Madras Agric. J., 103 (1-3): 1-4, March 2016

Effect of Plant Growth Regulators on Seed Germination Associated Traits under Salinity Stress in Rice

E. Kanmani, R. Sivakumar, V. Ravichandran, A. Senthil and P. Boominathan*
Department of Crop Physiology
Tamil Nadu Agricultural University, Coimbatore -3

Soil salinity is the major problem in arid and semi-arid regions, where rainfall is insufficient to leach salt and excess of sodium ion and it is one of the major constraints to the sustainability and expansion of rice cultivation. The depressive effect of salinity on germination could be related to a decline in endogenous levels of hormones. Presoaking seeds with optimal concentration of plant growth regulators (PGRs) has been shown to be beneficial to growth and yield of some crop species under saline conditions. It is extremely important to standardize the concentrations of PGRs as the activities changes drastically with concentrations. Therefore, we have used eight different concentrations of four PGRs along with absolute control and salinity stress treatments known to alter the germination *viz*., gibberellic acid, kinetin, salicylic acid and brassinosteriods and measured various physiological traits associated with germination. In case of germination percentage, brassinosteriods at 0.005 ppm was found to be the best as it registered highest germination percentage followed by gibberellic acid. Higher dry matter production was found in seeds treated with gibberellic acid and kinetin that recorded 37.4 % higher dry matter production than control. The critical enzyme in germination, a-amylase activity was higher in seeds treated with 500 ppm of gibberellic acid. These results strongly suggest that PGRs at optimum concentrations could be effectively used for improving germination associated traits under salinity condition in rice.

Key words: Plant growth regulators, Rice, Salinity stress

Rice is the world's most important food crop and a primary source of food for more than half the world's population. It is one of the important cereal crops ranks second in consumption, which supports more than three billion people and represents 50 to 80 per cent of the daily calorie intake. It has been estimated that the yield potential of major crops are usually lost due to unfavorable growing environments such as high salinity and drought. Soil salinity is the major problem in arid and semi-arid regions, where rainfall is insufficient to leach salt and excess of sodium ion down and out of root zone. Salinity happens to the major constraint to the sustainability and expansion of rice cultivation in areas where rice production has kept up with increasing demand from a growing population (Sritharan *et al.,*2006). Salinity is adversely affects plant growth and development, hindering seed germination, seedling growth and enzyme activity. The exogenous application of plant growth regulators produces benefit in alleviating the adverse effects of salt stress and also improves germination, growth, development, tillering, flowering and seed yields, yield quality (Egamberdieva, 2009).

Plant endogenous cytokinin content induced the germination and growth in rice. Kinetin acts as a direct free radical scavenger or it may involve in anti oxidative mechanism related to the protection of purine breakdown under salinity conditions (Senguttuvel *et al.,* 2012). Gibberellic acid is associated with various plant growth development processes such as seed germination, hypocotyls elongation, leaf expansion, floral initiation, uniform flowering, floral organ development and induction of some hydrolytic enzymes in the aleurone of cereal grains under salinity conditions. Pre-treatment with salicylic acid improved growth and resulted in higher resistance of plants to salinity, leading to increased germination percentage, seedling vigor index and growth parameters of the seedlings (Anwar *et al*., 2013).

With this background, the present study aimed at studying the influence of plant growth regulators on germination associated traits in rice under salinity and in addition to, finding out the optimum concentrations of plant growth regulators for increasing seedling growth and vigour.

Materials and Methods

The experiment was carried out in variety CO 51 with 160mM NaCl and varied concentrations of plant

*Corresponding author's email : boominathan.p@tnau.ac.in

growth regulators to study the germination associated traits by following method of Bor *et al.,*2004. The petriplates were placed in growth chamber with optimum light and aeration for twelve days after which various physiological traits associated with seedling growth were recorded.

Details of plant growth regulators and concentrations used

Treatments	Details			
T_1	Absolute control (without salinity)			
T_2	Salinity stress - 160mM NaCl (without PGRs)			
PGRs	Gibberellic acid (ppm)	Salicylic acid(ppm)	Kinetin(ppm)	Brassinosteriods(ppm)
T_3	10	5	1	0.0001
T_4	25	10	5	0.0005
T_5	50	25	10	0.001
T_6	100	50	25	0.005
T_7	150	75	50	0.01
T_8	200	100	75	0.05
T_9	250	150	100	0.1
T_{10}	500	200	150	0.5

Germination percentage

Fifty seeds of CO 51 were placed in petriplates and moistened with 160 mM NaCl and/or with respective concentrations of PGlRs. The germination percentage was recorded on 12 days after sowing (DAS). Number of seeds germinated was expressed as percentage for each treatment.

