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Research Article

PGPB Consortium Promotes Drought Tolerance in *Capsicum frutescens*

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ABSTRACT

Objectives: This study evaluated the potential of a bacterial consortium-*Bacillus cereus, Micrococcus luteus, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*-to enhance drought tolerance in *Capsicum frutescens* L. seedlings. **Materials and Methods:** Conducted from March to April 2025 at Cavite State University, the experiment followed a Randomized Complete Block Design (RCBD) with 150 seedlings grown under controlled watering and drought conditions. Morphological parameters were measured on Day 0 (baseline), Day 7 (after watering), Day 14 (after drought), and Day 17 (after recovery). **Results:** A marked decline in growth by Day 14 confirmed successful drought induction. By Day 17, significant improvements in plant height, root length, stem girth, total number of fully expanded leaves, leaf size, number of fully expanded leaves, and biovolume index were observed in inoculated treatments compared to controls. Although physiological traits and microbial load quantification did not show statistically significant differences, a clear biological trend was evident-plants inoculated with the consortium, under both drought and non-drought conditions, consistently showed higher values. **Conclusion:** These findings suggest that the bacterial consortium can enhance drought resilience in *C. frutescens* seedlings, supporting its potential as a biofertilizer for use in stress-prone agricultural systems in the Philippines. Recommendations include standardizing inoculation and sampling methods, increasing replicates, and incorporating additional physiological and biochemical stress indicators.

Keywords: Biofertilizer, *Capsicum frutescens*, Consortium, Drought.

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INTRODUCTION

Siling Labuyo (*Capsicum frutescens* L.) is an economically important chili well-adapted to the Philippines.[1,2] However, climate change and abiotic stresses-such as drought, water scarcity, and extreme temperatures-have reduced crop productivity and soil viability.[3-6]

Plant Growth-Promoting Bacteria (PGPB), particularly in consortia, have shown potential in enhancing drought tolerance. Strains from *Bacillus, Micrococcus, Klebsiella*, and *Pseudomonas* exhibit PGP traits that support plant growth under stress.[7-9] *Bacillus cereus* improves relative water content, chlorophyll content, and root length,[10] while *Pseudomonas aeruginosa* and *Micrococcus luteus* promote growth and offer biocontrol through antifungal activity.[11-15] *Klebsiella pneumoniae* contributes by fixing nitrogen and enhancing resistance to root-related diseases.[16,17] Together, these bacteria enhance drought tolerance by regulating hormones, sustaining nutrient levels, and strengthening plant defenses.[7]

This study aimed to evaluate the potential of a bacterial consortium-*Bacillus cereus, Micrococcus luteus, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*-in enhancing drought tolerance in *Capsicum frutescens* L. Specifically, it assessed seedling drought tolerance, examined morphological traits (plant height, root length, stem girth, leaf number and size, biovolume index), evaluated physiological responses (relative water and chlorophyll content), and quantified rhizosphere microbial load after drought stress.

MATERIALS AND METHODS

Research Design

This study employed a Randomized Complete Block Design (RCBD) to control variability by grouping treatments into three blocks, each with five plots. Treatments were randomly assigned, with each plot containing ten seedlings (Table 1).

Bacterial Strains and Culture Conditions

Four PGPB strains-*Bacillus cereus, Micrococcus luteus, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*-were sourced

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from the Philippine National Collection of Microorganisms (PNCM-UPLB). Using sterile loops under a biosafety cabinet, each strain was streaked on Nutrient Agar (NA) and incubated at 30ºC for 24 hr to promote optimal growth.[18,19]

In vitro Compatibility Testing of PGPB Strains

An *in vitro* compatibility assay was conducted to evaluate interactions among *B. cereus* (B1), *M. luteus* (B2), *K. pneumoniae* (B3), and *P. aeruginosa* (B4). Approximately 15 mL of sterilized NA was poured into 9 cm petri dishes and allowed to solidify. Each strain was cross-streaked following the method of Al-Daghari *et al.*,[20] and plates were incubated at 28±2ºC for 72 hr.

The test, performed in triplicates, aimed to detect antagonism (e.g., inhibition zones) or synergism (e.g., enhanced growth at intersections). Strains showing antagonistic activity, inconsistent growth, or potential pathogenicity were excluded.[4]

Field Trials under Polyhouse Conditions

Cultivation of Siling Labuyo Seedlings

Four-week-old seedlings were sourced from the Bureau of Plant Industry (BPI) to ensure authenticity and were transplanted to the Cavite State University Technology Demonstration Farm. The experiment was conducted in a controlled polyhouse with a translucent roof and exhaust fans to maintain adequate light and airflow.

