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Enhancement of sorghum (*Sorghum bicolor* (L.) Moench) growth performance by plant-growth promoting endophytes

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Abstract
A study was conducted to evaluate the endophytes effect on sorghum growth promotion under drought condition (PEG imposed). Sorghum root associated endophytes were isolated and characterized through partial 16S rRNA gene sequencing and validating the genera of isolates by BLAST analysis. Bacterial strains namely, *Acinetobacter pittii*, *Bacillus* sp. and *Pseudacidovorax intermedius* were inoculated with sorghum seeds TNS 661 and TKSV 1311. Results exhibited that endophytes had many positive effects on sorghum seedling in laboratory level experiments. Moreover, selected endophytes have the ability to enhance the growth potential such as germination percentage and germination rate index (GI) when plants were subjected to drought stress. Sorghum seeds treated with *Bacillus* sp. SR2 recorded an increased root length by 13% and *Acinetobacter pittii* VR2 and *Bacillus* sp. SR2 treatment significantly increased the shoot length of TNS 661 seedling as compared to uninoculated under drought condition. Similarly, TKSV 1311 seeds treated with *Pseudacidovorax intermedius* SR3 significantly recorded increased root: shoot ratio and biomass in terms of shoot fresh weight and total dry matter production of TNS 661. The results of this study concluded that when root endophytic bacteria interact with sorghum seedlings can subsidize to plant growth promotion as well as induced stress tolerance in sorghum.

Keywords: sorghum**,** endophytes, drought, growth promotion, biomass

Introduction
Agriculture facing diverse problem because of rising population and climatic changes, regarding climatic change, drought, high temperature and anthropogenic activities were constantly reducing the agriculture productivity and food availability to the increasing population. Globally expected in the year of 2100 global temperature would increase from 1.8 to 3.6°C. This climatic change may encourage the severe drought stress in several regions. When the plants transpired more amount of water which taken from the soil by help of roots cause deficiency in plant water requirement. Inadequate water supply is mainly caused by rainfall failure and/or decreased ground water table or Water Holding Capacity (WHC) of soil (Salehi-Lisar and Bakhshayeshan-Agdam, 2016) [26]. With the aim of regaining hydraulic status, plants adopting many strategies such as morpho-physiological and biochemical adjustments to balancing the water loss. Severe water loss inhibits the CO_2 assimilation by reduced stomatal aperture thus leads to decreasing photosynthetic rate and crop yield. Moreover, scientific peoples introduce breeding and biotechnological approaches to equalize the drought stress. Basically, these technologies were highly technical and labor intensive so it's quite difficult to apply. In alternate, currently microbial world communities PGPB play a major role in drought tolerance, bacteria with Plant Growth Promotion Capabilities (PGPB) are associated with root system presented in both root surface and endophytic region which can directly or indirectly assist the plant development in acute environment (Cassan *et al.*, 2009) [4]. Plant root and bacteria were mutualistic and beneficial associations and all the plant parts contain bacteria in their vascular bundles and inside the cell. Bacteria produced growth compounds such as exopolysaccharides, accumulation of osmolytes, siderophore and phytohormones indole-acetic acid (IAA), 1-aminocyclopropane- 1-carboxylic acid (ACC), cytokinins and gibberellins which can promote the plant growth and development that might be advantage to the host plant during their adverse condition (Ryan *et al.*, 2008) [25]. Several studies revealed that growth promoting bacteria clustered in host plant keep promise for plant growth promotion and eradicate the drought stress in several crops cited such as foxtail millet (Niu *et al.*, 2018) [21], maize (Vardharajula *et al.*, 2011) [30], wheat (Aslam *et al.*, 2018) [1], tomato and pepper (Mayak *et al.*, 2004) [17], pea (Zahir *et al.*, 2008) [32], sunflower (Sandhya *et al.*, 2009) [27] and sorghum (Govindasamy *et al.*, 2017) [14]. Hence, root colonizing bacteria grab the attention towards biotic and abiotic stress tolerance in plant crops through their root colonizing ability and production of antioxidant enzymes (Chauhan and Bagyaraj, 2015) [7]. Sorghum is a dual-purpose crop, widely cultivated for grain and husbandry feed stock purpose in worldwide, especially in Asian countries. Sorghum crops are deep rooted and extracting water from deeper layer of the soil even under water insufficient condition. Flowering (booting stage) is the critical stage for sorghum grain development, crop water requirement was more during the stage. Drought stress at this time can obstruct panicle exertion from the boot and lead to incomplete flowering which leads to grain yield loss. Therefore, sorghum treated with drought

