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Effect of PGPR on growth promotion of rice (*Oryza sativa* L.) under salt stress

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ABSTRACT

Large areas of the world are not usable from the agricultural perspective due to the constraints posed by nature and human activities making the soil less or unproductive. Soil salinity is a major abiotic stress worldwide. The development of salt-tolerant crops is not always an economical approach for sustainable agriculture, whereas microbial inoculation to alleviate salt stress is a better option because it minimizes production costs and environmental hazards. Plant Growth Promoting Rhizobacteria (PGPR) have been indicated as efficient growth enhancer of crop plants and the growth promoting effect under stress conditions has also been suggested to be beneficial for crop production. In this study two rice genotypes ADT43 and IR50 treated with PGPR (Pseudomonas strains PF1 and TDK1) were subjected to 100mM NaCl, following IRRI method under laboratory environment. The salt stress symptoms included stunted growth, poor root growth and the leaves from the tip turned to white. The results of the experiment indicated that the plant height, root length, dry weight of shoot and root were significantly increased due to Pseudomonas strain treatment even under salt stress whereas the plants grown without any treatment had less growth. ADT43 genotype treated with Pseudomonas strain TDK1 recorded better plant development under salt stress.

Key words: Rice, Stress, Salt tolerance, PGPR, Growth

INTRODUCTION

Salinity is an ever-present threat to crop yields, especially in countries where irrigation is an essential aid to agriculture. Although the tolerance of saline conditions by plants is variable, crop species are generally intolerant of one-third of the concentration of salts found in sea-water [6]. The plant growth is either depressed or entirely prevented due to excessive build up of salinity and / or alkalinity in the soil. Because many salts are also plant nutrients, high salt levels in the soil can upset the nutrient balance in the plant and interfere with the uptake of some nutrients and also cause osmotic stress. Among the various fungi and bacteria, PGPR play a significant role in the management of both biotic and abiotic stress. PGPR are a group of free living saprophytic bacterial microorganisms that live in the plant rhizosphere and colonize in the root system. They survive in seed or soil, multiply in the spermosphere in response to seed exudates rich in carbohydrates and amino acids [9] attach to root surface [13] and become endophytic by colonizing in root cortex region. PGPR generally provides the plant with a compound that is synthesized by the bacterium of facilitating the uptake of nutrients from the environment [8]. Plant growth benefits due to the addition of PGPR include increases in germination rates, root growth, yield including grain, leaf area, chlorophyll content, magnesium, nitrogen and protein content, hydraulic activity, tolerance to drought and salt stress, shoot and root weights and delayed leaf senescence [10]. The present study was conducted with the objective to study effect of PGPR treatments on morphological parameters under salt stress condition and to study effect of PGPR on improvement of source-sink relationship in two genotypes of rice under salt stress.

MATERIALS AND METHODS

The present study was carried out to screen two varieties of rice (*Oryza sativa* L.) for salinity tolerance, to understand the physiological and biochemical mechanisms of tolerance to salinity stress and observe the impact of PGPR for improving the salt tolerance. Experiment was conducted in the NLC Laboratory, Department of Crop Physiology, Tamil Nadu Agricultural University (TNAU), Coimbatore in the year 2010 using Completely Randomized Design (CRD) as the design of experiment. The set-up developed by IRRI includes Styrofoam floats (36.5cm x 26.5cm) having 100 holes (10mm x 10mm) and a nylon net bottom, placed on top pf a rectangular 18 litre plastic tray. However, the set-up can screen only a limited number of seedlings. Modifications were done to make this screening method applicable to evaluate sufficient number of seedlings. The set-up includes thermocole floats with 77 circular holes (1.75cm diameter) placed on rectangular 10 litre plastic trays.

Seeds were divided into four groups each group contained 250g. First set was soaked in water. Second set was soaked in $CaCl_2$ solution @ 50mM [1]. Third set was soaked in PF1 solution @ 2g per 10 litres of water. Fourth set was soaked in TDK1 solution @ 2g per 10 litres of water. Seeds were soaked for 12 hours. After 12 hours germinated seeds were transferred to sterilized sand bed and grown for 7 days. The most uniform seedlings were transferred to Yoshida's cultural solution (Yoshida *et al.*, 1976) through the holes of thermocole sheets after 7 days of sowing, which floated on the solution for 14 days. At 21st day of sowing 100mM salt stress (NaCl) was given. At 7 days after treating by NaCl, the plant samples were harvested and rinsed with distilled water and kept in refrigerator for physiological analysis. The shoot length of the seedling was measured from the base of the shoot to the longest leaf tip and the mean of the ten values were worked out from ten plants selected at random from each genotype. Root length was determined in ten plants selected at random from each genotype. The roots were removed carefully from the tray with minimum damage and their mean length from the base of the shoot to the tip of the longest root was measured. Number of leaves was determined by counting the leaves from the base to the tip of the plant and the mean value of ten plants selected at random from each genotype was worked out. Leaf Area (LA) for the whole sampling unit was measured by using Leaf Area Meter (Licor Model 3100) and expressed as cm^{-2} $plant^{-1}$. Plant samples were first shade dried, and then oven dried at 80°C for 24 hours. The dry weight of the whole plant was taken and expressed in g $plant^{-1}$. Using AGRES software, significance of the observed values was determined. Based on SEd and CD value (P=0.05) significance of the effect of *Pseudomonas* strains and chemical treatment with $CaCl_2$ was determined.

