of 50 seeds.

The statistical analyses were carried out in MINITAB environment (MINITAB ver.18). Data were presented as mean ±

standard error. The results were subjected to one-way analysis of variance (ANOVA). The level of significance was established at $P \leq 0.05$ at every instance for all the biochemical assays, throughout the study. In case a significant interaction was found among the factors (varieties × dosage), such instances were compared by Tukey's test (Manna and Bandyopadhyay 2017).

Results

Si reduced ROS accumulation under NaCl stress

Roots of plantlets of both the cultivars were stained with 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) that binds to ROS (predominantly H_2O_2). It was observed that a higher intensity of green fluorescence was produced in roots of both the cultivars under salt stress (Figs. 2 and 3). Intensity of fluorescence increased with increasing concentration of NaCl, from 25 to 100 mM. Basal level of green fluorescence and concomitantly ROS too was found to be higher in cv. Nonabokra in comparison to cv. MTU 1010. Silicon application decreased green fluorescence and the production of ROS as well in both the cultivars which was evident from the lesser intensity of green fluorescence in silicon supplemented roots of both cultivars.

Si retrieved GSH levels under NaCl stress

Increasing NaCl concentrations precipitated decrease of the GSH contents in both the cultivars. Roots of plantlets belonging to the susceptible cv. MTU 1010 showed a decrease of GSH contents by ~16 and 20%, respectively, when exposed to 25 and 50 mM NaCl, and a significant ($P \leq 0.05$) decrease of 43% under 100 mM NaCl treatment over water control. In the shoots of these plantlets, the decrease of GSH components registered was 14% under 25 mM NaCl treatment, whereas the GSH levels decreased significantly ($P \leq 0.05$) by ~25 and 29% under 50 and 100 mM NaCl concentrations, respectively, when compared to untreated control (Fig. 4a). In the roots of plantlets belonging to the salt tolerant cv. Nonabokra, GSH levels decreased slightly by ~8 and 12% under 25 and 50 mM NaCl treatments, respectively, and a significant ($P \leq 0.05$) decrease of 31% in GSH contents under 100 mM NaCl treatment was recorded when compared to water control. Similarly, in shoots of the cv. Nonabokra seedlings exposed to NaCl, GSH levels decreased by about 11, 17, and 21% under 25, 50, and 100 mM of NaCl treatments, respectively, over water control (Fig. 4b).

During joint application of NaCl in concentrations mentioned above along with 2 mM silicate, the magnitude of decrease in GSH level in roots of exposed plantlets of cv. MTU 1010 was

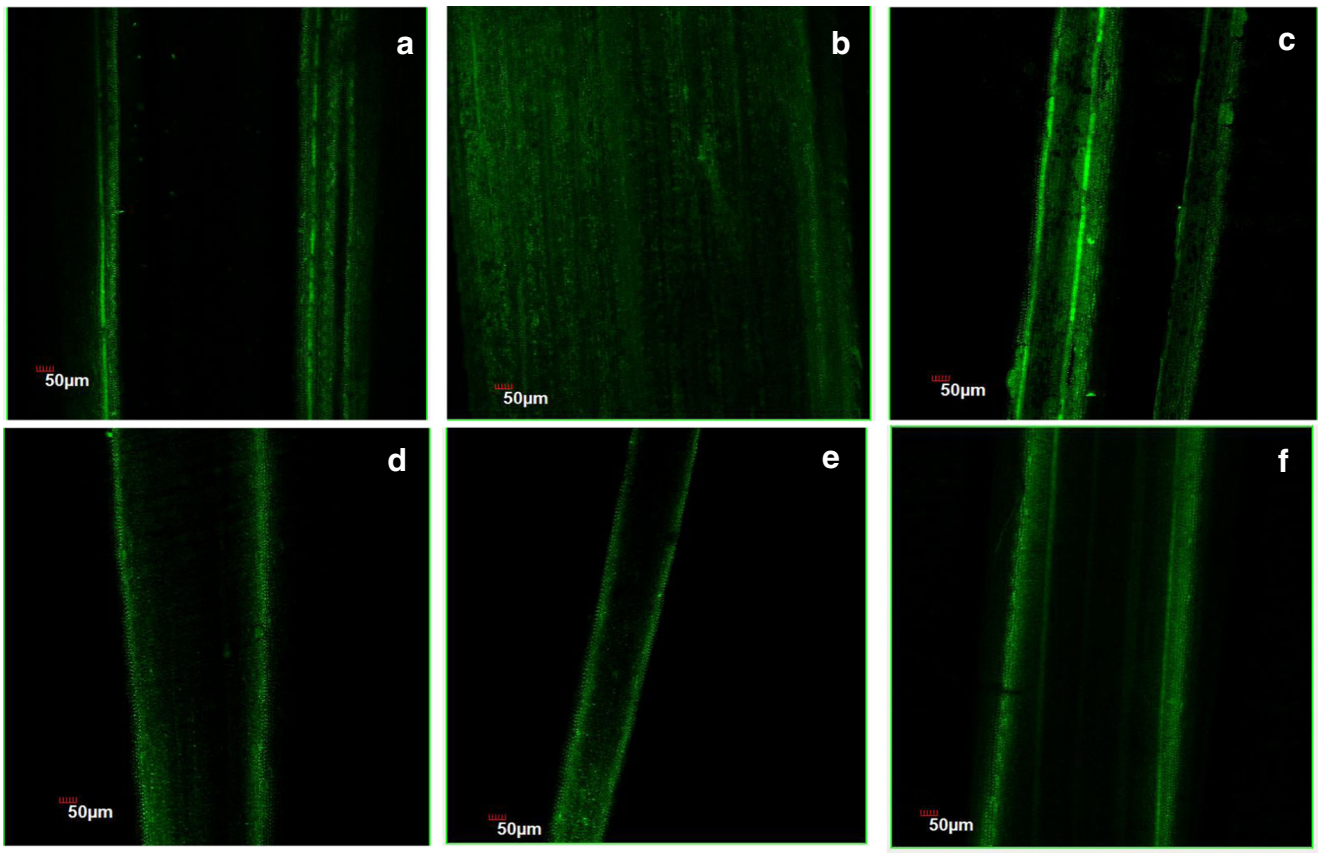

Fig. 2 H_2DCFDA staining images of ROS accumulation in roots of cv. MTU 1010. **a** Control. **b** 25 mM NaCl. **c** 100 mM NaCl. **d** 2 mM silicon. **e** 25 mM NaCl + 2 mM Si. **f** 100 mM NaCl + 2 mM Si

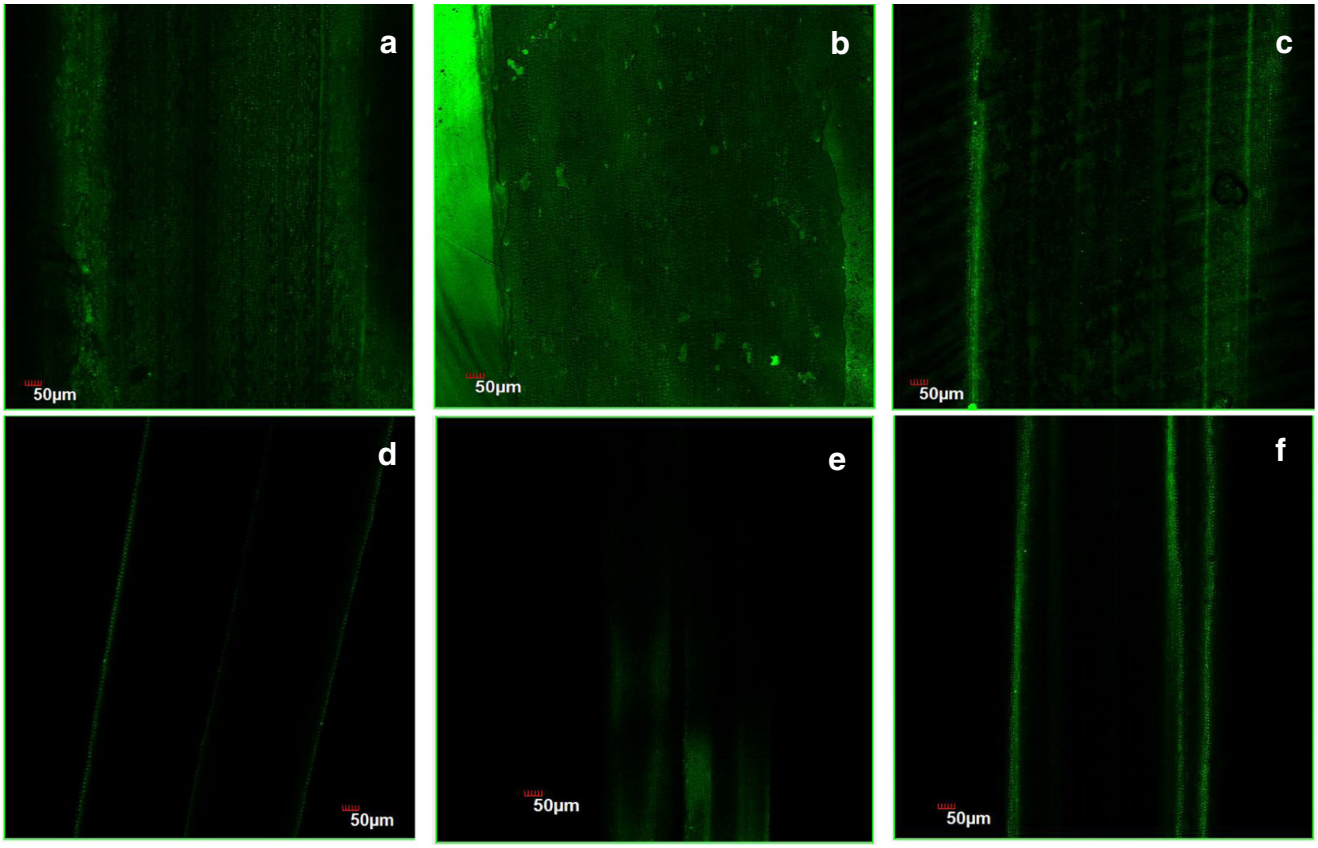

Fig. 3 H_2DCFDA staining images of ROS accumulation in roots of cv. Nonabokra. **a** Control. **b** 25 mM NaCl. **c** 100 mM NaCl. **d** 2 mM silicon. **e** 25 mM NaCl + 2 mM Si. **f** 100 mM NaCl + 2 mM Si

much less (by about 9, 13, and 27%, respectively, over water control), whereas in their shoots, the magnitude of decrease was also reduced by only 6, 19, and 26%, respectively, over water control. Similarly, in plantlets of cv. Nonabokra exposed to both NaCl and Si, the level of reduction in GSH contents was narrowed down both in roots and shoots, declining by only 4, 8, and 26% in roots and 5, 14, and 18% in shoots, when compared to water control, under the three doses of salt stress, respectively.

When monochlorobimane staining was used to monitor the root GSH level, it was observed that under NaCl stress, GSH content was prominently decreased. GSH levels were significantly restored in the salt-stressed plants of both the cultivars that had been co-treated with Si (Figs. 5 and 6).

Regression analysis results (Supplementary Tables 1 and 2) showed negative correlation of GSH content with NaCl concentration, whereas a positive correlation was found between application of silicon and increment in GSH contents even on salt exposure.

