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Effect of bacterial endophytes inoculation on morphological and physiological traits of sorghum (*Sorghum bicolor* (L.) Moench) under drought

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Abstract

Globally, drought stress causes the negative impact on agriculture production. However, microbial inoculation is the suitable cost-effective technology to attenuate the drought stress. The aim of this experiment was conducted to evaluate the impact of microbial inoculation on morpho-physiological characters of sorghum under moisture stress condition. A pot experiment with sorghum was conducted to access the role of previously identified bacterial endophyte strains. For comparison, two non-inoculated control treatments (T₁: absolute control and T₂: drought control) were maintained to compare the drought and inoculation effect. Drought was imposed at flowering stage and soil moisture was measure at frequent days. When the soil moisture attains close to zero, plants were rehydrated. During drought and rehydration period, plant morphological and physiological characters were evaluated. Drought stress drastically reduces the plant characters in control (T₂-uninoculated) plant. Whereas, in the presence of bacterial inoculation considerably reduces the drought effect also *Bacillus* sp. inoculation treatment promoted better recovery during their rehydration process than absolute control. Overall, *Bacillus* sp. inoculation was the most promising bacterium for possible field trials.

Keywords: Inoculation-*Bacillus* sp.-drought-morphological trait-physiological trait

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important cereal crop after maize. In worldwide ranking India secured sixth place in the sorghum production followed by Mexico and the production was 4.4 million metric tons. Sorghum crop was mostly grown under water deficit condition where other crop performance are very poor. Water use efficiency of sorghum mainly depends on their growth stages. Sorghum required 450 to 650 mm of water during their growing period, in that, flowering phase was the most sensitive stage which required water at 7-10 mm/day until after anthesis, in particular at the stage water stress can affect the fertilization and induce premature abortion (Assefa *et al.*, 2010) [2]. Grain yield is more sensitive to water stress at certain growth stages than the total water available through growing season (Garrity *et al.*, 1982) [8]. In addition to the drought tolerance cause the major yield loss in sorghum plant. For instance, drought may reduce the nutrient diffusion from soil to root and induces the nutrient deficiency in their aerial parts of sorghum thereby restricting transpiration rate and declining of active transport and membrane permeability (VIETS, 1972; Alam, 1999) [1, 29]. Breeding and biotechnological approaches were made more effective to develop drought tolerant plants but these methods were cost intensive. Now a day's most of the research outcome proved bacteria could help to overcome the negative effect of drought stress endowed with some plant growth promoting activities in plants.

Drought affects the crop growth of various crops exhibiting a reduction in yield such as barley (Samarah, 2005) [24], maize (Kamara *et al.*, 2003) [13], rice (Lafitte *et al.*, 2007) [17], wheat (Rampino *et al.*, 2006) [22] and sorghum (Assefa *et al.*, 2010) [2]. Drought stress changes the availability of water and soil nutrients alters the morpho and physiological activities like plant height, root characters, specific leaf weight, leaf area, biomass, Relative Water Content (RWC), Excised leaf water retention capacity and SPAD value. Drought stress caused decrease in the shoot length in *Lathyrus sativus* L. (Gheidary *et al.*, 2017) [9] of limiting nutrient absorption by the plant. Root plays a pivotal role in nutrient accumulation and water absorption, which is also associated with symbiotic microorganisms in plant rhizosphere. Photosynthetic production synthesis takes place in the leaf region. If the plant sense water stress, leaf area get declined which resulted in decreasing water loss by the process of

transpiration and inhibiting leaf expansion (Bangar *et al.*, 2019) [4]. Water deficit suppress the photosynthesis by the reduction of leaf area, increased stomata closure and leaf temperature leads to damage in the photosynthetic apparatus like, Chlorophyll and photosystem II, inhibition of photosynthetic activity ultimately reducing the plant productivity (Xiang *et al.*, 2013) [30].

The microorganisms play a vital role in plant growth and nutrient management in all the way through colonization in plant roots to promote plant growth directly and indirectly. Possible direct mechanism for drought tolerance induced by rhizobacteria includes: the PGPB acquired the resources from surrounding environment, for instance, nitrogen, phosphorus and iron, production of phyto hormones (abscisic acid, gibberellic acid, cytokinin's, and indole-3-acetic acid), ACC deaminase to reduce the ethylene level in the roots, induced systemic tolerance by bacterial compounds and bacterial exopolysaccharides.

