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Drought-Alleviating Effects of Endophytic Bacteria Isolated from Xerophytic Plants on *Capsicum annuum* L. Seedlings

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Abstract

In the current study, 51 endophytic bacteria were isolated from 5 different xerophytic plants. Their drought tolerance properties were screened in vitro, and from these, four endophytes with tolerance up to − 1.5 MPa water potential were further selected and identified as *Acinetobacter* sp. Eo3, *Pseudomonas* sp. Ni5, *Bacillus safensis* Ni7, and *Stenotrophomonas* sp. C3. Due to biosafety concern, *Acinetobacter* sp. Eo3 and *Pseudomonas* sp. Ni5 were excluded from further investigation, while *B. safensis* Ni7 and *Stenotrophomonas* sp. C3 were subjected to detailed study. The drought tolerance properties of these endophytes were evaluated in vivo using *Capsicum annuum* L. by analysing the growth parameters (leaf number, root number, shoot length, and plant fresh weight) as well as physiological and biochemical parameters (stomatal index, relative water content, chlorophyll content, and carbohydrate accumulation) of bacteria-treated and control seedlings. Here, treatment with *B. safensis* Ni7 and *Stenotrophomonas* sp. C3 was found to result in statistically significant enhancement ($P \leq 0.001$) of the measured parameters of plants when compared with the control groups. In the case of fresh weight itself, Ni7 and C3 treatment was found to result in values of 157.76 and 142.8 mg, respectively, and was statistically significant enhancement as the same for nutrient broth and distilled water control were 73.3 mg and 70.5 mg only. Additionally, the endophyte-treated seedlings displayed significant improvement in other growth parameters even under induced drought stress. These findings highlight the potential of xerophytic-derived bacterial endophytes to have significant role in mitigating the drought stress effects in plants with the promises for field application.

Introduction

Drought is a common stress factor that reduces the global agricultural production. Approximately 42% of the Indian land area is affected by drought, with 6% of this region experiencing extremely dry conditions, which substantially reduces the agricultural output. Climate change and global warming further accelerate the intensity of drought and its after effects [1]. As sessile systems, plants are continuously and directly subjected to the severity of drought stress and hence it is one of the most severe abiotic stress encountered by plants [2]. Numerous methods have been preconized to impart drought tolerance in agriculturally important crops that encompasses the traditional breeding, genetic engineering, and various agronomic cultural practices. Other technologies including molecular breeding and genome editing tools are still at its infancy and yet to make an impact on crop productivity especially the drought tolerance. Therefore, there is an urgent requirement of alternative strategies to impart drought tolerance in economically important crop plants [3]. Depending on the severity and duration of exposure, low-moisture stress in plants can lead to hampering of crop physiological as well as biochemical performance with cascade of phenotypes such as physiological wilting and retarded plant growth, reduced quality, and yield [4]. However, long-term drought stress can have catastrophic effects, resulting in the breakdown of chloroplasts and starch granules and altered photochemical and photorespiratory activities [5, 6].

Plant microbiome has recently been identified to significantly influence the plant growth and response to the stress factors. Various genera of bacteria, like *Bacillus*,

Four endophytic bacterial species 16S rRNA gene sequences were deposited in NCBI with the accession numbers *Acinetobacter* sp. Eo3 (OR290931), *Pseudomonas* sp. Ni 5 (OR290932), *Bacillus safensis* Ni 7(OR290933), and *Stenotrophomonas* sp. C3 (OR290934).

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Serratia, *Pseudomonas*, *Paenibacillus*, etc., have already been reported to get associated with plants as endophytes, rhizobacteria or phyllosphere organisms [7–9]. The functional contribution by beneficial bacteria can be considered to empower plants to mitigate the effects of abiotic stress factors. At the same time, the global market for microbial formulations to enhance plant growth and yield under biotic and abiotic stress conditions is increasing. Using microorganisms for plant stress management is effective as it is sustainable and environmentally friendly. Recent studies have also been reported the plants surviving in drought-prone areas to have systematically shaped microbiomes to retain the valuable colonizers [10]. Thus, it is presumed that endophytes colonizing in arid plants could have an advantage over others to adapt in the arid environment to confer beneficial effects to the cultivated plants. However, attempts are limited to exploit the endophytic bacteria of arid plants for mitigating the drought in agriculturally important crops. Given the impact of drought on plant growth and productivity, using endophytes to reduce the drought severity is a practicable, dependable, and reliable strategy [11]. In the current study, endophytes were isolated from xerophytic *Nerium indicum* L., *Euphorbia hirta* L., *Emblica officinalis* L., *Calotropis gigantea* L., and *Cereus hexagonus* (L.) Mill by expecting such microorganisms present in these plants to have the ability to confer resistance to biotic and abiotic stress factors along with their plant growth-promoting properties. Because, endophytes have already been reported to have an essential role in host health, food supply, and stress management executed through numerous ways [12, 13]. They can improve the growth characteristics of plants, allowing them to absorb more water [14–16]. Increased metabolism of specific amino acids, proteins, and other secondary metabolites [17, 18] has also been linked to the endophyte-mediated drought resistance in plants. In addition, the regulation of abscisic acid concentration [19, 20] and osmotic capacity [21–24] via the accumulation of osmolytes, such as carbohydrates, has been linked to drought resistance in endophyte-treated plants.

In the present study, *Capsicum annuum* L. was chosen to investigate the drought-alleviating mechanisms of the selected endophytes. This is an economically important crop and already known to be sensitive to drought, as its optimal growth and metabolism require adequate water. By inducing the colonization of potential endophytes on drought-sensitive plants, this research intends to improve their drought tolerance. Endophyte-based drought control will be a cost-effective and environmentally favourable method under current conditions. Based on the results of the study, the endophytes selected for the study may have significant agricultural implications to be exploited for managing arid stress in economically important plants.

Materials and Methods

Collection of Plant Samples

Stem and leaf samples from five different xerophytic plants were collected from the garden of the Department of Botany, Catholicate College, Pathanamthitta, Kerala, India. These included *Nerium indicum* L., *Euphorbia hirta* L., *Emblica officinalis* L., *Calotropis gigantea* L., and *Cereus hexagonus* (L.) Mill.