A total of 150 seedlings were used, divided into three replicates per treatment, with ten seedlings per replicate. Each seedling was planted in an 18.5×15 cm pot filled with pure loam soil. Distilled water (50 mL) was applied regularly. Plant height, stem girth, leaf size, and total leaf count were measured before, during, and after treatment. Root length was measured only at the end of the experiment to avoid disturbing plant development and microbial interaction.

Inoculation of Consortium using Root

Drenching Method

Uniform pre-grown seedlings-same in age, height, leaf number, and health-were measured for plant height, root length, stem girth, leaf size, and total leaf count prior to transplantation for baseline comparison. For treatments T_2 and T_4, roots were soaked for 30 min in LB broth containing a bacterial consortium (25 mL each strain). T_1 (negative control) and T_3 seedlings were soaked in uninoculated LB broth.[4] For T_0 (positive control), BioGroe™ from UPLB-BIOTECH was prepared by mixing 100 g of BioGroe™ with 100 mL of distilled water (1:1), and roots were dipped in the solution for 5 min.[21]

Soil Moisture Content

Prior transplantation and after drought stress induction, soil moisture content was measured using 6-in-1 RCYAGO Soil Meter. The probe was inserted vertically to a 5-inch depth, and moisture levels were recorded as percentages: 10-20% (extremely dry), 30-40% (dry), 50-60% (moderate), 70-80% (wet), and 90-100% (extremely humid). Each setup was measured three times, and the average was calculated to ensure accuracy.

Transplantation

Seedlings were transplanted in the morning to minimize transplant shock, using pure loam soil composed of 40% sand, 40% silt, and 20% clay. They were watered for seven days to promote bacterial root colonization under experimental conditions.[4]

Sampling and Measurement Timeline for Morphophysiological Parameters

Initial morphological measurements were taken on Day 0 to establish baseline data under controlled polyhouse conditions. After transplantation, seedlings underwent a 7-day acclimatization period (Day 1-7) with regular watering. From Day 8 to 14, drought treatments involved water withholding, while control plants continued receiving water. Morphological traits were monitored throughout this period. On Day 17, following a 3-day rewatering recovery phase, both morphological and physiological parameters-including relative water content and leaf chlorophyll content-were measured. Seedlings in treatments T_0 (BioGroe™), T_3 (uninoculated with drought), and T_4 (inoculated with drought) experienced a 7-day drought period only, as prolonged water stress would risk mortality in control groups.[22]

Morphological Parameters Assessment and Biovolume Index Determination

Immediately after the drought period, all plants from each treatment replicate were evaluated for morphological traits: plant height, root length, stem girth, leaf size, and total leaf count. Plant height was measured from the stem base to the tallest shoot using a ruler, while root length was taken from the stem base to the root tip. Stem girth was measured by wrapping a thread around the stem, cutting it to fit, and aligning it with a measuring tape. Leaf size was estimated by multiplying the length and width of the largest fully developed leaf. The biovolume index was then calculated using the following formula:

$$BI = plant\ height \times stem\ girth$$

Physiological Parameters Assessment

After drought stress, seedlings were rewatered daily (50 mL/plot) for seven days, after which two plants per replicate were randomly selected for this.[22]

Relative Water Content

On the eighth day after the drought period, three fully expanded young leaves per replicate were sampled for RWC analysis. Fresh Weight (FW) was measured immediately, Turgid Weight (TW) after 4 hr in distilled water, and Dry Weight (DW) after 24 hr at 70°C.[22] RWC was calculated as:

$$RWC\% = \left(\frac{FW - DW}{TW - DW}\right)(100)$$

Leaf Chlorophyll Content

On the eighth day after the drought period, chlorophyll content was determined following the method of Yusuf and Hamed.[23] Under dim light and on ice to prevent degradation, 0.15 g of young expanded leaves was ground with 3.0 mL of 80% acetone. The extract was centrifuged at 10,000 rpm for 10 min, and absorbance of the supernatant was measured at 663 nm and 645 nm using a spectrophotometer. Total chlorophyll content was calculated using the following formula:

$$TCC\ (mg/gmFW) = \frac{20.2\ A_{645} + 8.02\ A_{663}\ (x)(v)}{(1000)(w)(a)}$$

Wherein: w=fresh weight (g); v=acetone volume (mL); and a=1.0 cm.

Microbial Load Quantification

On the 14th day post-drought, rhizosphere soil samples were collected to assess microbial load and the effect of the bacterial consortium. Roots from ten seedlings per treatment were shaken in sterile saline to dislodge attached soil. Serial dilutions up to 10^{-5} were prepared, and aliquots were plated on Plate Count Agar (PCA), a general-purpose medium.

