

Enhancing drought tolerance in common bean by plant growth promoting rhizobacterium *Bacillus amyloliquefaciens*

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Abstract: The study was set to explore the implications of plant growth promoting rhizobacterium in common bean while acclimating to drought stress. *Bacillus amyloliquefaciens* Priest et al., 1987 (PPB6)-inoculated plants showed better morphological attributes and pod yield as compared to uninoculated drought-stressed plants. PPB6 enhanced photosynthesis efficiency under drought stress by means of improving photosynthetic pigments and photochemical efficiency (F_v/F_m) when compared to uninoculated drought-stressed plants. PPB6 increased leaf relative water content (59.67 %) and reduced proline accumulation ($0.63 \mu\text{mol g}^{-1}$ FM) under drought stress, compared to uninoculated drought-stressed plants (43.67 % and $1.42 \mu\text{mol g}^{-1}$ FM respectively). However, during drought stress, PPB6 led to produce comparatively lower level of hydrogen peroxide ($66.60 \mu\text{mol g}^{-1}$ FM), and lipid peroxidation product; malondialdehyde ($0.05 \mu\text{mol g}^{-1}$ FM), and electrolyte leakage (30.20 %) as compared to uninoculated drought-stressed plants ($136.25 \mu\text{mol g}^{-1}$ FM, $0.08 \mu\text{mol g}^{-1}$ FM and 55.93 % respectively). However, as compared to uninoculated drought-stressed plants, significant reduction of total phenolics and flavonoids, and activity of enzymatic antioxidants such as ascorbate peroxidase, glutathione S-transferase and peroxidase in both non-stressed uninoculated and PPB6-inoculated drought affected plants, suggesting the lowering of drought-induced oxidative damage by this bacterium. Here, we suggest PPB6 had the ability to mitigate drought effects in common bean plant. Besedilo izvlečka

Key words: drought, common bean, stress, antioxidant

Pospeševanje odpornosti na sušo pri navadnem fižolu z rizo-bakterijo *Bacillus amyloliquefaciens*, ki pospešuje rast rastlin

Izvleček: Raziskava je bila izvedena za preučevanje uporabe rizobakterij, ki pospešujejo rast rastlin pri prilaganju navadnega fižola sušnemu stresu. Z rizobakterijo *Bacillus amyloliquefaciens* Priest et al., 1987 (PPB6) inokulirane rastline so pokazale boljše morfološke parametre rasti in večji pridelek strokov v primerjavi z neinokuliranimi. PPB6 je povečala učinkovitost fotosinteze v sušnem stresu z izboljšanjem stanja fotosinteznih barvil in večjo fotokemično učinkovitostjo fotosinteze (F_v/F_m) v primerjavi z rastlinami, ki v sušnem stresu niso bile inokulirane. PPB6 je povečala relativno vsebnost vode (59,67 %) in zmanjšala akumulacijo prolina ($0,63 \mu\text{mol g}^{-1}$ FM) v razmerah sušnega stresa v primerjavi z neinokuliranimi rastlinami (43,67 % in $1,42 \mu\text{mol g}^{-1}$ FM). V sušnem stresu inokulirane rastline so imele manjšo vsebnost vodikovega peroksida ($66,60 \mu\text{mol g}^{-1}$ FM), manjšo vsebnost produktov peroksidacije lipidov kot je malondialdehid ($0,05 \mu\text{mol g}^{-1}$ FM) in manjše puščanje elektrolitov (30,20 %) v primerjavi z neinokuliranimi rastlinami rastočimi v razmerah sušnega stresa ($136,25 \mu\text{mol g}^{-1}$ FM, $0,08 \mu\text{mol g}^{-1}$ FM in 55,93 % respectively). V neinokuliranih rastlinah, ki so rastle v razmerah sušnega stresa je bilo ugotovljeno značilno zmanjšanje v vsebnosti celokupnih fenolov in flavonoidov kot tudi v aktivnosti antioksidacijskih encimov kot so askorbat peroksidaza, glutation S-transferaza in peroksidaza. Isti trend je bil opažen v neinokuliranih rastlinah, rastočih v nestresnih razmerah kot tudi v inokuliranih rastlinah, rastočih v sušnem stresu, kar vse nakazuje zmanjševanje od sušnega stresa povzročenih oksidacijskih poškodb z omenjeno rizo-bakterijo. Prepostavljamo, da ima rizobakterija PPB6 sposobnost blaženja učinkov suše pri navadnem fižolu.

Ključne besede: suša, navadni fižol, stres, antioksidant

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1 INTRODUCTION

The unprecedented and constant threats of global warming have been greatly involved in reducing crop yield worldwide by triggering the negative consequences of abiotic stresses. Among the abiotic stresses, drought is the most dangerous one, having tremendous impacts on crop productivity. It is anticipated that drought will likely to affect the crop yield of about 50 % of arable lands by 2050 (Akhtar *et al.*, 2021). Due to the fact of aberrant nature of world's climate, more severe and persistent droughts accompanied by less rainfall, are expected in the 21st century across many regions of the world (Schwalm *et al.*, 2017; Trenberth *et al.*, 2014). Many plant attributes such as growth, photosynthetic pigments, water and nutrient use efficiency, cellular and biochemical changes including enzymatic activities are greatly affected by drought stress which is responsible for ultimate reduction in crop yield (Rezayian *et al.*, 2018; Seleiman *et al.*, 2021). The reactive oxygen species (ROS)-induced damage to biomolecules is one of the major factors that limit plant growth under drought stress (Nadeem *et al.*, 2020). Hence, management of drought is a crucial challenge for increasing crop production to ensure food security.

Common bean (*Phaseolus vulgaris* L.), an annual crop belonging to the family Fabaceae, is one of the important grain legume crops in tropical and temperate countries for direct human consumption and comprises 50 % of the grain legumes consumed worldwide (McClean *et al.*, 2002). It is a dual-purpose crop that is grown as pulse (grain) and also consumed in the immature stage as a tender vegetable (Nazrul & Shaheb, 2016). Common bean cultivation has been drastically affected by drought stress across the globe (Assefa *et al.*, 2015). Management of drought by altering physico-chemical properties and developing drought tolerant varieties should be very imperative for enhancing yield. Conventional plant breeding techniques have allowed the development of high-yielding and drought-tolerant crop varieties but that is very time consuming and may lead to the loss of other desirable traits from the host's gene pool (Eisenstein, 2013). Agronomic management and exogenous application of several growth regulators such as melatonin, abscisic acid (ABA), methyl jasmonate, salicylic acid have been investigated to mitigate drought stress (Arnao & Hernandez-Ruiz, 2018; Mohi-Ud-Din *et al.*, 2021). Though these strategies have a potential scope, the application of such expensive regulators in farmers' field and performances of those is still questionable. Hence, drought management strategy by natural and less expensive way might cause suitable solution for this issue. Currently, plant-associated microbial communities including plant growth promoting rhizo-

