J Bangladesh Agril Univ 19(1): 53–60, 2021 https://doi.org/10.5455/JBAU.51377

ISSN 1810-3030 (Print) 2408-8684 (Online)

Journal of Bangladesh Agricultural University

Journal home page: http://baures.bau.edu.bd/jbau

Comparative Study of Purple Rice and Green Rice for Salt Stress Sensitivity

Most. Aynun Nahar[1], Mst. Nur-E-Nazmun Nahar[1]✉, Md. Yeasin Prodhan[1], Md. Anamul Hoque[2], Md. Shazadur Rahman[3]

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

[2]Department of Soil Science, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

[3]Department of Agricultural Chemistry, Hajee Mohammad Danesh Science and Technology University, Dinajpur 5200, Bangladesh

ARTICLE INFO

Article history
Received: 02 Feb 2021
Accepted: 14 Mar 2021
Published: 30 Mar 2021

Keywords
Purple rice, Anthocyanin, Proline, Catalase, Salt stress

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

ABSTRACT

Farmers of Bangladesh are cultivating an unidentified so-called purple rice (PR) having purple-colored leaf. Less information is available on PR in any areas of research in Bangladesh and the information about salt-stress tolerance potentiality of PR is absent. Here, we investigated salt stress-induced changes in PR to unravel its stress tolerance potentiality. The results of PR were compared with those of green rice (BRRI dhan28). The effects of salt stress (*e.g.,* 0 mM, 50 mM, 100 mM, and 150 mM NaCl) on both PR and BRRI dhan28 were observed. Under salt stress condition, the shoot and root lengths of PR and BRRI dhan28 were decreased compared to control but the percent reduction of shoot and root length of PR was significantly lowered than that of BRRI dhan28; the number of live leaves per tiller was higher in PR than BRRI dhan28; the plant height of PR was significantly lower than BRRI dhan28. The results of biochemical analysis showed that the proline content in PR was higher in non-stress condition but was lower at 100 mM and 150 mM NaCl stress compared to BRRI dhan28. Catalase activity and anthocyanin content were significantly higher in leaves of PR than those of BRRI dhan28 in both salt-stress and non-stress conditions. Moreover, total chlorophyll content was higher in PR than BRRI dhan28 in control condition. Taken together, these results suggest that PR rice has higher salt tolerance potentiality than green rice (BRRI dhan28).

Introduction

Green rice is chiefly cultivating in the world including Bangladesh. Recently, farmers of Bangladesh are growing a rare rice cultivar called purple rice (PR) that draws the attraction of farmers and media personnel for its beautiful leaf and stem color. This rice is rich in anthocyanin and has high antioxidant property (Jang and Xu, 2009). It is reported that the high amount of anthocyanin in leaf tissues allows the plant to develop resistance against several environmental stresses such as salinity (Eryılmaz, 2006; Kiełkowska *et al*., 2019).

Salinity is one of the major problems of rice production in Bangladesh. To our knowledge, no information is available regarding the stress-tolerance potentiality of this unknown PR. The sensitivity of rice to salinity varies with its different growth stages such as germination, seedling, and booting, etc. Therefore, the proposed study was aimed to investigate the salt stress-induced changes in PR to unravel its stress tolerance potentiality.

To understand salt stress tolerance capacity of a rice variety, it is essential to observe it's morphological and biochemical changes occurred when exposed to saline condition. The salt stress-induced morphological and biochemical changes in PR and BRRI dhan28 were investigated in this study. The root and shoot length reductions, height of plants and number of live leaves per tiller under salt stress condition were measured as morphological parameters. Accumulation of compatible solutes in plants is one of the main adaptive mechanisms to salt stress. Proline, a compatible solute, protects plants from damaging effects of various stresses (Ashraf and Foolad, 2007; Hossain *et al.*, 2014). It eliminates oxidative stress by triggering the antioxidant defense system that improves salt tolerance in plants (Patade *et al.*, 2014).

Salinity also regulates enzyme activities (Dubey, 1994) such as catalase that is a H_2O_2 scavenger in plants under salt stress conditions (Nor'aini *et al*., 1997; Cheeseman, 2006).

Cite This Article

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

Chlorophyll (chl) acts as a reactive oxygen species (ROS) scavenger under stress conditions by using defensive compounds of carbon (Agathokleous *et al.*, 2020; Hu *et al.*, 2015) and carotenoid also acts as an antioxidant that improves the defense mechanisms of plants under salt stress by eliminating ROS (Uarrota *et al.*, 2018). Here, the effects of salt stress on the accumulation of proline, levels of chl and carotenoid, as well as activity of catalase enzyme were examined in PR and BRRI dhan28 to test their stress tolerance potentiality.

