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BIOLOGICAL NITROGEN FIXATION IN SUGARCANE AND NITROGEN TRANSFER FROM SUGARCANE TO CASSAVA IN AN INTERCROPPING SYSTEM

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ABSTRACT

Investigation of biological nitrogen fixation (BNF) in sugarcane and quantification of fixed N transfer from sugarcane to other crops is important for commercial crop production systems. Biological N fixation of sugarcane and fixed N transfer from sugarcane to cassava in an intercropping system were determined with the ^{15}N isotope dilution technique. The experiment was conducted using a randomized block design including mono-cropping and intercropping of sugarcane and cassava with 2 replications. Results indicated that % Ndfa (^{15}N natural abundance N derived from atmosphere N_2) was 29.5 % and the N fixation rate was 11.3 g N/m^2 (113.1 kg N/ha) under the mono-sugarcane planting conditions. In the sugarcane-cassava intercropping system, the % Ndfa was 36.4 %, including 29.9 % in sugarcane for its growth utilisation and 6.5 % in cassava transferred from sugarcane, and the N fixation rate was 17.8 g N /m^2 (178.2 kg N/ha), in which 82.1 % was used for sugarcane growth and 17.9 % used for cassava growth. It was concluded that sugarcane plants received the same amount of N from biological fixation in both sole planting and intercropping systems, but it could fix more N and transfer a portion of fixed N to cassava crops in the sugarcane–cassava intercropping system.

KEYWORDS: Sugarcane, Cassava, Intercropping system, ^{15}N isotope dilution technique, Nitrogen-fixing percentage

INTRODUCTION

Nitrogen (N) application rate for sugarcane production in China usually reaches 500-700 kg/ha annually. This N rate is much higher than that in Brazil where approximately 60 kg/ha N for plant cane and 80-120 kg/ha for ratoon cane are applied annually. High N application leads to not only high production cost but also environmental issues. One of the possible reasons for low N application in Brazil is associated with the biological N fixation (BNF) of sugarcane. Döbereiner (1961) first discovered the phenomenon of biological N fixation in sugarcane and isolated the N fixation bacteria *Beijerinck*. Previous studies have proved that a certain amount of N in sugarcane plants comes from biological fixation and there exist BNF systems associated with bacteria inside sugarcane plants (Lin *et al*., 2008, 2012; Luo *et al*., 2013; Wei *et al*., 2014). It was reported that sugarcane plants could obtain 30-80 % of the total N required from BNF (Boddey *et al*., 1991; Döbereiner, 1997). The proportion of the sugarcane biologically fixed N varies substantially among varieties and locations with 25-80 % reported in Brazil (Boddey *et al*., 1991; Robert *et al*., 2000), 10-67 % in Mexico (Fuentes-Ramirez *et al*., 2004), 30 % in Thailand (Ando *et al*., 2004), and 31.3 % in China (Luo *et al*., 2013). However, all of these studies were conducted under a sugarcane mono-cropping system rather than under a two-crop intercropping system. Previous studies have shown that the biologically fixed N in leguminous plants can transfer to graminaceous crops in intercropping systems (Giller *et al*., 1991; Stern, 1993). Although sugarcane is a graminaceous crop for which BNF ability has been reported, it is not clear if sugarcane biologically-fixed N can be transported to other crops in intercropping systems. The objectives of this study were to investigate BNF in sugarcane when intercropped with cassava, to quantify fixed N transfer from sugarcane to cassava, and to provide a reference for research on further utilising the N fixation ability of sugarcane.