Ascorbate content increased upon Si application under NaCl stress

When the level of ascorbate was quantified under increasing concentrations of NaCl, a differential response was observed among the two cultivars. In roots of the susceptible cv. MTU 1010, treatment with 25 and 50 mM NaCl led to an insignificant decrease of ascorbate by ~ 17 and 35% and significant ($P \leq 0.05$) decrease by ~ 51% when exposed to 100 mM NaCl concentration, respectively, over water control. Similarly, shoots of cv. MTU 1010 plantlets when exposed to 25, 50, and 100 mM NaCl showed decreased ascorbate contents by ~ 15, 25, and 35%, compared to untreated control (Fig. 4c). In contrast, in plantlets of cv. Nonabokra, ascorbate content increased consistently at all doses of NaCl treatment. Roots from plantlets of cv. Nonabokra, 25, 50, and 100 mM NaCl treatments induced an increase by about 12, 33, and 52% of ascorbate content, compared to water control. In their shoots, ascorbate tends to increase linearly with salt concentration from about 3, 11, and 21% under 25, 50, and 100 mM NaCl concentrations, respectively, over water control (Fig. 4d).

Silicon administration along with NaCl altered the ascorbate content in both roots and shoots of the two cultivars of rice seedlings. In plantlets of the cv. MTU 1010, decrement in ascorbate content was narrowed down to ~ 5, 24, and 42% decrease roots and ~ 9, 21, and 27% decrease in shoots, under 25, 50, and 100 mM NaCl treatments, compared to water control. In plantlets of cv. Nonabokra, silicon supplementation further increased ascorbate contents from only salt-treated seedlings. In roots, ascorbate contents were elevated from 12 to 21% under 25 mM, from 33 to 41% in 50 mM NaCl and from 52 to 58% in 100 mM NaCl. In shoot, the increment escalated from 3 to 8, 11 to 14, and 21 to 25% under similar treatments.

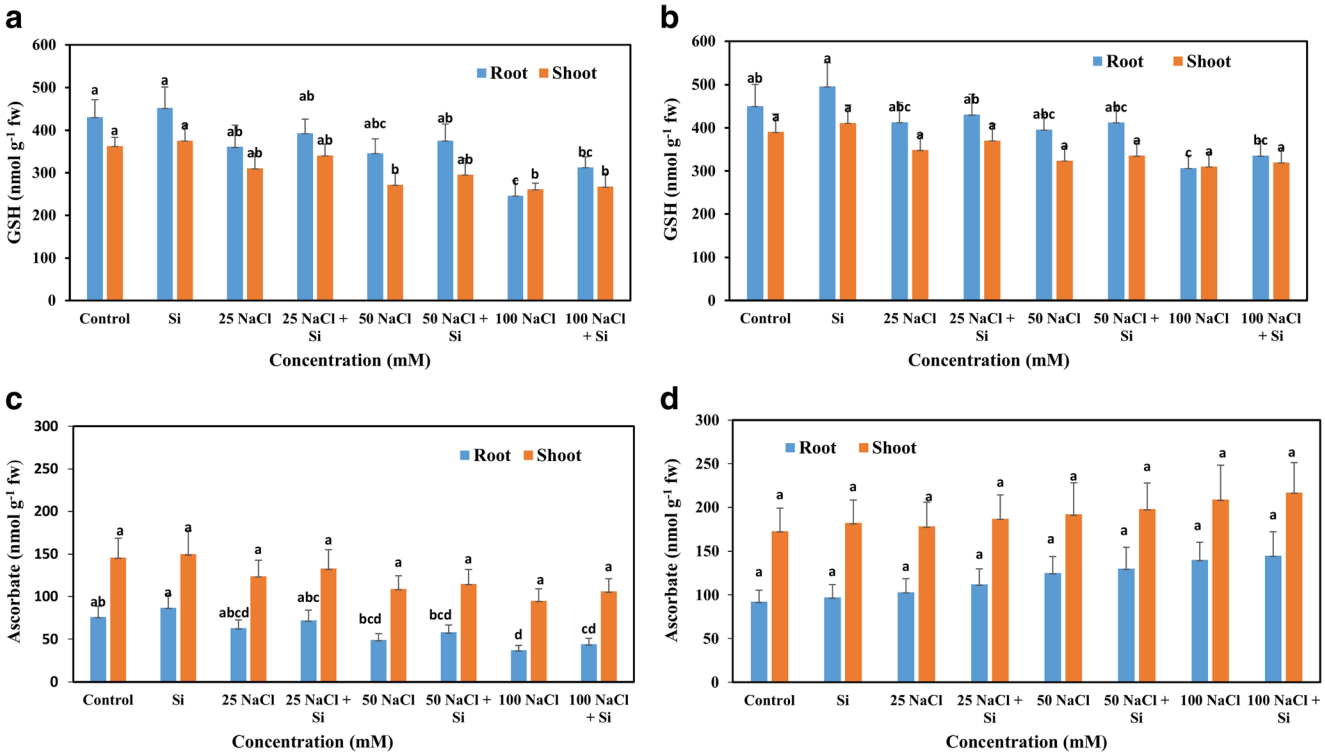

Fig. 4 Effect of silicon (2 mM) and NaCl stress (25, 50, and 100 mM) on GSH (**a** cv. MTU1010; **b** cv. Nonabokra) and ascorbate content (**c** cv. MTU1010; **d** cv. Nonabokra) in root and shoot of 21 days old rice seedlings. Vertical bars represent the standard error ($n = 3$). Different letters at the same time point represent significant differences ($P \leq 0.05$) between the treatments

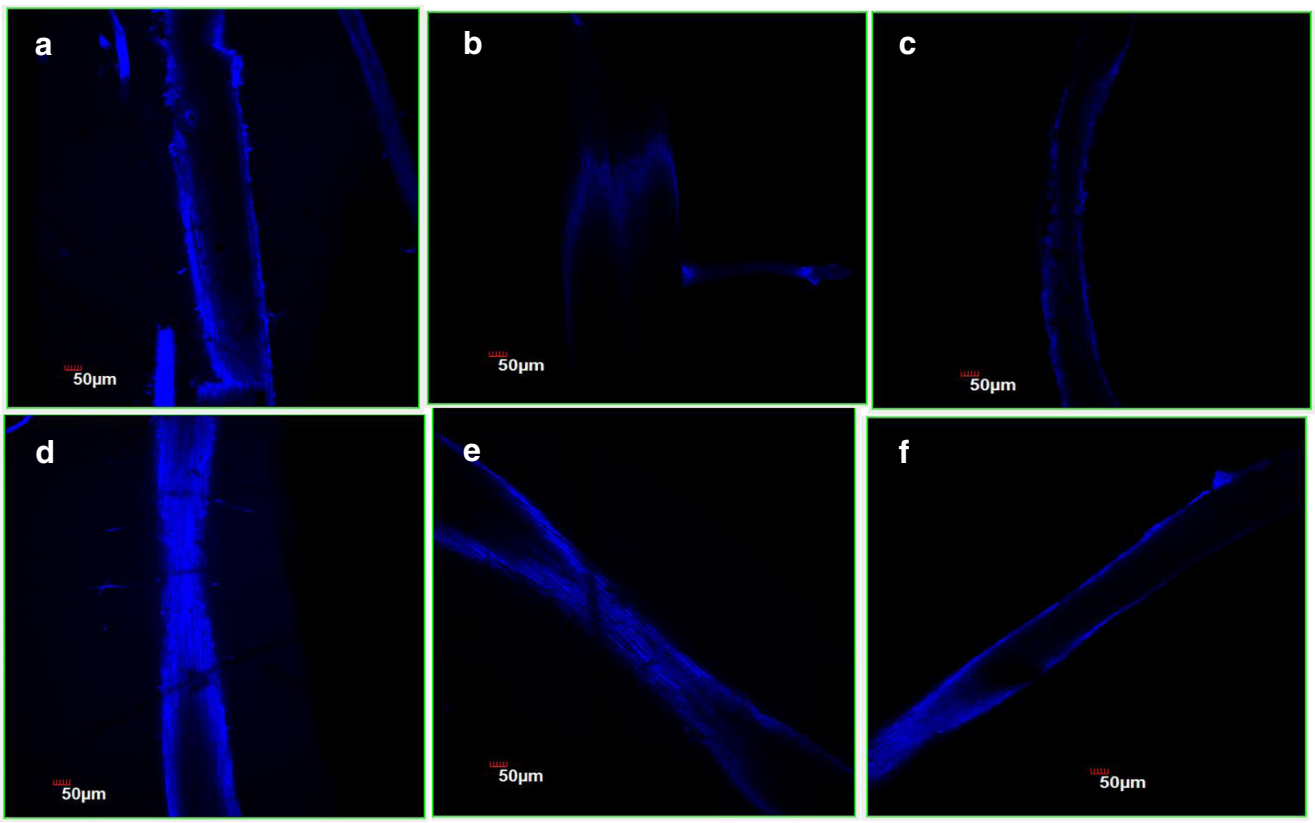

Fig. 5 Monochlorobimane staining images of reduced glutathione content in roots of cv. MTU 1010 (**a** control; **b** 25 mM NaCl; **c** 100 mM NaCl; **d** 2 mM silicon; **e** 25 mM NaCl + 2 mM Si; **f** 100 mM NaCl + 2 mM Si)

