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Plant growth-promoting bacteria improve growth and nitrogen metabolism in maize and sorghum

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Abstract Plant growth-promoting bacteria (PGPB) present potential to be used in agriculture supporting plant growth and improving physiological responses even in N-fertilized soils. Thus, this study aimed to evaluate PGPB inoculation, combined or not with N-fertilizer, on growth and N metabolism of maize and sorghum. A total of thirteen PGPB, with high variability in producing indole-3-acetic acid and nitrogenase activity were inoculated in maize and sorghum grown with and without N fertilization. The growth and physiological responses of plants were assessed 50 d after plant emergence. In general, PGPB increased maize and sorghum growth, N fixation efficiency and N metabolites as compared to non-inoculated plants. Particularly, IPACC55 and IPACC10 increased leaf area, chlorophyll, shoot dry mass, total N and symbiotic efficiency. The majority of PGPB increased the relative and N use efficiencies in maize. In addition, PGPB reduced free ammonia, while increased nitrate and soluble protein in maize

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and sorghum. The results also showed that inoculated maize and sorghum grown without N fertilization displayed higher plant biomass and relative and N use efficiencies, while that, in plants grown with N fertilization, the inoculation improved shoot dry mass, and symbiotic and N use efficiencies. As conclusion, PGPB positively influence N fixation and metabolism in maize and sorghum, even in N-fertilized soil. This indicates that PGPB can provide N to maize and sorghum and stimulate plant growth.

Keywords PGPB · N metabolism · Plant physiology · Non-legume plants

1 Introduction

Chemical fertilizers have contributed to support crop yield (Lu and Tian 2017). Particularly, N-fertilizers are the most important chemical input used in agriculture as the N is highly required by plants, affecting their growth and yield, and also increasing their photosynthetic rates (Sun et al. 2016; Bassi et al. 2018). Plants absorb N from the soil as nitrate or ammonium; nitrate is more available and absorbed by plants due to the adsorption of ammonium by soil cation exchange matrix or microorganisms (Bloom 2015). Since N requirement by plants is high, this nutrient is applied at high rates to avoid significant losses of yield (Bloch et al. 2020). However, application of high rates of N-fertilizers could promote soil, air and water pollution (Good and Beaty 2011). Particularly to soils, the intensive use of N-fertilizers has promoted acidification and degradation and consequently, these processes can decrease plant growth (Lu and Tian 2013; Tian and Niu 2015).

Some ecological and sustainable alternatives to support plant growth and decrease the dependence on N-fertilizers have been proposed in the last decade, such as the use of plant growth-promoting bacteria (PGPB) (Souza et al. 2015; da Silva et al., 2020). PGPB colonize plants rhizosphere, endosphere and phyllosphere, and can enhance their growth (Dong et al. 2019). These bacteria present direct strategies to promote plant growth, such as production of phytohormones and siderophores or N-fixing capacity (De La Torre-Ruiz et al. 2016; Compant et al. 2019). Indeed, previous studies have reported positive effects of PGPB on growth of important crops such as sugarcane, maize and soybean (Antunes et al. 2017; Breedt et al. 2017; Di Salvo et al. 2018; Santos et al. 2018; Aquino et al. 2019; Bavaresco et al., 2020). For instance, Breedt et al. (2017) reported increased maize growth varying from 24 to 34% after inoculation of *Paenibacillus*, *Bacilus*, and *Brevundimonas*. Recently, Di Salvo et al. (2018) inoculated some cereal crops with PGPB and observed improvement of plant growth. In sugarcane, Santos et al. (2018) found *Bacillus subtilis* improving its growth and yield. In addition, some PGPB are also recognized as a biological alternative to fix atmospheric N in cereals crops (Kuan et al. 2016; Ladha et al. 2016; Antunes et al. 2019). In maize, Kuan et al. (2016) found PGPB providing N from the atmosphere to plants and increasing their growth with a reduction of 30% in N-fertilizers.

PGPB also affect N metabolism in plants (Zeffa et al. 2019) such as the biosynthesis of important N compounds, i.e., chlorophylls, amino acids, nucleotides and proteins (Bloom 2015). For example, *Bacillus* sp. increased chlorophyll content in lima bean (Lima et al. 2016). In maize, *Azospirillum brasilense* increased leaf nitrate and ammonium, while *Bacillus* sp. decreased leaf amino acids and proteins (Calzavara et al. 2019). Therefore, these bacteria, especially those with capability on biological N fixation (BNF), exhibit potential to improve N nutrition in cereals (Ladha et al. 2016).

Maize and sorghum are important cereals cropped in several countries, being used as human food and animal feeding (Ribeiro et al. 2019). Maize is recognized as one of the most important crop species worldwide providing a source of energy to almost the total of population in Africa and the Americas (FAOSTAT Food Balance Sheets 2020). Sorghum is important for millions of people living in the subtropical and semi-arid regions of Africa, South America and Asia, being a source of food and fodder, mostly in the traditional, smallholder farming sector (Hariprasanna and Rakshit 2016). Therefore, the demand for maize and sorghum in response to growing populations will require high productivity with consequent increase on demand of chemical fertilizers, notably for N-fertilizers resulting in both an increase in costs and potential negative impact on the environment.

