Plant Archives Vol. 15 No. 1, 2015 pp. 479-487 *ISSN 0972-5210*

BIOCHEMICAL AND MOLECULAR ANALYSIS IN INDIAN MUSTARD VARIETY UNDER ARSENATE STRESS

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Abstract

Arsenic (As) is one of the most toxic environmental pollutant and damage plants by interfering negatively on plant metabolism. The present work was carried out to examine (i) morphological changes and As accumulation in *Brassica juncea* variety Pusa Bold, which was exposed to 0, 50, 150 and 300 µM of As(V) concentration for 24 and 96h duration (ii) Role of antioxidant enzymes and other stress related indicators (iii) Transcript profiling of genes related to metal stress. The results indicated that seed germination and root-shoot length decreased with increasing concentration of As(V). Total accumulation of As increased with increasing concentration and duration, being more in roots. The As(V) treated PB variety exhibited a greater level of SOD, CAT, APX, cysteine and proline content at 150 µM, even after 96h duration, showing their active involvement in the As(V) detoxification mechanism. In addition, MDA content, indicator of stress in plants were also found to be maximum at 150 µM As(V). RT-PCR analysis showed up-regulated expression of *MT-2, GR* and *GS* gene under As(V) stress, which suggests that PB variety was able to tolerate As damages through the activation of defence mechanisms and also having a substantial capacity to accumulate As.

Key words : Arsenate, *Brassica juncea*, Pusa Bold, RT-PCR, antioxidant enzymes.

Introduction

Arsenic (As) has become a global problem due to contamination of ground water, which is the primary source of drinking water and food, pose a serious health risk to the people. High levels of As in ground-water have been detected in many countries, but Bangladesh and West Bengal (India) are the most affected country in the world (Rosen and Liu, 2009). As is an element of interest due to the toxic properties of several As compounds. The major inorganic As species found in the environment are arsenite [As(III)] and arsenate [As(V)] and organic As species are monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), trimethylarsine oxide (TMAO), arsenobetaine, arsenosugars and arsenocholine (Tangahu *et al*., 2011). The inorganic As species arsenite [As(III)] and arsenate [As(V)] are the main phytoavailable forms of As and predominantly found in soil/water (Meharg and Hartley-Whitaker, 2002). These inorganic forms are interconvertible, depending on the redox condition and may be metabolized by plants from the inorganic to organic form. As(V) is the prevalent As species present in aerobic soils under normal pH conditions (pH 4-8). It is considered as a structural analogue of phosphate, with similar electron configurations, uncouples oxidative phosphorylation, inhibits ATPase, replaces P in DNA and inhibits DNA repair mechanism (Tripathi *et al*., 2007). As (V) and inorganic phosphate (Pi) compete for uptake through the same transport systems in As hyperaccumulators, As-tolerant non-hyperaccumulators, and As-sensitive non-accumulators (Rai *et al*., 2011 and Gupta *et al*., 2009).

The sessile nature of plants needs more protection and this enabled them to evolve unique mechanisms to cope with different stress factors. However, variations do exist in tolerance mechanisms in plants. In the process plants alter their physiology, metabolic mechanisms, gene expressions and developmental activities to cope-up with the stress effects. Several mechanisms to counter As-induced stress are reported in various model plants as they vary from species to species depending on their genetic background (Wang *et al*., 2002; Raab *et al*., 2005; Tripathi *et al*., 2007 and Gupta *et al*., 2009). Furthermore, many heavy metals including As stimulate the formation of free radicals, reactive oxygen species (ROS) and malondialdehyde (MDA) content (a product of membrane lipid peroxidation and an indicator of free radical

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production). The scavenging system controlling ROS includes both enzymatic and non-enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione, ascorbate and carotenoids (Srivastava *et al*., 2009; Ahmad *et al*., 2012; Ahmad and Gupta, 2013; Ali *et al*., 2014a, 2014b). In stress conditions the free radicals may increase, which will enhance the activities of these detoxifying enzymes. Therefore, increased activities of these enzymes may be considered as typical defence components against As toxicity.