Materials and Methods

Location of experiment

The experiment was carried out in the laboratory of the Department of Biochemistry and Molecular Biology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. A pot experiment was carried out from November 2018 to April 2019. In this experiment, PR (a new rice cultivar) and BRRI dhan28 were used.

Measurement of shoot and root lengths

Rice seeds were germinated in Petri dishes containing filter paper. Then the seeds were exposed to 0 mM, 50 mM, 100 mM, and 150 mM NaCl stress. The shoot and root lengths of seedlings were measured on 9 days after germination.

Treatments and transplantation of seedlings

Seedlings of PR and BRRI dhan28 were transplanted into plastic pots filled with 6 kg soil in each. Soil nutrient management was done according to Bhusan *et al*. (2016). Three seedlings of 30-day-old were transplanted in a single hill. At active tillering stage (35 days after transplanting; DAT), different concentrations of salinity (50 mM, 100 mM, and 150 mM) were applied. The morphological parameters such as plant height at 35 days after salt treatment (DAST) and number of live leaves/tiller at 15 and 35 DAST were measured. Leaf samples were collected at booting stage for analysis of biochemical parameters.

Measurement of anthocyanin content

The anthocyanin content was measured according to Lange *et al.* (1970). An aliquot amount of leaf sample was soaked in acidified methanol, immersed in boiling water for 1.5 minutes, and kept in the dark for 24 h at room temperature. The sample was centrifuged at 5,000 g for 40 minutes and the absorbance of supernatant was taken at 535 and 650 nm.

The amount of anthocyanin was calculated by following Rayleigh's formula:

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

Determination of chlorophyll and carotenoid contents

Chlorophyll content was measured according to Porra *et al.* (1989). An aliquot amount of fresh leaf was suspended in 80% acetone, mixed well, and kept at room temperature in the dark for 48 h. The absorbance of the supernatant was recorded at 645, 663, and 470 nm wavelengths using a spectrophotometer. The chl_a, chl_b, total chl, and total carotenoid contents were determined following the formula given by Arnon (1949):

$Chl_a = 12.21A_{663} - 2.81A_{646}$ (µg/ml of plant extract)
$Chl_b = 20.13A_{646}-5.03A_{663}$ (µg/ml of plant extract)
Total chl = $Chl_a + Chl_b$ (µg/ml of plant extract)
Total carotenoid = {(1000 A_{470} – 3.27 Chl_a - 104 Chl_b)/ 229} (µg/ml of plant extract)

Estimation of endogenous proline content

Proline content was measured according to Bates *et al.* (1973). Leaf sample was homogenized with 3% aqueous sulphosalicylic acid and centrifuged at 12,000 g for 10 minutes. The supernatant was then added to acid-ninhydrin and glacial acetic acid. After incubation at 100 ℃ for 1 h, the colored reaction mixture was extracted with toluene. The absorbance was recorded at 520 nm.

Determination of catalase activity

Aliquot amount of fresh leaf was homogenized with potassium phosphate buffer (pH = 8.0). The homogenate was centrifuged at 12000 rpm for 10 minutes and then supernatant was collected. Catalase (EC 1.11.1.6) activity was measured according to Aebi (1984). Ethylenediaminetetraacetic acid, H_2O_2, and potassium phosphate buffer (pH = 8.0) were taken in a tube and mixed well. The reaction was started by the addition of enzyme extract to the mixture. The changes in absorbance were recorded immediately at 240 nm wavelength at 30 seconds intervals for 2 minutes.

Statistical analysis

The significance of differences between mean values of all experiments was assessed by Tukey's test using R software. Differences at the level of $p \leq 0.05$ were considered as significant.

Results

Salinity-induced changes of phenotypic characters of purple rice and BRRI dhan28

The effects of salt stress on some morphological parameters of PR and BRRI dhan28 were observed. The shoot length reduction was increased with increasing salt stress in both PR and BRRI dhan28 (Fig. 1A). The shoot length reductions of PR at 50, 100, and 150 mM salt stress were 14.34%, 46.85%, and 69.93%, respectively, which was significantly lower than that of BRRI dhan28 (24.41%, 57.33%, and 85.13% at 50, 100, and 150 mM salt stress, respectively) (Fig. 1A).

