Journal of Dry Zone Agriculture, 2018, 4(1): 14 - 20

ISSN 2012-8673

Mitigation of Salt Stress in Rice through Seed Priming with Regulation of Defense Enzymes

Hemalatha, G.,[1] Renugadevi, J.[1] and Eevera, T.[2]

[1]Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.
[2]Department of Plant Breeding and Genetics, Anbil Dharmalingam Agricultural College and Research Institute, Tamil Nadu Agricultural University, Trichy, Tamil Nadu, India.

Abstract: In India, rice (*Oryza sativa* L.) is the major staple food crop providing 43 per cent of calorie requirement for more than 70 per cent of the population. Salinity is one of the major hurdles that cause serious hazards in agriculture thereby limiting agricultural productivity. Various reclamation methods had been followed to alleviate the salinity, among them most successful tool is to increase the productivity which is utilization of tolerance varieties. In addition to that, seed priming is very useful technique to mitigate the effect of salt stress. In this experiment, the rice seeds of salt tolerant (TNAU Rice TRY 3), salt sensitive (ADT (R) 49) and moderately salt tolerant (Co 43) varieties were primed with 0.25% hydrogen peroxide, 75 µM jasmonic acid, 80 µM Sodium nitroprusside (SNP) along with hydropriming and unprimed seeds as control. The performance of primed seeds was assessed under salt affected field. In addition to seed priming, foliar spray with hydrogen peroxide, jasmonic acid and SNP for respective priming treatments were also given to the seedlings on 15th day after sowing (DAS). The field emergence (%) 5th DAS, seedling length (cm), dry matter production (DMP), root volume (ml) and biochemical changes like proline and defense enzymes such as., peroxidase and catalase were observed. The results revealed that priming treatment enhanced the performance of salt sensitive variety in addition to the tolerance and moderately tolerant varieties. The improved performance of primed seeds under salt stress condition may be due to the regulated defense responses leading to systemic tolerance to subsequent salt stress exposure. Hence, from this study it is clearly envisaged that the seed priming with SNP was found to be suitable for enhancing the performance of rice seeds under salinity condition.

Keywords: Defense enzymes, rice, salt stress, sodium nitroprusside

---

Corresponding author: G. Hemalatha, e-mail: *hemi7654@gmail.com*

Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world, as it feeds nearly half of the planet's population and approximately three quarters of a billion of world's poorest people. In Asia, about 90 per cent rice is produced and consumed. In India rice covers about 23.3% of gross cropped area of the country and plays a vital role in the national food grain supply. It puts up 43% out of total food grain production and for total cereal production 46% of nation. India secures second position in production of rice among all rice producing countries, China leads the highest production. The world area, production and productivity in 2014-15 were 162.2 Mha, 483.3 million tonnes and 4.44 MT/ ha, respectively. In India, rice is being grown in 44.10 Mha area with production of 106.5 million tones and productivity of 3.52 MT/ha, respectively (USDA, 2016).

Agriculture is a complex sector involving different driving parameters (environment, economic and social). It is now well recognized that crop productivity is very sensitive to climate change with different effects according to region. Climate change is noticed as a serious threat to rice farming systems in Cauvery river basin regions of Tamil Nadu. The expected effect of climate change ranges from changing rainfall pattern to increasing salinity of the soil. Millions of hectares in the humid regions of South and South East Asia are technically suited for rice production, but are left uncultivated or grown with low yields because of salinity problem. Salinity is one of the major hurdles that cause serious hazards in agriculture thereby limiting agricultural productivity. It is estimated that 20 per cent of the irrigated land at global level is affected by salinity (Gupta and Huang, 2014). About 6.73 million hectares of salt affected soils exist in India. Out of which, 2.96 million hectares is saline and remaining 3.77 million hectares area is classified as sodic soils (Sharma and Chaudary, 2012). The quality and yield of rice is greatly affected by environmental stresses such as salinity, drought, heat and cold (Swain *et al*., 2017).

