Physiological and Molecular Responses Underlying Differential Arsenic Tolerance in *Phaseolus vulgaris*

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Abstract:

Arsenic (As) is the most toxic elements causing major health concern worldwide due to presence in food composites. The aim of this study was to evaluate the effects of Sodium arsenate ($As^{+5)}$ on *Phaseolus vulgaris* (consumed in the world, which have high nutritional quality; they are an excellent sources of starch and protein and are fairly good sources of dietary fibre, minerals, vitamins, and polyunsaturated fatty acids).When of *Him-I* variety seed were grown on treated sand with different conc. of sodium arsenate and the effect on physiological parameter such as shoot height, root length, leaf surface area, no. of seeds germinated , time period of germination was studied, it was found that there was increase in the aforesaid parameters up to 0.1Mm conc of arsenic, which was followed by decrease with further increasing As conc. (1 & 2Mm). Effect of Arsenic on Antioxidant enzymes such as, POD, CAT, SOD showed different patterns while normal sand and sand treated leaves were studied. POX activity was increased up to 1mM although CAT, SOD activity increased significantly up to 2Mmarsenic concentration. Present study is revealed that *P. vulgaris*(*Him-I*) variety can tolerate arsenic and could be used for phytoremediation but further investigation are necessary.

Key words: Arsenic, *phaseolus vulgaris*, sodium arsenate, antioxidant enzymes.

1. Introduction:

Arsenic (As) is ubiquitous most toxic element and present in all typesof soil. The Agency for Toxic Substances and Diseases Registry (ATSDR 2011) has classified Arsenic at 1st rankin the list of heavy metals on the basis of toxicity and its posing serious health concerns in South East Asiawhere elevated concentration of As, up to 3200 mg/l in drinkingwater has been reported (McCarty et al., 2011) against the safe limit of 10 mg/l recommended by World Health Organization (WHO).South East Asia especially the Bangladeshand West Bengal in India are worst affected areas by As contamination.Arsenic contaminated water serves as principal source of Asexposure followed by food.

Arsenate (AsV) and arsenite (AsIII) are two inorganic chemicalforms of As occurring in soil. Arsenate is chemically analogous tophosphate and thus competes with it for the uptake by the plantsthrough phosphate transporters (Zhao et al., 2010b). Arsenic is non-essential element for plant growth and development and hampers the plant growth in various ways (Singhet al., 2015). Severalphysiological functions of plant are deserter for As provoked toxicity.One of common mode of toxicity of As is the induction ofoxidative stress and disturbance of redox state leading to damage tomembranes, proteins, lipids and ultimately cell death (Srivastavaet al, 2007 and Srivastava et al, 2011).The root system is theprimary site of damages when As reaches phytotoxic levels.Compared to P, translocation of As to shoots is generally low.Typical As concentrations in aerial parts are <2 mg As/kg(O'Neill, 1995), and crop damage is usually expected before As reaches concentrations which are considered critical forhuman health.

Several antioxidant enzymes such as peroxidase, catalase, superoxide dismutase, GDH, GO-GAT and metabolites are involved in the defense pathways of plants against As-induced oxidative stress and accumulation in plant body. However, As accumulation by aboveground biomass of terrestrial plants may be limited by various factors. The different uptake behaviour and biotransformation ability of plant species can lead to tolerant and non-tolerant plant species to elevate As concentrations in soil, as reviewed by Meharg and Hartley-Whitaker, 2002.

Food crops are one of the important parts of our diet, and they may contain a number of essential and toxic metals such as arsenic (Yang et al. 2011; Waqas et al. 2015) depending on growing media characteristics. Thebioaccumulation of As in crop plants is potentially hazardous to public health, and this is of great environmentalconcern because As is known to be a carcinogen and apowerful co-mutagen (Patra et al.2004, WHO, 2012) causes several diseases. Vegetables are the major source of human exposure to heavy metal and contribute about 90 % of the total metal intake, while the rest 10 % intake occurs through dermal contacts and inhalation of contaminated dust (Martorell et al. 2011; Kim et al. 2009; Ferré-Huguet et al. 2008; Khan et al. 2014). *Phaseolus vulgaris* L. consumed in the world, which have high nutritional quality; they are an excellent sources of starch and protein and are fairly good sources of dietary fibre, minerals, vitamins, and polyunsaturated fatty acids.

