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Screening of PEG-tolerant bacteria for plant growth promotion and drought-stress alleviation in *Chrysanthemum*

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ABSTRACT

Drought stress severely limits the growth and productivity of *Chrysanthemum* and other ornamental crops. However, identifying bacterial isolates that combine polyethylene glycol (PEG)-induced osmotic stress tolerance with effective plant growth-promoting activity and the ability to alleviate drought stress remains challenging. Here, 45 bacterial isolates from harsh soils were screened for tolerance to PEG 6000-induced osmotic stress and subsequently evaluated for plant growth-promoting (PGP) traits and their effects on *Chrysanthemum* growth and recovery under water-withholding conditions. Three isolates, identified as *Serratia* sp. L5, *Bacillus* sp. NG1, and *Pseudomonas* sp. SN1, markedly promoted plant growth under well-watered conditions and improved plant performance during and after the water-withholding treatment. After re-watering, inoculation with these strains increased fresh plant biomass by 28.10%–69.42% compared with the non-inoculated control. All three strains produced indole-3-acetic acid but differed in other PGP traits, including putative nitrogen-fixing capacity, phosphate solubilization, and siderophore production. These findings demonstrate that combining PEG-induced osmotic stress screening with in planta functional evaluation can facilitate the selection of PEG-tolerant bacterial strains with plant growth-promoting activity and potential for drought-stress alleviation. The selected strains therefore represent promising candidates for further evaluation as microbial inoculants for *Chrysanthemum* production under water-limited conditions.

1. INTRODUCTION

Chrysanthemum is one of the most economically important ornamental crops in the global horticultural industry [1]. Owing to their diverse and highly valued ornamental traits, *Chrysanthemum* cultivars are widely cultivated as cut flowers, potted plants, landscape decorations, or materials for the medicinal and cosmetic industries [1,2]. However, *Chrysanthemum* is highly sensitive to environmental stresses, particularly drought, which threatens its global production [3]. Under drought conditions, root function is impaired, leading to cellular dehydration and alterations in plant morphology and physiological and biochemical processes [4]. In horticultural plants, studies have shown that drought stress reduces biomass and growth rate and directly compromises aesthetic traits such as flower number, size, color intensity, and longevity [5,6]. In *Chrysanthemum*, drought stress leads to significant reductions in biomass accumulation, flower quality, and other physiological processes, including photosynthesis, transpiration, and stomatal conductance [7]. Drought stress is an important environmental constraint that can limit the growth and production of *Chrysanthemum* [8]. Among the biological approaches proposed to mitigate drought stress, drought-tolerant plant growth-promoting rhizobacteria (PGPR) have emerged as a promising strategy for improving plant adaptation under water-deficit conditions. Diverse drought-tolerant PGPR have been reported as alleviators for various horticultural crops in different groups, including proteobacteria (diverse species of different genera), firmicutes, and actinobacteria [9]. These bacteria have been shown to enhance drought tolerance in horticultural crops through a wide range of mechanisms. Research indicates that drought-tolerant PGPR can enhance plant drought tolerance through multiple mechanisms, including modulating phytohormone production [10], inducing systemic tolerance [11], improving nutrient acquisition, and modulating stress-responsive pathways [10,12]. Consequently, these beneficial bacteria could directly or indirectly contribute to improved plant growth and productivity when plants are in water-deficit conditions. Although numerous drought-tolerant PGPR have been reported to enhance plant performance under drought conditions, identifying bacterial isolates with reliable plant growth-promoting (PGP) traits remains challenging. Among the available screening approaches, osmotic stress assays using polyethylene glycol (PEG) are widely employed for the preliminary screening of bacterial osmotic stress tolerance [11,13]. However, whether bacterial tolerance to PEG-induced osmotic stress alone reliably predicts PGP performance under drought remains unclear. Therefore, functional validation *in planta* is required to identify bacterial isolates with reliable potential to improve plant drought tolerance. In this context, the present study aimed to screen bacterial isolates from harsh soils for PEG-induced osmotic stress tolerance and subsequently evaluate

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their plant growth-promoting activities and ability to alleviate drought stress in *Chrysanthemum* under water-withholding conditions.

2. MATERIALS AND METHODS

2.1. Sample Collection and Plant Materials

Rhizosphere soil samples of various host plants were collected from different areas in Dien Bien, Vietnam (22°13'48.0"N, 102°42'25.2"E; 21°27'10.8"N, 103°15'46.8"E; and 21°23'34.8"N, 103°10'22.8"E).

Plant Materials: Uniform rooted cuttings of *Chrysanthemum* sp. "Cam Lua" were used in this study. The cuttings were obtained from mother plants originally propagated through tissue culture at the laboratory of Vietnam National University of Agriculture. The cuttings were rooted in sand for 7 days before the experiment. At the beginning of the experiment, the rooted cuttings were uniform in size, with a height of 4–5 cm and a root length of approximately 1 cm. The plants were maintained under natural daylight throughout the experimental period. No additional fertilizer was applied during the experiment.

2.2. Isolation and Screening of PEG-Tolerant Bacteria

Soil samples (1 g) were suspended in 9 ml of 0.85% (w/v) NaCl solution and serially diluted up to 10^{-7}. The diluted suspensions were subjected to an enrichment step in liquid Luria–Bertani (LB) medium supplemented with 15% (w/v) PEG 6000 to impose osmotic stress and incubated for 24 hours. Subsequently, 100 µl of the enriched cultures was spread onto LB agar plates containing 15% PEG 6000 and incubated at 30°C until colony formation. Distinct colonies were purified and further evaluated for their ability to tolerate osmotic stress by assessing biomass accumulation in LB medium supplemented with 20% PEG 6000, in comparison with growth in PEG-free LB medium (control). Cultures were incubated on a rotary shaker at 150 rpm for 48 hours at 30°C. Bacterial biomass was harvested by centrifugation, and the pellets were washed once with sterile RO water to remove residual culture medium and PEG 6000. The washed pellets were then collected by centrifugation and dried at 60°C to a constant weight. All experiments were conducted in triplicate for each strain. Osmotic stress responses were first assessed by comparing biomass accumulation under 20% PEG 6000 with that under PEG-free control conditions. Strains showing lower biomass accumulation under PEG than under the PEG-free control were considered sensitive to the applied osmotic stress. For preliminary screening, the response of each isolate was subsequently expressed as relative biomass increase, calculated as [(biomass under 20% PEG 6000 − biomass under PEG-free control) / biomass under PEG-free control] × 100. Isolates showing relative biomass increases of <100%, 100%–200%, and >200% were assigned to low, intermediate, and high relative biomass-increase categories, respectively. These categories were established as study-specific operational criteria based on the observed distribution of biomass responses and were not intended to represent statistically or biologically validated thresholds of PEG tolerance. Isolates showing a relative biomass increase of >200% were selected for subsequent plant growth-promotion assays.

