Silicon (2020) 12:535–545
https://doi.org/10.1007/s12633-019-00158-w

ORIGINAL PAPER

Impact of Exogenous Silicate Amendments on Nitrogen Metabolism in Wheat Seedlings Subjected to Arsenate Stress

Palin Sil[1] · Prabal Das[1] · Asok K. Biswas[1]

Received: 13 January 2019 / Accepted: 8 April 2019 / Published online: 25 April 2019

Abstract

Purpose We intended to investigate the response of arsenate on nitrogen metabolism in wheat seedlings and aimed to assess the efficacy of silicon amendments in modulating the metabolic disturbances caused by arsenate stress.

Methods The nitrogen metabolism of wheat cultivated in different levels of arsenate with or without silicate in a medium supplemented with modified Hoagland's solution for 21 days was studied. Experimental design was completely randomized with different arsenate concentrations (0, 25, 50 and 100 μM) with or without 5 mM silicate.

Results Arsenate treatment decreased growth along with decline in nitrate (NO_3^-) uptake and accumulation. Activities of nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS) as well as glutamate synthase (GOGAT) were lowered in the test seedlings. Decline in nitrite (NO_2^-) and amino acid contents were also evident along with an enhancement in the accumulation of toxic ammonia. Silicate supplementation under arsenate stress however, improved growth, repaired the arsenate-induced effects leading to an enhancement in nitrate (NO_3^-) uptake and consequently improved nitrite (NO_2^-) and amino acid contents as well. The total and soluble nitrogen contents were enhanced along with enhancements in activities of enzymes associated with nitrate metabolism while ammonia accumulation was lowered.

Conclusions Results therefore, imply the involvement of exogenous silicon amendments in relieving the metabolic alterations in nitrogen metabolism caused by arsenate stress that enabled wheat seedlings to adapt under arsenate excess and eventually promoted plant growth.

Keywords Arsenate · Growth · Nitrogen metabolism · Silicate · Wheat

1 Introduction

The element Arsenic (As) is one of the ubiquitous environmental contaminants and is classified as non threshold class 1 carcinogen [21, 31]. Ingression of arsenic into the environment occurs through natural as well as anthropogenic sources. Inhabitants of the South-east Asian region predominantly India and Bangladesh are severely affected by the emerging crisis of arsenic contamination. Concentrations of arsenic in arable land of Indo-Gangetic basin, deltaic areas of West Bengal, regions of Uttar Pradesh, Assam, Chhattisgarh and Bihar ranges from 3.34 to 105 mg kg^{-1} [28]. Our study area in Bengal basin is considered to be the most acutely arsenic infested geological province where arsenic concentration in water has been reported up to 3200 μg l^{-1} against the acceptable threshold of 10 μg l^{-1}, recommended by the WHO [4, 32]. Arsenic contamination in both soil and water has become a severe environmental hazard in agricultural areas where the metalloid accumulates in edible plant parts and finally reaches human body [10]. Consumption of agricultural produce from arsenic-contaminated soils together with use of arsenic contaminated water for drinking and irrigation forms a tangible route of arsenic exposure to the food chain that subsequently leads to the incidence of arsenic related disorders in the natives. Phyto availability of arsenic is dependent upon the concentrations of total and available phosphorus in the soil. Being a chemical analog of phosphate, arsenate uses various phosphate (Pi) channels in the root epidermis for entering the

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12633-019-00158-w) contains supplementary material, which is available to authorized users.

✉ Asok K. Biswas
asokbiswas2017@yahoo.com

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

plant cell while arsenite entry is mediated via aquaporin channels which are silicon transporters [1]. Arsenic, after its uptake as arsenate is converted to arsenite that induces production of reactive oxygen species (ROS). This generates a plethora of morphological, physiological, biochemical as well as molecular alterations that affects plant development, impedes diverse biochemical and metabolic pathways, disrupts plant water status, dismantles cellular integrity and disturbs the ionic homeostasis by limiting nutrient absorption [1, 28]. Arsenite further interacts with the functional groups of enzymes and replaces essential ions from ATP that damages proteins, lipids as well as nucleic acids and hampers various metabolic pathways, either as competitive inhibitors of Pi or by interfering with the activities of enzymes and eventually affects growth processes. Arsenic contamination in agricultural system is a constraint to crop productivity as well as quality. The way out in combating the impending crisis would be to develop tolerant crops or environmental remediation. Remediating environment using physical and chemical techniques seems to be impractical owing to the wide expanse of pollution. A reasonable farmer friendly alternative would be using chemicals that would minimize arsenic uptake and in turn mitigate arsenic-induced toxicity. Exogenous application of phosphate, silicate, nitric oxide, proline, salicylic acid and phytohormones have been reported to afford protection under heavy metal ion-stressed environment in different crop plants [39, 40, 43, 44, 49]. These substances reduce metal-induced toxicity by lowering metal uptake and resist membrane damage. Application of these chemicals modifies the expression of stress-related genes and improve antioxidative defense that quenches metal-induced ROS production [1]. Priming with phytohormones such as auxin, gibberallin, cytokinins and brassinosteroids decreases ROS generation together with lipid per oxidation and up regulates the anti oxidative defense that helps to restore growth [49].

The quasi essential element silicon (Si) has been documented to alleviate combinatorial environmental constraints in plants and has become a focus of interest in recent times. Silicon has significant role in mitigation of stress imposed by heavy metals and metalloids through either external or internal mechanisms [2, 23, 36, 40, 41, 50]. Silicon induced augmentation in antioxidant defense efficiently lowered oxidative damage and enhanced plant tolerance against toxic metal ions. Silicon supplementation also stabilized chloroplast structure, PSII integrity and increased pigment concentration that improved net photosynthesis under arsenate stress [41]. Additionally silicon treatment has been documented to augment the transcript levels of PsbY (Os08g02630), a vital protein involved in photosystem II (PSII) and improve the efficiency of electron transfer in PS II along with an up-regulation of PetC that codes Rieske Fe-S center-binding polypeptide of cytochrome bf complex. This improved the structural integrity of the chloroplast under zinc stress [47]. The endogenous jasmonic acid and salicylic acid contents reduced with silicon treatment in rice under heavy metal stress while abscisic acid first increased and then decreased after 14 days of treatment [17]. Silicon treatment also induced an increase in gibberellin contents in soybean under salinity stress [20] while delayed senescence by increasing cytokinin biosynthesis in *Sorghum* and *Arabidopsis* [30]. Employment of nanotechnology with Si nanoparticles (Si-NPs) also has promising applications in the agricultural sector and has the potential to develop improved varieties with high productivity resistant to several biotic and abiotic factors. The unique properties of Si-NPs help to control agricultural damage during climate change and/or abiotic stress and can be applied to revolutionize the prevalent technology against heavy metal toxicity to devise sustainable strategies to improve crop yield [37]. Silicon fertilization by natural silicates is low-cost, environment friendly and has the potential in restoring soil integrity. Silicate-fortified fertilizers has gained importance recently due to its beneficial effects on growth; photosynthesis and improves plant performance under stressed conditions [29].