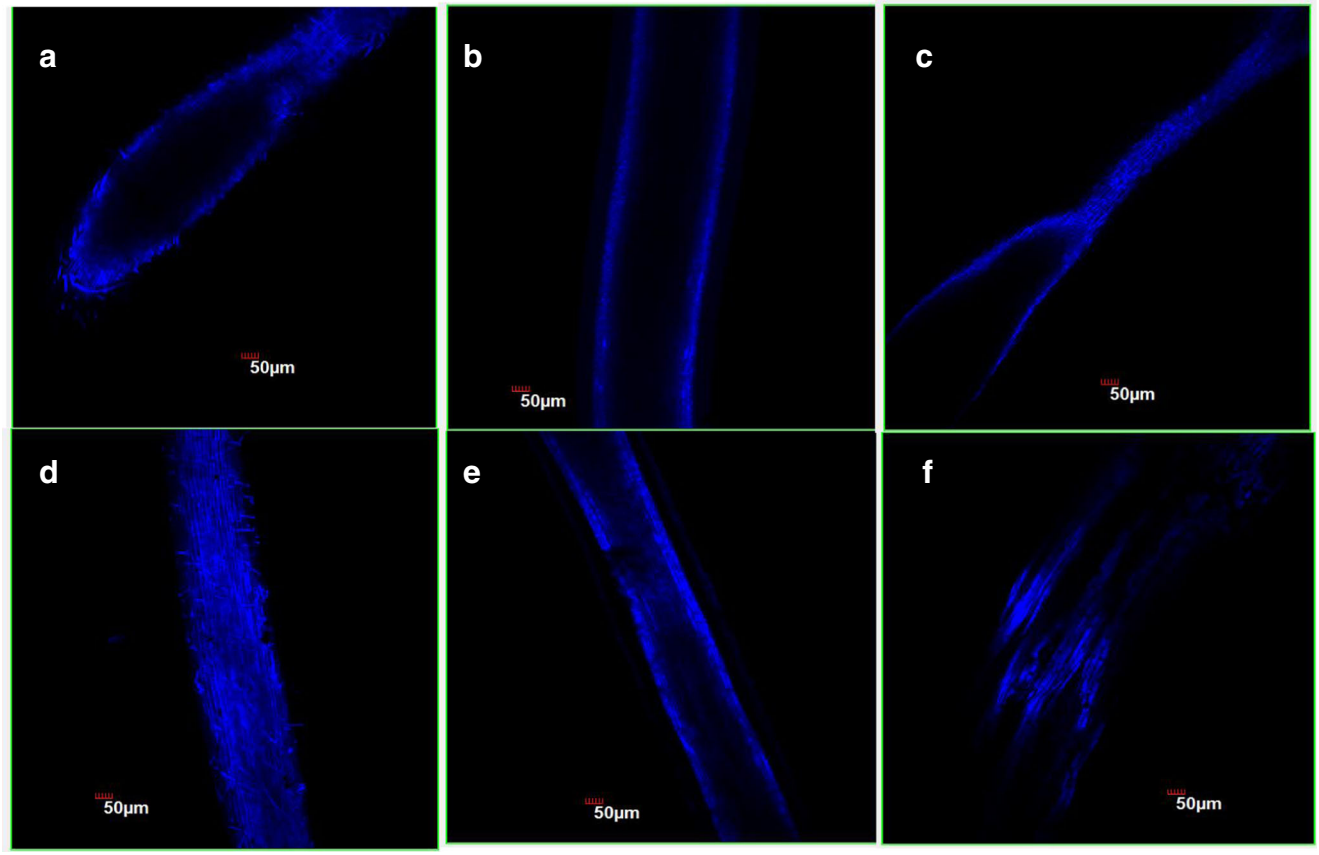

Fig. 6 Monochlorobimane staining images of reduced glutathione content in roots of cv. Nonabokra (**a** control; **b** 25 mM NaCl; **c** 100 mM NaCl; **d** 2 mM silicon; **e** 25 mM NaCl + 2 mM Si; **f** 100 mM NaCl + 2 mM Si)

Regression equation indicated that in both the cultivars, ascorbate content was positively correlated with silicon, but negatively correlated with NaCl concentrations in the case of cv. MTU 1010 only (Supplementary Tables. 1 and 2).

Reduced cysteine accumulation due to Si under NaCl stress

Cysteine is an essential amino acid of glutathione (GSH), and therefore, glutathione level is dependent on the supply of cysteine. In our experiment, both the rice cultivars showed enhanced cysteine accumulation under salt stress. Roots of plantlets of cv. MTU 1010 exhibited 20, 25, and 37% elevation in cysteine contents, while the shoots recorded increments of 12, 23, and 39% over water control under 25, 50, and 100 mM NaCl treatment, respectively, where changes in 100 mM concentration were statistically significant ($P \leq 0.05$) in both root and shoots (Fig. 7a). In the plantlets of cv. Nonabokra, elevation in cysteine contents was about 4, 9, and 22% in root samples followed by 9, 14, and 18% elevation in the shoot under above mentioned doses, respectively (Fig. 7b).

Co-application of Si and NaCl reduced the accumulation of cysteine in both the cultivars compared to the NaCl-treated seedlings. In the roots of cv. MTU 1010 seedlings, elevation in cysteine levels was recorded to be 9, 21, and 28% lower due to silicon treatment, while in shoot, the percentage of increase was reduced to 8, 13, and 33%. In cv. Nonabokra seedlings, increment in cysteine contents was also decreased and it was only 3, 7, and 20; and 3, 12, and 16% over control in roots and shoots, respectively.

Supplementary Tables 1 and 2 show the correlation coefficients between cysteine content of the roots and shoots of rice seedlings of both cultivars treated with NaCl and Si. The results indicated that the cysteine level was positively correlated with the NaCl concentration and negatively correlated with Si.

Si enhanced activity of GR under NaCl stress

Decrease in glutathione reductase activity was recorded under NaCl stress in roots and shoots of both the cultivars compared to water control. Seedlings of cv. MTU 1010 showed maximum decrease in the GR enzyme activity, whereas cv. Nonabokra showed the minimum. In the roots of cv. MTU 1010 seedlings, exposure to 25 and 50 mM NaCl led to a decrease of about 12 and 16% from water control, whereas 100 mM NaCl treatments led to a significant ($P \leq 0.05$) decrease of about 40% that was recorded from water control. In the shoots of cv. MTU 1010 seedlings, exposure to 25, 50, and 100 mM NaCl concentrations

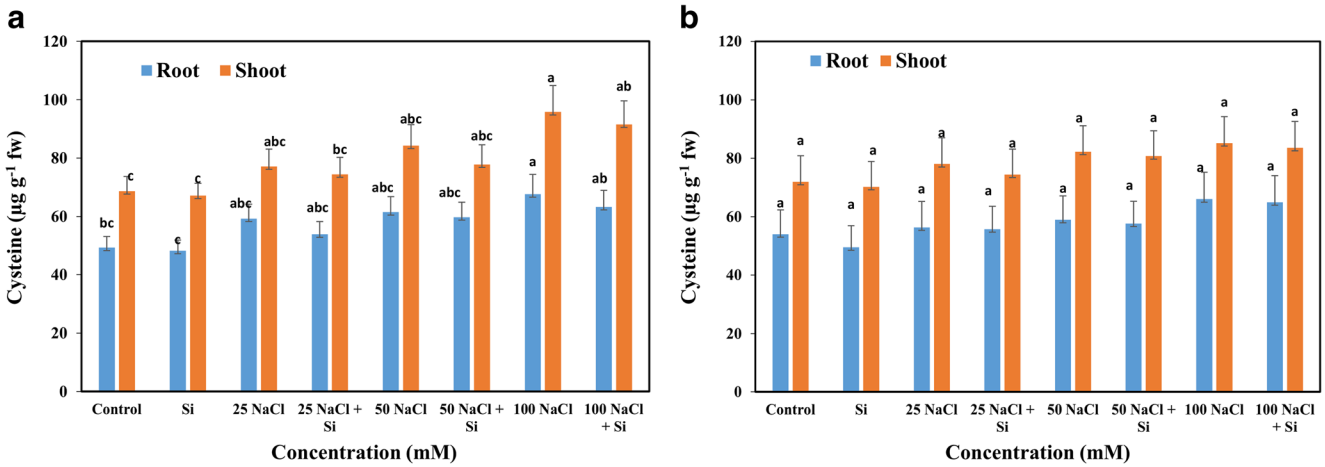

Fig. 7 Effect of silicon (2 mM) and NaCl stress (25, 50, and 100 mM) on cysteine content (**a** cv. MTU1010; **b** cv. Nonabokra) in root and shoot of 21 days old rice seedlings. Vertical bars represent the standard error ($n = 3$). Different letters at the same time point represent significant differences ($P \leq 0.05$) between the treatments

registered a significant ($P \leq 0.05$) decrease in enzyme activity which was about 12, 19, and 26% compared to water control (Fig. 8a). In the roots of cv. Nonabokra, an insignificant decrease of 9 and 14% in GR activity was recorded in 25 and 50 mM NaCl treatments, respectively, whereas a significant decrease ($P \leq 0.05$) of about 32% was documented in GR activity upon 100 mM NaCl treatment over water control. In the shoots of cv. Nonabokra seedlings, the activity of GR decreased by 9, 14, and 16% under the said doses of NaCl treatments, respectively, compared to untreated control (Fig. 8b).

Addition of silicon with salt resulted in the retrieval of GR activity in the both cultivars of rice. In cv. MTU 1010 seedlings,

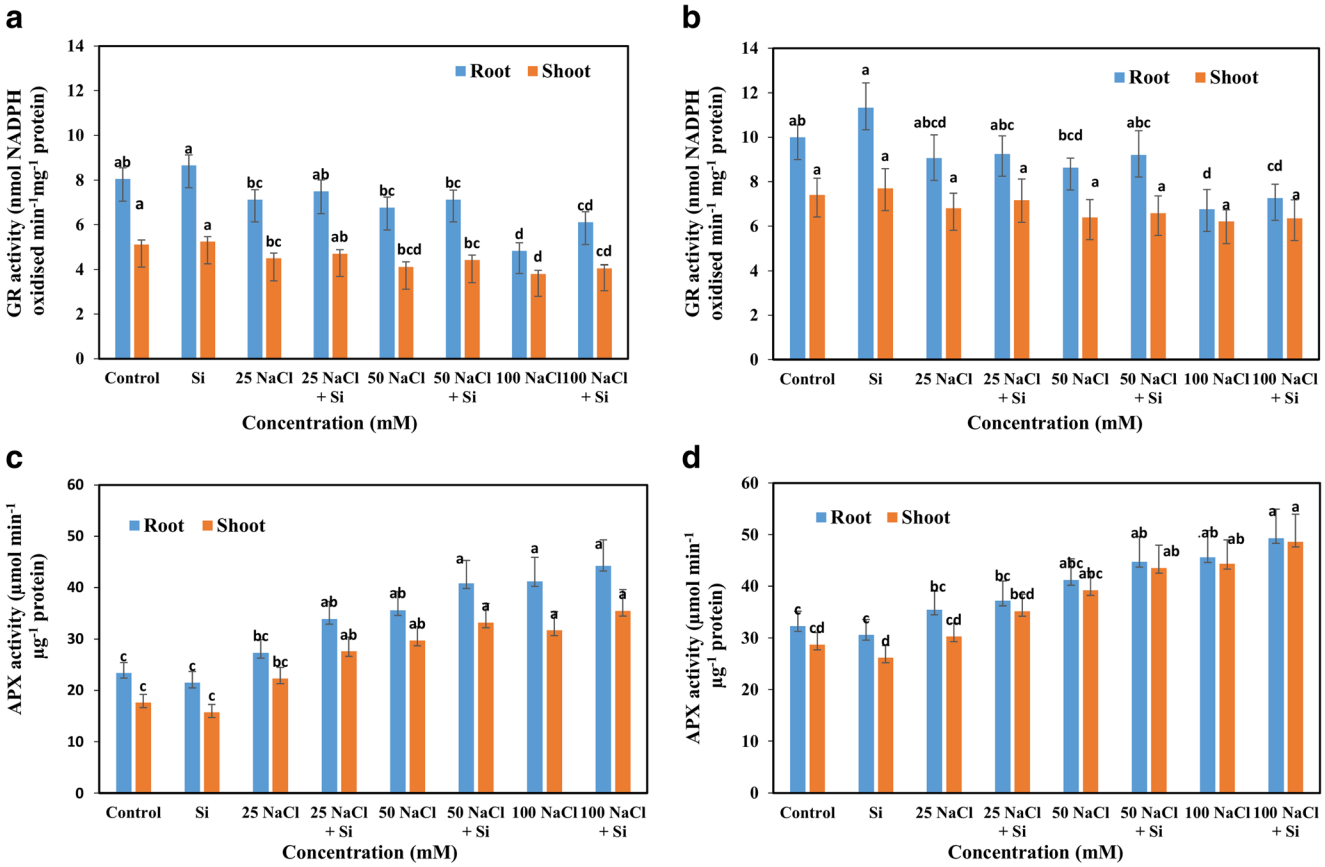

Fig. 8 Effect of silicon (2 mM) and NaCl stress (25, 50, and 100 mM) on GR activity (**a** cv. MTU1010; **b** cv. Nonabokra) and APX activity (**c** cv. MTU1010; **d** cv. Nonabokra) in root and shoot of 21-day-old rice seedlings. Vertical bars represent the standard error ($n = 3$). Different letters at the same time point represent significant differences ($P \leq 0.05$) between the treatments

salt-induced suppression of GR activity was reduced to 9, 11, and 24% in root and 8, 14, and 21% in shoot compared to control in 25, 50, and 100 mM NaCl treatments supplemented with silicon. Similarly, in cv. Nonabokra, downregulation of GR activity was checked partially and it was only 7, 8, and 27% lower in root and 3, 11, and 14% lower in shoot over untreated control.