bacteria (PGPR) have received increased attention for enhancing crop productivity and stress resistance (Glick, 2012). PGPR are soil born bacteria living in association with the plant roots (rhizosphere) which are involved in promoting plant growth and development through the secretion of various regulatory molecules (Vocciante *et al.*, 2022). PGPR have been implicated in increasing abiotic stress tolerance including drought in different crop plants (Gowtham *et al.*, 2022; Chieb & Gachomo, 2023). PGPR inoculation in plants can enhance the productivity of crops under drought stress through a variety of mechanisms (Bouremani *et al.*, 2023). For instance, PGPR-treated plants maintained relatively higher relative water content (RWC) compared to non-treated plants by enhancing synthesis of phytohormones and osmoprotectants, leading to the survival of plants under drought stress (Kudoyarova *et al.*, 2019; Chieb & Gachomo, 2023). Besides, PGPR have been reported to increase the stomatal conductance, photosynthetic rate, photochemical efficiency of pigment system II; PSII (*Fv/Fm*), and decrease the transpiration rate in plants, which simultaneously affect the structural and functional characteristics of photosynthetic apparatus (Martins *et al.*, 2018; Khan & Bano, 2019). Improvement of drought tolerance has also been reported in wheat plant by using growth promoting bacterium *Stenotrophomonas maltophilia* Palleroni & Bradbury 1993 (Kasim *et al.*, 2021). Furthermore, PGPR were investigated to increase the activity of ROS scavenging enzymatic and non-enzymatic antioxidants, thereby decreasing the excess ROS accumulation in drought affected plants (Chandra *et al.*, 2020; Ansari *et al.*, 2021). *Bacillus subtilis* (Ehrenberg 1835) Cohn 1872 and its sister species *Bacillus amyloliquefaciens* have been widely used as the beneficial agents for plant growth promotion, suppression of soil-borne diseases in agriculture and industrial purposes (Chen *et al.*, 2007; Fan *et al.*, 2012). *B. subtilis* and *B. amyloliquefaciens* have been investigated to improve drought tolerance in maize (Vardharajula *et al.*, 2011), rice (Tiwari *et al.*, 2017), wheat (Sood *et al.*, 2020), tomato (Gashash *et al.*, 2022) and soybean (Braz *et al.*, 2022). Although having such potentials, the role of *B. amyloliquefaciens* in the drought tolerance of common bean has been less clarified. For instance, *B. amyloliquefaciens* was investigated in abiotic stress tolerance of common bean by bacterial biofilm formation (Martins *et al.*, 2017). Another study using *B. amyloliquefaciens* as biostimulant did not confer tolerance to moderate level of drought stress in common bean (Galvão *et al.*, 2019). Although having this progress, the alteration of morphophysiological and biochemical processes by this bacterium is yet to be elucidated in drought-stressed common bean plants. Therefore, the investigation of which is of utmost importance for sustainable crop yield in climate

resilient agriculture. However, regulation of drought-induced oxidative stress by this bacterium is still to be clarified in common bean. Considering the facts, the present investigation was set to find out the implications of plant growth promoting rhizobacterium in common bean while acclimating to drought stress by means of morpho-physiological and biochemical analyses. The efforts suggest that *B. amyloliquefaciens* has the ability to mitigate the negative consequences of drought effects in common bean that might help in sustainable crop yield and future food security.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL MATERIALS, PREPARATION OF BACTERIAL SUSPENSION, INOCULATION AND SCREENING OF SUITABLE BACTERIAL STRAIN

Seeds of common bean (variety BARI Jharsheem-1) were collected from Bangladesh Agricultural Research Institute (BARI), Bangladesh. The bacterial strains such as *Bacillus subtilis* (PPB2), *Stenotrophomonas maltophilia* (PPB3), and *B. amyloliquefaciens* (PPB6) were collected from the department of Plant Pathology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, where the strains were isolated from the rhizosphere of different crop plants and found to be promising for having higher efficacy for the synthesis of indole acetic acid (IAA), and secondary metabolites (Islam et al., 2015; Masum et al., 2018). PPB2, PPB3 and PPB6 strains were cultured in yeast extract peptone dextrose agar media for 48 hours at 28 °C and stored in 4 °C temperature. From the stock culture, bacteria were grown in liquid culture for 48 hours at 28 °C on a rotary shaker (80-100 rpm). The cultured bacteria were centrifuged at 4000 rpm at 4 °C for 15 minutes. Then, the supernatant was removed and the pellet was re-suspended in distilled water and the volume was adjusted. Before sowing, the sterilized common bean seeds were inoculated with the bacterial suspensions and kept overnight in petri dishes. Then, seeds were sown in pots containing sterile soils with proper fertilization by maintaining three replicates. Treatment combinations at this level were uninoculated control, plants inoculated with three bacterial strains like PPB2, PPB3 and PPB6. When plants got three trifoliolate leaves (at 21 days after germination), the bacterial suspensions were again injected into the root zones and they were allowed to grow under ambient condition with proper fertilization and irrigation. PPB6 was screened out as the best bacterial strain based

on the effects of those bacterium on the growth and development of common bean.

2.2 IMPOSITION OF DROUGHT TREATMENTS AND MORPHOLOGICAL DATA COLLECTION

The treated and non-treated common bean seeds with PPB6 were allowed to grow in pots containing sterile soil with proper fertilizers by maintaining three replications. After 15 days of germination, some plants were thinned out and only five plants were kept to grow furtherly and PPB6 was inoculated at the root zone at 21 days after germination. The uninoculated plants were injected by same amount of deionized water. After 2nd injection (at 32 days after germination) with PPB6 suspension, the plants were supposed to either normal irrigated or drought-stressed conditions by stopping irrigation and maintaining 50 % field capacity as followed by Mohi-Ud-Din et al. (2021). The treatment combinations followed at this level were non-stressed uninoculated plant (Control), uninoculated drought-stressed plants (Drought) and Drought stressed plants inoculated with PPB6 (D+PPB6). The morpho-physiological data were collected upon getting the drought effects on plant (after 12 days of drought imposition). The experiments were done by maintaining at least three replications. During screening of bacteria, the phenotypic data were collected after harvesting. During drought treatment, the morphological data such as shoot length, number of leaflets, leaflet length, number of flowers and pods were collected after 12 days of drought imposition. The tri-foliolate leaves were numbered as per development as 1, 2, 3 etc. and data were collected consistently according to the number. The root, shoot and pod fresh and dry mass were noted after harvesting of common bean.