On the other hand, PGPB could be a sustainable and ecological alternative to support maize and sorghum growth, decreasing their demand to N-fertilizers. In addition, PGPB can improve some plants physiological traits such as chlorophyll content (Rojas-Tapias et al., 2012), amino acids, and proteins (Souza et al. 2015). Thus, it is necessary to evaluate potential PGPB to be used as inoculants to maize and sorghum, increasing their growth. In this study, we hypothesized that PGPB, combined with N-fertilizer, would be able to promote maize and sorghum growth, improving the N metabolism and efficiency related to plant growth. Thus, the aim of this study was to evaluate potential PGPB on the growth and N metabolism in maize and sorghum.

2 Material and methods

2.1 PGPB isolates

In this study, thirteen PGPB isolated by Antunes et al. (2017) from sugarcane tissues were selected according to their contrasting biochemical potential (Aquino et al. 2019) (Table 1). Particularly, these PGPB present high variability in producing indole-3-acetic acid (IAA) and reducing acetylene (a measurement of nitrogenase activity). Each PGPB were grown under orbital shaking (200 rpm; 25 °C; 72 h) in Erlenmeyer containing Tryptic Soy Broth liquid culture medium. The optimum growth was verified by measuring the optical density using a spectrophotometer at 540 nm, and a final concentration of 10^9 CFU mL^{-1} was considered for inoculation.

2.2 Experimental design

All PGPB were evaluated on maize and sorghum in a pot-experiment under greenhouse conditions. Two individual experiments were carried out to compare the responses of maize (*Zea mays* L.) cv. AG-1051, and sorghum (*Sorghum bicolor* L.) cv. Palo Alto N52K1009, to inoculation of PGPB. For each plant species, the treatments consisted of thirteen PGPB and one control without inoculation. These treatments were tested in two N levels (0% and 50% of total N required by plants). Both experiments were performed under a completely randomized blocks design in a factorial scheme (14 treatments × 2 levels of N) with five replications.

2.3 Experimental setup

The experiments were carried out in plastic pots containing 18 dm^3 of soil (83% sand, 11% silt, and 6% clay) (Table 2). Before sowing, the seeds were disinfected with 70% alcohol (30 s) followed by 2% sodium hypochlorite (60 s), and then washed with sterile distilled water. Seeds were inoculated with 1.0 mL of the cell suspensions containing PGPB. Ten days after sowing, one plant was left in each pot. All pots received the application of P_2O_5 (2.5 and 2.1 g per pot to maize and sorghum, respectively) and K_2O (2.1 and 2.4 g per pot to maize and sorghum, respectively). For treatments with 50% of N required by plants, it was applied ammonium sulfate (3.37 and

Table 1 Isolates of plant growth-promoting bacteria (BPCP) used in this study and their capability to produce indole-3-acetic acid (IAA) and perform N_2 fixation by the acetylene-reducing activity (ARA)

PGPB	Genus/Species*	IAA	ARA
IPACC01	*Bacillus* sp.	+ +	+ +
IPACC08	*Herbaspirillum seropedicae*	+	+ + +
IPACC07	*H. seropedicae*	+	+ + +
IPACC10	*Burkholderia* sp.	+ +	+ + +
IPACC26	*B. subtilis*	+ +	+ + +
IPACC29	*B. subtilis*	+	+ + +
IPACC36	*B. pumilus*	+ + +	+ + +
IPACC38	*Paenibacillus* sp.	+	+ + +
IPACC55	*Paenibacillus* sp.	+	+ + +
IPACC53	No identified	+ +	+
IPACC58	No identified	+	+
IPACC59	No identified	+	+
IPACF40	No identified	+	+ + +

*Identified according to NCBI byAntunes et al. (2017)

**IAA (mg L^{-1}) or ARA (nmol C_2H_4 h^{-1}): (+) 0 to 2.0; (+ +) 2.0 to 5.0; (+ + +) > 5.0

Table 2 Chemical characterization of the soil used in the experiments

Sample (cm)	pH H_2O	P mg kg^{-1}	K^+	Na^+	Mg^{+2} $cmol_c$ kg^{-1}	Ca^{+2}	Al^{+3}	H + Al	CEC	V (%)
0–20	5.7 ± 0.1	0.4 ± 0.1	14.0 + 1.3	3.5 ± 0.2	0.8 ± 0.1	1.3 ± 0.1	0.1 ± 0.1	1.1 ± 0.2	3.4 ± 0.3	66 ± 3.6

CEC cation exchange capacity, *V* base saturation

2.47 g per pot to maize and sorghum, respectively) at the sowing.

2.4 Plant measurements

The plants were collected 50 d after germination. Leaf area was measured using an automatic leaf area meter (Model LiCor LI-3000). Shoots were separated from roots, and both were oven-dried at 65 °C to determine shoot and root dry mass. Fresh leaves were collected for biochemical analysis. Total chlorophyll was quantified according to Lichtenthaler and Wellburn (1983). Free ammonia, nitrate, total free amino acids and soluble protein were quantified using aliquots of supernatant (extract) obtained in water bath at 100 °C (60 min). Free ammonia was measured by the phenol-hypochlorite method (Weatherburn 1967), while nitrate concentration was measured by the nitration of salicylic acid (Cataldo et al. 1975). Total free amino acids were quantified by ninhydrin (Yemm and Cocking 1955), while soluble protein was measured according to Bradford (1976). The N content in shoot was measured according to the semi-micro Kjeldahl method (Bremner and Mulvaney 1982).