However,bean plants (Phaseolus vulgaris L.) demonstratedrelatively good ability to translocate As to abovegroundbiomass compared to other species such as tomato[Carbonell-Barrachina et al, 1997). Therefore, we decided to investigate the potential morphological changes in bean plants.

The current work strives to find suitability of*P vulgaris* (a plants agriculturalcrops) to study the As behaviour in plant contaminated soil through morphological parameters. *P vulgaris* belong to the As-sensitive plants with low toleranceto elevated As concentrations under classical pot experimental conditions. Thus the current study investigates the role of different antioxidant system during AsV stress.

2. Material and methods

River sand was washed and sterilized properly. 1 kg sand transferred into each pot.20 seeds per pot were grown and primary excised leaves of seedlings were used for analysis. 200mg leaves cut into small segments and floated on 1/4th strength Hoagland's solution for 24 hrs at continuous light intensity of 40wm-2 at a temperature of 26±2ºC inside BOD.

2.1 Plant Growth

Seeds of *Him-I* variety of *Phaseolus vulgaris* were purchased from Himachal Pradesh Krishi Vishva Vidyalaya, rinsed in running water for 2 min. Seeds were surface sterilized with 0.1% $HgCl_2$ for 30 sec. and then washed thoroughly with autoclaved double distil water.

2.2 Metal Treatment

Treatment with metal at two levels in order to comparison of the same with the subsequent treatment plans.

- Excised young leaves from normal acid washed sand were cut into small segments and treated with metals of desired concentrations in continuous light for 24 hr.

- Acid washed sand was treated with different concentration (0, 0.001, 0.01, 0.1, 1 and 2mM) of Sodium Arsenate for 24 hr. The pot contained 0mM $Na_2HAsO_4.7H_2O$ were served as a control. Primary leaves of the seedling were kept aside for various analytical determinations.

2.3 **MorphologicalAssay**

For this study, acid washed sand treated with different concentration of metal was used. After growth of seedlings were harvested then shoot, root and leaves were separated for measurements. Seed germination and germination time were also counted. All values counted in at least three replicates of experiment (Ling T et al, 2010; Sharma et al 2009;Shrivastava et al 2016).

2.4 Antioxidant enzyme:

Peroxidase (POD) was estimated according to Maehly, et al 1954. POD catalyses the transformation of guaiacol to tetraguaiacol (brown product). The oxidation of guaiacol was measured by the increase in absorbance at 436 nm for 1 min. Activities of **catalase** (CAT) was assayed in fresh leaf tissue extracts by using a modification of the method of Zhou , 2001 and Zhang, 1990 as mentioned in Cui and Wang, 2006 with slight modification. Catalase activity was expressed as µmol of H_2O_2 decomposed per min per gram of fresh weight (µmol/min/g FW).Activity of **superoxide dismutase (SOD**) was measured as 50% reduction of nitroblue tetrazolium (NBT) as described by Beauchamp and Fridovich (1971). For extraction of SOD enzyme, leaves were homogenized with extraction buffer (0.1 M phosphate buffer containing 0.5 mM EDTA at pH 7.5) in a pre-chilled mortar and pestle. For enzyme assay, the reaction mixture consisting of methionine (200 mM), NBT (2.25mM), EDTA (3 mM), phosphate buffer (100 mM at pH 7.8) and sodium carbonate (1.5 M) was prepared and an enzyme extract was added. After adding riboflavin (0.4 ml) placed the tubes under 15 W fluorescent lamps for 15 minutes. During the estimation, a complete reaction mixture without enzyme served as control. The reaction was terminated by switching off the light and tubes were kept in dark. The OD was measured at 560 nm and one unit of enzyme activity was taken as the amount of enzyme, which reduced the absorbance reading to 50% as compared to control.

