Comparative study of silicon and selenium to modulate chloroplast pigments levels, Hill activity, photosynthetic parameters and carbohydrate metabolism under arsenic stress in rice seedlings

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Abstract
Arsenic (As) in groundwater severely harms global economic development by affecting growth and productivity of agricultural crops that causes human health risk. The comparative influence of silicon (Si) and selenium (Se) to modulate pigments levels, photosynthetic parameters using LI-6400XT Portable Photosynthesis System and carbohydrate metabolism under arsenate (As-V) stress in rice cv. MTU-1010 were evaluated. As(V) stress significantly decreased chlorophyll-a (32% on an average), chlorophyll-b (58% on an average), total chlorophyll (46% on an average), fluorescence intensity (31% on an average), carotene (39% on an average), xanthophyll (33% on an average), Hill activity (47% on an average) and the photosynthetic parameters, viz. intercellular CO₂ concentration (52% on an average), net photosynthesis (54% on an average), transpiration rate (36% on an average) and stomatal conductance (38% on an average) in the test seedlings. As(V)+Si treatments enhanced the stated occurrences more than As(V)+Se treatments in rice seedlings. Sugar contents, viz. reducing (85% on an average) and non-reducing sugar (61% on an average), were increased, but starch content (57% on an average) was decreased in only As(V)-treated rice seedlings. The activities of carbohydrate metabolizing enzymes were increased, while sucrose synthase activity was decreased due to As(V) toxicity in the test seedlings. Co-application of Si and As(V) as well as Se and As(V) showed ameliorative effects on sugar and starch contents along with the activities of carbohydrate metabolizing enzymes, but more potential effect was observed under combined application of Si and As(V) in rice seedlings. Thus, it is an important purpose of this paper to compare the ability of Se and Si to alleviate As(V) toxicity in rice seedlings which will be an effective approach to develop possible strategies in As-contaminated agricultural soil to improve normal growth and productivity of rice plants.

Keywords Arsenic · Rice · Silicon · Selenium · Chlorophyll · Photosynthesis · Li-COR · Carbohydrate metabolism

Introduction
Arsenic is a carcinogenic metalloid with the symbol ‘As’ and atomic number ‘33’. This phytotoxic metalloid, found naturally in soils (Smedley and Kinniburgh, 2002) belonging to Group 15 or V-A in the periodic table, exists in four oxidation states: (−3), 0, (+3) and (+5). Arsenite (As-III) and arsenate (As-V) are the two predominant oxidation states (WHO, 2001; IARC, 2004) of which As(III) is more toxic than As(V) for both humans and plants (Zhao et al., 2009). As toxicity inhibits various kinds of physiological as well as biochemical metabolisms in plant cells among those most severe conditions may occur during photosynthesis (Anjum et al., 2017; Srivastava et al., 2013). Rice is the major crop for nutrition in Pan-Asianic countries consumed worldwide by humans. Rice cv. MTU-1010 possesses high yielding capacity, and extensive amount of groundwater is needed for crop irrigation. So, As could easily get accumulated in different tissues including grains of the test cultivar by As-polluted groundwater.

Silicon (Si), a trace element present in the soil, belongs to Group 14 or IV-A in the periodic table. Plants can absorb Si as uncharged silicic acid (H₄SiO₄). Si possesses the ability to reduce the stress-induced toxic effects during Al, Cd...
and Zn stress in rice (Singh et al., 2011). The Si mediated transporter, \textit{Lsi1}, is a member of NIP subfamily that helps to transport Si in plant cells (Mitani et al. 2008). There is another transporter \textit{Lsi2}, known to efflux Si/As(III) towards xylem in plants (Zhao et al. 2009). According to Wattanapanyakul et al. (2011), Si fertilization is beneficial for plants. Therefore, Si administration will be a low-cost approach to combat As-induced toxicity by lessening As uptake and accumulation within plant cells. In our previous study, we also reported that in the presence of Si, As accumulation was reduced in rice seedlings (Das et al. 2018).

Selenium (Se) is a trace element and a Group 16 or VI-A metalloid in the periodic table. Se prevails in nature as both inorganic forms, viz. selenate \((\text{SeO}_4^{2-})\), selenite \((\text{SeO}_3^{2-})\), selenide \((\text{Se}^2-)\) and elemental Se, and organic forms, viz. selenocysteine \((\text{SeCys})\) and selenomethionine \((\text{SeMet})\) (Wu et al., 2015). \text{SeO}_4^{2-}\ is the most ubiquitous form of bioavailable Se in agricultural fields as well as more water soluble than \text{SeO}_3^{2-}\ (Missana et al., 2009). In previous studies, it has been demonstrated that at low concentration \((0–5 \mu\text{M})\), Se has the ability to protect plants from various environmental stresses, viz. cold (Chu et al., 2010), drought (Hasanuzzaman and Fujita, 2011) and also metal stresses (Kumar et al., 2012; Pandey and Gupta, 2015). Generally, Se concentration lower than 1 mg kg\(^{-1}\) in soils can enhance plant growth in non-accumulating plants. Se increases plant resistance against oxidative stress caused by generation of free radicals in soybean (Djanaguiraman et al., 2005). As(V) uptake in \textit{Pteris vittata} was suppressed by the addition of Se, indicating the antagonistic effects of selenium on arsenate uptake (Feng et al., 2009). Se application in the form of fertilizer will be a low-cost way to reduce the toxicity in rice grown in arsenic-prone soil due to its antagonistic nature.

In chloroplast, chlorophylls operate photosynthesis by assimilating and converting light energy into chemical energy to drive all kinds of cellular activities. But the formation and activation of this pigment is suppressed under stress-induced oxidative difficulties in the environment (Agathokleous et al., 2020). There is another pigment; carotenoids provide defence to chlorophyll during biotic and abiotic stresses (Drążkiewicz and Baszyński 2010). They have photoprotective role and protect the photosynthetic machinery from the harmful effects of free radicals. As stress impedes the biogenesis of chlorophyll that let down the potency of PS-II and thus interrupts the photosynthesis process (Bankaji et al., 2014). This metalloid contamination restricts the stomatal gateway from entering the CO\(_2\) which is correlated with the depletion in transpiration rate as there is limitation of gas interchange from environment to plants (Milivojevic et al., 2006; Anjum et al., 2016). If the stomata have been closed for a long time, water levels and its potentiality are being enhanced which results in the reduction of photosynthesis (Ohashi et al., 2006). In some previous reports, it was demonstrated that Si application enhanced the rate of photosynthesis in wild-type rice plant (Sanglard et al., 2014). Sil et al. (2019) also reported that Si was able to improve photosynthetic activity under As stress in wheat seedlings. On the other side, Se reduced the generation of oxidative stress by alleviating malondialdehyde levels and elevated the photosynthetic activity as well as accumulation of sugars in \textit{Solanum tuberosum} L. cv. Sante under both As and Cd stresses (Shahid et al. 2019). Therefore, the present investigation is emphasized on the comparative effect of Si and Se to lessen As-induced toxicity on the basis of pigment levels, photosynthetic parameters and carbohydrate metabolism in hydroponically grown rice seedlings.

A seedling in flourishing stage generally faces endangered situations concerning instability of carbohydrates in the cells which is a perfect phase to examine the impact during biotic and abiotic stresses (Hanley and May, 2005). During photosynthesis, plants promote carbohydrate production which is carried out to different parts in the form of soluble sugars or stored in the form of starch and sugar. It was demonstrated in the report of Rosa et al. (2009) that the growth and development of plant seeds to seedlings stage fundamentally rely on carbohydrate storage that is transported to different plant parts from the repository organ which is essential to balance osmotic equilibrium in plants. Higher levels of photosynthetic products in plant tissue can maintain the proficiency of plants to revive them under various kinds of environmental stresses (Bagheri and Sadeghipour, 2009; Naureen and Naqvi, 2010). Accumulation of sugars under metal toxicity predominantly protects the concentration of amino acids, nucleic acids and proteins and also provides osmo-protection in the plant cell. Assimilated carbon produces sugar which can modulate toxicity level as well as gene regulation during environmental stresses (Lemoine et al., 2013). Joint application of As(V) with Si and As(V) with Se was an attempt to reduce the metal-induced toxic effects by improving pigment levels and photosynthetic parameters and regulating carbohydrate metabolism to develop tolerance against toxicity.

