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Effect of seed priming in rice on physiological and biochemical constituents under sodic soil condition

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Abstract
Soil salinity and sodicity has become a severe threat to ensure food security in the developing world. Increasing salinity had significant impact on food production and more agricultural lands are expected to become salt affected due to climate change. Cereals contribute mainly to food production and growing grain crops on saline and sodic soils require adoption of different strategies for sustainable crop production. Based on the above view an experiment were undertaken at Anbil Dharmalingam Agricultural College and Research Institute, Navalurkuttapattu, Trichy to study the effect of seed priming on alleviating sodicity stress. The following two rice varieties TNAU Rice TRY 3 and I.W. Ponni seeds were primed with 1% KCl, 1% mixture of $CaSO_4$+$ZnSO_4$+$FeSO_4$, GA_3 20 ppm, +*Azophos* 1 % + *Pseudomonas fluorescens* 1.0 %, 1% mixture of KCl+ $CaSO_4$+$ZnSO_4$+$FeSO_4$ +$GA_3$20 ppm +*Azophos* 1.0 % + *Pseudomonas fluorescens* 1.0 % individually as well as combinations followed by foliar spray of 0.5% $ZnSO_4$, 2 % DAP and 2% cowpea pulse sprout extract at active tillering stage. Among the different combinations of priming tried, the seeds primed with 1% mixture of $CaSO_4$ + $ZnSO_4$ + $FeSO_4$ + GA_3 20 ppm + *Azophos* 1% followed by 0.5 % foliar spray of ZnSO4 at active tillering stage recorded the improved biochemical and physiological constituents such as proline content (744.5 µ g^{-1}**)**, soluble protein content (8.63 mg g^{-1}). Nitrate reductase (21.3 µ moles NO^{-}_{2} g^{-1} h^{-1}) chlorophyll (1.800 mg g^{-1}) and chlorophyll stability index (60.3).

Keywords: Sodicity, priming, proline, chlorophyll and chlorophyll stability index

Introduction
Rice is grown in more than 154 million hectares in the world in a wide range of ecosystems under varying temperatures and water regimes in India and it occupies an area of 44 million hectares. While the population of rice consumers is increasing at a rate of 1.8 per cent annually, the population grow at a rate of 1.5 per cent every year. Hence, the rice requirement by the year 2025 would be about 125 million tons [1]. A total of 800 million hectares of land throughout the world are salt affected either by salinity (397 million hectares) or by sodicity (434 million hectares). In Asia alone, 21.5 million hectares of land area is thought to be salt affected, of which 12 million hectares is due to saline and the remaining 9.5 million hectares is due to alkaline / sodic conditions. In India, approximately 8.6 million hectares of agricultural land is affected by varying degrees of salt related problems and of which about 3.4 million hectares is under sodic soils. Rice is susceptible to salt stress [2] particularly during the early seedling stage [3]. Salinity affects the seed germination by creating osmotic stress due to reduced water uptake or through ionic imbalance due to toxic effects of sodium (Na^{+}) and chloride (Cl^{-}) ions [4]. Salinity also upsets plant hormone level and reduces the utilization of seed reserves [5]. Salinity induced stress inhibited seed germination constraints to achieve uniform seedling stand in rice [6] and ultimately diminishes economic yield and quality of produce [7]. The exogenous application of osmolytes, osmoprotectants or plant hormones through foliar or seed is a good option to alleviate the adverse effects of salinity stress on crops [8]. To alleviate salt problem one of the seed enhancement techniques is seed priming.
Seed priming is a controlled hydration process that involves exposing seeds to low water potentials that restrict germination, but permits pre germinative physiological and biochemical changes to occur [9, 10]. Upon rehydration, primed seeds may exhibit faster rate of germination, more uniform emergence, greater tolerance to environmental stresses, and reduced dormancy in many species [11].

Micronutrient deficiencies are very common under salt stress owing to high pH [12] Foliar application might be better than soil application because ion imbalance and uptake problem happened under saline soil condition. Soil or foliar application of the three micronutrient Zn^{+2}, Fe^{+2}, Mn^{+2} partially alleviates the adverse effects of salinity on yield and yield components of salt stressed plants [13]. The research work under sodic soil in seed priming is negligible based on the above view the research work is undertaken to study the effect of priming in salt affected soil.