$$\text{Germination percentage} = \frac{\text{Number of germinated seedlings}}{\text{Number of seeds taken for germination}} \times 100$$

α-amylase activity

Three replicates of 500mg of pre-germinated seed samples were homogenized in 1.8ml of cold 0.02M sodium phosphate buffer (pH 6.0) and centrifuged at 20,000 rpm for 20 min to extract enzymes. To 0.1ml of enzyme extract, 1ml of 0.067 per cent starch solution was added. The reaction was stopped after 10 min of incubation at 25^oC by the addition of1ml of iodine HCl solution (60mg KI and 6mg I_2 in 100ml of 0.05N HCl). Change in colour was measured at 620 nm. The activity was calculated and expressed as mg maltose min^{-1} by following protocol of Paul *et al.* (1970).

$$\alpha\text{- Amylase activity} = \frac{\text{OD value}}{\text{Volume of sample taken}} \times \frac{1000}{500}$$

Seedling total dry matter production

Ten seedlings in each treatment from each replication were dried in an electric hot air oven at 80 ±2^oC for 48 hrs and the mean dry weight was expressed as mg seedling^{-1}. The data on various parameters were analyzed statistically as per the procedure suggested by Gomez and Gomez (1984).

Results and Discussion

Abiotic stress has been estimated that more than half of the yield potential of major crops is usually lost due to unfavorable growing environments such as drought or high salinity (Cortina and Culianez-Macia, 2005). This can be recovered to a certain extent with the applications of plant growth regulators (PGRs) that play an important role in several physiological and molecular processes of plants. In addition, PGRs used as potential tools to increase defense mechanisms against stress conditions (Nair *et al.,* 2009). Hence, different approaches were adopted to mitigate the effect of salinity and one of the options is use of plant growth regulators.

Effect of PGRs on germination percentage under salinity stress

Germination of seeds is one of the most crucial and decisive phases in the growth cycle of plant species since it determines plant establishment and final yield of the crops. To produce the crop satisfactorily under saline conditions, seeds must germinate and grow vigorously, pass through the salty layer of the soil and survive. Therefore, any treatment which could be used to improve seed germination and subsequent seedling establishment under saline conditions would be highly desirable.

The depressive effect of salinity on germination could be related to a decline in endogenous levels of hormones (Debez *et al.,* 2001). Concerted attempts have been made to mitigate the harmful effects of salinity by application of plant growth regulators. Presoaking seeds with optimal concentration of plant growth regulators has been shown to be beneficial to growth and yield of some crop species under saline conditions by increasing nutrient reserves through increased physiological activities. It is extremely important to standardize the concentrations of PGRs as various metabolic processes changes with concentrations especially during seedling stage. Therefore, we have used ten different concentrations for each PGR based on

Table 1. Effect of PGRs on germination percentage and seedling vigour under160 mM NaCl

Treatments	Brassinosteriods		Gibberellic acid		Salicylic acid		Kinetin	
	Germination percentage	Seedling vigour	Germination percentage	Seedling vigour	Germination percentage	Seedling vigour	Germination percentage	Seedling vigour
T_1	97.9	1514	97.9	1514	97.9	1514	97.9	1514
T_2	52.4	182	52.4	182	52.4	182	52.4	182
T_3	94.3	820	93.0	334	94.0	356	65.2	305
T_4	96.2	1144	94.1	385	96.2	460	69.3	386
T_5	94.1	997	93.4	448	94.8	511	75.4	412
T_6	90.4	931	90.2	514	91.7	621	77.3	446
T_7	87.2	758	85.1	527	90.3	624	82.2	459
T_8	85.3	656	81.7	654	84.9	629	85.2	484
T_9	70.1	469	70.1	554	85.6	917	89.4	534
T_{10}	65.3	372	65.2	475	81.2	672	87.5	560
Mean	65.3	440	66.9	543	68.9	673	63.9	667
S.Ed	0.72	5.3	0.81	4.9	0.86	5.6	0.66	2.9
CD(0.05)	1.46**	10.8**	1.65**	10.0**	1.76**	11.5**	1.34**	6.2**

literature to find out concentration at which best results can be obtained. Thus, the effects of different concentration of various PGRs on germination associated characters were measured.