In the present study, we have documented how exogenous Si and Se amendments are individually potential to improve the chloroplast pigments level, photosynthetic parameters and carbohydrate metabolic processes in rice cv. MTU-1010 seedlings during As(V) application. The current study will assist to develop some techniques in soil condition which will be a low-cost way to cultivate rice with improved growth and yield in As-subdued agricultural soils.

Material and methods

Plant material and chemical treatments

Rice seeds, collected from the State Agricultural Rice Research Station, Chinsurah, Hooghly, West Bengal, were...
esterilized with 5% NaOCl solution and washed with distilled water. Fifty seeds for each treatment were spread over in Petri dishes (11 cm in diameter) lined with filter papers containing water and incubated at 37 °C for 3 days in the dark for germination (2 replicates for each treatment, 24 Petri dishes for root and 24 Petri dishes for shoot for each experiment). The germinated seeds were transferred to Petri dishes lined with filter papers containing 25-μM, 50-μM and 75-μM sodium arsenate (Na₅H₂AsO₄·7H₂O; Loba Chemie, India) solutions (w/v) with or without 2-mM sodium silicate (Na₂SiO₃·9H₂O; Loba Chemie, India) and 5-μM sodium selenate (Na₂SeO₄; Loba Chemie, India) along with modified Hoagland solution (pH 6.5) (Hoagland and Arnon, 1950) for 3 weeks between 25 and 28 °C temperature under 16-h photoperiod (260 μmol m⁻² s⁻¹ PFD). The control set was composed of only modified Hoagland solution. In every alternate day, fresh solutions were added in each treatment with pH adjustment. Then the seedlings were harvested, and roots and shoots were separated for the following studies.

**Estimation of chlorophyll contents and its fluorescence intensity**

Plant leaves of 1 g obtained from each treatment was chopped finely and extracted in 20 ml 80% (v/v) alkaline (0.1 M Na₂CO₃) acetone. The chlorophyll contents were estimated at 645 nm and 663 nm, respectively, and calculated by following the formula described by Arnon (1949). The fluorescence intensity of the chlorophyll was tracked at an excitation wavelength 640 nm and an emission wavelength 680 nm by using a Hitachi-650–40 spectrofluorometer and expressed as μg chlorophyll g⁻¹ FW.

\[
\text{Chlorophyll – a : } [(OD663 - OD645)xV/1000x1/W] \\
\text{Chlorophyll – b : } [(OD645 - OD663)xV/1000x1/W] \\
\text{Total chlorophyll : } [(OD645 + OD663)xV/1000x1/W]
\]

where

| OD | Optical density |
| V  | (Final volume of 80% alkaline acetone-chlorophyll extract) |
| W  | Fresh weight of leaves (here, 1 g) |

**Estimation of carotenoid contents**

Carotene and xanthophyll contents were measured by following the method of Davies (1965). Pigmented alkaline acetone solution was mixed with 20 ml cyclohexane in a separating funnel. The hexane layer was washed, and xanthophyll was separated from the upper hexane layer containing carotene by extracting it with 20 ml 90% (v/v) methanol. Carotene and xanthophyll contents were estimated at 425 nm and 450 nm, respectively. The said contents were expressed as optical density g⁻¹ FW.

**Estimation of Hill activity**

Hill activity was estimated by following the method of Vishniac (1957). Plant leaves of 1 g obtained from each treatment was crushed in 5 ml sucrose phosphate buffer (0.5 M sucrose in 0.05 M sodium phosphate buffer, pH 6.2) and centrifuged at 1000 g at 4 °C for 10 min. The supernatant was collected and re-centrifuged at 5000 g at 4 °C for 15 min. Chloroplast suspension was prepared by dissolving pellets obtained after centrifugation in 5 ml sucrose-phosphate buffer. Reaction mixture containing 1 ml chloroplast suspension, 4 ml sucrose phosphate buffer and 0.5 ml 0.03% DCPIP was kept under bright sunlight for 30 min after recording initial absorbance at 610 nm, followed by measuring the absorbance of discoloured solution. Differences in OD values were recorded. The activity was calculated from a standard curve prepared with known concentration of DCPIP and expressed as μg DCPIP reduced g⁻¹ chlorophyll h⁻¹.

**Estimation of photosynthetic parameters**

Photosynthetic parameters in terms of internal CO₂ concentration (Cᵢ), net photosynthesis (Pᵣ), transpiration rate (Tᵣ) and stomatal conductance (Gₛ) were estimated from intact fully stretched leaves by using Infrared Gas Analyzer (IRGA) portable photosynthetic system (Li-COR 6400, NE, USA) having an attached LED light source (6400-02B) (Biswas et al., 2013). The experiment was conducted between 8 and 11 am. The environmental conditions, viz. air temperature (25 °C), relative humidity (85%), PPFD (900 μmol mol⁻² s⁻¹) and CO₂ concentration (300 μmol mol⁻¹), were maintained throughout the experiment.

**Estimation of carbohydrate contents**

**Reducing and non-reducing sugar contents**

Reducing sugar contents was estimated according to Miller (1972). Plant samples of 1 g obtained from each treatment was crushed in 5 ml 80% (v/v) ethanol and centrifuged at 2000 g for 20 min. The supernatant was collected and mixed with 0.5 ml 1% DNSA (3,5-dinitrosalicylic acid) reagent. The mixture was kept in boiling water bath for 5 min. After incubation, the reducing sugar contents were measured at 515 nm. Reducing sugar content was calculated from a
standard curve of glucose and data was expressed as mg g\(^{-1}\) FW.

Total soluble sugar contents were estimated by phenol sulphuric acid reagent method according to Dubois et al. (1956). Plant sample of 1 g from each treatment was homogenized in 5 ml 80\% ethanol. It was centrifuged at 2000 g for 20 min. Reaction mixture containing 1 ml supernatant, 5 ml conc. \(\text{H}_2\text{SO}_4\) and 0.05 ml 5\% phenol solution was incubated in water bath at 30 °C for 20 min. A yellow-orange colour was developed in the solution and measured at 490 nm. The said content was calculated from a standard curve and expressed as mg g\(^{-1}\) FW. Non-reducing sugar contents were estimated by subtracting the value of reducing sugar from the values of total sugar and expressed as mg g\(^{-1}\) FW.

**Starch contents**

The remaining pellet obtained during the estimation of total soluble sugar was suspended in 2.5 ml distilled \(\text{H}_2\text{O}\) to measure starch contents (McCready et al., 1950). The resultant suspension was mixed with 3.25 ml 52\% \(\text{HClO}_4\) and stirred continuously. The mixture was centrifuged at 2000 g for 20 min and supernatant was collected. The final volume of supernatant was made 50 ml by adding distilled water and filtered by using Whatman (No. 42) filter paper. To estimate starch contents, 1 ml filtrate was taken and measured following the method used for total soluble sugar contents. The starch contents were estimated in terms of glucose and 0.9 factor was used for the conversion of glucose to starch. Data was expressed as mg g\(^{-1}\) FW.

**Assay of carbohydrate metabolizing enzymes**

**Sucrose synthase (SS; EC 2.4.1.13) and sucrose phosphate synthase (SPS; EC 2.4.1.14) activities**

For assay of SS and SPS activities, the plant sample of 1 g from each treatment was extracted according to Hubbard et al. (1989) and assayed by Miron and Schaffer (1991). A total of 1-g plant tissue was crushed in 50-mM HEPES–\(\text{NaOH}\) buffer (pH 7.5) comprising 1-mM EDTA, 5-mM \(\text{MgCl}_2\), 0.05\% (v/v) Triton-X-100 and 2.5-mM DTT. The homogenate was centrifuged at 10,000 rpm for 10 min at 4 °C. For the assay of SPS activity, reaction mixture was prepared using 50-mM HEPES–\(\text{NaOH}\) buffer (pH 7.5), 15-mM \(\text{MgCl}_2\), 25-mM fructose-6-phosphate, 25-mM glucose-6-phosphate, 25-mM UDP-glucose and 0.1 ml enzyme extract in a total volume of 1 ml. The reaction mix was incubated at 37 °C for 30 min. After incubation, the reaction was stopped by adding 30\% KOH. The composition of reaction mix to assay SS activity was same as described in SPS activity except it consisted of 25-mM fructose instead of fructose-6-phosphate and was devoid of glucose-6-phosphate. The sucrose formed during SPS catalysed reaction and sucrose hydrolysed during SS catalysed reaction were measured by using the method by Vassey et al. (1991). The activity of SPS and SS were expressed as \(\mu\)mol sucrose formed/ hydrolysed mg\(^{-1}\) protein min\(^{-1}\), respectively.

