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RESEARCH ARTICLE

Arsenic Induced Alteration in Macromolecule Concentration and Antioxidant System in Two Improved Rice Varieties

Ritu Kamboj, Gajendra Singh Vishwakarma, Shilpa Sharma and Sunil Mittal*

Centre for Environmental Science and Technology, Central University of Punjab Bathinda, Punjab, India - 151001

Received: 21 Mar 2016

Revised: 28 April 2016

Accepted: 24 May 2016

*Address for correspondence

Sunil Mittal

Centre for Environmental Science and Technology

Central University of Punjab Bathinda,

Punjab, India - 151001

Email: sunil.cevs@gmail.com



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ABSTRACT

Arsenic contamination of rice has been highlighted as major issue throughout the world as it is a staple food for millions. The aim of study was to analyze the effect of different concentration of arsenic on the germination, physiology, macromolecules concentration and antioxidant enzymes in improved varieties of rice. *In vitro* study indicates that the lower concentration of arsenate had a stimulating effect on germination, chlorophyll content as well as respiratory content while an inhibitory effect at higher concentration. Roots were more affected than shoots. Further, the content of macromolecules (carbohydrate and protein) was elevated while the activities of their hydrolyzing enzymes (α , β amylase and protease) were declined on arsenic stress. The significant elevation in the activity of superoxide dismutase and peroxidase enzymes also proved the generation of reactive oxygen species due to the arsenic toxicity

Keywords: Arsenic; α -amylase; β -amylase; peroxidase; rice; superoxide dismutase

INTRODUCTION

Arsenic (As) is one of the most toxic metalloid naturally present in the environment (Fitz and Wenzel, 2002) with an average concentration of about 3 mg/kg in earth crust and about 1-8 μ g/L in sea water (Smedley and Kinniburgh, 2002). Arsenic contamination received higher attention worldwide because it causes considerable harmful effects to plants and humans, including inhibition of growth in plants (Stoeva et al., 2003), physiological disorders in humans (Stoeva et al., 2005). Arsenic contamination of groundwater has been reported throughout the world but the situation is severe in Asian countries like Bangladesh, India and China (Chowdhury et al., 2000; Williams et al., 2006). In these

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regions, arsenic in drinking water exceeds the WHO recommended value of 10µg/L (WHO 2001) and even 50µg/L specified in India and Bangladesh (Ahamed et al., 2006; Ahmed et al., 2004). This potentially toxic element exists in both inorganic and organic form in the soil where, inorganic form is more toxic than organic form (Smith et al., 1998). The rice crop is one of the major staple food throughout the world and has been reported as hyper accumulator of arsenic (Meharg et al., 2009). The rice crop is more prone to arsenic accumulation than other cereals as it is generally grown under flooded condition where arsenic mobility is high, thus posing a potential health risk to humans and animals (Xu et al., 2008). When rice plants are grown in arsenic treated soil, decrease in yield and fruit production, as well as in fresh and dry biomass of roots and shoots and morphological changes have been observed (Mokgalaka-Matlala 2008; Shaibur et al., 2008; Srivastava et al., 2009). Previous experiments carried out and the plethora of information obtained reveals that metabolism of plants is adversely affected under arsenic stress, which results in more accumulation of soluble sugars and starch in many plants species (Dubey and Singh 1999; Dubey 1997) Further, it was observed that when plants were exposed to inorganic arsenic, there was generation of reactive oxygen species as a result of oxidative stress (Flora 1999). A plant gets protection against the oxidative damage by antioxidant enzymes including ascorbate peroxidase, superoxide dismutase and glutathione reductase (Hartley-Whitaker et al., 2002).

In the present study, two improved rice varieties PR122 and PUSA1121 have been used to investigate the potential risks of arsenic. These two varieties are cultivated in Malwa region of Punjab, India which has been reported to have high concentration of As in irrigation water and soil ranging from 11.4- 688 ppb (Jain and Kumar, 2007). The selected rice varieties PR122 and PUSA1121 has not been studied previously for the effect of As. So, the main objective of this study was to investigate the alteration in growth parameters, effect on chlorophyll, effect on macromolecules and their hydrolyzing enzymes and effect on antioxidant enzymes under arsenic toxicity in both the varieties PR122 and PUSA1121.

MATERIALS AND METHODS

The experiment was performed at the Central University of Punjab, Bathinda (India). The seeds of rice were procured from Punjab Agricultural University; Ludhiana. Two varieties of rice PR122 and PUSA1121 were used in the study. Seeds were surface sterilized with 5% sodium hypochlorite and subsequently rinsed with distilled water. The arsenic solution was prepared by dissolving sodium arsenate (Loba chem Pvt Ltd) in distilled water. Four different concentrations of sodium arsenate i.e. 50µM, 100µM, 250µM, 500µM which is equivalent to 12µM, 24µM, 60µM and 120µM of arsenic, were used for experiment. Control was maintained by moistening the filter paper lined in the tray.