Based on the aforementioned data, the relative, symbiotic and N use efficiencies were calculated. The relative efficiency was calculated according to Bergensen et al. (1971) using non-inoculated plants fertilized with 50% of N. The interaction efficiency (inoculated plants) was quantified according to the formula: (total N in inoculated plants)—(total N in N-fertilized plants)/(total N in N-fertilized plants—total N in non-fertilized plants) × 100 (Brockwell et al. 1966). The N use efficiency for each treatment was calculated according to Siddiqi and Glass (1981).

2.5 Data analysis

All statistical analyses were carried out separately for maize and sorghum. Data were analyzed using analysis of variance (ANOVA), preceded by an *F* test, and means were compared by the Scott-Knott's test ($p \leq 0.05$). These statistical analyses (univariate tests) were performed using the statistical software R Studio (version 3.5.2). Principal component analysis (PCA) was performed with all standardized data, in order to identify the effects of PGPB on maize and sorghum. This analysis was performed using Past (v.4.02) (https://folk.uio.no/ohammer/past/).

3 Results

The analysis of variance showed individual and interactive effects by the inoculation and N-fertilization on the majority of variables determined in maize and sorghum (Table 3). In maize, the exceptions were free ammonia and N use efficiency, in N-fertilization, and total chlorophyll, in the interaction. Total free amino acids did not present significant differences for inoculation and N-fertilization. In sorghum, the exceptions were total chlorophyll, root dry mass, nitrate, total free amino acids and soluble proteins, in N-fertilization, while that total free amino acids did not vary significantly in the interaction. Interestingly, except for total free amino acids in maize (Scott–Knott's test; p = 0.07), PGPB influenced significantly all variables in maize and sorghum.

In general, PGPB promoted higher leaf area, chlorophyll and dry biomass (shoot and roots) in maize and sorghum as compared to non-inoculated plants (Figs. 1 and 2). Interestingly, some PGPB improved the plants performance without N fertilization when compared with those N-fertilized plants. Thus, IPACC55 promoted the highest leaf area in maize and sorghum grown without N fertilization as compared to N-fertilized plants (Fig. 1a). By the way, IPACC55 exhibited higher total chlorophyll (Fig. 1b) and shoot dry mass (Fig. 2a) in sorghum. In maize, IPACC10 promoted the highest leaf area and

Table 3 Analysis of variance (ANOVA) in double factorial scheme with an additional treatment performed with experimental data obtained from maize and sorghum plants

	Analysis of variance (F value)			Coefficient of variation (%)
	Inoculation (IN)	Nitrogen (NT)	Interaction (IN × NT)	
Maize variables				
Foliar area	26.6**	66.0**	7.51**	6.9
Total chlorophyll	24.9**	44.4**	1.6^{NS}	8.4
Shoot dry mass	9.4**	10.0**	4.8**	8.5
Root dry mass	7.1**	5.8**	7.0**	13.7
Total nitrogen	98.9**	129.4**	13.6**	3.8
Symbiotic efficiency	101.5**	130.0**	13.5**	12.6
Relative efficiency	9.3**	9.9**	4.8**	8.6
Nitrogen use efficiency	7.5**	2.8^{NS}	5.8**	18.8
Free ammonia	91.4**	0.15^{NS}	5.4**	8.8
Nitrate	36.1**	29.1**	11.0**	9.3
Total free amino acids	1.7^{NS}	2.5^{NS}	2.8**	8.7
Soluble proteins	13.2**	3.5**	3.3**	8.8
Sorghum variables				
Foliar area	38.8**	30.9**	10.0**	7.9
Total chlorophyll	11.7**	1.6^{NS}	4.4**	8.9
Shoot dry mass	87.6**	26.2**	6.8**	7.7
Root dry mass	21.5**	1.9^{NS}	7.9**	13.2
Total nitrogen	180.4**	112.6**	18.6**	3.3
Symbiotic efficiency	191.6**	119.5**	19.7**	7.8
Relative efficiency	87.6**	26.2**	6.7**	7.7
Nitrogen use efficiency	60.4**	5.3**	9.4**	15.1
Free ammonia	19.6**	1.8**	16.1**	8.7
Nitrate	77.4**	0.25^{NS}	14.9**	9.3
Total free amino acids	15.2**	3.7^{NS}	0.63^{NS}	8.9
Soluble proteins	8.6**	0.48^{NS}	0.89^{NS}	8.9
Degrees of freedom	13	1	13	–

**Significant at 5%. ^{NS}Non-significant. *CV* coefficient of variation

chlorophyll in plants grown without and with N fertilization, respectively.