Supplementary Tables 1 and 2 show the correlation coefficients between GR activity of the roots and shoots of rice seedlings of both cultivars treated with NaCl and Si. The results indicated that GR activity was negatively correlated with the NaCl concentration and positively correlated with Si.

APX activity increased on Si application under NaCl stress

It was observed that APX activity increased in the test seedlings irrespective of cultivars under all concentrations of salt treatments. However, when seedlings were grown under NaCl stress with Si supplementation, enzyme activity was further elevated. In the roots of cv. MTU 1010, APX activity showed an insignificant increase of about 17% in 25 mM NaCl and 2 mM Si, and significant increase ($P \leq 0.05$) of about 52 and 76% in 50 and 100 mM NaCl treatments, respectively, over water control. Similarly, in the shoots of cv. MTU 1010, APX activity showed an increase of about 27, 69, and 80% under 25, 50, and 100 mM NaCl treatments, respectively, over water control (Fig. 8c) where changes in 50 and 100 mM concentrations were statistically significant.

But, APX activity was further elevated to 45, 74, and 89% in roots and 57, 89, and 101% in shoots when Si was applied to salt-treated plants of MTU 1010 cultivar. In the roots of cv. Nonabokra, APX activity registered an insignificant increase of about only 10 and 28% in 25 and 50 mM NaCl treatment and significant ($P \leq 0.05$) increase of about 41% in 100 mM NaCl treatments, respectively, when compared to water control. Similarly, in the shoots of the cv. Nonabokra, APX activity showed an insignificant increase of about only 6% in 25 mM and significant one ($P \leq 0.05$) of about 37 and 54% in 50 and 100 mM NaCl treatments, respectively, over water control (Fig. 8d). Again, the increment percentage was escalated to 15, 38, and 53% in root and 23, 52, and 69% in shoots under 25, 50, and 100 mM NaCl-treated seedlings, respectively, with added silicon.

The regression analysis results indicated that both the NaCl and Si concentrations were positively correlated with APX activity (Supplementary Tables 1 and 2).

Si induced increase in GPx levels under NaCl stress

Glutathione peroxidase activity showed differential responses in the two rice cultivars under salt stress. Salt treatment of three doses (25, 50, and 100 mM) alone showed 9, 23, and 38% decline in glutathione peroxidase activity in roots, while the shoots recorded a decrease of about 8, 25, and 34% over water control (Fig. 9a), where values in 100 mM NaCl treatment were statistically significant ($P \leq 0.05$) both for root and shoot.

However, when seedlings were grown under salinity stress with Si supplementation, the reduction percentage of enzyme activity in root and shoot were 3, 15, and 29, and 4, 15, and 27%, respectively, compared to control. In cv. Nonabokra, GPx activity tended to increase linearly with the increasing NaCl concentrations. In roots, 25, 50, and 100 mM NaCl treatments registered an insignificant promotion of enzyme activity which was about 13, 23, and 32% over water control. Whereas in shoots of the same cultivar, 25, 50, and 100 mM NaCl led to an insignificant increase of GPx by about 9, 17, and 27% over untreated control (Fig. 9b). But, the promotion percentage was further escalated to 17, 28, and 41% in root and 13, 22, and 35% in shoot under similar doses of NaCl treatment supplied with additional silicon.

Positive correlation was found between GPx activity and Si application in both cultivars under salt stress (Supplementary Tables. 1 and 2).

GST activity decreased on Si application

Roots of cv. MTU 1010 exhibited 9, 24, and 37% elevation in glutathione S-transferase activity, while the shoots recorded an increment of 8, 22, and 33% over water control under 25, 50, and 100 mM NaCl treatment, respectively, where promotion in 50 and 100 mM concentrations was statistically significant ($P \leq 0.05$) in both root and shoots (Fig. 9c).

In NaCl and Si co-treated seedlings, the rate of increase in the enzyme activity was narrowed down and was about 4, 16, and 27, and 4, 14, and 26% higher in root and shoot, respectively, over untreated water control. In cv. Nonabokra root, the elevation in enzyme activity was 12, 29, and 38%, while in shoot, the increase in GST activity was 11, 24, and 36% over control under the same concentrations of NaCl treatment (Fig. 9d), only 100 mM NaCl treatment being statistically significant ($P \leq 0.05$). But, the elevation percentage was reduced to 6, 21, and 35, and 7, 21, and 33% in root and shoot when Si was applied to salt-treated plants of Nonabokra cultivar.

Supplementary Tables 1 and 2 show the correlation coefficients between the GST activity of the roots and shoots of rice seedlings treated with NaCl and Si. The results indicated that the Si concentration was negatively correlated with GST activity.

Discussion

The role of silicon in improving the inductive and facultative attributes of a plant has become well documented in recent times. Enhancement of growth, better yield, and quality has prompted the use silicon as a cheap and effective fertilizer. Wide availability, non-reactive nature, low cost, and being environmentally safe with no build up in the eco system make

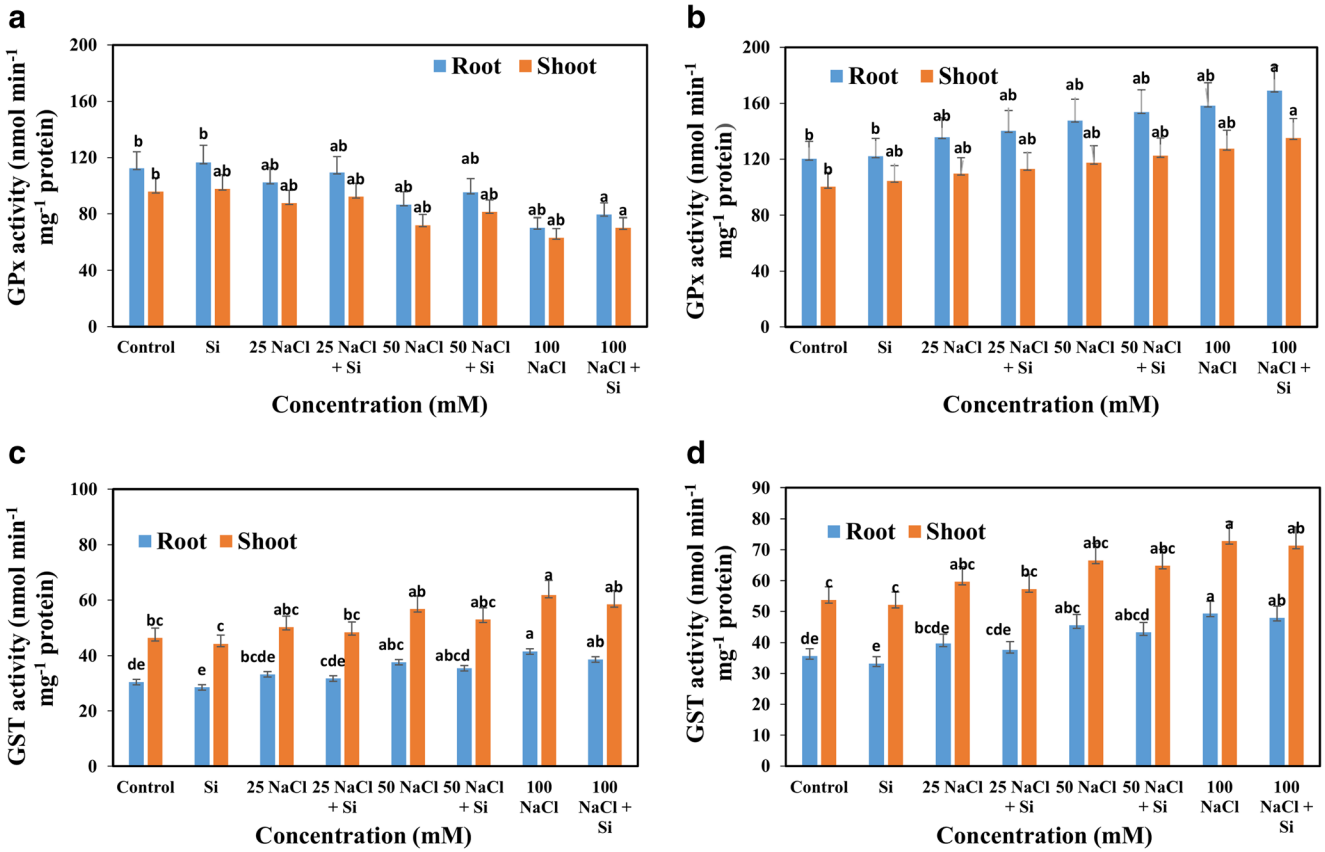

Fig. 9 Effect of silicon (2 mM) and NaCl stress (25, 50, and 100 mM) on GPx activity (**a** cv. MTU1010; **b** cv. Nonabokra) and GST activity (**c** cv. MTU1010; **d** cv. Nonabokra) in root and shoot of 21-day-old rice seedlings. Vertical bars represent the standard error ($n = 3$). Different letters at the same time point represent significant differences ($P \leq 0.05$) between the treatments

silicon choicest among other chemically synthesized fertilizers. However, soluble, absorbable forms of Si are scarce (Epstein 1994) with silicic acid, $Si(OH_4)$ being the only absorbable source of silicon for most plants (Ma and Yamaji 2006; Rios et al. 2017). Not only that, the role of exogenous silicon in priming a plant for inadvertent stress response is a topic of much enthusiasm (Ahmed et al. 2016). It has already been shown that silicon application improves photosynthetic output, nitrogen fixation, and so on (Coskun et al. 2016). The effect of silicon in alleviating both biotic and abiotic stress is also well-known (Meharg and Meharg 2015). Interaction between silicon and rice is a classic example where silicon enters the tissue and helps in bypassing the impending hyperosmotic shock and helps in getting rid of contents of unwanted ions. Silicon detains ROS-induced lipid peroxidation to maintain membrane integrity too. Most of these qualities of silicon are selective and only activates in case of abiotic, biotic stress, or in case of herbivory (Van Bockhaven et al. 2012).

Salt stress exerts its negative impact on plants by creating ionic imbalance and inducing over oxidation state in the exposed tissues. Phenotypic complications including rapid loss in dry weight, reduced length of root and shoot, and deformed setting of seeds are well-known markers of high saline growth conditions (Yan et al. 2013; Nxele et al. 2017). Rapid accumulation of high concentration of salts in these tissues beyond a threshold limit alters the inherent genetic plasticity of the plant species, which are manifested as specific stress-induced responses, in this case those related to salt tolerance. The spectrum of such responses varies highly among different plant species, due to which are defined as salt sensitive (glycophyte) or tolerant (halophyte) (Roy and Wu 2002). An upsurge in the reactive oxygen species is a major physico-chemical indicator of salinity stress, and immediately after ROS induction, components of the antioxidant defense system act to protect the plant from oxidative damage by scavenging ROS. According to Noctor and Foyer (1998), ascorbate-glutathione cycle has been deduced to be the most important intra-cellular antioxidant pathway for ROS scavenging, and studies conducted on the perturbations of physiological and biochemical parameters in rice plants exposed to increased salinity have confirmed that experiments where salt stress was induced by the application of various doses of NaCl under laboratory conditions could simulate the field conditions and are widely considered to be acceptable alternatives for assessing the performance of plants subjected to salt stress in field conditions (Ghosh et al. 2011).