Agriculture was faced many drought consequences in their production sector. Bacterial endophytes have the ability to resolve the food security problems through their plant growth promoting activities. With this context, we isolate and screen the drought tolerant bacterial endophytes from sorghum root and examine the plant promoting activities of isolates on sorghum plant under drought. In this stand, research work was carried out with the objective of study the morpho-physiological and drought tolerance traits influenced by the endophytic microbes in sorghum under drought.

Materials and Methods

Experiment design and endophytic materials inoculation

Sorghum seed variety CO 30 was kindly collected from Department of millets, Tamil Nadu Agricultural University, Coimbatore. Healthy seeds were surface sterilized with sodium hypochlorite for 10 min and washed with sterile distilled water. The seeds were soaked in sterile distilled water for over-night for sprouting. *Bacillus* sp. and *Pseudacidovorax intermedius* were grown in LB broth at 28°C for 16 h, and were pelletized by centrifugation at 6000 rpm for 20 min at room temperature. The sprouted sorghum seeds were biotized using cell pellet and allowed to shade dry. Seeds were grown in a container filled with organic soil and irrigated regularly with water under a 12 h photoperiod at temperatures ranging from 25 to 29°C and the relative humidity of 70-75% in the glasshouse, Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore.

Crop	: Sorghum (CO 30)
Treatments	: Five
T ₁	: Absolute control
T ₂	: Drought control
T ₃	: Seed biotization with <i>Bacillus</i> sp.
T ₄	: Seed biotization with <i>Pseudacidovorax intermedius</i>
T ₅	: Seed biotization with <i>Bacillus</i> sp. + <i>Pseudacidovorax intermedius</i>
Replication	: Four
Design	: CRD
Pot size	: 43cm length × 30cm top diameter
DOS	: 31.10.2020

Observations taken on various morphological and physiological aspects were studied during drought (DD) and drought recovery (DR) period. The soil moisture was measured by soil moisture sensor kit SM150 (HH150) up to the moisture level close to zero, this meter displays volumetric water content (% volume) and the values are

expressed in m³.m⁻³ (Data not shown). The absolute control plants were watered daily to maintain their field capacity (FC).

Morphological characters

Plant height

Plant height was measured from the ground level to the tip of the growing point and expressed as cm.

Absolute growth rate (AGR)

Absolute growth rate (AGR) of plant height was calculated using the equation:

$$AGR = FH - IH / \Delta t$$

Where FH corresponds to final height, IH corresponds to initial height, and Δt corresponds to time variation (Silva *et al.*, 2000) [26, 27], expressed in centimetres per day.

Root traits

Plants were uprooted from the pot and the root was taken with minimum damage during drought and drought recovery period. Roots were washed carefully and root traits of each pot were evaluated.

Root length

The plant was uprooted and the root was taken with minimum damage and the length from the base of the stem to the tip of the longest root was measured and expressed in cm.

Root volume

The root volume was estimated by water displacement method. Individual plant roots were immersed in known volume of water and the amount of water displaced was measured expressed in cc.

Biomass production

The bio mass of the whole plant (TDM) was measured. The plants fresh weight was measured first after that plants were shade dried and then oven dried at 72 °C for 48 hours. The dry weight of the whole plant was recorded and expressed as g plant⁻¹.

Specific leaf weight (SLW)

Specific Leaf Weight was calculated by using the formula of Pearce *et al.* (1969) [21] and expressed in mg cm⁻².

$$SLW = \frac{\text{Leaf dry weight per plant}}{\text{Leaf area per plant}}$$

Physiological parameters

Excised leaf water retention capacity (ELWRC)

ELWRC was measured as method of Lakshmi *et al.* (2009) [18]. Third leaf from top were excised and weights were taken at zero min, 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360 minutes. Then curves were drawn to record the moisture percent actually retained by the leaf when it was excised from the plant and expressed in %.

$$ELWRC = [\text{Initial weight at that specific time} / \text{initial weight}] \times 100$$

SPAD value

Chlorophyll meter from Minolta (model 502 of Minolta, Japan) was used to measure SPAD values. Measurements were taken from top most fully expanded leaf. Four readings

were taken from each replication and the average values were computed using the method described by Minolta (1989) [20].

Chlorophyll stability index (CSI)

Based on Kaloyereas (1958) [12] protocol chlorophyll stability index was estimated

$$CSI = \frac{\text{Total chlorophyll content (Treated)}}{\text{Total chlorophyll content (Control)}} \times 100$$

Statistical analysis

All statistical data analysis were carried out through SPSS statistical software package version 16.0. The data regarding growth promotion of endophytes on sorghum crop were analysed by the analysis of variance (ANOVA) and compared by Duncan's Multiple-Range Test (DMRT) with four replicates. All statistical tests were performed at the $P \leq 0.05$ level.

