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Research Article

Comparative Study of Arsenic-stress Sensitivity in Purple Rice and BRRI dhan28

Most. Aynun Nahar, Snigdha Rany Sharma, Md. Yeasin Prodhan and Mst. Nur-E-Nazmun Nahar✉

Department of Biochemistry and Molecular Biology, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh

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Correspondence
Mst. Nur-E-Nazmun Nahar
✉: nnnbhstu@gmail.com

ABSTRACT

Purple rice is an unidentified rice cultivar that has purple colour leaf. Some farmers of Bangladesh are showing interest to cultivate purple rice due to its ornamental look and high price of seeds. Hence, before occupying the green rice land by the purple rice, more information about purple rice is necessary to guide farmers properly. Less information about purple rice is available in any area of research except nutritional analysis. Purple rice leaf has high antioxidant properties which play role in stress tolerance. The salt-stress tolerance potentiality of purple rice compared to green rice has been reported. Therefore, as a part of unraveling the heavy-metal stress tolerance potentiality of purple rice, here, we aimed to find out the arsenic-stress tolerance potentiality of purple rice. Arsenic is a toxic metalloid that disrupts plant cells. The effects of different arsenate concentrations (*e.g.* 0 µM, 100 µM, and 500 µM) on purple rice and BRRI dhan28 were observed based on morpho-biochemical parameters. The results of purple rice were compared with those of green rice (BRRI dhan28). Arsenic stress reduced root length and plant height compared to control in both rice but the reductions in purple rice were lesser than those of BRRI dhan28. Proline, a compatible solute, content was higher in purple rice under both arsenic stress and non-stress conditions compared with BRRI dhan28 and the proline content was decreased in BRRI dhan28 in a concentration-dependent manner. The catalase (an antioxidant enzyme) activity was increased in both rice under arsenic stress conditions compared to control and purple rice showed more activity than BRRI dhan28 in response to arsenic stress. Arsenic stress increased the anthocyanin content (a purple-colored leaf pigment) in a concentration-dependent manner in purple rice than that of BRRI dhan28. BRRI dhan28 showed reduction of chlorophyll and carotenoid contents under arsenic stress compared to the both control and purple rice. The ability of less reduction of phenotypes in purple rice might be achieved by the higher activity of catalase and a higher level of anthocyanin content. Though proline is utilized in BRRI dhan28 under arsenic stress it was either just a symptom or influenced the antioxidant homeostasis without decreasing its concentration. Purple rice may have more potential to protect its cell reductions by arsenic stress than BRRI dhan28.

Introduction

The green-leaved rice plants are chiefly cultivated in Bangladesh. Recently, the farmers are showing interest in cultivating an unidentified so-called purple-leaved rice plant (purple rice) in some areas of Bangladesh (*e.g.,* Comilla, Gaibandha, and Gazipur districts) due to its ornamental look, high price of seeds and high nutritional value. Purple rice was cultivated in China many years ago as a food of the Chinese Emperors. The purple rice has already drawn the attention of the mass media and visitors. Less information is available on purple rice in many areas of research. Bangladeshi newspapers have published many reports on purple rice based on farmers' interviews that purple rice is less affected by diseases and the production of purple rice is equivalent to that of our local paddy. Hence, before spreading and occupying the green rice land by purple rice, more information about the purple rice is necessary to guide farmers properly.

Purple rice is rich in anthocyanin content and has high antioxidant properties (Jang and Xu, 2009). It is reported that the high amount of anthocyanin in leaf tissues allows the plant to develop resistance against environmental stresses (Eryılmaz, 2006; Kiełkowska *et al*., 2019). Therefore, it is believed that there are some differences present between green rice and purple rice in terms of stress mitigation potentiality. Heavy metal stress destroys rice production. The salt-stress tolerance potentiality of purple rice compared to green rice has been reported (Nahar *et al*., 2021). Arsenic is a

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toxic metal that is present in the soil and causes many physiological abnormalities in plants (Nahar *et al*., 2017) and reduces crop yield (Panaullah *et al*., 2009). In the future, arsenic may be a big problem because the depth of irrigation water is increasing day by day due to the decreasing groundwater level. To our knowledge, there is no information regarding the arsenic tolerance capacity of purple rice. Therefore, as a part of unraveling the heavy-metal stress tolerance capacity of purple rice, here, we aimed to find out the arsenic-stress tolerance potentiality of purple rice. The results of purple rice were compared with those of green rice (BRRI dhan28).

Arsenate (AsO_4-) and arsenite (AsO_3-) are two available forms of arsenic in the soil but plants can uptake only the arsenate form (Tripathi *et al.*, 2007). Arsenic induces the production of free radicals and reactive oxygen species (ROS) in plants (Singh *et al*., 2007). Various enzymatic and non-enzymatic antioxidants have been activated to fight against the lethal effects of arsenic-induced ROS synthesis (Liu *et al*., 2007; Singh *et al.,* 2013). Under environmental stresses, plants often produce ROS such as superoxide, hydrogen peroxide and hydroxyl radicals causing damage to DNA, proteins and lipids. Arsenic exposure leads to the generation of ROS through the conversion of arsenate to arsenite catalyzing by arsenate reductase, a process that rapidly occurs in plants (Flora, 1999; Mascher *et al.*, 2002). To minimize the harmful effects of ROS, plants have evolved an effective scavenging system composed of antioxidant molecules and antioxidant enzymes (Meharg, 1994). Catalase is an antioxidant enzyme that scavenges ROS from plants under stress conditions (Cheeseman, 2006). However, in some instances, this defensive mechanism becomes inadequate to withstand stressful conditions (Chandrakar *et al.*, 2016).

