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Silicon induced mitigation of TCA cycle and GABA synthesis in arsenic stressed wheat (*Triticum aestivum* L.) seedlings

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ABSTRACT

The metalloid arsenic (As) is considered to be biologically non-essential and major environmental concern to all organisms. The aim of our study was to evaluate whether silicon (Si) has ameliorative effects on growth, respiratory cycle and Gamma-Aminobutyric acid (GABA) synthesis in wheat (*Triticum aestivum* L. cv PBW 343) seedlings under arsenic stress. The experiments were performed in a completely randomized design with three repeats and two replicates for each treatment. The seedlings were subjected to different doses of arsenate As (V) (25, 50 and 100 μM) with or without silicate (5 mM) in modified Hoagland's nutrient media for 21 days. Arsenate stress decreased seedling growth, activities of respiratory cycle enzymes particularly dehydrogenases while enhanced the levels of oxidative stress markers, Krebs cycle intermediates and GABA including activities of enzymes associated with GABA synthesis. Arsenate treatment disturbed the phosphate contents and impaired the respiratory process. Irrespective of arsenate concentrations, silicate administration substantially modulated the toxic effects of the metalloid in the test cultivar. Thus, silicon supplementation may emerge as a beneficial strategy to reduce potential health risks and might help to mitigate arsenic- induced stress in wheat seedlings.

1. Introduction

Plants continually confront undesirable environmental conditions throughout their life that impairs their growth, development and productivity. Despite all other stresses, arsenic (As) stress is of considerable importance and has prominent effects on growth and agronomic yield. The metalloid arsenic is an environmental contaminant and has been classified as Class I carcinogen by International Agency for Research on Cancer (Martinez et al., 2011). Contamination of soil by arsenic has become a critical environmental concern throughout the world particularly in the South East Asian countries (Sharma 2012). Arsenic pollution in soil could lead to substantial metalloid accumulation in the plant body, thereby posing a menace to human health through biomagnification via food chain. Arsenic toxicity in the Ganga-Brahmaputra basin is an emerging crisis where As content in soil and water exceeds the permissible limit set by the WHO (World Health Organization) (Shrivastava et al., 2015). Escalating As concentrations have become one of the major adversities not only because it affects metabolic processes of plants but consequently affects the ecosystem. Arsenic uptake occurs through the plant root-system mostly as arsenate [As (V)] which is reduced to arsenite[As (III)]by arsenate reductase (Hartley–Whitaker and Meharg, 2002). Arsenite reacts with sulphydryl groups (–SH) of enzymes and proteins and inhibits cellular functions. At the metabolic level, As triggers generation of reactive oxygen species (ROS) which subsequently promotes oxidative stress. Being a chemical analog of phosphate, it disrupts phosphate-dependent reactions and competes with phosphate to form unstable arsenate ADP [As (V)-ADP] which hampers cellular adenosine tri phosphate (ATP) production and uncouples ATP synthesis from electron transport (Shrivastava et al., 2015). This causes detrimental changes in mitochondrial respiration including operation of electron transport chain (ETC). The presence of As at elevated level in the soil retards normal growth of plants and causes biomass reduction, decline in root and shoot growth, decrease in plant height (Choudhury et al., 2011), wilting and necrosis of leaf blades, reduction in leaf area as well as photosynthesis, lowers fruit and grain yield (Srivastava and Sharma, 2013).

Silicon (Si) is the most abundant metalloid in the soil. It is absorbed as uncharged silicic acid [$Si(OH)_4$], and is precipitated as amorphous silica throughout the plant (Ranganathan et al., 2006. The beneficial effects of Si in enhancing tolerance of plants to biotic and abiotic stresses in crops have been described (Luyckx et al., 2017). Silicon can also improve stress resistance of some plant species, especially to toxic metals and metalloids (Huang et al., 2012; Vaculik et al., 2012; Liu et al., 2017; Pontigo et al., 2017). The tricarboxylic acid (TCA) cycle is a metabolic focal point connecting pathways of respiration, photorespiration and nitrogen assimilation in photosynthetic and non-photosynthetic cells (Foyer et al., 2011). The cycle comprises of enzymes that link the product of pyruvate and malate oxidation with NADH generation for oxidation by the mitochondrial electron transport

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chain. Pyruvate, the final product of glycolysis is converted to acetyl coenzyme A by the pyruvate dehydrogenase enzyme complex. Regulation of this enzyme is supposed to be one of the major regulatory points in the cycle (Nunes-Nesi et al., 2013). TCA cycle is considered to be an amphibolic pathway since the intermediates of this cycle are variously utilized in several anabolic reactions. Organic acids like pyruvate, citrate, succinate, and malate are essential due to their involvement in a number of biochemical pathways that involve energy production, help in the formation of precursors for amino acid biosynthesis and ammonia assimilation. Intermediates of the TCA cycle also help in the biosynthesis of specific compounds required for conferring protection to the cellular machinery against environmental stress (Sweetlove et al., 2010).

Oxoglutaric acid moves out of the mitochondrial matrix to the cytosol where it is converted to glutamate by glutamate dehydrogenase (GDH). Gamma-aminobutyric acid (GABA) is synthesized from glutamate in cytosol by the action of glutamate decarboxylase (GAD). This four carbon non-proteinogenic amino acid moves to the mitochondria via an unknown transporter where it forms succinic semi-aldehyde (SSA) by the enzyme GABA transaminase (Palanivelu et al., 2003). The next step in the GABA shunt involves oxidation of SSA by SSA dehydrogenase to form succinate (Renault et al., 2010). Succinate is utilized in TCA cycle and as an electron donor for the electron transport chain. GABA metabolism has been connected with carbon–nitrogen (C/N) balance and ROS scavenging (Liu et al., 2011). It has been observed that GABA pathway in plants is stimulated by environmental changes (Kinnersley and Turano, 2000).

Wheat is a major food crop that provides one-fifth of the total calories for the world's population (Reynolds et al., 2010). Previous reports in wheat subjected to cadmium, lead, and arsenic stress indicate an increase in activities of respiratory enzymes at lower concentrations accompanied by a sharp decline at higher concentrations of these metals (Shao et al., 2011). Arsenic-induced increase in plant respiration has also been documented in *Pistia stratiotes*. Low ATP level due to the synthesis of unstable As-ADP triggered respiratory activity but futile cycles of As-ADP production compromised the overall respiratory process along with mitochondrial membrane disruption (Farnese et al., 2017). However, the effects of As on TCA cycle, GABA metabolism and Si-induced modulation of As toxicity in wheat seedlings are yet to be reported.

The objective of the present study was to investigate the damage in mitochondrial respiration elicited by As and consider the relevant ability of exogenous silicate in alleviating the As-induced damage in wheat seedlings grown in solution culture. To evaluate effects of arsenate stress in the test seedlings, we measured the level of stress markers, contents of arsenic, silicon, phosphorus, organic acids (OAs) as well as assayed the activities of respiratory cycle enzymes and also the GABA synthesizing enzymes along with GABA contents. The hypothesis was whether Si might alter As-induced oxidative stress by affecting As uptake and modulate the TCA cycle enzymes and intermediates in wheat seedlings.

2. Material and methods

2.1. Experimental material and treatments

Wheat (*Triticum aestivum* L. cv. PBW-343) seeds obtained from State Agricultural Research Station, Burdwan, West Bengal, were surface sterilized with sodium hypochlorite solution (5% v/v). About 100 seeds for each treatment were spread in Petri dishes (ϕ 20 cm) lined with filter papers. The seeds were allowed to germinate in dark and humid conditions for 48 h at 30 ± 2 °C. Germinating seeds were subjected to treatments with sodium arsenate ($Na_2HAsO_4.7H_2O$; Loba-Chemie, India) (w/v) solution with or without sodium silicate ($Na_2SiO_3.9H_2O$ Loba-Chemie, India) (w/v) solution and exposed to 16-h photoperiod, 260 μmol m^{-2} s^{-1} Photon flux density with 25 ± 2°, /15 ± 2° day/night temperatures for 21 days in presence of modified Hoagland's solution that lacked salts containing phosphorus (Zhu et al., 2006). The pH was adjusted to 5.5–6 with 0.1 (N) HCl or NaOH. The control comprised of only nutrient solution while nutrient solution supplemented with either $Na_2HAsO_4.7H_2O$ (25, 50 and 100 μM) and $Na_2SiO_3.9H_2O$ (5 mM) individually or in combination of $Na_2SiO_3.9H_2O$ (5 mM) and $Na_2HAsO_4.7H_2O$ (25, 50 and 100 μM) were the treatment sets. The arsenate concentrations used were environmentally relevant and comparable to field conditions (Choudhury et al., 2010). Solutions were renewed every alternate day. The seedlings were harvested after 21 days. Root and shoot of the seedlings were thoroughly rinsed with deionized water, weighed in equal quantities for each set and further stored at −20 °C for biochemical studies.

2.2. Experimental methods

2.2.1. Growth status measurement

About 10 seedlings were selected randomly from each set. The root and shoot length of these seedlings were measured. Average length of these 10 seedlings was calculated. The same procedure was repeated thrice in different seasons of the year. Results obtained from these three means were subjected to statistical analysis.