Germination studies

Forty seeds of each variety were soaked in their respective concentrations of arsenic i.e. 12µM, 24µM, 60µM and 120µM for 18 hours. After 18 hours, trays were lined with thin layer of sterilized cotton and one filter paper and moistened with 20mL of different concentration of arsenic and incubated at 35° temperatures under dry and hygienic conditions. On 7thday, the numbers of germinated seeds were counted and the results were expressed as percentage over control.

$$\text{Germination} = \frac{\text{Total no. of germinated}}{\text{Total no. seeds taken for}} \times 10$$

The seedlings were harvested on 7thday and the shoot and root length was measured using metric scale in centimeter. The roots and shoots were oven dried at 60° for 48 hours for dry weight biomass estimation.



**Sunil Mittal et al.****Chlorophyll content**

Total chlorophyll content was measured by spectrophotometric method (Hiscox and Israelstam 1979) and calculations were done by (Arnon, 1949) and modified by (Rani, 1991). For this 100mg of test plant material was taken in test tube and 4mL of DMSO reagent was added to it and kept in water bath for 1 hour. After cooling, absorbance was taken at wavelength of 645nm and 663nm using DMSO as blank. For estimation of percent cellular respiratory ability (Steponkus and Lanphear, 1967), 100mg of tissue from fully expanded leaves of test plant material was dipped in 1.5mL of freshly prepared TTC solution. The samples were incubated in the dark at room temperature for 18 hours during which the colour of the plant tissue in tubes turned red. After 18 hours, TTC solution was drained off from test tubes then rinsed twice with distilled water. After washing, samples were extracted with 5mL of absolute alcohol and kept in water bath for 20 minutes. After cooling, absorbance was recorded at 530nm and expressed in terms of dry weight equivalent. The cellular respiratory ability was expressed as a percent with respect to control.

Preparation of extracts

100mg of plant material (root and shoot) was homogenized in pestle mortar with 5mL of 0.1M of phosphate buffer (pH 7). The homogenates were centrifuged at 15,000 rpm for 15 minutes. The supernatant was used for determining the activities of total protein, carbohydrates, protease, α amylase, β amylase, superoxide dismutase and peroxidase.

Estimation of carbohydrate and protein content

For carbohydrate estimation (Loewus, 1952), 200 μ L freshly prepared plant extract was taken in test tube and made to a final volume of 1mL by adding 800 μ L distilled water. Added 4mL anthrone reagent in each test tube, simultaneously blank was prepared by adding distilled water and heated for eight minutes in boiling water bath. Cooled and when green to dark green colour appeared, the reading was taken on UV-Visible spectrophotometer at 630nm. For protein content (Lowery, 1951), 2.5mL of reagent C (50mL 2% sodium carbonate in 0.1N sodium hydroxide and 1mL of 0.5% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 1% sodium hydroxide) was taken in test tube and then 0.5mL of plant enzyme extract was added to it; simultaneously blank was prepared by adding distilled water. The samples were mixed thoroughly and then the reaction mixture was allowed to stand for 10 minutes at room temperature. After 10 minutes, 0.25mL of reagent D (Folin Ciocalteu's reagent) was added in the test tube and mixed properly. After 30 minutes, the reaction mixture in the test tube turned blue and the reading was taken on UV-Visible spectrophotometer at 660nm.

Assays for sugar metabolizing enzymes

α amylase activity was estimated (Muentz, 1977) (EC 3.2.1.1) by using starch as a substrate. 0.5mL of plant extract was taken in test tube and then 1mL of substrate solution was added to it, simultaneously blank was prepared by adding distilled water. The reaction mixture was incubated for half an hour and then 0.1mL of EDTA was added. 0.2mL reaction mixture was taken in another test tube and 3mL of iodine solution was added, absorbance was recorded on UV-Visible spectrophotometer at 630nm using starch (50 μ g/mL) as standard. β amylase activity was estimated by (Bernfeld, 1951) (EC 3.2.1.2) using maltose as a substrate. 0.5mL of plant extract was taken in a test tube followed by the addition of 0.7mL of substrate and 0.1mL of 0.1M EDTA, simultaneously blank was prepared by adding distilled water. The reaction mixture was incubated for half an hour at 30° followed by the addition of 1mL DNSA. Boiled the reaction mixture in water bath for 20 minutes and then 3mL of distilled water was added. The absorbance was recorded on UV-Visible spectrophotometer at 560nm using starch (50 μ g/mL) as standard.

Protease activity was estimated by (Basha and Beevers, 1975) (EC 3.4) using casein as a substrate. 0.5mL of enzyme extract was taken in test tube and 0.5mL of casein solution was added to it. The reaction mixture was incubated for 1



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hour at 37^o to which 1mL of TCA solution was added. It resulted in precipitation of the proteins. The reaction mixture was centrifuged at 10,000 rpm for 5 minutes. After centrifugation, supernatant was collected and Lowery method was performed for the estimation of enzyme activity. Specific activity was calculated against 50µg/mL tyrosine as standard and expressed as g/hour/mg protein.

Assays for antioxidant enzymes

For superoxide dismutase assay (SOD) (Marklund and Marklund, 1974) (EC 1.15.1.1), the reaction mixture was prepared by adding 1.5mL of Tris HCL buffer (pH 8.2) and 0.5mL of EDTA. 1mL of pyrogallol solution and 0.1mL of enzyme extract was added in a cuvette immediately and the absorbance was recorded spectrophotometrically at 420nm after every 30 seconds up to 3 minutes. A blank was prepared without pyrogallol solution. A unit of enzyme has been defined as the amount of enzyme causing 50% inhibition of auto-oxidation of pyrogallol observed in blank. For peroxidase assay (POD) (EC 1.11.1.7) (Shannon et al., 1966), 3mL of guaiacol was taken in test tube. 0.1mL of H₂O₂ and 0.1mL of enzyme extract was added in a cuvette immediately. The reaction was initiated by H₂O₂ addition and the absorbance was recorded spectrophotometrically at 470nm after every 30 seconds up to 3 minutes. A blank was prepared without H₂O₂ solution. Peroxidase activity has been expressed as change in absorbance/minute/g of tissue.

