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Influence of Seed Priming and Foliar Spray on Seed Germination, Seedling Growth, Total Carbohydrate and Protein Content of Resultant Rice (*Oryza Sativa* L.) Seeds Under Salinity

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ABSTRACT: A study was conducted to investigate the influence of seed priming and foliar spray of sodium nitroprusside (SNP), potassium nitrate (KNO_3), salicylic acid (SA) and gibberellic acid (GA_3) on seed germination, seedling growth, total carbohydrate and protein content of the resultant rice seeds produced under a saline block of ARS, Ganavathi, UAS, Raichur. Among the treatments imposed, seed priming (0.43 mM)+ foliar spray (150 ppm) with gibberellic acid (GA_3) significantly increased the total carbohydrate (81.3 mg g^{-1}) and protein content (8.6%) of the resultant seed. This treatment also recorded significantly high seed germination (95.5%), shoot length (9.51 cm), root length (16.9 cm), seedling dry weight (90.4 mg) and seedling vigor index I (2522) compared to hydropriming and control.. These results indicate the use of GA_3 in mitigating salt stress due to better seed germination and seedling growth by enhanced antioxidant activity, growth and assimilation of photosynthates. The other chemicals SNP,SA and KNO_3at specific doses positively affected seed quality attributes compared to control.

Keywords: Foliar spray, Gibberellic acid, Rice, Salinity, Seed germination, Seed priming

Rice is the staple food for 60 percent of the world's population and the second-largest crop grown in the world in terms of area and production, more than 90 percent of the world's rice is produced and consumed in Asia and more than 3 billion people in Asia meet out 30-75 percent of their energy requirement from rice [1]. Rice is a salt-sensitive crop, and the sensitivity varies with the growth stages. However, germination, early seedling and reproductive stages are considered most sensitive to salinity [2]. Salinity is the second most widespread abiotic stress in rice-growing regions after drought, which reduces crop productivity by impairing normal growth and metabolic processes. Salinity primarily causes high Na^+ ion toxicity and osmotic stress and secondarily leads to oxidative stress and generation of Reactive Oxygen Species (ROS) that are responsible for membrane damage, cell leakage and inhibition of photosynthetic efficiency, thereby affecting growth and productivity [3]. 'Over the years due to continuous and indiscriminate use of irrigation water in command area the normal soils are getting converted to saline soil. Hence, to meet the food requirement for the ever-growing Indian population, efficient strategies are required for effective utilization of even these saline lands for crop cultivation.

Among the various strategies, seed priming and foliar spray with chemicals or growth regulators are the most appropriate and efficient techniques in mitigating salt stress [4]. Out of the different chemicals, Sodium nitroprusside (SNP) is one of the most important donor of NO and regulates many plant physiological functions, and sequential cell death under salinity through enhanced antioxidant enzymatic activities [5]. In the same way, salicylic acid is an endogenous plant growth regulator found to generate a wide range of metabolic and physiological responses in plants thereby modifying the growth and development [6] of many crop plants. The potassium also plays a role in large number of enzymatically catalysed reactions, in water economy of plants such as stomatal resistance and transpiration, translocation of water, nutrients and sugars within the plants, balancing membrane potential and turgor, activating enzymes and regulating osmotic pressure [7] and making plants tolerant to various stresses. The growth promoter like gibberellic acid (GA3) is an important growth hormone which increases cell division in cambial zone, breaks seed dormancy, promotes seed germination, internodal length, hypocotyls growth, increases the size of leaves, enable greater photo-synthesis and also activates antioxidant systems [8].

Looking to the seriousness of salinity problem and the positive role of different chemicals and growth promoter like GA_3 the present investigation was carried out to evaluate and the effectiveness of these chemicals through seed priming and foliar spray methods in counteracting the salinity problem and to produce quality seed in terms of seed germination, seedling growth, total carbohydrate and protein content of freshly harvested seeds under salinity soils.

