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Growth enhancement of Brazilian green dwarf coconut seedlings by *Bacillus cereus* **in coconut fiber substrate is associated with changes in metabolism and reduction in fertilization**

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Abstract

The objective of this work was to evaluate the effects of application of *Bacillus cereus* combined with the use of substrate and reduction in conventional fertilization on the growth of green dwarf coconut seedlings in Brazil, the Santa Isabel nursery in the state of Pará, Brazil. The experiment design was completely randomized with four treatments: soil+100% fertilizati+on; soil+ 50% fertilization + *B. cereus*; coconut fiber+100% of fertilization; and coconut fiber+33% of fertilization plus *B. cereus*, with 5 replications. Biochemical tests were performed with *B. cereus* and in the field where the following were evaluated: biometrics, hormonal and nutritional profile compared by t Test ($P < 0.05$). Isolate *B. cereus* produced siderophores, IAA and phosphate solubilizer. In the field, the treatmwxent with inoculated coconut fiber and *B. cereus* stood out where nursery time was reduced by two months. Increments by 190% in IAA, 31% in GA3, and 17% in GA4 were found. This combination resulted in an increase by 98% in N, 42% in P, 82% in K, 103% in Ca, 68% in Mg, 84% in B, 41% in Fe, compared to control plants in coconut fiber+100% fertilization. Therefore, the use of *B. cereus* to obtain coconut seedlings is a technology that provides greater development in a shorter time in nursery and reduction in application of inputs.

Keywords: PGPR. Rhizosphere.Sustainable. agriculture.

1. **Introduction**

The coconut tree culture has great economic and social importance due to the value generated and added to its production chain. According to FAO [1], Indonesia stands out as the world's largest coconut producer, followed by the Philippines, India and Sri Lanka. Brazil is ranked fifth among the coconut producing countries in the world. Its production occupies and area of 215,700 hectares, yielding approximately 1,937,484 million fruits [2], out of which 1,485,184 correspond to the production of green dwarf and hybrid (SINDCOCO, 2018).

In order to achieve vigorous coconut palm seedlings, it is essential to consider obtaining good quality genetic material, adequate phyto-sanitary management, and better use of synthetic fertilizer, as the slow germination and pre-formation of seedlings is irregular, lasting three months at this stage (RABELO et al., 2006). Another important factor is the choice of substrate, in which its physical, chemical and biological characteristics must be considered (YAMANISHI et al., 2004).

The production of coconut seedlings has as its main limiting factor the duration of the cycle until transplanting to field, ranging from eight to 12 months, therefore, time, inputs and services are required. Fertilizers, fungicides, herbicides, labor, are some of the costs to be considered for obtaining seedlings (VINODHINI; DESHMUKH, 2017). The use of low efficiency synthetic fertilizers is largely related to the loss of leaching and evaporation into the atmosphere (TILMAN, 1998; GYANESHWAR et al., 2002). Thus the efficient use of synthetic fertilizers is important for productivity, combined with less damage to the environment (PAUNGFOO-LONHIENNE et al., 2019).

The use of microorganisms in agriculture is considered a viable and sustainable management, especially in search for the best use of synthetic fertilizers. The use of rhizobacteria in plants can promote growth (GANGE; GADHAVE, 2018). Some genera have already been described as growth promoters in coconut seedlings such *as Pseudomonas* sp. and *Bacillus* sp. (George et al., 2018). Rhizobacteria have the ability to alter anatomical characteristics, and provide better photosynthetic, hormonal and nutritional performance (LUCY; REED; GLICK, 2004; LWIN et al., 2012; SAMANIEGO et al., 2016). Rhizobacterial species such as *Bacillus* spp., *Pseudomonas* sp. and *Azuspirillum brasiliense* has the ability to make nutrients available in other ways, such as producing siderophores, solubilizing phosphate and mineralizing organic matter (AZIZ et al., 2012; SASIREKHAA; SHIVAKUMARB, 2016; NUMOTO et al., 2019). They also stimulate the synthesis of phytohormones such as indole acetic acid (AIA) and gibberellins that promote growth of the roots and the aerial part (PAHARI; MISHRA, 2017).

Thus, the objective of this work was to promote the growth of coconut seedlings based on the inoculation with *Bacillus* cereus, combined with different substrates and reduced synthetic fertilization.

Methodology

The field experiment was conducted on Reunidas Sococo commercial farm located in Santa Isabel do Pará-Brazil (1°13'26”S and 48°02'29” W).

Obtaining Isolate

Bacillus cereus isolate was obtained from rhizospheric soil samples taken from commercial coconut palm plantations through serial dilution according to Fillippi et al., (2011) and stored in the crop collection of the Plant Protection Laboratory- (LPP) at UFRA.

Identification of bacterial isolate

Isolate of *B. cereus* was cultured in Kado and Heskett (1970) culture medium 523 for 24 hours at 28°C. Microbial growth material was added to a micro-tube containing 567µL TE1x, followed by shaking. After that, 30µL of 10% SDS was added, followed by stirring. Then, 3 µl proteinase K was also added followed by shaking, and incubation for 60 minutes at 37°C. After this period, 100 µL 5 M NaCl was added, followed by stirring. Next, 80 µl CTAB/NaCl was added, followed by stirring and water bath at 65°C for 10 minutes. Finally, 780µL of isoamyl chloroform (24: 1) was added and stirred for 10 minutes and centrifuged for five minutes at 14,000 rpm. The supernatant was transferred to micro-tubes and 960µL of ice-cold isopropanol was added. Subsequently the micro-tube was inverted until the DNA was visible and centrifuged at 14,000 rpm for 25 minutes. The supernatant was discarded and 1mL of 70% ethanol was added, and centrifuged for 3 minutes at 14,000 rpm. Then, the supernatant was discarded and the pellet was dried for 20 minutes in a slide chamber. The DNA was re-suspended in 100 µL 0.1x TE and 6mg mL-1 ribonuclease, followed by incubation at 25°C for 12 hours (DOYLE; DOYLE, 1990). DNA was quantified through BioDrop µLite (Thermo Fisher Scientific, USA), resulting in a final concentration of 10 ngµL-1 and stored at -20°C.