3. Results and discussion:

Each experiment was repeated at least thrice and data presented are the average value and standard deviation value of findings. Statistical data collected from one-way ANOVA test software. (significance *$p<0.05$, **$p<0.01$).

3.1 Effect on Morphology

Bini et al. (2012) stated that high metal concentrations inplants have strong effect on plant morphology. Plants, underhigh metal stress, show clear symptoms of structural changesincluding absence of palisade structure and reduced leaf thickness. Growth inhibition is one of the important manifestations ofAs-induced toxicity in plants (Hartley – Whitaker et al, 2001;Talukdar, 2011). Significant inhibition of plant growthin the present *P vulgaris* seedlings subjected to As treatment for 7 days was in agreement with these reports. Result shows that shoot height increased up to 1mM then decreases and found highest at 0.1mM while root length also increased with As concentration then reduced at 1mM and found maximum at 0.01mM. Leaf surface area also increased up to 1mM when compared to control. Effect

of As on seed germination is more pronounced at higher concentration. percent germination at various concentrations are control 77%, 0.001mM 87%, 0.01mM 90%, 0.1mM 88%, 1mM 66%and 2mM 41%. The reduction of growth parameters were more pronounced in the roots compared to shoots. In 1mM there was only 2mM 41% germination found. Obviously, accumulation of As had more inhibitory effect on the roots of present *P. vulgaris* seedlings than on the shoots, agreeing well with earlier reports [Uchida et al. 2002; Ahmed et al. 2006; Singh et al. 2007; Talukdar 2011a,Yasmin et al, 2017) . The highest percentage of seed germination was observed in 0.01mM concentration compare to control (without treatment) then continuously decreased (Table 1).Decline in no of day of seed germination is observed at 0.01 and 0.1mM after this no of days incensed significantly. Results indicate that As induced toxicity on morphology was increased with increased concentrations. Heavy metalinduced structural changes in the plant parts were also reported in mung bean (Singh et al, 2007), pea (Rodríguez –Serrano et al, 2009) and in radish (Vitória, 2006).

Concentration of As(mM)	Shoot Height (cm)	Root Length (cm)	Leaf Surface Area (cm)	Germination No.	Growth in Days
Control	10.05± 1.28	5.95± 59.94	9.6	15.4	7.4
0.001	9.89 ± 2.48	5.67 ± 2.28	9.5	17.4	7.4
0.01	10.12 ± 2.09	7.79 ± 3.33	9.8	18	7
0.1	11.28 ± 0.98	7.78 ± 1.2	10.9	17.6	7
1	8.25 ± 1.94	5.25± 1.09	8.7	13.2	9.4
2	6.28 ± 0.98	2.15 ± 1.17	7.3	8.2	15

Table 1: Showing Effect of Arsenic on Mophology of *P. vulgaris (Him-I)*

3.2 The effect of As on antioxidants:

Antioxidants are defined as compounds that inhibit or delay the oxidation of othermolecules by inhibiting the initiation or propagation of oxidizing chain reactions. They arealso called as oxidation inhibitor [Jacob RA (1995)]. At any point of time, one antioxidant molecule canreact with single free radical and is capable to neutralize free radical(s) by donating one oftheir own electrons, ending the carbon-stealing reaction. Antioxidants prevent cell andtissue damage as they act as scavenger. A variety of components act against free radicals toneutralize them from both endogenous and exogenous origin (Vimala and Adenan, 1999).

Activity of peroxidase enzyme was increased significantly (*p<0.05, **p<0.01) up to 0.1mM when normal sand leaves were treated with Arsenic. Activity was also higher at 1mM when compared to control but it was not found significant. In sand treated leaves peroxidase activity increased significantly up to 2mM compare to its control but it was little inhibited after 0.1mM (Table 2).