2.3 ANALYSIS OF PHYSIOLOGICAL DATA

The percentage (%) of leaf relative water content (LRWC) was determined by following the procedure of Shivakrishna et al. (2018). Briefly, leaves were collected from each treatment and weighed immediately to record the fresh mass (FM) and then placed in petri dishes containing distilled water for 4 h, at room temperature to record the turgid mass (TM). The leaves were dried in an oven at 80 °C for 24 h to obtain dry mass (DM). Then LRWC (%) was calculated using the formula; $RWC = (FM - DM) / (TM - DM) \times 100$. Chlorophylls and carotenoids content were estimated using the method described by Porra et al. (1989). Briefly, leaf pigments were extracted in 80 % (v/v) acetone followed by filtered with

Whatman filter papers. The absorbance of the supernatants was taken with a UV-visible spectrophotometer at 663, 646, and 470 nm. The quantification of the pigments was done according to the formula of Lichtenthaler & Welburn (1983). SPAD values of the leaves were also recorded with a Chlorophyll Meter (Model: SPAD-502, Minolta Co., Ltd., Tokyo, Japan) after 12 days of drought imposition. Maximum photochemical efficiency of PSII in terms of F_v/F_m in the fresh leaf samples was determined according to the method of Yaghoubian *et al.* (2016) with slide modification. Briefly, after dark acclimation of leaves for 15 minutes, the minimum (F_o) and maximum fluorescence intensity (F_m) were measured in leaves using a portable fluorometer. The variable fluorescence (F_v) and maximum photochemical efficiency of PSII (F_v/F_m) were calculated using equation I ($F_v = F_m - F_o$) and II ($F_v/F_m = (F_m - F_o)/F_m$) respectively. The proline content of fresh leaf was measured spectrophotometrically at 520 nm using the acid ninhydrin assay according to Bates *et al.* (1973).

2.4 MEASUREMENT OF ELECTROLYTE LEAKAGE, HYDROGEN PEROXIDE (H_2O_2) AND MALONDIALDEHYDE (MDA) CONTENT

Electrolyte leakage of the damaged tissue in the leaf was measured by following the protocols as followed by Ghosh *et al.* (2021). The fresh leaf tissues (0.04 g) were homogenized in 1.5 ml of 5 % (w/v) trichloroacetic acid (TCA). After centrifugation at $11,500 \times g$ for 10 minutes, the supernatant was used to determine H_2O_2 and malondialdehyde (MDA). The H_2O_2 content was determined spectrophotometrically at 390 nm according to the procedure of Ghosh *et al.* (2021). The H_2O_2 concentration was calculated using the extinction coefficient of $0.28 \mu M^{-1} cm^{-1}$ and expressed as $\mu mol g^{-1} FM$. The MDA content was determined spectrophotometrically at 532 nm and 600 nm according to the procedure of Ghosh *et al.* (2021). The MDA content was calculated using an extinction coefficient of $155 mM^{-1} cm^{-1}$ and expressed as $\mu mol g^{-1} FM$.

2.5 DETERMINATION OF NON-ENZYMATIC ANTIOXIDANTS ACTIVITY

Phenolic content of the methanolic extracts was determined spectrophotometrically according to the Folin-Ciocalteu method (Singleton *et al.*, 1999). The absorbance of reaction solutions was measured spectrophotometrically at 765 nm and quantification was done according to the formula of Abdul-Hafeez *et al.* (2014).

Then, the results were compared to a standard curve of gallic acid solutions and expressed as micrograms of gallic acid equivalent per gram fresh leaf mass ($\mu g g^{-1} FM$). The flavonoids content was measured using the aluminum-chloride colorimetric assay (John *et al.*, 2014). The absorbance of the extracts and standard solutions was measured at 510 nm using a spectrophotometer. The measurements were expressed as micrograms of quercetin equivalent (QE) per gram of fresh leaf mass ($\mu g g^{-1} FM$).

2.6 ANALYSIS OF ENZYMATIC ANTIOXIDANTS

0.3 g of leaf tissue was crushed well with an ice-cold mortar and pestle in 1 ml of the extraction buffer containing 50 mM ice-cold K-P buffer (pH 7.0), potassium chloride (100 mM), ascorbate (1 mM), β -mercaptoethanol (5 mM) and glycerol (10 % v/v). The homogenized plant materials were then centrifuged at 11,500 rpm at 4 °C for 12 minutes and the supernatant was used as a soluble protein solution for enzyme activity. The protein concentration of the samples was determined by the method of Bradford (Bradford, 1976) using BSA as a protein standard. The activity of ascorbate peroxidase (APX) was determined according to the protocol used by Nakano & Asada (1981). APX activity was estimated with the extinction coefficient of $2.8 mM^{-1} cm^{-1}$ and determined as $\mu mol min^{-1} mg^{-1} protein$. The activity of catalase (CAT) was determined by using the procedure of Hasanuzzaman *et al.* (2014). CAT activity was calculated with an extinction coefficient of $39.4 M^{-1} cm^{-1}$ and determined as $\mu mol min^{-1} mg^{-1} protein$. Glutathione S-transferase (GST) activity was measured by following the procedure of Hossain *et al.* (2010). GST activity was calculated with an extinction coefficient of $9.6 mM^{-1} cm^{-1}$ and determined as $nmol min^{-1} mg^{-1} protein$. Peroxidase (POD) was measured by the method followed by Hemeda and Klein (1990). POD activity was estimated with an extinction coefficient of $26.6 mM^{-1} cm^{-1}$ and determined as $nmol min^{-1} mg^{-1} protein$.

2.7 STATISTICAL ANALYSIS

All the experiments were conducted by following CRD (Completely Randomized Design) with three replications. Statistical analysis was performed using Statistix 10 software. The least significant difference (LSD) was analyzed at 5 % level of significance.

