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Mitigation of drought stress in wheat (*Triticum aestivum* L.) by inoculation of drought tolerant *Bacillus paramycoides* DT-85 and *Bacillus paranthracis* DT-97

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ABSTRACT

Drought as an environmental stressor poses threat to crop yields and consequently jeopardizes agricultural sustainability. Microbes harboring in the roots of native plants having ability to promote plant growth can offer a promising tool to combat the drought stress in plants. In this context, drought tolerant *Bacillus* strains were isolated from the rhizospheric soils from hot arid regions of Jaisalmer district of Rajasthan, India. On screening 120 isolates, two isolates DT-85 and DT-97 identified as *Bacillus paramycoides* and *Bacillus paranthracis*, respectively, using 16S rRNA gene sequencing, showed tolerance to high osmotic stress (10–30% polyethylene glycol 6000), salinity (5–15%), and temperature (45°C). Strain DT-97 exhibited efficient plant growth promoting traits such as production of (1) phosphate, (2) siderophores (SPI 2.93), (3) exopolysaccharide (216–244%), (4) indole-3-acetic acid (49–59%), (5) ammonia, and (6) gibberellic acid (110.5%) in comparison to strain DT-85 under both normal watered and drought stress conditions. Drought stress conditions reduced the root-shoot length, leaf area, and chlorophyll content of wheat crops on inoculation of *B. paramycoides* DT-85 and *B. paranthracis* DT-97 resulted in mitigation of drought stress by enhanced production of drought combating molecules like superoxide dismutase, peroxidase, catalase and proline. *B. paranthracis* DT-97 showed better plant growth promoting attributes and thus can be a used as a bioinoculant for mitigating drought in wheat crop.

1. INTRODUCTION

Drought is a fast increasing abiotic stress threatening the food security around the world [1] posing vast economic losses [2,3]. Drought stress is an impediment to the crop production by decreasing the growth of plants and thereby leading to loss of crop [4]. By the year 2050, more than half of the agriculture land globally will be affected by the drought and will not allow plant growth [5] and consequently make it insufficient to support the estimated population of 9.1 billion [6,7]. This condition will be aggravated by a long-lasting drought as a consequence of climate change globally [8]. In the arid regions, it is predicted that due to climate change, drought stress increases because of decreased availability of water and ultimately as an outcome, yield of crop reduces [9,10].

Plants develop various strategies to combat the stresses of environment like drought, salinity, temperature, and heavy metals [11]. Particularly under drought stress, plants adjust biological processes such as tissues permeability for movement of water, adjustment of osmosis, and up regulation of system of antioxidant [12]. Some researchers have developed the genetically modified plants having drought tolerant potential by genetic manipulation techniques whereas other researchers employed plant growth promoting rhizobacteria (PGPR) which help the plants to facilitate water absorption and nutrients uptake by profuse root system under drought stress [13,14].

In the past few years, the use of PGPR for inoculation in crop in lieu of chemical fertilizers has fascinated the farmers for development of sustainable agricultural practice [15]. Various mechanisms are being employed by the PGPR to assist in the nutrient uptake, growth, and yield of plants [16]. The PGPR such as *Aeromonas punctata*, *Azospirillum brasilense*, *Bacillus megaterium*, *Pseudomonas fluorescens*, and *Serratia marcescens* stimulated the production of plant hormones directly, while some other PGPR promoted the growth of plants by making available minerals and nitrogen in the soil under drought conditions [17-19]. Now, reports are supporting inoculation of native PGPR in dry areas on plants to increase the drought tolerance [20]. PGPR colonize the plant root system and support the growth and yield of plants upon inoculation of seed or soil [21,22]. These colonized microbes release metabolites directly which stimulate plant growth. Several other mechanisms exhibited by PGPR include release of plant

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hormones [23,24] such as indole-3-acetic acid (IAA) [25], cytokinin and gibberellic acid (GA) [26], increased N_2 fixation [27], phosphate solubilization [28], absorption of other nutrients, production of siderophores [29], and control of phytopathogens by synthesis of antibiotics such as phenzines, iturin, fengycin, and surfactin [30].

It has been reported that plant–PGPR interactions improve seed germination, root development/architecture [31], shoot and root weights [32-35], leaf area, chlorophyll content, protein content, nutrient uptake, and yields of corn [34], wheat [23,32], vegetables, and potato [36]. Usually, beneficial microbes can enhance tolerance to plants against abiotic stresses. Wheat is considered as a nutritionally and economically staple crop throughout the world [37,38]. Wheat grain contains 8–12% protein, 55% carbohydrate and constitutes 20% of daily human diet [39] around the world making it most important food crop. Hence, it is a good model crop to study influence of PGPR on growth in abiotic stresses. Since mitigation of drought is a problem globally, the present work was undertaken to characterize putative bacilli isolated from the villages of Jaisalmer, Rajasthan, India for assessing their PGP traits as well as drought tolerance potential and evaluating their effect on plant growth and drought tolerance induction in wheat crop.

2. MATERIALS AND METHODS

2.1. Sampling Site

The rhizosphere soils of local wheat crop and other wild plant (*Argemone mexicana*) were collected from four different villages (Roopsi, Lodurva, Baramsar and Sam (26.9107°N and 70.9144°E and 26.9798°N, 70.7525°E) of Jaisalmer district of Rajasthan, India. A total of 20 rhizosphere soil samples were collected in zipped polyethene bags and stored at 4°C until further analysis.