Statistical analysis

All the experiments were performed in a completely randomized block design and performed twice. Three replicates were maintained for each treatment. The data collect from dose response study were subjected to one way ANOVA with Tukey's test.

RESULTS**Effect of arsenic on germination, root and shoot length and biomass dry weight**

Arsenic toxicity was evaluated by carrying out germination studies in seeds of rice varieties and the interpretation of results showed significant decrease in germination with the increase in arsenic concentration (Table 1 and 2). Plant growth in terms of root and shoot length was significantly inhibited at 12-120µM arsenic treatment. In addition, biomass (DW) significantly declined in the range of 22%- 69% for PR122 and 23%-63% for PUSA1121 at 12-120µM arsenic treatment.

Effect of arsenic on total chlorophyll content

Presence of arsenic had an inhibitory effect on growth and photosynthetic pigments in rice seedlings. Chlorophyll content of PR122 declined by 19%, 21%, 34% and 70% in response to 12, 24, 60 and 120µM arsenic treatments, respectively, over the control (Fig. 1). Initially, PUSA1121 showed an elevation of 10% in chlorophyll content at 12µM arsenic concentration, that further declined by 4%, 6% and 25% at 24-120 µM arsenate treatment, as compared to control (Fig. 1). Chlorophyll content in PR122 was more affected as compared to PUSA1121.

Effect of arsenic on percent cellular respiration

The percent cellular respiration content decreased by 11% to 54% with the increase in arsenic concentration from 12-120µM, while PUSA1121 initially showed an increase of 12% at 12µM arsenic treatment and then, decreased to 21%, 46% and 60% for 24, 60, 120µM arsenic treatments (Fig. 2). These results led to the conclusion that lower concentration of arsenic has stimulating effect on cellular respiration content while higher concentration has inhibitory effect on cellular respiration content.



**Sunil Mittal et al.****Effect of arsenic on carbohydrate content and the activity of their hydrolyzing enzymes - α and β amylase in roots and shoots**

Carbohydrate content in PR122 roots increased from 18%-39% in response to 12-60 μ M arsenic treatment while PUSA1121 showed an increase of 55%, 31% and 45% over the control, at 12, 24 and 60 μ M arsenic treatments (Fig. 3a). Similarly, shoots of PR122 showed an elevation in carbohydrate content by 18%-91% while PUSA1121 showed an increase of 110%, 80%, 94% and 104% over the control, with the increasing arsenic concentration (12-120 μ M) (Fig. 3b). In contrast, α -amylase activity showed a decline in roots as well as shoots of PR122. The decline was reported to be 25-68% in roots (12-60 μ M) (Fig. 3a) and 24-79% in shoots in response to (12-120 μ M) arsenic treatments of PR122 (Fig. 3b). Similarly, β -amylase activity declined by 27%-77% in roots and 35%-88% in shoots in response to (12-60 μ M) arsenic treatment (Fig. 3a, b). Roots and shoots of PUSA1121 showed 20%-74% and 14%-71% decline in α -amylase activity over the control, for μ M and 12-120 μ M arsenic concentration, respectively. Similar trend was observed for PR122 where the decline in β -amylase activity of roots (12-60 μ M) and shoots (12-120 μ M) was reported to be 27%-77% and 35%-88% respectively, over the control.

Effect of arsenic on protein content and the activity of hydrolyzing enzyme - protease in roots and shoots

Protein content increased in roots and shoots of both the rice varieties. Protein content in roots of PR122 and PUSA1121 varieties increased by 6%-29% and 19%-22%, respectively, over the control, for 12-60 μ M arsenic treatments (Fig. 4a). Increasing trend in protein content was also reported in shoots of PR122 and PUSA1121, with an increase of 14%-62% and 17%-31% over the control, for 12-120 μ M arsenic concentrations, respectively (Fig. 4b). Roots of both the varieties i.e., PR122 and PUSA1121 showed decline in protease activity by 45%-85% and 53%-89% over the control, at 12-60 μ M arsenic treatment (Fig. 4a). Likewise, protease activity in shoots was reported to decline by 40%-90% and 44%-91% over the control for PR122 and PUSA1121, at 12-120 μ M arsenic treatments, respectively (Fig. 4b).

Effect of arsenic on SOD (Superoxide dismutase) activity in roots and shoots

Excess of arsenic altered the activity of antioxidant enzymes. The increase was significant at all treatments as compared to the control. SOD activity increased in roots of both the varieties i.e. PR122 and PUSA1121 by 47%- 67% and 80%- 133%, in response to 12, 24 and 60 μ M arsenic treatments, respectively, over the control (Fig. 5a). Shoots of both the varieties revealed an increasing trend in SOD activity, whereby the increase for PR122 and PUSA1121 was about 110%-212% and 86-154% respectively, as compared to control, at 12-120 μ M arsenic treatments (Fig. 5b).