Plants accumulate large quantities of different types of compatible solutes in response to different stresses (Serraj and Sinclair, 2002). Proline is accumulated in plants under stress (Serraj and Sinclair, 2002) and its accumulation may be a part of stress signal influencing adaptive responses. It protects plants against different abiotic stresses and performs different cellular processes such as osmotic regulation, energy production, nutrient assimilation etc. (Aggarwal *et al*., 2011; Yingli *et al*., 2011; Szepesi *et al*., 2018). Endogenous proline scavenges free radicals and reduces arsenic accumulation and thus fights against arsenic stress Yadav *et al*. (2014). Proline scavenges free radicals and ROS (Ozturk *et al*., 2010; Hossain *et al*., 2014; Nahar *et al*., 2017). Up-regulation of the components of antioxidant defense system offered by proline protects plants against arsenic-induced oxidative damage. The roles of proline and antioxidant enzyme activity are changed under abiotic stress conditions. It is also reported that the proline accumulation in plants under stress conditions is not related to the amelioration of stress but it is just a symptom (Liu and Zhu, 1997) and did not show any protective value (Moftah and Michel, 1987). Plant phenotypes such as root-shoot length, plant height, and yield etc. reduce under stress conditions but the reduction is higher in sensitive plants (Barrachina *et al*., 1995; Knauer *et al*., 1999). The biochemical and morphological parameters are interrelated. The decision can be made whether the variety is sensitive or tolerant by observing the variation of phenotypic data under stress and non-stress conditions.

The present study was designed to observe the consequences of arsenic stress on the morpho-biochemical parameters such as root length, plant height, proline level, amount of different plant pigments, and antioxidant enzyme activity in both rice to unveil their arsenic-stress tolerance capability.

Materials and Methods

Place of study

The experiment was performed in pots at the Agriculture Farm of Hajee Mohammad Danesh Science and Technology University from the period October 2018 to September 2019. A cultivar of botanically unidentified purple rice and a variety of commonly growing green rice (BRRI dhan28) were used throughout the experiment to compare their results.

Measurement of root length

Rice seeds were sterilized with 2.5% sodium hypochlorite. Seeds were washed and soaked overnight with distilled water and then placed on filter paper in the petri dish for germination. Total 15 seeds were placed per petri dish. Filter papers were moistened with 10 mL of 0 µM, 100 µM, and 500 µM arsenic solutions to develop different levels of arsenic stress. The root lengths of seedlings were measured after the 9th day of germination. Percent root length reduction was measured according to the following formulae-

Percent root length reduction = [{(Root length taken at control condition- root length taken at the respective treatment) × 100} ÷ Root length taken at control condition]

Seedling transplantation and application of treatments

Plastic pots having 6 kg of soil in each were used for the transplantation of rice seedlings (purple rice and BR28). Soil fertilization was done followed by Bhusan *et al*. (2016). A single hill contained three seedlings (30-day-old). Seedlings were exposed to various arsenic stresses such as 0 µM, 100 µM, and 500 µM at 35 days after transplanting at active tillering stage. The same experiment was repeated three times in response to each treatment.

Measurement of plant height

After 30 days of arsenic treatment, the plant height was measured. A measuring scale (cm) is used to observe the height. The height of the tiller was measured from soil level to the tallest leaf.

Measurement of endogenous proline content

The leaf sample was collected for biochemical analysis 30 days after arsenic treatment. The proline content was measured according to the method of Bates *et al.* (1973). The leaf sample was homogenized with sulphosalicylic acid (3%) followed by centrifugation at 12,000g for 10 to 12 minutes and then the supernatant was mixed with acid-ninhydrin and glacial acetic acid. The toluene that contained chromophore was separated after incubating 1 h at 100°C. The absorbance was taken at 520 nm.

Measurement of activity of catalase

Potassium phosphate buffer (pH:8) was used to homogenate the leaf sample followed by homogenization at 12000 rpm for 10 minutes. The supernatant was collected. The activity of catalase enzyme was measured as followed by Aebi (1984). Mixing of ethylenediaminetetraacetic acid, hydrogen per-oxide and buffer solution was done and the enzyme extract was added to the mixture to start the reaction. The absorbance alterations were taken at 240 nm instantly for 2 minutes at 30-second intervals using a UV-Vis Spectrophotometer (UV-2000PRO; Shanghai Yoke Instrument Co. Ltd.; China).

Assessment of anthocyanin content

The anthocyanin content was determined followed by the method of Lange *et al.* (1971). Requisite amount of fresh leaf was taken in a tube and acidified methanol was added. The sample tube was immersed in boiling water for 1.5 minutes and kept at room temperature for 24 h in dark. The absorbance of supernatant was recorded at 535 nm and 650 nm wavelength. Anthocyanin content was calculated as per Rayleigh's formula:

Amount of anthocyanin (Corrected A_{535}) = $A_{535} - 2.2\ A_{650}$ [A= Absorbance]

Assessment of chlorophyll and carotenoid contents

Chlorophyll (chl) and carotenoid contents were assessed according to (Porra *et al.,* 1989). Required amount of fresh leaf was suspended in 80% acetone. The sample was kept at room temperature for 48 h in the dark. A UV-Vis Spectrophotometer (UV-2000PRO; Shanghai Yoke Instrument Co. Ltd., China) was used to take absorbance at 663, 645, and 470 nm wavelengths. The contents of different pigments were calculated according to Arnon's formula (1949).

Statistical analysis

Tukey test was performed to assess the significance of differences of mean values using R software. Level of probability up to 5% was considered as significant.

Results

Effects of arsenic stress on root length and plant height of purple rice *and BRRI dhan28*

The first experiment was designed to examine whether any difference existed in the morphological traits between purple rice and BRRI dhan28 in response to arsenic stress. The different arsenic concentrations such as 0 µM, 100 µM, and 500 µM were applied on the young roots of rice seedlings to measure the root length reduction.