Indian mustard (*Brassica juncea*), a fast growing high biomass plant is a good accumulator of heavy metals and is considered as a potential candidate to be a model plant for phytoremediation (Chaturvedi, 2006; Ahmad and Gupta, 2013). Although, lots of work has been done in different varieties of *Brassica* under As stress, still we need to explore more varieties, which will help in the selection of As tolerant variety. It is not possible to block entirely the entry of As species into crop plants because of their shared transport systems with essential and beneficial elements. However, the adverse effect of As can be reduced by selecting different tolerant crop plant species. In order to devise new strategies to improve tolerance and to understand how plants take up and metabolize As by using different detoxification mechanism to counter the problem of food-chain contamination, the present study was conducted to see the physiological, biochemical and molecular responses in Indian mustard (*Brassica juncea*) variety Pusa Bold (PB), which is known as high yielding oil crop plant, grow in many region of north-western India.

Materials and Methods

Plant material and treatment conditions

Seeds of *B. juncea* var. PB were obtained from Indian Agriculture Research Institute (IARI), Pusa, New Delhi, India. Seeds were surface sterilized in 3% H_2O_2 and washed with distilled water prior to germination. Equal numbers of seeds (25) soaked in distilled water for 24 h in the dark were transferred to petri plates containing moist cotton bed and watered with Hoagland nutrient medium with and without As [arsenate, As(V)], prepared using salt Na_2HAsO_4. After 2 days in the dark, petri plates were transferred to a 16-h photoperiod culture room with a day/night temperature of 25±2°C. The nutrient solution was aerated continuously and replaced weekly. After 7 days, the number of germinated seedlings was counted and root–shoot length was measured using a metric scale. Fourteen-day-old plants were treated with different concentrations (50, 150 & 300 µM) of As(V) for 24- and 96-h exposure time under controlled condition as mentioned above. To prevent the oxidation/reduction process, the nutrient solution containing As was replaced every two days post-treatment. After harvesting, each plant was separated into leaf and roots, washed thoroughly with distilled water and made into two subsamples, one stored in -80°C and the other kept in an oven for drying for 72 h. Plants treated without metals served as controls.

As accumulation

Equal amounts of dried leaf and root samples (ca. 500 mg) were digested by 1 ml of concentrated HNO_3. The digestion tubes were heated at 180°C for 1 h and then 200°C to evaporate the samples to dryness. The residue was taken up in 10 ml of 10% (w/v) HCl containing 10% (w/v) KI and 5% (w/v) ascorbic acid. Total As concentration in the samples was determined on an atomic absorption spectrophotometer (AA6800, Shimadzu) coupled to a GBC hydride generation system using external calibration. Three replicates were taken for each sample and the mean values were obtained on the basis of calculation of those three replicates. A reagent blank and standard reference plant material (peach leaves, a plant standard certified by the National Institute of Standards and Technology) were included to verify accuracy and precision of the digestion procedure and subsequent analysis. The detection limit of As was 0.001 ppm.

Translocation factor (TF) was calculated using the following formula:

TF = [As] shoot / [As] root

Where, [As] shoot = As concentration of shoot (mg/kgDW), [As root] = As concentration in root (mg/kgDW).

Isolation and quantification of total chlorophyll and protein

Total chlorophyll was isolated according to Arnon (1949). Leaf tissue were homogenized in 80% ice chilled acetone using mortar and pestle and centrifuged at 10,000 g for 10 min. The absorption in the supernatant was recorded at wavelengths 663 and 645 nm. Total protein estimation was carried out following Bradford's (1976) method, with bovine serum albumin (BSA) as a standard.