Rice is regarded as one of the more salt-sensitive crops, which is certainly true when grain yield is considered (Khatun *et al*., 1995). This problem can be overcome by either changing the growth environment suitable for the normal growth of the plants or selecting the crop or changing genetic architecture of the plant so that it can be grown in problematic areas. Breeding crop varieties with in-built salt tolerance is considered as the most promising, economical and socially acceptable approach when compared to soil engineering and amelioration processes. Development of high yielding salt tolerant cultivars will considerably improve rice production under salt stress conditions. Seed priming is one of the reclamation method to mitigate the illness of salt stress and also improves the uniformity and rate of emergence of seedlings (Bakht *et al*., 2010). Preferably, seed priming with inorganic salts as halopriming is effective to improve their germination and crop establishment even under salt stress conditions (Sivritepe *et al*., 2003). Several researchers claimed that, halopriming chemicals repairs the plant metabolism and also scavenging the Reactive Oxygen Species (ROS) in the plants enzymatic and non-enzymatic defence systems like peroxidase (Pox), catalase (CAT), superoxide dismutase (SOD), proline etc. These defense systems protect the plant cells against the ROS damage (Hossain, 2006). In this regard, to study the rice seeds with various response to salinity condition were given with seed priming and foliar spray with H_2O_2, Jasmonic acid and SNP is effective and incomparison with hydropriming and unprimed control was carried out to find out the effect of various halopriming in order to mitigate the salt stress effect in rice plants by the regulation of defense enzymes.

Materials and Methods

Materials

Experiments were carried out in research field of Anbil Dharmalingam Agricultural College and Research Institute, Tamil Nadu Agricultural University, Trichy, Tamil Nadu, India. The seeds of three different rice varieties of salt tolerant (TNAU Rice TRY 3), salt sensitive (ADT (R) 49) and moderately salt tolerant (Co 43) varieties were obtained from the Department of Plant Breeding and Genetics, Anbil Dharmalingam Agricultural College & Research Institute (ADAC & RI), Trichy, Agricultural and Engineering College & Research Institute, Kumulur and Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore respectively and taken for the study and these seeds were primed with 0.25% hydrogen peroxide, 75 µM jasmonic acid, 80 µM SNP along with hydropriming and unprimed seeds as control. The foliar spray treatments were also given with the above said chemicals for the respective priming treatments.

Methods

The healthy non-dormant, homogenous seeds of rice were pre-treated in the respective solution for the duration of 12 hours and the seeds were removed from the solutions and shade dried at room temperature to bring back its original moisture content of 13% for assessing the seed quality parameters.

Treated seeds were evaluated for their performance in the sodic soil by conducting the field trial. The field emergence were recorded on 5th Days After Sowing(DAS) and seedling length also recorded by measuring the length of the root and shoot for each treatment on a meter scale and for estimating the dry matter production, the seedlings were placed in paper bag individually and air-dried for 24 h. Then, the seedlings were kept in a hot air oven maintained at 85 ± 2 °C for 24 h and cooled in a desiccator, weighed and expressed in mg seedling^{-1}.

The roots of randomly selected ten seedlings from each plot were scooped along with soil and washed in running water carefully. Then the roots were detached from the nodal base of seedlings. The excess moisture on the surface of the roots was removed by wiping with blotter paper. Then, the roots of each seedling were placed in a measuring cylinder containing known volume of water. By measuring the increase in water column, root volume was assessed and the mean expressed in cc seedling^{-1}.

The amino acid, proline content was estimated in fully expanded leaves at flowering stage following the method of Bates *et al*. (1973) and expressed in $\mu g\ g^{-1}$ on fresh weight basis. Peroxidase activity was assayed as increase in optical density due to the oxidation of guaiacol to tetraguaiacol (Castillo *et al*., 1984). Catalase assay is based on the absorbance of H_2O_2 at 240 nm in UV-range. A decrease in the absorbance is recorded over a time period as described by Aebi (1984).

Spectrum analysis was done by UV-Vis spectroscopy to identify the functional group which is formed in the treatments compared with unprimed seeds.

Statistical analysis

The data obtained from each of the experiments were subjected to an analysis of variance and treatment differences tested for significance (p= 0.05) after Gomez and Gomez (1984). Wherever necessary, the per cent values were transformed to arc-sine values before analysis. The critical differences (CD) were calculated at 5 and 1 per cent probability level.

