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Efficacy of *Bacillus velezensis* TCR11 Encapsulated in Alginate - Biochar to Promote Growth and Physiological Characteristics of *Vigna unguiculata*

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Abstract Environmental pollution poses a growing threat to worldwide food security and the sustainability of agricultural systems. Encapsulation of bacterial cells presents a promising biotechnological approach to improve the efficacy of biofertilizers and enhance crop productivity. This study evaluated the impact of encapsulated beads containing plant beneficial bacteria (PBB) and biochar (BC) on the growth, photosynthetic pigments, protein content, and relative water content (RWC) of *Vigna unguiculata*. Out of 24 bacterial isolates, *Bacillus velezensis* strain TCR11 was selected for its superior seedling growth promotion and its ability to produce key plant beneficial metabolites including indole-3-acetic acid, siderophores, and P-solubilizing compounds. BC was produced from sawdust and bamboo under pyrolysis conditions of 400°C, 550°C, and 700°C. Among all tested BC, the bamboo-derived biochar produced at 550°C (BB2) demonstrated superior performance by significantly promoting plant growth and enhancing the viability of TCR11, making it the optimal matrix for encapsulation with sodium alginate (SA). Pot experiments demonstrated that the encapsulated beads containing TCR11, SA, and BB2 significantly improved *V. unguiculata* shoot and root length, biomass accumulation, pigment concentration, protein levels, and RWC compared to untreated controls. Moreover, this treatment increased TCR11 colonization in the rhizosphere of *V. unguiculata*, indicating that BC not only contributed its own benefits but also created a favourable microenvironment that supported the survival and activity of the encapsulated PBB. This likely facilitated the synthesis of growth-promoting metabolites, leading to enhanced plant performance. In conclusion, bamboo-derived BC (BB2) proved to be an effective additive for PBB encapsulation. Combined with PBB, it significantly improved the growth and physiological responses of *V. unguiculata*, highlighting its potential for sustainable agricultural applications.

Keywords: *Encapsulation, Biochar, Vigna Unguiculata, Bacillus velezensis, Relative Water Content, Rhizosphere*

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1. Introduction

Plant beneficial bacteria (PBB) have long been utilized to enhance plant health and productivity, thereby contributing to food and feed security for the growing worldwide population [1]. Their significance lies in their ability to improve nutrient uptake, modulate physiological responses, enhance stress tolerance, and suppress plant pathogens ultimately leading to increased crop yields [2]. It is well established that PBB promote plant growth through various mechanisms, such as the synthesis of phytohormones that regulate cell division, cell elongation, and stimulate root development [3]. They also synthesize iron-chelating siderophores, which enhance Fe availability and its uptake [4], and solubilize P, thereby increasing its bioavailability in the soil [5]. However, despite their proven potential, the efficiency of these PBB is often compromised under field conditions due to diverse abiotic and biotic stresses [6].

To improve the efficiency of PBB, several strategies have been developed to create a favourable microenvironment that supports sustained nutrient availability and offers physical protection over time. One widely adopted technique involves immobilizing PBB cells within biocompatible polymers, such as sodium alginate (SA) [7]. To enhance the structural and functional performance of these encapsulated formulations, additives like skim milk powder, clay, humic acids etc., are often incorporated into SA matrices [8]. These composite systems have shown improved stability and effectiveness in promoting plant growth [9]. According to [10], the synergistic utilization of humic substances and PBB has been shown to enhance PBB colonization in the rhizosphere while simultaneously improving plant

resilience to abiotic stress and optimizing nutrient uptake. Complementary findings by [11] revealed that the incorporation of skim milk powder into SA-based encapsulation systems markedly increased the survival rates of *Bacillus subtilis* and *Pseudomonas corrugate*, maintaining viable populations even after six months of storage under ambient conditions. In a related study, [12] demonstrated that skim milk supplementation in carrier-based PBB formulations not only stabilized PBB viability but also enhanced field performance, resulting in improved root colonization and increased crop productivity. Collectively, these studies emphasize the critical importance of incorporating additives into SA-based encapsulation formulations to develop resilient and effective PBB inoculants that enhance sustainable crop management.

Biochar (BC), a carbon-dense material synthesized via pyrolysis of biomass, is widely recognized as a sustainable and environmentally friendly soil amendment that mitigates ecological risks while enhancing plant growth [13,14]. Its high surface area and porous internal structure enable the retention of essential nutrients including N, P, K, and Ca, thereby improving soil health and supporting plant development [15]. Additionally, BC exhibits distinctive physico-chemical properties, including abundant oxygen-containing functional groups, elevated cation exchange capacity (CEC), and superior water-holding ability, all of which contribute to enhanced crop productivity [16]. For example, a recent study by [17] reported that peanut shell-derived BC increased nutrient availability in soils and thus root biomass, and water retention in *Schefflera arboricola*. These effects were attributed to the BC's porous architecture and nutrient-rich composition, which collectively improved nutrient use efficiency and promoted overall plant growth. Similarly, [18] found that the combined application of *P. hysterophorus* BC and PBB significantly enhanced plant growth and physiological responses, including chlorophyll content, photosynthetic efficiency, and root development. Moreover, this integrated approach improved key soil physico-chemical properties such as pH, organic carbon content, and nutrient availability (N, P, K), which collectively contributed to higher crop yields. These insights prompted us to investigate the role of BC as an additive in SA-based encapsulation systems, aiming to improve the efficiency and performance of encapsulated PBB. We hypothesize that integrating BC into the SA encapsulation matrix will enhance the viability of PBB and stimulate the expression of their plant growth promoting (PGP) traits, thereby improving overall plant growth and productivity.

The objectives of this study were to: (1) screen and evaluate the suitability of PBB and BC for the preparation of encapsulated beads, and (2) assess their impact on growth parameters, pigment biosynthesis, protein accumulation, and relative water content (RWC) in *Vigna unguiculata*.