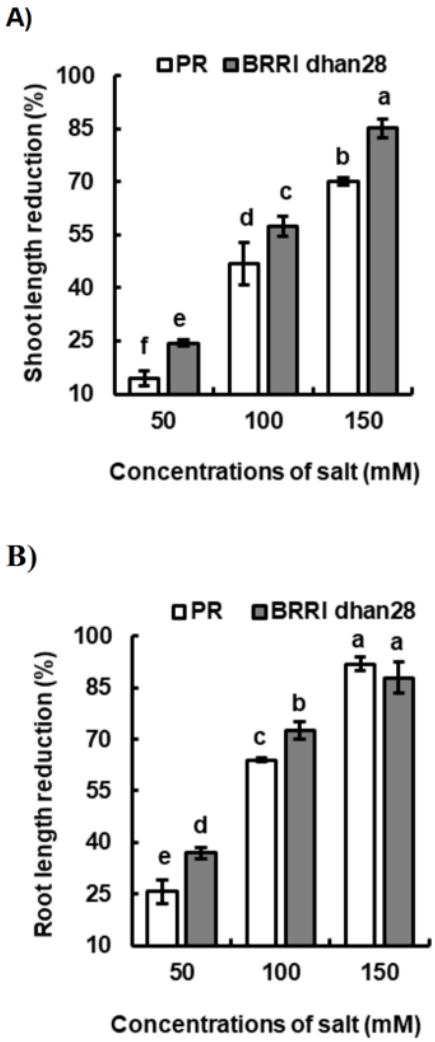

Figure 1. Effects of salt stress on shoot length (A) and root length (B) of purple rice (PR) and BRRI dhan28 at 9th day of germination in response to 50, 100, and 150 mM NaCl. Error bars represent standard errors. Values indicated by the same letter do not differ significantly at 5% level of significance as determined by Tukey test.

The root length reduction was also increased in both PR and BRRI dhan28 under salt stress but the reduction rate was significantly lower in PR (25.65% and 63.90%) than those of BRRI dhan28 (36.91% and 72.55%) at 50 mM and 100 mM salt stress, respectively (Fig. 1B). Salinity caused significant difference in plant height of PR at 35 days after transplanting (DAT) (Fig. 2). Plant height of PR was significantly lower than BRRI dhan28 under both non-stressed (control) and stressed conditions. The plant heights were 27.59, 26.38, 26.39, and 28.34 cm for PR rice and 29.71, 30.49, 30.92, and 32.66 cm for BRRI dhan28 at 0, 50, 100, and 150 mM NaCl, respectively.

At 50 mM and 100 mM NaCl conditions, the number of live leaves/tiller of PR was higher than BRRI dhan28 at both 15 and 35 days after salt treatment (DAST) (Fig. 3). On the other hand, BRRI dhan28 contained increased number of live leaves/tiller than PR at 35 DAST but decreased at 15 DAST at 150 mM salt conditions. The number of live leaves/tiller was higher in PR than BRRI dhan28 under control as well as salt-stressed conditions both at 15 and 35 DAST (Fig. 3).

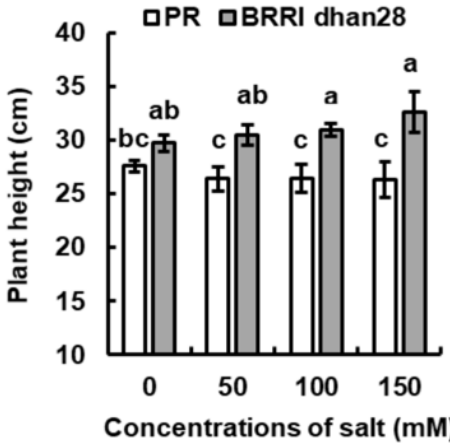

Figure 2. Plant height of purple rice (PR) and BRRI dhan28 in response to 0, 50, 100, and 150 mM salt stresses at 35 days after salt treatment. Error bars represent standard errors. Values indicated by the same letter do not differ significantly at 5% level of significance as determined by Tukey test.

