Gesunde Pflanzen (2018) 70:185–194
https://doi.org/10.1007/s10343-018-0430-3

ORIGINAL ARTICLE

Exogenous Silicon Protects *Brassica napus* Plants from Salinity-Induced Oxidative Stress Through the Modulation of AsA-GSH Pathway, Thiol-Dependent Antioxidant Enzymes and Glyoxalase Systems

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Received: 13 April 2018 / Accepted: 16 July 2018 / Published online: 21 August 2018

Abstract

Although silicon (Si) has showed its potential role in mitigating abiotic stress-induced damages in many plant species its role in coordinated induction of antioxidant defense is yet to be elucidated. Therefore, we studied rapeseed (*Brassica napus*) seedlings applied with exogenous Si for changes occurring in antioxidant defense and glyoxalase systems. Seedlings (12-day-old) grown semi-hydroponically were exposed to Si (silicon dioxide, SiO_2; 1 mM) solely and in combination with NaCl (100 and 200 mM) for 48 h. Salinity created oxidative damage by increasing H_2O_2 and malondialdehyde (MDA) contents resulting in disruption of antioxidant defense system and in arousing methylglyoxal (MG) toxicity by the down-regulation of glyoxalase enzyme activities. Exogenous Si treatment showed reduction of both H_2O_2 and MDA contents and up-regulation of antioxidant components including the activities of related enzymes (APX, MDHAR, DHAR, GR, GST, GPX and CAT) and the contents of AsA and GSH. Enhanced activities of glyoxalase I (Gly I) and glyoxalase II (Gly II) detoxified the toxic MG. Thus, this study clearly indicates that Si improved plant tolerance to salinity stress through enhancement of both antioxidant defense and glyoxalase systems that led to reduced oxidative damage and MG toxicity.

Keywords Ionic toxicity · Osmotic stress · Plant nutrients · Reactive oxygen species · Salinity · Trace element

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Exogenes Silizium schützt *Brassica-napus*-Pflanzen vor salzinduziertem oxidativen Stress durch die Modulation des AsA-GSH-Zyklus, thiolabhängiger antioxidativer Enzyme und der Glyoxalase-Systeme

Zusammenfassung

Obwohl Silizium (Si) seine potenzielle Rolle bei der Abschwächung abiotischer stressinduzierter Schäden bei vielen Pflanzenarten gezeigt hat, ist seine Rolle bei der koordinierten Induktion der antioxidativen Abwehr noch zu klären. Daher untersuchten wir Rapskeimlinge (*Brassica napus*), die mit exogenem Si behandelt wurden, auf Veränderungen in der antioxidativen Abwehr und der Glyoxalase-Systeme. Keimlinge (12 Tage alt), die semi-hydroponisch gezüchtet wurden, wurden 48 h Si (Siliciumdioxid, SiO_2; 1 mM) ausgesetzt – allein und in Kombination mit NaCl (100 und 200 mM). Die Salinität verursachte oxidative Schäden durch die Erhöhung des H_2O_2- und Malondialdehyd (MDA)-Gehalts, was zu einer Störung des antioxidativen Abwehrsystems und zur Stimulation der Methylglyoxal (MG)-Toxizität durch die Herunterregulierung der Glyoxalase-Enzymaktivitäten führte. Die exogene Si-Behandlung zeigte eine Reduktion der H_2O_2- und der MDA-Gehalte sowie eine Hochregulierung der antioxidativen Komponenten einschließlich der Aktivitäten verwandter Enzyme (APX, MDHAR, DHAR, GR, GST, GPX und CAT) und der AsA- und GSH-Gehalte. Verstärkte Aktivitäten von Glyoxalase I (Gly I) und Glyoxalase II (Gly II) entgifteten das toxische MG. Somit zeigt diese Studie deutlich, dass Si die Toleranz von Pflanzen gegenüber Salzstress durch die Steigerung der antioxidativen Abwehr und der Aktivität der Glyoxalase-Systeme verbesserte, was zu einer verringerten oxidativen Schädigung und MG-Toxizität führte.

Schlüsselwörter Ionische Toxizität · Osmotischer Stress · Pflanzennährstoffe · Reaktive Sauerstoffspezies · Salzgehalt · Spurenelement

Introduction

Abiotic stress has become the utmost threat for crop production in this age of climate change. Salinity, being a dreadful form of abiotic stress, has shown multidimensional effects on crop life including damaged growth and reduced yield. Initially, salinity affects the vascular transportation of solutes and electrochemical gradients which leads to osmotic imbalance (Manivannan et al. 2016) and also creates ionic imbalance and toxicity (Rahman et al. 2016). Higher amount of ions like Na^+ and Cl^- produced due to salt stress adds another dimension of injury to plant survival (Hasanuzzaman et al. 2012; Hussain et al. 2013; Assaha et al. 2015). The osmotic and ionic imbalance secondarily cause oxidative stress in plants which eventually results in detrimental effects on plant morphological, physiological and/or biochemical attributes (Mahmood et al. 2016).