2.2. Isolation and Screening of Putative Bacilli for Tolerance to Drought, Temperature and Salt Stresses

Isolation of aerobic *Bacillus* isolates from rhizosphere soil samples was carried out by employing serial dilution method on nutrient agar (NA) medium (Hi Media). The pure *Bacillus* isolates were preserved on the NA slants in refrigerator at 4°C and in 20% glycerol and kept in thefreezer (-80°C). For screening of drought tolerant bacilli, NA medium supplemented with 5–8% polyethylene glycol-6000 (PEG-6000) was used to screen the tolerant isolates followed by screening at higher concentrations of PEG-6000 in nutrient broth medium at concentration of 10%, 15%, 20%, 25%, and 30% PEG-6000 [40,41]. The growth of bacteria on these media is indicative of positive for drought tolerance on particular level. The selected drought tolerant isolates were grown at 25–55°C temperature range to assess magnitude of temperature tolerance. Similarly, salt tolerance was studied by growing the same selected drought tolerant isolates in media supplemented with sodium chloride (0–15%).

2.3. Screening of Drought Tolerant *Bacillus* Isolates for PGP Traits

The selected drought tolerant isolates were assessed for the PGP attributes like production of siderophores, IAA, GA, exopolysaccharide (EPS), aminocyclopropane-1-carboxylic acid (ACC) deaminase, ammonia, and phosphate solubilization employing standard methods. Two bacterial isolates were screened for inorganic phosphate solubilization ability as per method given by Pikovskaya [42]. Siderophore produced by *Bacillus* isolates was estimated by CAS- shuttle assay [43]. IAA production ability of the drought tolerant isolates was estimated as per the methodology described by Brick *et al*. [44] and expressed as µg IAA/ml. IAA production by isolates without PEG 6000 supplementation was taken as control treatment. The experiment was performed by growing the isolates DT-85 and DT-97 in Luria Bertani (LB) broth supplemented with 0%, 10%, 20%, and 30% PEG 6000. GA production by two isolates in normal and water stress conditions was assessed by method given by Borrow *et al*. [45]. Briefly, GA was estimated in ethyl acetate extracted GA from culture medium and measuring absorbance at 680 nm. The experiment was performed by growing the isolates DT-85 and DT-97 in LB broth supplemented with 0 %, 10%, 20%, and 30% PEG 6000. ACC deaminase activity of the selected bacilli was assayed as per Penrose and Glick [46]. Development of brownish to yellow color with Nessler's reagent in peptone water containing drought tolerant isolates confirms ammonia production [47]. Hydrogen cyanide (HCN) production was determined qualitatively as per method given by Kremer and Souissi [48]. Two *Bacillus* isolates were assayed for antifungal activities against *Fusarium chlamydosporium, Fusarium oxysporum* f. sp. *ciceris*, *Macrophomina phaseolina* and *Sclerotium rolfsii* using potato dextrose agar employing dual culture confrontation technique [49].

2.4. Identification of Drought Tolerant Putative *Bacillus* Isolates

Two drought tolerant bacilli showing promising PGP traits were subjected to identification on the basis of morphological and biochemical characteristics [47], and 16S rRNA gene sequence. The genomic DNA of the two drought tolerant isolates DT-85 and DT-97 was extracted by the help of Nucleopore Quick Gel Recovery Kit of Genetix Biotech Asia Pvt. Ltd, New Delhi, India. The 16S rRNA gene was amplified by PCR using the universal primers 27F1 (5-AGAGTTTGATCMTGGCTCAG-3) and 1494Rc (5-TACGGCTACCTTGTTAC GAC-3). The purification of 16S rRNA gene and its sequencing was carried out by National Centre for Microbial Resource, Pune, India. The 16S rRNA gene sequences were aligned with closely related species using BLASTn program based on percentage of similarity of sequence (>99%) accessible at data base of Gen Bank. The MEGA 8 software was used for the construction of phylogenetic tree on the aligned datasets using the neighbor-joining method (NJ) applied in this software/program [50]. The nucleotide sequences data was submitted to NCBI GenBank database. The bacterial isolates DT-85 and DT-97 were identified as *Bacillus paramycoides* and *B. paranthracis* and these bacilli were banked in National Agriculturally Important Microbial Culture Collection (NAIMCC) with accession number of NAIMCC-B-02941 and NAIMCC-B-02232

2.5. Evaluation of *Bacillus* Isolates DT-85 and DT-97 in Pot Culture

The effect of two drought tolerant isolates namely DT-85 and DT-97 on growth of wheat plants (variety HD-2967) was assessed in pot under net house conditions in Research Farm of ICAR-National Bureau of Agriculturally Important Microorganisms (ICAR-NBAIM), Mau during December 2018 - March 2019. Specifically, wheat seeds were surface sterilized by 1% sodium hypochlorite and dipped in broth of isolates for 1 h. The seeds bacterized with two isolates were sown in the plastic pots of size (30 cm × 30 cm × 26 cm) carrying 4 kg of non-sterilized soil. Pots were divided into three treatment groups (1) control without inoculum, (2) DT-85 treated (3) DT-97 treated. Each treatment group was kept at two conditions: watered and water stressed condition (half dose water). All the experiments were performed in six replications. Rhizosphere soils were collected from plants after

45 and 75 days of plant growth and plants were also harvested. Phenotypic attributes like root, shoot length, leaf area and fresh and dry weight were estimated. Leaves harvested from plants were kept at 4°C for assaying biochemical parameters like chlorophyll, superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and proline. The leaf chlorophyll content was estimated as per method given by Arnon (1967) [51]. Reactive oxygen species (ROS) scavenging enzymes CAT and SOD were assayed in different experimental conditions. CAT activity was assayed by recording the decomposition of H_2O_2 at 240 nm as described by Aebi (1974) [52]. For SOD activity (U mg^{-1} protein) one unit of activity was taken as an amount of protein required to inhibit 50% initial reduction of nitro blue tetrazolium in light condition [53]. Leaf samples (200 mg) in 3% (w/v) were used to extract proline using aqueous sulfosalicylic acid and quantified by ninhydrin method as given by Bates *et al*. [54]. POD activity was determined spectrophotometrically at 436 nm using the reaction mixture containing 10-µl of enzyme solution and 2.99 ml of 50 mM sodium phosphate buffer (pH 6.0) containing 18.2 mM guaiacol and 4.4 mM hydrogen peroxide (H_2O_2) as substrates [55].