2.2.2. Proline content

Proline content was determined according to Bates et al. (1973). One (1) g of tissue was extracted with 5 ml of 0.1 M sulpho salicylic acid and centrifuged at 5000*g* for 30 min. To 2 ml of supernatant, 5 ml each of glacial acetic acid and 140 mM ninhydrin were added and shaken vigorously. The mixture was heated in a boiling water bath followed by cooling and the chromophore obtained was extracted in 10 ml of toluene. Absorbance of the extract was measured at 520 nm. The proline contents were calculated from standard curve and expressed as μg g^{-1}fw.

2.2.3. Malondialdehyde content (lipid peroxidation)

Lipid peroxidation according to Hodges et al. (1999) was measured in terms of malondialdehyde (MDA) production. One (1) g of tissue was homogenized in 4 ml of 1% TCA (Trichloro acetic acid) solution (*w/v*) and centrifuged at 10,000*g* for 10 min. The supernatant was mixed with 1 ml of 0.5% TBA (Thiobarbituric acid) in 20% TCA (w/v) and was incubated in boiling water bath for 30 min. The reaction was terminated by placing the tube in an ice bath followed by re-centrifugation at 10,000*g* for 5 min and the absorbance was recorded at 532 nm. The values for non-specific absorption at 600 nm were subtracted. The amount of MDA-TBA complex present was calculated using an extinction coefficient (o) of 155 mM^{-1} cm^{-1} and expressed as m M g^{-1}fw.

2.2.4. H_2O_2 content

H_2O_2 content was measured as described by Thurman et al. (1972). Plant tissue was extracted with 5% TCA at 4 °C followed by centrifugation at10, 000 *g* for 10 min. 2 ml of the supernatant was mixed with 0.5 ml of 50% TCA, 0.5 ml 10 mM ferrous ammonium sulphate, 0.3 ml 2.5 M potassium thiocyanate, and 1.7 ml of distilled water. The absorbance of the mixture was measured at 480 nm. The H_2O_2 content was expressed as μmol peroxide g^{-1}fw.

2.2.5. Determination of Arsenic, Silicon and Phosphorus content

After harvesting, plant samples (roots and shoots) were dried at 80 °C in an oven for 48 h. Dried samples were treated with Conc HNO_3 (70%) and Conc HCl (35%) in the ratio of 3:1 for approximately 5 min and the vessels were sealed. These were then inserted in separate cabins in the rotor and placed for microwave digestion (CEM MARS) to the required temperature. After digestion, the contents were cooled and the volume was made up to 25 ml in a standard flask with deionized water. The samples were stored in plastic containers to prevent loss of elements by absorption and quantitatively determined by inductively

coupled plasma-optical emission spectroscopy (ICP-OES) (PE optima 5300 DV). The concentration of elements was expressed in mg^{-1}kg dw.

2.2.6. Determination of TCA cycle intermediates

One (1) gof shoot and root samples from each set were homogenized in 5 ml of 0.2 M phosphate buffer (pH 7.4) and centrifuged at 10,000 rpm for 15 min at 4 °C. The supernatant was deproteinized with 5% TCA and filtered.

Pyruvic acid was estimated according to Sadasivam and Manickam (2008). The assay mixture consisted of 1.0 ml deproteinized supernatant, 2.0 ml of 0.2 M phosphate buffer, 0.5 ml of 0.02% DNPH (2,4-dinitrophenylhydrazine). The mixture was incubated for 30 min at 37 °C then 5.0 ml of 0.8 N sodium hydroxide was added followed by another incubation of 10 min at room temperature. The mixture without plant sample was used as blank. The optical density (OD) of the brown colored product was measured in a spectrophotometer at 510 nm. Amount of pyruvic acid present in the samples was calculated from a standard curve using known concentrations of sodium pyruvate. The quantity of the total pyruvic acid was expressed as mg g^{-1}fw.

Citric acid content was determined following the method of Saffran and Denstedt (1948). The reaction mixture comprised of 1.0 ml filtrate, 5 ml of analytical grade acetic anhydride and 1.3 ml of analytical grade pyridine. The mixture was kept in a water bath for 30 min at 32 ± 0.5 °C. The blank contained 1.0 ml distilled water instead of the sample. The OD was measured at 405 nm in a Hitachi U-2000 spectrophotometer. A standard curve was prepared using known concentrations of citric acid. The citric acid content was expressed as mg g^{-1}fw.

Succinic acid content was measured according to Valle et al. (1978). Plant tissues were crushed in liquid nitrogen and homogenized in a buffer containing 0.4 M mannitol, 1 mM EDTA (ethylenediamine-N,N, N′,N′-tetraacetic acid) and 50 mM Tris pH 8.2. The homogenate was fractionated by centrifugation at 0 °C for 10 min at 2000 *g*. The resulting supernatant was centrifuged at 20,000 *g* for 20 min. The pellet containing a brown tightly packed lower layer was washed and re suspended in the homogenization buffer comprised of the mitochondrial fraction. The suspension was re centrifuged for 20 min at 20,200 g. The washed pellet was re suspended in potassium phosphate buffer pH 7.6. The assay mixture comprised of 0.5 ml buffer containing 0.1 M KH_2PO_4 (Potassium dihydrogen phosphate),5 mM EDTA,5 mM sucrose, pH 7.6,0.2 ml of 0.5% INT(2 p-iodophenyl −3- p-nitrophenyl - 5- phenyl tetrazolium chloride), different concentrations of standard succinate solution or an experimental sample containing succinate and distilled water to produce a final volume of 0.9 ml. The tubes were placed in ice and 0.1 ml mitochondrial suspension was added followed by the unknown or standard succinate solutions to make a final volume of 1 ml. The tubes were kept further in ice for 15 min followed by incubation at 37 °C for 60 min. The reaction was terminated by addition of 1 ml. of 10% (*w*/*v*) TCA. The red formazan obtained was cooled in ice and extracted in ethyl acetate (4 ml). Absorbance was recorded at 490 nm. Amount of succinate present was calculated from the standard curve and was expressed as mg g^{-1}fw.

Malic acid content was estimated according to Hummel (1949). The reaction mixture contained 0.5 ml of filtrate, 1.0 ml of 1 N HCl, 0.1 ml of 0.1% DNPH (2,4-dinitrophenylhydrazine) and 0.5 ml of 10% calcium chloride. The reaction mixture was incubated at room temperature for 30 min, followed by addition of 0.3 ml of 5 N ammonium hydroxide and 6 ml of absolute alcohol. Tubes were kept undisturbed at room temperature for 12 h to complete precipitation. After recentrifugation at 5000 rpm for 15 min, the supernatant was poured off. The tubes were dried in an oven at 105 °C for 15 min to remove moisture. About 3 ml of 0.08% orcinol sulfuric acid mixture was added to the dried pellets and the contents were thoroughly mixed with a glass rod and further heated to 100 °C for 10 min. The mixtures were cooled and diluted to 10 ml with concentrated sulfuric acid. The fluorescence was measured using a Hitachi-650-40 spectrofluorometer against blank. The amount of total malic acid was expressed as mg g^{-1}fw.

2.2.7. Assay of Pyruvate dehydrogenase activity

Pyruvate dehydrogenase (E.C. 1.2.4.1) activity was assayed according to Williams and Randall (1979). Plant samples were homogenized in 1.5 ml of 50 mMTris HCl buffer (pH 7.8) containing 0.7 M sucrose, 57 mM β-mercaptoethanol, 2 mM EDTA,0.5% (*w*/*v*) BSA (Bovine serum albumin) and centrifuged at 10,000 rpm for 20 min at 4 °C. The assay mixture contained 0.2 ml supernatant, 0.2 ml of 50 mM Tris HCl (pH 8.0) and 0.1 ml each of 5 mM magnesium chloride, 0.12 mM CoA (coenzyme A), 2.6 mM cysteine HCl and 1.5 mM pyruvate making a total volume 1.0 ml. The OD of the reaction mixture was recorded in a Hitachi U-2000 spectrophotometer with respect to blank at 340 nm. The absorbance was further noted every 60 s for 2 min after addition of 0.2 ml of 1.4 mM NAD (Nicotinamide adenine dinucleotide (oxidized)). An increasing OD showed the amount of NADH (Nicotinamide adenine dinucleotide (reduced)) produced per minute. A standard curve with known concentrations of NADH was prepared from which the enzyme activity was calculated and expressed as μmol NADH formed mg^{-1} protein min^{-1}.

2.2.8. Assay of Citrate synthase activity

Citrate synthase (EC 2.3.3.1) activity was determined according to the method described by Srere (1969). Samples were homogenized in a buffer containing 0.1 mol/L Tris–HCl buffer (pH 8.0), 0.1% (v/v) Triton X-100, 2% (w/v) PVP (Polyvinyl polypyrrolidone), and 10 mmol/L iso-ascorbic acid. The extracts were centrifuged for 5 min at 15,000 rpm at 4 °C, and the supernatant was assayed for enzyme activity. The reaction mixture comprised of 0.1 ml 1 mM DTNB (5′-Dithiobis-2-Nitrobenzoic Acid), 0.03 ml of 10 mM acetyl CoA and 0.05 ml supernatant. Initial absorbance was recorded followed by addition of 0.05 ml 10 mM OAA (Oxalo acetate) and final absorbance was recorded at 412 nm in a Hitachi U-2000 spectrophotometer. Enzyme activity was calculated as μg citric acid formed mg^{-1} protein min^{-1}.