Results

Plant height

Plant height of sorghum plants were significantly different,

the result was given in the figure (1.A). Absolute control (Non stress) recorded the maximum plant height (176.50, 183.02 cm). Drought impose during flowering significantly reduced the plant height in uninoculated control T₂ but bacterial inoculation (*Bacillus* sp.) T₃ (161.80, 168.67 cm) positively enhanced the plant height in both condition when compared to uninoculated control T₂ (138.07, 144.57 cm). It was followed by combination of both isolates (*Bacillus* sp. + *Pseudacidovorax intermedius*) in T₅ (158.75, 165.22 cm). The results were significantly different but statistically on par with each other.

Absolute growth rate (AGR)

AGR was a measure to know the total growth per unit time. Among the treatments, (*Bacillus* sp.) T₃ (0.62 cm day⁻¹) achieved maximum AGR followed by T₅ (0.59 cm day⁻¹) and T₁ (0.59 cm day⁻¹) and the results were on par with each other. Uninoculated control T₂ (0.56 cm day⁻¹) recorded the lowest AGR than other treatments. The results were statistically not-significant (Figure 1.B).

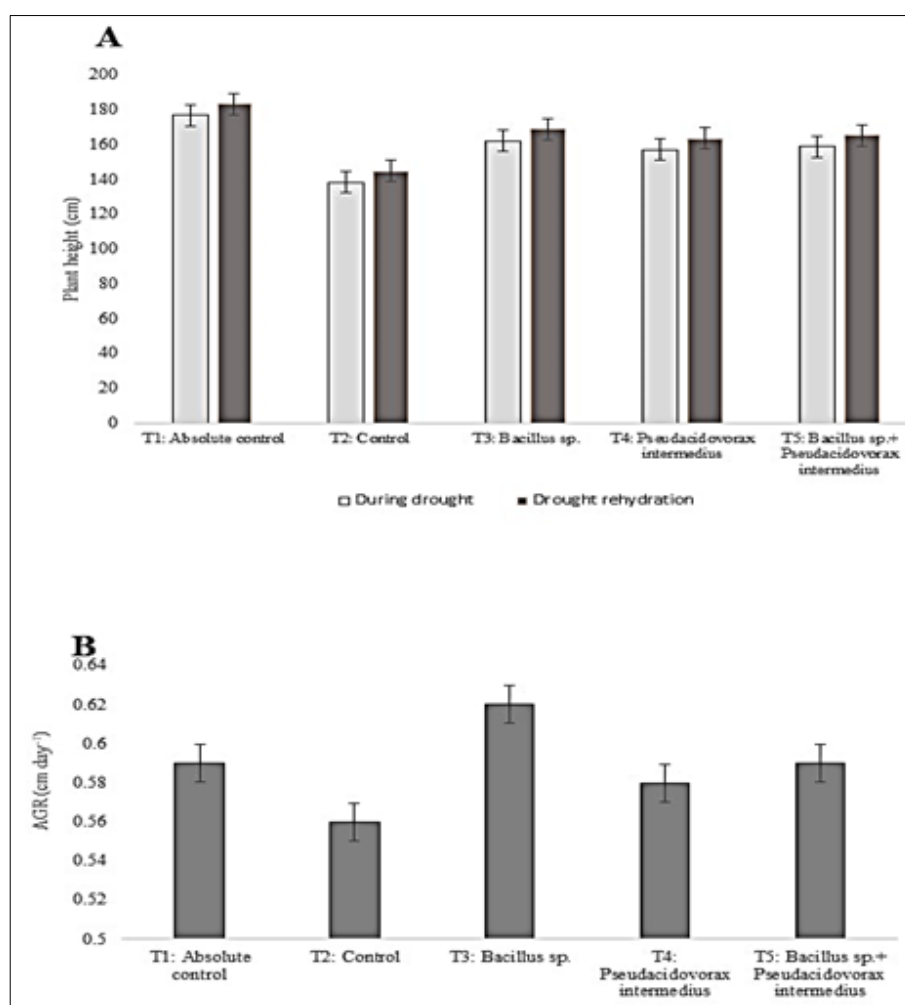


Fig 1: Endophytes effect on morphological characters. (A) Plant height, (B) Absolute growth rate (AGR). All values are mean of four replicates \pm S.E.