PCA was prepared by dissolving 23.5 g of dehydrated powder in 1 L of distilled water, heated to boiling, then sterilized by autoclaving at 121°C for 15 min. The medium was cooled to 45-50°C before use.[24] Plates were incubated at 35°C for 24-48 hr, allowing colonies to form. Viable bacterial counts were expressed as Colony-Forming Units (CFU) per mL of dry soil, using colony counts within the 30-300 range.

Data Analysis

Field data were analyzed using ANOVA and Tukey's test ($p<0.05$) in JASP to assess the drought tolerance effect of the bacterial consortium on *C. frutescens* L.

RESULTS

In vitro Compatibility Testing of Drought-Tolerant PGPB Strains

The *in vitro* cross-streak compatibility test among *B. cereus, M. luteus, K. pneumoniae*, and *P. aeruginosa* showed no visible signs of antagonism after 72 hr of incubation at 28±2°C among different combinations or replicates of cross-streaks. As shown in Figure 1, all streaked bacteria grew consistently without forming any clear zones of inhibitions at the intersections points. Growth at the points of intersections was continuous and unaffected, indicating a lack of competitive or inhibitory interactions.

Soil Moisture Content

Figure 2.1 shows that on Day 0, soil moisture content ranged narrowly from 28.33% (T_4) to 29.33% (T_0), all within the "dry" range (30-40%), indicating well-drained soil. Despite the small range, ANOVA showed a significant difference (F=4.999, p=0.026). Tukey's HSD test revealed T_0 differed significantly from T_4, while T_1, T_2, and T_3 were statistically similar. This minor variation highlights the importance of checking moisture levels before stress induction.

Table 1: RCBD polyhouse experimental layout.

Block	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5
1	T_3	T_1	T_4	T_0	T_2
2	T_4	T_2	T_3	T_1	T_0
3	T_1	T_0	T_2	T_3	T_4

Treatments: T_0 - Inoculated with BioGroe™, with drought stress (Positive Control). T_1 - Uninoculated, with proper water (Negative Control). T_2 - Inoculated, with proper water. T_3 - Uninoculated, with drought stress. T_4 - Inoculated, with drought stress.

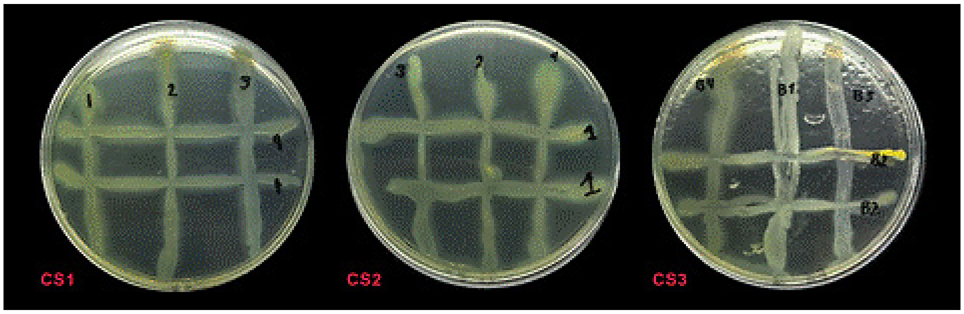


Figure 1: *In vitro* compatibility testing of PGPB strains.

Morphological Parameters Evaluation

Plant Height

As shown in Figure 2.2, at Day 0, plant heights across treatments were similar, ranging from 40.90 cm (T_0) to 41.24 cm (T_1), with no significant differences (F=1.524; *p*=0.283). By Day 7, after regular watering, slight height increases were seen, but differences remained non-significant (F=0.896; *p*=0.509). On Day 14, drought stress led to height reductions in T_0 (39.97 cm) and T_3 (36.75 cm), while T_2 (42.27 cm) and T_4 (43.92 cm) maintained or increased height, suggesting a possible early benefit of microbial treatment. Despite these trends, results were still statistically non-significant (F=2.211; *p*=0.158). By Day 17, microbial inoculation showed visible benefits, especially under adequate watering, as plants in treated groups exhibited better height recovery and overall vigor compared to untreated controls.

Root Length

As shown on Figure 2.3, at Day 0, all treatments showed similar root lengths, with no significant differences (F=1.232; *p*=0.357; CV=2.21%), indicating uniform initial development. By Day 17, root lengths increased across treatments, with T_2 showing the highest at 15.98 cm, followed by T_0 (15.13 cm), T_4 (14.94 cm), T_1 (13.57 cm), and T_3 (11.71 cm). A significant difference was observed among treatments (F=3.899; *p*=0.048).