Singlet oxygen ($^1O_2$), superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^\bullet$) etc. are some of the reactive oxygen species (ROS) generated under as a response to saline condition. These ROS are considered responsible for the oxidation of lipids, amino acids and nucleic acids, damages at cellular level and even programmed cell death (Mahmud et al. 2017). To mitigate these injurious effects of ROS, an efficient antioxidant defense system is developed within plant cells (Apel and Hirt 2004). The components of this defense system act simultaneously to scavenge and/or detoxify these ROS and help to save plants from the damages caused by oxidative stress (Hasanuzzaman et al. 2014a). Methylglyoxal (MG), another compound produced under abiotic stress conditions including salinity, has been reported to be highly reactive and cytotoxic when produced in a higher amount (Yadav et al. 2008). Two thiol-dependent enzymes i.e. glyoxalase I (Gly I) and glyoxalase II (Gly II) form a well-organized glyoxalase system which detoxifies this toxic MG by some reactions in order (Rahman et al. 2016; Hasanuzzaman et al. 2017a, 2017b). Coordinated action and up-regulation of both the antioxidant defense and glyoxalase system components are needed to attain significant tolerance in plants against the oxidative stress and this was supported by several research findings (Nahar et al. 2015; Rahman et al. 2016; Mahmud et al. 2017).

Silicon (Si) ranks second in abundance within the earth crust and mostly exists as silica (SiO_2) or silicate (SiO_4^{4-}) as it shows strong affinity to oxygen (O_2) (Hasanuzzaman et al. 2014b). Silicon has certain potential to improve plant growth and development under adverse conditions, yet it is considered as nonessential for plant production (Shahzad et al. 2017). Si-induced reduction of ROS in plants is the crucial effect of exogenous Si against abiotic stress which has been demonstrated by several lines of study (Hashemi et al. 2010; Hasanuzzaman et al. 2017c). In addition, decreased electrolytic leakage, malondialdehyde (MDA) content and toxic ions (e.g., Na^+) accumulation was observed when Si applied exogenously under salt stress condition (Kim et al. 2017). Si-induced modifications in both apoplastic and symplastic components were also studied to evaluate the prospects to develop salinity tolerance in plants (Coskun et al. 2016). A number of experiments reported that the exogenous Si application mediated regulation of antioxidant defense system components in different crops

(Torabi et al. 2015; Manivannan et al. 2016). However, Si-induced coordinated induction of antioxidant defense and other related systems have hardly been studied. Our hypothesis was that the Si-mediated activation of glutathione (GSH) synthesis may have played an important role in the MG detoxification process by the up-regulation of Gly I and Gly II activities. Based on the above discussed notions, we designed this experiment to investigate the effect of Si applied exogenously on *Brassica napus* seedlings influencing the contents of non-enzymatic and enzymatic components of the antioxidant defense and glyoxalase systems under salinity condition.

Materials and Methods

Plant Materials and Stress Treatments

Uniform rapeseed (*Brassica napus* L. cv. BINA sharisha 3) seeds were washed for several times to obtain clean seeds. Six layers of filter papers were lined on Petri plates (9 cm) which were used for sowing seeds. Initially 60 seeds were sown from where 40 seedlings were kept for further analysis. Seedlings were kept in growth chambers maintaining controlled conditions with 100 µmol photon $m^{-2} s^{-1}$ light, 25 ± 2 °C temp and 65–70% RH. The growing medium of seeds was semi-hydroponic containing Hyponex solution (10,000-fold diluted) which was added when necessary. After 12 days of sowing, silicon dioxide, SiO_2 (1 mM) and NaCl (100 and 200 mM) were added in the solution; alone or in combination. The first leaves were harvested and used for quantifying different parameters after 48 h of treatment exposure. Only Hyponex solution was used for control plants to grow in. The experiment was laid out following completely randomized design (CRD) and the same experiments were replicated for three times.

Measurement of H_2O_2 Content

The procedure used by Yu et al. (2003) was followed to estimate the levels of H_2O_2. After homogenizing and extracting the harvested leaves in K-P buffer (50 mM, pH 6.5), the supernatant was mixed with $TiCl_4$ (0.1%) in H_2SO_4 (20%). This solution was allowed to set for 10 min in normal temperature and centrifuged again before reading the absorbance at 410 nm. The unit used for expressing the result is nmol $H_2O_2\,g^{-1}$ fresh weight.

Measurement of MDA Content

The method of Heath and Packer (1968) was followed to determine the MDA content (an indicator of lipid peroxidation). Trichloroacetic acid (TCA) (5%) was used as extraction buffer and 0.5% thiobarbituric acid (TBA) (in 20% TCA) was used as reaction mixture. After the extraction and occurrence of reaction, the solution was exposed to boiling at 95 °C temp. It was boiled for 30 min and then cooled on ice, before reading the absorbance at both 532 and 600 nm. The later absorbance was deducted from the former one to attain the precision of non-specific turbidity. The unit used for expressing the result is nmol MDA g^{-1} fresh weight.