3 RESULTS AND DISCUSSION

3.1 EFFECTS OF GROWTH PROMOTING RHIZO-BACTERIA ON GROWTH AND YIELD OF COMMON BEAN

Three bacterial strains, PPB2, PPB3, and PPB6 were applied for showing their effects on the growth and yield performances of common bean. Among the bacterial strains, plants inoculated with PPB6 showed best performances on all the morphological characteristics studied here such as shoot length, leaflet length, number of flowers, number of pods, and pod fresh mass and dry mass (Table 1). Although plants inoculated with PPB6 showed more or less similar performances to uninoculated control plants, the length of leaflets were significantly increased by PPB6. The leaflet length of plants treated with uninoculated control, PPB2, PPB3, PPB6 was 14.67 cm, 14.67 cm, 16.00 cm, 16.67 cm respectively (Table 1). Previous investigations with *Bacillus* species produced improved phenotypes in rice seedlings as compared to uninoculated control (Awlachev & Mengistie, 2022). This was because of the improvement of the plant's growth by *Bacillus* spp by means of enhancing availability of nitrogen, phosphorus, potassium and iron in soil (Xue et al. 2021). In addition, plant growth could be promoted by *Bacillus* species by engaging several essential phytohormones like auxin; indole acetic acid (IAA), cytokinins (CKs) and gibberellic acids (GAs) (reviewed in Tsotetsi et al. 2022). The PGPR also promote volatile organic carbon (VOCs) which can modify the hormonal signal and enhance cell division (reviewed in Tsotetsi et al. 2022). Moreover, upon inoculation, PGPR induce spermidine synthesis which was claimed for plant growth via expansin and reduction of ethylene levels (Xie et al. 2014). In our studies, as compared to uninoculated control, plants inoculated with PPB6 produced better phenotypes particularly in the enhancement of leaflet length which should have impacts on the better pod yield of the plant.

The larger leaflet by PPB6 may be due to the accumulation of higher phytohormones, and VOCs and spermidine in the leaf. Therefore, for having relatively better performances of PPB6 (Table 1), we screened out this bacterium for further drought studies in common bean.

3.2 IMPROVEMENTS OF PLANT'S PHENOTYPE AND POD YIELD BY PPB6 UNDER DROUGHT STRESS

The phenotypic parameters of common bean plants have been significantly altered by drought stress. Plant's height in terms of shoot length was found to be the highest (48.67 cm) in non-stressed uninoculated control (Control) plants followed by the drought-stressed plants inoculated with PPB6; D+PPB6 (44.67 cm) and uninoculated drought-stressed (Drought) plants (32.33 cm) (Fig. 1A & Fig. 1B). Likewise, others phenotypic data such as shoot fresh and dry mass, root fresh and dry mass, number of flowers, number of pods, and fresh and dry mass of pods were recorded as best in the control plants followed by the plants of D+PPB6 and drought. (Figs. 1C-1J).

Plants inoculated with effective PGPR strains could maintain near normal shoot growth rates, resulting in increased crop productivity. For instance, Vardharajula et al. (2011) inoculated growth-promoting *Bacillus* spp in drought-stressed maize and found that all the PGPR treated plants showed significantly greater shoot compared to non-treated plants. Similarly, Lim & Kim (2013) showed that pepper plants treated with *Bacillus licheniformis* (Weigmann 1898) Chester 1901 K11 produced higher biomass than non-treated plants. PGPR were reported to enhance shoot growth under drought stress of mungbean (Sarma & Saikia, 2014), and maize (Naseem & Bano, 2014). Alongside, root is also vital organ for plant which could perceive and senses the changes in soil moisture (Amtmann et al., 2022). Naveed et al. (2014) reported that maize plants inoculated with *Burkholderia phytofirmans* (Sessitsch et al. 2005) Sawana et al. 2015 significantly increased root biomass in Mazurka and Ka-

Table 1: Effect of different growth promoting bacterial strains on the growth and yield performances of common bean plants treated with uninoculated control and *Bacillus subtilis* (PPB2), *Stenotrophomonas maltophilia* (PPB3), and *Bacillus amyloliquefaciens* (PPB6).

| Treatment | Shoot Length (cm) | Leaflet Length (cm) | No. of Flowers | No. of Pods | Pod Fresh Mass (g) | Pod Dry Mass (g) |
|-----------|-------------------|---------------------|----------------|-------------|--------------------|------------------|
| Control | 32.00 a | 14.67 b | 23 ab | 18.67 ab | 29.60 ab | 2.36 a |
| PPB2 | 28.67 b | 14.67 b | 19 b | 15.33 b | 23.40 b | 2.27 a |
| PPB3 | 31.33 a | 16.00 a | 21 ab | 14.67 b | 32.35 ab | 2.80 a |
| PPB6 | 33.00 a | 16.67 a | 25 a | 20.33 a | 39.93 a | 3.36 a |

Different letters in the table show significant differences at $p < 0.05$.

leo cultivars, respectively under drought stress. Besides, *Pseudomonas aeruginosa* (Schröter 1872) Migula 1900 was found to increase the number of pods per plant in *Vigna radiata* (L.) R. Wilczek under drought stress (Uzma *et al.*, 2022). Our results regarding less compromization of shoot and root biomass, and pod yield in common bean plants inoculated with PPB6 under drought stress are very consistent to those findings (Fig. 1). Although, *Bacillus* species are well documented to show response to abiotic stress tolerance, the molecular mechanism and signaling of those are still remains enigmatic. Enhanced synthesis of IAA in plants should be vital for approaching drought tolerance issue. For instance, *Pseudomonas tolaasii* Paine 1919 and *Pseudomonas fluorescens* (Flügge 1886) Migula, 1895 maintained better plant growth under cadmium stress by engaging IAA (Dell'Amico *et al.* 2008). However, *Bacillus* species could enhance expression of several genes related to the synthesis of IAA, phytase and siderophores which have tremendous roles in stress acclimation, and nutrient availability in soil respectively (reviewed in Luo *et al.* 2022). The species also conferred the expression of transcripts of late embryogenesis abundant (LEA) and dehydrin (DHN) under salt and heat stresses (Tiwarly *et al.* 2017) suggesting to

have roles in drought stress. Therefore, based on those findings, it is assumed that upon inoculation, PPB6 may enhance the expression of those genes in common bean while acclimating to drought stress and lead to better phenotypes than that of uninoculated drought-stressed plants.

3.3 AMELIORATION OF DROUGHT-INDUCED PHOTOSYNTHETIC DAMAGE BY PPB6

Because maintenance of chlorophyll content is crucial for plants while acclimating to drought stress, we determined Chl a, Chl b, total Chl content of common bean under different treatment conditions. Chl a content of plants of control, drought and D+PPB6 was 0.45, 0.16 and 0.30 mg g⁻¹ FM respectively and Chl b of those treatments was 0.09, 0.003 and 0.07 mg g⁻¹ FM respectively by D+PPB6 and drought (Fig. 2C). We also measured non-destructive chlorophyll index (SPAD) values of common bean and highest SPAD values was recorded in control followed by D+PPB6 and drought (Fig. 2D). We found varied level of carotenoids content under control (0.16 mg g⁻¹ FM), drought (0.07 mg g⁻¹ FM) and D+PPB6 (0.11