Stem Girth

At Day 0, values were nearly uniform across treatments (0.78-0.79 cm), with no significant differences (F=1.353; *p*=0.331) (Figure 2.4). By Day 7, slight increases were observed, but differences remained non-significant (F=1.225; *p*=0.373). Significant changes emerged by Day 14 during drought stress (F=76.404; *p*=0.000) and persisted after rewatering on Day 17 (F=15.024; *p*=0.001), indicating a clear treatment effect.

Total Number of Fully Expanded Leaves

On Day 0, a significant difference in the number of fully expanded leaves was observed among treatments (*p*=0.004; CV=4.75), as shown in Figure 2.5. By Day 7, all treatments showed increased leaf numbers with no significant difference, indicating uniform growth. On Day 14, drought stress led to a decrease in leaf count, with T_4 highest (5.87) and T_0 lowest (2.60); differences were significant (*p*=0.023; CV=22.43%). By Day 17, T_4 remained highest (6.60), but differences were no longer significant (*p*=0.393; CV=25.08%).

Leaf Size

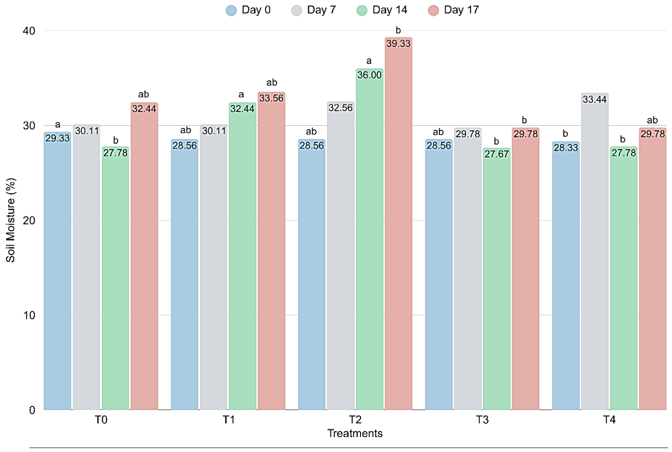
As shown in Figure 2.6, there were no significant differences in leaf size among treatments at Day 0 (F=0.499; *p*=0.738). By Day 7, slight increases were observed across all groups following consistent watering, but differences remained non-significant (F=2.051; *p*=0.180). On Day 14, after drought stress was applied, significant differences emerged (F=29.806; *p*<0.001). By Day 17, following rewatering, T_2 showed a marked advantage with a leaf size of 2466.78 cm^2-significantly larger than all other treatments (F=29.806; *p*<0.001).

Biovolume Index

After measuring plant height and stem girth, biovolume was calculated by multiplying both values. As shown in Figure 2.7, no significant differences were observed on Day 0 and Day 7 (F=1.414 and 1.098; *p*=0.313 and 0.420; CV=0.86% and 2.36%, respectively).

By Day 14 (drought period), significant differences emerged (F=119.288; *p*=0.000; CV=6.97%). T_0, T_3, and T_4 showed declines in biovolume, while T_1 slightly increased, and T_2 (inoculated+proper watering) showed a sharp rise to 80.77 cm^3.

On Day 17, following rewatering, biovolume increased across all treatments. T_2 remained highest (109.64 cm^3), followed



Means followed by a common letter are not significant at 5% Tukey's Test.

Figure 2.1: Soil Moisture Content (%) across different treatments.

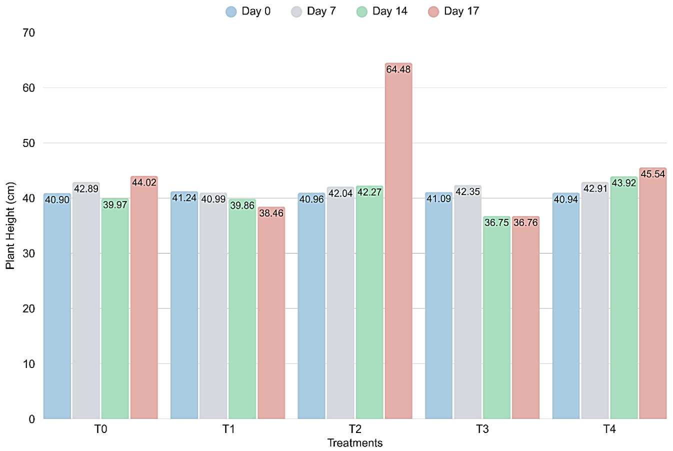


Figure 2.2: Plant height (cm) across different treatments.