Antioxidant enzyme activity (SOD, CAT and APX)

Frozen plant leaves (0.4 – 0.8 g) were homogenized in ice cold extraction buffer (pH 7.5) containing 50 mM HEPES, 0.4 mM EDTA, 5 mM $MgCl_2$, 10% glycerol, 1% PVP, 2 mM DTT and 1 mM PMSF (Gregenheimer, 1990). The homogenate was centrifuged (14,000g) at 4°C for 20 min. The supernatant was used for enzyme activity superoxide dismutase (SOD) and catalase (CAT). SOD

activity (SOD, EC 1.15.1.1) was measured by monitoring the inhibition of photo chemical reduction of nitro blue tetrazolium (NBT) according to Dhindsa *et al*. (1981). CAT activity (CAT, EC 1.11.1.6) was determined by monitoring the disappearance of H_2O_2 by the method of Aebi (1983). Ascorbate peroxidase (APX) was extracted in leaf samples (0.4 – 0.8 g) homogenizing in medium containing 100 mM Phosphate buffer (pH 7.3), 1 mM EDTA, 1% PVP and 1 mM ascorbate. The rate of H_2O_2-dependent oxidation of AA was determined in a reaction mixture containing 0.1 M Phosphate buffer (pH 7.0), 0.5 mM ascorbic acid and 100 µl enzyme extract. Activity of APX was measured according to Nakano and Asada (1981) by monitoring the rate of H_2O_2-dependent oxidation of AA followed by measuring the decrease in absorbance at 290 nm (extinction coefficient of 2.8 mM^{-1} cm^{-1}).

Stress-related parameters (cysteine, MDA and proline)

Plant leaves were homogenized in 5% chilled per chloric acid and centrifuged at 10,000g for 10 min at 4°C. Cysteine content was measured in supernatant using acid ninhydrin reagent at 560 nm according to the method of Gaitonde (1967). Malondialdehyde (MDA) content was estimated following Heath and Packer (1968) by reaction with thiobarbituric acid (TBA). The amount of MDA was calculated from the difference in absorbance at 532 and 600 nm using an extinction coefficient of 155 mM^{-1} cm^{-1}. The level of proline was measured by following Bates *et al*. (1973). Plant leaves (0.5 g) were crushed in 3% sulfosalicylic acid and centrifuged at 4,000g for 10 min. The supernatant (2 ml) was reacted with ninhydrin and acetic acid, incubated for 1 h at boiling temperature. The mixture was extracted with toluene, and the absorbance was measured at 520 nm from the organic phase. The amount of proline was calculated from a standard curve as µmol/g FW.

Total RNA extraction and semiquantitative RT-PCR analysis of selected genes

Total RNA was extracted from leaves of As treated and control 14-day-old plants using TRI reagent (Sigma-Aldrich) according to the manufacturer's protocol. Concentration and quality of RNA was verified by spectrophotometry and on a 1% agarose gel, three bands corresponding to ribosomal RNA (28s, 18s and 5s) were apparent. Total RNA was pre treated with RNAse-free DNAse (Ambion) before using for semiquantitative reverse transcription-polymerase chain reaction (RT-PCR). A First-strand cDNA synthesis was carried out with 5 µg of total RNA using oligo dT primer (Promega, USA) and PowerScript reverse transcriptase™ (BD Biosciences, USA) following the manufacturer's instruction and used as template for polymerase chain reactions. Independent polymerase chain reaction (PCR) reactions with equal amounts of cDNA were performed using primers of different genes. PCR conditions used were initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 15 s, annealing at 54°C for 15 s and extension at 72°C for 1 min, with final extension at 72°C for 10 min. The PCR product after 30 cycles was selected to check the differential expression of the transcripts. The identity of each RT-PCR product was confirmed by direct sequencing: PCR products were resolved on a 1.2% (w/v) agarose gel for size verification, purified using MinElute Gene Extraction kit (Qiagen, Germany) according to manufacturers protocol, and then sequenced using Big Dye terminator chemistry version 3.1 on an ABI 3730 × l DNA sequencer (Applied Biosystem, Foster City, CA USA).

Statistical analysis

Each treatment was performed in triplicates. Data were analyzed using analysis of variance (ANOVA) to evaluate whether the means were significantly different, taking $P<0.05$ as the significant level.

Results

Effect of As (V) on seed germination and root–shoot growth

The effect of As(V) was analyzed on seed germination and root-shoot growth of *B. juncea* var. PB. The percent germination decreased with increasing concentration of As(V). Similarly the decrease in root and shoot length were also observed with increasing As(V) concentration. Root/Shoot length reduced by 27%, 61%, 70.0% / 23%, 54%, 63% after treatment with for As(V).