The UFRA19 isolate was identified by using the 16S rDNA region gene with the aid of 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'ACCTTGTTACGACTT-3') primers (LANE, 1991). The PCR amplification reaction was composed of 1X Master Mix 2X (Promega) (0.05 U µL-1 Taq DNA polymerase, 4 Mm MgCl2 reaction buffer, 0.4 Mm of each DNTP); 10 µM of each primer and 50 ng DNA. Amplification of the 16S rDNA region was performed in a thermal cycler (Eppendorf MasterCycler Nexus Hamburg, Germany) with the following steps: initial denaturation at 94°C for 4 minutes; 25 cycles of 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute; and final extension at 72°C for 7 minutes.

Reactions were purified using 5 µL PCR product plus 2 µL Exo-sap enzyme (Exonuclease / Alkaline Phosphatase). Samples were purified through thermal cycler, performed at 37°C for 4 minutes, followed by an incubation period at 80°C for 1 minute to irreversibly inactivate both enzymes. After the purification reaction, sequencing was carried out in an automated sequencer ABI3730 owned by the Laboratory of Bioinformatics and Evolutionary Biology of the Federal University of Pernambuco (LABBE-UFPE).

DNA sequence analysis and assembly of the UFRA19 isolate contigs was performed with the aid of the Staden Package (STADEN et al., 1998). The nucleotide sequence of the UFRA19 bacteria was

compared to the isolate sequences available in the National Center for Biotechnology Information (NCBI) database using the BLASTn software (https://www.ncbi.nlm.nih.gov). Afterwards, all sequences were aligned (MEGA). Bayesian Inference (IB) analysis was performed by means of Mr. Bayes v.3.2.6 (RONQUIST et al., 2012) implemented in CIPRES (https://www.phylo.org/portal2/home.action) using the best nucleotide replacement model selected according to Aikake's Information Criterion (AIC) through Mr. Modeltest 2.3 (Nylander 2004), using 1,000,000,000 generations of Markov Chain Monte Carlo (MCMC), with sampling every 1,000 and 10,000 generations. Subsequent probabilities were calculated after discarding the first 25% generations. All trees obtained from individual genes and concatenated through the IB method were visualized through Fig Tree 1.4.1 software. (http://tree.bio.ed.ac.uk/software/figtre).

Biochemical Test

Detection of Indoleacetic Acid (AIA) Production

The *B. cereus* isolate was grown in Luria Bertani (LB) medium under 100 rpm agitation and incubated at 28°C for 78 hours. After this period, 3 mL of the suspension was centrifuged at 4°C for 10 minutes at 4000 rpm (MOUSTAINE et al., 2017). Then 90 µL of supernatant and 60 µL of Salkowski reagent were added into another micro-tube, and then incubated in the dark for 30 minutes to determine the occurrence of a change in media color (GORDON; WEBER, 1951).

Detection of Siderophores production

Isolate of *B. cereus* was inoculated into test tubes containing 10 mL TSL (1:10 diluted) medium (3 g in 1000 mL distilled water) and incubated at 28°C under agitation at 114 rpm for 24 hours. Subsequently, the tube containing the bacterial suspension was centrifuged for 10 minutes at 12,000 rpm. Then 1 mL of the added supernatant was transferred into another tube containing 1 mL of blue chrome S (BCS) solution. Fifteen minutes after mixing, the isolate converts the dark blue color to yellow when the siderophore is produced (SCHWYN; NEILANDS, 1987).

Phosphate Solubilization Detection

Isolate of *B. cereus* was grown in NBRIP growth medium, according to Nautiyal (1999), containing 10 g glucose, 2.5 g Ca3 (PO4) 25 g MgCl2 W6H2O, 0.25 g MgSO4W7H2O, 0.2 g KCl, and 0.1g (NH4) 2SO4, pH = 7.0 and addition of 1.5% agar in triplicate. The plates were incubated for 14 days at 28°C, then the presence of halo was evaluated, which characterizes phosphate solubilization.

Experimental Design

Seedlings of Brazilian green dwarf Coconut (Cocos nucifera L.) at one month of age were used in a completely randomized design with four treatments: T1: soil and 100% of conventional fertilization; T2: soil + 50% of conventional fertilization and application of *B. cereus*; T3: coconut fiber + 100% of conventional fertilization; T4: coconut fiber + 33% of conventional fertilization + application of *B. cereus*, with 5 repetitions.

Fertilization

Conventional fertilization was applied to one Brazilian green dwarf coconut seedling over the eight-month cycle: 18 g Urea, 240 g SFS (18% P2O5), 60 g KCL (60% K2O), 30 g MgO (30 % Mg) and 3g Ulexite (10% B), the application of synthetic fertilization occurs in three plots at the first, third and fifth months according to Lins and Viegas (2008).

Chemical characterization of coconut fiber soil and substrate

The seedlings were grown in a 40 x 40- polyethylene bag on soil substrate with the following chemical characterization: pH (CaCl2) 3.9; 0.012g Kg -1 Ca; 0.03 g Kg-1 Mg; 0.019 g Kg-1 K; 0.004 g Kg-1 P and 0.8% organic matter (OM). The chemical characterization of coconut fiber substrate (Golden-Mix type 4-amafribra) was, as it follows: 0.086 g Kg-1 N; 0.264 g Kg-1 P; 0.580 g Kg-1 K; 0.128 g Kg-1 Ca; 0.447 g Kg-1 Mg; 272.86mg S; 42.25mg Na; 0.703 g Kg-1 B; 0.12 g/kg Cu; 0.5mg Fe; 0.6mg Mn; 0.78mg Zn and 92.43% OM.