2.2.9. Assay of Isocitrate dehydrogenase activity

Isocitrate dehydrogenase (E.C. 1.1.1.41) activity was determined following the method of Zhou et al. (2012).). One (1) g each of plant samples of were ground in liquid nitrogen and homogenized in 1.5 ml of 50 mM HEPES buffer (pH 7.5) containing 10 mM β-mercaptoethanol and 5% PVP The homogenates were centrifuged at 14,500 *g* for 20 min at 4 °C. The assay mixture contained 40 mM HEPES buffer (pH 8.2), 2 mM sodium isocitrate, 800 mM NAD, 200 mM manganese sulphate and 0.1 ml supernatant containing enzyme extract making a final volume of 0.5 ml. The initial absorbance as well as the increase in absorbance of each reaction mixture was measured in a Hitachi U-2000 spectrophotometer at340 nm for 2 min. The enzyme activity was expressed as μmol NADH formed mg^{-1} protein min^{-1}.

2.2.10. Assay of Oxoglutarate dehydrogenase activity

Oxoglutarate dehydrogenase (E.C. 1.2.4.2) activity was determined according to Millar et al. (1999) with slight modifications. Samples were ground in liquid nitrogen followed by homogenization in 50 mM MOPS (3- *N*-morpholino propane sulfonic acid) HCl Buffer, (pH 7.5) and centrifuged at 10,000 rpm for 20 min at 4 °C. The enzyme activity was measured by determining amount of NADH formation at 340 nm in a reaction medium containing 75 mM TES-KOH (pH 7.5), 0.05% (*w*/*v*) Triton X-100, 0.5 mM magnesium chloride, 2 mM NAD+, 0.12 mM lithium- CoA, 0.2 mM thiamine pyrophosphate, 2.5 mM cysteine HCl, 1 mM AMP (Adenosine monophosphate), 1 mM sodium-2-oxoglutarate and 3 units of lipoamide dehydrogenase. The enzyme activity was expressed as μmol NADH formed mg^{-1} protein min^{-1}.

2.2.11. Assay of Succinate dehydrogenase activity

Succinate dehydrogenase (E.C. 1.3.5.1) activity was determined according to Green and Narahara (1980). Samples were ground in liquid nitrogen and homogenized in 1.5 ml of 4mMTris HCl (pH 7.5) buffer containing 0.19 M sucrose. The homogenates were centrifuged at

10,000 rpm for 15 min at 4 °C. The assay mixture comprised 0.05 ml each of 0.19 M sucrose, 0.1MTris HCl (pH 7.5), 10 mM sodium azide, 8 mM INT [2-(p-iodophenyl)-3-(pnitrophenyl)- 5-(phenyl tetrazolium chloride)], 0.1 ml of 0.5 M sodium succinate, 0.1 ml of distilled water and 0.1 ml supernatant. The mixture was incubated at 30 °C in a water bath for 10 min. 95% alcohol was added to each tube, mixed thoroughly and kept in an ice bath for 10–15 min followed by centrifugation at 8000 rpm at room temperature for 10 min. The absorbance was recorded at 458 nm using a Hitachi U-2000 spectrophotometer. Basal reduction of INT was determined in control tubes where succinate was absent. The enzyme activity was expressed as μmol INT reduced mg^{-1} protein min^{-1}.

2.2.12. Assay of Fumarase activity

Fumarase activity (EC 4.2.1.2) was determined according to Bergmeyer et al. (1974). One (1) g of plant samples from each treatment were ground in liquid nitrogen and homogenized in 100 mM potassium phosphate buffer, pH 7.6 at 25 °C. The homogenates were centrifuged at 10,000 *g* for 20 min at 4 °C. Conversion of malate was determined by change in absorbance at 240 nm resulting in fumarate production by the enzyme. The assay mixture comprised of 70 mMphosphate buffer pH 7.4, 0.5 ml of the enzyme extract and 50 m M malate. Increase in absorbance at 240 nm was recorded at 10 s interval for 60 s and fumarase activity was expressed as A_{240} μg^{-1} protein min^{-1}.

2.2.13. Assay of Malate dehydrogenase activity

Malate dehydrogenase (E.C. 1.1.1.37) activity was determined according to Kumar et al. (2000). One (1) g of plant sample from each set was homogenized in 3 ml of 50mMTris HCl (pH 8.0) buffer containing 50 mM magnesium chloride, 5 mM β-mercaptoethanol and 1 mM EDTA and centrifuged at 10,000 rpm for 20 min at 4 °C. The assay mixture comprised 0.5 ml of 5 mM OAA, 0.5 ml of 10 mM magnesium chloride, 1.3 ml of 0.1 M Tris HCl buffer (pH 7.8) and 0.2 ml of the enzyme extract. The initial absorbance of the reaction mixture was noted using a Hitachi U-2000 spectrophotometer at 340 nm followed by addition of 0.5 ml 0.4 mM NADH. The absorbance was further recorded every 60 s for at least 2 min. Enzyme activity was expressed as μmol NADH oxidized mg^{-1} protein min^{-1}.

2.2.14. Glutamate dehydrogenase and Glutamate decarboxylase enzyme assays

Plant samples were ground in liquid nitrogen and homogenized with two volumes of homogenization buffer at 4 °C. Homogenization buffer contained 5 mM EDTA, 0.1 mM Tris– HCl, 10 mM dithiothreitol, 1 mM pyridoxal-5-phosphate hydrate, and 1% (*w*/*v*) PVP (pH 7.8). Homogenates were centrifuged at 10,000 *g* for 10 min at 4 °C. Supernatant containing the protein extract were used in GDH and GAD activity assays. All spectrophotometric analyses were conducted on a Hitachi U-2000 spectrophotometer.

GDH (E.C.1.4.1.2) activity assay was performed according to Akihiro et al. (2008). 200 μl of the extract was mixed with the assay mixture containing 50 mM ammonium sulphate, 13 mM α-ketoglutarate, 0.25 mM NADPH (Nicotinamide adenine dinucleotide phosphate) and 1 mM calcium chloride, 100 mMTris–HCl buffer (pH 8.0). Absorbance differences were determined in samples at 340 nm and GDH activity was expressed as μmol NADH mg^{-1} protein min^{-1}.

GAD (E.C. 4.1.1.15) activity was assayed according to Bartyzel et al. (2003). 100 μl of the above extract was mixedwith reaction mixture for decarboxylation. Decarboxylation was performed at 30°C for 1 h with the reaction mixture that contained; 3 mM l-glutamate; 20 μM pyridoxal phosphate, 50 mM potassium phosphate (pH 5.8). Samples were pre-incubated with the reaction mixture without glutamate for 10 min at 30 °C. The reaction was stopped by addition of 0.1 mL of 0.5 M HCl and samples were centrifuged at 12,500×*g* for 10 min. Derivatization of the samples was done with ninhydrin solution 0.35% (*w*/*v*). GABA content of the samples was determined spectrophotometrically by comparing with standard GABA values. GAD enzyme activity was calculated as mg GABA formed mg^{-1} protein.

2.2.15. Estimation of GABA content

GABA contents were measured using the method of Kitaoka and Nakano (1969). Plant samples were homogenized with 80% ethanol, shaken thoroughly, and filtered. The filtrate was boiled to remove ethanol followed by addition of 1 ml distilled water and centrifuged at 10000 rpm for 10 min. The floating portion on top was aspirated, and 0.2 ml of 0.2 M borate buffer and 1.0 ml of 6% phenol were added. For the standard curve, GABA solution (0.1–0.3 ml) was added to test tubes together with 0.2 ml of borate buffer and 1.0 ml of phenol reagent. The solutions were mixed thoroughly, cooled and 0.4 ml of 10–15% sodium hypochlorite was added, and the solution was shaken for 1 min, and cooled again. Then the solution was boiled in a water bath for 10 min, and allowed to cool. Optical density was recorded at 630 nm by Hitachi U-2000 spectrophotometer, with ethanol as blank. GABA contents were quantified by comparing the reading with the standard GABA content curve ($y = 0.049 + 10.14\ x$).

2.2.16. Protein estimation

Protein contents were estimated according to Lowry et al. (1951) using bovine serum albumin (BSA, Sigma) as standard.

2.2.17. Statistical Analysis

The experiments were carried out in a CRD (completely randomized design) with three repeats and two replicates in each treatment. Each repeat comprised a single petridish containing an average of 100 seeds. The data and significant differences among the mean values were compared by descriptive statistics (±SE) followed by Student's t-test using Graph pad software and further subjected to regression analyses using Minitab statistical software, version 18. Origin Pro 8.0 was used for data processing and figures.

3. Results

3.1. Effect on growth

Arsenate treatment significantly altered root and shoot growth of the test seedlings (Fig. 1A). The root lengths decreased by about 52%, 69%and 74% under 25 μM, 50 μM, and 100 μM arsenate treatments respectively. Similarly, for shoots, there was a decrease in length by about 27%, 37% and 44% under same concentrations of arsenate treatments respectively over control. Joint treatment of silicate (5 mM) along with same concentrations of arsenate narrowed down the rate of decrease to about 43% in root and 17% in shoot on an average with respect to control (Figs. 1B and 2).

3.2. Effect on Proline content

Arsenate treatment considerably increased the proline content in both root and shoot of the test seedlings. Proline content increased by about 42%, 78% and 125% in root and by about 23%, 63% and 88% in shoot under 25 μM, 50 μM, and 100 μM arsenate treatments respectively. Co-application of silicate along with arsenate also caused an increase in proline content but less than arsenate treatment alone that were 20%,50%,105% in root and 4%,36%,75% in shoot over control under 25 μM, 50 μM, and 100 μM arsenate treatments respectively (Table 1).