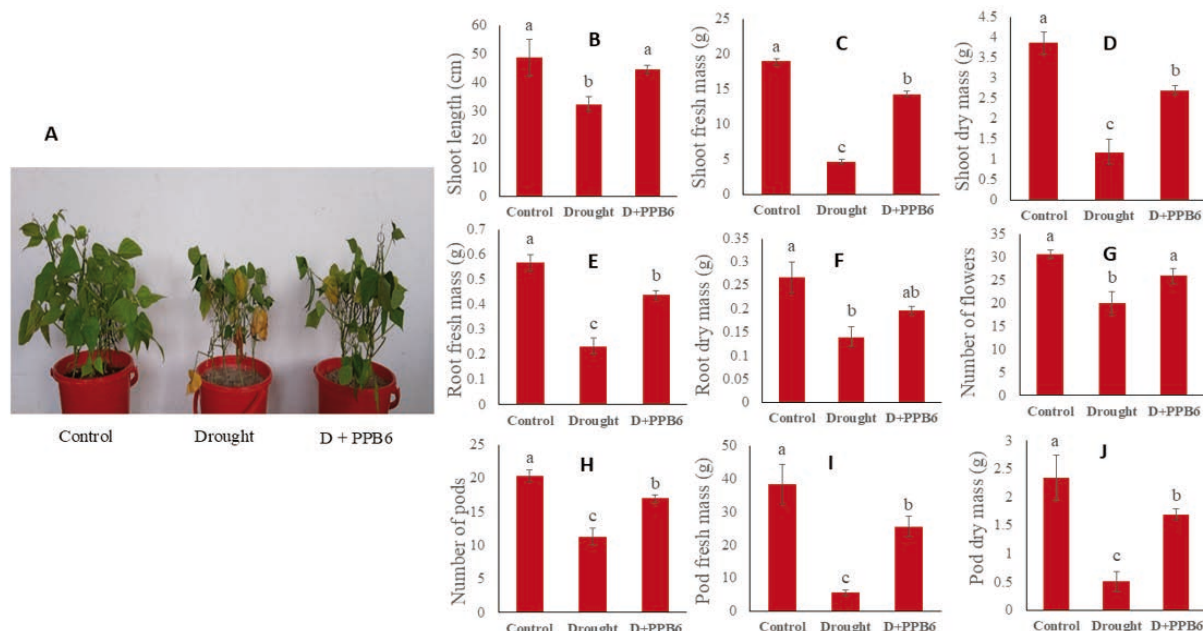


Figure 1: Alteration of morphological characteristics of common bean by *Bacillus amyloliquefaciens* (PPB6) under drought stress. Phenotypic appearance of common bean plants under uninoculated non-stressed condition (Control), uninoculated drought-stressed condition (Drought) and drought-stressed inoculated with PPB6; D+PPB6 (A). Effect of PPB6 on shoot length (B), shoot fresh mass (C), shoot dry mass (D), root fresh mass (E), root dry mass (F), number of flowers (G), number of pods (H), pod fresh mass (I) and pod dry mass (J) under drought stress. Error bars indicate standard error. Different letters on the bars show significant differences at $p < 0.05$.

mg g⁻¹ FM) conditions (Fig. 2E). We also recorded data of maximum photochemical efficiency of PSII (*Fv/Fm*) and found significant reduction of that in plants treated with drought (0.73) as compared with D + PPB6 (0.78) and control (0.79) (Fig. 2F).

The decreased chlorophyll content by drought is considered as the typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation (Anjum et al., 2011). Many investigations confirmed that PGPR could increase chlorophyll content by increasing stomatal conductivity, rate of photosynthesis, water and nutrient uptake, and protect chlorophyll degradation under drought stress (Liu et al., 2019). For instance, *Bacillus* strains were reported to increase Chl *a*, Chl *b* under drought stress in *Zea mays* L. and *Triticum aestivum* L. (Ilyas et al., 2020; Saleem et al., 2021). Furthermore, *B. amyloliquefaciens* was reported to increase Chl *a*, Chl *b* in *Medicago sativa* L. under drought stress (Han et al., 2022). Along with those, our results of relatively higher chlorophyll content under D+PPB6 as compared to uninoculated drought stress (Figs. 2A-2C) suggest the conspicuous role of *B. amyloliquefaciens* in the recovery of chlorophyll pigments in drought-stressed common bean. SPAD values were also reported to be enhanced during drought stress condition (He et al., 2019)

which is compatible to our findings where higher SPAD value was recorded in drought-stressed plants supplemented with *B. amyloliquefaciens* (Fig. 2D). Alongside, carotenoids, as one of the major classes of secondary metabolites boost up the antioxidant machineries to counter face oxidative stress in plants (Polapally et al., 2022). *Bacillus* strains has been reported to increase carotenoid under drought stress in crop plants (Ilyas et al., 2020; Saleem et al., 2021) which is very compatible to our findings (Fig. 2E). During photosynthesis, abiotic stress quietly alters the maximum quantum yield of PSII having impacts on crop yield. Maximum photochemical efficiency of PSII (*Fv/Fm*) is used as a sensitive indicator of plant photosynthetic performance (Lobos et al., 2012) and found to be greatly reduced by drought stress (Zhuang et al., 2020). The recent efforts indicated that PGPRs including *B. amyloliquefaciens* are able to improve *Fv/Fm* in plants under drought stress (Asghari et al., 2020; Petrillo et al. 2022). Consistently, *B. amyloliquefaciens* (PPB6) could improve *Fv/Fm* under drought stress (Fig. 2F). Thus, *B. amyloliquefaciens* could be the potent mitigator of drought stress effects in common bean by enhancing photosynthetic pigments like chlorophyll and carotenoids, and improving photochemical quantum yield of PSII.