Materials and Methods

The study was conducted to find out the effect of seed priming on biochemical constituents of rice seeds. Genetically pure rice seeds of two varieties *viz*. TNAU Rice TRY 3 and Improved White Ponni obtained from the Department of Plant Breeding and Genetics, ADAC&RI, Trichy formed the basic materials for the study. The bio-control agent *Pseudomonas fluorescens* obtained from the Department of Plant Protection, ADAC&RI, Trichy and liquid bio-fertilizer *Azophos* obtained from the Department of Agricultural Microbiology, TNAU, Coimbatore were used for this study. The Soil type is Clay loam, the sieved soil were analysed for Mechanical composition viz. Coarse sand (%) 4.04, Fine sand (%) 5.03, Silt (%) 21.00,Clay (%)68.50 and Chemical composition viz. pH- 9.5 EC (dSm^{-1}) 0.45 ESP (%)20.31 Exc. Ca+Mg (c mol kg^{-1}) 14.12, Exc. Na (c mol kg^{-1}) 10.14, Available nitrogen (kg ha^{-1}) 189, Available phosphorus (kg ha^{-1}) 15.33 Available potassium (kg ha^{-1}) 364. The Experiment were laid out in Split design with Main plot consisting of two varieties M_1 - TNAU Rice TRY 3 M_2- Improved White Ponni. Sub plot; S_1 - Control S_2 -Seed priming with 1 % mixture of $CaSO_4$+$ZnSO_4$+$FeSO_4$ S_3 -Seed priming with 1% mixture of $CaSO_4$+$ZnSO_4$+$FeSO_4$ + GA_3 20 ppm +*Azophos* 1.0 % S_4 - Seed priming with 1% mixture of KCl + $CaSO_4$+ $ZnSO_4$+ $FeSO_4$ + GA_3 20 ppm+*Azophos* 1.0 % S_5 -Seed priming with 1% mixture of KCl + $CaSO_4$+ $ZnSO_4$+ $FeSO_4$ + GA_3 20 ppm + *Azophos* 1.0% + *Pseudomonas fluorescens* 1.0 % S_6 - Seed priming with 1% mixture of $CaSO_4$ + $ZnSO_4$ + $FeSO_4$ + foliar spray of 0.5% $FeSO_4$ S_7- Seed priming with 1% mixture of $CaSO_4$ + $ZnSO_4$ + $FeSO_4$ + GA_3 20 ppm + *Azophos* 1% + foliar spray of 0.5% $ZnSO_4$ S_8 -Seed priming with 1% mixture of KCl + $CaSO_4$ + $ZnSO_4$ + $FeSO_4$ + GA_3 20 +*Azophos* +1% foliar spray of 2 % DAP

S_9 -Seed priming with 1 % mixture of KCl + $CaSO_4$ + $ZnSO_4$ + $FeSO_4$ + GA_3 20 ppm +*Azophos* 1% +*Pseudomonas fluorescens* 1% + foliar spray of 2% cowpea pulse sprout extract with three replications, spacing of 20 x 10 cm with plot size -3 x 3m.

Chlorophyll content (Chlorophyll 'a', Chlorophyll 'b', and total chlorophyll)

For estimation of chlorophyll 250 mg of leaf sample was taken and transferred to a pestle and mortar. The sample was macerated with 10 ml of 80% acetone, centrifuged the content at 3000 rpm for 10 minutes. The optical density measured at 645, 652 and 663 nm by an Optima UV-VIS spectrophotometer (Model SP-3000). The chlorophyll content of the sample was expressed as mg g^{-1} of fresh weight [14].

Formula for calculation of Chlorophyll 'a', 'b' and Total Chlorophyll

$$\text{Chlorophyll 'a'} = (12.7 \times OD \text{ at } 663) - (2.69 \times OD \text{ at } 645) \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll 'b'} = (22.9 \times OD \text{ at } 645) - (4.68 \times OD \text{ at } 663) \times \frac{V}{1000 \times W}$$

$$\text{Total Chlorophyll} = \frac{OD \text{ at } 652 \times 1000}{34.5} \times \frac{V}{1000 \times W}$$

Where,

OD = Optical Density
V = Final volume of supernatant (25 ml)
W = Weight of the leaf sample taken in gram

Chlorophyll stability index

Chlorophyll stability index (CSI) in leaf was estimated by spectrophotometric method as suggested by Kolyroeas [15]. Soluble protein content of leaf was estimated as per the method [16]. and expressed as mg g^{-1} fresh weight and Proline content of the leaf was estimated as per the method of Bates *et al.* [17] and expressed as µg g^{-1} fresh weight. Nitrate reductase activity was estimated in fully expanded functional leaves [18] and the enzyme activity was expressed as µ moles NO^{-}_{2} g^{-1} h^{-1}.