Suspension Preparation

The bacterial suspension was adjusted in a 550 nm Spectrophotometer (Å550 = 0.1 corresponding to 108 CFU·mL-1) (FILIPPI et al., 2011), and the volume adjusted to 300 mL per pot. Two applications were performed per seedling.

Assessments

Biometry

The biometric evaluations of coconut seedlings were performed at two different periods. For seedlings grown on soil substrate, the evaluation was performed when they were eight months old and six months old for seedlings grown on coconut fiber substrate, due to root extravasation in coconut fiber

166 treatment. The following were evaluated: plant height, stem diameter, fresh mass of the aerial part and roots
167 and dry mass of the aerial part and roots.

168 Hormonal profile

169 Concentration of the hormones AIA, GA3, GA4 and ACC, phytohormone ethylene precursor, was
170 determined following MUNNE´-BOSCH (2011). For the determination, 300 mg of fresh tissue from the
171 second leaf of the plants was stored in liquid nitrogen, soon after, the material was lyophilized and
172 macerated in liquid nitrogen. Then, 40 mg dry mass was weighed and 400 µL of extraction solvents
173 (methanol: isopropyl alcohol: acetic acid 20: 79: 1) were added. Samples were vortexed 4 times for 20s (on
174 ice), sonicated for 5 minutes, placed on ice for 30 min, and then centrifuged at 13,000 rpm for 10 min at
175 4°C. After centrifugation, 350 µL of supernatant was removed and transferred to another micro-tube.
176 Approximately 300 µL of the extract obtained in flasks was added and from these, 5 µL were injected into
177 the NuBioMol LC/MS system (Biomolecule Analysis Center - UFV, Brazil). A chromatography column
178 (Agilent Eclipse) was used (RRHD, 1.8, lm, 2.1 9 50 mm) with a flow rate of 0.3 mLmin-1 coupled to a
179 triple quadrupole QQQ mass spectrometer (Agilent). Mass spectra were alternately negative/positive
180 operated according to retention time for each hormone standard and sample was scanned through Multiple
181 Reaction Monitoring (MRM) using the following mass transitions: JA 209> 59; SA 137> 93; ABA 263>
182 153; ACC 106> 56. The generated mass spectra were processed using MassHunter Software to obtain the
183 extracted ion chromatograms (XIC) of each transition and area values, indicative of the abundance of each
184 hormone. A curve pattern of each hormone over a concentration range from 0.1 to 300 ng mL-1 was used
185 to convert the XIC area values into ng g-1 of plant tissue (MUNNE´-BOSCH, 2011). Molecular mass
186 spectra analysis was obtained with the aid of Skyline software.

187

188 Nutritional content of dry matter shoot

189 In order to determine the phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and iron
190 (Fe) contents, the plant material was dried and ground and subsequently subjected to nitro-perchloric
191 digestion. Boron (B) was analyzed after dry digestion of the samples by the Azometrinah method. For
192 determination of nitrogen (N) contents, the plant material was submitted to sulfuric digestion
193 (MALAVOLTA; VITTI; OLIVEIRA, 1997).

194 Statistical analysis

Differences among means of the treatments were evaluated by the t Test ($p < 0.01$). All data were analyzed using R software version 3.5.1. (R, 2019).

3 Results

Identification of bacterial isolate

The UFRA19 isolate sequence was compared in GenBrank by means of the BLASTn tool. The isolate showed 99.79% identity with the genus *Bacillus* (ATCC14579T). Based on the construction of the phylogenetic tree, it was possible to identify the species of the genus *Bacillus*, the isolate showed similarity to *B. cereus* species. The sequence was deposited in Genbank as UFRA19 with accession number MN393059 (Figure 1).