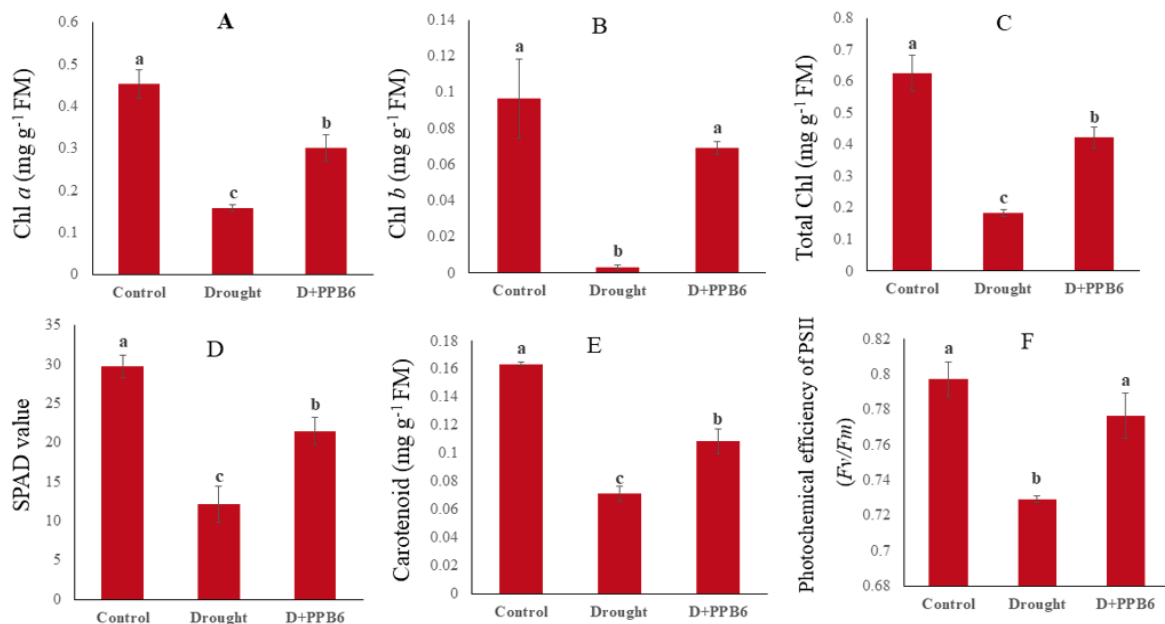


Figure 2: Effects of *Bacillus amyloliquefaciens* (PPB6) on the physiological parameters of common bean under drought stress. Effect of PPB6 on Chl *a* (A), Chl *b* (B), total chlorophyll (total Chl) (C), SPAD value (D), carotenoid contents (E) and photochemical efficiency of PSII (*Fv/Fm*) (F) under drought stress. Error bars indicate standard error. Different letters on the bars show significant differences at $p < 0.05$.

3.4 REGULATION OF OSMOTIC ADJUSTMENT BY PPB6 IN DROUGHT-STRESSED COMMON BEAN PLANTS

Since maintenance of cell turgor, osmotic adjustment and cellular homeostasis are crucial for acclimating drought stress, we measured percentage of leaf relative water content (LRWC) in leaf and found that D + PPB6 maintained more relative water content (59.67 %) as compared to uninoculated drought-stressed plants (43.67 %) (Fig. 3A). However, the highest percentage of LRWC was maintained by control treatment (78 %) (Fig. 3A). Since, cell turgor and osmotic adjustment are highly regulated by the accumulation of osmolytes, we measured proline content in common bean plants under different treatments. Proline content of plants under control, drought, and D + PPB6 was 0.18 $\mu\text{mol g}^{-1}$ FM, 1.42 $\mu\text{mol g}^{-1}$ FM, 0.63 $\mu\text{mol g}^{-1}$ FM respectively (Fig. 3B). The LRWC is vital for the regulation of cell expansion, growth and development while acclimating to drought stress (Ashraf, 2010). PGPR-treated plants maintained relatively higher LRWC compared to non-treated plants under drought stress (Bouremani *et al.*, 2023) and the results of which are compatible to our findings where common bean plants inoculated with PPB6 showed higher LRWC as compared to uninoculated drought-stressed plants (Fig. 3A). Proline accumulation is quite complex for explaining its role during plant-microbs interaction.

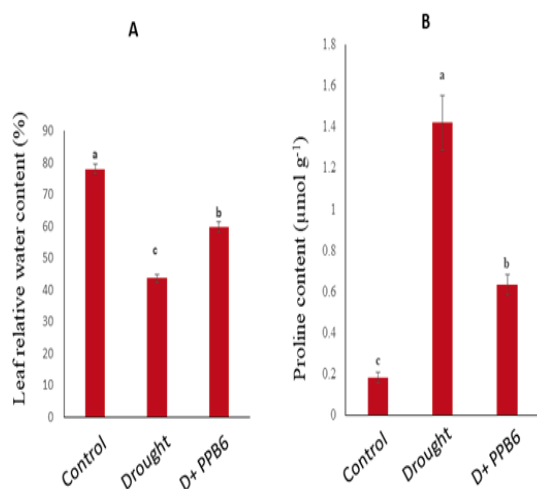


Figure 3: Effects of *Bacillus amyloliquefaciens* (PPB6) on leaf relative water and proline content of common bean subjected to non-stressed uninoculated condition (Control), uninoculated drought-stressed condition (Drought) and drought-stressed condition inoculated with PPB6 (D + PPB6). Error bars indicate standard error. Different letters on the bars show significant differences at $p < 0.05$.

Various aspects of the regulation of proline accumulation by PGPR is due to the differences of mode of action of the bacterial species, intensity of drought and differential ontogenic responses of the plants. For instance, PGPR like *P. fluorescens*, *Burkholderia* sp., *Mitsuaria* sp. and *B. amyloliquefaciens* showed more proline accumulation in wheat, maize and *Arabidopsis* under drought stress (Vardharajula *et al.*, 2011; Huang *et al.*, 2017; Chandra *et al.*, 2018). Contrary, PGPR like *Alcaligenes faecalis* Castellani & Chalmers 1919, *Proteus penneri* Hickman *et al.* 1982, and *Pseudomonas aeruginosa* were claimed to inhibit proline accumulation in plants under drought stress (Naseem & Bano, 2014), and the findings of which is consistent to our findings where PPB6 reduced proline accumulation under drought stress as compared to uninoculated drought-stressed plants (Fig. 3B). Because PPB6 could help the plants for having osmotic balance by enhancing relative water content, the inoculated plants did not cause substantial proline accumulation under drought stress.

3.5 RECOVERY OF PLANT'S TISSUE DAMAGE BY PPB6 UNDER DROUGHT STRESS

Because drought stress cause oxidative damage leading to tissue death, we measured tissue damage by means of determining electrolyte leakage, ROS; H_2O_2 and lipid peroxidation product; MDA. Electrolyte leakage of the common bean under control, drought, and D+PPB6 was 27.80 %, 55.93 %, 30.20 % respectively (Fig. 4A). H_2O_2 content of plants of control, drought and D+PPB6 was 46.47, 136.25 and 66.60 $\mu\text{mol g}^{-1}$ FM respectively (Fig. 4B) and MDA content of those treatments was 0.03, 0.08 and 0.05 $\mu\text{mol g}^{-1}$ FM respectively (Fig. 4C). Enhanced occurrence of ROS during abiotic stresses causes detrimental effects to cell membrane by increasing lipid peroxidation products leading to higher electrolyte leakage from damaged tissue (Ghosh *et al.*, 2022; Huang *et al.*, 2019). Higher electrolyte leakage in plant tissue indicates the negative consequences to membrane permeability and stability of cell membrane (Abdelaa *et al.*, 2021). Different *Bacillus* strains could maintain cell membrane stability by means of lowering electrolyte leakage in maize (Vardharajula *et al.*, 2011). Similarly, other PGPR were also involved to protect drought-induced membrane damage by means of lowering electrolyte leakage (Bouremani *et al.*, 2023; Chiappero *et al.*, 2019). Consistently, our result with PPB6 in drought-stressed common bean supports the above-mentioned statements and clarified the role of PPB6 in cell membrane stability under drought stress. Enhanced accumulation of H_2O_2 , as the crucial member of ROS causes lipid peroxidation and