The data was analysed through analysis of variance (ANOVA) technique for factorial controlled randomized design and presented at 5% level of significance (P = 0.05) by the procedure prescribed by Panse and Sukhatme [19].

Results and Discussion

Emergence and establishment are the two basic requirements for quality seeds as they offer scope not only for uniformity in the field stand, but also for full exploitation of yield potential of crops. Pre sowing seed quality enhancement causes early enhanced germination and growth rate of seedlings. At active tillering stage, TNAU Rice TRY 3 recorded higher chlorophyll content of 1.537 mg g^{-1} over- Improved White Ponni (1.404 mg g^{-1}). Among the sub plot treatments, Seed priming with 1% mixture of $CaSO_4$ + $ZnSO_4$ + $FeSO_4$ + GA_3 20 ppm + *Azophos* 1% + foliar spray of 0.5% $ZnSO_4$ (S_7) recorded higher chlorophyll content of 1.800 mg g^{-1} followed by Seed priming with 1 % mixture of KCl + $CaSO_4$ + $ZnSO_4$ + $FeSO_4$ + GA_3 20 ppm +*Azophos* 1% +*Pseudomonas fluorescens* 1% + foliar spray of 2% cowpea pulse sprout extract (S_9) (1.703 mg g^{-1}) while control (S_1) recorded the lowest total chlorophyll content (1.225 mg g^{-1}). Interaction of main plot with sub plot treatments was significant. M_1S_7 and M_1S_9 recorded higher total chlorophyll content of 1.940 mg g^{-1} and 1.770 mg g^{-1} while M_2S_1 recorded (1.173 mg g^{-1}) (Table 1). Salinity induced chlorophyll reduction and consequently the reduction in photosynthesis is well established by many researchers in green gram; (20&21) in rice. The reduction in chlorophyll content under salinity stress to suppression of enzymes responsible for synthesis of chlorophyll under higher salt concentrations [22]. Increased chlorophyllase activity and interference of salt ions with *de novo* synthesis of structural component proteins of chloroplast are also known to influence

the chlorophyll content under salinity stress [23, 24, 25]. TNAU Rice TRY 3 variety registered higher total chlorophyll content than I.W. Ponni. This increase in chlorophyll content might be attributed to increase chlorophyll synthesis by PGR present in the priming treatments, promotes cell division and cell expansion in expanding leaves [26].

TNAU Rice TRY 3 recorded higher chlorophyll stability index of 63.5 over I.W. Ponni with 47.3. Among the sub plot treatments, Seed priming with 1% mixture of $CaSO_4$ + $ZnSO_4$ + $FeSO_4$ + GA_3 20 ppm + *Azophos* 1% + foliar spray of 0.5% $ZnSO_4$ (S_7) recorded higher chlorophyll stability index of 60.3, followed by Seed priming with 1 % mixture of KCl + $CaSO_4$ + $ZnSO_4$ + $FeSO_4$ + GA_3 20 ppm +*Azophos* 1% +*Pseudomonas fluorescens* 1% + foliar spray of 2% cowpea pulse sprout extract (S_9) recorded 58.8 whereas, control recorded 49.3 (Table 2). Chlorophyll stability index (CSI) is an important parameter for screening of plant cultivars for abiotic stresses including salinity [27]. Significant variation was recorded between two varieties as TNAU Rice TRY 3 recorded higher value for CSI indicating its tolerance for salt stress.

TNAU Rice TRY 3 recorded higher soluble protein content of 9.57 mg g^{-1} over I.W. Ponni with 6.13 mg g^{-1}. Among the sub plot treatments, S_7 recorded higher soluble protein content of 8.63 mg g^{-1} followed by S_9 with a value of 8.50 mg g^{-1} while control recorded the lowest soluble protein of 7.15 mg g^{-1}. The interaction of main plot with sub plot treatments was found to be significant. M_1S_7 recorded higher soluble protein content of 10.40 mg g^{-1} followed by M_1S_8 and M_1S_9 with 10.10 mg g^{-1} each whereas control M_2S_1 recorded the lowest soluble protein content of 5.40 mg g^{-1}(Table 3).

