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PLANT GROWTH PROMOTING RHIZOBACTERIA INDUCED MODULATION OF PHYSIOLOGICAL RESPONSES IN RICE UNDER SALT AND DROUGHT STRESSES

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Abstract

Effects of *Pseudomonas sp.* on physiology of *Oryza sativa* (L.) var. KS-282 were evaluated under induced drought stress and salinity stress conditions. For seed inoculation, sterilized seeds were immersed in *Pseudomonas* broth culture for 2 to 4 hours, whereas control seeds were soaked in distilled water for the same time period before being sowed in autoclaved, sand-soil mixture. Drought stress was induced by withholding water supply for 10 days, while salinity stress was induced by irrigating with 200 mM NaCl. Plants were harvested at the vegetative stage. Both the stresses significantly decreased the relative water content of leaves, root area, total soluble leaf proteins, chlorophyll content of leaves, osmotic potential of leaves whereaas electrolyte leakage, proline content of leaves and superoxide dismutase activity were significantly increased. The decrease in moisture content of the rhizosphere soil was much less marked in salt-stressed plants. The PGPR ameliorated the adverse effects of drought and salt stress on all parameters but at different magnitude. Generally, PGPR were more effective under drought stress. The percentage decrease in soil moisture content and relative water content (RWC) were higher under drought stress. The drought stress induced a decrease in protein production, and an increase in proline was similar under both stresses however, the PGPR induced production of protein was 50% lower in saline condition but was equally effective for proline production. The antioxidant enzyme superoxide dismutase (SOD) activity was higher under drought stress than that under salt stress, and PGPR further augmented it. It is inferred from the present finding that the effect of drought is more pronounced in rice and that the ameliorative effectiveness of PGPR used as bioinoculant differs under different stresses for a plant grown under similar condition.

Key words: Drought stress; Salinity stress; *Oryza sativa,* Rhizospheric PGPR.

Introduction

Abiotic stress conditions, particularly drought and salinity, are detrimental to major crop growth and productivity (Zhang *et al*., 2016). Both drought and salinity stress result in osmotic stress conditions that lead to cell homeostasis disruption, redox imbalance, impaired photosynthesis, and cellular energy depletion (Ma *et al*., 2020).

Rice (*Oryza sativa*) provides staple food for more than half of the world's population (Tiwari *et al*., 2020; Khush 2005). Rice is mostly grown under irrigation, therefore, intermittent water stress at critical stages may result in considerable yield reduction and crop failure (Nugroho *et al*., 2018). Severe abiotic stresses, particularly drought and salinity, often threaten the principle rice-growing areas in Asia that account for more than 85% of rice production (Yang *et al*., 2019). Rice plant has developed few adaptations to osmotic stress as a result of drought and salinity (Matsunami *et al*., 2020), but the level of tolerance can be enhanced by the use of beneficial microorganisms associated with root pertaining positive impacts on growth, physiology, and yield indices under stress conditions (Pascale *et al*., 2020).

Both drought and salinity stress lead to the overproduction of reactive oxygen species (ROS), including superoxide, hydroxyl radicals, and hydrogen which are the root cause of oxidative damage to important biological compounds like DNA, lipids, and proteins and ultimately cell death (Hasanuzzaman *et al*., 2020). To cope with oxidative burst, plant activates their antioxidant defense system, including antioxidant enzymes like Peroxidases (POD), Catalases (CAT), and Superoxide dismutase (SOD) (Dumont & Rivoal, 2019).

The plant growth-promoting rhizobacteria (PGPR) are economical, environment-friendly, and sustainable choices as they are involved in increasing plant tolerance to abiotic stresses such as salinity and drought by promoting some physical and chemical changes (Forni *et al*., 2017). Under stress conditions the plant growth-promoting rhizobacteria colonize the rhizosphere soil and promote plant growth either through biosynthesis of various plant hormones or modulation of nutrient uptake (Naseem *et al*., 2018). There are studies that reveal the effectiveness of PGPR for providing stress tolerance by the production of antioxidant enzymes and proline content (Fazal & Bano, 2016). Cardinale *et al*., (2015) reported that under abiotic stress conditions, various PGPR strains of *Pseudomonas* resulted in better vegetative growth, improved grain yield and better photosynthetic efficiency, while Hong *et al*., (2016) suggested that PGPR strains could help to alleviate the detrimental effects of salinity in Paddy rice by promoting seed germination, plant survival rate, plant biomass, nutrient uptake, and antioxidant defense system. Other studies reported that *Pseudomonas*-induced dehydration stress tolerance is directly associated with enhanced water retention capacity and better regulation of carbon sources (Kumari *et al*., 2016).