Effect of arsenic on POD (Peroxidase) activity in roots and shoots

Significant increase was observed in the activity of peroxidase (POD) in roots and shoots of both the rice varieties. Percent enhancement in POD activity in roots of PR122 and PUSA1121 was observed to be 5%-48% and 28%-38% over the control, respectively, at 12-60 μ M arsenic treatment (Fig. 6a). Similarly, at 12-120 μ M arsenic concentration, percent elevation in POD activity in shoots of both the varieties i.e., PR122 and PUSA1121 was observed to be 36%-99% and 3%-23%, respectively, over the control (Fig. 6b).

DISCUSSION

Arsenic exposure significantly affects the growth parameters and physiology of rice seedlings. In the present study, different concentrations of arsenic were studied on two rice varieties i.e. PR122 and PUSA1121. It was observed that both the varieties were radically affected in response to the increased concentration of arsenic. Germination studies revealed that low concentration of arsenic stimulates the growth of rice seedlings, whereas higher concentration inhibits the seed germination. Moreover, similar results were also reported by (Hoffmann and Schenk 2011; Shri et



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al., 2009) in rice varieties. The inhibitory effect was also observed in root and shoot length as compared to control in both the rice varieties. The effect was more prominent on root length than shoot height since roots are the first site of contact with the heavy metals. Earlier, (Choudhury et al., 2011; Das et al, 2004; Abedin et al., 2002a; Abedin et al., 2002b) reported the pronounced inhibition in roots than shoots. It was also observed that as the plant height decreases, it automatically affects biomass of plant, and in turn the dry biomass of plant decreased with increased concentration of arsenic. (Panaullah et al., 2009; Pigna et al., 2009; Srivastava et al., 2009) also reported reduction in dry biomass at higher concentration of arsenic. It was observed that arsenic interferes with plant metabolic processes such as phosphorylation and ATP synthesis and thus no ATP are formed which inhibits the plant growth and ultimately plant death (Garg and Singla 2011). The effect of arsenic was studied on photosynthetic pigments of the test plants PR122 and PUSA1121 and the results showed that reduction in chlorophyll content was more significant at higher concentration of arsenic than at lower level of arsenic. Similar results were also reported by various researchers (Azad et al., 2013; Hoffmann and Schenk 2011; Rahman et al., 2007; Shaibur et al., 2006). The reason is attributed to the fact that As binds with –SH base of protein after it enters and gets accumulated in the cell which ultimately leads to disruption of chloroplast, due to which chlorophyll content is reduced. Cellular respiratory ability declined in both the rice varieties i.e. PR122 and PUSA1121. Similar results were reported by Shao et al., 2011; Singh et al., 2007. Excess of heavy metals disturbed the physiological and biochemical processes of plant hence disturbing the cellular respiratory ability of the plant. According to (Shao et al., 2011), each plant has its own protective mechanism towards stress so they can adjust according to the environmental changes. Carbohydrate content increased with the increase in the concentration of arsenic in both the varieties i.e. PR122 and PUSA1121. The results obtained are in accordance with previous studies of Choudhury et al., 2010; Dubey and Singh, 1999; Dubey 1997. A study led by Dubey and Singh 1999 states the fact that plant needs more energy to combat metallic stress which, in turn is provided by the production of more soluble sugars. Thus, to overcome cellular dehydration, the cells produce and accumulate more soluble sugars under As stress, thus resulting in increased carbohydrate content. However, carbohydrate content was more in roots than shoots thus affecting the roots more than shoots. High levels of arsenic in plants also affect the hydrolyzing enzymes amylases and proteases. Starch is the main reserve food material of plants and mobilization of starch reserves depends on α and β amylase and their hydrolyzed products are absorbed by scutellum which is used by growing embryo for the growth of seedlings. Presence of any environmental stress may disturb the mobilization of reserves which could significantly affect germination (Kaneko et al., 2002). The activities of both α and β amylase declined in both the varieties i.e. PR122 and PUSA1121 with increased concentration of arsenic. Same trend of observation was reported by (Jha and Dubey, 2005; Jha and Dubey, 2004). High content of arsenic affects the protein levels of the plant. Proteins of cells will not be used up due to reduced plant growth but will accumulate which results in increase in protein content of seedling (Yu et al., 1995). Another reason for increase in protein content was decrease in activity of protease enzyme. Protease activity decreased with increased concentration of sodium arsenate in both varieties PR122 and PUSA1121. The decreased activity of protease indicates that the plant is inefficient to hydrolyze proteins under arsenic stress (Bhattacharya et al., 2013; Mishra and Dubey, 2006). Significant increase was observed in superoxide dismutase (SOD) and peroxidase (POD) activity with increased arsenic. Similar observations were reported by (Bhoomika et al., 2013; Dave et al., 2013; Geng et al., 2006). Plant gets protection against the ROS by releasing scavenging enzymes superoxide dismutase (SOD) and peroxidase (POD).