Isolation of Endophytic Bacteria from Selected Plant Samples

According to the previous methodology, stem and leaves of selected plants were used to isolate the endophytic bacteria [25]. Here, the plant parts were cleaned with distilled water and then treated with Tween 80 for 10 min. After that, the samples were treated with 2% sodium hypochlorite for 10 min, followed by 70% alcohol for 30 s. The plant parts were finally washed several times with sterile distilled water, and the last wash was plated onto nutrient agar (NA) medium as control. The surface-sterilized plant samples were further macerated, and the extract was subjected to serial dilution and plating. In addition, the surface-sterilized plant materials were also directly inoculated on nutrient agar. All the plates were further incubated for 3–4 days at room temperature and observed periodically. Morphologically distinct colonies obtained from these were selected, purified, and used for further studies.

Screening of Endophytic Bacterial Isolates for Drought Tolerance Properties

Screening of the isolates for drought stress tolerance was performed with trypticase soy broth (TSB) supplemented with various concentrations of poly ethylene glycol (PEG) 6000 to provide the varying water potentials of − 0.25, − 0.5, − 0.75, − 1, − 1.25, and − 1.5 MPa (Megapascal). The overnight grown cultures of all the isolated endophytic bacteria with the adjusted optical density (OD) of 0.1 at 600 nm were inoculated into the above media. Growth of the isolates at various stress levels was then estimated by measuring the OD at 600 nm after incubating it at 28 °C for 24 h [26]. The relative growth rate of each isolate at maximum water-stressed condition (− 1.5 MPa) was then calculated [27].

Identification of Selected Endophytic Bacteria

After in vitro drought tolerance analysis, all the selected endophytic bacteria were subjected to various morphological

and biochemical tests as per Bergey's manual of systematic bacteriology. For the biochemical characterization, a combination of 12 biochemical tests (HiAssorted™ KB002, HiMedia, Mumbai, India) were used. These tests were based on the principle of change in pH, change in colour, and utilization of the substrate by bacterial isolates. For molecular identification, genomic DNA was extracted from selected bacterial isolates using HiPurA® Bacterial Genomic DNA Purification Kit (MB505-50PR), HIMEDIA. The presence of genomic DNA was further confirmed by agarose gel electrophoresis. The genomic DNA extracted was used for PCR using the universal primers specific to 16S rRNA, such as 16S F (5′- GAG TTT GAT CCT GGC TCA G-3′) and 16S R (5′-GAT ATT ACC GCG GCG CCT G-3′) [25]. The formation of PCR products was confirmed by agarose gel electrophoresis followed by sequencing at AgriGenome, Kakkanad, Cochin, Kerala. The sequence data thus obtained were further subjected to analysis in EzBioCloud. The sequence data of type strains of each isolates was collected from LPSN database and used for the phylogenetic analysis using the Maximum likelihood method with 1000 bootstraps by MEGA X [28].

In planta Drought Tolerance Analysis on *C. annuum* L. Seedlings Through the Supplementation of Selected Bacteria

After the biochemical and molecular identification of biologically active endophytes, two of the four selected endophytes were omitted due to biosafety concerns and the other two were selected for further investigation. To study the in vivo drought tolerance effects of the selected endophytes, seeds of *C. annuum* were surface-sterilized using 1% sodium hypochlorite solution for 10 min followed by treatment with 70% ethanol for 30 s. The seeds were then washed several times with sterile distilled water, soaked in sterile distilled water for three days, and allowed to germinate [25]. Here, four distinct experimental groups were used. The first and second groups were kept as the negative controls, in which the seeds were treated with distilled water and uninoculated nutrient broth, respectively. The third group comprised of seeds primed with Ni7 and the fourth group included seeds primed with C3. Each group consisted of ten seeds, organized into triplicates. For the third and fourth groups, Ni7 and C3 were used at a concentration of 10^8 CFU/mL for the treatment. Here, the germinated seedlings of *C. annuum* were dipped in respective bacterial cultures of third and fourth groups for 2 h. In the same way, seeds of first and second groups were treated with respective controls for 2 h. All the treated and control seedlings were planted in grow bags containing sterile soil. Following four weeks of growth under normal conditions, a drought period was induced by withholding the watering for 11 days. After the induced drought period, the plants were harvested and subjected to assessment for various growth parameters, including leaf number, root number, shoot length, and fresh weight. Moreover, physiological attributes such as stomatal index, relative water content, chlorophyll content, and carbohydrate levels were also evaluated [25].

Physiological and Biochemical Analyses

Physiological and biochemical parameters such as stomatal index, relative water content, chlorophyll content, and carbohydrate accumulation were checked for the treated plants.

For the analysis of the stomatal index, leaf materials obtained from each treatment sets including the controls were gently cleaned with running tap water to eliminate the dust and debris. The bottom epidermal layer was further carefully pulled off with fingertips, followed by staining with safranine solution and microscopic examination. The number of stomata present in the microscopic field was then counted three times using various preparations of the same experimental set and the stomatal index was calculated as described before [29].

Stomatal index $(\%) = S/(S+E) \times 100$.

S is number of stomata in the microscopic field, E is number of epidermal cells in the microscopic field.

At the same time, relative water content of plant samples was analysed by using the previously described method [30],

RWC = (Fresh weight − Dry weight)/(Turgid weight − Dry weight) × 100.

Fresh weight: the weight of the sample immediately after the harvest, Dry weight: weight of the sample after drying. Turgid weight: weight of the sample after soaking it in water for 3/4 h.

For the chlorophyll estimation, 500 mg of dried leaf tissue was suspended in 2 mL of DMSO (dimethyl sulfoxide) followed by incubation for 20 min at 60 °C in a water bath. The supernatant was then collected, and 3 mL of DMSO was further to the residue. After the processing described above, the first and second supernatants were pooled and made up to 10 mL with DMSO and the absorbance was measured at 663, and 645 nm with DMSO as the control [31]. The chlorophyll concentration was estimated using the following equation as described below [32].

$$\text{Chlorophyll a/tissue (mg/g)} = 12.7\ (A_{663}) - 2.69\ (A_{645}) \times V/1000 \times W,$$

$$\text{Chlorophyll b/tissue (mg/g)} = 22.9\ (A_{645}) - 4.68\ (A_{663}) \times V/1000 \times W,$$

$$\text{Total chlorophyll/tissue (mg/g)} = 20.2\ (A_{645}) + 8.02\ (A_{663}) \times V/1000 \times W.$$

A is absorbance at specific wavelength, V is final volume of chlorophyll extract in DMSO, W is fresh weight of tissue extracted.