2.6. Statistical Analysis

Statistical analyses were performed using GraphPad Prism (Version 9; CA USA) for obtaining p values. All the data were analyzed by analysis of variance and the means were compared with Tukey's test at $P < 0.05$. Graphics and standard deviation of the data were performed using Microsoft Office Excel 2007.

3. RESULTS

3.1. Isolation and Screening of Putative Bacilli for Tolerance to Drought, Temperature and Salt Stresses

Bacillus isolates were isolated in PEG (10–30%) supplemented media from rhizosphere soil samples collected from wheat crop and *A. mexicana* from drought affected rain-fed fields of four villages of Jaisalmer district, Rajasthan, India. Total 120 morphologically distinct *Bacillus* isolates were recovered; purified and pure cultures were preserved for further characterization. The drought tolerance ability of all 120 isolates was tested, of which 113 isolates were able to survive 5–8% of PEG-6000. Under pressure of 15%, 20%, 25%, and 30% of PEG-6000, the 19, 19, 17, and 7 isolates, respectively, showed the tolerance. Selected isolates were grown at different temperature 37–65°C and 5%, 10%, 15%, and 20% sodium chloride concentration. Isolate DT-85 grew at 45°C and in the range of 5–10% of sodium chloride while isolate DT-97 survived at temperature 45°C and grew in the range of 5–15% of sodium chloride [Table 1].

3.2. Screening of Drought Tolerant *Bacillus* Isolates for PGP Traits

The bacilli were subjected for screening their PGP attributes, namely, ammonia production, phosphate solubilization, siderophores production, IAA, GA3, and EPS production. Siderophores production index (SPI) was calculated based on halo diameter and colony diameter of each isolate [Table 2]. It was observed that isolates DT-85 and DT-97 have SPI of 2.68 and 2.93, respectively. Further siderophore production of both the isolates was determined at experimental stress condition of 10–30% PEG added medium. The siderophores production for isolate DT-97 seems to be increased to 151.6%, 163.9%, and 283.56% at 10%, 20%, and 30% PEG (6000), respectively, which were significantly ($P < 0.001$) more than at control non-stressed (0% PEG6000) conditions. Similarly, significant increase in siderophores production was observed for DT-85 isolate by 127%, 150%, and 77.68% ($P < 0.001$) in 10%, 20%, and 30% PEG supplemented media than in non-stress conditions [Figure 1a].

Isolate DT-85 increased EPS production by 105.0%, 102.1%, and 160% ($P < 0.0001$) at 10%, 20%, and 30% PEG stressed conditions, respectively, as compared to non-stressed control. Similarly, isolate DT-97 increased EPS production by 244.55%, 244.8%, 216%, and 202% ($P < 0.0001$) at 10%, 20%, and 30% PEG (6000) stressed conditions, respectively, as compared to non-stressed control. This clearly reflects that the EPS production was significantly higher in isolate DT-97 than DT-85 and production of EPS was found to be higher under stress conditions [Figure 1b]. IAA production by isolate DT-85 increased non-significantly by 20.53%, 13.61%, and 27.53% ($P > 0.05$) at 10%, 20%, and 30% PEG conditions, respectively. Isolate DT-97 showed significant increase of 49.3%, 59%, and 55.18% ($P < 0.001$) in IAA level at 10%, 20% PEG, and 30% PEG, respectively [Figure 1c]. Both the isolates DT-85 and DT-97 exhibited maximum production of 4.5 µg/ml and 6.03 µg/ml GA, respectively, at a drought stress of 20% PEG which is 22.9% and 110.5% ($P < 0.001$) more in comparison to non-stressed control (0% PEG). It is noted that isolate DT-97 synthesized more amount of GA than isolate DT-85 during drought stress [Figure 1d]. Both the selected bacilliwere found positive for ammonia production, but were negative for HCN production. Bacterial isolates DT-85 and DT-97 were recorded positive for phosphate solubilization when tested in Pikovskayas broth supplemented with 0.1% tricalcium phosphate [Table 3]. For isolate DT-85, no significant ACC deaminase production was found but isolate DT-97 showed positive response for ACC deaminase. Isolate DT-85 did not show any significant antifungal activity while isolate DT-97 showed antifungal activity against *M. phaseolina* [Table 3].

3.3. Identification of Drought Tolerant Isolates

The isolates DT-85 and DT-97 were found Gram positive, motile, aerobic, non-acid fast, and endospore forming bacteria. Biochemical characterization confirmed that isolates DT-85 and DT-97 were found positive for CAT, methyl red (MR) tests, starch hydrolysis, and protease tests while negative for VP, oxidase, xylanase, and indole production. Further, isolate DT-85 was found negative for nitrate reduction, whereas positive for cellulase production and isolate DT-97 showed positive result for nitrate reduction and negative for cellulase production [Table 4]. NJ tree constructed based on the 16S rRNA gene sequences showed the relationship with *Bacillus* group members and specifically isolate DT-85 was closely related to *B. paramycoides* NH24A2 (T) (99.01%), whereas DT-97 was related to *B. paranthracis* (99.01%).

