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RESEARCH ARTICLE

Impact of drought-tolerant rice apoplastic fluid endophyte (*Sphingobium yanoikuyae* MH394206) on the morphological and physiological characteristics of rice (CO51) grown in moisture deficit condition

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ABSTRACT

A pot culture study was conducted to evaluate the effect of rice apoplastic fluid ACCD (aminocyclopropane-1 carboxylate deaminase)containing endophyte, *Sphingobium yanoikuyae* MH394206 on moisture stress alleviating potential in rice (CO51). Impact of this organism was compared with plant growth promoting bacterial inoculants such as *Methylorubrum* sp, (pink pigmented facultative methylotroph-PPFM) and *Bacillus altitudinis* FD48. The effect was assessed in terms of changes in growth and morpho-physiological characteristics of rice grown in 75% moisture stress. Seed application of these bacterial cultures enhanced plant growth and stress-related parameters like total phenolic content, proline accumulation and activity of antioxidant enzymes such as peroxidase (PO), catalase (CAT) and polyphenol oxidase (PPO) compared to uninoculated moisture stressed control. The effect was more significant (P>0.05%) due to *Sphingobium yanoikuyae* MH394206. Further studies are needed to find out the mechanism of plant growth promotion under stress by *Sphingobium yanoikuyae* MH394206.

Keywords: *Moisture stress, Sphingobium yanoikuyae, relative water content, antioxidant enzymes*

INTRODUCTION

Global warming and climate change increased the area under drought. Rainfall data over the past one century indicated that there has been an occurrence of severe drought in India once in every eight to nine years. We are also currently witnessing high temperature, unpredicted rainfall and drought. This alarming situation warrants reorientation of our research programme to cope up with these extreme conditions. Drought is expected to cause serious growth-related problems for crops on more than 50% of the earth's arable lands in the year 2050 (Vinocur and Altman, 2005). Therefore, there is a new interest to find solutions to various water-related problems such as drought and reduce the impact in food security in the world (Alexanratos and Bruinsma, 2012). Rice (*Oryza sativa*) is a very important staple food for more than one half of the world's population. Rice cultivation is highly dependent on the availability of water, so drought during the sensitive flowering stage severely affects grain yield (Ramegowda *et al*., 2014).

Presently the plant-associated endophytic microbial communities have received increased attention for enhancing the stress tolerance capacity of the crops (Mayak *et al*., 2004). Inoculation of beneficial microorganisms with agricultural crops not only promotes plant growth and development but also stress tolerance capacity of the crop (Marulanda *et al*., 2007). Beneficial, symbiotic interaction between the plants and microbes can protect the plants from various abiotic stresses (Mascher, 2007). According to Timmusk and Wagner(1999), inoculation of*Arabidopsis thaliana* with *Paenibacillus polymyxa* increased drought tolerance capacity by inducing the drought-responsive genes. Greater accumulation of proline in plants inoculated with microbes indicated the plant tolerance to higher water stress (Gusain *et al*., 2015). The plants treated with a consortium of various endophytic strains enhanced survival of plants under water stress condition through the multiple distinct drought-response pathways (Khan *et al*., 2016).

Bacterial inoculants generally improve the nutritional, morphological, physiological and biochemical response of the plant to any external stimuli and thus impart resistance to biotic and abiotic stresses. Plants inoculated with plant growth

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promoting rhizobacteria (PGPR) strains and exposed to water stress showed better water status than control plants, alleviated drought stress by using alternative mechanisms, and higher yields under drought conditions (Compant *et al*., 2010). Like any abiotic factor, drought also induced ethylene production in plant tissues which leads to abnormal growth of a plant (Bresson *et al*., 2013). Inoculation of crops with ACC deaminase-containing PGPR may assist plant growth by alleviating deleterious effects of stress ethylene.

Mitigation of the oxidative damage and sustaining plant growth under stress is correlated with the enhancement of antioxidant enzymes like POD, SOD, CAT and PPO. Hence, activities of these enzymes may serve as a suitable indicator to evaluate the degree of drought tolerance in crop plants.