For the carbohydrate accumulation analysis, quantification was done using Anthrone method. Here, anthrone reagent was made by dissolving the anthrone powder in concentrated H_2SO_4 at a ratio of 2 g anthrone for 1 L conc. H_2SO_4. For the analysis, 1 g of plant tissue was extracted and resuspended in 10 mL of distilled water. 5 mL of anthrone reagent was added to 1 mL of the test sample while the blank was prepared by 1 mL distilled water with 5 mL of reagent. The standard solution was composed of 1 mL glucose (200 g/mL) solution and 5 mL of the reagent. Following the experiment, the optical density (OD) at 620 nm was measured. From the standard curve plotted with known concentrations of glucose, the carbohydrate concentration was calculated as described before [33].

Statistical Analysis

Statistical analysis of variance (One-way ANOVA) was performed using GraphPad Prism 5.0. The significant difference among all the experimental data were compared with Dunnett's multiple comparison tests [34].

Results

Isolation of Endophytic Bacteria from Different Plant Samples

In the study, a total of 51 distinct bacterial isolates were obtained from *N. indicum* (8), *E. hirta* (8), *E. officinalis* (12), *C. gigantea* (4), and *C. hexagonus* (19) through serial dilution and plating. The absence of microbial growth in the control plates after one week of incubation further confirmed the obtained isolates as endophytes.

Screening of Endophytic Bacterial Isolates for Drought Tolerance

All the 51 bacterial isolates were screened in vitro for the drought tolerance property by culturing it in trypticase soy broth supplemented with different concentrations of PEG 6000. Here, four isolates (Eo3, Ni5, Ni7, and C3) could be observed to tolerate up to − 1.5 MPa water potential during their growth in the selected medium. The growth was calculated by taking the OD at 600 nm and the relative growth of each isolate was calculated by comparing their growth in non-stressed medium (Fig. 1). Here the isolate Eo3 was found to have maximum relative growth under stressed condition among the four, followed by Ni7. The isolate C3 was shown to have growth comparable to Ni5. The difference between growth under non-stressed and a maximum stressed condition was chosen as measurement for the drought tolerance activity [35].

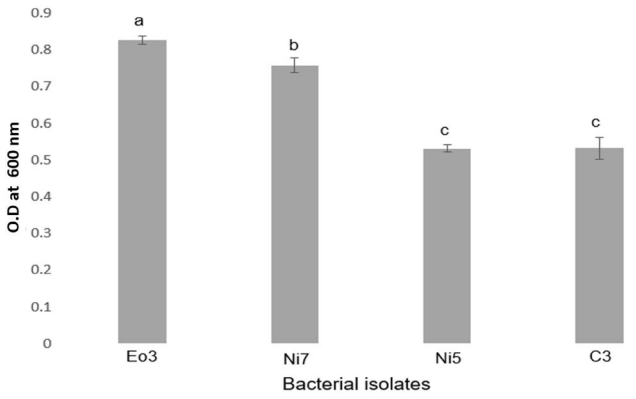

Fig. 1 Effect of in vitro drought stress on the growth of selected bacterial isolates. The impact of in vitro drought stress (− 1.5 MPa) on the growth of bacterial isolates Eo3, Ni7, Ni5, and C3 in trypticase soy broth was studied by supplementing with PEG 6000. The relative growth of each isolate under stressed and non-stressed conditions was analyzed by measuring the O.D. at 600 nm. The values are the means of three replicates ± standard deviation ($n = 3$)

Identification of Selected Endophytic Bacteria

The isolates Eo3, Ni5, and C3, which were found to be Gram-negative and the Gram-positive Ni7 were selected for further identification. Here, oxidase and catalase tests were found to be positive for Ni5 and Ni7, and both were negative for C3. However, Eo3 was found to be oxidase-negative and catalase-positive. The identification of selected endophytic bacteria was confirmed further by sequencing of 1500 bp region of its 16S rRNA gene. The sequence data were further used for the identification using EzBioCloud (https://www.ezbiocloud.net/), and from this, Eo3 was found to have 99.57% similarity with *Acinetobacter variabilis* NIPH2171, Ni5 to have 100% identity with *Pseudomonas otitidis* MCC10330, the Ni7 with 100% identity to *Bacillus safensis* FO-36b and C3 with 99.11% similarity to *Stenotrophomonas maltophilia* MTCC 434 (Table 1). All the sequence data were submitted to NCBI GenBank under the accession numbers OR290931, OR290932, OR290933, and OR290934, respectively, for *Acinetobacter* sp. Eo3, *Pseudomonas* sp. Ni5, *B. safensis* Ni7, and *Stenotrophomonas* C3 sp. Further, phylogenetic analysis has been done with selected type strains from LPSN using the Maximum likelihood method with1000 bootstraps by MEGA X (Fig. 2).