2.3. Evaluation of the Plant Growth-Promoting Activities of PEG-Tolerant Bacterial Isolates

To evaluate the PGP effects of the selected bacterial strains on *Chrysanthemum*, 19 bacterial cultures were applied to soil during cultivation, and plant growth parameters were assessed after 4 weeks. The soil used in the experiment was a mixture of soil and coconut coir (1:1 w/w) and was sterilized prior to use. Each planting pot contained 200 g of the soil mixture. The experiment consisted of 20 treatments, including the control treatment with no bacterial inoculation and 19 treatments, each inoculated with a selected bacterial strain. Each treatment consisted of 10 pots, with two *Chrysanthemum* plants per pot (20 plants per treatment). The pot was considered the experimental unit. For statistical analysis, data from the two plants grown in the same pot were averaged and treated as one biological replicate ($n = 10$). Bacterial strains were initially cultured in liquid LB medium on a rotary shaker at 150 rpm and 35°C for 24 hours. The cultures were then subcultured into 100 ml fresh LB medium at a 1:100 (v/v) ratio and incubated under the same conditions to obtain actively growing cells. Bacterial cells were harvested by centrifugation at 10,000 rpm for 10 minutes, and the resulting biomass was resuspended in sterile water to an optical density of $OD_{600} = 0.5$. A volume of 2 ml of the bacterial suspension was applied to each pot. Bacterial inoculation was performed twice during the 4-week cultivation period. After 4 weeks, plant growth parameters were evaluated, including fresh biomass (mg plant^{-1}), dry biomass (mg plant^{-1}), plant height (cm), and root length (cm). Plant height and root length were measured using ImageJ software.

2.4. Evaluation of Drought-Stress Alleviation by Selected Bacterial Strains in *Chrysanthemum*

To evaluate the effects of the selected bacterial strains (L5, NG1, and SN1) on *Chrysanthemum* under water-withholding conditions, plants were grown and inoculated with bacterial strains during the first 2 weeks of cultivation. During the first 2 weeks of cultivation, each pot was irrigated daily with 30 ml of RO water. After that, irrigation was withheld for 15 consecutive days to impose water-deficit conditions. All pots contained the same amount of growth substrate, were maintained under identical greenhouse conditions, and were subjected to the water-withholding treatment simultaneously to minimize variation in water-deficit exposure among treatments. Soil and bacterial culture were prepared as described previously in the testing of growth promotion activity (section 2.3). The experiment consisted of four treatments, including the control (no bacterial inoculation) and three bacterial inoculation treatments (L5, NG1, and SN1). Each treatment consisted of 10 pots containing two plants per pot (20 plants per treatment). The experiment was conducted in three independent biological replications, resulting in a total of 60 plants per treatment. The pot was considered the experimental unit. Bacterial inoculation was performed by applying 2 ml of bacterial suspension ($OD_{600} = 0.5$) per plant during weeks 1 and 2. During the water-withholding treatment, greenhouse conditions were maintained at 30°C–32°C with a relative humidity of 75%–80%. After 15 days of water withholding, the plants were re-watered and allowed to recover for 16 days. The evaluated parameters included wilting rate (%), recovery rate (%), mortality rate (%), plant morphology, and fresh biomass (mg plant^{-1}). Data from the three independent biological replicates were used for statistical analysis. For fresh biomass, the biomass values of all 20 plants within each treatment in each independent biological replicate were averaged to obtain one biological observation, resulting in three observations per treatment ($n = 3$). For each independent biological replicate, wilting, recovery, and mortality percentages were calculated based on all 20 plants within each treatment. The results are presented as mean ± SD for percentage data and mean ± SE for fresh biomass ($n = 3$). For the longitudinal wilting data, a two-way repeated-measures analysis of variance (ANOVA) was performed, with bacterial treatment and assessment time as factors and time treated as the repeated-measures factor. The treatment × time interaction was included in the model, and the Greenhouse–Geisser correction was applied when the assumption of sphericity was not met. Dunnett's multiple-comparison test was

subsequently used to compare each bacterial treatment with the non-inoculated control at each assessment time. Adjusted p values < 0.05 were considered statistically significant.

2.5. Morphological and Biochemical Characteristics

Three selected bacterial strains were examined for colony morphology on nutrient agar medium, Gram staining, and biochemical traits related to plant-promotion activity. The indole acetic acid (IAA) production was quantified using the Salkowski colorimetric assay [14]. Bacterial strains were cultured in LB broth supplemented with 100 mg/l L-tryptophan and incubated at 30°C for 72 hours in the dark with shaking at 200 rpm. Culture supernatants were obtained by centrifugation (10,000 rpm, 10 minutes) and mixed with Salkowski reagent. After incubation in the dark for 30 minutes, absorbance was measured at 530 nm, and IAA concentration (μg ml^{-1}) was determined using a standard curve prepared with commercial IAA. The nitrogen-fixing ability of the selected bacterial strains was preliminarily assessed based on their growth on nitrogen-free Burk's solid medium [15]. The phosphate solubilization of selected bacteria was assessed by spot inoculating the bacterial strains onto Pikovskaya's agar medium and incubating at 35°C for 5 days [16]. Phosphate-solubilizing strains were qualitatively identified by the formation of a clear (white) halo zone surrounding the bacterial colonies. Qualitative siderophore production by the bacterial strains was determined following the method of Louden *et al.* [17]. The strains were cultured in liquid LB medium, and after 24 hours, the bacterial suspension was streaked onto Chrome Azurol S agar plates and incubated at 35°C for 24 hours. Strains capable of producing siderophores induced a color change in the medium from blue to yellow-orange around the colonies, indicating iron chelation.