In light of the above, studies aimed at searching for new potent endophytic beneficial bacterial strain(s) for rice cultivation under drought are underway. Accordingly, bacterial endophytes were isolated from leaf apoplast of drought resistant and normal rice cultivars. Diverse bacterial genera with various plant growth promoting abilities were isolated from tested rice cultivars. Some of the bacteria exhibited relatively greater ACCD activity, produced more phytohormones such as IAA & GA_3 and also showed tolerance to extreme water stress. One among them was *Sphingobium yanoikuyae* MH394206 with greater ACCD activity, a substantial quantity of IAA and GA_3production and ability to tolerate water potential of -5.5MPa.In the current study, we aimed to evaluate and compare the impact of this most potent apoplast endophyte with our previously reported phylloplane, *Methylorubrum* sp. (PPFM) and endophytic bacteria (*Bacillusaltitudinis* FD48).

MATERIAL AND METHODS

Bacterial cultures

For this study, the bacterial culture (*S. yanoikuyae* MH394206.) used was obtained from the Algal laboratory of Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore. Other two cultures (*Methylorubrum* sp. (PPFM) &*B. altitudinis*FD48), were kindly provided by the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore. These bacteria were cultured in specific broth (AMS – PPFM, *B. altitudinis*FD48 and *S. yanoikuyae* - (LB broth) at 28°c with 120 rpm until the culture reached log phase with approximately 10^7 to10^8cfu mL^{-1}. The characteristics of the bacterial cultures are detailed in Table 1.

Rice cultivar

Ruling rice cultivar CO51 seeds were provided by the Paddy Breeding Station (PBS), Tamil Nadu Agricultural University, Coimbatore.

Pot experiment for drought treatment

A pot culture experiment was conducted in the Department of Agricultural Microbiology, TNAU, Coimbatore to study the effect of the rice apoplastic fluid endophytic bacterium (*S. yanoikuyae* MH394206) on the rice cultivar CO51 grown in moisture-stressed condition. For comparison, two more bacterial inoculants used as drought stress mitigators were (pink pigmented facultative methylotroph (PPFM) *Methylorubrum* sp and *B. altitudinis*FD48 also included. The treatments included 1. Control (Uninoculated with water logged condition), 2. 75% moisture stress + seed inoculation with pink pigmented facultative methylotroph (PPFM), 3. 75% moisture stress + seed inoculation with *S. yanoikuyae* MH394206,4. 75% moisture stress + seed inoculation with *B. altitudinis* FD48, and 5. Control (Uninoculated 75% moisture stressed condition).

This experiment was conducted between December 2018 and March 2019 with day temperature ranging from 28 to 35^{0}C, Relative humidity 85%, photoperiod 12/12 h day and night cycle. The soil used was collected from the wet land field of Department of Farm Management, Tamil Nadu Agricultural University, Coimbatore. The texture of the soil was clay loam, pH 6.9, and EC 0.71 dSm^{-1}. Nutrient content of the soil was as follows organic carbon 0.71 (%), total nitrogen 220 kg ha^{-1}, available phosphorus17.5 kg ha^{-1} and potassium 630 kg ha^{-1}content. This soil was air dried, mixed uniformly, and autoclaved twice at 121°C with 15 lbs. pressure for 30 min. Each pot was filled with 4 kg sterile soil and the pots were saturated with water and kept overnight. Then the field capacity of the soil was assessed gravimetrically. Seeds were surface sterilized with sodium hypochlorite (0.5%) for 3 min and washed with distilled water twice and soaked overnight in sterile distilled water for imbibition and pre-germination. Meanwhile, the bacterial cultures were allowed to grow up to log phase so as to reach cell concentration of 10^8 CFU mL^{-1}; then centrifuged at 6,000 rpm for 6 min and then the cell pellet was washed with 0.2Mphosphate buffer (pH7.0) and suspended in 1 ml sterile distilled water. Pre-germinated seeds were treated with bacterial cell suspension @ 1ml/25seeds. One hour after seed treatment, the seeds were sown @ 10 seeds per pot and thinned down to 6 seedlings after 15 days of sowing. The experimental design was completely randomized with four replications. The pots were irrigated regularly using tap water. After 45 days of sowing, drought was induced by imposing 75% moisture stress. This condition was achieved by maintaining the soil moisture status at

25% which was below field capacity. The stress was maintained until its harvest stage. The crop growth was monitored regularly and observations were recorded. The response of rice to moisture stress and microbial inoculation was studied by analyzing the morphological and physiological characteristics of plants after 25 days of stress induction.