2. Materials and Methods

2.1. Isolation and Characterization of PBB

Bacterial strains were isolated from tannery effluent-contaminated soils in Trichy, Tamil Nadu, India, using the serial dilution plating technique [19]. To identify potential PBB, a roll towel assay was performed using *V. unguiculata*. Seeds were surface-sterilized with 75% ethanol for 1 minute, followed by 1% sodium hypochlorite for 5 minutes, and washed three times with sterile deionized water. Each isolate was cultured overnight in Luria-Bertani (LB) broth at 28 ± 2°C with continuous shaking. After 18 hours, cultures were centrifuged at 5000 rpm for 10 minutes, and the cells separated were resuspended in sterile distilled water to an OD_{600} of 1.0 (~10^8 CFU/mL). Sterilized seeds were soaked in the bacterial suspension for 2 hours under aseptic conditions, while control seeds were treated with sterile water. Both sets were placed on moist blotting paper and incubated in a growth chamber for 7 days. Germination rate, shoot and root length, and vigour index were recorded [20].

Isolate TCR11, which exhibited strong PGP potential, was selected for further characterization. This included assessment of IAA production [21], siderophore synthesis [22], phosphate solubilization [23], ammonia generation [24], and hydrogen cyanide (HCN) production [25]. The strain's tolerance to heavy metal [26] and drought stress [27] was also evaluated. Molecular identification was performed via 16S rRNA gene sequencing [28].

2.2. Preparation and Screening of Biochar

Biochar was produced from bamboo and sawdust via slow pyrolysis at 400°C, 550°C, and 700°C under limited oxygen conditions following standard method [29]. The BC was ground and sieved to <2 mm particles for use. Its impact on *V. unguiculata* growth and survival of the PBB strain TCR11 was assessed to determine suitability for encapsulation.

For pot trials, soil collected from Bharathiar University was amended with BC at 0% or 5% (w/w). Seeds were sown in 1 kg of BC treated or untreated soil and maintained at 30°C under a 16/8 h light/dark cycle. After 20 days, plants were evaluated for shoot length (SL), root length (RL), fresh weight (FW), and dry weight (DW). To determine DW, the harvested biomass was oven-dried at 80°C for 48 hours.

To assess BC's effect on TCR11 growth, 5% (w/v) BC was added to tryptone soya broth. Cultures grown to $OD_{600} = 1.0$ were inoculated (0.1 mL) into each treatment and incubated at 28 ± 2°C with shaking (125 rpm). Growth was assessed every 12 hours for 72 hours. Samples were serially diluted in phosphate-buffered saline, plated on LB agar, and incubated at 30°C for 24 hours to determine colony-forming units (CFUs).

Bamboo BC produced at 550°C (BB2), which exhibited superior effectiveness in promoting plant growth and supporting TCR11 viability, was chosen for subsequent investigation.

2.3. Encapsulation

Prior to encapsulation, the PBB strain TCR11 was tagged with ampicillin resistance (300 mg/L) to enable monitoring of its colonization efficiency in the rhizosphere. This antibiotic-based tagging technique was

adapted from the protocol established by [30]. For encapsulation, TCR11 was grown in LB broth for 18 hours at 28 ± 2°C with continuous shaking. Post incubation, the cells were separated by centrifugation at 5000 rpm for 7 minutes and resuspended in sterile distilled water, adjusting the final concentration to 4.8×10^5 CFU/mL. The bacterial suspension was then combined with a sterile solution containing 2% SA and 5% BB2 in a 1:5 ratio. This mixture was extruded dropwise through a sterile 25 mL syringe into a 2% calcium chloride solution, facilitating bead formation via ionic crosslinking. After one hour of curing, the beads were collected by filtration, washed with sterile distilled water to remove residual calcium, and dried using sterile absorbent paper.

2.4. Pot experiment and plant analysis

A greenhouse experiment was carried out to assess the impact of free and encapsulated PBB on *V. unguiculata* growth and biochemical traits. Soil collected from Bharathiar University was sterilized through autoclaving at 121°C for 30 minutes over five consecutive days. Six treatments were prepared: 1. Untreated – seeds without TCR11, SA and BB2, 2. SA – uninoculated SA microcapsules, 3. SA + BB2 – uninoculated SA and BB2 microcapsules, 4. F-TCR11 – free TCR11 suspension, 5. TCR11 + SA – TCR11 encapsulated in SA, 6. TCR11 + SA + BB2 – TCR11 encapsulated in SA and BB2. *V. unguiculata* seeds were sown in pots containing 600 g of autoclaved soil. Each seed received either 1 mL of F-TCR11 bacterial suspension (7.2×10^6 CFU/mL) or 1 g of encapsulated beads. The pots were kept in a controlled environment at 30°C with a 16-hour light and 8-hour dark cycle. After 60 days of growth, plants were uprooted and measured for SL, RL, FW, and DW.

To evaluate the colonization ability of TCR11 in the rhizosphere of *V. unguiculata*, 1 g of soil was suspended in sterile distilled water and spread on LB agar containing 300 mg/L ampicillin. The plates were incubated at 37°C for 4–5 days, after which CFUs were recorded as per the method outlined by [30].

Chlorophyll and carotenoid contents were measured following [31]. Briefly, fresh leaf tissue (0.1 g) was homogenized in 6 mL of 80% ethanol. After centrifugation, the supernatant was analyzed spectrophotometrically at 663 nm, 645 nm (chlorophyll a and b), and 510 nm, 480 nm (carotenoids) using a UV-1800 spectrophotometer.

Protein content was determined following the procedure outlined by [32]. Briefly, fresh leaf tissue (0.1 g) was homogenized in phosphate buffer (pH 7.5), centrifuged, and the supernatant was diluted. To this, 1 mL of reagent (a 50:1 mixture of Solution 1: 2% Na_2CO_3, 1% $NaK(C_4H_4O_6)$, 0.4% NaOH; and Solution 2: 0.5% $CuSO_4 \cdot 5H_2O$) was added and incubated for 10 minutes. Then, 0.1 mL of Folin–Ciocalteu reagent was introduced. After 30 minutes, absorbance was recorded at 660 nm, and protein levels were estimated using a BSA calibration curve ranging from 20 to 640 µg.

Relative water content (RWC) in plant leaves was determined by calculating the difference between fresh, turgid, and dry weights. Fresh leaf samples were first immersed in distilled water for 12 hours in the dark to obtain turgid weight (TW). The same samples were then oven-dried at 72°C for 12 hours to determine dry weight (DW). RWC was derived using the formula:

$$RWC\ (\%) = [(FW - DW) / (TW - DW)] \times 100$$

Where, FW represents fresh weight, TW is turgid weight, and DW is dry weight.