3.3. Effect on Malondialdehyde content

A gradual increase in MDA content in the test seedlings exposed to arsenate indicated an enhanced rate of lipid peroxidation. The

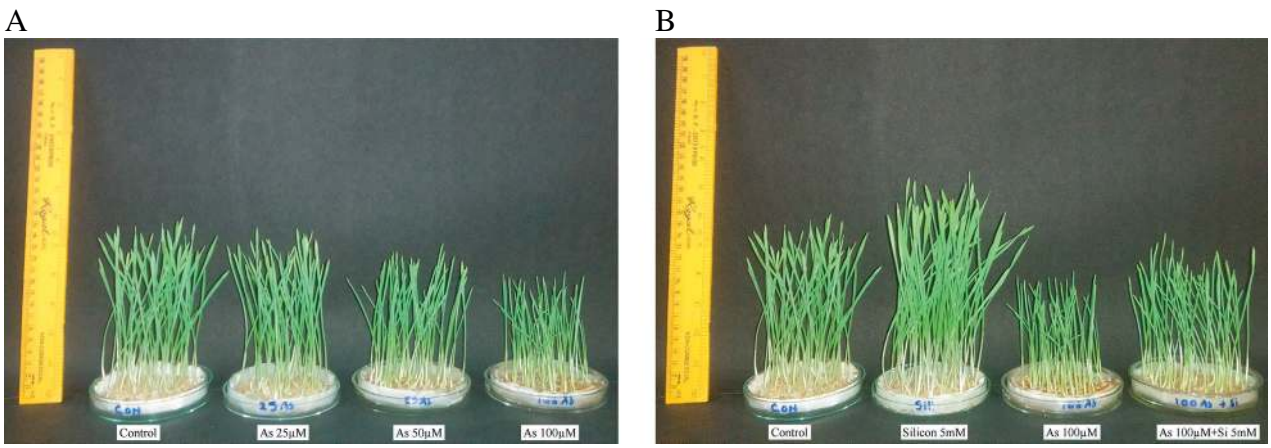

Fig. 1. Effect of arsenate either applied individually (A) or in combination with silicate (B) on the growth of 21 days old wheat (cv. PBW343) seedlings.

MDA content increased by about 27, 42 and 56% in root under 25, 50 and 100 μM arsenate treatments, respectively, whereas in the shoot at similar treatments, the MDA content augmented by 15, 23 and 38%. Furthermore, supplementation of silicate along with arsenate narrowed the increase in MDA to 13, 14 and 27% in root and by about 8, 14 and 23% in shoot with respect to control at 25 μM As + 5 mM Si,50 μM As + 5 mM Si and 100 μM As + 5 mM Si treatments, respectively (Table 1).

3.4. Effect on H_2O_2 content

The H_2O_2 content escalated by 55, 118 and 136% in root at 25, 50 and 100 μM arsenate treatments, respectively, while in shoot the content enhanced by 21, 33 and 58% under similar doses of arsenate with respect to control. Supplementation of silicate along with arsenate enhanced the level of H_2O_2 by 27, 82 and 109% in root, and by about 12, 19 and 36% in shoot at 25 μM As + 5 mM Si,50 μM As + 5 mM Si, and 100 μM As + 5 mM Si treatments with respect to control (Table 1).

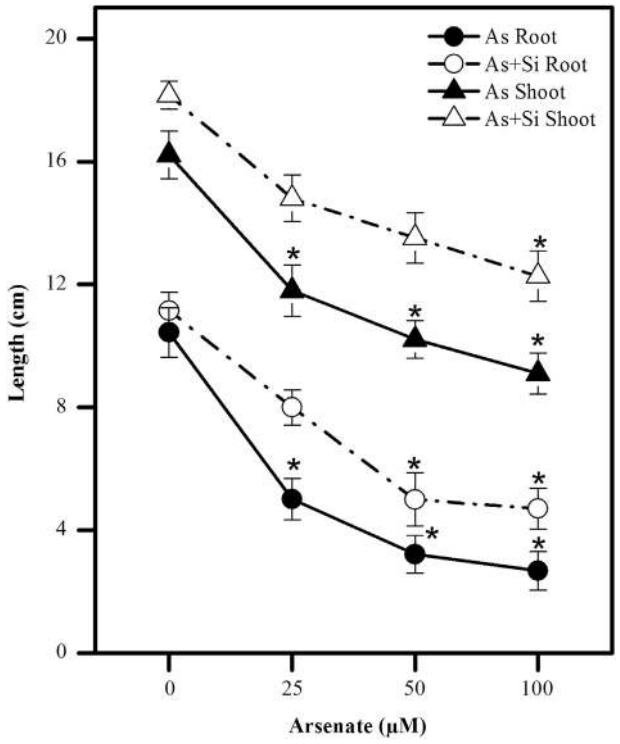

Fig. 2. Effect of arsenate and silicate either applied individually or in combination on the growth of 21 days old wheat (cv. PBW343) seedlings. Each data point is the mean ± SE of three repeats with two replicas in each treatment, * indicates statistically significant at $p \leq .05$ respectively compared to control.

3.5. Effect on arsenic, silicon and phosphorus content

As accumulation in root and shoot of the test cultivar were dependent on the dose of arsenate treatments, the contents increased with increasing concentrations of arsenate in the media (Table 2). In control plants, As content was below detection level in both root and shoot. Roots had higher As contents irrespective of arsenate doses compared to shoots. Silicon utilization in arsenic containing media restricted the translocation and reduced As contents in both root and shoot of the test seedlings.

The seedlings treated with only silicon registered highest Si accumulation in both root and shoot, but Si contents in the arsenate treated test seedlings gradually declined with increasing doses of arsenate. However, in As + Si treated seedlings, the contents of Si were enhanced when compared to the only arsenate treated seedlings (Table 2).

The phosphorus (P) contents in the test seedlings decreased with the application of increasing arsenate concentrations (Table 2). The control set registered higher P contents in both root and shoot compared to the only arsenate treated sets. Silicate application along with arsenate enhanced the P contents in both root and shoot with respect to the only arsenate treated sets.

3.6. Effect on intermediates of TCA cycle

3.6.1. Pyruvate content

Arsenate treatment increased pyruvate contents in both root and shoot of the test cultivar. In root, pyruvate contents increased by about 91% at 25 μM, 161% at 50 μM and 207% at 100 μM arsenate concentration, whereas in shoot the said contents increased by about 19% at 25 μM, 31% at 50 μM and 70% at 100 μM arsenate treatment with respect to control (Fig. 3). However, joint application of silicate along with arsenate caused an increase in pyruvate content that was less than arsenate treatment alone. In root, the pyruvate content was increased by 73% at 25 μM As + Si, 86% at 50 μM As + Si and 142% at 100 μM As + Si treatments, while in shoot it was only 5% at 25 μM As + Si, 10% at 50 μM As + Si and 38% at 100 μM As + Si concentrations.

3.6.2. Citrate content

Citrate contents of arsenate treated wheat seedlings increased with respect to control in both root and shoot (Fig. 3). In shoot, an increment in citrate contents of about 13, 27 and 34% was recorded, while in root the citrate contents were 20, 53 and 71% at 25, 50 and 100 μM arsenate

Table 1
Effect of arsenate and silicate either applied individually or in combination on Proline, MDA and H_2O_2 content of 21 days old wheat (cv. PBW343) seedlings.

Treatment	Proline [μg g^{-1}fw]		MDA [mM g^{-1}fw]		H_2O_2 [μM g^{-1}fw]	
	Root	Shoot	Root	Shoot	Root	Shoot
Control	120 ± 7.94	160 ± 5.51	4.38 ± 0.38	4.03 ± 0.32	11 ± 2.08	10.30 ± 1.07
As25 μM	170 ± 7.57*	190 ± 5.29*	5.57 ± 0.38	4.65 ± 0.43	17 ± 2.65	12.50 ± 1.29
As 50 μM	214 ± 8.72*	260 ± 8.62*	6.20 ± 0.58	4.96 ± 0.23	24 ± 3.06*	13.75 ± 0.66
As 100 μM	270 ± 7.21*	300 ± 7.94*	6.82 ± 0.46*	5.58 ± 0.37*	26 ± 3.79*	16.25 ± 1.09
Silicate(5 mM)	110 ± 6.43	148 ± 6.11	4.03 ± 0.29	3.72 ± 0.24	8 ± 1.15	9.50 ± 0.81
As 25 μM + Si	144 ± 6.93	166 ± 6.11	4.96 ± 0.33	4.34 ± 0.29	14 ± 2.65	11.50 ± 0.51
As 50 μM + Si	180 ± 7.57*	218 ± 8.08*	5.00 ± 0.32	4.60 ± 0.20	20 ± 2.65	12.25 ± 0.82
As100μM + Si	246 ± 7.81*	280 ± 7.37*	5.58 ± 0.31	4.96 ± 0.20	23 ± 2.89*	14.00 ± 1.04*

Each data point is the mean ± SE of three repeats with two replicas in each treatment, * indicates statistically significant at $p \leq 0.05$ respectively compared to control.

treatments respectively. Citrate content also increased in As + Si treated roots but less than arsenate treatment alone and was 14% on an average with respect to control. In shoot, however, at 25 μM As + Si treatment the citrate content decreased by about 6% whereas 50 μM As + Si and 100 μM As + Si treatments registered an increase of about 8%, on an average with respect to control.

