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Effect of Arsenic on Biochemicals and Antioxidant Enzymes in Two Species of *Marchantia* L. (Marchantiophyta): Role of enzymes in stress acclimatization

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Abstract: The study compares the effect of Arsenic (As) on some physiological metabolites and anti-oxidant enzymes in two thalloid liverworts *Marchantia paleacea* Bertol. and *Marchantia papillata* subsp *grossibarba* (Stephani) Bischel, having different morphological characteristics. The plants were administered with different concentrations of As ranging from 5 ppm to 10 ppm. Accumulation of As in the plant bodies increased with As concentration in both the species. Chlorophyll a, Chlorophyll b, carotenoids and protein content were found to increase in *M. paleacea* but decreased in *M. papillata* subsp. *grossibarba*. Malondialdehyde content was observed to increase in *M. papillata* subsp. *grossibarba* and *M. paleacea* (except at 10 ppm in *M. paleacea*). The activities of superoxide dismutase and ascorbate peroxidase were observed to increase with increasing As concentrations in both the species, but the activity was greater in *M. paleacea*. The metabolite glutathione was also observed to increase with increasing As concentration in both the species. The two taxa of *Marchantia* seem to follow different strategy for As stress tolerance. *M. paleacea* strategy is of the excluder type and *M. papillata* of the accumulator type. From this study it is evident that *M. paleacea* and *M. papillata* subsp *grossibarba* were able to survive and reproduce even at a very high As concentration but *M. paleacea* is more tolerant as compared to *M. papillata* subsp *grossibarba*.

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1. Introduction

Bryophytes have been widely used as 'biomonitors' of environmental pollution (Brown DH, 1984; Carballeira et al., 2001; Nath et al., 2010a, Nath et al., 2010b; Rosa and Giuseppe, 2009; Sahu et al., 2014; Sun et al. 2009; Vincent et al., 2001). The vast diversity in their habitat makes these plants a very practical object for monitoring the pollution from metals and other xenobiotics. The uptake mechanisms of elements and nutrients in vascular plants and bryophytes are vastly different. Vascular plants meet their nutritional requirements mainly by absorbing them from the soil through their developed root system and foliar systems, whereas, bryophytes obtain their nutrition by absorbing the substances dissolved in air moisture through their thallus surface. Bryophyte has been reported to act as sink for various heavy metals, thereby accumulating metals many times the concentration found in the associated substrates (Nath et al., 2010a; Rosa and Giuseppe, 2009; Sun et al.,. 2009; Tyler, 1970). They possess a counter gradient mechanism by which they accumulate metal ions. The high metal accumulating capacity of the bryophytes can be attributed to their high surface to volume ratio and frequent absence of the cuticle (Carginale et al., 2004). The high metals accumulation capabilities of bryophytes make this group of plants an ideal candidature for phytoremediation.

Excessive accumulation of As by plant leads to stress condition (Carbonell-Barrachina et al., 1998; Sinha et al., 2010). In the presence of As, reactive oxygen species (ROS) are induced in the cytoplasm of plants (Singh et al., 2006; Srivastava et al., 2005); and the harm caused by ROS is known as oxidative stress (Alscher et al., 1997). It has been reported that As exerts its toxicity through excess production of ROS, namely superoxide ($O_2^{\bullet-}$), hydroxyl ($OH^{\bullet}$), and peroxyl ($ROO^{\bullet}$) radicals and hydrogen peroxide (Hughes, 2002; Kitchen and Ahmad, 2003). The binding of inorganic As to thiol groups of proteins and cellular non-protein thiols such as glutathione

(GSH) is another mechanism through which As imparts its toxicity (Chouchane and Snow, 2001). Under stress, plant responds by regulating the antioxidant enzymes production inside the organelles. Superoxide dismutase (SOD) is one of the early induced enzymes, and is responsible for the dismutation of the active superoxide radicals to hydrogen peroxide and water (Bowler et al., 1992). The conversion of H_2O_2 into water in peroxisomes is carried out by catalase (CAT), while that in cytosol and chloroplasts by ascorbate–glutathione cycle, which involves Ascorbate peroxidase (APX), ascorbate (AsA), reduced glutathione (GSH), and glutathione reductase (GR) (Noctor and Foyer, 1998). The APX is the catalyst in the conversion of H_2O_2 into water using AsA as substrate, while GSH and GR are involved in the regeneration of AsA. Numerous studies have been carried out on oxidative stress and defense mechanism in plants under heavy metal stress (Chaudhary and Panda, 2004; Sinha et al., 2010), but very few reports are available on the stress tolerance in bryophytes (Panda, 2003).