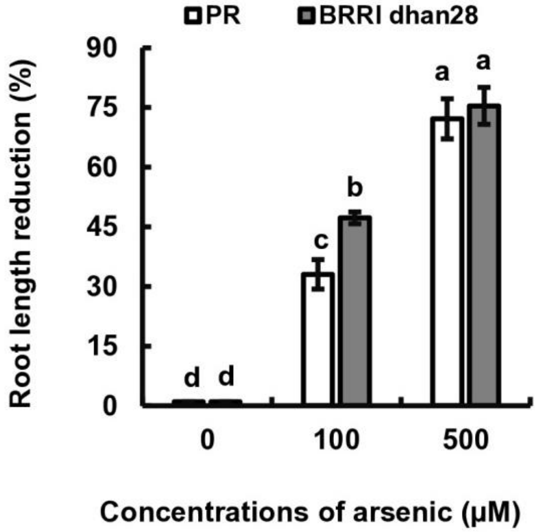

Fig. 1. Arsenic stress-induced effects on root length of purple rice (PR) and BRRI dhan28 in response to different arsenic concentrations. SE showed by error bars. Values indicated by the different letters differ significantly. Level of probability was considered at 5% level as assessed by Tukey test.

The results showed that root length reductions were increased with the increase in arsenic concentrations. purple rice showed less reduction in root length (33.04%) than that of BRRI dhan28 (47.26%) at 100 µM arsenic concentration but did not show any significant difference at 500 µM arsenic concentration because the maximum reduction was achieved for both rice cultivars at the later high concentration (Fig. 1).

The stressing effects of arsenic were also examined on the plant heights of both rice (Fig. 2). BRRI dhan28 showed more plant height than purple rice at non-stress condition. The heights of both rice plants were decreased gradually with the gradual increase of arsenic concentrations (Fig. 2a). Though no significant difference was found between the plant height of purple rice and BRRI dhan28 under 100 µM and 500 µM arsenic stress conditions, but compared to control, the reduction of plant height was more in BRRI dhan28 than purple rice as the BRRI dhan28 was taller than purple rice before stress (Fig. 2b).

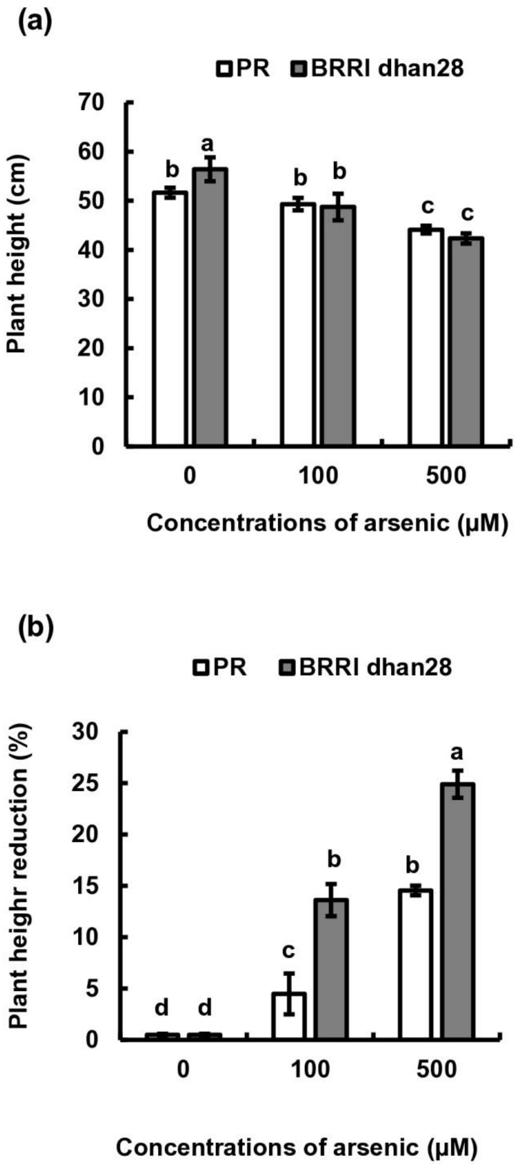

Fig. 2. Arsenic stress-induced changes on plant height of purple rice (PR) and BRRI dhan28 in response to different arsenic concentrations. Plant height (a), and percent reduction of plant height (b) compared to control. SE showed by error bars. Values indicated by the different letters differ significantly. Level of probability was considered at 5% level as assessed by Tukey test.

The above results suggest that the root length and plant height reductions were more in BRRI dhan28 than those of purple rice under arsenic stress.

Effects of arsenic stress on endogenous proline level of purple rice and BRRI dhan28

Proline is a compatible solute that is accumulated in plants and plays crucial role in response to different stresses (Serraj and Sinclair, 2002; Ozturk *et al.,* 2010; Hossain *et al.*, 2014; Nahar *et al.*, 2017). Therefore, here we tested whether any changes occurred on proline level in both purple rice and BRRI dhan28 under arsenic stress. The results revealed that the constitutive proline content in purple rice was higher than BRRI dhan28 (Fig. 3).

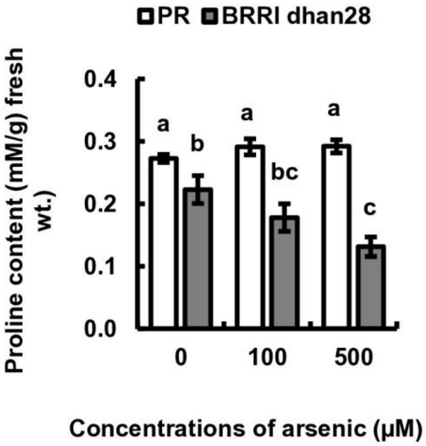

Fig. 3. Arsenic stress-induced changes of proline content in purple rice (PR) and BRRI dhan28 in response to different arsenic concentrations. SE showed by error bars. Values indicated by the different letters differ significantly. Level of probability was considered at 5% level as assessed by Tukey test.

