Nutr Cycl Agroecosyst (2020) 117:93–101
https://doi.org/10.1007/s10705-020-10051-3

ORIGINAL ARTICLE

Biological nitrogen fixation in field-grown sorghum under different edaphoclimatic conditions is confirmed by N isotopic signatures

Benaia Gonçalves de França Barros · Ana Dolores Santiago de Freitas · José Nildo Tabosa · Maria do Carmo Catanho Pereira de Lyra · Adália Cavalcanti do Espirito Santo Mergulhão · Aleksandro Ferreira da Silva · Wagner da Silva Oliveira · Paulo Ivan Fernandes-Júnior · Everardo Valadares de Sá Barretto Sampaio

Received: 4 July 2019 / Accepted: 2 February 2020 / Published online: 19 February 2020

Abstract The association between sorghum and N_2-fixing bacteria has been assessed only under limited conditions. We investigated biological nitrogen fixation (BNF) in situ in fifteen sorghum genotypes with dry or succulent culm types under five edaphoclimatic conditions. One randomized block experiment was established in each of five locations, from the humid to the semiarid regions of Pernambuco state, Brazil. BNF was estimated using the ^{15}N natural abundance method by comparing the average $\delta^{15}N$ value of each sorghum genotype with those of the reference species. High levels of productivity, up to 22 Mg shoot biomass ha^{-1} in the 3-month cycle, were obtained where rainfall was high, and up to 5 Mg ha^{-1} was obtained under low rainfall. The nitrogen contents showed a similar pattern as biomass production, and the genotypes with the highest productivity accumulated from 200 to 300 kg N ha^{-1}. BNF ranged from 55 to 78% of plant N in one location and from 36 to 56% in another location, but BNF did not occur in the other three locations. Although the factors that blocked effective symbiosis were not determined, symbiosis was not influenced by P or K availability. The proportion of N_2 fixation was similar in the grain-producing, dry culm genotypes and in the sugar-rich, succulent culm genotypes. The sorghum genotypes fixed N_2, reaching up to 218 kg ha^{-1} N, without inoculation with diazotrophs. Therefore, sorghum has a high potential to fix atmospheric N_2, but the factors that block N_2 fixation must be identified for crop management planning.

Keywords Natural abundance technique · C4 photosynthetic system · Energy crop · *Sorghum bicolor* (L.) Moench · Diazotrophs

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10705-020-10051-3) contains supplementary material, which is available to authorized users.

B. G. F. Barros · E. V. S. B. Sampaio
Departamento de Energia Nuclear, Centro de Tecnologia, Universidade Federal de Pernambuco, Av. Professor Luís Freire, n° 1000, Recife, PE, Brazil

A. D. S. Freitas (✉) · A. F. Silva · W. S. Oliveira
Departamento de Agronomia, Universidade Federal Rural de Pernambuco, R. Dom Manoel de Medeiros, s/n, Recife, PE, Brazil
e-mail: anadoloressantiagodefreitas@gmail.com

J. N. Tabosa · M. C. C. P. Lyra · A. C. E. S. Mergulhão
Instituto Agronômico de Pernambuco, IPA, Avenida General San Martin, n° 1371, Recife, PE, Brazil

P. I. Fernandes-Júnior (✉)
Embrapa Semiárido, BR 428, km 152, s/n, Petrolina, PE, Brazil
e-mail: paulo.ivan@embrapa.br

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most cultivated cereal in the world (FAO 2010). It is a multipurpose species that produces forage, grain and sugar (Tuinstra 2008; Davila-Gomez et al. 2011; Mathur et al. 2017). Sorghum grain is usually produced in genotypes with short, dry culms, and sugar and forage are produced in genotypes with tall, succulent, sugar-rich culms. The production of alcohol production from the fermentable sugars and cellulose in the sugar-rich culm varieties is also an increasing field of interest (Guigou et al. 2011; Fernandes et al. 2014). As a plant with the C4 photosynthetic pathway, sorghum is highly efficient in converting solar radiation, water and nitrogen into aboveground biomass under a wide range of soil and climatic conditions (Albrizio and Steduto 2005; Steduto and Albrizio 2005). Sorghum is particularly useful as a crop adopted to areas where rainfall is insufficient for most traditional tropical humid grain or sugar-producing crops, such as maize and rice, or sugarcane and palm, respectively (Ananda et al. 2011; Vasilakoglou et al. 2011).

Sorghum, similar to other Poaceae species, may associate with several diazotrophic bacteria, mainly the genera *Azospirillum* (Puri et al. 2017), *Herbaspirillum* (Monteiro et al. 2012), *Gluconacetobacter* (Yoon et al. 2016), *Burkholderia* (Silva et al. 2018), *Stenotrophomonas*, *Bacillus* (Antunes et al. 2019) and *Bradyrhizobium* (Hara et al. 2019), which are capable of introducing atmospheric N_2 via biological nitrogen fixation (BNF). Many reports of effective BNF in Poaceae species have been published in the last decade (Morais et al. 2012; Baptista et al. 2014; Alves et al. 2015) but other studies could not find evidence of N_2 fixation in the same Poaceae species (Biggs et al. 2002). In fact, many Poaceae species were used as reference species in studies of BNF in legume species, including sorghum (Kurdaly 2009). In this last study, the ^{15}N signal of sorghum was similar to that of sunflower, both considered as non-fixing species.

