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Research Article

Plant growth-promoting rhizobacteria mediated moisture stress alleviation in the early stages of Rice (*Oryza sativa* L.) variety CO 51

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Abstract

Drought is one of the abiotic stresses that have a significant impact on agricultural growth across the world. Plant growth-promoting rhizobacteria (PGPR) inoculation in rice plants may be a viable and environmentally acceptable method of sustaining the development and yield of drought-stressed rice plants. The current study focused on the alleviation of drought in the early stages of rice variety CO 51 using PGPR isolated from the rhizosphere of xerophytes. The seeds were treated with bio inoculants and subjected to different moisture stress levels (10%, 20% and 30%) using PEG 6000. The seeds treated with bio inoculants exhibited higher germination percentage and growth traits such as shoot length root length and fresh weight, especially seeds treated with *Bacillus velezensis* VKSB5 (MT729963), and *Bacillus altitudinis* MLSB2 (MT729964) over uninoculated plants. This was found to be due to the increased proline accumulation and antioxidant activity in these seedlings, which plays a major role in drought alleviation by altering the osmotic potential and by its ROS scavenging mechanism. Hence this study provides evidence for the effective drought ameliorating ability of these cultures during the initial growth stages of rice. Further studies can contribute to the development of effective bio-inoculants for the mitigation of drought in rice.

Keywords: *Bacillus,* Drought, Moisture stress, Plant growth-promoting rhizobacteria, Rice

INTRODUCTION

Rice is a field crop that requires more water for growth; drought stress is regarded as the greatest impediment to rice growth, productivity, and yield (Mumtaz *et al.*, 2020). In many parts of the world, the supply of irrigation water for agriculture, particularly rice cultivation, is challenged not only by a worldwide scarcity of water resources (Cai *et al.*, 2020) but also by rising urban and industrial demand (Boretti and Rosa, 2019). Rice farming takes significantly more water than other crops worldwide; it has been estimated that irrigated rice consumes over 40% of global water utilised specifically for irrigation (Hoekstra *et al.*, 2011).

Water deficiency during germination leads to a decrease or even total suppression of seedling emergence and stand establishment as a primary limitation affecting agricultural output worldwide (Kaya *et al.*, 2006). Drought stress inhibits seed germination and seedling establishment owing to a decrease in water potential, which leads to a decrease in water intake (Farooq *et al.*, 2009). Plant growth-promoting rhizobacteria (PGPR) might play a key role in the mitigation of drought-induced harmful effects on plants among the numerous drought-relief techniques (Vurukonda *et al.*, 2016). These beneficial bacteria invade plant rhizospheres/endo-rhizospheres and enhance plant development via a variety of direct and indirect processes (Grover *et al.*, 2011).

In this context, the present study focussed on the alleviation of moisture stress in the initial stages of rice variety CO 51 to promote germination and seedling establishment using plant growth-promoting rhizobacteria (PGPR) isolated from the rhizosphere of xerophytes and characterization of the physiological and biochemical responses to drought stress in rice seedlings under laboratory conditions.

MATERIALS AND METHODS

In vitro assessment of plant growth of rice under induced drought stress

The bacterial strains *Bacillus aryabhattai* APSB18 (MT729997), *Bacillus velezensis* VKSB5 (MT729963), and *Bacillus altitudinis* MLSB2 (MT729964) were used in this study and were obtained from the Insects ecology laboratory, Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, where it was previously isolated from the rhizosphere of xerophytes (Karvembu *et al.*, 2021). The standard in this study was *Bacillus altitudinis* FD48, which was obtained from the Biocatalysts Laboratory, Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore. As indicated by Sandhya *et al.* (2009), drought-resistant PGPR derived from the rhizosphere of xerophytes was utilised to evaluate the potential in relieving drought stress effects in host plant rice (*Oryza sativa* L.) variety CO 51. The Seeds were surface sterilized and colonized with (10^8 cells/g) of the drought-tolerant strains, shade dried and placed in a sterile germination sheet. The moisture stress was imposed using Polyethylene Glycol (PEG 6000 MW). Twenty-five seeds were gently placed on a wet paper towel, making sure they did not touch, and a moistened second paper towel was carefully placed over the seeds. The paper towels and a polythene sheet beneath them were then lightly folded into a tube and secured with a rubber band. The rolls were placed in containers of different PEG concentrations. Drought stress was imposed using different concentrations viz., 10%, 20% and 30% of PEG 6000, respectively, in Hoagland's nutrient solution. The entire experimental setup was exposed to light and darkness at 12 h intervals. After 15 days of drought exposure, germination percentage, root length, shoot length, and fresh weight were measured in water-stressed seedlings and their unstressed controls. The proline and antioxidant enzyme estimations were done in seedlings. The experiment was laid in a completely randomized design with three replications.