CONCLUSION

From the present study, it can be concluded that different concentration of arsenic affects growth and physiology of PR122 and PUSA1121 rice varieties. Arsenic in lower concentrations may have growth promoter effects. Roots accumulate more arsenic than shoots. Lower concentration of arsenic had a stimulating effect on chlorophyll content as well as respiratory content while an inhibitory effect at higher concentration. Carbohydrate and protein content was increased with increase concentration of arsenic while the activity of their hydrolyzing enzymes α , β amylase and protease was decreased with increased concentration of arsenic. Significant increased was observed in the activity of SOD and POD at higher concentration of arsenic.





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ACKNOWLEDGEMENTS

This work was supported financially by a research grant (20-26(12)/2012(BSR) from University Grants Commission (UGC), New Delhi. The authors are grateful to Punjab Agriculture University, Ludhiana for providing rice seeds to carry out entire work.

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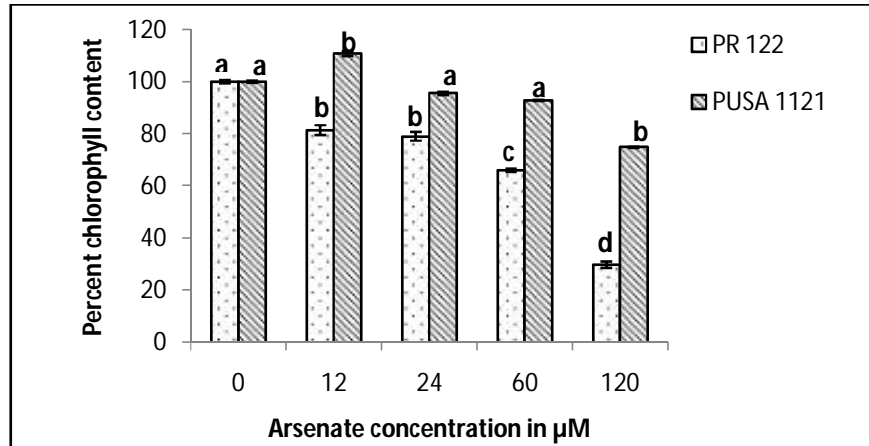


Fig. 1 Percent chlorophyll content of rice cvs.PR122 and PUSA1121. Data were analyzed by one way Anova, post hoc multiple comparison Tukey’s test. Means followed by the same letter (within the variety) are insignificant at the 0.05% level and S.E represents standard error.

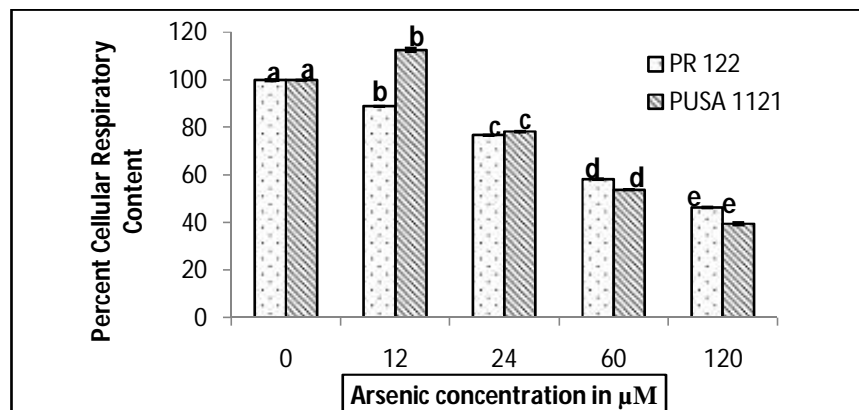


Fig. 2 Percent cellular respiratory content of rice cvs.PR122 and PUSA1121. Data were analyzed by one way Anova, post hoc multiple comparison Tukey’s test. Means followed by the same letter (within the variety) are insignificant at the 0.05% level and S.E represents standard error.





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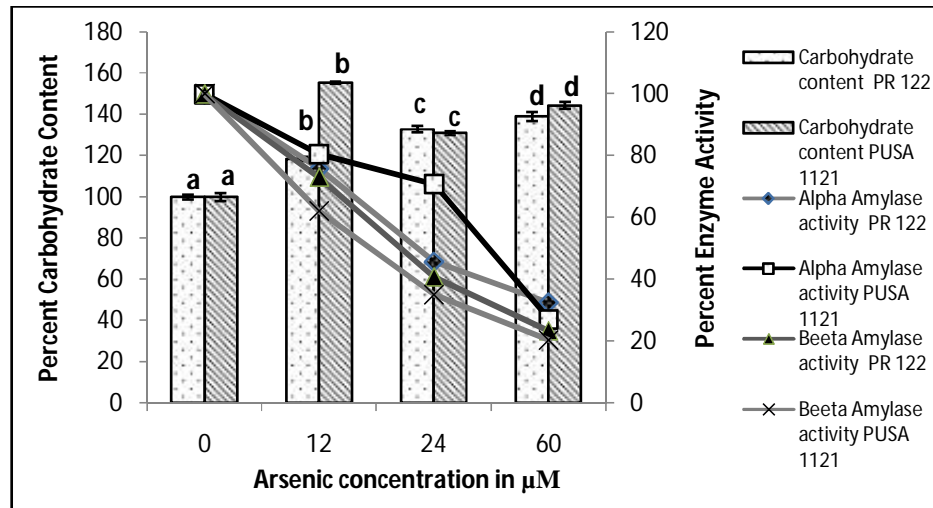


Fig. 3a Carbohydrate content and the activity of their hydrolyzing enzymes - α and β *amylase* in roots of rice cvs.PR122 and PUSA1121. Data were analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter (within the variety) are insignificant at the 0.05% level and S.E. represents standard error.