In both plants, PGPB increased shoot and roots biomass than non-inoculated plants (Fig. 2). In maize grown without N fertilization, IPACC01, IPACC08, IPACC26, IPACC29, IPACC38, IPACC55 and IPACC58 increased the shoot dry mass; while IPACC07, IPACC10, IPACC38, IPACC53, and IPACF40 increased the root dry mass. In N-fertilized maize, IPACC29, IPACC53, IPACC55, and IPACC59 increased the plant biomass. In sorghum grown without N fertilization, IPACC07, IPACC26, IPACC38, and IPACC55 increased the roots biomass as compared to non-inoculated plants. Interestingly, IPACC55, in sorghum, and IPACC38, in both plants, promoted the highest roots growth when they were grown without N fertilization (Fig. 2).

In maize, IPACC55 and IPACC10 increased the total N and symbiotic efficiency in maize grown with and without N fertilization, respectively (Fig. 3a and b). In sorghum, IPACC29 and IPACC38 increased the total N and interaction efficiency in N-fertilized plants. Interestingly, IPACC07 increased the total N and interaction efficiency in plants grown without N

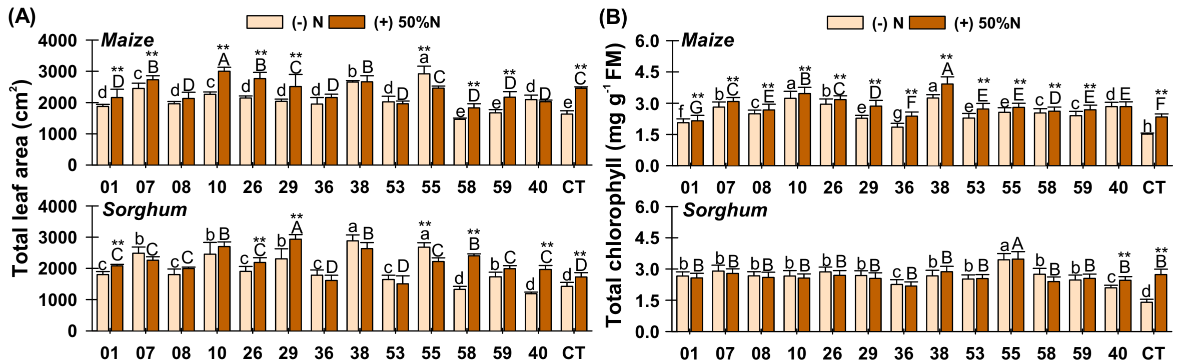

Fig. 1 Total leaf area (**a**) and total chlorophyll (**b**) of maize and sorghum plants inoculated with PGPB (IPACC01, IPACC07, IPACC08, IPACC10, IPACC26, IPACC29, IPACC36, IPACC38, IPACC53, IPACC55, IPACC58, IPACC59 and IPACF40) and supplied or not with nitrogen at 50% of full fertilization. *CT* plants non-inoculated, *FM* fresh mass. Different lowercase letters indicate the significant differences among plants when non-supplied with N, while capital letters show the significant differences among plants when supplied with 50% of full fertilization. The double asterisk (**) indicates significant differences between non-fertilized and fertilized plants in each treatment (Scott–Knott' test; $p \leq 0.05$)

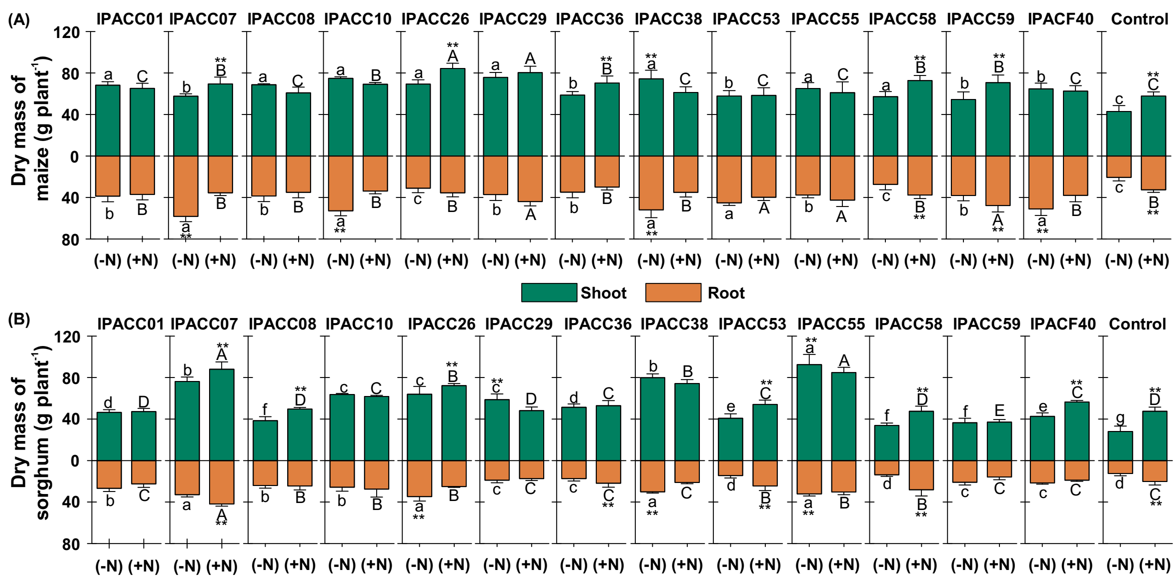

Fig. 2 Shoot and root dry mass of maize (**a**) and sorghum (**b**) plants inoculated with PGPB (IPACC01, IPACC07, IPACC08, IPACC10, IPACC26, IPACC29, IPACC36, IPACC38, IPACC53, IPACC55, IPACC58, IPACC59 and IPACF40) and supplied or not with nitrogen at 50% of full fertilization. Control = plants non-inoculated. Different lowercase letters indicate the significant differences among plants when non-supplied with N, while capital letters show the significant differences among plants when supplied with 50% of full fertilization. The double asterisk (**) indicates significant differences between non-fertilized and fertilized plants in each treatment (Scott–Knott' test; $p \leq 0.05$)