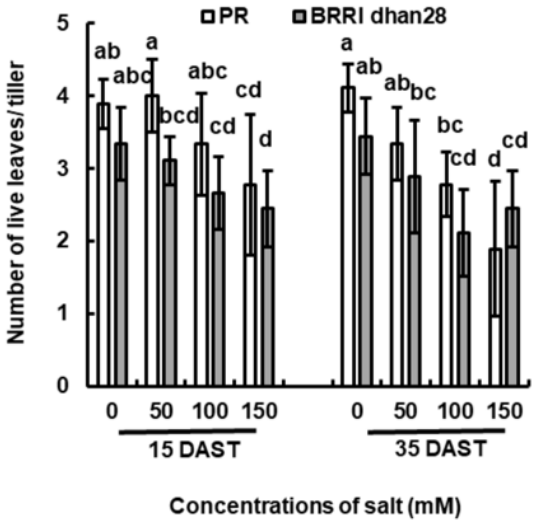

Figure 3. Effects of salt stress on number of live leaves per tiller of purple rice (PR) and BRRI dhan28. Number of live leaves per tiller was counted at 15 and 35 days after salt treatment (DAST). Error bars represent standard errors. Values indicated by the same letter do not differ significantly at 5% level of significance as determined by Tukey test.

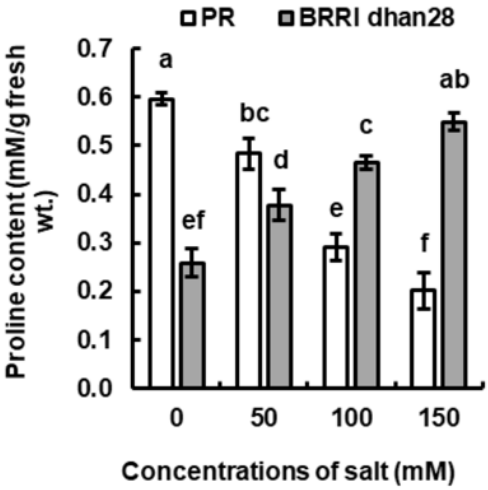

Figure 4. Salt stress-induced changes of proline content in purple rice (PR) and BRRI dhan28 in response to 0, 50, 100, and 150 mM NaCl. Error bars represent standard errors. Values indicated by the same letter do not differ significantly at 5% level of significance as determined by Tukey test.

Effects of NaCl on endogenous proline content

Proline functions as an antioxidant to protect cells against various abiotic stresses such as salt stress (Hasegawa *et al.*, 2000; Okuma *et al.*, 2004; Banu *et al.*, 2010). In our study, PR showed higher proline content (0.60 mM/g fresh wt.) than BRRI dhan28 (0.26 mM/g fresh wt.) under controlled condition (Fig. 4). On the contrary, PR showed decreased (*i.e.* 0.6, 0.48, 0.29, and 0.20 mM/g fresh wt.) and BRRI dhan28 showed increased (*i.e*. 0.26, 0.38, 0.46, and 0.55 mM/g fresh wt.) proline content with increasing salinity level (*i.e.* at 0, 50, 100, and 150 mM salinity), respectively (Fig. 4). At control and 50 mM NaCl, the proline content in PR was more than BRRI dhan28 but lower at 100 and 150 mM NaCl. Accumulation of endogenous proline did not show any fixed trend rather it varied with the increase of salt concentration (Fig. 4).

Effects of NaCl on catalase activity

To investigate whether salinity influenced antioxidant defense system, major ROS-scavenging antioxidant enzyme such as catalase activity was determined at NaCl stress in PR and BRRI dhan28. Catalase activity was significantly affected in response to salinity stress. The activity of catalase enzyme was significantly higher in PR (1.1 and 0.9 µmole/min/g fresh wt.) than BRRI dhan28 (0.4 and 0.1 µmole/min/g fresh wt.) in both control as well as salt stress (100 mM) conditions, respectively (Fig. 5).

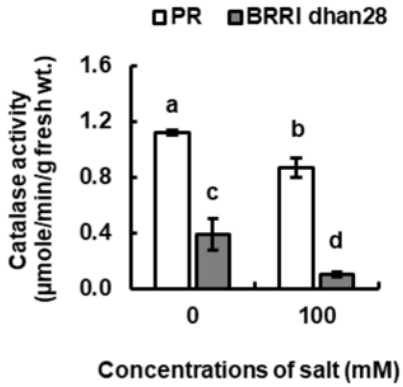

Figure 5. Activity of catalase in purple rice (PR) and BRRI dhan28 in control and 100 mM salt stress conditions. Error bars represent standard errors. Values indicated by the same letter do not differ significantly at 5% level of significance as determined by Tukey test.