Root length and root volume

Irrespective of the treatment's drought stress reduced the root length of sorghum plant. Even though, inoculation of endophytes slightly increased the root length of plants during their drought (DD) and drought rehydration (DR) period (Figure 2.A). During drought period (*Bacillus* sp.) T₃ (28.42

cm plant⁻¹) significantly recorded increased root length followed by (*Pseudacidovorax intermedius*) T₄ (27.87 cm plant⁻¹) when compared to uninoculated control T₂ (27.45 cm plant⁻¹). Similarly, in drought rehydration period minimum root length reduction was observed in (*Bacillus* sp.) T₃ (29.10 cm plant⁻¹) followed by (*Pseudacidovorax intermedius*) T₄

(28.35 cm plant⁻¹) but uninoculated T₂ (28.22 cm plant⁻¹) plant root length reduction was more when compared to inoculated plants. The results were significantly on par with each other. Data on root volume of sorghum plants were recorded at both DD and DR condition. There was a quite variation observed between treatments (Figure 2.B). Among the treatments, absolute control (non-stress) T₁ recorded less root volume from 3.70 cc plant⁻¹ to 3.85 cc plant⁻¹ but drought stress however improved the volume of root. According to the results, root volume in bacterial inoculation of (*Bacillus* sp.) T₃ (6.90 cc plant⁻¹ to 7.30 cc plant⁻¹) showed significant increase followed by (*Bacillus* sp. + *Pseudacidovorax intermedius*) T₅ (4.02 cc plant⁻¹ to 4.22 cc plant⁻¹) in both drought and drought rehydration conditions. The result of T₃ was significantly different but other treatments were statistically on par.

Total dry matter production (TDMP)

TDMP represent the photosynthetic efficiency of the plant (Figure 2.C). The effect of drought and bacterial inoculation on TDMP was studied and it revealed that the bacterial inoculation could record significant influence in increasing the TDMP in both drought and rehydration conditions. Comparing the inoculated treatments, maximum TDMP was found in T₃ (bacterial isolate *Bacillus* sp. inoculated plants) (83.85 g plant⁻¹) and the minimum in T₂ (uninoculated control plants) (69.51 g plant⁻¹) under drought condition. Whereas, in drought rehydration condition, T₃ (bacterial isolate *Bacillus* sp. inoculated plants) registered higher TDMP (95.31 g plant⁻¹) followed by T₅ (*Bacillus* sp. + *Pseudacidovorax intermedius* inoculated plants) (89.81 g plant⁻¹).

