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Arsenic induced physiological changes in improved varieties of rice grown in Malwa region of Punjab

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ABSTRACT

Rice is a major staple food throughout the world. However metal contamination of rice has been recognized as a new disaster on large scale. Two rice cultivars viz PR 116 and PR 118 were grown in vitro to study the effect of increasing arsenic concentration (50, 100, 250 and 500 μM) on germination, root and shoot length, biomass dry weight, chlorophyll content and per cent cellular respiration. Results showed reduction in all these parameters thereby proving the toxic nature of arsenic. Arsenic accumulation in roots and shoots of both the varieties was monitored by atomic absorption spectrophotometer (AAS) and it was concluded that arsenic is translocated to above ground tissues and ultimately reaches the grain. The status of arsenic concentration of rice and its affect on human health need to be further investigated.

Keywords: Arsenic; rice; concentration; toxic

INTRODUCTION

Arsenic concentration in earth crust is about 3 mg/kg and 1-8 $\mu\text{g/l}$ in sea water (Cullen and Reimer 1989, Smedley and Kinniburgh 2002). Arsenic is present both in inorganic as well as organic form. However organic form is less toxic as compared to inorganic form. Arsenate (+5) is the main form present under aerobic conditions whereas under anaerobic conditions arsenite (+3) is commonly found (Smith et al 1998). Toxicity and solubility of arsenite is more than arsenate (Oremland and Stolz 2003). Human population gets

exposed to this heavy metal by ingestion of plants produced in the contaminated areas (Dudka and Miller 1999). Crop cultivation for human or livestock consumption can lead to the uptake and accumulation of this metal in edible plant parts with a resulting risk to human and animal health (Gupta and Gupta 1998, Lim et al 2008). Toxicity symptoms of arsenic on plants include decrease in plant height, reduction in leaf area and photosynthesis, wilting and necrosis of leaf blades (Carbonell-Barrachina et al 1999). Average concentration of arsenic in rice has been reported to be about 0.5 mg/kg

(Zhao et al 2010) and per day consumption of rice is 200 g (Zhu et al 2008). As a result the amount of ingested arsenic exceeds the daily limit which has been set for drinking water.

Arsenic in low concentrations induces stimulatory effect on the plant growth while at higher concentrations exerts negative effect on the plant growth which includes reduction in grain yield, grain weight and root biomass (Panauallah et al 2009).

Thus the present study was conducted to find out the effect of different concentrations of arsenic on germination, seedling growth of PR 116 and PR 118 varieties of rice in terms of per cent germination, shoot length, root length and biomass dry weight, arsenic accumulation in roots and shoots of the two varieties, total chlorophyll content and per cent cellular respiratory ability.

MATERIAL and METHODS

Germination studies: Rice seeds of cultivars PR 116 and PR 118 procured from Punjab Agriculture University, Ludhiana were grown in vitro in plastic trays lined with filter paper and adsorbent cotton. Seeds were sterilized with 5 per cent sodium hypochlorite solution and rinsed with

distilled water thrice. 60 seeds were placed in each tray and treated with different concentrations of arsenate (50, 100, 250 and 500 μ M). Trays were incubated at 35°C under dry and hygienic conditions. Germinated seeds were counted after 7 days (Abedin and Meharg 2002). Germination was considered to be complete after the emergence of plumule and radicle. Root and shoot length was measured using metric scale. Biomass dry weight was estimated by drying the roots and shoots at 60°C for 48 hours.

Determination of arsenic accumulation in plant material by atomic absorption spectrophotometer (AAS): Plant material (100 mg) was digested in concentrated HNO_3 at 70°C and was preserved and analysed by graphite furnace atomization (GFA) method. Accumulated arsenic in roots and shoots was measured by standard graph of arsenic.

Chlorophyll estimation (Hiscox and Israeltam 1979): Shoots (100 mg) were taken in test tube and 4 ml of DMSO reagent was added to it. The mixture was kept in a water bath for 1 hour. Absorbance was taken at the wavelength of 645 nm and 663 nm. Blank was prepared using DMSO. Total chlorophyll content was calculated according to the method of Arnon (1949) modified by Rani and Kohli (1991).

$$\text{Total chlorophyll content } (\mu\text{g/ml}) = 6.45 \times A_{663} + 17.72 \times A_{645} \dots\dots A$$

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$$\text{Total chlorophyll content } (\mu\text{g/ml}) = \frac{A \times \text{volume of DMSO (ml)}}{\text{Dry weight of tissue}}$$

