



RESEARCH ARTICLE

# Isolation and molecular characterization of *Bacillus megaterium* ATCC 14581 and its effect on nitrogen fertilizer use reduction, growth and yield of baby corn

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## Abstract

Despite the known benefits of biofertilizers, there remains a limited understanding of effective indigenous strains specifically adapted to baby corn cultivation. This study isolated, characterized and evaluated an indigenous strain, *Bacillus megaterium* ATCC 14581, from the baby corn rhizosphere to assess its potential as a plant growth-promoting rhizobacterium biofertilizer. Morphological, biochemical and molecular analyses confirmed the identity and nitrogen-fixing capacity of this strain. Laboratory assays demonstrated significant nitrogenase activity alongside effective phosphate solubilization and indole-3-acetic acid (IAA) production, indicating multifunctional plant growth-promoting traits. Field experiments conducted with varying chemical nitrogen fertilizer (CNF) reduction levels (0, 25, 50 and 75 %) and bacterial inoculation revealed that inoculation with strain ATCC 14581 significantly improved baby corn growth parameters, including biomass, cob length and yield components compared to non-inoculated controls. Importantly, the strain enabled up to a 50 % reduction in CNF use without compromising yield, thereby contributing to nutrient efficiency. Inoculated plants showed increased protein and phosphorus levels in edible corn cobs. The strain showed good adaptability to environmental stresses, including temperature, pH and salinity, supporting its practical application. These results underscore the potential of strain ATCC 14581 as a sustainable biofertilizer that can help reduce CNF input, improve soil fertility and promote climate-smart agriculture in baby corn production systems. Further research on large-scale application and long-term soil health impact is recommended to optimize field implementation.

**Keywords:** ATCC 14581; baby maize; *Bacillus megaterium*; biofertilizer; fixation; inoculation

## Introduction

Baby corn (*Zea mays* L. var. *rugosa*) is an immature maize ear harvested within a few days of silk emergence, before pollination occurs. Owing to its tender texture, pleasant flavor and high nutritional value, baby corn has emerged as a high-value vegetable crop with significant commercial potential in tropical and subtropical regions (1). It has gained popularity not only in domestic markets but also as a key export commodity for high-end restaurants, hotels and the food processing industry. From an agronomic perspective, baby corn is attractive because of its short growth cycle (approximately 50–70 days from sowing to harvest) and compatibility with multiple cropping systems, allowing farmers to obtain three to four harvests annually from the same land area (2). Nutritionally, baby corn is low in calories and fat, yet rich in protein, dietary fiber, vitamins (A, B-complex and C) and essential minerals such as phosphorus, potassium and magnesium (3). Furthermore, because it is harvested at an early stage with husk intact, baby corn is typically free from pesticide residues, making it a safe choice for health-conscious consumers. In rural economies, it provides farmers with a steady source of income due to frequent harvests and consistent market demand (4).

Despite these advantages, the sustainable production of baby corn faces considerable challenges. Climate variability, characterized by prolonged droughts, unpredictable rainfall patterns and periodic heat waves, directly impact crop growth and yield (5). Baby corn is especially sensitive to soil moisture and temperature stress during tasseling and silking. High temperatures during these stages can reduce silk emergence, impair pollination and lead to poor ear development (6). In addition to climatic stress, current production practices in many regions contribute to soil degradation and declining productivity. Intensive cultivation with minimal fallow periods, combined with excessive use of chemical fertilizers and pesticides, has led to nutrient depletion, soil acidification and reduced organic matter content (7). Overreliance on synthetic nitrogen (N), phosphorus (P) and potassium (K) fertilizers increases production costs while disturbing soil nutrient balance and contaminating water resources through runoff and leaching (8, 9). Continuous application of pesticides disrupts beneficial microbial communities, particularly those involved in nutrient cycling, organic matter decomposition and disease suppression (10). Over time, these factors reduce soil fertility and biological activity, ultimately diminishing yields and farm profitability (11).

Addressing these issues requires a shift toward more sustainable, resource-efficient and environment friendly crop management strategies. One promising approach is the use of plant growth-promoting rhizobacterium (PGPR), beneficial bacteria that colonize plant roots and enhance growth through multiple mechanisms (12, 13). These include fixing atmospheric nitrogen, solubilizing insoluble phosphates, producing phytohormones such as indole-3-acetic acid (IAA), enhancing root architecture for improved water and nutrient uptake and inducing systemic resistance against pathogens (14, 15).

For maize and other cereals, PGPR inoculation has been shown to significantly improve plant growth, yield and nutrient uptake, even under reduced fertilizer input conditions (16, 17). Nitrogen-fixing species such as *Azospirillum* spp. and *Azotobacter* spp. can supplement nitrogen requirements, reducing the dependence on synthetic fertilizers while supporting healthy vegetative growth (18). Phosphate-solubilizing bacteria, including *Bacillus* spp. and *Pseudomonas* spp., can convert unavailable forms of phosphorus into plant-accessible forms, improving nutrient efficiency, especially in phosphorus-deficient soils (19). Moreover, integrating PGPR with organic amendments such as farmyard manure, compost or vermicompost has been shown to yield synergistic benefits (20, 21). Organic inputs not only supply essential nutrients and improve soil structure but also serve as habitats and nutrient source for beneficial microbes, thereby enhancing PGPR survival and activity in the rhizosphere (22). Such integrated nutrient management approaches have improved soil microbial biomass, enzyme activity and nutrient cycling in various cropping systems (23). For baby corn, this could mean more resilient plants, higher yields and improved quality even under challenging environmental conditions (24).

A critical step in developing effective PGPR-based biofertilizers is the isolation and characterization of indigenous strains from the rhizosphere of target crops. Native strains are adapted to local soil and climatic conditions, often exhibiting superior colonization ability and performance compared to non-native or commercial strains (25-27). Identifying new nitrogen-fixing and phosphorus-solubilizing bacteria from baby corn rhizospheres can therefore provide a foundation for tailored biofertilizer products that enhance crop performance while restoring soil health. Given the pressing need to reduce agrochemical use, improve soil fertility and maintain profitability in baby corn cultivation, research on isolating and evaluating native PGPR strains holds significant promise. Such work directly supports the principles of climate-smart and regenerative agriculture, which aim to boost productivity while minimizing environmental footprints and restoring ecosystem functions (28, 29).

