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Research Article

Plant growth-promoting endophytic bacteria on maize and sorghum[1]

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ABSTRACT

Plant growth-promoting bacteria (PGPB) are found in plant tissues and promote plant growth by secretion of hormones and enzymes, or by facilitating the nutrient uptake. This study assessed forty PGPB isolates to determine their effects on maize and sorghum growth. These isolates were also compared with uninoculated plants, as negative (-N; without N fertilization) and positive (+N; with N fertilization) controls. Plant height, stem diameter, shoot and root dry mass, leaf N accumulation and chlorophyll content were evaluated. For both the maize and sorghum, the height, stem diameter and shoot dry mass in plants inoculated with PGPB were similar to those of uninoculated plants supplied with N, and the responses for root mass were higher than in plants supplied with N. However, the PGPB isolates did not promote N accumulation and chlorophyll content similar to those of uninoculated plants supplied with N. The IPACC26 and IPACC30 isolates, both identified as *Bacillus subtilis*, resulted in better responses for plant growth and N accumulation than the other isolates.

KEYWORDS: *Bacillus subtilis*, nitrogen accumulation, chlorophyll.

RESUMO

Bactérias endofíticas promotoras de crescimento em milho e sorgo

Bactérias promotoras de crescimento em plantas (BPCP) são encontradas em tecidos vegetais e promovem o crescimento vegetal por meio da secreção de hormônios e enzimas, ou auxiliando a absorção de nutrientes. Avaliaram-se quarenta isolados de BPCP para determinar seus efeitos sobre o crescimento de milho e sorgo. Estes isolados foram também comparados com plantas não inoculadas, como controles negativos (-N; sem fertilização nitrogenada) e positivos (+N; com fertilização nitrogenada). A altura, diâmetro de caule, massas secas da parte aérea e raízes, acúmulo de N e conteúdo de clorofila foram avaliados. Em ambos o milho e o sorgo, a altura, diâmetro do caule e a massa seca da parte aérea em plantas inoculadas com BPCP foram similares aos das plantas não inoculadas e fertilizadas com N, e as respostas da massa das raízes foram maiores que das plantas fertilizadas com N. Entretanto, os isolados de BPCP não promoveram acúmulo de N e conteúdo de clorofila semelhantes aos das plantas não inoculadas e fertilizadas com N. IPACC26 e IPACC30, ambos identificados como *Bacillus subtilis*, apresentaram melhores respostas para o crescimento das plantas e acúmulo de N do que os outros isolados.

PALAVRAS-CHAVE: *Bacillus subtilis*, acúmulo de nitrogênio, clorofila.

INTRODUCTION

Plant growth-promoting bacteria (PGPB) are microorganisms that can improve the plant performance in many different ways (Palacios et al. 2014). These bacteria directly or indirectly promote the plant growth by secretion of hormones and enzymes, or by facilitating the uptake and accumulation of nutrients such as nitrogen (N) and phosphorus (P) (Ahemad & Kibret 2014). They can also protect plants from pathogens, improve the soil structure and degrade pollutants (Hayat et al. 2010).

Several bacteria genera are classified as PGPB, including *Bacillus*, *Pseudomonas*, *Rhizobium*, *Bradyrhizobium* and *Azospirillum*. *Bacillus* and *Pseudomonas* influence the plant growth mainly by the synthesis of plant hormones (e.g., auxin, cytokinin and gibberellins) and production of siderophores and

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antibiotics (Adesemoye et al. 2008). On the other hand, *Rhizobium* and *Bradyrhizobium* are involved in the biological N fixation in leguminous plants (Sulieman & Tran 2014), while *Azospirillum* acts on non-leguminous plants (Santi et al. 2013) and can also synthetize phytohormones such as indole-3-acetic acid (Fukami et al. 2018). The inoculation with rhizobacteria can enhance the plant development and productivity. Studies have shown that *Bacillus* and *Pseudomonas* increase the soybean and wheat growth (Araujo 2008, Sharma et al. 2011). Similarly, *Azospirillum*, *Rhizobium* and *Bradyrhizobium* promote the growth of sugarcane (Schultz et al. 2014), common bean (Chekanai et al. 2018) and soybean (Ulzen et al. 2016).