The macronutrient nitrogen is indispensible for plant growth, and forms an integral part of nucleic acids and proteins. An adequate quantity of this element is essential for plants due to its involvement in coenzymes, photosynthetic pigments, polyamines and secondary metabolites formation [54]. Nitrate (NO_3^-), the predominant form of nitrogen (N) in the soil is bioavailable and mostly assimilated by plants. After nitrate reductase (NR) mediated reduction of nitrate (NO_3^-) to nitrite (NO_2^-) in cytoplasm, it is transported to chloroplast and further reduced to NH_4^+ by the enzyme nitrite reductase (NiR) [11]. Highly toxic ammonium (NH_4^+) is rapidly assimilated into glutamine and glutamate through the glutamine synthase-glutamate synthase cycle (GS/GOGAT cycle) by the synergistic actions of glutamine synthetase (GS) and glutamate synthase (GOGAT), glutamate dehydrogenase (GDH), alanine amino transferase and aspartate amino transferase and assimilated to nucleic acids, amino acids, proteins, chlorophyll and different metabolites, essential for growth and development [22, 42]. Several studies have shown that the enzymes involved in N metabolism exhibit species, cultivar and tissue specific variations. Additionally, the duration and intensity of stress also affects N metabolism [11, 22].

Heavy metal phytotoxicity is associated with a decrease in absorption and assimilation of nitrogen [7]. Previous work disclosed a significant decrease in nitrate uptake and transport along with inhibitory effects on NR and NiR activities under copper, cadmium and aluminium stress in plants [3, 7, 38, 55]. Besides, the GS-GOGAT cycle and GDH pathways have been shown to be significantly induced under copper treatment [25].

In spite of several research advancing our perception regarding nitrogen metabolism in plants, information concerning the influence of exogenous silicate on nitrogen

metabolism in wheat seedlings under arsenate stress remains to be documented. Although wheat contributes significantly to the food security of the natives, comprehensive study on physio- biochemical changes due to heavy metal stress in wheat is still meagre. Deleterious effects of heavy metals in arid and semi arid lands cause substantial yield loss of this cereal crop that affects human health. The current research was therefore, devised to examine the efficacy of silicate supplementation under arsenate stress on parameters related to growth and metabolism of nitrogen in wheat and develop our insight to the sequence of various metabolic reactions in relation to varying arsenic concentrations amended with silicate. Here, we hypothesized that silicate supplementation could mitigate the toxic effects of arsenate by improving nitrogen metabolism. Such information would provide a better understanding of nitrogen metabolism in arsenate stressed wheat seedlings and may assist in determining the impact of exogenous silicate in combating the stress.

2 Material and Methods

2.1 Plant Material and Experimental Conditions

Caryopses of the wheat cultivar (*Triticum aestivum* L. cv. PBW-343) procured from the State Agricultural Research Station, Burdwan, West Bengal, India were surface sterilized with sodium hypochlorite (5% *v*/v) and germinated in petri dishes ($\phi = 20$ cm) under dark and humid conditions for 48 h at 30 ± 2 °C. Germinated plantlets were treated with different levels (0, 25 μM, 50 μM and 100 μM concentrations) of sodium arsenate (Na_2HAsO_4, $7H_2O$; Loba-Chemie, India) in presence or absence of (5 mM) sodium silicate (Na_2SiO_3, $9H_2O$ Loba-Chemie, India) under 16 h photoperiod (260 μmol m^{-2} s^{-1} PFD) and relative humidity of 70% for 21 days in modified Hoagland's solution [56]. The pH of the medium was maintained within 5.5–6. Solutions were renewed on alternate days. Experiments were undertaken in a completely randomized design thrice and each treatment had two replications. After the definite period, plant samples were collected, weighed in required quantities and preserved at −20 °C for biochemical studies.

2.2 Seedling Growth

About 10 seedlings were chosen arbitrarily from every treatment. Both root and shoot lengths were measured and numbers of lateral roots were also counted. This procedure was repeated thrice. Results obtained from these three means were analyzed statistically.

2.3 Total, Soluble Nitrogen, Nitrate and Nitrite Contents

Total and soluble nitrogen contents were estimated following Vogel [51] and were expressed as mg nitrogen g^{-1} dw and mg nitrogen g^{-1} fw respectively. Nitrate content was estimated following Cataldo et al. [6] while nitrite content was determined according to Snell and Snell [46] and was expressed as μg nitrate g^{-1} fw and μg nitrite g^{-1} fw respectively.

2.4 Estimation of NR and NiR Activities

Activity of nitrate reductase (NR, EC 1.6.6.6) was assayed by the method of Hageman and Reed [13] and the activity of nitrite reductase (NiR, EC 1.6.6.4) was determined following Mendez and Vega [33]. The activities were expressed in terms of μmol of NO_2^- produced/reduced mg^{-1} protein min^{-1}.

2.5 Free Amino Acid Content

Free amino acid level was assayed by the method of Lee and Takahashi [19] and expressed as μg amino acid g^{-1}fw.

2.6 Dissolved Ammonia Content

Ammonia content was estimated following Hoshida et al. [14] and expressed as μg ammonia g^{-1}fw.

2.7 Assay of Glutamine Synthetase and Glutamate Synthase Activities

Fresh tissue was homogenized in the ratio 1/10 with 50 mM potassium phosphate buffer (*w*/*v*) (pH 7.5), containing 2 mM EDTA, 1.5% soluble casein (w/v), 2 mM dithiothreitol, and 1% insoluble polyvinylpyrrolidone (w/v). Centrifugation was performed at 3000 g for 5 min at 2 °C followed by further centrifugation at 30000 g at 2 °C for 20 min. The supernatant was used to determine the enzyme activities.

Glutamine synthetase (GS) [EC 6.3.1.2] activity was assayed according to Canovas et al. [5] and represented as n mol glutamic hydroxamate formed per mg^{-1} protein min^{-1}. Activity of glutamate synthase (GOGAT) [1.4.1.13] was estimated by the protocol of Chen and Cullimore [8] and was calculated as nmol NADH oxidized mg^{-1} protein min^{-1}. Protein estimation for all the enzyme assays were performed according to Lowry et al. [24] taking bovine serum albumin as standard.

2.8 Statistical Analyses

The data presented are the averages obtained from experiments conducted thrice, each time with two replicates per treatment and reported as mean ± SE. p value ≤0.05 was

considered to be statistically significant according to student's t test. Regression analysis was carried out using Minitab 18 environment. Microsoft Excel 2010 was used for figures.

3 Results

Arsenate treatments diminished growth and inhibition was greater in root compared to that of shoot. The inhibition was by 51%, 66% and 83% in root and by 28%, 38% and 48% in shoot under 25 μM, 50 μM and 100 μM arsenate treatments compared to control (Figs. 1 and 2). Silicate amendments in the arsenate treated samples also inhibited root as well as shoot growth but to a lesser extent compared to the only arsenate treatments. The root lengths decreased by 33%, 52% and 64% while shoot length decreased by 9%, 21% and 40% under 25 + Si, 50 + Si and 100 + Si doses respectively. Equations $Y_r = 8.986–0.07758$ As +0.317 Si, (R square = 80.91%) and $Y_s = 15.336–0.07739$ As +0.4550 Si, (R square = 88.21%) explained the regression of arsenate and silicate treatment on growth in root and shoot respectively.

Arsenate treatments also decreased the number of lateral roots (Fig. 3). This decrease was by 21%, 32% and 57% under 25 μM, 50 μM and 100 μM arsenate treatments respectively over control. Silicate treatment along with arsenate increased the number of the lateral roots in comparison to the only arsenate treated sets. The lateral roots decreased by 7%, 14% and 43% in the 25 + Si, 50 + Si and 100 + Si treatments respectively over control. Equation $Y_r = 9.117–0.05410$ As +0.3000 Si, (R square = 88.32%) demonstrated the regression of arsenate and silicate treatment on the number of lateral roots in the test cultivar.

