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Research paper

Synergistic influence of selenium and silicon supplementation prevents the oxidative effects of arsenic stress in wheat

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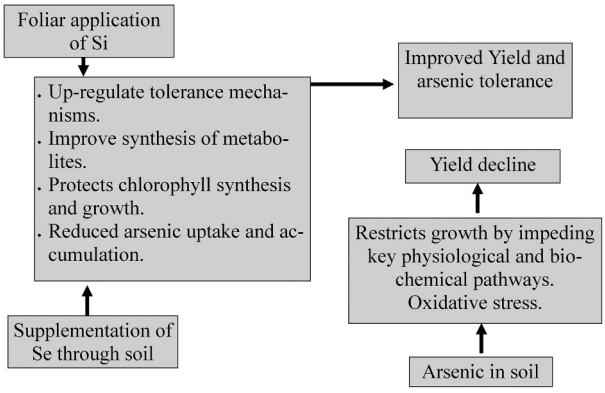
HIGHLIGHTS

- As restricted growth, chlorophyll synthesis and nitrate reductase activity.
- Supplementation of Se and Si alleviated the oxidative effects of arsenic.
- Se and Si up-regulated tolerance mechanisms to counter the damaging effects of arsenic stress.

GRAPHICAL ABSTRACT



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ABSTRACT

Influence of supplementation of selenium (Se, 1 and 5 μM) and silicon (Si, 0.1 and 0.5 mM) was investigated in wheat under arsenic (30 μM As) stress. Plants grown under As stress exhibited a significant decline in growth parameters however, Se and Si supplementation mitigated the decline significantly. Treatment of Se and Si alleviated the reduction in the intermediate components of chlorophyll biosynthesis pathway and the content of photosynthetic pigments. Arsenic stressed plants exhibited increased reactive oxygen species accumulation and the NADPH oxidase activity which were lowered significantly due to Se and Si treatments. Moreover, Se and Si supplementation reduced lipid peroxidation and activity of lipoxygenase and protease under As stress. Supplementation of Se and Si significantly improved the antioxidant activities and the content of cysteine, tocopherol, reduced glutathione and ascorbic acid. Treatment of Se and Si alleviated the reduction in nitrate reductase activity. Exogenously applied Se and Si mitigated the reduction in mineral elements and reduced As accumulation.

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Hence, supplementation of Se and Si is beneficial in preventing the alterations in growth and metabolism of wheat under As stress.

1. Introduction

Rapid industrialisation has significantly increased the concentration of several metal(loid)s in environment which has imparted considerable ecological changes affecting the growth, distribution and yield productivity of plants [98,39,70]. Among the key heavy metal(loid)s are included cadmium, lead, mercury, chromium, arsenic (As) etc which are continuously added to soil and water [13,2,63]. Continuous use of As in agrochemicals like fungicides and pesticides etc has resulted in increasing its concentration in agricultural land thereby significantly affecting the growth of crops grown on such soils [70]. In addition, As is added to soil from sedimentary rock leaching, smelting and mining [24, 88]. After entering into the plants, As affectively disturbs the metabolism by binding with the sulfhydryl groups of proteins and enzymes [119]. Plant growth is hampered by As due to its negative impact on cellular division, germination and viability, root growth, photosynthesis, mineral uptake and assimilation etc [10,58,75,79]. Contamination of ground water with As has been reported in many countries including China therefore making its obvious availability for the irrigation purposes [93]. Uptake of toxic metal ions generates oxidative damage in plants hence affecting growth and development [10]. Plants have mechanisms that are up-regulated to overcome the adverse effects of stress triggered oxidative damage [6,15,91].

Selenium (Se) is essential redox-active micronutrients [109], found in damp and alkaline soils, and both natural and human activities add Se to soil [36,71]. Earlier, Se was considered as poisonous however, several studies have revealed its beneficial effects when present at optimal concentrations [15,27,32,33]. Certain key properties of Se including covalent binding ability with metals, strong coordination with metals and its structural stability with different oxidative states render Se highly specialized and integrative in biological functions [38]. Predominant forms of Se in plants include selenite (SeIV) and selenate (SeVI) [97]. Due to its similarity with sulfur, plants uptake Se through sulfur transporters and is also assimilated via sulfur assimilating pathway [50,71]. It has been reported that supplementation of Se to plants improves growth and alleviates the negative impact of different stress factors like drought [117], salinity [33], heavy metals [15] etc. Improved tolerance to stresses due to exogenous Se supplementation is ascribed to its positive impact on the tolerance mechanisms [15,33].

Silicon (Si) is one of the most abundant elements mostly existing as silicates. Si is found in plants however, is considered as non essential nutrient, nevertheless several studies have shown its key role in several processes from growth to stress tolerance [106]. Plants absorb Si via aquaporin channels as silicic acid under pH below than 9 [78,81]. Plants show signifcant difference in Si accumulation making 0.1 to 10% of their total dry weight and among angiosperms plants belonging to Poales and Arecales accumulate substantial concentrations of Si in shoot [77]. Among the Poales, plants belonging to *Gramineae* accumulate greater Si concentrations [78]. Foliar application of Si improves plant growth by increasing enzyme functioning, photosynthesis and mineral uptake [61]. Accumulation of Si at higher concentrations cannot harm plants [106]. Application of Si mitigates the ill impact of stresses by modulating the tolerance mechanisms like osmolyte accumulation, antioxidant activity and also reducing the uptake of harmful ions and metals [26,51,61,85]. However interactive role of Se and Si in alleviating the adverse effects of As has not been reported.

Wheat (*Triticum aestivum* L) is a significant cereal plant grown throughout world for its seeds as food and grass for animal fodder. There has been a significant variation in the concentration of As in agricultural soils of China thereby can have considerable impact on the potential yield of the crops grown [46]. Pollution of agricultural fields directly affects the crop growth and productivity hence threatening the global food security. With this backdrop, it was hypothesized that exogenous supplementation of Se and Si can be beneficial in preventing the damaging effects of As in wheat by modulating the tolerance mechanisms.

2. Material and methods

Seeds of wheat (*Triticum aestivum* L.) were disinfected by 0.001% $HgCl_2$ for 5 min. Seeds were washed with distilled water and blot dried. Sowing of sterilized seeds was done in pots filled with acid washed sand. After germinations, seedlings were allowed to grow for one week and after thinning ten seedlings per pot were maintained. All pots were supplied Hoagland nutrient (150 mL) solution on alternate days. Two weeks (fifteen days) after germination pots were divided into different groups and were irrigated with either normal Hoagland solution or Hoagland solution supplemented with sodium arsenite (AsIII; 30 μM $NaAsO_2$) or Hoagland solution supplemented with sodium selenate (1 and 5 μM Na_2SeO_4). Foliar application of silicon (Si; 0.1 and 0.5 mM Na_2SiO_3) at the rate of 10 mL per pot was also started on same day. Treatments continued for another twenty days on every alternate day. The overall treatments in the experimental setup can be summarized as: (1) Control (Hoagland solution), (2) 30 μM As, (3) 30 μM As + 1 μM Se, (4) 30 μM As + 5 μM Se (5) 30 μM As + 0.1 mM Si (6) 30 μM As + 0.5 mM Si, (7) 30 μM As + 1 μM Se + 0.1 mM Si, (8) 30 μM As + 5 μM Se + 0.1 mM Si, (9) 30 μM As + 1 μM Se + 0.5 mM Si, (10) 30 μM As + 5 μM Se + 0.5 mM Si. Four pots for each treatment were kept in complete randomized block design. Twenty days after treatments of As, Se and Si, several growth, physiological and biochemical parameters were worked out using standard protocol described below.

2.1. Plant height, fresh and dry weight

A scale was used to measure the height. After uprooting the plants fresh weight of root and shoot was taken immediately while as for determination of dry weight same tissue was dried in oven for 48 h at 70 °C.

2.2. Determination of intermediates of chlorophyll biosynthesis and photosynthetic pigments

Photosynthetic pigments were estimated according to Arnon [16]. The content of glutamate 1-semialdehyde (GSA) was estimated following Kannangara and Schouboe [65], while as δ-Amino levulinic acid (ALA) was determined according to Harel and Klein (1972). Estimation of prototoporphyrin IX (Proto IX), Mg-prototoporphyrin IX (Mg-Proto IX) and protochlorophyllide (Pchlide) was carried in accordance with Hodgins and Huystee [55].

2.3. Estimation of osmolytes

Proline was extracted in sulphosalicylic acid and separated using toluene after reacting the supernatant with ninhydrin reagent. Absorbance was recorded at 520 nm [21]. Grieve and Grattan's [48] method was used for glycine betaine while as free sugars were estimated following method of Fong et al. [40]. Free amino acids were determined using ninhydrin method as described by Ahanger et al. [4].

2.4. Measurement of lipid peroxidation, hydrogen peroxide and superoxide

Methods of Heath and Packer [53], Velikova et al., [111] and Yang et al., [115] were used for measuring lipid peroxidation, hydrogen peroxide (H_2O_2) and superoxide ($O_2^{\bullet-}$) respectively.

2.5. Measurement of protease, lipoxygenase and NADPH oxidase activity

Activity of protease (EC 3.4.21.40) was measured following Green and Neurath [47]. Lipoxygenase (LOX; EC, 1.13.11.12) was assayed according to Doderer et al. [31]. For measuring the activity of NADPH oxidase method described by Sagi and Fluhr [94] was followed. Leaf plasma membranes were isolated using two-phase aqueous polymer partition system according to Larsson et al. [74] as described by Jiang and Zhang [62]. Reduction of 3′-[1-[phenylamino-carbonyl]− 3, 4-tetrazolium]-bis(4-methoxy-6-nitro)benzenesulfonic acid hydrate (XTT) by $O_2^{\bullet-}$ was measured in an assay mixture containing Tris–HCl buffer (50 mM; pH 7.5), 0.5 mM XTT, 100 μM NADPH and membrane proteins. Addition of NADPH initiated the reaction and XTT reduction was determined at 470 nm.

2.6. Determination of activity of nitrate reductase

Nitrate reductase activity was measured following Srivastava [107].

2.7. Assay of antioxidant enzyme activities

One gram fresh tissue was extracted in 100 mM phosphate buffer (pH 7.8) containing PVP, EDTA and PMSF. Extract was centrifuged at 12,000 g and supernatant was used in enzyme assay. Superoxide dismutase (SOD) activity was measured following Bayer and Fridovich [22]. Activity of catalase (CAT) and glutathione S-transferase (GST) were measured following the protocol described by Aebi [1] and Hasanuzzaman and Fujita [52] respectively. Activity of ascorbate peroxidase (APX) was assayed according to Nakano and Asada [87]. Method of Nakano and Asada [87] was used for measuring the activity of dehydroascorbate reductase (DHAR) and monodehydroascorbate reductase (MDHAR) was assayed according to Hossain et al. [56]. Foyer and Halliwell's [41] method was employed to measure the activity of glutathione reductase (GR; EC 1.6.4.2).

2.8. Estimation of ascorbate, reduced glutathione, α-tocopherol and cysteine

The content of ascorbate and reduced glutathione was estimated according to Mukherjee and Choudhuri [84] and Ellman [34] respectively. For tocopherol determination, protocol described by Backer et al. [20] was used however, cysteine was determined according to Gaitonde [45].

2.9. Total phenol content and total antioxidant activity

Phenols were estimated following Malick and Singh [80]. Method of Shimada et al. [100] described by Ahanger and Agarwal [3] was used for determining the total antioxidant activity. Activity was measured as percent DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging.

2.10. Estimation of ions and arsenic

Content of nitrogen was estimated using micro-Kzeldahl method [59]. Atomic absorption spectrophotometer was used for determination of P, K, S and Mg content. Content of arsenic (As) was estimated by digesting the tissue in HNO_3 and was estimated by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500 USA).

2.11. Statistical analysis

Data given is mean (± SE) of four replicates. Duncan's Multiple Range Test was performed using ANOVA for determining the least significant difference (LSD) among the mean values at $p < 0.05$.

3. Results

Treatment of Se and Si at both concentrations increased growth, however obvious amelioration was observed due to their higher concentrations i.e., 5 μM Se and 0.5 mM Si. Relative to control, plant height (50.19%), shoot fresh weight (51.78%), shoot dry weight (66.26%), root fresh weight (57.72%) and root dry weight (56.68%) decreased due to As stress. Treatment of 5 μM Se and 0.5 mM Si were affective in mitigating the decline caused by As stress with maximal mitigation observed in plants treated with As + 5 μM Se + 0.5 mM Si. Relative to control, decline in plant height, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight was only 12.01%, 14.95%, 19.47%, 29.06% and 25.64% in As + 5 μM Se + 0.5 mM Si treated plants (Fig. 1).

Plants stressed with As exhibited a significant decline in the content of GSA, δ-ALA, Proto IX, Mg-Proto IX and protochlorophyllide however, supplementation of Se and Si proved beneficial in assuaging the reduction considerably. Compared to control, observed decline was 48.44% for GSA, 46.59% for δ-ALA, 51.71% for Proto IX, 52.26% for Mg-Proto IX and 51.56% for protochlorophyllide in As stressed plants. However, supplementation of Se and foliar application of Si at both concentrations, individually as well as combinedly, resulted in mitigation of the decline. Relative to control, decline in GSA, δ-ALA, Proto IX, Mg-Proto IX and protochlorophyllide was only 31.36%, 27.25%, 35.18%, 30.47% and 32.04% in As + 5 μM Se and 31.98%, 26.03%, 34.93%, 31.96% and 33.78% in As + 0.5 mM Si treated plants. However, plants treated with As + 5 μM Se + 0.5 mM Si showed a decline of only 8.54% for GSA, 7.78% for δ-ALA, 9.76% for Proto IX, 11.28% for Mg-Proto IX and 9.32% for protochlorophyllide over control (Fig. 2).