2.5. Statistical Analysis

All experiments were carried out in triplicate, and data are expressed as mean ± standard deviation. Treatment effects were evaluated using one-way ANOVA, followed by Tukey's HSD post hoc test to compare group means at a significance level of $p < 0.05$. Statistical analysis was performed using SPSS software (v25.0).

3. Results and Discussion

3.1. Isolation and Characterization of PBB

During the initial isolation process, 24 morphologically distinct bacterial strains were obtained from tannery effluent-contaminated soils. To identify efficient PBB, all isolates were evaluated for their PGP potential using an in vitro roll towel assay. Among the tested strains, TCR11 exhibited the highest enhancement in vigor index, showing a 183% increase compared to the uninoculated control (Table 1). The ability of PBB to stimulate plant growth is often attributed to the production of key metabolites such as IAA, siderophores, and P-solubilizing compounds. Generally, IAA enhances cell elongation, division, and root development, thereby improving water and nutrient uptake [33]. Similarly, siderophores, which are iron-chelating compounds produced by rhizosphere microbes, facilitate iron acquisition by forming stable complexes with iron, thus enhancing its availability to plants [34]. Additionally, certain PBB strains promote P uptake by converting insoluble forms into bioavailable ones through the secretion of organic and inorganic acids, ultimately supporting robust root growth and increased crop productivity [35]. Extensive research has demonstrated a significant correlation between enhanced plant growth and the biosynthesis of plant growth-promoting (PGP) metabolites by plant-beneficial bacteria (PBB) [33,34,35,36]. For instance, [36] reported that the enhanced growth of tomato and watermelon was closely associated with the synthesis of various PGP compounds such as IAA, siderophores and P- solubilizing metabolites. To validate this association, the isolate TCR11 identified as the most effective strain based on its highest vigor index in *V. unguiculata* was selected for further characterization. Remarkably, TCR11 demonstrated the ability to synthesize IAA at a concentration of 14.98 ± 0.34 µg/ml, produce siderophores at 24.62 ± 1.37%, and solubilize P at 16.12 ± 0.66 µg/ml (Table 2). Additionally, the strain tested positive for ammonia and catalase production. These findings support earlier reports that the biosynthesis of PGP metabolites plays a crucial role in enhancing plant growth and development [33,34,35]. Since the survival of inoculated PBB in the rhizosphere of new environments is often challenged by various abiotic stresses, we evaluated the

tolerance of the strain TCR11 to heavy metals, and polyethylene glycol (PEG)-induced drought conditions. The strain TCR11 demonstrated notable resistance to heavy metals, including Zn up to 200 mg/L, Cd up to 150 mg/L, Cu up to 200 mg/L, Cr up to 500 mg/L, Ni up to 200 mg/L, and Pb up to 1000 mg/L. Additionally, it exhibited drought tolerance under PEG-induced stress up to 60%. This enhanced resistance to heavy metals and drought may be attributed to the origin of the strain, as it was isolated from tannery effluent-contaminated, drought-prone soil conditions that likely exerted selective pressure, enabling the strain to adapt and survive under extreme stress [37]. Similar findings were reported by [38] who observed that bacterial strains isolated from heavy metal contaminated soils exhibited high tolerance to heavy metal stress due to their acquired resilience in such environments. Analysis of the partial 16S rRNA sequence through the online BLAST program, followed by phylogenetic tree construction (Figure 1), revealed that the PBB TCR11 belongs to the genus Bacillus. The sequenced fragment comprising 1325 base pairs, was taxonomically classified as *Bacillus velezensis* and has been deposited to GenBank under the accession number MH718826.1.

Table 1. Effect of selected bacteria on vigor index of *V. unguiculata*

Strain Name	Vigor Index
Control	426 ± 28.65^{so}
CRT01	468 ± 29.04^{mno}
CRT02	566 ± 30.47^{njklm}
CRT03	319 ± 20.28^{lmno}
CRT04	459 ± 22.51^{no}
CRT05	618 ± 32.66^{ijk}
CRT06	589 ± 34.41^{jkl}
CRT07	737 ± 33.73^{fgh}
CRT08	600 ± 36.88^{ijk}
CRT09	731 ± 41.64^{fgh}
CRT10	774 ± 38.77^{efg}
TCR11	1288 ± 40.89^{a}
TACR12	1123 ± 38.2^{b}
TACR13	905 ± 41.68^{cd}
TACR14	942 ± 39.64^{c}
TACR15	734 ± 37.22^{fgh}
TACR16	832 ± 30.82^{def}
TACR17	485 ± 20.19^{mno}
TACR18	903 ± 32.44^{cd}
TACR19	842 ± 29.08^{cfe}
TACR20	744 ± 28.83^{efgh}
TACR21	540 ± 30.11^{klmn}
TACR22	697 ± 21.67^{ghi}
TACR23	663 ± 26.44^{hij}
TACR24	812 ± 25.09^{def}

Bars (±) represent standard deviations of three replicates. Bars indexed with the same letter are not significantly different between treatments according to the HSD Tukey test at $p < 0.05$.