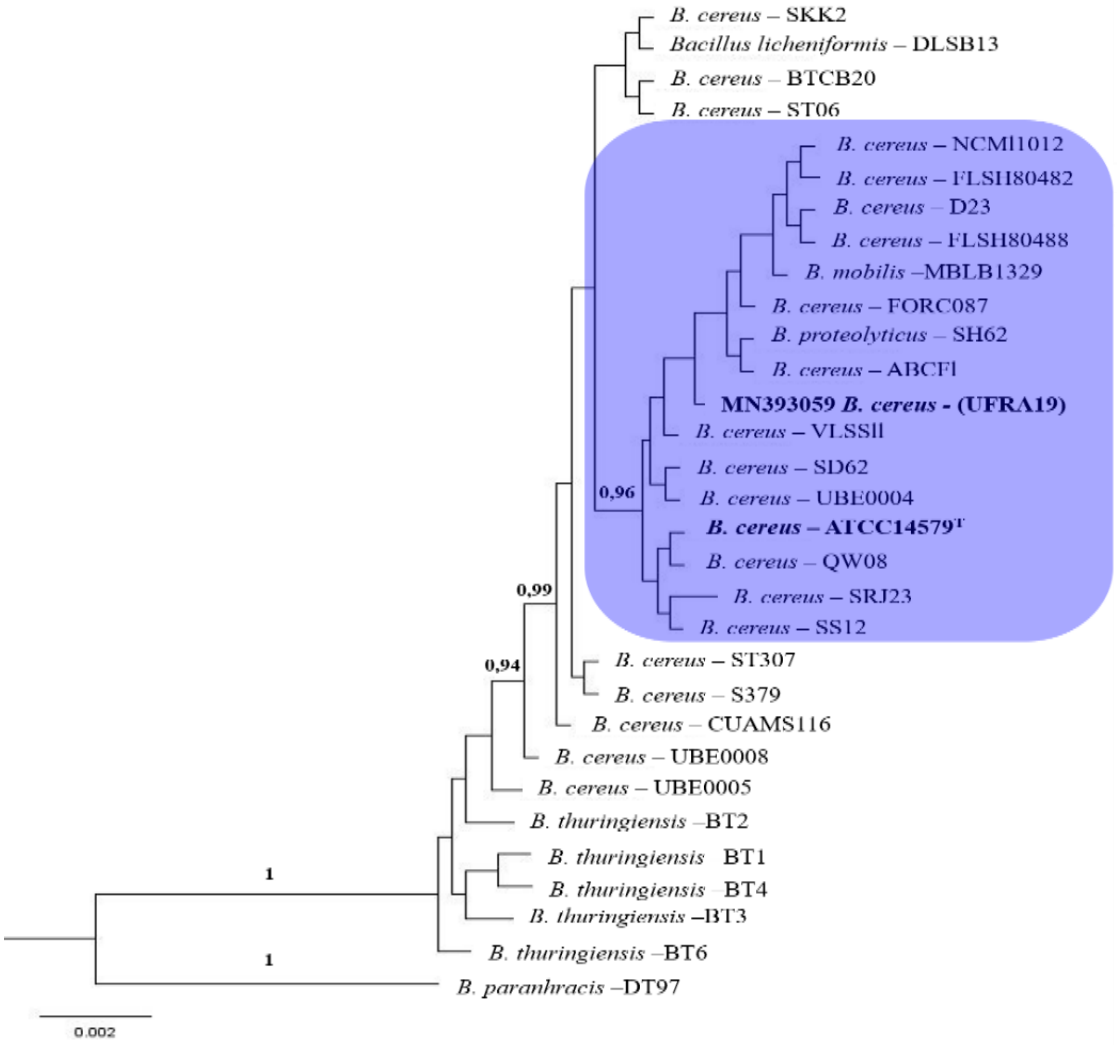


Fig 1 **Molecular phylogenetic analysis of B.cereus isolated from coconut rhizosphere. The maximum**

likelihood method was employed to build a tree. At values ≥70 of the maximum likelihood bootstrap are shown. (*) insulated type *B. Cereus-ATCC14579.*

In vitro biochemical test

Phosphate solubilization was identified from *B. cereus* isolate in the evaluation of the in vitro test where halo formation occurred in the peripheral region of the rhizobacteria colonies. When the isolate was exposed to CAS solution, dark blue color was converted into yellow color, indicating that it is capable of producing siderophores. It was possible to identify that the isolate produces IAA from Salkowski's reagent reaction (Figure 2).

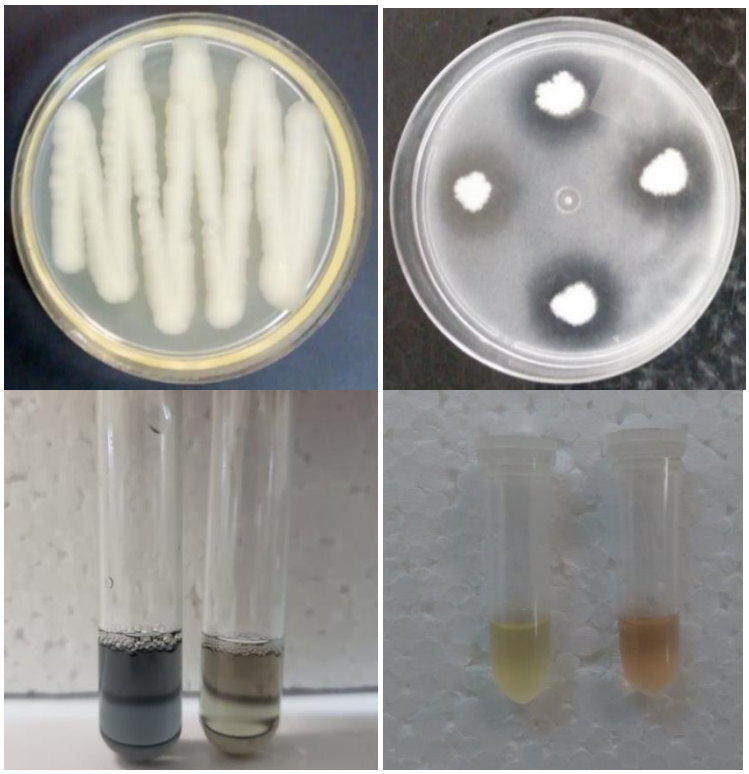
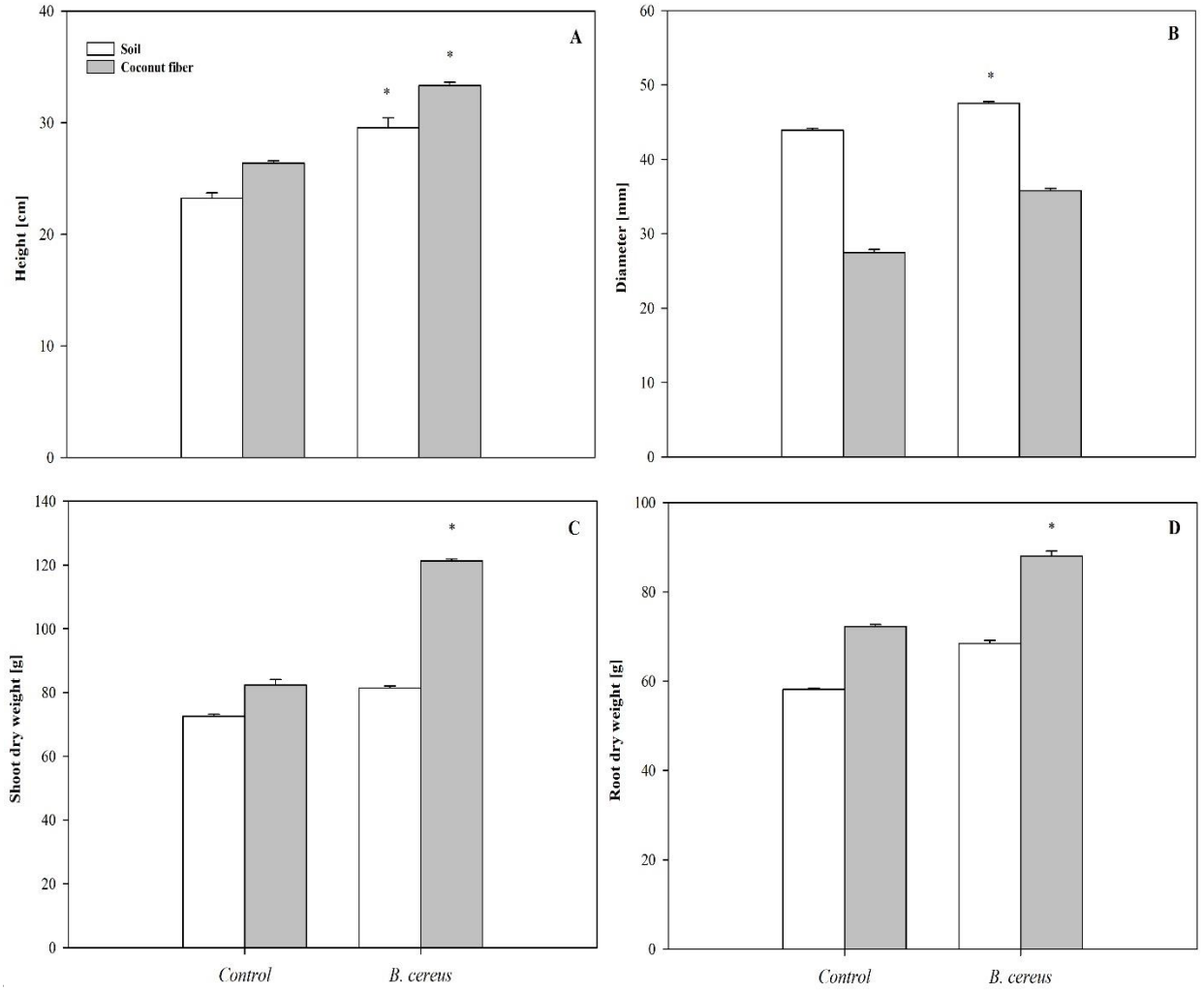