Determination of AsA and GSH

Ascorbate (AsA) and GSH contents were measured by homogenizing 0.5 g fresh rapeseed leaves in 3 ml of metaphosphoric acid solution (5%) containing ethylenediaminetetraacetic acid (EDTA; 1 mM). After centrifuging this supernatant at 11,500 × g (for 15 min) the AsA content was estimated spectrophotometrically at 265 nm using different reacting solutions (Huang et al. 2005). Neutralization of supernatant was done with potassium-phosphate (K-P) buffer (0.5 M, pH 7.0), and then assayed with ascorbate oxidase (AO; 0.5 unit) in K-P buffer (100 mM, pH 7.0). The content of GSH pool was quantified at 412 nm following the procedure described by Yu et al. (2003) with some changes (Paradiso et al. 2008). Neutralization of supernatant with K-P buffer (0.5 M, pH 7.0) was done, and then mixed with 5,5′-dithio-bis 2-nitrobenzoic acid (DTNB), nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione reductase (GR) and then assayed for total GSH content. Oxidized glutathione (GSSG) content was measured by the reaction of 2-vinylpyridine to remove the GSH. Final calculation of AsA, GSH and GSSG was done with the help of standard curves plotted from concentrations of AsA, GSH and GSSG, which were known. The content of GSSG was deducted from total GSH content, and the result was considered as the reduced GSH content.

Enzyme Extraction

Rapeseed leaves (0.5 g fresh weight) were homogenized, extracted and centrifuged according to Bradford (1976) and accordingly bovine serum albumin (BSA) was used as a protein standard for the determination of protein content. This supernatant was also used for the enzyme assays (Ascorbate peroxidase, APX; monodehydroascorbate reductase, MDHAR; dehydroascorbate reductase, DHAR; GR; glutathione peroxidase, GPX; glutathione *S*-transferase, GST and catalase, CAT).

Enzyme Assay

The activity of APX was quantified following Nakano and Asada (1981) explained method using K-P buffer (pH 7.0)

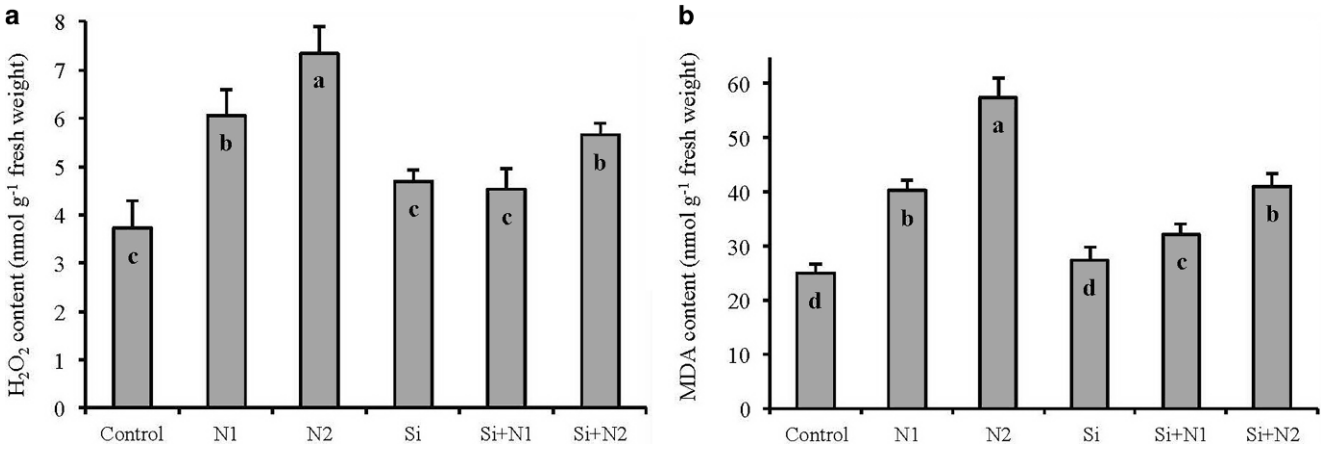

Fig. 1 H_2O_2 (**a**) and MDA (**b**) contents in rapeseed leaves induced by Si under salt stress. N_1, N_2, Si, Si+N_1 and Si+N_2 indicate 100 mM NaCl, 200 mM NaCl, 1 mM SiO_2, 100 mM NaCl+SiO_2, 200 mM NaCl+SiO_2, respectively. Values sharing the same letter do not differ significantly at $P \leq 0.05$

and AsA, at a concentration of 50 and 0.5 mM, respectively along with H_2O_2 and EDTA of 0.1 mM concentration.

The activity of MDHAR was measured following Hossain et al. (1984) explained procedure using Tris–HCl buffer (pH 7.5), NADPH and AsA at a concentration of 50 mM, 0.2 mM and 2.5 mM, respectively along with AO of 0.5 unit.

The activity of DHAR was estimated following Nakano and Asada (1981) described method using K-P buffer (pH 7.0), GSH and dehydroascorbate (DHA) at a concentration of 50 mM, 2.5 mM and 0.1 mM, respectively.

The activity of GR was determined following Cakmak et al. (1993) described procedure using K-P buffer (pH 7.8) and NADPH at a concentration of 0.1 M and 0.2 mM, respectively along with EDTA and GSSG of 1.0 mM concentration.

The activity of GST was estimated following Booth et al. (1961) described procedure with inputs from Hossain et al. (2009) using Tris–HCl buffer (pH 6.5), GSH and 1-chloro-2,4-dinitrobenzene (CDNB) at a concentration of 100 mM, 1.5 mM and 1.0 mM, respectively.