BNF in sorghum has been subjected to few studies, some conducted in vitro (Stein et al. 1997) and under greenhouse conditions (Carvalho et al. 2017). Under field conditions, only two experiments have reported sorghum BNF using the ^{15}N natural abundance technique. The first study tested a single genotype planted as green manure for irrigated crops and found that more than 70% of plant N was derived from BNF (Ferreira Neto et al. 2017). The other experiment tested three genotypes inoculated with *Burkholderia* spp. and *Herbaspirillum* ssp. strains and found a higher proportion of N_2 fixation in the forage genotype than in the grain and sweet genotypes (Santos et al. 2017). The lower fixation of the sugar-rich type seems to contradict the hypothesis that the higher provision of easily accessible carbohydrates would benefit the association with effective diazotrophic bacteria. The limited and contradictory results indicate that BNF studies with different sorghum types and dozens of sorghum genotypes should be conducted, preferentially under distinct environmental conditions.

These studies are critical for establishing the advantages of N_2 fixation, which could partially or even totally substitute for the application of nitrogen fertilizers. This advantage is important for sorghum crops, which are usually grown in areas where rainfall scarcity and low capital availability prevent mineral fertilization, such as large parts of Africa and the semiarid Brazilian Northeast Region.

Considering the large number of available genotypes, the general absence of the practice of inoculation and the extensive range of agro-climatic conditions under which sorghum is planted, we investigated BNF in 15 genotypes that were not yet tested for BNF potential, grown in the field in five municipalities from humid to semiarid regions in Pernambuco state, Brazil. We hypothesized that (1) different genotypes behave differently under different edaphoclimatic conditions and (2) the succulent, sugar-rich culm genotypes show greater BNF than genotypes with lower culm sugar and water concentrations (dry culms).

Methods and materials

Fifteen sorghum genotypes, including varieties and progenies of different purposes (grain, forage, sugar, or biomass for bioenergy) and culm types (dry or succulent), from the sorghum breeding program of the Instituto Agronômico de Pernambuco (IPA, Agronomic Institute of Pernambuco) and Empresa Brasileira de Pesquisa Agropecuária (Embrapa, Brazilian Agricultural Research Corporation) were evaluated in five municipalities with different edaphoclimatic conditions in Pernambuco state (Tables S1 and S2).

The three experiments in the subhumid and semiarid municipalities (Caruaru, Belo Jardim and Serra Talhada) were conducted during the rainy season (March to June) in 2014. In Caruaru, the experiment was rainfed (214.8 mm during the growth cycle), but in the other two municipalities, the experiments were irrigated due to the scarce rains. The experiments in the humid municipalities (Itambé and Goiana) were conducted in the same year, from July to November and were rainfed (656.1 mm and 876.6 mm, respectively). The average monthly temperatures along the growing cycle were similar to the annual averages (Table S2), while the daily variations were larger than the monthly variations, mainly in the subhumid and semiarid municipalities, where they reached up to 10 °C.

One randomized block experiment was established in each municipality, with three replications and plots with four 6 m long rows, spaced at 0.8 m. One extra plot in each block was planted with sunflower (*Helianthus annuus* L) in Goiana, Itambé and Serra Talhada to be used as reference plants. In Caruaru and Belo Jardim, due to logistical problems, these extra plots were not planted, and spontaneous species growing along the border of the experiments (five plants per species) were used as the reference plants.

Soil samples were collected in each location before planting and were analyzed (Table S3) according to the methodology recommended by Embrapa (2009). Dolomitic lime was applied (700–1500 kg ha^{-1}) based on the soil analyses at each site. The experimental areas were plowed and harrowed and, in all sites except Belo Jardim, were fertilized in doses ranging from 8 to 15 kg ha^{-1} of N in the form of urea, 25 to 45 kg ha^{-1} of P_2O_5 in the form of triple superphosphate, and 36 to 66 kg ha^{-1} of K_2O in the form of KCl. The application rates were based on the soil tests at each site. Sorghum seeds were distributed in 50 mm deep furrows, and the stands were thinned to 12 plants m^{-1} when the average plant height was 100 mm.

All the aboveground biomass in the central area of each plot (8 m^2) was harvested 90 days after planting, chopped in a forage processor, weighed and subsampled. The subsamples were oven-dried, weighed and analyzed for total N concentration (Table S4) by the Kjeldahl method (Embrapa 2009) after digestion with sulfuric acid and hydrogen peroxide (Thomas et al. 1967). The N contents were calculated by multiplying the concentrations by the biomass, except at Belo Jardim, where the biomass data were lost.

At harvest, leaf material from five plants in the border rows of each plot was collected for biological nitrogen fixation (BNF) estimation. In Goiana, Itambé and Serra Talhada, leaf material was also collected from five sunflower plants in each extra plot. In Caruaru and Belo Jardim, where sunflower was not planted, the leaf material to be used as a reference was collected from five plants of the spontaneous species *Herissantia tiubae* (K. Schum.) Brizicky, Malvaceae, and other unidentified non-N-fixing species in Caruaru and *H. tiubae* and *Panicum maximum* Jacq., Poaceae, in Belo Jardim. The leaf ^{15}N signals were assumed to be similar to the signals of the culms, considering the short cycle of the plants and the intense circulation of N within each plant (Shearer and Kohl 1989).