Plants treated with As exhibited a decline of 48.62% in chlorophyll a, 46.80% in chlorophyll b, 47.09% in total chlorophylls and 35.84% in carotenoids over control. Supplementation of Se and Si mitigated the decline induced by As to significant levels. Higher concentrations of Se and Si alleviated the decline more obviously as compared to lower concentrations however, combined treatments were much affective than the individual ones. Relative to control, decline in chlorophyll a was 11.59%, chlorophyll b was 9.79%, total chlorophylls was 14.85% and carotenoids was 1.66% in plants treated with As + 5 μM Se + 0.5 mM Si (Fig. 3).

Treatment of Se and Si alleviated the oxidative damage induced by As stress to significant levels. Relative to control, H_2O_2, $O_2^{\cdot}$ and lipid peroxidation increased by 166.25%, 122.78% and 144.81% respectively due to As stress. Plants treated with As + 5 μM Se exhibited only 84.42%, 51.26% and 79.29% increase in H_2O_2, $O_2^{\cdot}$ and lipid peroxidation while as, As + 0.5 mM Si treated plants showed 84.22%, 52.53% and 75.78% increase respectively compared to control. Combined Se and Si application mitigated the adverse effects of As by causing maximal decline in H_2O_2, $O_2^{\cdot}$ and lipid peroxidation. In As + 5 μM Se + 0.5 mM Si treated seedlings the decline in H_2O_2, $O_2^{\cdot}$ and lipid peroxidation was 50.92%, 49.14% and 48.49% respectively over the As stressed plants (Fig. 4).

Activity of protease, lipoxygenase and NADPH oxidase increased by 101.24%, 153.58% and 162.18% in As stressed plants. However, treatment of Se and Si to As stressed plants resulted in reduced activities of protease, lipoxygenase and NADPH oxidase with obvious decline due to their combined treatment. Relative to As stressed plants, the activity of protease, lipoxygenase and NADPH oxidase was reduced by 18.33%, 21.10% and 30.87% in As + 5 μM Se, by 20.05%, 22.87% and 29.08% in As + 0.5 mM Si and by 33.63%, 41.96% and 51.15% in As + 5 μM Se + 0.5 mM Si treated plants (Fig. 5).

Arsenic stress resulted in enhancement in the activity of antioxidant

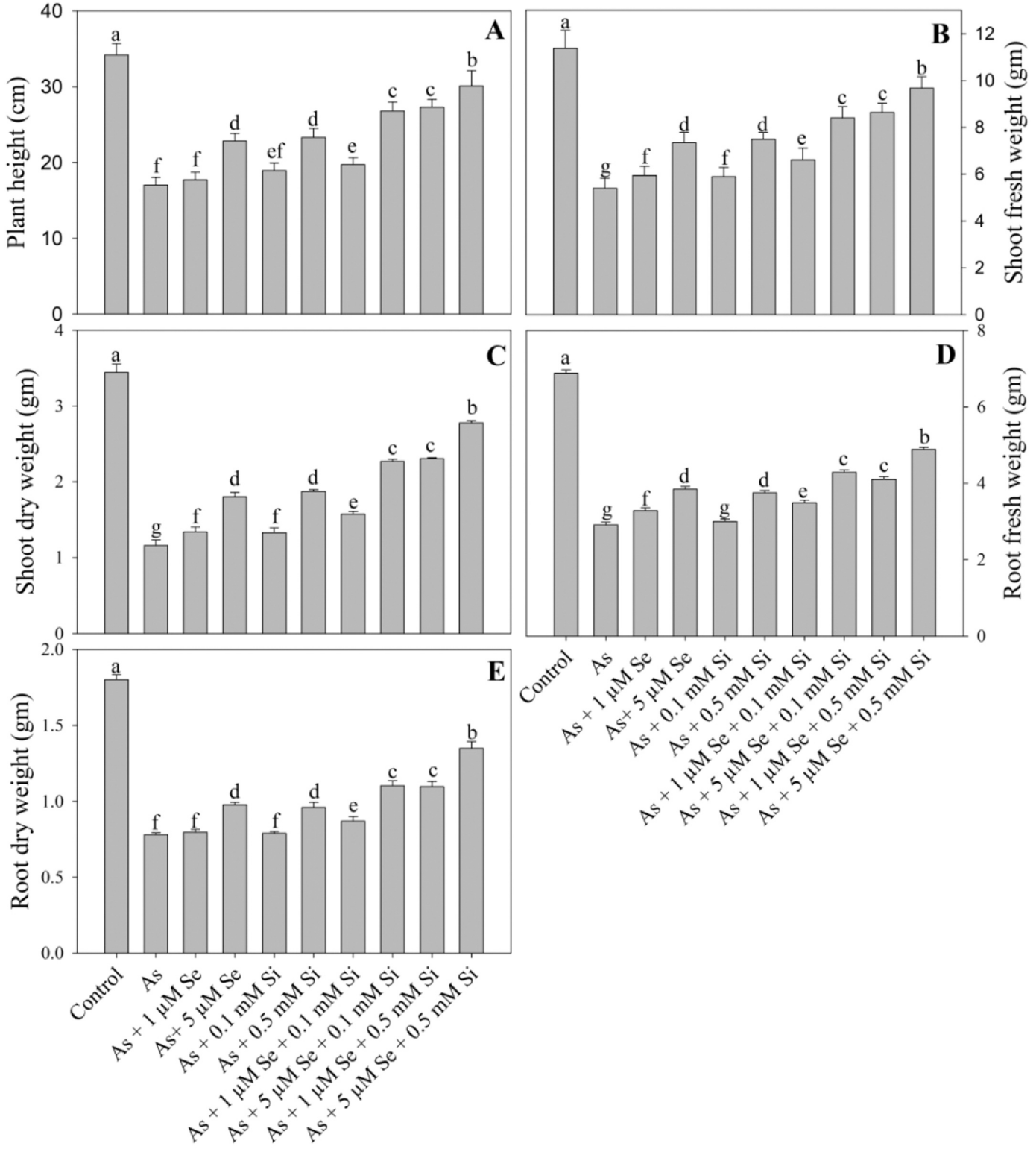

Fig. 1. Effect of individual and combined supplementation of different concentrations of selenium (Se; 1 and 5 μM) and silicon (Si; 0.1 and 0.5 mM) on (A) plant height, (B) shoot fresh weight, (C) shoot dry weight, (D) root fresh weight and (E) root dry weight in *Triticum aestivum* L. under arsenic stress (30 μM). Values presented are mean (± SE) of four replicates and different letters on bars represent the significant difference at $P < 0.05$.

enzymes assayed and it was observed that exogenous application of Se and Si to As stressed plants caused further increase. Compared to control, percent increase in SOD was 34.67%, CAT was 28.44%, GST was 40.95%, APX was 29.50%, DHAR was 23.04%, MDHAR was 62.65% and GR was 44.95% due to As stress. The activities of SOD, CAT, GST, APX, DHAR, MDHAR and GR further improved by 28.73%, 68.98%, 72.02%, 19.12%, 31.55%, 33.16% and 21.92% respectively in As + 5 μM Se treated plants over the As stressed plants. However, foliar application of 0.5 mM Si to As stressed plants caused a further increase of 33.79%, 33.80%, 19.70%, 23.66%, 27.81%, 37.49% and 21.24% in SOD, CAT,

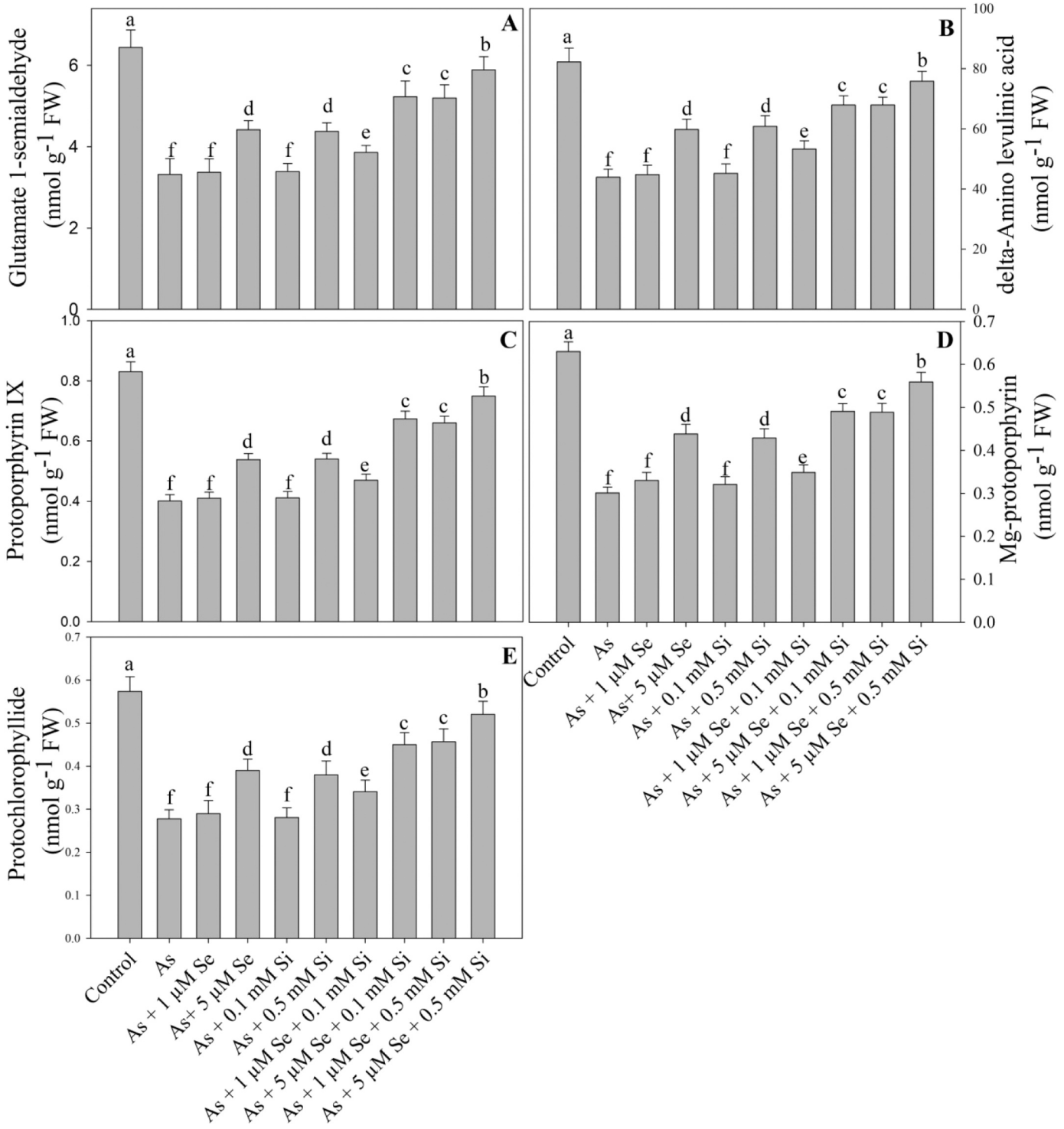

Fig. 2. Effect of individual and combined supplementation of different concentrations of selenium (Se; 1 and 5 μM) and silicon (Si; 0.1 and 0.5 mM) on content of (A) Glutamate 1-semialdehyde, (B) δ-Amino levulinic acid, (C) Prototoporphyrin IX, (D) Mg-prototoporphyrin IX and (E) protochlorophyllide (Pchlide) in *Triticum aestivum* L. under arsenic stress (30 μM). Values presented are mean (± SE) of four replicates and different letters on bars represent the significant difference at $P < 0.05$.

GST, APX, DHAR, MDHAR, and GR activity over the As stressed plants. Relative to control, maximal increase of 103.09% for SOD, 107.42% for CAT, 127.07% for GST, 101.24% for APX, 111.39% for DHAR, 195.71% for MDHAR, and 116.62% for GR was observed in plants treated with As + 5 μM Se + 0.5 mM Si (Fig. 6). Plants stressed with As showed increased accumulation of AsA (10.34%), GSH (12.98%), tocopherol (43.26%) and cysteine (19.14%) compared to control. Application of Se and Si further increased their concentration with maximum increase observed due to higher concentration, individually as well as combinedly. Maximal increase of 47.19%, 41.91%, 123.07% and 99.28% in AsA, GSH, tocopherol and cysteine respectively was observed in As + 5 μM Se + 0.5 mM Si treated plants over the control (Fig. 7).