Salinity, by and large, depressed protein synthesis, accelerated their degradation and disturbed the soluble amino acid protein ratio in plants (28, 29, 30 & 31). Breakdown of polysomes accompanied by an increased level of NRase was demonstrated during salt stress (32 & 33). Moderate salinity in some cases might increase total protein but innumerable eports revealed a decrease in protein and nucleic acid content in root, leaves and embryo axis of mung bean, chickpea, pea and cowpea under NaCl and Na_2SO_4 salinity stress [34].

Under sodicity condition, at active tillering stage TNAU Rice TRY 3 recorded higher proline content of 791.6µg g^{-1}. Among the sub plot treatments, S_7 recorded higher proline content of 744.5 µg g^{-1} which was on par with S_9 and S_8 treatment, while control recorded 685.7µg g^{-1}. (Table 4). The interaction of main plot with sub plot treatments was significant. M_1S_7 recorded higher proline content of 826.7µg g^{-1} followed by M_1S_9 (819.7µg g^{-1}) while M_2S_1 recorded lower proline content of 623.0 µg g^{-1}. The study also revealed a considerable decline in soluble protein content of the two rice varieties TNAU Rice TRY 3 and I.W. Ponni grown under sodicity condition without receiving any priming treatments. However, the varieties which were imposed with priming treatments, responded differentially by showing enhanced level of soluble protein. Under sodicity condition, seeds primed with 1 % mixture of $CaSO_4$ + $ZnSO_4$ + $FeSO_4$ + GA_3 20 ppm + *Azophos* along 1 % with 0.5 % foliar spray of $ZnSO_4$ at active tillering stage recorded higher soluble protein over control. The soluble protein content of plants is a deciding factor of drymatter accumulation thereby increasing the yield and could be attributed to the efficiency of RuBpcase at stress level [35].

Proline accumulation is a universal response of plants to various stresses. Proline acts as an osmolyte and helps the plants to maintain tissue water potential under all kinds of stresses. Proline, as an osmoprotectant, is largely confined to the cytoplasm and is mostly absent from the vacuole [36]. It plays a key role in the cytoplasm as a scavenger of free radicals as well as a mediator in osmotic adjustment and also increases the solubility of sparingly soluble proteins. [37, 38, 39] Free proline accumulation was generally more in the most tolerant cultivars, suggesting its correlation with salinity tolerance of the plants [40]. The proline accumulation was maximum in TNAU Rice TRY 3 than I.W. Ponni under sodicity condition. The priming treatments 1 % mixture of $CaSO_4$ + $ZnSO_4$ + $FeSO_4$ + GA_3 20 ppm +*Azophos*1 % along with 0.5 % foliar spray of $ZnSO_4$ help to increasing the level of proline accumulation. The maximum proline accumulation could be attributed to non-incorporation of free amino acid, proline into protein synthesis due to salt stress or the breakdown of the existing protein molecules into various constituent amino acids [41]. M_1 recorded higher nitrate reductase activity of 22.5 µ moles No_2 $g^{-1}h^{-1}$over M_2 with 11.6 µ moles No_2 $g^{-1}h^{-1}$. Among the sub plot treatments, S_7 recorded 21.3 µ moles No_2 $g^{-1}h^{-1}$ followed by S_9 with 19.5 µ molesNo_2 $g^{-1}h^{-1}$whereas control recorded 14.2 µ moles No_2 $g^{-1}h^{-1}$. Among the interaction treatments, M_1S_7 and M_1S_9 recorded 28.4 and 25.2 µ moles No_2 $g^{-1}h^{-1}$ while M_2S_1 recorded 8.8 µ moles No_2 $g^{-11}h^{-1}$(Table 5.)

In plants, nitrogen assimilation is regulated by the activation of the enzyme, nitrate reductase. This enzyme plays a constructive role in nitrogen utilization by the plants through nitrogen metabolism and which is the most affected enzyme by salinity. Salinity may strongly affect the overall nitrate assimilation process because nitrate is required to induce nitrate reductase, the key enzyme of nitrate assimilation process [42]. Sodicity stress reduced nitrate reductase activity in leaves of the two rice cultivars, with more adverse effect on I.W. Ponni. The priming treatments, particularly PGRs play their beneficial role in improving the overall biochemical processes, which are generally inhibited by stress conditions. Under sodicity condition seeds primed with 1 % mixture of $CaSO_4$ + $ZnSO_4$ + $FeSO_4$ + GA_3 20 ppm +*Azophos* 1 % along with foliar spray of 0.5 % $ZnSO_4$ per cent increase over the control. The decreased NRase activity in salt sensitive rice cultivars is possibly due to the inhibition of enzyme induction under salinization [43].