The proline content was also higher in purple rice than BRRI dhan28 in response to 100 µM and 500 µM arsenic stress but proline content gradually decreased in BRRI dhan28 with the increase of arsenic concentration (*i.e.* 0.22, 0.18, and 0.13 mM/g fresh wt. at 0, 100, and 500 µM arsenate, respectively). The proline content was decreased in BRRI dhan28 but did not change in purple rice under arsenic stress.

Effects of arsenic stress on the activity of catalase enzyme of purple rice and BRRI dhan28

Catalase is one of the major antioxidant enzymes that scavenge ROS. The activity of catalase enzyme was measured in both purple rice and BRRI dhan28 under arsenic stress.

The activity was statistically similar in both rice (*i.e.*0.48 and 0.35 µmole/min/g fresh wt., respectively) under non-stress condition but purple rice rice showed higher activity (0.78 and 1.26 µmole/min/g fresh wt.) than BRRI dhan28 (0.64 and 0.98 µmole/min/g fresh wt.) under 100 µM and 500 µM arsenic stress, respectively (Fig. 4). Both purple rice and BRRI dhan28 showed increased catalase activity under arsenic stress compared to control but the increase was higher in purple rice than BRRI dhan28 (Fig. 4).

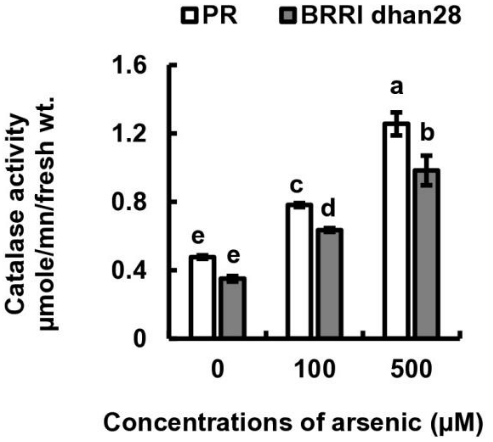

Fig. 4. Activity of catalase in purple rice (PR) and BRRI dhan28 in response to different arsenic concentrations. SE showed by error bars. Values indicated by the different letters differ significantly. Level of probability was considered at 5% level as assessed by Tukey test.

Effects of arsenic stress on leaf pigments of purple rice and BRRI dhan28

Purple rice has high antioxidant properties due to its high anthocyanin content (Jang and Xu, 2009) which allows the plant to develop resistance against abiotic stresses (Eryılmaz, 2006; Kiełkowska *et al.*, 2019).

In this study, anthocyanin content was only detected in purple rice but not in BRRI dhan28 (Fig. 5). Purple rice showed a notable change in the amount of anthocyanin at 100 µM (0.95 O.D. (optical density), 535 nm) and 500 µM (1.01 O.D., 535 nm) arsenic stress compared to control (0.84 O.D., 535 nm) (Fig.5).

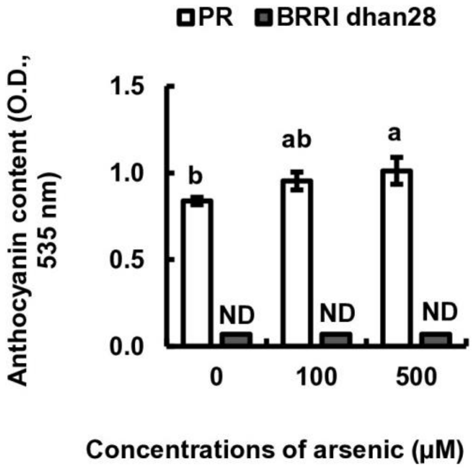

Fig. 5. Anthocyanin content in leaves of purple rice (PR) and BRRI dhan28 in response to different arsenic concentrations. O.D. (optical density) at 535 nm. ND, not detected. SE showed by error bars. Values indicated by the different letters differ significantly. Level of probability was considered at 5% level as assessed by Tukey test.

Here, we also examined the effects of arsenic stress on the chlorophyll and carotenoid contents (Table 1) which are also the indicators for determining abiotic stresses.

At non-stress condition (0 µM), the total chlorophyll content was higher in purple rice (33.74 µg/ml of plant extract) than BRRI dhan28 (29.68 µg/ml of plant extract) and total carotenoid content was also higher in purple rice (7.10 µg/ml of plant extract) than BRRI dhan28 (6.40 µg/ml of plant extract) (Table 1). At 500 µM arsenic stress condition, the higher amount of total chlorophyll (36.70 µg/ml of plant extract) and total carotenoid (7.59 µg/ml of plant extract) contents were also found in purple rice than BRRI dhan28 (26.08 µg/ml of plant extract and 5.98 µg/ml of plant extract, respectively) (Table 1).

Table 1. Effects of arsenic stress on pigment contents of purple rice (PR) and BRRI dhan28. Values indicated by the different letters differ significantly. Level of probability was considered at 5% level as assessed by Tukey test

Arsenic concentrations (µM)	Chl$_a$ content (µg/ml of plant extract)		Chl$_b$ content (µg/ml of plant extract)		Tchl content (µg/ml of plant extract)		Tcar content (µg/ml of plant extract)	
	PR	BRRI dhan28	PR	BRRI dhan28	PR	BRRI dhan28	PR	BRRI dhan28
0	26.67 ± 1.12^{ab}	23.87 ± 1.49^{bc}	7.07 ± 0.64^{b}	5.81 ± 0.50^{c}	33.74 ± 0.78^{a}	29.68 ± 1.94^{b}	7.10 ± 0.17^{ab}	6.40 ± 0.47^{bc}
100	22.25 ± 1.34^{cd}	22.06 ± 0.82^{cd}	5.93 ± 0.64^{bc}	$5.29\ \pm0.35^{c}$	28.19 ± 1.68^{b}	27.35 ± 1.16^{b}	6.08 ± 0.13^{c}	6.04 ± 0.46^{c}
500	27.94 ± 0.82^{a}	20.41 ± 1.06^{d}	8.76 ± 0.62^{a}	5.67 ± 0.68^{c}	36.70 ± 1.44^{a}	26.08 ± 1.69^{b}	7.59 ± 0.24^{a}	5.98 ± 0.43^{c}

Discussion

As a part of unraveling the heavy-metal stress tolerance potentiality of purple rice rice, here we investigated the arsenic-stress tolerance capability of purple rice. In previous, our research group reported the salt-stress tolerance potentiality of purple rice. The experiments were conducted to examine the effects of different levels of arsenic stress such as 0 µM, 100 µM and 500 µM on the morphological and biochemical traits of purple rice. All the results were compared with the same parameters of BRRI dhan28 as a representative variety of green rice which is a commonly cultivated variety in Bangladesh.