Content of total phenols increased by 10.94% in As stressed plants and exogenous supplementation of Se and Si caused further increase

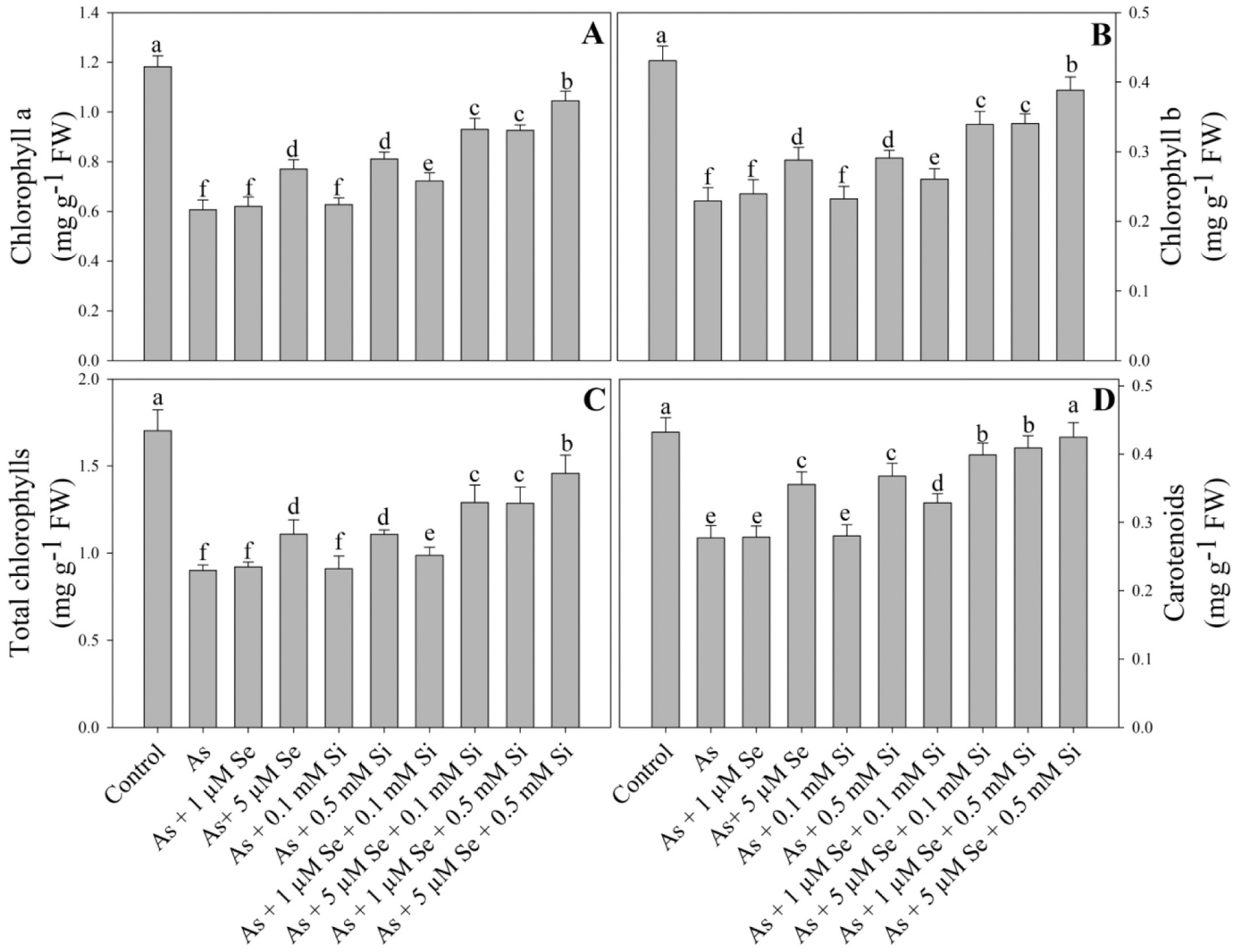

Fig. 3. Effect of individual and combined supplementation of different concentrations of selenium (Se; 1 and 5 µM) and silicon (Si; 0.1 and 0.5 mM) on content of (A) chlorophyll a, (B) chlorophyll b, (C) total chlorophylls and (D) carotenoids in *Triticum aestivum* L. under arsenic stress (30 µM). Values presented are mean (± SE) of four replicates and different letters on bars represent the significant difference at $P < 0.05$.

resulting in maximal increase of 39.30% due to As + 5 µM Se + 0.5 mM Si over control (Fig. 8 A). Total antioxidant activity assayed as percent DPPH radical scavenging was increased due to As stress by 21.73%. Exogenous treatment of Se and Si caused further increase in total antioxidant activity. Relative to control, an increase of 43.73% in As + 5 µM Se, 48.26% in As + 0.5 mM Si and 86.93% in As + 5 µM Se + 0.5 mM Si treated plants was observed in total antioxidant activity (Fig. 8B). Activity of NR was decreased by As stress by 52.40% while as treatment of Se and Si resulted in amelioration of the decline to significant levels. Relative to control, decline in NR activity was only 24.60% in As + 5 µM Se + 0.5 mM Si treated plants (Fig. 8 C).

Plants stressed with As exhibited significant increase in the content of sugars (29.34%), proline (42.32%), glycine betaine (27.23%) and free amino acids (4.37%) over the control. Exogenous Se and Si further increased the accumulation of these osmolytes exhibiting highest increase of 138.92%, 153.95%, 112.96% and 97.24% in sugars, proline, glycine betaine and free amino acids respectively in As + 5 µM Se + 0.5 mM Si treated plants over control (Fig. 9).

Content of As was reduced due to treatment of Se and Si in shoot as well as root at all concentrations and combinations. Relative to As stressed plants, maximal decline in As was 52.60% and 53.34% in shoot and root due to As + 5 µM Se + 0.5 mM Si treatment (Fig. 10 A and B). Relative to control, content of N, P, K, S and Mg reduced by 44.10%, 45.45%, 49.97%, 44.37% and 56.15% respectively due to As stress. However, exogenous supplementation of Se and Si assuaged the reduction to substantial levels. Compared to As treated plants, N, P, K, S and Mg increased by 24.50%, 20.33%, 37.40%, 16.54% and 52.48% respectively in As + 5 µM Se treated plants and by 25.28%, 20.62%, 38.37%, 18.19% and 45.70% respectively in As + 0.5 mM Si treated plants. However in As + 5 µM Se + 0.5 mM Si treated plants content of N, P, K, S and Mg increased by 55.46%, 46.32%, 72.13%, 46.87% and 93.66% respectively over the As stressed plants (Fig. 10C-G).

4. Discussion

In recent past there has been an alarming increase in soil pollution which has resulted in significant decline in the growth and yield of crop plants [98]. Efficient management strategies can be affective in alleviating the stress mediated damage and thereby can be beneficial for crop productivity and sustainable food security. In this backdrop, the potential of exogenous supplementation of Si and Se was tested in mitigating the damaging effects of As stress in wheat. Arsenic reduced growth parameters like height, fresh and dry weight. Supplementation of Si and Se proved beneficial in alleviating the decline considerably, however plants treated combinedly with Si and Se exhibited better alleviation. Earlier, in wheat [75] and rice [101] decline in growth

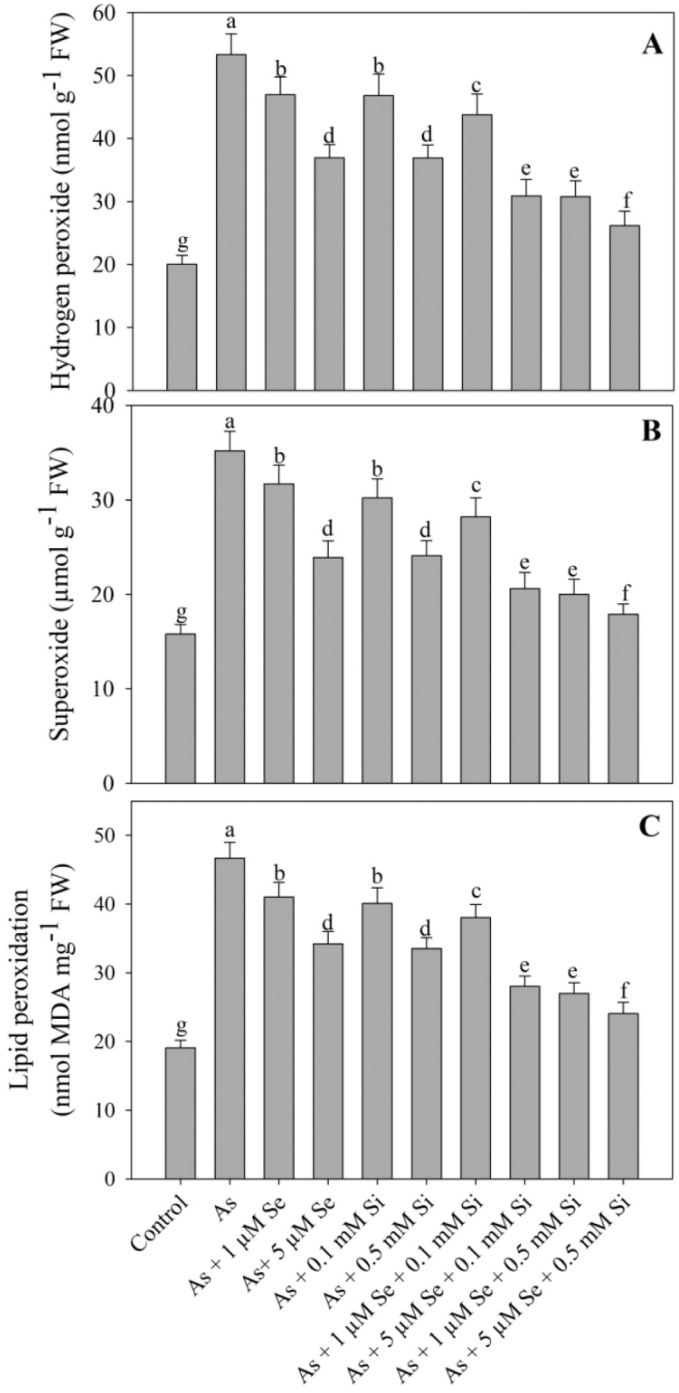

Fig. 4. Effect of individual and combined supplementation of different concentrations of selenium (Se; 1 and 5 μM) and silicon (Si; 0.1 and 0.5 mM) on content of (A) hydrogen peroxide, (B) superoxide and (C) lipid peroxidation in *Triticum aestivum* L. under arsenic stress (30 μM). Values presented are mean (± SE) of four replicates and different letters on bars represent the significant difference at $P < 0.05$.

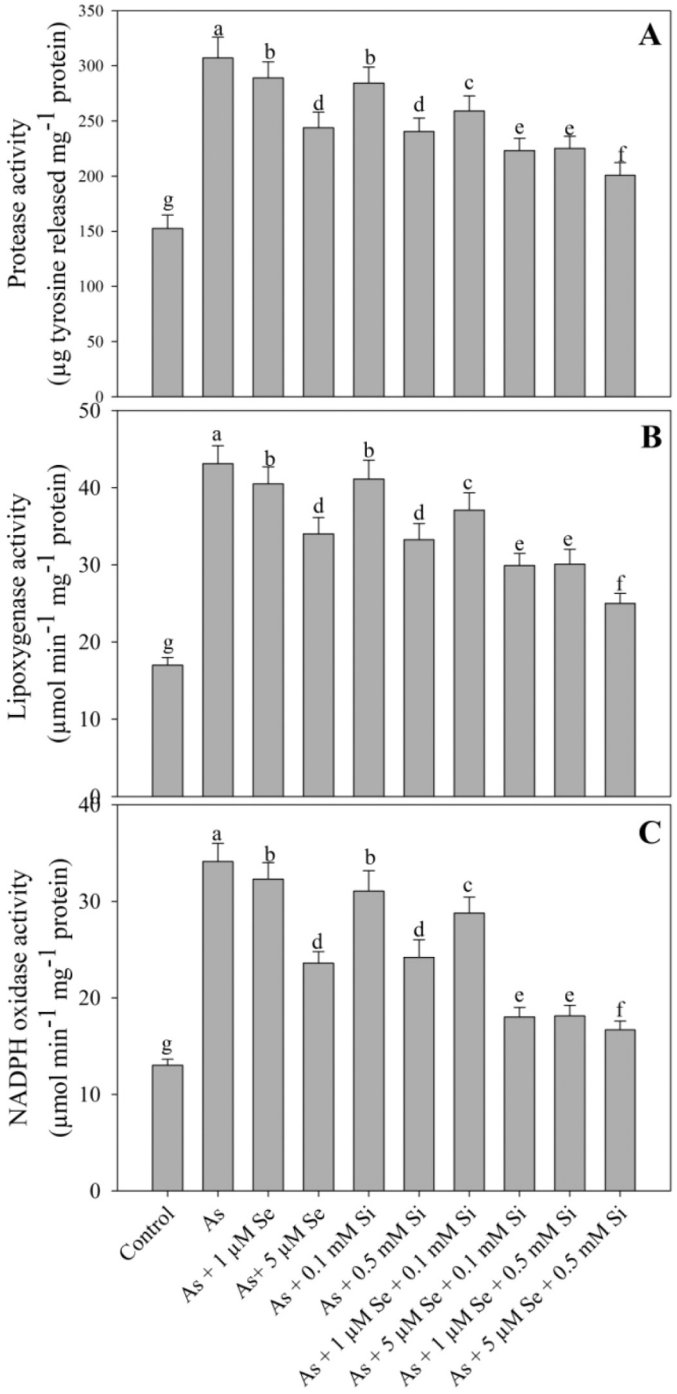

Fig. 5. Effect of individual and combined supplementation of different concentrations of selenium (Se; 1 and 5 μM) and silicon (Si; 0.1 and 0.5 mM) on the activity of (A) protease, (B) lipoxygenase and (C) NADPH oxidase in *Triticum aestivum* L. under arsenic stress (30 μM). Values presented are mean (± SE) of four replicates and different letters on bars represent the significant difference at $P < 0.05$.