Per cent cellular respiration (Steponkus and Lenphear 1967): Leaf tissue (100 mg) of test plant material was dipped in 1.5 ml of freshly prepared triphenyl tetrazolium chloride (TTC) solution. Sample was incubated at room temperature in dark for 18 hours. After incubation TTC solution was drained off from test tubes which were rinsed with distilled water twice. Extraction of samples was carried out with 5 ml of absolute alcohol and the extracted samples were kept in water bath for 20 minutes. Absorbance was recorded at 530 nm and expressed in terms of dry weight equivalent. The cellular respiratory ability was expressed as per cent with respect to control.

RESULTS

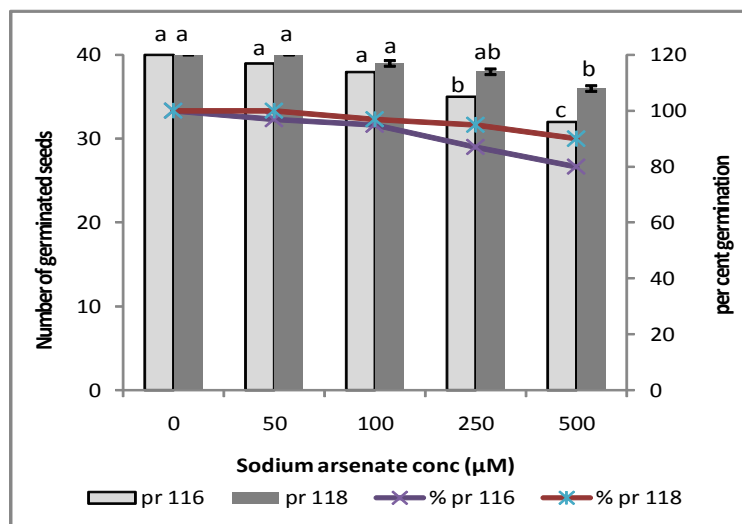
Germination: Data were analyzed by one way ANOVA post hoc multiple comparison Tukey's test (Fig 1). 100% germination was reported in control. Arsenic exposure did not affect the seeds of improved varieties (PR 116 and PR 118) of rice to a greater extent. Decline in per cent germination for PR 116 was 3, 5, 13 and 20 at 50, 100, 250 and 500 μM arsenic treatment respectively over the control. However no decline in germination was observed up to 50 μM concentration. Thereafter 3, 5 and 10 per cent decline was reported at 100-

500 μM concentration. It shows that heavy metal exposure had negligible effect on improved varieties of rice.

Shoot length: Data were analyzed by one way ANOVA post hoc multiple comparison Tukey's test (Fig 2). Gradual decrease in shoot length was observed at higher concentrations (250 and 500 μM). However lower concentrations had no significant effect on rice seedlings of both the varieties. Decline in shoot length for PR 116 was 3, 13, 18 and 24 per cent whilst for PR 118 it was 0.5, 9, 16 and 25 per cent at 50, 100, 250 and 500 μM respectively over the control.

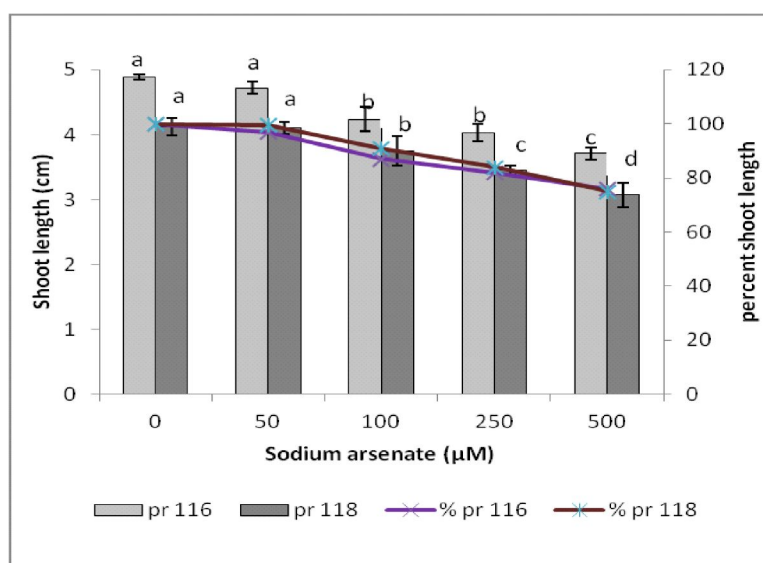
Root length: Data were analyzed by one way ANOVA post hoc multiple comparison Tukey's test (Fig 3). Significant reduction in root length was reported for both the varieties. As compared to control decline in root length for PR 116 and PR 118 ranged from 47-93 and 39-96 per cent respectively at 50-500 μM treatment.