Result and Discussion

The physiological parameters of field emergence, seedling length, drymatter production and root volume and also biochemical parameter like proline and enzymes like POX and CAT were analysed in the rice nursery after 20 days of stress conditioned field. The study to alleviate effect of salt stress through seed priming and foliar spray indicated that the seeds haloprimed with SNP at 80 µM recorded increased ranges of all the parameters when compared to hydroprimed and unprimed seeds. In this regard, the seeds haloprimed with SNP at 80 µM recorded increased ranges of germination (83-89%), seedling length (24-30 cm), drymatter production (1-1.3 g seedlings^{-1}) and root volume (1.17-1.22 cc seedling^{-1}) than the other priming treatments irrespective of varieties. Compared with hydropriming and unprimed seeds the primed seeds performed better. Seed priming and foliar spray with SNP at 80 µM recorded increased germination of 21 per cent than unprimed and hydroprimed seeds for the salt sensitive variety ADT (R) 49 followed by other priming treatments such as H_2O_2 and Jasmonic acid. According to Zadeh and Naeini (2007) who reported that salinity changes the morphological characters of rice. In this study, the effectiveness of SNP may be due to increased activity of POX, CAT and proline. The SNP at higher concentration act as stress inducer and at lower concentration stimulate the activity of defense enzymes and thereby improving the emergence and establishment of seedlings under salt stress condition (Hayat *et al*., 2014).

SNP having its capability of producing Nitrous Oxide (NO) seems to depend on its interaction with sulfhydryl-containing molecules. Cysteine and glutathione are the sulfhydryl-containing amino acid which is already present in the rice seeds (Louris and Sandra, 2005). Actually, the reaction between SNP and thiols was described several decades ago (Scagliarini and Swinehart, 1967) and is used as a test for their identification, but still, SNP is the most widely studied of the iron nitrosyl compounds. The effect has been justified through UV-Vis spectroscopy (Figure 1).

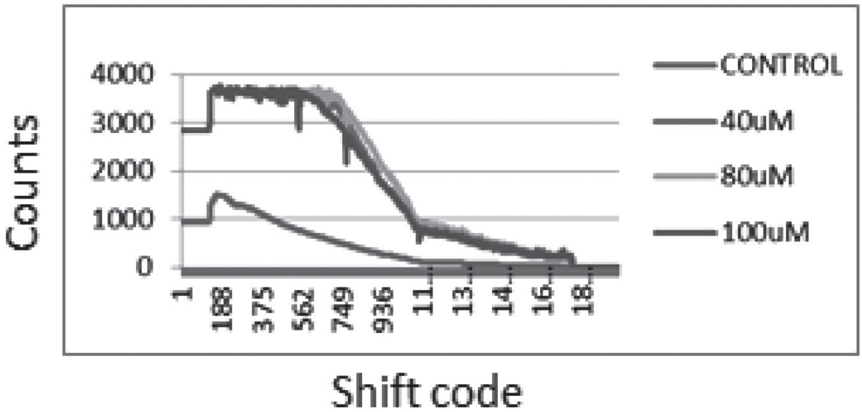

Figure 1: Interaction between the SNP and glutathione amino acid portion of the paddy seed identified through Raman spectrum

SNP which is reacted with the glutathione amino acid which is already present in the rice seeds finally formed the temporary nitric oxide storage compound like s-nitrosoglutathione (Figure 2) which slowly release NO during germination. Periodical release of NO at lower rate in-turn trigger the defense related enzymes within the seed under salt stress condition.

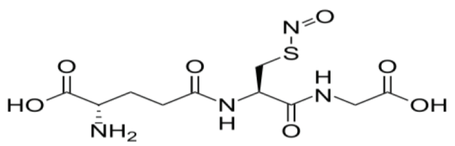

Figure 2: Temporary nitric oxide storage compound

With reference to the varieties, all the varieties responded well to seed priming and foliar spray treatments. However, the performance of salt sensitive variety ADT (R) 49 has been improved to an extent of 18% increased germination than the unprimed seeds. The moderately salt sensitive variety Co 43 recorded 17% improved germination. Salt tolerant variety TNAU Rice TRY 3 recorded 15% increased germination. Hence the priming treatments improved the performance of salt sensitive variety. Similar results were found in hot pepper, which enhanced tolerance of seedlings raised from haloprimed seeds may be due to induced resistance by NaCl (Amjad, 2007). In addition to that seed priming with H_2O_2 played an important role in signal transduction for abiotic stress tolerance, although H_2O_2 is toxic at high concentrations. Meanwhile Seed priming with H_2O_2 having the capacity to enhance the multi-resistance to heat, drought, chilling and salt stress (Uchida *et al*., 2002). Seeds primed with Jasmonic acid have proved effective in improving plant stress tolerance but it depends on the type of species and concentration (Creelman and Mullet, 1995).