The objective of the present investigation is to study the: (i) accumulation of As; and (ii) induction of oxidative stress and the role of antioxidant enzymes in stress detoxification in *M. paleacea* Bertol. and *M. papillata* subsp. *grossibarba* treated with arsenite. The two species were considered because of their differences in their morphology.

2. Materials and Methods

2.1. Experimental design

Marchantia paleacea species were collected from Jeolikote, Nainital, India, and *M. papillata* subsp. *grossibarba* (Stephani) Bischel. from Moss house of National Botanical Research Institute, Lucknow. The collected samples were grown under moist conditions by spraying them regularly with distilled water in Petri dishes containing 10g garden soil, for acclimatization. The plants were kept at room temperature and in natural day-light.

M. paleacea and *M. papillata* subsp. *grossibarba* were treated twice with 5 and 10 µg/g As as Sodium arsenite ($NaAsO_2$). The first and second dose of treatment was given after 7 and 14d of acclimatization respectively. One set of experiment without As treatment was taken as the control. All experiments were carried out in four replicates. After one month (30d), plant material was taken for biochemical and enzymatic estimations.

2.2. Metal Analysis

Plant samples were thoroughly washed first in running tap water, followed by deionized water to remove any soil particles adhered to plant surfaces and oven dried at 80^0C for 24 hrs. The oven dried samples (100 mg) were digested in HNO_3:$HClO_4$ in 3:1 ratio (v/v) on a temperature-controlled digestion block and metal contents was estimated using atomic absorption spectrophotometer (GBC, Avanta Σ) (Sinam et al, 2012).

2.3. Estimation of chlorophyll, protein and lipid peroxidation

The chlorophyll content in the fresh thalli were extracted by chilled 80% acetone (v/v) and was estimated by the method of Arnon (1949) and calculated using the formula given by Macchachlan and Zalic (1963) and carotenoid content by the method of Duxbury and Yantsch (1956). The protein content in the thalli of the plant was estimated by the method of Lowry et al. (1951).

The lipid peroxidation in the thalli was measured in terms of malondialdehyde (MDA) content, determined by thiobarbituric acid (TBA) reaction following the method of Heath and Packer (1968).

2.4. Estimation of antioxidant enzymes

2.4.1. Enzyme extraction

Thalli (200 mg) were homogenized in 2 ml of 100 mM potassium phosphate buffer, pH 7.5 containing 1 mM of EDTA in presence of pinch of polyvinyl polypyrrolidone (PVP). The homogenate was centrifuged at 12000g for 15 min at 4 ºC. This supernatant was used to measure the activities of superoxide dismutase (EC 1.15.1.11), ascorbate peroxidase (EC 1.11.1.11) and guaiacol peroxidase (EC 1.11.1.7).

2.4.2. Estimation of enzymatic activities

SOD activity was measured in the thalli by the method of Nishikimi et al. (1972). APX activity was measured in the thalli by the method of Nakano and Asada (1981). GPX activity was measured in the thalli following the method of Curtis (1971), modified by Kato and Shimizu (1987). CAT activity was measured following the method of Chandlee and Scandalios (1984).

2.5. Statistical Analysis

The experiment was performed in completely randomized block design. All dataset were subjected to one way analysis of variance (ANOVA) followed by Duncan's Multiple range test (DMRT, at $p<0.05$) for multi-comparisons of mean (Gomez and Gomez, 1984). All results are means of three reading with corresponding standard deviation. Index letters indicate statistical differences between the means. Statistical analyses were performed using SPSS software (16.0).