Table 1: Growth response of bacterial isolates under drought, temperature, and salt stress conditions

Isolates	Concentration of PEG 6000				Temperature at 45°C	Salt Tolerance at 5–15%
	0%	10%	20%	30%		
DT-85	++	++	++	++	+	++
DT-97	++	++	++	++	+	++

Table 2: Siderophore producing index (SPI) of *Bacillus paramycoides* DT-85 and *Bacillus paranthracis* DT-97

Isolates	Halo diameter (cm)	Colony Diameter (cm)	SPI
DT-85	2.7 cm	1.6 cm	2.68
DT-97	2.9 cm	1.5 cm	2.93

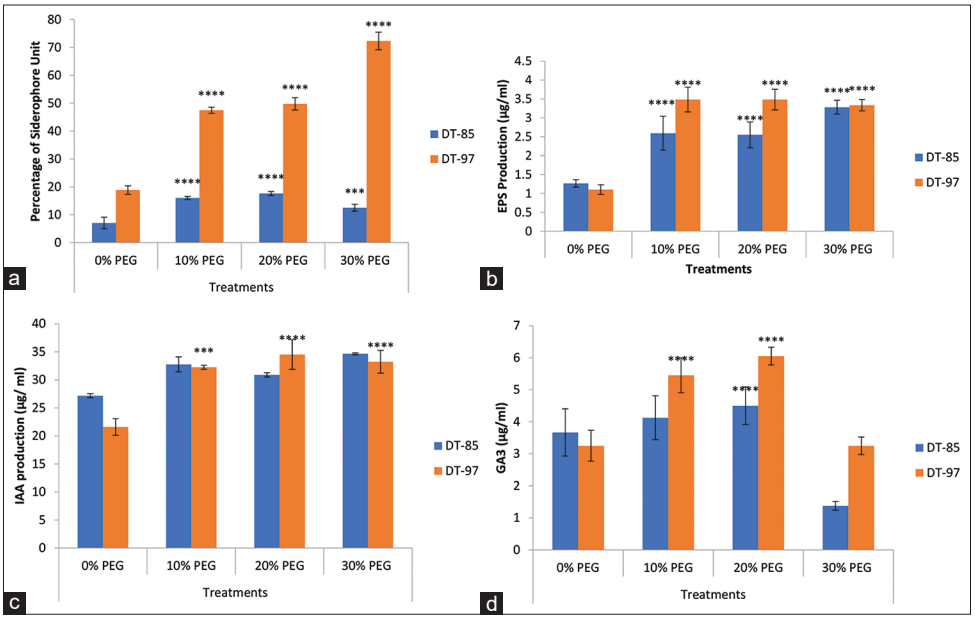

Figure 1: Plant growth promoting traits and drought tolerance potential of *Bacillus paramycoides* DT-85 and *B. paranthracis* DT-97 under watered and drought stress conditions (10-30% polyethylene glycol [PEG]). (a) Siderophore units (b) Exopolysaccharide (µg/ml); (c) Indole-3-acetic acid (µg/ml); (d) Gibberellic acid 3 (µg/ml). Comparison is made with non-stressed (0%PEG) throughout the experiment. Data show the means±standard deviation of three replicates. Significant at the *(P<0.05) and **(P<0.01), ***(P<0.001), ****(P<0.0001) probability levels, respectively. NS: Not significant

Table 3: Plant growth promoting traits of *Bacillus paramycoides* DT-85 and *Bacillus paranthracis* DT-97

Isolates	Ammonia production	Phosphate solubilization	Antifungal activity	ACC deaminase activity
DT-85	Positive	Positive	Negative	Negative
DT-97	Positive	Positive	Positive	Positive

ACC: Aminocyclopropane-1-carboxylic acid

Table 4: Morphological and biochemical characteristics of *Bacillus paramycoides* DT-85 and *Bacillus paranthracis* DT-97

Test	DT-85	DT-97
Gram staining	Gram positive	Gram positive
Motility test	Motile	Motile
Growth in air	Aerobic	Aerobic
Acid fastness	Non-acid fast	Non-acid fast
Endospore formation	+	+
Catalase test	+	+
Oxidase test	N	N
Nitrate reduction test	N	+
Starch hydrolysis test	+	+
MR test	+	+
VP test	N	N
Protease test	+	+
Cellulase test	+	N
Xylanase test	N	N
Indole production test	N	N

The sequences were submitted to GenBank with accession number MW959784 and MK547282 for DT-85 and DT-97 isolates, respectively [Figure 2].

3.4. Evaluation of Isolates DT-85 and DT-97 in Pot Culture

Two drought-tolerant isolates DT-85 and DT-97 were tested for their plant growth promotion and drought tolerance potential. The results emanated are given hereunder.

3.4.1. Growth parameters

Drought tolerant isolates DT-85 and DT-97 showed a significant ability to mitigate the effect of drought on wheat seedlings by improving their growth as discernible by improved root and shoot length. Uninoculated drought control group showed decrease in root length as compared to uninoculated control watered plants during both 45 and 75 days. Root and shoot length of wheat plants improved significantly with inoculation of isolates DT-85 and DT-97 after 45 days, and 75 days of sowing [Figure 3a and b]. In drought conditions, after 45 days, root length of wheat plant inoculated with isolate DT-85 and DT-97 was 73% and 130% (P < 0.0001) more in root length respectively as compared to drought control. While after 75 days growth period with drought treatment insignificant change with DT-85 inoculation but 39% (P < 0.001) increased root length with isolate DT-97 inoculated plants was recorded. Similarly, 18.57% (P < 0.001) decrease in shoot length of plants in drought stress in 45 days treated plants was recorded in comparison to watered control plants. An insignificant (P > 0.001) and significant 40% (P < 0.001) shoot length increase was observed in DT-85 and DT-97 isolates, respectively, in inoculated plants after 45 days growth as compared to drought control. For 75 days treated plants, 22.57% decrease in shoot length was recorded in comparison to watered control plants and 22.8% and 22.7% (P < 0.001) increase in shoot length was recorded as compared to uninoculated drought stress control. The plants inoculated with isolate DT-97 showed better growth and shoot length even in drought condition and hence proved that DT-97 effectively promoted the shoot growth of the plant under drought stress conditions.