MATERIALS & METHODS

Planting and field management

The experiment was conducted on the farm of the Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences/Sugarcane Research Center, Chinese Academy of Agricultural Sciences, Nanning, China. The soil type was a red laterite with former crop of maize. The topsoil had the following characteristics: organic matter 17.66 g/kg, total N 1.68 g/kg, total P 0.66 g/kg, total K 10.96 g/kg, available N 132 mg/kg, available P 51 mg/kg, available K 185 mg/kg, NH_4^+-N 21.25 mg/kg, and NO_3^--N 14.88 mg/kg, and pH 6.36. The N-fixing sugarcane variety in this study was B8, provided by the Sugarcane Research

Institute, Guangxi Academy of Agricultural Sciences, and the non-N_2 fixing reference cassava variety was HN8, provided by the Cash Crop Research Institute, Guangxi Academy of Agricultural Sciences. Three treatments were: sugarcane-cassava intercropping, sugarcane mono-cropping, and cassava mono-cropping. A randomized block design was employed with two replications and the mono-cropping treatments were used as the controls. Each plot had 6 rows 3 m long and 1 m row spacing. The mono-cropping plot was planted to a single crop of sugarcane or cassava only. For the mono-cropping sugarcane, cane setts with 2 buds were used and 10.5×10^4 buds/ha were planted. For the mono-cropping cassava, stem setts with 4 buds were used and 1.0×10^4 plants/ha were planted. In the intercropping plots, sugarcane and cassava were planted in every other row. The planting date was 5 March 2008. The seedcane setts were treated with the 1 % solution of 50 % wettable powder of carbendazim prior to planting. A rate of 45 kg/ha of 3 % granular carbonfuran was applied in the furrow at planting to control insects. After planting and watering, atrazine solution was applied on the soil surface for weed control followed by plastic film mulching in the furrow. Fertiliser $(^{15}NH_4)_2SO_4$ with 15.15 % of isotopic abundance was applied to all treatments at a rate of 3 g per row on 4 May and covered with soil. No any other fertiliser was applied in the growing season. Other field management was done based on commercial sugarcane production practices in China.

Sampling and measurements

All dry matter, including dry leaf, sheath (sugarcane), and petiole (cassava), was collected from the central rows of each plot. Crops were harvested on 30 Dec. 2008 and different parts, *i.e*. root, stem, leaf and sheath of sugarcane, and root, tuber, stem, leaf and petiole of cassava, were separated and weighed. The tissue samples were immediately placed in a dryer at 105ºC for 30 min, then dried at 75ºC to constant weight.

The dry samples were ground and sieved. A given amount of subsample from each ground sample was used to analyse total N and ^{15}N isotopic abundance. Samples included the roots (mixed roots), stalks (node 3~4 from top), leaves and sheaths (leaf 2~3 for both) of sugarcane; and the root mixture (root: tuber root = 1:1 in weight), stems (nodes and internodes 3~4 from top), leaves and petioles (green parts for both) of cassava. Total N and ^{15}N isotopic abundance were measured (Yao, 1988, Yao *et al*., 2004; Urquiaga *et al*., 1992) in a laboratory at the Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China.

Calculation for N fixation in sugarcane

BNF calculations using the isotope dilution method were based on Yao (1988), Boddey *et al.* (1995), and Urquiaga *et al.* (1992). The following calculation formulae were used:

- ^{15}N-atom per cent excess (A%E) = Sample ^{15}N isotopic abundance - 0.3663%;
- The weighted arithmetic average of A%E = Sample A%E × Total N/Total N in whole plant;
- ^{15}N natural abundance N derived from atmosphere N_2 (% Ndfa) in sugarcane under the mono-cropping conditions = (1 - A%E mono-sugarcane / A%E mono-cassava), and under the intercropping condition, % Ndfa = (1 – A%E intercropping sugarcane/A%E mono-cassava);
- % N transfer from sugarcane to cassava (%NTFL) = 1 – A%E intercropping cassava/A%E mono-cassava;
- ^{15}N natural abundance N derived from fertiliser N (% Ndff) = Plant sample A%E – Fertiliser sample A%E; ^{15}N natural abundance N derived from soil N (% Ndfs) = 1 - %Ndff; Fertiliser use efficiency (%) = % Ndff × Plant total N/Fertilised N × 100; and Plant total N = Total dry weight of plant (all parts) × % Total N.