Brassinosteriods (BRs) are implicated in plant responses to abiotic environmental stresses and to undergo profound changes in plants. In this study, among the BRs concentrations tried, 0.005ppm (T_4) recorded maximum germination per cent which is 83 percent higher than salt stress (Table 1). It is also evident that high concentrations of BRs inhibited germination percentage. The increase in germination might be due to the activation or suppression of key enzymatic reactions, induction of protein synthesis and the production of various chemical defense compounds. Gibberellic acid (GA), which comes under the naturally occurring growth hormone, regulates the various plant growth and development and processes. In this study, maximum germination was noticed in treatment at GA 100 ppm (T_5) which is 80 per cent higher than control. This might be due to the activation of hydrolytic enzymes necessary for seed germination and low absorption of Na^+.

Salicylic acid (SA), a plant phenolic is considered as a hormone like endogenous regulator and its role in the defense mechanisms against biotic and a biotic stress has been well documented. In the present study, SA 100 ppm (T_4) recorded higher germination (96.2%). Similar observation was made by El-Tayeb (2005) where, they showed that SA treatment increases germination percentage under salinity conditions. Kinetin can also enhance resistance to salinity and high temperature in plants. Seed priming with kinetin is reported to increase the salinity tolerance. In this present study, higher germination percentage was obtained in 150 ppm of kinetin (T_9). This might be due to its antagonistic effect to ABA which inhibits seed germination. Among the four PGRs studied, 0.005 ppm BRs was found maximum germination percentage followed by gibberellic acid.

Effects of PGRs on seedling vigour

Seedling vigour is the product of germination percentage and seedling length (Table 1). The seedling vigour was found to be high in absolute control (1514) while it was low in salinity stress without PGRs (182). Among the PGRs treated seedlings, the seedling vigour was maximum in (T_4) of BRs while minimum in T_3 of kinetin. Seedling vigour was declined steadily with increase in salinity level which might be due to consequences of the intake of toxic ions and changes in certain enzymatic and hormonal activities as reported by Amin *et al*. 1996. Moreover, mobilization of seed reserves, which occurs during early seed germination, is crucial because it supplies substrate for proper functioning of different metabolic processes that are essential for growth of the embryonic axis.

Effects of PGRs on dry matter production

Dry matter production of seedling is the manifestations of the physiological efficiency of the germinating seeds which depends on the seed vigour and seedling growth. Among the treatments, 0.0001ppm brassinosteriods, 500ppm gibberellic acid, 200 ppm salicylic acid and 200ppm kinetin showed maximum dry matter production (Table 2). Among the PGRs, gibberellic acid and kinetin recorded 37.4 % higher dry matter production compared to control. Reversal of the harmful effects of NaCl on dry matter production due to exogenous gibberellic acid could be attributed to stimulation of α-amylase activity. This result is in accordance with the findings of Misratia *et al.* (2013).

Effects of PGRs on a-amylase activity

Starch mobilization of seed reserves that occurs during early seed germination, is crucial because it supplies substrates for the proper functioning of different metabolic processes essential to growth of the embryonic axis. In present study, 0.005 ppm of brassinosteriods (T_5), 500 ppm of gibberellic acid (T_9), 150 ppm of salicylic acid (T_9) and 1 ppm of kinetin (T_3) showed higher alpha amylase activity

Table 2. Effect of PGRs on Total Dry Matter (TDM) (g) and α-amylase activity (mg maltose min^{-1}) under 160 mM NaCl

Treatments	Brassinosteriods		Gibberellic acid		Salicylic acid		Kinetin	
	TDM	α-amylase activity	TDM	α-amylase activity	TDM	α-amylase activity	TDM	α-amylase activity
T_1	0.15	48.4	0.15	48.4	0.15	48.4	0.15	48.4
T_2	0.11	27.0	0.11	27.0	0.11	27.0	0.11	27.0
T_3	0.14	35.0	0.11	29.5	0.10	30.2	0.12	39.0
T_4	0.15	33.1	0.12	32.0	0.11	34.0	0.13	37.0
T_5	0.14	31.5	0.12	34.3	0.12	36.7	0.13	36.1
T_6	0.14	30.1	0.13	35.4	0.13	40.1	0.14	34.7
T_7	0.13	29.5	0.15	37.5	0.14	42.2	0.14	32.8
T_8	0.13	28.1	0.14	39.7	0.14	44.0	0.14	29.0
T_9	0.12	27.9	0.15	44.5	0.14	43.0	0.14	29.0
T_{10}	0.12	27.8	0.15	42.3	0.14	36.0	0.15	28.5
Mean	0.1	31.3	0.13	36.7	0.1	37.6	0.13	37.1
S.Ed	0.001	0.7	0.001	0.64	0.001	0.86	0.57	0.87
CD (0.05)	0.002**	1.38**	0.002**	1.3**	0.002**	1.79**	1.17**	1.8**

under salt stress (Table 3). Among the four PGRs, 500 ppm of gibberellic acid was found to be the best as it recorded the maximum (64.8%) α-amylase activity over the control. Similar findings was reported by Murtaza and Asghar (2012) in pea, where gibberellic acid reduced the NaCl inhibition of α-amylase activity under salt stress.