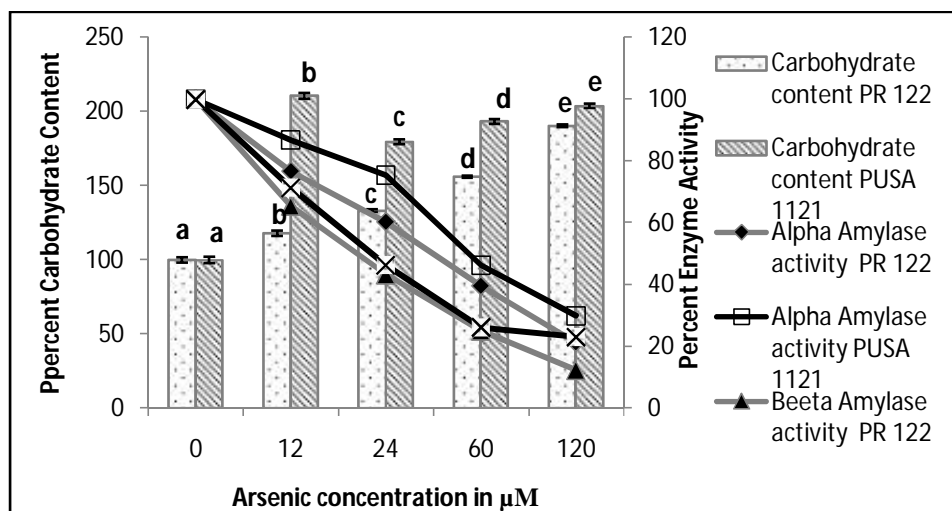


Fig. 3b Carbohydrate content and the activity of their hydrolyzing enzymes - α and β *amylase* in shoots of rice cvs.PR122 and PUSA1121. Data were analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter (within the variety) are insignificant at the 0.05% level and S.E. represents standard error.





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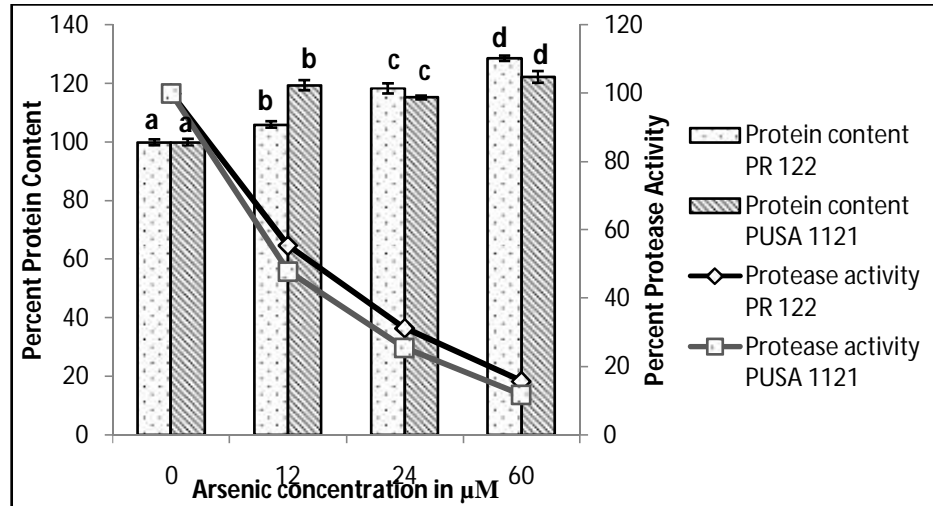


Fig. 4a Protein content and the activity of their hydrolyzing enzymes-protease in roots of rice cvs.PR122 and PUSA1121. Data were analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter (within the variety) are insignificant at the 0.05% level and S.E. represents standard error.

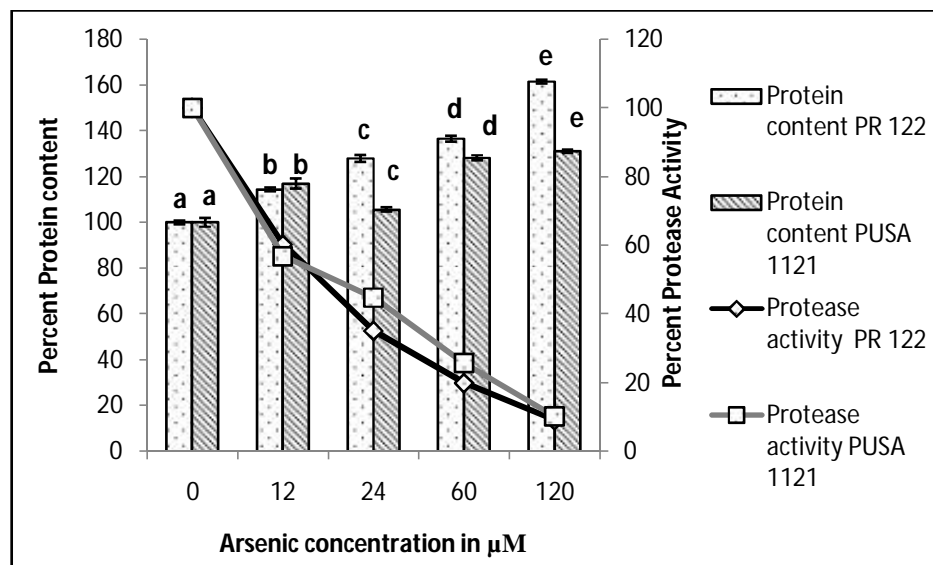


Fig. 4b Protein content and the activity of their hydrolyzing enzymes-protease in shoots of rice cvs.PR122 and PUSA1121. Data were analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter (within the variety) are insignificant at the 0.05% level and S.E. represents standard error.