The study results supported that plants inoculated with both the isolates increased in leaf area than control in both watered and drought condition. Leaf area of control drought plants was 26% less than

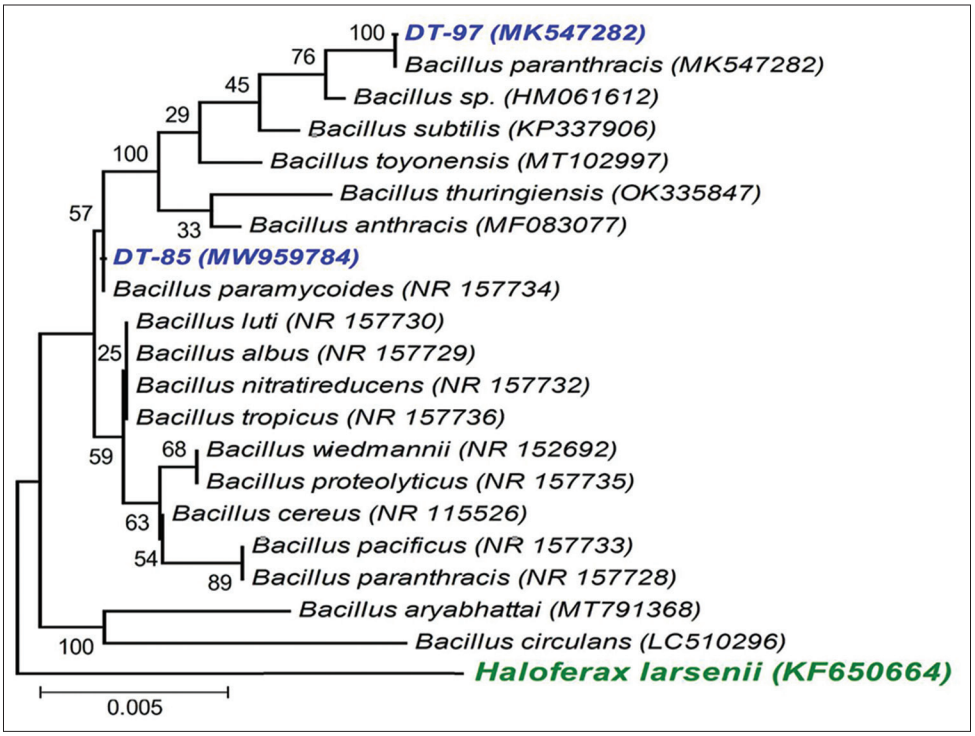

Figure 2: Phylogenetic tree constructed with the neighbor-joining method based on 16S rRNA gene sequences of (a) *Bacillus paramycoides* DT-85 and (b) *Bacillus paranthracis* DT-97 and other related taxa. Bootstrap values with more than 50% are shown on the nodes as percentages of 1000 replicates.

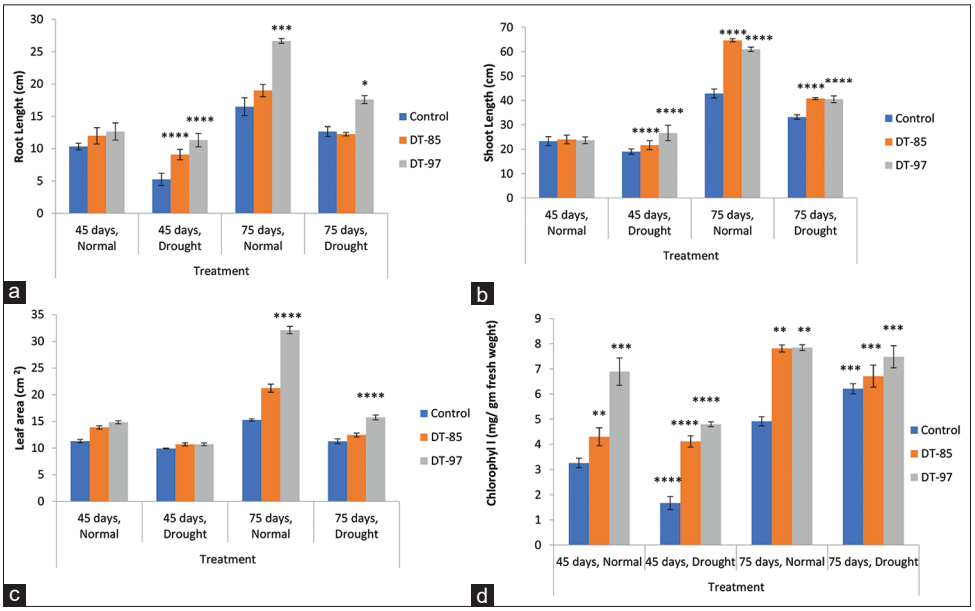

Figure 3: Effect of *Bacillus paramycoides* DT-85 and *B. paranthracis* DT-97 on plant attributes (a) root length (cm); (b) shoot length (cm); (c) leaf area (cm^2); (d) total chlorophyll content (mg/g fresh weight) of wheat crop (HD-2967) after 45 days and 75 days of sowing under water and drought conditions. Data represent means±SD, n=6. Significance levels at * (P<0.05), ** (P<0.01), ***(P<0.001), ****(P<0.0001).

watered control group plants in 75 days treatment groups. Isolate DT-85 treated plants showed insignificant increase of 11% in both 45 days and 75 days treatment groups while DT-97 inoculated plants showed a significant increase of leaf area by 40% ($P < 0.001$) as compared to uninoculated drought control after 75 days of growth. Of two isolates, DT-97 showed better growth promotion under drought stress in terms of leaf area [Figure 3c].

