RESEARCH ARTICLE

Kinetics of Arsenic Accumulation and its Impact on Biochemical Responses of *Brassica juncea*

Sudhakar Srivastava[1*], Ashish K. Srivastava[2], Penna Suprasanna[2]

DOI: 10.18811/ijpen.v6i01.03

Abstract

Arsenic (As) contamination of the environment is a widespread problem with the situation at its worst in the South Asian region of West Bengal, India, and Bangladesh. In order to cope up with the problem and to be able to engineer plants in near future, there is a need to thoroughly understand the kinetics of As uptake and its impact on responses of plants. In this work, *Brassica juncea* (L.) Czern was used as a model system to understand kinetic interaction between As uptake and transport to the shoot and corresponding biochemical responses. The seedlings of *B. juncea* were exposed to 100 µM arsenite [As(III)] in hydroponics for different time points of 1, 4, and 24 hours. The As concentration was found to show a gradual increase in different tissues with time and the level of As followed the order: lower root > upper root > shoot > top leaves at all the times points. However, the level of hydrogen peroxide (H_2O_2) and the activities of NADPH oxidase, superoxide dismutase (SOD) and ascorbate oxidase (AO) were increased even at 1 h in lower shoot and leaves. The expression of transporters genes, NIP1;1, NIP2;1, NIP5;1 and NIP6;1 was found significantly up-regulated at 24 hours. This work establishes a kinetic relationship between As accumulation and ensuing biochemical responses in *B. juncea*.

Keywords: Arsenic; Indian mustard, NADPH Oxidase, NIPs.

International Journal of Plant and Environment (2020); **ISSN:** 2454-1117 (Print), 2455-202X (Online)

Introduction

Arsenic (As) is a naturally occurring element widely distributed in the earth's crust, having properties of both metal and a non-metal. Arsenic can be found in rock, soil, water, air, and the earth biosphere (Shukla *et al*., 2020). The sources include both anthropogenic inputs and natural biogeochemical processes (Awasthi *et al*., 2017). There are many forms, or species of As, which are broadly categorized as inorganic and organic (ATSDR, 2000). The major forms of As are inorganic arsenite [As(III)] and arsenate [As(V)]. Arsenate is an analog of inorganic phosphate, and it interferes with the essential cellular processes like oxidative phosphorylation and ATP synthesis. Arsenite binds to sulfhydryl groups of proteins and has detrimental effects on general protein functioning (Finnegan and Chen, 2012; Awasthi *et al*., 2017; Majumdar and Bose, 2017, 2018). Arsenate is taken up and transported in plants by phosphate transporters (Catarecha *et al*., 2007; Cao *et al*., 2017; Ye *et al*., 2017). Arsenite is able to enter root cells mainly through nodulin26-like intrinsic proteins (NIPs; Lindsay and Maathuis, 2017; Awasthi *et al*., 2017; Majumdar and Bose, 2018) of aquaglyceroporins.

Roots are usually the first tissue to be exposed to As and therefore root response to As and changes in root architecture play a crucial role in As stress tolerance (Srivastava *et al*., 2019). With increasing exposure duration and translocation of As to shoot tissues, As severely inhibits plant growth by slowing or arresting cellular expansion and by reducing photosynthetic efficiency (Garg and Singla, 2011; Mishra *et al*., 2014). One of the major mechanisms of As toxicity is the increased production of reactive oxygen species (ROS) and consequent damage to lipids, proteins and DNA (Srivastava *et al*., 2011; Gupta *et al*., 2018; Gautam *et al*., 2020). An increase in the activity of pro-oxidant enzymes like NADPH oxidase and ascorbate oxidase (AO) occurs for regulated increase in ROS production to mediate As stress signaling (Srivastava *et al*., 2011; Gupta *et al*., 2013). The fine-tuning of scavenging of ROS is achieved through the role of enzymes like superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and molecules like glutathione (GSH) and ascorbate (Srivastava *et al*., 2007). When the level of ROS increases uncontrollably, stress responses are evident (Gautam *et al*., 2020). Arsenic detoxification is achieved through its complexation via thiol (-SH) containing ligands including GSH, and phytochelatins (PCs) and subsequent transport of complexed As to the vacuoles (Song *et al*., 2014).

Considerable information has come to light about As induced stress responses in a number of plant systems like rice, Indian mustard, as well as aquatic plants like *Hydrilla verticillata* (Srivastava *et al*., 2007, 2009; Chauhan *et al*., 2017; Awasthi *et al*., 2018; Pandey and Gupta, 2018). Despite this knowledge resource, there is still paucity of information, especially on the temporal and spatial dynamics of As uptake. In this regard, it is interesting to study how kinetics of As uptake and translocation

[1]Plant Stress Biology Laboratory, Institute of Environment and Sustainable Development, Banaras Hindu University, Varanasi-221005, Uttar Pradesh, INDIA

[2]Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai-400084, Maharashtra, INDIA

***Corresponding author:** Dr. Sudhakar Srivastava, Plant Stress Biology Laboratory, Institute of Environment and Sustainable Development, Banaras Hindu University, Varanasi-221005, Uttar Pradesh, India; Mobile: +91-9369607936; Email: sudhakar.srivastava@gmail.com; sudhakar.iesd@bhu.ac.in

How to cite this article: Srivastava, S., Srivastava, A.K. and Suprasanna, P. (2020). Kinetics of Arsenic Accumulation and its Impact on Biochemical Responses of Brassica juncea. International Journal of Plant and Environment, 6(1): 28-34.