Therefore, the present study was undertaken to isolate and identify efficient nitrogen-fixing and phosphate-solubilizing bacterial strains from the rhizosphere of baby corn and evaluate their effects alone and in combination with reduced chemical nitrogen fertilizer (CNF) use on the growth, yield and nutrient uptake of baby corn grown in degraded soils. The goal is to develop sustainable cultivation practices that enhance crop productivity, improve soil health and reduce reliance on synthetic inputs, thereby contributing to long-term agricultural sustainability and farmer livelihoods.

## Materials and Methods

### Collection and treatment of baby corn soil and root samples

Thirty baby maize root samples were collected from cultivated fields in the Cho Moi commune, An Giang Province, Vietnam, an area originally characterized by alluvial soils. However, due to the construction of embankments and prolonged monoculture of baby corn, the soil has become degraded and nutrient deficient. Intact roots were gently uprooted, placed in sterile polyethylene bags and immediately transported to the laboratory under cool conditions. For bacterial isolation, root surfaces were surface sterilized. Initially, roots were thoroughly rinsed under running tap water to remove adhering soil particles and debris. The samples were then immersed in 70 % ethanol for 3 min, followed by treatment with 2.5 % sodium hypochlorite for 5 min to minimize microbial contaminants on the surface. The final sterilization step involved briefly dipping the roots in 70 % ethanol, followed by repeated rinsing with sterile distilled water to remove residual disinfectants. Sterilization effectiveness was verified by placing treated root segments onto nutrient agar (NA) plates; the absence of bacterial growth after incubation confirmed successful surface sterilization (30, 31).

### Isolation and identification of strain ATCC 14581

To isolate strain ATCC 14581, surface-sterilized root samples were homogenized and serially diluted up to  $10^8$ . From each dilution, 0.1 mL of the suspension was spread onto yeast mannitol agar (YMA) plates and incubated at 32 °C for 60 hr. Among the resulting colonies, ten bacterial isolates exhibiting morphological characteristics consistent with the genus *Bacillus* were selected for further investigation (32). Molecular identification of the ten selected isolates was conducted through 16S rRNA gene sequencing. Amplification of the target gene was carried out using the universal primers 786F (5'-CGAAAGCGTGGGGAGCAAACAGG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (33). The obtained sequences were aligned and compared with reference sequences in the GenBank database using the BLASTN tool. Phylogenetic analysis was performed in MEGA version 11, with bootstrap analysis conducted using 1000 replicates (34-36). Ambiguous alignment positions were removed using the pairwise-deletion option (37). One isolate was identified as strain ATCC 14581, showing 100 % similarity with reference strains. The sequences obtained were deposited in the NCBI GenBank database and used for phylogenetic tree construction.

### Ammonia production, nitrogenase activity and fixation assay

Ammonia production by the identified strain ATCC 14581 was qualitatively evaluated by inoculating single colonies into peptone water and subsequently incubating them on NA at 30 °C for 60–80 hr. Ammonia presence was confirmed by a color shift from brown to yellow upon addition of Nessler's reagent (38). Nitrogenase activity was determined using the acetylene reduction assay (39). Initially, strain ATCC 14581 colonies were cultivated in YMA broth for 24 hr prior to transfer into nitrogen-free liquid medium, with a parallel uninoculated control included. Under incubation at 30 °C and shaking at 160 rpm, the culture's optical density at 600 nm ( $OD_{600}$ ) reached 0.8 (40). Strain ATCC 14581 was then grown in nitrogen-free medium supplemented with 0.05 % malate as the primary carbon source and incubated at 30 °C under static conditions for a predetermined duration. After

incubation, cultures were centrifuged at 3000 rpm for 1 min to pellet the cells and the resulting supernatant was collected for nitrogen analysis. Quantification of fixed or released nitrogen was performed, enabling precise determination of nitrogen levels under nitrogen-limiting conditions (41).

### Soluble phosphate quantification

The strain ATCC 14581 was cultured in liquid National Botanical Research Institute's (NBRI) phosphate buffer medium at room temperature with shaking at 120 rpm (42). Soluble phosphate content was determined using the ascorbic acid-ammonium molybdate-potassium antimony tartrate reagent, based on the principle that dissolved phosphate in the medium reacts with ammonium molybdate under acidic conditions to form a yellow phosphomolybdate complex. The Oniani colorimetric method, measured at a wavelength of 880 nm, was employed on days 5, 10, 15 and 20 of incubation to quantify the amount of phosphate solubilized.

### Indole acetic acid quantification

The strain ATCC 14581 was cultured in liquid isolation medium supplemented with 100 mg L<sup>-1</sup> tryptophan at room temperature with shaking at 120 rpm. Indole-3-acetic acid production was quantified using Salkowski's reagent (R2) and colorimetric measurements were taken at a wavelength of 530 nm on days 2, 4, 6 and 8 of incubation (43).

### Quantification of thermal, salt and pH adaptation

The evaluation of the thermal adaptability of strain ATCC 14581 was conducted following a two-step procedure. Initially, four test tubes containing YMA liquid medium were prepared for each temperature level (four replicates). Thus, a total of 16 tubes were used for the four tested temperatures (25, 30, 35 and 45 °C). Subsequently, colonies of strain ATCC 14581 were inoculated into the YMA medium and incubated at the four respective temperatures (25, 30, 35 and 45 °C). Each temperature condition was replicated four times and colony growth was monitored daily for one week (31). To assess the salt tolerance of the strain ATCC 14581, a series of YMA agar tubes were prepared with NaCl concentrations of 1.0, 2.0, 3.0, 4.0 and 5.0 %. Colonies of strain ATCC 14581 were inoculated into each medium and incubated at 28 °C. Each treatment was repeated four times and colony growth was evaluated after seven days (31, 44).

The pH tolerance of *B. megaterium* was assessed by culturing the strain in a standard growth medium (NB or YMA liquid medium) adjusted to a series of pH values. The pH levels tested were 5.0, 6.0, 7.0, 8.0 and 9.0, adjusted using sterile 1 N HCl or 1 N NaOH prior to autoclaving. An actively growing bacterial culture was standardized to an optical density (OD<sub>600</sub>) of approximately 0.1 ( $\approx 10^8$  CFU mL<sup>-1</sup>) and inoculated into tubes or flasks containing the prepared media. Cultures were incubated at 30–32 °C for 24–48 hr under shaking conditions (120–150 rpm).