Under salinity and drought stresses, the plant faces almost similar types of growth and physiological hindrances, but only a few researches have been conducted to highlight plant responses against dual stresses. This study reveals the physiological responses of rice exposed to drought and salt stress for a similar period and to evaluate the potential of *Pseudomona*s sp. used as bio-inoculant.

Materials and Methods

Seeds of rice (*Oryza sativa)* var. KS-282 were collected from National Agricultural Research Centre (NARC), Islamabad. Seeds were sterilized with ethanol (95%). Afterwards, seeds were shaken in 10% chlorox for 2-3 min and subsequently washed 2-3 times with autoclaved distilled water. About 4-6 seeds were germinated in pots placed in growth chamber maintained at 14 h photoperiod with day /night temperature of 28/22°C with 75-80% humidity. After four weeks of normal growth, the seedlings were exposed to drought stress by withholding irrigation for 10 days. For salinity stress, 4 weeks -old plants were irrigated with 200 mM of NaCl. Both controlled and stressed rice seedlings were harvested at the vegetative stage and were stored at -80C for further experimental work. For each treatment, three independent biological replicates were used.

Preparation of inoculum: For seed coating the *Pseudomonas* sp. (Acc No KF30719) was captured on LB agar plate at 37°C. After that a single purified colony of *Pseudomonas* sp. was inoculated in 100 ml LB broth and shaken for 48 h. After centrifugation at 10,000 rpm for 10 min, the pellet was separated and suspended in distilled water to make volume up to 1ml. Later, for seed inoculation, sterilized seeds were immersed in *Pseudomonas* Brath culture for 2- 4 hours, whereas control seeds were soaked in distilled water for same time period before being sowed (11×8 cm^2 Pots) in autoclaved sand-soil 1:3 mixture.

Osmotic potential of leaves: To determine the osmotic potential of the cell sap, a freezing point osmometer (Reobling Messtechnike, Berlin,Germony) was used and the method of Capell & Dorffling (1993) was applied.

Relative water content of leaves: Relative water content (RWC) of rice leaves was measured by the method of Jin *et al*., (2017). After maintaining the leaf in distilled water under light at 22°C until it reached a consistent weight and became fully turgid, the turgid leaf weight was calculated (typically after 4 h). After storing the turgid leaf in an oven for 16 hours at 80°C, the dry weight of the leaf was measured. The following formula was used to compute the relative water content (RWC): RWC (%) = (FW - DW)/(TW - DW), where FW stands for fresh weight, DW stands for dry weight, and TW stands for turgid weight.

Electrolytic leakage (EL): The EL was determined according to the method described by Fan & Sokorai (2005). Leaf samples were washed consecutively with tap and distilled water, respectively. Leaf discs (0.1g) were made for treated and control leaves and dipped in distilled water (10 ml) in a test tube. Thereafter, test tubes were kept in water bath at 40°C for 30 min and at 100°C for 10 min. Electrical conductivity (C1) was recorded on both temperatures by electrical conductivity meter and electrolyte leakage was calculated by formula,

$$EL=C1/C2\times100$$

Chlorophyll and protein contents of leaves: Chlorophyll meter SPAD (Minolta Reading SPAD 502) (Ghimire *et al*., 2015) was used to check the chlorophyll content of leaves, while protein content was determined using the Lowry *et al*., (1951) method.