**Acid invertase (AI; EC 3.2.1.26) activity**

Acid invertase activity was measured according to Borkowska and Szczersza (1991). Plant sample of 1 g obtained from each treatment was crushed in 5 ml 10-mM sodium acetate buffer (pH 4.6) containing 3.3-mM \(\text{MgCl}_2\), 1-mM EDTA and 1-mM PMSF (phenylmethylsulfonyl fluoride), centrifuged at 10,000 rpm for 20 min at 4 °C. The composition of the reaction mixture was 10-mM sodium acetate buffer (pH 4.6), 0.4 M sucrose and 0.25 ml enzyme extract to make the final volume 1 ml. The reaction mix was incubated at 30 °C for 30 min and the reaction was stopped by adding 0.5 M \(\text{Na}_2\text{HPO}_4\). The reducing sugar produced was measured according to Nelson (1944) and Somogyi (1945). The enzyme activity was expressed as \(\mu\)mole sucrose hydrolysed mg\(^{-1}\) protein min\(^{-1}\).

**Starch phosphorylase (SP; EC 2.4.1.1) activity**

Starch phosphorylase activity was measured by following the method of Dubey and Singh (1999). Plant sample of 1 g from each treatment was homogenized in 5 ml 50-mM citrate buffer (pH 6) containing 1-mM EDTA, 5-mM \(\beta\)-mercaptoethanol and 1-mM PMSF, centrifuged at 10,000 rpm for 20 min at 4 °C. The composition of the reaction mixture was 50-mM citrate buffer (pH 6), 5\% soluble starch (w/v), 0.1-mM glucose-1-phosphate and 0.2 ml enzyme extract to make the final volume 1 ml. The reaction was terminated after 10 min by adding 5\% TCA. The mixture was centrifuged and the phosphoryorous contents in the supernatant were estimated according to Fiske and Subbarow (1925). The enzyme activity was expressed as \(\mu\)mole of Pi liberated mg\(^{-1}\) protein min\(^{-1}\).

**Protein estimation**

The protein contents of enzyme extracts were estimated according to Lowry et al. (1951). The reaction mixture was composed of 0.1 ml enzyme extract, 0.9 ml distilled water and 5 ml Lowry solution and kept for 10 min at room temperature. Then 0.5 ml Folin-Ciocalteu reagent was added to it and incubated for 20 min in the dark. The absorbance was recorded at 660 nm and calculated from a standard curve prepared using bovine serum albumin (BSA) as standard.
Statistical analysis

All the experimental values were means from three independent experiments, each with two replicates in each treatment and the results demonstrated as means ± SE. Statistical analysis was performed by using SPSS software (PASW Statistics 18: Version 18.0.0.282). The correlation (r) coefficient values were predicted by using Pearson’s correlation analysis. Different treatment effects, viz. As and/or Si application, including control, were compared by testing the significance of their mean differences via Tukey’s HSD (honest significant difference) tests from ANOVA showed that the said effect was statistically significant in chlorophyll-a with p value 0.034 and in chlorophyll-b with p value 0.012 under only 75 µM As(V) treatment. The reduction in total chlorophyll contents was statistically significant under 50 µM, 75 µM and Se + 75 µM As(V) treatments with p values 0.028, 0.001 and 0.029, respectively. The consequence of fluorescence intensity was significant under 50-µM, 75-µM As(V) and Se + 75-µM As(V) treatments with p = 0.006, p < 0.001 and p = 0.007, respectively.

Results

Influence of As(V) with or without Si and Se on pigment contents

Chlorophyll contents and fluorescence intensity

The levels of chlorophyll-a, chlorophyll-b, total chlorophyll and fluorescence intensity of chlorophylls were linearly decreased in As(V)-treated rice seedlings over control. Upon Si and Se application in As(V)-treated seedlings, the chlorophyll contents and fluorescence intensity were increased than As(V) treatments alone. Under 25-µM, 50-µM and 75-µM As(V) treatments, chlorophyll contents were decreased, viz. chlorophyll-a by about 24%, 30% and 42%; chlorophyll-b by about 50%, 53% and 73%; total chlorophyll by about 38%, 42% and 59%; and fluorescence intensity of chlorophyll by about 13%, 31% and 51%, respectively, over control (Table 1). During co-application of As(V) and Si, chlorophyll-a contents decreased by about 11%, 15% and 19%; chlorophyll-b contents decreased by about 29%, 31% and 39%; total chlorophyll contents reduced by about 21%, 24% and 30%; the fluorescence intensity of chlorophyll decreased by about only 2%, 10% and 16%, respectively, over control (Table 1). Se supplementation in As(V)-treated seedlings also reduced the chlorophyll contents along with the fluorescence intensity but less than As(V) treatments alone and improvement was less than As(V) + Si–treated seedlings. Under combined application with As(V) and Se, chlorophyll contents decreased, viz. chl-a by about 16%, 22% and 32%; chl-b by about 39%, 43% and 51%; total chlorophyll by about 28%, 33% and 42%; and the fluorescence intensity of chlorophylls by about 8%, 21% and 30%, respectively, over control (Table 1). The multiple comparison analysis using Tukey’s HSD (honest significant difference) tests from ANOVA showed that the said effect was statistically significant in chlorophyll-a with p value 0.034 and in chlorophyll-b with p value 0.012 under only 75 µM As(V) treatment. The reduction in total chlorophyll contents was statistically significant under 50 µM, 75 µM and Se + 75 µM As(V) treatments with p values 0.028, 0.001 and 0.029, respectively. The consequence of fluorescence intensity was significant under 50-µM, 75-µM As(V) and Se + 75-µM As(V) treatments with p = 0.006, p < 0.001 and p = 0.007, respectively.

Carotenoid contents

The carotene and xanthophyll contents were linearly declined in As(V)-treated test seedlings, while upon Si and Se applications in As(V)-treated test seedlings, the said contents were increased than As(V) treatments alone. Carotene contents were decreased by about 25%, 32% and 59% (Table 1), whereas xanthophyll contents were decreased by about 19%, 28% and 52% (Table 1) under 25-µM, 50-µM and 75-µM As(V) treatments, respectively, over control. During joint application of As(V) and Si, both carotene and xanthophyll contents were decreased but less than As(V) treatment alone that were by about 8%, 16% and 20% for carotene and by about 3%, 10% and 23% for xanthophyll, respectively, over control. Se supplementation in As(V)-treated seedlings, both carotene and xanthophyll contents, were reduced but less than only As(V) treatment and not less than in As(V) + Si–treated seedlings. Thus, under co-application of As(V) and Se, carotene contents were reduced by about 19%, 24% and 31% while xanthophyll contents were reduced by about 13%, 17% and 35%, respectively, over control. The depletion was significant in carotene under 50-µM (p < 0.006), 75-µM (p < 0.001) and Se + 75-µM (p = 0.009) As(V) treatments and in xanthophyll only under 75-µM As(V) treatments with p value 0.004.

Influence of As(V) with or without Si and Se on Hill activity

Hill activity was reduced in response to As(V) treatments in the test seedlings. The rates of reduction in the said activity were about 38%, 43% and 59% under 25-µM, 50-µM and 75-µM As(V) treatments, respectively, over control, whereas under co-application of Si + As(V), the said reduction was altered and much less decrease in Hill activity was recorded that were only 3%, 7% and 11%, respectively, over control. Se supplementation in As(V)-treated seedlings also reduced the said activity but more than As(V) + Si treatments. During joint application of Se and As(V), Hill activity was
Table 1  Influence of increasing concentrations of As(V) with or without Si and Se on chlorophyll contents, fluorescence intensity of chlorophyll and carotenoid contents in 3-week-old rice seedlings. Multiple comparisons of the means ± SE (n = 6, i.e. three experiments with two replicates each) were conducted with the Tukey–Kramer HSD test. Treatments having significantly different effects (at 5% level) are marked by different alphabets.