Bacterial growth at each pH level was evaluated by measuring optical density (OD<sub>600</sub>) using a spectrophotometer. In parallel, viable counts (CFU mL<sup>-1</sup>) were determined via serial dilution and plating on NA. The pH range supporting growth was defined as the range in which cultures exhibited visible growth, while the optimum pH was determined as the value yielding the highest OD<sub>600</sub> and CFU counts (45).

### Quantification of soluble phosphorus

The bacterial isolates were cultured in liquid NBRIP medium at room temperature with shaking at 120 rpm. Soluble phosphorus was quantified using the ascorbic acid-ammonium molybdate potassium antimony tartrate reagent, based on the principle that phosphorus, once solubilized in the medium, reacts with ammonium molybdate under acidic conditions to form a yellow phosphomolybdate complex (46). The colorimetric method was employed at a wavelength of 880 nm on days 5, 10, 15 and 20 of incubation to determine the amount of phosphorus released into the medium (47).

### Density augmentation of strain ATCC 14581

The strain ATCC 14581 was cultured in sterilized YMA liquid medium at 32 °C for 60 hr. After incubation, the cultures were centrifuged at 6000 rpm for 5 min to obtain cell pellets and the supernatant was discarded. The harvested bacterial pellets were washed with sterile physiological saline and the cell density was standardized to approximately 10<sup>8</sup> CFU mL<sup>-1</sup>. Each bacterial suspension was then diluted to prepare a 1.0 % (v/v) inoculum for subsequent evaluation of plant growth-promoting traits. In the field experiment, 100 mL of the inoculum was applied directly to maize seeds before sowing (48).

### Experimental design and baby corn variety

A field experiment was conducted in Cho Moi commune, An Giang province. The experimental site is situated on a river islet, which is characterized by low rainfall and humidity during the dry season, from October to April. Throughout the experimental period, ambient temperatures ranged from 22 to 30 °C, with overall cool and stable weather conditions. The experiments were arranged in a randomized complete block design (RCBD) consisting of seven treatments and four replications. Treatments combined two main factors: inoculation with strain ATCC 14581 (applied at a concentration of 10<sup>8</sup> CFU mL<sup>-1</sup>) or no inoculation and different nitrogen reduction rates (0, 25, 50 and 75 %) compared with the standard nitrogen dose of 350 kg ha<sup>-1</sup>. The amounts of chemical fertilizers, nitrogen reduction levels and weight reductions for each treatment are detailed in Table 1.

The baby corn variety CP.468, with a growth duration of approximately 95 to 115 days, was obtained from Red Pine International, Vietnam. It is suitable for flat land with well-drained soil and exhibits good adaptability to diverse environments. Notably, it possesses resistance to leaf and ear diseases, which is

**Table 1.** Treatments of strain ATCC 14581 inoculation and CNF rates in the field experiment

Treatments	Strain ATCC 14581 (10 <sup>8</sup> CFU mL <sup>-1</sup> )	Nitrogen reduction (kg ha <sup>-1</sup> )		Chemical fertilizer (kg ha <sup>-1</sup> )
		Reduction rates (%)	Reduction weight (kg ha <sup>-1</sup> )	
BN1	No	0	350	400 P <sub>2</sub> O <sub>5</sub> -80 K <sub>2</sub> O
BN2	No	25	262.5	
BN3	No	50	175	
BN4	No	75	87.5	
BN5	Yes	25	262.5	
BN6	Yes	50	175	
BN7	Yes	75	87.5	

one of its key advantages. Three pre-germinated seeds of baby corn inoculated with strain ATCC 14581 were sown per hole. The experiment consisted of seven treatments, each replicated four times, covering a total experimental area of 560 m<sup>2</sup> (2 m wide × 10 m long × 4 replicates × 7 treatments). Seeds were sown at a spacing of 25 cm × 30 cm, three seeds per hole and thinned to one healthy plant per hole for 15 days after sowing (DAS). All treatments received chemical fertilizers according to the rates specified and supplied by Binh Dien Fertilizer Company (Vietnam) (Table 1). A total of 28 soil samples were collected from the experimental plots 15 days before sowing and analyzed, with the results presented in Table 2. Agronomic parameters, including plant height, number of tillers and chlorophyll content were recorded at 15 and 30 DAS. Yield attributes, such as marketable ear yield and grain nutrient composition, were analyzed at harvest. Plant and soil analyses were conducted at the Laboratory of An Giang University, Vietnam.

### Analysis methods

Soil texture was determined using the Robison method (49). The pH of the soil was assessed in a 1:2.5 soil-to-distilled water suspension. Organic carbon was analyzed following the Walkley–Black method and total nitrogen (N) was quantified using the Kjeldahl procedure (50). Available phosphorus (P) was measured through the Olsen extraction technique, while exchangeable potassium (K<sup>+</sup>) was determined using the forced BaCl<sub>2</sub> exchange method (51, 52).

### Data collection and analysis

Agronomic traits, yield attributes, ear and cob production and seed nutritional characteristics were measured at harvest for both cropping seasons. The data obtained was statistically processed using Statgraphics XV and Microsoft Excel. An analysis of variance (ANOVA) was conducted and differences among treatment means were assessed using the least significant difference (LSD) test at a  $p \leq 0.05$ .

## Results and Discussion

### Biochemical and genetic profiling of strain ATCC 14581

Of the 30 baby corn root samples, a total of 60 pure bacterial colonies were isolated, averaging two colonies per root sample. Following an initial screening aimed at identifying *Bacillus* species, 10 colonies meeting the selection criteria were retained. All isolated colonies that shared a common morphological feature, grew and developed under microaerophilic conditions in semi-solid YMA medium, forming a thin film approximately 2–5 mm below the medium surface. Colonies of strain ATCC 14581 were Gram-positive, rod-shaped bacteria with rounded ends, motile and spore-forming, measuring approximately 2.0–5.0 μm in length and 0.6–0.8 μm in width. Under the microscope, they were typically observed singly or in short chains microscope (32). Morphologically, prior to molecular confirmation, *B. thaonhiensis*

colonies exhibited a deep pink coloration upon Gram staining, consistent with Gram-positive bacteria. This staining pattern arises from the thick peptidoglycan layer in the bacterial cell wall, which retains the crystal violet dye even after decolorization with alcohol. On YMA medium, the colonies displayed typical strain ATCC 14581 morphology (Fig. 1).