Proline content of leaves: Free proline content of fresh leaves of *Oryza sativa* was determined by the method of Bates *et al*., (1973).

$$\text{Proline content (mg/g)} = \frac{\text{K value}\times \text{Dilution factor} \times \text{Absorbance}}{\text{Weight of the sample}}$$

Superoxide dismutase assay: The SOD activity was determined by measuring the absorbance at 560 nm using the method of Dhindsa *et al*., (1981). One unit SOD activity was defined as the amount of enzyme which reduced the absorbance reading by 50% as compared to the control (lacking enzyme).

Soil moisture contents assay: Soil moisture content was determined at the time of sample collection. For the soil moisture assay, 20g of soil was taken at a uniform depth of 6 inches below the soil surface. After recording their fresh weight, the soil was dried in the oven at 70°C for 72 hours for dry weight calculation. Thereafter the following formula was applied for the calculation of % Soil moisture content.

$$\text{Soil moisture content (\%)} = \frac{\text{Fresh weight of the soil-dry soil weight}}{\text{dry soil weight}} \text{ x } 100$$

Root length analysis: For root length analysis three independent biological replicate were for each treatment were harvested and their root length analysis was performed, using ImageJ software.

Statistical analysis

Graph Pad Prism 7 was used to do a Two-way ANOVA with Tukey's multiple comparison test. The asterisk represents significant difference between control and treated groups. For each group, three biological replicates were used.

Results

Relative water (RWC) and electrolyte leakage of rice leaves: Salinity stress resulted in a significant decrease (20%) in the relative water content of leaves (Fig. 1A). The decrease was further augmented (83.5% of the control) under drought stress condition. *Pseudomonas* sp. completely overcame this inhibition in RWC based on the type of stress, like under drought stress *Pseudomonas* inoculation resulted in a significant increase (57%) while under salinity stress, the significant increase was reduced to 19.4%. *Pseudomonas* inoculation was also found to be effective under control condition and resulted in almost 11% increase in relative water content over control.

Both the stress conditions resulted in significant increase in leaf electrolytic leakage, being a little higher under drought stress as shown in (Fig. 1B). *Pseudomonas sp*. inoculation overcame stress induced increase in electrolyte leakage under both salinity as well as drought stress conditions. Under unstress condition *Pseudomonas* sp. inoculation also resulted in 7.7% decreased electrolytic leakage as that of control.

Root area and chlorophyll content of rice leaves: An increase in root area was significant (17.7%) in inoculation treatment of *Pseudomonas* sp. (Fig. 2A). Drought stress resulted in a significant decrease in root area by 58.3% over that of control. However, *Pseudomonas* sp. had significantly increased the root area by 43.5%. Salinity stress didn't markedly decrease the root area.

Pseudomonas sp. significantly (15.7%) enhanced chlorophyll content of leaves over control under unstressed condition (Fig. 2B). Inoculation with *Pseudomonas* sp. showed increased chlorophyll content of leaves by 14.3% and 9.7%, respectively, over that of drought and salinity stress. Significant (21.7% and 16.6%) decrease in chlorophyll content of leaves was recorded under both drought stress and salinity stress. *Pseudomonas sp*. resulted in a significant increase in chlorophyll content under both salinity and drought stress condition and completely overcame the inhibitory effect of these stress conditions.