fertilization. The majority of PGPB increased the relative and N use efficiencies in maize (Fig. 3c and d). Plants grown without N fertilization increased their relative efficiency by inoculation of IPACC01, IPACC08, IPACC10, IPACC26, IPACC29, IPACC38, IPACC55 and IPACF40. In N-fertilized maize, IPACC26 or IPACC29 promoted the highest values of relative efficiency (Fig. 3c). Regarding to N use efficiency, IPACC01, IPACC07, IPACC10, IPACC29, IPACC38, and IPACF40 promoted better performance in plants grown without N fertilization. However, in N-fertilized plants, IPACC26, IPACC29, and IPACC59 were most effective. IPACC55 promoted the highest values of relative efficiency and N

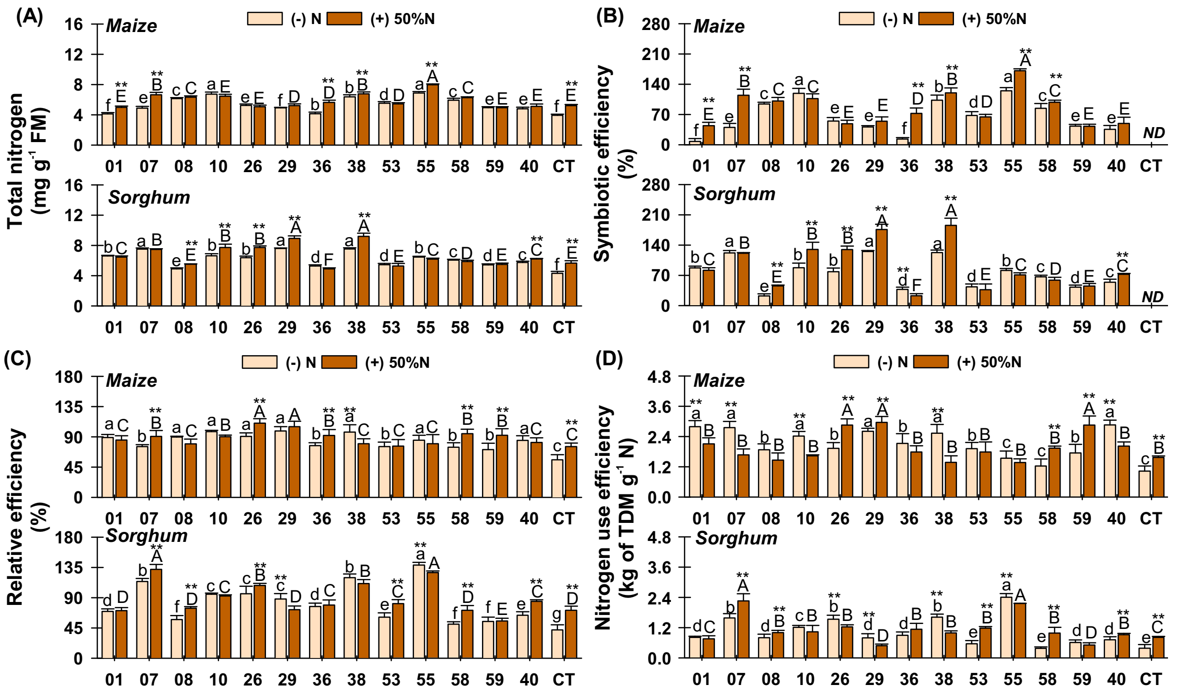

Fig. 3 Total nitrogen (**a**), symbiotic efficiency (**b**), relative efficiency (**c**) and nitrogen use efficiency (**d**) of maize and sorghum inoculated with PGPB (IPACC01, IPACC07, IPACC08, IPACC10, IPACC26, IPACC29, IPACC36, IPACC38, IPACC53, IPACC55, IPACC58, IPACC59 and IPACF40) and supplied or not with nitrogen at 50% of full fertilization. *CT* plants non-inoculated. *ND* non-determined. *FM* fresh mass. *TDM* total dry mass. Different lowercase letters indicate the significant differences among plants when non-supplied with N, while capital letters show the significant differences among plants when supplied with 50% of full fertilization. The double asterisk (**) indicates significant differences between non-fertilized and fertilized plants in each treatment (Scott-Knott' test; $p \leq 0.05$)

use efficiency in both plants grown with and without N fertilization; while IPACC07 increased these parameters in N-fertilized plants.