All these leaf materials were dried and ground separately. Subsamples were placed in capsules and inserted into a Delta V Advantage mass spectrometer to obtain the isotopic ratios of N. The isotopic ratios were determined according to recognized international standards. Reference materials (atropine, yeast extract and soil standard 502–308, LECO Corporation) were included in all analytical runs. The natural abundance of ^{15}N in the samples (Table S5) was expressed using the delta notation ($\delta^{15}N$), which represents the parts per thousand deviations (‰) in relation to the ^{15}N and ^{14}N ratio (R) in atmospheric N_2 (Junk and Svec 1958; Mariotti 1983):

$$\delta = \left(R_{\text{sample}}/R_{\text{reference}} - 1\right) \times 1000$$

The proportion of nitrogen derived from the atmosphere (%Ndfa) was estimated by comparing the average $\delta^{15}N$ value of each sorghum genotype ($\delta^{15}N_{\text{sorghum}}$) with those of the reference species ($\delta^{15}N_{\text{reference}}$) in each experiment, provided that the difference was statistically significant ($p < 0.05$), using the formula (Shearer and Kohl 1986):

$$\%\text{Ndfa} = 100\left[\left(d^{15}N_{\text{reference}} - d^{15}N_{\text{sorghum}}\right) / \left(d^{15}N_{\text{reference}} - B\right)\right]$$

where B is the ^{15}N abundance of the sorghum when the plants rely entirely on BNF from atmospheric N_2 (Amarger et al. 1979). In the absence of a known value for sorghum, the B value was assumed to be zero, as has been done for the estimation of Poaceae fixation (Morais et al. 2012; Baptista et al. 2014; Alves et al.

2015). The amounts of fixed N were estimated by multiplying %Ndfa by the N concentrations and the total aboveground biomass.

The normality and homogeneity of the data for biomass, N concentration, N amount and fixed N of the sorghum genotypes from each experiment were tested by the Shapiro–Wilk and Levene tests. The N concentration data were submitted to arcsine transformation. The data were submitted to analysis of variance, and the averages were compared by the Tukey test at the 0.05 probability level. To compare the $\delta^{15}N$ signals of the sorghum genotypes with those of the reference species, a *T* test was used at the same probability level. The same test was used to compare the $\delta^{15}N$ signals of the succulent and dry culm genotype groups. The correlation between the %Ndfa and biomass productivity was calculated. All tests were performed using Sisvar version 4.8 (Ferreira 2008).

Results

Biomass production

The biomass productivity data confirmed that sorghum can be a highly efficient crop under both high and low water availability. The irrigated plants in Serra Talhada and the rainfed plants in Itambé and Goiana, where 656 and 877 mm of rain fell during the growth cycle, produced up to 22 Mg ha^{-1} (Table 1). These levels of productivity in a growth cycle of only 90 days are only matched by those of maize. In Caruaru, the productivity was above 2 Mg ha^{-1} and reached 5 Mg ha^{-1}, which is also high considering the severe water limitation caused by the low rainfall (215 mm).

A few genotypes produced significantly more shoot biomass than the others, but the high-producing genotypes were not the same in all municipalities, except for variety SF 11 (dry culm), which had the highest productivity in three of the municipalities (Table 1). The productivity of most of the genotypes was intermediate and statistically similar in all municipalities, and the only genotype with consistent lower productivity was progeny IPA P-F6 08 (succulent culm). The different performances of the progenies indicate the potential for selection.

Nitrogen uptake and fixation

The nitrogen concentrations varied from 9 to 30 g kg^{-1} dry matter (Table S4) and were not correlated with biomass productivity. These concentrations are within the usual range under conditions where N availability is not deficient. The total nitrogen accumulation followed the pattern of biomass production, since the production variation was larger than the concentration variation, with the total N accumulation in more productive genotypes reaching from 200 to 300 kg ha^{-1} (Table 2).

The $\delta^{15}N$ signals of the reference plants were high (> 5.80‰), with little variation (CV < 18%) within each municipality, allowing good estimates of the biological N_2 fixation of the sorghum genotypes (Table S5). In the municipality where one grass (*Panicum maximum*) and one nongrass (*Herissantia tiubae*) spontaneous species were used as reference plants, the signals of the two species were similar, indicating the absence of fixation in the grass species. The absence of significant differences in the $\delta^{15}N$ signals of all sorghum genotypes and the reference species in Itambé, Belo Jardim and Serra Talhada also indicated the absence of BNF. On the other hand, differences larger than 2‰, most of which were statistically significant, indicate active BNF in most genotypes in Goiana and Caruaru. In Goiana, more than 50% of the plant nitrogen of the fixing genotypes was derived from the atmosphere (%Ndfa), reaching 78% in variety SF 11 (Table 3), the one with the highest biomass productivity (Table 2). In Caruaru, the %Ndfa was somewhat lower than that in Goiana but still ranged from 36 to 56%. Considering the low water availability and relatively low biomass productivity in Caruaru, these %Ndfa correspond to 11 to 18 kg N ha^{-1} from BNF, while in Goiana, with high productivity, BNF contributed 65 to 265 kg N ha^{-1} (Table 3). Despite these high contributions, there was no correlation between %Ndfa and biomass productivity. These contributions are underestimated because they referred only to the aboveground parts of the plants; fixed N was certainly incorporated into the roots (not harvested), and some may have been transferred to the soil.