MATERIAL AND METHODS

The field experiment was conducted at Agricultural Research Station, Gangavthi, University of Agricultural Sciences, Raichur, Karnataka, during *kharif* 2019-20. The experimental site was located between 15°15′4′′ N latitude and 76°31′40′′ E longitude at an altitude of 419 m above mean sea level. The experimental plot was medium black and clay texture, alkaline in soil reaction (8.35) and low in electrical conductivity (4.1 dS m^{-1}). The experiment consisted of ten treatments *viz.*T_1- control; T_2- hydro priming; T_3- seed priming with sodium nitroprusside (SNP) @ 80 µM; T_4 - T_3 + foliar spray with SNP 100 µM; T_5- seed priming with salicylic acid (SA) @ 200 µM; T_6- T_5 +foliar spray with SA 200 ppm; T_7- seed priming with potassium nitrate (KNO_3) @ 0.75%; T_8 - T_7 + foliar spray with $KNO_3$10 mM; T_9- seed priming with gibberellic acid (GA_3) @ 0.43 mM; T_{10}- T_9 +foliar spray with GA_3 150 ppm. The seed of variety RNR 15048 (salt-sensitive) were primed in the respective chemicals and growth promoter (GA_3) for 12 hours [9] as per the above treatment details. The primed seeds were sown in the nursery and then were transplanted on 21 days after sowing. Later on, two foliar sprays one before anthesis(50 DAT) and other after anthesis (80 DAT) were given. The experiment was laid out in the randomized block design with three replications. Once the crop was matured the freshly harvested seeds from each treatment were used for assessing various seed quality parameters.

Germination

The seeds harvested from each treatment were subjected to seed germination by following between paper method [10]. Four replicates of 100 seeds each were placed on wet germination paper uniformly and incubated at 25 ± 2 °C temperature and 90 ± 5 percent relative humidity. The final count was taken on 14^{th} day. The number of normal seedlings from each replication was counted and the mean germination were expressed as percentage.

Seedling growth parameters

At the time of germination, ten normal seedlings were selected at random from each replication and used for measuring root and shoot length. The seedlings used for growth measurement were dried in a hot air oven at 80°C for 24 h. The Dry weight was recorded and the mean values were expressed in milligram. The seedling vigour index-I was calculated using the formula as suggested by Abdul-Baki and Anderson [11].

Vigour index-I = Germination (%) × Mean seedling length (cm)

Total carbohydrate content

The total carbohydrate content was measured according to Hedge and Hofreiter [12]. Seed sample (100mg) were mixed with 5.0 ml of 2.5 N HCl in a boiling tube and hydrolysed by keeping it in a boiling water bath for three hours. After cooling the volume was made to 100 ml with distilled water and centrifuged. The supernatant was mixed with 4ml of anthrone reagent and heated for 8 minutes. The carbohydrate content was measured by taking absorbance at 630 nm and by using glucose standard expressed as mg g^{-1}.

Protein content

The protein content of seeds was measured as per micro kjeldhal procedure [13]. One g sample was kept in the digestion flask, with a little quantity of catalyst mixture (K_2SO_4 + $CuSO_4$), and 10 ml of 96 per cent sulphuric acid for complete digestion. The digested sample was distilled using 40 per cent of NaOH solution along with 2 ml of distilled water. The liberated ammonia collected in a boric acid solution containing a drop of double indicator was titrated with 0.1 N H_2SO_4. The color change at the endpoint is from green to pink. The protein per cent in seeds was estimated by multiplying the nitrogen content by a factor 6.