2.6. Molecular Identification of Selected Bacterial Strains

For genomic DNA extraction, a single colony of each bacterial strain was inoculated into 5 ml of LB broth and incubated at 35°C for 48 hours. Cells were harvested by centrifugation at 10,000 × g for 5 minutes and subsequently used for genomic DNA extraction. Genomic DNA from the three selected bacterial strains was extracted using an adapted protocol based on the method described by Masoomi-Aladizgeh *et al.* [18]. Two modifications were introduced to adapt the procedure for bacterial cells: the incubation step at 65°C during cell lysis was extended to 30 minutes, and an additional extraction with phenol:chloroform:isoamyl alcohol (25:24:1, v/v/v) was performed. The organic solvent was added to the cell lysate at a 1:1 (v/v) ratio [19]. The resulting genomic DNA was used as the template for PCR amplification. The 16S rRNA gene was amplified using the universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). PCR amplification was performed in a 30 μl reaction mixture containing 15 μl of 2× PCR Master Mix, 0.5 μM of each primer, approximately 50 ng of genomic DNA, and nuclease-free water. The PCR program consisted of an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 53°C for 30 seconds, and extension at 72°C for 90 seconds, with a final extension at 72°C for 10 minutes. The amplified products were purified and sequenced by 1st BASE (Singapore). The resulting 16S rRNA gene sequences were deposited in GenBank under accession numbers PZ501659.1 (L5), PZ501658.1 (NG1), and PZ501657.1 (SN1). The sequences were compared with reference sequences available in the NCBI GenBank database using BLAST. Reference sequences of closely related species were selected for phylogenetic analysis. Multiple sequence alignment was performed using MUSCLE implemented in MEGA version 12. Phylogenetic relationships were inferred using the maximum likelihood method. The best-fit nucleotide substitution model was selected based on the Bayesian information criterion, with K2+I, HKY+G, and K2+G selected for L5, NG1, and SN1, respectively. The reliability of the inferred phylogenetic trees was assessed using 1,000 bootstrap replicates. Gaps and missing data were treated using the partial deletion option with a site coverage cutoff of 95%. *Proteus mirabilis* DSM4479 (AJ301682.1), *Paenibacillus polymyxa* DSM36 (NR117727.2), and *Acinetobacter calcoaceticus* ATCC23055 (Z93434.1) were used as outgroups for L5, NG1, and SN1, respectively. Phylogenetic analyses were conducted using MEGA version 12.

2.7. Data Analysis

Data were expressed as mean ± SE or SD, as indicated in the corresponding tables and figures. Statistical analyses were performed using GraphPad Prism version 9.3.1. For growth-promotion assays under well-watered conditions, differences among treatments were analyzed using one-way ANOVA followed by Tukey's multiple-comparison test. For post-rewatering fresh biomass, differences among treatments were analyzed using one-way ANOVA followed by Tukey's multiple-comparison test. For longitudinal wilting data, a two-way repeated-measures ANOVA was performed with bacterial treatment and assessment time as factors, followed by Dunnett's multiple-comparison test for comparisons with the non-inoculated control at each assessment time. Adjusted p values < 0.05 were considered statistically significant.

3. RESULTS

3.1. Screening of Bacterial Isolates for PEG Tolerance

A total of 45 bacterial isolates obtained from harsh soils were evaluated for their ability to grow under high osmotic stress induced by 20% PEG 6000 (Supplementary Table S1). Relative biomass increase was calculated by comparing biomass production under 20% PEG 6000 with that in PEG-free LB medium. The isolates showed substantial variation in relative biomass increase, ranging from 55.84% to 655%. Based on the study-specific operational screening criteria, 18 isolates showed a relative biomass increase of <100%, 8 isolates showed an increase of 100%–200%, and 19 isolates showed an increase of

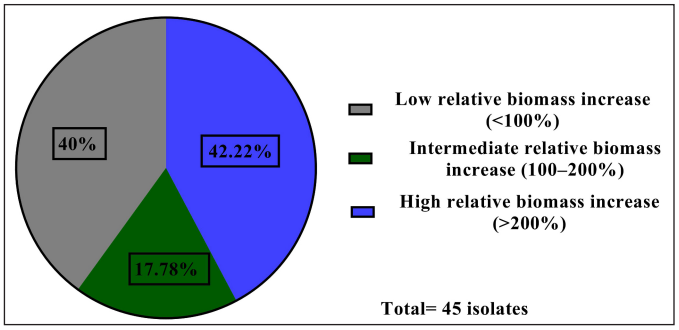

Figure 1. Distribution of the 45 bacterial isolates according to relative biomass increase under 20% PEG 6000. Relative biomass increase was calculated as [(biomass under 20% PEG 6000—biomass under PEG-free control conditions) / biomass under PEG-free control conditions] × 100. The three categories represent study-specific operational screening criteria based on the observed relative biomass responses and were not intended as statistically or biologically validated thresholds of PEG tolerance.

>200% (Fig. 1). The 19 isolates showing a relative biomass increase of >200% were selected for subsequent plant growth-promotion assays.

3.2. Plant Growth-Promoting Activity of PEG-Tolerant Bacterial Isolates

The 19 isolates showing a relative biomass increase of >200% were screened for their PGP ability on *Chrysanthemum* plantlets under pot conditions. Comparing growth parameters between the control plants (no added bacteria) and treatment plants (supplemented with bacteria), only three bacterial strains were found to enhance the growth of *Chrysanthemum* (Table 1, Fig. 2), whereas the remaining 16 isolates showed no statistically significant differences for any of the measured growth parameters (Supplementary Table S2). The strains that promoted growth of *Chrysanthemum* plants after 4 weeks of cultivation were L5, NG1, and SN1. In detail, all three

Table 1. The effect of selected bacterial strains on *Chrysanthemum* growth under well-watered conditions (after 4 weeks of cultivation).