The succulent and dry culm types did not differ in the %Ndfa or amount of fixed N in the two municipalities where fixation occurred (Table 3), contradicting our second hypothesis. In Caruaru, both groups

Table 1 Dry-matter production (Mg ha^{-1}) of fifteen sorghum genotypes grown in 2014 in five municipalities in Pernambuco state, Brazil. Means of three replicate plots

Genotypes	Goiana	Itambé	Caruaru	Serra Talhada
IPA 8602589	4.61 cd	8.46 bcd	1.49 b	8.49 bcde
SF 11	14.52 a	17.40 a	6.42 a	22.15 a
IPA 730 1011	5.33 cd	6.92 d	3.22 ab	8.49 bcde
IPA 8602600	7.27 cd	9.36 bcd	3.16 ab	9.07 bcde
BR 506	8.71 abcd	13.54 abc	2.30 ab	8.57 bcde
IPA 467-4-2	13.45 ab	18.26 a	1.80 b	12.07 bc
IPA 2502	7.65 bcd	10.07 bcd	2.92 ab	6.57 cde
IPA P-F6 01	9.45 abc	16.58 a	6.37 a	10.07 bcde
IPA P-F6 02	8.37 bcd	13.88 abc	5.48 ab	10.74 bcd
IPA P-F6 03	5.74 cd	8.09 bcd	3.91 ab	8.91 cde
IPA P-F6 04	8.35 bcd	14.29 ab	3.81 ab	7.16 bcde
IPA P-F6 05	6.81 cd	8.85 bcd	3.81 ab	13.32 b
IPA P-F6 06	6.42 cd	8.36 bcd	3.56 ab	12.82 bc
IPA P-F6 07	4.91 cd	7.71 cd	2.74 ab	5.41 de
IPA P-F6 08	3.15 d	8.97 bcd	2.69 ab	4.08 e

Values followed by the same letter in the column are not significantly different at the 0.05 level (Tukey test)

had one genotype that did not fix N, while in Goiana, only one genotype of the succulent group did not fix N, but it was not the same genotype that did not fix N in Caruaru.

Discussion

Sorghum is highly productive under dry and wet conditions

The high aboveground biomass productivity obtained under both irrigated and rainfed conditions confirms the potential of sorghum genotypes to produce grains and sugar or alcohol in the Northeast Region of Brazil. Several genotypes with biomass production of above ten and up to 22 Mg ha^{-1} could be selected to be planted in the humid zone, particularly in its border area where rainfall is lower and sugarcane productivity is frequently limited by low water availability (Sampaio et al. 1984). Even more important was the productivity in the subhumid and dry zones, up to 5 Mg ha^{-1}, indicating that sorghum can outcompete maize, the main crop in these zones, which is more sensitive to water stress (Sampaio et al. 2004). The distribution of these two crops in the African Sahel, the origin of sorghum, indicates that the introduced maize was only competitive where water availability was relatively high.

Table 2 Nitrogen accumulation (kg ha^{-1}) of fifteen sorghum genotypes grown in 2014 in five municipalities in Pernambuco state, Brazil

Genótipo	Goiana	Itambé	Caruaru	Serra Talhada
IPA 8602589	127 c	182 abc	23 b	122 b
SF 11	339 a	321 abc	98 a	273 a
IPA 730 1011	160 abc	146 c	57 ab	121 b
IPA 8602600	168 abc	221 abc	42 ab	126 b
BR 506	194 abc	313 abc	33 ab	116 b
IPA 467-4-2	320 ab	334 ab	27 b	148 b
IPA 2502	196 abc	231 abc	44 ab	113 b
IPA P-F6 01	229 abc	322 ab	100 a	142 b
IPA P-F6 02	210 abc	350 a	84 ab	156 b
IPA P-F6 03	140 bc	190 abc	61 ab	133 b
IPA P-F6 04	200 abc	328 ab	60 ab	120 b
IPA P-F6 05	167 abc	222 abc	66 ab	177 ab
IPA P-F6 06	184 abc	160 bc	62 ab	182 ab
IPA P-F6 07	128 c	182 abc	25 b	82 b
IPA P-F6 08	94 c	243 abc	46 ab	77 b

Means of three replicate plots

Values followed by the same letter within columns are not significantly different at the 0.05 level (Tukey test)

Biological N_2 fixation is high at some sites

The high productivity in the three municipalities with sufficient water availability and with N concentrations within the normal range for sorghum aboveground biomass resulted in high levels of plant N accumulation that were above 100 kg ha^{-1} in most genotypes (Tables 1 and 2) and that in all cases were above the amount of N applied as fertilizer (45 kg ha^{-1} at most). Even in Caruaru, the municipality with low rainfall, where productivity was lowest, most of the genotypes accumulated more N than the amount applied as fertilizer; they certainly accumulated more N than the amount derived from the fertilizer, since under field conditions, the efficiency of N fertilizer utilization rarely exceeds 50% (Martinez-Feria et al. 2018; Yang and Udvardi 2018). The accumulation was also higher than that measured in the shoots because the belowground biomass was not included in the measurements.