tolerant endophytic bacteria may add the partial drought tolerance mechanism to facing the unfavourable condition. Several studies contribute that sorghum as a source of tolerant PGPB isolates and inoculation with drought condition showing positive responses by their PGP activities (Mareque *et al.*, 2018; Schlemper *et al.*, 2018; Govindasamy *et al.*, 2020) [16, 29, 13]. Even though, very less number of studies have demonstrated in PGPB inoculation with sorghum under drought (Santana *et al.*, 2020) [28]. Within this aim, collecting the root material from drought prone areas and isolating the endophytes which is most drought tolerance capability can be employed for imparting the drought tolerance ability in crops like sorghum. Additionally, to assess the plant growth promoting characters such as proline accumulation, exopolysaccharide production and phytohormonal activity (cytokinin and gibberellins) of selected endophytes and to know the inoculation effect of isolates (*Acinetobacter pittii*, *Bacillus* sp and *Pseudacidovorax intermedius*) on sorghum growth and development under moisture deficit condition had been attempted in the study.

Materials and Methods

Endophyte evaluation study was done at Department of Crop Physiology laboratory, Tamil Nadu Agricultural University (TNAU), Coimbatore (11°N latitude and 77°E longitude). Materials used for the isolation was completely autoclaved and for media preparation analytical reagent (AR) grade M/s HiMedia and M/s Sigma Chemicals were used.

Bacterial strain and growth of isolates under drought stress

Bacterial strains *Acinetobacter pittii*, *Bacillus* sp. and *Pseudacidovorax intermedius* were isolated from sorghum root material of Vellacholam and COFS 29 and their plant growth promoting characters such as proline, exopolysaccharides (EPS), protein, phytohormonal activity and 1-aminocyclopropane-l-carboxylic acid (ACC) deaminase were analyzed (Data not shown). The experiment was conducted here to evaluate the ability of endophytes induced drought tolerance in sorghum seedling. Endophytes were grown in luria–bertani broth (LB) with and without polyethylene glycol PEG 6000 (-1 MPa) under an ideal environment (120 rpm at 28˚C).

16S rRNA gene amplification and Sequencing

Cetyl Trimethylammonium Bromide (CTAB) is a widely used method to isolate genomic DNA and the presence of DNA conformed by 1 % agarose gel electrophoresis. Universal primer 16S rRNA region, 27F (5' AGA GTT TGA TCM TGG CTC AG 3') and 1492R (5' CGG TTA CTT TGT TAC GAC TT 3') primer was used (Weisburg *et al.*, 1991) [31] for their amplification. 10 µl of Polymerase Chain Reaction (PCR) hold 5µl of master mix ($MgCl_2$, buffer and Taq), 1 µl of reverse and forward primer each, 2µl of cell lysate template and 2 µl of DI water and the reaction was performed under standard protocol (an initial denaturation 94˚C for 5 min; 40 cycles at 95 ˚C for 1 min, 55 ˚C for 1 min, and 72 ˚C for 2 min and final cycle at 72 ˚C for 10 min). Purified PCR product was subjected to 16S rRNA partial gene sequencing to identify the strain specificity and submitted to the National Centre for Biotechnology Information database to get their accession number.

Sorghum promoting activities by endophytes under drought

Sorghum drought screening

Sorghum seeds were procured from Department of Millets, TNAU, Coimbatore. PEG 6000 concentration was standardized from sorghum drought screening experiment. Based on, seedling health characters such as germination percentage, root and shoot length characters, - 0.6 MPa PEG concentration was decided to be employed in the future experiments.