Additionally, biological N fixation measurements with total N difference methods and with tracer difference methods were taken according to Ma *et al.* (1983). Nitrogen fixation with total N difference = Total N in N_2 fixing crop –Total N in non N_2 fixing crop and N_2 fixation with tracer difference = (Total N in N_2 fixing crop – Fertiliser N in N_2 fixing reference crop) – (Total N in non N_2 fixing crop –Fertiliser N in non N_2 fixing reference crop). The experimental data were analysed statistically using Excel and DPS (Data Processing System) Nepal software. The Student *t* test was used to determine differences between the intercropping and mono-cropping systems for each crop.

RESULTS

Effect of intercropping on economic and biological yields in sugarcane and cassava

Economic yield (fresh stalk yield) and biological yield (dry weight yield) of sugarcane were not significantly different in intercropping than mono-cropping systems (Table 1).

Intercropping significantly decreased cassava economic (fresh tuber) and biological (dry weight) yields compared to mono-cropping (Table 1) with a 30.6 % decrease in fresh tuber yield (P<0.05) and 29.2 % decrease in whole plant dry weight (P<0.01). The dry weights of tubers and stems of intercropped cassava decreased 29.8 and 34.5 % (P<0.05 for both), respectively, compared to those of mono-cropped cassava.

TABLE 1: Economic and biological yields in sugarcane and cassava under mono-cropping or intercropping systems

Crop		Economic yield (Fresh, kg/m^2)	Biological yield (Dry weight, g/m^2)				
			Root	Tuber	Stem	Leaf	Whole plant
Sugarcane	Mono-cropping	10.23 a	42.07 a	–	3163.10 a	1291.75 a	4496.92 a
	Intercropping	10.87 a	44.70 a	–	3616.49 a	1313.04 a	4974.23 a
Cassava	Mono-cropping	1.47 a	7.29 a	529.65 a	856.56 a	96.70 a	1490.20 A
	Intercropping	1.02 b	7.24 a	371.63 b	561.19 b	114.45 a	1054.51 B
Total	Mono-cropping	11.70 a	49.36 a	529.65 a	4019.66 a	1388.45 a	5987.12 a
	Intercropping	11.89 a	51.93 a	371.63 b	4177.68 a	1427.49 a	6028.74 a

Note: Means followed by different uppercase letters within a column and cropping system are significant at $P \leq 0.01$ and by different lowercase letters are significant at $P \leq 0.05$.

When yield data were combined across sugarcane and cassava, there were no significant differences between intercropping and mono-cropping in either economic yield or biological yield except for cassava dry tuber weight as mentioned above (Table 1).

Effect of intercropping on plant total N in sugarcane and cassava

Among plant parts, obviously, leaf blades had the highest and leaf sheaths/petioles had lowest N contents, and N content in stems was slightly higher than that in roots for both sugarcane and cassava (Table 2). For sugarcane and cassava, there were no significant differences between intercropping and mono-cropping in total N content in any plant part, except for cassava stems where mono-cropping had significantly higher N content than the intercropping ($P<0.05$).

TABLE 2: Total N distribution in different parts of sugarcane and cassava plants under mono-cropping or intercropping conditions

Crop		Total N content (%)				Total N amount (g/m^2)			
		Root + Tuber	Stem	Leaf	Sheath/ Petiole	Root+ Tuber	Stem	Leaf + Sheath/ Petiole	Whole Plant
Sugarcane	Mono-cropping	0.61 a	0.87 a	1.17 a	0.47 a	0.26 a	27.49 b	10.59 a	38.33 B
	Intercropping	0.55 a	0.96 a	1.10 a	0.57a	0.25 a	34.57 a	10.90 a	45.71 A
Cassava	Mono-cropping	1.21 a	1.54 a	2.79 a	0.91 a	6.50 a	13.19 A	1.79 a	21.48 a
	Intercropping	1.31 a	1.39 b	2.92 a	1.01 a	4.92 b	7.80 B	2.25 a	14.97 b
Total	Mono-cropping	—	—	—	—	6.75 a	40.68 b	12.38 a	59.81 a
	Intercropping	—	—	—	—	5.17 b	42.36 a	13.15 a	60.68 a

Note: Means followed by different uppercase letters within a column and cropping system are significant at $P \leq 0.01$ and by different lowercase letters are significant at $P \leq 0.05$.