Estimation of proline in rice seedlings

The methodology devised by Bates *et al.* (1973) was utilized for assessing the total proline content in leaves produced under moisture stress. Leaf samples (500 mg) were homogenized in 3% sulfosalicylic acid. After filtration, 2 mL homogenate mixture, 2 mL acid ninhydrin, and 2 mL glacial acetic acid were mixed to estimate proline. The reaction mixture was then incubated for an hour at 100°C in a boiling water bath and then cooled in an ice bath to terminate the process. Total proline was separated with 4 mL of toluene and vigorously mixed. After the separation of the chromophore layer, the absorbance at 520 nm was measured against a blank. The total proline was determined using a proline standard graph, and it was given in milligrams per gram of fresh weight.

Estimation of antioxidant enzyme production

For the Superoxide dismutase (SOD), Catalase (CAT), Peroxidase (POD) and Ascorbate peroxidase (APX) estimation, 500 mg of leaf sample was homogenized in 5 mL ice-cold buffer containing 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA (ethylene diamine tetra acetic acid), and 1% (w/v) PVP (polyvinyl pyrrolidone) with an ice-cold pestle and mortar. The homogenate was centrifuged for 30 minutes at 4°C at 10,000 rpm. The enzyme assay was performed using the supernatant obtained. Ascorbate peroxidase (APX) activity was assessed according to the procedure by Nakano and Asada (1987). Catalase (CAT) activity was estimated by the method described by Azevedo *et al.* (1998). Superoxide dismutase (SOD) activity was estimated by the nitro blue tetrazolium (NBT) method devised by Beauchamp and Fridovich (1971) and Peroxidase (POD) activity by the method devised by Hammerschmidt *et al.* (1982).

RESULTS

The germination traits (germination percentage, shoot length, root length and fresh weight) rice variety CO 51 decreased with an increase in moisture stress (Table 1). There was no significant difference in germination percentage under non-stressed conditions among the treated seeds. As the moisture stress increased, seeds treated with bio inoculants exhibited higher germination than non-treated seeds, among which seeds treated with *Bacillus altitudinis* (C2) and *Bacillus velezensis* (C4) treated seeds showed the highest germination percentage of 72% under 30% PEG concentration. The root and shoot length were greatly reduced by 30% PEG but were greater in PGPR-treated seedlings. The maximum shoot length (7.5 cm) was observed in *B. altitudinis* (C2) treated seedlings and the maximum root length (13.11 cm) was observed in *B. velezensis* (C4) treated seedlings at 30% PEG respectively. The fresh weight was found to be maximum (53 mg $plant^{-1}$) in seedlings treated with *B. altitudinis* (C2) at 30% PEG which was lower than the standard used. The proline accumulation showed an increasing trend towards moisture stress (Fig. 1). The *B. altitudinis* (C2) primed seedlings accumulated the maximum proline of 31.51 µg g^{-1} FW at 30% PEG.

The antioxidant enzyme activity also showed a similar

Table 1. Effect of PGPR inoculation on the germination and growth parameters of rice seedlings under different moisture stress conditions