Experimental details

Laboratory experiment was conducted in Complete Randomized Design (CRD) with four replications to assess the isolates effect on sorghum growth and development. Two different types of pre-released sorghum seeds *viz.*, TNS 661 and TKSV 1311 were used. 16hr grown LB broth bacterial cultures (OD 600=0.6) are centrifuged at 10000 rpm for 20 min and pellets were applied on disinfected, overnight soaked sorghum seeds and allowed to shade dry. Petri dish were completely sanitized with 3% sodium hypochlorite and washed three times with sterile water, germination sheet was used as a bed material for seed germination. Twenty seeds of each variety were transferred to the plastic petri dish and 5ml of distilled water was added to each plate. After 24hr, 10ml of -0.6 Mpa PEG solution was pour to each related treatment. Germination count was taken in alternate days up to 7 days. On the 8th day, germinated seeds were taken out from the petri dish for assessing their germination components according to International Seed Testing Association ISTA (2008). Petri dish was maintained under room temperature with optimum light intensity.

Growth characters of sorghum seedling

Germination Percentage (GP) was calculated as total number of germinated seeds divided by total number of seed used multiplied into 100.

$$GP = \frac{Number\ of\ total\ germinated\ seeds}{Total\ number\ of\ seed\ tested} x100$$

Germination rate index (GI) was calculated by the formula given by (Ellis *et al.*, 1981).

$$GI = [(No. of\ germinated\ seeds)/(Days\ of\ first\ count)] + \ldots + [(No. of\ germinated\ seeds)/(Days\ of\ final\ count)]$$

The Seedling vigour index (SVI) was calculated as shoot and root length into germination percentage divided by 100.

$$SVI = Germination\ (\%)\ x\ Seedling\ length\ (cm)/100$$

Root: shoot ratio (RSR) calculated as root length divided by shoot length into 100.

$$RSR = \frac{Root\ length}{Shoot\ length} x100$$

Root length (RL) and shoot length (SL) measured in cm, fresh weight of shoot and root was calculated. Root and shoot dry weight were evaluated for total dry matter production (TDMP). Root and shoot dry weight were obtained after drying at 70ºC for 48 h.

Statistical analysis

All statistical data analysis was carried out through SPSS statistical software package version 16.0. The PGPR characters and data regarding growth promotion of endophytes on sorghum seedling 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

Genetic identity of PGP endophytes

Endophytes of selected isolates were subjected to quantify their plant growth promoting traits and to assess the phylogenetic identity of isolates, partial 16S rRNA gene sequencing of these bacterial endophytes. Sequentially, Basic Local Alignment Search Tool (BLAST) analysis was performed to validate the genera of selected isolates. Phylogenetic identity of isolates was given in the following table (1).

Table 1: Phylogenetic identity of selected endophytes isolated from sorghum roots

Isolate name	Gene Bank submission (NCBI acc. no.)	Percent Identity (%)
Acinetobacter pittii strain VR2	MN744689	96
Bacillus sp. strain SR2	MN744707	93
Pseudacidovorax intermedius starin SR3	MN508430	94

Endophytes improve sorghum growth characters and report drought tolerance

Sorghum seeds of two pre-released TNS 661 and TKSV 1311 seeds were bacterized with three different drought tolerant endophytes *viz.*, *Acinetobacter pittii* VR2, *Bacillus* sp. SR2, and *Pseudacidovorax intermedius* SR3 showed moderate effect on growth character of sorghum seedlings under PEG imposed condition. Drought stress significantly affect the germination percentage of sorghum seeds in both varieties. In the case of endophytes seed treated with TNS 661, exhibited positive results on germination percentage. However, drought stress decreases the germination percentage up to 34%. As a result of seeds treated with *Acinetobacter pittii* VR2 showed 15% increased germination percentage than control under PEG imposed condition. On the other hand, there was no significant effect on germination percentage in TKSV 1311 seeds but T_8 showed on par effect with control under drought (Fig. 1).