Salt stresses affect plants in mainly two pathways: osmotic stress and ionic stress (Wang et al. 2015). Excess Na^+ and Cl^- ions cause osmotic stress in the plant, whereas their hyper

accumulation leads to degeneration in metabolic status. The plant under salt stress engages various methodologies to segregate excess Na^+ into vacuoles and reduce transpiration and other water loss (Mäser et al. 2002). However, frequently ever increasing ion fluxes induce massive damage to the salt ravaged plants. Mechanistically speaking, hyperosmotic and ionic Na^+ switches present in a plant, comprised of the two component salinity indicator and litigator response cascades, are necessary for this response (Blumwald 2000), though molecular characterization of such control mechanism has been elusive till date. Ca^{2+} channels orchestrate a very vital role in the hyperosmotic component (Harmon et al. 2000). Root cytosolic Ca^{2+} channels of *Arabidopsis* were found to be activated soon after ionic disharmony, thus increasing cytosolic Ca^{2+} (Munns and Tester 2008), giving insight into the intricate physiological reactions that take place in the inter/intracellular environment (Tester and Davenport 2003). Salinity-induced ROS-mediated calcium signaling too has a similar effect, downregulating kinases, thus degrading gene regulation and protein synthesis (Deinlein et al. 2014). Common stress mitigating transcription factors are functional in case of salinity stress too; genes for ion uptake and osmolyte synthesis are upregulated through these transcription factors (Geng et al. 2013). Mitigation of salinity stress leads to higher accumulation of many of these osmolytes viz. proline, glycine betaine, mannitol, and sorbitol (Agarwal et al., 2013). Hormonal regulation too comes into foreplay for mitigation mainly by changes in geotropism. Interplay of hormones like ABA and AUX (Duan et al. 2013; Galvan-Ampudia et al. 2013) take part in halotropism (Deinlein et al. 2014), but in the course of action, root growth is hampered and growth is subdued. Membrane transporters like K^+ are most vital in maintaining a healthy Na^+ flux and maintain the K^+/Na^+ steady state in case of salt stress (Schoeder et al. 2013). Osmolytes particularly polyamines and proline have beneficial roles in salt stress response (Verslues et al. 2006), proline is synthesized rapidly on detection of hyperosmotic response (Szabados and Savoure 2010) and acts as ROS scavenger bringing in stabilization of membrane potential and protein function in the face of high salt intensity (Ashraf and Foolad 2007; Verbruggen and Hermans 2008). In fact, salinity affects photosynthetic output quite adversely, and deterioration in photosynthetic pigments and electron transport chain is directly correlated to increase in salinity beyond amendments (Parida et al. 2004; Sun et al. 2016; Mbarki et al. 2018) and fortifies the mechanical strength of the cell wall by silification, by binding with hemicellulose, thus creating a barrier against further damage (Hossain et al. 2002). Silicon also blocks bypass transpirational flow to check osmotic stress (Gong et al. 2005). Further, indirect proton pump activation (Liang et al. 2007) and polyamine production alleviates salinity-stressed cells (Berberich et al. 2015), whereas by deteriorating lipid peroxidation, Si helps in maintaining membrane integrity (Khoshgoftarmanesh et al. 2014). The spike in antioxidant machineries on silicon application benefits the cell in stress mitigation too. However, all these mechanisms are specifically tested on particular model systems, universality of which remains to be checked (Coskun et al. 2016).

Rice is a well-known glycophyte or salt-sensitive species. However, local cultivars which exhibit varying degrees of salt tolerance are known (Reddy et al. 2017). Except salt-tolerant rice cultivars, salt-sensitive rice cultivars showed remarkable morphological as well as biochemical alterations even under mild salt stress (in our study, it is 25 mM NaCl). In our previous study (Das et al. 2016), we have focused on regulation of growth, antioxidants, and sugar metabolism in rice cv. MTU 1010 only subjected to salt stress and its partial amelioration with the application of silicon. But, in the present study, we have focused on comparative analyses of two rice cultivars, viz. MTU 1010 and Nonabokra under salt stress and also their response towards applied silicon. Moreover, the parameters which we have studied here are totally different from the previous one, giving emphasis on ascorbate-glutathione cycle and ROS scavenging. The present work is a continuation of the previous study indeed, but in a more elaborative manner.

The present study was designed to assess silicon-induced amelioration of salinity damage in rice plants exposed to three doses of NaCl, viz. 25, 50, and 100 mM, to mimic the actual field conditions ranging from low saline soil to high salt-affected soils. In the present investigation, we initially examined performance of two rice cultivars, one of which MTU1010 is a salt susceptible cultivar and the other Nonabokra is a salt-tolerant cultivar, with respect to the glutathione cycle. The second and most important part of the work was to study whether the salt-induced physiological perturbations could be relieved form salt-induced injury by exogenous silicon application. This work also gave interesting insights into the comparative assessment of physiology of salt-induced stress in the two cultivars. While standardizing the experiments, we have observed that 2 mM Si had the most remarkable effect on counteracting salt-induced damage in rice plants of both cultivars. We have grown rice seedlings in soil-less culture with Hoagland's nutrient medium supplied with three doses of NaCl, viz. 25, 50, and 100 mM with or without Si. These concentrations actually mimic the low, medium, and high salinity in field condition. From our obtained results, it is evident that silicon application is useful in partial amelioration of salt-induced damages of all the three doses, but the level of promotion was more vigorous under 50 and 100 mM concentrations of NaCl compared to 25 mM NaCl treatment which was very mild to induce any significant stress responses (Supplementary Fig. 1 and Supplementary Table 3).

Histochemical detection of hydrogen peroxide by H_2DCFDA dye showed that the salt-tolerant cv. Nonabokra maintained a higher basal level of H_2O_2 in both roots and shoots when compared to the salt-sensitive cv. MTU 1010.

H_2O_2 plays the role of secondary messenger which in turn activates different stress-mediated signaling pathways at genetic and physiological levels (Salwa et al. 2015). From the present study, it could be inferred that higher basal level of H_2O_2 in the roots and shoots of cv. Nonabokra primes it for adaptation to salinity, by constitutive activation of various stress-mediated signaling pathways. Interestingly, at 100 mM NaCl treatment, the level of increase in H_2O_2 content was much greater in the roots and shoots of cv. MTU 1010, as compared to those of cv. Nonabokra. It can be argued that since the salt-sensitive cv. MTU1010 seedlings had undergone higher ROS-induced damage than seedlings of cv. Nonabokra, at high salt stress, they accumulated high levels of ROS, such as H_2O_2, under the same doses of salinity. Application of exogenous silicon decreased this accumulation of ROS in both the cultivars in all the salt concentrations tested, which were evident from the staining images. Higher constitutive level of ROS in the salt-tolerant cultivar compared to the sensitive one of rice was previously observed through H_2DCFDA dye staining (Kaur et al. 2016). Silicon was found to lower the production of H_2O_2 in water-stressed tomato plants (Shi et al., 2016).

Interestingly, the basal level of GSH and ascorbate was found to be significantly higher in roots and shoots of the seedlings of cv. Nonabokra than in cv. MTU 1010. It is known that under adverse environmental conditions, GSH levels get depleted in sensitive genotypes and increase in tolerant genotypes, as was observed of mung bean (Tausz et al. 2004). In our study, however, the GSH content considerably decreased in roots and shoots of both the cultivars on salt exposure. The magnitude of depletion of GSH was more in the seedlings of cv. MTU 1010 than in cv. Nonabokra. In our study, it was observed that increasing salt stress decreased the ascorbate content in cv. MTU 1010, whereas ascorbate content increased in cv. Nonabokra under the same conditions. Increased ascorbate content recorded under salinity stress in cv. Nonabokra could possibly be attributed efficient recycling via the Asada–Halliwell–Foyer pathway and/or its inherent enhanced biosynthesis in the salt-tolerant cultivar. Application of exogenous silicon increased ascorbate and GSH levels in both the cultivars in the presence of the salt too. Other workers reported an elevation of GSH and ascorbate level in water-stressed tomato supplemented with silicon (Shi et al., 2016) that supports observations from our study. Enhancement of these non-enzymatic antioxidants due to addition of silicon contributed in alleviating salt-induced toxicity in rice seedlings.

Formation of cysteine is a central step required for incorporation of reduced sulfur into organic compounds such as GSH (Noctor and Foyer 1998). In our study, the cysteine content in the roots and shoots of the seedlings of two rice cultivars increased under NaCl stress when compared to their control sets. This increment was higher in seedlings of cv. MTU 1010 than in those of cv. Nonabokra. Higher cysteine content in both the cultivars, especially in the salt-sensitive cv. MTU 1010, upon NaCl stress, may be explained as a consequence of the reduction in formation of GSH under salt stress. It was mentioned earlier that exposure to salt stress decreased GSH contents in seedlings of both the rice cultivars, hence net cysteine availability increased. Interestingly, Si addition in the presence of enhanced salts decreased cysteine content in both the cultivars when compared to the salt-stressed seedlings for all the concentrations tested. This result was concomitant with the increase in GSH content of the seedlings under silicon supplementation and hence may be a consequence of more of the endogenous cysteine pool being utilized for increased biosynthesis of thiol-related compounds, like glutathione. Increased glutathione may subsequently be involved in maintaining cellular redox homeostasis by quenching of ROS.

Glutathione alternates between reduced (GSH) and oxidized (GSSG) forms, with the help of GR activity at the cost of NADPH (Noctor et al., 2012, b). Values of GR activity were found to be 10 and 7.41 nmol NADPH oxidized min^{-1} mg^{-1} protein in root and shoot of control seedlings of cv. Nonabokra, whereas in the control seedlings of cv. MTU 1010, these values were 8.05 and 5.11 nmol NADPH oxidized min^{-1} mg^{-1} protein in root and shoot, respectively. Our study revealed that GR activity diminished in both the cultivars with a greater magnitude of decrease observed in the seedlings of the salt-sensitive cv. MTU 1010 and probably indicated its lesser efficacy in continuing active ascorbate glutathione cycle under salt stress. Reduction in GR activity was reported previously in the roots of salt-stressed mung bean seedlings (Saha et al. 2015). Silicon augmentation elevated the GR activity irrespective of cultivars which may enhance the rate of oxidation of NADPH to NADP+, resulting in increased availability of NADP+ to capture electrons generated from photosynthetic electron transport chain and converted back to its reduced form NADPH that finally carries electrons to the Calvin cycle. Moreover, elevation of GR activity under silicon application could be implicated in sustaining the redox status of rice seedlings possibly by regeneration of GSH from GSSG that in fact could be corroborated with increased GSH levels in the silicon supplemented seedlings as mentioned earlier. Upregulation of GR activity thus enhanced the antioxidant capacity of the salt-stressed seedlings by maintaining sufficient GSH pools, thereby improving their tolerance to salt stress. Similar increment of GR activity upon silicon application in drought-stressed wheat was reported earlier (Gong et al., 2005).