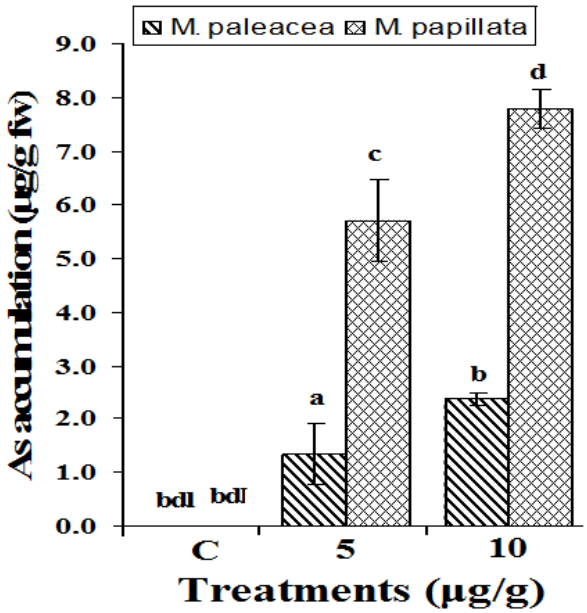

Fig. 1. Accumulation of As in the thalli of *M. paleacea* and *M. papillata* subsp *grossibarba* after 30d of exposure. All the values are means of four replicates. Values marked with similar alphabets are not significantly (DMRT, p<0.05) different.

3. Results and Discussion

3.1. Effect on As accumulation

The sensitivity of bryophytes to heavy metals has been reported in numerous earlier investigations (Chaudhury and Panda, 2004, Sahu et al., 2010). Arsenic accumulation was increased with increasing concentration in both the species after the treatment with different levels of As (Fig. 1). A significant (p<0.05) 3.5 folds (appox.) higher accumulation of As was observed in *M. papillata* subsp. *grossibarba* in comparison to *M. paleacea* at all the treatments levels. The average bioaccumulation factor (Biomass concentration/Soil concentration) for *M. paleacea* and *M. papillata* subsp. *grossibarba* was 0.25 and 0.96 respectively. Bryophytes are known to accumulate high As in its biomass as compared to other group of plants (Carginale et al., 2004; Koch et al., 2000), the uptake of As in the thalloid liverworts may have been caused by high surface area as well as high cation exchange capacity (Tyler, 1990). The higher As accumulation observed in *M. papillata* subsp. *grossibarba* may be attributed to the open type of pores present in its thallus which facilitates in metal/nutrient uptake; in contrast to the cruciate type of pore present in *M. paleacea*, which have less nutrient exchange capacity.