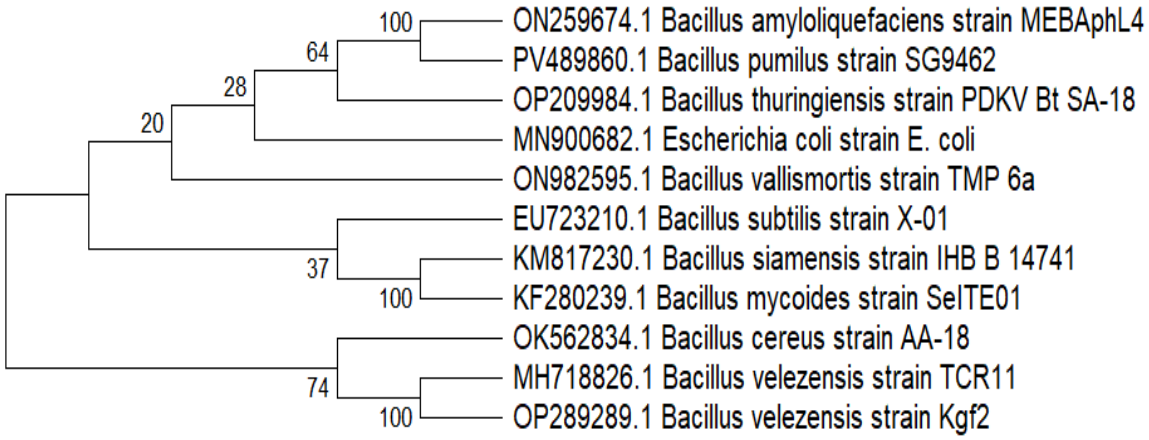


Figure 1. Phylogenetic analysis of strain TCR11 based on partial 16S rRNA sequences. Evolutionary relationships between TCR11 and closely related sequences obtained from the NCBI database were inferred using the neighbor- joining algorithm implemented in MEGA version 11.2.1.7

Table 2. Abiotic stress tolerance and plant growth-promoting features of *B. velezensis* TCR11

Characteristics	TCR11
Metal tolerance level (mg/L)	
Zn	200
Cd	150
Cu	200
Cr	500
Ni	200
Pb	1000
Drought resistance (%)	
PEG	60
In- vitro Plant growth- promotion *	
Shoot length	73
Root length	95
Plant Growth Promoting Traits IAA (µg/ml)	14.98 ± 0.34
Siderophore production (%)	24.62 ± 1.37
Phosphate solubilisation (µg/ml)	16.12 ± 0.66
Catalase	+
Ammonia production	+

Values are means ± standard deviations of three samples. +, positive results of the test. Indole 3-acetic acid (IAA), Polyethylene Glycol (PEG). * Percent increase as compared to un-inoculated plants

Table 3. Effect of BC on the growth of *V. unguiculata*

BC	Shoot length (cm)	Root length (cm)	Fresh weight (g/plant)	Dry weight (g/plant)
Control	20.01 ± 1.85^{d}	19.3 ± 145^{c}	1.1 ± 0.07^{c}	0.22 ± 0.01^{d}
BB1	23.03 ± 2.09^{bcd}	26.5 ± 2.09^{b}	1.25 ± 0.09^{bc}	0.3 ± 0.02^{cd}
BB2	30.67 ± 1.86^{a}	34 ± 2.86^{a}	2.46 ± 0.75^{a}	0.74 ± 0.06^{a}
BB3	27.63 ± 1.74^{ab}	31.7 ± 3^{ab}	1.68 ± 0.06^{abc}	0.53 ± 0.04^{abc}
SB1	20.29 ± 1.86^{cd}	25.6 ± 2.11^{bc}	2.03 ± 0.07^{ab}	0.36 ± 0.02^{cd}
SB2	26.36 ± 2^{abc}	28.2 ± 1.89^{ab}	1.82 ± 0.05^{abc}	0.61 ± 0.05^{ab}
SB3	24.33 ± 2.2^{bcd}	29.8 ± 2.12^{ab}	1.25 ± 0.03^{bc}	0.43 ± 0.01^{bcd}

BB1: Bamboo BC pyrolyzed at 400°C, BB2: Bamboo BC pyrolyzed at 550°C, BB3: Bamboo BC pyrolyzed at 700°C, SB1: Sawdust BC pyrolyzed at 400°C, SB2: Sawdust BC pyrolyzed at 550°C, SB3: Sawdust BC pyrolyzed at 700°C. Error bars (±) indicate the standard deviation from three replicates. Treatments marked with the same letter do not differ significantly, based on Tukey's HSD test at $p < 0.053$.

3.2. Preparation and Screening of BC for Encapsulation

Effective bioformulations must be carefully designed to promote the survival and establishment of inoculated microorganisms in the rhizosphere. Ensuring microbial persistence is crucial for the consistent release of PGP substances such as IAA, siderophores, and P-solubilizing compounds [3]. Beyond maintaining microbial viability, these formulations should enhance plant growth and improve soil quality by stimulating beneficial microbial interactions and optimizing nutrient dynamics [8]. In this study, to identify the most suitable BC for encapsulation, BCs derived from various agro-waste biomasses at different pyrolysis temperatures were evaluated for their compatibility with *V. unguiculata* and selected PBB strain TCR11 by assessing their effect on plant growth and microbial viability.

3.2.1. Effect of BC on the Growth of *V. unguiculata*

The effect of BC amendment on *V. unguiculata* growth was evaluated by measuring SL, RL, FW and DW. Overall, BC application demonstrated a positive influence on plant development, with noticeable improvements across all growth parameters. As documented in numerous studies, BC used as a soil amendment has a positive influence on plant growth due to its large surface area, well-developed pore structure, abundance of oxygen-containing functional groups, and high CEC [13,18]. These properties enhance nutrient availability and retention, thereby improving crop performance by directly modifying soil physicochemical conditions [39,40,41]. However, compared to sawdust-derived BC, bamboo-derived BC consistently promoted greater shoot and root elongation, along with increased biomass accumulation. This improvement may be ascribed to the physico-chemical properties of BC such as pH, surface area, porosity, and nutrient composition which directly affect soil fertility, microbial activity, and consequently plant growth [39]. Previous studies have revealed that bamboo BC is typically rich in essential nutrients including K, Ca and Mg, which enhance nutrient availability and stimulate plant growth [42,43,44]. In contrast, BC derived from hardwoods or low-nutrient feedstocks tends to exhibit lower PGP efficiency due to limited nutrient content and reduced microbial support [45]. Notably, among the bamboo BC tested, the BC produced at 550°C (BB2) resulted in the highest plant growth enhancement, with SL increasing by 53%, RL by 76%, FW by 123%, and DW by 229% compared to the control. It is well established that BC produced at moderate to high pyrolysis temperatures (450–550°C) possess improved structural integrity, higher surface area, and enhanced porosity, which contribute to

better water retention and nutrient exchange in the soil [44]. For example, wheat straw-derived BC produced at 550°C significantly increased water-stable aggregates and available water content, improving soil structure and moisture conservation by up to 166% over untreated soil [46]. Similarly, [47] reported that water hyacinth BC produced at 550°C enhanced plant height and biomass in maize, likely due to increased N and P availability and improved CEC. These findings also suggest that BC produced at 550°C contains functional groups such as carboxylic acids and amines, along with a high nutrient load [48], and exhibits increased pore volume and spatial structure [49], creating an optimal environment for plant growth. Although our findings clearly demonstrate the significant impact of bamboo BC produced at 550°C on the growth of *V. unguiculata*, comprehensive physico-chemical characterization and molecular-level analysis are essential to elucidate the precise mechanisms responsible for its PGP effects.

