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SHORT COMMUNICATION

Arsenite and arsenate impact the oxidative status and antioxidant responses in *Ocimum tenuiflorum* L

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Abstract Biochemical responses of *Ocimum tenuiflorum* plants were studied upon exposure to arsenite (AsIII) and arsenate (AsV) for 1 to 10 d. Plants accumulated significant amounts of As in leaves (662 μg g^{-1} dry weight; DW and 412 μg g^{-1} DW in response to 100 μM AsIII and AsV exposure, respectively after 10 d). Consequently, fresh weight and growth of plants declined in a concentration dependent manner. Further, total chlorophyll and carotenoid contents also declined while oxidative stress markers increased, particularly on longer durations. Various antioxidant enzymes and thiols (cysteine and glutathione; GSH) showed significant and variable increases upon exposure to AsV and AsIII with the response being comparatively better in response to AsV. Proline increased significantly upon exposure to both AsIII and AsV. Plants thus tolerated high As concentrations through induced antioxidant machinery.

Keywords Antioxidants · Arsenic · *Ocimum* · Oxidative stress

Introduction

Arsenic (As) is a naturally occurring ubiquitous highly toxic metalloid. Arsenate (AsV) and arsenite (AsIII) are the two main inorganic chemical forms occurring in soils (Singh et al. 2015). Arsenate is chemically analogous to phosphate and competes with it for uptake by the plants. The uptake of AsIII occurs through aquaglyceroporins of various classes due to its existence mostly as a neutral molecule (Zhao et al. 2010; Kumar et al. 2015). One of common mode of toxicity of AsV and AsIII is the induction of oxidative stress and disturbance of redox state leading to damage to membranes, proteins, and lipids and ultimately cell death (Srivastava et al. 2007, 2011). Several antioxidant enzymes and metabolites are involved in the defence pathways of plants against As-induced oxidative stress. The enzymatic antioxidants include superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), and guaiacol peroxidase (GPX), while major non-enzymatic antioxidants are proline, ascorbate, and thiols (Sharma 2012). Many of the medicinal plants have been found to accumulate significant amount of As (Tripathi et al. 2012). In our earlier study (Siddiqui et al. 2013), we compared three species of *Ocimum* (*O. tenuiflorum, O. basilicum* and *O. Gratissimum*) for As accumulation and found that these species were able to accumulate significant amounts of As in roots, stems and leaves. It was also found that As was not removed from the tissues during the process of steam distillation; essential oils was thus free from As. This study evaluated the antioxidant responses of *O. tenuiflorum* during exposure to AsV and AsIII to understand the mechanism of As tolerance.

Material and methods

Seeds of *O. tenuiflorum* L. (obtained from CSIR-CIMAP, Lucknow) were grown and seedlings were maintained as reported earlier (Siddiqui et al. 2013). After 30 d of sowing, plants having approximately same height and weight were carefully uprooted, washed and transferred to 250 ml conical

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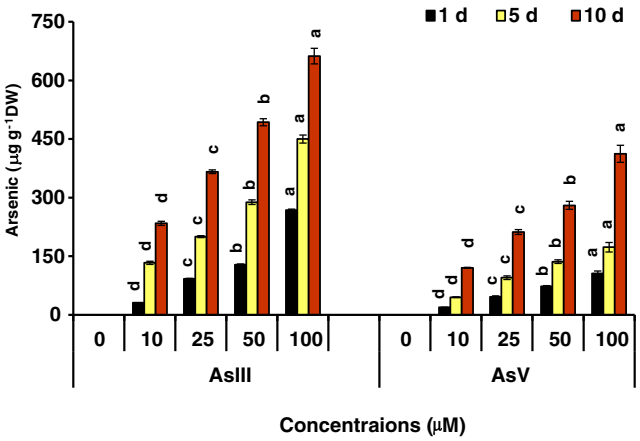