Due to high affinity for H_2O_2, ascorbate peroxidase (APX) scavenges it in a reaction that utilizes ascorbate as an electron donor (Sharma and Dubey 2004). From the present study, it was clear that seedlings of cv. Nonabokra had much higher basal APX level compared to those of cv. MTU 1010. In the water control seedlings of cv. Nonabokra, APX activity was

found to be 32.3 and 28.7 μmol min^{-1} mg^{-1} protein in root and shoot, respectively, while in the case of water control seedlings of cv. MTU 1010, these values were 23.4 and 17.6 μmol min^{-1} mg^{-1} protein in root and shoot, respectively. Enhancement of APX activity due to salinity was reported earlier in wheat (Esfandiari and Gohari 2017) and in tomato (Gharsallah et al. 2016). Addition of Si further increased the APX activity in both the cultivars suggesting that under salinity and under a combination of salinity and Si, APX impart tolerance by detoxifying H_2O_2 in rice seedlings.

Glutathione peroxidase (GPx), another important H_2O_2 scavenging enzyme of ascorbate-glutathione cycle, is localized in different cellular organelles like mitochondria, peroxisome, and chloroplast. Due to its stronger affinity for its substrate H_2O_2, it donates an e- to H_2O_2 and helps in its degradation, thus protecting against stress (Brigelius-Floh'e and Floh'e 2003). In the present study, differential expression pattern of enzyme activity was noted between the two cultivars under salinity. Salt-tolerant cultivar Nonabokra showed upregulation of GPx activity at all doses of NaCl, whereas in the sensitive cultivar MTU 1010, GPx activity declined. Increase in the enzyme activity in the tolerant cultivar is probably due to inherently enhanced synthesis of antioxidant enzymes and ensures effective ROS scavenging at all doses of NaCl. On the other hand, decline in the enzyme activity in the sensitive one indicates inefficient ROS detoxification process in the plant making it vulnerable to ROS-induced damages. However, silicon-supplemented seedlings of both the cultivars showed upregulation of the GPx enzyme activity compared to salinity-stressed seedlings alone, level of increment being lower in the tolerant cultivar Nonabokra. Hence, we can hypothesize that the Si-induced increase in GPx activity in the stressed seedlings is useful in mitigating the excess accumulation of H_2O_2. Silicon-induced increase in GPX activity in salt-stressed cucumber was reported earlier (Khoshgoftarmanesh et al. 2014).

Glutathione S-transferase (GST) is an enzyme group that removes different genotoxic and cytotoxic compounds by GSH conjugation (Cummins et al. 2011). Increment in GST activity in cv. Nonabokra under salt stress as observed in the present study supports an earlier report that drought-tolerant rice genotypes showed higher GST activity compared to the sensitive ones under water stress (Pyngrope et al. 2013). Such a difference in enzyme activity can be explained by the upregulation of the antioxidant defense system in salt-tolerant cv. Nonabokra against salinity-induced oxidative damages. NaCl-stressed seedlings supplemented with exogenous Si belonging to both the cultivars showed a decrease in GST activity when compared to only NaCl-treated seedlings indicating that addition of Si helps in reduced accumulation of genotoxic and cytotoxic compounds. Hence, to detoxify lesser amount of accumulated genotoxic compounds eventually, GST activity was decreased in both the cultivars.

It is clear that the damaging effect of salt stress was more drastic in sensitive cv. MTU 1010 compared to tolerant cv. Nonabokra. Silicon nutrition had partially ameliorated this damage in both the cultivars which is evident from our results (Supplementary Fig. 1 and Supplementary Table 3). But, it is also noteworthy that the percentage of amelioration, i.e., the changes between only NaCl-treated and NaCl+Si-treated plants of every dose was found to be more prominent in cv. MTU 1010 rather than cv. Nonabokra. So, we have concluded that silicon has more pronouncedly ameliorated the adverse effects of salt stress in cv. MTU 1010 rather than cv. Nonabokra.

In general, NaCl stress is harmful to plants, especially glycophytes like rice, as it induces oxidative stress, and tolerance to such stress depends upon the regulation of enzymatic

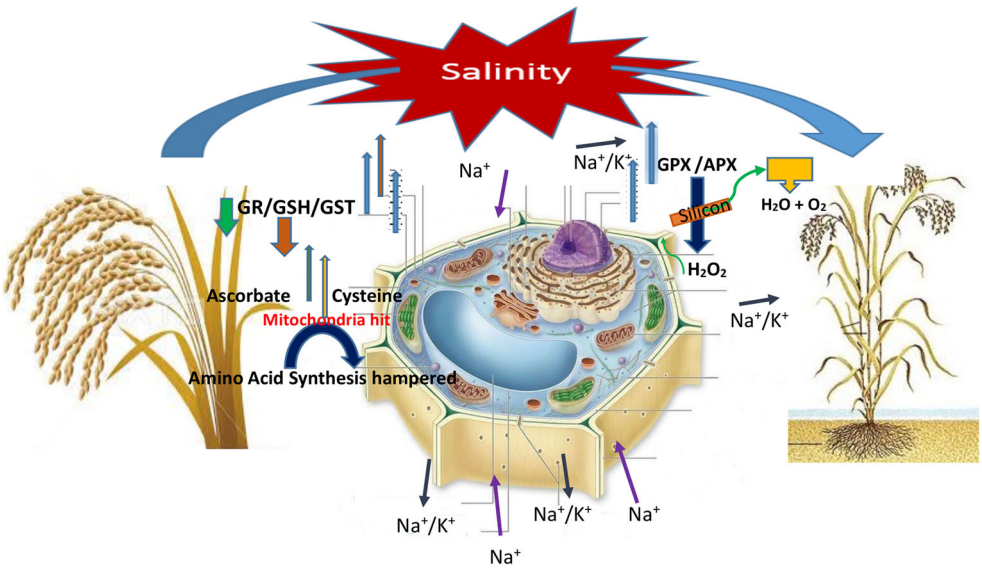

Fig. 10 Schematic representation of silicon-induced alteration of AsA-GSH cycle in rice seedlings

as well as non-enzymatic anti-oxidation cascade. Decrease of or insufficient activity of the antioxidative system under salinity indicates sensitivity of the particular variety towards salt stress. From our studies, it is clear that under salt stress, cv. Nonabokra undeniably proved its potential to tolerate salinity-induced oxidative stress with its efficient upregulation of the antioxidant machinery, specifically the ascorbate-glutathione cycle. On the other hand, cv. MTU 1010 was evidently more sensitive under similar conditions showing less efficient strategies related to the ascorbate-glutathione cycle. Moreover, exogenous application of silicon was proved to be its beneficial for both the varieties by inducing enzymatic as well as non-enzymatic anti-oxidation cascade. This apparently proves the plausibility of utilizing Si to reduce the accumulation of ROS and ameliorating the inevitable damage of salinity to rice plant at cellular levels (Fig. 10). Studies conducted in laboratory, inducing a short-term but strong salt stress condition by using various doses on NaCl, enable a larger number of cultivars to be screened within a limited period of time, which helps to reduce time as well as develop a more cost-effective alternative to expensive field trials. Nevertheless, a short time NaCl exposure under laboratory conditions to rice plants may not decipher the actual scenario in reality for the plants which were subjected to long-term salt stress in field condition, and the composite effects of various metals in field conditions must be satisfied as well. Still, our study on the comparison of physiological responses of antioxidation pathway between two rice cultivars under salt stress would help in deciphering the underlying mechanisms of salt tolerance in rice. Although salt tolerance nature of a genotype is not always correlated to its capacity of detoxifying ROS, still many comparative studies based on salt-sensitive and salt-tolerant genotypes have revealed a positive relation between elevation of antioxidant enzymes activities and salt tolerance (Sekmen et al. 2007). This work may eventually help us in setting selection criteria for rice varieties under salinity or other abiotic factors which induce oxidative stress. More research is needed on the long-term effect of silicon on salt-stressed rice plants before making it as a large-scale agricultural benefit. We intend to do such research in field condition in the near future.

Conclusion

The effect of silicon on antioxidant machinery, osmoprotectants or as regulators of physiological process under salinity, was documented previously in other articles but here studied in two contrasting rice cultivars differing in their salt tolerance mechanism. In conclusion, the present study reveals that Si treatment had the desired effect on mitigation of the negative effects of salinity on rice. The sensitive cultivar MTU 1010 was evident as more respondent to exogenous silicon than the tolerant cultivar Nonabokra. It can also be concluded that enhancement of AsA-GSH pathway by exogenous silicon even in the sensitive cultivar is an impressive point, and these results deserve further researches on protective role of silicon on salinity-induced signaling cascades. Since the response of Si-augmented plants in the field condition is different from that in laboratory, extensive field experiments must be carried out before setting any recommendations for farmers.

Acknowledgements The authors would like to acknowledge the facilities provided by the Department of Botany (CAS Phase VI, VII), University of Calcutta, DBT-IPLS facility, University of Calcutta for providing access to confocal microscopy, and DST-FIST for infrastructural support. IM appreciates UGC-GOI for CSIR-NET Fellowship scheme.

Authors' contribution IM and PD contributed equally to the work, they designed the experiments and carried them out, and they also co-wrote the paper and analyzed the results. MB and AKB tediously checked the experimental findings and analyzed the results. The authors have approved of publication, and there is no conflict of interest. All the authors equally approve of publication.

Compliance with ethical standards

Conflict of interest The authors would like to declare that there is no conflict of interest, and no commercial or financial help has there been involved in the study.

References

Agarwal PK, Shukla PS, Gupta K, Jha B (2013) Bioengineering for salinity tolerance in plants: state of the art. Mol Biotechnol 54(1): 102–123. https://doi.org/10.1007/s12033-012-9538-3

Ahmed M, Qadeer U, Ahmed ZI, Hassan FU (2016) Improvement of wheat (Triticum aestivum) drought tolerance by seed priming with silicon. Arch Agron Soil Sci 62(3):299–315. https://doi.org/10.1080/03650340.2015.1048235

Ali M (2013) The Greenhouse Effect. In: The greenhouse effect. Climate change impacts on plant biomass growth. Springer, Dordrecht, p 13–27. https://doi.org/10.1007/978-94-007-5370-9

Amani A.L., Fatma, M El-Demerdash., 2017. The Ameliorative Effects of Silicon on Salt-Stressed Sorghum Seedlings and Its Influence on the Activities of Sucrose Synthase and PEP Carboxylase. J Plant Physiol Pathol 2017, 5:2 doi: https://doi.org/10.4172/2329-955X.1000164

Anonymous (2016) Arsenic in rice and rice products risk assessment report. U.S. food and drug administration. Available at http://www.fda.gov/Food/FoodScienceResearch/RiskSafetyAssessment/default.htm. Date accessed 29 June 2018.