Source of support: Nil

Conflict of interest: None

Submitted: 16/12/2019 **Accepted:** 25/01/2020 **Published:** 31/01/2020

within plants is correlated to the responses of plants in various plant tissues. In the present study, time- and tissue-dependent responses of Indian mustard [*Brassica juncea* (L.) Czern.] were investigated. Among the plants of the *Brassica* species, *B. juneca* has become as a candidate system in a number of metal related studies owing to its metal hyperaccumulator potential and significant tolerance to a number of metals like As, Cd, Zn and Pb (Srivastava *et al*., 2009; Rizwan *et al*., 2018).

Materials and Methods

Plant material and Treatment

The seeds of *B. juncea* var. TPM-1 were surface sterilized with 30% ethanol for 3 minutes and then washed thoroughly with distilled water to remove the traces of ethanol. The seeds were soaked in water for 6 hours and then kept overnight under dark for germination. The seeds were placed in theromcol float with holes filled with 0.4% agarose. Once the seeds were germinated, the floats were transferred to 500 ml beaker containing ½ strength Murashige and Skoog nutrient medium. The seedlings were grown in a Plant growth chamber (Sanyo, Japan) with a temperature of 25 ± 2°C, 125 µM photon m^{-2} s^{-1} of illumination, with a 12 hours photoperiod, a relative humidity of 70% and vigorous aeration to maintain good growth. The medium was changed every third day. After 14 d, seedlings subjected to 100 µM As(III) ($NaAsO_2$; Sigma) for three-time points (1, 4, and 24 hours). The sets without As were maintained for the control treatment. At harvesting, seedlings were cut into four segments – lower root (LR; 1 cm from tip), upper root (UR), shoot (Sh; young stem plus lower leaves), and topmost leaves (TL) (as depicted in Fig. 1). The harvested tissues were cut directly in pestle mortar in liquid nitrogen and were homogenized to a fine powder. The tissues were stored at -80°C until further analysis. For weight measurements, a separate set was used. The fresh weight of seedlings was recorded, and dry weight was taken after drying at 80°C for 2 to 3 days to reach a constant weight.

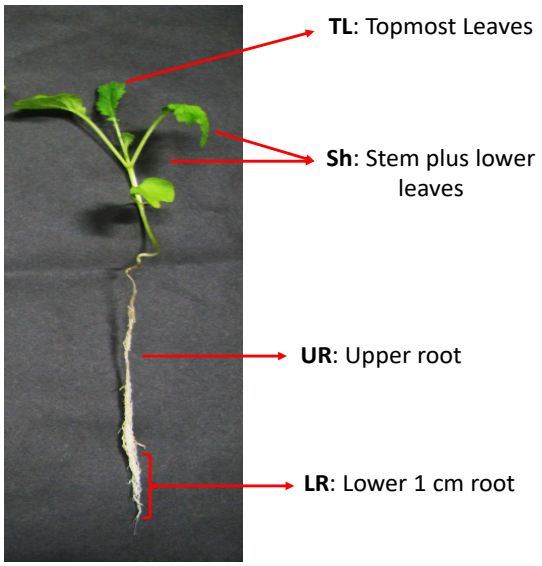

Fig. 1: Representation of *Brassica* seedlings cut into four different parts.

Arsenic Estimation

To determine the total content in the plants, plants were cut into four segments as mentioned above and oven-dried to constant weight (Srivastava and D'Souza, 2010). The samples were subjected to acid digestion by using concentrated HNO_3 on a heating block 180°C for 1 h and subsequently at 200°C for 45-60 min so as to evaporate the samples to dryness. Arsenic concentrations were determined on an atomic absorption spectrophotometer (GBC 906AA, Australia) coupled to a hydride generation system (HG3000). In each analytical batch, reagent blanks and spiked samples were included in the acid digestion to assess the accuracy of the chemical analysis. Analytical data quality was ensured through repeated analysis of standard reference materials. The translocation factor (TF) of As from root (LR + UR) to Sh (= As concentration in Sh / As concentration in root) and from Sh to TL (= As concentration in TL/As concentration in Sh) was calculated.

Histochemical Staining by using 3, 3'-diaminobenzidine (DAB)

For the detection of H_2O_2 *in vivo,* plant samples were stained with 3, 3'-diaminobenzidine (DAB) as detailed in Srivastava *et al*. (2011). DAB is oxidized by H_2O_2 in the presence of peroxidases, to generate a dark brown precipitate. To perform DAB staining, a solution of 0.5 mg ml^{-1} DAB (pH 3.8) was prepared in 10 mM MES buffer (pH 6.5). Leaves (2nd leaves from top) and roots were dipped in 20 ml of DAB solution in scintillation vials and were vacuum infiltrated 3 times in dark with the interval of 5 min each. After infiltration, the plantlets were incubated in the dark overnight. The next day, leaves and roots were placed carefully on transparency sheets and scanned.