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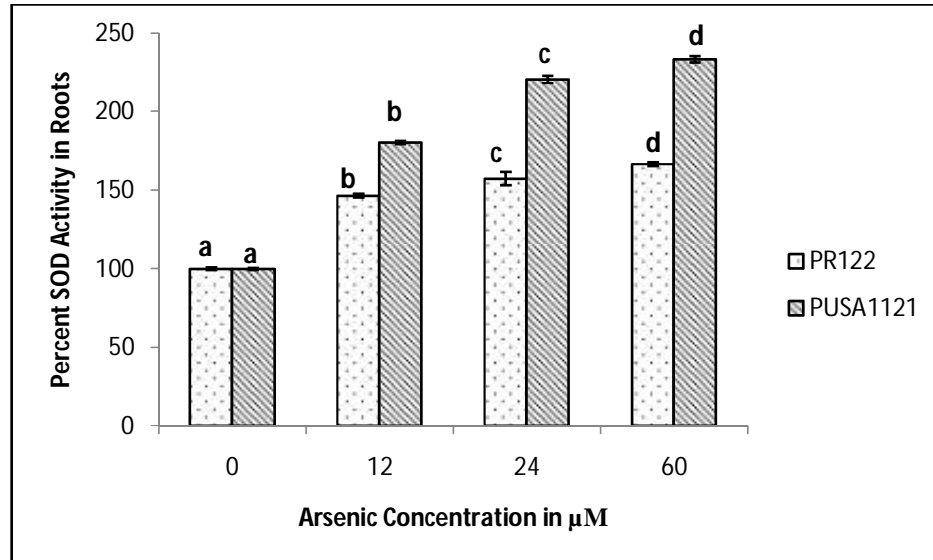


Fig. 5a Percent SOD activity in roots of rice cvs. PR122 and PUSA1121. Data were analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter (within the variety) are insignificant at the 0.05% level and S.E. represents standard error.

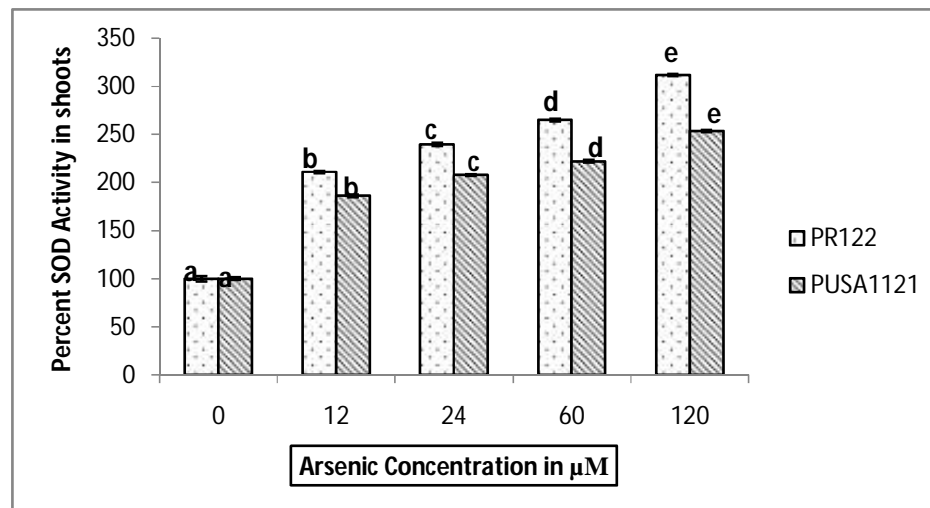


Fig. 5b Percent SOD activity in shoots of rice cvs. PR122 and PUSA1121. Data were analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter (within the variety) are insignificant at the 0.05% level and S.E. represents standard error.





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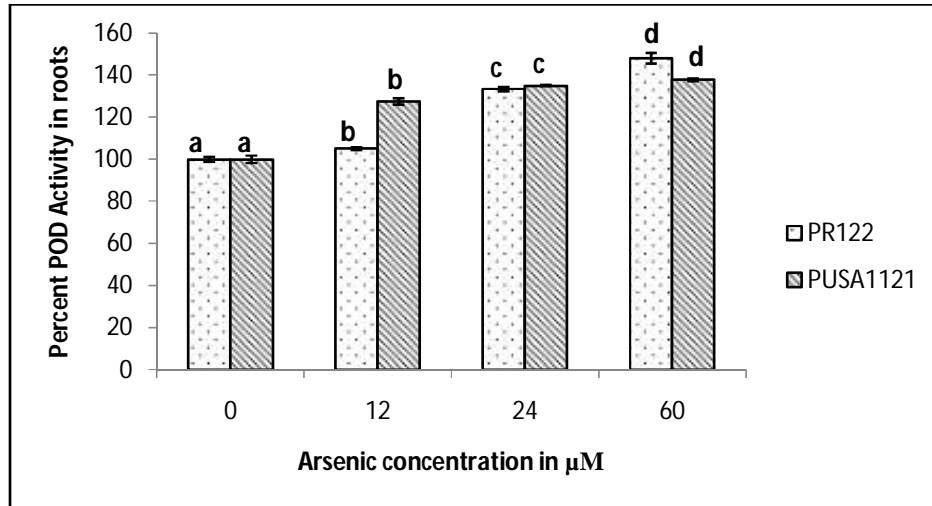