Ando K, Honma M, Chiba S, Tahara S, Mizutani J (1988) Glutathione transferase from Mucor javamicus. Agric Biol Chem 52(1):135–139

Ashraf M, Foolad M (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exp Bot 59(2): 206–216. https://doi.org/10.1016/j.envexpbot.2005.12.006

Berberich T, Sagor GHM, Kusano T (2015) Polyamines in plant stress response. In: Polyamines. Springer, Tokyo, pp 155–168

Blumwald E (2000) Sodium transport and salt tolerance in plants. Curr Opin Cell Biol 12(4):431–434. https://doi.org/10.1016/S0955-0674(00)00112-5

Brigelius-Floh'e RL, Floh'e L (2003) Is there a role of glutathione peroxidases in signaling and differentiation? Biofactors 17(1–4):93–102

Coskun D, Britto DT, Huynh WQ, Kronzucker HJ (2016) The role of silicon in higher plants under salinity and drought stress. Front Plant Sci 7:1072. https://doi.org/10.3389/fpls.2016.01072

Cummins I, Dixon DP, Freitag-Pohl S, Skipsey M, Edwards R (2011) Multiple roles for plant glutathione transferases in xenobiotic detoxification. Drug Metab Rev 43(2):266–280. https://doi.org/10.3109/03602532.2011.552910

Cuong XT, Ullah H, Datta A, Hanh CT (2017) Effects of silicon-based fertilizer on growth, yield and nutrient uptake of rice in tropical zone of Vietnam. Rice Sci 24(5):283–290. https://doi.org/10.1016/j.rsci.2017.06.002

Das P, Seal P, Biswas AK (2016) Regulation of growth, antioxidants and sugar metabolism in rice (Oryza sativa L.) seedlings by NaCl and its reversal by silicon. Am J Plant Sci 7(03):623–638. https://doi.org/10.4236/ajps.2016.73055

Deinlein U, Stephan AB, Horie T, Luo W, Xu G, Schroeder JI (2014) Plant salt-tolerance mechanisms. Trends Plant Sci 19(6):371–379. https://doi.org/10.1016/j.tplants.2014.02.001

Duan L, Dietrich D, Ng CH, Chan PMY, Bhalerao R, Bennett MJ, Dinneny JR (2013) Endodermal ABA signaling promotes lateral root quiescence during salt stress in Arabidopsis seedlings. Plant Cell 25(1):324–341. https://doi.org/10.1105/tpc.112.107227

Epstein E (1994) The anomaly of silicon in plant biology. Proceedings of the National Academy of Sciences 91(1):11–17

Epstein E, Bloom AJ (2005) Inorganic components of plants Mineral nutrition of plants: principles and perspectives, 2nd edn. Sinauer Associates, Inc., Massachusetts, p 44–45

Esfandiari E, Gohari G (2017) Response of ROS-scavenging systems to salinity stress in two different wheat (*Triticum aestivum* L.) cultivars. Not Bot Horti Agrobo 45(1):287–291. https://doi.org/10.15835/nbha45110682

Faisal M, Saquib Q, Alatar AA, Al-Khedhairy AA, Hegazy AK, Musarrat J (2013) Phytotoxic hazards of NiO-nanoparticles in tomato: a study on mechanism of cell death. J Hazard Mater 250:318–332

Folkard A, Marco CSW (2001) Responses of field-grown irrigated rice cultivars to varying levels of floodwater salinity in a semi-arid environment. Field Crops Res 70:127–137. https://doi.org/10.1016/S0378-4290(01)00128-9

Gaitonde MK (1967) A spectrophotometric method for the direct determination of cysteine in the presence of other naturally occurring amino acids. Biochem J 104(2):627–633

Galvan-Ampudia CS, Julkowska MM, Darwish E, Gandullo J, Korver RA, Brunoud G et al (2013) Halotropism is a response of plant roots to avoid a saline environment. Curr Biol 23(20):2044–2050. https://doi.org/10.1016/j.cub.2013.08.042

Geng Y, Wu R, Wee CW, Xie F, Wei X, Chan PMY, Tham C, Duan L, Dinneny JR (2013) A spatio-temporal understanding of growth regulation during the salt stress response in Arabidopsis. Plant Cell 25(6):2132–2154. https://doi.org/10.1105/tpc.113.112896

Genisel M, Erdal S, Kizilkaya M (2015) The mitigating effect of cysteine on growth inhibition in salt-stressed barley seeds is related to its own reducing capacity rather than its effects on antioxidant system. Plant Growth Regul 75(1):187–197

Gharsallah C, Fakhfakh H, Grubb D, Gorsane F (2016) Effect of salt stress on ion concentration, proline content, antioxidant enzyme activities and gene expression in tomato cultivars. AOB Plants 8: plw055. https://doi.org/10.1093/aobpla/plw055

Ghosh N, Adak MK, Ghosh PD, Gupta S, Gupta DS, Mandal C (2011) Differential responses of two rice varieties to salt stress. Plant Biotechnol Rep 5(1):89–103. https://doi.org/10.1007/s11816-010-0163-y

Gong HJ, Zhu XY, Chen KM, Wang SM, Zhang CL (2005) Silicon alleviates oxidative damage of wheat plants in pots under drought. Plant Sci 169:313–321. https://doi.org/10.1016/j.plantsci.2005.02.023

Gupta RK, Abrol IP (1990) Salt-affected soils: their reclamation and management for crop production. In: Adv Soil Sci. Springer, New York, p 223–288. doi: https://doi.org/10.1007/978-1-4612-3322-0_7

Haghighi M, Pessarakli M (2013) Influence of silicon and nano-silicon on salinity tolerance of cherry tomatoes (Solanum lycopersicum L.) at early growth stage. Sci Hortic 161:111–117. https://doi.org/10.1016/j.scienta.2013.06.034

Harmon AC, Gribskov M, Harper JF (2000) CDPKs—a kinase for every Ca2+ signal? Trends Plant Sci 5(4):154–159. https://doi.org/10.1016/S1360-1385(00)01577-6

Hasanuzzaman M, Hossain MA, Fujita M (2011) Nitric oxide modulates antioxidant defense and the methylglyoxal detoxification system and reduces salinity-induced damage of wheat seedlings. Plant Biotechnol Rep 5(4):353–365. https://doi.org/10.1007/s11816-011-0189-9

Hasanuzzaman M, Hossain MA, da Silva JAT, Fujita M, (2012) Plant response and tolerance to abiotic oxidative stress: antioxidant defense is a key factor. In: Crop stress and its management: perspectives and strategies. Springer, Dordrecht, p 261–315. doi.https://doi.org/10.1007/978-94-007-2220-0_8

Hossain MT, Mori R, Soga K, Wakabayashi K, Kamisaka S, Fujii S et al (2002) Growth promotion and an increase in cell wall extensibility by silicon in rice and some other Poaceae seedlings. J Plant Res 115(1):0023–0027. https://doi.org/10.1007/s102650200004 https://www.fda.gov/downloads/Food/FoodScienceResearch/RiskSafetyAssessment/UCM486543.pdf

Ibrahim EA (2016) Seed priming to alleviate salinity stress in germinating seeds. J Plant Physiol 192:38–46. https://doi.org/10.1016/j.jplph.2015.12.011

Kamencic H, Lyon A, Paterson PG, Juurlink BH (2000) Monochlorobimane fluorometric method to measure tissue glutathione. Anal Biochem 286(1):35–37. https://doi.org/10.1006/abio.2000.4765

Karan R, Subudhi PK (2012) A stress inducible SUMO conjugating enzyme gene of a grass halophyte *Spartina alterniflora* (SaSce9) enhances salinity and drought stress tolerance in *Arabidopsis*. BMC Plant Biol 12:187. https://doi.org/10.1186/1471-2229-12-187

Kaur N, Dhawan M, Sharma I, Pati PK (2016) Interdependency of reactive oxygen species generating and scavenging system in salt sensitive and salt tolerant cultivars of rice. BMC Plant Biol 16(1):131. https://doi.org/10.1186/s12870-016-0824-2.

Khoshgoftarmanesh AM, Khodarahmi S, Haghighi M (2014) Effect of silicon nutrition on lipid peroxidation and antioxidant response of cucumber plants exposed to salinity stress. Arch Agron Soil Sci 60:639–653. https://doi.org/10.1080/03650340.2013.822487

Li H, Zhu Y, Hu Y, Han W, Gong H (2015) Beneficial effects of silicon in alleviating salinity stress of tomato seedlings grown under sand culture. Acta Physiol Plant 37:71–80. https://doi.org/10.1007/s11738-015-1818-7

Liang Y, Sun W, Zhu YG, Christie P (2007) Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: a review. Environ Pollut 147(2):422–428. https://doi.org/10.1016/j.envpol.2006.06.008

Liu P, Yin L, Wang S, Zhang M, Deng X, Zhang S, Tanaka K (2015) Enhanced root hydraulic conductance by aquaporin regulation accounts for silicon alleviated salt-induced osmotic stress in Sorghum bicolor L. Environ Exp Bot 111:42–51. https://doi.org/10.1016/j.envexpbot.2014.10.006

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193(1):265–275

Lutts S, Kinet JM, Bouharmont J (1995) Changes in plant response to NaCl during development of rice (*Oryza sativa* L.) varieties

differing in salinity resistance. J Exp Bot 46:1843–1852. https://doi.org/10.1093/jxb/46.12.1843

Ma JF, Yamaji N (2006) Silicon uptake and accumulation in higher plants. Trends Plant Sci 11(8):392–397. https://doi.org/10.1016/j.tplants.2006.06.007

Manna I, Bandyopadhyay M (2017) Engineered nickel oxide nanoparticle causes substantial physicochemical perturbation in plants. Front Chem 5:92. https://doi.org/10.3389/fchem.2017.00092

Mäser P, Eckelman B, Vaidyanathan R, Horie T, Fairbairn DJ, Kubo M et al (2002) Altered shoot/root Na+ distribution and bifurcating salt sensitivity in Arabidopsis by genetic disruption of the Na+ transporter AtHKT1. FEBS Lett 531(2):157–161. https://doi.org/10.1016/S0014-5793(02)03488-9

Maurel C, Santoni V, Luu DT, Wudick MM, Verdoucq L (2009) The cellular dynamics of plant aquaporin expression and functions. Curr Opin Plant 12(6):690–698. https://doi.org/10.1016/j.pbi.2009.09.002biology

Maurel C, Boursiac Y, Luu DT, Santoni V, Shahzad Z, Verdoucq L (2015) Aquaporins in plants. Physiol Rev 95(4):1321–1358. https://doi.org/10.1152/physrev.00008.2015

Mbarki S, Sytar O, Cerda A, Zivcak M, Rastogi A, He X, Zoghlami A, Abdelly C, Brestic M (2018) Strategies to mitigate the salt stress effects on photosynthetic apparatus and productivity of crop plants. In: Salinity responses and tolerance in plants, vol 1. Springer, Cham, p 85–136

Meharg C, Meharg AA (2015) Silicon, the silver bullet for mitigating biotic and abiotic stress, and improving grain quality, in rice? Environ Exp Bot 120:8–17. https://doi.org/10.1016/j.envexpbot.2015.07.001

Mukherjee SP, Choudhuri MA (1983) Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in Vigna seedlings. Physiol Plant 58(2):166–170

Muller M, Tausz M, Guttenberger H, Grill D (1999) Histochemical localization of glutathione in plant tissues: a comparison of two fluorescence staining methods. PHYTON-HORN 39(3):69–74

Munns R, Gilliham M (2015) Salinity tolerance of crops—what is the cost? New Phytol 208:668–673. https://doi.org/10.1111/nph.13519