In the humid municipality of Goiana, except in one genotype, over 54% of plant N, and as much as 78% of plant N, originated from symbiotic fixation (Table 3); these percentages are equivalent to 65 to 218 kg N ha^{-1}. The genotype with a different pattern (BR506) had a high signal of δ^{15}N, similar to those of the reference nonfixing species; therefore, there was no evidence of biological fixation. In Caruaru, the proportion of N originating from N_2 fixation was lower than that in Goiana but still high for most genotypes (34–56%), despite water limitation. Due to this limitation, which resulted in low biomass and N accumulation (Tables 1 and 2), the amounts of fixed N in the aboveground biomass were only 11–48 kg ha^{-1} (Table 3). Two genotypes had no evidence of fixation, but neither of them was the same genotype that did not show fixation in Goiana; that genotype showed a good fixation level in Caruaru (48% Ndfa). These results confirm that noninoculated sorghum can derive high proportions of N from association with fixing bacteria (Ferreira Neto et al. 2017) and that without water limitation, sorghum can incorporate large quantities of N into the plant-soil system. These amounts may represent an important economic contribution and make production viable under the minimum input system that prevails in the Northeast Region of Brazil (Tiessen et al. 2001).

Comparisons of these proportions of N derived from the atmosphere with those of sorghum planted elsewhere are limited by the low number of published results, explained by the fact that fixation in sorghum plants was only confirmed a few years ago (Ferreira Neto et al. 2017; Santos et al. 2017). The %Ndfa of one sorghum genotype planted as a green manure crop together with several other species (Ferreira Neto et al. 2017), including legume species, was as high (79%) as the highest levels in Goiana. The amounts fixed in the manure crop were lower, but a direct comparison cannot be made since the sorghum was not planted as a single crop. The three genotypes (grain, forage and sugar-rich) inoculated with selected diazotrophic bacteria in the work of Santos et al. (2017) had lower %Ndfa (< 32%) than those in Goiana and

Table 3 Proportion of nitrogen in the plant derived from the atmosphere (% Ndfa) and amount of nitrogen fixed in fifteen sorghum genotypes grown in 2014 in Goiana and Caruaru municipalities, Pernambuco state, Brazil

Genotypes	Goiana		Caruaru	
	Ndfa (%)	Fixed nitrogen (kg ha^{-1})	Ndfa (%)	Fixed nitrogen (kg ha^{-1})
IPA 8602589	57.1 a	71 c	0	0
SF 11	78.1 a	265 a	49.2 a	48 a
IPA 730 1011	57.6 a	97 bc	35.5 a	19 a
IPA 8602600	61.4 a	96 bc	42.7 a	19 a
BR 506	0	0	47.9 a	16 a
IPA 467-4-2	67.0 a	218 ab	39.8 a	11 a
IPA 2502	61.8 a	115 bc	46.9 a	22 a
IPA P-F6 01	61.4 a	144 abc	42.3 a	42 a
IPA P-F6 02	57.0 a	129 abc	44.8 a	37 a
IPA P-F6 03	66.5 a	97 bc	56.0 a	35 a
IPA P-F6 04	54.9 a	109 bc	43.7 a	30 a
IPA P-F6 05	74.5 a	108 bc	40.6 a	22 a
IPA P-F6 06	69.9 a	130 abc	0	0
IPA P-F6 07	61.4 a	75 bc	49.3 a	12 a
IPA P-F6 08	69.1 a	65 c	47.8 a	25 a

Means of three replicate plots

Values followed by the same letter in the column are not significantly different at the 0.05 level (Tukey test)

Caruaru, and only the grain genotype responded to the inoculation. The forage genotype had significantly higher %Ndfa than the grain and forage genotypes, contrary to the results in Goiana and Caruaru, in which the two groups of grain and sugar-rich genotypes did not differ. However, in both groups, some genotypes had higher %Ndfa than others, and comparison with the single genotype per group in the work of Santos et al. (2017) provides little additional information regarding group performance.

No N_2 fixation occurred at other sites

Other Poaceae, especially sugarcane, can reach the same high %Ndfa (Urquiaga et al. 2012; Baptista et al. 2014) as registered in Goiana while showing no N_2 fixation in other places (Biggs et al. 2002). All these %Ndfa determined for Poaceae species could be altered if the B value of the calculation were found to be different from 0. However, if the B value is similar to those of most legume species (approximately—1‰), the differences would not be very large, given that the signals of the reference species are relatively high (5.87 and 9.95‰).