| Treatments          | Xanthophyll (A<sub>450</sub> g<sup>-1</sup> FW) | Carotene (A<sub>425</sub> g<sup>-1</sup> FW) | Fluorescence intensity (arbitrary units) | Total chlorophyll mg g<sup>-1</sup> FW | Chlorophyll-a (mg g<sup>-1</sup> FW) | Chlorophyll-b (mg g<sup>-1</sup> FW) | Treatments          |
|---------------------|-----------------------------------------------|---------------------------------------------|----------------------------------------|----------------------------------------|----------------------------------------|----------------------------------------|---------------------|
| Control             | 1.143 ± 0.062<sup>a</sup>                      | 2.306 ± 0.136<sup>a</sup>                   | 81.64 ± 2.25<sup>b</sup>               | 0.199 ± 0.020<sup>b</sup>              | 0.111 ± 0.014<sup>a</sup>              | 0.089 ± 0.007<sup>a</sup>               | Control             |
| 2-mM Si             | 1.166 ± 0.250<sup>b</sup>                      | 2.391 ± 0.069<sup>a</sup>                   | 85.69 ± 3.2<sup>d</sup>                | 0.206 ± 0.001<sup>b</sup>              | 0.116 ± 0.006<sup>a</sup>              | 0.091 ± 0.006<sup>a</sup>               | 2-mM Si             |
| 5-µM Se             | 1.160 ± 0.138<sup>b</sup>                      | 2.338 ± 0.092<sup>b</sup>                   | 82.49 ± 4.32<sup>b</sup>               | 0.199 ± 0.003<sup>b</sup>              | 0.108 ± 0.007<sup>b</sup>              | 0.090 ± 0.007<sup>b</sup>               | 5-µM Se             |
| Si + 25-µM As(V)    | 0.931 ± 0.032<sup>b</sup>                      | 1.727 ± 0.128<sup>b</sup>                   | 71.43 ± 5.96<sup>c</sup>               | 0.123 ± 0.015<sup>b</sup>              | 0.056 ± 0.016<sup>b</sup>              | 0.068 ± 0.007<sup>b</sup>               | Si + 25-µM As(V)    |
| Se + 25-µM As(V)    | 1.11 ± 0.072<sup>b</sup>                       | 2.125 ± 0.099<sup>b</sup>                   | 80.34 ± 1.47<sup>c</sup>               | 0.158 ± 0.016<sup>b</sup>              | 0.079 ± 0.012<sup>c</sup>              | 0.079 ± 0.009<sup>c</sup>               | Se + 25-µM As(V)    |
| Se + 50-µM As(V)    | 0.993 ± 0.015<sup>b</sup>                      | 1.874 ± 0.073<sup>b</sup>                   | 74.95 ± 1.56<sup>c</sup>               | 0.142 ± 0.015<sup>b</sup>              | 0.053 ± 0.013<sup>b</sup>              | 0.074 ± 0.006<sup>c</sup>               | Se + 50-µM As(V)    |
| Si + 50-µM As(V)    | 0.827 ± 0.022<sup>b</sup>                      | 1.560 ± 0.211<sup>b</sup>                   | 56.70 ± 6.50<sup>g</sup>               | 0.114 ± 0.016<sup>b</sup>              | 0.068 ± 0.017<sup>c</sup>              | 0.062 ± 0.005<sup>b</sup>               | Si + 50-µM As(V)    |
| Se + 75-µM As(V)    | 1.033 ± 0.041<sup>b</sup>                      | 1.938 ± 0.072<sup>c</sup>                   | 73.41 ± 4.22<sup>c</sup>               | 0.152 ± 0.017<sup>d</sup>              | 0.076 ± 0.014<sup>d</sup>              | 0.075 ± 0.008<sup>c</sup>               | Se + 75-µM As(V)    |
| 75 µM-As(V)         | 0.946 ± 0.045<sup>b</sup>                      | 1.764 ± 0.129<sup>c</sup>                   | 64.36 ± 1.36<sup>b</sup>               | 0.133 ± 0.014<sup>d</sup>              | 0.064 ± 0.013<sup>e</sup>              | 0.070 ± 0.005<sup>d</sup>               | 75 µM-As(V)         |
| Si + 75-µM As(V)    | 0.546 ± 0.031<sup>b</sup>                      | 0.956 ± 0.050<sup>b</sup>                   | 40.23 ± 5.11<sup>e</sup>               | 0.082 ± 0.023<sup>e</sup>              | 0.031 ± 0.018<sup>d</sup>              | 0.052 ± 0.008<sup>b</sup>               | Si + 75-µM As(V)    |
| Se + 75 µM As(V)    | 0.880 ± 0.032<sup>b</sup>                      | 1.851 ± 0.123<sup>f</sup>                   | 68.90 ± 3.30<sup>c</sup>               | 0.140 ± 0.015<sup>f</sup>              | 0.068 ± 0.009<sup>e</sup>              | 0.072 ± 0.009<sup>d</sup>               | Se + 75 µM As(V)    |
Influence of As(V) with or without Si and Se on photosynthetic parameters

Internal CO₂ concentration

Internal CO₂ concentration was adversely affected under As(V) stress and decreased by about 48%, 50% and 59% in 25-µM, 50-µM and 75-µM As(V) treatments, respectively, over control. In case of As(V) + Si and As(V) + Se treatments, the said level was increased than As(V) treatments alone that were clearly observed with less reduction over control (Fig. 1b). The said concentration was reduced under As(V) + Si treatment, by about 16%, 24% and 27%, respectively, over control. Se supplementation in As(V)-treated rice seedlings reduced the said concentration but more than As(V) + Si–treated seedlings that were by about 21%, 30% and 38%, respectively, over control. Tukey’s HSD multiple comparisons analysis showed that the rate of reduction was significant under 25-µM (p = 0.043), 50-µM (p = 0.026) and 75-µM (p = 0.006) As(V) treatments in the test cultivar.

Net photosynthesis

Net photosynthesis rate was also hampered in the test seedlings under As(V) stress. It was linearly decreased by about 43%, 54% and 66% in 25-µM, 50-µM and 75 µM As(V) treatments, respectively, over control. During joint application with Si and As(V), decrease in net photosynthesis was less than As(V) treatment alone that were by about 5%, 10% and 21%, respectively, over control. When Se was applied in As(V)-treated test seedlings, the net photosynthesis rate was also reduced but more than As(V) + Si–treated seedlings that were by about 16%, 30% and 36%, respectively, over control (Fig. 1c). The rate of reduction was statistically significant only under 75-µM As(V) treatment with p value 0.025.

Transpiration rate

Transpiration rate of the test seedlings decreased by As(V) application. The rates of reduction were about 24%, 33% and 50% in 25-µM, 50-µM and 75-µM As(V) treatments, respectively, over control. During joint application with Si and As(V), rates of transpiration were reduced less than As(V) treatments alone that were by about 7%, 14% and 23%, respectively, over control. During co-application with As(V) and Se, the transpiration rate was reduced more than As(V) + Si treatment but less than only As(V)-treated seedlings that were by about 14%, 26% and 32%, respectively, over control (Fig. 1d). The effect was highly significant under 75 µM As(V) treatment with p value 0.007.

Stomatal conductance

The stomatal conductance was decreased by about 21%, 40% and 52% in 25-µM, 50-µM and 75-µM As(V)-treated seedlings, respectively, over control. During joint application with Si and As(V), the rate of stomatal conductance was increased than As(V) treatments alone that were by about 3%, 12% and 21%, respectively, over control. During joint application with Se and As(V), the stomatal conductance was decreased than As(V) + Si treatment but less than only As(V) treatments that were by about 13%, 21% and 33%, respectively, over control (Fig. 1e). The effect was statistically significant under 75-µM As(V) treatment with p value 0.017.

Influence of As(V) with or without Si and Se on reducing, non-reducing and total soluble sugar contents

Reducing sugar

Reducing sugar contents was increased in both root and shoot of the test seedlings. In root, the said contents were increased by about 68%, 86% and 93%, while in shoot they were increased by about 70%, 92% and 99% under 25-µM, 50-µM and 75-µM As(V) treatments, respectively, over control. The reducing sugar contents were increased more in shoot than root. During joint application with Si and As(V), the said contents were increased less than As(V) treatments alone that were by about 10%, 28% and 40% in root and 15%, 29% and 43% in shoot under Si and said concentrations of As(V) treatments, respectively, over control (Table 2). Se supplementation in As(V)-treated test seedlings enhanced the reducing sugar contents by about 40%, 61% and 71% in root and 41%, 63% and 77% in shoot, respectively, over control (Table 2). The increments were significant under 25-µM (p = 0.031 in root and 0.008 in shoot), 50-µM (p = 0.003 in root and < 0.001 in shoot), 75-µM (p = 0.001 in root and < 0.001 in shoot), Se + 50-µM (p = 0.021 in shoot) and Se + 75-µM (p = 0.022 in root and 0.003 in shoot) As(V) treatments in the test cultivar.

