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SHORT COMMUNICATION

A preliminary study on quantification of biological nitrogen fixation in sugarcane grown in Sevanagala in Sri Lanka

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Abstract: The extent of nitrogen(N) fixation in sugarcane grown in reddish brown earth soils in Sevanagala was determined using the difference in ^{15}N abundance in the top visible dewlap leaves of sugarcane (variety CO 775, SL 8306) compared to those of neighbouring weeds. Results indicate that biological N_2 fixation contributed to an average of 18% of the total N in sugarcane in the study site.

Keywords: Biological nitrogen fixation, ^{15}N natural abundance technique, sugarcane.

INTRODUCTION

Nitrogen (N) is one of the important plant nutrients necessary for sugarcane production. Over the next few decades, due to depletion of petroleum reserves and increased production costs of other fuels, price rise of inorganic nitrogenous fertilizers may be inevitable. Hence it becomes imperative to substitute inorganic nitrogen by a cheap source which can at least partially meet the crop requirements.

If N_2 fixing bacteria inhabit the rhizosphere of any plant or occur endophytically in the plant tissues, it would be a symbiotic association similar to that found in legumes and rhizobia. It has been found in Brazil and few other countries where sugarcane is grown, that endophytic nitrogen fixing bacteria associated with some sugarcane varieties supply a considerable amount of nitrogen. A variety of N_2-fixing (diazotrophic) bacteria (*Beijerinckia, Bacillus, Klebsiella, Enterobacter, Erwinia, Azospirillum, Herbaspirillum* and *Gluconaacetobacter*) have been isolated from the rhizosphere, roots, stems, and leaves of sugarcane[1, 2].

Some sugarcane varieties grown in Brazil obtain over 60% of total plant N (equivalent to over 200 kg N/ha/ year) from biological nitrogen fixation (BNF) [2]. Further, sugarcane varieties CB 45-3 and SP 70-1143 were found to fix 170−210 kg N/ha/year when grown with ample phosphorus(P) and foliar application of molybdenum (Mo) (500g/ha) and these varieties have given 180 t/ha even when grown in very low fertile soils without any nitrogen fertilization. A previous study[3] estimated that average N input to sugarcane plant by BNF was 28% to 33% of the total N in the plant when grown in a research field in central Thailand. Sugarcane grown in some sites in Brazil, Philippines and Miyako Island in Japan was found to fix 33 % of N by BNF [4] .

The ^{15}N natural abundance technique appears to provide a better quantitative measure of BNF associated with legume or actinorhizal tree species in natural ecosystems, plantations or agro forestry systems over the various other methods such as total N difference method, acetylene reduction assay, and other ^{15}N methodologies[5]. As a result of isotope discrimination effects that occur during soil formation, most soils have slightly higher ^{15}N abundance than the atmosphere. Because of this difference, nitrogen fixing plants have been found to have a lower ^{15}N enrichment than non fixing ones and this has been the basis for the ^{15}N natural abundance technique. With the development of automated high precision continuous flow isotope ratio mass spectrometers, the use of ^{15}N natural abundance version of the isotope ratio has become a more accurate technique[5,6]. The application of this technology to estimate BNF associated with sugarcane has been thoroughly discussed[6].

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Sugarcane is grown in Sri Lanka with low N fertilizers compared to other countries, yet it gives a substantial yield. In addition, some N fertilizer trials and NPK fertilizer trials conducted in Sri Lanka show no significant results in both cane and sugar yield [7]. This may be attributed to the presence of BNF in Sri Lankan sugarcane varieties. Therefore, this study was undertaken to quantify the extent of BNF associated with sugarcane cultivated in the Sevanagala sugarcane plantation in Sri Lanka.