Biomass dry weight: Data were analyzed by one way ANOVA post hoc multiple comparison Tukey's test (Fig 4). Studies reported significant effect of arsenic exposure on dry biomass in both the rice varieties. Lowest treatment (50 μM) showed 27 and 16 per cent while highest



Means followed by the same letter columns are not significantly different by Tukey's test at 0.05% level

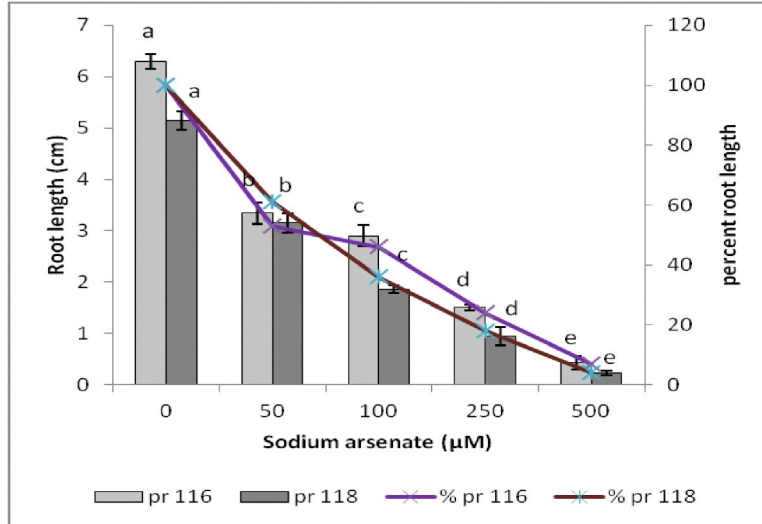
Fig 1. Germination of rice seedlings grown on different arsenate concentrations



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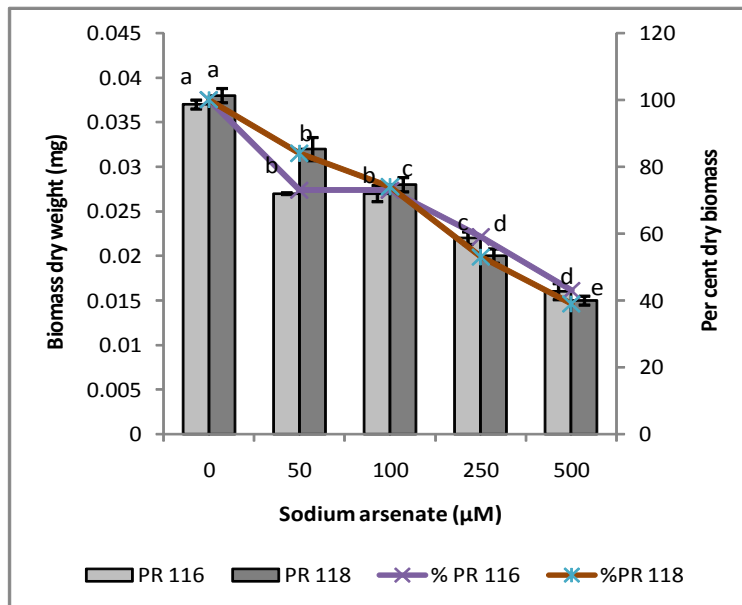
Fig 2. Shoot length of rice seedlings grown on different arsenate concentrations

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Means followed by the same letter columns are not significantly different by Tukey's test at 0.05% level

Fig 3. Root length of rice seedlings grown on different arsenate concentrations



Means followed by the same letter columns are not significantly different by Tukey's test at 0.05% level

Fig 4. Biomass (DW) of rice seedlings grown on different arsenate concentrations

treatment (500 μ M) showed 57 and 61 per cent decline in biomass for PR 116 and PR 118 respectively over the control.