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681

Murgia I, Tarantino D, Vannini C, Bracale M, Carravieri S, Soave C (2004) Arabidopsis thaliana plants overexpressing thylakoidal ascorbate peroxidase show increased resistance to Paraquat-induced photooxidative stress and to nitric oxide-induced cell death. Plant J 38(6):940–953. https://doi.org/10.1111/j.1365-313X.2004.02092.x

Murtaza G, Ghafoor A, Owens G, Qadir M, Kahlon UZ (2009) Environmental and economic benefits of saline-sodic soil reclamation using low-quality water and soil amendments in conjunction with a rice–wheat cropping system. J Agron Crop Sci 195(2):124–136. https://doi.org/10.1111/j.1439-037X.2008.00350.x

Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22(5):867–880

Noctor G, Foyer CH (1998) Ascorbate and glutathione: keeping active oxygen under control. Annu Rev Plant Physiol Plant Mol Biol 49:249–279. https://doi.org/10.1146/annurev.arplant.49.1.249

Noctor G, Gomez L, Vanacker H, Foyer CH (2002) Interactions between biosynthesis, compartmentation and transport in the control of glutathione homeostasis and signalling. J Exp Bot 53(372):1283–1304. https://doi.org/10.1093/jexbot/53.372.1283

Noctor G, Mhamdi A, Chaouch S, Han Y, Neukermans J, MárquezGarcía B, Queval G, Foyer CH (2012) Glutathione in plants: an integrated overview. Plant Cell Environ 35:454–484. https://doi.org/10.1111/j.1365-3040.2011.02400.x

Nxele X, Klein A, Ndimba BK (2017) Drought and salinity stress alters ROS accumulation, water retention, and osmolyte content in sorghum plants. S Afr J Bot 108:261–266. https://doi.org/10.1016/j.sajb.2016.11.003

Parida AK, Das AB, Mittra B (2004) Effects of salt on growth, ion accumulation, photosynthesis and leaf anatomy of the mangrove, Bruguiera parviflora. Trees 18(2):167–174. https://doi.org/10.1007/s00468-003-0293-8

Pyngrope S, Bhoomika K, Dubey RS (2013) Reactive oxygen species, ascorbate-glutathione pool, and enzymes of their metabolism in drought-sensitive and tolerant indica rice (*Oryza sativa* L.) seedlings subjected to progressing levels of water deficit. Protoplasma 250:585–600. https://doi.org/10.1007/s00709-012-0444-0

Rafi M.M., Epstein, E., 1999. Silicon absorption by wheat (*Triticum aestivum L.*). Plant Soil; 211, 223–230. doi: https://doi.org/10.1023/A:1004600611582.

Ranieri A, Castagna A, Baldan B, Soldatini GF (2001) Iron deficiency differently affects peroxidase isoforms in sunflower. J Exp Bot 52(354):25–35

Rasool S, Ahmad A, Siddiqi TO, Ahmad P (2013) Changes in growth, lipid peroxidation and some key antioxidant enzymes in chickpea genotypes under salt stress. Acta Physiol Plant 35:1039–1050. https://doi.org/10.1007/s11738-012-1142-4

Reddy INBL, Kim BK, Yoon IS, Kim KH, Kwon TR (2017) Salt tolerance in rice: focus on mechanisms and approaches. Rice Sci 24(3):123–144. https://doi.org/10.1016/j.rsci.2016.09.004

Rios JJ, Martínez-Ballesta MC, Ruiz JM, Blasco B, Carvajal M (2017) Silicon-mediated improvement in plant salinity tolerance: the role of aquaporins. Front Plant Sci 8(948). https://doi.org/10.3389/fpls.2017.00948

Romero-Aranda MR, Jurado O, Cuartero J (2006) Silicon alleviates the deleterious salt effect on tomato plant growth by improving plant water status. J Plant Physiol 163(8):847–855. https://doi.org/10.1016/j.jplph.2005.05.010

Roy M, Wu R (2002) Over-expression of S-adenosylmethionine decarboxylase gene in rice increased polyamine level and enhances NaCl stress tolerance. Plant Sci 163:987–992. https://doi.org/10.1016/S0168-9452(02)00272-8

Saha P, Mukherjee A, Biswas AK (2015) Modulation of NaCl induced DNA damage and oxidative stress in mungbean by pretreatment with sublethal dose. Biol Plant 59(1):139–146. https://doi.org/10.1007/s10535-014-0460-3

Salwa AO, Mona GD, Salman SR (2015) Comparative study between the physiological role of hydrogen peroxide and salicylic acid in alleviating the harmful effect of low temperature on tomato plants grown under sandponic culture. Sci Agric 9:49–59. https://doi.org/10.15192/PSCP.SA.2015.1.9.4959

Schroeder JI, Delhaize E, Frommer WB, Guerinot ML, Harrison MJ, Herrera-Estrella L et al (2013) Using membrane transporters to improve crops for sustainable food production. Nature 497(7447):60–66. https://doi.org/10.1038/nature11909

Sedlak J, Lindsay RH (1968) Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 25:192–205

Sekmen AH, Turkan I, Takio S (2007) Differential responses of antioxidative enzymes and lipid peroxidation to salt stress in salt-tolerant *Plantago maritima* and salt-sensitive *Plantago media*. Plant Physiol 131:399–411. https://doi.org/10.1111/j.1399-3054.2007.00970.x

Sharma P, Dubey RS (2004) Ascorbate peroxidase from rice seedlings: properties of enzyme isoforms, effects of stresses and protective roles of osmolytes. Plant Sci 167:541–550. https://doi.org/10.1016/j.plantsci.2004.04.028

Shi Y, Zhang Y, Han W, Feng R, Hu Y, Guo J, Gong H (2016) Silicon enhances water stress tolerance by improving root hydraulic conductance in Solanum lycopersicum L. Front Plant Sci 22:196–215. https://doi.org/10.3389/fpls.2016.00196

Singh VP, Tripathi DK, Kumar D, Chauhan DK (2011) Influence of exogenous silicon addition on aluminium tolerance in rice seedlings. Biol Trace Elem Res 144(1–3):1260–1274. https://doi.org/10.1007/s12011-011-9118-6

Smith IK, Vierheller TL, Thorne CA (1988) Assay of glutathione reductase in crude tissue homogenates using 5, 5′-dithiobis (2-nitrobenzoic acid). Anal Biochem 175(2):408–413

Sun ZW, Ren LK, Fan JW, Li Q, Wang KJ, Guo MM, Wang L, Li J, Zhang GX, Yang ZY, Chen F (2016) Salt response of photosynthetic electron transport system in wheat cultivars with contrasting tolerance. Plant Soil Environ 62:515–521. https://doi.org/10.17221/529/2016-PSE

Szabados L, Savoure A (2010) Proline: a multifunctional amino acid. Trends Plant Sci 15(2):89–97. https://doi.org/10.1016/j.tplants.2009.11.009

Tang X, Mu X, Shao H, Wang H, Brestic M (2015) Global plant-responding mechanisms to salt stress: physiological and molecular levels and implications in biotechnology. Crit Rev Biotechnol 35(4):425–437. https://doi.org/10.3109/07388551.2014.889080

Tausz M, Sircelj H, Grill D (2004) The glutathione system as a stress marker in plant ecophysiology: is a stress response concept valid? J Exp Bot 55:1855–1862. https://doi.org/10.1093/jxb/erh194

Tester, M., & Davenport, R. (2003). Na+ tolerance and Na+ transport in higher plants. Ann Bot, 91(5), 503–527. https://doi.org/10.1093/aob/mcg058g

Tripathi DK, Singh VP, Prasad SM, Chauhan DK, Dubey NK (2015a) Silicon nanoparticles (SiNp) alleviate chromium (VI) phytotoxicity in Pisum sativum (L.) seedlings. Plant Physiol Biochem 96:189–198. https://doi.org/10.1016/j.plaphy.2015.07.026

Tripathi DK, Singh VP, Prasad SM, Chauhan DK, Dubey NK, Rai AK (2015b) Silicon-mediated alleviation of Cr (VI) toxicity in wheat seedlings as evidenced by chlorophyll florescence, laser induced breakdown spectroscopy and anatomical changes. Ecotoxicol Environ Saf 113:133–144. https://doi.org/10.1016/j.ecoenv.2014.09.029

Tripathi DK, Singh S, Singh VP, Prasad SM, Chauhan DK, Dubey NK (2016a) Silicon nanoparticles more efficiently alleviate arsenate toxicity than silicon in maize cultiver and hybrid differing in arsenate tolerance. Front Environ Sci 4(46). https://doi.org/10.3389/fenvs.2016.00046

Tripathi DK, Singh VP, Prasad SM, Dubey NK, Chauhan DK, Rai AK (2016b) LIB spectroscopic and biochemical analysis to characterize lead toxicity alleviative nature of silicon in wheat (Triticum aestivum L.) seedlings. J Photochem Photobiol B Biol 154:89–98. https://doi.org/10.1016/j.jphotobiol.2015.11.008

Ullah H, Datta A, Shrestha S, Ud Din S (2017) The effects of cultivation methods and water regimes on root systems of drought tolerant (RD6) and drought-sensitive (RD10) rice varieties of Thailand. Arch Agron Soil Sci 63(9):1198–1209. https://doi.org/10.1080/03650340.2016.1266077

Van Bockhaven J, De Vleesschauwer D, Höfte M (2012) Towards establishing broad-spectrum disease resistance in plants: silicon leads the way. J Exp Bot 64(5):1281–1293. https://doi.org/10.1093/jxb/ers329

Verbruggen N, Hermans C (2008) Proline accumulation in plants: a review. Amino Acids 35(4):753–759. https://doi.org/10.1007/s00726-008-0061-6

Verslues PE, Agarwal M, Katiyar-Agarwal S, Zhu J, Zhu JK (2006) Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. Plant J 45(4):523–539. https://doi.org/10.1111/j.1365-313X.2005.02593.x

Wang S, Liu P, Chen D, Yin L, Li H, Deng X (2015) Silicon enhanced salt tolerance by improving the root water uptake and decreasing the ion toxicity in cucumber. Front Plant Sci 6:759. https://doi.org/10.3389/fpls.2015.00759

Yan K, Shao H, Shao C, Chen P, Zhao S, Brestic M, Chen X (2013) Physiological adaptive mechanisms of plants grown in saline soil and implications for sustainable saline agriculture in coastal zone. Acta Physiol Plant 35(10):2867–2878. https://doi.org/10.1007/s11738-013-1325-7

Zhang D, Tong J, He X, Xu Z, Xu L, Wei P, Huang Y, Brestic M, Ma H, Shao H (2016) A novel soybean intrinsic protein gene, GmTIP2; 3, involved in responding to osmotic stress. Front Plant Sci 6:1237. https://doi.org/10.3389/fpls.2015.01237

Zhu Z, Wei G, Li J, Qian Q, Yu J (2004) Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (Cucumis sativus L.). Plant Sci 167(3):527–533. https://doi.org/10.1016/j.plantsci.2004.04.020

Zhu M, Zhou M, Shabala L, Shabala S (2017) Physiological and molecular mechanisms mediating xylem Na^+ loading in barley in the context of salinity stress tolerance. Plant Cell Environ 40:1009–1020. https://doi.org/10.1111/pce.12727