Treatments (inoculated with strain)	Plant height (cm) ± SE	Number of leaves per plant ± SE	Fresh biomass (mg plant^{-1}) ± SE	Dry biomass (mg plant^{-1}) ± SE
Control (water)	12.50^{a} ± 0.66	9.70^{a} ± 0.47	1989^{a} ± 100	328^{a} ± 20
T1 (inoculated L5)	17.91^{c} ± 0.49	12.00^{b} ± 0.44	3650^{b} ± 280	555^{b} ± 40
T2 (inoculated NG1)	14.91^{b} ± 0.50	10.40^{a} ± 0.30	3503^{b} ± 160	637^{c} ± 50
T3 (inoculated SN1)	14.55^{b} ± 0.49	10.30^{a} ± 0.36	3421^{b} ± 210	556^{b} ± 30

Means marked with different letters are significantly different from one another at $p < 0.05$, $n = 10$.

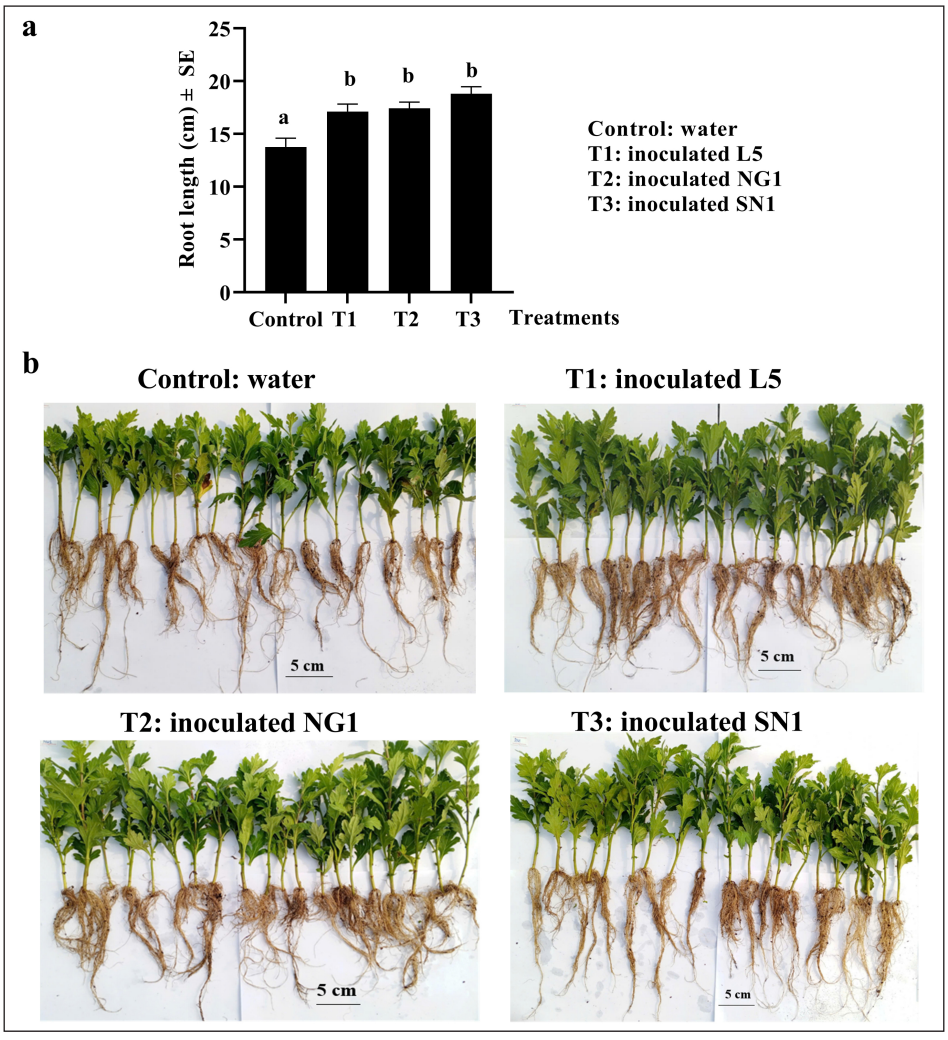

Figure 2. Effect of selected bacterial strains on root length (a) and plant morphology (b) of *Chrysanthemum* under well-watered conditions after 4 weeks of cultivation. Treatments included the non-inoculated control and plants inoculated with strains L5 (T1), NG1 (T2), or SN1 (T3). Bars represent the mean ± SE ($n = 10$ biological replicates). Different letters indicate significant differences among treatments according to one-way ANOVA followed by Tukey's multiple comparison test ($p < 0.05$).

strains significantly stimulated stem elongation, with strain L5 showing the greatest effect, resulting in a mean plant height of 17.91 ± 0.49 cm per plant. Regarding the number of leaves per plant, only plants treated with strain L5 showed a significant increase compared with the control and the NG1- and SN1-treated plants. In addition, both the fresh biomass and the dry biomass of plants grown in soil supplemented with bacterial cultures were higher than those of the control, and these differences were statistically significant ($p < 0.05$). Interestingly, the treatment with strain NG1 increased plant dry biomass more than those with treatments of strains L5 and SN1. Furthermore, plants inoculated with strains L5, NG1, or SN1 showed increased root length and distinct root morphological characteristics compared with the control (Fig. 2). Notably, *Chrysanthemum* plants treated with strain L5 exhibited a distinct root architecture, characterized by a greater number of lateral roots compared with the other treatments (Fig. 2b).