parameters including height, fresh and dry weight due to As stress has been reported. Treatment of As induces oxidative damage, restricts mineral uptake and interferes with key cellular and metabolic processes thereby leading to significant growth retardations and yield decline [10, 118,120]. Individually, supplementation of Si [61] and Se [15] alleviated the detrimental effects of cadmium stress however, combined impact under As stress has not been reported. Growth improvement and alleviation of damaging effects of As due to Se and Si treatments can be ascribed to improved chlorophyll and photosynthesis, antioxidant activity and mineral uptake concomitant with reduction in the As accumulation [11]. Maintaining increased root and shoot weight under As stress depicts the importance of Se and Si treatments. Exogenous supplementation of Se and Si were imperative in mitigating the decline in the photosynthetic pigments with their combined treatments being more affective. Plants stressed with As showed significant reduction in the intermediates of chlorophyll synthesis pathway which has not been

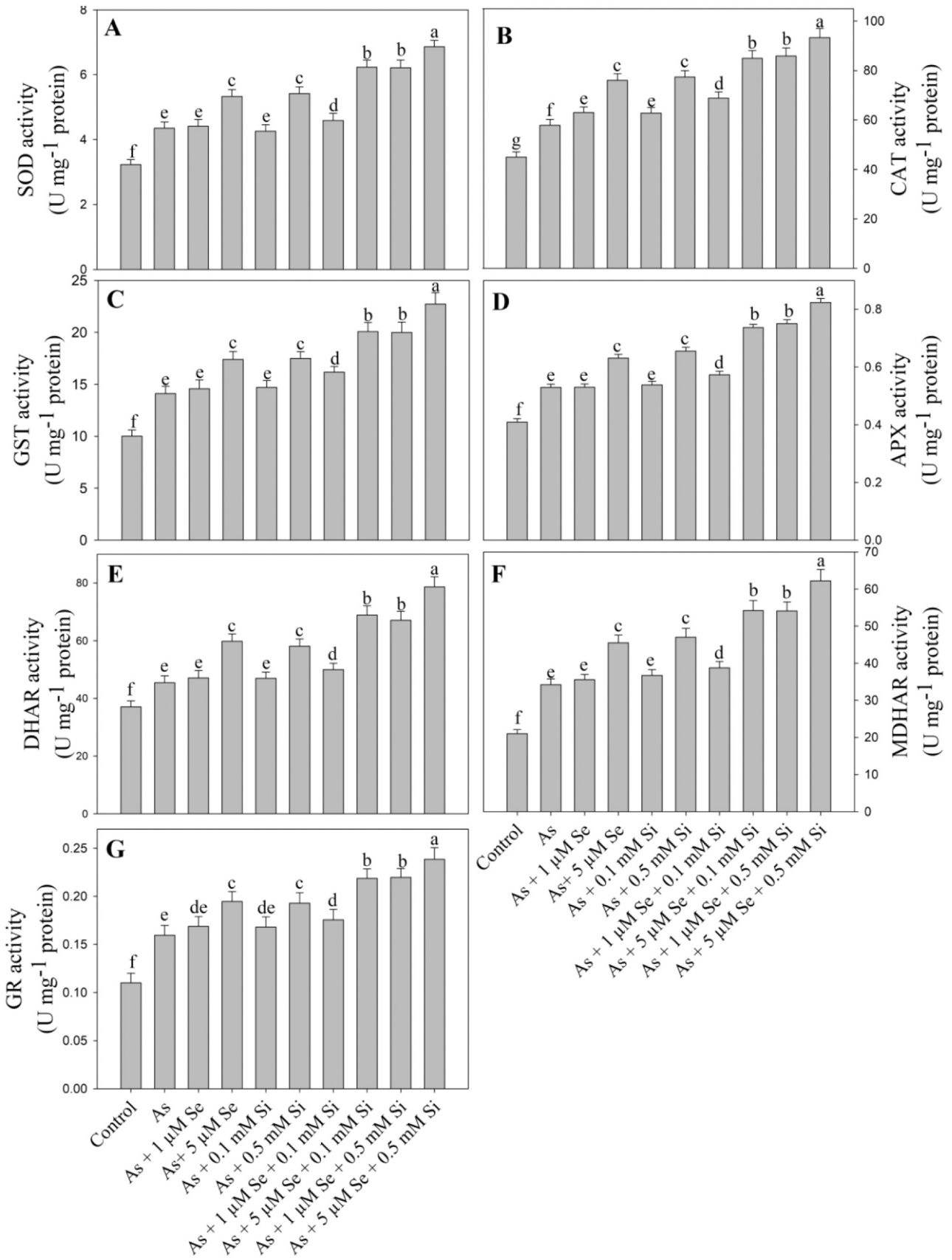

Fig. 6. Effect of individual and combined supplementation of different concentrations of selenium (Se; 1 and 5 μM) and silicon (Si; 0.1 and 0.5 mM) on the activity of (A) superoxide dismutase, (B) catalase, (C) glutathione S-transferase, (D) ascorbate peroxidase, (E) dehydroascorbate reductase, (F) monodehydroascorbate reductase and (G) glutathione reductase in *Triticum aestivum* L. under arsenic stress (30 μM). Values presented are mean (± SE) of four replicates and different letters on bars represent the significant difference at $P < 0.05$.

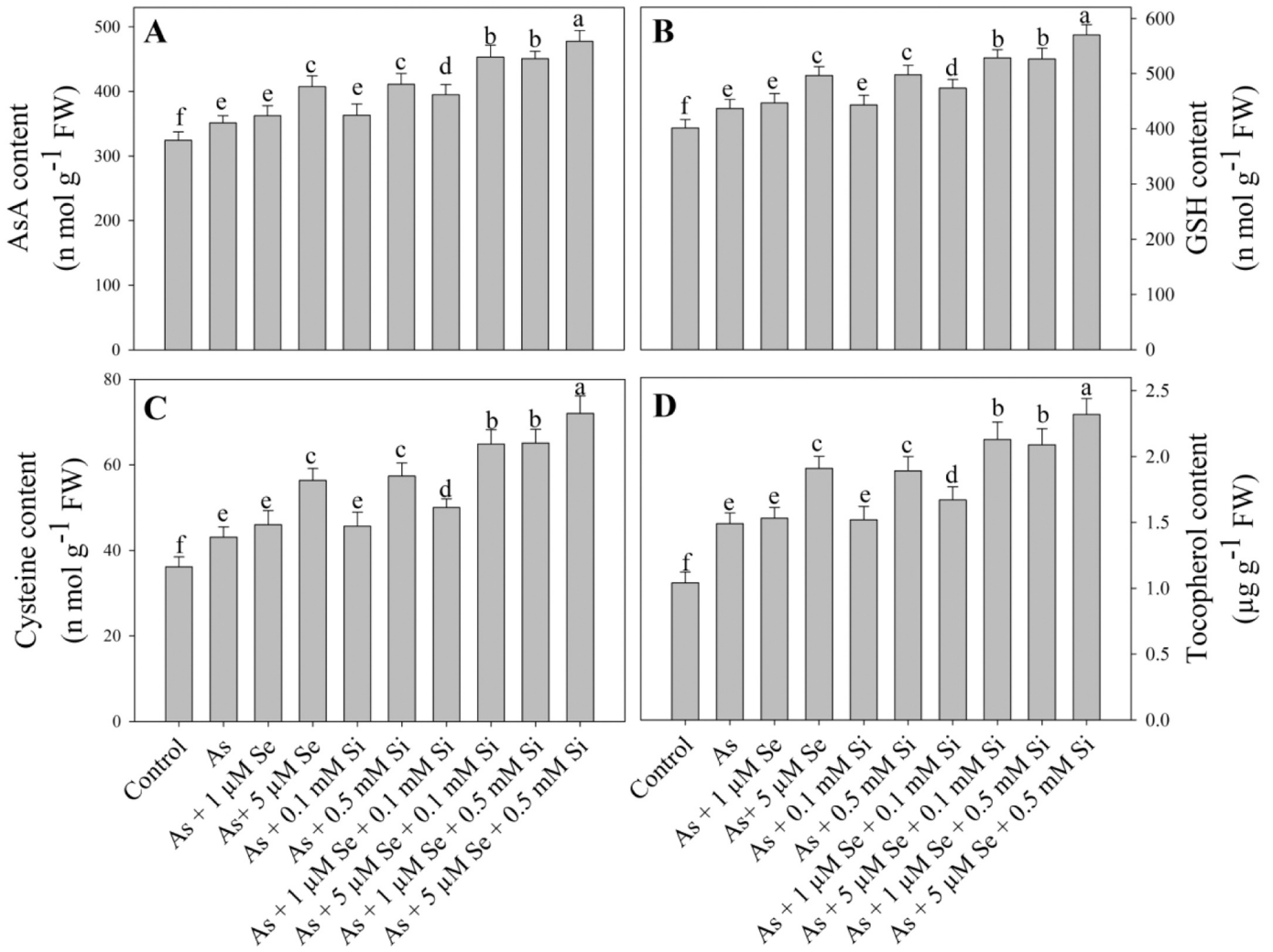

Fig. 7. Effect of individual and combined supplementation of different concentrations of selenium (Se; 1 and 5 µM) and silicon (Si; 0.1 and 0.5 mM) on content of (A) ascorbate, (B) reduced glutathione, (C) cysteine and (D) tocopherol in *Triticum aestivum* L. under arsenic stress (30 µM). Values presented are mean (± SE) of four replicates and different letters on bars represent the significant difference at $P < 0.05$.

reported. Stresses including nickel [90], salinity [14] and water deficit [29] caused significant reduction in the synthesis of chlorophyll intermediates reflecting in considerable decline in chlorophyll synthesis. Reduced contents of GSA, δ- ALA, Proto IX, Mg-Proto IX and protochlorophyllide due to stresses directly results from the decline in the enzyme activities mediating the conversions and the expression of the genes coding these enzymes [29,92]. Increased synthesis of these intermediate molecules directly influences the chlorophyll synthesis and photosynthetic performance of plants [14]. Reduced chlorophyll synthesis in As stressed plants has been reported [11,112,118]. Exogenous treatment of Se [23] and Si [116] alleviated the decline in chlorophyll pigments to considerable extent under As stress, however combined effects have not been reported. In present study also it was seen that exogenous treatment of Se and Si significantly ameliorated the As induced decline in chlorophyll pigments. Carotenoids are important pigment molecules and act as antioxidant as well thereby its increased synthesis in Se and Si treated plants may help in prevention of photoinhibition and also the oxidative effects of reactive oxygen species (ROS) to photosynthetic machinery [28,42].

Amelioration of decline in growth of As stressed plants by Se and Si supplementation may also be ascribed to greater uptake of key mineral ions with concomitant decline in the As accumulation. Arsenic stress drastically reduces the uptake and assimilation of key mineral nutrients thereby impeding the normal growth and metabolism [118,37]. Elements like N, P, K, S and Mg are very important for smooth metabolic functioning and have key roles in alleviating the negative impact of adverse environmental conditions [4,69]. However, application of Se and Si were affective in alleviating the As mediated decline in the uptake of these nutrients. Earlier, Bhadwal and Sharma [23] has also reported that Se treatment alleviated the decline in uptake of nutrient elements in rice plants under As stress. Similarly, supplementation of Si to rice mitigated the decline in N, P, K, Mn, Zn, Fe and Cu under As stress and this increased uptake of nutrients was correlated with increased expression of transporter genes with concomitant decline in As transporters [67]. Recently, in *Spinacia oleracea*¸ Saleem et al., [96] has also observed significant decline in As uptake due to Si supplementation concomitant with increased uptake of beneficial elements. It has been reported that plant species maintaining significantly increased mineral uptake and assimilation better counter the undesirable effects of stresses [5,17]. Supplementation of Si and Se may have also influenced the expression and activity of transporter genes involved in maintaining the mineral uptake with significant decline in As transporters. Moreover, reduced As accumulation is also ascribed to increased phytochelatin synthesis and thiolic ligands thereby preventing its excess accumulation and hence lessening the toxic effects [73]. Maintaining, higher nitrate reductase (NR) activity under As stress due to Se [23] and Si [67] treatment contributed to significant increase in the nitrogen content. However effect of combined Se and Si on NR activity and mineral uptake