The stressing effects of arsenic were observed on the root length and plant height of both rice. Root length is one of the important morphological attributes that act as an index in response to abiotic stresses such as arsenic stress. Accumulation of toxic metals in roots causes injury to cells and ultimately retards plant growth (Talukdar, 2012). Here we found decreased root length in both purple rice and BRRI dhan28 under arsenic stress (Fig. 1). At 100 µM arsenic stress, the root length reduction of purple rice (33.04%) was lower than BRRI dhan28 (47.26%) compared to control (Fig.1) but no significant difference was found in both purple rice (72.14%) and BRRI dhan28 (75.40%) at 500 µM arsenic stress because the root growth was stopped for both rice at higher concentration. Shri *et al.* (2009) also reported the stopping of root growth of plants at 500 µM arsenic stress. Here we also found that the reduction of plant height was higher in BRRI dhan28 than purple rice under arsenic stress compared to control (Fig. 2). Based on the above results, the lesser reduction of root length and plant height of purple rice suggested that arsenic stress is less harmful for the phenotypic traits of purple rice compared to BRRI dhan28. It is reported that the reduction of root length and shoot length of purple rice was lower than those of BRRI dhan28 under salt stress (Nahar *et al*., 2021). It is also reported that the reduction of plant phenotypes such as root-shoot length, plant height, yield etc. is higher in sensitive plants (Barrachina *et al.*, 1995; Knauer *et al.*, 1999). BRRI dhan28 is a salt-sensitive plant. Therefore, it might be other heavy-metal sensitive plant.

To dig into the differences between arsenic-induced changes in purple rice and BRRI dhan28, the effects of arsenic stress on the biochemical parameters such as proline, catalase activity, and plant pigments of both rice were observed. Proline is an important compatible solute which plays diversified roles in plants. There is no clear consensus is devised about the role of proline in plants. The mechanism by which proline ameliorates heavy metal stresses in plants is still unclear. The diversified role (positive, negative and no effect) of proline has been reported in previous studies regarding the mitigation of stresses in plants. For example, proline mitigates salt stress (Hoque *et al*., 2007) and cadmium stress in BY-2 cells (Islam *et al*., 2009). However, the negative role of proline was also reported in some cases *e.g.,* proline shows toxicity to plants (Hellmann *et al.*, 2000; Deuschle *et al*., 2001) and proline inhibits the growth of saltgrass (*Distichlis spicata*) (Rodriguez and Heyser, 1988). In the present study, our data showed that the proline content was higher in purple rice than in BRRI dhan28 without arsenic stress and proline was decreased in BRRI dhan28 with the increase of arsenic concentration but did not change in purple rice. It is also reported that the accumulation of proline in plants under stress condition is not associated with the mitigation of stress but it is just a symptom (Liu and Zhu, 1997) and did not show any protective value (Moftah and Michel, 1987). Though proline mitigates heavy metal stresses in plants but it is not a suitable osmoticum for the mitigation of arsenic stress (Nahar *et al.,* 2017). The utilization of proline in stress-tolerance variety and in stress-susceptible variety is different. The previous reports with our findings suggest that the mitigatory role of proline might depend on some conditions such as type of stress, and plant genotypes. In this study (Fig.3), though proline is catabolized in BRRI dhan28 under arsenic stress but it was either just a symptom (Liu and Zhu, 1997) or influenced the antioxidant homeostasis without decreasing its concentration. Whether proline is involved or how proline protected the root length and shoot length reduction in purple rice without changing its concentration needs to be further investigated.

Catalase is an important antioxidant enzyme that plays role under stress conditions. Catalase is a heme-containing enzyme found in different parts of plants and is capable of breaking down H_2O_2 into water and molecular oxygen (Noctor and Foyer, 1998; Sharma (2012). It is one of the most important H_2O_2 scavengers (Mhamdi *et al*., 2010 and Hasanuzzaman *et al.,* 2012). It protects the cell with energy-efficient mechanism to remove hydrogen peroxide. Higher activity of catalase has been shown in arsenic-tolerant plants than in arsenic-sensitive plants (Srivastava *et al.,* 2005; Mylona *et al.*, 1998). In contrast, arsenic-induced decline in catalase activity has also been reported in Mung bean and *Taxithelium nepalense* (Singh *et al.,* 2007). Compared to the control, increased catalase activity was observed in both purple rice and BRRI dhan28 under arsenic stress; but purple rice showed higher (0.781 and 1.256 µmole/min/g fresh wt.) catalase activity than BRRI dhan28 (0.635 and 0.983 µmole/min/g fresh wt.) at 100 and 500 µM arsenic, respectively (Fig.4). Increased level of catalase activity under arsenic stress indicated that catalase is highly expressive in rice and takes part in ROS detoxification (Nath *et al.,* 2014). Higher level of catalase activity in purple rice might indicate the more ROS detoxification capacity of purple rice than BRRI dhan28.