3.3. Effects of Selected Bacterial Strains on *Chrysanthemum* Under Water-Withholding Conditions

To investigate the effects of the selected bacterial strains on *Chrysanthemum* under water-withholding conditions, plants were grown under well-watered conditions with or without bacterial inoculation for 2 weeks. Irrigation was subsequently withheld for 15 consecutive days. Quantitative assessments of wilting were performed on days 9, 12, and 15 after irrigation was withheld (Fig. 3). On day 9, 23.33% of the control plants were wilted, whereas no wilting was recorded in any of the bacterial-inoculated treatments. On day 12, the wilting rate of the control increased to 33.33%, while no wilting was observed in plants inoculated with L5, NG1, or SN1. By day 15, the wilting rate in the control reached 66.67%, whereas the bacterial-inoculated treatments showed significantly lower wilting rates of 40.00% for L5 and 33.33% for both NG1 and SN1 (Fig. 3). These results indicate that inoculation with the selected bacterial strains

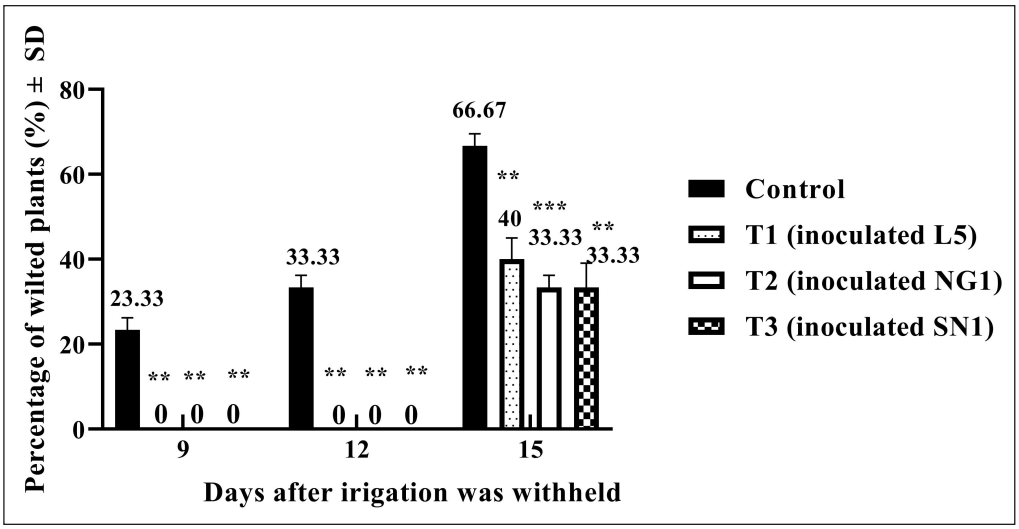

Figure 3. Percentage of wilted *Chrysanthemum* plants during the water-withholding treatment. Values represent mean ± SD ($n = 3$ independent biological replicates). Each biological replicate comprised 10 pots per treatment, with two plants per pot, and wilting percentage was calculated from the 20 plants within each biological replicate. Asterisks indicate significant differences from the non-inoculated control within each assessment time, based on Dunnett's multiple-comparison test (**$p < 0.01$, ***$p < 0.001$).

Table 2. Effect of bacterial strains on the recovery ability and biomass of *Chrysanthemum* after 16 days of re-watering.

Treatments	Recovery rate ± SD (%)	Mortality rate ± SD (%)	Fresh biomass (mg plant^{-1}) ± SE	Biomass increase compared to control (%)
Control (water)	86.67 ± 2.89	13.33 ± 2.89	2420^{a} ± 140	-
T1 (inoculated L5)	100	0	3610^{c} ± 150	49.17
T2 (inoculated NG1)	100	0	3100^{b} ± 160	28.10
T3 (inoculated SN1)	100	0	4100^{d} ± 280	69.42

Means marked with different letters are significantly different from one another at $p < 0.05$, $n = 3$.

Table 3. Plant growth promotion characteristics of selected bacterial strains.

Number	Bacterial strains	Nitrogen fixation	Phosphate solubilization	Siderophore production	IAA production (µg ml^{-1}) ± SD
1	L5	+	+	+	70.30 ± 1.34
2	NG1	–	+	+	17.68 ± 0.78
3	SN1	+	–	–	60.61± 2.42

Note: (+) : Positive; -: Negative.