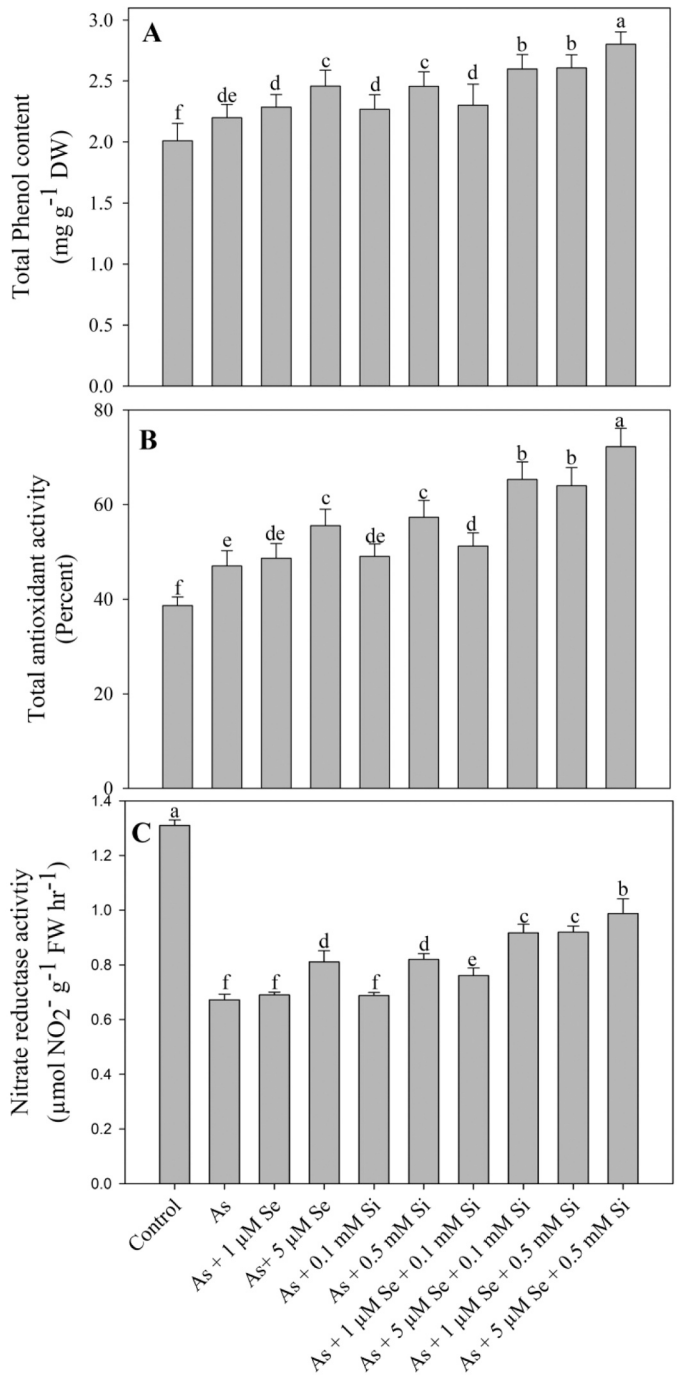

Fig. 8. Effect of individual and combined supplementation of different concentrations of selenium (Se; 1 and 5 µM) and silicon (Si; 0.1 and 0.5 mM) on content of (A) total phenols, (B) total antioxidant activity and the activity of (C) nitrate reductase in *Triticum aestivum* L. under arsenic stress (30 µM). Values presented are mean (± SE) of four replicates and different letters on bars represent the significant difference at $P < 0.05$.

under As stress has not been reported.

Growth reductions in As stressed plants were obviously reflected in significant increase in the parameters of oxidative damage including ROS, lipid peroxidation and the activities of enzymes like lipoxygenase, protease and NADPH oxidase. Generation of ROS and the lipid peroxidation increased under As stress in different plants [10,101,35,52]. Increased activity of NADPH oxidase under cadmium [60], nickel [105] and As [49] stress resulted in greater accumulation of ROS reflecting in increased oxidative damage. Application of Se and Si significantly reduced the activities of lipoxygenase, protease and NADPH oxidase with their combined application imparting more obvious impact. Lipoxygenase catalyse the oxidation of polyunsaturated fatty acids into fatty acid hydroperoxides thereby triggering several downstream stress responsive mechanisms [103]. Treatment of Se reduced the expression of lipoxygenase in As stressed rice plants [73]. Proteases mediate breakdown of peptide bonds causing irreversible posttranslational protein modifications and in addition proteolysis derived peptides regulate the ROS signalling under stressful conditions [99]. Reduced protease and lipoxygenase activity due to supplementation of protectants reflects in improved stress adaptation [8,9,86]. Recently, Karimi et al. [66] has demonstrated that Si supplementation reduced ROS accumulation in As stressed *Isatis cappadocica* thereby causing reduction in oxidative damage. Reduced accumulation of ROS due to Se [15] and Si [61] significantly protects the functioning of photosynthetic machinery.

Reduction in the oxidative effects of As by the treatment of Se and Si in wheat was evident due to their combined application. Arsenic stressed plants exhibited a significant enhancement in the activities of antioxidant enzymes. Activities of SOD, GST, CAT and the ascorbate-glutathione cycle enzymes enhanced significantly by Se and Si application thereby making the elimination of ROS much quicker. Similar to our results, in *Vicia faba* [10], *Solanum lycopersicum* [43] and *Oryza sativa* [83] activity of antioxidant enzymes improved under As stress. Further enhancement due to supplementation of Se has been reported by Kumar et al., [73] in *Oryza sativa*. Exogenous supplementation of Si [61] and Se [15] up-regulated the functioning of antioxidant system, including the ascorbate-glutathione cycle components thereby, significantly alleviating the deleterious effects of cadmium in *Pisum sativum* and *Solanum lycopersicum*. Exogenous Se and Si induced up-regulation of antioxidant system under stressful conditions is evident as significant improvement in chlorophyll synthesis and membrane functioning. It has been reported that exogenous supplementation of Se and Si induced strengthening of antioxidant system protects the photosynthesis and other metabolic functions against the oxidative influence of ROS [15,61,110]. Both Se and Si significantly enhanced GSH and AsA which are involved in regulation of several functions including redox homeostasis, ROS scavenging and regulation of tolerance mechanisms [12,25,104,114]. In addition GSH is key component of phytochelatin synthesis [114]. Reduced glutathione, ascorbic acid, cysteine and tocopherol are among the key non-enzymatic antioxidants and maintain redox homeostasis to protect major cellular functions under stress [120,30]. Exogenous treatment of Se [68] and Si [102] resulted in increased cysteine content in *Triticum aestivum* and *Brassica juncea* under cadmium and lanthanum stress. Increased functioning of antioxidant system and the contents of non-enzymatic antioxidants due to Si treatments prevents the As mediated growth damage in rice [67]. Increase in their concentrations due to supplementation of Se and Si can benefit wheat seedling to better withstand the adverse effects of As stress.

It was observed that wheat plants stressed with As showed increase in the total phenol content. Phenols are important plant secondary compounds actively implicated in several key mechanisms including germination, photosynthesis, stress signalling, ROS scavenging and stress tolerance [113,7]. Increase in the content of phenols due to Se has been reported in *Allium sativum* [18], *Avena sativa* [54] and *Echium amoenum* [64] under different stresses. Similarly, Si increased phenol content in buckwheat [19], *Salvia officinalis* [89] and *Eruca sativa* [57]. Kofronova et al., [72] has reported that tobacco genotypes exhibiting significant increase in phenols, GSH and ascorbate exhibited greater tolerance to As stress. Greater secondary metabolite synthesis results due to increased functioning of enzymes catalyzing their biosynthesis [76,91]. Treatment of Se and Si mediated enhancement in the metabolite contents resulted in increased total antioxidant activity thereby contributing to assuaging of the As induced oxidative stress. Increased total antioxidant activity reflects the potential to avert the damage to sensitive cellular organelles and pathways [3]. In addition the content of proline, sugars, free amino acids and glycine betaine improved

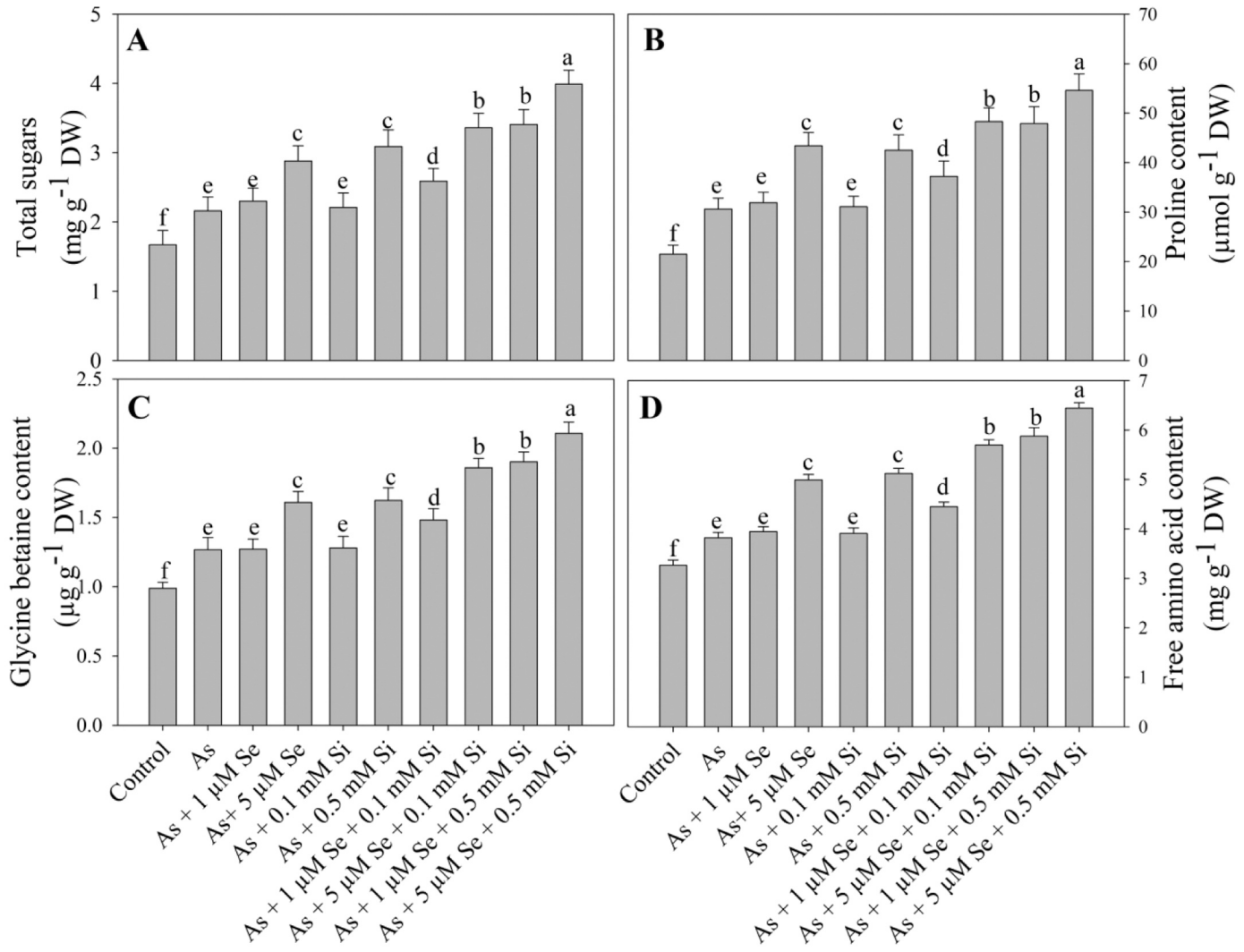

Fig. 9. Effect of individual and combined supplementation of different concentrations of selenium (Se; 1 and 5 μM) and silicon (Si; 0.1 and 0.5 mM) on content of (A) total sugars, (B) proline, (C) glycine betaine and (D) free amino acids in *Triticum aestivum* L. under arsenic stress (30 μM). Values presented are mean (± SE) of four replicates and different letters on bars represent the significant difference at $P < 0.05$.

significantly due to supplementation of Se and Si. Compatible osmolytes regulate growth under stresses by protecting photosynthesis, enzyme activity, membranes, maintaining redox homeostasis, mediating ROS scavenging and stress signalling [121,44]. In corroboration to the results, earlier increased osmolytes including glycine betaine, sugars, proline and free amino acids due to As stress has been reported (Pavlik et al., 2010; [11,95,82]). Supplementation of Se [10,95] and Si (Zahedi eta al. 2020; [108]) further increased the content of osmolytes to mediate more protection to cellular metabolism by maintaining relative water content, protecting enzyme functioning and plant tolerance against As. Further studies can be beneficial in understanding the exact mechanisms.

5. Conclusion

Exogenous Se and Si proved beneficial in protecting wheat against the adverse effects of As. Combined treatment of higher concentrations of Se and Si were more affective in alleviating the decline in growth and chlorophyll synthesis. Alleviation of arsenic stress induced oxidative damage was reflected in reduced ROS concentration, lipid peroxidation and the activity of protease, LOX and NADPH oxidase. Improved tolerance to As in Se and Si treated plants can be attributed to improved antioxidant system functioning and greater osmolyte and redox components synthesis. Further, increased phenol content together with osmolyte accumulation was evident as increased total antioxidant activity in Se and Si treated plants. Combined treatment of Se and Si can be exploited to prevent the damaging effects of As to wheat.

Environmental Implications

Arsenic (As) is one of the toxic metal ions affecting the growth and productivity of plants all over the world. The concentration of As in agricultural soils is increasing day by day and China is also facing the same problem. This has serious consequences on global food security and the health of humans. As in soil alters soil health thereby hindering the normal growth of plants and if taken by humans (through food or water) can cause serious damage to several organs. As polluted areas have been declared unsafe for crop cultivation thereby management strategies can be worthwhile for sustainable growth. In the backdrop, present manuscript addresses the implication of arsenic (As) on the growth, chlorophyll synthesis, enzyme functioning and oxidative damage in wheat.