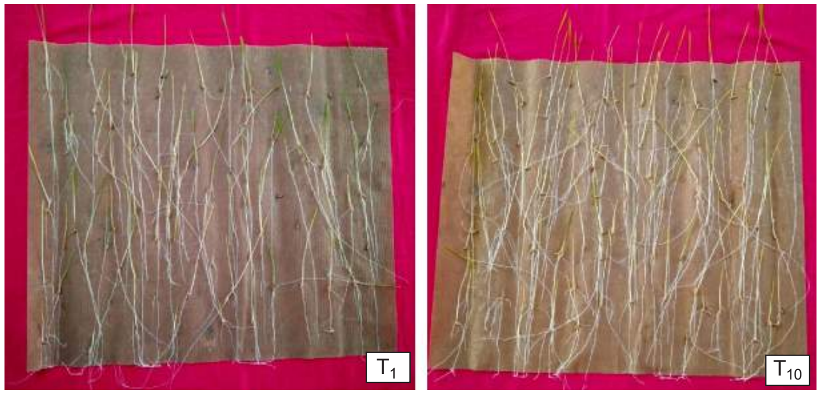


Plate 1. Seed germination of the resultant seed as influenced by seed priming and foliar spray with GA_3 in comparison with contorl (T_1- control and T_{10}- seed priming (0.43 mM)+foliar spray(150 ppm) with GA_3

Table 1. Influence of seed priming and foliar spray on seed germination (%) and seedling growth parameters of resultant rice seeds produced under salinity

Treatment	Germination (%)	Root length (cm)	Shoot length (cm)	SDW (mg)	SVI-I
T_1	91.0	14.8	8.48	72.5	2115
T_2	92.4	15.4	8.81	73.7	2236
T_3	92.6	16.0	9.07	74.8	2321
T_4	94.3	16.5	9.35	81.6	2434
T_5	93.6	16.3	9.25	78.2	2388
T_6	95.0	16.6	9.43	87.3	2473
T_7	93.1	16.2	9.16	77.4	2360
T_8	94.8	16.6	9.38	84.4	2463
T_9	94.0	16.4	9.26	78.7	2413
T_{10}	95.5	16.9	9.51	90.4	2522
S.Em. ±	0.56	0.36	0.18	3.33	39
C.D. 0.01	1.64	1.06	0.54	9.71	114

The data collected from the experiments were analyzed statistically [14] using Fisher's analysis of variances technique.

RESULTS AND DISCUSSION

Germination

Salinity induces numerous disorders in seeds due to mineral imbalance and toxicity during germination and reduces water uptake due to low osmotic potential of the medium [15]. Among all the treatments, seeds harvested from seed priming with gibberellic acid (GA_3) @ 0.43mM + foliar spray with GA_3 (@ 150ppm) recorded significantly highest seed germination (95.5%) compared to the lowest germination (91%) recorded in the control (Plate 1 and Table 1).

This might be due to stimulation of α amylase activity in the germinating seed with better cell division and elongation [16-17] due to GA_3 application thus, positively improving [16] the seed germination process (Plate 1).

Seedling growth parameters

Salinity reduced the length of root and shoot may be due to toxic effects of the Na^+ and Cl^- used and unbalanced nutrient uptake by the seedlings [24]. In the present investigation, significantly better seedling growth (Plate 2) in terms of root length, shoot length, seedling dry weight and seed vigor index I, (16.9 cm, 9.51 cm, 90.4 mg and 2522, respectively) were observed due to GA_3 treatment compared to other treatments (Table 1). This might be due to externally supplied GA_3 increase the mobilization of reserved food material needed for degradation of the cells surrounding radical by both better cell division and expansion [18] thus speeds up the germination process by promoting seedling elongation in cereals [25]. It was also evident from the work of [26-28] that foliar application

Plate 2. Seedling growth of the resultant seed as influenced by seed priming and foliar spray with GA_3 in comparison with contorl (T_1- control and T_{10}- seed priming (0.43 mM)+foliar spray(150 ppm) with GA_3