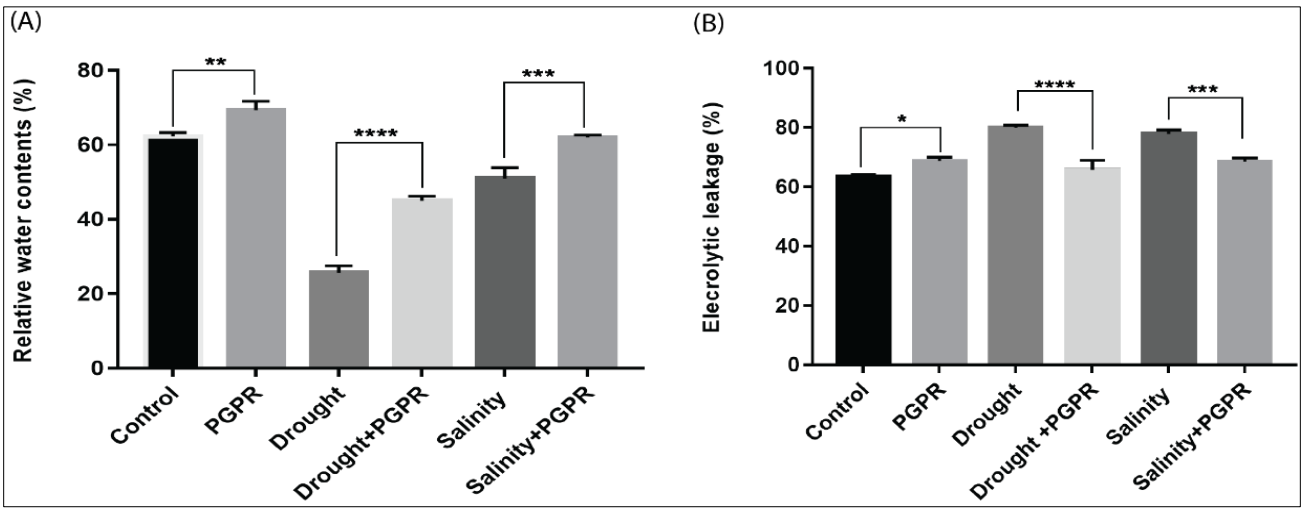

Fig. 1. Effect of *Pseudomonas sp.* on relative water content and electrolyte leakage of *Oryza sativa* (Ks-282) leaves under drought and salt stress conditions. The asterisk represents significant difference between control and treated groups. For each treatment, three biological replicates were used.
Control = Without any treatment or stress; PGPR = Inoculated with *Pseudomonas* sp. (Acc No KF307196); Drought = 10 days of drought stress; Drought + PGPR = Inoculated with *Pseudomonas* sp. (Acc No KF307196) and exposed to 10 days of drought stress; Salinity = Exposed to 200mM salinity stress (NaCl); Salinity + PGPR = Inoculated with *Pseudomonas* sp. (Acc No KF307196) and exposed to 200mM salinity stress.