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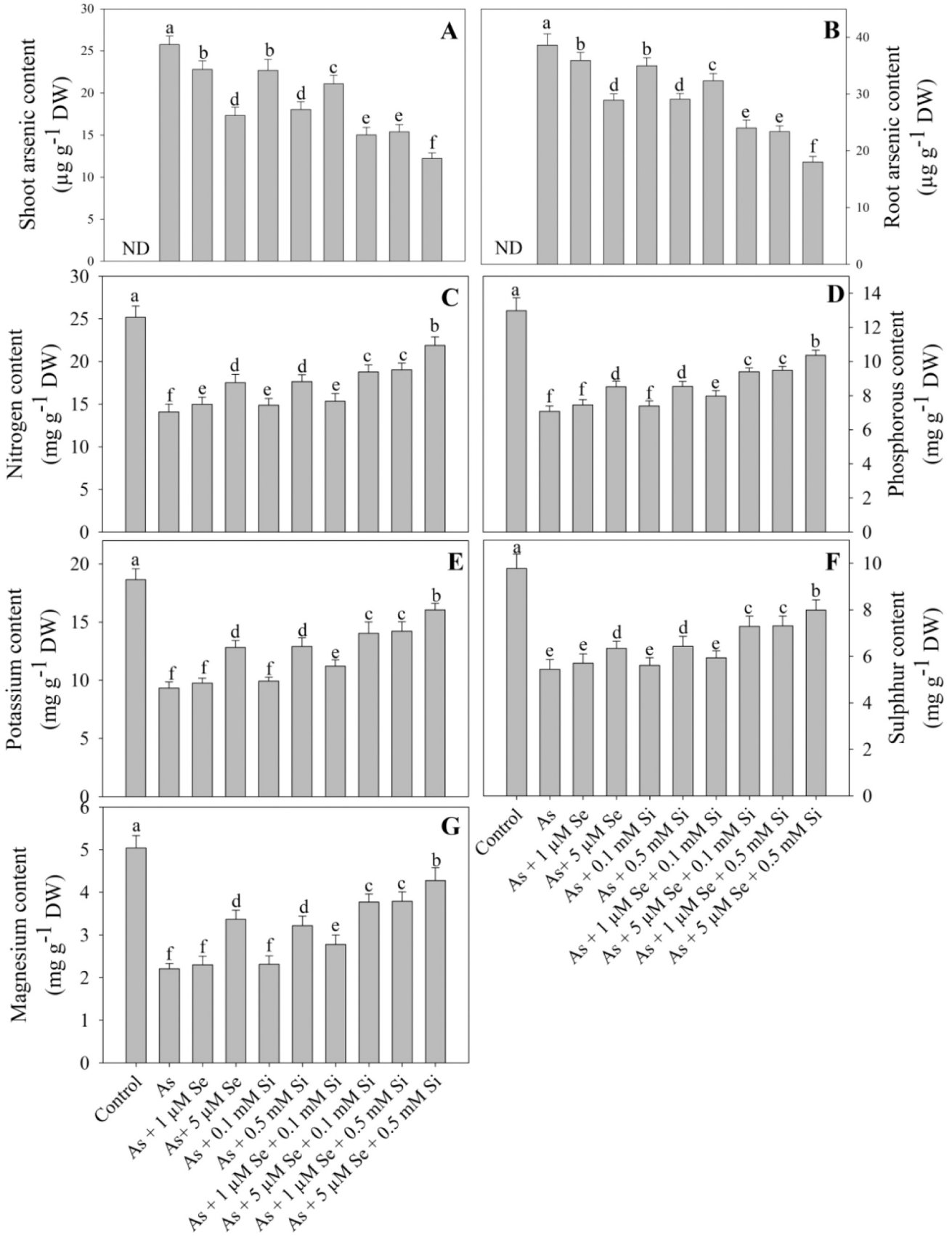

Fig. 10. Effect of individual and combined supplementation of different concentrations of selenium (Se; 1 and 5 μM) and silicon (Si; 0.1 and 0.5 mM) on content of (A) shoot arsenic, (B) root arsenic, (C) nitrogen, (D) phosphorous, (E) potassium, (F) sulphur and (G) magnesium in *Triticum aestivum* L. under arsenic stress (30 μM). Values presented are mean (± SE) of four replicates and different letters on bars represent the significant difference at $P < 0.05$.

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CRediT authorship contribution statement

Alsahli Abdulaziz Abdullah: Funding acquisition, Visualization, Writing – original draft, Writing – review & editing. **Ahanger Mohammad A Abass:** Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Qin Cheng:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft. **Lian Huida:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Validation, Writing – original draft. **Zhang Bo:** Formal analysis, Software, Writing – original draft. **He Zhan:** Methodology, Resources, Software, Visualization, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

All data generated have been included in the manuscript.

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References

[1] Aebi, H., 1984. Catalase in vitro. Meth Enzymol 105, 121–126.
[2] Aftabtalab, A., Rinklebe, J., Shaheen, S.M., Niazi, N.K., Moreno-Jimenez, E., Schaaler, J., Knorr, K.H., 2022. Review on the interactions of arsenic, iron (oxy)(hydr)oxides, and dissolved organic matter in soils, sediments, and groundwater in a ternary system. Chemosphere 286 (2), 131790. https://doi.org/10.1016/j.chemosphere.2021.131790.
[3] Ahanger, M.A., Agarwal, R.M., 2017. Salinity stress induced alterations in antioxidant metabolism and nitrogen assimilation in wheat (*Triticum aestivum* L) as influenced by potassium supplementation. Plant Physiol Biochem 449–460.
[4] Ahanger, M.A., Agarwal, R.M., Tomar, N.S., Shrivastava, M., 2015. Potassium induces positive changes in nitrogen metabolism and antioxidant system of oat (*Avena sativa* L cultivar Kent). J Plant Interact 10 (1), 211–223.
[5] Ahanger, M.A., Tittal, M., Mir, R.A., Agarwal, R.M., 2017. Alleviation of water and osmotic stress-induced changes in nitrogen metabolizing enzymes in *Triticum aestivum* L. cultivars by potassium. Protoplasma 254 (5), 1953–1963.
[6] Ahanger, M.A., Tomar, N.S., Tittal, M., Argal, S., Agarwal, R.M., 2017. Plant growth under water/ salt stress: ROS production; antioxidants and significance of added potassium under such conditions. Physiol Mol Biol Plants 23 (4), 731–744.
[7] Ahanger, M.A., Bhat, J.A., Siddiqui, M.H., Rinklebe, J., Ahmad, P., 2020. Integration of silicon and secondary metabolites in plants: a significant association in stress tolerance. J Exp Bot 71 (21), 6758–6774. https://doi.org/10.1093/jxb/eraa291.
[8] Ahanger, M.A., Aziz, U., Alsahli, A.A., Alyemeni, M.N., Ahmad, P., 2020. Combined kinetin and spermidine treatments ameliorate growth and photosynthetic inhibition in *Vigna angularis* by up-regulating antioxidant and nitrogen metabolism under cadmium stress. Biomolecules 10, 147. https://doi.org/10.3390/biom10010147.
[9] Ahanger, M.A., Qin, C., Maodong, Q., Dong, X.X., Ahmad, P., Abd_Allah, E.F., Zhang, L., 2019. Spermine application alleviates salinity induced growth and photosynthetic inhibition in *Solanum lycopersicum* by modulating osmolyte and secondary metabolite accumulation and differentially regulating antioxidant metabolism. Plant Physiol Biochem 144, 1–13.
[10] Ahmad, A., Khan, W.U., Shah, A.A., Yasin, N.A., Naz, S., Ali, A., Tahir, A., Batool, A.I., 2021. Synergistic effects of nitric oxide and silicon on promoting plant growth, oxidative stress tolerance and reduction of arsenic uptake in Brassica juncea. Chemosphere 262, 128384. https://doi.org/10.1016/j.chemosphere.2020.128384.
[11] Ahmad, P., Alam, P., Balawi, T.H., Altalayan, F.H., Ahanger, M.A., Ashraf, M., 2020. Sodium nitroprusside (SNP) improves tolerance to arsenic (As) toxicity in *Vicia faba* through the modifications of biochemical attributes, antioxidants, ascorbate-glutathione cycle and glyoxalase cycle. Chemosphere. https://doi.org/10.1016/j.chemosphere.2019.125480.
[12] Al-Huqail, A.A., Ali, H.M., Kushwaha, B.K., AL-Huqail, A.A., Singh, V.P., Siddiqui, M.H., 2020. Ascorbic acid is essential for inducing chromium (VI) toxicity tolerance in tomato roots. J Biotech 322, 66–73. https://doi.org/10.1016/j.jbiotec.2020.07.011.
[13] Al-Makishah, N.H., Taleb, M.A., Barakat, M.A., 2020. Arsenic bioaccumulation in arsenic-contaminated soil: a review, 2020 Chem Pap 74, 2743–2757. https://doi.org/10.1007/s11696-020-01122-4.
[14] Al-Mushhin, A.A.M., 2022. Interactive effect of potassium and spermidine protects growth, photosynthesis and chlorophyll biosynthesis in Vigna angularis from salinity induced damage by up-regulating the tolerance mechanisms. Not Bot Horti Agrobot Cluj-Napoca 50 (1), 12607. https://doi.org/10.15835/nbha50112607.
[15] Alyemeni, M.N., Ahanger, M.A., Wijaya, L., Alam, P., Bhardwaj, R., Ahmad, P., 2018. Selenium mitigates cadmium-induced oxidative stress in tomato (*Solanum lycopersicum* L.) plants by modulating chlorophyll fluorescence, osmolyte accumulation, and antioxidant system. Protoplasma 255 (2), 459–469.
[16] Arnon, D.I., 1949. Copper enzymes in isolated chloroplast polyphenol oxidase in *Beta vulgaris*. Plant Physiol 24, 1–15.
[17] Asgher, M., Khan, N.A., Khan, M.I.R., Fatma, M., Masood, A., 2014. Ethylene production is associated with alleviation of cadmium-induced oxidative stress by sulfur in mustard types differing in ethylene sensitivity. Ecotoxicol Environ Saf 106, 54–61. https://doi.org/10.1016/j.ecoenv.2014.04.017.
[18] Astaneh, R.K., Bolandnazar, S., Nahandi, F.Z., Oustan, S., 2018. Effect of selenium application on phenylalanine ammonia-lyase (PAL) activity, phenol leakage and total phenolic content in garlic (*Allium sativum* L.) under NaCl stress. Inf Process Agric 5 (3), 339–344. https://doi.org/10.1016/j.inpa.2018.04.004.
[19] Azad, M.O.K., Park, B.S., Adnan, M., Germ, M., Kreft, I., Woo, S.H., Park, C.H., 2021. Silicon biostimulant enhances the growth characteristics and fortifies the bioactive compounds in common and Tartary buckwheat plant. J Crop Sci Biotechnol 24, 51–59. https://doi.org/10.1007/s12892-020-00058-1.
[20] Backer, H., Frank, O., de Angells, B., Feingold, S., 1980. Plasma tocopherol in man at various times after ingesting free or ocetylaned tocopherol. Nutr Rep Int 21, 531–536.
[21] Bates, L.S., Waldre, R.P., Teare, I.D., 1973. Rapid determination of free proline for water stress studies. Plant Sci 39, 205–207.
[22] Bayer, W.F., Fridovich, J.L., 1987. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal Biochem 161, 559–566.
[23] Bhadwal, S., Sharma, S., 2022. Selenium alleviates physiological traits, nutrient uptake and nitrogen metabolism in rice under arsenate stress. Environ Sci Pollut Res 29, 70862–70881. https://doi.org/10.1007/s11356-022-20762-5.
[24] Bhattacharya, S., Sharma, P., Mitra, S., Mallick, I., Ghosh, A., 2021. Arsenic uptake and bioaccumulation in plants: a review on remediation and socio-economic perspective in Southeast Asia. Environ Nanotech Monit Manag 15, 100430. https://doi.org/10.1016/j.enmm.2021.100430.
[25] Celi, G.E.A., Gratao, P.L., Dantas, M.G., Lanza, B., dos Reis, A.R., 2023. Physiological and biochemical roles of ascorbic acid on mitigation of abiotic stresses in plants. Plant Physiol Biochem 202, 107970. https://doi.org/10.1016/j.plaphy.2023.107970.
[26] Chang, Y., Cui, H., Wang, Y., Li, C., Wang, J., Jin, M., Luo, Y., Li, Y., Wang, Z., 2023. Silicon spraying enhances wheat stem resistance to lodging under light stress. Agronomy 13 (10), 2565. https://doi.org/10.3390/agronomy13102565.
[27] Chen, H., Cheng, Q., Chen, Q., Ye, X., Qu, Y., Song, W., Fahad, S., Gao, J., Saud, S., Xu, Y., Shen, Y., 2022. Effects of selenium on growth and selenium content distribution of virus-free sweet potato seedlings in water culture. Front Plant Sci 13. https://doi.org/10.3389/fpls.2022.965649.
[28] Collini, E., 2019. Carotenoids in photosynthesis: the revenge of the "accessory" pigments. Chem 5 (3), 494–495. https://doi.org/10.1016/j.chempr.2019.02.013.
[29] Dalal, V.K., Tripathy, B.C., 2012. Modulation of chlorophyll biosynthesis by water stress in rice seedlings during chloroplast biogenesis. Plant Cell Environ 35, 1685–1703. https://doi.org/10.1111/j.1365-3040.2012.02520.x.
[30] de Queiroz, A.R., Hines, C., Brown, J., Sahay, S., Vijayan, J., Stone, J.M., Bickford, N., Wuellner, M., Glowacka, K., Buan, N.R., Roston, R., 2023. The effects of exogenously applied antioxidants on plant growth and resilience. Phytochem Rev 22, 407–447. https://doi.org/10.1007/s11101-023-09862-3.
[31] Doderer, A., Kokkelink, I., van der Veen, S., Valk, B., Schram, A., Douma, A., 1992. Purification and characterization of two lipoxygenase isoenzymes from germinating barley. Biochim Biophys Acta 112, 97–104.
[32] Du, B., Luo, H., He, L., Zhang, L., Liu, Y., Mo, Z., Pan, S., Tian, H., Duan, M., Tang, X., 2019. Rice seed priming with sodium selenate: effects on germination, seedling growth, and biochemical attributes. Sci Rep 9, 4311. https://doi.org/10.1038/s41598-019-40849-3.
[33] Elkelish, A.A., Soliman, M.H., Alhaithloul, H.A., El-Esawi, M.A., 2019. Selenium protects wheat seedlings against salt stress-mediated oxidative damage by up-regulating antioxidants and osmolytes metabolism. Plant Physiol Biochem 137, 144–153. https://doi.org/10.1016/j.plaphy.2019.02.004.
[34] Ellman, G.L., 1959. Tissue sulphydryl groups. Arch Biochem Biophys 82, 70–77.
[35] Emamverdian, A., Ding, Y., Hasanuzzaman, M., Barker, J., Liu, G., Li, Y., Mokhberdoran, F., 2023. Insight into the biochemical and physiological mechanisms of nanoparticles-induced arsenic tolerance in bamboo. Front Plant Sci 14, 1121886. https://doi.org/10.3389/fpls.2023.1121886.
[36] Etteieb, S., Magdouli, S., Komtchou, S.P., Zolfaghari, M., Tanabene, R., Brar, K.K., Calugaru, L.L., Brar, S.K., 2021. Selenium speciation and bioavailability from