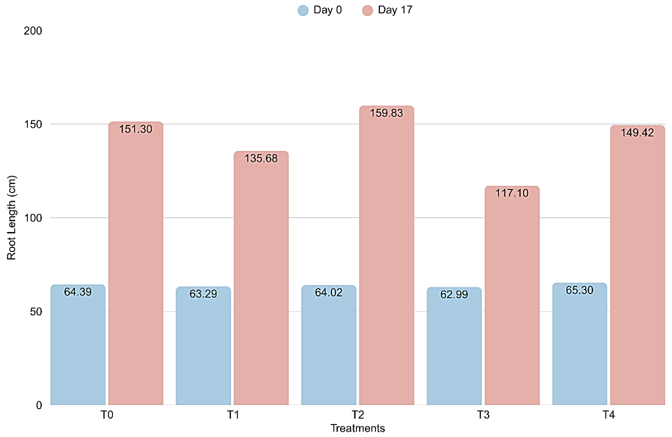


Figure 2.3: Root length (cm) across different treatments.

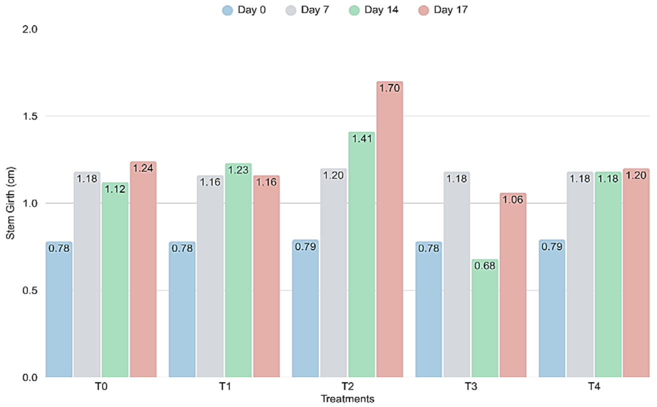
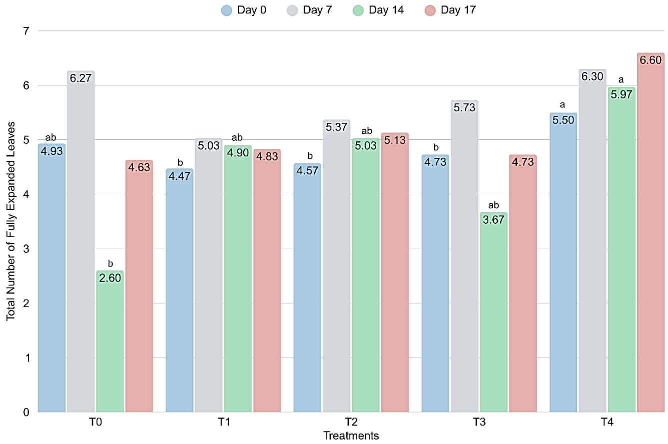
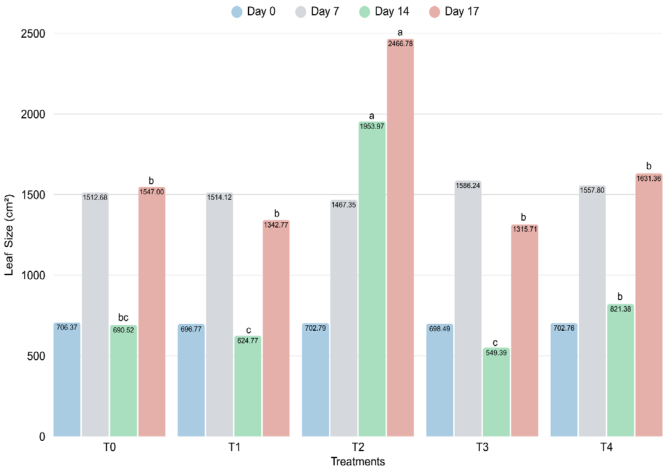


Figure 2.4: Stem Girth (cm) across different treatments.



Means followed by a common letter are not significant at 5% Tukey's Test.

Figure 2.5: Total Number of Fully Expanded Leaves across different treatments.



Means followed by a common letter are not significant at 5% Tukey's Test.

Figure 2.6: Leaf Size (cm^2) across different treatments.