Morphological characteristics

Standard procedures were followed for recording leaf area, shoot and root length and plant biomass. The leaf area was estimated by measuring the length (L) and width (W) at mid of the fully expanded third leaf using the formula, Leaf area = L * W * K, where K is a correction factor. The shoot length (cm) and root length (cm) were measured manually; shoot (g) and root (g) biomass were recorded gravimetrically (Palaniswamy *et al*., 1975). Root volume was measured through water displacement technique.

Physiological characteristics

Relative water content (RWC)

The relative water content was determined in flag leaves of the plants of each treatment by following the protocol of Smart and Bingham, (1974).

RWC (%) = (Fresh weight – Dry weight) / (Soaked weight – Dry weight) × 100

Photosynthetic pigment

The leaf samples were randomly collected from each replication and the total chlorophyll content was estimated (Arnon, 1949).

Total soluble sugars (TSS)

The total soluble sugars were determined based on the method of phenol-sulphuric acid. About 0.5 g fresh leaf was homogenized in 1 mL deionized water using pestle and mortar. The extract was filtered and to the filtrate, 0.5 mL (5%) phenol and 4.5 mL concentrated sulphuric acid (98%) were added and incubated for 1h. Then, the optical density of the resultant solution was measured at 485 nm in a Spectrophotometer (Biochrom 2100). The result was expressed as mg g^{-1} fresh weight of the sample(Dubois *et al*., 1956).

Total phenolic content

About 0.5g fresh leaf tissue was homogenized with 1mL of 80 % methanol. The extract was centrifuged at 10,000 rpm for 10 min. and the supernatant was collected. Total phenolic content in the extract was determined spectrophotometrically according to the Folin-Ciocalteu method (Singleton and Rossi, 1965) with minor changes. Gallic acid (GA) of different concentration (10 to 100 ppm) was used as a standard. About 0.2 mL plant extract was taken in a test tube and to this 1mL distilled water and 1mL Folin-Ciocalteu reagent (1N) were added and incubated for 3 min. After incubation, 1mL of 20 % sodium carbonate was added and mixed thoroughly and the test tube with the mixture was kept in boiling water for 1 min and then brought to room temperature and incubated for one hour. The absorbance was taken at 725 nm and expressed as mg of GA equivalents per gram fresh weight (mg GA g^{-1} fresh weight).

Assay of antioxidant enzymes

Peroxidase (PO)

One gram leaf sample was homogenized with liquid nitrogen (-196°c). To 100 mg homogenized leaf powder 5 mL of 0.01 M sodium phosphate buffer, pH 6.0 containing 0.5 M sucrose was added and mixed thoroughly.The suspension was kept in an ice bath for 1 h with intermittent stirring and then centrifuged at 7500 rpm for 20 min. at 4°c. The resultant supernatant was used as a source of soluble peroxidase (Hammerschmidt *et al*., 1982). The peroxidase assay was done using guaiacol as a hydrogen donor. The reaction mixture consisted of 2.9 ml of a mixture containing 0.25% (v/v) guaiacol in 0.01 M sodium phosphate buffer, pH 6.0, and 0.1 M H_2O_2. The enzyme extract (0.1 ml) was added to initiate the reaction which was followed by measuring absorbance colorimetrically at 470 nm. The boiled enzyme was used as a blank. The peroxidase activity was expressed as an increase in the absorbance at 470 nm $min^{-1}g^{-1}$ of fresh tissue.

Polyphenol oxidase (PPO)

Polyphenol oxidase enzyme activity was estimated using the procedure of Mayer *et al*. (1965). To one gram liquid nitrogen homogenized leaf powder, 5 mL phosphate buffer (pH 6.5) was added, mixed thoroughly and centrifuged at 10,000 rpm for 10 min. at 4°c. The supernatant was used as a source of enzyme. To 200 µL of enzyme extract, 1.5 mL of 0.1 M sodium phosphate buffer (pH 6.5) and 200 µl of 0.01 M catechol were added and the absorbance was measured immediately at 490 nm. The mixture was incubated at room temperature for one hour and the absorbance was taken at 490 nm. The activity was expressed as a change in absorbance at 490 nm $min^{-1}g^{-1}$ of fresh tissue.