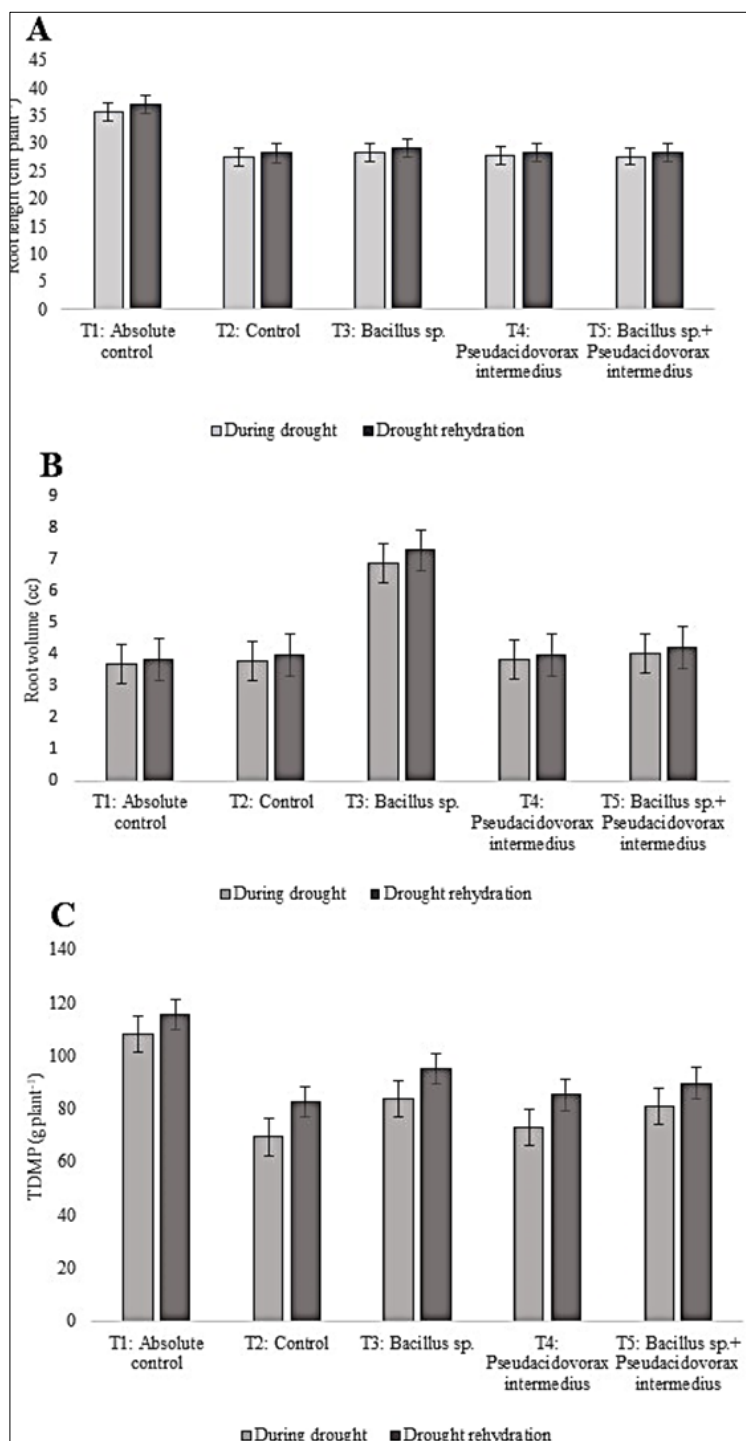


Fig 2: Endophytes effect on morphological characters. (A) Root length, (B) Root volume, (C) Today dry matter production (TDMP). All values are mean of four replicates \pm S.E.

Specific leaf weight (SLW)

Specific leaf weight gets reduced when plants are exposed to the drought stress (Figure 3.A). T₃ (bacterial isolate *Bacillus* sp. inoculated plants) (4.07 mg cm⁻²) ranked first in terms of SLW, followed by T₁ (3.97 mg cm⁻²) and (*Bacillus* sp. + *Pseudacidovorax intermedius* inoculated plants) T₅ (3.63 mg cm⁻²) had the lowest SLW during drought stage. Although,

there was a significant difference observed in SLW with different bacterial inoculation. Whereas, in rehydration stage (*Bacillus* sp.) T₃ (4.25 mg cm⁻²) followed by (*Bacillus* sp. + *Pseudacidovorax intermedius*) T₅ (4.05 mg cm⁻²) attained higher SLW and (uninoculated control) T₂ projected low SLW (3.89 mg cm⁻²).