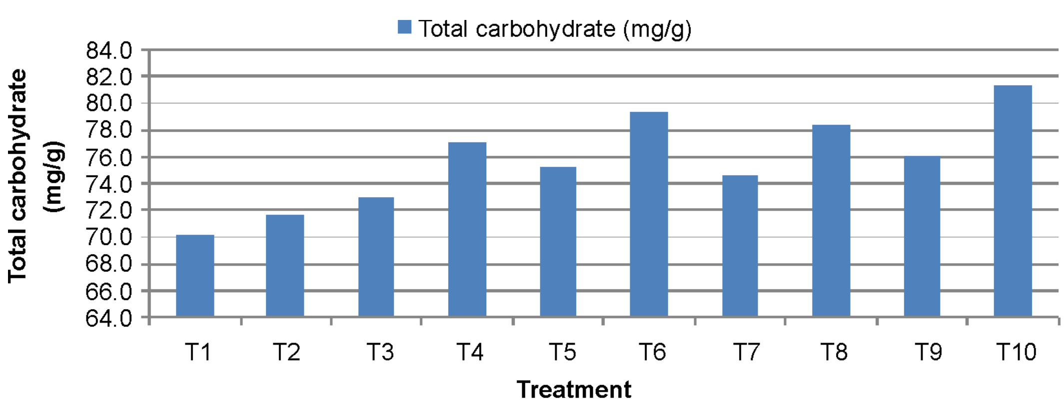
of GA_3 significantly increased root and shoot length of crop plants under saline condition.

Total carbohydrate content

Carbohydrates are important solutes that get accumulated in cytosol under salt stress and utilized to maintain the osmotic homeostasis of cells. The accumulation of sugar at stress conditions acts as a protective mechanism which prevents the sodium entry via cell [29]. In the present study, seed priming with gibberellic acid (GA_3) @ 0.43 mM + foliar spray with GA_3 150 ppm recorded higher carbohydrate content (81.3 mg g^{-1}) compared to control (70.2 mg g^{-1}) (Figure 1A). It may be attributed that GA_3 enhanced the synthesis of carbohydrate through better chlorophyll content and reduced oxidative damage of carbohydrate through better antioxidant system thereby imparting stress tolerance [30].

Protein content

Oxidative damage of protein by the production of reactive oxygen species (ROS) due to salinity reduces protein content [17]. Among all the treatments, fresh seeds harvested from the GA_3 treatment recorded significantly higher protein content (8.6%) compared to hydropriming (7.1%) and control (7%) (Figure 1B). It may be due to gibberellic acid's role in protecting the protein from denaturation due to oxidative damage and enhanced protein synthesis by reducing free amino acid accumulation and activating antioxidant systems due to application of GA_3 under salinity [30].



(A)

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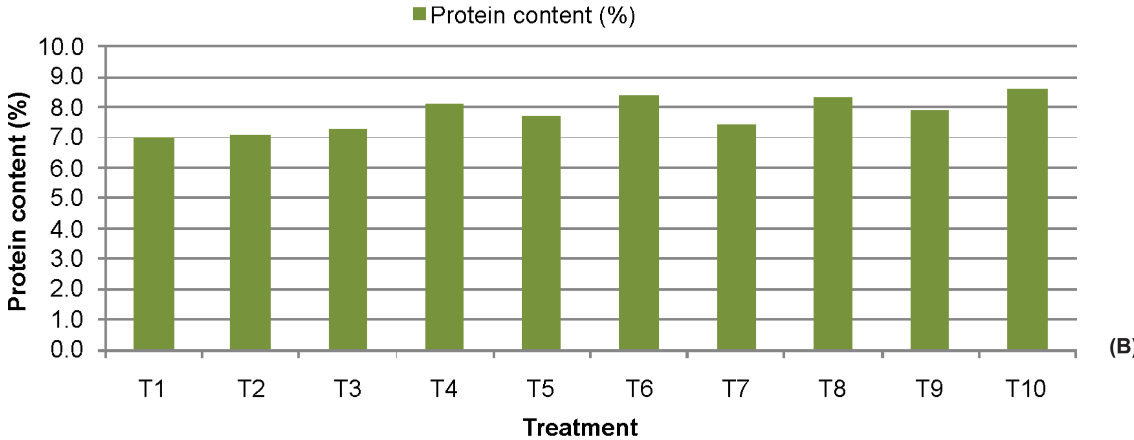