Catalase (CAT)

Catalase activity was assayed spectro photometrically as described by Azevedo *et al*. (1998). One gram plant tissue was ground to a fine powder using liquid nitrogen in a pestle and mortar. In order to extract the enzyme from the leaf powder, 3 ml of 100 mM potassium phosphate extraction buffer (pH7) was added and mixed thoroughly. The homogenate was centrifuged at 10,000 rpm for 20 min and the supernatant was used as a source of enzyme. The assay mixture comprised of 1 mL of

100 mM potassium phosphate buffer (pH 7.5) and 2.5 µl H_2O_2 (30% solution) and 100 µl of enzyme extract. The activity was measured immediately by monitoring the degradation of H_2O_2 at 240 nm at one min. interval, the enzyme-free reaction mixture served as blank. The activity was expressed as changes in absorbance at 240 nm $min^{-1}g^{-1}$ of fresh tissue.

Estimation of proline

The proline content was estimated in plant tissue according to the procedure of Bates *et al*. (1973). Approximately 0.5g leaf tissue was ground with 10 mL of 3% aqueous sulphosalicylic acid using pestle and mortar. The homogenate was centrifuged at 8000 rpm for 10 min. The supernatant was collected and used for proline estimation. The reaction mixture consisted of 2 mL plant extract, 2 mL acid ninhydrin and 2 mL of glacial acetic acid. This mixture was placed in a water bath at 100 ºC for 1 h. Then the reaction mixture was cooled to room temperature by placing in an ice bath for 10 min. Later, 4 mL toluene was added to the test tube containing reaction mixture and mixed vigorously with a test tube stirrer for 20-30s. The supernatant layer was collected and optical density was measured at 520 nm. Toluene was used as a blank. The proline content was expressed as µmoles of proline g^{-1} of fresh tissue.

Statistical analysis

All the experimental data were subjected to one-way analysis of variance (ANOVA) and the results are expressed as mean with standard error (mean ± SE). Duncan's multiple range test (DMRT) at $P < 0.05$ was used to compare the mean values. The software package used was SPSS version (16.0).

RESULTS AND DISCUSSION

Morphological characteristics

Inoculation of rice with moisture stress mitigating bacterial inoculants improved shoot and root lengths (cm), root volume (mL) root/shoot ratio and shoot and root dry biomass (g) significantly ($p < 0.05$) compared to uninoculated moisture stressed treatment (Table-2) and leaf area (cm^2) (Fig.1A) Irrespective of the bacterial inoculants, plants grown under moisture stressed condition recorded comparatively lower shoot and root length and biomass production over plants grown in waterlogged uninoculated condition. However, among the three bacterial inoculants, the highest shoot (41.98 cm) and root (27.3 cm) length were observed in plants treated with *S. yanoikuyae* MH394206under moisture stress treatment.

Table 1. Biochemical characteristics of the bacterial strains used in this experiment

Bacterial strains	ACCD activity (n moles mg^{-1} protein h^{-1})	IAA (g/mL)	GA_3 (g/mL)	Proline (g/mL)	Reference
Methylorubrum sp	Positive	ND	ND	ND	Akila, 2019
*S. yanoikuyae*MH394206	210 (0.34)	15.15 (0.142)	549.50 (8.87)	21.00 0.415	Nivitha, 2018
Bacillus altitudinis FD48	Positive	10.27 (0.24)	10.67 0.25	6.81 (0.16)	Sowmya, 2019

Data represent the mean ± SE from four replicates. ND-Not Determined

Table 2. Effects of drought stress and bacterial inoculants on rice root and shoot growth

Treatment	Shoot length (cm)	Shoot fresh weight(g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight(g)	Root dry weight (g)	Root Volume(mL)
T1	47.43(2.46)^{a}	16.219(0.25)^{a}	5.629(0.14)^{a}	31(0.86)^{a}	13.04(0.23)^{a}	3.78(0.39)^{a}	12(0.34)^{a}
T2	40.83(1.52)^{bc}	8.811(0.30)^{c}	3.763(0.46)^{b}	24.1(0.33)^{c}	11.35(0.36)^{b}	2.82(0.30)^{b}	7(0.37)^{c}
T3	41.98(0.86)^{b}	10.21(0.16)^{b}	3.615(0.23)^{b}	27.3(0.38)^{b}	9.75(0.22)^{c}	2.11(0.14)^{c}	9(0.18)^{b}
T4	38.41(1.16)^{bc}	8.247(0.53)^{d}	2.923(0.37)^{c}	22(0.75)^{d}	6.90(0.40)^{d}	1.43(0.26)^{d}	6(0.11)^{d}
T5	32.62(0.36)^{c}	7.103(0.26)^{e}	2.015(0.07)^{d}	17(0.57)^{e}	5.47(0.33)^{e}	1.28(0.16)^{d}	4(0.26)^{e}