Treatment		**Germination %**	**Shoot Length (cm)**	**Root Length (cm)**	**Vigour Index**	**Fresh weight (mg plant^{-1})**
C1	0% PEG	96 ±0.31^{a}	9.7 ±0.27^{b}	17.5 ±0.09^{d}	2611.2 ±120.69^{b}	69 ±0.78^{c}
C2		100 ±4.88^{a}	12.5 ±0.37^{a}	20.5 ±0.6^{a}	3300 ±73.07^{a}	79 ±1.19^{a}
C3		100 ±4.93^{a}	11.7 ±0.22^{a}	18.4 ±0.25^{cd}	3010 ±49.42^{a}	73 ±1.97^{bc}
C4		100 ±4.52^{a}	11.8 ±0.42^{a}	20.01 ±0.31^{ab}	3181 ±39.9^{a}	74 ±0.67^{b}
C5		100 ±1.01^{a}	12.8 ±0.6^{a}	19.2 ±0.33^{bc}	3200 ±142.93^{a}	77 ±1.61^{ab}
C1	10% PEG	84 ±4.01^{a}	7.9 ±0.15^{d}	15 ±0.49^{c}	1923.6 ±4.85^{d}	62 ±1.98^{b}
C2		96 ±0.32^{a}	11.4 ±0.34^{a}	18.5 ±0.02^{a}	2870.4 ±21.76^{a}	73 ±2.59^{a}
C3		92 ±4.13^{a}	8.4 ±0.21^{d}	16.5 ±0.72^{bc}	2290.8 ±49.76^{c}	67 ±1.36^{ab}
C4		96 ±4.62^{a}	9.2 ±0.07^{c}	17.6 ±0.33^{ab}	2572.8 ±53.71^{b}	69 ±3.13^{ab}
C5		96 ±3.75^{a}	10.1 ±0.07^{b}	16.9 ±0.58^{b}	2592 ±59.34^{b}	71 ±1.71^{a}
C1	20% PEG	64 ±2.78^{b}	6.4 ±0.23^{c}	10.8 ±0.14^{c}	1100.8 ±7.37^{c}	55 ±0.02^{b}
C2		84 ±1.34^{a}	9.1 ±0.36^{a}	14.9 ±0.24^{a}	2016 ±90.51^{a}	67 ±0.59^{a}
C3		80 ±3.01^{a}	7.8 ±0.24^{b}	12.1 ±0.54^{b}	1592 ±21.94^{b}	62 ±2.59^{a}
C4		80 ±1.13^{a}	8.5 ±0.14^{ab}	15.3 ±0.23^{a}	1904 ±60.82^{a}	65 ±3.08^{a}
C5		84 ±2.24^{a}	8.7 ±0.02^{a}	14.5 ±0.13^{a}	1948.8 ±80.85^{a}	65 ±2.3^{a}
C1	30% PEG	56 ±1.14^{b}	5.6 ±0.18^{c}	7.12 ±0.08^{c}	712.32 ±29.48^{c}	42 ±1.3^{c}
C2		72 ±2.95^{a}	7.5 ±0.03^{a}	12.25 ±0.47^{a}	1422 ±17.59^{a}	53 ±2.04^{ab}
C3		68 ±2.6^{a}	6.1 ±0.23^{c}	10.22 ±0.47^{b}	1109.76 ±8.43^{b}	49 ±0.48^{b}
C4		72 ±0.91^{a}	6.8 ±0.07^{b}	13.11 ±0.24^{a}	1433.52 ±74.29^{a}	52 ±0.61^{ab}
C5		72 ±0.07^{a}	7.1 ±0.22^{ab}	12.54 ±0.28^{a}	1414.08 ±63.22^{a}	55 ±2.69^{a}
SEd		4.252	0.374	0.535	91.364	2.64
CD		8.584	0.749	1.102	189.001	5.407
CV		6.14	5.061	4.404	5.420	5.116

C1- Uninoculated Control, C2- *Bacillus altitudinis*, C3- *Bacillus aryabhattai*, C4- *Bacillus velezensis*, C5- *Bacillus altitudinis* FD48.Values are mean (±standard error) (n=3) and values followed by the same letter in each moisture stress are not significantly different from each other on the observation day as determined by DMRT (p≤0.05).