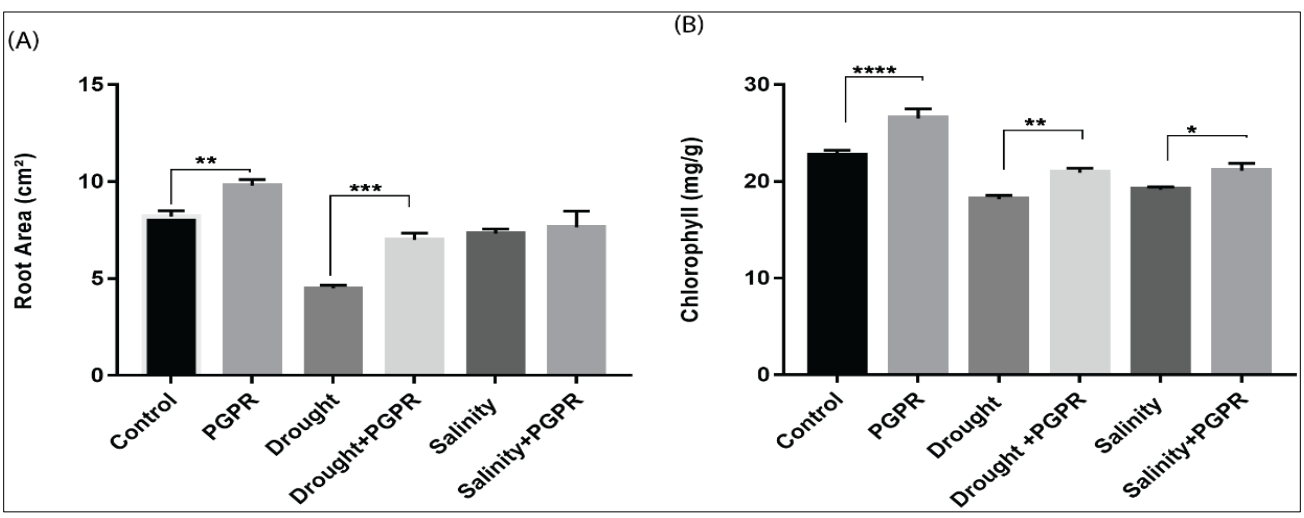

Fig. 2. Effect of *Pseudomonas sp.* on root area and chlorophyll content of *Oryza sativa* (Ks-282) leaves under drought and salt stress conditions. The asterisk represents significant difference between control and treated groups. For each treatment, three biological replicates were used.
Control = Without any treatment or stress; PGPR = Inoculated with *Pseudomonas* sp. (Acc No KF307196); Drought = 10 days of drought stress; Drought + PGPR = Inoculated with *Pseudomonas* sp. (Acc No KF307196) and exposed to 10 days of drought stress; Salinity = Exposed to 200mM salinity stress (NaCl); Salinity + PGPR = Inoculated with *Pseudomonas* sp. (Acc No KF307196) and exposed to 200mM salinity stress.

Protein and proline content of rice leaves: Both the drought and salinity stress resulted in significant (45.9% and 35.89%) decrease in the total leaf protein content (Fig. 3A). With respect to salinity stress, the decrease was higher under drought stress. Under controlled growth conditions, *Pseudomonas* inoculated plants showed significant (7.89%) increase in protein content over control. Plants inoculated with *Pseudomonas* sp. exhibited significantly higher (71%) protein content over control in leaves under drought stress while in saline conditions, *Pseudomonas* inoculated plants showed significant (17.9%) increase in total protein contents.

Both drought and salinity stress conditions resulted in significant increase in proline production by 133% and 132%, respectively, over control (Fig. 3B). *Pseudomonas* inoculation improved proline production by 31.7% and 38.25% respectively over drought and salinity stresses.

Salinity treatment increased the proline production significantly over control; however, drought stress resulted in 3% more proline production over salinity stress. Under control condition, inoculation with *Pseudomonas* was the proline production significantly increased over that of control.

Super oxide dismutase (SOD) activity of rice leaves: Drought stress condition exhibited significantly higher SOD activity than control. Similarly, salinity stress condition also displayed significant increase in SOD activity over control, as shown in (Fig. 4). Inoculation with *Pseudomonas* had no effect on SOD activity over control however, under stress condition of drought and salinity, *Pseudomonas* inoculation had increased SOD activity by 29% and 31.8% respectively.

Moisture content of rhizospheric soil and osmotic potential of rice leaves: The moisture content of soil (Table 1) was decreased significantly under both drought and salinity. Inoculation with *Pseudomonas* sp. resulted in significant increase in soil moisture content both under drought and salinity stress conditions. Along with stress conditions, *Pseudomonas* inoculation significantly increased the moisture content of soil under unstressed conditions too.

Pseudomonas sp. as bio-inoculant improved the osmotic potential of rice leaves (Table 1). There was a 25% decrease in osmotic potential under drought stress which was counteracted by *Pseudomonas* inoculation and the value was significantly higher than control. However, salinity stress alone had no marked effect on the osmotic potential of leaves over control. Collectively our results suggested that *Pseudomonas* inoculation conferred both drought and salinity stress tolerance by modulating proline biosynthetic and antioxidant enzymes activity. Both of which acted as ROS quenchers and protected the plant from oxidative stress damage.