The content of N compounds varied between inoculated maize and sorghum grown (Fig. 4). In general, PGPB reduced free ammonia in maize (Fig. 4a). In sorghum, lowest values of free ammonia were found in N-fertilized plants and inoculated with IPACC01, IPACC07, IPACC26, IPACC29 and IPACF40. Plants grown without N fertilization and inoculated with IPACC04, IPACC26, IPACC59 and IPACF40 showed lowest values of free ammonia (Fig. 4a). In N-fertilized maize, IPACC01, IPACC08, IPACC10, IPACC26, IPACC59 or IPACF40 increased the content of nitrate (Fig. 4b). In sorghum, IPACC01 and IPACC07 increased the content of nitrate in plants grown without and with N fertilization, respectively (Fig. 4b).

The values of total free amino acids varied between maize and sorghum (Fig. 4c). In N-fertilized maize and inoculated with PGPB, no differences were found in total free amino acids as compared to non-inoculated ones; however, plants grown without N fertilization and inoculated with IPACC07, IPACC10, IPACC29 and IPACC38 displayed higher values of total free amino acids (Fig. 4c). In sorghum grown with and without N fertilization, IPACC29 and IPACC55 increased the total free amino acids, respectively (Fig. 4c). IPACC07 or IPACC55 promoted higher soluble proteins in maize, while IPACC29 or IPACC38 increased the soluble protein in sorghum (Fig. 4d). Non-inoculated plants displayed lower content of soluble protein in maize and sorghum grown with or without N fertilization (Fig. 4d).

Through the PCA both inoculated and non-inoculated plants were clustered separately, showing that all PGPB promoted distinct effect those found in non-inoculated plants, mainly in plants grown without N fertilization (Fig. 5). The two principal axes explained 66.1% and 73.9% of the total variation in maize and

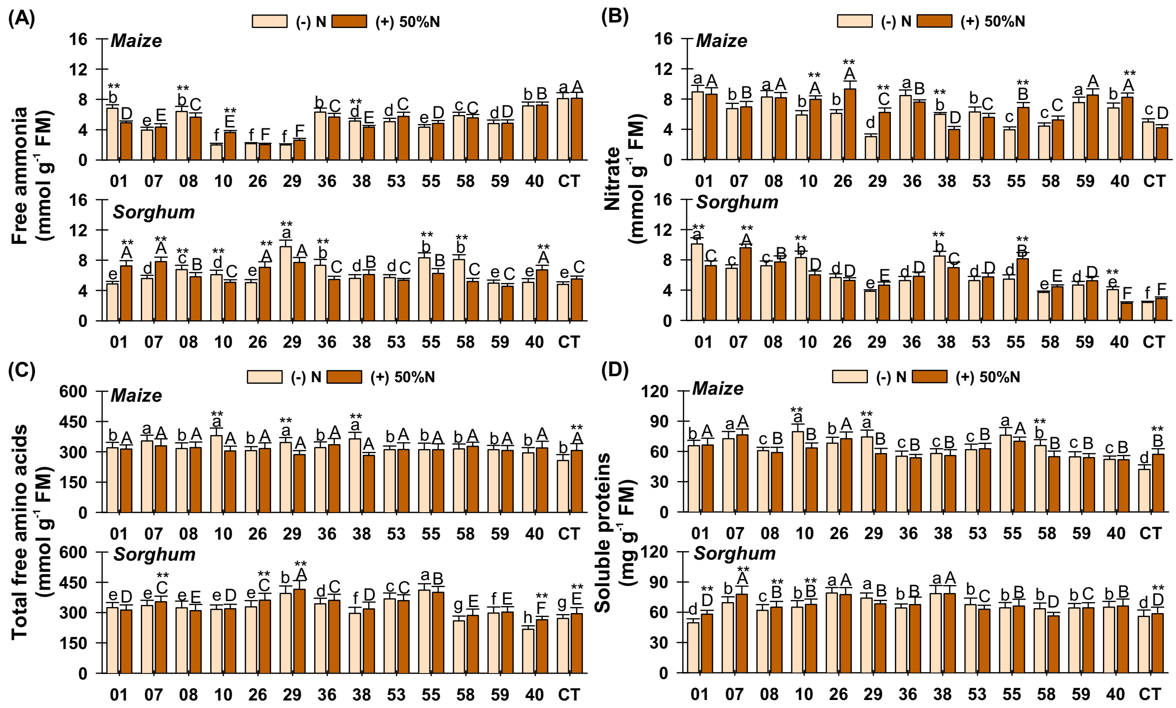

Fig. 4 Free ammonia (**a**), nitrate (**b**), total free amino acid (**c**) and soluble proteins (**d**) of maize and sorghum inoculated with PGPB (IPACC01, IPACC07, IPACC08, IPACC10, IPACC26, IPACC29, IPACC36, IPACC38, IPACC53, IPACC55, IPACC58, IPACC59 and IPACF40) and supplied or not with nitrogen at 50% of full fertilization. CT: plants non-inoculated; FM: fresh mass. Different lowercase letters indicate the significant differences among plants when non-supplied with N, while capital letters show the significant differences among plants when supplied with 50% of full fertilization. The double asterisk (**) indicates significant differences between non-fertilized and fertilized plants in each treatment (Scott-Knott' test; $p \leq 0.05$)

sorghum grown without N fertilization, respectively (Fig. 5a and 5c). In N-fertilized plants, the two principal axes explained 57.8% and 69.5% of the total variation in maize and sorghum, respectively (Fig. 5b and d). Inoculated maize and sorghum grown without N fertilization displayed higher plant biomass and relative and N use efficiencies (loadings > 0.4). In N-fertilized plants, the inoculation improved the shoot dry mass and symbiotic and N use efficiencies (loadings > 0.5) (Fig. 5d).