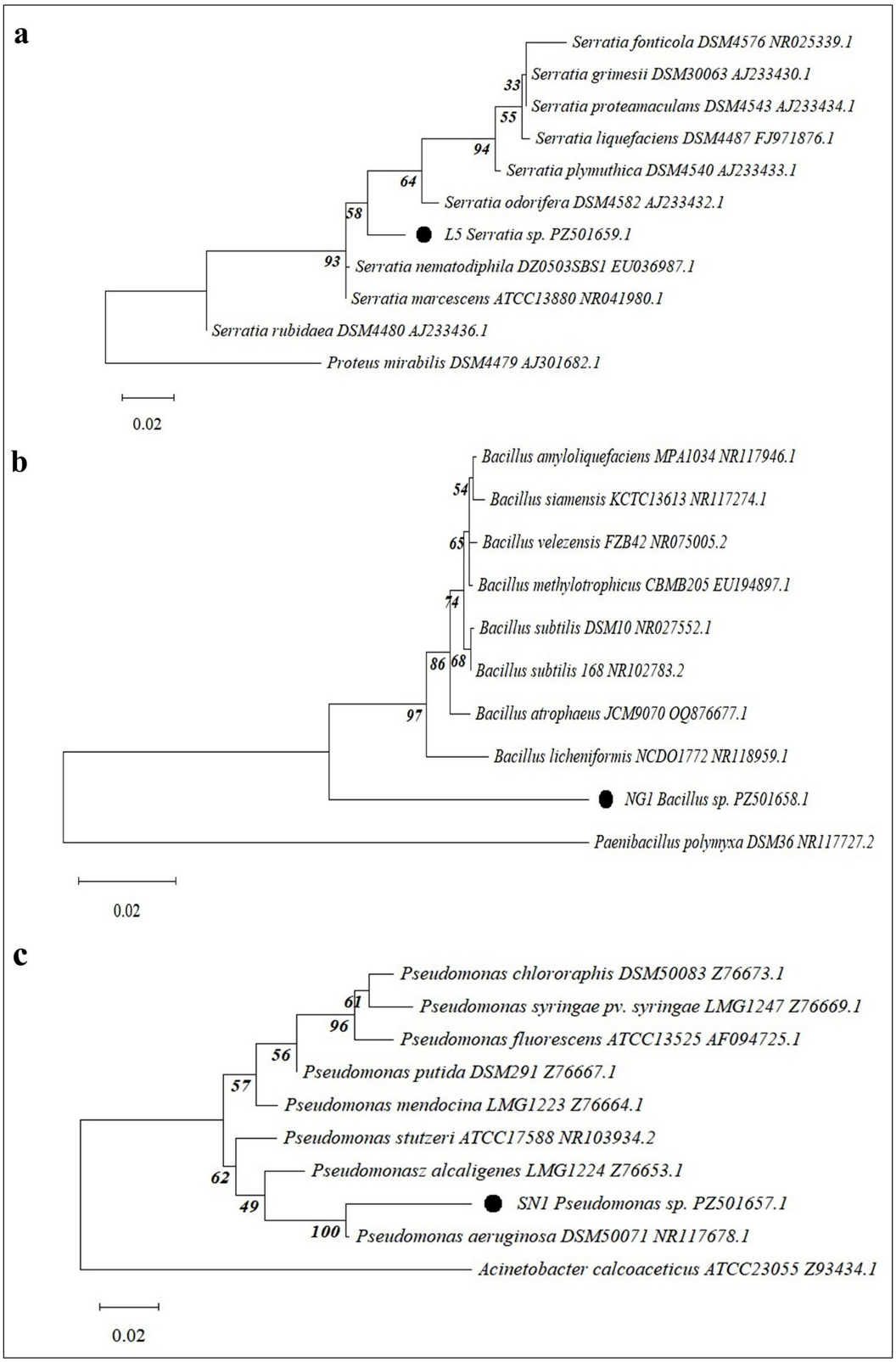

Figure 4. Phylogenetic analysis of the selected bacterial isolates based on 16S rRNA gene sequences. Phylogenetic trees were constructed using the maximum likelihood method for *Serratia* sp. L5 (a), *Bacillus* sp. NG1 (b), and *Pseudomonas* sp. SN1 (c). The trees were inferred using the K2+I, HKY+G, and K2+G nucleotide substitution models for L5, NG1, and SN1, respectively, with 1000 bootstrap replicates. Bootstrap values (%) are shown at the corresponding nodes. *Proteus mirabilis* DSM4479 (AJ301682.1), *Paenibacillus polymyxa* DSM36 (NR117727.2), and *Acinetobacter calcoaceticus* ATCC23055 (Z93434.1) were used as outgroups for panels (a), (b), and (c), respectively. The black circles indicate the isolates obtained in this study. The scale bar represents 0.02 nucleotide substitutions per site.

delayed wilting and reduced the proportion of wilted plants during water withholding.

The recovery ability of the plants after drought stress and subsequent re-watering was also evaluated (Table 2). In the control treatment, 86.67% of the plants survived and recovered, whereas 13.33% died and failed to recover. In contrast, all plants inoculated with L5, NG1, or SN1 exhibited a 100% recovery rate after re-watering. Furthermore, plants inoculated with L5, NG1, and SN1 showed significantly higher fresh biomass than the non-inoculated control after 16 days of re-watering. Specifically, fresh biomass increased by 49.17%, 28.10%, and 69.42% following inoculation with L5, NG1, and SN1, respectively (Table 2).

3.4. Plant Growth-Promoting Traits of Selected Bacterial Strains

To further characterize the three selected bacterial strains, their PGP traits, including nitrogen fixation, phosphate solubilization, siderophore production, and IAA production, were assessed. The results in Table 3 showed that all three strains had different characteristics and exhibited at least two biochemical traits associated with PGP activity. Bacterial strain L5 had all four tested traits and exhibited the highest IAA production (70.30 µg ml^{-1}) among the 3 bacterial strains. The strain NG1 displayed phosphate-solubilizing activity, siderophore production, and IAA production. In contrast, the strain SN1 had only nitrogen-fixing activity and IAA production.

3.5. Identification of the Selected Bacterial Strains

Phylogenetic analysis based on 16S rRNA gene sequences confirmed that L5, NG1, and SN1 belonged to the genera *Serratia*, *Bacillus*, and *Pseudomonas*, respectively (Fig. 4). L5 was positioned within the *Serratia* lineage and was phylogenetically close to *S. odorifera*, although the relevant node showed moderate bootstrap support (58%). NG1 was located within the *Bacillus* lineage but formed a distinct branch from the reference species included in the analysis. SN1 clustered with *P. aeruginosa* DSM50071 with strong bootstrap support (100%). Because the phylogenetic analyses were based on the 16S rRNA gene alone, the three isolates were conservatively designated as *Serratia* sp. L5, *Bacillus* sp. NG1, and *Pseudomonas* sp. SN1.

4. DISCUSSION

In this study, a sequential screening strategy was employed to identify PEG-tolerant bacterial strains with plant growth-promoting and drought-stress-alleviating activities. PEG-induced osmotic stress was used as an initial screening criterion because PEG 6000 reduces water availability in culture media, providing a controlled approach for evaluating bacterial osmotic-stress tolerance [20]. However, PEG-based screening primarily evaluates the ability of bacterial isolates to tolerate osmotic stress and does not fully capture the complex conditions of the plant–soil system under drought. In soil, drought-induced changes in water availability can alter microbial communities and soil processes, which may influence plant performance [21]. This limitation was also reflected in our results. The three selected strains promoted *Chrysanthemum* growth in the separate well-watered screening experiment and improved plant performance during and after the water-withholding treatment. These findings highlight the importance of combining laboratory osmotic stress screening with plant-based evaluation to identify potential bacterial strains. Therefore, PEG tolerance should be interpreted as an indicator of bacterial osmotic adaptation rather than a reliable predictor of PGP performance under drought conditions.