Fig. 6a Percent POD activity in roots of rice cvs. PR122 and PUSA1121. Data were analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter (within the variety) are insignificant at the 0.05% level and S.E. represents standard error.

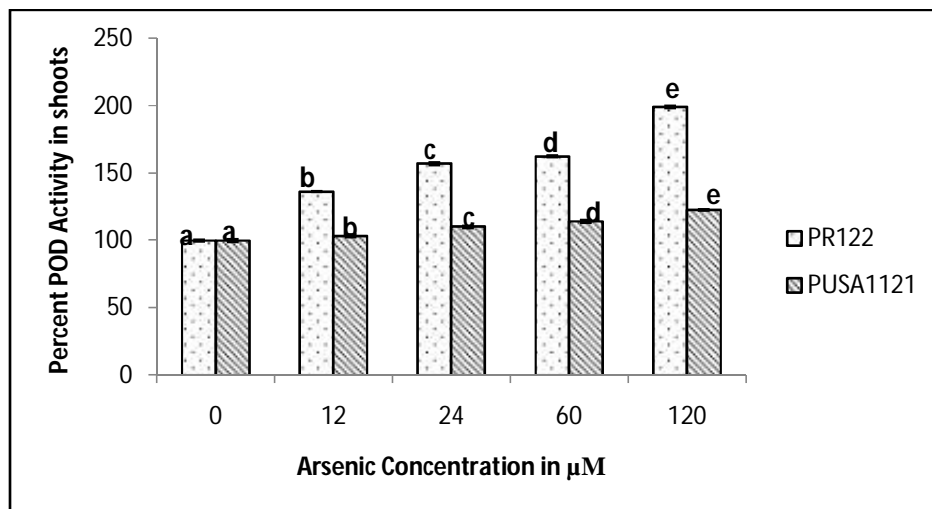


Fig. 6b Percent POD activity in shoots of rice cvs. PR122 and PUSA1121. Data were analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter (within the variety) are insignificant at the 0.05% level and S.E. represents standard error.





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Table 1 Effect on germination; shoot length, root length and dry weight in PR122 and PUSA1121 with increasing the concentration of arsenic

Conc. (µM)	PR122				PUSA1121			
	Germination	Shoot length (cm)	Root length (cm)	Dry weight (g)	Germination	Shoot length (cm)	Root length (cm)	Dry weight (g)
00	40.00 ^a ±0.00	4.84 ^a ±0.09	6.26 ^a ±0.19	0.036 ^a ±0.0007	40.00 ^a ±0.00	7.41 ^a ±0.16	7.94 ^a ±0.36	0.043 ^a ±0.0001
12	39.00 ^a ±0.33	4.53 ^a ±0.10	3.05 ^b ±0.18	0.028 ^b ±0.0008	40.00 ^a ±0.00	6.41 ^b ±0.18	5.15 ^b ±0.25	0.033 ^b ±0.0008
24	38.33 ^{ab} ±0.33	3.91 ^b ±0.15	2.32 ^c ±0.03	0.025 ^b ±0.0007	38.66 ^a ±0.33	5.47 ^c ±0.22	2.68 ^c ±0.14	0.028 ^c ±0.0008
60	36.33 ^b ±0.88	3.67 ^c ±0.06	0.62 ^d ±0.20	0.016 ^c ±0.0009	36.00 ^b ±0.57	4.49 ^d ±0.07	1.35 ^d ±0.16	0.020 ^d ±0.0004
120	30.66 ^c ±0.88	2.70 ^d ±0.12	0.21 ^e ±0.02	0.011 ^d ±0.0011	30.66 ^c ±0.88	3.36 ^e ±0.12	0.21 ^e ±0.02	0.016 ^e ±0.0008

Data were analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter (within the variety) are insignificantly at the 0.05% level and S.E represents standard error.

Table 2 Percentage germination, shoot length, root length and dry weight in PR122 and PUSA1121 with increasing the concentration of arsenic

Conc. (µM)	PR 122				PUSA 1121			
	Germination	Shoot length	Root length	Dry weight	Germination	Shoot length	Root length	Dry weight
0	100	100	100	100	100	100	100	100
12	97.5	93.59	48.72	77.77	100	86.5	64.86	76.74
24	95.82	80.78	37.06	69.44	96.65	73.81	33.75	65.11
60	90.82	75.82	9.9	44.44	90	60.59	17	46.51
120	76.65	55.78	3.35	30.55	76.65	45.34	2.64	37.2