3.6.3. Succinate content

Succinate content of arsenate treated test seedlings increased with respect to control in both root and shoot (Fig. 3). In the shoot, an increment of about 39, 53 and 66% was noted while in root, the contents were 16, 24 and 40% at 25, 50 and 100 μM arsenate treatments, respectively. Succinate content of the As + Si treated seedlings also increased but was less compared to arsenate treatment alone and was about 18% on an average in root and 38% on an average in shoot with respect to control.

3.6.4. Malate content

Arsenate exposure increased malate content in both root and shoot of test seedlings. In root, an increase of about 22, 38 and 69% was recorded at 25, 50 and 100 μM arsenate treatments respectively, while in shoot, the increments were about 39, 53 and 68% with respect to control at similar levels of arsenate treatments (Fig. 3). Co-application of silicate along with 25, 50 and 100 μM arsenate narrowed the level of increase on an average by about 40% in root with respect to control. In shoot, however, at 25 μM As + Si treatment the malate content decreased by about 13% and increased by about 32 and 43% at 50 μM As + Si and 100 μM As + Si treatments, respectively.

3.7. Effect on respiratory enzymes

3.7.1. Pyruvate dehydrogenase (PDH)

The toxic effect of arsenate on test seedlings depicted a progressive decrease in activity of PDH in both root and shoot over control (Fig. 4). The enzyme activity declined by about 10, 12 and 16% in root at 25, 50 and 100 μM arsenate treatment, respectively, while in shoot, it reduced by 9, 14 and 19% under similar concentrations. The seedlings treated jointly with arsenate and silicate also revealed a decline in the enzyme activity by 9, 10 and 14% in root at 25 μM As + Si, 50 μM As + Si and 100 μM As + Si treatments, respectively, whereas in shoot, under similar treatments the activity decreased by 2, 4 and 16%.

3.7.2. Citrate synthase (CS)

Arsenate treatment on the test seedlings demonstrated a progressive increase in the activity of CS in both root and shoot over control (Fig. 5A). The activity of the enzyme increased on an average by about 21, 38 and 47% in root at 25, 50 and 100 μM arsenate treatment, respectively, while in the shoot, it enhanced by about 15, 22 and 30% under similar concentrations of arsenate. The seedlings treated jointly with arsenate and silicate revealed an increase of the enzyme activity on an average by about 8% in root and 17% in shoot.

3.7.3. Isocitrate dehydrogenase (IDH)

The activity of isocitrate dehydrogenase was reduced in both root and shoot when subjected to arsenate toxicity. In the shoot, the decrease was about 8% on an average while in the root, the decrease was 6% on an average with respect to control (Fig. 5B). However, joint application of silicate along with arsenate registered a rise in enzyme activity by about 7% at 25 μM As + Si treatment in shoot, whereas the activity decreased on an average by about 3% at 50 μM As + Si and 100 μM As + Si treatments. In root, the enzyme activity decreased by about 3% on an average over control under similar treatments.

3.7.4. Oxoglutarate dehydrogenase (OGDH)

A progressive decrease in activity of OGDH was noted in response to arsenate treatment in both root and shoot over control (Fig. 5C). The enzyme activity declined by about 23, 55 and 64% in root at 25, 50 and 100 μM arsenate treatment, respectively, while in shoot it reduced by about 61, 71 and 74% under similar concentrations of arsenate. The enzyme activity declined by about 9, 30 and 61% in root, and by about 49, 62 and 69% in shoot under similar concentrations of arsenate supplemented with silicate which was less compared to arsenate treatment alone.

3.7.5. Succinate dehydrogenase (SDH)

SDH activity declined by about 9, 12 and 17% over control in shoot at 25, 50 and 100 μM concentrations of arsenate respectively (Fig. 5D). However, the decrease in enzyme activity was narrowed down on an

Table 2
Effect of arsenate and silicate either applied individually or in combination on Arsenic, Silicon and Phosphorus content of 21 days old wheat (cv. PBW343) seedlings.

Treatment	Arsenic [mg kg^{-1} dw]		Silicon [mg kg^{-1} dw]		Phosphorus [g kg^{-1} dw]	
	Root	Shoot	Root	Shoot	Root	Shoot
Control	<0.1	<0.1	38.12 ± 3.63	35.47 ± 2.94	11.66 ± 1.89	20.61 ± 0.61
As 25 μM	111.39 ± 8.41*	6.60 ± 0.87*	32.89 ± 3.67	29.27 ± 3.71	8.84 ± 1.50	19.80 ± 0.77
As 50 μM	113.47 ± 8.50*	9.40 ± 1.63*	23.41 ± 3.49*	25.62 ± 3.29	7.59 ± 1.56	18.14 ± 1.33
As 100 μM	128.00 ± 8.79*	15.20 ± 2.78*	19.66 ± 3.38*	23.09 ± 3.23*	6.98 ± 1.21*	15.43 ± 1.32*
Silicate(5 mM)	<0.1	<0.1	43.63 ± 4.13	47.98 ± 3.81	12.36 ± 1.07	21.79 ± 1.90
As 25 μM + Si	61.62 ± 6.12	3.00 ± 0.55	40.20 ± 3.29	37.04 ± 3.85	11.13 ± 1.15	20.42 ± 0.60
As 50 μM + Si	100.42 ± 7.13	4.80 ± 0.832	35.02 ± 3.32	35.50 ± 3.14	10.68 ± 0.36	19.20 ± 0.80
As 100 μM + Si	118.80 ± 7.85*	8.90 ± 1.21*	32.60 ± 3.55	32.00 ± 3.07	10.32 ± 0.59	18.60 ± 0.61

Each data point is the mean ± SE of three repeats with two replicas in each treatment, * indicates statistically significant at $p \leq 0.05$ respectively compared to control.

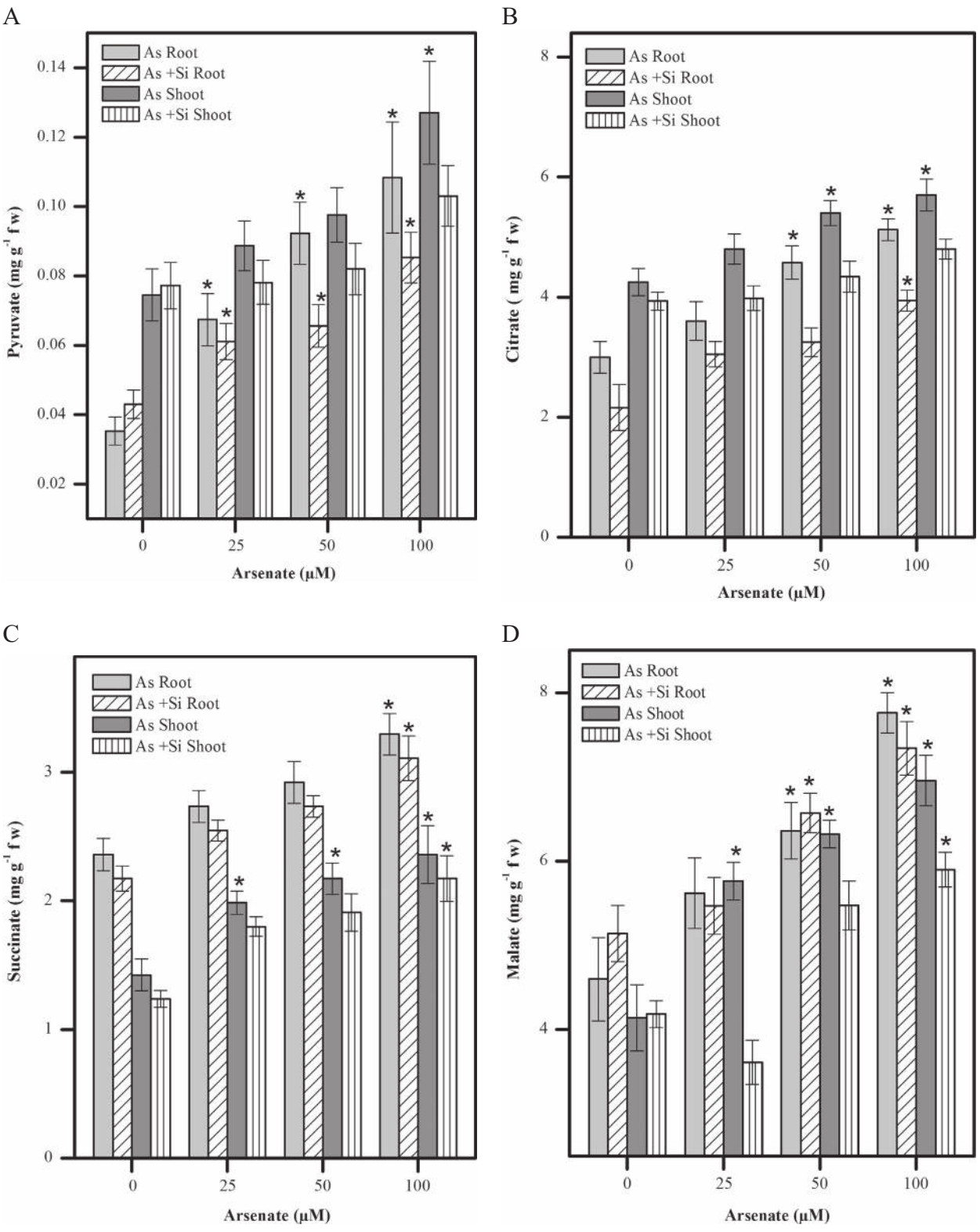

Fig. 3. Effect of arsenate and silicate either applied individually or in combination on OA content (A – Pyruvate, B – Citrate, C – Succinate, D – Malate) of 21 days old wheat (cv. PBW343) seedlings. Each data point is the mean ± SE of three repeats with two replicas in each treatment, * indicates statistically significant at $p \leq 0.05$, respectively, compared to control.

average to 10% with respect to control when silicate was administered along with arsenate. Similarly, in root, a decline in enzyme activity was by about 32% on an average over control. The level of decrease in enzyme activity was narrowed to about 25% on an average over control when silicate along with arsenate was applied.