Catalase is one of the most efficient antioxidant enzymes and it plays an important role in maintaining the redox homeostasis of the cell (Shaw, 1995). A concentration dependent activity of catalase was observed from 0.001 to 2mM in normal sand leaves treated with As (Table 3). It was highest and significant at 2mM. Sand treated leaves shows enhanced activity throughout but slightly inhibited at 1mM and found significant except 0.001mM.

Superoxide dismutase shows the almost same tradition like peroxidase, the activity of SOD was significant in all concentrations. Activity of enzyme in increases significantly in leaf treated up to 2mM compare to control. Activity is highly pronounced in sand treated leaves and increased over all concentrationsand found most significant among all above parameters (Table 4).

Concentration of Arsenic (mM)	Normal sand (mg enz./ml original sol/protein) Mean± SD	Sand treated with Arsenic concentrations (mg enz./ml original sol/protein) Mean± SD
Control	3.65±0.2	3.82±0.28
0.001	5.25±0.91*	5.11±0.45**
0.01	4.19±0.17*	6.21±0.24**
0.1	4.63±0.19**	6.77±0.07**
1	3.78±0.41	5.55±0.33**
2	3.35±0.35	4.45±0.26**

Table 2: Showing Effect of Arsenic on Peroxidase activity in*P. vulgaris(Him-I)*(mg enz./ml original sol/protein) Mean± SD

Concentration of Arsenic (mM)	Normal sand ((µmol/min/g FW) Mean± SD	Sand treated with Arsenicconcentrations (µmol/min/g FW) Mean± SD
Control	8.87±1.34	10.47±3.44
0.001	9.56±2.13	17.78±3.15
0.01	9.29±1	16.55±1.43*
0.1	10.55±1.02	25.99±1.92**
1	12.77±0.89**	25.57±3.9**
2	15.48±4.68	31.8±2.77**

Table 3: Showing Effect of Arsenic on Catalase activity in*P. Vulgaris (Him-I*)(µmol/min/g FW) Mean± SD

Concentration of Arsenic (mM)	Normal sand (units/g/ fresh weight) Mean± SD	Sand treated with Arsenic concentrations (units/g/ fresh weight) Mean± SD
Control	27.04±2.48	30.14±3.9
0.001	36.78±1.36**	42.81±3.37**

0.01	42.13±1.7**	42.87±2.21**
0.1	47.37±3.71**	52.36±3.76**
1	49.43±4.95**	66.18±2.57**
2	47.06±3.02**	79.01±4.77**

Table 4: Showing Effect of Arsenic on SOD activity in*P. Vulgaris (Him-I*)(units/g/ fresh weight) Mean± SD

4. Conclusion

As it explored, in this study the Arsenic stress causes morphology effect on p vulgaris at higher concentration. In conclusion, the present study revealed that the upper limit of As tolerance in p vulgaris seedlings is 0.1mM when seeds were grown in treated sand. Seedlings bio-physiological responses decreased with higher concentrations of As, suggesting its phyto-toxic effects and shows that *Him-I*variety has tolerance mechanism which helps it to grow at higher contaminated sand as well. There still plenty of unknown aspects regarding arsenic's genotoxicity, namely, the mechanistic, target and extent of its effects in plants.

At 1mM concentrations of arsenic caused increase in shoot height, root lengths, Leaf surface area of *P. vulgaris* seedlings, as compared to control. Study showing moderated determined resistance to As contamination. A coordinated increase in anti-oxidative enzyme activity was noted with an increase in As concentrations in the tissues, peroxidase increased up to 1mM, while catalase and SOD increased in up to 2mM conc. This indicates that *p vulgaris* may have a detoxification mechanism to cope with such a high concentrations of As. Thus, the direction of the plant response depends on the metal concentration and the intensity of the stress. Present study is revealed that *P. vulgaris* (*Him-I*) variety can tolerate arsenic and could be used for phytoremediation but further investigation are necessary.

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