Anthocyanin acts as an antioxidant in plants (Maulani *et al.,* 2019). Plants that have anthocyanin-containing plant parts such as leaves, stems or roots become resistant to different environmental stresses (Kiełkowska *et al.,* 2019). In this study, only purple rice showed anthocyanin content (Fig. 5) which is in line with the results of Chin *et al.* (2016) and Nahar *et al.* (2021) who reported that green rice did not contain anthocyanin. Here, purple rice showed increased anthocyanin content with the increase of arsenic stress. It is reported that the presence of high anthocyanin increases the stress endurance capability of crop plants (Eryılmaz, 2006 and Kiełkowska *et. al*., 2019). Therefore, the presence of higher amount of anthocyanin in purple rice indicates its more antioxidant potentiality than BRRI dhan28.

Chlorophyll and carotenoid contents are important abiotic stress indicators. The total chlorophyll and carotenoid contents were higher in purple rice than BRRI dhan28 at both non-stress and 500 µM arsenic stress condition (Tab. I). Previous report suggests that arsenic stress causes a reduction in chlorophyll content of rice and eggplants (Singh *et al.,* 2015; Rahman *et al.,* 2015; Gaikwad, *et al.,* 2020 and Mahajan *et al.,* 2023). BRRI dhan28 also showed reduction of chlorophyll and carotenoid contents under arsenic stress compared to both control and purple rice. Though the chlorophyll and carotenoid content were higher in purple rice compared to BRRI dhan28 but no significant change was observed in 0 µM and 500 µM in either genotype suggesting that these green pigments were not involved in arsenic tolerance in this study.

Conclusion

The ability of less reduction of root length and plant height in purple rice was might be due to higher antioxidant potentiality that was achieved by the higher activity of catalase and levels of anthocyanin. In purple rice under arsenic stress, whether proline is involved or just a symptom or accelerated the antioxidant system needs to be further investigated. The previous salt stress report and present arsenic stress data suggest that purple rice may have more potential to protect its cell destruction by abiotic stress than BRRI dhan28. To further elucidate the arsenic stress tolerance potentiality of purple rice, molecular analysis such as expression of stress-responsive genes, measurement of DNA damage and H_2O_2, and up-regulation of the components of antioxidant defense system is required.

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References

Aebi, H. 1984. Catalase in vitro. Methods in enzymology,105:121-126. http://dx.doi.org/10.1016/S0076-6879(84)05016-3

Aggarwal, M., Sharma, S., Kaur, N., Pathania, K., Kaushal, R., Kaur, K., Singh, A., Srivastava, Nayyar, H. 2011. Exogenous Proline Application Reduces Phytotoxic Effects of Selenium by Minimising Oxidative stress and Improves Growth in Bean (*Phaseolus vulgaris* L.) Seedlings. *Biological Trace Element Research*, 140:354–367. https://doi.org/10.1007/s12011-010-8699-9

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology,* 24(1): 1-15. https://doi.org/10.1104/pp.24.1.1

Bates, L.S., Waldren, R.P. and Teare, I.D. 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil*, 39: 205–207. https://doi.org/10.1007/BF00018060

Barrachina, A.C., Carbonell, F.B. and Beneyto, J.M. 1995. Arsenic uptake, distribution, and accumulation in tomato plants: effect of arsenite on plant growth and yield. *Journal of plant nutrition*, *18*(6):1237-1250. https://doi.org/10.1080/01904169509364975

Bhusan, D., Das, D.K., Hossain, M., Murata, Y., Hoque, M.A. 2016. Improvement of salt tolerance in rice (*Oryza sativa* L.) by increasing antioxidant defense systems using exogenous application of proline. *Australian Journal of Crop Science*, 10(1): 50-56.

Chandrakar, V., Dubey, A. and Keshavkant, S. 2016. Modulation of antioxidant enzymes by salicylic acid in arsenic exposed Glycine max L. *Journal of soil science and plant nutrition*, 16(3): 662-676. http://dx.doi.org/10.4067/S0718-95162016005000048

Cheeseman, J.M. 2006. Hydrogen peroxide concentrations in leaves under natural conditions. *Journal of Experiment Botany*, 57 (10): 2435-2444. https://doi.org/10.1093/jxb/erl004

Chin, H.S., Wu, Y.P., Hour, A.L., Hong, C.Y., Lin, Y.R. 2016. Genetic evolutionary analysis of purple leaf sheath in rice. Rice, 9 (8): 1-14. https://doi.org/10.1186/s12284-016-0080-y

Deuschle, K., Funck, D., Hellmann, H., Däschner, K., Binder, S. and Frommer, W.B. 2001. A nuclear gene encoding mitochondrial Δ1-pyrroline-5-carboxylate dehydrogenase and its potential role in protection from proline toxicity. *The Plant Journal*, 27(4): 345-356. doi.org/10.1046/j.1365-313X.2001.01101.x

Eryılmaz, F. 2006. The relationships between salt stress and anthocyanin content in higher plants. Biotechnology and Biotechnological Equipment, 20:47–52. https://doi.org/10.1080/13102818.2006.10817303

Flora, S.J. 1999. Arsenic-induced oxidative stress and its reversibility following combined administration of n-acetylcysteine and meso 2, 3–dimercaptosuccinic acid in rats. Clinical and Experimental Pharmacology and Physiology, *26*(11): 865-869. doi.org/10.1046/j.1440-1681.1999.03157.x

Gaikwad, D.J., Pal, A., Mondal, S. 2020. Effect of arsenic toxicity on chlorophyll content and antioxidative enzymes activites in rice seedlings. *International Journal of Environment and Climate Change*, 10(12):411–421.