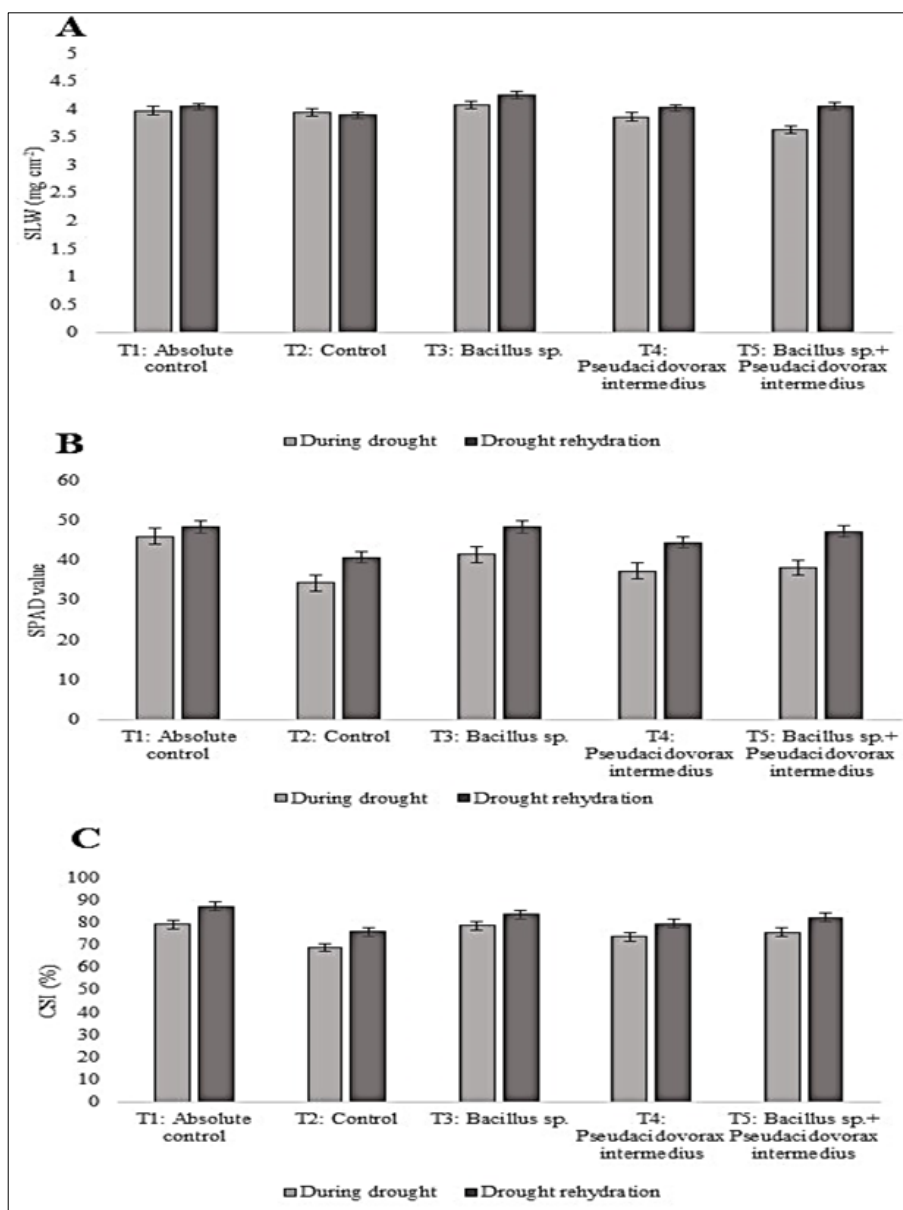


Fig 3: Endophytes effect on physiological characters. (A) Specific leaf weight (SLW). (B) Value, (C) Chlorophyll stability index (CSI). All values are mean of four replicates \pm S.E.

Excised leaf water retention capacity (ELWRC)

Significant differences in water holding capacity of excised sorghum leaves among the DD (Figure 4. A) and DR (Figure 4. B) condition were revealed by ELWRC. Bacterial inoculation treatments retained higher water content when compared to uninoculated control plant T₂ (43.70 to 42.65 %) under drought and rehydration condition respectively. DD treatment result viz., treatment (*Bacillus* sp. +

Pseudacidovorax intermedius) T₅ (63.35%) maintained higher water status followed by (*Pseudacidovorax intermedius*) T₄ (56.72%) and (*Bacillus* sp.) T₃ (54.30%). Whereas in rehydration condition, (*Pseudacidovorax intermedius*) T₄ (57.80%) plant held lowest moisture content and (*Bacillus* sp.) T₃ (62.27%) retained highest moisture content within inoculated treatment.