Non-reducing sugar

Non-reducing sugar contents were also increased in both root and shoot of the test seedlings. In root, non-reducing sugar contents were increased by about 36%, 50% and 73%, while in shoot they were increased by about 49%, 75% and 85% under 25-µM, 50-µM and 75-µM As(V) treatments, respectively.
treatments, respectively, over control. During joint application with Si and As(V), the said contents were increased less than As(V) treatments alone. The rates of increments were by about 9%, 14% and 22% in root and 17%, 24% and 33% in shoot under Si and said concentrations of As(V) treatments, respectively, over control (Table 2). During

Fig. 1 Influence of increasing concentrations of As(V) with or without Si and Se on Hill activity (a) and photosynthetic parameters, viz. internal CO₂ concentration (b), net photosynthesis (c), transpiration rate (d) and stomatal conductance (e) in 3-week-old rice seedlings. Multiple comparisons of the means ± SE (n = 6, i.e. three experiments with two replicates each) were conducted with the Tukey–Kramer HSD test. Treatments having significantly different effects (at 5% level) are marked by different alphabets.
Table 2 Influence of increasing concentrations of As(V) with or without Si and Se on reducing sugar, non-reducing sugar and starch contents in 3-week-old rice seedlings. Multiple comparisons of the means \( \pm SE \) (\( n = 6 \), i.e. three experiments with two replicates each) were conducted with the Tukey–Kramer HSD test. Treatments having significantly different effects (at 5% level) are marked by different alphabets, separately for root and shoot samples.

| Treatments       | Reducing sugar (mg g\(^{-1}\) FW) | Non-reducing sugar (mg g\(^{-1}\) FW) | Starch (mg g\(^{-1}\) FW) |
|------------------|-----------------------------------|--------------------------------------|--------------------------|
|                  | Root                               | Shoot                               | Root                     | Shoot                   |
| Control          | 0.52 ± 0.04\(^d\)                 | 0.77 ± 0.05\(^d\)                  | 4.86 ± 0.19\(^c\)       | 7.83 ± 0.97\(^b\)      |
| 2-mM Si          | 0.56 ± 0.05\(^d\)                 | 0.82 ± 0.13\(^d\)                  | 4.98 ± 0.84\(^c\)       | 8.26 ± 2.04\(^b\)      |
| 5-µM Se          | 0.57 ± 0.06\(^bc\)                | 0.79 ± 0.08\(^c\)                  | 4.97 ± 0.41\(^c\)       | 8.29 ± 1.63\(^b\)      |
| 25-µM As(V)      | 0.87 ± 0.08\(^abc\)               | 1.30 ± 0.07\(^abc\)                | 6.60 ± 0.19\(^abc\)     | 11.64 ± 1.49\(^ab\)    |
| Si + 25-µM As(V) | 0.57 ± 0.023\(^ed\)               | 0.88 ± 0.06\(^d\)                  | 5.29 ± 0.18\(^bc\)      | 9.16 ± 0.71\(^ab\)     |
| Se + 25-µM As(V) | 0.73 ± 0.045\(^abc\)              | 1.08 ± 0.12\(^bc\)                 | 5.94 ± 0.26\(^bc\)      | 10.25 ± 0.10\(^ab\)    |
| 50-µM As(V)      | 0.96 ± 0.10\(^b\)                 | 1.47 ± 0.08\(^b\)                  | 7.31 ± 0.52\(^ab\)      | 13.71 ± 1.43\(^ab\)    |
| Si + 50-µM As(V) | 0.66 ± 0.035\(^bcd\)              | 0.99 ± 0.05\(^d\)                  | 5.52 ± 0.25\(^bc\)      | 9.70 ± 0.88\(^ab\)     |
| Se + 50-µM As(V) | 0.83 ± 0.08\(^abc\)               | 1.25 ± 0.09\(^ab\)                 | 6.16 ± 0.26\(^bc\)      | 11.21 ± 0.68\(^ab\)    |
| 75-µM As(V)      | 1.00 ± 0.10\(^a\)                 | 1.53 ± 0.09\(^a\)                  | 8.40 ± 0.67\(^a\)       | 14.47 ± 1.38\(^a\)     |
| Si + 75-µM As(V) | 0.73 ± 0.039\(^bcd\)              | 1.09 ± 0.09\(^bcd\)                | 5.94 ± 0.40\(^abc\)     | 10.40 ± 0.87\(^ab\)    |
| Se + 75-µM As(V) | 0.88 ± 0.08\(^ab\)                | 1.36 ± 0.08\(^ab\)                 | 6.91 ± 0.09\(^ab\)      | 11.91 ± 0.69\(^ab\)    |

Total soluble sugar

Total soluble sugar contents were enhanced in both root and shoot under As(V) treatments. In root, the said contents were increased by about 39%, 54% and 75%, while in shoot they were increased by about 51%, 77% and 86% under 25-µM, 50-µM and 75-µM As(V) treatments, respectively, over control. During joint application with Si and As(V), the total sugar contents were increased less than As(V) treatments alone. The rates of increments were by about 9%, 15% and 24% in root and 17%, 24% and 34% in shoot under Si and said concentrations of As(V) treatments, respectively, over control. When Se was applied in As(V)-treated test seedlings, the said contents were increased more than Si + As(V) treatments but increased less than As(V) treatments alone that were by about 24%, 30% and 45% in root and 32%, 45% and 54% in shoot, respectively, over control. The effect was significant under 25-µM (\( p = 0.036 \) in root), 50-µM (\( p = 0.001 \) in root and 0.036 in shoot), 75-µM (\( p = 0.001 \) in root and 0.013 in shoot) and Se + 75-µM (\( p = 0.010 \) in root) As(V) treatments in the test cultivar.

Influence of As(V) with or without Si and Se on starch contents

Starch contents were decreased in both root and shoot of the test seedlings under As(V) stress. Starch contents were decreased by about 46%, 52% and 62% in root and by about 55%, 60% and 69% in shoot under 25-µM, 50-µM and 75-µM As(V) treatments, respectively, over control. During joint application with Si and As(V), the starch contents were decreased less than As(V) treatments alone and rates of reductions were by about 4%, 12% and 21% in root and 25%, 29% and 32% in shoot, respectively, over control (Table 2). During Se supplementation in As(V) exposed test seedlings, the said levels were decreased by about 19%, 28% and 33% in root and 39%, 42% and 46% in shoot, respectively, over control (Table 2). The effect was significant under 25-µM (\( p = 0.001 \) in shoot), 50-µM (\( p = 0.044 \) in root and < 0.001 in shoot), 75-µM (\( p = 0.009 \) in root and < 0.001 in shoot), Se + 25-µM (\( p = 0.034 \) in shoot), Se + 50-µM (\( p = 0.017 \) in shoot) and Se + 75-µM (\( p = 0.007 \) in shoot) As(V) treatments in the test cultivar.

Influence As(V) with or without Si and Se on carbohydrate metabolizing enzymes

Sucrose synthesizing enzyme

Sucrose phosphate synthase (SPS; EC 2.4.1.14) activity SPS activity was increased in both root and shoot under As(V) stress in rice seedlings. The enzyme activity was significantly increased under As(V) treatments alone. In root, the  

\[ \text{Influence of As(V) with or without Si and Se on carbohydrate metabolizing enzymes} \]

Sucrose synthesizing enzyme

Sucrose phosphate synthase (SPS; EC 2.4.1.14) activity SPS activity was increased in both root and shoot under As(V) stress in rice seedlings. The enzyme activity was significantly increased under As(V) treatments alone. In root, the
SPS activity was increased by about 28%, 53% and 76%, while in shoot the said activity was increased by about 46%, 66% and 92% under 25-µM, 50-µM and 75-µM As(V) treatments, respectively, over control. During joint application with Si and As(V), the said activity was increased less than As(V) treatments alone. The rates of increments were about 10%, 13% and 16% in root and 10%, 21% and 31% in shoot, respectively, over control. Under Se and different concentrations of As(V), the enzyme activity was increased less than As(V) treatments alone but increased more than Si + As(V) treatments that were by about 16%, 30% and 34% in root and 26%, 36% and 52% in shoot, respectively, over control (Fig. 2).