If N_2 fixation were confirmed in some municipalities, the absence of N_2fixation in some of the genotypes in Goiana and Caruaru and all the genotypes in the other three municipalities casts doubts about the possible generalization of the beneficial effects of this symbiosis. Large variations in the fixation proportions of C4 Poaceae are common (Biggs et al. 2002; Baptista et al. 2014; Carvalho et al. 2017). Effective symbiosis is dependent on the presence of diazotrophic bacteria and the appropriate environmental conditions for integrated functioning. Endophytic bacteria, such as those that form symbiotic relationships with sorghum, commonly belonging to several genera, such as *Azospirillum*, *Herbaspirillum*, *Gluconacetobacter, Enterobacter, Bacillus* and *Paraburkholderia* (Roncato-Maccari et al. 2003; Coelho et al. 2009; Yoon et al. 2016; Silva et al. 2018; Antunes et al. 2019), may occur within seeds or may migrate from the soil; in both cases, they invade roots and stems (Luna et al. 2010). If the seeds did not carry the bacteria, the absence of these bacteria in the soil could be the cause of the lack of fixation in Belo Jardim, Itambé and Serra Talhada. If the seeds contained the bacteria, considering that the seeds came from the same sources for all experiments, diazotrophic bacteria would be available at all sites. In this case, and if bacteria were present in all soils, the growing conditions hampered fixation in Belo Jardim, Itambé and Serra Talhada. It is not known what the limiting condition is, since temperatures were similar and the large difference in water availability (Table S2) did not impede fixation in Goiana and Caruraru (Table 3). However, the limiting condition could be a nutrient deficiency. Phosphorus deficiencies seem to limit BNF in native legume plants under nonfertilized conditions (Silva et al. 2017), but it is unlikely that P or K deficiencies blocked fixation since all experiments were fertilized to the recommended levels. The only consistent differences in the soil characteristics from the sites where fixation occurred and did not occur were lower Ca and silt concentrations (Puri et al. 2017) in the former (Table S3). The importance of Ca for BNF of legume plants has been repeatedly demonstrated (O'Hara 2001; Weisany et al. 2013), and it is likely that it is also important for N_2 fixation in Poaceae species, even though endophytic N_2 fixation is different from N_2 fixation through nodule formation. However, fixation occurred in the areas with lower Ca concentrations, and the correlation would indicate that somewhat higher Ca concentrations would block N_2 fixation. No references were found relating higher soil Ca concentrations to the inhibition of N_2 fixation. Additionally, no reference was found relating lower silt concentrations to the absence of effective symbiotic N_2 fixation.

Until a clearer knowledge of the causes of the lack of N_2 fixation in some genotypes and at some sites is gained, the benefits of symbiotic N_2 uptake may remain a random perspective and cannot be counted on while planning crop management. Since fixation can reduce the need for N fertilization and result in large economic gains, studies of N_2 fixation with more genotypes and at more sites are urgently needed. These studies should address possible nutrient deficiencies, especially micronutrient deficiencies, different forms of inoculation and the complex field of plant and bacteria interactions.

Conclusions

Sorghum has high potential to fix atmospheric N_2 even without inoculation with symbiotic bacteria. The potential is similar for grain, forage and sugar-producing genotypes, but some genotypes within each group performed better than others, indicating the possibility of selection for genotypes that are more efficient at N_2 fixation. However, the absence of N_2 fixation in all genotypes at some sites and in a few genotypes at sites where other genotypes showed N_2 fixation indicates that factors not accounted for could block effective symbiosis. These factors must be identified before fixation can be depended on for crop management planning.

Acknowledgements The authors thank the Brazilian Council for Scientific and Technological Development (CNPq; Projeto Universal 2012, Grant Number 472997/2012-2), the Projeto "Pesquisas integradas para a garantia da sustentabilidade hídrica, alimentar e energética no Bioma Caatinga" (PEGASUS 19-2017-Nexus, Grant Number 441305/2017-2), the Fundação de Amparo à Ciência e Tecnologia de Pernambuco (FACEPE; Grant Number APQ-0199-5.01/15) and the Brazilian Agricultural Research Corporation (Embrapa Grant Number 20.18.03.066.00.00) for financial support. The second and last two authors are research fellows of CNPq.

References

Albrizio R, Steduto P (2005) Resource use efficiency of field-grown sunflower, sorghum, wheat and chickpea I. Radiation use efficiency. Agric Forest Meteorol 130:254–268. https://doi.org/10.1016/j.agrformet.2005.03.009

Alves GC, Videira SS, Urquiaga S, Reis VM (2015) Differential plant growth promotion and nitrogen fixation in two genotypes of maize by several *Herbaspirillum* inoculants. Plant Soil 387:307–321. https://doi.org/10.1007/s11104-014-2295-2

Amarger N, Mariotti A, Mariotti F, Durr JC, Bourguignon C, Lagacherie B (1979) Estimate of symbiotically fixed nitrogen in field grown soybeans using variations in ^{15}N natural abundance. Plant Soil 52:269–280

Ananda N, Vadlani PV, Prasad PVV (2011) Evaluation of drought and heat stressed grain sorghum (*Sorghum bicolor*) for ethanol production. Ind Crop Prod 33:779–782. https://doi.org/10.1016/j.indcrop.2011.01.007

Antunes GR, Santana SRA, Escobar IEC, Brasil MS, Araújo GGL, Voltolini TV, Fernandes-Júnior PI (2019) Associative diazotrophic bacteria from forage grasses in the Brazilian semiarid region are effective plant growth promoters. Crop Pasture Sci 70:899–907. https://doi.org/10.1071/CP19076

Baptista RB, Morais RF, Leite JM, Schultz N, Alves BJR, Boddey M, Urquiaga S (2014) Variations in the ^{15}N natural abundance of plant-available N with soil depth: their influence on estimates of contributions of biological N_2 fixation to sugar cane. Appl Soil Ecol 73:124–129. https://doi.org/10.1016/j.apsoil.2013.08.008

Biggs IM, Stewart GR, Wilson JR, Critchley C (2002) ^{15}N natural abundance studies in Australian commercial sugarcane. Plant Soil 238:21–30