Conclusion

With the rapid growth in population consuming rice as staple food and the deteriorating soil and water quality around the globe, there is an urgent need to understand the response of this important crop towards these environmental stresses. Among many, germination is one of the processes affected due to salinity stress in rice. Although the role of PGRs in mitigating the abiotic stress including salinity has been known for long time, the exact concentrations leading to improved germination associated traits has been critical for practical utility, which has been addressed in this study.

Acknowledgement

We sincerely thank Professor and Head, Department of Crop Physiology, TNAU, Coimbatore for providing laboratory facilities including growth chambers for carrying out this study.

References

Anwar, S., Iqbal, M., Raza, S.H. and Iqbal, N. 2013. Efficacy of seed preconditioning with 744 salicylic and ascorbic acid increasing vigor of rice (*Oryza sativa* L.) seedling. *Pak. J. Bot.,* **745 (45):** 157-162

Amin, W., Saif-ul-malook, A., Mumtaz,S.H. and Hafeez1 .M. 2014. Combining ability analysis and effect of seed priming on seedling traits in Sunflower (*Helianthus annus*). *Report and Opinion.,* **6:** 19-30

Bor, M., Seckin, B., Ozgur, R., Yilmaz, O., Ozdemir, F. and Turkan, I. 2009. NaCl pre-treatments mediate salt adaptation in melon plants through antioxidative system. *Acta Physiologiae Plantarum,* **31(3)**: 655-659

Cortina,C. and Culiáñez-Macià, F.A. 2005. Tomato abiotic stress enhanced tolerance by trehalose biosynthesis. *Plant Sci.,* **169 (1):** 75-82

Debez, A., Chaibi,W. and Bouzid, S. 2001. Effect du NaCl et de regulatoeurs de croissance sur la germination d' *Atriplex halimus L. Cah Agric.,* **10:**135-138

Egamberdieva, D. 2009. Alleviation of salt stress by plant growth regulators and IAA producing bacteria in wheat. *Acta Physiol. Plant,* **31:**861-864

El-Tayeb, M.A.2005. Response of barley grains to the interactive effect of salinity and salicylic acid. *Plant Growth Regul.,* **45:** 215-224.

Farahbakhsh, H. and Saiid, M.S. 2011. Effects of foliar application of salicylic acid on vegetative growth of maize under saline conditions. *African J. Plant Sci.,***5(10):** 575-578

Gomez, K. A. and. Gomez. A. A. 1984. Statistical procedures for agricultural research. (2nd Ed.) John Wiley and sons**,** NewYork, USA. p. 680

Misratia, K.M., Ismail, M.R., Hakim, M.A., Musa, M.H. and Puteh, A. 2013. Effect of salinity and alleviating role of gibberellic acid (GA3) for improving the morphological, physiological and yield traits of rice varieties. *Aust. J. Crop Sci.,* **7:** 1682-1692

Nair, V.D., Gopi, R., Mohankumar,M., Kavina, J. and Panneerselvam, R. 2009. Effect of triadimefon: a triazole fungicide on oxidative stress defense system and eugenol content in *Ocimum tenuiflorum* (L.). *Acta Physiol Plant.,* **34:** 599-605

Paul, A.K., Mukherjee, S. and Sicar, S .1970. Metabolic changes in rice seeds during storage. *Indian J. Agric sci.,* **40**: 1031-1036

Senguttuvel, P., Vijayalakshmi, C., Thiyagarajan, K., Sritharan, N., Geetha, S., KannanBapu, J.R. and Viraktamath, B.C. 2012. Differential response of rice seedlings to salt stress in relation to antioxidant enzyme activity and membrane stability index. *Archives of Agronomy and Soil Science*, 1:1-13.

Shakirova, F.M., Sakhabutdinova, A.R., Bezrukova, M.V., Fatkhutdinova, R.A. and Fatkhutdinova, D.R. 2003. Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. *Plant Sci.,* **164:** 317-322

Sritharan, N and Mallika Vanangamudi. 2006. Screening of rice genotypes for salt tolerance. *Plant Archives*, **6(2**)**:** 815-818.

Received after revision : February 24, 2016 ; Accepted : March 14, 2016