In Planta Drought Tolerance Analysis on *C. annuum* L. Seedlings Through the Supplementation of Selected Bacteria

From the isolated bacteria, *B. safensis* Ni7 and *Stenotrophomonas* C3 sp. were selected for further detailed analysis. The isolates *Acinetobacter* sp. Eo3, *P. otitidis* Ni5 were omitted from further study due to biosafety concern

Table 1 Summary of molecular identification of selected endophytic bacteria with their NCBI accession number and percentage of similarity with their closest relative in EzBioCloud

Name of the isolate	Identified as (with NCBI accession number)	Top-hit strain (EzBioCloud)	Percentage of similarity (%)
Eo3	*Acinetobacter* sp. Eo3 (OR290931)	*Acinetobacter variabilis* NIPH2171	99.57
Ni5	*Pseudomonas* sp. Ni5 (OR290932)	*Pseudomonas otitidis* MCC10330	100
Ni7	*Bacillus safensis* Ni7 (OR290933)	*Bacillus safensis* FO-36b	100
C3	*Stenotrophomonas* sp. C3 (OR290934)	*Stenotrophomonas maltophilia* MTCC 434	99.11

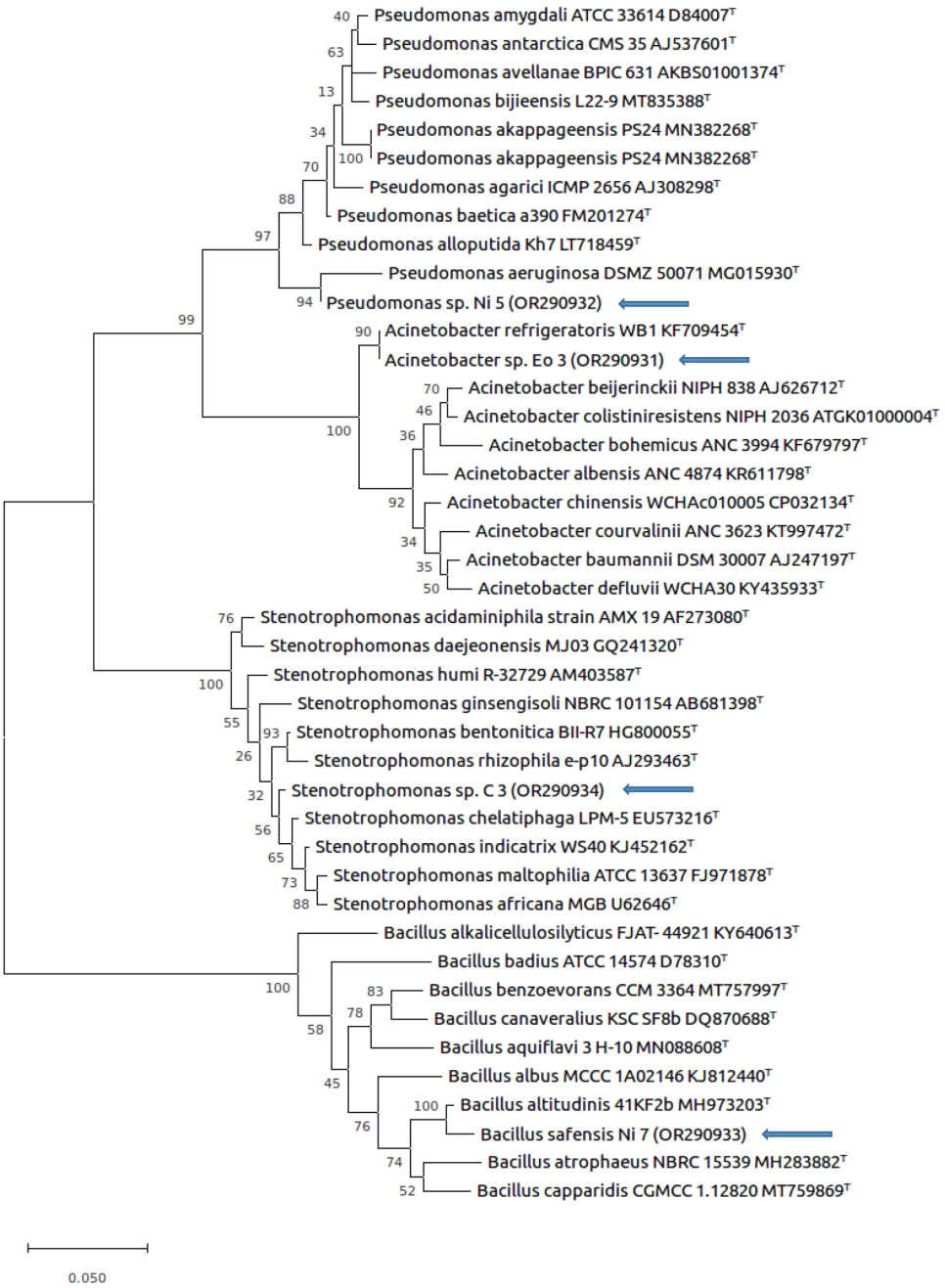

Fig. 2 Phylogenetic analysis of 16S rRNA gene sequences of selected bacterial isolates. Here, analysis of 16S rRNA gene sequences of Ni5, Eo3, C3, and Ni7 (represented with arrow mark) was carried out along with sequences of type strains retrieved from LPSN (represented with superscript T). The analysis was conducted with MEGAX using maximum-likelihood method with 1000 boot-strap replicates

with them. Drought was induced in 4-week-old *C. annuum* seedlings for 11 days. After this, the control plants were observed to become withered and desiccated, whereas the *B. safensis* Ni7 (Ni7) and *Stenotrophomonas* C3 sp. (C3), treated plants were not significantly damaged (Fig. 3). Upon comparison with the distilled water (DW) and nutrient broth (NB)-treated control plants, endophyte-treated plants had a considerable increase in their fresh weight. Regarding the leaf number, Ni7-treated seedlings showed an average value of 5.4 and was 5.6 for the C3 treated. These were statistically significant (degree of freedom, DF = 3, *F* value, $F = 57.90$, and *P* value, $P < 0.001$) when compared with the 4.1 and 3.33 observed for the NB, and DW treated control plants. The highest number of roots was also observed for the Ni7 treatments, with the statistically significant value of 4.8 (degree of freedom, DF = 3, *F* value, $F = 23.48$, and *P* value, $P < 0.001$). The values for the same was 3.03 for the distilled water-treated plants and 3.6 for NB-treated plants. For the shoot length, the Ni7 and C3-treated seedlings showed values of 6.82 and 6.61 cm (degree of freedom, DF = 3, *F* value, $F = 37.42$, and *P* value, $P < 0.001$) and was significantly high when compared to the 5.15 and 5.88 cm values obtained for the DW- and NB-treated plants, respectively. The overall fresh weight of the seedlings was also significantly high for Ni7 treated (157.76 mg), and C3 treated (142.8) seedlings (degree of freedom, DF = 3, *F* value, $F = 86.81$, and *P* value, $P < 0.001$). Because the same for DW- and NB-treated seedlings were having values of only 70.5 and 73.3 mg, respectively (Fig. 4a and b).