Fig. 1 Arsenic accumulation in plants of *O. tenuiflorum* after 1, 5 and 10 d of exposure to different concentrations of arsenite and arsenate. Values represent the mean of three biological replicates±S.D. Two-way ANOVA was significant at $p \leq 0.01$. Different letters indicate significantly different values for a specific duration and As species (DMRT, $p \leq 0.05$)

flasks containing 10 % Hoagland solution for one week in laboratory conditions (light:dark, 14:10 h, temperature 28± 2 °C, with 115 µmol m^{-2} s^{-1} illumination provided through fluorescent tube lights). Experiments were set up in triplicate and each replicate contained five plants. Plants were treated with different concentrations of AsV (0–100 µM; Na_2HAsO_4) and AsIII (0–100 µM; $NaAsO_2$) for 10 d. At 1, 5, and 10 d, plants were washed with double distilled water, blotted to remove water, and leaves were separated (4th leaves from the top), which were used for the study of various parameters.

Total As in the plant material was estimated after digestion of oven-dried plants (100 mg) following the protocol of Srivastava and D'Souza (2010). Photosynthetic pigments (chlorophylls and carotenoids) were estimated following the procedure of Arnon (1949) and Duxbury and Yentsch (1956). For the determination of relative water content (RWC), fresh weight, saturated weight and dry weights were measured as described by Slavik (1974). The rate of superoxide radicals ($O_2^{\cdot -}$) production, the level of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) were measured following the protocols of Chaitanya and Naithani (1994), Pick (1986), and Heath and Packer (1968), respectively as described earlier (Mishra et al. 2008a; Srivastava et al. 2011). For assay of antioxidant

Fig. 2 Effect of different concentrations of arsenate and arsenite on growth pattern of plants after 10 d of exposure

enzymes, proteins were extracted and protein contents of the supernatant was measured by the protocol of Lowry et al. (1951). The activities of SOD, APX, GR, GPX and CAT were assayed by following the protocols of Beauchamp and Fridovich (1971), Nakano and Asada (1981), Smith et al. (1988), Hemeda and Klein (1990), and Aebi (1974), respectively as detailed earlier (Mishra et al. 2008a). Proline content was estimated by following the method of Bates et al. (1973). Among thiolic compounds, cysteine and glutathione (GSH) levels were measured according to the method of Gaitonde (1967) and Hissin and Hilf (1976), respectively. Experiments were performed in a complete randomized block design involving five treatments and two durations. A two-way analysis of variance was performed to confirm the validity of the data. Duncan's Multiple Range Test (DMRT) was done to determine the significant difference between treatments.

Results and discussion

O. tenuiflorum plants showed significant As accumulation in concentration and duration dependent manner with the maximum being 662 and 412 μg g^{-1} DW in response to 100 μM AsIII and AsV exposure, respectively after 10 d (Fig. 1). The higher accumulation of As observed in plants exposed to AsIII as compared to AsV could be attributed to differential rate of As accumulation due to variable mechanisms involved in their uptake (Zhao et al. 2010). Srivastava et al. (2007) reported higher uptake of AsIII in *Hydrilla verticillata* than AsV. The significant potential of plants for As accumulation is in coherence with earlier reports of accumulation of metals viz., Cu, Cd, and Cr by *O. tenuiflorum* plants (Rai et al. 2004). Growth of the plants was affected by exposure to both AsV and AsIII (Fig. 2). Fresh weight of plants was not significantly affected after 1 d. However at 5 and 10 d, fresh weight showed a