Sucrose hydrolysing enzymes

Sucrose synthase (SS; EC 2.4.1.13) activity SS activity was decreased in both root and shoot of the test seedlings under As(V) stress. In root, the enzyme activity was decreased by about 39%, 44% and 69%, while in shoot it was decreased by about 42%, 54% and 76% under 25-µM, 50-µM and 75-µM As(V) treatments, respectively, over control. During joint application with Si and increasing concentrations of As(V), the enzyme activity was decreased but less than As(V) treatments alone that were by about 9%, 16% and 27% in root and 13%, 22% and 35% in shoot, respectively, over control. SS activity was also decreased less than As(V)-treated test seedlings that were about 14%, 34% and 37% in root and 23%, 27% and 45% in shoot under Se and different concentrations of As(V) treatments, respectively, over control (Fig. 3).

Acid invertase (AI; EC 3.2.1.26) activity AI activity was increased in both root and shoot under As(V) stress in rice seedlings. In root, the enzyme activity was increased by about 33%, 51% and 69%, while in shoot it was increased by about 43%, 72% and 92% under 25-µM, 50-µM and 75-µM As(V) treatments, respectively, over control. During joint application with Si and As(V), the enzyme activity was increased less than As(V) treatments alone that were by about 9%, 15% and 23% in root and 10%, 21% and 30% in shoot, respectively, over control. AI activity was increased more under Se supplementation in As(V) treatments as compared to As(V) + Si–treated seedlings. The rate of increments were by about 14%, 25% and 38% in root and 21%, 34% and 42% in shoot under Se and different concentrations of As(V), respectively, over control (Fig. 4).

Starch hydrolysing enzymes

Starch phosphorylase (SP; EC 2.4.1.1) activity SP activity was increased under As(V) treatments in both root and shoot of the test seedlings. In root, the enzyme activity was increased by about 46%, 60% and 73%, while in shoot, it was increased by about 52%, 78% and 90% under 25-µM, 50-µM and 75-µM As(V) treatments, respectively, over control. During joint application with both Si + As(V) and Se + As(V), the said activity was increased less than As(V) treatments alone but the enzyme activity was increased more in Se + As(V) treatments than Si + As(V)–treated seedlings. The rate of increments were about 14%, 21% and 27% in root and 23%, 28% and 35% in shoot under Si and different concentrations of As(V) treatments, respectively, over control.
The enzyme activity was also increased less than only As(V) treatments that were about 27%, 36% and 45% in root and 29%, 48% and 57% in shoot under Se + As(V) treatments, respectively, over control in the test cultivar (Fig. 5).

**Interrelations between the experimental factors**

The Pearson correlations between different experimental factors, in presence of As(V) and/or Si and Se, are presented in Fig. 6 as a heatmap; see Table S1 in the supplementary file for the correlation values along with $p$ values for their significance. A strong positive correlation was observed among chlorophyll contents, carotenoid contents, Hill activity and all the photosynthetic parameters. In the present study, chlorophyll-a ($r = 0.893$), chlorophyll-b ($r = 0.605$) and total chlorophyll ($r = 0.764$) were positively correlated with internal CO$_2$ concentration, and correlation was significant at $p < 0.001$ in all cases. Fluorescence intensity was also positively correlated with total chlorophyll contents ($r = 0.700$, $p < 0.001$). The latter one was positively correlated with
The net photosynthesis rate at the significance level \( p = 0.005 \). The rate of photosynthesis showed high positive correlation with transpiration rate \( (r = 0.813, p < 0.001) \) and stomatal conductance \( (r = 0.806, p < 0.001) \); the latter two were also highly correlated among themselves \( (r = 0.963) \) with \( p < 0.001 \).

There were a highly positive correlation of total soluble sugar with reducing \( (r = 0.910, p < 0.001) \) and non-reducing sugar \( (r = 0.999, p < 0.001) \) contents. But starch contents were negatively correlated with reducing sugar \( (r = -0.814, p < 0.001) \), non-reducing sugar \( (r = -0.812, p < 0.001) \) and total soluble sugar contents \( (r = -0.821, p < 0.001) \). Besides these, sucrose phosphate synthase (SPS) activity was positively correlated with non-reducing sugar with the correlation being significant at \( p = 0.019 \). But sucrose synthase (SS) activity was negatively correlated with non-reducing sugar contents \( (r = -0.500, p = 0.002) \). A negative correlation also occurred between starch contents and starch phosphorylase activity \( (r = -0.613, p < 0.001) \).

**Comparison of Si- and Se-based treatment effects**

For Si supplementation in As(V)-treated rice seedlings, the significance of corresponding treatments effects on individual parameters were obtained by performing two-way ANOVA as per our experimental design. The resulting \( p \) values associated with the main effects of Si in the tests of between-subject effects indicated the strength of the treatment effects. The same was also obtained for Se supplementation, and the \( p \) values obtained in both the cases were reported in Table 3 for root and shoot separately. These were used to compare the treatment effects of Si and Se supplementation; the results clearly showed significantly stronger effects of Si in ameliorating As(V)-induced toxicity as compared to Se. In particular, the treatment effects of Se supplementation on chlorophyll-a, chlorophyll-b, total chlorophyll, fluorescence intensity of the chlorophyll, Hill activity, transpiration rate, stomatal conductance, non-reducing sugar, total soluble sugar and carbohydrate metabolizing enzymes were not at all statistically significant at 95% level, whereas the effects of Si supplementation are highly significant on all the parameters (except only for sucrose synthase, acid invertase and starch phosphorylase in shoot).

**Discussion**

**Influence on chloroplast pigments, Hill activity and photosynthetic parameters**

Chlorophylls are accountable for photosynthesis by absorbing lights and transforming it into chemical energy leading to subsequent ATP formation (Zhou et al., 2018). If there is any deviation in chlorophyll levels, it directly affects the photosynthesis rate and plant growth (Zhang et al., 2011). It has already been noted in the literature that, during As(III) and As(V) accumulation in different rice cultivars, increase in the ROS production and deficiency in various enzymatic and non-enzymatic antioxidant activities lead to the generation of oxidative stress (Das et al., 2018; Majumder et al., 2018, 2019). Das et al. (2018) also demonstrated that accumulation of As(V) and As(III) decreased the growth and
biomass contents in rice seedlings. In the present study, chlorophyll-a, chlorophyll-b, total chlorophyll contents along with fluorescence intensity, i.e. photosynthetic energy conversion indicator; carotenoid content, viz. carotene and xanthophyll; Hill activity in terms of photolysis of water; and all photosynthetic parameters, viz. internal CO₂ concentration, net photosynthesis, transpiration rate and stomatal conductance were significantly decreased under all concentrations of As(V) in the rice seedlings (Table 1). The reductions in the said parameters were due to the generation of ROS under As(V) stress that disrupts all kinds of physiological, biochemical and metabolic activities in plant cells. Chlorophyll contents were also decreased under Cu stress in bamboo (Jiang et al., 2013). This reduction in pigment contents occurs mainly by the reduced synthesis of PBG (porphobilinogen), an intermediate compound for chlorophyll biosynthesis (Cenkci et al., 2010). According to Padmaja et al. (1990), the reduction in chloroplast pigments during As toxicity apparently obstructed the δ-aminolevulinic acid dehydratase activity and protochlorophyllide reductase activity which induce the chlorophyll formation in plant cell. As stress decreased the chlorophyll fluorescence, efficiency of photosynthesis in lettuce (Gusman et al., 2013) and potentiality of photosystem-II in *Spartina densiflora* (Mateos-Naranjo et al., 2012). According to Dresler et al. (2014), the depletion in total chlorophyll contents along with

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**Fig. 6** Heatmap of the correlations among different experimental factors
the enhancement in chlorophyll a/b ratio was the results of enzyme inactivation required for the synthesis of chlorophyll pigments under Cd and Cu stresses in Zea mays seedlings which is in accordance with our study where chlorophyll a/b ratio was enhanced under As stress in rice seedlings (Tables 1 and 4). During the applications of As(V) + Si and As(V) + Se, the chlorophyll a/b ratios were decreased indicating the diminution of stress in the test seedlings. Similar fact was also observed in Ocimum basilicum under Cu, Ni and Zn stresses where chlorophyll levels were reduced triggering low quantum yield (Georgiadou et al., 2018).