Maize and sorghum are two important cereal crops in Brazil, with a production of about 102 million tons, in 2019 (IBGE 2019). Usually, maize and sorghum producers plant these crops in soils with nutrient deficits and, therefore, PGPB could contribute to the enhancement of plant biomass and nutrient uptake. Studies in Brazil have shown positive effects of PGPB on plant growth and yield in maize (Araujo & Guerreiro 2010) and sorghum (Schlemper et al. 2018). However, it is important to select suitable bacteria isolates and evaluate their growth-promoting abilities.

In this study, several PGPB that were isolated from sugarcane and presented high biochemical and plant growth-promotion abilities (Antunes 2016) were evaluated for their ability to promote growth in maize and sorghum. As sugarcane belongs to the gramineae group as maize and sorghum, the hypothesis is that PGPB isolated from sugarcane would promote the maize and sorghum growth.

MATERIAL AND METHODS

The study was conducted at the Universidade Federal do Piauí, in Teresina, Piauí state, Brazil, from March to June 2018. A total of 40 PGPB isolates (Table 1), obtained in leaves and stalks of sugarcane by Antunes et al. (2017), were inoculated into seeds of sorghum (*Sorghum bicolor* cv. Palo Alto N52K1009) and maize (*Zea mays* cv. AG-1051). Therefore, two separate experiments (sorghum and maize), in a completely randomized design, with three replicates, were conducted in a growth chamber, to assess the reaction of both crops to the isolates (treatments). These isolates were also compared with uninoculated negative (-N; without N fertilization) and positive (+N; with N fertilization) controls.

To prepare the inoculants, the PGPB isolates were individually grown in Erlenmeyer flasks

Table 1. Plant growth-promoting bacteria (PGPB) isolates used in this study as inoculants for maize and sorghum.

PGPB	Genus[1]	PGPB	Genus[1]
IPACC01	*Bacillus* sp.	IPACF18	*Bacillus* sp.
IPACC06	*Paenibacillus* sp.	IPACF20	*Bacillus megaterium*
IPACC07	*Herbaspirillum seropedicae*	IPACF21	*Bacillus subtilis*
IPACC10	*Burkholderia* sp.	IPACF41	*Bacillus methylotrophicus*
IPACC14	*Paenibacillus* sp.	IPACF42	*Bacillus methylotrophicus*
IPACC23	*Paenibacillus* sp.	IPACF44	*Burkholderia* sp.
IPACC24	*Bacillus subtilis*	IPACF45	*Bacillus methylotrophicus*
IPACC25	*Bacillus subtilis*	IPACF46	*Paenibacillus* sp.
IPACC26	*Bacillus subtilis*	IPACF47a	*Paenibacillus* sp.
IPACC29	*Bacillus subtilis*	IPACF47b	*Paenibacillus* sp.
IPACC30	*Bacillus subtilis*	IPACF48	*Brevibacillus agri*
IPACC33	*Pseudomonas* sp.	IPACF62	*Paenibacillus* sp.
IPACC34	*Bacillus pumilus*	IPACF65	*Bacillus megaterium*
IPACC35	*Bacillus* sp.	IPACF66	*Pseudomonas* sp.
IPACC36	*Bacillus pumilus*	IPACC53	Not identified
IPACC38	*Paenibacillus* sp.	IPACC58	Not identified
IPACC49	*Burkholderia* sp.	IPACC59	Not identified
IPACC55	*Paenibacillus* sp.	IPACF17	Not identified
IPACC56	*Pseudomonas* sp.	IPACF40	Not identified
IPACF16	*Bacillus* sp.	IPACF60	Not identified

[1] Classified by Antunes et al. (2017).

containing 50 mL of liquid culture medium (Tryptic Soy Broth - TSB at 25 %) and incubated under orbital shaking (200 rpm and 31 ºC) for 72 h. The bacterial growth was verified by measuring the optical density using a spectrophotometer (wavelength of 540 nm), and a final concentration of 10^9 CFU mL^{-1} was considered for inoculation.