Enzymatic Assays

For enzymatic assays also, the plants were divided into four segments, as mentioned above, and each segment was ground in liquid nitrogen. The samples were used for protein extraction in 20 mM HEPES (pH 7.0) containing 1 mM EDTA, 1 mM EGTA, 1 mM phenylmethyl sulfonyl fluoride (PMSF), 2.5% polyvinylpyrrolidone (PVP) and 5% glycerol under chilled conditions. The homogenate was centrifuged at 10000 g for 10 minutes and the supernatant was used for enzyme assay. The protein content in the supernatant was assayed following Lowry *et al*. (1951). The activity of ascorbate oxidase (AO; EC 1.10.3.3) was determined by measuring the decrease in absorbance at 265 nm due to ascorbate oxidation (ε = 2.8 mM^{-1} cm^{-1}) as described previously (Yamamoto *et al*., 2005). NADPH-dependent $O_2^{\bullet-}$ generation by the enzyme NADPH oxidase (NOX; EC 1.6.3.1) was measured using nitro blue tetrazolium (NBT) as an electron acceptor (Bielski *et al*., 1980), whose reduction was monitored at 530 nm. Monoformazan concentrations (and therefore $O_2^{\bullet-}$ concentrations) were calculated using an extinction coefficient of 12.8 mM^{-1} cm^{-1}. The reaction mixture consisted of Tris buffer (50 mM Tris-HCl, pH 7.4), 5 mM NBT, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM NADPH, and a suitable aliquot of enzyme extract. The selective reduction of NBT by $O2^{\bullet-}$ was calculated from the difference in the NBT reduction rate in the presence and absence of SOD (50-100 units mL^{-1}; Sigma). No NBT reduction with NADPH was observed in the absence of protein fractions. The activity of

superoxide dismutase (SOD; EC 1.15.1.1) was assayed following the protocol of Beauchamp and Fridovich (1971).

Real-Time PCR Expression Profiling of Transporter Genes

For real-time RT-PCR analysis of NIP transporters, gene sequences of *NIP1;1, NIP2;1, NIP5;1* and *NIP6;1* were downloaded from *Brassica rapa* database (BRAD; www.brassicadb.org), and primers of intron-exon boundary were then prepared (Table 1). Primer optimal concentrations of target and reference genes were determined with serial dilutions of cDNA obtained from 10 µg of RNA isolated from *B. juncea*. Real-time quantitative RT-PCR was carried out using a Corbett rotor gene 3000 (Corbett Life Science, www.corbettlifescience.com/; Srivastava *et al.,* 2009). The actin gene was amplified in parallel with the target gene, allowing for gene-expression normalization and providing quantification. The procedure detailed in Srivastava *et al*. (2009) was followed for real-time PCR expression of selected genes. A relative expression ratio plot was generated using the software REST-MCS (Pfaffl *et al*., 2002).

Statistical Analysis

Statistical treatments included one way ANOVA and Duncan's multiple range test (DMRT) to assess the variability of the data and to know significant differences between treatments. Statistical analysis was performed by using Statistical Package for the Social Sciences (SPSS) version 16.0.

Results

Arsenic Accumulation Occurred in Time-dependent Manner

The As accumulation showed a gradual increase over different time points, from 1 h to 24 h in *Brassica* seedlings in all the studied tissues. As accumulation at 1 h was very high in LR (110.08 µg g^{-1} dw) and UR (121.35 µg g^{-1} dw) as compared to that of Sh (3.17 µg g^{-1} dw) and TL (0.09 µg g^{-1} dw) (Fig. 2). With an increase in duration, there was an enhanced translocation of As from root to shoot tissue. At 24 hours, the As concentration in LR, UR, Sh, and TL was 202.85 µg g^{-1} dw, 187.5 µg g^{-1} dw, 97.5 µg g^{-1} dw, and 7.16 µg g^{-1} dw, respectively (Fig. 2). The translocation factor of As was calculated at each time point. The TF for root to Sh increased from 0.01 at 1-hour to 0.12 at 4-hour and 0.25 at 24 hours. The TF values for Sh to TL were 0.03, 0.03, and 0.07 at 1, 4, and 24-hour, respectively. Hence, the translocation of As from roots to Sh showed a marked increase from 1 h to 4 h while As translocation to TL was very low.

Table 1: Sequences of primers used for real-time PCR analysis.

S.N.	*Primer name*	*Primer sequence from 5' to 3'*
1.	*NIP1;1* For	TCGGTGGTTGTAAACATGCAAAACG
2.	*NIP1;1* Rev	CGCCTGCAGATCCGATCCCACCG
3.	*NIP2;1* For	AGCAACCATGGTAATGTTGTTGTA
4.	*NIP2;1* Rev	ACAGCTGGATTGAAATGTGCGGAGA
5.	*NIP5;1* For	CACGGGGATGAGAGTTGATTCAA
6.	*NIP5;1* Rev	GCTGTCGCGGTGAATATCAAGATG
7.	*NIP6;1* For	GAGTTGGCGGGAATAGCGGTAG
8.	*NIP6;1* Rev	AATGGCTGGACCTAGTGTTCTTACA
9.	*Actin* For	CTCCTGCCATGTATGTCGCTATCC
10.	*Actin* Rev	AAGGTCCAAACGCAGAATAGCAT

Arsenic Exposure Resulted in an increase in H_2O_2 and Induced Responses of Prooxidant and Antioxidant Defense

H_2O_2 accumulation was histochemically detected in the root and leaf tissues by using DAB staining (Fig. 3A-H). The accumulation

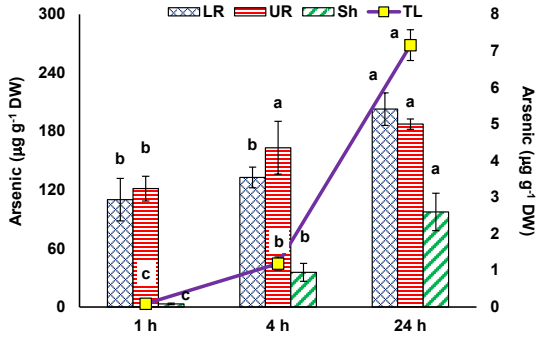

Fig. 2: Arsenic accumulation in LR, UR, Sh, and TL of *B. juncea* seedlings exposed to 100 µM As(III) for 1, 4, and 24-hour. All the values are means of three replicates ±SD. ANOVA was found significant at 95% significant level. Different symbols indicate significantly different values for a particular tissue (DMRT ≤0.05).