Carotenoids are the non-enzymatic antioxidant that provides defence towards chlorophylls against oxidative damage (Kenneth et al., 2000; Hou et al., 2007; Sharma et al., 2012). In our study, carotenoid levels were reduced under As(V) stress in rice seedlings (Table 1). This observation was supported by Rastgoo and Alemzadeh (2011) in Aeluropus littoralis under Ag, Cd, Co and Pb stresses. In the study of Zhou et al. (2018), it was also reported that carotenoid levels were reduced in Acorus calamus during Sb stress. They reported that there is a positive correlation between the reduction of carotenoid levels and the less activity of photosystem-II due to lack of electron transfer under stressed condition.

Hill activity, i.e. photolysis of water, was also decreased under As(V) stress. Both Si and Se administrations increased the said activity as compared to As(V) treatments alone. But As(V) + Si treatments enhanced the Hill activity more than As(V) + Se treatments in the test cultivar (Fig. 1). In the report of Yang et al. (2009), it has been demonstrated that the demotion in Hill activity affected phosphorylation, reduced NADP+ and suppressed the concentration of CO2 in the seedlings of Cucumis sativus under nitrate stress. According to them, the reduction in Hill activity restricted the phosphorylation of ADP; as a result, assimilation of CO2 and the rate of photosynthesis were affected causing low growth in the said seedlings due to nitrate toxicity. Elevated level of Ni decreased the Hill activity in Zea mays significantly (Ghasemi et al., 2012). Allakhverdiev et al. (2000) stated that the dysfunction in electron transport chain which is accommodated by PS-II may repress the Hill activity in plants.

The generation of ROS in chloroplast may cause oxidative damage in both inner and outer membrane (Wang and Blumwald, 2014). In the study of Schneider et al. (2013), it was reported that the production of ROS during As stress depleted the rate of carbon accumulation in Leucaena leucocephala. In our study, the accumulation and/or activity of photosynthetic attributes reduced under As(V)-stressed environment in rice seedlings (Fig. 1). Si administration in As(V)-treated rice seedlings increased the photosynthetic activities more than Se administration in As(V)-treated seedlings. Photosynthetic gas exchange and chlorophyll fluorescence were also alleviated due to copper toxicity in Avicennia germinans (Mendoza et al., 2013). The alleviation in net photosynthesis rate downregulates the Rubisco activity and thus hampers the activities of respiratory cycle enzymes under As toxicity (Farnese et al., 2017); the closing

| Experimental parameters | Chlorophyll-a | Chlorophyll-b | Total chlorophyll | Fluorescence intensity | Carotene | Xanthophyll | Hill activity | Internal CO2 concentration | Net photosynthesis | Transpiration rate | Stomatal conductance | Reducing sugar | Non-reducing sugar | Total soluble sugar | Starch | Sucrose phosphate synthase | Sucrose synthase | Acid invertase | Starch phosphorylase |
|-------------------------|---------------|---------------|-------------------|------------------------|----------|-------------|--------------|---------------------------|-------------------|-----------------|------------------|---------------|------------------|------------------|---------|-------------------------|------------------|------------|------------------------|
| Root | 0.039 | 0.035 | 0.010 | 0.000 | 0.000 | 0.016 | 0.007 | 0.002 | 0.000 | 0.000 | 0.000 | 0.004 | 0.001 | 0.000 | 0.000 | 0.000 | 0.012 | 0.030 | 0.000 |
| Shoot | 0.080 | 0.086 | 0.147 | 0.064 | 0.012 | 0.030 | 0.067 | 0.023 | 0.024 | 0.100 | 0.068 | 0.130 | 0.004 | 0.008 | 0.003 | 0.017 | 0.072 | 0.278 | 0.094 |

Table 3: p values corresponding to the test of between-subject (main) effects in the two-way ANOVA performed separately for Si and Se supplementation.

Table 4: Influence of As(V) with or without Si or Se on chlorophyll a/b ratio.
of stomatal aperture is also responsible for the reduction of photosynthetic activities (Sanglard et al., 2014). In some previous literatures, it has been documented that the optimal yield of rice mainly relies on the rate of photosynthesis which is correlated with the stomatal conductance (Ambavaram et al., 2014; Stuerz et al., 2014). In the present study, the reduction in stomatal conductance is highly correlated with the reduction in the transpiration rate that may be due to the superfluous water loss form the tissues of As(V)-treated rice seedlings (Fig. 6) which is in accordance with the study of Gusman et al. (2013). They reported that in *Lactuca sativa*, when the rhizosphere was affected by As(V) and As(III) treatments, the capability of water consumption as well as internal CO₂ level, stomatal conductance and transpiration rate were decreased. Low internal CO₂ concentration was also noted in *Zea mays* under As and Cd stresses which is associated with the closing of stomata and less photosynthetic rate (Anjum et al., 2016).

We have also studied the relationship among basic physiological parameters by comparing their values across all the treatment levels. We observed a strong linear relationship between chlorophyll-b and total chlorophyll so that either one can be predicted quite accurately from the other; the relation between chlorophyll-a and total chlorophyll is also linear but relatively weaker (Fig. 7a). Similarly strong linear relations of stomatal conductance with net photosynthesis and transpiration rate were observed with the latter being stronger (Fig. 7b). According to Deng et al. (2003), the opening of stomata mainly regulates the transpiration rate in plants. Thus, under As(V) stress, as stomatal conductance decreased, i.e. opening of stomata were getting hampered due to As(V) toxicity, the transpiration rate was also decreased. Therefore, the reduction in chlorophyll-a, chlorophyll-b, chlorophyll a/b ratio, total chlorophyll, carotenoids, Hill activity and all the photosynthetic attributes due to As(V) toxicity might be connected with the less accumulation of carbon that consistently reduced both growth parameters and biomass contents in the test cultivar due to production of ROS under As(V) stress (Das et al., 2018). In plants, ROS production restricted the signalling pathways by weakening the PS-II activity that inhibited less photosynthesis rate by triggering photoinhibition (Gururani et al., 2015).

Further, three-dimensional (3D) scatter matrix was used to explore the influence of As(V), Si and Se in the array of wide dimension on different physiological attributes. 3D scatterplot in Fig. 8a represents a pronounced disparity in the range showing the influence on total chlorophyll in y axis, net photosynthesis rate in x axis and Hill activity in z axis under As(V), with or without Si and Se, treated rice seedlings. Si treatments showed the effect at a higher range as compared to only As(V) treatments and As(V) + Se treatments, indicating greater ameliorative power of Si supplementation in increasing total chlorophyll contents, increasing Hill activity and, hence, elevating net photosynthesis rate in the presence of As(V). Another 3D scatterplot (Fig. 8b) showed differential effects on total chlorophyll (y axis), internal CO₂ concentration (x axis) and fluorescence intensity of chlorophyll (z axis) under As(V), with or without Si and Se, treated rice seedlings, where similar trend was observed for As(V) + Si and As(V) + Se treatments.

Both silicon and selenium application in As(V)-treated seedlings resulted in an enhancement in mentioned...
parameters but Si was more potent than Se in improvement of photosynthesis machineries. Vacuík et al. (2020) reported that Si suppressed the gene expressions, viz. HMA2, OVPI, NRAMP5 and LCT1, that act as transporters for metal uptake so that metal accumulation was reduced in plant cells. Si also reduced total As contents, As(III) contents and oxidative stress markers in rice seedlings under As(V) stress (Das et al., 2018). Etienne et al. (2020) stated that in Brassica napus, Si plays a vital role in the regulation of photosynthetic activities by transcriptomic alterations of DEGs (differentially expressed genes) in shoots by exogenous application of 1.7-mM sodium silicate. Silva et al. (2012) also demonstrated that Si application increased chlorophyll content and gas exchange parameters in two Lycopersicon esculentum cultivars that were exposed to drought. Si addition significantly increased fluorescence intensity of chlorophyll, photosynthesis rate and gas conductance in Fragaria sp. under water-deficit conditions (Safoora et al., 2018). Si improves tolerance capacity by enhancing photosynthetic efficiency in plant cell wall (Ali et al., 2013). Se significantly increased chlorophyll-a, chlorophyll-b and carotenoid content in Lycium chinense leaves (Dong et al., 2012). In the study of Kadhim (2017), it was also reported that Se caused an increase in chlorophyll and carotenoid content of Cucumis melo and Phaseolus aureus Roxb. under Cd treatments. Under salinity stress, Se nanoparticles (n) in low dose (nSe 10 µM) enhanced the photosynthetic efficiency by elevating the performance index, the quantum yield of PS-II and the potentiality of water-splitting complex in strawberry cv. Gaviota (Soleymanzadeh et al., 2020). Se nanoparticles in the range of 10 to 30 mg l⁻¹ exhibited acute toxic effects and alleviated the growth of roots and leaves in Capsicum annum while nSe in the range between 0.5 and 1 mg l⁻¹ exhibited promotion in the said parameters and also regulated the expression of WRKY1 and bZIP transcription factors in DNA methylation techniques (Sotoodehnia-Korani et al., 2020). In the study of Neysanian et al. (2020), it was also reported that 3 mg l⁻¹ nSe application not only increased the growth, biomass contents and fruit development but also triggered the expression of bZIP transcription factor as well as miR172 gene regulation in tomato plants.