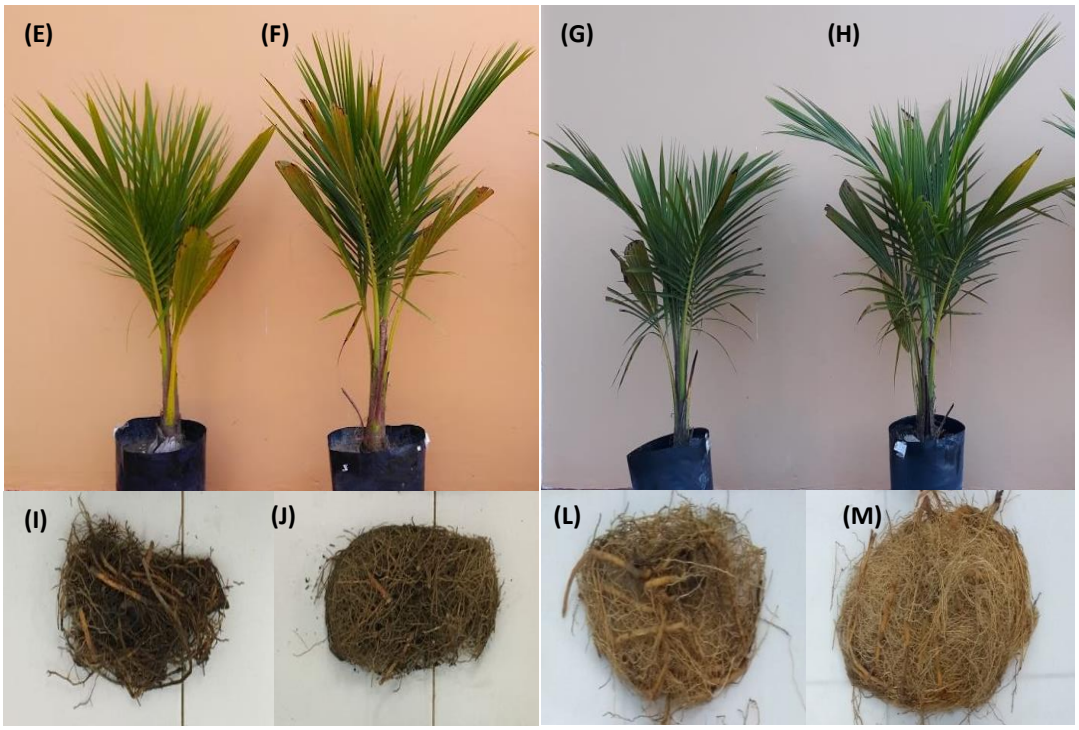
Fig 2 In vitro biochemical test. *B.cereus* in petri dish (A), phosphate solubilization detection (B), siderophores production (C) and Indolacetic Acid (EIA) production (D).

Biometry

Coconut palm seedlings grown in soil and inoculated with *B. cereus* increased the seedling height by 27% compared to seedlings on the same substrate without inoculation. Brazilian green dwarf coconut seedlings grown in coconut fiber combined with inoculation of *B. cereus* provided positive increases by 26% in height, by 47% in biomass of the aerial part and root biomass by 28% (Figure 3).


A
Soil
Coconut fiber
Height [cm]
B
Diameter [mm]
C
Shoot dry weight [g]
D
Root dry weight [g]
Control
B. cereus
Control
B. cereus

2.


(E)
(F)
(G)
(H)
(I)
(J)
(L)
(M)

224

Fig 3 Biometry of coconut seedlings. Height (A), stem diameter (B), aerial dry mass (C), root dry mass (D), change in soil substrate with 100% conventional fertilization and without inoculation of *B. cereus* (E, I), change in soil substrate with 50% conventional fertilization and inoculation of *B. cereus* (F, J), seedlings in coconut fiber substrate with 100% conventional fertilizing and without *B. cereus* inoculation (G, L), changes in coconut fiber substrate with 33% conventional fertilization and *B. cereus* inoculation. (H, M) (*) Small letters and equals do not differ significantly from each other at test t ($p<0,01$).