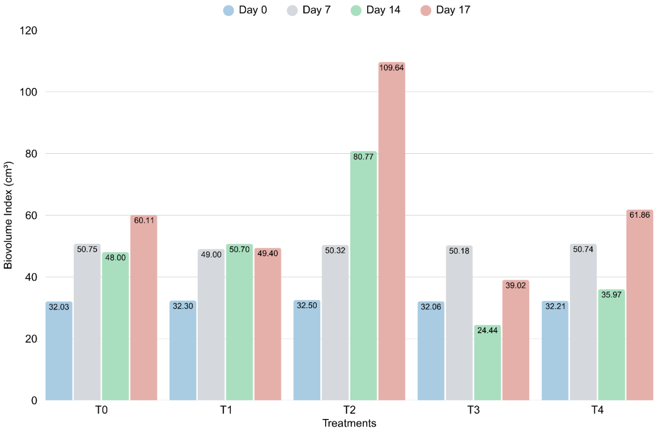


Figure 2.7: Biovolume Index (cm^3) across different treatments.

Figure 2: Morphological Parameters across different treatments.

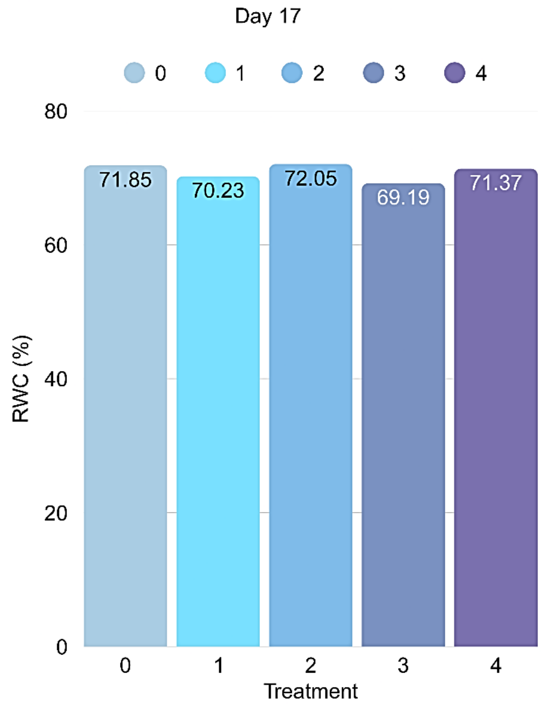


Figure 3.1: Relative Water Content (%) across different treatments.

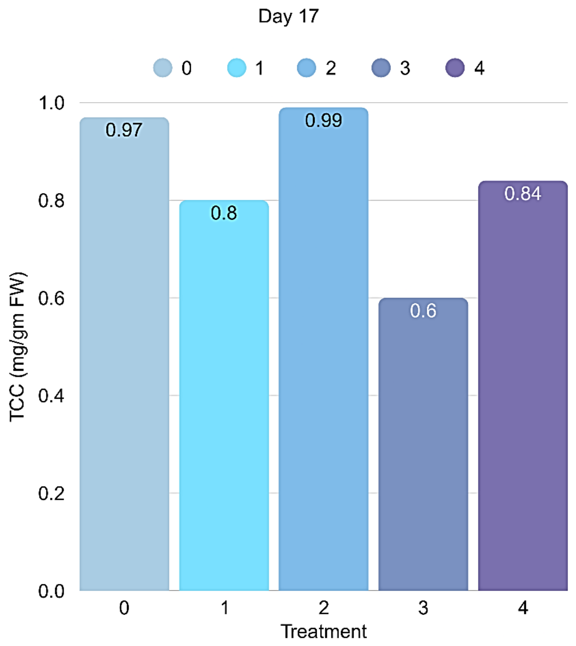


Figure 3.2: Total Chlorophyll Content (mg/gm FW) across different treatments.

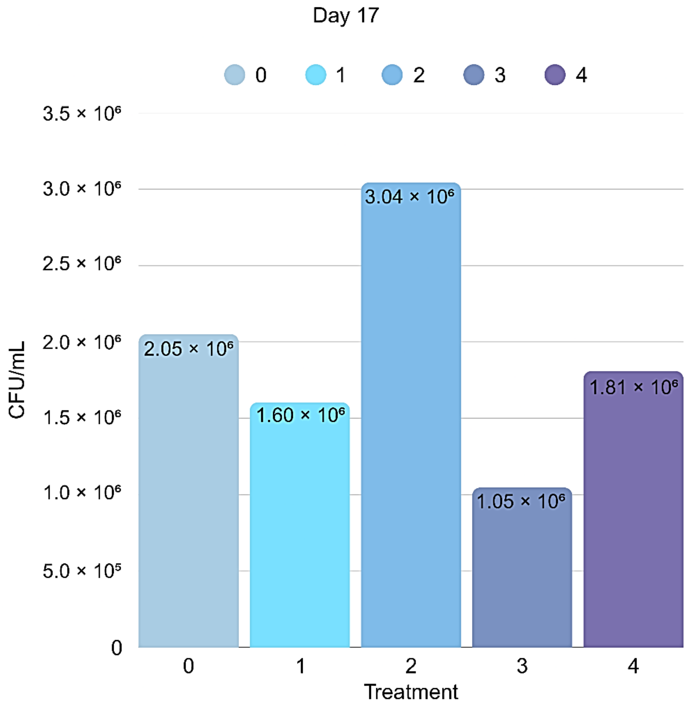


Figure 3.3: Microbial Load (CFU/mL) across different treatments.