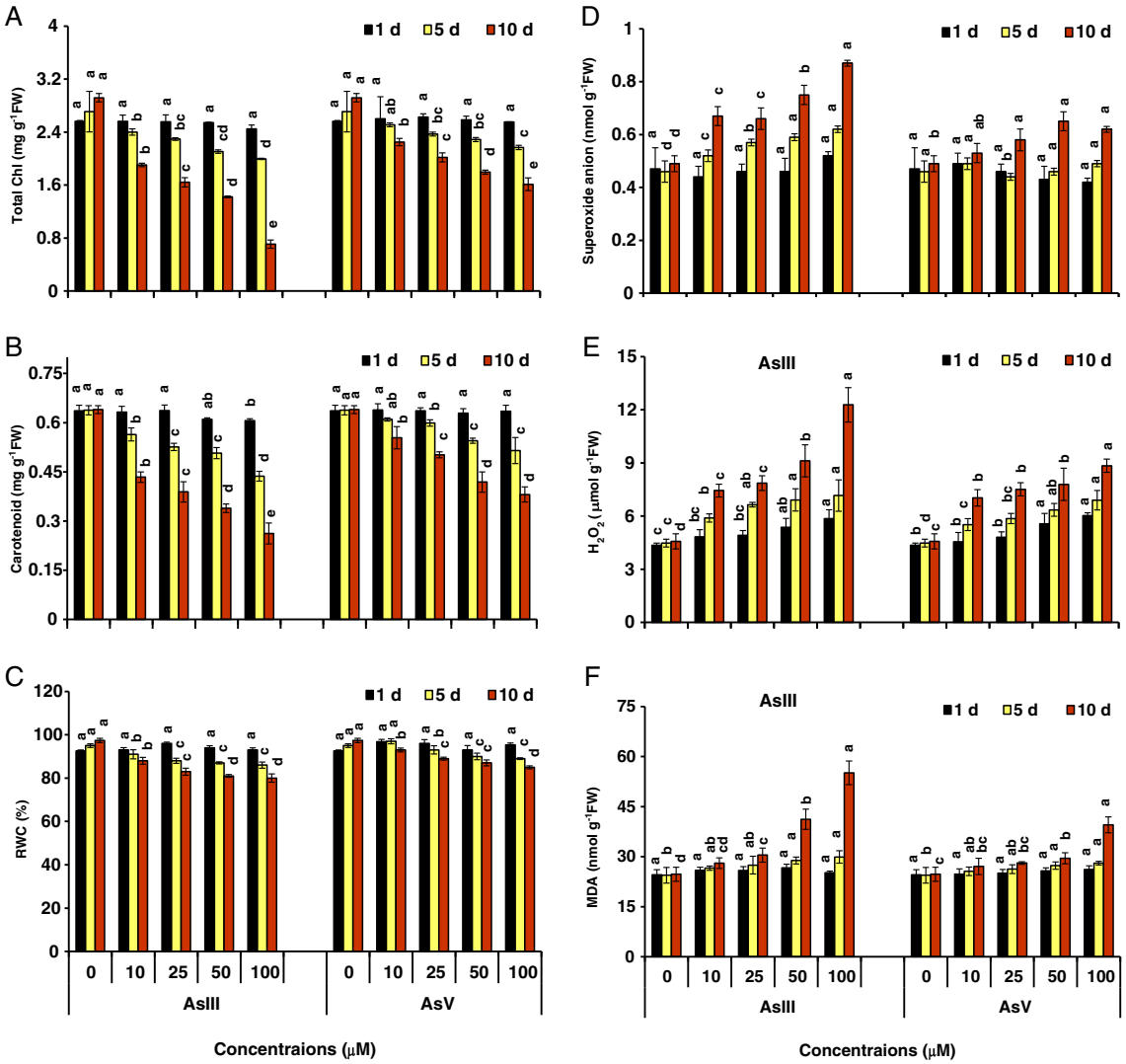

Fig. 3 Effect of different concentrations of arsenite and arsenate on total chlorophyll (**a**), carotenoid (**b**), RWC (**c**), superoxide anion (**d**), H_2O_2 (**e**) and MDA (**f**) levels in leaves of *O. tenuiflorum* after 1, 5 and 10 d. Values represent the mean of three biological replicates±S.D. Two-way ANOVA was significant at $p \leq 0.01$. Different letters indicate significantly different values for a specific duration and As species (DMRT, $p \leq 0.05$)

declining trend (Data not shown). The reduction in fresh weight by As at high concentrations might be attributed to hampered uptake of nutrients like P, Cu, Mn, Fe etc. (Srivastava et al. 2007).