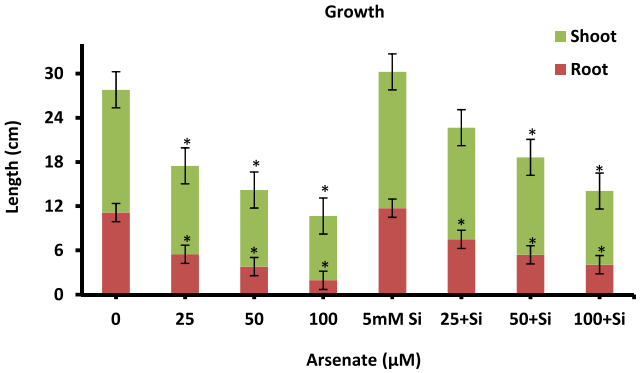

Fig. 2 Effect of arsenate and or silicate on growth in 21 days old wheat (cv PBW 343) seedlings. Each data point is the mean ± SE with 3 replicates. * Indicates statistically significant at $p \leq 0.05$ respectively as compared to control

3.1 Total, Soluble Nitrogen, Nitrate and Nitrite Contents

The total and soluble nitrogen contents decreased in both root as well as shoot of the arsenate challenged seedlings in comparison to control. The total nitrogen contents declined by 8%,19% 24% in root and by 14%, 21% 29% in shoot under 25,50 and 100 μM arsenate treatments respectively while decline in soluble nitrogen contents was 10%, 20%, 23% in root and 7%, 19%, 46% in shoot under identical treatments (Table 1). Silicon supplementation along with arsenate narrowed down the decrease in total nitrogen contents by 2%, 9%, 14% in root and 4%, 9%, 14% in shoot. The decrease in soluble nitrogen contents was also narrowed down by 2%, 4%, 9% in root and by 4%, 12%, 17% in shoot under similar treatments. However, both total and soluble nitrogen contents in the silicon supplemented seedlings were greater than the individual arsenate treated seedlings. Regression equations

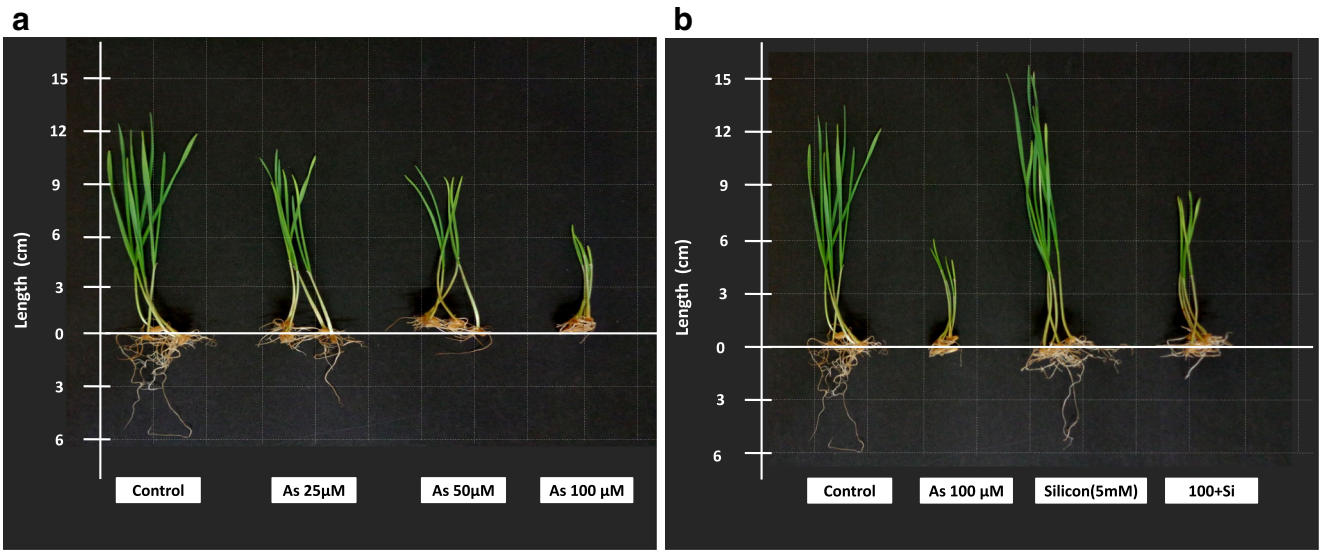

Fig. 1 Effect of arsenate either applied individually (**a**) or in combination with silicate (**b**) on the growth of 21 days old wheat (cv. PBW343) seedlings

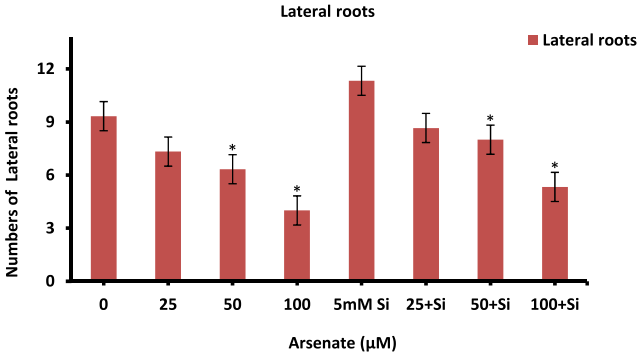

Fig. 3 Effect of arsenate and or silicate on number of lateral roots in 21 days old wheat (cv PBW 343) seedlings. Each data point is the mean ± SE with 3 replicates. * Indicates statistically significant at $p \leq 0.05$ respectively as compared to control

$Y_r = 3.802–0.00884$ As +0.0685 Si, (R square = 55.82%) and $Y_s = 5.147–0.01290$ As +0.1200 Si, (R square = 64.76%) explained the consequence of arsenate and silicate treatment on total nitrogen contents in root and shoot of the test seedlings respectively whereas effect of arsenate and silicate treatment on soluble nitrogen contents in both root and shoot correspondingly fitted to the equations $Y_r = 3.455–0.00693$ As +0.0830 Si, (R square = 61.72%) and $Y_s = 4.655–0.01623$ As +0.1020 Si, (R square = 71.59%).

Arsenate treatments reduced nitrate contents in both root and shoot. Nitrate contents decreased by about 8%, 22%, 43% in root and by 13%, 20, 33% in shoot under 25 μM, 50 μM and 100 μM arsenate treatments respectively in comparison to control (Table 1). Silicate supplementation along with arsenate also decreased nitrate contents but was less than arsenate treatments alone by about 3%, 11%, 34% in root and by 6%, 15%, 28% in shoot under identical concentrations. The equations $Y_r = 188.33–0.7960$ As +2.95 Si, (R square = 84.83%) and $Y_s = 164.13–0.5057$ As +1.350 Si, (R square = 74.69%) explained the relationship between nitrate content under arsenate and silicate treatment in root and shoot respectively. Nitrite contents equally decreased by about 18%, 26%, and 35% in root and 14%, 28%, 41% in shoot under 25 μM, 50 μM and 100 μM arsenate treatments respectively compared to control (Table 1). Application of silicate in combination with arsenate also confirmed a decrease, but to a relatively lesser extent than arsenate treatment alone by about 9%, 21%, 30% in root and about 4%, 20%, 25% in shoot under 25 + Si, 50 + Si and 100 + Si treatments respectively with respect to control. The variation in nitrite contents under different concentrations of arsenate and silicate in root and shoot fitted to the equations $Y_r = 162.98–0.5651$ As +1.85 Si, (R square = 77.15%) and $Y_s = 144.10–0.5394$ As +3.00 Si, (R square = 76.97%).