Physiological and Biochemical Analyses

Besides the above-mentioned morphological parameters, the selected physiological and biochemical parameters were also analysed for the treated plants (Table 2). Here, Ni7-treated seedlings were found to have the highest stomatal index of 21.72, while the same for C3-treated, DW-treated, NB-treated seedlings were 18.37, 15.009, and 18.4, respectively. The Ni7-treated seedlings could be least affected by the induced drought stress when compared with others by maintaining their internal water content. In others, the stomata might have decreased to reduce the water loss through stomatal openings under the induced drought stress condition. The Ni7-treated seedlings also exhibited the highest RWC of 61.43. In contrast, the DW-treated seedlings exhibited the lowest RWC of 30.31 and the same for C3 and NB-treated seedlings were 48.97 and 43.41, respectively. The results of chlorophyll estimation under drought stress indicated the seedlings treated with Ni7 to have higher photosynthetic activity (0.4041 mg/g) followed by C3-treated seedlings with chlorophyll content of 0.3206 mg/g. At the same time, NB-treated and DW-treated seedlings showed a chlorophyll content of 0.2591 and 0.2002 mg/g, respectively. Carbohydrate accumulation was also analysed in the study for each experimental set. Since the carbohydrates can function as osmolytes under stress conditions, its accumulation might provide mechanistic insight into the drought resistance observed in the study. Here, Ni7-treated seedlings were found to have high accumulation of carbohydrates (930 µg/ml), followed by C3 treated seedlings (926 µg/ml). At the same time, DW-treated seedlings showed the lowest

Fig. 3 Drought-alleviating effects of selected bacteria on *C. annuum* seedlings. Here, bacterial treatment was observed to provide drought tolerance to plants **A** control seedlings treated with distilled water, **B** control seedlings treated with nutrient broth, **C** seedlings treated with *Bacillus safensis* Ni7, and **D** seedlings treated with *Stenotrophomonas* C3 sp

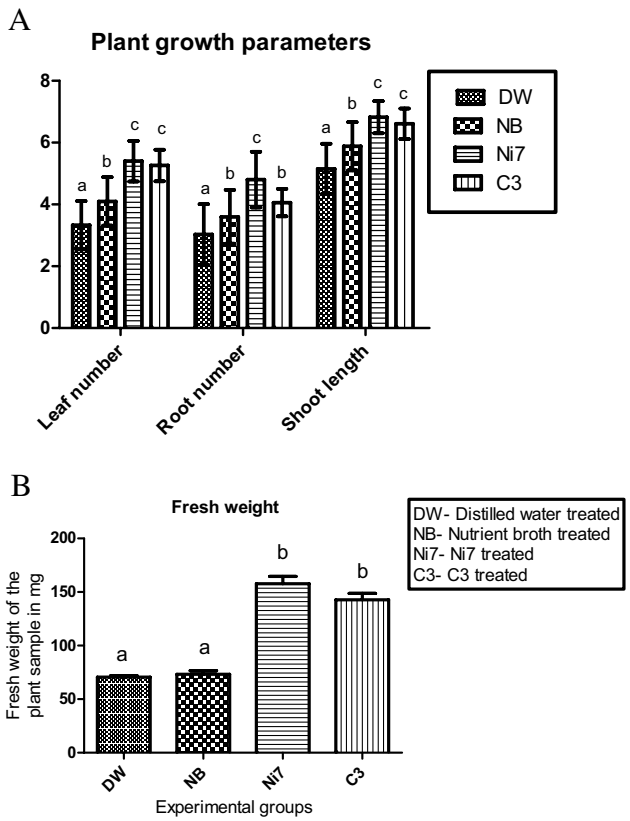

Fig. 4 **A** and **B** Statistical analysis of growth parameters of *C. annuum* seedlings treated with selected bacterial isolates. The seeds were treated as different experimental groups such as **DW** (Distilled water), **NB** (Nutrient broth), **Ni7** (*B. safensis* Ni7), and **C3** (*Stenotrophomonas* sp. C3) under drought condition. **A** One-way ANOVA analysis of leaf number, root number, and shoot length of bacteria-treated and control *C. annuum* seedlings and **B** one-way ANOVA analysis of fresh weight of *C. annuum* seedlings treated with bacteria in comparison with control

concentration of carbohydrates (690 µg/ml). For NB-treated seedlings (850 µg/ml), it was also lower than the Ni7 and C3 treatments. From the results of the study, *B. safensis* Ni7 can be considered to have enhanced efficiency than the *Stenotrophomonas* C3 sp. in providing the drought tolerance to *C. annuum*.

Discussion

Biotic and abiotic stress factors are the most influential environmental factors affecting the agricultural productivity worldwide. Due to the climatic changes, drought has been one of the common abiotic stresses that negatively influence the plant growth. The conventional breeding and genetic engineering methods which are routinely being employed to mitigate the negative effects of drought stress on plants are not sufficient to successfully manage the stress under field conditions. Therefore the application of endophytic bacteria, which have already been demonstrated to have protective effects on various plant species have immense applications to be exploited for the same. Hence, the study has been designed to isolate drought-resistant endophytes from various xerophytic plants in order to analyse the translation of their drought-protective properties to sensitive plants. Drought stress could reduce the soil water content, which further increases the salt concentration and, consequently, the osmotic stress and ion toxicity in plants. Drought can also severely affect the plant physiology, leaf structure, nutrient uptake, photosynthetic activity, and seedling germination [17, 36]. Various strategies have been employed by plants to deal with the drought-induced stress. As part of this, plants have been reported to induce a cascade of events involving signal transduction, induction of stress-responsive genes, activation or inactivation of functional proteins, and responses in specific cell organelles such as chloroplasts, mitochondria, and peroxisomes [36]. Plants have also been reported to secrete stress hormones and reactive oxygen species (ROS) to regulate the cellular physiology, which allows plants to function normally [37, 38]. However, only limited reports are available on the use of beneficial microbes for plant drought management. It is already acknowledged that plant microbial communities play a crucial role in maintaining or enhancing the plant growth and fitness under diverse environmental conditions. However, using beneficial microbes for the drought management has received little attention [38]. Studies have reported the remarkable potential of endophytes in drought stress mitigation and growth promotion in plants. A recent study reported that the