Table 1 : Effect of As(V)on seed germination and root-shoot length of *B. juncea* var. Pusa Bold.

As(V) Conc.	PB		
	Seed germination (%)	Root length (cm)	Shoot length (cm)
C	100	2.20±0.25	2.44±0.13
50µM	68	1.61±0.08	1.89±0.08
150µM	64	0.86±0.15	1.12±0.12
300µM	60	0.66±0.11	0.90±0.24

C-control, PB- Pusa Bold.

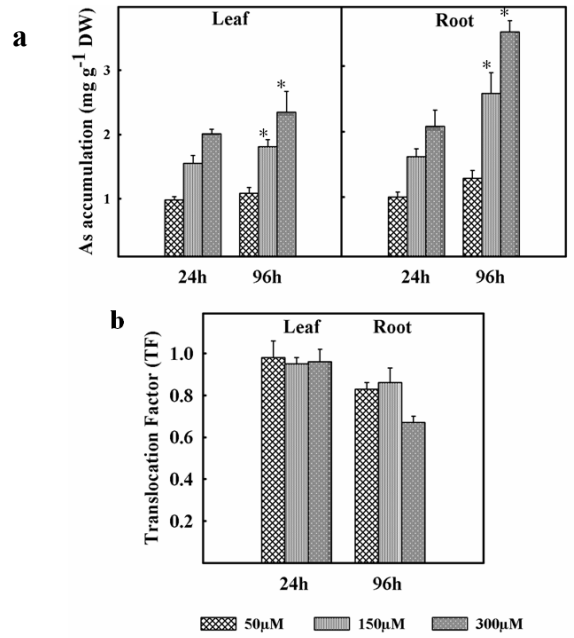

Fig. 1 : Effect of increasing As(V) exposure on As accumulation in leaf and root **(a)** and TF **(b)** of *Brassica juncea var*. PB. The data represented as an average of three independent experiments (± S.E., n = 3). An asterisk (*) represents the difference between arsenic accumulation in leaves and roots are statistically significant (P<0.05).

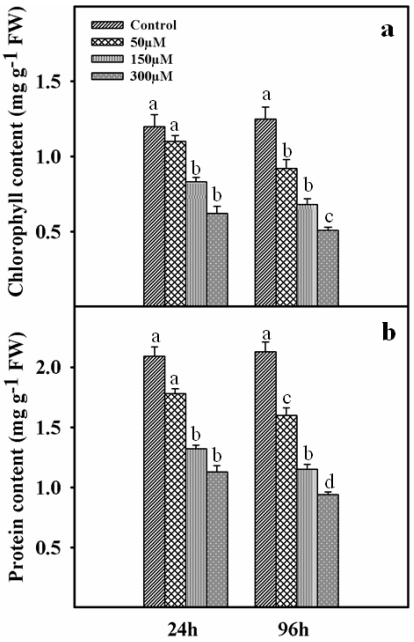

Fig. 2 : Effect of increasing As(V) concentration on chlorophyll **(a)** and protein content **(b)** in leaves of 14 days old seedlings of PB variety after 24h and 96h duration. All values are means of triplicates (±S.E., n=3). Different letters on the bars represent significant changes (P<0.05) in the treatments as compared to their control.

Accumulation of As in leaves and roots of *B. juncea*

As was found to accumulate in As(V) treated root and leaves of PB variety (fig. 1a) at 50, 150 and 300µM concentration for 24 and 96h duration. Accumulation of As increased with increasing concentration and duration, being more in roots as compared to leaves. Based on the results presented in fig. 1a, total As content observed in root of PB variety was 1.0, 1.62 and 2.08 mg/kg DW, while in leaves, it was 0.98, 1.55 and 2.01 mg/kg DW after 24h duration. Maximum accumulation was more both in leaves and roots after long (96h) duration at 300µM.