Hasanuzzaman, M., Hossain, M.A., Teixeira da Silva, J.A., Fujita, M. 2012. Plant responses and tolerance to abiotic oxidative stress: antioxidant defense is a key factor. In: V. Bandi, A.K.Shanker, C. Shanker, M. Mandapaka (Eds), Crop Stress and Its Management: Perspectives and Strategies, pp. 261–315. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-2220-0_8

Hellmann, H., Funck, D., Rentsch, D. and Frommer, W.B. 2000. Hypersensitivity of an Arabidopsis sugar signaling mutant toward exogenous proline application. Plant Physiology, *122*(2): 357-368. https://doi.org/10.1104/pp.122.2.357

Hossain, M.A., Hoque, M.A., Burritt, D.J. and Fujita, M. 2014. Proline protects plants against abiotic oxidative stress: Biochemical and Molecular Mechanisms. In: P. Ahmad (Ed.), Oxidative Damage to Plants: Antioxidant Networks and Signaling. pp. 477-522.

Hoque, M.A., Banu, M.N.A., Okuma, E., Amako, K., Nakamura, Y., Shimoishi, Y., Murata, Y. 2007.Exogenous proline and glycinebetaine increaseNaCl-induced ascorbate–glutathione cycle enzyme activities, and proline improves salt tolerance more than glycinebetaine in tobacco Bright Yellow-2 suspension-cultured cells. *Journal of Plant Physiology,* 164:1457–1468. doi: 10.1016/j.jplph.2006.10.004

Islam, M.M., Hoque, M.A., Okuma, E., Banu, M.N.A., Shimoishi, Y., Nakamura, Y. and Murata, Y. 2009. Exogenous proline and glycinebetaine increase antioxidant enzyme activities and confer tolerance to cadmium stress in cultured tobacco cells. *Journal of plant physiology*, *166*(15):1587-1597. https://doi.org/10.1016/j.jplph.2009.04.002

Jang, S. and Xu, Z. 2009. Lipophilic and hydrophilic antioxidants and their antioxidant activities in purple rice bran. *Journal of Agricultural and Food Chemistry,* 57 (3): 858-862. https://doi.org/10.1021/jf803113c

Kiełkowska, A. Grzebelus, E. Krzyścin, A.L., Maćkowska, K. 2019. Application of the salt stress to the protoplast cultures of the carrot (*Daucus carota* L.) and evaluation of the response of regenerants to soil salinity. Plant Cell, Tissue and Organ Culture, 137:379-395. https://doi.org/10.1007/s11240-019-01578-7

Knauer, K. Behra, R. and Hemond, H. 1999. Toxicity of inorganic and methylated arsenic to algal communities from lakes along an arsenic contamination gradient. Aquatic Toxicology, *46*(3-4): 221-230. https://doi.org/10.1016/S0166-445X(98)00131-3

Lange, H., Shropshire, W.Jr., Mohr, H. 1971. An analysis of phytochrome-mediated anthocyanin synthesis. Plant Physiology, 47 (5): 649-655. https://doi.org/10.1016/j.ecoenv.2007.11.016

Liu, J. and Zhu, J.K.1997. Proline accumulation and salt-stress-induced gene expression in a salt-hypersensitive mutant of Arabidopsis. Plant Physiology, 114(2): 591-596. doi: 10.1104/pp.114.2.591

Liu, X.L., Zhang, S.Z., Shan, X.Q., Christie, P. 2007. Combined toxicity of cadmium and arsenate to wheat seedlings and plant uptake and antioxidative enzyme responses to cadmium and arsenate co-contamination. Ecotoxicology and Environmental Safety, 68:305-313. https://doi.org/10.1016/j.ecoenv.2006.11.001

Mahajan, M., Nazir, F., Jahan, B., Siddiqui, M.H., Iqbal, N., Khan, M.I.R. 2023. Salicylic acid mitigates arsenic stress in Rice (*Oryza sativa*) via Modulation of Nitrogen–Sulfur Assimilation, Ethylene Biosynthesis, and Defense systems. Agriculture, 13(7):1293. https://doi.org/10.3390/agriculture13071293

Mascher, R., Lippmann, B., Holzinger, S. and Bergmann, H. 2002. Arsenate toxicity: effects on oxidative stress response molecules and enzymes in red clover plants. Plant Science, 163(5): 961-969. https://doi.org/10.1016/S0168-9452(02)00245-5

Maulani, R.R., Sumardi, D., Pancoro, A. 2019. Total flavonoids and anthocyanins content of pigmented rice. Drug Invension Today, 12(2): 369-373.

Meharg, A.A. 1994. Integrated tolerance mechanisms: constitutive and adaptive plant responses to elevated metal concentrations in the environment. Plant, Cell and Environment, 17(9): 989-993. https://doi.org/10.1111/j.1365-3040.1994.tb02032.x

Mhamdi, A., Queval, G., Chaouch, S., Vanderauwerav, S., Breusegem, F van., Noctor, G. 2010. Catalase function in plants: a focus on *Arabidopsis* mutants as stress-mimic models. *Journal of Experimental Botany*, 61(15): 4197-4220.
https://doi.org/10.1093/jxb/erq282

Moftah, A.E., Michel, B.E.1987. The effect of sodium chloride on solute potential and proline accumulation in soybean leaves. Plant Physiology, 83(2): 238-240. doi: 10.1104/pp.83.2.238

Mylona, P.V., Polidoros, A.N. and Scandalios, J.G. 1998. Modulation of antioxidant responses by arsenic in maize. Free Radical Biology and Medicine, 25(4-5): 576-585. https://doi.org/10.1016/S0891-5849(98)00090-2

Nahar, M.A., Nahar, M.N.N., Prodhan, M.Y., Hoque, M.A., Rahman, M.S. 2021 Comparative Study of Purple Rice and Green Rice for Salt Stress Sensitivity. *Journal of Bangladesh Agricultural University*, 19(1): 53–60. https://doi.org/10.5455/JBAU.51377