Conclusion

The present study revealed that the TNAU Rice TRY 3 maintained better seedling growth under salinity conditions. This might be attributed to the inherent mechanism of tolerant variety in the regulation of entire germination process. The performance of moderately salt tolerant and salt sensitive were improved due to the seed priming treatment and foliar spray with SNP at 15 DAS and as well as with $H_2O_2$and Jasmonic acid. The performance due to the formation of S-nitrosoglutathione in the paddy seed which trigger the defense related enzymes within the seed under salt stress condition which was confirmed through UV-Vis Spectroscopy.

References

Aebi, M. 1984. Catalase in vitro. Methods in Enzymology, 105:121-126.

Amjad, M., Ziaf, K., Iqbal, Q., Ahmad, I., Riaz, M.A. and Saqib, Z.A. 2007. Effect of seed priming on seed vigour and salt tolerance in hot pepper. Pakistan Journal of Agricultural Sciences, 44(3):408-416.

Bakht, J., Shafi, M. and Shah, R. 2010. Effect of various priming sources on yield and yield components of maize cultivars, Pakistan Journal of Botany, 42(6):4123-4131.

Bates, L., Waldren, R.P. and Teare, I.D. 1973. Rapid determination of free proline for water-stress studies. Plant and Soil, 39:205-207.

Castillo, F.I., Penel, I. and Greppin, H. 1984. Peroxidase release induced by ozone in sedum album leaves. Plant Physiology, 74:846-851.

Gomez, K.A. and Gomez, A. A. 1984. Statistical Procedures for Agricultural Research, 2nd Edition, John Wiley and Sons.

Gupta, B. and Huang, B. 2014. Mechanism of salinity tolerance in plants: Physiological, biochemical and molecular characterization. International Journal of Genomics, doi:10.1155/2014/701596.

Hayat, S., Yadav, S., Alyemeni, M.N. and Ahmad, A. 2014. Effect of sodium nitroprusside on the germination and antioxidant activities of tomato (Lycopersicon esculentum Mill). Bulgarian Journal of Agricultural Science, 20:156-160.

Hossain, M.M. 2006. Effect of salinity level on growth and yield of advanced mutant rice in Boro season. M.S. Thesis, Dept. Crop Bot., Bangladesh Agricultural University, Mymensingh.

Khatun, S., Rizzo, C.A. and Flower, T.J. 1995. Genotypic variation in the effect of salinity on fertility in rice. Plant and Soil, 173(2):239-250.

Creelman, R. A. and Mullet, J.E. 1995. Jasmonic acid distribution and action in plants: Regulation during development and response to biotic and abiotic stress. Proceedings of the National Academy of Sciences of the United States of America, 92:4114-4119.

Sharma, D.K. and Chaudhari, S.K. 2012. Agronomic research in salt-affected soils of India: An Overview. Indian Journal of Agronomy, 5(7):175-185.

Scagliarini, G. and Swinehart, J.H. 1967. The nitroprusside ion. Coordination Chemistry Reviews, 2:385-402.

Sivritepe, N., Sivritepe, H.O. and Eris, A. 2003. The effects of NaCl priming on salt tolerance in melon seedlings grown under saline conditions. Scientia Horticulturae, 97:229-237.

Swain, D.M., Sahoo, R.K. and Tuteja, N. 2017. RGG1 transgenic rice plants and heir physiological characteristics in relation to salinity stress. Oryza, 54(1):13-20.

Uchida, A., Jagendorf, A.T., Hibino, T., Takabe, T. and Takabe, T. 2002. Effect of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. Plant Science, 163:515-523.

USDA. 2016. Rice Outlook, Economic Research Service /RCS-16J/October 14, 2016. pp, 1 – 25. http://www.ers.usda.gov/ media/2150132/rice-outlook-october-2016.pdf

Zadeh, H.M. and Naeini, M.B. 2007. Effects of salinity stress on the morphology and yield of two cultivars of canola (*Brassica napus* L.), Journal of Agronomy, 6:409-411.