Figure 1. Influence of seed priming and foliar spray on total carbohydrate (A) and protein content (B) in resultant seeds of rice under salinity (T_1- control; T_2- hydro priming; T_3- seed priming with sodium nitroprusside (SNP) @ 80 µM; T_4 - T_3 + foliar spray with SNP 100 µM; T_5- seed priming with salicylic acid (SA) @ 200 µM; T_6- T_5 +foliar spray with SA 200 ppm; T_7- seed priming with potassium nitrate (KNO_3) @ 0.75%; T_8- T_7+foliar spray with $KNO_3$10 mM; T_9- seed priming with gibberellic acid (GA_3) @ 0.43 mM; T_{10}- T_9 +foliar spray with GA_3 150 ppm)

CONCLUSION

Results illustrated that seed priming (0.43 mM) and foliar spray (150 ppm) with gibberellic acid plays an important role in alleviating salt stress by producing quality seeds with better germination and seedling vigor in rice. In the present study, gibberellic acid treatment increased total carbohydrate and protein content in seeds may be due to reduced oxidative damage, osmotic adjustment and improved physiological processes. Therefore, gibberellic acid can be effectively used in mitigating salt stress in rice seed production.

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REFERENCES

1. KHUSH GS (2004). Harnessing science and technology for sustainable rice-based production systems. *Proceedings of FAO Rice Conference "Rice is life" International Rice Community Newsletter,* **53**: 17-23.
2. HOANG TML, TRAN TN, NGUYEN TKT, WILLIAMS B, WURM P, BELLAIRS S AND MUNDREE S (2016). Improvement of salinity stress tolerance in rice: challenges and opportunities. *Agronomy,* **6**(4): 1-23.
3. JISHA KC, VIJAYA KUMARI K AND PUTHUR JT (2013). Seed priming for abiotic stress tolerance: an overview. *Acta Physiologiae Plantarum*, **35**(5): 1381-1396.
4. ASHRAF M, ATHAR HR, HARRIS PJC AND KWON TR (2008). Some prospective strategies for improving crop salt tolerance. *Advances in Agronomy,* **97**: 45-110.
5. SHAMSUL H, MORI M, PICHTEL J AND AHMAD A (2010). Nitric oxide in plant physiology. *Wiley Blackwell*, 1-16.
6. HAYAT S, HASAN SA, MORI M, FARIDUDDIN Q AND AHMAD A (2010). Nitric oxide: chemistry, biosynthesis and physiological role. Nitric oxide in plant physiology. *GmbH and Co. KGaA, WILEY-VCH Verlag, Weinheim, Germany,* 15-21.
7. KAYA C, TUNA A AND YOKAS I (2009). Salinity and water stress. *Netherlands: Springer*, **44**: 45-50.
8. NEELAMBARI (2016). The effects of ascorbic and gibberellic acid on metabolism of wheat (*Triticum aestivum L.*) at seedling stage under saline conditions. *International Journal of Agricultural Science*, **6**(6): 307-316.
9. HEMALATHA G, RENUGADEVI J AND EEVERA T (2017). Seed priming to alleviate the effect of salinity stress in rice. *International Journal of Chemical Studies,***5**(6): 1140-1143.
10. ISTA (2013). International Rules of Seed Testing. *Seed Science and Technology*, **27**:25-30.
11. ABDUL-BAKI AA AND ANDERSON JD (1973). Vigour determination by multiple criteria. *Crop Science,* **13**: 630-637.
12. HEDGE IE AND HOFREITER BT (1962). Carbohydrate chemistry 17 (Eds Whistler RL and Be Miller, JN), vol.17, pp: 420, Academic Press, New York.
13. JACKSON ML (1973). Soil Chemical Analysis. Prentice Hall of India private limited, pp: 10-144, New Delhi, India.
14. SUNDARAJAN N, NAGRAJU S, VENKTATARAMAN S AND JAGANATH MH (1972) Design and analysis of field experiments. *University of Agricultural Sciences, Bangalore*.
15. ALMANSOURI M, KINET M AND LUTTS S (2001). Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). *Plant and Soil*, **231**: 243-254.
16. BASSIOUNI SMA, ZAYEDA BA, MOHAMED AE AND OMAR AM (2011). Effect of pre- sowing seed and seedling treatments on growth and yield of egyptian hybrid rice under saline soil conditions. *Journal of Agricultural Research,* **37**(2): 270-281.
17. PARVANEH R AND HOSEINI SM (2015). Evaluation of germination percentage and some physiologic factors under

salinity stress and gibberellic acid hormone (GA_3) treatments in wheat (*Triticum aestivum* L.). *International Journal of Advanced Research in Biological Sciences,* **2**(2): 122-131.