Nutritional Content

The inoculation of *B. cereus* combined with the soil substrate provided green dwarf coconut seedlings an increase by 23% in nitrogen, 49% in boron and 12% in magnesium contents. The application of *B. cereus* in coconut fiber showed a significant increase in macronutrients: 98% in N, 42% in P, 82% in K, 68% in Mg and 163% in Ca. It also increased B micronutrients by 68% and Fe by 84% when compared to seedlings grown on coconut fiber substrate without *B. cereus* inoculation (Figure 4).

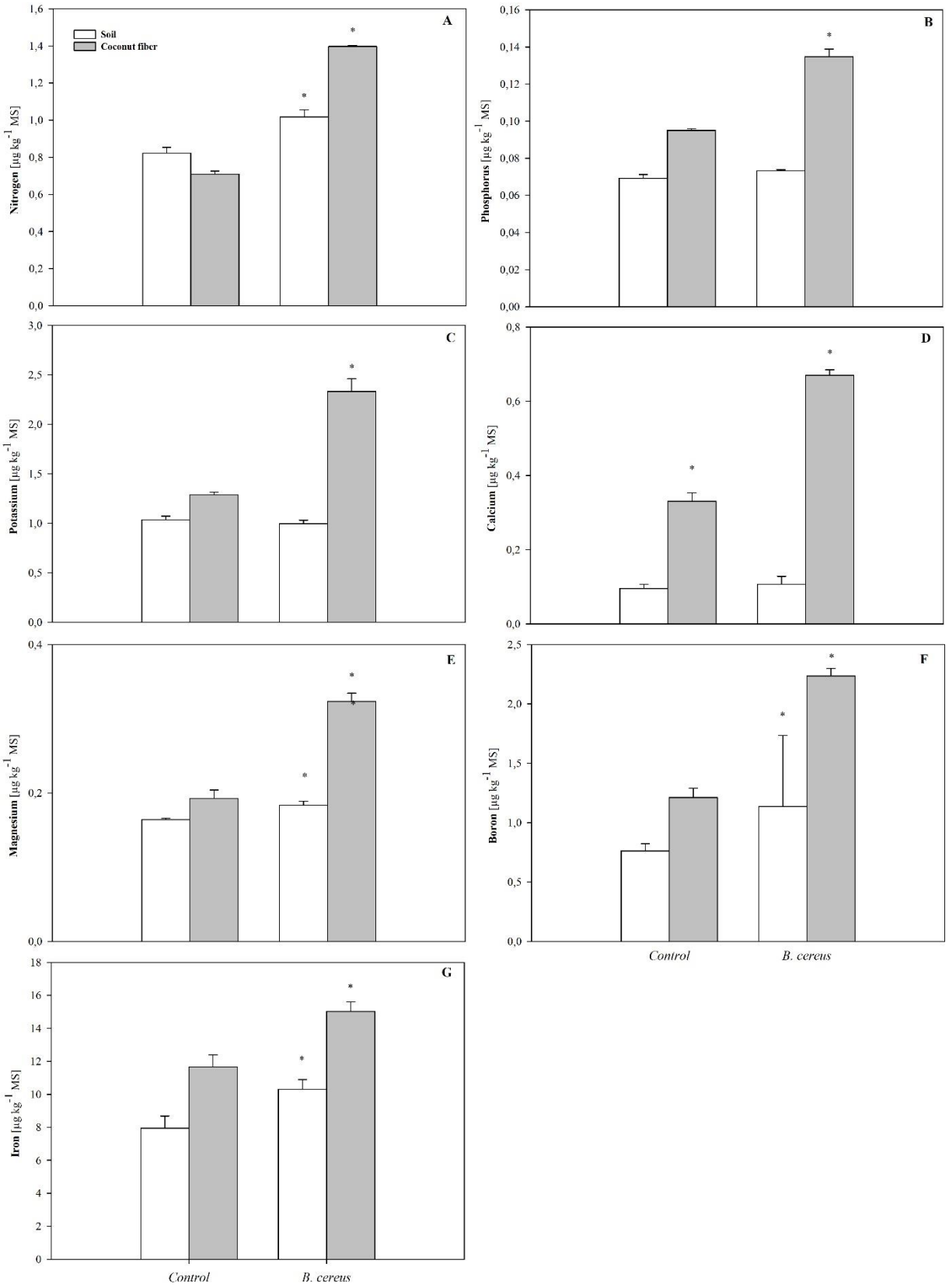


Fig 4 Nutritional content of coconut palm seedlings. Nitrogen (A), phosphorus (B), potassium (C), calcium (D), magnesium (E), boron (F) and iron (G) contents, seedling in soil substrate with 100% conventional fertilization and without *B. cereus* inoculation, seedling in soil substrate with 50%

conventional fertilization and without *B. cereus* inoculation, seedling on coconut fiber substrate with 100% conventional fertilization and without *B. cereus* inoculation and seedling on coconut fiber substrate with 33% conventional fertilization and without *B. cereus* inoculation. (*) Small letters and equals do not differ significantly from each other at test t ($p<0,01$).

Hormonal profile

The use of *B. cereus* combined with the soil resulted in an increase in GA4 by 60% in Brazilian dwarf coconut palm seedlings compared to seedlings in soil without *B. cereus* inoculation. The inoculation of *B. cereus* in seedlings grown in coconut fiber provided increments by 190% in IAA, 31% in GA3 and 17% in GA4, when compared to the treatment of coconut fiber with no application of *B. cereus* (Figure 5).