The final selected colony was subjected to biochemical testing and identification through sequencing, confirming it as genus *Bacillus* and species *megaterium*. Among the 10 pure isolates, the colony exhibiting the most distinctive morphological and Gram staining characteristics was selected for sequencing. Strain ATCC 14581 showed 100 % sequence similarity with reference strain *B. megaterium* CACC 157, thereby confirming its taxonomic identity (Fig. 2). Subsequently, the morphology and physiological characteristics of this strain were examined in greater detail to evaluate its nitrogen-fixing potential prior to field experimentation.

Table 3 shows that the VITEK 2 system (BioMérieux, France) identified the bacterial strain with a 95 % probability and this result was subsequently corroborated by illumina-based genetic analysis. The system was also used to characterize the morphological and biochemical traits of the four selected strains. The identification of strain ATCC 14581 relied chiefly on morphological criteria and partly on the VITEK 2 (Table 3). Additionally, phylogenetic analysis based on the 16S rRNA gene sequence with a total branch length of 0.01 confirmed 100 % genetic similarity to the reference sequence of strain ATCC 14581 (Fig. 2). Recent literature underscores that accurate bacterial identification through the integration of morphological, biochemical and molecular approaches is essential for selecting strains with strong potential in agricultural applications particularly for nitrogen fixation and plant growth promotion (53). This dual-level confirmation phenotypic and genotypic greatly enhances the reliability of selecting strains for subsequent field trials. Rigorous multi-method identification ensures taxonomic precision and supports the strain's suitability for developing sustainable biofertilizers (54). In practice, using strains such as strain ATCC 14581 that have been validated with comprehensive techniques minimizes misidentification risk and maximizes the consistency of their performance across varied agricultural conditions (55, 56). The identification of strain ATCC 14581 combining morphological, biochemical and molecular methodologies aligns with best practices in microbial taxonomic verification.

### Evaluation of ammonia activity and levels

To evaluate the ability of the acetylene (C<sub>2</sub>H<sub>2</sub>) reduction process into ethylene (C<sub>2</sub>H<sub>4</sub>), measurements were conducted using an acetylene injection system. A short lag phase was observed immediately after acetylene introduction, followed by a steady reaction throughout the 72 hr incubation period. Both nitrogenase enzyme activity and the N concentration produced increased progressively over time (Fig. 3). Specifically, nitrogenase activity

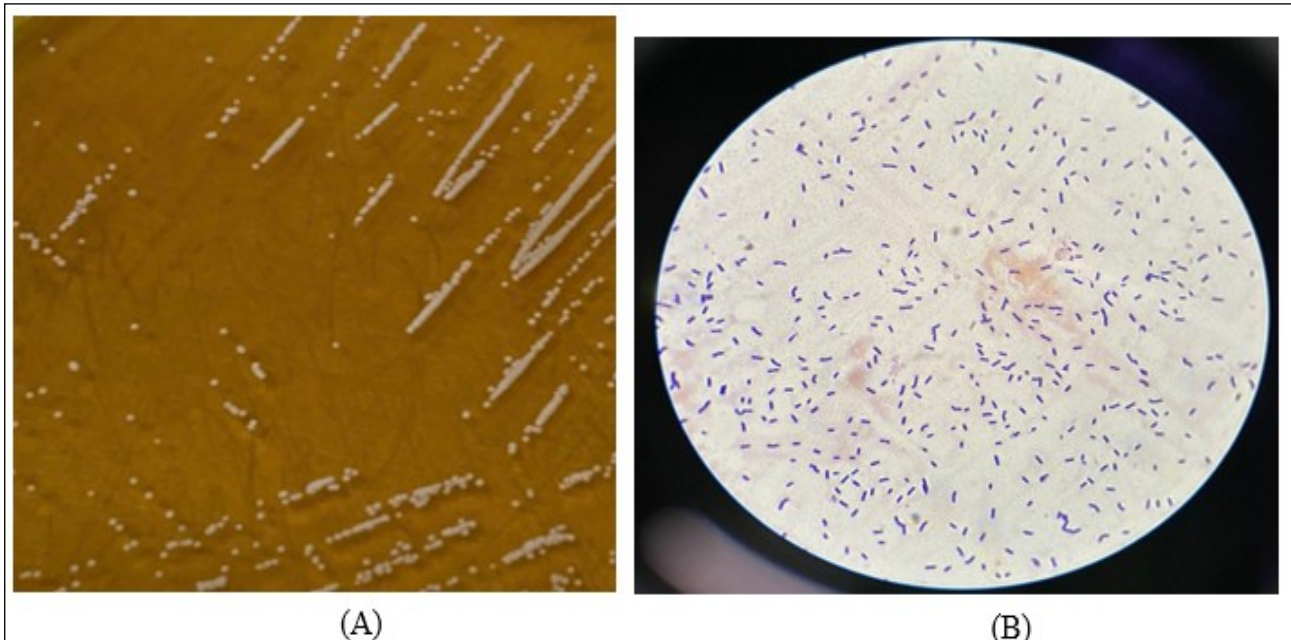
**Table 2.** Physicochemical soil properties prior to the experiment (n = 28)

Properties	Values	Properties	Values
<b>Soil deep (0-20 cm)</b>			
pH (1g soil / 2.5 ml H <sub>2</sub> O)	5.1	CEC cmol <sup>+</sup> kg <sup>-1</sup>	22.0
SOM (%)	4.70	Silt (%)	15.6
Exchangeable K (meq 100 g <sup>-1</sup> )	0.27	Clay (%)	2.00
Total N (%)	0.26	Sand (%)	82.4
Available P (mg P 100 g <sup>-1</sup> )	24.8	Texture	Loamy sand