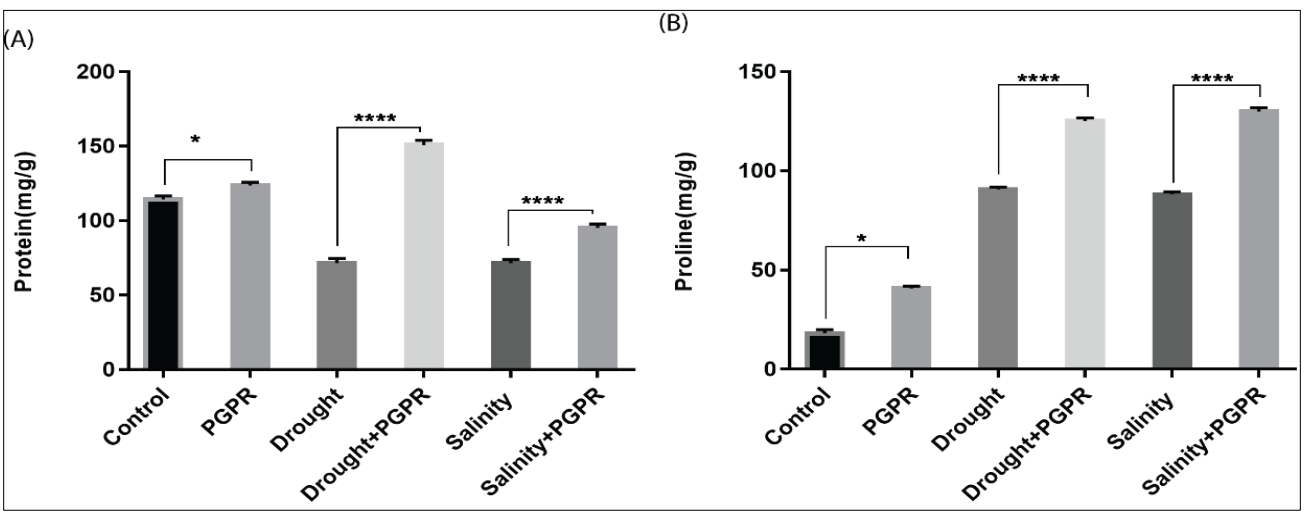

Fig. 3. Effect of *Pseudomonas sp.* on protein and proline content of *Oryza sativa* (Ks-282) leaves under drought and salt stress conditions. The asterisk represents significant difference between control and treated groups. For each treatment, three biological replicates were used.

Control = Without any treatment or stress; PGPR = Inoculated with *Pseudomonas* sp. (Acc No KF307196); Drought = 10 days of drought stress; Drought + PGPR = Inoculated with *Pseudomonas* sp (Acc No KF307196) and exposed to 10 days of drought stress; Salinity = Exposed to 200 mM salinity stress (NaCl); Salinity + PGPR = Inoculated with *Pseudomonas* sp (Acc No KF307196) and exposed to 200mM salinity stress.

Table 1. Effect of *Pseudomonas* sp. on soil moisture content and osmotic potential of *Oryza sativa* (Ks-282) leaves under drought and salt stress.

Treatments	Soil moisture content (%)	Osmotic potential of leaves (milliosmole)
Control	26.94B	-0.160 AB
PGPR	33.6A	-0.165 BC
Drought	5.26 F	-0.299 F
Drought + PGPR	8.95E	-0.170C
Salinity	17.64D	-0.250 E
Salinity + PGPR	25C	-0.180 CD

The alphabets represent significant differences between control and treated groups. For each treatment, three biological replicates were used

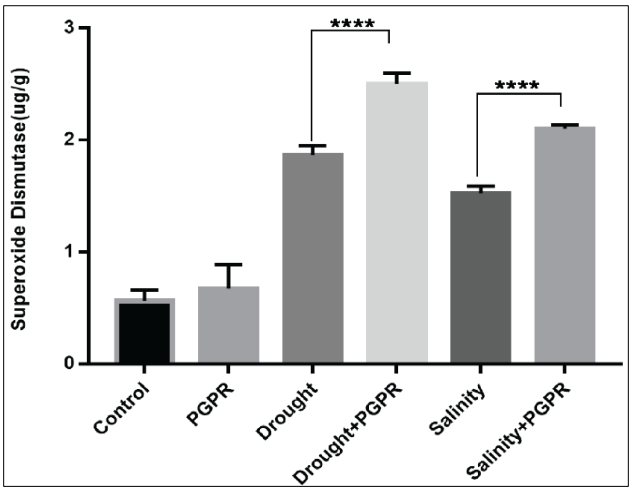

Fig. 4. Effect of *Pseudomonas sp.* on Superoxide dismutase (SOD) activity of *Oryza sativa* (Ks-282) leaves under drought and salt stress conditions. The asterisk represents significant difference between control and treated groups. For each treatment, three biological replicates were used.
Control = Without any treatment or stress; PGPR = Inoculated with *Pseudomonas* sp. (Acc No KF307196); Drought = 10 days of drought stress; Drought + PGPR = Inoculated with *Pseudomonas* sp. (Acc No KF307196) and exposed to 10 days of drought stress; Salinity = Exposed to 200mM salinity stress (NaCl); Salinity + PGPR = Inoculated with *Pseudomonas* sp (Acc No KF307196) and exposed to 200mM salinity stress.