METHODS AND MATERIALS

Ginigalpallassa area of the Sevanagala sugarcane plantation was selected for this study. According to a previous study[6], ^{15}N natural abundance technique was used to quantify the BNF in sugarcane. Allotments of 13 farmers, which were closely located, were selected by considering the age (7 – 8 months after planting or ratooning), type of the crop (plant or ratoon), and the variety of the crop. Soils of all these allotments were reddish brown earth (Rhodustalf). In each allotment, 15 Top Visible Dewlap (TVD) cane leaf samples were randomly collected and bulked by allotments separately. Simultaneously, 2−3 types of weed samples (non leguminous) in each allotment were collected, and the whole shoots of weeds were bulked by botanical species, separately for each allotment. Bulked samples were dried at 65 ^{0}C for about 72 h and ground using a mill to a fine powder. ^{15}N abundance of these samples was determined using continuous flow isotope ratio mass spectrometry. These samples were analyzed for ^{15}N by the International Atomic Energy Authority (IAEA) in Austria. The % N_2 derived from N fixation (%Ndfa) was calculated for each allotment as follows [4].

%Ndfa = (1 - $\delta^{15}N$ value of sugarcane/ $\delta^{15}N$ value of neighbouring plants) X 100

In this estimation, isotopic fractionation during N fixation by associated bacteria was assumed to be zero. When the reference plants (weeds) had ^{15}N value lower than sugarcane, %Ndfa was assumed to be zero[4]. An average value of %Ndfa was obtained for Ginigalpallasse area of the Sevanagala plantation.

RESULTS

The N content, ^{15}N abundance [$\delta\ ^{15}N$(‰)] and the % N derived from N_2 fixation (% Ndfa) of plant crops of each cane allotment are shown in Table 1 and the above values for the ratoon crops are shown in Table 2. As a whole, the $\delta\ ^{15}N$ (‰) value of sugarcane was lower than those of neighbouring weeds in seven allotments. BNF appears to have not taken place in sugarcane grown in allotments C-07-29, C-07-51, D-02-330 and D-02-324 while cane in other allotments have fixed N_2 as shown in Tables 1 and 2. The $\delta\ ^{15}N$(‰) value of sugarcane and neighbouring weeds showed too much variation. The possible average contribution of N_2 fixation to total N in sugarcane was about 18% within a range of 0 - 77% concerning both plant and ratoon crop. However, in this study ratoon cane has shown higher average BNF ability (22.3%) than the plant crop (8%).

DISCUSSION AND CONCLUSION

These results show that a considerable level of nitrogen fixation occurs in the study site though the variation is too large. If the number of sample sites was increased,

Table 1: ^{15}N natural abundance, % N of TVD leaves and weeds, % N derived from N_2 fixation of plant crops

Location	Cane variety	Growth cycle	Reference plant	$\delta\ ^{15}N$(‰)	%Ndfa	% N
C-07-51	CO 775	Plant crop		7.58		1.44
			Borreria hispida	2.60	0	2.74
			Euphorbia hirta	2.62	0	2.34
			Crassocephalum crepidioides	4.21	0	2.91
C-06- 46	CO 775	Plant crop		3.65		1.31
			Borreria latifolia	2.82	0	1.99
			Ageratum conyzoides	4.10	11	2.93
C-03-09	CO 775	Plant crop		4.62		1.57
			Echinochloa colonum	9.28	51	1.55
			Euphorbia hirta	5.09	10	1.21
D-02-330	CO 775	Plant crop		7.28		1.63
			Scoporia oauls	7.15	0	2.31
			Solanum nigrum	5.76	0	3.11

variation would be less. In a similar study conducted in several areas in the Philippines and three sites in Miyako Island (Japan), it has been found that BNF contributed to an average of 33% within a range of 0−72% [4]. Moreover, another study[6] using a similar technique found that 60% of plant N in sugarcane in a commercial plantation was derived from BNF. In addition, Shotaro *et al*.[3] have estimated BNF in sugarcane cultivated in central Thailand to be 28−33% using cassava as reference plants.

A previous study[6] pointed out that optimum fertilization with P, potassium (K) and micronutrients and year-round irrigation are important to occur high BNF in sugarcane. In the present study, only NPK fertilizers were used and year-round irrigation was not practised.