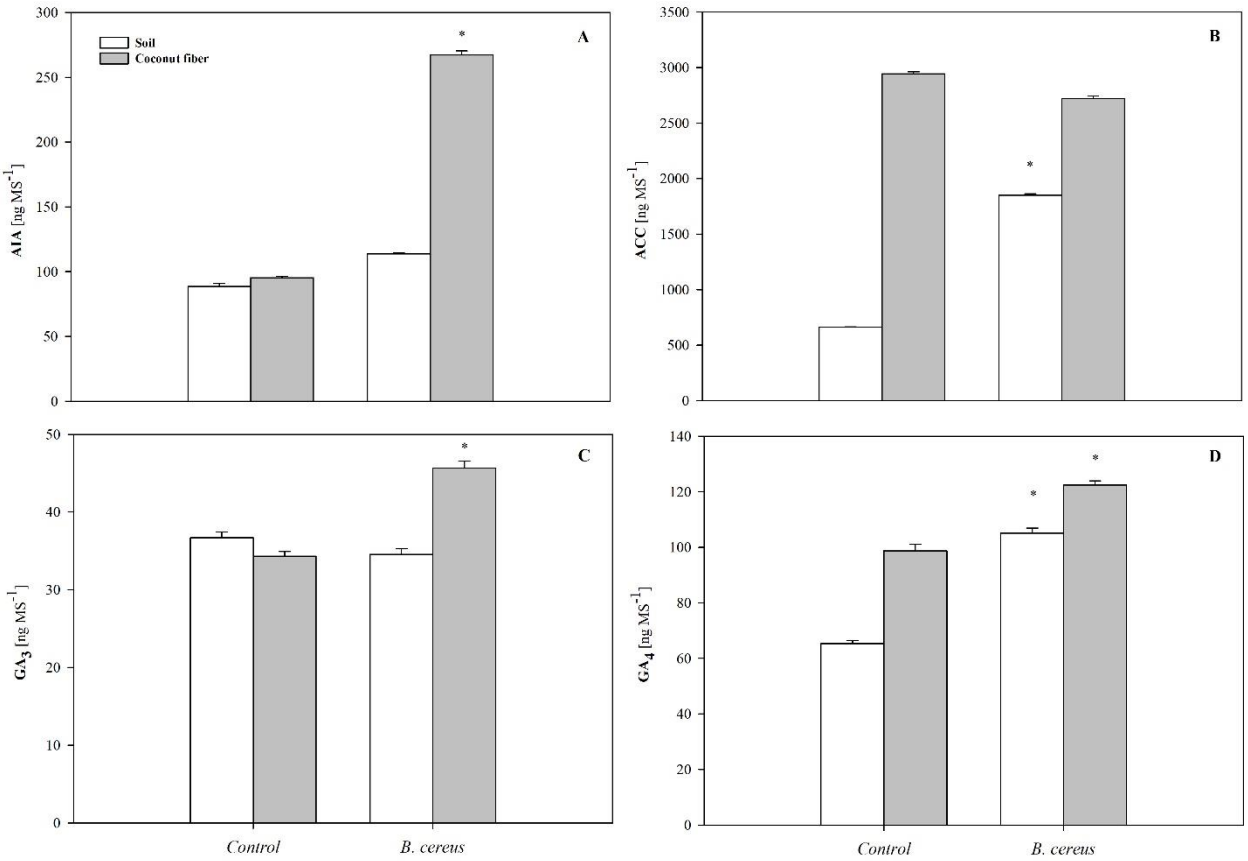


Fig 5 Quantificação de fito-hormônios. Indolacetic Acid (AIA) (A), aminocyclopropane-1-carboxylic acid (ACC) (B), Gibberellic Acid GA3 (C) Gibberellic Acid GA4 (D), seedling in soil substrate with 100% conventional fertilization and without *B. cereus* inoculation, seedling in soil substrate with 50% conventional fertilization and without *B. cereus* inoculation, seedling on coconut fiber substrate with 100%

conventional fertilization and without *B. cereus* inoculation and seedling on coconut fiber substrate with 33% conventional fertilization and without *B. cereus* inoculation. (*) Small letters and equals do not differ significantly from each other at test t ($p<0,01$).

4. **Discussão**

Rhizobacteria inoculated in coconut fiber in reduced conventional fertilization treatment improve growth, change in hormonal and nutritional levels in Brazilian green dwarf coconut seedlings.

Based on the analysis of the 16S rDNA region, *B. cereus* isolate was identified in this study. Sequencing of 16S rDNA region has aided in the distinction between bacterial groups (PATWARDHAN et al., 2014) since it is a gene found in all bacteria. Sequences of 16S rDNA region of unknown bacteria can be compared with sequences of bacteria deposited in databases (CLARRIDGE, 2004; PATWARDHAN; RAY; ROY, 2014). The isolates of this genus are described as growth promoters in other studies (NIU et al., 2011; HASSAN et al., 2018).

The combination of coconut fiber and *B. cereus* inoculation resulted in an increase in the growth of coconut seedlings followed by hormonal changes and increased nutritional content, besides a 67% reduction in synthetic fertilizer dose. The higher growth in coconut fiber substrate compared to soil can be attributed to the characteristics of high porosity and moisture absorption, besides favoring the greater adaptation of *B. cereus* in coconut fiber. These factors allowed the coconut seedlings to a rapid root formation and subsequent increase in nutrient absorption efficiency (GLICK, 2012).

The greatest root growth of coconut seedlings inoculated with *B. cereus* and grown on coconut fiber substrate is the result of the positive interaction initiated from the release of root exudates that attract the bacteria to the root hairs where infection begins (SHARMA; KUMAWAT; KAUR, 2016; DOORNBOS; LOON; BAKKER, 2012). Inoculation with *B. cereus* in coconut fiber substrate promoted changes in root accumulation in seedlings resulting in an increase in volume and quantity of thin roots as can be seen in Figure 3. These results might be due to the increase in indoleacetic acid concentration (IAA) from *B. cereus* inoculation. Such phytohormone is responsible for modulating the differentiation and elongation of lateral roots, as well as increasing the number of root hairs, therefore promoting greater nutrient absorption

(BHARDWAJ, 2014; TURAN et al., 2014). This enhanced growth resulting from *B. cereus* inoculation may demonstrate the ability of this isolate to "manage" the activity of enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, which regulates ethylene synthesis by cleaving its acid precursor, 1-aminocyclopropane-1-carboxylic acid (ACC) (BELIMOV et al., 2009; SIDDIKEE et al., 2011), thus decreasing the negative effects on plant growth and allowing plants to develop a better root system (GLICK et al., 1999).