4 Discussion

In this study, potential PGPB were inoculated in maize and sorghum that were grown with and without N fertilization. In line with the hypothesis, PGPB were able to promote plant growth and improve N metabolism and efficiency in maize and sorghum. It is an interesting finding since N is the most required nutrient by maize and sorghum and can confirm that these selected PGPB could be used as potential inoculant to these crops. In addition, the inoculation of PGPB is an ecological and sustainable strategy to increase plant performance and, at the same time, reduce the dependence on N-fertilizers. Interestingly, all PGPB used in this study were previously inoculated in sugarcane and showed high potential for increasing the growth and N fixation (Antunes et al. 2017). Therefore, this study presents potential PGPB to be indicated in further field studies to find a potential inoculant to be recommended to maize and sorghum.

In general, all PGPB increased the growth and N metabolism in maize and sorghum. However, there were differences between PGPB on the responses of plants, even when the plants were grown without N fertilization as compared to those fertilized ones. Therefore, IPACC55 can be highlighted as a potential PGPB by increasing leaf area in both plants, while promoted higher chlorophyll content and shoot dry

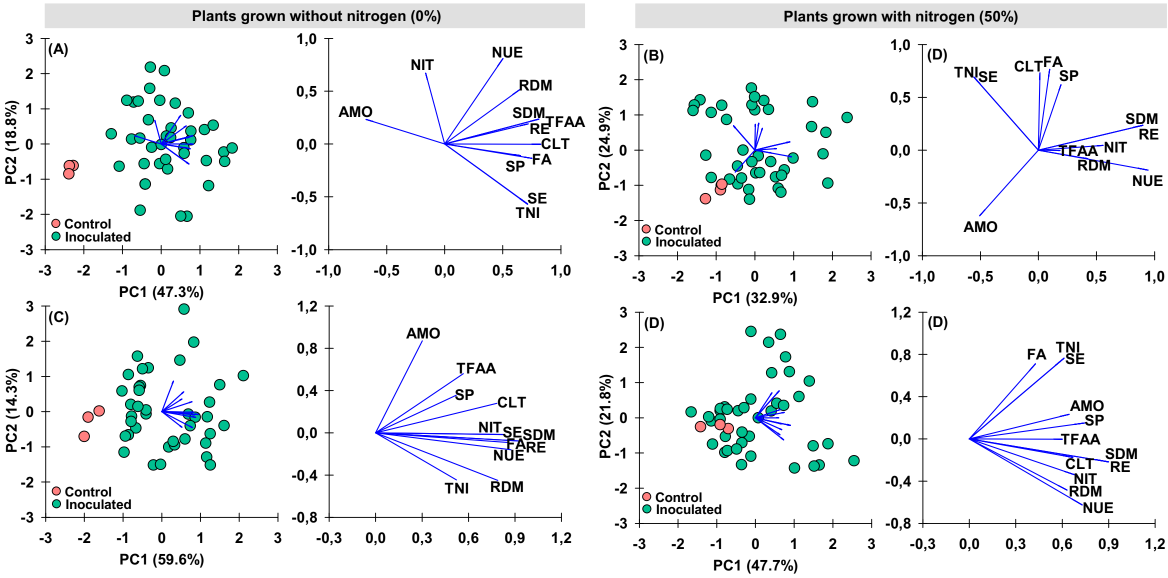

Fig. 5 Principal component analysis of maize (**a**, **b**) and sorghum (**c**, **d**) plants inoculated with PGPB (IPACC01, IPACC07, IPACC08, IPACC10, IPACC26, IPACC29, IPACC36, IPACC38, IPACC53, IPACC55, IPACC58, IPACC59 and IPACF40) and supplied or not with nitrogen at 50% of full fertilization. *Control* Plants non-inoculated. *FA*: foliar area. *CLT*: total chlorophyll. *SDM*: shoot dry mass. *RDM*: root dry mass. *AMO*: free ammonium. *NIT*: nitrate. *TFAA*: total free amino acids. *SP*: soluble protein. *TNI*: total nitrogen. *RE*: relative efficiency. *SE*: symbiotic efficiency. *NUE*: nitrogen use efficiency

mass in sorghum. On the other hand, IPACC10 has promoted higher leaf area and chlorophyll content in maize. Leaf area and chlorophyll are important parameters related to photosynthesis and plant growth, and previous studies have reported high leaf area associated with chlorophyll content, CO_2 assimilation rates, and growth of maize (Li et al. 2015) and sorghum (Silva et al. 2019). Leaf growth, chlorophyll content, and CO_2 assimilated by plants are severely reduced under N deficiency (Sun et al. 2016; Silva et al. 2019). Although in this study we did not estimate the CO_2 assimilation rate, our results suggest IPACC55 and IPACC10 as potential to promote maize and sorghum growth, since they could induce higher CO_2 assimilation rates. Interestingly, IPACC55 also contributed for increasing the root growth in maize, while IPACC38 stimulated more roots in both plants.