3.2 Activities of Nitrogen Assimilatory Enzymes

Arsenate treatment decreased nitrate reductase (NR) activity in the test cultivar (Fig. 4). The decrease was more prominent in root than shoot. The enzyme activity decreased by about 34%, 48% and 52% in root whereas, in shoot, the decrease was by about 26%, 32% and 38% under 25 μM, 50 μM and 100 μM arsenate treatments correspondingly over control. Administration of silicate together with arsenate narrowed down the level of decline in NR activity by about 22%, 39% 42% in root and 7%, 16% 28% in shoot over control which was again greater than arsenate treated samples. The regression equations $Y_r = 10.880–0.05954$ As +0.260 Si, (R square = 70.44%) and $Y_s = 6.225–0.02229$ As +0.1700 Si, (R square = 71.79%) represented variation in NR activity under different doses of arsenate and silicate treatments in root and shoot respectively.

Likewise, nitrite reductase activity (NiR) also declined under arsenate treatments significantly in both root and shoot (Fig. 5). NiR activity declined by 29%, 42%, 46% in root and by 14%, 26%, 29% in shoot under 25 μM, 50 μM and 100 μM arsenate treatments respectively with respect to the control. Supplementation of silicate with arsenate also decreased enzyme activity by 21%, 35%, 44% in root and by 3%, 19%, 26% in

Table 1 Effect of arsenate and/or silicate on total, soluble nitrogen, nitrate and nitrate contents in root and shoot of 21 days old wheat (cv PBW 343) seedlings

Treatment	Total N_2 content [mg g^{-1} dw]		Soluble N_2 content [mg g^{-1} fw]		Total nitrate content [μg g^{-1} fw]		Total nitrite content [μg g^{-1} fw]	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Control	3.92 ± 0.32	5.46 ± 0.27	3.64 ± 0.19	4.80 ± 0.22	190 ± 10.63	170 ± 8.47	172 ± 6.35	152 ± 6.44
As 25 μM	3.60 ± 0.20	4.69 ± 0.25	3.27 ± 0.15	4.48 ± 0.24	174 ± 7.83	148 ± 7.94	141 ± 7.68*	130 ± 7.31
As50μM	3.16 ± 0.19	4.30 ± 0.23*	2.90 ± 0.13*	3.90 ± 0.19*	148 ± 7.36*	136 ± 6.91*	128 ± 6.32*	110 ± 6.51*
As100μM	2.18 ± 0.18	3.88 ± 0.21*	2.80 ± 0.15*	2.60 ± 0.15*	102 ± 5.73*	114 ± 7.68*	112 ± 6.98*	90 ± 5.92*
Silicate(5 mM)	4.24 ± 0.25	5.82 ± 0.37	3.92 ± 0.20	4.98 ± 0.31	194 ± 10.46	168 ± 8.06	178 ± 6.22	160 ± 8.11
As 25 μM + Si	3.84 ± 0.17	5.26 ± 0.24	3.56 ± 0.14	4.62 ± 0.25	184 ± .8.89	160 ± 9.31	156 ± 6.56	146 ± 7.68
As 50 μM + Si	3.57 ± 0.18	4.98 ± 0.25	3.49 ± 0.15	4.24 ± 0.20	169 ± 7.95	145 ± 6.15	136 ± 7.92*	122 ± 6.63*
As 100 μM + Si	3.28 ± 0.16	4.67 ± 0.24	3.30 ± 0.14	3.98 ± 0.19	126 ± 6.64*	122 ± 4.80*	120 ± 6.35*	114 ± 7.35*

Each data point is the mean ± SE with 3 replicates. * Indicates statistically significant at $p \leq 0.05$ respectively as compared to control

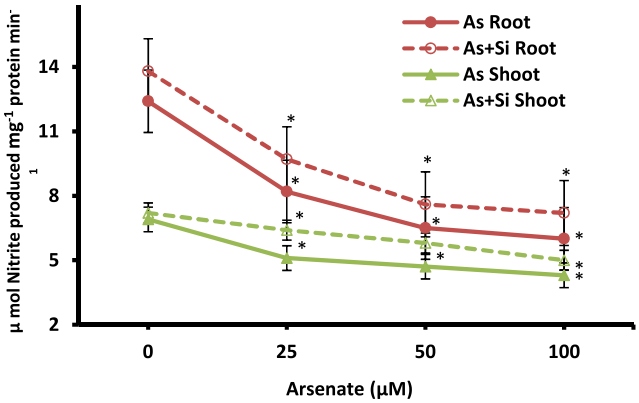

Fig. 4 Effect of arsenate and or silicate on nitrate reductase (NR) activity in root and shoot of 21 days old wheat (cv PBW 343) seedlings. Each data point is the mean ± SE with 3 replicates. * Indicates statistically significant at $p \leq 0.05$ respectively as compared to control

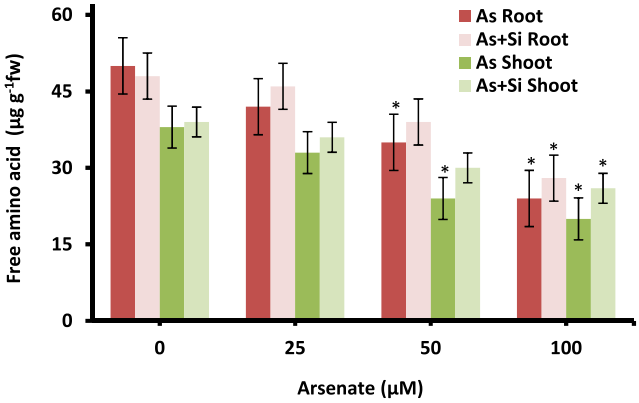

Fig. 6 Effect of arsenate and/or silicate on free amino acid contents in root and shoot of 21 days old wheat (cv PBW 343) seedlings. Each data point is the mean ± SE with 3 replicates. * Indicates statistically significant at $p \leq 0.05$ respectively as compared to control

shoot over control but was more than the only arsenate treated seedlings. Alterations in NiR activity in root and shoot under different doses of arsenate and silicate can be fitted to the equations $Y_r = 9.888–0.04829$ As +0.1250 Si, (R square = 75.19%) and $Y_s = 5.563–0.01743$ As +0.0750 Si, (R square = 70.54%) respectively.

3.3 Free Amino Acid Contents

Amino acid contents also reduced in the test cultivar under arsenate stress (Fig. 6). The amino acid levels decreased by about 16%, 30% and 52% in root while in shoot the level of decrease was by about 13%, 37% and 47% under 25 µM, 50 µM and 100 µM arsenate treatments respectively with respect to control. On the other hand, in silicate supplemented seedlings, decrease in amino acid contents narrowed down by about 8%, 22% and 44% in root and 5%, 21% and 32% in shoot compared to control and was greater than the corresponding arsenate treated sets. Alteration in amino acid levels in root and shoot under the influence of arsenate and silicate can be demonstrated by the equations $Y_r = 47.95–0.2331$ As +0.500 Si, (R square = 82.78%) and $Y_s = 35.70–0.1589$ As +0.800 Si, (R square = 70.69%).