Effects of NaCl on leaf pigments

Chlorophyll content is an important index for determining salt stress (Munns, 1993). Different salinity stress showed variation in chl and carotenoid contents in PR and BRRI dhan28 (Fig. 6). Chlorophyll-a content of PR was decreased (3.94 µg/ml of plant extract) at 50 mM, increased (17.27 µg/ml of plant extract) at 100 mM and similar (6.26 µg/ml of plant extract) at 150 mM salt stress compared to control (7.31 µg/ml of plant extract).

Chlorophyll-b content of PR was statistically similar (0.85µg/ml of plant extract) at 50 mM and increased at 100 mM, and 150 mM (5.25 and 2.48 µg/ml of plant extract, respectively) salt stress, compared to control (1.48 µg/ml of plant extract). The total chl and total carotenoid contents of PR significantly increased at 100 mM salinity but statistically similar at 50 and 150 mM salt concentrations compared to control. In BRRI dhan28, the contents of chl_a (4.86, 11.83, 15.03, and 16.08 µg/ml of plant extract), chl_b (1.05, 2.25, 2.93, and 3.07 µg/ml of plant extract), total chl (5.91, 14.08, 17.96, and 19.15 µg/ml of plant extract), and total carotenoid (1.7, 3.64, 4.36, and 4.54 µg/ml of plant extract) were increased at all salt concentrations (*i.e.* at 50, 100, and 150 mM salinity) compared to control. Total chl and total carotenoid content of PR were slightly higher than BRRI dhan28 at control and 100 mM salt concentration (Fig. 6).

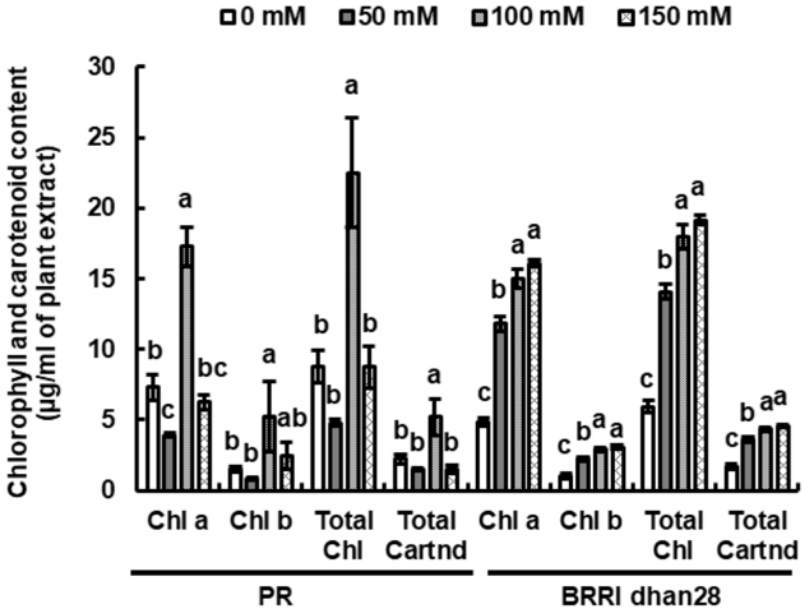

Figure. 6. Salinity-induced changes of pigment contents in leaves of purple rice (PR) and BRRI dhan28. The contents of chlorophylls and carotenoids were measured in response to 0, 50, 100, and 150 mM salt stresses. Error bars represent standard errors. Values indicated by the same letter do not differ significantly at 5% level of significance as determined by Tukey test.

Effects of salinity on anthocyanin content

Anthocyanin content is an important component of plant antioxidant activity. Here we measured the anthocyanin content and found that it was present in PR but not detected in BRRI dhan28. Anthocyanin content was significantly decreased in response to 100 mM (0.46 O.D., 535 nm) and 150 mM (1.25 O.D., 535 nm) NaCl compared to control (1.59 O.D., 535 nm) (Fig. 7).

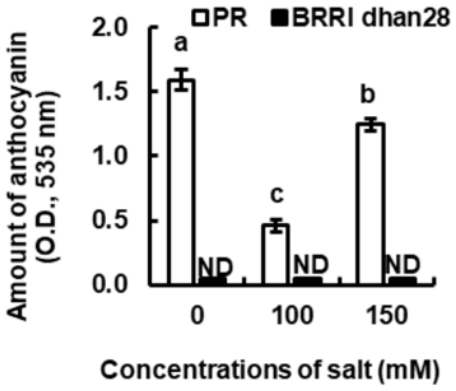

Figure 7. Salt stress-induced changes of anthocyanin content in leaves of purple rice (PR) and BRRI dhan28 in response to 0, 100, and 150 mM salt stresses. The anthocyanin content was expressed as O.D. (optical density) 535 nm. The ND indicated the anthocyanin content was not detected. Error bars represent standard errors. Values indicated by the same letter do not differ significantly at 5% level of significance as determined by Tukey test.