Figure 3: Physiological Parameters across different treatments.

by T_4 (61.86), T_0 (60.11), T_1 (49.40), and T_3 (39.02), with results remaining statistically significant (F=20.667; *p*=0.000; CV=16.14%).

Evaluation of the Physiological Parameters

Relative Water Content

Relative Water Content (RWC) was measured to evaluate the seedlings' water-retention under drought and recovery, with statistical results shown in Figure 3.1. RWC values ranged narrowly from 69.19% to 72.05%, with T_2 (inoculated, non-stressed) showing the highest mean (72.05%), followed by T_0, T_4, T_1, and T_3 (lowest at 69.19%). Despite these variations, no significant differences were found:

(F=0.035; *p*=0.997)

Total Chlorophyll Content

The Total Chlorophyll Content (TCC) of Siling Labuyo leaves were assessed to evaluate the effect of the PGPB consortium on photosynthetic capacity under drought. As shown in Figure 3.2, treatments showed slight variations, with mean values ranging from 0.60 to 0.99 mg/g FW. T_2 (inoculated, well-watered) had the highest TCC (0.99 mg/g), followed by T_0 (0.97 mg/g) and T_4 (0.84 mg/g). T_1 had a slightly lower mean (0.80 mg/g), while T_3 (uninoculated, drought-stressed) recorded the lowest (0.60 mg/g). Despite these trends, differences were not statistically significant:

(F=0.520; *p*=0.724)

Quantification of Microbial Load in the Rhizosphere

The microbial load of the five treatments were quantified through the Colony Forming Unit (CFU) method, as shown on Figure 3.3.

As presented in Day 17, T_2 recorded the highest mean value (3.04×10^6) microbial load, followed by T_0, T_4, T_1, and T_3, which had the lowest mean value (1.60×10^6).

However, despite the visible differences in treatment means, the Analysis of Variance (ANOVA) revealed that the treatments had no statistically significant effect on microbial load, with an F-value of 0.804 and a P-value of 0.555-both exceeding the standard 0.05 threshold. Additionally, a Coefficient of Variation (CV) of 74.19% was obtained, indicating a high degree of variability within the data.

DISCUSSION

Microbial inoculation is a proven strategy for enhancing plant tolerance to abiotic stresses, including drought. In this study, the microbial consortium-*Bacillus cereus, Micrococcus luteus, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*-significantly influenced the morphological development of *C. frutescens* under both drought and well-watered conditions. Treatments T_2 (inoculated, well-watered) and T_4 (inoculated, drought-stressed) exhibited superior growth, indicating that microbial symbiosis helps alleviate water stress.

T_2 recorded the highest root length (15.98 cm), leaf size (2466.78 cm^2), and biovolume index (109.64 cm^3), while T_4 maintained relatively high values despite drought. These improvements align with previous findings that Plant Growth-Promoting Bacteria (PGPB) enhance root architecture and water uptake under stress.[24] The observed benefits may be attributed to bacterial production of phytohormones (e.g., IAA, cytokinins)[25] and exopolysaccharides that enhance water retention and root-soil contact.[26]

Though physiological metrics like Relative Water Content (RWC) and total chlorophyll content showed no significant differences, inoculated treatments trended higher (RWC up to 72.05%, chlorophyll up to 0.99 mg/g FW), suggesting modest enhancements in water status and photosynthetic capacity. These trends echo findings that small increases in RWC aid turgor maintenance[27] and that higher chlorophyll levels may result from bacterial antioxidants like catalases and superoxide dismutases.[28]

Microbial quantification showed increased CFUs in inoculated groups (up to 3.04×10^6 CFU/mL in T_2), supporting effective rhizosphere colonization, though not statistically significant. Still, higher microbial density often correlates with stronger growth promotion.[29] The consortium's diverse traits-including ACC deaminase activity, phosphate solubilization, and osmolyte production-likely contributed to stress mitigation.[30]

Bacterial inoculation may also aid osmotic adjustment through increased proline and glycine betaine levels,[31] and improve nutrient uptake under drought, especially phosphorus, via phosphate-solubilizing microbes.[32] These mechanisms collectively explain the improved morphological recovery under stress.