The level of total chlorophyll showed some increase at 1 d, however it showed a declining trend with an increase in exposure concentration and duration (Fig. 3a). Carotenoid content also demonstrated a significant declining trend at 5 and 10 d with increase in AsIII and AsV concentrations (Fig. 3b). The reduction in photosynthetic pigments could also be attributed to reduced growth or vice versa. A slight insignificant increase in RWC was recorded at 1 d thereafter it showed declining trend. The maximum decrease in RWC was recorded at 100 μM at 10 d of treatment (13 % in AsV and 18 % in AsIII) (Fig. 3c). The decrease in RWC might be due to the As toxicity causing wilting and plasmolysis in plant cells. A disturbance to water uptake mechanisms of plants mediated through aquaporins could also have contributed to decline in RWC (Srivastava et al. 2013).

The level of $O_2^{\cdot -}$ and H_2O_2 showed gradual increase with increase in concentration and duration in both AsV and AsIII treated plants. The maximum increase in level of $O_2^{\cdot -}$ (44 and 65 %) and H_2O_2 (68 and 97 %) upon exposure to AsV and AsIII, respectively was at 100 μM at 10 d (Fig. 3d and e). MDA content also increased progressively in a concentration and duration dependent manner (Fig. 3f). Higher toxicity to plants exposed to AsIII supposedly resulted from its rapid influx in addition to its higher reactivity as has been proposed earlier (Srivastava et al. 2007). Arsenic mediated increase in the level of ROS and MDA levels has been reported earlier in various aquatic (*Hydrilla verticillata*; Srivastava et al. 2011, *Ceratophyllum demersum*; Mishra et al. 2008a) and terrestrial (*Oryza sativa*; Dave et al. 2013a) plants.