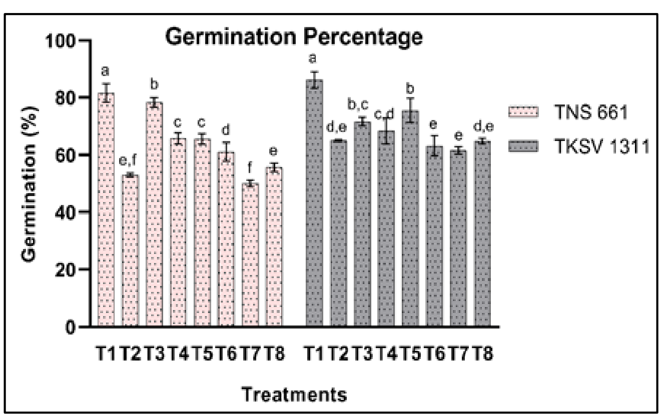

Fig 1

Fig. 1 Growth promotion of seed inoculated endophytes on sorghum germination percentage under stress and non-stress condition. TNS 661 and TKSV 1311- Sorghum varieties. T_1 Absolute control (non-stress) T_2 Control (Stress without inoculation) T_3 Seed treated with *Acinetobacter pittii* (non-stress) T_4 Seed treated with *Bacillus* sp. (non-stress) T_5 Seed treated with *Pseudacidovorax intermedius* (non-stress) T6 Drought + Seed treated with *Acinetobacter pittii* T_7 Drought + Seed treated with *Bacillus* sp. T_8 Drought + Seed treated with *Pseudacidovorax intermedius*. Values are mean of four replications ± S.E. Values with different letters are significantly different at $P \leq 0.05$

Germination rate index (GI) was calculated and presented in below (Fig. 2A). In that, T_7 significantly maximized @ 10% of GI percentage over control in TNS 661 followed by T_6 which was statistically on par with control. Unfortunately, there was no significant effect on GI in endophytes inoculated with TKSV 1311 seedlings under PEG imposed condition. Germination count was taken on alternate days *viz.*, 3, 5 and 7 days after germination. However, drought stress eventually reduced by 26-50% in germination count in TNS 661 and TKSV 1311 during their alternate days over absolute control. On the other hand, bacterial seed treatment enhances the germination counts when seeds were exposed to moisture stress, especially bacterization of *Bacillus* sp. SR2 increased the germination count from 4 to 24% in 3 and 5 days respectively, and during 7 days after germination T_6 performed well (15%) followed by T_8 (6%). In TKSV 1311, bacterial inoculation didn't show any significant results except T_8 which was statistically on par with negative control (T_2) (Fig. 2B).

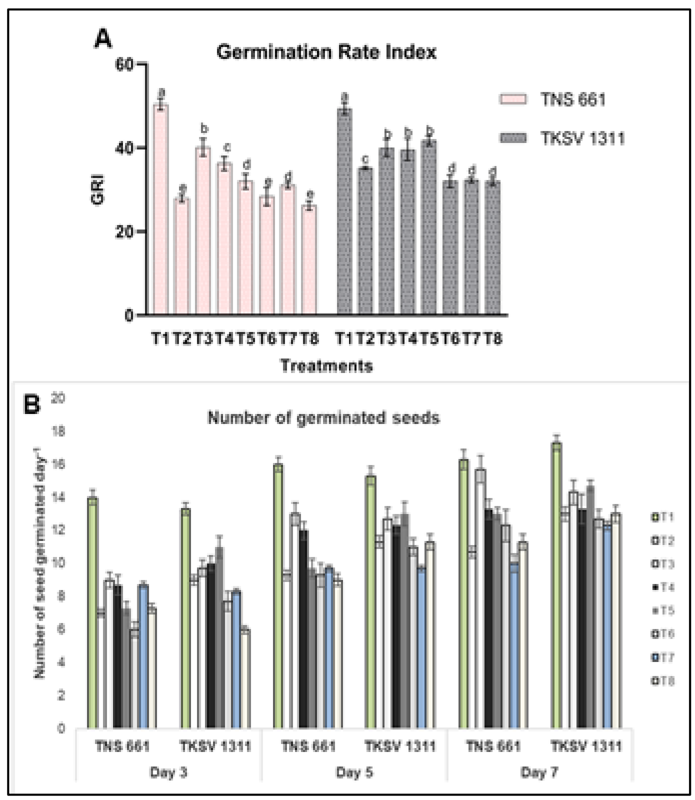

Fig 2

Fig. 2 Growth promotion of seed inoculated endophytes on sorghum seedlings under stress and non-stress condition. TNS