Conclusion

Under sodic soil condition the seeds primed with 1% mixture of $CaSO_4$ + $ZnSO_4$ + $FeSO_4$ + GA_3 20 ppm + *Azophos* 1% followed by 0.5 % foliar spray of ZnSO4 at active tillering stage recorded the improved physiological and biochemical constituents.

Table 1: Effect of seed priming and foliar spray on Total chlorophyll (mg g^{-1}) in leaf at active tillering stage in rice varieties of TNAU Rice TRY 3 and I. W. Ponni under sodicity condition.

Treatments	Sodicity			
	M_1		**M_2**	**Mean**
S_1	1.277		1.173	1.225
S_2	1.351		1.230	1.300
S_3	1.454		1.360	1.407
S_4	1.372		1.262	1.320
S_5	1.592		1.479	1.535
S_6	1.401		1.308	1.354
S_7	1.940		1.650	1.800
S_8	1.680		1.540	1.610
S_9	1.770		1.635	1.703
Mean	1.537		1.404	
	M	S	M x S	S x M
SEd	0.020	0.021	0.034	0.026
CD (P=0.05)	0.084	0.043	0.103	0.061

Table 2: Effect of seed priming and foliar spray on chlorophyll stability index (%) in leaf at active tillering stage in rice varieties of TNAU Rice TRY 3 and I. W. Ponni under sodicity conditions.

Treatments	Sodicity			
	M_1		**M_2**	**Mean**
S_1	55.6		42.3	49.3
S_2	62.0		44.6	53.3
S_3	63.5		46.5	55.0
S_4	61.5		44.8	53.1
S_5	65.3		48.3	56.8
S_6	62.5		45.6	54.0
S_7	68.3		52.3	60.3
S_8	66.7		50.3	58.5
S_9	66.5		51.1	58.8
Mean	63.53		47.30	
	M	S	M x S	S x M
SEd	0.800	1.080	1.645	1.321
CD (P=0.05)	3.442	2.197	NS	NS

Table 3: Effect of seed priming and foliar spray on soluble protein activity (mg g^{-1}) in leaf at active tillering stage in rice varieties of TNAU Rice TRY 3 and I.W. Ponni under sodicity condition.

Treatments	Sodicity			
	M_1		**M_2**	**Mean**
S_1	8.90		5.40	7.15
S_2	8.70		5.70	7.22
S_3	9.60		6.10	7.83
S_4	8.80		6.20	7.52
S_5	10.0		6.20	8.12
S_6	9.40		5.60	7.50
S_7	10.40		6.90	8.63
S_8	10.10		6.40	8.25
S_9	10.10		6.70	8.50
Mean	9.57		6.13	
	M	S	M x S	S x M
SEd	0.12	0.15	0.24	0.19
CD (P=0.05)	0.51	0.31	0.67	0.44

Table 4: Effect of seed priming and foliar spray on proline activity (µg g^{-1}) in leaf at active tillering stage in rice varieties of TNAU Rice TRY 3 and I.W. Ponni under sodicity condition.

Treatments	Sodicity			
	M_1		**M_2**	**Mean**
S_1	748.3		623.0	685.7
S_2	762.0		634.3	698.2
S_3	787.7		645.0	716.3
S_4	784.3		629.7	707.0
S_5	796.3		653.7	725.0
S_6	788.3		653.7	721.0
S_7	826.7		662.3	744.5
S_8	810.3		655.7	733.0
S_9	819.7		662.3	741.0
Mean	791.6		646.6	
	M	S	M x S	S x M
SEd	9.91	5.81	12.6	7.11
CD (P=0.05)	42.6	11.83	46.2	16.73

Table 5: Effect of seed priming and foliar spray on nitrate reductase activity (µ moles NO^{-}_{2} g^{-1} h^{-1}) in leaf at active tillering stage in rice varieties of TNAU Rice TRY 3 and I.W. Ponni under sodicity condition.

Treatments	Sodicity			
	M_1		**M_2**	**Mean**
S_1	19.6		8.8	14.2
S_2	20.5		9.8	15.2
S_3	21.1		11.6	16.4
S_4	19.8		9.6	14.7
S_5	23.3		12.7	18.1
S_6	20.7		11.0	15.9
S_7	28.4		14.2	21.3
S_8	24.0		13.4	18.7
S_9	25.2		13.7	19.5
Mean	22.5		11.6	
	M	S	M x S	S x M
SEd	0.25	0.53	0.74	0.64
CD (P=0.05)	1.08	1.07	1.80	1.51

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