To evaluate the phytoextraction ability of PB variety, data presented in fig. 1a was used for the calculation of translocation factor (TF) from root to leaves (fig. 1b). The TF values were higher during 24h exposure period as compared to 96h, maximum being at 300µM As(V) (0.96).

Effect of As(V) on chlorophyll and protein content

Leaves of As(V) treated PB variety showed dose and duration dependent inhibition in chlorophyll and protein content (fig. 2a). Decline in chlorophyll content was observed at all the tested concentrations as compared to the controls. A significant inhibition in chlorophyll content (52%) was observed at 300µM As(V) after 96h. Similarly, decrease in protein content with increasing concentration and duration was observed in PB variety. The protein content was 55% lower at 300µM after 96h duration relative to the control (fig. 2b). Overall, the level of both total chlorophyll and protein content showed dose dependent inhibition in leaves of PB variety.

Effect of As(V) on antioxidant enzymes (SOD CAT and APX) and stress related parameters (cysteine, proline and MDA content)

Increased generation of ROS due to As treatment in plants is associated with enhanced activities of scavenging enzymes like SOD, CAT and APX. The effect of different concentration of As(V) on SOD, CAT and APX activity in leaves of *B. juncea* var. PB were observed (fig. 3 a-c). It can be seen in fig. 3a that there was no significant changes in the SOD activity at 50 µM for both durations as compared to their control. However, it increased with increasing As(V) treatment, maximum being at 150µM (275%) after 96h duration. Effects of As(V) on CAT activity are presented in fig. 3b, which displayed increasing trend at higher concentration range (150 & 300 µM). Maximum CAT activity was observed at 150µM (246%) after 96h duration as compared to their control. Similarly, highest level of stimulation in APX activity (176%) was observed at 150 µM As(V) after 96h duration (fig. 3c). Overall, the activities of these

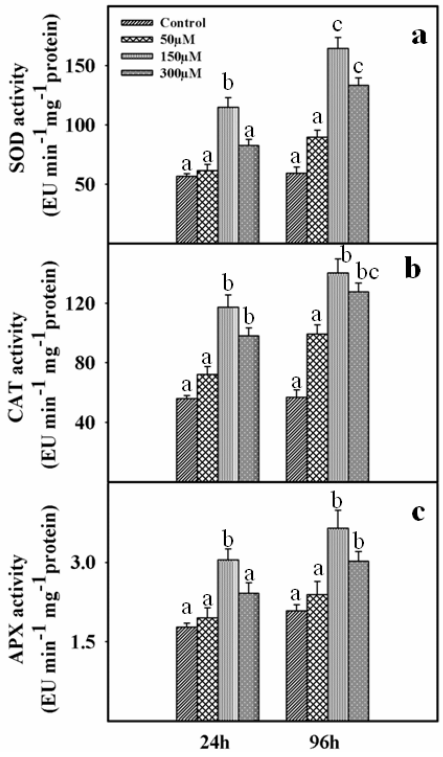

Fig. 3 : Effect of different concentration of As(V) on the activity of SOD **(a)**, CAT **(b)** and APX **(c)** in leaves of *Brassica juncea var* PB. All values are means of triplicates (±S.E.,n=3). Different letters on the bars represent significant changes (P<0.05) in the treatments as compared to their control.

enzymes were significantly stimulated at 150μM As(V) for both the durations. Prolonged exposure to higher concentration (300 μM) of As(V) resulted in a decrease in activity for each of the studied enzymes.

In order to examine further tolerance towards As(V) treatment, cysteine, proline and malondialdehyde (MDA) content were investigated as stress related parameters. All the three parameters depicted significant increase in their contents following exposure to As(V) at given durations (fig. 4 a-c).