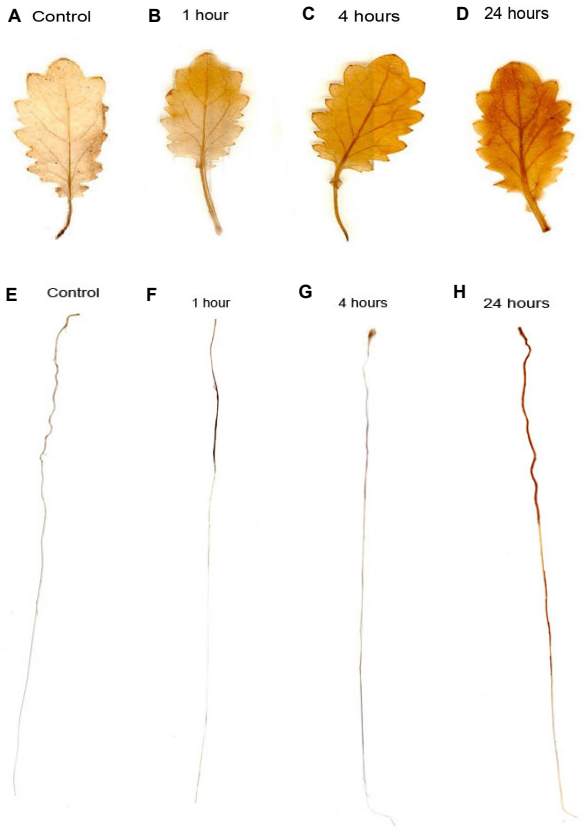

Fig. 3: Effect of 100 µM As(III) exposure on hydrogen peroxide accumulation in 2nd leaves and roots of *B. juncea* seedlings at 1-hour (B, F), 4-hour (C, G) and 24-hour (D, H). Control leaf (A) and root (E) is also shown.

of H_2O_2 was observed from 1-hour onwards and was continued to increase until 24 hours. Thus, As exposure resulted in H_2O_2 accumulation even at 1-hour in 2nd leaves from top in seedlings, where As accumulation was very low. Among enzymes, SOD showed a highly significant increase in all tissues on all time points. At the early time point of 1-hour, SOD showed an increase of 36, 61, 58, and 40% in LR, UR, Sh, and TL, respectively, as compared to corresponding control for each tissue (Fig. 4A).

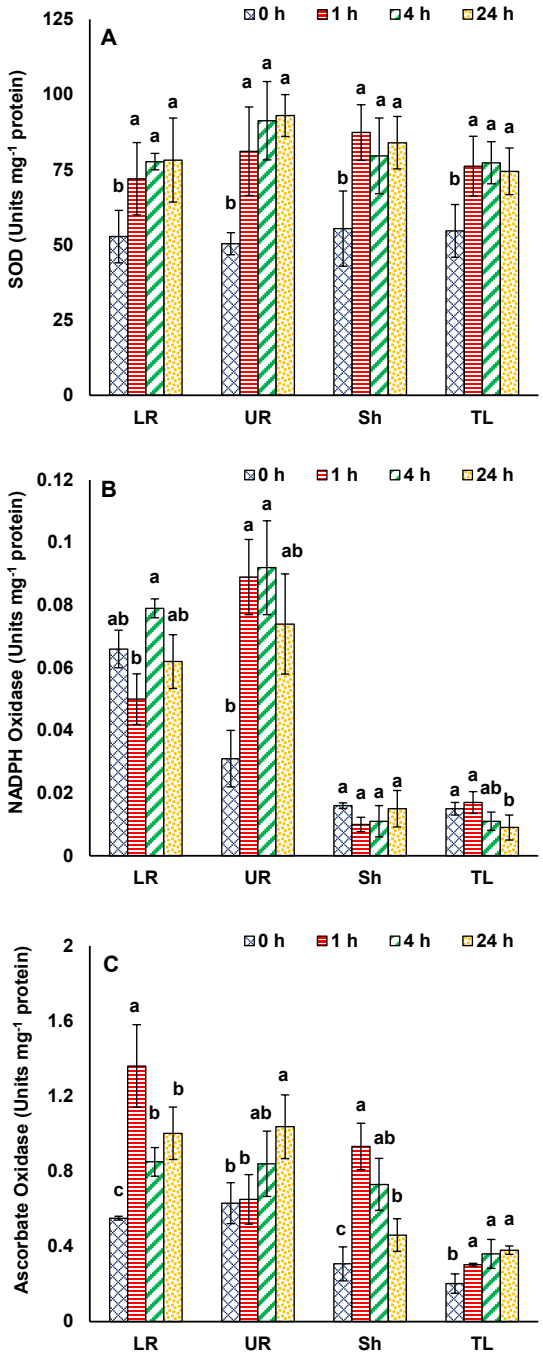

Fig. 4: Effect of 100 µM As (III) exposure on the activity of SOD (A), NADPH oxidase (B), and AO (C) of *B. juncea* seedlings at 1, 4, and 24 hours. All the values are means of three replicates ±SD. ANOVA was found significant at 95% significant level. Different symbols indicate significantly different values for a particular tissue (DMRT ≤0.05).