3.7.6. Fumarase

Arsenate treatment increased the activity of fumarase in the test cultivar. In the shoot, an increase of about 18, 27, and 72% was registered at 25, 50 and 100 µM As concentrations (Fig. 5E). This increase was narrowed down by about 14, 20 and 34% with respect to control in

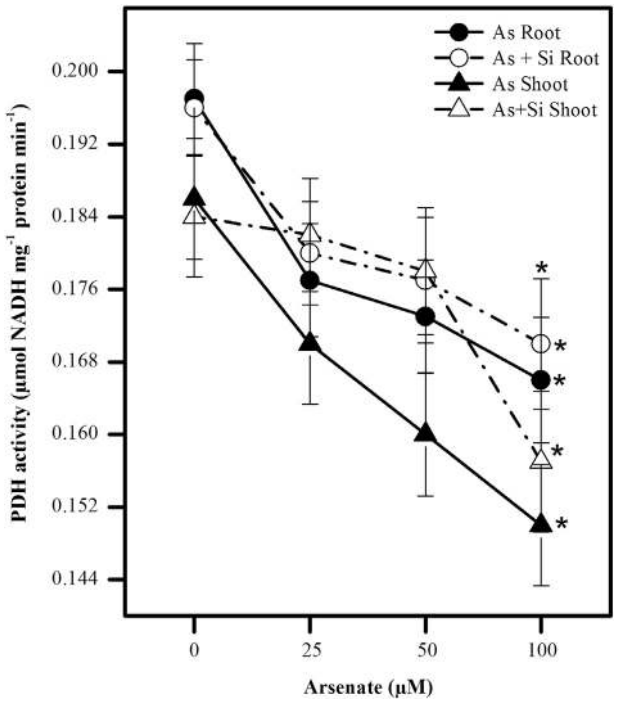

Fig. 4. Effect of arsenate and silicate either applied individually or in joint combination on PDH activity of 21 days old wheat (cv. PBW343) seedlings. Each data point is the mean ± SE of three repeats with two replicas in each treatment, * indicates statistically significant at $p \leq 0.05$ respectively compared to control.

the respective As + Si treated sets. Similarly, in root, the activity was enhanced by about 3, 42 and 51% at 25, 50 and 100 μM arsenate concentrations, respectively while application of silicate along with arsenate raised the enzyme activity by about 1 and 20 and 31% in 25 μM As + Si, 50 μM As + Si and 100 μM As + Si treatments above control, respectively.

3.7.7. Malate dehydrogenase (MDH)

The activity of MDH decreased in response to arsenate treatment (Fig. 5F). In shoot, a decrease of about 13, 18 and 21% was documented at 25, 50 and 100 μM arsenate concentrations whereas, treatment of silicate along with arsenate the decrease was narrowed down to 10, 14 and 18% with respect to control. Similarly, in root, the activity declined by about 24, 29 and 38% at 25, 50 and 100 μM arsenate treatment, respectively. A decrease in the activity of the enzyme by about 2, 14 and 21% was noted in 25 μM As +Si, 50 μM As +Si and 100 μM As +Si treatments respectively.

3.8. Effect on glutamate dehydrogenase

The activity of GDH increased significantly in response to arsenate treatment (Fig. 6A). The enzyme activity increased by about 32, 58 and 63% in the shoot at 25 μM As, 50 μM As, and 100 μM As treatments, respectively, over control. In root, the increment was 4, 10 and 15% under similar doses of arsenate treatments. Supplementation of silicate along with arsenate resulted in an enhancement in the enzyme activity by about 25% in the shoot and 5% in the root on an average with respect to control.

3.9. Effect on glutamate decarboxylase

GAD activity significantly increased with increasing concentrations of arsenate in the test cultivar (Fig. 6B). In shoot, the increment was about 181, 244 and 213%, whereas in root, the increment was about 35, 75 and 108% over control at 25, 50 and 100 μM arsenate treatments respectively. The activity of the enzyme increased by about 77% in shoot and 51% in root on an average with respect to control when seedlings were supplemented with silicate along with similar doses of arsenate.

3.10. Effect on GABA content

GABA content escalated in response to arsenate treatment in the test seedlings with respect to control (Fig. 7). In the shoot, 25 μM arsenate treatment led to an increase of about 6% in GABA, while at 50 and 100 μM treatments the GABA content enhanced by about 26 and 62%, respectively. Joint application of silicate along with arsenate at 25, 50 and 100μM concentrations narrowed down the increase in GABA content by 2, 11 and by about 20%, respectively with respect to control. Similarly, in root, the GABA content increased on an average by about 11% under 25, 50 and 100 μM arsenate treatments which was narrowed down to about 6% on an average in As + Si supplemented sets.

4. Discussion

The aim of our study was to evaluate the relevance of Si supplementations in mitigating As stress. Results obtained provide insight to the influence of Si in wheat seedlings during As stress. Imposition of arsenate toxicity retarded normal growth and development in the test seedlings (Fig. 1A). Shoot growth of seedlings was less affected compared to that of root (Fig. 2). Roots being directly exposed to arsenate in the nutrient media appear to be the most vulnerable part of the plant (Abedin and Meharg, 2002). Along with decrease in root growth, reductions in length and number of root hairs were noted. Similar observations have been reported in different species (Hartley–Whitaker et al., 2001; Choudhury et al., 2011). In the present study, the toxic effect of arsenate could be ameliorated to some extent by supplementation of silicate. It is evident from several studies (Vaculık et al., 2012; Tripathi et al., 2015; Pontigo et al., 2017) that application of Si exerts beneficial effects on plant growth by alleviating biotic as well as abiotic stresses including diseases, pests, lodging, drought, salt and nutrient imbalances.

The present study utilized proline, MDA and H_2O_2 content as indicators of oxidative damage. Proline maintains cytoplasmic osmoticum and protects cellular membranes as well as proteins from denaturation. The proteinogenic amino acid is a metal chelator and inhibits ROS mediated apoptosis under stress (Gill and Tuteja, 2010). Enhanced proline content in our study is consistent to previous report in rice under arsenate treatment (Choudhury et al., 2011). Proline accumulation under environmental stress has been correlated with the decrease in mitochondrial respiration (Rastagoo and Alemzadeh, 2011). The hydrocarbon fragments like ketones and MDA formed during membrane lipid peroxidation reacts with thiobarbituric acid (TBA) forming thiobarbituric acid-reactive substances (TBARS). TBARS formation is an indicator of free radical formation in plant tissues. Escalation in MDA contents indicates membrane damage in response to arsenate toxicity that subsequently results in oxidative stress. Our results are in agreement to previous reports in bean and rice under arsenate toxicity (Stoeva et al., 2005; Choudhury et al., 2011). A remarkable increase in H_2O_2 level in the test seedlings signifies inadequate enzymatic defense. H_2O_2 functions as a second messenger and modulates the expression of antioxidant enzymes by inducing oxidative damage (Foreman et al., 2003). A positive correlation between arsenate treatment and proline, MDA as well as H_2O_2 accumulation was prominent in our study. However, co-application of silicate along with arsenate led to substantive decline of these stress markers (Table 1) indicating alterations in the activities of antioxidative enzyme by Si amendments functioned to scavenge ROS. Silicon amendments have been documented to increase plant defense by reducing lipid peroxidation, electrolyte leakage and up-regulating the activities of antioxidant enzymes (Khaliq et al., 2015).