661 and TKSV 1311- Sorghum varieties. A). Germination rate index; B). Number of germinated seeds. Description of the treatment details T_1 to T_8 was already given in Fig. 1 (Please see Fig. 1 description). Values are mean of four replications ± S.E. Values with different letters are significantly different at P≤0.05.

In TNS 661, inoculation of endophytes significantly improved the root length under drought situation (Fig 3A), sorghum seed treated with *Bacillus* sp. SR2 increase of 13% of root length, followed by *Acinetobacter pittii* VR2 (2%). PGPB inoculation with TKSV 1311 showed 4% increase in root length over control seedlings. Further, drought stress drastically reduces the shoot length of TNS 661 sorghum seedlings, even though bacterial seed treatment significantly enhances the shoot length from 35-48% when the seeds treated with *Acinetobacter pittii* VR2 and *Bacillus* sp. SR2 under moisture stressed condition. *Bacillus* sp. SR2 inoculated seedlings recorded maximum shoot length (7-13%) when compared with uninoculated (absolute control) seedlings in non-stressed condition of both pre-released seedlings. However, T_7 showed highest percentage in shoot length (6%) when compared with both endophyte treatments and control followed by T_6 but it was statistically on par with control in TKSV 1311 (Fig. 3B).

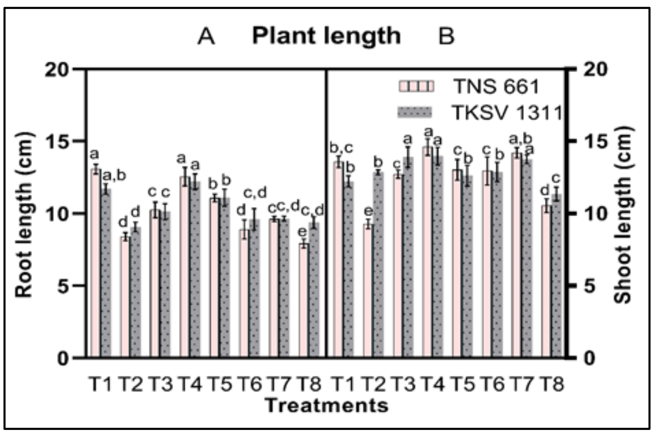

Fig 3

Fig. 3 Growth promotion of seed inoculated endophytes on sorghum root and shoot length under stress and non-stress condition. TNS 661 and TKSV 1311- Sorghum varieties. A). Root length; B). Shoot length. Description of the treatment details T_1 to T_8 was already given in Fig. 1 (Please see Fig. 1 description). Values are mean of four replications ± S.E. Values with different letters are significantly different at P≤0.05.

The ratio of root and shoot was calculated and drought stress drastically reduced the RSR over absolute control (Fig. 4A). Treatment of *Pseudacidovorax intermedius* SR3 showed 15% increase in RSR value of TKSV 1311 under PEG imposed condition and this result was significantly different from negative control. But there were no statistical differences observed in TNS 661 even when the seeds were treated with endophytes in both normal and drought environments. It has been reported that seed treatment of *Acinetobacter pittii* VR2 registered maximum of 54% in SVI followed by *Bacillus* sp. SR2 treated seedlings (36%) when associated with drought stress in uninoculated seedlings (T_2). In TKSV 1311, *Bacillus* sp. SR2 inoculated treatment (T_7) showed significant result but were statistically on par with control and T_6 (Fig. 4B).