Influence on carbohydrate metabolism

The carbon fixed during photosynthesis is required for the synthesis of starch which is accumulated in chloroplast and is transferred to different plant parts to provide energy in the cells. If carbon metabolism is affected due to metal stress, it greatly hampers the nutrition worth of plants (Wahid et al., 2007). Carbohydrates produced during photosynthesis play crucial role to provide energy for cellular metabolism and also regulate osmotic potential and defending biomolecules (Muller et al., 2011). In our study, both reducing and non-reducing sugar contents were enhanced, while starch contents were declined under As(V) stress in rice seedlings. In some previous literatures, it is noted that accumulation of sugars plays a vital role as free radical scavenger, generated due to oxidative stress, and regulates oxidative pentose phosphate pathway to actuate ROS (Van den Ende and Valluru, 2009; Hu et al., 2012; Peshev and Van den Ende, 2013). Reducing sugars control cell division while non-reducing sugars control differentiation of cell and cell maturity that favours the optimal growth by cell development (Eveland and Jackson, 2011; Sami et al., 2016). In our

Fig. 8 3D scatterplot of (a) total chlorophyll, net photosynthesis and Hill activity and (b) total chlorophyll, internal CO₂ concentration and fluorescence intensity of chlorophyll under different treatment levels in 3-week-old rice seedlings
study, the enhancement levels in sugar levels were an effort to enhance tolerability in the rice seedlings under As(V)-stressed environment by accommodating the breakdown of starch levels (Table 2). It was also found that both reducing and non-reducing sugar contents were highly correlated with total soluble sugar contents with $r = 0.910$ and 0.999, respectively, and the correlation was significant at $p < 0.001$, whereas the former two sugar contents were also highly correlated with each other ($r = 0.888$, $p < 0.001$). Elevated level of sugar contents in the test cultivar restricted the net photosynthesis rate that might be the results of low expressions of genes leading to feedback inhibition during photosynthesis (Hammond and White, 2011). Enhanced level of reducing sugar was also reported in the study of Roychoudhury et al. (2012) under Cd toxicity in rice leaves to stabilize the cell membrane and to protect osmotic imbalance. Reducing sugar contents was also increased under Cd-stressed almond to provide defensive responses (Nada et al., 2007). According to Hu et al. (2012), the activities of antioxidant enzymes were enhanced by glucose amendments in wheat seedlings.

Carbohydrates are stored as the form of starch in plant cells. But under various kinds of biotic and abiotic stressed environments, the breakdown of starch causes the deposition of soluble sugars in plant cells which can accumulate as storage form to supply carbon and to accomplish the fundamental metabolic activities required for proper growth and development of plants (Hurry et al. 1995; Stitt and Zeeman, 2012). In our study, the sugar contents were negatively correlated with the starch contents (Fig. 9a) where $r = -0.821$ and the correlation was significant at $p < 0.001$. The breakdown of starch occurs when net photosynthesis is restricted and insufficient to maintain optimal growth under As(V)-challenged condition (Fig. 1c and Table 2). So, it is supposed that the accumulation of carbon in plant cells and its implementation by the breakdown of starch is necessary for maintaining the growth of plants under stressed environments. We have also studied the relationship among total soluble sugar, starch and internal CO$_2$ concentration by comparing their values across all the treatment levels. A strong linear relationship was observed between starch contents and internal CO$_2$ concentration (Fig. 9a) so that either one can be predicted quite accurately from the other; the relation between total soluble sugar and internal CO$_2$ concentration is also linear but negative (Fig. 9b).

The synthesis of sucrose is controlled by sucrose phosphate synthase (SPS) activity governed by various kinds of environmental stresses (Krause et al., 1998). Enhancement in SPS activity in As(V)-treated seedlings was correlated with the high accumulation of sucrose under As(V) treatments which is also noted in the study of Yang et al. (2001). They reported that increased activity of SPS was closely associated with the accumulation of sucrose in rice leaves under water stress. Starch is degraded by starch phosphorylase (SP) activity which triggers the formation of glucose-1-phosphate by reversible phosphorylation of α-glucans (Salisbury and Ross, 1991). In our study, sucrose synthase (SS) activity was decreased while activity of acid invertase (AI) was increased in As(V)-applied rice seedlings. Acid invertase produces glucose and fructose, while sucrose synthase forms UDP glucose and fructose (Rosa et al., 2009). Dubey and Singh (1999) demonstrated that the increment in acid invertase activity under metal toxicity favours the hexose formation which is required to balance osmoregulation in

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**Fig. 9** Plots representing the relations of (a) starch with total soluble sugar and (b) total soluble sugar, starch and internal CO$_2$ concentration in 3-week-old rice seedlings
Plant tissue. Joint application of Si with As(V) and Se with As(V) resulted in a significant modulation in sugar accumulation and an elevation of starch contents, but Si executes better performance than Se in the test seedlings by enhancing tolerance mechanism under stress condition. An increased level of chloroplast pigment content in plants under Si supplementation leads to high accumulation of starch which improves growth and development of plants during stressed environments (Kang et al., 2014). In the present study, a positive correlation occurred between non-reducing sugar contents and SPS activity with \( p = 0.019 \), but a negative correlation was observed between SPS activity and SS activity with \( p < 0.001 \). The same concurrence also occurred in the study of Verma et al. (2010) in Saccharum officinarum. SP activity was positively correlated with the soluble sugar contents in rice seedlings \( (r = 0.544, \ p = 0.001) \) but negatively correlated with starch remobilization \( (r = -0.613, \ p < 0.001) \) (Fig. 6). This phenomenon was also reported in the study of Yang et al. (2001) in rice under water stress. Thus, the activities of carbohydrate metabolizing enzymes were reversed during co-application of Si and Se with As(V) in the test seedlings with better performance with Si proving that Si was more capable than Se to sustain osmotic potential and enhance tolerability in rice seedlings during As(V)-challenged environment (Fig. 10).

**Conclusion**

Our study illustrated that As(V) stress greatly affected all the physiochemical attributes investigated, viz. the levels of chlorophyll-a, chlorophyll-b, total chlorophyll, fluorescence intensity of chlorophyll, Hill activity, carotenoid levels (carotene and xanthophyll) and photosynthetic parameters like internal CO\(_2\) concentration, net photosynthesis, transpiration rate and stomatal conductance, leading to an adaptation by regulating carbohydrate metabolism in the tested seedlings of rice cv. MTU-1010. Si treatment along with As(V), however, promoted the said parameters more than Se supplementation in As(V)-treated rice seedlings. Enhanced accumulation of As (see Supplementary Table S2) was the crucial features for the generation of ROS which was, in turn, responsible for enhancement of the oxidative stress markers; this possibly leads to reduced photosynthetic efficacy in As(V)-treated rice seedlings. Further, the contents of reducing sugar, non-reducing sugar and total soluble sugar were increased, but the starch content was decreased, to maintain osmotic potential in cell cytoplasm under As(V) toxicity in rice seedlings. Activities of carbohydrate metabolizing enzymes were also interrupted that ultimately caused an ionic imbalance due to As(V) stress in rice seedlings. The activities of sucrose phosphate synthase, starch phosphorylase and acid invertase were enhanced, whereas sucrose synthase activity was decreased to produce tolerance.

**Fig. 10** Schematic representation of Si/Se mediated alterations of chloroplast pigment levels, photosynthetic attributes and carbohydrate metabolism in rice seedlings
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Author contribution SD conceived the study, analysed the experiments and experimental data statistically and wrote the manuscript. AKB provided supervision during the experimental design and gave the paper in its final shape.

Data availability Data can be obtained from the first author upon request.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

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