The experimental units consisted of pots (0.8 dm^3) containing 0.7 dm^3 of sterile sand (pH 6.8) that were autoclaved (120 ºC at 101 kPa) for 60 min. The autoclaving procedure was repeated three times. Sorghum and maize seeds were surface disinfected with alcohol (70 %) for 30 s, followed by sodium hypochlorite (2 %) for 60 s, and then washed with sterile distilled water. Seeds were directly inoculated with 1 mL aliquots of the cell suspensions containing the PGPB isolates. Plants were grown under a photoperiod cycle of 14 h of light at 28 ºC and 10 h of darkness at 20 ºC. Every 3 days, 2 mL of the Hoagland's nutrient solution (Hoagland & Arnon 1950), without N, were added to each pot. The positive control (+N) received 50 mg pot^{-1} and 75 mg pot^{-1} of N for sorghum and maize, respectively, in accordance with their individual N requirements. These rates were applied, after diluting with water, five times during the experiment (10 mL pot^{-1}). Sorghum and maize were maintained for 30 and 45 days, respectively, before being harvested. The plant height and stem diameter were measured. Shoots and roots were dried at 65 ºC to a constant mass, to determine their dry mass. The N content in leaves was evaluated using the Kjeldahl method (Bremner & Mulvaney 1982) and the chlorophyll content was estimated using spectrophotometric methods (Lichtenthaler & Wellburn 1983).

Analyses of variance and F-tests at 5 % of significance were performed to estimate and test the effects of the isolates on both crops (maize and sorghum). Data normality was verified by the Shapiro-Wilk test, and treatment means were compared by using the Scott-Knott test, also at 5 % of significance.

RESULTS AND DISCUSSION

The PGPB treatment showed significant effects on all the evaluated variables in maize and sorghum (Table 2). The PGPB isolates had similar or higher growth effects on maize and sorghum, if compared to the growth of uninoculated plants supplied with N.

Thus, the majority of the isolates promoted an increased height in maize and sorghum similar to that of plants supplied with N (Figure 1). Notably, 17 PGPB isolates promoted a higher stem diameter in maize than in uninoculated maize plants supplied with N (Figure 2).

Few isolates were able to promote an increase in the shoot dry mass in maize (Figure 3A), while the majority of the isolates promoted similar increases in the sorghum shoot dry mass, if compared to that in uninoculated plants supplied with N (Figure 3B). Interestingly, the majority of the isolates promoted an increased root growth (Figure 4A), while few isolates promoted an increase in the sorghum root growth (Figure 4B) higher than that of plants supplied with N. Notably, the inoculation of the isolates IPACC24 (*B. subtilis*) in maize and IPACC59 (unidentified) in sorghum yielded the highest ability to promote the root growth.

These results indicate that the evaluated PGPB promoted a similar or even an increased growth of maize and sorghum, when compared to plants that received only N fertilization. Since a low N

Table 2. Analyses of variance and F-test values for treatment effects on growth variables[1] in maize and sorghum trials, with plants inoculated or not with plant growth-promoting bacteria.

Effect	df [2]	Height	Diameter	SDW	RDW	Nitrogen	Chlorophyll
	Maize						
Treatment	41	60.9*	0.37*	0.14*	0.24*	65.10*	1.49*
Error	84						0.08
CV (%)		12.1	6.80	17.60	15.80	10.50	15.70
	Sorghum						
Treatment	41	11.7*	0.98*	0.09*	0.15*	47.10*	0.49*
Error	84						
CV (%)		10.8	10.10	27.90	17.50	9.48	5.91

[1] Height: plant height; diameter: stem diameter; SDW: shoot dry weight; RDW: root dry weight; Nitrogen: N accumulation; Chlorophyll: chlorophyll content. [2] df: degrees of freedom. * Significant values ($p \leq 0.05$).