Losses caused by synthetic fertilization may occur due to volatilization, leaching, adsorption, resulting in low plant uptake (ISWAS; LADHA; DAZZO, 2000; GYANESHWAR et al., 2002). The use of *B. cereus* combined with coconut fiber with 33% of conventional fertilization promoted satisfactory use of nutrients. Also, it allowed identifying greater accumulation of evaluated macro- and micro-nutrients. Previous studies show that inoculation with *Bacillus aryabhattai* S10 and *B. subtilis* ZM63 in corn plants promoted phosphate solubilization by raising P levels by 90% (AHMAD et al., 2019). Inoculation with *Bacillus* sp. PZ-1 in mustard showed efficiency in siderophores production and aided nutrient absorption (YU et al., 2017). In addition to the rhizobacteria itself, which triggers mechanisms of organic matter mineralization, phosphate solubilization and production of siderophores making nutrients available for absorption and promoting greater nutrient absorption by plant roots (ARIF et al., 2017; CHERIF et al., 2017).

The inoculation with *B. cereus* combined with coconut fiber substrate with 33% of conventional fertilization promoted a 30 to 570% increase in nutrient content. Higher levels of N may be the result of ability of *B. cereus* in biodegrading organic matter and make nutrients available, in addition to promoting smaller losses through leaching (PAUNGFOO-LONHIENNE et al., 2019). Inoculation with *B. megaterium* SNji (BmeSNji) and Azospirillum brasilense 65B (Abr65B) in wheat, enhanced N uptake, promoting accumulation in plant biomass (NGUYEN et al., 2019).

Coconut seedlings inoculated with *B. cereus* and fertilized with 33% of conventional fertilization showed higher P content. Positive results were also recorded in sugarcane with the use of *Bacillus* subtilis combined with B. pumilus and chickpea with the inoculation of *Pseudomonas* sp. (SANTOS; KANDASAMY; RIGOBELO, 2018; ZAHEER et al., 2019). These rhizobacteria genera have the ability to make P available from phosphate solubilization, which consists in releasing protons and secondary metabolites such as organic acids or chelating cations that follow phosphate anion, besides the production of phosphatases and

phytases enzymes that mineralize organic material by hydrolysis of H3PO4 esters and anhydrides (BISHOP et al., 1994; RICHARDSON, 1994).

The seedlings inoculated with *B. cereus* in coconut fiber also induced the highest K absorption as it was observed in wheat inoculated with *B. cereus*, with an increaser greater than 25% in K content and dry matter (HASSAN et al., 2018). The higher availability of K in rhizobacteria-inoculated plants can occur through solubilization, from the production of organic acids such as tartaric and citrus, and polysaccharides, as recorded for *B. mucilaginosus* and *B. Aphicu species* (RICHARDS; BATES, 1989; LIN et al., 2002), mechanisms that may have occurred with *B. cereus* isolates inoculated in coconut seedlings.

Other nutrients such as Fe, Mg, Ca, B, and Mn may also be limiting to plant growth. When coconut palm seedlings were inoculated with *B. cereus* in coconut fiber substrate, a high micronutrient content was obtained. Bacteria may have made iron available through the production of organic acids or siderophores (KLOEPPER et al., 1980; NEILANDS, 1995; AHMED; HOLMSTROM, 2014), which are low molecular weight molecules that act as iron chelators (BULGARELLI et al. al., 2013). Contents of Ca, Mg, B and Mn may have increased in *B. cereus*-inoculated coconut seedlings due to its ability to mineralize organic compounds, as observed in raspberry with inoculation of *Bacillus* M3 alone or in combination with *Bacillus* OSU-142, which besides increasing the contents of P, Fe, Ca, Mg and Mn, also increased the growth and yield (ORHAN et al., 2006).

In seedlings inoculated with *B. cereus* and grown on coconut fiber substrate plus 33% of conventional fertilization showed higher accumulation of dry matter in the aerial part, nutritional content and concentration of GA3 and GA4 gibberellins. This result may have occurred as a consequence of the increase in root development that stimulated the release of new leaves in the aerial part. The greater synthesis of gibberellin in *B. cereus*-stimulated leaf tissue may be due to direct production of gibberellin through conjugation of gibberellins and or change in the inactive status of gibberellins into active (LUCANGELI; BOTTINI, 1997; PICCOLI et al., 1997; CASSÁN et al., 2001). Similar to alder (Alnus glutinosa) plants, in which the use of *Bacillus* sp. produced several isomers of gibberellins (GA1, GA3, GA4 and GA20) that were responsible for stem elongation (GUTIERREZ MUNERO et al., 2001).

The use of coconut fiber substrate associated with *B. cereus* showed to be a new strategy for coconut tree seedling production systems. Among the factors that may have contributed positively to the growth and development of the seedlings, the high content of about 92% of organic matter found in coconut fiber stands

out. The organic matter in this substrate, after mineralization and decomposition processes may strongly in the supply and release of macro and micronutrients, besides allowing the proper temperature and moisture retention of the substrate, favorable environment for the microbial activities microorganisms, which in turn are the primary decomposition agents of organic matter, facilitating the bioavailability of nutrients to seedlings.

5. **Conclusion**

The advantages obtained from inoculation with B. cereus in coconut fiber, as a positive effect on the accumulation of macro and micronutrients, a higher concentration of phytohormones and a greater accumulation of biomass, combined with a reduction in conventional fertilization by 67%, indicate that this technology may be an important tool in the production system of green dwarf coconut tree seedlings in Brazil.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' contribution

All authors acted directly in the development of the study, writing and review.

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Authors' contribution

All authors acted directly in the development of the study, writing and review.