mine discharge to the environment: a field study in Northern Quebec, Canada. Environ Sci Pollut Res 28, 50799–50812.
[37] Faizan, M., Sehar, S., Rajput, V.D., Faraz, A., Afzal, S., Minkina, T., Sushkova, S., Adil, M.F., Yu, F., Alatar, A.A., Akhter, F., Faisal, M., 2021. Modulation of cellular redox status and antioxidant defense system after synergistic application of zinc oxide nanoparticles and salicylic acid in rice (*Oryza sativa*) plant under arsenic stress. Plants 10 (11), 2254. https://doi.org/10.3390/plants10112254.
[38] Fernandes, J., Hu, X., Smith, M.R., Go, Y.M., Jones, D.P., 2018. Selenium at the redox interface of the genome, metabolome and exposome. Free Radic Biol Med 127, 215–227.
[39] Finnegan, P.M., Chen, W., 2012. Arsenic toxicity: the effects on plant metabolism. Front Physiol 3, 182. https://doi.org/10.3389/fphys.2012.00182.
[40] Fong, J., Schaffer, F.L., Kirk, P.L., 1953. The ultramicrodetermination of glycogen in liver. A comparison of the anthrone and reducing-sugar methods. Arch Biochem Biophy 45 (2), 319–326.
[41] Foyer, C.H., Halliwell, B., 1976. The presence of glutathione and glutathione reductase in chloroplast: a proposed role in ascorbic acid metabolism. Plant 133, 21–25.
[42] Frank, H.A., Brudvig, G.W., 2004. Redox functions of carotenoids in photosynthesis. Biochemistry 43, 8607–8615.
[43] Ghorbani, A., Pishkar, L., Roodbari, N., Pehlivan, N., Wu, C., 2021. Nitric oxide could allay arsenic phytotoxicity in tomato (Solanum lycopersicum L.) by modulating photosynthetic pigments, phytochelatin metabolism, molecular redox status and arsenic sequestration. Plant Physiol Biochem 167, 337–348. https://doi.org/10.1016/j.plaphy.2021.08.019.
[44] Ghosh, U.K., Islam, M.N., Siddiqui, M.N., Khan, M.A.R., 2021. Understanding the roles of osmolytes for acclimatizing plants to changing environment: a review of potential mechanism. Plant Signal Behav 16 (8), 1913306. https://doi.org/10.1080/15592324.2021.1913306.
[45] Giatonde, M.K., 1967. A spectrophotometric method for the direct determination of cysteine in the presence of other naturally occurring aminoacids. Biochem J 104, 627–633.
[46] Gong, Y., Qu, Y., Yang, S., Tao, S., Shi, T., Liu, Q., Chen, Y., Wu, Y., Ma, J., 2020. Status of arsenic accumulation in agricultural soils across China (1985–2016). July 2020 Environ Res 186, 109525. https://doi.org/10.1016/j.envres.2020.109525.
[47] Green, N.M., Neurath, H., 1954. Proteolytic enzymes. In: Neurath, H., Vailey, K. (Eds.), The Proteins, Vol. II. Part B. Academic Press, New York, pp. 1057–1198.
[48] Grieve, C.M., Grattan, S.R., 1983. Rapid assay for determination of water soluble quaternary ammonium compounds. Plant Soil 70, 303–307.
[49] Gupta, D.K., Inouhe, M., Rodríguez-Serrano, M., Romero-Puertas, M.C., Sandalio, L.M., 2013. Oxidative stress and arsenic toxicity: Role of NADPH oxidases. Chemosphere 90 (6), 1987–1996. https://doi.org/10.1016/j.chemosphere.2012.10.066.
[50] Gupta, M., Gupta, S., 2017. An overview of selenium uptake, metabolism, and toxicity in plants. Front Plant Sci 7, 2074.
[51] Habibi, G., 2016. Effect of foliar-applied silicon on photochemistry, antioxidant capacity and growth in maize plants subjected to chilling stress. Acta Agric Slov 107, 33–43.
[52] Hasanuzzaman, M., Fujita, M., 2013. Exogenous sodium nitroprusside alleviates arsenic-induced oxidative stress in wheat (*Triticum aestivum* L.) seedlings by enhancing antioxidant defense and glyoxalase system. Ecotoxicology 22, 584–596. https://doi.org/10.1007/s10646-013-1050-4.
[53] Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chlorplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125, 189–198.
[54] Hegab, R.H., Eissa, D., Abou-Shady, A., 2022. Effects of foliar application of selenium and potassium-humate on oat growth in Baloza. North Sinai, Egypt Sci Rep 12, 15119. https://doi.org/10.1038/s41598-022-19229-x.
[55] Hodgins, R.R., Huystee, R.B.V., 1986. Rapid simultaneous estimation of protoporphyrin and Mg-porphyrins in higher plants. J Plant Physiol 125, 311–323.
[56] Hossain, M.A., Nakano, Y., Asada, K., 1984. Monodehydroascorbate reductase in spinach chloroplasts and its participation in the regeneration of ascorbate for scavenging hydrogen peroxide. Plant Cell Physiol 25, 385–395.
[57] Hussain, I., Ayub, A., Nayab, A., Ashraf, M.A., Ashraf, M.A., Hussain, S., Siddiqui, M.H., Sabir, M.A., Zulfiqar, U., Khan, T.H., 2023. Exogenous application of silicon and zinc attenuates drought tolerance in *Eruca sativa* L. through increasing chlorophyll pigments, osmoprotectants, and modulating defense mechanisms. J Plant Growth Regul. https://doi.org/10.1007/s00344-023-11116-7.
[58] Imran, M., Ashraf, M., Awan, A.R., 2021. Growth, yield and arsenic accumulation by wheat grown in a pressmud amended salt-affected soil irrigated with arsenic contaminated water. Ecotox Environ Saf 224, 112692. https://doi.org/10.1016/j.ecoenv.2021.112692.
[59] Jackson, M.L., 1973. Soil Chemical Analysis. Prentice Hall of India Pvt. Ltd,, New Delhi.
[60] Jakubowska, D., Janicka-Russak, M., Kabała, K., Migocka, M., Reda, M., 2015. Modification of plasma membrane NADPH oxidase activity in cucumber seedling roots in response to cadmium stress. Plant Sci 234, 50–59. https://doi.org/10.1016/j.plantsci.2015.02.005.
[61] Jan, S., Alyemeni, M.N., Wijaya, L., Alam, P., Siddique, K.H., Ahmad, P., 2018. Interactive effect of 24-epibrassinolide and silicon alleviates cadmium stress via the modulation of antioxidant defense and glyoxalase systems and macronutrient content in *Pisum sativum* L. Seedlings. BMC Plant Bio 18, 146. https://doi.org/10.1186/s12870-018-1359-5.
[62] Jiang, M., Zhang, J., 2002. Involvement of plasma-membrane NADPH oxidase in abscisic acid- and water stress-induced antioxidant defense in leaves of maize seedlings. Planta 215, 1022–1030. https://doi.org/10.1007/s00425-002-0829-y.
[63] Jimenez-Jimenez, P.A., Diaz, X., Silva, M.L.N., Vega, A., Curi, N., 2023. Assessing and understanding arsenic contamination in agricultural soils and lake sediments from Papallacta Rural Parish, northeastern ecuador, via ecotoxicology factors, for environmental embasement. Sustainability 15 (5), 3951. https://doi.org/10.3390/su15053951.
[64] Kalaei, M.H.R., Abdossi, V., Danaee, E., 2022. Evaluation of foliar application of selenium and flowering stages on selected properties of Iranian Borage as a medicinal plant. Sci Rep 12–12568. https://doi.org/10.1038/s41598-022-16241-z.
[65] Kannangara, C.G., Schouboe, A., 1985. Biosynthesis of d-ALA in greening barley leaves. VII. Glutamate-1-semialdehyde accumulation in gabaculine treated leaves. Carlsberg Res Commun 50, 179–191.
[66] Karimi, N., Mortezazadeh, A., Souri, Z., Farooq, M., 2023. Silicon application alleviates arsenic toxicity in isatis (*Isatis cappadocica* Desv.) by modulating key biochemical attributes and antioxidant defense systems. J Plant Growth Reg. https://doi.org/10.1007/s00344-023-11079-9.
[67] Khan, E., Gupta, M., 2018. Arsenic–silicon priming of rice (*Oryza sativa* L.) seeds influence mineral nutrient uptake and biochemical responses through modulation of Lsi-1, Lsi-2, Lsi-6 and nutrient transporter genes. Sci Rep 8, 10301. https://doi.org/10.1038/s41598-018-28712-3.
[68] Khan, M.I.R., Nazir, F., Asgher, M., Per, T.S., Khan, N.A., 2015. Selenium and sulfur influence ethylene formation and alleviate cadmium-induced oxidative stress by improving proline and glutathione production in wheat. J Plant Physiol 173, 9–18. https://doi.org/10.1016/j.jplph.2014.09.011.
[69] Khan, M.I.R., Nazir, F., Maheshwari, C., Chopra, P., Chhillar, H., Sreenivasulu, N., 2023. Mineral nutrients in plants under changing environments: A road to future food and nutrition security. Plant Genome 2023, e20362. https://doi.org/10.1002/tpg2.20362.
[70] Khan, Z., Thounaojam, T.C., Upadhyaya, H., 2022. Arsenic stress in rice (*Oryza sativa*) and its amelioration approaches. Plant Stress 4, 100076. https://doi.org/10.1016/j.stress.2022.100076.
[71] Khan, Z., Thounaojam, T.C., Chowdhury, D., Upadhyaya, H., 2023. The role of selenium and nano selenium on physiological responses in plant: a review. Plant Growth Regul 100, 409–433. https://doi.org/10.1007/s10725-023-00988-0.
[72] Kofronova, M., Hrdinova, A., Maskova, P., Tremlova, J., Soudek, P., Petrova, S., Pinkas, D., Lipavska, H., 2020. Multi-component antioxidative system and robust carbohydrate status, the essence of plant arsenic tolerance. Antioxidants 9, 283. https://doi.org/10.3390/antiox9040283.
[73] Kumar, A., Singh, R.P., Singh, P.K., Awasthi, S., Chakrabarty, D., Trivedi, P.K., Tripathi, R.D., 2014. Selenium ameliorates arsenic induced oxidative stress through modulation of antioxidant enzymes and thiols in rice (*Oryza sativa* L.). Ecotoxicology 23 (7), 1153–1163. https://doi.org/10.1007/s10646-014-1257-z.
[74] Larsson, C., Widell, S., Kjellbom, P., 1987. Preparation of high-purity plasma membranes. Method Enzymol 148, 558–568. https://doi.org/10.1016/0076-6879(87)48054-3.
[75] Li, C., Feng, S., Shao, Y., Jiang, L., Lu, X., Hou, X., 2007. Effects of arsenic on seed germination and physiological activities of wheat seedlings. J Environ Sci 19 (6), 725–732. https://doi.org/10.1016/S1001-0742(07)60121-1.
[76] Lian, H., Qin, C., Shen, J., Ahanger, M.A., 2023. Alleviation of adverse effects of drought stress on growth and nitrogen metabolism in mungbean (*Vigna radiata*) by sulphur and nitric oxide involves up-regulation of antioxidant and osmolyte metabolism and gene expression. Plants 12, 3082. https://doi.org/10.3390/plants12173082.
[77] Ma, J.F., Takahashi, E., 2002. Soil, fertilizer, and plant silicon research in Japan. Elsevier Science,, Amsterdam, The Netherlands.
[78] Ma, J.F., Yamaji, N., 2006. Silicon uptake and accumulation in higher plants. Trends Plant Sci 11 (8), 392–397. https://doi.org/10.1016/j.tplants.2006.06.007.
[79] Mahdieh, S., Ghaderian, S.M., Karimi, N., 2013. Effect of arsenic on germination, photosynthesis and growth parameters of two winter wheat varieties in Iran. J Plant Nut 36 (4), 651–664. https://doi.org/10.1080/01904167.2012.754036.
[80] Malick, C.P., Singh, M.B., 1980. Plant enzymology and histoenzymology. Kalyani Publishers,, New Delhi, p. 286.
[81] Mandlik, R., Thakral, V., Raturi, G., Shinde, S., Nikolić, M., Tripathi, D.K., Sonah, H., Deshmukh, R., 2020. Significance of silicon uptake, transport, and deposition in plants. J Exp Bot 71 (21), 6703–6718. https://doi.org/10.1093/jxb/eraa301.
[82] Mishra, V., Singh, V.P., 2021. Implication of nitric oxide and hydrogen sulfide signalling in alleviating arsenate stress in rice seedlings. Environ Pollut 291, 117958. https://doi.org/10.1016/j.envpol.2021.117958.
[83] Mousavi, S.R., Niknejad, Y., Fallah, H., Tari, D.B., 2020. Mousavi SR, Methyl jasmonate alleviates arsenic toxicity in rice. Plant Cell Rep 39 (8), 1041–1060. https://doi.org/10.1007/s00299-020-02547-7.
[84] Mukherjee, S.P., Choudhuri, M.A., 1983. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. Physiol Plant 58, 166–170.
[85] Mustafa, T., Sattar, A., Sher, A., Allah, S., Ijaz, M., Irfan, M., Butt, M., Cheema, M., 2021. Exogenous application of silicon improves the performance of wheat under terminal heat stress by triggering physio-biochemical mechanisms. Sci Rep 11, 23170. https://doi.org/10.1038/s41598-021-02594-4.
[86] Nahar, K., Hasanuzzaman, M., Alam, M.M., Rahman, A., Suzuki, T., Fujita, M., 2016. Polyamine and nitric oxide crosstalk: Antagonistic effects on cadmium toxicity in mungbean plants through upregulating the metal detoxification,