The activity of GPX was quantified following the procedure mentioned in Elia et al. (2003) using EDTA and NaN_3 of 1 mM concentration, NADPH, GSH and H_2O_2 at a concentration of 0.12 mM, 2.0 mM and 0.6 mM, respectively and single unit of GR. All of these were mixed in 100 mM of K-P buffer (pH 7.0) as an input from Hasanuzzaman and Fujita (2013) explained method.

The activity of CAT was determined following the method mentioned in Hossain et al. (2009) using K-P buffer (pH 7.0) and H_2O_2 at a concentration of 50 and 15 mM, respectively.

Statistical Analysis

One-way analysis of variance (ANOVA) was performed. Mean differences were compared and tested by Fisher's Least Significant Difference (LSD) using CoStat software (CoHort Software, Monterey, CA) from three replications. Differences at $P \leq 0.05$ were considered significant.

Results

Accumulation of ROS and Oxidative Damage

Results revealed that salt stress increased the H_2O_2 content in salt stressed rapeseed plants. Lipid peroxidation level (denoted by MDA content) markedly increased in plants exposed to salt. These are the signs for oxidative damage in rapeseed seedlings caused by salt stress. Gradual rise of H_2O_2 (63 to 98%) and MDA (60 to 129%) levels with the increased dose (100 to 200 mM NaCl) of salinity shows that high salt dose privileged more oxidative damage, compared to control. Silicon addition with salt stress reversed the adverse consequences of salt stress by decreasing levels of both MDA and H_2O_2 (compared to seedlings exposed to salt stress only) (Fig. 1).

AsA and GSH Levels

The vital antioxidant AsA dropped by 17% due to mild salt stress which decreased by 44% under severe salt stress. Co-application of Si with salt treatments arouses the content of AsA, compared to salt treatment alone (Fig. 2a). GSH level was found to increase with salt stress. Its level further up-regulated in Si co-treated salt stresses. The level of GSH increased after supplemental Si

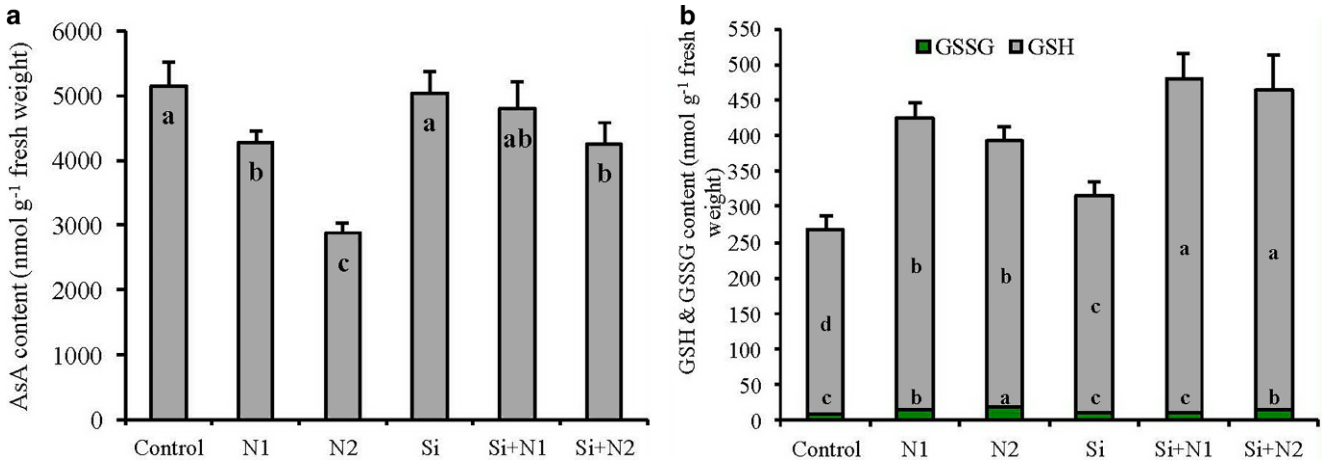

Fig. 2 AsA (**a**), GSH and GSSG (**b**) contents in rapeseed leaves induced by Si under salt stress. N_1, N_2, Si, Si+N_1 and Si+N_2 indicate 100 mM NaCl, 200 mM NaCl, 1 mM SiO_2, 100 mM NaCl+SiO_2, 200 mM NaCl+SiO_2, respectively. Values sharing the same letter do not differ significantly at $P \leq 0.05$