membrane injury in plants (Sachdev et al., 2021). Lipid peroxidation product; MDA is formed by the reaction of free radicals and lipid and alter the structure of cell membrane and its stability (Woźniak et al., 2006). PG-PRs like *P. fluorescens* and *Pseudomonas palleroniana* Gardan, et al.2002 decreased H_2O_2 and MDA content in wheat under drought stress (Chandra et al., 2018). Along with those findings, the significant reduction of H_2O_2 and MDA by PPB6 under drought stress as compared to uninoculated drought-stressed plants in this study (Figs. 4B & 4C), suggesting the potential role of this bacterium in maintaining cell membrane stability that could complement of the maintenance of higher LRWC, lower proline content and higher photosynthetic activity by PPB6 under drought stress (Figs. 2 & 3).

3.6 REGULATION OF ANTIOXIDANT ACTIVITIES BY PPB6 UNDER DROUGHT STRESS

Plants boost up non-enzymatic antioxidants to confront drought-induced oxidative damage. Therefore, we determined the activity total phenolic and flavonoid contents. Total phenolics contents of common bean under control, drought and D+PPB6 was 313.59, 508.54 and 325.68 $\mu\text{g g}^{-1}$ FM respectively (Fig. 5A). Flavonoid content of those conditions was 624.15, 2279.66 and 1070.76

$\mu\text{g g}^{-1}$ FM respectively (Fig. 5B). In addition, enzymatic antioxidants also play a crucial role in enhancing plant's tolerance to drought-induced oxidative damage (Hasanuzzaman et al., 2020). Therefore, we measured the activity of enzymatic antioxidant such as APX, CAT, GSTs and POD in common bean under different treatments. APX activity of plants of control, drought and D+PPB6 was 2.06, 3.52 and 2.67 $\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein respectively (Fig. 5C). CAT activity of those treatments was 56.62, 6.333 and 20.18 $\mu\text{mol min}^{-1} \text{mg}^{-1}$ respectively, and GST activity of those was 169.48, 236.14 and 189.45 $\text{nmol min}^{-1} \text{mg}^{-1}$ respectively, and POD activity of those was 13.09 and 25.94 and 15.17 $\text{nmol min}^{-1} \text{mg}^{-1}$ respectively (Figs. 5D-5F).

Modulation of antioxidant activities by PGPR is quite complex and varies depending on the types of abiotic stresses, bacterial strains, plant species and growth conditions. For instance, phenolics and flavonoids content in plant were found to be increased under drought stress upon application of PGPR as compared to untreated plants (Chandra et al., 2019; Azizi et al., 2021). Contrary, *Azospirillum brasilense* Tarrand, Krieg & Döbereiner, 1978 produced no effect on total phenolic contents (Asghari et al., 2020), and *Pseudomonas putida* Trevisan, 1889 showed reduced flavonoid content in *Glycine max* (L.)Merr. under drought stress. Accordingly, our findings showed reduced accumulation of both phe-

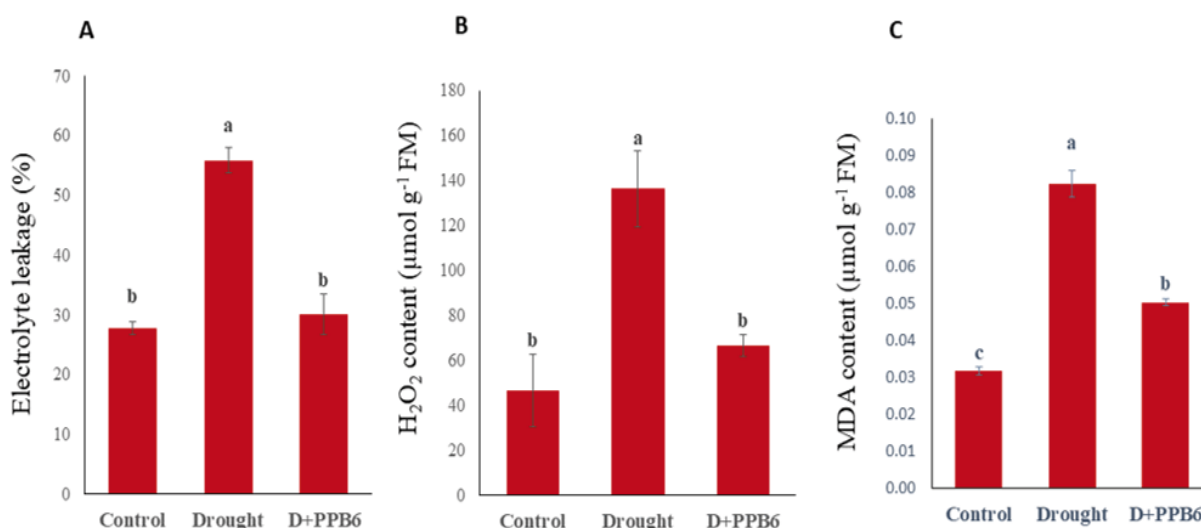


Figure 4: Effects of *Bacillus amyloliquefaciens* (PPB6) on the recovery of tissue damage caused by drought stress in common bean. Percentage (%) of electrolyte leakage (A), H_2O_2 content (B), and lipid peroxidation product; malondialdehyde (MDA) content (C) of common bean subjected to non-stressed uninoculated condition (Control), uninoculated drought-stressed condition (Drought) and drought-stressed condition inoculated with PPB6 (D + PPB6). Error bars indicate standard error. Different letters on the bars show significant differences at $p < 0.05$. efficiency of PSII (F_v/F_m) (F) under drought stress. Error bars indicate standard error. Different letters on the bars show significant differences at $p < 0.05$.