NADPH oxidase activity showed a significant decrease in LR at 1 h while an increase at 4 hours as compared to control (Fig. 4B). In Sh, NADPH oxidase did not show a significant change while in TL, NADPH oxidase activity showed a significant decline at 4-hour (27%) and 24 h (40%) in comparison to control. Only UR depicted a significant increase in NADPH oxidase activity on all-time points in comparison to control, which were 187, 197, and 138% at 1, 4, and 24 hours, respectively (Fig. 4B). Similar to SOD, AO also showed a significant increase in all tissues on all time points except in UR at 1-hour (Fig. 4C). The maximum increase in AO activity in LR (147%) and Sh (204%) was observed at 1-hour in comparison to control while the maximum increase was seen at 24 h in UR (65%) and TL (88%).

Transporter Genes Showed Significant Arsenic-induced Change in Expression

The expression level of four transporter genes (*NIP1;1, NIP2;1, NIP5;1* and *NIP6;1)* depicted significant changes in tissue- and time-dependent manner. The gene expression was analyzed in root and shoot tissues as a whole with no further separation. An expression change of > or <1-log2-fold was considered to be significant. At 4 hours, only *NIP1;1* showed significant down-regulation in roots while other genes were not altered significantly (Fig. 5). At 24 h, *NIP1;1* expression was up-regulated significantly (4.88-fold) while *NIP2;1* was down-regulated (-1.51-fold). *NIP5;1* and *NIP6;1*were not significantly regulated in roots. In shoot, the expression of *NIP1;1* was not detected while other three genes (*NIP2;1, NIP5;1* and *NIP6;1*) showed significant up-regulation at both 4, and 24 hours. The up-regulation in *NIP2;1, NIP5;1* and *NIP6;1*was 4.6-, 6.7- and 6.0-fold, respectively at 24 h (Fig. 5).

Discussion

Arsenic accumulation in plants is determined by several factors related to soil and plant; the latter is related to As uptake by roots, its translocation, and distribution and relocation in different plant tissues (Zheng *et al*., 2011). As induced physiological, biochemical, and molecular responses have received great attention (Srivastava *et al*., 2009; 2016; Du *et al*., 2020). Insights into temporal and spatial dynamics of As stress responses will provide valuable information about the transport and

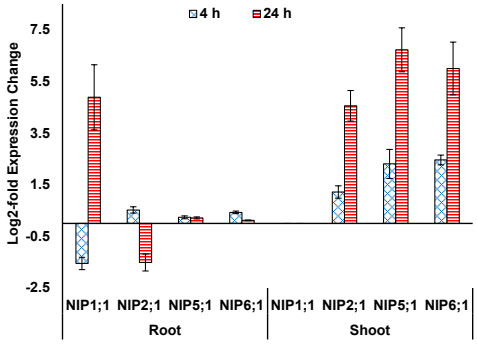

Fig. 5: Effect of 100 µM As(III) exposure on the expression of various transporter genes of *B. juncea* seedlings at 4 and 24 hours. The X-axis represents the value of control plants. All the values are means of three replicates ±SD.

accumulation. This study demonstrated that stress responses occur in tissue- and time-dependent manner in association with As accumulation changes. As accumulation in roots was found to occur rapidly, reaching to high level even at 1-hour. However, the translocation of As to Sh and TL was rather slow. The increase in As translocation from roots to Sh occurred with an increase in time and therefore, the fold-difference in root (LR / UR) and shoot As decreased from about 22-fold at 1 h to 2-fold at 24 h. Although As translocation also increased from Sh to TL with time, it could be seen that As translocation to TL was restricted to a great extent. This appears to be due to the fact that plants attempted to protect the most sensitive and young tissues, i.e., TL from As damage. A graded level of As concentration from roots to grains is known to be a phenomenon in rice plants (Shrivastava *et al*., 2020). In *B. juncea* also, a significant difference in As concentration in root and shoot has been observed (Pathare *et al*., 2013). Thus, roots are presumably more resistant to As accumulation than leaves. The uptake and transport of As are mediated by various transporters. The As in As(III) form is mostly taken up and transported through nodulin26-type intrinsic proteins (NIPs) of the aquaglyceroporin family. The role of a number of NIPs transporters in As(III) uptake and transport has been elucidated. NIP1;1 from *A. thaliana* is an important transporter involved in As(III) uptake, and its mutation has been found to enhance As tolerance of plants (Kamiya and Fujiwara, 2009; Ji *et al*., 2017). Another homolog of NIP1;1, i.e., NIP5;1, was also found to mediate As transport as mutant lines of *nip5;1* had reduced As content in *Arabidopsis* (Kamiya and Fujiwara, 2009). Bienert *et al*. (2008) expressed a number of genes of NIPs in yeast and examined their As(III) transport potential. In this study, AtNIP5;1 and AtNIP6;1 were found to mediate bidirectional As(III) transport in yeast; however AtNIP2;1was not found to mediate As(III) transport (Bienert *et al*., 2008). However, in rice, OsNIP2;1 (Lsi1) is a major transporter involved in As(III) uptake and transport (Ma *et al*., 2008; Awasthi *et al*., 2017). In this study, the studied transporters showed a significant change in expression. At 24 h, NIP1;1 in roots and NIP2;1, NIP5;1 and NIP6;1 in shoots showed significant up-regulation; >=4.6-fold for each gene. As accumulation data shows a significant increase in uptake as well as translocation of As from root to shoot, which was correlated to the up-regulation observed in transporter genes. Thus, in *B. juncea,* NIP1;1, NIP2;1, NIP5;1 and NIP6;1 might have a role in As(III) uptake and transport, which needs to be studied in future.