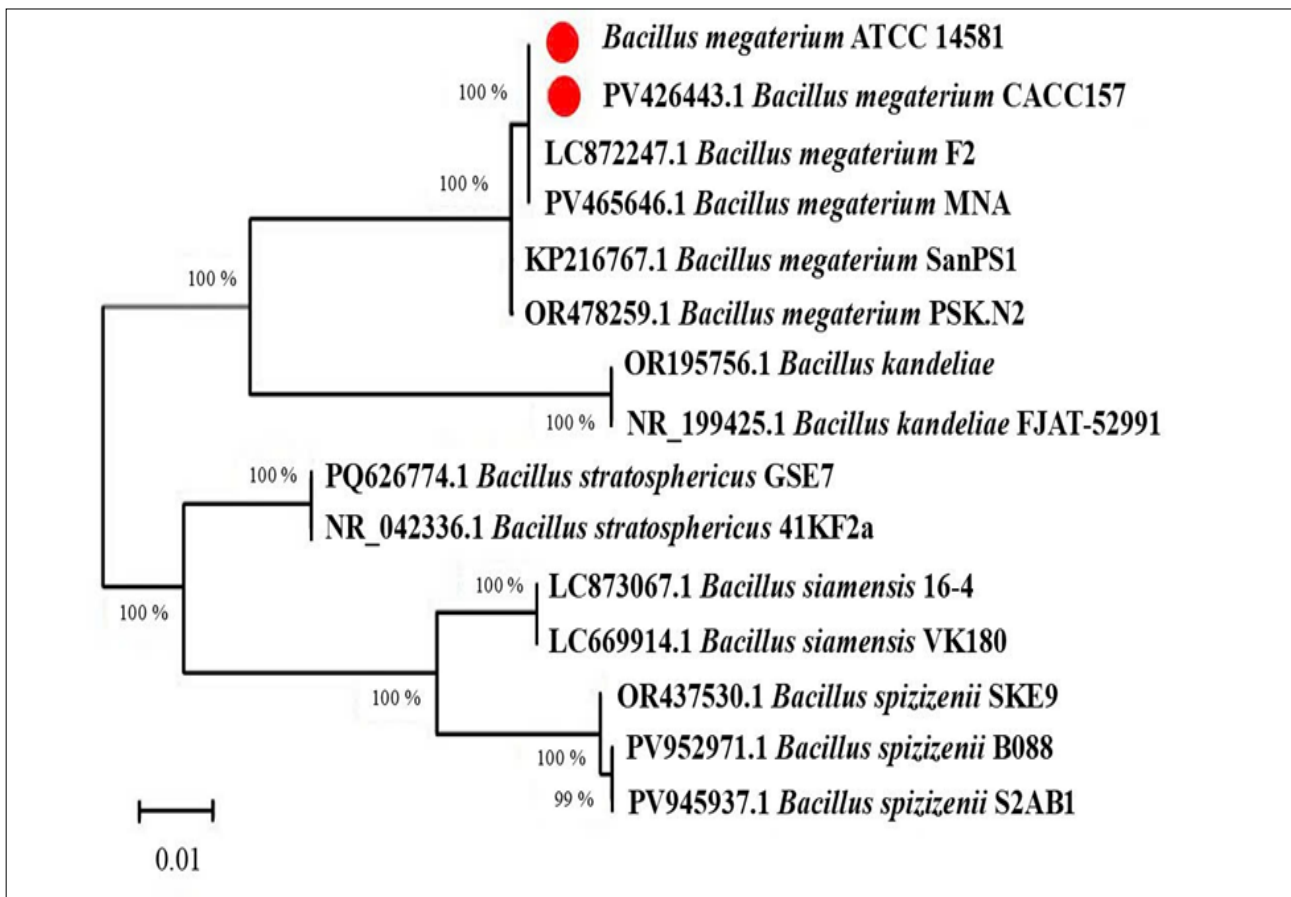
**Table 3.** The chemical characterization of strain ATCC 14581 using the VITEK 2 analyzer

Biochemical test	Strain ATCC 14581	Biochemical test	Strain ATCC 14581
Starch hydrolysis	+	NaCl (1 - 5 %)	++
Cyclodextrin	+	pH (5.0 - 9.0)	++
A-mannosidase	-	Temperature (20 - 45 °C)	++
D-glucose	-	D - mannitol	+
D-galactose	-	Citrate use	+
D-ribose	-	β - glucosidase	+
Catalase	++	Mannitol	+
Oxidase	-	Raffinose	+

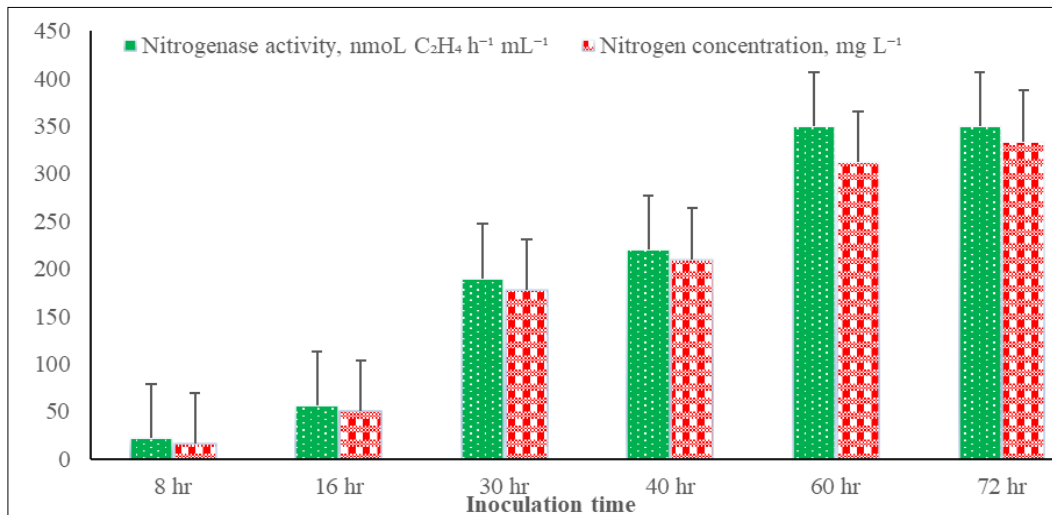
(-): negative reaction; (+): weak reaction; (++): strong reaction.



**Fig. 1** (A): Colonies of the reference strain ATCC 14581 grown on the culture medium; (B): Micrograph of strain ATCC 14581 observed under a light microscope at 100× magnification.