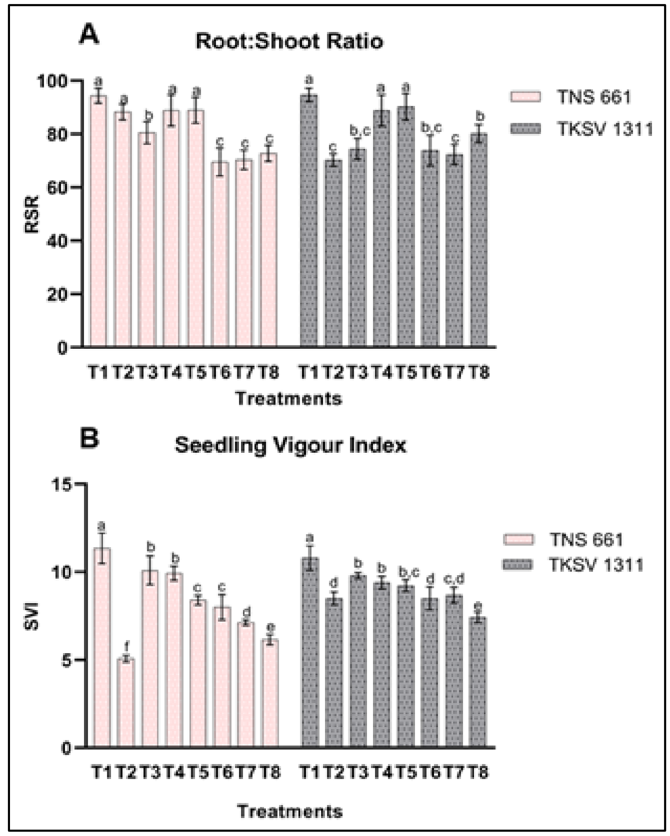

Fig 4

Fig. 4 Growth promotion of seed inoculated endophytes on sorghum seedlings under stress and non-stress condition. TNS 661 and TKSV 1311- Sorghum varieties. A). Root: Shoot ratio; B). Seedling vigour index. Description of the treatment details T_1 to T_8 was already given in Fig. 1 (Please see Fig. 1 description). Values are mean of four replications ± S.E. Values with different letters are significantly different at P≤0.05. Drought stress significantly reduced the fresh weight of root and shoot (Fig. 5A and B). It has been reported that, endophytic bacteria treatment resulted in improvement on root fresh weight when the TNS 661 seedlings which were subjected to drought stress but the results were statistically on par with control. On the other hand, shoot fresh weight was significantly high in T_8 (37%) and T_7 (14%) seedlings followed by T_6 under drought-imposed condition when compared to T_2. Increased Total Dry Matter Production (TDM) was noticed when plants were not exposed to drought stress, inoculation of seeds with *Acinetobacter pittii* VR2 strain (T_3) significantly enhanced the TDM as compared to absolute control (T_1) followed by T_5. Under drought, bacterial inoculation of *Pseudacidovorax intermedius* SR3 (T_8) significantly increased the TDM followed by seed treatment of *Acinetobacter pittii* VR2 (T_6) over uninoculated drought control (T_2). In TKSV 1311, seed treatment with *Acinetobacter pittii* VR2 (T_6) and *Bacillus* sp. SR2 (T_7) showed significant difference in root fresh weight over control (T_2) but statistically on par with each other under drought condition. Under drought + inoculated condition, T8 showed significant results in shoot fresh weight as compared with T_2, T_7 and T6 but T_2 was statistically on par with T_7 and non-significant with T_6. Under PEG imposed condition all the three endophytes were significantly produce higher TDM than control (Fig. 5C).