It is known that photosynthesis is affected to a great extent upon As exposure and As-induced loss of pigments and photosynthetic efficiency have been suggested as the major mechanism of As stress in plants (Mishra *et al*., 2014). The loss of photosynthetic efficiency is also related to the outburst of ROS and associated toxicity (Srivastava *et al*., 2011). A study by Rai *et al*. (2014) demonstrated that enhanced photosynthesis plays a role in As tolerance in *Artemisia annua*. Hence, As accumulation kinetics was clearly indicative of plant response through restriction of most of the As in root tissues allowing very little As reaching to TL. However, the biochemical responses, well known to be associated to stressed conditions (*i.e.*, increased H_2O_2 production and SOD activity) (Hartley-Whitaker *et al*., 2001; Karimi *et al*., 2019) were observed even at 1 hour in TL when As concentration in TL was very low (0.09 µg g^{-1} DW). The increase in SOD was 36% in LR, while 39% in TL. At the same time, As concentration was 1223-fold higher in LR (110.08 µg g^{-1} DW) than that in TL (0.09 µg g^{-1} DW). Hence, the response of plants appeared to be connected to the signaling of As stress to TL at a very early time. Plants appeared to have sensed As entry into roots and transferred this information to the TL to prepare it for tackling the incoming As stress (Huber and Bauerle, 2016; Ko and Helariutta, 2017). Hydrogen peroxide is a well-known messenger molecule that transfers stress signals to long distances under stress conditions (Cuypers *et al*., 2016; Sies, 2017). Therefore, the increased level of H_2O_2 observed in TL at 1 hour may either be attributable to increased production in TL itself or to transport of H_2O_2 from roots and Sh tissues.

A significant increase in SOD, which is responsible for dismutation of superoxide radicals to H_2O_2 might have also contributed to H_2O_2accumulation (Culotta *et al*., 2006). Tissue-specific responsive nature of *B. juncea* was also evident through variable increases in NADPH oxidase and AO on different time points (Fig. 4). NADPH oxidase enzyme has also been suggested as a source of extracellular H_2O_2 generation (Qin *et al*., 2004; Steinite *et al*., 2004). NADPH oxidase uses cytoplasmic NADPH to transfer an electron to molecular O_2 to form superoxide radicals, which are then dismutated to H_2O_2 (Yang *et al*., 2007). In this study, NADPH oxidase, as well as SOD activities, were significantly increased in UR, and these two might have acted in a concerted manner to enhance H_2O_2 production. AO catalyzes the reduction of oxygen to water with the consumption of ascorbate. This affects ascorbate pool of cells and affects H_2O_2 quenching through the ascorbate-glutathione cycle (De Tullio *et al*., 2013). The activity of AO was also significantly increased in all tissues on all-time points; specific mention to be made of LR at 1 h. Hence, various enzymatic responses and H_2O_2 accumulation visualized at 1 h in TL indicate that plants induced H_2O_2 production through concerted changes in response to As stress. This appeared to be a signaling mechanism of plants for tolerance to As stress. It has also been proposed that AO induced ascorbate oxidation and consequent accumulation of dehydroascorbate plays a role in the signaling of stress (De Tullio *et al*., 2013). An active involvement of SOD, NADPH oxidase, and AO in As stress responses have been demonstrated in *Hydrilla verticillata* (Srivastava *et al*., 2007, 2011). Besides, the role of a pro-oxidant enzyme, glycolate oxidase, which is involved in increased production of H_2O_2, has been shown to play a crucial role in As stress responses of *Arabidopsis thaliana* (Gupta *et al*., 2013). Hence, with the beginning of As infiltration in roots, plants regulate both pro-oxidant and antioxidant enzymes so as to finely regulate H_2O_2 levels for the purpose of signaling the stress to distant tissues (TL).

In conclusion, the present study shows that spatial and temporal profiling of As in *Brassica* provides new insights into As accumulation. The data on the differential pattern overtime points and different tissues indicate towards the identification of important early check-points. Expression profiling of transporter genes showed a good correlation with As accumulation and tissue distribution.

References

ATSDR (Agency for Toxic substances and Disease Registry) 2000. Toxicological profile for arsenic: Health effects chapter. US

Department of Health and Human Services, Atlanta, GA, USA, pp. 244. http://www.atsdr.cdc.gov/toxpro2.html#Final.

Awasthi, S., Chauhan, R., Srivastava, S. and Tripathi, R.D. 2017. The journey of arsenic from soil to grain in rice. *Frontiers in Plant Science* **8**: 1007.

Beauchamp, C. and Fridovich, I. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry* **44**: 276-287.

Bielski, B.H.J., Shlue, G.G. and Bajuk, S. 1980. Reduction of nitro blue tetrazolium by CO_2^- and O_2^- radicals. *The Journal of Physical Chemistry* **84**: 830-833.

Bienert, G.P., Thorsen, M., Schüssler, M.D., Nilsson, H.R., Wagner, A. and Tamás, M.J., 2008. A subgroup of plant aquaporins facilitate the bi-directional diffusion of $As(OH)_3$ and $Sb(OH)_3$ across membranes. *BMC Biology* **6**: 26.

Cao, Y., Sun, D., Ai, H., Mei, H., Liu, X. and Sun, S. 2017. Knocking out OsPT4 gene decreases arsenate uptake by rice plants and inorganic arsenic accumulation in rice grains. *Environmental Science and Technology* **51**: 12131-12138.

Catarecha, P., Segura, M.D., Franco-Zorrilla, J.M., García-Ponce, B. and Lanza, M. 2007. A mutant of the Arabidopsis phosphate transporter PHT1; 1 displays enhanced arsenic accumulation. *Plant Cell* **19**: 1123-1133.