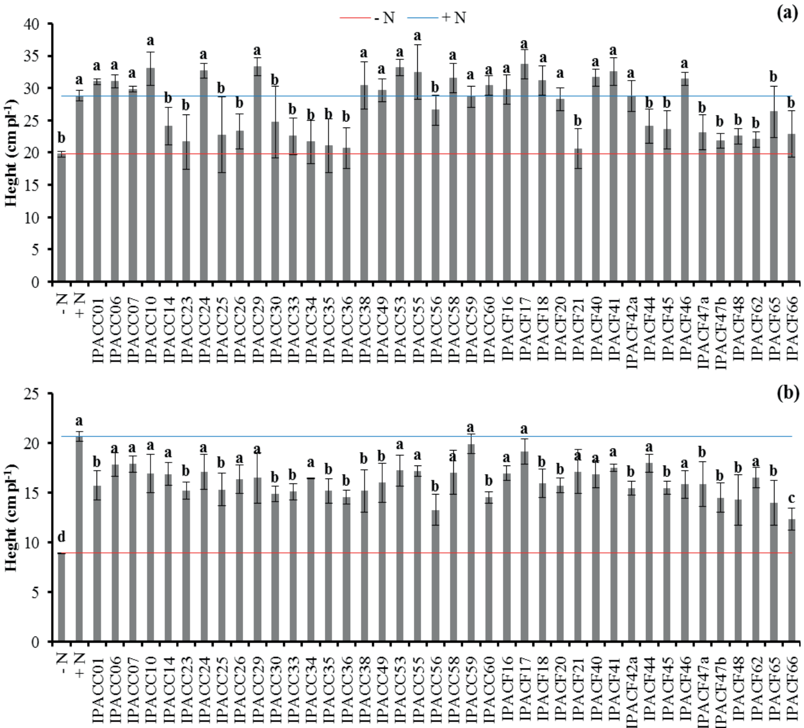

Figure 1. Plant height of maize (a) and sorghum (b) inoculated with plant growth-promoting bacteria isolates. Means followed by the same letter do not differ significantly at 5 % by the Scott-Knott test. -N: uninoculated plants without N fertilization; +N: uninoculated plants with N fertilization.

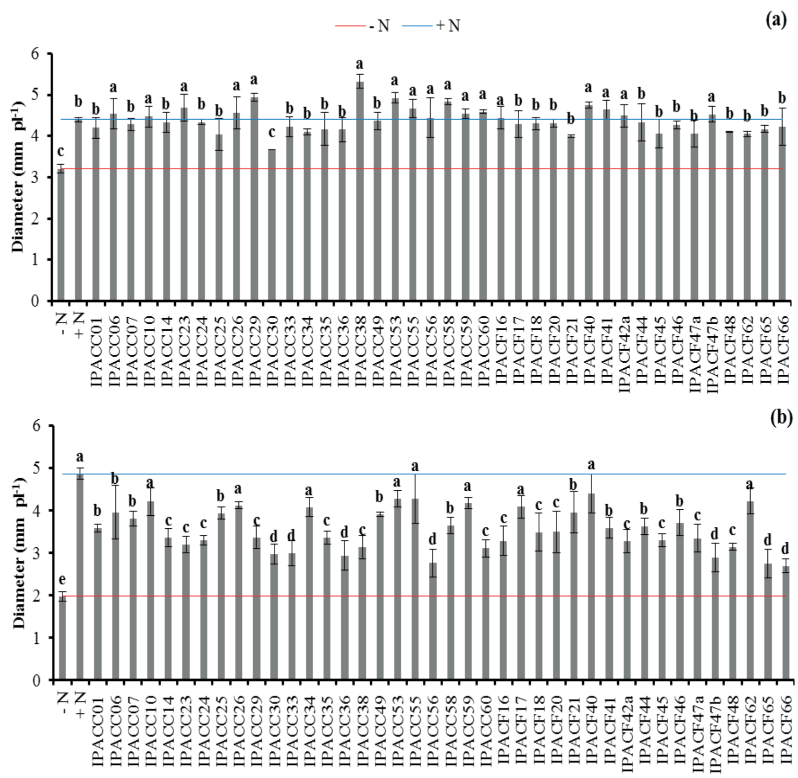

Figure 2. Stem diameter of maize (a) and sorghum (b) inoculated with plant growth-promoting bacteria isolates. Means followed by the same letter do not differ significantly at 5 % by the Scott-Knott test. -N: uninoculated plants without N fertilization; +N: uninoculated plants with N fertilization.