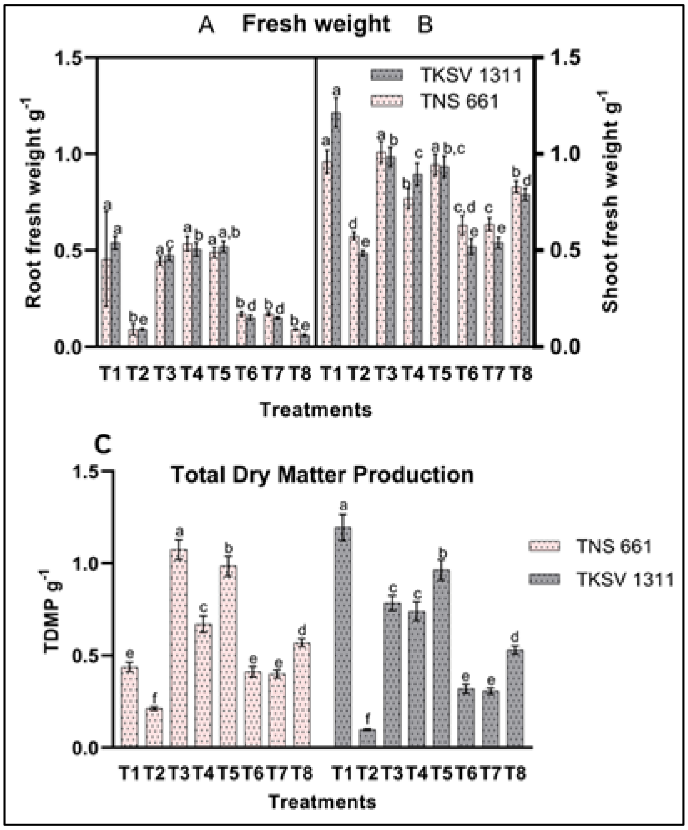

Fig 5

Fig. 5 Growth promotion of seed inoculated endophytes on sorghum biomass under stress and non-stress condition. TNS 661 and TKSV 1311- Sorghum varieties. A). Root fresh weight; B). Shoot fresh weight; C). Total dry matter production. Description of the treatment details T_1 to T_8 was already given in Fig. 1 (Please see Fig. 1 description). Values are mean of four replications ± S.E. Values with different letters are significantly different at P≤0.05.

Discussion

Drought stress constantly affected the plant growth and development. Hence, PGPB inoculation positively upregulated the plant metabolism to increase the crop yield under adverse environment (Chanway and Holl, 1994) [6]. Root-microbe association and metabolite production was mainly be contingent on the edaphic factors and genetic characters of plant (Govindasamy *et al.*, 2017) [14]. This study revealed that sorghum endophytes identified with different PGP traits were evaluated to know their potential to promote growth characters on sorghum seedlings under drought and non-stress environment.

Germination percentage of both endophytes treated and non-treated sorghum seedlings were calculated. In the study, seeds treated with *Acinetobacter pittii* VR2 showed maximum germination percentage, many literatures previously reported that seed treatment of PGP producing organisms increase the germination both biotic and abiotic stress (Raju *et al.*, 1999; Nain *et al.*, 2012) [24]. The scientific understanding of plant growth promotion was bacterial producing compounds like GA and IAA might have played an important role on seed germination and radical length (Maiyappan *et al.*, 2010) [15]. Likewise, sorghum seed treated with *Pseudomonas fluorescens* improved seed germination (Raju *et al.*, 1999) [24]. Increased GA might trigger the enzyme activity of α-amylase which was responsible for early germination through maximizing the availability of starch assimilation. Moreover, improved auxin biosynthesis could increase the seedling vigor and germination rate (Bharathi *et al.*, 2004) [2]. In this context, biofertilizer treatment with *Pantoea agglomerans* and *P. putida* in *Onobrychis sativa* L. plants showed highest germination rate when compared with uninoculated treatment at 0.4 FC level but there was no significant effect on germination percentage (Delshadi *et al.*, 2017) [10]. Among the treatments, T7 showed the highest germination rate under PEG imposed condition, endophytes treatment could had enhanced the speed and early germination of seedling when plants were subjected under adverse conditions. Several studies reported that growth promoting bacteria's namely, *Azotobacter* spp. *Bacillus* spp. and *Pseudomonas* spp. had significant effect on germination, germination rate and seedling vigor of Chilli, Pearl millet, Cow pea, *Bromus tomentellus*, *Onobrychis sativa*, *Avena sativa*, wheat and *Onobrychis sativa* L. respectively (Raj *et al.*, 2003; Nezarat and Gholami, 2009; Nain *et al.*, 2012; Delshadi, 2015; Nuncio-Orta *et al.*, 2015; Delshadi *et al.*, 2017) [23, 20, 19, 9, 22, 10].