18. KRISHANA K (2019).The effect of exogenous application of GA_3 to mitigate the salt induced damages in rice cultivars during germination. *International Journal for Research in Applied Science and Engineering Technology,* **7**(1): 2321-9653.

19. AFZAL I, BASRA SM, AHMAD NAZIR, CHEEMA MA, WARRAICH EA AND KHALIQ A (2002). Effect of priming and growth regulator treatments on emergence and seedling growth of hybrid maize (*Zea mays* L.). *International Journal of Agriculture and Biology*, **4**(2): 303-306.

20. BAHRANI A AND POURREZA J (2012). Gibberellic acid and salicylic acid effects on seed germination and seedlings growth of wheat (*Triticum aestivum* L.) under salt stress condition. *World Applied Sciences Journal*, **18**(5): 633-641.

21. GHODRAT V AND ROUSTA MJ (2012). Effect of priming with gibberellic acid (GA_3) on germination and growth of corn (*Zea mays* L.) under saline conditions. *International Journal of Agriculture and Crop Sciences*, **4**(13): 882-885.

22. YOUNESI O AND MORADI A (2014). Effect of priming of seeds of *Medicago sativa* 'bami' with gibberellic acid on germination, seedlings growth and antioxidant enzymes activity under salinity stress. *Journal of Horticultural Reserach*, **22**(2): 167-174.

23. TSEGAY BA AND ANDARGIE M (2018). Seed priming with gibberellic acid (GA_3) alleviates salinity induced inhibition of germination and seedling growth of *Zea mays* L., *Pisum sativum* Var. abyssinicum A. Braun and *Lathyrus sativus* L. *Journal of Crop Science and Biotechnology*, **21**(3): 261-267.

24. JAMIL M, LEE DB, JUNG KY, ASHRAF M, LEE SC, RHA ES (2006). Effect of salt (NaCl) stress on germination and early seedling growth of four vegetables species. *Journal of Central European Agriculture*, **7**(2): 273-282.

25. ROOD SB, BUZZELL RI, MAJOR DJ AND PHARIS RP (1990) Gibberellins and heterosis in maize: quantitative relationship. *Crop Science,* **30**: 281-286.

26. KHAZEH A, KHAZEH Z, JABARI HT, EYMUR M AND HASHEMYBAGHA M 2015, Effect of gibberllic acid (GA_3) foliar spray on some physiological traits and mount of pigments in *Brassica napus* L. *International Journal of Biosciences,* **6**(3): 54-61.

27. DAI LY, ZHU HD, YIN KD, DU JD AND ZHANG YX (2017) Seed priming mitigates the effects of saline-alkali stress in soybean seedlings. *Chilean Journal of Agricultural Research*, **77**(2): 118-125.

28. CHAUHAN A, ABUAMARAH BA, KUMAR A, VERMA JS, GHRAMH HA, KHAN KA AND ANSARI MJ (2019). Influence of gibberellic acid and different salt concentrations on germination percentage and physiological parameters of oat cultivars. *Saudi Journal of Biological Sciences*, **26**(6): 1298-1304.

29. FERNANDO E, CECILIA P, MIRIAM B, JUAN G, GONZALEZ A (2000) Effect of NaCl on germination, growth and soluble sugar. *Botanical bulletin of Academia Sinica*, **12**: 27-34.

30. NEELAMBARI PK AND MANDAVIA C (2018). Three effective role of growth stimulators in mitigating the adverse effect of salinity stress on wheat (*Triticum aestivum* L.) at seedling stage. *Journal of Pharmacogan and Phytochem*istry, **1**: 723-729.