**Fig. 2.** Phylogenetic tree of strain ATCC 14581 isolated from corn roots with the sequences from previously selected strains. Using MEGA 11 software with corresponded to 0.01 of the scale bar per nucleotide position. Numbers on the branches were bootstrap percentages.



**Fig. 3.** Variations in nitrogenase activity (nmol C<sub>2</sub>H<sub>4</sub> h<sup>-1</sup> mL<sup>-1</sup>) and nitrogen concentration (mg L<sup>-1</sup>) of the strain ATCC 14581 across different inoculation times.

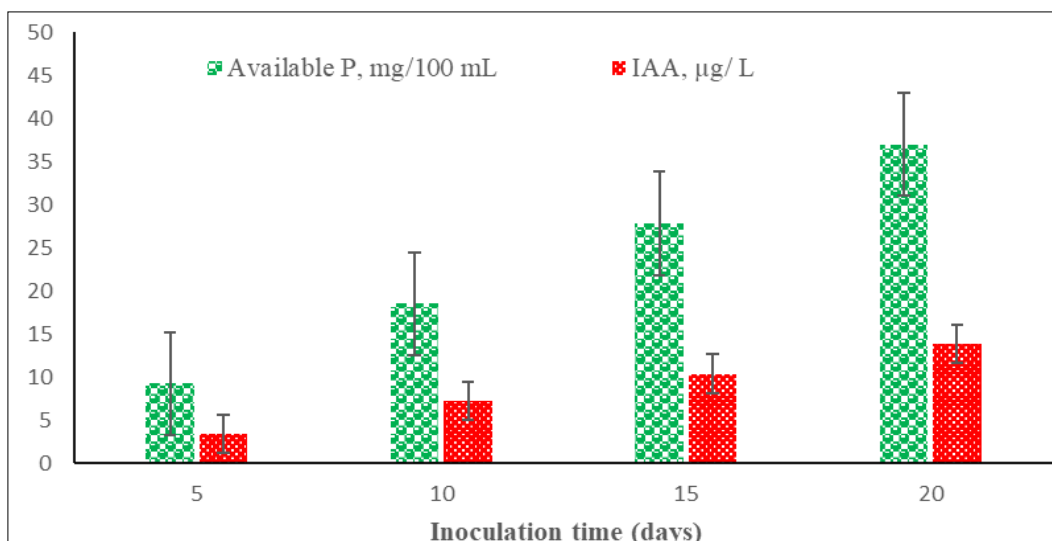
started at a low level (approximately 25 nmol C<sub>2</sub>H<sub>4</sub> h<sup>-1</sup> mL<sup>-1</sup> after 8 hr) and increased steadily, reaching a maximum of around 312 nmol C<sub>2</sub>H<sub>4</sub> h<sup>-1</sup> mL<sup>-1</sup> after 72 hr. Similarly, the nitrogen concentration, determined using the Kjeldahl method, rose from below 12 mg L<sup>-1</sup> at 8 hr to over 334 mg L<sup>-1</sup> at 72 hr. These findings confirm that strain ATCC 14581 actively participates in biological N fixation, with clear evidence of sustained nitrogenase activity over time (57). The strong correlation between ethylene production and nitrogen accumulation indicates that this species maintains robust nitrogen-fixing capacity under laboratory conditions, consistent with previous reports on highly efficient diazotrophic bacteria (58).

#### Phosphate solubilization capacity and IAA production

Both phosphate solubilization (mg 100 mL<sup>-1</sup>) and IAA concentration (μg L<sup>-1</sup>) of the strain ATCC 14581 increased steadily over the incubation period, with a short initial lag phase, followed by a progressive rise toward a plateau at later time points (Fig. 4). This pattern is consistent with the physiology of *Bacillus* spp., in which the exponential growth phase is accompanied by secretion of organic acids (e.g., citric, gluconic and lactic) and/or phosphatase enzymes that facilitate the release of PO<sub>4</sub><sup>3-</sup>. Concurrently, the tryptophan-dependent pathway accelerates IAA biosynthesis during the late growth phase. The trends gradually increase with time reported for multifunctional plant growth-

promoting bacteria (Fig. 4). The solubilization of insoluble phosphate by *Bacillus* is primarily driven by organic acid production, which lowers local pH and chelates cations (Ca<sup>2+</sup>, Fe<sup>3+</sup> and Al<sup>3+</sup>) to release H<sub>2</sub>PO<sub>4</sub><sup>-</sup> and HPO<sub>4</sub><sup>2-</sup>; phosphatase/phytase enzymes, which hydrolyze organic phosphorus compounds and siderophore production, which competes for Fe<sup>3+</sup>, indirectly freeing Fe-bound phosphate. The steady increase in soluble P suggests that organic acid production plays a prominent role, as its accumulation is often aligned with late exponential or stationary growth phases (55).

Indole-3-acetic acid synthesis in *Bacillus* is generally tryptophan dependent. As biomass increases, enzymes of the indole-3-pyruvate and indole-3-acetamide pathways become more active, resulting in the progressive accumulation of IAA over time (Fig. 4). Indole-3-acetic acid promotes root system development (elongation and lateral root formation) and enhances P uptake by increasing root surface area. This synergy creates a positive feedback loop between phosphate availability and root growth. The parallel trends suggest a positive correlation between soluble P release and IAA accumulation. Physiologically, exogenous IAA stimulates root exudation of carbon-rich compounds, which can enhance bacterial metabolism and organic acid secretion, thereby further increasing P solubilization.



**Fig. 4.** Variations in phosphate solubilization (mg 100 mL<sup>-1</sup>) and IAA concentration (μg L<sup>-1</sup>) of the strain ATCC 14581 across different inoculation times.

Such dual-function interactions are advantageous in plant and microbe associations, enabling the host plant to access nutrients more efficiently (29, 31). *Bacillus* strains exhibiting dual functionality P solubilization and IAA production offer combined benefits by increasing the pool of plant-available phosphorus while stimulating root growth, particularly in soils with high levels of insoluble P (acidic, calcareous or iron/aluminum-rich soils). The selection of this strain ATCC 14581 for biofertilizer formulations aimed at reducing chemical phosphate fertilizer use and improving phosphorus use efficiency (29).