Discussion

To find out the salt-stress tolerance potentiality of purple rice (PR), a new cultivar, here we examined the effects of salt stress on PR, and the results were compared with BRRI dhan28 which is a commonly cultivating rice variety in Bangladesh. Morphological traits of crops such as shoot and root lengths provide important indications of plants response to salt stress. In the present study, both PR and BRRI dhan28 showed increasing trend of shoot and root lengths reduction with the increase of salt stress but PR represented the lower rate of root and shoot lengths reduction than BRRI dhan28 (Fig. 1A and B).

These results were alike with the findings of Djanaguiraman *et al.* (2003) and Hakim *et al.* (2010). Report suggests that the suppression of shoot and root growth are the most important evidence of salt tolerance (Tuna *et al.*, 2008). Salt stress reduced the lengths of root and shoot in both PR and BRRI dhan28 but the percent reduction in PR was lower, suggesting the more salt stress tolerance potential of the PR than BRRI dhan28. The number of live leaves/tiller was significantly higher in PR than BRRI dhan28 under control as well as salt stress condition (Fig. 3). The higher number of live leaves/tiller in PR may be due to higher salt-stress tolerance capability than BRRI dhan28.

Plant antioxidant defense system is inhibited by salt stress, whereas proline enhanced this defense system against NaCl-induced damage (Hossain and Fujita, 2010; Hasanuzzaman *et al.*, 2014). In response to stress, proline is accumulated in plants and then plays role to mitigate stress. Therefore, when plants have high endogenous proline content, it may start its functions quickly to cope with the adverse situations.

In PR, the endogenous proline content was high in control condition but was low in stress condition (Fig. 4). The lower amount of proline under stress condition might be the utilization of proline in the mechanism of stress mitigation. In BRRI dhan28, the proline content was low in control condition but was high in stress condition (Fig. 4). This is due to the fact that salt-sensitive genotype needs to synthesize high levels of proline for osmotic adjustment under saline conditions (Bhushan *et al.,* 2016). Similar to the present study, Bhushan *et. al., (*2016) also reported that salt-tolerant rice possessed higher proline content than that in salt-sensitive, and the higher amount of proline was also found in salt-sensitive IR-28 rice than salt-tolerant Pokkali (a land-race rice variety) under salt stress (Demiral and Türkan, 2005). The accumulation of proline in plant is correlated with improved salinity tolerance (Ashraf and Foolad, 2007; Hasanuzzaman et al., 2014). The more proline content in PR under control condition suggests its higher salt-tolerance potentiality than BRRI dhan28.

The metabolism of H_2O_2 is mainly depends on the antioxidant enzyme activity such as catalase which is the most efficient H_2O_2 scavenging enzyme (Hasanuzzaman *et al.,* 2012 and Mhamdi *et al.,* 2010). Salinity stress regulates the antioxidant enzyme activities. Here, the catalase activity was higher in PR than in salt-sensitive BRRI dhan28 both at control and saline conditions (Fig. 5). The increased catalase activity was reported in salt-tolerant rice variety than salt-sensitive variety under salt stress condition (Demiral and Turkan, 2005; El-Shabrawi *et al.,* 2010; Hasanuzzaman *et al.,* 2011, 2012, 2014; Ghosh *et al.,* 2011).

Moreover, the catalase activity was decreased under salt stress compared to control in both PR and BRRI dhan28 but the decrease was much higher in BRRI dhan28 than PR (Fig. 5). The higher catalase activity in PR suggested its higher capability of scavenging H_2O_2 under salt stress.

The chlorophyll (chl) content is an important index for determining salt stress (Munns, 1993). The total chl content of PR was significantly increased at 100 mM salt stress (25.51 µg/ml of plant extract) compared to control (8.79 µg/ml of plant extract) (Fig. 6). Total carotenoid content of PR also had similar findings as total chl content (Fig. 6). The total chl and carotenoid contents of salt-sensitive BRRI dhan28 were increased with the increase of salt stress compared to control (Fig. 6). The decrease in chl content at 50 mM in PR might occur due to pigment degradation. Hasanuzzaman *et al.* (2014) also found a decrease in total chl content under salt stress than control. Krishnamurathy *et al.* (1987) reported that chl content was increased with increasing salt stress, which is similar with our results for PR as well as salt-sensitive BRRI dhan28.