Sugarcane had 19.3 % higher ($P<0.01$) total plant N in the intercropping than in the mono-cropping system (Table 2). The total N amount in sugarcane stems was 24.3 % higher ($P<0.05$) in the intercropping than in the mono-cropping. There were no differences between the two cropping systems in total amount of N in sugarcane roots or leaves. For cassava, the total amounts of N in stems, tubers, and whole plant were 40.9, 24.3, and 30.3 % ($P<0.05$-0.01) lower, respectively, in intercropping than in mono-cropping. When data were combined across sugarcane and cassava to determine cropping system differences, total N in stems of the intercropping system were significantly higher than that of the mono-cropping, but the amount of N in roots (+tubers) for the intercropping was significantly lower than that for the mono-cropping ($P<0.05$). No significant differences between the two cropping systems were detected in total amounts of N in leaves and whole plants.

Nitrogen fixation in sugarcane and N transfer from sugarcane to cassava

Results of ^{15}N isotope tracing showed that the sugarcane plant had substantial N-fixing ability when cassava was used as a reference crop in intercropping (Tables 3 and 4). For the entire sugarcane crop, BNF contributed 29.5 % of total accumulated N or 11.31 g/m^2 under mono-cropping, and 36.4 % of total accumulated N or 17.82 g/m^2 (including the transferred N) under intercropping, suggesting a higher N fixing efficiency under intercropping than under mono-cropping.

TABLE 3: The ^{15}N A%E in sugarcane and cassava, biological N_2 fixation by sugarcane, and fixed N transfer from sugarcane to cassava

Crop		^{15}N A%E					N_2 fixation		N transfer	
		Root	Stem	Leaf	Sheath/ Petiole	Whole plant*	% Total N	Amount (g/m^2)	% Total N	Amount (g/m^2)
Sugarcane	Mono-cropping	0.16	0.06	0.08	0.07	0.06	29.50	11.31	—	—
	Intercropping	0.22	0.06	0.05	0.06	0.06	31.99	14.62	—	—
Cassava	Mono-cropping	0.15	0.06	0.10	0.10	0.09	—	—	—	—
	Intercropping	0.10	0.06	0.05	0.04	0.07	—	—	21.42	3.19

*Data are the weighted arithmetic average of A%E of all parts of the plant.

TABLE 4: Biological N fixation (BNF) efficiency in sugarcane/ cassava intercropping system

Total N amount in intercropping (g/m^2)		BNF by sugarcane Amount (g/m^2)	%	BNF N % in total N
Total N in intercropped sugarcane plant	45.71±0.09	14.62±3.07	82.1	29.9
N transferred from sugarcane to cassava	3.19±0.70	3.19±0.70	17.9	6.5
Total	48.90±0.79	17.82±3.77	100.0	36.4

Under mono-cropping, the N from BNF by sugarcane was used for its own growth, but a part of fixed N was transferred to cassava under intercropping (Table 4). In the present study, 82.1 % of the N from BNF by sugarcane was used for its own growth while 17.9 % of the fixed N was transferred to cassava. The contribution of BNF to total N in sugarcane alone was fairly stable, which was 29.5 and 29.9 % in mono-cropping and intercropping, respectively. In sugarcane/cassava intercropping systems, 21.4 % of the N in cassava came from BNF by sugarcane.

Plant N sources in sugarcane/cassava intercropping

TABLE 5: Nitrogen in sugarcane and cassava crops from different sources of fertilizer, soil, and biological N fixation

Crop		Total N in crop (g/m^2)	Crop N % from					Fertilizer use efficiency (%)	Crop N amount (g/m^2) from				
			Ferti-liser	Soil	Fixed N				Ferti-liser	Soil	Fixed N		
					For itself	Trans-fer	Total				For itself	For trans-fer	Total
Sugar-cane	Mono-cropping	38.33	0.42	70.07	29.50	—	29.50	16.28	0.16	26.86	11.31	—	11.31
	Intercropping	48.90	0.44	63.08	29.90	6.53	36.43	21.43	0.21	30.85	14.62	3.19	17.82
Cassava	Mono-cropping	21.48	0.60	99.40	—	—	—	12.94	0.13	21.35	—	—	—
	Intercropping	14.97	0.47	78.10	—	21.42	—	7.09	0.07	11.69	—	3.19	—