Cysteine content increased with increasing concentration of As(V) (fig. 4a). It increased by 239% at 150μM after 96h duration, however, it decreased at 300 μM As(V), but remain higher as compared to their control. The 150μM As(V) concentration enhanced proline content by 337% after 96h duration (fig. 4b). No difference in proline content was observed at lower concentration (50μM) at 24 h duration. MDA content also increased with increasing concentration of As(V). It increased slightly when the concentration was lower, but increased maximum at 150μM after 96h duration (fig. 4c). Overall, with increase in exposure duration, the level of cysteine, proline and MDA content declined at higher

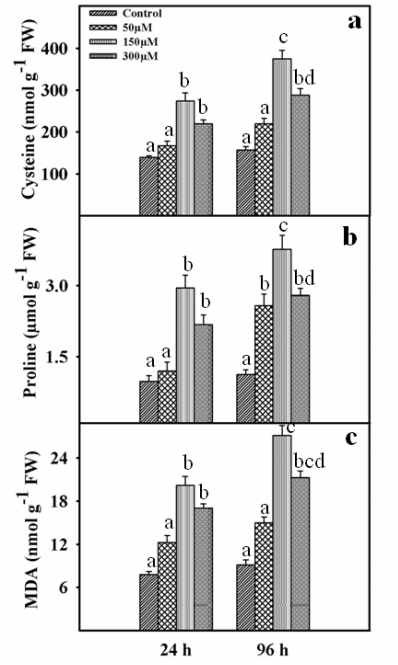

Fig. 4 : Effect of different concentration of As(V) on the content of cysteine **(a)**, proline **(b)** and MDA **(c)** in leaves of *Brassica juncea var* PB**.** All values are means of triplicates (± S.E., n = 3) Different letters on the bars represent significant changes (P<0.05) in the treatments as compared to their control.

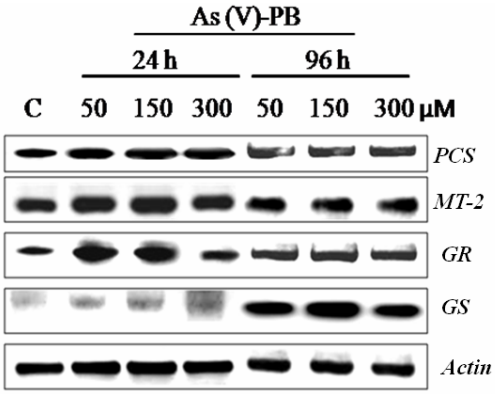

Fig. 5 : Semi-quantitative RT-PCR of *PCS, MT-2, GR* and *GS* gene. RNA was isolated from the leaves of *Brassica juncea* (var. PB) exposed to 50, 150 and 300μM As(V) for 24h and 96h. *Actin* was used as an internal control. The experiment was repeated twice with the similar results.

concentration (300μM), but remained higher than control values at all exposures of As(V).

Effect of As(V) on gene expression analysis

Transcriptional analysis of selected genes, phytochelatins synthase (*PCS*), metallothionine-2 (*MT-2*), glutathione reductase (*GR*) and glutathione synthetase (*GS)* was performed in leaves of PB variety at 50, 150 and 300μM As(V) for 24 and 96h period (fig. 5). The

expression level of various transcripts was recorded in comparison to their controls. Up-regulation of *MT2* and *GR* gene was maximum at 150µM after 24h and *GS* was more at 150µM As(V) after 96h as compared to their controls. However, no significant up or down regulation was observed for *PCS* gene at all the tested concentration and duration of As(V) as compared to their controls.

Discussion

As is known to affect vegetative response such as germination, root/shoot ratio etc. which are considered as one of the best indicator of metal toxicity (Abedin and Meharg, 2002). In the present study, seeds of PB variety was exposed to different As(V) concentration for 7 days to study the preliminary response of As treated plant. Results showed inhibition in seed germination and root/shoot ratio at given As concentration, though inhibition was more at higher concentration. Similar kind of study in response to As exposure in plants including mustard has been reported by a number of investigators (Hong *et al*., 2011; Ahmad *et al*., 2012; Ahmad and Gupta, 2013). Abedin and Meharg (2002) reported that germination and early seedling growth of rice decreased significantly with increasing concentrations of As. The inhibition was stronger in root than in the shoot is due to the fact that plant roots were the first point of contact for As in the nutrient media (Wang *et al*., 2002).