Data represent the mean ± SE from four replicates. Different letters in the same column indicate significant differences according to Duncan's test ($P < 0.05$). The treatment include T_1-Control (Uninoculated with water logged condition), T_2- 75% moisture stress + seed inoculation with (PPFM), T_3-75% moisture stress + seed inoculation with S. yanoikuyae MH394206,T_4-75% moisture stress + seed inoculation with B. altitudinis FD48, and T_5-Control (Uninoculated 75% moisture stressed condition).

With respect to root biomass, the performance of PPFM and *S. yanoikuyae* MH39420 was on par with each other. The leaf area did not vary significantly among bacterial treatments. Inconsistent with the shoot and root lengths, root volume and root/shoot ratio were significantly greater for *S. yanoikuyae* MH394206treated plants. In fact root/shoot ratio of *S. yanoikuyae* MH394206received plants was similar to plants grown in waterlogged and uninoculated condition. Plants grown in moisture stress without bacterial inoculation recorded the lowest value for all the above parameters. The results indicated that the vegetative growth of rice under moisture deficit condition increased significantly due to bacterial inoculants; the effect was quite significant due to *S. yanoikuyae* MH394206. This could be due to its plant growth promoting abilities like ACCD activity, antioxidants, and siderophores and phytohormones production. It has been observed that gibberellic acid production by *S. yanoikuyae* MH394206 enhanced

almost 10 times in moisture deficit situation (Data not shown). Similar plant growth enhancement effects by bacteria were reported in several earlier studies(Compant*et al*., 2005; Turan, *et al*., 2014; Karlidag, *et al*., 2010) Due to the greater growth promoting effect by *S. yanoikuyae* MH394206on rice in moisture-stressed condition, it is logical to include this bacterium in the plant growth promoting bacterial (PGPB) group.