Anthocyanin, a phenolic compound, plays an important role in plant as antioxidant (Maulani *et al.,* 2019). Previous report suggests that anthocyanin is not present in green rice (Chin *et al*., 2016). In our study, anthocyanin was only present in PR but not detected in BRRI dhan28. The anthocyanin content in PR was significantly decreased at 100 mM and 150 mM salt stress compared to control (Fig. 7). It is reported that the synthesis of anthocyanin and its localization in plant stem, root and leaf tissues makes the plant resistant to several environmental stresses (Eryılmaz, 2006; Kiełkowska *et al*., 2019). The previous reports with our findings suggest the presence of anthocyanin as an indicator of stress tolerance of crop plants. The increased amount of anthocyanin in PR suggests its more tolerance capability against salt stress.

Conclusion

Purple rice maintained higher antioxidant enzyme activity than BRRI dhan28 under salt stress and non-stress conditions and also contained higher endogenous proline than BRRI dhan28 in control condition. By considering the higher amount of anthocyanin, more catalase activity, higher amount of constitutive proline, lesser root and shoot length reductions, more live leaves per tiller in PR than BRRI dhan28, it is suggested that PR may have more salt tolerance capacity than BRRI dhan28. Purple rice is less sensitive to salt stress compared with BRRI dhan28. However, further research is required based on molecular analysis to confirm the stress tolerance potentiality of PR.

Acknowledgements

Thanks to the Ministry of Science and Technology, Government of the People's Republic of Bangladesh for the financial support of the research.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

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

Agathokleous, E., Feng, Z.Z. and Penuelas, J. 2020. Chlorophyll hormesis: Are chlorophylls major components of stress biology in higher plants? Science of the Total Environment, 726: 138637. https://doi.org/10.1016/j.scitotenv.2020.138637

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

Ashraf, M. and Foolad, M.R. 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany, 59(2): 206-216. https://doi.org/10.1016/j.envexpbot.2005.12.006

Banu, M.N.A., Hoque, M.A., Watanable-Sugimoto, M., Islam, M.M., Uraji, M., Matsuoka, K., Nakamura, Y., Murata, Y. 2010. Proline and glycine betaine ameliorated NaCl stress via scavenging of hydrogen peroxide and methylglyoxal but not superoxide or nitric oxide in tobacco cultured cells. Bioscience, Biotechnology and Biochemistry, 74(10): 2043–2049. https://doi.org/10.1271/bbb.100334

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

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.

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

Demiral, T. and Turkan, I. 2005. Comparative lipid peroxidation, antioxidant defence systems and proline content in roots of two rice cultivars differing in salt tolerance. Environmental and Experimental Botany, 53(3): 247–257. https://doi.org/10.1016/j.envexpbot.2004.03.017

Djanaguiraman, M., Ramadass, R., Durga-Devi, D. 2003. Effect of salt stress on germination and seedling growth in rice genotypes. Madras Agricultural Journal, 90 (1-3): 50-53.

Dubey, R.S. 1994. Protein synthesis by plants under stressful conditions. In: Handbook of Plant and Crop Stress, Pessaraki, M. (Ed.) Marcel Dekker, New York. pp. 277-299.

El-Shabrawi, H., Kumar, B., Kaul, T., Reddy, M.K., Singla-Pareek, S.L., Sopory, S.K. 2010. Redox homeostasis, antioxidant defense, and methylglyoxal detoxification as markers for salt tolerance in Pokkali rice. Protoplasma, 245(1): 85-96. https://doi.org/10.1007/s00709-010-0144-6

Eryılmaz, F. 2006. The relationships between salt stress and anthocyanin content in higher plants. Biotechnology & Biotechnological Equipment, 20(1): 47-52. https://doi.org/10.1080/13102818.2006.10817303

Ghosh, N., Adak, M.K., Ghosh, P.D., Gupta, S., Gupta, D.N.S., Mandal, C. 2011. Differential responses of two rice varieties to salt stress. Plant Biotechnology, 5: 89-103. https://doi.org/10.1007/s11816-010-0163-y