Arsenic accumulation in roots and shoots: Arsenic accumulation in roots was higher than shoots. Average arsenic concentration in roots of PR 116 and PR 118 was 410.11 and 428.38 mg/kg respectively. Similarly shoots of PR 116 and PR 118 accumulated 36.06 and 30.41 mg/kg of arsenic respectively. Arsenic accumulation in above ground tissue (shoot) showed the translocation of this heavy metal into aerial parts (Fig 5).

Chlorophyll content: Data were analyzed by one way ANOVA post hoc multiple comparison Tukey's test (Fig 6). Photosynthetic pigments were inhibited in presence of arsenic. Reduction in chlorophyll content was observed in both the varieties. In PR 116 chlorophyll content decreased to 92, 80, 60 and 44 per cent at 50, 100, 250 and 500 μ M respectively over the control. Similarly decrease in chlorophyll content for PR 118 was observed to be 94, 84, 63 and 54 per cent respectively at 50-500 μ M arsenic treatment over the control.

Per cent cellular respiration: Data were analyzed by one way ANOVA post hoc multiple comparison Tukey's test (Fig 7). 100 per cent cellular respiration was reported in control of both the rice varieties. In PR 116 cellular respiration was reduced

to 84, 71, 59 and 46 per cent at 50-500 μ M arsenic treatment respectively over the control. Similar results were observed with PR 118 showing 83-39 per cent reduction at 50-500 μ M arsenic treatment as compared to control.

DISCUSSION

Growth and physiology of rice seedlings was affected upon arsenate exposure. The study was carried out to investigate the effect of heavy metal (arsenic) exposure on two improved varieties of *O sativa* L viz PR 116 and PR 118 and led to the conclusion that each parameter was affected with increased arsenic. To test metal toxicity germination is not the commonly used technique. Vegetative response such as root length, shoot height and total biomass are used to study the resistance of plants towards metal toxicity. In the present study germination of rice seedlings was not affected to a considerable extent in response to metal toxicity. Arsenic in higher concentration (500 μ M) inhibited the seed germination. The results are in accordance to the work done by previous researchers (Abedin and Meharg 2002, Shri et al 2009) in rice varieties. The results showed inhibitory effect of arsenic on root length and shoot height. Effect of arsenic was more conspicuous on roots as compared to shoots since roots are the first site of contact with arsenic. Similar results were shown by Das et al (2004) and Choudhury et al (2011)