Three selected strains, *Serratia* sp. L5, *Bacillus* sp. NG1, and *Pseudomonas* sp. SN1, consistently promoted *Chrysanthemum* growth under both well-watered and drought conditions (Tables 1 and 2). Among the PGP traits evaluated in the present study, IAA production was the only characteristic shared by all three strains (Table 3). IAA is a key phytohormone that modulates root architecture, enabling plants to develop more extensive root systems for water and nutrient acquisition under water-limited conditions [22]. Consistent with this role, inoculated plants exhibited significantly greater root length and lateral root formation than the non-inoculated control, accompanied by increases in plant height and biomass (Table 1 and Fig. 2). Similar responses have been reported in other crops, where bacterial IAA production promoted root development and improved plant performance under water-limited conditions [11,23]. In the present study, the shared ability of all three strains to produce IAA, together with the observed enhancement of root development, suggests a possible association between bacterial IAA production and the growth-promoting effects. However, the present data do not establish a causal mechanism, and additional studies investigating bacterial colonization, endogenous phytohormone dynamics, and other physiological responses will be required to confirm the underlying mechanisms.

Despite sharing IAA production, the three selected isolates differed in their other PGP characteristics, indicating diversity in their potential PGP functions (Table 3). *Serratia* sp. L5 and *Bacillus* sp. NG1 exhibited phosphate-solubilizing and/or siderophore-producing abilities, indicating that these isolates possess additional PGP characteristics. Phosphate solubilization and siderophore production are important PGP traits that can improve nutrient acquisition and contribute to plant tolerance to drought stress through multiple indirect mechanisms [11,24]. In addition, *Serratia* sp. L5 exhibited growth on nitrogen-free Burk's medium, suggesting a potential nitrogen-fixing capacity and a broader repertoire of PGP traits. However, the phenotypic assays performed in this study provide only preliminary evidence of these traits and do not demonstrate their functional expression in planta. Interestingly, although *Pseudomonas* sp. SN1 exhibited only putative nitrogen-fixing ability and IAA production among the traits evaluated, it produced the greatest increase in plant biomass after drought recovery. This finding suggests that the biochemical traits evaluated in the present study alone cannot fully explain the superior performance of this strain, indicating that additional bacterial traits and mechanisms not examined in this study may also contribute to its performance under drought conditions. Collectively, these observations suggest that the performance of individual strains under drought conditions is likely influenced by the combined action of multiple bacterial traits, including mechanisms that were not evaluated in the present study.

Overall, the present study highlights the value of a stepwise screening strategy for identifying PEG-tolerant bacterial strains with consistent plant growth-promoting and drought-stress-alleviating activities. By integrating laboratory-based osmotic stress screening with the evaluation of PGP traits and functional validation in planta, this approach enabled the identification of bacterial strains with demonstrated effects on plant performance under water-withholding conditions. Beyond identifying promising PGPR candidates for further evaluation in *Chrysanthemum* cultivation, these findings emphasize that successful selection of beneficial microorganisms should not rely on osmotic stress tolerance or individual PGP traits alone, but rather on their overall functional performance in association with the host plant. An additional limitation of the present study is that the water-withholding experiment did not include concurrent well-watered controls for each bacterial treatment. Therefore, the present design does not allow direct statistical testing of a water

regime × bacterial treatment interaction. The separate well-watered screening experiment was conducted independently and should not be interpreted as a factorial control. Accordingly, the effects observed in the water-withholding experiment should be interpreted as differences among bacterial treatments under a common water-deficit regime rather than as evidence of a statistically tested interaction between bacterial inoculation and water regime. Nevertheless, this study was limited to preliminary screening and greenhouse validation. Therefore, the physiological and biochemical mechanisms underlying bacterial-mediated drought-stress alleviation were beyond the scope of the present study and remain to be elucidated. In addition, although a sterilized soil–coconut coir substrate was used throughout the greenhouse experiment, soil physicochemical properties, including pH, electrical conductivity, nutrient status, organic carbon content, and water-holding capacity, were not determined in the present study. These factors may influence plant–microbe interactions and should be considered in future investigations.

5. CONCLUSION

This study highlighted the value of integrating PEG-induced osmotic stress screening with functional evaluation *in planta* for the selection of bacterial strains with plant growth-promoting and drought-stress-alleviating activities. Using this approach, three bacterial isolates, namely, *Serratia* sp. L5, *Bacillus* sp. NG1, and *Pseudomonas* sp. SN1, were selected as promising candidates after consistently enhancing *Chrysanthemum* growth and improving plant recovery following the water-withholding treatment. Although all three isolates shared the ability to produce IAA, they differed in other PGP characteristics, suggesting that their beneficial effects may result from the combined contribution of multiple functional traits rather than any single characteristic. The present study was limited to preliminary screening and greenhouse validation, and therefore did not investigate the detailed physiological and biochemical mechanisms underlying bacterial-mediated drought-stress alleviation. Future studies should elucidate these mechanisms, including plant physiological responses and additional bacterial functional traits, evaluate the effectiveness of the selected strains under field conditions, and conduct comprehensive taxonomic and biosafety assessments before their practical application as microbial inoculants for sustainable *Chrysanthemum* production under water-limited environments.

6. AUTHOR'S CONTRIBUTION

All authors made substantial contributions to the 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 the article to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All authors are eligible to be authors as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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8. CONFLICTS OF INTEREST

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

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All the data are available with the authors and shall be provided upon request.

11. PUBLISHER'S NOTE

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12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that they have not used artificial intelligence (AI) tools for writing and editing of the manuscript, and no images were manipulated using AI.

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SUPPLEMENTARY MATERIAL

Supplementary Table S1. PEG 6000-induced osmotic stress tolerance screening of 45 bacterial isolates.