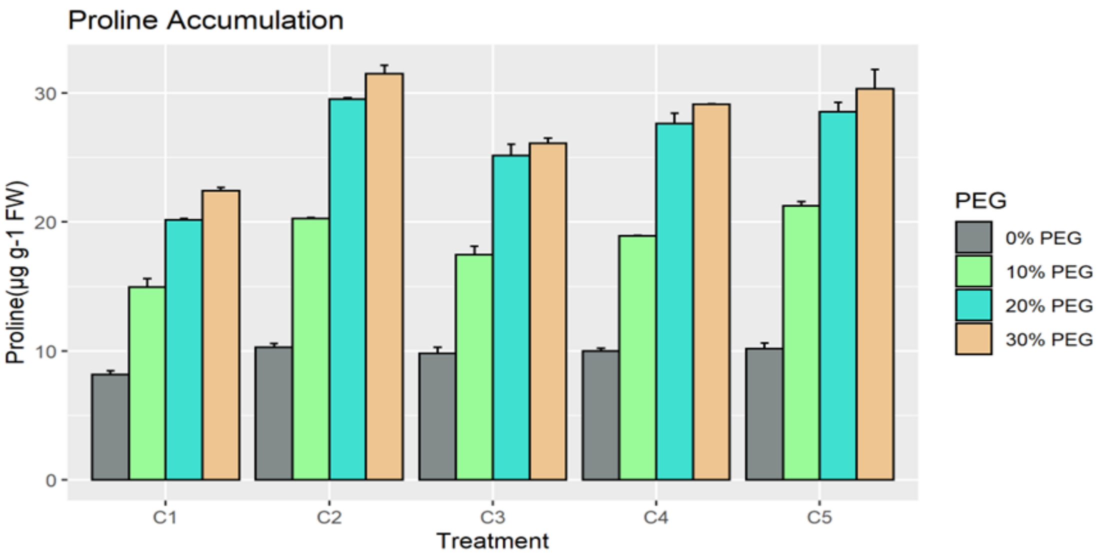

Fig. 1. *Proline accumulation in Rice CO51 under moisture stress. C1- Uninoculated Control, C2- Bacillus altitudinis, C3- Bacillus aryabhattai, C4- Bacillus velezensis, C5- Bacillus altitudinis FD48*

trend as proline accumulation. The activity increased to 20% PEG concentration and later decreased to 30% PEG concentration (Fig. 2). At 30% PEG the SOD, POD and APX activity was highest in *B. altitudinis* (C2) primed seedlings which were 0.62 U min^{-1} g^{-1} FW, 18.47 U min^{-1} g^{-1} FW and 9.21 U min^{-1} g^{-1} FW respectively. In the case of CAT activity, maximum activity of 15.89 U min^{-1} g^{-1} FW was observed in *B. velezensis* (C4) treated seedlings.

DISCUSSION

Drought is one of the most significant abiotic factors that have a negative impact on plant performance and has a negative impact on biomass and yield (Fahad *et al.*, 2017). Drought-tolerant PGPR has been shown to improve plant development and reduce drought stress (Khan and Bano, 2019). Hence in this study, drought-tolerant PGPR isolated from the rhizosphere of xero-