Carvalho EX, Menezes RSC, Freitas ADS, Sampaio EVSB, Simões Neto DE, Tabosa JN, Primo DC, Queiroz RO (2017) The ^{15}N natural abundance technique to assess the potential of biological nitrogen fixation (BNF) in some important C4 grasses. Aust J Crop Sci 11:1559–1564. https://doi.org/10.21475/ajcs.17.11.12.pne729

Coelho MRR, Marriel IE, Jenkins SN, Lanyon CV, Seldin L, O'Donnell AG (2009) Molecular detection and quantification of *nifH* gene sequences in the rhizosphere of sorghum (*Sorghum bicolor*) sown with two levels of nitrogen fertilizer. Appl Soil Ecol 42:48–53. https://doi.org/10.1016/j.apsoil.2009.01.010

Davila-Gomez FJ, Chuck-Hernandez C, Perez-Carrillo E, Rooney WL, Serna-Saldivar SO (2011) Evaluation of bioethanol production from five different varieties of sweet and forage sorghums (*Sorghum bicolor* (L) Moench). Ind Crop Prod 33:611–616. https://doi.org/10.1016/j.indcrop.2010.12.022

Embrapa—Empresa Brasileira de Pesquisa Agropecuária (2009) Manual de análises químicas de solos, plantas e fertilizantes, 2nd ed. In: Embrapa Solos, Embrapa Informática Agropecuária, Brasília

FAO—Food and Agriculture Organization of the United Nations (2010) FAOSTAT. http://www.faostat.org/site/567/. Accessed on 18 March 2015

Fernandes G, Braga TG, Fischer J, Parrella RAC, Resende MM, Cardoso VL (2014) Evaluation of potential ethanol production and nutrients for four varieties of sweet sorghum during maturation. Renew Energy 71:518–524. https://doi.org/10.1016/j.renene.2014.05.033

Ferreira DF (2008) SISVAR: um programa para análises e ensino da estatística. Rev Symp 6:36–41

Ferreira Neto RA, Freitas ADS, Giongo V, Camargo PB, Menezes RSC, Sampaio EVSB (2017) Nitrogen fixation of Poaceae and Leguminoseae in a green manure experiment in the Brazilian semiarid region. Aust J Crop Sci 11:1559–1564. https://doi.org/10.21475/ajcs.17.11.11.pne726

Guigou M, Lareo C, Pérez LV (2011) Bioethanol production from sweet sorghum: evaluation of post-harvest treatments on sugar extraction and fermentation. Biomass Bioenerg 35:3058–3062. https://doi.org/10.1016/j.biombioe.2011.04.028

Hara S, Morikawa T, Wasai S, Kasahara Y, Koshiba T, Yamazaki K, Fujiwara T, Tokunaga T, Minamisawa K (2019) Identification of nitrogen-fixing *Bradyrhizobium* associated with roots of field-grown sorghum by metagenome and proteome analyses. Front Microbiol. https://doi.org/10.3389/fmicb.2019.00407

Junk G, Svec HV (1958) The absolute abundance of the nitrogen isotopes in the atmosphere and compressed gas from various sources. Geochim Cosmochim Acta 14:234–243

Kurdaly K (2009) Growth and nitrogen fixation in Dhaincha/Sorghum and Dhaincha/sunflower intercropping systems using ^{15}Nitrogen and ^{13}Carbon natural abundances techniques. Commun Soil Sci Plant Anal 40:2995–3014. https://doi.org/10.1080/00103620903261577

Luna MF, Galar ML, Aprea J, Molinari ML, Boiardi JL (2010) Colonization of sorghum and wheat by seed inoculation with *Gluconacetobacter diazotrophicus*. Biotechnol Lett