Table 2 Summary of physiological parameters such as stomatal index, relative water content, total chlorophyll content, and total carbohydrates in *C. annuum* L. seedlings for different experimental groups: **DW** (Distilled water treated), **NB** (nutrient broth treated), **Ni7** (*B. safensis* Ni7 treated), and **C3** (*Stenotrophomonas* sp. C3 treated) under induced drought conditions

Treatments	Stomatal index	Relative water content	Total chlorophyll	Total carbohydrates
DW	15.87 ± 4.67^{a}	30.21 ± 0.82^{a}	0.1964 ± 0.0031^{a}	689 ± 8.60^{a}
NB	19.26 ± 4.79^{b}	40.19 ± 2.31^{b}	0.2055 ± 0.0011^{b}	859 ± 6.64^{b}
Ni7	21.80 ± 3.47^{c}	60.81 ± 0.81^{c}	0.4100 ± 0.0128^{c}	930 ± 2.44^{c}
C3	18.59 ± 1.98^{b}	48.21 ± 0.53^{d}	0.3254 ± 0.0034^{d}	926 ± 3.74^{c}

property of *Paenibacillus polymyxa* and *Fusarium oxysporum* to alleviates drought stress and enhances plant growth, make them suitable candidates for utilization as biofertilizers [39]. According to another study, it is recommended to inoculate *Festuca ovina* seeds with *Azotobacter* and *Pseudomonas* in order to enhance their growth and development characteristics, particularly under drought conditions [40].

The current study evaluated two endophytic bacterial strains, *B. safensis* Ni 7 and *Stenotrophomonas* C 3 sp., for their drought tolerance effects in *C. annuum* L. seedlings, as both were demonstrated to have in vitro drought tolerance. A recent study has reported that *Bacillus wiedmannii,* a rhizobacterium isolated from the wheat rhizosphere, exhibits remarkable potential in enhancing the growth properties, including germination percentage (PG), germination rate (GV), and seed vigour index (SV), in wheat plants under water-deficit conditions [41]. The application of *Cronobacter* Y501, a plant growth-promoting rhizobacterium, has been found to optimize various growth attributes of maize, including biomass, plant height, and root viability in drought environments. The bacterial inoculation also facilitated the recovery of chlorophyll content, reduction in MDA accumulation, and activation of SOD, catalase, and peroxidase [42]. The potential of endophytic bacteria to adapt to the altered osmotic conditions is a crucial factor in determining their ability to survive and support plant growth under harsh environments. The accumulation of suitable solutes/osmolytes such as carbohydrates, glutamate, proline, and glycine during their growth in PEG 6000-containing media might have accounted for the drought tolerance of *B. safensis* Ni7 and *Stenotrophomonas* C3 sp. as per previous report [43]. Exopolysaccharides, oxidase, carbonic anhydrase, and catalase synthesis by bacteria under the water-stressed conditions have already been reported previously [44–46]. Several endophytic bacterial strains isolated from *Ananas comosus*, such as *Bacillus* sp., *Providencia* sp., and *Staphylococcus* spp., have also been demonstrated to enhance the drought tolerance, growth, and disease resistance in *Vigna radiata* [26, 45]. Endophytic bacterial strains isolated from other plant species have also been demonstrated to confer drought tolerance up to − 1.02 matric potential [26]. Four endophytic bacteria isolated in the current study such as *B. safensis* Ni7, *Stenotrophomonas* C3 sp., *Acinetobacter* sp. Eo3, and *P. otitidis* Ni5 have also been shown to have drought tolerance up to − 1.5 MPa. The *in planta* analysis on 4-week-old *C. annuum* seedlings using the selected bacteria *B. safensis* Ni7 and *Stenotrophomonas* sp. C3 further confirmed their superior drought tolerance properties. Here, the control plants began to wilt on the third day under simulated drought conditions, whereas the bacteria-treated plants remained healthy. In a previous study, *S. maltophilia* was identified for enhancing the drought tolerance in the *Gemiza-9* wheat cultivar [47].

Plants have already been reported to use diverse mechanisms for drought stress management. The changes in the root architecture are considered to be one of the primary strategies used by plants for increasing the drought resistance [46]. To adapt to the changing environment, roots can alter their morphology and structural properties [36, 48]. Osmotic adjustment is another strategy used by plants to overcome the destruction caused by the drought stress. Here, the active accumulation of carbohydrates and organic or inorganic solutes is one of the essential physiological responses considered to be activated under drought stress [49]. Hence, the concentration of carbohydrates accumulated in treated plants was also calculated in the present study. Here, endophyte-treated plants were found to accumulate more carbohydrates than the control plants. The relative water content of plants has also been studied to determine their ability to retain water. Compared to other seedlings, those treated with *B. safensis* Ni7 was observed to have a higher RWC, and it might be one of the most effective criteria to evaluate the plant tissue under water deficiency [50].

From the results of the current study, drought resistance and plant growth-promoting properties of endophytic bacteria might have been favoured the plant growth under the induced drought stress especially in the case of *B. safensis* Ni7. Further research is required to identify the active drought resistance mechanisms induced in endophyte-treated plants to translate these for field application.

Conclusions

The potential role of bacterial endophytes has been less investigated with respect to drought stress management. Thus, the current study has demonstrated the endophytic bacterial strains to have the potential to alleviate the drought stress in *C. annuum* seedlings through diverse mechanisms. Here, two endophytic bacteria isolated from the xerophytic plants were found to have the ability to protect plants from drought stress. By treating the isolated bacteria with *C. annuum* seedlings, the seedlings were found to withstand the drought conditions. Based on the morphological and physiological characteristics of endophyte-treated and drought protected plants, there is great scope for translating these to manage the drought tolerance in drought-sensitive plants under field conditions. However, future investigations at the omics level are required to unravel the global mechanisms involved to explore their potential in other crops for the fulfilment of the goal of sustainable crop production.

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Data Availability All data and material were transparent in the study.