#### Effect of strain ATCC 14581 and CNF rates on baby corn yield traits

Table 4 indicates a clear trend of positive effects from ATCC 14581 inoculation on fresh yield components such as silk, husk, cob and ear weight particularly under optimal CNF reduction conditions. Treatments BN5 and BN6, achieved the highest values across all yield components, with silk yields of  $0.56 \text{ t ha}^{-1}$  and  $0.57 \text{ t ha}^{-1}$ , husk yields of  $2.26 \text{ t ha}^{-1}$  and  $2.27 \text{ t ha}^{-1}$ , cob yields of  $2.91 \text{ t ha}^{-1}$  and  $2.95 \text{ t ha}^{-1}$  and total ear yields of  $7.46 \text{ t ha}^{-1}$  and  $7.56 \text{ t ha}^{-1}$ , respectively. These treatments significantly outperformed all other nitrogen-reduction treatments (from BN1 to BN4 and BN7) and even matched or exceeded the non-reduction control, demonstrating that bacterial inoculation can compensate for reduced nitrogen input.

Compared to BN1, which likely represents the chemical fertilizer control without nitrogen reduction, BN5 and BN6 increased total ear yield by approximately 9–11 %, suggesting that the synergistic effect of beneficial bacteria with a reduced nitrogen regime supports better nutrient uptake and plant growth. This aligns with previous findings where PGPR improved maize yield under reduced CNF conditions by enhancing nitrogen use efficiency and root architecture (13, 15). On the contrary, treatments BN2, BN3 and BN4 which also applied nitrogen reduction but likely without optimal bacterial strains or combinations recorded significantly lower yields (ear yields of

$4.29 - 4.73 \text{ t ha}^{-1}$ ), indicating that the beneficial effect depends on the specific bacterial strain and application strategy. BN7, although better than BN2, BN3 and BN4, still underperformed compared to BN5 and BN6, reinforcing that not all bacterial treatments yield equivalent benefits. The positive performance of BN5 and BN6 under reduced nitrogen suggests that certain bacterial strains may enhance nutrient solubilization (especially N and P), stimulate phytohormone production such as IAA and improve water and nutrient uptake efficiency, thereby maintaining or increasing yield despite reduced synthetic nitrogen application (26). This is consistent with sustainable agriculture strategies aiming to reduce CNF use without sacrificing yield, which can contribute to both economic and environmental benefits (24).

Table 5 demonstrates that ATCC 14581 inoculation can significantly enhance yield parameters of baby corn even under optimal nitrogen reduction conditions. Treatments BN5 and BN6 exhibited the highest plant biomass, tassel length, cob length and cob diameter, surpassing both the control (BN1) and other N-reduction treatments. BN6 achieved the highest value across all parameters, with plant biomass of  $4.95 \text{ t ha}^{-1}$ , tassel length of  $0.76 \text{ cm}$ , cob length of  $10.0 \text{ cm}$  and cob diameter of  $1.97 \text{ cm}$ . BN5 showed a similar trend with slightly lower but statistically comparable results ( $p \leq 0.01$ ). Compared to BN1 (no nitrogen reduction), BN6 increased cob length by approximately 7.7 % and cob diameter by 25.5 %, indicating that bacterial inoculation not only compensates for reduced nitrogen but may also improve morphological traits linked to market quality. In contrast, nitrogen-reduction treatments without optimal bacterial support (BN2, BN3 and BN4) resulted in significantly reduced performance, particularly in cob diameter (as low as  $1.0 \text{ cm}$  in BN4) and plant biomass ( $2.62 \text{ t ha}^{-1}$  in BN4). BN7, although higher than BN2, BN3 and BN4, still fell short of BN5 and BN6, suggesting that both bacterial strain specificity and nitrogen management are critical for maximizing yield benefits.

The superior performance of BN5 and BN6 aligns with

**Table 4.** Effect of strain ATCC 14581 inoculation and CNF reduction levels on fresh yield components

Treatments	Fresh yield ( $\text{t ha}^{-1}$ )			
	Silk	Husk	Cob	Ear
BN1	$0.52 \pm 0.01^b$	$2.09 \pm 0.05^b$	$2.66 \pm 0.08^e$	$6.82 \pm 0.19^b$
BN2	$0.36 \pm 0.02^d$	$1.45 \pm 0.07^d$	$1.84 \pm 0.08^{de}$	$4.73 \pm 0.20^d$
BN3	$0.34 \pm 0.02^e$	$1.36 \pm 0.05^e$	$1.71 \pm 0.09^d$	$4.39 \pm 0.43^{de}$
BN4	$0.32 \pm 0.0^e$	$1.29 \pm 0.06^e$	$1.68 \pm 0.08^c$	$4.29 \pm 0.39^e$
BN5	$0.56 \pm 0.02^a$	$2.26 \pm 0.07^a$	$2.91 \pm 0.11^b$	$7.46 \pm 0.49^a$
BN6	$0.57 \pm 0.01^a$	$2.27 \pm 0.06^a$	$2.95 \pm 0.08^a$	$7.56 \pm 0.40^a$
BN7	$0.43 \pm 0.01^c$	$1.70 \pm 0.01^c$	$2.13 \pm 0.12^a$	$5.47 \pm 0.36^c$
F test	**	**	**	**
CV(%)	22.9	22.9	23.6	23.6

\*\* $: p \leq 0.01$ ; CV (%) denotes the coefficient of variation; the symbol ( $\pm$ ) represents the standard deviation of the mean based on four replications; identical letters within the same column for each parameter indicate no statistically significant difference among the means.