Accumulation of total As in both root and leaves increased with increasing As(V) concentration and followed the trend, root>leaves. More accumulation was observed at higher concentration and duration. Results of the present study showed that As accumulation was more in roots, but for 24h exposure period there was no significant difference between leaves and root As accumulation at all the concentration. In this study, we also observed that exposure period is an important factor for As accumulation as the uptake pathways, storage forms, influence of phosphorus uptake and also due to the involvement of transporter or the long distance translocation of metal from root to shoot (Lombi *et al*., 2002; Wang *et al*., 2002 and Duman *et al*., 2010). In general, As translocation has been found to be low in most of the plants except few hyper-accumulators (Meharg and Hartley-Whitaker, 2002). Metal accumulating plants have the ability to take up metals effectively into their tissues. Translocation factor shows the efficiency of plant to transport metal from root to shoot or leaves. Our results showed more TF in at higher concentration of As(V). This may be due to the ability of plant to make As-PC complex, which also influence the TF for different concentration and exposure time (Raab *et al*., 2007). Hong *et al*. (2011) reported 1.9 TF value for As(V) treated *Cucumis sativus* in hydroponic culture medium. More accumulation of As in roots shows the most prevalent mechanism of tolerance in plants (Burlo *et al*., 1999).

Arsenate-dose dependent inhibition was observed for both chlorophyll and protein content in leaves of PB variety. At longer duration (96h) decrease in chlorophyll content is a sign of absence of adaptive adjustments of pigment synthesis to high As levels. Previous studies also showed that decrease in chlorophyll content may be due to the inhibition of chlorophyll biosynthesis brought about by As mediated inhibition of δ-amino levulinic acid dehydratase reported by Srivastava *et al*. (2009), Ahmad and Gupta (2013) or may also be due to the per-oxidative breakdown of pigment and chloroplast membrane lipids by ROS. Total protein content in organisms is an important indicator of reversible and irreversible metabolic changes. The protein content declined with increasing concentration of As(V), however, this decrease was not significant at lower As(V) concentration (50µM) for shorter duration (24h). These findings are similar with the previous studies that increase/decrease might be due to induction of stress proteins/adverse effects of ROS or increase in protease activity (Palma *et al*., 2002).

Plants have a defense mechanism to cope up with the damage caused by ROS. A comparison of the oxidative damage caused by As(V) was examined by measuring the activities of SOD, CAT and APX. Results showed J-shaped concentration response trend, where the activity of all the enzymes increased with As concentration up to a maximum threshold. A similar finding with As was also observed in number of plants treated with As (Srivastava *et al*., 2007; Gupta *et al*., 2009; Ahmad *et al*., 2012; Ahmad and Gupta, 2013). Superoxide dismutase is known as a prominent biomarker of defense against oxidative stress in plants. The increase in SOD might protect PB variety from oxidative injury under As stress possible explanation for the decrease in SOD activity at higher concentration may be due to the production of H_2O_2 in different cellular compartments or unspecific enzyme degradation. Hydrogen peroxide, product of SOD is toxic to plant cell and further detoxified by CAT and APX, which broke H_2O_2 into H_2O and O_2. Overall data showed that CAT and APX activities also increase/decrease in a similar manner as we observed for SOD. Increase is attributed to the substrate increase of CAT and APX and decrease may be caused by inactivation or degradation by peroxisomal protease (Cakmak, 2000 and Duman *et al*., 2010).