3.2.2. Effect of biochar on the growth of PBB

Before selecting an appropriate material for the encapsulation matrix, it is crucial to assess whether the candidate substances can support microbial viability or, at the very least, do not exert inhibitory effects on the target microorganisms [50]. BC, due to its diverse physico-chemical properties such as chemical composition, feedstock origin, and the presence of water-soluble organic compounds has been reported to either stimulate or suppress bacterial growth depending on its characteristics and the microbial strain involved [51]. In the present study, we investigated the influence of BC on the growth of *B. velezensis* TCR11 in tryptone soya broth. The results demonstrated a consistent stimulatory effect across all BC treatments, with significantly enhanced bacterial proliferation compared to the untreated control (Figure 2). This suggests that BC, irrespective of its source and pyrolysis temperature, can serve as a favourable amendment for promoting microbial growth. The observed enhancement in TCR11 proliferation is likely attributable to BC's carbon source, high surface area, porous architecture, strong adsorption capacity and high-water retention. These properties facilitate microbial colonization by providing protective microhabitats and improving the retention of moisture and nutrients essential for microbial metabolism and replication [52]. However, among the BC tested, BB2 exhibited the most pronounced positive effect, achieving a peak cell density of 5.64×10^5 CFU/mL within 24 hours of incubation. This may be linked to its pyrolysis temperature of 550°C, which is known to enhance the structural stability and chemical functionality of BC [46]. BC pyrolyzed at elevated temperatures typically exhibit lower concentrations of volatile organic compounds and higher fixed carbon content, both of which contribute to a more stable and nutrient-rich environment conducive to microbial proliferation [46,51]. Our findings align with previous reports indicating that high-temperature BC can improve microbial viability and activity by minimizing toxic residues and enhancing nutrient availability [53]. Although the positive response of *B. velezensis* TCR11 to BB2 confirms its compatibility with the strain, further investigations such as detailed characterization of BC properties and molecular-level studies on BC–PBB interactions are necessary to elucidate the mechanisms through which BC enhances bacterial growth.

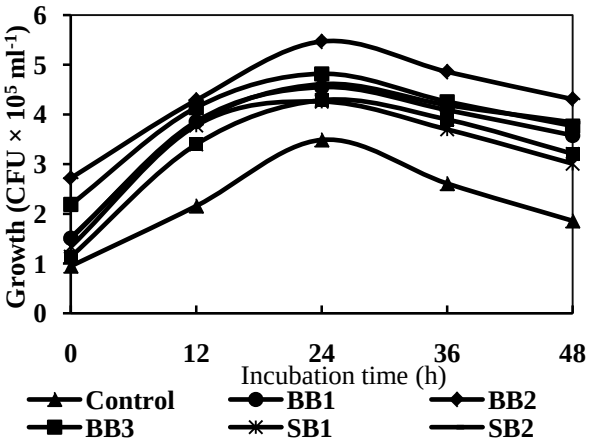
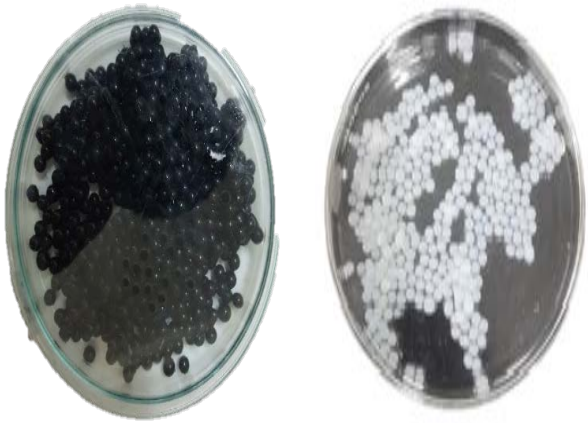


Figure 2. Impact of BC on the growth of *B. velezensis* TCR11 in tryptone soya broth. BB1: Bamboo BC pyrolyzed at 400°C, BB2: Bamboo BC pyrolyzed at 550°C, BB3: Bamboo BC pyrolyzed at 700°C, SB1: Sawdust BC pyrolyzed at 400°C, SB2: Sawdust BC pyrolyzed at 550°C, SB3: Sawdust BC pyrolyzed at 700°C. Error bars (±) indicate the standard deviation from three replicates. Treatments marked with the same letter do not differ significantly, based on Tukey's HSD test at $p < 0.05$

3.3. Encapsulation of *B. velezensis* TCR11 in SA and BC macrobeads



(a) (b)

Figure 3. TCR11 encapsulated beads with BC (a) and without BC (b)

This study employed ionotropic gelation to synthesize BC–alginate composite beads. SA solutions with or without BC and TCR11, were dispensed dropwise into a $CaCl_2$ solution, resulting in the formation of small, spherical, insoluble macrobeads with size ranging from 1 mm to 2 mm. Beads appeared white in the absence of BC and black when BC was incorporated (Figure 3). The gelation mechanism is driven by ionic exchange, where Ca^{2+} replace Na^+ and interact with $–COO^-$ on the guluronic acid segments of alginate. This interaction forms a robust hydrogel matrix, often described by the "egg-box" model of calcium–alginate crosslinking [54]. Similarly, BC enhances the structural and functional integrity of the composite through its surface-active groups including –OH, –COOH, and –C=O which participate in hydrogen bonding and electrostatic

interactions with the alginate polymer [55]. The viable count of *B. velezensis* TCR11 in TCR11+SA and TCR11+SA+BB2 beads was found to be 3.9 × 10^5 CFU/g and 4.62 × 10^5 CFU/g, respectively, indicating that the incorporation of BC did not affect the encapsulation efficiency of the beads.