3.4 Dissolved Ammonia Contents

Dissolved ammonia contents significantly increased with arsenate treatment in the seedlings (Fig. 7). The increase was by about 32%, 82% and 103% in root and by about 26%, 74% and 95% in shoot respectively under 25, 50 and 100 µM arsenate treatments with respect to control. Administration of arsenate along with silicate also increased ammonia contents but to a lesser extent compared to individual arsenate treatments that were by about 6%, 44% and 82% in root and 10%, 46%, 82% in shoot under 25 + Si, 50 + Si and 100 + Si treatments respectively in comparison to control. Regression equations $Y_r = 37.52 + 0.3423$ As - 1.650 Si, (R square = 85.44%) and $Y_s = 41.82 + 0.3697$ As - 1.250 Si, (R square = 85.02%) illustrated the changes in ammonia contents in root as well as in shoot under different doses of arsenate and with or without silicate respectively.

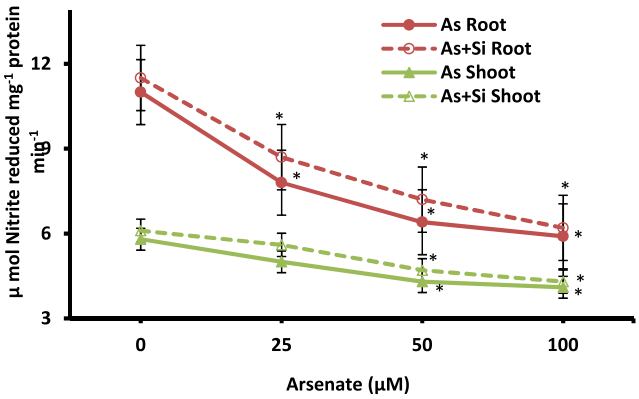

Fig. 5 Effect of arsenate and or silicate on nitrite reductase (NiR) activity in root and shoot of 21 days old wheat (cv PBW 343) seedlings. Each data point is the mean ± SE with 3 replicates. * Indicates statistically significant at $p \leq 0.05$ respectively as compared to control

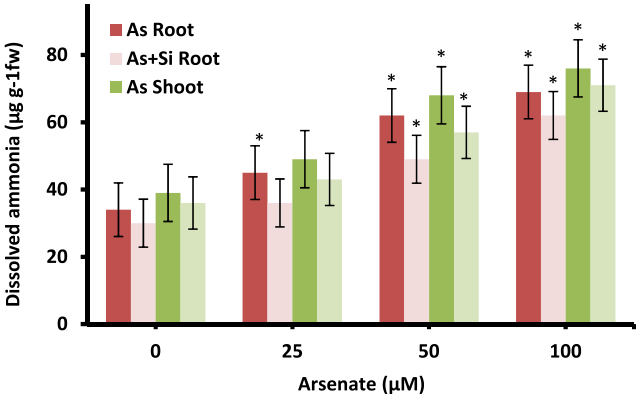

Fig. 7 Effect of arsenate and/or silicate on dissolved ammonia contents in root and shoot of 21 days old wheat (cv PBW 343) seedlings. Each data point is the mean ± SE with 3 replicates. * Indicates statistically significant at $p \leq 0.05$ respectively as compared to control

3.5 Activities of Ammonia Assimilatory Enzymes

Glutamine synthetase (GS) activity decreased by 21%, 45% and 52% in root and by 20%, 31% and 42% in shoot respectively at 25, 50 and 100 μM arsenate treatments with respect to control (Fig. 8). Supplementation of silicate in conjunction with arsenate also decreased the enzyme activity by about 14%, 38% and 48% in root and 8%, 13% and 33% in shoot under the same treatments. The decline in GS activity under different concentrations of arsenate and silicate in both root and shoot can be fitted to the regression equations $Y_r = 2.670–0.01417$ As +0.0200 Si, (R square = 81.73%) and $Y_s = 3.724–0.01482$ As +0.0850 Si, (R square = 81.65%).

GOGAT activity similarly, decreased by about 12%, 19% and 38% in root and by 19%, 22% and 38% in shoot under 25, 50 and 100 μM arsenate treatments respectively with respect to control (Fig. 9). The decrease in GOGAT activity narrowed down by about 4%, 13% and 27% in root and 13%, 17% and 25% in shoot under 25 + Si, 50 + Si and 100 + Si treatments respectively over control. Decrease in GOGAT activity in response to different concentrations of arsenate and silicate in root and shoot can be explained by the regression equations $Y_r = 2.5583–0.00933$ As +0.0435 Si, (R square = 77.28%) and $Y_s = 2.9463–0.00849$ As +0.0275 Si, (R square = 66.73%) respectively.

4 Discussion

Arsenic provoked metabolic alterations in the test seedlings were manifested by suppression in plant growth. The root growth was more affected compared to that of shoot. Moreover, reductions in growth of lateral roots were also noted but silicate amendments helped to revive the arsenate

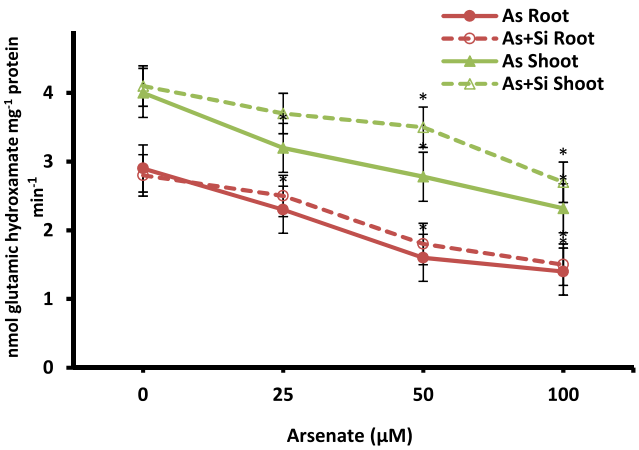

Fig. 8 Effect of arsenate and/or silicate on glutamine synthetase (GS) activity in root and shoot of 21 days old wheat (cv PBW 343) seedlings. Each data point is the mean ± SE with 3 replicates. * Indicates statistically significant at $p \leq 0.05$ respectively as compared to control

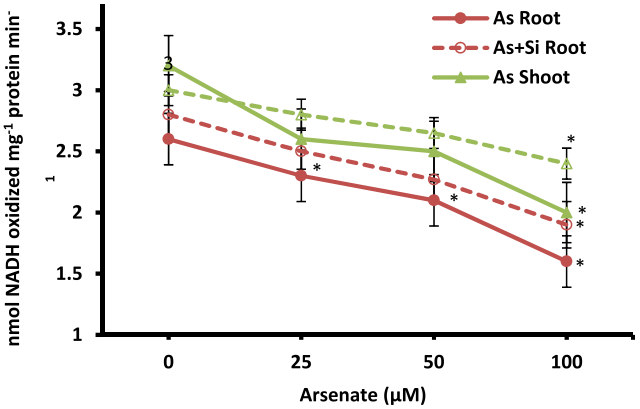

Fig. 9 Effect of arsenate and/or silicate on glutamate synthase (GOGAT) activity in root and shoot of 21 days old wheat (cv PBW 343) seedlings. Each data point is the mean ± SE with 3 replicates. * Indicates statistically significant at $p \leq 0.05$ respectively as compared to control

imposed alterations to considerable extents and there was restoration in root as well as shoot growth. Heavy metal stress has been documented to enhance ethylene and abscisic acid production along with a decline in endogenous auxin that decreased growth [35]. Rapid increase in abscisic acid production under heavy metal stress caused stomatal closure that inhibited photosynthesis and eventually reduced growth. Silicon treatment however, lowered the production of abscisic acid as well as ethylene and increased cytokinin biosynthesis in *Oryza sativa*, *Arabidopsis* and *Sorghum* under heavy metal stress [17, 30]. In the present study similarly, silicate treatment possibly rescued the test seedlings from arsenate imposed effects by regenerating the hormonal imbalance and improved growth. Nitrogen is a fundamental component of almost all bio molecules and a prerequisite for normal plant growth and development. The nitrogen content in plant represents its uptake and nutrition status and can be correlated with plant growth and biomass [18]. The decrease in total and soluble nitrogen content in our study similarly, decreased growth as well as biomass and is a physiological response of the test seedlings in response to arsenate stress.