nolics and flavonoids by PPB6 under drought stress as compared to uninoculated drought-stressed plants (Figs. 5A & 5B). The role of enzymatic antioxidants by PGPR is also quite ambiguous while acclimating to drought stress. Although CAT is essential for catalyzing H_2O_2 into water and oxygen (Vitolo, 2021) and having tremendous role in abiotic stresses, but the activity of CAT is found to be reduced sometimes by drought stress (Sofo *et al.*, 2005; Mohi-Ud-Din *et al.*, 2021). The fact is indicating that the activity of CAT is little bit unstable and depending on the intensity of drought and growth phases of plants. Likewise, APX is also crucial and needed to accumulate more in plant for protecting chloroplasts and other cell constituents from oxidative damage (Asada, 1992). Along with those, GSTs and POD were found to be involved in scavenging ROS and reducing oxidative damage in plants (Khan *et al.*, 2014; Kumar & Trivedi, 2018). Although, PGPR have been reported to increase the activity of enzymatic antioxidants under drought stress (Chandra *et al.*,

2021; Akhter *et al.*, 2021), many investigations claimed that PGPR including *Bacillus* species reduce the enzymatic antioxidant activity in drought-stressed plants. For instance, *B. amyloliquefaciens*, *B. licheniformis*, *B. subtilis*, *Bacillus thuringiensis* Berliner 1915, and *Paenibacillus favisporus* decreased APX and CAT activity, and *A. faecalis*, *P. penneri* and *P. aeruginosa* decreased the CAT and POD activity in maize, and *Bacillus cereus* Frankland & Frankland 1887 and *Planomicrobium chinense* Dai *et al.* 2005 reduced the activity of CAT and POD in wheat under drought stress (Vardharajula *et al.*, 2011; Naseem & Bano, 2014; Khan & Bano 2019). The results of those are consistent to our studies like reduced activities of APX, GST and POD by PPB6 under drought-stress. (Figs. 5C, 5E and 5F). The varied level of both non-enzymatic and enzymatic antioxidant partitivities by PGPR is likely due to the plant species, ontogeny of plants, bacterial strains and intensity of drought stress. The previous findings also demonstrated that PGPR could tend to reduce the

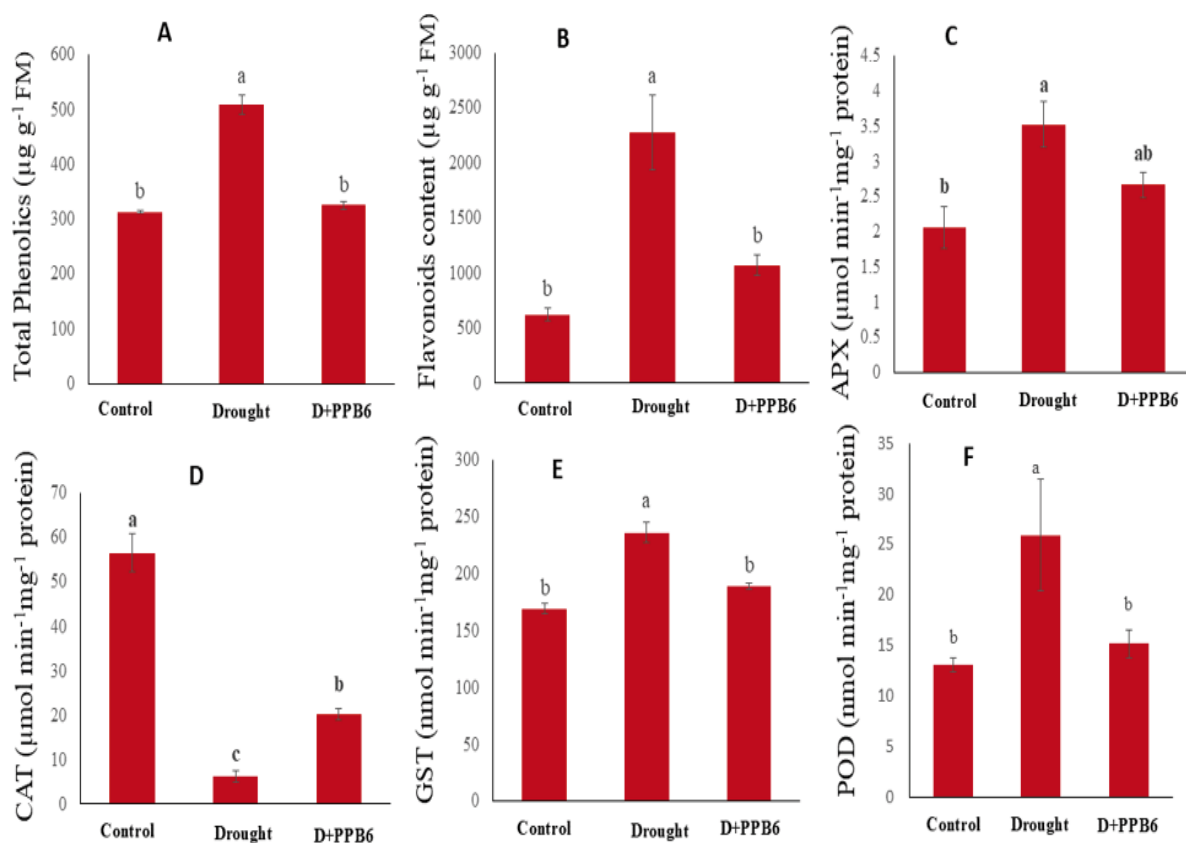


Figure 5: Effects of *Bacillus amyloliquefaciens* (PPB6) on the antioxidant activities of common bean under drought stress. Total phenolics (A) and flavonoids (B) content, and the activities of ascorbate peroxidase; APX (C), catalase; CAT (D), glutathione S-transferase; GST, (E) and peroxidase; POD (F) in common bean subjected to non-stressed uninoculated condition (Control), uninoculated drought-stressed condition (Drought) and drought-stressed condition inoculated with PPB6 (D+PPB6). Error bars indicate standard error. Different letters on the bars show significant differences at $p < 0.05$.

drought stress effects and hence to lessen the activity of antioxidant enzymes (Han & Lee, 2005). Our findings are very compatible to that where PPB6 could produce lower ROS, higher membrane stability and lower level of antioxidant activities as compared to uninoculated drought-stressed plants (Figs. 4 & 5).

4 CONCLUSION

The present study concluded that PPB6 could increase the efficiency of photosynthesis, and maintain cell membrane stability by increasing relative water content and decreasing ROS effects in drought-stressed common bean plant. PPB6 inoculated plants perceived lower drought effects which was evidenced by the reduction of proline and antioxidant enzymes. The comparable occurrence of ROS and antioxidant activities in drought-stressed plants inoculated with PPB6 and non-stressed uninoculated plants suggesting the partial complementation of drought-effects by this bacterium. The findings might help for understanding the implications of *B. amyloliquefaciens* in drought tolerance of common bean. The knowledge of this study will further assist for molecular characterization of common bean plant inoculated with *B. amyloliquefaciens* under drought stress.

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