3.4. Effect of PBB Macrobeads on *V. unguiculata*

3.4.1. Plant Growth

Table 4. Effect of PBB microbeads on the growth of *V. unguiculata*

Treatment	Shoot length (cm)	Root length (cm)	Fresh weight (g/plant)	Dry weight (g/plant)
UNTREATED	17.62 ± 1.46^{b}	12.16 ± 1.13^{c}	3.02 ± 0.4^{d}	0.98 ± 0.06^{e}
SA	18.62 ± 1.16^{b}	15.42 ± 1.24^{bc}	4.12 ± 0.45^{cd}	1.45 ± 0.07^{cd}
SA+BB2	22.46 ± 2.19^{b}	24.6 ± 2.16^{a}	6.55 ± 0.62^{ab}	2.1 ± 0.12^{b}
F-TCR11	21 ± 2.95^{b}	18 ±1.68^{b}	4.22 ± 0.29^{cd}	1.78 ± 0.05^{bc}
TCR11+SA	22.06 ± 2.14^{b}	17.66 ± 1.76^{b}	4.93 ± 0.32^{bc}	1.81 ± 0.13^{bc}
TCR11+SA+BB2	32.48 ± 2.08^{a}	28.11 ± 2.44^{a}	6.68 ± 1.14^{a}	3.27 ± 0.13^{a}

Bars (±) represent standard deviations of three replicates. Bars indexed with the same letter are not significantly different between treatments according to the HSD Tukey test at $p < 0.05$. SA- sodium alginate, BB2- bamboo BC produced at 550°C, TCR11- *B. velezensis.*

Table 4 illustrates the impact of free and encapsulated *B. velezensis* TCR11 macrobeads on the growth of *V. unguiculata*. The findings reveal that both free and encapsulated forms of PBB significantly enhanced plant growth traits compared to the untreated control. This improvement may be ascribed to the synergistic or independent effects of TCR11 and BB2. PBB are known to improve plant growth through multiple mechanisms, including the synthesis of IAA, siderophores, and solubilization of P [56]. These traits collectively promote root development, increase the bioavailability of nutrients such as Fe and P, and facilitate their uptake by plants [57]. For instance, [58] recently reported that inoculation with a PBB *B. pumilus* JIZ13 led to enhanced plant growth, biomass accumulation, and nutrient acquisition, primarily through the synthesis of key metabolites associated with growth promotion. Likewise, BC has been widely recognized as an effective soil amendment that enhances key physico-chemical properties, including soil porosity, moisture retention, and nutrient-holding capacity, thereby contributing to improved plant development. [59] assessed the impact of poultry manure BC on the growth of *Cicer arietinum, Zea mays, Glycine max and Vigna radiata* in alkaline soils. Their findings revealed significant improvements in plant growth and related parameters, primarily attributed to the BC's ability to increase soil water-holding capacity and optimize the root-zone environment. Interestingly, the highest growth response in our study was observed in the TCR11+SA+BB2 treatment, which led to increases of 84% in SL, 131% in RL, 121% in FW, and 177% in DW compared to the untreated control plants. These outcomes indicate that the synergistic interaction between PBB and BC significantly enhanced overall plant development. In addition to their individual benefits, BC likely created a favourable microenvironment that supported the survival and activity of the encapsulated PBB in the rhizosphere, thereby facilitating the synthesis of key PGP metabolites [60].

3.4.2. TCR11 Colonization

To validate the relationship between BC and the survival of *B. velezensis* TCR11 in the rhizosphere, the colonization of TCR11 in the rhizosphere of *V. unguiculata* was assessed (Figure 4). While colonization was evident across all treatments, the highest levels were recorded in the TCR11+SA+BB2 treatment (4.67× 10^5 CFU/g), followed by TCR11+SA (2.89× 10^5 CFU/g), and free TCR11 (2.08× 10^5 CFU/g), suggesting that BB2 provides a favourable microenvironment for the survival and establishment of TCR11 in the rhizosphere. These results are consistent with those of [61], who found that BC application enhances soil physico-chemical properties including pH, moisture retention, and nutrient availability thereby increasing the abundance of *Pseudomonas sp.* TP27 in the rhizosphere. BC is known to supply essential nutrients like P and N, which promote microbial proliferation and facilitate root colonization [62]. Furthermore, BC improves carbon availability, stimulates microbial activity, enhances soil structure, and supports overall plant health [63]. [64] also demonstrated that wheat straw BC significantly increased rhizospheric bacterial populations in winter wheat, attributing this effect to its porous architecture and nutrient-rich composition that favour microbial habitation and proliferation. The enhanced colonization (Figure 4) and improved plant growth (Table 4) observed in the TCR11+SA+BB2 treatment, compared to free TCR11, confirm that the synergistic interaction between BB2 and TCR11 plays a pivotal role in optimizing plant growth.