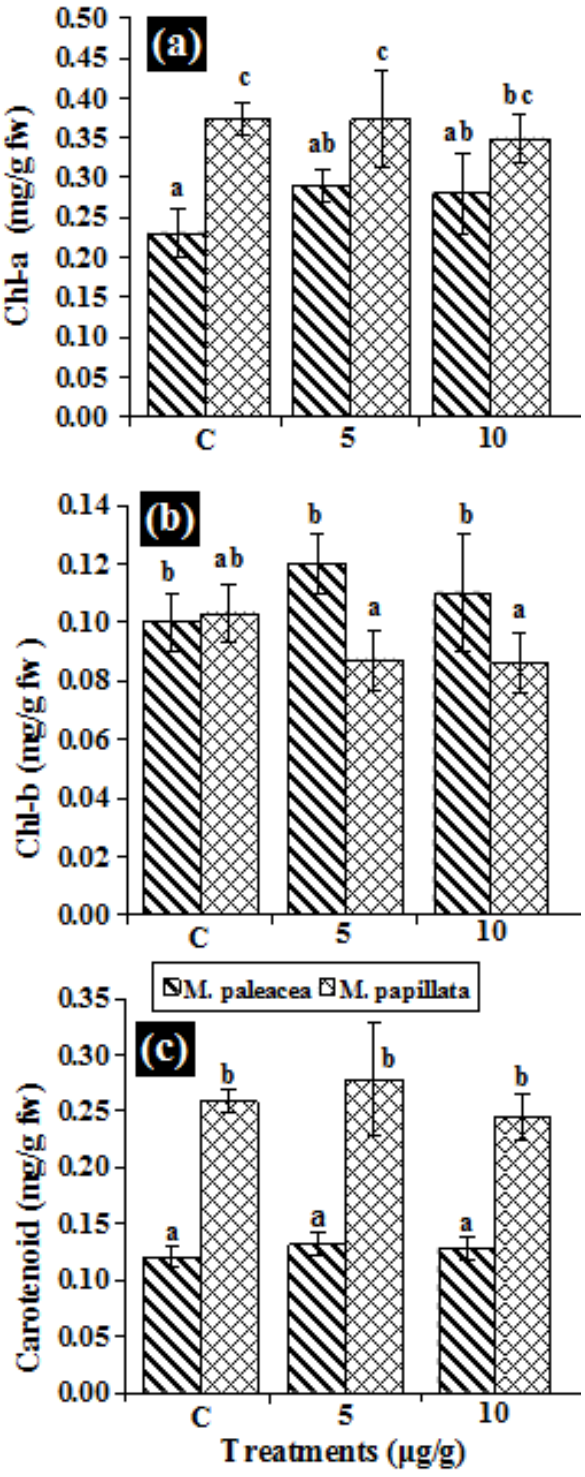

Fig. 2. Effect on chlorophyll-a (a), chlorophyll-b (b) and carotenoid (c) contents in the thalli of *M. paleacea* and *M. papillata* subsp *grossibarba* after 30d. All the values are means of four replicates. Values marked with similar alphabets are not significantly (DMRT, p<0.05) different.