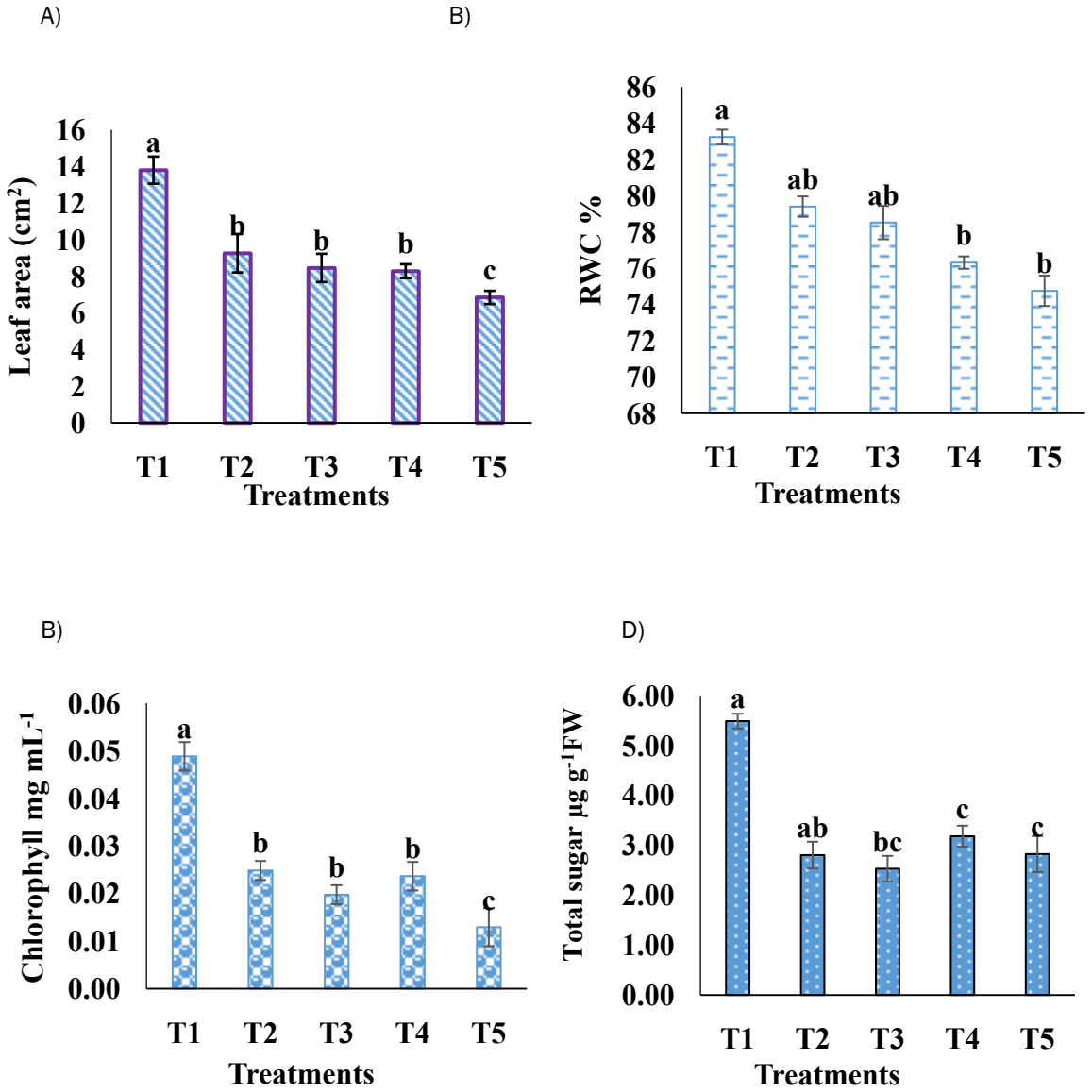

Figure 1. Leaf area (A), RWC (B), chlorophyll(C) and TSS (D) content of the leaf. Data represent the means ± SE of four independent replication. Different letters on the graph indicate significant differences according to Duncan's test (P < 0.05). The treatments include T_1-Control (Uninoculated with water logged condition), T_2- 75% moisture stress + seed inoculation with (PPFM), T_3-75% moisture stress + seed inoculation with S. yanoikuyae MH394206,T_4-75% moisture stress + seed inoculation with B. altitudinis FD48, and T_5-Control (Uninoculated 75% moisture stressed condition).

Physiological characteristics

The relative water content of leaf is an important indicator of water status in plants; it reflects the balance between water supply to the leaf tissue and transpiration rate (Lugojan *et al*., 2011). Results of the current study indicated, a significant reduction in the relative water content of the leaf exposed to moisture stress. But the reduction was insignificant due to the application of PPFM and *S. yanoikuyae* MH394206 (Fig.1B). Under drought stress conditions the solute concentration in the root zone may be increased which decreased the permeability of the roots and reduced water uptake by the roots as a result declined leaf relative water content. The maximum relative water content of 83.2 % was