antioxidant defense and methylglyoxal detoxification systems. Ecotox Environ Saf 126, 245–255.
[87] Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach-chloroplasts. Plant Cell Physiol 22, 867–880.
[88] Patel, K.S., Pandey, P.K., Martin-Ramos, P., Corns, W.T., Varol, S., Bhattacharya, P., Zhu, Y., 2023. A review on arsenic in the environment: contamination, mobility, sources, and exposure. RSC Adv 2023 (13), 8803–8821. https://doi.org/10.1039/D3RA00789H.
[89] Pirooz, P., Amooaghaie, R., Ahadi, A., Sharififar, F., Torkzadeh-Mahani, M., 2022. Silicon and nitric oxide synergistically modulate the production of essential oil and rosmarinic acid in *Salvia officinalis* under Cu stress. Protoplasma 259, 905–916. https://doi.org/10.1007/s00709-021-01708-z.
[90] Qin, C., Shen, J., Ahanger, M.A., 2022. Supplementation of nitric oxide and spermidine alleviates the nickel stress induced damage to growth, chlorophyll metabolism, and photosynthesis by up-regulating ascorbate–glutathione and glyoxalase cycle functioning in tomato. Front Plant Sci 13, 1039480. https://doi.org/10.3389/fpls.2022.1039480.
[91] Qin, C., Lian, H., Alqahtani, F.M., Ahanger, M.A., 2023. Chromium mediated damaging effects on growth, nitrogen metabolism and chlorophyll synthesis in tomato can be alleviated by foliar application of melatonin and jasmonic acid priming. Sci Hortic 323, 112494. https://doi.org/10.1016/j.scienta.2023.112494.
[92] Qin, C., Ahanger, M.A., Zhou, J., Ahmed, N., Wei, C., Yuan, S., Ashraf, M., Zhang, L., 2020. Beneficial role of acetylcholine in chlorophyll metabolism and photosynthetic gas exchange in *Nicotiana benthamiana* seedlings under salinity stress. Plant Biol 22 (3), 357–365.
[93] Rodriguez-Lado, L., Sun, G., Berg, M., Zhang, Q., Xue, H., Zheng, Q., Johnson, C. A., 2013. Groundwater arsenic contamination throughout China. Science 341 (6148), 866–868. https://doi.org/10.1126/science.1237484.
[94] Sagi, M., Fluhr, R., 2001. Superoxide production by plant homologues of the gp91phox NADPH oxidase. Modulation of activity by calcium and by tobacco mosaic virus infection. Plant Physiol 126, 1281–1290.
[95] Sahay, S., Khan, E., Praveen, A., Panthri, M., Mirza, Z., Gupta, M., 2020. Sulphur potentiates selenium to alleviate arsenic-induced stress by modulating oxidative stress, accumulation and thiol-ascorbate metabolism in *Brassica juncea* L. Environ Sci Pollut Res 27, 11697–11713. https://doi.org/10.1007/s11356-019-07520-w.
[96] Saleem, M.H., Mfarrej, M.F.B., Alatawi, A., Mumtaz, S., Imran, M., Ashraf, M.A., Rizwan, M., Usman, K., Ahmad, P., Ali, S., 2023. Silicon Enhances Morpho–Physio–Biochemical Responses in Arsenic Stressed Spinach (*Spinacia oleracea* L.) by Minimizing Its Uptake, 2023 J Plant Growth Regul 42, 2053–2072. https://doi.org/10.1007/s00344-022-10681-7.
[97] Schiavon, M., Pilon-Smits, E.A., 2017. Selenium biofortification and phytoremediation phytotechnologies: a review. J Environ Qual 46, 10–19.
[98] Sharma, A., Kapoor, D., Gautam, S., Landi, M., Kandhol, N., Araniti, F., Ramakrishnan, M., Satish, L., Singh, V.P., Sharma, P., 2022. Heavy metal induced regulation of plant biology: Recent insights. Physiol Plant 174 (3). https://doi.org/10.1111/ppl.13688.
[99] Sharma, P., Gayen, D., 2021. Plant protease as regulator and signaling molecule for enhancing environmental stress-tolerance. Plant Cell Rep 40, 2081–2095. https://doi.org/10.1007/s00299-021-02739-9.
[100] Shimada, K., Fujikawa, K., Yahara, K., Nakamura, T., 1992. Antioxidative properties of xanthone on the auto oxidation of soybean in cylcodextrin emulsion. J Agr Food Chem 40, 945–948.
[101] Shri, M., Kumar, S., Chakrabarty, D., Trivedi, P.K., Mallick, S., Misra, P., Shukla, D., Mishra, S., Srivastava, S., Tripathi, R.D., Tuli, R., 2009. Effect of arsenic on growth, oxidative stress, and antioxidant system in rice seedlings. Ecotoxicol Environ Saf 72 (4), 1102–1110. https://doi.org/10.1016/j.ecoenv.2008.09.022.
[102] Siddiqui, M.H., Mukherjee, S., Al-Munqedhi, B.M.A., Kumar, R., Kalaji, H.M., 2023. Salicylic acid and silicon impart resilience to lanthanum toxicity in *Brassica juncea* L. seedlings. Plant Growth Regul 100, 453–466. https://doi.org/10.1007/s10725-021-00787-5.
[103] Singh, P., Arif, Y., Miszczuk, E., Bajguz, A., Hayat, S., 2022. Specific roles of lipoxygenases in development and responses to stress in plants. Plants 11 (7), 979. https://doi.org/10.3390/plants11070979.
[104] Singh, S., Parihar, P., Singh, R., Singh, V.P., Prasad, S.M., 2016. Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics, and ionomics, 2016 Front Plant Sci 6. https://doi.org/10.3389/fpls.2015.01143.
[105] Sirhindi, G., Mir, M.A., Abd-Allah, E.F., Ahmad, P., Gucel, S., 2016. Jasmonic acid modulates the physio-biochemical attributes, antioxidant enzyme activity, and gene expression in *Glycine max* under nickel toxicity. Front Plant Sci 7, 591. https://doi.org/10.3389/fpls.2016.00591.
[106] Sogarwal, A., Kumari, N., Sharma, V., 2022. Role of silicon in abiotic stress tolerance in wheat. Cereal Res Commun. https://doi.org/10.1007/s42976-022-00346-2.
[107] Srivastava, H.S., 1974. In vivo activity of nitrate reductase in maize seedlings. Indian J Biochem Biophys 11, 230–232.
[108] Teixeira, G.C.M., de Prado, R.M., Rocha, A.M.S., de Oliveira filho, A.S.B., da Sousa Junior, G.S., Gratao, P.L., 2022. Action of silicon on the activity of antioxidant enzymes and on physiological mechanisms mitigates water deficit in sugarcane and energy cane plants (|). Sci Rep 12, 17487. https://doi.org/10.1038/s41598-022-21680-9.
[109] Titov, A.F., Kaznina, N.M., Karapetyan, T.A., Dorshakova, N.V., Tarasova, V.N., 2022. Role of selenium in plants, animals, and humans. Biol Bull Rev 12, 189–200.
[110] Ulhassan, Z., Gill, R.A., Huang, H., Ali, S., Mwamba, T.M., Ali, B., Huang, Q., Hamid, Y., Khan, A.R., Wang, J., Zhou, W., 2019. Selenium mitigates the chromium toxicity in *Brassicca napus* L. by ameliorating nutrients uptake, amino acids metabolism and antioxidant defense system. Plant Physiol Biochem 145, 142–152. https://doi.org/10.1016/j.plaphy.2019.10.035.
[111] Velikova, V., Yordanov, I., Edreva, A., 2000. Oxidative stress and some antioxidant systems in acid rain treated bean plants, protective role of exogenous polyamines. Plant Sci 151, 59–66.
[112] Vezza, M.E., Alemano, S., Agostini, E., Talano, M.A., 2022. Arsenic Toxicity in Soybean Plants: Impact on Chlorophyll Fluorescence, Mineral Nutrition and Phytohormones. J Plant Growth Regul 41, 2719–2731. https://doi.org/10.1007/s00344-021-10469-1.
[113] Yadav, B., Jogawat, A., Rahman, M.S., Narayan, O.P., 2021. Secondary metabolites in the drought stress tolerance of crop plants: A review. Gene Rep 23 (2021), 101040. https://doi.org/10.1016/j.genrep.2021.101040.
[114] Yadav, S.K., 2010. Heavy metals toxicity in plants: an overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. S Afr J Bot 76, 167–179.
[115] Yang, H., Wu, F., Cheng, J., 2011. Reduced chilling injury in cucumber by nitric oxide and the antioxidant response. Food Chem 127 (3), 1237–1242. https://doi.org/10.1016/j.foodchem.2011.02.011.
[116] Yang, Z., Chen, Z., He, N., Yang, D., Liu, M., 2022. Effects of Silicon and Iron Application on Arsenic Absorption and Physiological Characteristics of Rice (*Oryza sativa* L.). Bull Environ Contam Toxicol 108, 1046–1055. https://doi.org/10.1007/s00128-022-03476-9.
[117] Zahedi, S.M., Moharrami, F., Sarikhani, S., Padervand, M., 2020. Selenium and silica nanostructure-based recovery of strawberry plants subjected to drought stress. Sci Rep 10, 17672. https://doi.org/10.1038/s41598-020-74273-9.
[118] Zemanova, V., Popov, M., Pavlíková, D., Kotrba, P., Hnilicka, F., Ceska, J., Pavlik, M., 2020. Effect of arsenic stress on 5-methylcytosine, photosynthetic parameters and nutrient content in arsenic hyperaccumulator *Pteris cretica* (L.) var. Albo-lineata. BMC Plant Biol 20, 130. https://doi.org/10.1186/s12870-020-2325-6.
[119] Zhang, J., Hamza, A., Xie, Z., Hussain, S., Brestic, M., Tahir, M.A., Ulhassan, Z., Yu, M., Allakhverdiev, S.I., Shabala, S., 2021. Arsenic transport and interaction with plant metabolism: Clues for improving agricultural productivity and food safety. Environ Pollut 290, 117987. https://doi.org/10.1016/j.envpol.2021.117987.
[120] Zulfiqar, F., Ashraf, M., 2022. Antioxidants as modulators of arsenic-induced oxidative stress tolerance in plants: an overview. In: J Hazard Mat, 427, 127891. https://doi.org/10.1016/j.jhazmat.2021.127891.
[121] Zulfiqar, F., Ashraf, M., 2023. Proline alleviates abiotic stress induced oxidative stress in plants. J Plant Growth Regul 42, 4629–4651. https://doi.org/10.1007/s00344-022-10839-3.