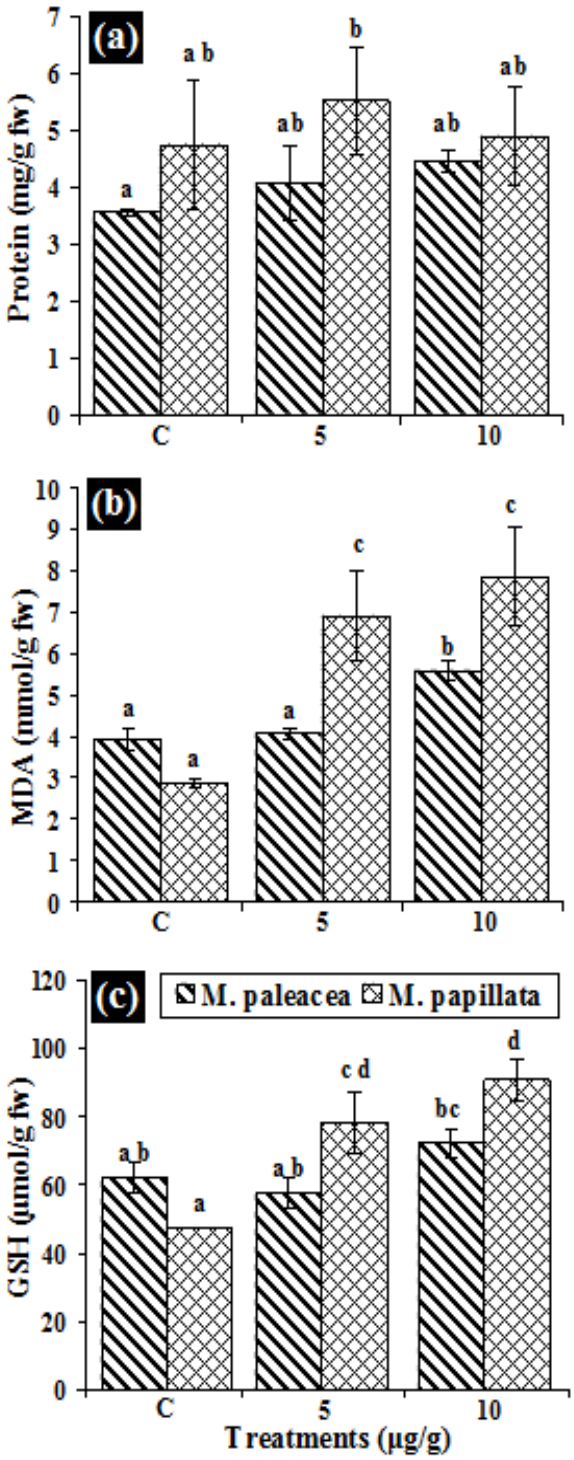

Fig. 3. Effect Arsenite on protein (a), MDA (b), and GSH (c) contents in the thalli of *M. paleacea* and *M. papillata* subsp *grossibarba* after 30d. All the values are means of four replicates. Values marked with similar alphabets are not significantly (DMRT, $p<0.05$) different.

Carotenoid content (Fig. 2c) was observed to increase non-significantly ($p<0.05$) with increasing As dose in *M. paleacea*, and also in *M. papillata* subsp. *grossibarba*, except at 5 µg/g As treatment. The effect of As were not so detrimental on the photosynthetic pigments as evidenced from its morphological appearance during the study.

3.3. Effect on protein, MDA and GSH content

Protein content (Fig. 3a) was observed to increase non-significant ($p<0.05$) in both the plant of *M. paleacea* and *M. papillata* subsp. *grossibarba* as compared to their respective control. Arsenic is reported to stimulate plant growth at low concentration (Carbonell-Barrachina et al. 1997). The reason for this enhancement is still unknown; it has been suggested that the growth benefit in plants at low As concentration may be due to stimulation of Pi uptake (Tu and Ma, 2003).

Malondialaldehyde (MDA) is cytotoxic product of lipid peroxidation and an indicator of free radical production in plant cell due to metal stress (Chun-Xi et al., 2007; Sinam et al., 2011). The formation of MDA was considered as a measure of lipid peroxidation, which indicated the oxidative stress in the liverwort induced by As. MDA content (Fig. 3b) was found to increase non significantly ($p< 0.05$) at lower dose and significantly by 42 % at higher dose of As in *M. paleacea*. Whereas, significant ($p<0.05$) increase of 141 and 173 % was observed in *M. papillata* subsp. *grossibarba* at both the treatment respectively, as compared to its control. In this study, the increase of MDA content in thalloid liverworts could be considered as an apparent reflection of mild oxidative damage caused by As stress, which are known to induce the production of H_2O_2 and O_2^- (Srivastava et. al., 2005).

Non enzymatic antioxidant GSH content (Fig. 3c) showed an increase with increase in concentration of As in both the species as compared to control, except for the slight decrease in *M. paleacea* at 5 ppm. At the 5 ppm As level, the increase was significant ($p< 0.05$) only in *M. papillata* subsp. *grossibarba*, however, at the highest treatment level the increase was significant (*M. paleacea* – 16% and *M. papillata* subsp. *grossibarba* – 100%) for both the plants as compared to their respective control. The increase in GSH concentration apparently renders the plants substantially more resistant to different stresses (May and Leaver, 1993; Marrs, 1996; Nocter and Foyer, 1998;). The reactive cysteine residue in GSH is able to keep thiol group containing proteins in their native state during stress condition.