RESULTS AND DISCUSSION

The present investigation was about studying the influence of PGPR in imparting salinity tolerance in rice genotypes and to elucidate the information on morphological and physiological mechanisms of salinity tolerance. The statistically analyzed data of various experiments on the influence of two *Pseudomonas* strains PF1 and TDK1 on salinity tolerance are presented in appropriate table and figures and results are presented here under.

Plant height

Rice is sensitive to salt stress during seedling stage [5]. The plant height was found to be significantly increased due to treatment effect even under salt stress and was found to be varied between two genotypes studies. Shoot length of 28 days old seedlings showed significant increase in all the varieties subjected to sodium chloride stress. Significant increase of shoot length was due to the effect of treatments and genotypic variation. Between the genotypes, ADT43 treated with TDK1 strain registered the highest mean plant height (33.10cm $plant^{-1}$). *Pseudomonas* inoculation on the plant height resulted in taller plants with a lower increase in stem diameter, probably due to an increase in cell division and cell elongation [4]. The genotype IR50 had comparatively lower plant height (32.48cm $plant^{-1}$) with *Pseudomonas* strain TDK1 seed treatment under salt stress because of genotypic difference. Treatments PF1 and $CaCl_2$ were also found better in improving the plant height significantly over control. $CaCl_2$ has positive effect on ameliorating adverse effects of salt stress. This was supported by Afzal *et al.* [1] who explained that seed treatment with $CaCl_2$ would counteract the salinity induced growth inhibition in wheat seedlings.

Root length

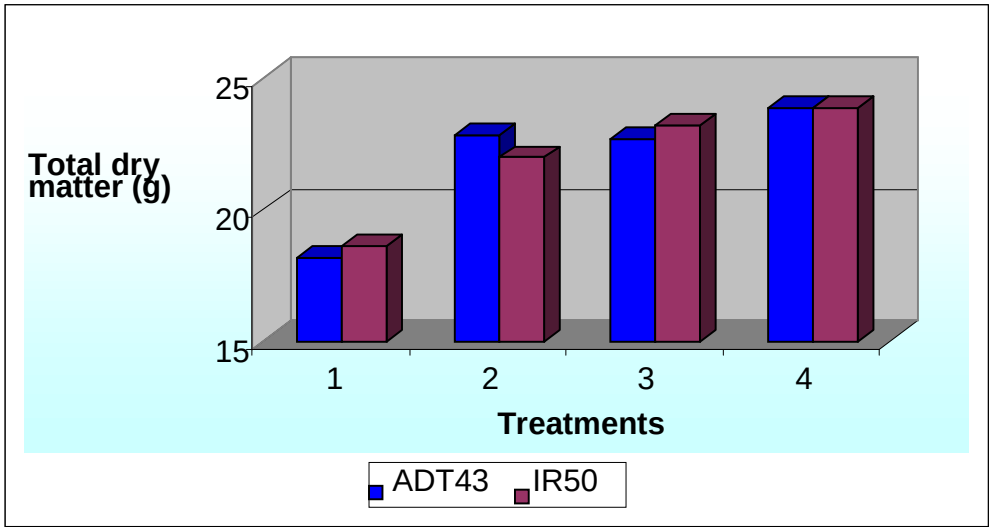
During the initial phase of salinity, the osmotic effect predominates and induces water stress due to the high salt concentration in the root medium. In the present study, root length of two genotypes was influenced by the *Pseudomonas* and was found to be significantly varying. Root length of ADT43 increased from 11.16cm (no treatment) to 14.42cm (*Pseudomonas* strain TDK1 seed treatment) grown under salt stress condition (100mM NaCl

salt stress). Variation for root length between two rice genotypes was significant. Ali *et al.* [2] reported that tolerant lines of rice had higher root and shoot ratio at the seedling stage thus providing a clue about salt tolerance potential of a genotype. Among all the treatments, *Pseudomonas* strain TDK1 treated plants showed very less reduction of root length indicating Pseudomonas strain TDK1 increases salt tolerance of the plants.