Arsenic accumulation in the test seedlings linearly increased with increment of arsenate concentration in the growth media (Table 2). Roots accumulated more As and restricted its entry to shoots. Higher retention of As in roots has been attributed to the conversion of As (V) to As (III)

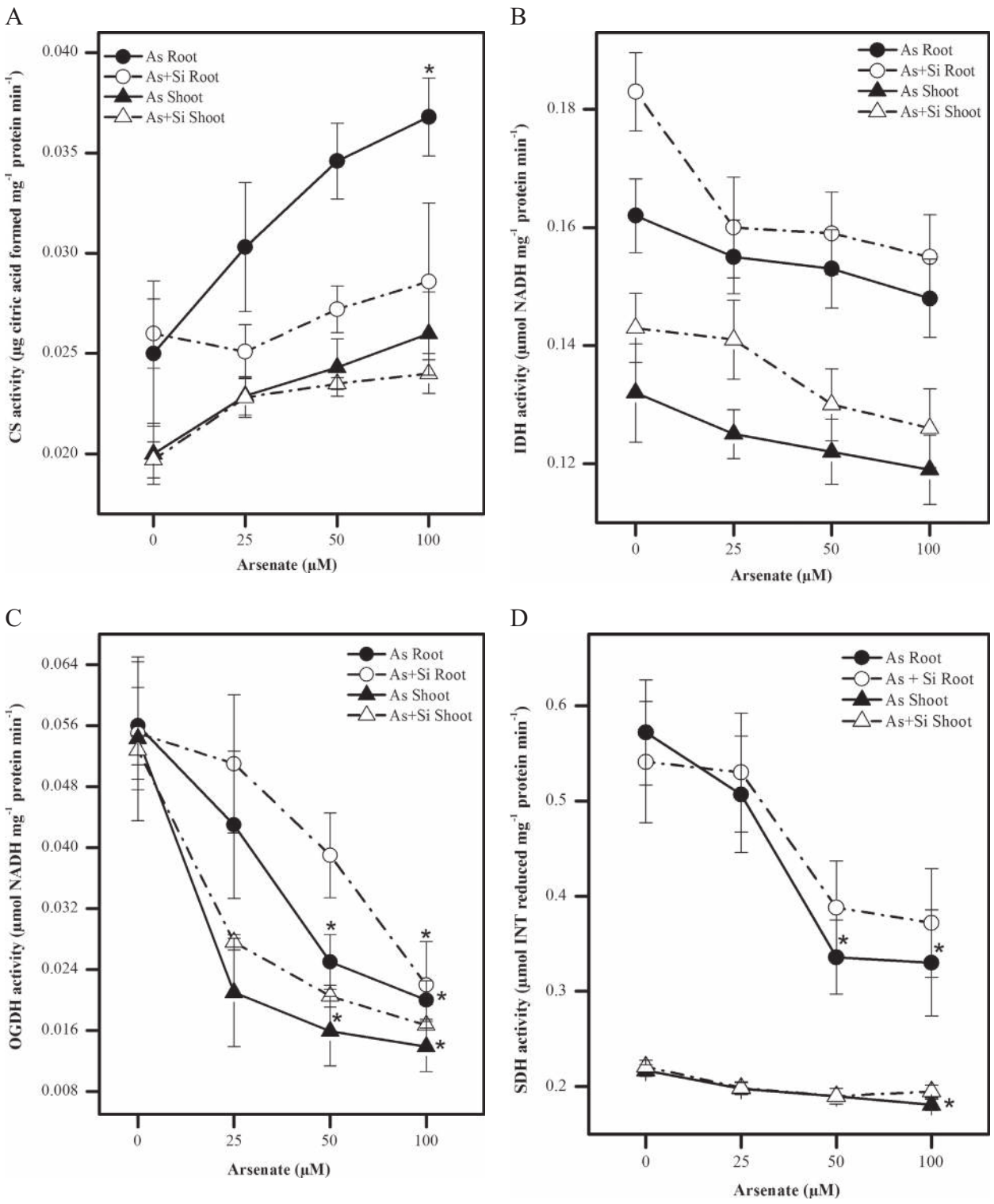

Fig. 5. Effect of arsenate and silicate either applied individually or in combination on activities of TCA cycle enzymes (A – CS, B – IDH, C – OGDH, D – SDH, E – Fumarase, F – MDH) in 21 days old wheat (cv. PBW343) seedlings. Each data point is the mean ± SE of three repeats with two replicas in each treatment, * indicates statistically significant at $p \leq 0.05$ respectively compared to control.

and its subsequent compartmentalization in root vacuoles as As (III)–PCs (arsenite–phytochelatins) (Li et al., 2015). Conversely, Si amendments lowered As contents when compared to arsenate treatment alone which might be a strategy for Si-induced alleviation of As toxicity in the test cultivar. After As(V) uptake via phosphate transporters in root, immediate reduction to As(III) occurs and competitive inhibition between As (III) and silicic acid also reduces As (III) uptake (Fleck et al., 2013). Si-induced lignin deposition in cell wall has been documented to restrict and reduce root to shoot translocation of metal ions followed by its co-precipitation to metal-silicate complexes that lowers the availability of metal in the tissues (Bharwana et al., 2013). Such Si-induced suberization as well as lignification in exodermermis and endodermis of root along with Si deposition enhanced the endogenous Si contents in the Si treated seedlings compared to the non-Si treated

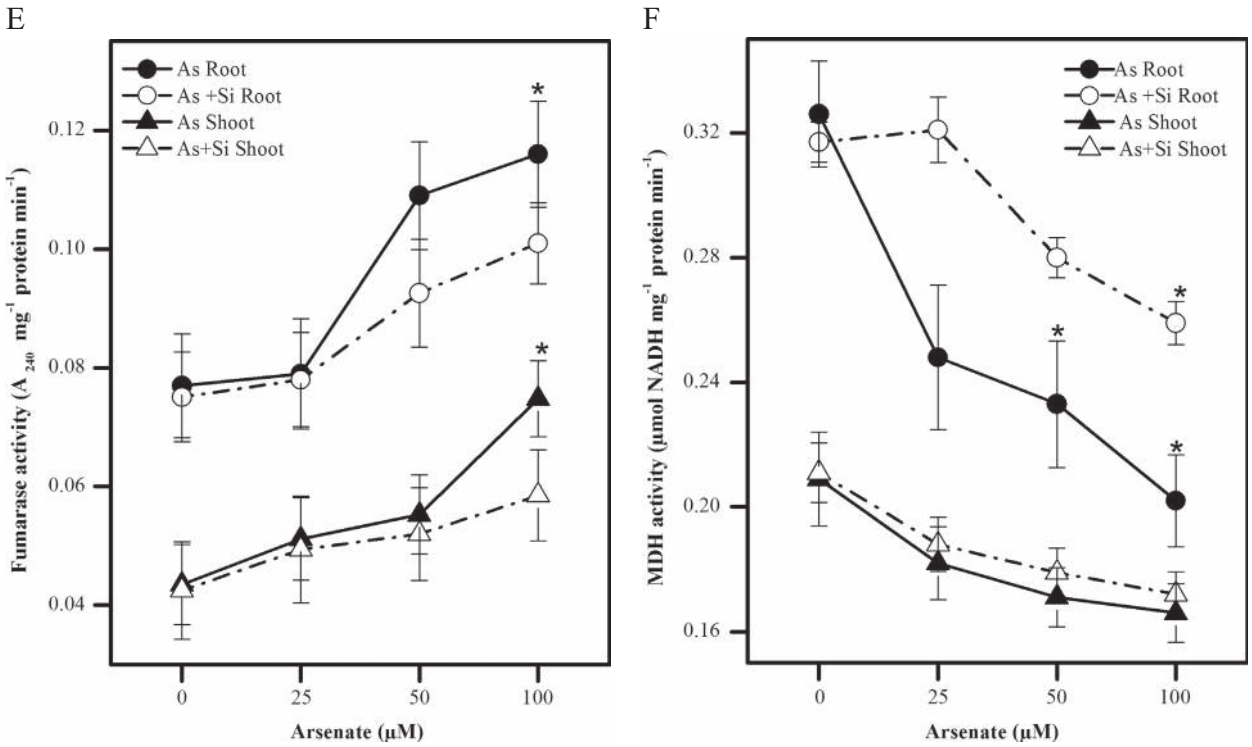

Fig. 5 (*continued*).

ones. This provided strength and counteracted entry of As to the xylem and lowered As contents in the cultivar. Such Si mediated restriction of As transport from root to shoot also increases the probability of decreasing As contents in the grains too.

Phosphorus is the principal element for energy transfer and protein metabolism. Replacement of phosphorus with As (V) lowered the phosphorus contents under arsenate stress. Chemical similarity between As (V) and phosphate led to the formation of unstable ADP-As complexes which slowed down the respiratory process (Shrivastava et al., 2015). After conversion of As (V) to As (III) within the cell, As (III) decreases the activities of phosphorolytic enzymes as well as ATPase which decreases the phosphorus pool in plants and consequently lowers phosphorus content (Mishra and Dubey, 2007). Si treatment however, restricted the entry as well as translocation of As and probably enhanced the activities of phosphorolytic enzymes and ATPase leading to an increase in phosphorus contents as was evidenced from ICP studies.

OAs of Krebs cycle are indicators of stress and their accumulation is a physiological mechanism that helps to maintain ionic balance and stabilize pH under stress (Xu et al., 2013). Arsenate treatment increased pyruvate, citrate, succinate as well as malate content in the test seedlings (Fig. 3). OAs like citrate and malate secreted by plant roots can form stable complexes with metals in the rhizosphere that prevents binding of metals to cellular compartments resulting in detoxification of metals (Ma et al., 2001). Such OA accumulation occurs in response to the deficiency of inorganic ions which causes a deficit of negative charge (Hui, 2012). These organic solutes protect cellular structures and biomolecules from ROS and toxic effect of ions. However, silicate induced drop in the content of OAs to a comparable value may be probably due to increased extrusion of OA in the rhizosphere to chelate arsenic and restrict its entry to the plant thereby lowering the endogenous content of OAs and can be considered a potential mechanism in amelioration of As toxicity.