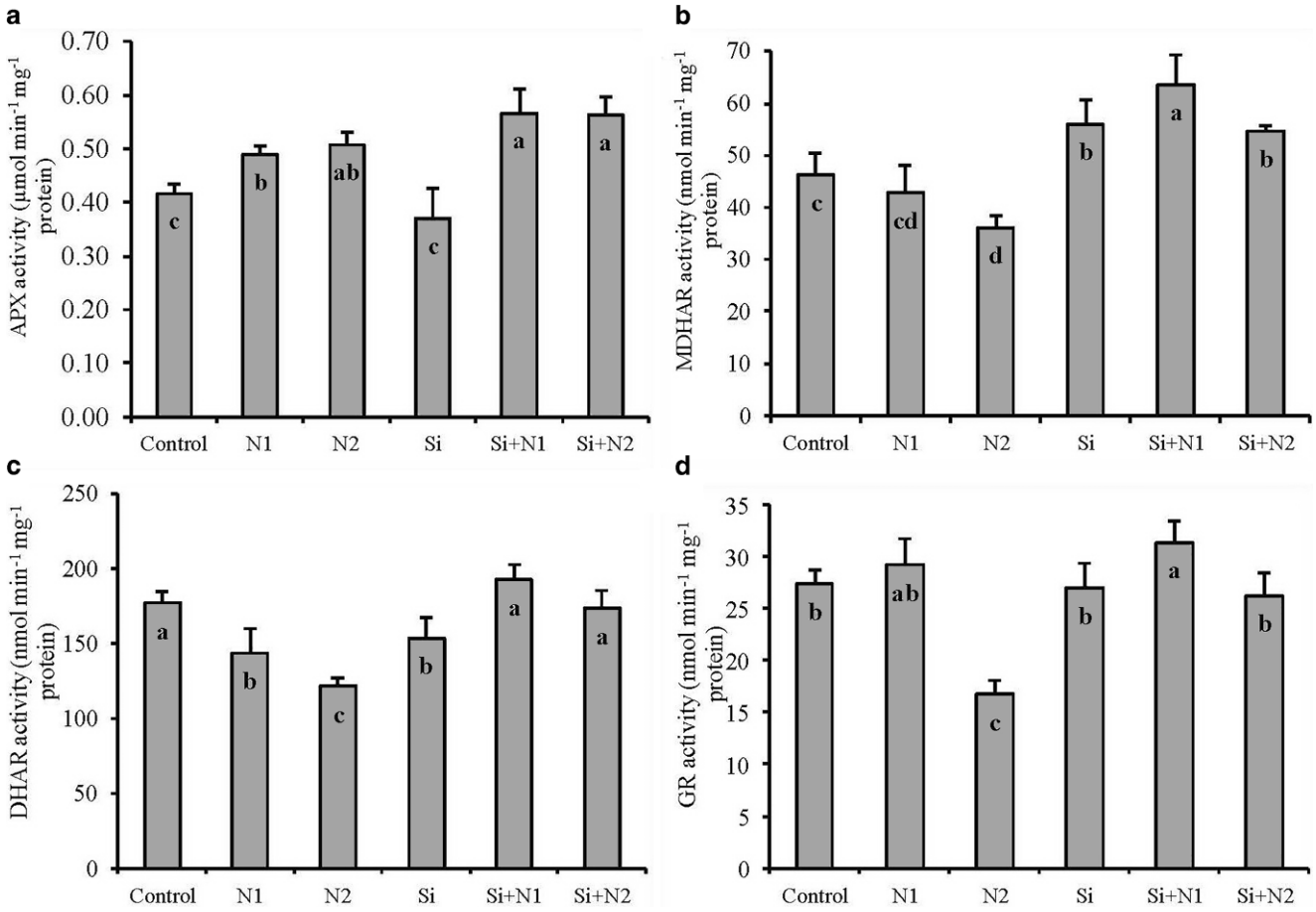

Fig. 3 Activities of APX (**a**), MDHAR (**b**), DHAR (**c**) and GR (**d**) in rapeseed leaves induced by Si under salt stress. N_1, N_2, Si, Si+N_1 and Si+N_2 indicate 100 mM NaCl, 200 mM NaCl, 1 mM SiO_2, 100 mM NaCl+SiO_2, 200 mM NaCl+SiO_2, respectively. Values sharing the same letter do not differ significantly at $P \leq 0.05$