Table 1: Mean performance of morphological traits of two rice genotypes with *Pseudomonas* strains and $CaCl_2$ treatment under salt stress

Traits	No treatment		$CaCl_2$		PF1		TDK1	
	ADT43	IR50	ADT43	IR50	ADT43	IR50	ADT43	IR50
Plant height (cm)	24.98	23.06	27.30	24.90	28.16	28.20	33.10	32.48
Root length (cm)	11.16	11.38	11.80	12.64	13.14	13.16	14.42	13.80
Fresh leaves (no.)	2	1	2	2	4	3	4	3
Dry leaves (no.)	4	4	3	3	2	2	1	2
Leaf area (cm^2)	73.13	71.77	82.40	81.64	84.70	82.78	89.66	88.82
SDW (g)	13.67	13.17	14.69	14.08	14.54	15.02	15.53	15.72
RDW (g)	4.50	5.42	8.14	7.89	8.11	8.13	8.34	8.14
TDMA (g)	18.17	18.59	22.83	21.96	22.65	23.15	23.87	23.86

Figure 1: Effect of salt stress on total dry matter accumulation in two rice genotypes



Treatments:
1. No external seed treatment
2. $CaCl_2$ treatment
3. Pseudomonas strain PF1 treatment
4. Pseudomonas strain TDK1 treatment

Number of fresh and dry leaves

Al-Maskari *et al.* [3] reported that number of leaves in lettuce was reduced significantly with increasing salinity levels. In the present experiment, untreated rice genotype IR50 exhibited highest number of dry leaves (4 leaves $plant^{-1}$) and ADT43 genotype treated with Pseudomonas strain TDK1 exhibited highest number of fresh leaves (4 leaves $plant^{-1}$) indicating that the *Pseudomonas* strains are effective in maintaining leaf turgor and chlorophyll content due to which plants produce more number of fresh leaves. This was supported by Yildrim *et al.* [15] who reported that salt stress significantly decreased fresh leaf number of the radish plants compared with the non-saline conditions. However, plants treated with PGPR had more number of fresh leaves than the controls under both salt stress and absence of salt stress. TDK1 had the same effect on both the genotypes under salt stress as both ADT43 and IR50 had 1 dry leaf per plant. Untreated ADT43 genotype (2 leaves $plant^{-1}$) had the lower dry leaves than IR50 (4 leaves $plant^{-1}$).

Leaf area

Leaf area is primarily related to photosynthesis by light perception. In this study, leaf area of two genotypes showed a significant variation. ADT43 genotype with *Pseudomonas* strain TDK1 which recorded a leaf area of 89.66cm^2

and IR50 with same seed treatment with TDK1 strain 88.82cm^2 both registering higher values for leaf area. These were followed by ADT43 with PF1 treatment (84.70cm^2). Among all four *Pseudomonas* strain treatments and two genotypes, IR50 plants grown with no external seed treatment recorded the lowest leaf area (71.77cm^2). It indicates that leaf area is significantly increased due to the Pseudomonas strain treatment effect. This result is supported by Gholami *et al*. [7] who stated that leaf size was increased due to PGPR application (*Azospirillum* and *Pseudomonas*) in maize seedlings.

Shoot and root dry weight (SDW and RDW)