Among the key respiratory enzymes of plant metabolism, dehydrogenases are considered to be important in generating reducing power. Inhibition of dehydrogenase activity during stress may be one of the possible reasons for decreased growth (Bouthour et al., 2012). PDH complex, considered to be the primary target of arsenate toxicity is inhibited by arsenic which directly binds to the dihydrolipoyl group of transacetylase and depletes the mitochondrial NADH pool leading to oxidative stress (Shen et al., 2013). A decrease in activity of PDH as well as IDH was evident in the arsenate treated seedlings (Figs. 4 and 5B) also corresponded to pyruvate and citrate accumulation indicating impairment of the TCA cycle. Similar inhibition of PDH and IDH activity was reported earlier in pea (Bansal et al., 2002). Higher concentrations of arsenic, cadmium and lead have been documented to down regulate IDH activity due to a decrease in expression of IDH isozymes in wheat. This eventually affected respiration and disturbed metabolism (Shao et al., 2011). An increase in the activity of CS along with a decrease in activity of IDH led to citrate accumulation. Inhibition of OGDH in the present study occurred since arsenite inhibited OGDH complex by direct binding (Shen et al., 2013). SSDH has a key role in mitochondrial metabolism and participates in both TCA cycle and electron transport chain. Arsenate-induced decline of SDH activity in the present study (Fig. 5D) also corresponded to succinate accumulation in the seedlings. ROS production during arsenate stress inactivates the antioxidant enzymes causing H_2O_2 accumulation that oxidizes the thiol groups of enzymes and reduces SDH activity. Another possibility of succinate accumulation might have been from GABA via GABA shunt as anaplerotic replenishment to the TCA cycle. Similar reduction in SDH activity under hypoxic conditions was due to decreased expression of SDH isozymes f1 and f2 in cucumber (He et al., 2015). MDH catalyzes the interconversion of oxaloacetate (OAA) and malate and has diverse roles in the metabolism of plants. Escalation in fumarase activity along with an inhibition of MDH activity (Figs. 5E and F) could be correlated with malate accumulation in the test cultivar. Such subdued effect of MDH was

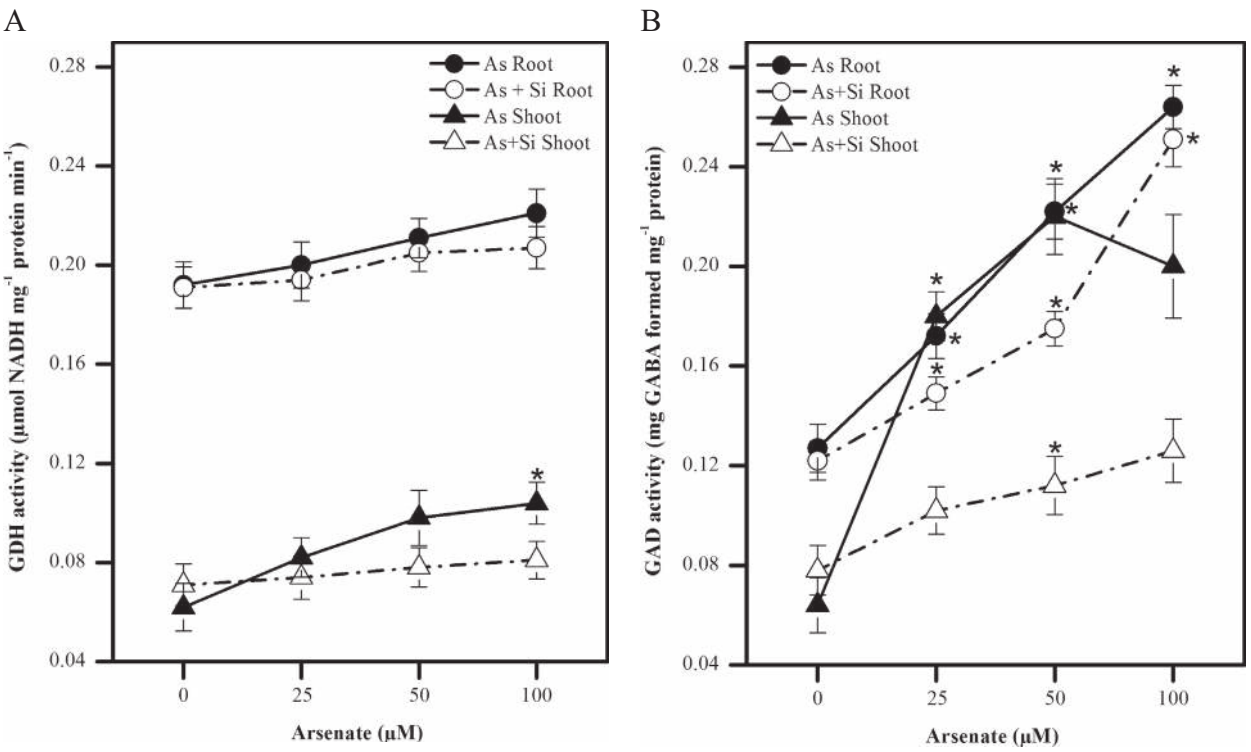

Fig. 6. Effect of arsenate and silicate either applied individually or in combination on activities of GABA synthesizing enzymes (A – GDH, B – GAD) in 21 days old wheat (cv. PBW343) seedlings. Each data point is the mean ± SE of three repeats with two replicas in each treatment, * indicates statistically significant at $p \leq 0.05$, respectively, compared to control.

put forward in rice and tobacco (Kumar et al., 2000; Bouthour et al., 2012). The decline in MDH activity disrupts cellular redox, inhibits respiration and alters carbon metabolism (Bouthour et al., 2012). Si supplementation along with arsenate increased the activities of PDH, IDH, OGDH, SDH and MDH in comparison to the arsenate treated doses possibly because it lowered the arsenate uptake and improved the phosphate balance in the test seedlings. This Si-induced alteration in enzymatic activity possibly helped to alleviate the detrimental consequences imposed by arsenate.

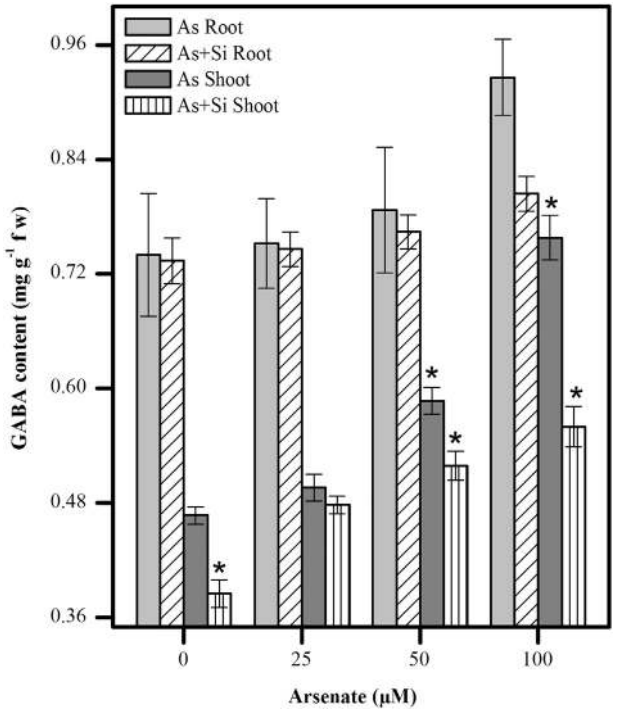

Fig. 7. Effect of arsenate and silicate either applied individually or in combination on GABA contents of 21 days old wheat (cv. PBW343) seedlings. Each data point is the mean ± SE of three repeats with two replicas in each treatment, * indicates statistically significant at $p \leq 0.05$, respectively, compared to control.

GABA has extensively been reported during biotic as well as in abiotic stresses (Bouche and Fromm, 2004). Enhancement in GABA contents by arsenate treatment coincides with GABA accumulation under adverse environmental conditions (Bartyzel et al., 2003; Bor et al., 2009; Liu et al., 2011). Although the predominant pathway for GABA biosynthesis is through GAD activity, polyamine degradation during stress may also cause GABA accumulation. GABA has a role in regulation of C/N balance, buffering of cytosolic pH, osmoregulation and oxidative stress management (Akcay et al., 2012). A positive correlation between the activities of GABA synthesizing enzymes viz., GDH as well as GAD and arsenate treatment (Figs. 6A and B) enhanced GABA contents (Fig. 7) while its subsequent transport to the mitochondria resulted in succinate accumulation. However, administration of silicate along with arsenate modulated the arsenate imposed effects considerably leading to a decrease in activities of GABA synthesizing enzymes as well as GABA content resulting in less succinate accumulation.

5. Conclusion

Arsenic stress adversely affected normal growth and physiological attributes of wheat seedlings. Arsenic contents in the test cultivar increased while phosphorus contents decreased along with increasing concentrations of arsenate treatments. Silicon amendments reduced arsenic content and restored the phosphorus content substantially. The levels of oxidative stress markers, respiratory cycle intermediates and the activities of GABA synthesizing enzymes along with GABA contents decreased appreciably while activities of respiratory enzymes were also revived with silicon supplementations. This Si-induced alteration of TCA

cycle and GABA synthesis in arsenate stressed wheat seedlings may provide a viable approach to employ silicon as a potential mitigator of arsenate contamination. The present study points out that enhanced Si content with silicate supplementation not only obstructs entry of arsenate but also modulates the respiratory cycle as well as GABA synthesis by improving the availability of phosphorus and is the first such report in wheat seedlings under arsenate toxicity. Administration of silicate enriched fertilizers in arsenic contaminated soils may therefore, form the starting point to reduce possible health risks associated with arsenic contamination. This would have positive implications in agro biological systems with proper field trials.

Author Contributions

AKB conceived the study, participated in its design and coordination, helped during preparation of the manuscript. PS performed the experiments, did statistical analysis and drafted the manuscript. PS, PD analyzed the data. All authors read and approved the final manuscript.

Declaration of Interests

The authors declare that they have no competing interests.

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