3.4.2. Physiological parameter

In 45 days grown plants subjected to drought stress a decrease in total leaf chlorophyll content was found to the tune of 46% less as compared to leaf chlorophyll content in watered plants. Isolates DT-85 and DT-97 inoculated plants showed 142% and 182 % ($P < 0.0001$) increase as compared to uninoculated drought stressed plants. A decrease of 21.7% ($P < 0.01$) was recorded in plants subjected to drought stress

as compared to watered plants grown for 75 days. Isolates DT-85 and DT-97 had increased total chlorophyll content of plant by 37.03% and 52.9% ($P < 0.001$) as compared to plant under drought stress without inoculation. This suggests that isolate DT-97 supports plant growth in drought conditions with more chlorophyll content [Figure 3d].

3.4.3. Biochemical parameters

3.4.3.1. ROS scavenging enzymes

The antioxidant capacity of inoculated plants under drought stress was evaluated by studying SOD and CAT activities in leaves. SOD activity also exhibited by significant increase of 334 %and 295% after 45 days and 75 days of growth, respectively, in DT-85 inoculated drought stress group, respectively, as compared to drought stressed plants without inoculation with isolates. Similarly, isolate DT-97 inoculated plants exhibited 120% and 246% increase after 45 days and 75 days of growth, respectively, under drought stress as compared to uninoculated drought stress [Figure 4a]. A significant increase of 45% ($P < 0.01$) and 66% ($P < 0.001$) in CAT activity by isolates DT-85 and DT-97, respectively, under drought stress of 45 days was recorded as compared to control drought plants. While in 75 days treatment group, isolate DT-97 showed 61.74% increase ($P < 0.001$) and DT-85 inoculated plants showed 71.39% ($P < 0.0001$) increase in CAT activity in comparison to uninoculated drought control plants [Figure 4b]. POD significantly increased by 54% ($P < 0.001$) and 22.84% on inoculation of isolates DT-85, DT-97 under water stress as compared to watered controlled plants without inoculation while 63% and 58% increase ($P < 0.001$) was noted for DT-85 and DT-97, respectively, under drought stress as compared to drought control after 45 days. Similarly, 75 days of treatment showed significant increase of 44.65% and 53.69% ($P < 0.001$) by DT-85 and D-97 inoculation, respectively, in comparison of drought control under drought stress condition [Figure 4c].

3.4.3.2. Free proline content

Proline, an important osmolyte, accumulates during stress conditions. DT-85 inoculated plants increased 47% ($P < 0.0001$) and 96% ($P < 0.0001$) of proline content after 45 and 75 days, respectively, as compared to plants with no inoculation under drought stress conditions. The isolate DT-97 inoculated plants exhibited significant 23% and 86% increase ($P < 0.0001$) under drought condition in comparison to uninoculated drought conditions at 45 and 75 days of growth, respectively [Figure 4d].

4. DISCUSSION

The employment of rhizobacterial strains possessing stress-adaptive traits as bioinoculants has been found to gain interest now- a-days [56]. They demonstrate a crucial role in improving plant growth during stress and simultaneously maintain soil fertility and health. Drought is the most deleterious abiotic stress that adversely affects the growth and development of plant. The investigation of present study supports that *Bacillus* possesses the ability to assist the crops to ameliorate adverse drought effects. The PGPR stimulate plant growth, biocontrol and nutrient uptake potential that are desirable for mitigation of abiotic stress [Figure 5]. In addition, many PGPR have been found to be distributed in extreme conditions of high temperature, drought, acidity, alkalinity, salinity, and heavy metal toxicity. Hence, effective bacterial strains with growth promoting traits could be isolated from native environment affected with stress to develop bioinoculants for such stress environments.

The present study described the PGP traits of *B. paramycoides* DT-85 and *B. paranthracis* DT-97 that could enhance the plant growth and development under drought stress by synthesizing siderophore, EPS and IAA. Further pot experiment results presented in this study helped to determine effectiveness of the selected strains for promoting plant growth under drought stressed and non-stressed conditions. Our study is in agreement with reports of many researchers. For example, Jochum *et al*. [57] reported that *Bacillus* sp. possess stress tolerant capacity and aids for the growth of cotton. Saad and Abo-Koura [58] also reported that *B. paranthracis* strain could promote plant growth