The present study documented a decrease in both NO_3^- and NO_2^- content in the test cultivar and was negatively correlated with arsenate treatments. Our findings are in line with previous reports in spinach, tobacco and gourd where NO_3^- and NO_2^- content declined under heavy metal stress [27, 53, 55]. Such reduction in NO_3^- contents of both root and shoot might be ascribed to the reduced uptake and transport of NO_3^- that decreased nitrogen assimilation and lowered synthesis of amino acids under arsenate imposed stress in the test seedlings. Again decreased uptake of NO_3^- under arsenate stress may be attributed to the competition between NO_3^- and other anions that caused imbalance on plasma membrane permeability [52].

Nitrate reductase catalyzes the reduction of NO_3^- to NO_2^- and its activity is inducible by its substrate NO_3^-. This

reaction is vital in nitrate assimilation and eventually affects nitrogen status and plant growth [12]. Low availability of NO_3^- in the test seedlings owing to arsenate stress may be directly correlated with the reduction in NR activity that eventually decreased NO_2^- contents and lowered NiR activity too. Additionally, arsenate induced production of reactive oxygen species (ROS) might have lowered NR and NiR activities. These free radicals possibly damaged the enzymatic proteins and decreased their activities under stress. Conversion of arsenate to arsenite favours ROS formation as arsenite binds with sulfhydryl groups in proteins. Disturbance in ROS homeostasis initiates membrane damage and subsequently disrupts metabolism. Further, to minimize the toxicity of excess ammonia, reduction of NO_3^--N to NH_4^+-N was controlled by decreasing NR and NiR activities and this also reduced NO_3^- uptake. Such decline in nitrogen assimilation under arsenate stress consequently lowered synthesis of amino acids. Similar to our study arsenic induced suppression in activities of both NR and NiR decreased protein total protein content in *Vigna radiata*, increased susceptibility to ROS attack and suppressed growth [15].

Supplementation of exogenous silicate decreased arsenate availability, improved NO_3^- uptake and increased NO_3^- contents in the test cultivar. Increased availability of substrate (NO_3^-) enhanced the activity of NR and therefore caused more NO_2^- production in the test seedlings which subsequently escalated the activity of NiR in the study. This subsequently elevated the total as well as soluble nitrogen contents and increased the amino acid level leading to improved growth in the test seedlings (Fig. 10). Silicon deposits in the form of silica have been documented to afford mechanical protection while the presence of sodium metasilicate in growth media induced a rise in the rhizospheric pH that decreased heavy metal availability [26]. Si-induced immobilization of toxic metals in the root along with co-precipitation of metal-silicate complexes in the root apoplast not only restricted metal entry to the root but also limited translocation of toxic metals to shoot in maize, rye grass and wheat [9, 36, 40]. This indicated existence of some metal detoxification/ compartmentation mechanisim in the test seedlings. To facilitate the vacuolar sequestration through the interaction of tonoplast-localized transporters and ion chelators under heavy metal stress, plants often increase the synthesis of phytochelatins (PCs). A major strategy for arsenic detoxification following the reduction of arsenate to arsenite involves binding of arsenite to PCs followed by its sequestration to vacuoles [21]. Such PC-mediated vacuolar sequestration of accumulated arsenic in roots has been documented to protect the rice plants from arsenic toxicity at tissue level [49]. However, an up regulation in PC and reduced glutathione contents was observed under joint treatments of silicon with chromium that suggested involvement of silicon in modulating vacuolar sequestration of chromium in rice plants under chromium stress. At cellular level, silicon effectively restricted chromium in roots through PC-mediated vacuolar sequestration and reduced translocation to shoots [34]. Such Si-mediated enhancements in metal-PC complexes enhanced vacuolar sequestration that helped to reduce the metal-elicited effects and revived growth. Further, silicon amended plants have been reported to exude phenolic compounds with antioxidant and/or structural function that have metal-chelating abilities and contribute to detoxification of metals [36].

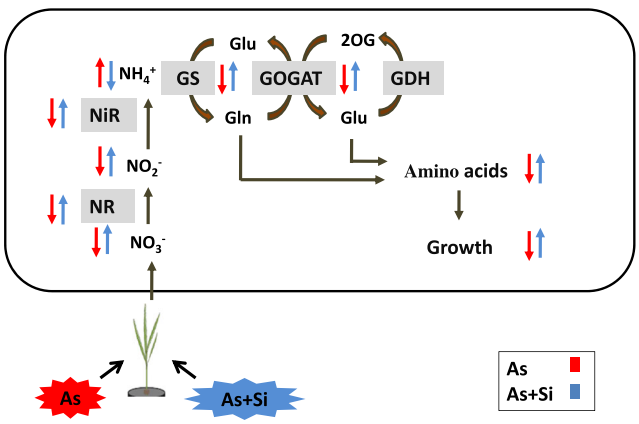

Fig. 10 Schematic representation of silicon-mediated alteration of growth and nitrogen metabolism in wheat seedlings

In plants, the GS/GOGAT cycle is the principal pathway for assimilation of ammonia. Glutamine synthetase (GS), an enzyme of this cycle catalyzes the ATP – dependant assimilation of ammonium to glutamine followed by its transformation to glutamate by NADH-dependent glutamate synthase (NADH-GOGAT) [57]. Incorporation of ammonium to glutamine and glutamate is crucial for plant development, as other amino acids and nitrogenous compounds are synthesized from these amino acids [18]. Arsenate inflicted stress lowered the activities of these enzymes leading to accumulation of ammonia in the test cultivar. Reduction in GS and GOGAT activity could be due to stress-induced, oxidative modifications of these enzymatic proteins [11]. Arsenate stress has been documented to inhibit the activities of nitrogen assimilatory enzymes along with a decrease in affinity towards their respective substrates that eventually suppressed nitrogen assimilation and impaired growth in crop plants [16]. However, another pathway for ammonia assimilation catalyzed by a mitochondrial NADH-GDH enzyme involves reductive amination of oxoglutarate to glutamate [47]. A perceptible increase in ammonia also caused stimulation of NADH-GDH activity under arsenate stress as was observed in our previous study [40]. Such induction of GDH activity could also be attributed to greater amino acid catabolism that enhanced ammonia level during stress.

On the other hand, silicon has the ability to lower the effects of arsenate inflicted stress and were positively correlated with the activities of GS and NADH-GOGAT. This enhanced

utilization of NH_4^+-N levels, lowered ammonia contents. Our previous work indicated Si-induced restriction of arsenate uptake lowered oxidative stress and reduced lipid peroxidation in the test cultivar. Additionally, silicon-mediated augmentation in polyamine synthesis improved the intrinsic ionic balance that reduced membrane lipid peroxidation and boosted antioxidative defense to an appreciable extent [40, 41]. Thus silicon supplementation probably interacted with excess cellular ROS and induced defense mechanisms that assisted in enduring arsenate stress and increased the activities of GS and GOGAT as well. Consequent enhancement in amino acid pool restored morpho-physiological parameters that improved growth in the experimental cultivar (Fig. 10).