Code Availability Not applicable.

Declarations

Conflict of interest The authors declare no conflicts of interest.

Ethical Approval All aspects of the study were performed in accordance with the ethical standards of the Institutional Research Committee.

Informed Consent Informed consent was obtained from all individual participants included in the study.

Consent for Publication Authors confirm that this work is original and has not been published elsewhere nor is currently under consideration for publication elsewhere.

References

1. Allen CD, Breshears DD, McDowell NG (2015) On underestimation of global vulnerability to tree mortality and forest die-off from hotter drought in the Anthropocene. Ecosphere 6(8):art129. https://doi.org/10.1890/ES15-00203.1
2. Shahzad MA et al (2016) Drought stress and morphophysiological responses in plants. 452–467. https://doi.org/10.1002/9781119054450.ch27
3. Ngumbi E, Kloepper J (2016) Bacterial-mediated drought tolerance: current and future prospects. Appl Soil Ecol 105:109–125. https://doi.org/10.1016/j.apsoil.2016.04.009
4. Mathur P, Roy S (2021) Insights into the plant responses to drought and decoding the potential of root associated microbiome for inducing drought tolerance. Physiol Plant 172(2):1016–1029. https://doi.org/10.1111/ppl.13338
5. Yan G et al (2018) Responses and mechanisms of soil greenhouse gas fluxes to changes in precipitation intensity and duration: a meta-analysis for a global perspective. Can J Soil Sci 98(4):591–603. https://doi.org/10.1139/cjss-2018-0002
6. Shao RX et al (2016) Changes in chloroplast ultrastructure in leaves of drought-stressed maize inbred lines. Photosynthetica 54(1):74–80. https://doi.org/10.1007/s11099-015-0158-6
7. Heenan-Daly D et al (2021) Volatile compounds from *Bacillus*, *Serratia*, and *Pseudomonas* promote growth and alter the transcriptional landscape of *Solanum tuberosum* in a passively ventilated growth system. Front Microbiol. https://doi.org/10.3389/fmicb.2021.628437
8. Elbouazaoui A et al (2022) Biocontrol activity of *Bacillus*, *Paenibacillus* and *Pseudomonas* against *Fusarium* wilt of chickpea in Morocco. Acta Agric Scand B 72(1):847–859. https://doi.org/10.1080/09064710.2022.2100819
9. Nair MI, Jayachandran K (2017) A novel strain of *Pantoea eucrina* endophyte of *Murraya koenigii* with squalene cyclase activity. LIFE Int J Health Life Sci 3(2):161–177. https://doi.org/10.20319/lijhls.2017.32.161177
10. Shahzad R et al (2018) What is there in seeds? Vertically transmitted endophytic resources for sustainable improvement in plant growth. Front Plant Sci. https://doi.org/10.3389/fpls.2018.00024
11. Kaushal M, Wani SP (2016) Rhizobacterial–plant interactions: strategies ensuring plant growth promotion under drought and salinity stress. Agric Ecosyst Environ 231:68–78. https://doi.org/10.1016/j.agee.2016.06.031
12. Lau JA, Lennon JT, Heath KD (2017) Trees harness the power of microbes to survive climate change. Proc Natl Acad Sci USA 114(42):11009–11011. https://doi.org/10.1073/pnas.171541711
13. Lau JA, Lennon JT (2012) Rapid responses of soil microorganisms improve plant fitness in novel environments. Proc Natl Acad Sci USA 109(35):14058–14062. https://doi.org/10.1073/pnas.1202319109
14. Li X et al (2019) Dark septate endophytes improve the growth of host and non-host plants under drought stress through altered root development. Plant Soil 439(1–2):259–272. https://doi.org/10.1007/s11104-019-04057-2
15. Ferus P, Barta M, Konôpková J (2019) Endophytic fungus *Beauveria bassiana* can enhance drought tolerance in red oak seedlings. Trees 33(4):1179–1186. https://doi.org/10.1007/s00468-019-01854-1
16. Naveed M et al (2014) Increased drought stress resilience of maize through endophytic colonization by *Burkholderia phytofirmans* PsJN and *Enterobacter* sp. FD17. Environ Exp Bot 97:30–39. https://doi.org/10.1016/j.envexpbot.2013.09.014
17. Li J et al (2017) Influence of drought stress on photosynthetic characteristics and protective enzymes of potato at seedling stage. J Saudi Soc Agric Sci 16(1):82–88. https://doi.org/10.1016/j.jssas.2015.03.001
18. Ghaffari MR et al (2019) Root endophytic fungus *Piriformospora indica* improves drought stress adaptation in barley by metabolic and proteomic reprogramming. Environ Exp Bot 157:197–210. https://doi.org/10.1016/j.envexpbot.2018.10.002
19. Cohen AC et al (2015) *Azospirillum brasilense* ameliorates the response of *Arabidopsis thaliana* to drought mainly via enhancement of ABA levels. Physiol Plant 153(1):79–90. https://doi.org/10.1111/ppl.12221
20. Ruiz-Lozano JM et al (2016) Arbuscular mycorrhizal symbiosis induces strigolactone biosynthesis under drought and improves drought tolerance in lettuce and tomato. Plant Cell Environ 39(2):441–452. https://doi.org/10.1111/pce.12631
21. Ullah A et al (2019) Drought tolerance improvement in plants: an endophytic bacterial approach. Appl Microbiol Biotechnol 103(18):7385–7397. https://doi.org/10.1007/s00253-019-10045-4
22. Kang Q et al (2018) CURatio: genome-wide phylogenomic analysis method using ratios of total branch lengths. IEEE/ACM Trans Comput Biol Bioinform. https://doi.org/10.1109/TCBB.2018.2878564
23. He L, Hatier JHB, Matthew C (2017) Drought tolerance of two perennial ryegrass cultivars with and without AR37 endophyte. NZ J Agric Res 60(2):173–188. https://doi.org/10.1080/00288233.2017.1294083
24. Hahn H et al (2008) Neotyphodium fungal endophytes confer physiological protection to perennial ryegrass (*Lolium perenne* L.) subjected to a water deficit. Environ Exp Bot 63(1–3):183–199. https://doi.org/10.1016/j.envexpbot.2007.10.021
25. Jasim B et al (2013) Isolation and characterization of plant growth promoting endophytic bacteria from the rhizome of *Zingiber officinale*. 3 Biotech 4(2):197–204. https://doi.org/10.1007/s13205-013-0143-3
26. Sandhya V et al (2017) Endophytes from maize with plant growth promotion and biocontrol activity under drought stress. Russ Agric Sci 43(1):22–34. https://doi.org/10.3103/S1068367417010165