Drought stress expressed considerable reduction on the root and shoot length. It had been reported that bacterization of *Bacillus* sp. SR2 strain during drought stress concurrently increased the root and shoot length of both sorghum cultivars. Growth compounds such as auxin and GA produced by endophytes might had provided favorable environment for plant growth and development when plants were subjected to drought stress (Nadeem *et al.*, 2014) [18]. Authors find that interaction effects of bio-fertilizer and drought stress had significant effect on the shoot length at different levels of drought stress, authors also elucidated that the mechanism behind this root and root length was bacteria increasing the root development to acquire nutrients and water, thereby enticing the plant nutrients, finally the plant nutrients' uptake expressed in the plant shoot growth (Davoodifard *et al.*, 2012) [8]. *Bacillus* sp. RM-2 enhanced the percent emergence (94.75 %), plumule (3.08 cm) and radicle (5.95 cm) length in solid agar plates and also it performed well on root and shoot length of cow pea plant in pot and field level experiment too when compared with control plant (Nain *et al.*, 2012) [19]. The changes in root and shoot length was believed due to the production of auxin might be produced by bacterial inoculation. Amino cyclopropane-1-carboxylate deaminase (ACC) production was another drought tolerant mechanism produced by bacteria. ACC deaminase reduces the endogenous ethylene level in plant through the inhibition of ACC-oxidase enzyme, thereby counterbalancing the ethylene negative effect on root growth (Glick *et al.*, 2007) [12]. Maize seedling treated with *Azotobacter* and *Pseudomonas* exhibited increased plant height (Zahir *et al.*, 2004) [33].

In the present study, treatments of endophytes showed gradual increase in root and shoot fresh weight. Whereas drought stress reduces the total dry matter production. However, results articulated there was no significant effect on root fresh weight but shoot fresh weight significantly increased. Similarly, finger millet seeds inoculated with *Pseudomonas putida* DPB15 strain significantly increase the root and shoot fresh weight about 49.8% and 48.6% than control plant (Chandra *et al.*, 2018) [5]. Although, *Pseudacidovorax intermedius* SR3 and *Bacillus* sp.

SR2 treatment showed highest shoot fresh weight as compared with uninoculated. Likewise, *Bacillus* sp. and Ochrobactrum sp. EB-165 strains resulted in improved root and shoot characters and high dry biomass production in maize and sorghum seedlings under drought condition (Vardharajula *et al.*, 2011; Govindasamy *et al.*, 2020) [30, 13]. Similarly, Chandra *et al.* (2018) [5] who reported that ACC deaminase-producing PGPB significantly recorded higher root and shoot dry weight of finger millet both in drought-stressed and non-stress conditions, as compared with non-inoculated controls. Root length and dry matter accumulation was positively correlated with each other, because of increased root length plants were able to acquire more volume of water and nutrient during their unfavorable condition which is the believed to be the hypothesis behind the total dry matter increase when plants were inoculated with growth promoting bacteria under drought stress. Bhattacharyya *et al.* (2012) [3] who reported that IAA and GA produced by bacterial inoculation was an efficient technology to increase dry biomass and yield due to increased nutrient uptake.

Conclusion

Sorghum root associated endophytes isolated from different cultivars were characterized based on the phylogenetic identity with different plant growth promoting attributes. Based on our exploration, selected endophytes showed copious growth responses on sorghum seedlings to induce drought tolerance. In addition to germination percentage, it had increased the germination rate and especially the speed of germination too in selected sorghum cultivar. Moreover, endophyte inoculation concurrently enhances the root and shoot characters and thus helping the plants to withstand drought environments. The experiment had helped to understand the activity of PGP bacteria on sorghum growth under drought-imposed condition. In the forthcoming situations of drought tolerance and bacteri, information requires to know the molecular mechanism of PGP mediated drought tolerance and their meticulous mechanism in sorghum drought tolerance which is generally to cultivated in resource poor and dryland areas.

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