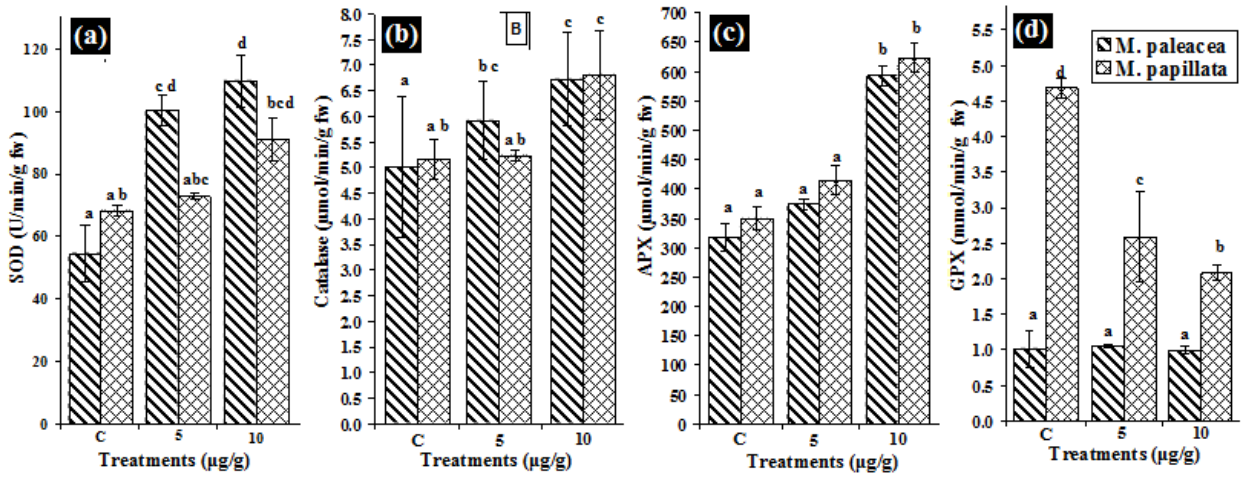

Fig. 4. Effect of Arsenite on the SOD (a), Catalase (b), APX (c) and GPX (d) activities in the thalli of *M. paleacea* and *M. papillata* subsp *grossibarba* after 30d. All the values are means of three replicates. Values marked with similar alphabets are not significantly (DMRT, p<0.05) different.

3.4. Effect on Arsenic on activity of SOD, Catalase, APX and GPX

The activity of SOD (Fig. 4a) was observed to increase significantly (p<0.05) by 84 and 101% in *M. paleacea* and non significantly (p<0.05) by 7 and 33% in *M. papillata* subsp. *grossibarba* as compared to their respective control when treated with 5 and 10 µg/g As respectively. SOD is a key enzyme in protecting the cell against oxidative stress. It is responsible for the degradation of superoxide radicals and for the subsequent production of H_2O_2 and O_2. The activity of CAT (Fig. 4b) was also observed to increased significantly (p<0.05) by 18 and 34% with increasing As dose in *M. paleacea*. However, in *M. papillata* subsp. *grossibarba* the activity was observed significant (32%) only at the highest As concentration. In both the *Marchantia* species the activity of APX (Fig. 4c) was observed significant (p<0.05) only at the highest in concentration of As (*M. paleacea* – 87%, *M. papillata* - 78%). The activity of GPX (Fig. 4d) in *M. paleacea* did not reveal any significant changes; However, in *M. papillata* the activity was observed to decreased significantly (p<0.05) by 45 and 56% at 5 and 10 µg/g As respectively. An increase in SOD and CAT activity with a simultaneous decrease in GPX activities in both the species was found when treated with As. The increase in SOD activity indicated the plant's better ability to degrade the superoxide radical which in this case was exhibited by *M. paleacea*. Decrease in GPX activity by heavy metal ions could result from the attack caused by metal ion-induced reactive oxygen species (ROS), which may be possible in case of non-redox metals causing elevated lipid peroxidation, indirectly resulting in free radical production (Verma and Dubey, 2003).

4. Conclusion

In the present study, both the species of *Marchantia* did not show any morphological changes and physical injury due to As stress. Both the species were able to accumulate relatively high concentration of As in its biomass without affecting the growth. The defense strategies of the plants from As induced stress were identical but more efficient in *M. paleacea*, as evident from the effect on protein, MDA content and more efficient antioxidant enzymes activities. The two variety of *Marchantia* seems to follow different strategy for stress tolerance from As; *M. paleacea* strategy is of the excluder type and *M. papillata* of the accumulator type. From this study it can be concluded that *M. paleacea* and *M. papillata* subsp *grossibarba* were able to survive and reproduce even at a very high As concentration and *M. paleacea* is more tolerant to As as compared to *M. papillata* subsp *grossibarba*.

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Competing Interests: The authors declare that there is no potential conflict of interest.

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