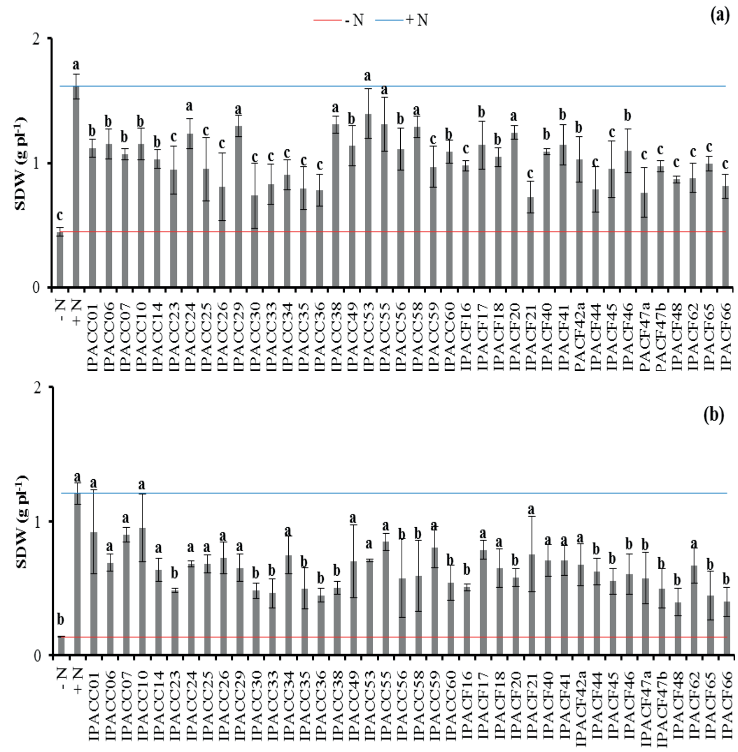

Figure 3. Shoot dry weight (SDW) of maize (a) and sorghum (b) inoculated with plant growth-promoting bacteria isolates. Means followed by the same letter do not differ significantly at 5 % by the Scott-Knott test. -N: uninoculated plants without N fertilization; +N: uninoculated plants with N fertilization.

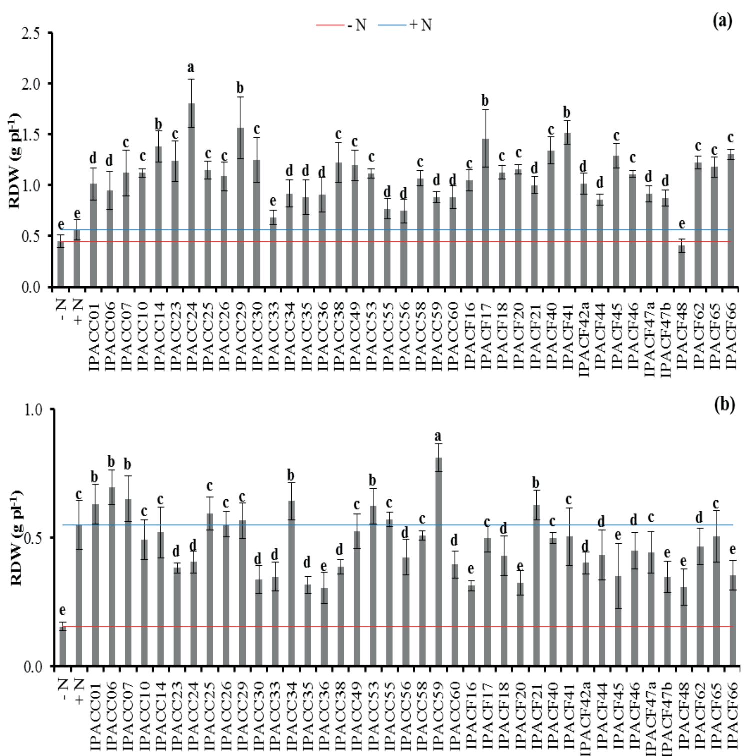

Figure 4. Root dry weight (RDW) of maize (a) and sorghum (b) inoculated with plant growth-promoting bacteria isolates. Means followed by the same letter do not differ significantly at 5 % by the Scott-Knott test. -N: uninoculated plants without N fertilization; +N: uninoculated plants with N fertilization.

availability is usually the main factor that limits the growth and yield of crops (Bhattacharjee et al. 2008), these results are important for tropical regions where these PGPB could present a potential alternative to decrease the use of N fertilizers. The promotion of plant growth by PGPB is already known and it involves, among others, biochemical capabilities, production of phytohormones such as indole-3-acetic acid (IAA) and the process of biological nitrogen fixation (Souza et al. 2015). Indeed, these PGPB isolates presented a high capability of producing IAA and performing the biological N fixation in sugarcane (Antunes et al. 2017), and could also promote the maize and sorghum growth. Previous studies have shown positive growth responses in maize and sorghum, when inoculated with PGPB (Schlemper et al. 2018, Widawati & Suliasih 2018). Similarly, Arruda et al. (2013) observed that the inoculation of maize with different bacteria significantly promoted root (50-68 %) and shoot (25-54 %) growth.