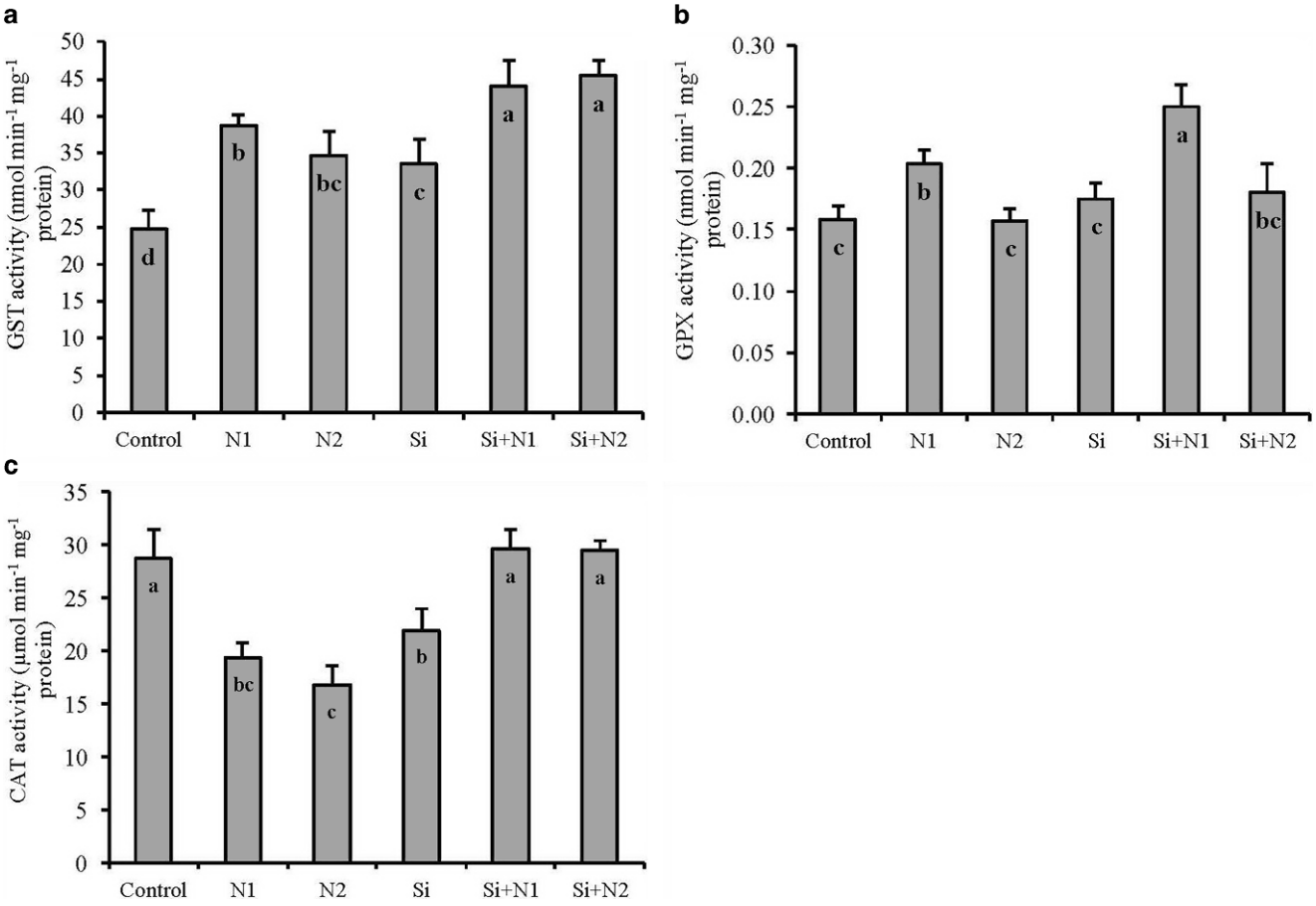

Fig. 4 Activities of GST (**a**), GPX (**b**) and CAT (**c**) in rapeseed leaves induced by Si under salt stress. N_1, N_2, Si, Si+N_1 and Si+N_2 indicate 100 mM NaCl, 200 mM NaCl, 1 mM SiO_2, 100 mM NaCl+SiO_2, 200 mM NaCl+SiO_2, respectively. Values sharing the same letter do not differ significantly at $P \leq 0.05$

in mild and severe salt treated rapeseed seedlings which were 14 and 21% higher, compared to mild and severe salt treated seedlings without Si, respectively. The oxidized form of GSH—GSSG—mounted noticeably in salt stressed seedlings. The content of GSSG increased by 54 and 116% due to mild and severe levels of stress, respectively, compared to control plants. Salt stressed seedlings provided with additional Si showed significant decrease of GSSG level, compared to salt stress alone (Fig. 2b).

Enzymes of AsA-GSH Cycle

Ascorbate-glutathione cycle enzyme activities were noticed to respond differentially under saline condition. Ascorbate peroxidase activity increased but the MDHAR, DHAR and GR activities reduced due to salt imposition, compared to control. Silicon supplementation showed its additive effects by increasing the activities of these enzymes in both levels of stress (except the APX activity as Si increased its activity under mild salt stress only), compared to seedlings exposed to salt stress only. Addition of Si increased APX activity by 16 and 11% under mild salt stress, compared to salt treatment without Si (Fig. 3a). Silicon co-treatment with mild and severe salt stress increased MDHAR activity by 49 and 52%, and DHAR activity by 34 and 43%, respectively, compared to salt stress alone (Fig. 3b,c). Si addition with mild salt stress increased GR activity by 8%, compared to salt stress alone (Fig. 3d).

Thiol-Dependent Antioxidant Enzymes, GPX and GST

The thiol-dependent antioxidant enzyme GPX showed slightly increased activity under mild salt stress whereas the activity decreased under severe salt stress, if compared with control. Another thiol-dependent enzyme, GST, showed increased activity due to both mild and severe salt stress levels, compared to control. When adding Si to stress treatments the activity of GST increased by 13 and 31% in mild and severe stress, respectively, compared to only salt treatments (Fig. 4a). Glutathione peroxidase activity increased only in mild stress after Si addition which was 23% higher, compared to salt stress alone (Fig. 4b).