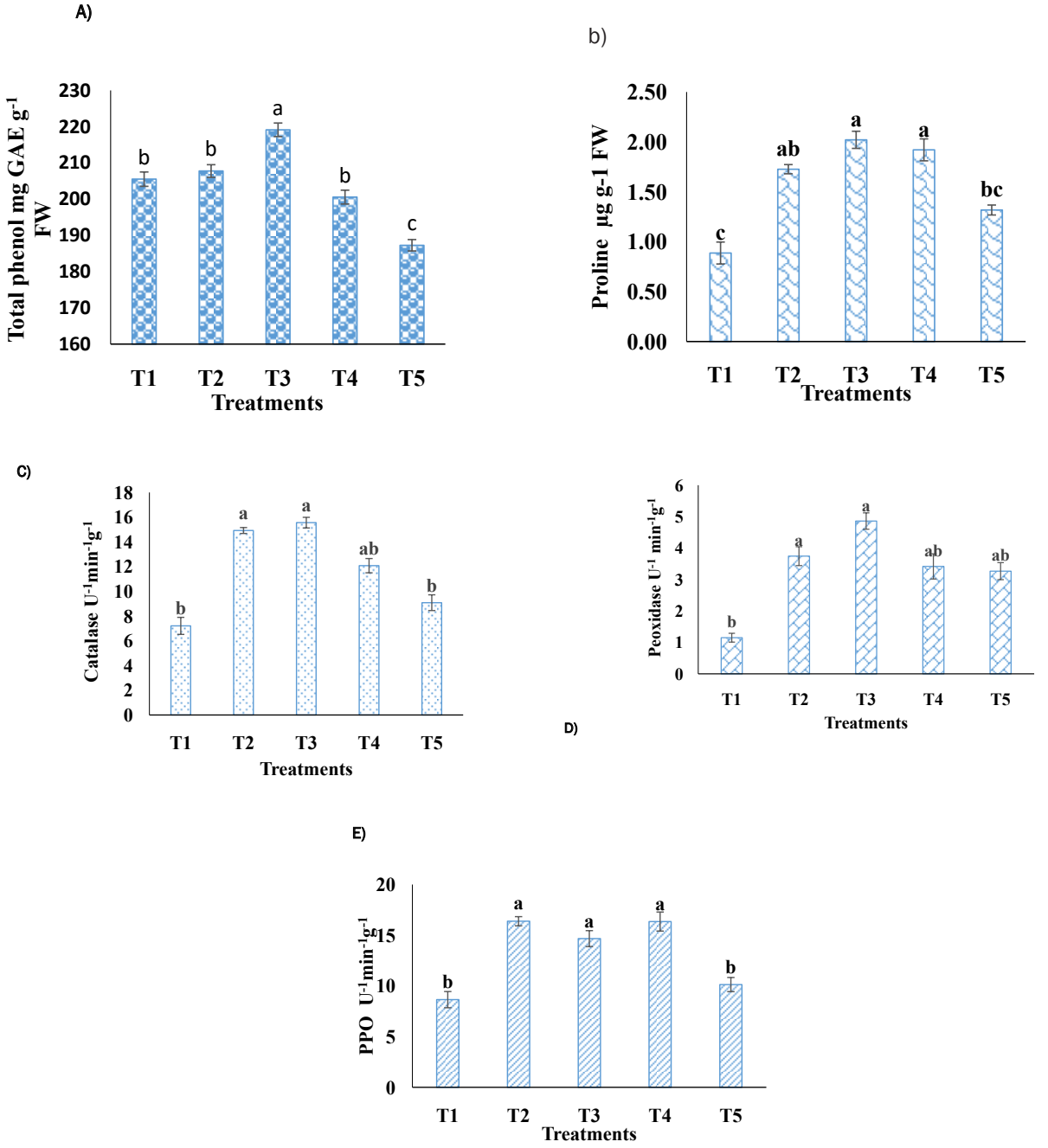

Figure 2. Total phenol (A), proline(B), peroxidase (C), catalase (D) and polyphenol oxidase(E)of leaf. Data represent the means ± SE of four independent replication. Different letters on the graph indicate significant differences according to Duncan's test (P < 0.05). The treatments include T1-Control (Uninoculated with water logged condition), T2- 75% moisture stress + seed inoculation with (PPFM), T3-75% moisture stress + seed inoculation with *S. yanoikuyae* MH394206, T4-75% moisture stress + seed inoculation with B. altitudinis FD48, and T5-Control (Uninoculated 75% moisture stressed condition)

observed in flooded condition (T1). The percent increase over stressed treatment was 6, 5 and 2 % respectively due to PPFM (T2), *S. yanoikuyae* MH394206 (T3)and *B. altitudinis* FD48 (T4). There is no significant difference between T2 and T3 under drought stress condition. The total chlorophyll content of rice reduced due to moisture stress (Fig.1C). Bacterial inoculation augmented chlorophyll content significantly compared to uninoculated moisture stressed treatment (T5). The highest chlorophyll content among bacterial inoculants was recorded in *S. yanoikuyae* inoculated plants which were a 31% increase over uninoculated moisture stressed control. However, there was no significant difference among bacterial inoculants.

Total soluble sugar content (TSS) was estimated after drought enforcement with five different treatments (Fig.1D). The TSS content reduced drastically due to bacterial inoculation except for T4. T1 registered maximum TSS (5.48 μg^{-1}g fresh weight) followed by T4 (3.18 μg^{-1}g FW). The total phenol content of the plant increased significantly due to bacterial application in moisture deficit conditions. The plants inoculated with *S. yanoikuyae* MH394206 had the highest total phenolic content (207.76 mg GAE g^{-1} FW), while the lowest phenolic content (187.28 mg GAE g^{-1} FW) was recorded in moisture-stressed un-inoculated(T5) treatment (Fig.2A). Natural phenolic exert their beneficial health effects mainly through their antioxidant activity. These compounds are capable of removing free radicals, chelating metal catalysts, activating antioxidant enzymes, and inhibiting oxidases (Heim *et al*., 2002). Chamam *et al*. (2013) showed that *Azospirillum* sp. was able to modulate the phenolic compounds in rice. Results showed that the proline content of leaf enhanced significantly during stress (Fig.2B). It had increased further in treatments receiving bacterial inoculum. Maximum proline content of 2.02 $\mu g\ g^{-1}$ FW was evidenced in *S. yanoikuyae* MH394206 treated plants which were 53% more over uninoculated and moisture stressed plants. The proline content of the plants grown in flooded condition was the least (0.89 $\mu g\ g^{-1}$ FW). The results indicated that proline has been accumulated as osmolyte during moisture stress.