Interestingly, root growth was the parameter that presented positive and significant responses to the isolates, even when comparing with the responses of uninoculated plants supplied with N. Thus, some isolates enhanced the root growth in maize and sorghum. The increased root growth may be attributed to the synthesis of plant growth-regulating substances, such as IAA, produced by these isolates (Antunes et al. 2017), which coordinates the developmental processes in plants and promotes a higher root development by increasing the lateral and adventitious root formation (Duca et al. 2014). This significant increase in root growth promoted by some PGPB may be important for maize and sorghum growth, since roots anchor plants and absorb and conduct nutrients and water (Maloof 2004). Youseif (2018) observed a significant increase in root growth in maize with inoculation of 49 PGPB isolates and attributed it to the high ability of synthetizing IAA. According to Vikram et al. (2007), the IAA produced by the bacteria may positively influence the development of the root system, improving the nutrient absorption required for plant growth.

Neither the PGPB isolates nor the uninoculated plants supplied with N promoted a N accumulation and chlorophyll content. However, the majority of isolates promoted a N accumulation in maize (Figure 5A) and sorghum (Figure 5B), if compared to uninoculated plants without N.

The isolates IPACC23 (*Paenibacillus* sp.), IPACC26 (*B. subtilis*), IPACC30 (*B. subtilis*), IPACC33 (*Pseudomonas* sp.), IPACF44 (*Burkholderia* sp.), IPACF47a (*Paenibacillus* sp.) and IPACF48 (*Brevibacillus agri*) presented the most efficient N accumulation in maize, while IPACC26 (*B. subtilis*) and IPACC30 (*B. subtilis*) were more efficient in sorghum. Similarly, the majority of the isolates promoted an increased chlorophyll content in maize (Figure 6A) and sorghum (Figure 6B). For both crops, IPACC26 (*B. subtilis*) and IPACF66 (*Pseudomonas* sp.) presented the highest increase in the chlorophyll content.

These results show that both the maize and sorghum could benefit from the increased N accumulation and chlorophyll content promoted by the inoculation with PGPB. However, the isolates could not increase the N accumulation and chlorophyll content as in plants supplied with N. On the other hand, since the inoculated plants did not receive additional N, the accumulation of N resulted from biological N fixation. Although these isolates did not present similar capabilities to increase the N accumulation and chlorophyll content as it happened after the N fertilization, they show a potential as N supplements to plants.

According to Kuan et al. (2016), PGPB may provide a biological alternative to fix N from the atmosphere. These endophytic bacteria colonize plant tissues, such as roots, stem and leaves, and can fix N for use by plants (James et al. 1997). The inoculation with PGPB in maize and sorghum leads to a significantly increased N accumulation (Alagawadi & Gaur 1992, Kuan et al. 2016). In this study, several PGPB isolates contributed to an increased chlorophyll content in maize and sorghum, suggesting an indirect effect, since the chlorophyll content is directly correlated with the N accumulation in plants (Liu et al. 2012). Previous studies have shown a significant effect of PGPB on the chlorophyll content in maize (Almaghrabi et al. 2014) and wheat (Turan et al. 2012). Consistently with the results of previous studies, *B. subtilis* was the most efficient in promoting the N accumulation and, consequently, increased the chlorophyll content in maize (Lima et al. 2015, Pupathy & Radziah 2015) and sorghum (Das et al. 2010). The isolates that promoted the highest N accumulation in maize and sorghum also presented a high ability to fix N in sugarcane (Antunes et al. 2017). This confirms that *Bacillus* can fix N from the atmosphere (Mollica et al. 1985). Studies have shown that *Pseudomonas* species can also fix molecular N and, thus, stimulate the