**Table 5.** Effects of strain ATCC 14581 and CNF levels on the yield parameters of baby corn

Treatments	Plant biomass	Tassel	Cob length	Cob diameter
	( $\text{t ha}^{-1}$ )		(cm)	
BN1	$4.87 \pm 0.07^a$	$0.67 \pm 0.01^b$	$9.28 \pm 0.02^b$	$1.57 \pm 0.09^b$
BN2	$3.26 \pm 0.35^b$	$0.53 \pm 0.03^d$	$8.66 \pm 0.17^c$	$1.3 \pm 0.08^c$
BN3	$3.16 \pm 0.13^b$	$0.45 \pm 0.05^e$	$8.59 \pm 0.09^c$	$1.18 \pm 0.13^c$
BN4	$2.62 \pm 0.07^c$	$0.36 \pm 0.01^f$	$8.16 \pm 0.01^c$	$1.0 \pm 0.14^d$
BN5	$4.92 \pm 0.13^a$	$0.68 \pm 0.02^b$	$9.72 \pm 0.79^{ab}$	$1.88 \pm 0.10^a$
BN6	$4.95 \pm 0.09^a$	$0.76 \pm 0.01^a$	$10.0 \pm 0.49^a$	$1.97 \pm 0.10^a$
BN7	$3.3 \pm 0.15^b$	$0.59 \pm 0.05^c$	$9.26 \pm 0.27^b$	$1.48 \pm 0.10^b$
F test	**	**	**	**
CV (%)	24.7	23.2	7.81	23.6

\*\* $: p \leq 0.01$ ; CV (%) denotes the coefficient of variation; the symbol ( $\pm$ ) represents the standard deviation of the mean based on four replications; identical letters within the same column for each parameter indicate no statistically significant difference among the means.

previous studies showing that PGPR can enhance nitrogen uptake efficiency, stimulate phytohormone production and improve root architecture, resulting in better nutrient acquisition and cob development even with reduced nitrogen fertilizer inputs (43). Moreover, improvements in cob diameter and length are particularly important for baby corn, where market acceptance is closely tied to uniform size and shape (18, 23). These findings reinforce the potential of integrating bacterial inoculation into nitrogen management strategies to achieve yield stability or improvement while reducing reliance on synthetic fertilizers, thereby supporting sustainable and environmentally friendly agricultural practices (29, 56).

### Effect of strain ATCC 14581 and CNF rates on nutrient composition of edible cob

Table 6 reveals that bacterial inoculation, particularly with strain ATCC 14581, significantly improved certain nutrient quality parameters of baby corn under optimal nitrogen reduction. Treatments BN5 and BN6 stood out for their higher lipid and phosphorus contents compared with both the full-nitrogen control (BN1) and the reduced-N treatments without bacterial support (BN2, BN3 and BN4). BN6 achieved the highest lipid content (0.31 %) and phosphorus concentration (29.5 mg kg<sup>-1</sup>), closely followed by BN5 (0.30 % lipid and 29.4 mg kg<sup>-1</sup> phosphorus). Both treatments exceeded BN1's phosphorus content by approximately 8–9 %, indicating that bacterial inoculation can enhance mineral accumulation even with reduced nitrogen fertilizer. This is consistent with the role of PGPR in mobilizing and solubilizing nutrients such as phosphorus through organic acid production and enzyme secretion (11, 26). Although protein content differences among treatments were statistically non-significant ( $p > 0.05$ ), BN6 and BN5 still recorded slightly higher protein levels (2.42 and 2.36 %, respectively) than BN1 (2.31 %), suggesting a tendency toward improved nitrogen assimilation efficiency under bacterial inoculation. In contrast, BN2, BN3 and BN4, which received reduced nitrogen without optimal bacterial inoculation, had lower lipid (0.17–0.19 %) and phosphorus levels (16.75–18.8 mg kg<sup>-1</sup>), underscoring the importance of the microbial partner in nutrient uptake.

Interestingly, the moisture (humidity) content was significantly lower in BN6 (75.3 %) than in other treatments (> 82%), which could be associated with a higher concentration of dry matter and improved maturity kernel traits often linked with better market quality and storability (18, 56). Overall, these findings confirm that PGPR inoculation, combined with optimal nitrogen reduction, can maintain or even improve the nutritional quality of baby corn while reducing synthetic fertilizer dependence. This supports sustainable agricultural practices that meet both yield

and quality targets (57, 58).

### Conclusion

In conclusion, the indigenous strain ATCC 14581 demonstrates significant potential as a biofertilizer for baby corn cultivation. Its strong nitrogen-fixing ability, phosphate solubilization and IAA production significantly enhance plant growth, nutrient uptake and yield, even under reduced nitrogen fertilizer applications. Field experiments demonstrated confirmed that inoculation with this strain can effectively lower chemical nitrogen use by up to 50 % without compromising yield. These findings support the development of sustainable, climate-smart agricultural practices that enhance soil fertility and productivity in baby corn production. Further research should explore large-scale applications and long-term soil health impacts.

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### Authors' contributions

NVC, NNPT, TTL and TLKT were responsible for conducting the experiments. NVC prepared the initial draft of the manuscript. NNPT, TLKT and TTL contributed to the conceptualization, study design and overall coordination. All authors read and approved the final manuscript.

### Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interest to declare.

**Ethical issues:** None

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**Table 6.** Effects of strain ATCC 14581 and CNF levels on nutrient composition of the baby corn edible cob

Treatments	Humidity	Lipid (%)	Protein	Phosphorous mg kg <sup>-1</sup>
BN1	84.9 ± 6.05 <sup>a</sup>	0.26 ± 0.01 <sup>b</sup>	2.31 ± 0.19	27.1 ± 0.71 <sup>b</sup>
BN2	86.3 ± 1.83 <sup>a</sup>	0.19 ± 0.01 <sup>d</sup>	2.25 ± 0.11	18.8 ± 0.89 <sup>d</sup>
BN3	86.3 ± 1.21 <sup>a</sup>	0.18 ± 0.01 <sup>de</sup>	2.12 ± 0.12	17.7 ± 0.71 <sup>e</sup>
BN4	86.6 ± 1.89 <sup>a</sup>	0.17 ± 0.01 <sup>e</sup>	2.01 ± 0.09	16.75 ± 0.76 <sup>e</sup>
BN5	82.6 ± 2.72 <sup>a</sup>	0.30 ± 0.01 <sup>a</sup>	2.36 ± 0.21	29.4 ± 0.87 <sup>a</sup>
BN6	75.3 ± 5.74 <sup>b</sup>	0.31 ± 0.02 <sup>a</sup>	2.42 ± 0.47	29.5 ± 0.78 <sup>a</sup>
BN7	86.3 ± 2.34 <sup>a</sup>	0.22 ± 0.01 <sup>c</sup>	2.28 ± 0.55	22.2 ± 0.08 <sup>c</sup>
F test	**	**	ns	**
CV (%)	5.9	23.2	13.3	23.0

\*\* $p \leq 0.01$ ; CV (%) denotes the coefficient of variation; the symbol ( $\pm$ ) represents the standard deviation of the mean based on four replications; identical letters within the same column for each parameter indicate no statistically significant difference among the means.

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