No	Isolate	Relative biomass increase (%) ± SD	Operational screening category
1	L1	157.80 ± 4.80	Intermediate relative biomass increase
2	L2	74.96 ± 2.67	Low relative biomass increase
3	L3	141.1 ± 13.75	Intermediate relative biomass increase
4	L4	132.3 ± 8.92	Intermediate relative biomass increase
5	L5	273.5 ± 3.83	High relative biomass increase
6	L6	73.07 ± 4.99	Low relative biomass increase
7	L7	83.7 ± 6.13	Low relative biomass increase
8	SN1	270.7 ± 4.45	High relative biomass increase
9	SN2	128.1 ± 5.87	Intermediate relative biomass increase
10	SN3	77.25 ± 8.52	Low relative biomass increase
11	SN4	59.45 ± 3.44	Low relative biomass increase
12	SN5	178.3 ± 6.94	Intermediate relative biomass increase
13	SN6	153.8 ± 3.47	Intermediate relative biomass increase
14	SN7	84.28 ± 7.12	Low relative biomass increase
15	NG1	228.6 ± 6.48	High relative biomass increase
16	NG2	386.9 ± 8.44	High relative biomass increase
17	NG3	265.9 ± 8.12	High relative biomass increase
18	NG4	223.6 ± 4.80	High relative biomass increase
19	NG5	72.04 ± 4.70	Low relative biomass increase
20	NG6	189.6 ± 2.93	Intermediate relative biomass increase
21	Đ1	55.84 ± 4.46	Low relative biomass increase
22	Đ2	75.91 ± 5.32	Low relative biomass increase
23	Đ3	655 ± 3.80	High relative biomass increase
24	Đ4	453.6 ± 8.70	High relative biomass increase
25	Đ5	68.83 ± 3.35	Low relative biomass increase
26	Đ6	114.5 ± 5.95	Intermediate relative biomass increase
27	H1	99.07 ± 5.32	Low relative biomass increase
28	H2	402.3 ± 3.80	High relative biomass increase
29	H3	89.31 ± 5.85	Low relative biomass increase
30	H4	80.2 ± 2.93	Low relative biomass increase
31	H5	523.3 ± 13.22	High relative biomass increase
32	H6	322.2 ± 7.59	High relative biomass increase
33	H7	73.62 ± 4.29	Low relative biomass increase
34	H8	502.8 ± 7.59	High relative biomass increase
35	H9	88.64 ± 2.64	Low relative biomass increase
36	H10	89.05 ± 3.80	Low relative biomass increase
37	SĐ1	71.6 ± 13.22	Low relative biomass increase
38	SĐ2	334.3 ± 17.22	High relative biomass increase
39	SĐ3	257.1 ± 18.94	High relative biomass increase
40	SĐ4	644.9 ± 7.59	High relative biomass increase
41	SĐ5	248.6 ± 8.60	High relative biomass increase
42	SĐ6	97.27 ± 5.80	Low relative biomass increase
43	SĐ7	308.3 ± 7.04	High relative biomass increase
44	SĐ8	299.4 ± 9.26	High relative biomass increase
45	SĐ9	281.53 ± 2.64	High relative biomass increase

Note: Relative biomass increase was calculated as [(biomass under 20% PEG 6000—biomass under PEG-free control) / biomass under PEG-free control] × 100. Operational screening categories were defined as low relative biomass increase (<100%), intermediate relative biomass increase (100%–200%), and high relative biomass increase (>200%).

Supplementary Table S2. Effects of bacterial inoculation on *Chrysanthemum* growth under non-stressed conditions after 4 weeks of treatment.

Treatments (inoculated with strain)	Plant height (cm) ± SE	Number of leaves/plant ± SE	Fresh biomass (mg plant^{-1}) ± SE	Dry biomass (mg plant^{-1}) ± SE
Control (water)	12.50^{a} ± 0.66	9.70^{a} ± 0.47	1989^{a} ± 100	328^{a} ± 20
L5	17.91^{c} ± 0.49	12.00^{b} ± 0.44	3650^{b} ± 280	555^{b} ± 40
NG1	14.91^{b} ± 0.50	10.40^{a} ± 0.30	3503^{b} ± 160	637^{c} ± 50
SN1	14.55^{b} ± 0.49	10.30^{a} ± 0.36	3421^{b} ± 210	556^{b} ± 30
Đ3	12.13^{a} ± 0.34	9.75^{a} ± 0.34	1926^{a} ± 80	312^{a} ± 10
Đ4	12.09^{a} ± 0.34	9.75^{a} ± 0.41	1977^{a} ± 20	329^{a} ± 20
NG2	12.09^{a} ± 0.35	9.15^{a} ± 0.56	1926^{a} ± 70	320^{a} ± 10
NG3	12.18^{a} ± 0.31	9.30^{a} ± 0.52	2313^{a} ± 60	384^{a} ± 30
NG4	12.34^{a} ± 0.22	9.56^{a} ± 0.21	2086^{a} ± 40	357^{a} ± 30
H2	12.06^{a} ± 0.35	9.60^{a} ± 0.31	2340^{a} ± 170	376^{a} ± 40
H5	11.82^{a} ± 0.24	9.25^{a} ± 0.39	2085^{a} ± 60	327^{a} ± 30
H6	12.04^{a} ± 0.32	9.65^{a} ± 0.26	1973^{a} ± 40	325^{a} ± 30
H8	11.94^{a} ± 0.42	9.84^{a} ± 0.41	2124^{a} ± 50	342^{a} ± 10
SĐ2	12.15^{a} ± 0.37	10.15^{a} ± 0.37	1802^{a} ± 60	316^{a} ± 10
SĐ3	12.32^{a} ± 0.28	10.37^{a} ± 0.51	2088^{a} ± 40	355^{a} ± 30
SĐ4	11.84^{a} ± 0.31	9.65^{a} ± 0.38	1934^{a} ± 60	335^{a} ± 20
SĐ5	12.60^{a} ± 0.41	10.10^{a} ± 0.37	2258^{a} ± 60	358^{a} ± 30
SĐ7	11.74^{a} ± 0.41	9.60^{a} ± 0.30	1875^{a} ± 90	255^{a} ± 20
SĐ8	12.17^{a} ± 0.37	9.85^{a} ± 0.42	1970^{a} ± 60	364^{a} ± 30
SĐ9	12.44^{a} ± 0.12	9.52^{a} ± 0.50	1997^{a} ± 40	324^{a} ± 30

Means followed by different letters within the same column are significantly different from each other ($p < 0.05$, $n = 10$ pots per treatment).