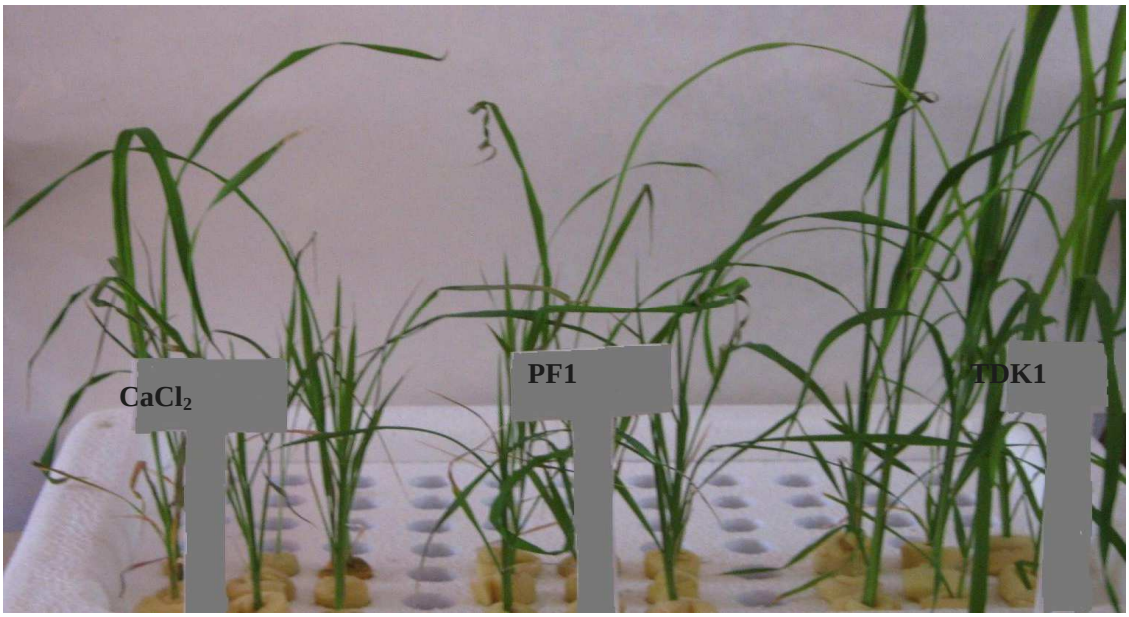
Welfare *et al*. [14] observed that salinity caused a substantial reduction in shoot and root dry weight in all varieties, but the effect on root growth was proportionately less than on shoot growth and increased root/shoot ratio, although this effect was more pronounced in some varieties than the others. *Pseudomonas* treatments had significant effect on shoot dry weight of two rice genotypes. Shoot dry weight increased from 13.67g (with no seed treatment) to 15.53g (*Pseudomonas* strain TDK1 seed treatment) in ADT43 genotype under salt stress (100mM NaCl). The highest shoot dry weight (15.72g plant^{-1}) was observed in IR50 genotype with *Pseudomonas* strain TDK1 seed treatment under salt stress and at the same time lowest shoot dry weight was also observed in this genotype (13.17g) grown without any seed treatment. Highest root dry weight was found in ADT43 genotype (8.34g) with Pseudomonas strain TDK1 treatment and the lowest root dry weight was also observed in ADT43 genotype without any seed treatment (4.50 g) under salt stress condition. This result is supported by Mafia *et al*. [11] who observed that there was a significant increase in shoot and root dry weight of *Eucalypts urophylla* upon inoculation with *Pseudomonas fulva* Ca. Sturz *et al*. [12] enumerated an increase in shoot and root weight of potatoes upon inoculation with *Pseudomonas* isolated from a weed.

Total Dry Matter Accumulation (TDMA)

Total dry matter accumulation is an outcome of the photosynthetic activity of the plant after respiratory loss. It was observed that TDMA increased with *Pseudomonas* strain TDK1 seed treatment (23.87g and 23.86g in ADT43 and IR50 respectively) compared to the plants grown without any chemical or biological treatment. In other words, the plants grown from the seeds soaked in just water did not perform as good as the plants treated with *Pseudomonas* strains or $CaCl_2$ which signifies that even though the salt stress was present, the *Pseudomonas* rescued the stress effects producing significant dry matter in rice. Lowest TDMA (18.17g) was noticed in case of ADT43 (no external treatment) grown under salt stress.

Figure 2: Rice plants treated with *Pseudomonas* strain TDK1 in Yoshida solution culture

Figure 3: ADT43 genotype in Yoshida solution culture grown under 100 MM salt stress



CONCLUSION

Rice is very sensitive to salt stress at seedling stage. Yield is drastically reduced under salt stress. Mitigation of salt stress at the seedling stage could minimize the yield loss at later stages as the rice plant is reported to be the most susceptible to salinity at vegetative period than the reproductive period. The seed treatment with plant growth promoting rhizobacteria (PGPR) could help to overcome the salt stress at seedling stage. The growth parameters such as plant height, root length, and leaf area were significantly increased due to *Pseudomonas* strain treatments. Leaves remained fresher in the *Pseudomonas* strain treated plants compared to non-treated plants. ADT43 genotype treated with *Pseudomonas* strain TDK1 performed better than IR50 genotype. Shoot dry weight, root dry weight and total dry matter accumulation (TDMA) showed to have significantly increased in *Pseudomonas* strain treated plants. IR50 genotype with *Pseudomonas* strain TDK1 seed treatment recorded highest shoot dry weight whereas ADT43 genotype treated with the same *Pseudomonas* strain recorded highest root dry weight. The salt tolerance rice genotypes generated through the treatments of two *Pseudomonas* strains identified in this study can be effectively used in the salt affected areas through further confirmation studies.

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