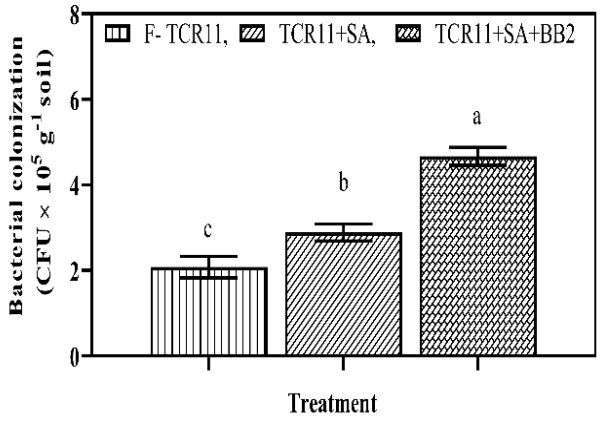
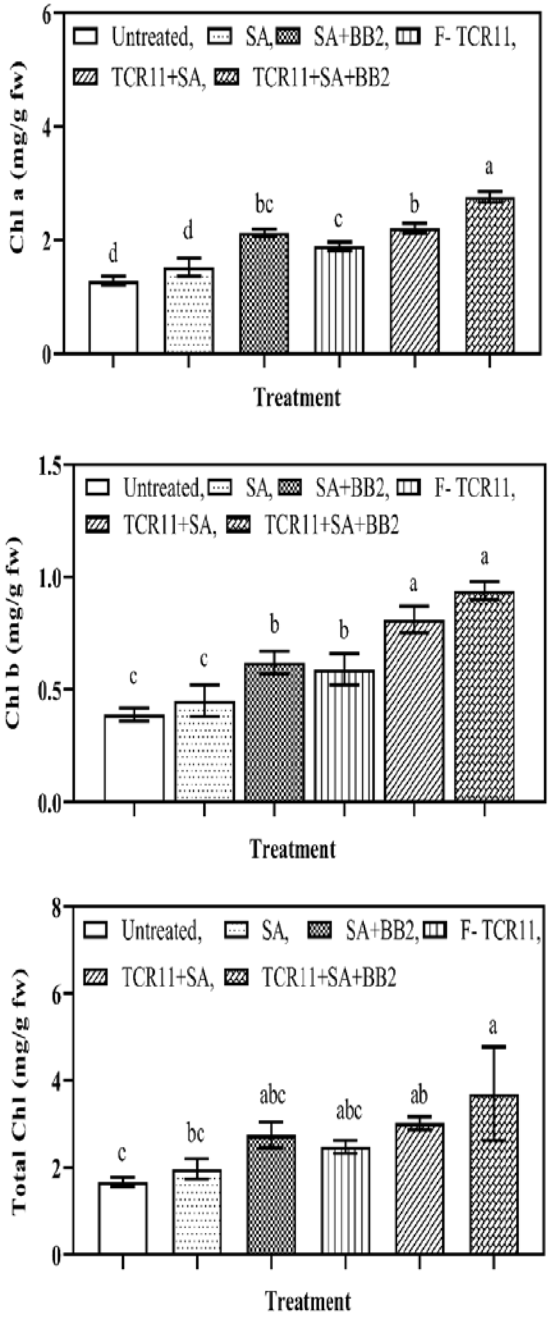


Figure 4. Colonization efficiency of *B. velezensis* TCR11 in the rhizosphere of *V. unguiculata.* The results are expressed as mean values with standard deviations, based on three independent replicates. Treatments labeled with identical letters indicate no statistically significant variation, as assessed by Tukey's Honest Significant Difference (HSD) test at a 5% significance level ($p < 0.05$). SA– sodium alginate; BB2 – bamboo BC pyrolyzed at 550°C; TCR11 – *B. velezensis*

3.4.3. Pigment Contents in *V. unguiculata*

Since plant health is closely linked to pigment concentration and overall photosynthetic efficiency, the impact of free and encapsulated *B. velezensis* TCR11 on pigment content in *V. unguiculata* was assessed. Plants treated with either free or encapsulated TCR11 showed elevated pigment levels compared to the untreated control (Figure 5). Notably, the TCR11+SA+BB2 treatment resulted in a marked increase, with chlorophyll a rising by 116%, chlorophyll b by 141%, total chlorophyll by 121%, and carotenoids by 98%. This enhancement can be attributed to the role of BC in improving soil structure, porosity, and water-holding capacity, along with its potential to retain and supply essential nutrients such as N, Mg, and Fe [65]. These favourable conditions support both plant and microbial activity, stimulate root development, and enhance nutrient uptake ultimately promoting chlorophyll biosynthesis [66]. Our findings align with those of [67], who reported that wheat-straw BC significantly augmented chlorophyll a, chlorophyll b, and total chlorophyll content in wheat, attributing the improvement to enhanced nutrient uptake. Similarly, [68] found that maize straw BC application in peanut crops elevated leaf N levels, which were positively associated with increased chlorophyll fluorescence and photosynthetic efficiency. In support of this, [28] recently demonstrated that coconut husk BC-amended soils facilitated colonization of *Enterobacter hormaechei* which produce various PGP metabolites, resulting in elevated chlorophyll and carotenoid levels in *Ricinus communis*. In our study, the observed increase in pigment levels aligns with the overall enhancement in plant growth (Table 4) under the TCR11+SA+BB2 treatment, suggesting that TCR11 and BB2 work synergistically to enhance pigment biosynthesis. This effect is likely mediated through improved soil physico-chemical properties and greater nutrient uptake by plants. However, further research focusing on detailed soil characterization is warranted to confirm these interactions and underlying mechanisms.



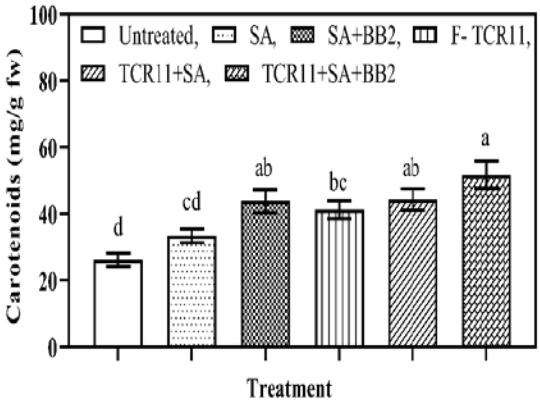


Figure 5. Impact of free and encapsulated TCR11 on pigment levels in *V. unguiculata*. Mean values ± SD from three replicates are shown for (a) chlorophyll a, (b) chlorophyll b, (c) total chlorophyll, and (d) carotenoids. Identical letters indicate no significant difference (Tukey's HSD, $p < 0.05$). SA – sodium alginate; BB2 – bamboo BC pyrolyzed at 550°C; TCR11 – *B. velezensis*; chl – chlorophyll; fw – fresh weight