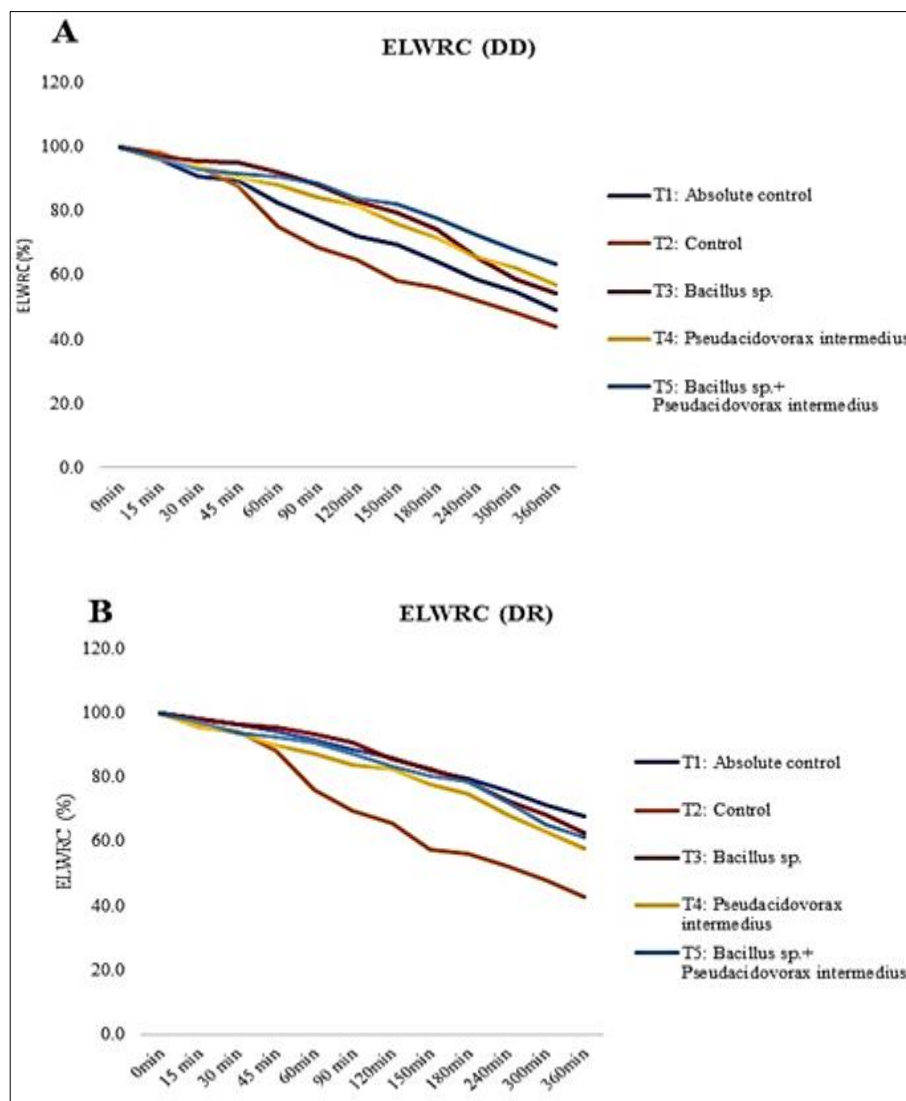


Fig 4: Excised leaf water retention capacity (ELWRC). (A) During drought (DD) condition, Drought rehydration (DR) condition. All values are mean of four replicates \pm S.E.

SPAD value

Chlorophyll index values were recorded and presented in Figure (3.B). Data exhibited that during drought and bacterial inoculation, the treatments were statistically significant. Among the treatments with inoculation, bacterial isolate *Bacillus* sp. inoculated plants T₃ recorded higher chlorophyll index of 41.35 during drought condition which is statistically on par with T₁ followed by *Bacillus* sp. + *Pseudacidovorax intermedius* inoculated plants T₅ (38.07). The lowest values were registered in uninoculated control T₂ plants (34.27) followed by plants inoculated with *Pseudacidovorax intermedius* (37.17) under drought condition. In further, drought rehydration results on chlorophyll content were not significantly different.

Chlorophyll stability index (CSI)

Like RWC, higher CSI also played an important role in drought tolerance (Figure 3.C). Overall CSI performance was significantly different between treatments. T₂ (plant with uninoculation) plants during drought CSI was below 75% (68.82%) that means plants were susceptible to drought. Even though, plants treated with bacterial inoculation moderately gave the tolerance to drought in T₁ (79.21%) and (*Bacillus* sp.) T₃ (78.49%) treatments during drought condition, which were on par with each other except (*Pseudacidovorax intermedius*) T₄, it registered below 75% CSI (73.62%).

Similarly, rehydration results also were similar as that of above results. After absolute control, (*Bacillus* sp.) T₃ (83.64%) and (*Bacillus* sp. + *Pseudacidovorax intermedius*) T₅ (82.28%) showed moderate drought tolerance which were on par with each other.

Discussion

Bacterial seed treatment has been considered as an effective tool to deliver the beneficial effect into the plant and ease the plant from stressful environment, soaking of seed in a bacterial suspension is considered as a seed treatment and many studies revealed that inoculation of microbes were able to promote crop growth characters such as germination, root, shoot and vigour index, biomass in rice, *Brassica napus* and maize plants respectively (Choi *et al.*, 2016; Lally *et al.*, 2017; Rozier *et al.*, 2017) [7, 19, 23]. From this study results of plant height, AGR, root length and root volume revealed that microbial inoculation of *Bacillus* sp. promoted the beneficial effect on the above-mentioned parameters and marginally reduced the dead tissue and increased the number of leaves. The outcome was already proven by many researchers (Klopper *et al.*, 2004; Santana *et al.*, 2020) [14, 26] who stated that ESA 11 (*Enterobacter* sp.) strain increase the plant height and ESA 13 (*Bacillus* sp.) bacteria increased the number of leaves and reduced the dead tissue content in sorghum plant during drought condition. Plants with PGPR treatment