Cysteine is a part of metal binding peptides (phytochelatin), which play a major role in detoxification of metal ions (Raab *et al*., 2007a; Ahmad and Gupta, 2013). The increase observed in cysteine may be attributed to the involvement of sulfur assimilation pathway or from the stimulation of sulfate transporters involved into glutathione and PCs (Srivastava *et al*., 2009). Less amount of cysteine content at higher concentration of As(V) may be due to the use of less defense machinery to cope up at this level. Proline accumulation also followed the same trend as MDA and cysteine content. Increased proline level shows osmotic adjustment, protection by the chelation of heavy metals and scavenging hydroxyl radicals, which is disturbed in the presence of heavy metals. Our finding supports the earlier report of significant accumulation of proline in mustard varieties (Ahmad and Gupta, 2013). Oxidative stress often results in a radical chain reaction that degrades membrane lipids by peroxidation and plant cell membranes are generally considered to be primary sites of metal injury. Malondialdehyde (MDA) content reflects the degree of damage of cell membrane and considered as a final decomposition products of lipid peroxidation (Mkandawire *et al*., 2004 and Srivastava *et al*., 2007). Presented data showed increase in MDA content in As(V) treated leaves of PB variety. High levels of MDA activity might be expected to correspond with high level of antioxidative enzymes activity (Hartley-Whitaker *et al*., 2001) indicating severe membrane damage and inhibiting cell process, which could have led to the inhibition in antioxidant enzymes activity at higher As concentration. It is reported that at higher concentration of As, plants are not able to protect themselves through antioxidant enzyme system (Duman *et al*., 2010 and Mishra *et al*., 2011).

Transcript levels of *PCS*, *MT-2*, *GR* and *GS* were analyzed by semi-quantitative RT-PCR using RNA isolated from As(V) treated leaves of PB variety. Phytochelatins are produced from GSH and catalyzed by the enzyme phytochelatin synthase (*PCS*) and its up-regulation is reported in the presence of various metals (Na and Salt, 2011). Metallothionein (MT) gene also codes for a metal binding protein. Glutathione reductase (*GR*) protect the cell against oxidative damage and maintain high GSH/GSSG ratio (Foyer and Noctor, 2005) and Glutathione synthetase (*GS*) is involved in glutathione biosynthesis pathway and responsible for addition of glycine to γ-EC to form GSH (Na and Salt, 2011). All these genes plays crucial role in the detoxification of heavy metals including As via chelation and sequestration. Our results indicated As(V) stress up-regulated the expression level of *MT-2*, *GR* and *GS* genes, which suggest that these genes are probably involved in the detoxification of As(V). However, no significant changes in case of *PCS*, suggested that this gene might not be able to serve as a defense tool to combat As(V)-induced oxidative damage in PB variety. Though, these enzymes are constitutively present, but its activity depends upon different concentration of metal supplied. At the same time, it is also important to maintain glutathione and other antioxidants levels for the survival of plants. Previous work on the involvement of *MT-2*, *Hsp-70*, *GR*, *PCS* have been reported in tomato, *Brassica* and rye grass under As and Cd stress (Goupil *et al*., 2009 and Mishra *et al*., 2011).

In the present study, we observed that up-regulation or down regulation of these genes were more affected at 150μM and 300μM As(V) after 96h duration. These findings are consistent as plant showed stimulation in antioxidant enzymes (SOD, CAT and APX) and stress related parameters (cysteine, proline and MDA) up to 150μM, even after 96h exposure period. Although, the inhibitory effect on some basic parameters (chlorophyll and protein) and growth of plant affected at the given concentration for 7 days. Level of gene transcripts, antioxidative enzymes and other stress related parameters showed a combinatorial type of tolerance mechanism to provide protection against As(V) stress. Overall, previous work and our results indicate that the stable or increased expression of stress related genes may confer As(V) tolerance on PB variety of *Brassica* plant.

Conclusion

The present investigation indicate that As(V) directly or indirectly leads to the formation of ROS and PB variety of *Brassica* have ability to adapt to the As(V) induced damages through the involvement of antioxidant enzymes and other stress related parameters. The study also showed that PB variety tolerated As(V) up to 300μM, even after 96h exposure period and accumulated substantial amount of As. The existence of tolerant mechanisms by this variety and substantial capacity for As accumulation appear to have potential for remediating moderately As-contaminated soil/water. Further research is needed to determine toxic effects of As(V) and genes linked to As tolerance in *Brassica* cultivars in the field. Furthermore, this information will help in the genetic improvement of *Brassica* plant for enhanced As tolerance.

Author contributions

MG conceived idea, designed and executed experiments, analyzed data and drafted the manuscript.

MAA carried out the experiments and analyzed data. CP carried out the gene expression study and prepared the manuscript.

Acknowledgements

The authors wish to thank Ministry of Environment and Forest, New Delhi, India for financial support.

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