The catalase activity increased significantly in bacteria inoculated treatments compared to uninoculated moisture stressed condition (Fig.2C). Maximum activity of 14.25 $unit^{-1}\ min^{-1}g^{-1}$ was shown by plants inoculated with PPFM which was 21.3% greater than the moisture stressed control (T5). The performance of *S .yanoikuyae* MH394206 (14.22 $unit^{-1}min^{-1}g^{-1}$) was on par with PPFM. Among bacterial treatments, the activity was least due to *B. altitudinis* FD48 (12.07 $unit^{-1}min^{-1}g^{-1}$). The result revealed that peroxidase activity increased during moisture stress (Fig.2D). The activity increased considerably when treated with various bacterial cultures. In consistent with catalase, maximum peroxidase activity was recorded in plants treated (T3) with *S. yanoikuyae*MH394206 (4.86 ± 0.36 $units^{-1}min^{-1}g^{-1}$) followed by PPFM (T2) treatment ($3.74\pm0.60 units^{-1}$ $min^{-1}g^{-1}$). However, the variation between these two treatments was statistically insignificant. As expected, the activity was least in plants grown under flooded condition (T1). Similar to the other two antioxidant enzymes, polyphenol oxidase activity increased due to moisture stress (Fig.2E). Bacterial inoculation further enhanced antioxidant activity. Among bacteria, *Methylorubrum* sp (PPFM) treated plants showed maximum ($16.7\pm0.45 units^{-1}min^{-1}g^{-1}$) activity, followed by *S .yanoikuyae*MH394206(15.67 ±0.78 $unit^{-1}min^{-1}g^{-1}$). However, the activity was insignificant among bacterial treatments. Increase in antioxidant activities would mitigate the ill effects of free radicals generated during moisture stress. It has been found that strawberry plants inoculated with multi traits PGPR strains showed high antioxidant enzymes activity which contributed to enhancing the plant protection against drought stress(Erdogan *et al*., 2016). Reduction of oxidative stress was correlated with drought stress tolerance induced by plant-beneficial bacteria (Compant *et al*., 2010).

In the present study, chlorophyll, anti-oxidant enzymes (CAT, POD, PPO) activity and total phenolic content were greater in bacteria inoculated treatments compared to plants grown in moisture-stressed and un–inoculated condition. Thus increased tolerance to moisture stress may be correlated with increase dantioxidant enzymes activity and total phenolics content. One of the interesting findings of this study is that among the three bacterial inoculants, the apoplastic fluid endophyte significantly improved the growth and drought tolerating capacity of rice in pot culture conditions.

Formulae

Leaf area (cm^2)=L*W*K
L – Length (cm)
W – Width (cm)
K – Correction factor

$$2.\ \mathbf{RWC}\ \% = \frac{\mathbf{Fresh\ weight - Dry\ weight}}{\mathbf{Soaked\ weight - Dry\ weight}} * \mathbf{100}$$

RWC – relative water content

CONCLUSION

Application of *S. yanoikuyae* MH394206, *Methylorubrum* sp. (PPFM), and *Bacillus altitudinis* FD48 improved rice growth significantly in water-stressed condition by altering the morphological and physiological characteristics of the plant. Improved plant phenolic content and enzyme activities (CAT, POD &PPO) as a result of inoculant application would be useful markers for the bacterial effect on the strategies of drought tolerance in the plants. The effect was pronounced due to the treatment of rice with apoplastic fluid associated bacteria *S.yanoikuyae* MH394206.Hence, this may serve as a potential bacterial inoculant for drought abatement. However, further studies are imperative to assess the effect in field condition.

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