Nahar, M.N.E.N., Islam, M.M., Hoque, M.A., Yonezawa, A., Prodhan, M.Y., Nakamura, T., Nakamura, Y., Munemasa, S., Murata, Y. 2017. Exogenous proline enhances the sensitivity of Tobacco BY-2 cells to arsenate. Bioscience Biotechnology and Biochemistry, 81:1726–1731. https://doi.org/10.1080/09168451.2017.1340088

Nath, S., Panda, P., Mishra, S., Dey, M., Choudhury, S., Sahoo, L., Panda, S.K. 2014. Arsenic stress in rice: redox consequences and regulation by iron. Plant Physiology and Biochemistry, 80:203210. http://dx.doi.org/10.1016/j.plaphy.2014.04.013

Noctor, G., Foyer, C.H. 1998. Ascorbate and glutathione: keeping active oxygen under control. Annual Review of Plant Physiology and Plant Molecular Biology, 49: 249–279. http://dx.doi.org/10.1146/annurev.arplant.49.1.249

Ozturk, F., Duman, F., Leblebici, Z., Temizgul, R. 2010. Arsenic accumulation and biological responses of watercress (*Nasturtium officinale* R. Br.) exposed to arsenite. *Environmental and Experimental Botany, 69*:167–174. https://doi.org/10.1016/j.envexpbot.2010.03.006

Panaullah, G.M., Alam, T., Hossain, M.B., Loeppert, R.H., Lauren, J.G., Meisner, C.A., Ahmed, Z.U., Duxbury, J.M. 2009. Arsenic toxicity to rice (*Oryza sativa* L.) in Bangladesh. Plant Soil, 317:31–39.

Porra, R. J., Thompson, W. A., Kriedemann, P. E. 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll II standards by atomic absorption spectroscopy. Biochimica Biophysica Acta-Bioenergetics, 975 (3): 384–394. https://doi.org/10.1016/S0005-2728(89)80347-0

Rahman, A., Mostofa, M.G., Alam, M.M., Nahar, K., Hasanuzzaman, M., Fujita, M. 2015. Calcium mitigates arsenic toxicity in rice seedlings by reducing arsenic uptake and modulating the antioxidant defense and glyoxalase systems and stress markers. Biomed Research International, 2015:1–12. https://doi.org/10.1155/2015/340812

Rodriguez, M.M. and Heyser, J.W. 1988. Growth inhibition by exogenous proline and its metabolism in saltgrass (*Distichlis spicata*) suspension cultures. Plant cell reports, 7: 305-308.

Serraj, R.A.C.H.I.D. and Sinclair, T.R. 2002. Osmolyte accumulation: can it really help increase crop yield under drought conditions?. Plant, cell & environment, 25(2): 333-341. doi.org/10.1046/j.1365-3040.2002.00754.x

Sharma, I. 2012. Arsenic induced oxidative stress in plants. Biologia, 67:447–453. doi:10.2478/s11756-012-0024-y

Shri, M., Kumar, S., Chakrabarty, D., Trivedi, P.K., Malick, S., Mishra, P., Shukla, D., Mishra, S., Srivastava, S., Tripathi, R.D. and Tuli, R. 2009. Effect of arsenic on growth, oxidative stress, and antioxidant system in rice seedling. Ecotoxicology and Environmental Safety, 72: 1102–1110. https://doi.org/10.1016/j.ecoenv.2008.09.022

Singh, H.P., Batish, D.R., Kohali, R.K. and Arora, K. 2007. Arsenic-induced root growth inhibition in mung bean (*Phaseolus au-reus* Roxb) is due to oxidative stress resulting from enhanced lipid peroxidation. Plant Growth Regulation, 53: 65–73. https://doi.org/10.1007/s10725-007-9205-z

Singh, V.P., Srivastava, P.K., Prasad, S.M. 2013. Nitric oxide alleviates arsenic-induced toxic effects in ridged Luffa seedlings. *Plant Physiology and Biochemistry*, 71:155–163.

Singh, M., Singh, V.P., Dubey, G., Prasad, S.M. 2015. Exogenous proline application ameliorates toxic effects of arsenate in *Solanum melongena* L. seedlings. *Ecotoxicology and Environmental Safety*, 117:164-173. https://doi.org/10.1016/j.ecoenv.2015.03.021

Srivastava, M., Ma, L.Q., Singh, N. and Singh, S., 2005. Antioxidant responses of hyper-accumulator and sensitive fern species to arsenic. *Journal of Experimental Botany*, *56*(415), pp.1335-1342.

Szepesi, Á., Szőllősi, R. 2018. Mechanism of proline biosynthesis and role of proline metabolism enzymes under environmental stress in plants. In: Plant Metabolites and Regulation Under Environmental Stress. Edited by Ahmad, P., Ahanger, M.A., Singh, V.P., Tripathi, D.K., Alam, P., Alyemeni, M.N.: Academic Press, p. 337–53.

Talukdar, D. 2012. Exogenous calcium alleviates the impact of cadmium-induced oxidative stress in *Lens culinaris* Medic. seedlings through modulation of antioxidant enzyme activities. *Journal of Crop Science and Biotechnology*, 15:325–334.

Tripathi, R.D., Srivastava, S., Mishra, S., Singh, N., Tuli, R., Gupta, D.K., Maathuis, F.J. 2007. Arsenic hazards: strategies for tolerance and remediation by plants. Trends in Biotechnology, 25:158-165. https://doi.org/10.1016/j.tibtech.2007.02.003

Yadav, G., Srivastava, P.K., Singh, V.P., Prasad, S.M. 2014. Light intensity alters the extent of arsenic toxicity in *Helianthus annuus* L. Biological Trace Element Research, 158:410–421.

Yingli, Y., Yang, L., Yuanyuan, Y., Zhang, Xueling, X., Wei, J., You, J., Wenrui, W., Wang, L., Ruxia, R., Shi, A. 2011. Comparative antioxidative responses and proline metabolism in two wheat cultivars under short term lead stress. Ecotoxicology and Environmental Safety, 74: 4–8.