Chauhan, R., Awasthi, S., Tripathi, P., Mishra, S., Dwivedi, S., Niranjan, A., Mallick, S., Tripathi, P., Pande, V. and Tripathi, R.D. 2017. Selenite modulates the level of phenolics and nutrient element to alleviate the toxicity of arsenite in rice (*Oryza sativa* L.). *Ecotoxicology and Environmental Safety* 138: 47-55.

Culotta, V.C., Yang, M., and O'Halloran, T.V. 2006. Activation of superoxide dismutases: putting the metal to the pedal. *Biochimica Biophysica Acta* **1763**: 747-758.

Cuypers, A., Hendrix, S., Amaral dos Reis, R., De Smet, S., Deckers, J. and Gielen, H. 2016. Hydrogen peroxide, signaling in disguise during metal phytotoxicity. *Frontiers in Plant Science* **7**: 470.

De Tullio, M.C., Guether, M. and Balestrini, R. 2013. Ascorbate oxidase is the potential conductor of a symphony of signaling pathways. *Plant Signaling and Behavior* **8**: e23213.

Du, F., Liu, P., Wang, K., Yang, Z. and Wang, L., 2020. Ionomic responses of rice plants to the stresses of different arsenic species in hydroponics. *Chemosphere* **243**: 125398.

Finnegan, P.M. and Chen W. 2012. Arsenic toxicity: the effects on plant metabolism. *Frontiers in Physiology* **3**: 182.

Garg, N. and Singla, P. 2011. Arsenic toxicity in crop plants: physiological effects and tolerance mechanisms. *Environmental Chemistry Letters* **9**: 303-321.

Gautam, A.,Pandey, A.K. and Dubey, R.S. 2020. *Azadirachta indica* and *Ocimum sanctum* leaf extracts alleviatearsenic toxicity by reducing arsenic uptake and improvingantioxidant system in rice seedlings. *Physiology and Molecular Biology of Plants* **26**: 63-81.

Gupta, D.K., Inouhe, M., Rodriguez-Serrano, M., Romero-Puertas, M.C. and Sandalio, L.M. 2013. Oxidative stress and arsenic toxicity: role of NADPH oxidases. *Chemosphere* **90**: 1987-1996.

Gupta, K., Mishra, K., Srivastava, S. and Kumar, A. 2018. Cytotoxic assessment of chromium and arsenic using chromosomal behavior of rot meristem in *Allium cepa* L. *Bulletin of Environmental Contamination and Toxicology* **100**: 803-808.

Hartley-Whitaker, J., Ainsworth, G. and Meharg, A.A. 2001. Copper- and arsenate-induced oxidative stress in Holcus lanatus L. clones with differential sensitivity. *Plant Cell and Environment* **24**: 713-722.

Huber, A.E. and Bauerle, T.L. 2016. Long-distance plant signaling pathways in response to multiple stressors: the gap in knowledge. *Journal of Experimental Botany* **67**: 2063-2079.

Ji, R., Zhou, L., Liu, J., Wang, Y., Yang, L. and Zheng, Q. 2017. Calcium-dependent protein kinase CPK31 interacts with arsenic transporter *AtNIP1;1* and regulates arsenite uptake in *Arabidopsis thaliana*. *PloS One* **12**: e0173681.

Kamiya, T. and Fujiwara, T. 2009. *Arabidopsis* NIP1;1 transports antimonite and determines antimonite sensitivity. *Plant and Cell Physiology* **50**: 1977-1981.

Karimi, N., Vakilipak, F., Souri, Z., Farooq, M.A. and Akhtar, J. 2019. The role of selenium on mitigating arsenic accumulation, enhancing growth and antioxidant responses in metallicolous and non-metallicolous population of *Isatis cappadocica* Desv. and *Brassica oleracea* L. *Environmental Science and Pollution Research* **26**: 21704-21716.

Ko, D. and Helariutta, Y. 2017. Shoot-root communication in flowering plants. *Current Biology* **27**: R973-R978.

Lindsay, E.R. and Maathuis, F.J.M. 2017. New molecular mechanisms to reduce arsenic in crops. *Trends in Plant Science* **22**: 1016-1026.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randal, E.J. 1951. Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* **193**: 265-275.

Ma, J.F., Yamaji, N., Mitani, N., Xu, X.Y., Su, Y.H., McGrath, S.P. and Zhao, F.J. 2008. Transporters of arsenite in rice and their role in arsenic accumulation in rice grain. *Proceedings of National Academy of Sciences USA* **105**: 9931-9935.

Majumdar, A. and Bose, S. 2017. Toxicogenesis and metabolism of arsenic in rice and wheat plants with probable mitigation strategies. In: Arsenic: Risks of exposure, behavior in the environment and toxicology. In: Knezevic, R. (Ed.), Nova Science Publishers, New York, United States, pp. 149-166.

Majumdar, A. and Bose, S. 2018. A glimpse on uptake kinetics and molecular responses of arsenic tolerance in rice plants. In: Hasanuzzaman, M., Nahar, K., Fujita, M. (Eds.), Mechanisms of arsenic toxicity and tolerance in plants, Springer, Singapore, pp. 299-315.

Mishra, S., Stark, H.J. and Kupper, H. 2014. A different sequence of events than previously reported leads to arsenic-induced damage in *Ceratophyllum demersum* L. *Metallomics* **6**: 444-454.

Pandey, C. and Gupta, M. 2018. Selenium amelioration of arsenic toxicity in rice shows genotypic variation: A transcriptomic and biochemical analysis. *Journal of Plant Physiology* **231**: 168-181.