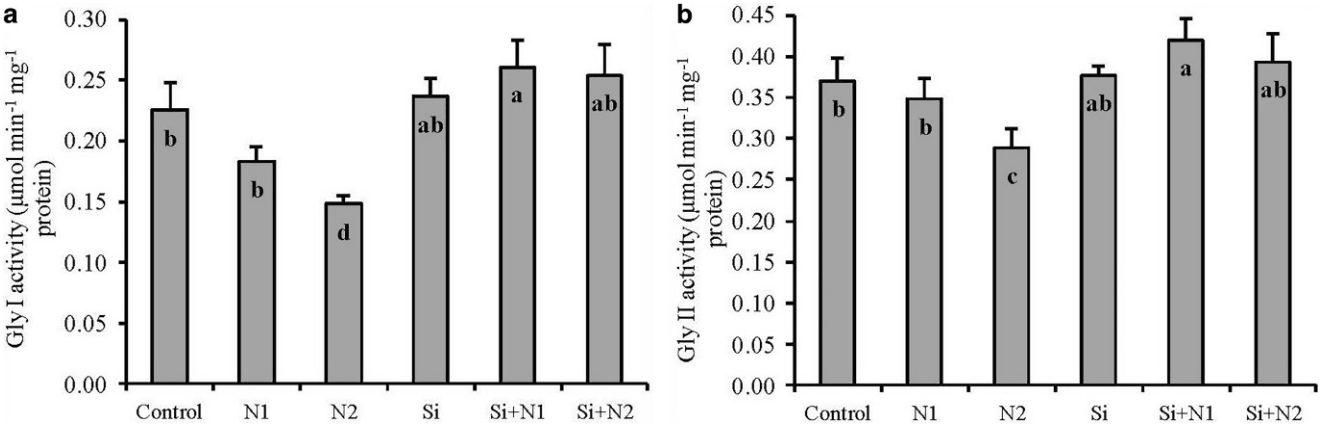

Fig. 5 Activities of glyoxalase system enzymes, Gly I (**a**) and Gly II (**b**) in rapeseed leaves induced by Si under salt stress. N_1, N_2, Si, Si+N_1 and Si+N_2 indicate 100 mM NaCl, 200 mM NaCl, 1 mM SiO_2, 100 mM NaCl+SiO_2, 200 mM NaCl+SiO_2, respectively. Values sharing the same letter do not differ significantly at $P \leq 0.05$

Activity of CAT

Activity of the vital H_2O_2 scavenging enzyme, CAT, dropped significantly due to salt imposition. Mild and severe salt stress decreased its activity by 32 and 41%, respectively, compared to control. Exogenous Si supplementation restored and increased CAT activity to a great extent which was 53 and 75% higher than its activity in mild and severe salt stress, respectively, compared to only salt treatments (Fig. 4c).

Enzymes of Glyoxalase System

Saline stress disrupted the MG detoxification system. The activity of glyoxalase enzymes decreased in salt imposed rapeseed seedlings, compared to control seedlings. Glyoxalase I activity upheld by 47 and 72% after applying Si in mild and severe salt treatments, respectively, compared to only salt stress treatments (Fig. 5a). Silicon supplementation also increased the activity of Gly II by 20 and 36% in mild and severe salt stress, respectively, when compared to seedlings exposed to salt stress only (Fig. 5b).

Discussion

High salt concentration increases ionic toxicity and osmotic or physiological drought stress. Both of these interrupt normal movement of stomata, decreases CO_2 concentration to the fixation site of Calvin cycle and due imbalanced electron transport ROS are overproduced (Hasanuzzaman et al. 2013). Salt stress reduces efficiency of antioxidant defense enzymes which further increases ROS accumulation in plant cell (Rahman et al. 2016). In our experiment, when exposed to salt, the seedlings of *B. napus* showed an excessive H_2O_2 production which was responsible for the disruption of the membrane properties as designated by high rise of MDA level, a product of lipid peroxidation. The membrane damage indicates the oxidative stress created by salt stress. Related advantageous role of Si is evident in our study. Exogenous Si co-treatment prevented production of excess ROS and the oxidative damage due to adverse effects of salt stress. Exogenous Si also enhanced antioxidant system that also prevented ROS accumulation and oxidative damage in rapeseed seedlings. These results are supported by previously published results with exogenous Si application in cucumber plants under salt stress (Khoshgoftarmanesh et al. 2014).

Production of ROS is a spontaneous and inevitable occurrence in plant cell. An efficient antioxidant defense system can scavenge and keep a balance state of ROS accumulation and in contrast, declining the efficiency of this system may cause irreparable damage of components of plant cell and even cause death. Therefore, enhanced antioxidant defense system i.e. increased levels of non-enzymatic antioxidants and enhanced activities of enzymes are pivotal for an improved oxidative stress tolerance under different abiotic stresses (Hasanuzzaman et al. 2017a).

Ascorbate, which is one of the simplest, most copious and effective primary antioxidant protecting cells against oxidative stress works in AsA-GSH cycle together with GSH (Hasanuzzaman et al. 2017a). After sudden salt imposition to the growth medium of rapeseed plants their AsA levels dropped down irrespective of the dose that is comparable with the findings of other research (Nahar et al. 2016). This dropdown of AsA was responsible for raising the H_2O_2 and the oxidative damage of cell. Ascorbate is disproportionate to DHA and monodehydroascorbate (MDHA) while scavenging ROS. Due to the activity of DHAR and MDHAR enzymes, respectively, AsA can be regenerated through the AsA-GSH cycle (Hasanuzzaman et al. 2017b). Then, when Si was added to the salt solution

of rapeseed seedlings it alleviated the stress effects raising the AsA level and reducing the oxidative injury. The raise of AsA in Si-added salt treatment was associated with enhanced MDHAR and DHAR activities those proficiently recycled back AsA.