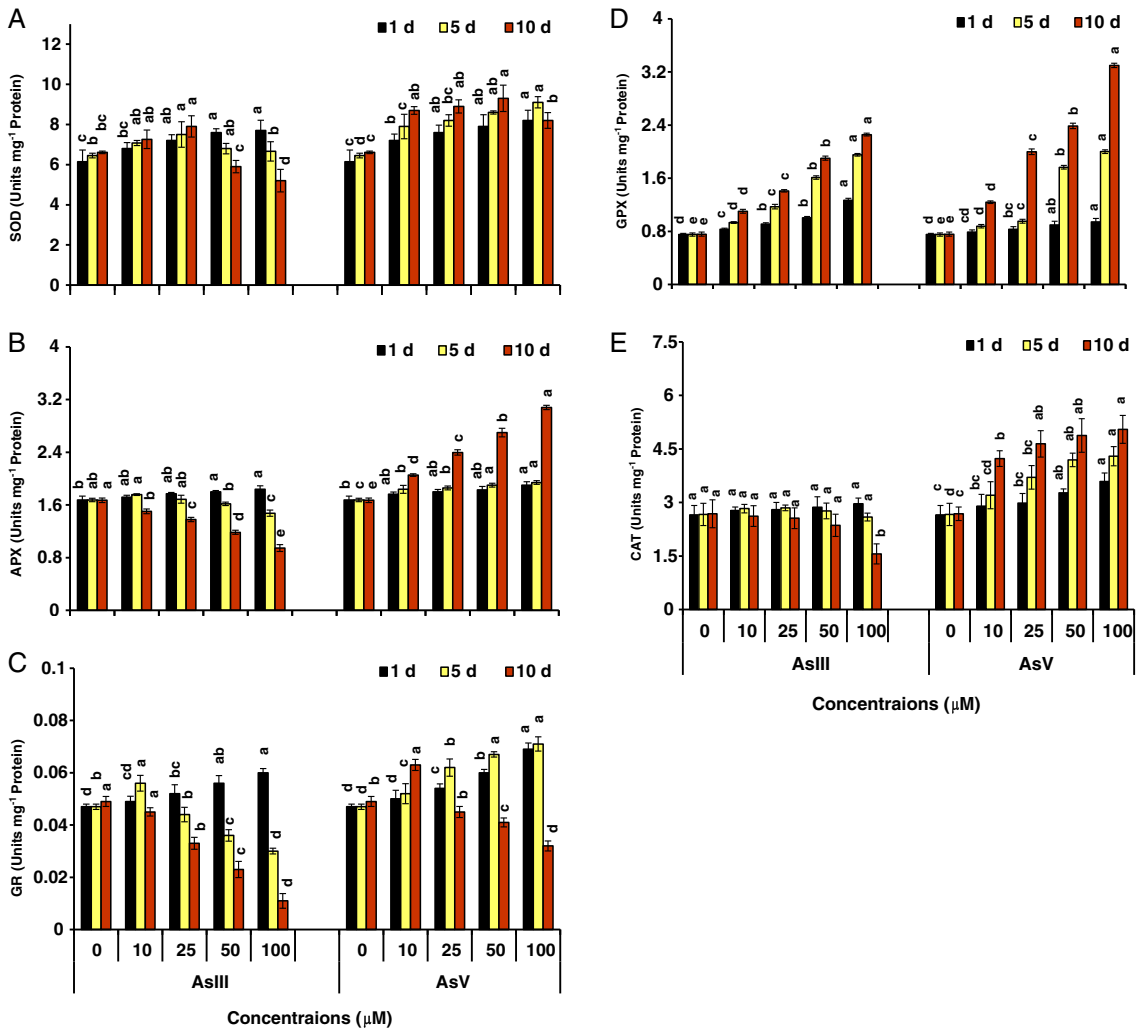

Fig. 4 Effect of different concentrations of arsenite and arsenate on the activity of SOD (**a**), APX (**b**), GR (**c**), GPX (**d**) and CAT (**e**) in leaves of *O. tenuiflorum* after 1, 5 and 10 d. Values represent the mean of three biological replicates±S.D. Two-way ANOVA was significant at $p \leq 0.01$. Different letters indicate significantly different values for a specific duration and As species (DMRT, $p \leq 0.05$)

In plants exposed to AsV, significant increase in SOD in comparison to control was noticed up to 100 μM until 10 d. In plants exposed to AsIII, SOD activity increased up to 25 μM after 10 d. Higher exposures of AsIII (50 and 100 μM) caused a decline in SOD activity at 10 d (Fig. 4a). APX activity increased at all concentrations and durations in response to AsV while it increased significantly only at 50 and 100 μM at 1 d in AsIII exposed plants (Fig. 4b). GR activity showed significant maximum increase of 56 % at 50 μM AsV after 10 d. In AsIII exposed plants, GR activity increased significantly at all concentrations after 1 d (Fig. 4c). At 10 d, GR activity declined progressively with increasing AsIII concentration. GPX activity showed concentration duration dependent increase in response to both metalloid species (Fig. 4d). In plants exposed to AsV, CAT showed increase at all concentrations and durations with the maximum being after 10 d at 100 μM (81 %). In AsIII-exposed plants, the CAT activity did not show any significant increase (Fig. 4e). Significant increases in SOD and APX along with GR would have maintained the function of the ascorbate-glutathione cycle to combat enhanced generation of ROS quite effectively in AsV-exposed plants (Srivastava et al. 2007; Dave et al. 2013a). However, in AsIII-exposed plants, ascorbate-glutathione cycle might not have been effective beyond 1 d due to imbalance among the activities of SOD, APX and GR. This was presumably responsible for observed higher increase in various ROS. Further, H_2O_2 detoxification through GPX and CAT activities was again more effective in plants exposed to AsV than AsIII. In rice plants, Dave et al. (2013a) found that AsV and AsIII induced differential responses of antioxidant enzymes owing to variable accumulation of As.