3.4.4. Protein Contents in *V. unguiculata*

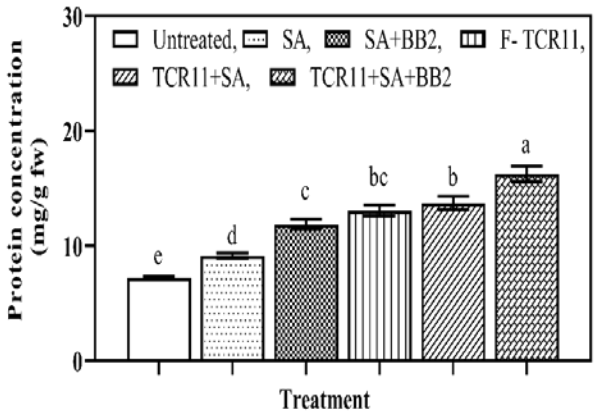


Figure 6. Influence of free and encapsulated TCR11 on protein levels in *V. unguiculata*. Values are shown as means ± SD from three replicates. Treatments with matching letters are not significantly different (Tukey's HSD, $p < 0.05$). SA – sodium alginate; BB2 – bamboo BC pyrolyzed at 550°C; TCR11 – *B. velezensis*; fw – fresh weight

Numerous prior investigations have demonstrated that BC amendments significantly improve soil physico-chemical characteristics, enhance microbial diversity, and stimulate microbial activity [69,70,71]. These effects collectively facilitate more efficient nutrient cycling and contribute to increased protein content in plants. Accordingly, the present study assessed the influence of both free and encapsulated PBB on protein accumulation in *V. unguiculata*. Consistent with their positive effects on plant growth and pigment contents, both free and encapsulated PBB significantly increased leaf protein content in *V. unguiculata*. Notably, the TCR11+SA+BB2 treatment resulted in a marked increase in protein content by 126% compared to the untreated control (Figure 6). This enhancement is likely attributable to the effects of BC, which promotes microbial survival and activity, thereby improving root architecture, nutrient cycling, bioavailability and uptake leading to enhanced plant growth and protein synthesis. Additionally, BC influence on soil physical, chemical, and biological properties contributes to increased water and nutrient retention, reduced nutrient leaching, and improved nutrient availability [28]. As a soil conditioner, BC preserves essential nutrients such as N, P and micronutrients, with N playing a direct role in protein biosynthesis [72]. Similarly, [73] demonstrated that corn straw BC application elevated total and ammonium N levels and enhanced microbial biomass N, factors closely associated with protein synthesis in plants. Supporting this, [74] reported that BC significantly increased PBB abundance and improved N-related soil parameters, resulting in higher crop yields. Similarly, [75] observed that corn straw BC application improved root morphology and increased soil nitrate and ammonium concentrations, thereby enhancing N availability and promoting plant growth.

3.4.5. Relative Water Contents in *V. unguiculata*

Relative water content (RWC) is a vital physiological metric used to assess the internal water status of plant tissues. It reflects the degree of cellular hydration and is closely tied to the availability of soil moisture, influencing *plant health* and agricultural yield [76]. In the present study, untreated *V. unguiculata* exhibited the lowest RWC, indicating suboptimal water retention. In contrast, treatments involving SA+BB2 and TCR11+SA+BB2 improved plant water status, as evidenced by increased RWC values (Figure 7). Notably, the treatment of TCR11+SA+BB2 resulted in a marked 11% increase in RWC, underscoring the synergistic role of TCR11 and BB2 in enhancing plant hydration. This improvement in RWC may be attributed to enhanced root development facilitated by PBB, which expand the root surface area and improve water absorption from the soil [77]. PBB also modulate physiological processes by activating aquaporins (water channel proteins) in root cells [78], regulating stomatal conductance [79], and promoting the accumulation of osmolytes like proline and soluble sugars [80], factors that collectively contribute to improved plant water retention. Furthermore, BC has been shown to significantly enhance PBB survival and activity in the rhizosphere by creating a conducive microenvironment. This interaction stimulates root proliferation and improves root architecture, thereby increasing the plant's capacity for water uptake [53]. Recent findings also suggest that the synergistic effect of BC and microbial communities not only expands root surface area but also enhances soil moisture retention and hydraulic conductivity [52]. Consequently, plants treated with these amendments exhibit superior water uptake efficiency and elevated

RWC in their tissues. Overall, the observed increase in RWC can be correlated with the cumulative effects of PBB and BC, which help maintain water balance and contribute to improved plant growth.

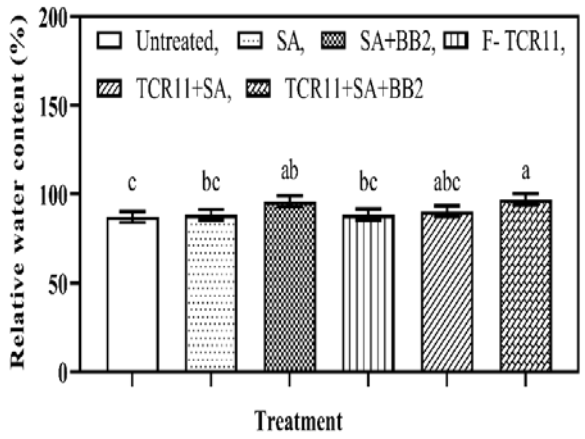


Figure 7. Influence of free and encapsulated TCR11 on relative water content in *V. unguiculata*. Data are shown as mean ± SD from three replicates. Treatments sharing the same letter are not significantly different (Tukey's HSD, $p < 0.05$). SA – sodium alginate; BB2 – bamboo BC pyrolyzed at 550°C; TCR11 – *B. velezensis*

4. Conclusion

The results of our study demonstrate that both free and encapsulated forms of *B. velezensis* TCR11 significantly enhanced various traits in *V. unguiculata*, including shoot length, root length, fresh weight, dry weight, leaf pigment content (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids), total protein levels and relative water contents, compared to the untreated control. These improvements can be ascribed to the combined beneficial effects of BC and PBB. Notably, macrobeads containing both BC and PBB (TCR11+SA+BB2) were found to be more effective in promoting plant growth. This suggests that the encapsulated BC within the beads provides a conducive microenvironment for the survival and activity of *B. velezensis* TCR11, thereby enhancing the physiological responses of plants through the production of PGP metabolites. To further elucidate the underlying mechanisms, a molecular-level investigation is warranted to uncover how BC and PBB-based beads contribute to plant development. Current research efforts are focused on evaluating the efficacy of TCR11 and BB2 formulations under field conditions to validate their role in improving crop performance. This research deepens insight into the interactions among plant, PBB and BC, while contributing to the design of eco-friendly bioformulations that support improved plant development and agricultural yield.

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