27. Dubey A et al (2021) Bacterial root endophytes: characterization of their competence and plant growth promotion in soybean (*Glycine max* (L.) Merr.) under drought stress. Int J Environ Res Public Health 18(3):931. https://doi.org/10.3390/ijerph18030931
28. Sreejith S et al (2020) Healthy broilers disseminate antibiotic resistance in response to tetracycline input in feed concentrates. Microb Pathog 149:104562. https://doi.org/10.1016/j.micpath.2020.104562
29. Zhu C et al (2021) A deep learning-based method for automatic assessment of stomatal index in wheat microscopic images of leaf epidermis. Front Plant Sci 12:716784. https://doi.org/10.3389/fpls.2021.716784
30. Soltys-Kalina D et al (2016) The effect of drought stress on the leaf relative water content and tuber yield of a half-sib family of 'Katahdin'-derived potato cultivars. Breed Sci 66(2):328–331. https://doi.org/10.1270/jsbbs.66.328
31. Liang Y et al (2017) A nondestructive method to estimate the chlorophyll content of *Arabidopsis* seedlings. Plant Methods 13:26. https://doi.org/10.1186/s13007-017-0174-6
32. Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24(1):1–15. https://doi.org/10.1104/pp.24.1.1
33. Koehler LH (2002) Differentiation of carbohydrates by anthrone reaction rate and color intensity. Anal Chem 24(10):1576–1579. https://doi.org/10.1021/ac60070a014
34. Kim H-Y (2015) Statistical notes for clinical researchers: post hoc multiple comparisons. Restor Dent Endod 40(2):172. https://doi.org/10.5395/rde.2015.40.2.172
35. Sharath S et al (2021) The role of phyllosphere bacteria in improving cotton growth and yield under drought conditions. Front Agron. https://doi.org/10.3389/fagro.2021.680466
36. Ma Y, Dias MC, Freitas H (2020) Drought and salinity stress responses and microbe-induced tolerance in plants. Front Plant Sci. https://doi.org/10.3389/fpls.2020.591911
37. Aeron A et al (2019) Revisiting the plant growth-promoting rhizobacteria: lessons from the past and objectives for the future. Arch Microbiol 202(4):665–676. https://doi.org/10.1007/s00203-019-01779-w
38. Li J et al (2019) Arbuscular mycorrhizal fungi alleviate drought stress in C3 (*Leymus chinensis*) and C4 (*Hemarthria altissima*) grasses via altering antioxidant enzyme activities and photosynthesis. Front Plant Sci. https://doi.org/10.3389/fpls.2019.00499
39. Ogbe AA et al (2023) Growth-promoting characteristics of fungal and bacterial endophytes isolated from a drought-tolerant mint species *Endostemon obtusifolius* (E. Mey. ex Benth.) N. E. Br. Plants 12(3):638. https://doi.org/10.3390/plants12030638
40. Rigi F, Saberi M, Ebrahimi M (2023) Improved drought tolerance in *Festuca ovina* L. using plant growth promoting bacteria. J Arid Land 15(6):740–755. https://doi.org/10.1007/s40333-023-0015-6
41. Karimzad L et al (2023) Drought tolerance induction in wheat by inoculation of seeds with a novel growth-promoting bacteria. Biocatal Agric Biotechnol 47:102594. https://doi.org/10.1111/ppl.13362
42. Gao X et al (2023) Effect of the plant growth promoting *Rhizobacterium*, *Cronobacter* sp. Y501, for enhancing drought tolerance in maize (*Zea mays* L.). J Soil Sci Plant Nutr 23(2):2786–2797. https://doi.org/10.1007/s42729-023-01234-2
43. Vardharajula S et al (2011) Drought-tolerant plant growth promoting *Bacillus* spp.: effect on growth, osmolytes, and antioxidant status of maize under drought stress. J Plant Interact 6(1):1–14. https://doi.org/10.1080/17429145.2010.535178
44. Vardharajula S, Sk AZ (2014) Exopolysaccharide production by drought tolerant *Bacillus* spp. and effect on soil aggregation under drought stress. J Microbiol Biotechnol Food Sci 4(1):51–57. https://doi.org/10.15414/jmbfs.2014.4.1.51-57
45. Aslam A et al (2018) Effect of carbonic anhydrase-containing endophytic bacteria on growth and physiological attributes of wheat under water-deficit conditions. Plant Prod Sci 21(3):244–255. https://doi.org/10.1080/1343943X.2018.1465348
46. Sandhya V et al (2009) Alleviation of drought stress effects in sunflower seedlings by the exopolysaccharides producing *Pseudomonas putida* strain GAP-P45. Biol Fertil Soils 46(1):17–26. https://doi.org/10.1007/s00374-009-0401-z
47. Kasim WA et al (2021) Enhancement of drought tolerance in *Triticum aestivum* L. seedlings using *Azospirillum brasilense* NO40 and *Stenotrophomonas maltophilia* B11. Bull Natl Res Cent. https://doi.org/10.1007/s00374-009-0401-z
48. Aeron A et al (2020) Revisiting the plant growth-promoting rhizobacteria: lessons from the past and objectives for the future. Arch Microbiol 202(4):665–676. https://doi.org/10.1007/s00203-019-01779-w
49. Ilyas M et al (2020) Drought tolerance strategies in plants: a mechanistic approach. J Plant Growth Regul 40(3):926–944. https://doi.org/10.1007/s00344-020-10174-5
50. Pour-Aboughadareh A et al (2019) Effect of water deficit stress on seedling biomass and physico-chemical characteristics in different species of wheat possessing the D genome. Agronomy 9(9):522. https://doi.org/10.3390/agronomy9090522