stimulated root growth and modify root architecture, which can lead to increased total root surface area and better water and nutrient absorption from the deeper layer of soil, as well as positive effects on overall plant growth. Plant height of *Bacillus* sp. inoculated plant increased up to 16% than uninoculated, plant height might be due to as for the findings *Bacillus* sp. was able to produce ACC deaminase enzyme that ACC deaminase activity reducing ethylene levels in the plants and maximizing cell elongation, as a result, length of plant got increased. This mechanism of PGP usually affects root hair development, resulting in structurally improved rooting systems. In additional evidence, bacterial strains can produce phytohormone IAA, as a result of these IAA production in this strain may also have been involved in the growth promotion. Harman and his co-workers (Harman *et al.*, 2004)^[10] have been conducting research on *Trichoderma* strains effect on maize plants for 5-10 years and have the results documented that the strain enhanced the root development, root hair formation and favoured deeper root system in maize. Plant biomass production is an important factor for the better yield achievement. Inoculation of bacterial strain *Bacillus* sp. and *Pseudacidovorax intermedius* effectively increased the total biomass production of the sorghum plant in both drought and drought rehydration condition respectively. Similar conclusion was reported by Santana *et al.* (2020)^[26] in sorghum plants, who reported, PGPB inoculation produce better effect on leaf dry matter (*Rhizobium* sp.), stem dry matter (*Enterobacter* sp.), shoot dry matter (*Rhizobium* sp.) and total dry matter content (*Rhizobium* sp.) respectively. The current experiment data revealed that *Bacillus* sp. treatment had a more capability of producing more amount of biomass in shoot and leaf, proposing that multiple mechanisms may have been the key contributors to this finding. There will be a stronger canopy architecture for the interception of both direct and dispersed radiant energy for carbon assimilation with higher plant height, which would result in improved accumulation of biomass in spite of stressful condition. In this study it was observed that bacterial inoculation had a significant effect on specific leaf weight.

The physiological processes within sorghum plant cells are disrupted by environmental factors that influence sorghum growth efficiency. Physiological traits are most important desirable characters to know the tolerance capacity of the plant, thereby improving these traits can attain more yield in sorghum. ELWRC, chlorophyll content (SPAD value) and CSI are some of the important physiological characteristics of plants, which were analysed in this experiment. ELWRC results are positively correlated with RWC of a plant. Several rainfed crops, including sorghum (Santamaria *et al.*, 1990)^[25], castor (Babita *et al.*, 2010)^[3], and pearl millet (Henson *et al.*, 1983)^[11] have been demonstrated with a beneficial interaction between ELWRC and osmotic changes. In hypothesis, Maintenance of higher RWC content could have subsidized to sustain higher moisture content even after excision. The rapid stomatal response to excision could also be responsible for the ELWRC. Improved water extraction from the soil might have been helped by improved root growth. Also, in the validation of bacterial inoculation treatment which increased the root growth has been documented. Results of this study did not substantiate with any previous findings of bacterial inoculations increase in the ELWRC results.

Chlorophyll molecule is an important pigment for the plant photosynthesis and green ness of leaves. Drought imposed at flowering stage significantly decreased the chlorophyll

content. In addition, SPAD index values also strongly corresponded with total chlorophyll content. According to Usuda (1995)^[28], the chlorophyll index (SPAD) for leaves is proportional to the chlorophyll amount per unit region. Bacterial inoculated plants obtained higher SPAD index values than uninoculated control. From these findings, it can be arrived at a clear conclusion that bacterial inoculation could help the chlorophyll biomolecules from oxidative damage and helps the plants to normal functioning under stressful environment. Chandra; Srivastava, and Sharma (2018)^[5, 6]; Kour *et al.* (2020) and Chandra^[5, 6, 15]; Srivastava; Glick; *et al.* (2018)^[5, 6] who had already reported that PGPB inoculation had a great impact on chlorophyll content and increased the chlorophyll biosynthesis in various stressful environment in various crops, such as wheat, foxtail millet and finger millet respectively.

CSI is another indicator for plant tolerance to stress. Bacterial inoculation gave the moderate tolerance capacity over uninoculated control expressed in CSI. The CSI was negatively correlated with membrane electrolytic leakage and MDA content. From this, results concluded that endophytic inoculation positively enhanced the CSI and might have reduced the chloroplast membrane damage and reducing the lipid peroxidation. Kumar *et al.* (2017)^[4, 15, 16] reported that drought stressed rice plants inoculated with *B. altitudinis* FD48 showed 69.23% CSI.

Conclusion

The study revealed that considering the bacterial distribution and the importance of endophytic association with plant in agriculture system. Endophytic inoculation made a broad range of positive effect on physiological and morphological characters of sorghum plant, there is a need for further studies to identify the genomic mechanisms to elucidate the functional properties of endophytes in plant system.

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