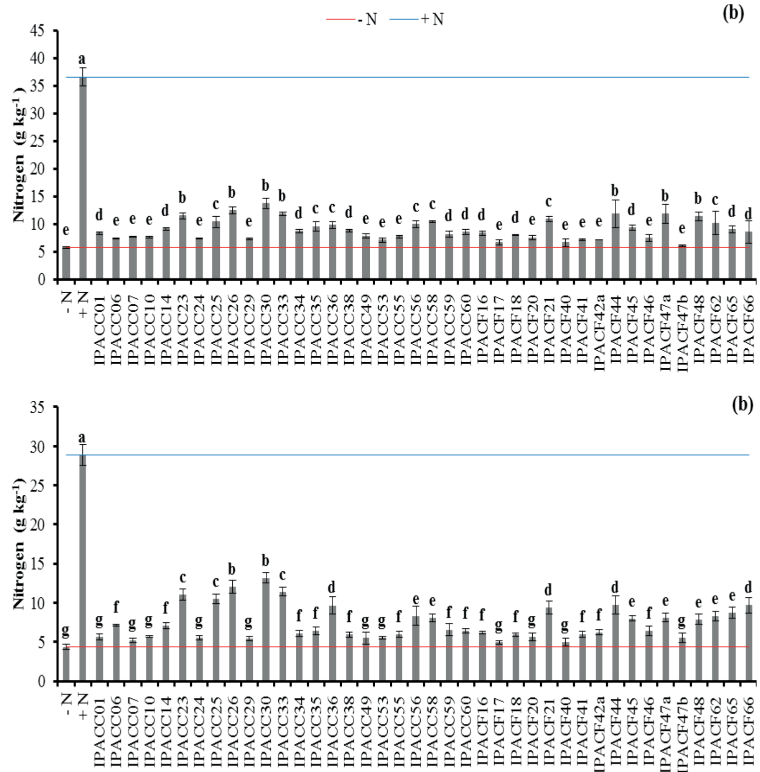

Figure 5. Nitrogen accumulated in maize (a) and sorghum (b) shoot inoculated with plant growth-promoting bacteria isolates. Means followed by the same letter do not differ significantly at 5 % by the Scott-Knott test. -N: uninoculated plants without N fertilization; +N: uninoculated plants with N fertilization.

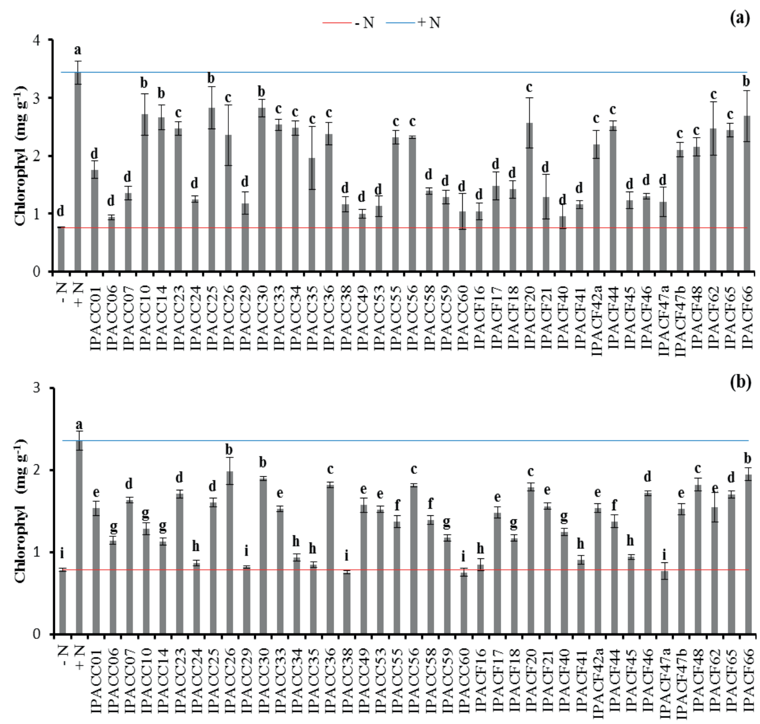

Figure 6. Chlorophyll content in maize (a) and sorghum (b) shoot inoculated with plant growth-promoting bacteria isolates. Means followed by the same letter do not differ significantly at 5 % by the Scott-Knott test. -N: uninoculated plants without N fertilization; +N: uninoculated plants with N fertilization.

accumulation of N and chlorophyll in maize (Kifle & Laing 2016) and sorghum (Praveen et al. 2012). Finally, our results show that these isolates present a potential for promoting maize and sorghum growth and, consequently, yield. Therefore, the inoculation with these isolates may be an ecological alternative for decreasing the dependence on N fertilizers.

CONCLUSIONS

1. Plant growth-promoting bacteria (PGPB) isolates may potentially be used as biological inoculants to increase the N accumulation and growth of maize and sorghum;
2. Most of the tested PGBP isolates promote growth in maize and sorghum;
3. The IPACC26 and IPACC30 isolates, both identified as *Bacillus subtilis*, have a better effect on the N accumulation in maize and sorghum.

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