32:1071–1076. https://doi.org/10.1007/s10529-010-0256-2
Mariotti A (1983) Atmospheric nitrogen is a reliable standard for natural $\delta^{15}N$ abundance measurements. Nature 303:685–687
Martinez-Feria RA, Castellano MJ, Dietzel RN, Helmers MJ, Liebman M, Huber I, Archontoulis SV (2018) Linking crop- and soil-based approaches to evaluate system nitrogen-use efficiency and tradeoffs. Agric Ecosyst Environ 256:131–143. https://doi.org/10.1016/j.agee.2018.01.002
Mathur S, Umakanth AV, Tonapi VA (2017) Sweet sorghum as biofuel feedstock: recent advances and available resources. Biotechnol Biofuels 10:146. https://doi.org/10.1186/s13068-017-0834-9
Monteiro RA, Balsanelli E, Wassem R, Brusamarello-Santos LCC, Schmidt MA, Tadra-Sfeir MT, Pankievicz VCS, Cruz LM, Chubatsu LS, Pedrosa FO, Souza EM (2012) *Herbaspirillum*-plant interactions: microscopical, histological and molecular aspects. Plant Soil 356:175–196. https://doi.org/10.1007/s11104-012-1125-7
Morais RF, Quesada DM, Reis VM (2012) Contribution of biological nitrogen fixation to Elephant grass (*Pennisetum purpureum* Schum.). Plant Soil 356:23–34. https://doi.org/10.1007/s11104-011-0944-2
O'Hara GW (2001) Nutritional constraints on root nodule bacteria affecting symbiotic nitrogen fixation: a review. Aust J Exp Agric 41:417–433
Puri A, Padda KP, Chanway CP (2017) Nitrogen-fixation by endophytic bacteria in agricultural crops: recent advances. In: Amanullah K, Fahad S (eds) Nitrogen in agriculture—updates. Intech, Rijeka, pp 73–94. https://doi.org/10.5772/intechopen.71988
Roncato-Maccari LDB, Ramos HJO, Pedrosa FO, Alquini Y, Chubatsu LS, Yates MG, Rigo LU, Steffens MBR, Souza EM (2003) Endophytic *Herbaspirillum seropedicae* expresses nif genes in gramineous plants. FEMS Microbiol Ecol 45:39–47. https://doi.org/10.1016/S0168-6496(03)00108-9
Sampaio EVSB, Salcedo IH, Bettany J (1984) Dinâmica de nutrientes em cana-de-açúcar. I. Eficiência na utilização de uréia ($-^{15}N$) em aplicação única ou parcelada. Pesq Agropec Bras 19:943–949
Sampaio EVSB, Tiessen H, Antonino ACD, Salcedo IH (2004) Residual N and P fertilizer effect and fertilizer recovery on intercropped and sole-cropped corn and beans in semiarid northeast Brazil. Nutr Cyc Agroecosys 70:1–11. https://doi.org/10.1023/B:FRES.0000049356.83427.93
Santos CLR, Alves GC, Macedo AVM, Fiori FG, Pereira W, Urquiaga S, Reis VM (2017) Contribution of a mixed inoculant containing strains of *Burkholderia* spp. and *Herbaspirillum* ssp. to the growth of three sorghum genotypes under increased nitrogen fertilization levels. Appl Soil Ecol 113:96–106. https://doi.org/10.1016/j.apsoil.2017.02.008
Shearer G, Kohl DH (1986) N_2-fixation in field settings: estimations based on natural ^{15}N abundance. Aust J Plant Physiol 13:699–756
Shearer G, Kohl DH (1989) Natural ^{15}N abundance as a method of estimating the contribution of biologically fixed nitrogen to N_2-fixing systems: potential for non-legumes. In: Skinner FA, Boddey RM, Fendrik I (eds) Nitrogen fixation with non-legumes. Kluwer Academic Publishers, Dordrecht, pp 289–299
Silva AF, Freitas ADS, Costa TL, Fernandes-Júnior PI, Martins LMV, Santos CERS, Menezes KAS, Sampaio EVSB (2017) Biological nitrogen fixation in tropical dry forests with different legume diversity and abundance. Nutr Cycl Agroecosyst 107:321–334. https://doi.org/10.1007/s10705-017-9834-1
Silva JF, Silva TR, Escobar IEC, Fraiz ACR, Santos JWM, Nascimento TR, Santos JMR, Peters SJW, Melo RF, Signor D, Fernandes-Júnior PI (2018) Screening of plant growth promotion ability among bacteria isolated from field-grown sorghum under different managements in Brazilian drylands. World J Microbiol Biotechnol 34:186. https://doi.org/10.1007/s11274-018-2568-7
Steduto P, Albrizio R (2005) Resource use efficiency of field-grown sunflower, sorghum, wheat and chickpea II. Water use efficiency and comparison with radiation use efficiency. Agric Forest Meteorol 130:269–281. https://doi.org/10.1016/j.agrformet.2005.04.003
Stein T, Hayen-Schneg N, Fendrik I (1997) Contribution of BNF by *Azoarcus* sp. BH72 in *Sorghum vulgare*. Soil Biol Biochem 29:969–971
Thomas RL, Sheard RW, Moyer JR (1967) Comparison of conventional and automated procedures for nitrogen, phosphorus, and potassium analysis of plant material using a single digestion. Agron J 59:240–243
Tiessen H, Sampaio EVSB, Salcedo IH (2001) Organic matter turnover and management in low input agriculture of NE Brazil. Nutr Cycl Agroecosyst 61:99–103. https://doi.org/10.1023/A:1013384730492
Tuinstra MR (2008) Food-grade sorghum varieties and production considerations: a review. J Plant Interact 3:69–72. https://doi.org/10.1080/17429140701722770
Urquiaga S, Xavier RP, Morais RF, Batista RB, Schultz N, Leite JM, Sá JM, Barbosa KP, Resende AS, Alves BJR, Boddey RM (2012) Evidence from field nitrogen balance and 15N natural abundance data for the contribution of biological N2 fixation to Brazilian sugarcane varieties. Plant Soil 356:5–21. https://doi.org/10.1007/s11104-011-1016-3
Vasilakoglou I, Dhima K, Karagianiannidis N (2011) Sweet sorghum productivity for biofuels under increased soil salinity and reduced irrigation. Field Crop Res 120:38–46. https://doi.org/10.1016/j.fcr.2010.08.011
Weisany W, Raei Y, Allahverdipoor KH (2013) Role of some of mineral nutrients in biological nitrogen fixation. Bull Environ Pharmacol Life Sci 2:77–84
Yang J, Udvardi M (2018) Senescence and nitrogen use efficiency in perennial grasses for forage and biofuel production. J Exp Bot 69:855–865. https://doi.org/10.1093/jxb/erx241
Yoon V, Tian G, Vessey JK, McFie SM, Dangi OM, Kumer AK, Tian L (2016) Colonization efficiency of different sorghum genotypes by *Gluconacetobacter diazotrophicus*. Plant Soil 398:243–256. https://doi.org/10.1007/s11104-015-2653-8