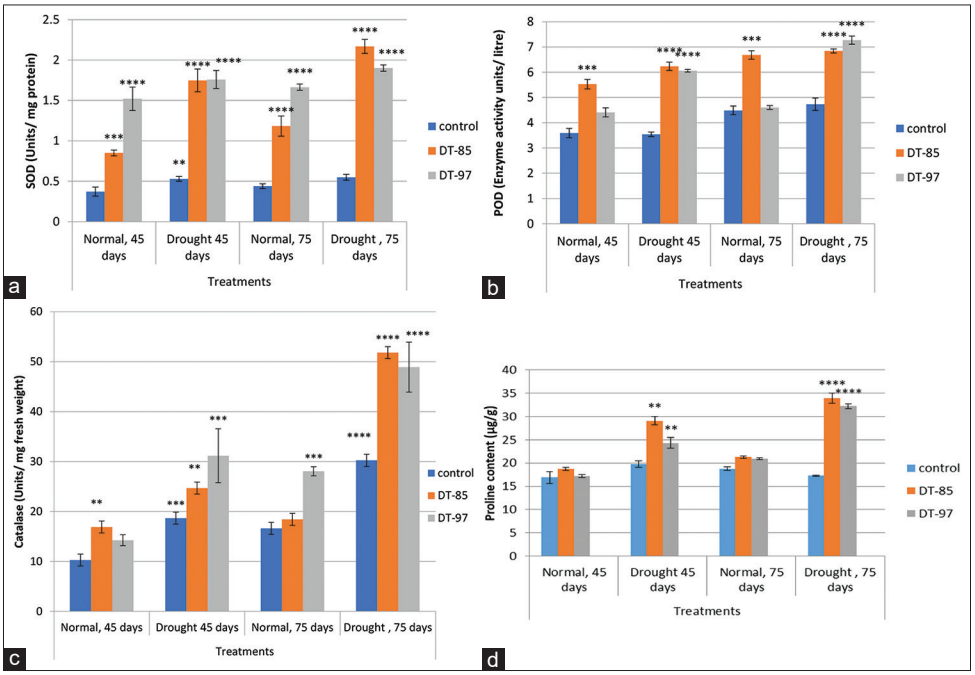

Figure 4: Effect of *Bacillus paramycoides* DT-85 and *Bacillus paranthracis* DT-97 on (a) superoxide dismutase (U/mg Protein); (b) catalase (Units/mg fresh weight), (c) peroxidase (Enzyme activity units/lit); (d) proline (µg/g) in leaves of wheat crop (HD-2967) after 45 days and 75 days of sowing under water and drought conditions. Data represent means±SD, n=6. Significance levels at (P<0.05), **(P<0.01), ***(P<0.001), ****(P<0.0001).

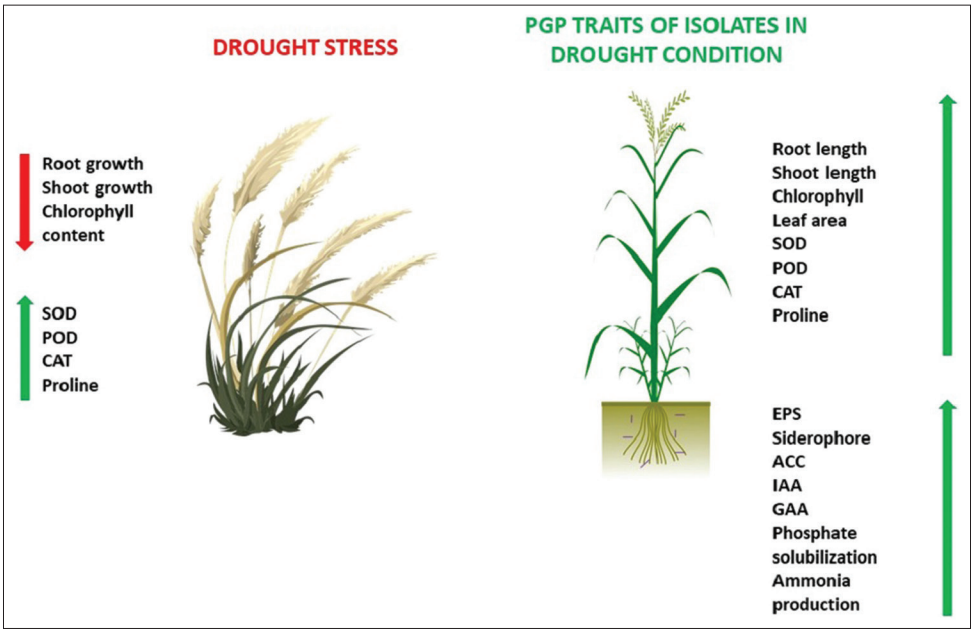

Figure 5: Graphical Abstract: Mitigation of drought stress by plant growth promoting *Bacillus* spp

against drought in sorghum plant. Further, Li *et al*. [59] reported that promotion of growth in wheat could be achieved by PGPR due to its drought tolerant activity.

Many documented studies are known that suggests drought stress has adverse effect on the plant growth and so it is important to screen the drought tolerance potential of the bacterial strain under investigation. Two *Bacillus* strains in the study could tolerate a wide range of PEG (10–30%) supplemented osmotic stress in *in vitro* conditions which state that the bacteria could be able to promote the plant growth under low water activity. Similar to our work study Paul *et al*. [60] and Zia *et al*. [61] also reported *Bacillus* species could eventually lead towards survival under low water activity by inducing tolerance to drought stress in wheat crop. These isolates possessed cultural characteristics such as Gram positive, motile, aerobic, non-acid fast, and endospore formation which enable it to survive and promote plant growth under drought conditions. Presence of CAT enzyme removes harmful H_2O_2 by converting to oxygen and water and hence prevents the plants from cellular damage. These reports are corroborated with report of Malleswari and Bagyanarayana [62] and Nawaz *et al*. [63], wherein they showed that *Bacillus* contribute plant growth promotion and biochemical changes in wheat crop under drought condition. These characteristics protect plant against stress induced damages by enhanced water relations, solute accumulation, and calcium and potassium acquisition and decreased antioxidant enzymatic activity [64].

The present study assessed two *Bacillus* strains for IAA, GA, siderophores, EPS, ACC deaminase production and phosphate solubilization traits under drought stress condition and watered conditions. Both species of *Bacillus* in our study showed siderophore production that has been confirmed by siderophores index value of 2.93 and 2.68 for DT-97 and DT-85 strains, respectively. Various siderophores bearing strains possess PGP potential since it makes the unavialable (insoluble) iron present in soil for plants. Without this it is difficult for the plants to absorb iron from soil due to low solubility. Thus, siderophores production would chelate iron and enable availability to bacteria and plant [65]. Past studies documented that rhizosphere bacteria secrete iron chelating siderophores that influence the mobilization of Fe, Cu, and Zn from soil to plant. The study observed the increase of plant growth with the production of EPS. This production helps the bacteria to survive in stress environments and endure to sustain in stressful conditions [66]. The EPS producing *Bacillus cereus* are able to maintain high moisture content in the soil and thereby support plant growth under drought conditions [67].