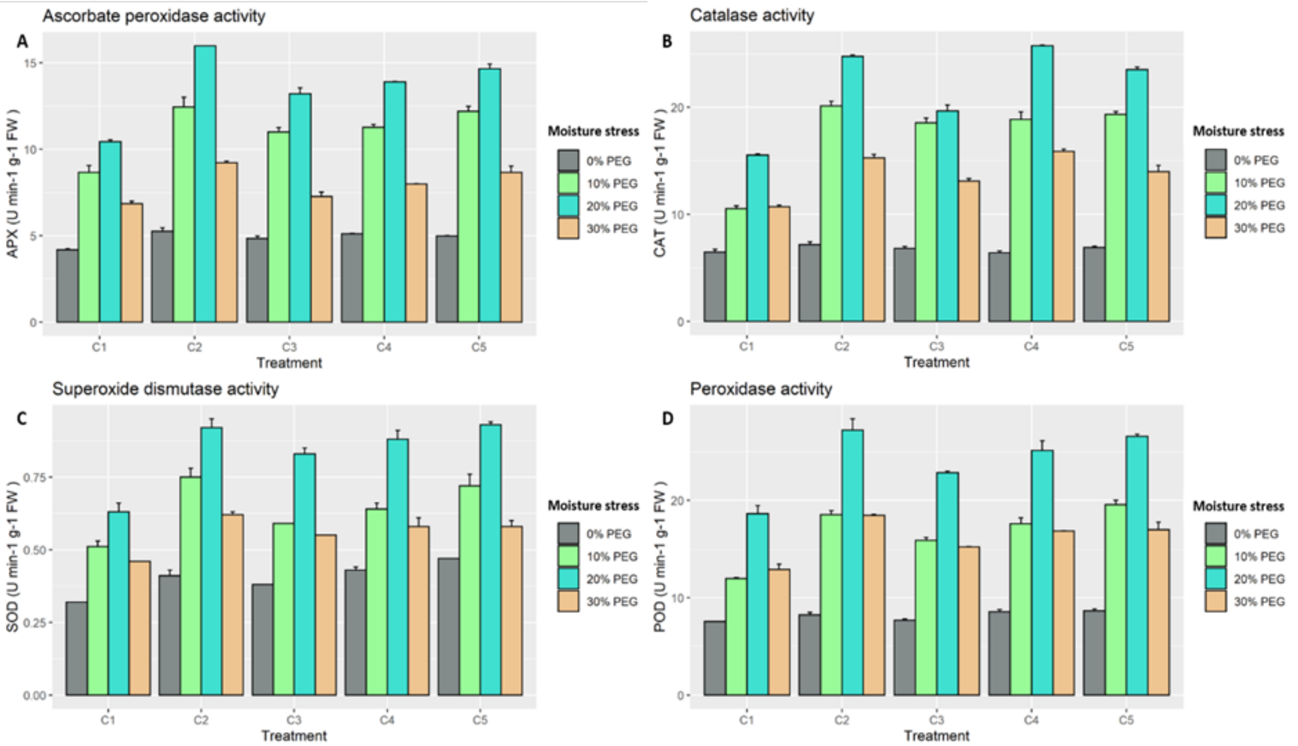

Fig. 2. *Antioxidant enzyme activity induced in Rice CO51 under moisture stress. A. Ascorbate Peroxidase (APX) activity induced in Rice CO51 under moisture stress, B. Catalase (CAT) activity induced in Rice CO51 under moisture stress, C. Superoxide Dismutase (SOD) activity induced in Rice under moisture stress, D. Peroxidase (POD) activity induced in Rice CO51 under moisture stress. C1- Uninoculated Control, C2- Bacillus altitudinis, C3- Bacillus aryabhattai, C4- Bacillus velezensis, C5- Bacillus altitudinis FD48*

phytes were utilized to alleviate drought stress in the early stages of rice. Drought stress causes low osmotic potential, which prevents water intake and slows or inhibits seed germination and seedling development (Kaya *et al.*, 2006). Similarly, in this study, moisture stress greatly reduced the seed germination with a maximum of 30% PEG concentration. But the PGPR primed seeds exhibited higher germination than the uninoculated control. These findings are consistent with those of Li *et al.* (2019), who observed a significant increase in the germination of wheat and cucumber seeds when treated with *Paenibacillus beijingensis* BJ-18 and *Bacillus sp*. L-56. Likewise, *Bacillus megaterium* MU2 showed a better effect by increasing root length by 33% as compared to the non-treated plants under water-deficit stress (Rashid *et al.*, 2021). Likewise, in the current study, the seedlings primed with *Bacillus altitudinis* (C2) showed 29% increased growth in shoot length and the seedlings primed with *Bacillus velezensis* (C4) showed 59% increased root length over uninoculated plants at 30% PEG concentration respectively. The study by Jabborova *et al.* (2021) further confirmed these results where, under drought conditions, co-inoculation of *B. japonicum* USDA 110 and *P. putida* NUU8 in soybean seeds (*Glycine max* L. Merr.), when compared to the control, dramatically increased root length by 56% and shoot length by 33% in drought challenged conditions. Plants also accumulate diverse organic solutes in response to external osmotic pressure changes to manage environmental factors. To counteract osmotic pressure, plants often accumulate organic compounds such as proline and soluble sugar (Moaveni, 2011). Proline, an organic solute, is well recognised for its osmotic adaptation activity and function in enhancing stress responses by inhibiting cellular membranes and enzyme integrity (Kumar *et al.*, 2017). In addition to the role played by proline in ROS scavenging, it can also be the major source of energy and nitrogen during drought stress metabolism (Xia *et al.*, 2020). The current work exhibited an increase in proline content with an increase in moisture stress with the maximum in seedlings treated with *B. altitudinis* (C2), which showed a 34% increase in proline accumulation over uninoculated control at 30% PEG concentration. This was in accordance with the results of Rashid *et al.* (2021) where, in comparison to non-inoculated water-stressed wheat variety “NARC11” plants, the bacterial strain *Bacillus megaterium* MU2 inoculation resulted in enhanced proline production. Previous research has shown that stressful situations may effectively increase the activity of enzymes such as POD, SOD, CAT, and APX, which are well associated with the scavenging ability of ROS and serve as important defensive measures when dealing with stressful environmental factors (Petrov *et al.*, 2015). Under drought stress and inoculation-induced changes in antioxidant activity have been described by Zhang *et al.* (2020). In the present investigation, the antioxidant enzyme activity increased with an increase in moisture stress up to 20% PEG concentration and later decreased at 30% PEG concentration. The antioxidant enzyme activity was higher in PGPR primed seedlings over uninoculated seedlings. The seedlings primed with *B. altitudinis* (C2) showed the maximum SOD, POD and APX activity which was 30%, 36% and 29% higher than uninoculated seedlings at 30% PEG. While CAT activity was highest in *B. velezensis* (C4) treated seedlings which exhibited 39% higher activity than unprimed seedlings at 30% PEG.

Conclusion

The present investigation concluded that the bacterial cultures *B. altitudinis* MLSB2 (MT729964) and *B. velezensis* VKSB5 (MT729963) proved to have a promising role in improving plant performance under drought conditions. Further investigation of the mechanisms involved in drought mitigation may result in an effective bioinoculant for drought mitigation in rice.

Conflict of interest

The authors declare that they have no conflict of interest.

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