5 Conclusions

Being sessile organisms, plants cannot escape environmental adversities. The present study was largely focused to ameliorate the arsenic imposed undesirable consequences on growth and metabolism and is a documentation to confirm the regulation of nitrogen metabolism under arsenic stress by silicon supplementation in the test cultivar. The work clearly demonstrates that arsenate imposition caused marked perturbations in nitrogen metabolism by hampering the enzyme activities of nitrogen and ammonium assimilatory pathways in the test seedlings. Reduction in nitrate, nitrite and free amino acid levels was accompanied by accumulation of ammonia as well. Supplemental silicon in the form of silicate restored the arsenate induced damaging effects to an appreciable extent. Application of silicate with arsenate elevated the nitrogen, nitrate, nitrite as well as amino acid contents in the test seedlings. Dissolved ammonia contents were lowered and there was a considerable enhancement in the activities of nitrate reductase, nitrite reductase, glutamine synthetase and glutamate synthase that helped to withstand the arsenate-imposed implications in the test seedlings and created conditions for better growth. Results obtained from our study therefore, points towards the positive influence of silicate amendments in ameliorating the detrimental consequences of arsenate imposition in wheat seedlings. Thus the use of silicate in suitable concentrations may provide an eco friendly as well as a low cost alternative to cultivate wheat in arsenic contaminated soil and might have encouraging outcomes in agricultural systems with appropriate field tryouts.

Acknowledgements The authors are grateful to the University Grants Commission, New Delhi, India; for financial assistance and Centre of Advanced Study, Department of Botany, University of Calcutta, India for infrastructural facilities in completion of the work. The authors also acknowledge the assistance of Prof. Uttam Bandopadhyay, Department of Statistics, University of Calcutta, India for regression analysis.

References

1. Abbas G, Murtaza B, Bibi I, Shahid M, Niazi NK, Khan MI, Amjad M, Hussain M, Natasha (2018) Arsenic uptake, toxicity, detoxification, and speciation in plants: physiological, biochemical, and molecular aspects. Int J Environ Res Public Health 15:59
2. Adrees M, Ali S, Rizwan M, Zia-ur-Rehman M, Ibrahim M, Abbas F, Farid M, Qayyum MF, Irshad MK (2015) Mechanisms of silicon-mediated alleviation of heavy metal toxicity in plants: a review. Ecotoxicol Environ Saf 119:186–197
3. Azmat R, Khan N (2011) Nitrogen metabolism as a bioindicator of Cu stress in *Vigna radiata*. Pak J Bot 43:515–520
4. Basu A, Saha D, Saha R, Ghosh T, Saha B (2014) A review on sources, toxicity and remediation technologies for removing arsenic from drinking water. Res Chem Intermed 40(2):447–485
5. Canovas FM, Canton FR, Gallardo F, Garcia-Gutierrez A, De Vincente A (1991) Accumulation of glutamine synthetase during early development of maritime pine (Pinus *pinaster*) seedlings. Planta 185:372–378
6. Cataldo DA, Haroon M, Schrader LE, Youngs VL (1975) Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commun Soil Sci Plant Anal 6:71–80
7. Chaffei C, Gouia H, Ghorbel Mohamed H (2003) Nitrogen metabolism in tomato plants under cadmium stress. J Plant Nutr 26(8): 1617–1634
8. Chen FL, Cullimore JV (1988) Two isoenzymes of NADH-dependent glutamate synthase in root nodules of *Phaseolus vulgare* L. purification, properties and activity changes during nodule development. Plant Physiol 88:1411–1417
9. Da silva AJ, Nascimento CW, Gouveia-Neto ADS, Silva Jr EA (2015) Effects of silicon on alleviating arsenic toxicity in maize plants. Rev Bras Ciênc Solo 39:289–296
10. Farooq MA, Islam A, Ali B, Najeeb U, Mao B, Gill RA, Yan G, Siddique KHM, Zhou W (2016) Arsenic toxicity in plants: cellular and molecular mechanisms of its transport and metabolism. Environ Exp Bot 132:42–52
11. Gangwar S, Singh VP (2011) Indole acetic acid differently changes growth and nitrogen metabolism in *Pisum sativum* L. seedlings under chromium (VI) phytotoxicity: implication of oxidative stress. Sci Hortic 129:321–328
12. Gao S, Liu KT, Chung TW, Chen T (2013) The effects of NaCl stress on *Jatropha* cotyledon growth and nitrogen metabolism. J Soil Sci Plant Nutr 13(1):99–113
13. Hageman RH, Reed AJ (1980) Nitrate reductase from higher plants. Methods Enzymol 69:270–280
14. Hoshida H, Tanaka Y, Hibino T, Hayashi Y, Tanaka A, Takabe T, Takabe T (2000) Enhanced tolerance to stress tolerance in transgenic rice that overexpresses chloroplast glutamine synthetase. Plant Mol Biol 43:103–111
15. Ismail GSM (2012) Protective role of nitric oxide against arsenic-induced damages in germinating mung bean seeds. Acta Physiol Plant 34:1303–1311
16. Jha AB, Dubey RS (2004) Arsenic exposure alters activity behaviour of key nitrogen assimilatory enzymes in growing rice plants. Plant Growth Regul 43:259–268
17. Kim YH, Khan AL, Kim DH, Lee SY, Kim KM, Waqas M et al (2014) Silicon mitigates heavy metal stress by regulating P-type heavy metal ATPases, Oryza sativa low silicon genes, and endogenous phytohormones. BMC Plant Biol 14:1
18. Kusano M, Fukushima A, Redestig H, Saito K (2011) Metabolomic approaches toward understanding nitrogen metabolism in plants. J Exp Bot 62:1439–1453