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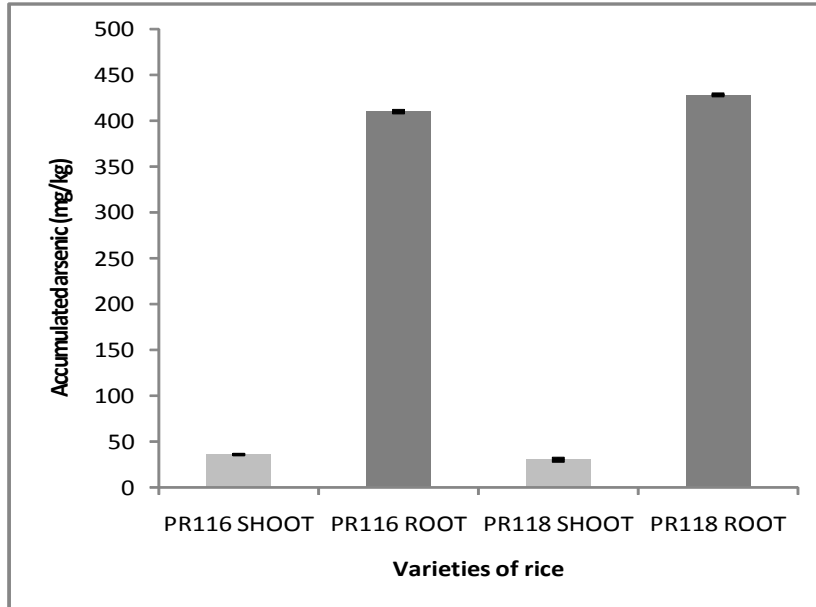
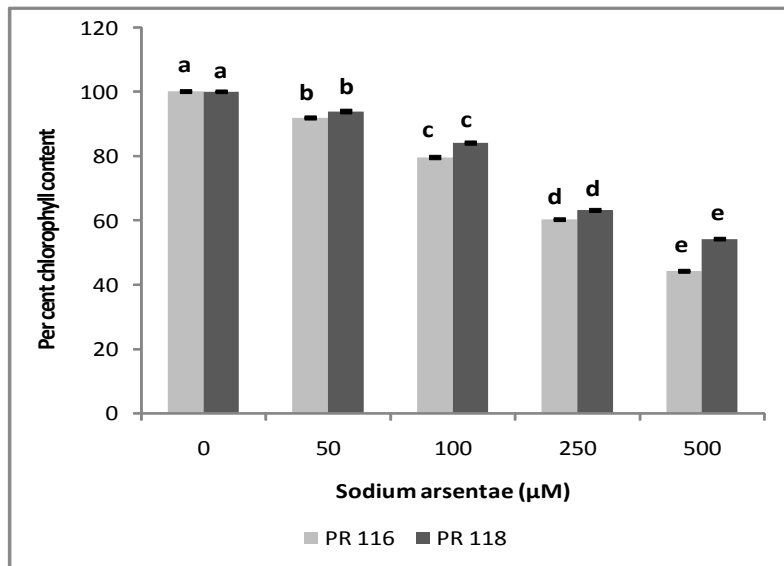
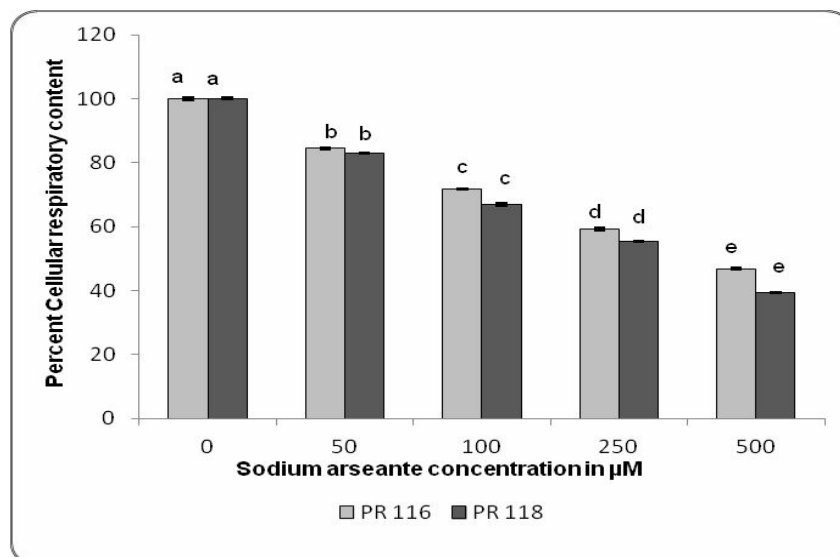


Fig 5. Arsenic accumulation in rice seedlings grown on different arsenate concentrations



Means followed by the same letter columns are not significantly different by Tukey's test at 0.05% level

Fig 6. Chlorophyll content of rice seedlings grown on different arsenate concentrations



Means followed by the same letter columns are not significantly different by Tukey's test at 0.05% level

Fig 7. Per cent cellular respiratory content of rice seedlings grown on different arsenate concentrations

where the inhibition effect was more on roots than shoots. Moreover decrease in plant height leads to biomass reduction. Pigna et al (2008) and Panaullah et al (2009) reported biomass reduction at higher concentration of arsenate. The above findings are based upon the fact of arsenic interference with plant metabolic processes such as phosphorylation and ATP synthesis. ATP production gets reduced which leads to plant growth inhibition and ultimately to plant death (Garg and Singla 2011). Root and shoot tissue of test plants were tested for arsenic accumulation. Results showed more arsenic accumulation in roots compared to shoots. Similar findings were

reported by Rahman et al (2007) showing arsenic accumulation 20 to 75 times higher in roots than shoots. Similar results were supported by previous findings of Liu et al (2004), Liu et al (2006) and Lee et al (2013).

Photosynthetic pigments (chlorophyll content) of the test plants PR 116 and PR 118 showed reduction which was more significant at higher arsenic concentration than at lower level of arsenic. Shaibur et al (2006) and Azad et al (2013) reported similar observations in their studies showing reduced chlorophyll content in plants under arsenic stress. The possible

reason could be that As binds with thiol group of protein and gets accumulated in the cell which is responsible for chloroplast disruption and leads to reduced chlorophyll content.

Results of cellular respiratory ability were reported to be in accordance with the findings of Singh et al (2007) and Shao et al (2011). Physiological and biochemical processes were disturbed in presence of heavy metals thereby altering the cellular respiratory ability of the plant.

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