Glutathione is a low molecular and water soluble tripeptide γ-glutamyl cysteinyl glycine (γ-Glu-Cys-Gly). Glutathione reduces oxidative stress scavenging ROS of various forms including H_2O_2, $^1O_2$, $O_2^{\bullet-}$ and OH. Without acting as direct scavenger of ROS, the functions of GSH in retaining cellular redox balance, and stress signal transfer and adaptation are imperative in plants (Foyer and Noctor 2005; Hasanuzzaman et al. 2017b). Salt-induced rise in GSH levels of the rapeseed seedlings of the present study is comparable to the findings of earlier study (Nahar et al. 2016). After participating in ROS detoxification process, GSH is transformed into GSSG for which the level of GSSG also increased under salt stress that is an indicator of stress. Nonetheless, a stress tolerant plant efficiently recycles GSSG into GSH immediately so that plants become instantly ready to detoxify ROS again. After inclusion of exogenous Si to the salt media the GSH level became much improved (with a noticeable decrease of GSSG), compared to the only salt treatment. The reason behind this increased GSH level was the enhanced activity of GR (a GSH recycling enzyme) by Si addition.

The modes of alteration of AsA and GSH pool as well as the functioning of enzymes involved in AsA-GSH cycle are in the same line with the observation and findings of previous research. Exogenous Si addition rose AsA and GSH level in salt affected sunflower plant which relaxed the oxidative stress from that plant (Ali et al. 2013). High silica uptake capacity of rice considerably enhanced the efficiency of antioxidant enzymes including the activity of APX under salt stress (Farooq et al. 2016). Likewise, Si use increased GSH content, APX activities in sorghum grown under saline condition (Kafi et al. 2011). Improved activities of three AsA-GSH cycle enzymes i.e., APX, MDHAR and GR with enhanced levels of AsA and GSH with diminishing oxidative stress were noticed in Si added cucumber suffered from chill-induced oxidative damage (Jiao-jing et al. 2009).

Glutathione peroxidase catalyzes H_2O_2 and organic hydroperoxides detoxification reaction that is dependent on GSH. The thiol/disulfide or NADPH/$NADP^+$ balance is maintained by GPX that plays roles in regulation of redox homeostasis which protects cells from oxidative damage. Glutathione peroxidase shows its activity during growth and development (Bela et al. 2015). The activity of GPX of rapeseed plants slightly increased with mild salt stress but declined with severe stress. Exogenous Si application with mild salt stress increased its activity whereas in severe stress, Si failed to up-regulate its activity. In contrary, in salt stressed rice plant, high Si uptake capacity was associated with an increased GPX activity and declined oxidative stress (Farooq et al. 2016). Plant GSTs are multifunctional enzymes catalyzing conjugation of xenobiotic substrates with GSH (Dixon et al. 2010). The peroxidase activity of GST reduces damage caused by oxidative stress (Gill and Tuteja 2010). Compared to control treatment, GST activity was up-regulated in *B. napus* seedlings when exposed to salt stress. It's activity further upheld again by exogenous Si addition with salt stress. Debona et al. (2014) showed similar pattern of Si induced enhancement of GST activity in wheat leaves.

Catalase plays important role in signal perception, metabolism and resistance development in plants (Shugaev et al. 2011; Su et al. 2014). Salt stress declined the activity of CAT that increased H_2O_2 accumulation. When Si was supplemented with NaCl the CAT activity increased which dismutated H_2O_2 to H_2O and relaxed the cellular environment from oxidative stress. Exogenous Si addition increased CAT activity that was observed in salt affected sunflower (Ali et al. 2013), sorghum (Kafi et al. 2011) and in lettuce (Milne et al. 2012).

The high reactive nature of MG damages plant cell under salt stress. Methylglyoxal is also accountable for increasing ROS (Hasanuzzaman et al. 2017a). Its cytotoxic effects have been reported through breaking down the cellular proteins and DNA (Yadav et al. 2005). The enzymes Gly I and Gly II are the components of glyoxalase system and these enzymes catalyze the conversion of highly active MG to a safe form D-lactate, where GSH is performing its function as a co-factor (Yadav et al. 2005; El-Shabrawi et al. 2010). In this experiment, the Gly I and Gly II activities of salt distressed rapeseed seedlings decreased with the rise of salt doses pointing toward the disrupted MG detoxification system and increased MG-induced damage (Nahar et al. 2016). When those salt affected plants were treated with Si, the leaf RWC was retrieved and improved more than that of salt treated plants only. NaCl treatment demonstrated the clear picture that Si proficiently up-regulated the Gly I and Gly II activities with a concomitant increase of GSH level which are accountable for detoxifying cellular MG.

Conclusion

Although it is not essential for the life of plants in general, Si has many important roles in the physiological process of many crop plants. In recent studies, Si was found to play vital role in plant's defense against abiotic stress. In our study, we found out that Si effectively mitigated the oxidative stress produced due to salinity by up-regulating the antioxidant enzyme activities and maintaining the higher amount of non-enzymatic antioxidant. Moreover, Si also

enhanced the glyoxalase system enzyme activities. Further research should be focused on the transport and regulation of Si in different plant species facing abiotic stress.

Acknowledgements We acknowledge Ms. Khursheda Parvin, Assistant Professor, Department of Horticulture, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh for the critical reading and editing of the manuscript.

Conflict of interest M. Hasanuzzaman, K. Nahar, M.M. Rohman, T.I. Anee, Y. Huang and M. Fujita declare that they have no competing interests.

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