In the present study, there were three major plant N sources in sugarcane/cassava intercropping systems: soil, fertiliser and BNF by sugarcane (Table 5). Soil N was the dominant N source which provided 63.1-70.1 % of total N for sugarcane and 78.1-99.4 % for cassava. Amounts of N from soil in sugarcane and cassava were 10.0 and 21.4 %, respectively, and were lower in the intercropping than in the mono-cropping. These results suggested that intercropping could reduce the N absorption from soil by both sugarcane and cassava because of BNF by sugarcane and the fixed N transfer from sugarcane to cassava. There was a low proportion of N from fertiliser in plant total N, which accounted for 0.42 - 0.43 % for sugarcane and 0.47 - 0.60 % for cassava. For sugarcane, the N from fertiliser was 3.2 % higher while, for cassava, that was 21.4 % lower in the intercropping than in the mono-cropping.

Comparison of sugarcane N fixation amounts estimated by different methods

Three different methods of the total N difference, the tracer difference and the ^{15}N isotope dilution were used to estimate amounts of sugarcane fixed N (Table 6). The estimated amounts of sugarcane N were very close between the total N difference method and the tracer difference method. However, the estimated N fixation from the ^{15}N isotope dilution method was considerably lower than that from the other two methods.

TABLE 6: Sugarcane N fixation amount (g/m^2) estimated by 3 different methods

Sugarcane crop	Total N difference	Tracer difference	^{15}N isotope dilution
Mono-cropping	16.85	16.82	11.31
Intercropping	33.93	33.79	17.82

DISCUSSION

Previous studies reported that BNF in sugarcane was affected by many factors such as sugarcane variety, growth stage and status, organ and position, and environment (Urquiaga *et al*., 1992; Boddey *et al*., 1995; Döbereiner, 1997; Robert *et al*., 2000; Lin *et al*., 2008, 2012; Yang *et al*., 2008; Luo *et al*., 2013; Wei *et al*., 2014). In the present study, the BNF of sugarcane provided 29.5 % of total N for plant growth under mono-cropping, which was similar to the result reported by Luo *et al*. (2012) who found a 31.3 % BNF after accumulation of N fixing bacteria into tissue culture derived sugarcane plants. The present study found that sugarcane/cassava intercropping improved BNF efficiency in sugarcane. There was a considerable amount of fixed N transfer from sugarcane to cassava under intercropping. Giller *et al.* (1991) and Stern *et al.* (1993) also reported BNF N transferred to non N fixing plants from N fixing plants in intercropping or mixed planting systems. The mechanism of the BNF N transfer is not clear yet, but the BNF N may be released to soil after the dead roots of the N fixing plants decompose, and then the released N could be absorbed by the non N fixing plants (Markus *et al*., 1994). Results of the present study indicated that the BNF N transfer from sugarcane to cassava did not change the BNF N contribution to sugarcane itself, but benefited the intercropped crop. It has been reported that ^{15}N isotope dilution is the most accurate method in estimating N fixation amounts compared to total N difference, and tracer difference methods, and that over-estimation often occurs when the total N difference method is used (Yao *et al*., 2004). The tracer difference method should be better than the total N difference method because the fertiliser N is subtracted in estimation. In the present study, however, fertiliser use efficiency was low and it may be why the estimated N fixation amounts were similar between the total N difference method and the tracer difference method. Based on the estimation by the ^{15}N isotope dilution method, sugarcane N fixation was 113.1 kg N/ha for mono-cropping and 178.2 kg N/ha for intercropping under the present experimental conditions. These results are similar to those in China (Luo *et al*., 2013; Yang *et al*., 2008) and in Thailand (Ando *et al*., 2004), but lower than those in Brazil (Urquiaga *et al*., 1992; Döbereiner, 1997). Screening for more efficient N fixing bacteria strains and sugarcane genotypes is ongoing in China.

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