Discussion

Several research studies have reported that plant growth-promoting rhizobacteria (PGPR) induces abiotic stress tolerance associated with enhanced production of stress-related phytohormones, osmolytes, and antioxidants (Ma *et al*., 2020). While working on drought stress, Yasmin *et al*., (2013) documented that PGPR induced drought tolerance in maize through maintaining the hydration status of the plant, improved root proliferation, and nutrient uptake. Similarly, in cucumber plant, PGPR stimulated drought and salinity stress tolerance through modulation of antioxidant defense, better plant growth, reduced electrolytic leakage and balanced ion homeostasis (Bharti *et al*., 2016). *Pseudomonas sp.* ultimately overcame the inhibiting effect of drought on soil moisture content, as revealed in the Table 1. The maintenance of higher soil moisture content demonstrates PGPR promotion of the root system, which results in increased uptake of nutrients and water deeper down the soil. As soil moisture content decreased, so did leaf turgidity, which was followed by a significant fall in the leaf relative water content. *Pseudomonas sp*. significantly increased the RWC of leaves of control plant. These effects were further stimulated under stresses. The notable increase in RWC following the inoculation of *Pseudomonas* sp. insinuates the positive role of PGPR, as previously reported by Qin *et al*., (2016).

Drought and salinity stresses ultimately affect the osmotic potential of leaves (Gharbi *et al.,* 2019). Osmotic potential was significantly decreased under salinity stress as well as under drought conditions. However, *Pseudomonas* sp. significantly enhanced the osmotic potential of leaves under stresses. Decrease in soil moisture content and RWC are associated with induction of osmotic stress. In order to cope with osmotic stress, plants synthesize various kinds of osmolytes (Liang *et al*., 2013). Osmolytes like proline plays an important role in abiotic stress tolerance by acting as a metal chelator, activator of various stress responsive signaling pathways and an antioxidative defense molecule (Slabbert & Kruger 2014). Proline also plays a vital role in abiotic stress tolerance by sustaining plant cell turgor, alleviating electrolytic leakage and excessive production of reactive oxygen species (Chun *et al*., 2018). In the present study, we observed a significantly enhanced production of proline in plants subjected to drought and salinity. However, this increase was significantly high in *Pseudomonas* inoculated rice seedlings suggesting that, *Pseudomonas* (PGPR) might induce stress tolerance through activation of proline biosynthesis. While elucidating heat and drought stresses, Cvikrova *et al*., (2013) reported that enhanced proline production could rescue tobacco plants against damages induced by these stresses.

Drought and salinity stress result in over generation of reactive oxygen species that cause lipid peroxidation and damage to cell membrane (Hasanuzzaman *et al*., 2020). To neutralize the effect of these reactive oxygen species, plants have evolved antioxidant defense systems like SOD that result in ROS detoxification (Dumont & Rivoal, 2019). In our study, a significant increase in SOD activity was observed which was particularly higher under 10 days of drought stress. PGPR application resulted in a significant increase in SOD, thus suggesting that *Pseudomonas* sp. inoculation triggered enhanced antioxidant defense and protected the plant from ROS-induced oxidative damage. The enhanced antioxidant defense during salinity stress was also reported by Zhang *et al.,* (2022); Ashraf & Foolad (2007). *Pseudomonas sp.* also proved effective in increasing the root area of rice as its inoculation resulted in proliferated root system. Proliferated root system is an important criterion for stress tolerance as earlier suggested by Palta & Turner (2019).

Conclusions

Pseudomonas sp. proved more efficient in imparting both salinity and drought stress tolerance to *Oryza sativa*. *Pseudomonas* inoculation stress tolerance is closely linked with better proliferation of root system, maintenance of plant turgidity, regulation of osmotic potential and protection from oxidative stress via the production of proline and SOD.

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