Pathare, V., Srivastava, S. and Suprasanna, P. 2013. Evaluation of effects of arsenic on carbon, nitrogen, and sulfur metabolism in two contrasting varieties of *Brassica juncea*. *Acta Physiologiae Plantarum* **35**: 3377-3389.

Pfaffl, M.W., Horgan, G.W. and Dempfle, L. 2002. Relative expressions of tware tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Research* **30**: e36.

Qin, W.M., Lan, W.Z. and Yang, X. 2004. Involvement of NADPH oxidase in hydrogen peroxide accumulation by *Aspergillus niger* elicitor-induced *Taxus chinensis* cell cultures. *Journal of Plant Physiology* **161**: 355-361.

Rai, R., Pandey, S., Shrivastava, AK and Rai, S.P. 2014. Enhanced photosynthesis and carbon metabolism favor arsenic tolerance in *Artemisia annua,* a medicinal plant as revealed by homology-based proteomics. *International Journal of Proteomics* **2014**: 163962.

Rizwan, M., Ali, S., Zia-ur-Rehman, M., Rinklebe, J., Tsang, D.C.W. and Bashir, A. 2018. Cadmium phytoremediation potential of *Brassica* crop species: A review. *Science of the Total Environment* **631-632**: 1175-1191.

Shrivastava, A., Barla, A., Majumdar, A., Singh, S. and Bose, S. 2020. Arsenic mitigation in rice grain loading via alternative irrigation byproposed water management practices. *Chemosphere* **238**: 124988.

Shrivastava, A., Barla, A., Singh, S., Mandraha, S. and Bose, S. 2018. Arsenic contamination in agricultural soils of Bengal deltaic region of West Bengal and its higher assimilation in monsoon rice. *Journal of Hazardous Materials* **324**: 526-534.

Shukla, A., Awasthi, S., Chauhan, R. and Srivastava, S. 2020. The status of arsenic contamination in India. In: Srivastava, S. (Ed.), Arsenic in drinking water and food. Springer, Singapore, pp. 1-12.

Sies, H. 2017. Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: Oxidative eustress. *Redox Biology* **11**: 613-619.

Song, W.Y., Mendoza-Cózatl, D.G., Lee, Y., Schroeder, J.I., Ahn, SN and Lee, H.S. 2014. Phytochelatin-metal(loid) transport into vacuoles shows different substrate preferences in barley and *Arabidopsis*. *Plant Cell and Environment* **37**: 1192-1201.

Srivastava, S. and D'Souza, S.F. 2010. Effect of variable sulfur supply on arsenic tolerance and antioxidant responses in *Hydrilla verticillata* (L.f.) Royle. *Ecotoxicology and Environmental Safety* **73**: 1314-1322.

Srivastava, S., Akkarakaran, J.J., Sounderajan, S., Shrivastava, M. and Suprasanna, P. 2016. Arsenic toxicity in rice (*Oryza sativa* L.) is

influenced by sulfur supply: Impact on the expression of transporters and thiol metabolism. *Geoderma* **270**: 33-42.

Srivastava, S., Mishra, S., Tripathi, R.D., Dwivedi, S., Trivedi, P.K. and Tandon, P.K. 2007. Phytochelatins and antioxidant systems respond differentially during arsenite and arsenate stress in *Hydrilla verticillata* (L.f.) Royle. *Environmental Science and Technology* **41**: 2930-2936.

Srivastava, S., Pathare, V., Sounderajan, S. and Suprasanna, P. 2019. Nitrogen supply influences arsenic accumulation and stress responses of rice (*Oryza sativa* L.) seedlings. *Journal of Hazardous Materials* **367**: 599-606.

Srivastava, S., Srivastava, A.K., Suprasanna, P. and D'Souza, S.F. 2009. Comparative biochemical and transcriptional profiling of two contrasting varieties of *Brassica juncea* L. in response to arsenic exposure reveals mechanisms of stress perception and tolerance. *Journal of Experimental Botany* **60**: 3419-3431.

Srivastava, S., Suprasanna, P. and D'Souza, S.F. 2011. Redox state and energetic equilibrium determine the magnitude of stress in *Hydrilla verticillata* upon exposure to arsenate. *Protoplasma* **248**: 805-815.

Steinite, I., Gailite, A. and Iveinsh, G. 2004. Reactive oxygen and ethylene are involved in the regulation of regurgitantinduced responses in bean plants. *Journal of Plant Physiology* **161**: 191-196.

Yamamoto, A., Bhuiyan, N.H., Waditee, R., Tanaka, Y., Esaka, M., Oba, K., Jagendorf, A.T. and Takabe, T. 2005. Suppressed expression of the apoplastic ascorbate oxidase gene increases salt tolerance in tobacco and Arabidopsis plants. *Journal of Experimental Botany* **56**: 1785-1796.

Yang, Y., Xu, S., An, L. and Chen, N. 2007. NADPH oxidase-dependent hydrogen peroxideproduction, induced by salinity stress, may beinvolved in the regulation of total calcium in roots of wheat. *Journal of Plant Physiology* **164**: 1429-1435.

Ye, Y., Li, P., Xu, T., Zeng, L., Cheng, D. and Yang, M. 2017. *OsPT4* contributes to arsenate uptake and transport in rice. *Frontiers in Plant Science* **8**: 2197.

Zheng, M.-Z., Cai, C., Hu, Y., Sun, G.-X., Williams, P.N. and Cui, H.-J. 2011. Spatial distribution of arsenic and temporal variation of its concentration in rice. *New Phytologist* **189**: 200-209.