Our study also observed IAA production by *B. paranthracis* DT-97 which has been associated with induction of cell division and cell elongation with the subsequent outcomes for plant development. Apart from that IAA serve as signaling molecule required for the plant organ development and growth coordination [68]. IAA could promote the production of chlorophyll in the leaves as compared to uninoculated control plants [69]. Further, Kenneth *et al*. [70] studied that IAA is effective in mycorrhizal growth and plants even at higher level of metal toxicity. *B. paranthracis* DT-97 also produced more amounts of IAA and GA than *B. paramycoides* DT-85. In general, gibberellin promotes node elongation and internode formation, increase in leaf size, and shoot length [71]. In addition, GA induced stem elongation, leaf expansion, promoted flowering and seed germination. Kenneth *et al*. [70] reported that GA production is significant in the maintenance of long-term sustainability and soil fertility. Similarly, reports suggested that more GA production for the promotion of cultivating wheat in drought stress area [72]. Isolates DT-85 and DT-97 were found positive for ammonia production to a great extent because of the availability of nitrogen to roots and thereby plant could produce nitrate for uptake. In this context, Singh and Jha [73] found that strains producing ammonia synergistically impact on the plant growth and plant nutrition. Isolate DT-97 also showed ACC deaminase production which is a known plant growth promoting trait needed for root development Sood *et al*. [74] screened ten morphologically distinct PGPR from rhizosphere of maize roots and showed that two *Bacillus* strains had high ACC deaminase activity.

In general, plant inoculated with the isolates DT-85 and DT-97 increased in shoot length, root length, chlorophyll content and leaf area under drought stress. The predominant adaptations for overcoming the drought stress includes the modifications in root architecture and shoot

growth inhibition, however short shoots interfere directly with the crop yield during the drought stress conditions. Various crops subjected to water stress conditions produce large number and deeper roots that help plants to cope with stress conditions more efficiently [75]. Similar to our study, Akhtar *et al*. [76] reported that spore forming *Bacillus licheniformis* influence growth and physiology of maize under drought and non-stressed conditions. In this study, DT-97 and DT-85 increased chlorophyll content even in drought stress. Rashid *et al*. [77] reported the effectiveness of *B. megaterium* and *B. licheniformis* in increasing germination index of 11–46%, fresh weight from 35 to 192%, seedling vigor index of 11–151% and dry weight of 58–226% of wheat under drought. Further, these strains effectively colonized the roots of wheat and increased plant growth, biomass, photosynthetic pigments, osmolytes, and relative water content. *Bacillus* inoculation displayed improved tolerance by improving the relative water content to 59% and 70% of chlorophyll a and b as well as carotenoid, 117% of proline content and 136% protein content. In addition, defense-related antioxidant enzyme activities were also upregulated [77].

Accumulation of ROS damages the membrane integrity, chlorophyll content effecting the development and yield of plant [78]. This increased ROS is suppressed by battery of SOD and CAT in the system. Our study found a higher concentration of SOD, CAT in DT-85 and DT-97 strains inoculated plants. Earlier studies also reported the similar result [79] wherein increased level help scavenging ROS under drought stress. Similarly, Chakraborty *et al*. [80] reported *Bacillus safensis* W10 or *Ochrobactrum pseudogregnonense* IP8 improved the activity of SOD and CAT which helped to combat the oxidative stress and improved root and shoot under biomass water stress. Further, to maintain the cellular function, plant tends to synthesize osmolytes to adjust to the water deficit conditions [81]. Such osmolytes maintain the turgor pressure and cell volume for uninterrupted function of metabolic activities under drought stress. Our results with high percentage of proline accumulation during drought stress in inoculated plants supports the results from other researchers [17,82]. Besides *Bacillus* species, many other microbes having PGP traits also involved in stress alleviation, for example, *Pseudomonas libanensis* EU-LWNA-33 have solubilized a substantial amount of P under the water-deficient conditions [83]. Similarly, *Acinetaobacter calcoaceticus* EU- LRNA-72 and *Penicillium* sp. EU-FTF-6 enhanced accumulation of sugars, glycine betaine, proline, and decreasing lipid peroxidation to mitigate drought stress in foxtail millet [84]. In other study, *Streptomycetes laurentii* EU-LWT$_3$-69 isolated from Himalayan region had solubilized phosphate [85]. In summary, this investigation reports that plant inoculated with *B. paramycoides* DT-85 and *B. paranthracis* DT-97 increased in shoot length, root length, chlorophyll content, and leaf area of wheat by mitigating drought stress [Figure 5].

5. CONCLUSION

It is well known that drought stress adversely influence the growth and yield of plants in this study. Inoculation of drought tolerant bacteria favored promotion of wheat crop growth and development under drought stress. *B. paranthracis* DT-97 exhibited better plant growth promotion in terms of root and shoot length, leaf area, chlorophyll content and oxidative enzyme probably because of high production of EPS, IAA, and ACC deaminase as compared to *B. paramycoides* DT-85. Overall, results suggest that the drought tolerance has been found more evident in wheat crop inoculated with *Bacillus* species and could serve as an effective biofertilizer and bioenhancer for the cultivation of wheat in semi-arid and arid regions.

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7. AUTHORS' CONTRIBUTIONS

All the authors have made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work.

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The authors report no financial or any other conflicts of interest in this work.

10. ETHICAL APPROVALS

Not applicable.

11. DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

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