All PGPB used in this study were selected according to their biochemical capabilities (Antunes et al. 2017). Particularly, IPACC10 (*Burkholderia* sp.) presents high capability to produce IAA, while IPACC55 and IPACC38 (*Paenibacillus* sp.) have high acetylene-reduction activity, i.e., nitrogenase activity. These features could explain the higher maize growth after inoculation of IPACC10, and the values of total N and symbiotic efficiency in maize and sorghum after inoculation of IPACC55 and IPACC38, respectively, even when plants were grown with N fertilization. *Burkholderia* and *Paenibacillus* are well recognized plant growth promoters with high performance on N fixation. Particularly, *Burkholderia* presents high efficiency on nitrogenase activity and N fixation in non-legumes plants, being identified as plant growth promoter in maize and sorghum (Perin et al. 2006; Aquino et al. 2019). *Paenibacillus* presents *nifH* gene encoding Fe protein of nitrogenase and shows nitrogenase activity (Liu et al. 2019), being potentially suggested to be used as biofertilizer to some crops such as maize, wheat and sugarcane (Hao and Chen 2017; Aquino et al. 2019).

This study also highlights IPACC07 and IPACC08, identified as *Herbaspirilum seropedicae*, in contributing with N to plants. *H. seropedicae* is a N-fixing bacterium associated to maize, sugarcane and sorghum (Monteiro et al. 2008). Previous studies have reported increases on the growth and N fixation in maize and sorghum after inoculation of *H. seropedicae* (Ribaudo et al. 2006), *Paenibacillus* sp. (Aquino et al. 2019) and *Burkholderia* sp. (Schlemper et al. 2018). Since plant development is influenced by N availability, these bacteria presented high efficiency in fixing N and, thus, provided adequate N levels when this nutrient is absent or poorly available (Carvalho et al. 2014). On the other hand, maize inoculated with IPACC10, IPACC26 and IPACC29 decrease their free ammonium content (∼ 70%) when compared to uninoculated plants. It also occurred to N-fertilized maize which presented a decrease in free ammonium content. These isolates were identified as *Burkholderia* sp. (IPACC10) and *B. subtilis* (IPACC26 and IPACC29). Ammonium or nitrate are the major inorganic N forms metabolized in plant cells (Nacry et al. 2013) and the inoculation with these bacteria increased the growth and N fixation in maize and sorghum, while presented the ability to convert nitrate or ammonium in other metabolites necessary to plant development (Lee et al. 2020). Considering that these isolates presented the ability to fix N, adequate levels of ammonia in these plants can reflect the higher N-fixation rate, greater activity of ammonium assimilation pathway, and other metabolic reactions involving ammonium that have been stimulated with symbiosis (Carvalho et al. 2014). These results are corroborated by increased leaf area, biomass, chlorophyll, and concentration of amino acids and proteins, and greater nitrogen use efficiency. Therefore, these results indicate that there was a greater flow of C and N in these plants, i.e., free ammonia was efficiently incorporated into organic molecules or structures.

Interestingly, N-fertilized plants inoculated with PGPB showed lower concentration of free ammonia, while increased their content of nitrate. It suggests higher availability of nitrate in rhizosphere, and an unbalance between uptake and reduction of this compound (Liu et al. 2014; Bloom 2015). PGPB can induce an increase in lateral root development and therefore improve the nitrate uptake and plant's N status. In fact, inoculated plants exhibited a larger root system. Previously, *Azospirillum brasilense* increased the concentration of nitrate in plants grown with N (Calzavara et al. 2019). The results have shown that PGPB increased plant biomass, chlorophyll and total N, and it suggests positive influence on photosynthesis and N uptake by roots. In addition, the reduction in nitrate assimilation is associated to the translocation and accumulation of this compound in vacuoles. This plant strategy is important to maintain a favorable water status in its tissues (Wang et al. 2014).

Finally, this study has shown that these selected PGPB present potential in promoting maize and sorghum growth with the possibility to reduce the dependence on N-fertilizers used in both crops. It is particularly interesting since these crops are important to smallholders that, usually, do not use chemical fertilizers. These results reinforce the positive benefit to maize and sorghum by inoculation with these potential PGPB. As a technology with low economic cost, easy application, environmentally friendly, and potentially responsive by maize and sorghum, the further recommendation of these PGPB could be a strategy to be used for farmers.

5 Conclusion

In this study, PGPB positively influenced the plant growth and N metabolism in maize and sorghum, even when plants were grown with N. This indicates that these PGPB can provide N to maize and sorghum and stimulate the plant growth. Positively, IPACC07, IPACC08, IPACC10, IPACC38 and IPACC55 presented potential to be indicated as PGPB in maize and sorghum. This study suggests that these PGPB may be useful to improve N nutrition, biomass and yield in maize and sorghum, and further studies should be done under field conditions. In addition, further studies could explore the possible use of these PGPB in combination to find some additive or synergistic responses.

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Author contributions JPAA, SMBR, and MRA carried out the experiments; JELA and ASFA designed the experiments; JELA, ASFA, AB, FAN analyzed data, discussed results and wrote the paper.

Declarations

Conflict of interest The authors declare no conflicts of interest.

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