Among non-enzymatic antioxidants, proline content increased in a concentration duration dependent manner upon exposure to AsV and AsIII with the maximum increases being at 100 μM at 10 d (Fig. 5a). This appears to be an additional support to tackle oxidative and water stress in AsV-exposed plants, while an important secondary mechanism in AsIII-exposed seedlings. The increase in free proline content upon As exposure has been observed earlier in rice (Dave et al. 2013a) and *Spinacia oleracea* (Pavlik et al. 2010) plants. Proline has a well demonstrated role as an osmoprotectant, scavenger of free radicals, protector of cytoplasmic enzyme and stabilizer of membranes (Matysik et al. 2002). Cellular thiolic compounds also play important role in inducing resistance in plants against free radicals (Dave et al. 2013b). In addition, they are the most important regulator of free As concentrations in cell due to their role in As complexation (Liu et al. 2010). In this study, cysteine and GSH showed variable increases with their maximum increases being observed after 5 d in response to both AsV and AsIII (Fig. 5b and c). The synthesis and consumption of cysteine and GSH were thus altered probably due to impact of As on enzymes of thiolic metabolism (Mishra et al. 2008b; Dave et al. 2013b).

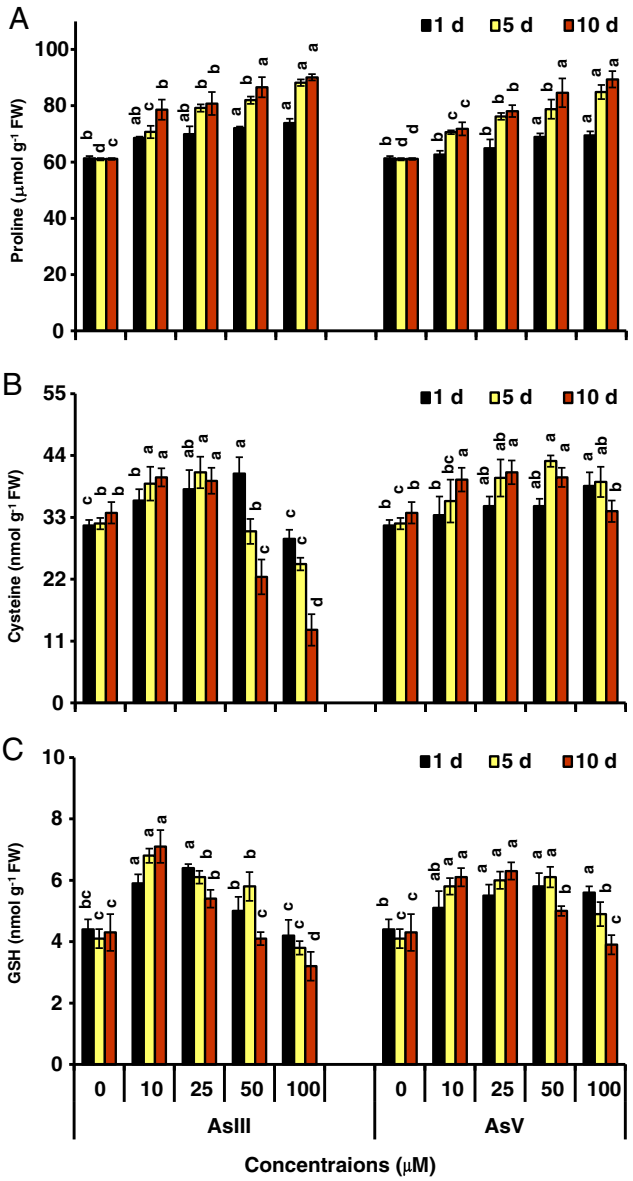

Fig. 5 Effect of different concentrations of arsenite and arsenate on the level of proline (**a**), cysteine (**b**) and GSH (**c**) in leaves of *O. tenuiflorum* after 1, 5 and 10 d. Values represent the mean of three biological replicates±S.D. Two-way ANOVA was significant at $p \leq 0.01$. Different letters indicate significantly different values for a specific duration and As species (DMRT, $p \leq 0.05$)

Thiol compounds supposedly played a role in As complexation up to a certain extent as per the capacity of the plants (Mishra et al. 2008b) beyond which most of the accumulated As remained free and caused toxicity.

In conclusion *O. tenuiflorum* plants tolerated higher concentration of AsV and AsIII than normally present in contaminated areas through the induced activity of antioxidant defence system. Significant toxicity appeared only at higher exposure concentrations of 50 and 100 μM. However, our results are laboratory based and before exploiting the results in field, a pilot field study is recommended.

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