19. Lee YP, Takahashi T (1966) An improved colorimetric determination of amino acids with the use of ninhydrin. Anal Biochem 14:71–77
20. Lee SK, Sohn EY, Hamayun M, Yoon JY, Lee IJ (2010) Effect of silicon on growth and salinity stress of soybean plant grown under hydroponic system. Agrofor Syst 80:333–340
21. Li N, Wang J, Song WY (2015) Arsenic uptake and translocation in plants. Plant Cell Physiol 0:1–10
22. Liang CG, Chen LP, Wang Y, Liu J, Xu GL, Li T (2011) High temperature at grain-filling stage affects nitrogen metabolism enzyme activities in grains and grain nutritional quality in rice. Rice Sci 18:210–216
23. Liang Y, Nikolic M, Bélanger R, Gong H, Song A (2015) Silicon in agriculture. Springer, Dordrecht. https://doi.org/10.1007/978-94-017-9978-2
24. Lowry OM, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with folin phenol reagent. J Biol Chem 193:265–275
25. Ludewig U, Neuhäuser B, Dynowski M (2007) Molecular mechanisms of ammonium transport and accumulation in plants. FEBS Lett 581:2301–2308
26. Luyckx M, Hausman JF, Lutts S, Guerriero G (2017) Silicon and plants: current knowledge and technological perspectives. Front Plant Sci 8:411
27. Maaroufi Dguimi H, Debouba M, Ghorbel MH, Gouia H (2009) Tissue-specific cadmium accumulation and its effects on nitrogen metabolism in tobacco (*Nicotiana tabaccum, Bureley* v. Fb9). C R Biol 332:58–68
28. Mallick S, Sinam G, Sinha S (2011) Study on arsenate tolerant and sensitive cultivars of *Zea mays* L. differential detoxification mechanism and effect on nutrients status. Ecotoxicol Environ Saf 74(5): 1316–1324
29. Manivannan A, Ahn YK (2017) Silicon regulates potential genes involved in major physiological processes in plants to combat stress. Front Plant Sci 8:1346
30. Markovich O, Steiner E, Kouøil S, Tarkowski P, Aharoni A, Elbaum R (2017) Silicon promotes cytokinin biosynthesis and delays senescence in *Arabidopsis* and *Sorghum*. Plant Cell Environ 40:1189–1196. https://doi.org/10.1111/pce.12913
31. Martinez VD, Vucic EA, Becker-Santos DD, Gil L, Lam WL (2011) Arsenic exposure and the induction of human cancers. J Toxicol 2011:1–13
32. McCarty KM, Hanh HT, Kim KW (2011) Arsenic geochemistry and human health in South East Asia. Rev Environ Health 26(1): 71–78
33. Mendez JM, Vega JM (1981) Purification and molecular properties of nitrite reductase from *Anabaena* sp. 7119. Physiol Plant 52(1):7–14. https://doi.org/10.1111/j.1399-3054.1981.tb06026.x
34. Nazmul Huda AKM, Haque MA, Zaman R, Swaraz AM, Kabir AH (2017) Silicon ameliorates chromium toxicity through phytochelatin-mediated vacuolar sequestration in the roots of *Oryza sativa* (L.). Int J Phytoremediation 19(3):246–253
35. Neto LB, Paiva ALS, Machado RD, Arenhart RA, Pinheiro MM (2017) Interactions between plant hormones and heavy metals responses. Genet Mol Biol 40(10):373–386
36. Pontigo S, Godoy K, Jiménez H, Gutiérrez-Moraga A, Mora ML, Cartes P (2017) Silicon-mediated alleviation of aluminum toxicity by modulation of Al/Si uptake and antioxidant performance in ryegrass plants. Front Plant Sci 8. https://doi.org/10.3389/fpls.2017.00642
37. Rastogi A, Tripathi DKT, Yadav S, Chauhan DK, Živčak M, Ghorbanpour M, El-Sheery NI, Brestic M (2019) Application of silicon nanoparticles in agriculture. Biotech 9:90
38. Rodrigues da Cruz FJ, Da Silva Lobato AK, Lobo da Costa RC, Dos Santos Lopes MJ, Borges Neves HK, De Oliveira Neto CF, Lima da Silva MH, Dos Santos Filho BG, De Lima Jr JA, Okumura RS (2011) Aluminum negative impact on nitrate reductase activity, nitrogen compounds and morphological parameters in sorghum plants. Aust J Crop Sci 5(6):641–645
39. Saha J, Majumder B, Mumtaz B, Biswas AK (2017) Arsenic induced oxidative stress and thiol metabolism in two cultivars of rice and its possible reversal by phosphate. Acta Physiol Plant 39:263
40. Sil P, Das P, Biswas AK (2018) Silicon induced mitigation of TCA cycle and GABA synthesis in arsenic stressed wheat *(Triticum aestivum* L.) seedlings. S Afr J Bot 119:340–352
41. Sil P, Das P, Biswas S, Mazumdar A, Biswas AK (2019) Modulation of photosynthetic parameters, sugar metabolism, polyamine and ion contents by silicon amendments in wheat (*Triticum aestivum* L.) seedlings exposed to arsenic. Environ Sci Pollut Res. https://doi.org/10.1007/s11356-019-04896-7
42. Singh N, Ma LQ, Vu JC, Raj A (2009) Effects of arsenic on nitrate metabolism in arsenic hyperaccumulating and non-hyperaccumulating ferns. Environ Pollut 157:2300–2305
43. Singh M, Singh VP, Dubey G, Prasad SM (2015) Exogenous proline application ameliorates toxic effects of arsenate in *Solanum melongena* L. seedlings. Ecotoxicol Environ Saf 117:164–173
44. Singh AP, Dixit G, Kumar A, Mishra S, Singh PK, Dwivedi S, Trivedi PK, Chakrabarty D, Mallick S, Pandey V, Dhankher OP, Tripathi RD (2016) Nitric oxide alleviated arsenic toxicity by modulation of antioxidants and thiol metabolism in rice (Oryza sativa L.). Front Plant Sci 6:1272
45. Skopelitis DS, Paranychianakis NV, Paschalidis KA (2006) Abiotic stress generates ROS that signal expression of anionic glutamate dehydrogenases to form glutamate for proline synthesis in tobacco and grapevine. Plant Cell 18:2767–2781
46. Snell FD, Snell CT (1949) Colorimetric methods of analysis. Van Nostrand, New York
47. Song A, Li P, Fan F, Li Z, Liang Y (2014) The effect of silicon on photosynthesis and expression of its relevant genes in rice (Oryza sativa L.) under high-zinc stress. PLoS One 9:e113782
48. Song WY, Yang HC, Shao HB, Zheng AZ, Brestic M (2014) The alleviative effects of salicylic acid on the activities of catalase and superoxide dismutase in malting barley (*Hordeum vulgare* l.) seedling leaves stressed by heavy metals. Clean: Soil, Air, Water 42(1): 88–97
49. Sytar O, Kumari P, Yadav S, Brestic M, Rastogi A (2018) Phytohormone priming: regulator for heavy metal stress in plants. J Plant Growth Regul. https://doi.org/10.1007/s00344-018-9886-8
50. Tripathi DK, Singh VP, Prasad SM, Dubey NK, Chauhan DK, Rai AKV (2016) LIB spectroscopic and biochemical analysis to characterize lead toxicity alleviative nature of silicon in wheat (*Triticum aestivum* L.) seedling. J Photochem Photobiol B 154: 89–98
51. Vogel AI (1961) Colorimetric estimation of nitrogen by Nessler's reagent. In: a textbook of quantitative inorganic analysis. Langman and Green, India
52. Wang XP, Geng SJ, Ri YJ, Cao DH, Liu J, Shi DC, Yang CW (2011) Physiological responses and adaptive strategies of tomato plants to salt and alkali stresses. Sci Hortic 130:248–255

53. Wu X, Liu C, Qu C, Huang H, Liu X, Chen L, Su M, Hong F (2008) Influences of lead (II) chloride on the nitrogen metabolism of spinach. Biol Trace Elem Res 121:258–265
54. Zhang Y, Hu XH, Shi Y, Zou ZR, Yan F, Zhao YY, Zhang H, Zhao JZ (2013) Beneficial role of exogenous spermidine on nitrogen metabolism in tomato seedlings exposed to saline-alkaline stress. J Am Soc Hortic Sci 138(1):38–49
55. Zhang L, He X, Chen M, An R, An X, Li J (2014) Responses of nitrogen metabolism to copper stress in *Luffa cylindrica* roots. J Soil Sci Plant Nutr 14(3):616–624
56. Zhu YG, Geng CN, Tong YP, Smith SE, Smith FA (2006) Phosphate (Pi) and arsenate uptake by two wheat (*Triticum aestivum*) cultivars and their doubled haploid lines. Ann Bot 98(3):631–636
57. Zilli CG, Balestrasse KB, Yannarelli GG, Polizio AH, Santa- Cruz DM, Tomaro ML (2008) Heme oxygenase up-regulation under salt stress protects nitrogen metabolism in nodules of soybean plants. Environ Exp Bot 64:83–89