Consistent with prior studies, multi-strain inoculants provided greater drought resilience than single strains.[33] However, unlike some reports, our physiological parameters lacked statistical significance-possibly due to the short experimental duration, controlled greenhouse conditions, and limited replication, which may have constrained variability detection.

Ecologically and agronomically, microbial consortia offer dual benefits-enhancing growth under optimal and stressful conditions alike-which is crucial for *C. frutescens*, a drought-sensitive crop. Field application, however, must account for soil variability, native microbial competition, and environmental fluctuations that affect inoculant survival.

Study limitations include the 17-day observation window, a narrow focus on physiological and microbial metrics, and the controlled setting. Future research should include longer trials, biochemical stress markers (e.g., MDA, antioxidants, osmolytes), and field tests across varied agroecological zones.

In conclusion, the study supports the role of microbial consortia in improving drought tolerance in *C. frutescens* by enhancing root growth, maintaining biomass under stress, and supporting rhizosphere colonization-positioning them as a promising tool in sustainable drought management.

CONCLUSION

The study confirmed successful drought induction in *C. frutescens* seedlings, as reflected in their drought tolerance assessments. By Day 14 (drought phase) and Day 17 (recovery), significant differences in morphological traits (e.g., plant height, root length, stem girth, leaf size, number of fully expanded leaves, biovolume index) demonstrated the microbial consortium's role in enhancing drought tolerance and recovery. Inoculated treatments showed slightly higher Relative Water Content and Total Chlorophyll Content, and higher CFUs, though these were not statistically significant. Overall, the findings highlight the potential of microbial consortia in mitigating drought stress and promoting sustainable agriculture amid climate challenges.

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ABBREVIATIONS

PGPB: Plant growth-promoting bacteria; **RCBD:** Randomized Complete Block Design; **PNCM-UPLB:** Philippine National Collection of Microorganisms; **BPI:** Bureau of Plant Industry; **NA:** Nutrient Agar; **LB:** Luria-Bertani broth; **RWC:** Relative Water Content; **FW:** Fresh weight; **TW:** Turgid weight; **DW:** Dry weight; **TCC:** Total Chlorophyll Content; **CFU:** Colony-forming units; **PCA:** Plate Count Agar; **JASP:** Jeffrey's Amazing Statistics Program; **ANOVA:** Analysis of Variance; **CV:** Coefficient of variation; **BI:** Biovolume index; **HSD:** Tukey's Honestly Statistical Difference test; **IAA:** Indole-3-acetic acid.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

BCC: Conceptualization, methodology design, fieldwork execution, laboratory analyses, data collection, statistical analysis, visualization, and manuscript drafting. **JD:** Conceptualization, planning of laboratory and field assays, fieldwork execution, financial management, data collection, and manuscript drafting. **AAP:** Conceptualization, methodology design, fieldwork execution, laboratory analyses, procurement of materials and reagents, data collection, and manuscript drafting. **LT:** Supervision, guidance on experimental design, data interpretation, and critical review of the manuscript.

SUMMARY

The study was conducted from March to April 2025 at the CvSU Technology Demonstration Farm and Interdisciplinary Research Building, Cavite State University - Don Severino delas Alas Campus, to evaluate the potential of a bacterial consortium (*Bacillus cereus, Micrococcus luteus, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*) in promoting drought tolerance in *C. frutescens* L. Specifically, it aimed to assess drought tolerance of seedlings, evaluate consortium effects on morphological parameters (plant height, root length, stem girth, total leaves, leaf size, biovolume index), physiological responses (relative water content, leaf chlorophyll), and quantify rhizosphere microbial load under drought stress.

A Randomized Complete Block Design was used. Bacterial strains were cultured, tested for compatibility, and applied as a consortium. During field trials, 150 seedlings were root-drenched and transplanted into pots with loam soil. Morphological parameters and soil moisture were measured before, during, and after treatment. After one week of rewatering, two plants per replicate were sampled for physiological assessments and microbial load quantification.

Drought stress was successfully induced, as reflected by a sharp decline in morphological parameters by Day 14. Most morphological traits showed significant differences between treatments after seven days of drought and following rewatering, demonstrating the consortium's effect on drought tolerance. Physiological parameters (RWC, chlorophyll) and microbial load were higher in inoculated plants, though differences were not statistically significant.

The study demonstrated that the bacterial consortium enhances drought tolerance in *C. frutescens* through significant morphological improvements and generally favorable physiological responses. Future studies should standardize inoculation and sampling, increase replicates, and include additional physiological and biochemical stress indicators.

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