Hakim, M.A., Juraimi, A.S., Begum, M., Hanafi, M.M., Ismail, M.R., Selamat, A. 2010. Effect of salt stress on germination and early seedling growth of rice (*Oryza sativa* L.). African Journal of Biotechnology, 9 (13): 1911– 1918. https://doi.org/10.5897/AJB09.1526

Hasanuzzaman, M., Hossain, M.A., Fujita, M. 2011. Nitric oxide modulates antioxidant defense and the methylglyoxal detoxification system and reduces salinity-induced damage of wheat seedlings. Plant Biotechnology, 5: 353-365. https://doi.org/10.1007/s11816-011-0189-9

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

Hasanuzzaman, M., Alam, M.M., Rahman, A., Hasanuzzaman, M., Nahar, K., Fujita, M. 2014. Exogenous proline and glycine betaine mediated upregulation of antioxidant defense and glyoxalase systems provides better protection against salt-induced oxidative stress in two rice (*Oryza sativa* L.) varieties. BioMed Research International, 2014: 757219. https://doi.org/10.1155/2014/757219

Hasegawa, P.M., Bressan, R.A., Zhu, J.K., Bohnert, H.J. 2000. Plant cellular and molecular responses to high salinity. Annual Review of Plant Physiology and Plant Molecular Biology, 51: 463–499. https://doi.org/10.1146/annurev.arplant.51.1.463

Hossain, M.A. and Fujita, M. 2010. Evidence for a role of exogenous glycine betaine and proline in antioxidant defense and methylglyoxal detoxification systems in mung bean seedlings under salt stress. Physiology and Molecular Biology of Plants, 16 (1): 19–29. https://doi.org/10.1007/s12298-010-0003-0

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. https://doi.org/10.1016/B978-0-12-799963-0.00016-2

Hu, X., Makita, S., Schelbert, S., Sano, S., Ochiai, M., Tsuchiya, T., Hasegawa, S.F., Hortensteiner, S., Tanaka, A., Tanaka, R. 2015. Reexamination of chlorophyllase function implies its involvement in defense against chewing herbivores. Plant Physiology, 167 (3): 660-670. https://doi.org/10.1104/pp.114.252023

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

Krishnamurathy, R., Anbazhagan, M., Bhagwat, K.B. 1987. Effect of sodium chloride toxicity on chlorophyll break down in rice. Indian Journal of Agricultural Sciences, 57 (8): 567-570.

Lange, H., Shropshire, W.Jr., Mohr, H. 1970. An analysis of phytochrome-mediated anthocyanin synthesis. Plant Physiology, 47 (5): 649-655. https://doi.org/10.1016/j.ecoenv.2007.11.016

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

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.

Munns, R. 1993. Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. Plant, Cell & Environment, 16 (1): 15-24. https://doi.org/10.1111/j.1365-3040.1993.tb00840.x

Nor'aini, M.F., Robert, P.F., Roy, H.B. 1997. Salinity, oxidative stress and antioxidant responses in shoot cultures of rice. Journal of Experimental Botany, 48 (307): 325-331. https://doi.org/10.1093/jxb/48.2.325

Okuma, E., Murakami, Y., Shimoishi, Y., Tada, M., Murata, Y. 2004. Effects of exogenous application of proline and betaine on the growth of tobacco cultured cells under saline conditions. Soil Science and Plant Nutrition, 50 (8): 1301-1305. https://doi.org/10.1080/00380768.2004.10408608

Patade, V.Y., Lokhande, V.H., Suprasanna, P. 2014. Exogenous application of proline alleviates salt induced oxidative stress more efficiently than glycine betaine in sugarcane cultured cells. Sugar Technology, 16: 22-29. https://doi.org/10.1007/s12355-013-0261-6

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

Tuna, A.L., Kaya, C., Higgs, D., Murrillo-Amador, B., Aydemir, S., Girgin, A.R. 2008. Silicon improves salinity tolerance in wheat plants. Environmental and Experimental Botany, 62(1): 10-16. https://doi.org/10.1016/j.envexpbot.2007.06.006

Uarrota V.G., Stefen D.L.V., Leolato L.S., Gindri D.M., Nerling D. 2018. Revisiting carotenoids and their role in plant stress responses: from biosynthesis to plant signaling mechanisms during stress. In: D. Gupta, J. Palma, F. Corpas (eds), Antioxidants and Antioxidant Enzymes in Higher Plants. Cham: pp 207-232. Springer International Publishing. https://doi.org/10.1007/978-3-319-75088-0_10