Due to limited facilities, only a limited number of samples were analyzed for $\delta^{15}N$ values. Weed samples were not replicated and hence, it was not possible to estimate the statistical significance of BNF for each variety and for each allotment. However, the average BNF value estimated from this study for this area is accurate and it is a considerable quantity in terms of N fertilizer.

Table 2: ^{15}N natural abundance, % N of TVD leaves and weeds, % N derived from N fixation

Location	Cane variety	Growth cycle	Reference plant	$\delta^{15}N$(‰)	%Ndfa	% N
C-06-288	CO 775	2nd ratoon		1.66		1.24
			Eleutheranthera ruderalis	2.96	44	1.87
			Corchorus aestuans	2.80	41	2.16
			Scoporia oauls	7.43	77	1.86
C-07-29	SL 8306	1st ratoon		5.90		1.46
			Aeschynomene indika	-0.52	0	4.10
			Mitracarpus hirtus	2.39	0	2.92
			Varnonia cinerea	1.91	0	2.63
C-05-126	CO 775	6th ratoon		3.96		1.45
			Digitaria ischaemum	3.84	0	1.59
			Unknown	7.2	45	1.58
			Commelina benghalensis	4.59	14	3.04
C-06-129	CO 775	6th ratoon		2.48		1.22
			Corchorus aestuans	5.06	51	2.54
			Ageratum conyzoides	3.26	24	2.93
D-02-547	SL 8306	3rd ratoon		4.73		1.68
			Amaranthus viridis	5.45	14	3.82
			Cleome viscosa	5.46	13	3.94
D-02-535	SL 8306	1st ratoon		4.50		1.45
			Atylosia scara baeoides	1.50	0	2.10
			Cucumis callosus	4.64	4	2.66
			Hibiscus lobatus	3.47	0	2.43
D-02-324	CO 775	15th ratoon		3.71		1.80
			Tridex procubens	2.80	0	2.02
			Ipomoea pestigridis	2.66	0	2.40
			Phaseolus lathyroides	-1.12	0	3.39
D-04- 1563	CO 775	3rd ratoon		3.09		1.39
			Scoporia oauls	7.43	58	1.86
			Solanum nigrum	5.76	46	3.11
D-04-138	CO 775	1st ratoon		3.55		1.45
			Phylanthus niruri	5.76	38	3.12
			Mikania scandense	6.31	44	1.97

Application of ^{15}N natural abundance technique and other independent techniques to quantify BNF have been discussed thoroughly in a previous study[5]. According to this study, ^{15}N natural abundance technique is the best tool to quantify the BNF in an agro-ecosystem. It has been recommended to use a number of different, preferably botanically diverse reference plants; however two - three non nitrogen reference plants have also been accepted to quantify the BNF associated with sugarcane[6]. Similarly, two - three reference plants were used in this study. Since BNF potential of sugarcane depends on genotype , it is suggested to investigate the BNF potential of all commercial sugarcane varieties in the country. This preliminary study examines whether sugarcane grown in Sri Lanka has the ability to fix N_2 from atmosphere. As the study was carried out only in Sevanagala, it is important to quantify the extent of BNF associated with sugarcane cultivated in other sugarcane growing areas of the country.

In almost all countries in the world where sugarcane is grown, BNF ability of sugarcane is used for sustainable sugarcane production. The findings reported in the present study can be used to improve the N fertilizer recommendation for sugarcane production, to select varieties for breeding programmes and to isolate diazotrophic bacteria for the production of bio fertilizer. Some N fertilizer trials conducted in certain areas of this plantation had not shown any significant difference in the yield[7] (even in 1:2:3 N in the trial). Based on the results of this study, this non significant response may be attributed to the presence of BNF associated with sugarcane in the area.

Several attempts to isolate nitrogen fixing bacteria from sugarcane showed *Gluconoacetobacter diazotrophicus* as the most common species[8]. This species is able to fix nitrogen in a medium of high sugar concentration, low pH and in the presence of nitrate. *Gluconoacetobacter diazotrophicus* has been found in all parts of the sugarcane plant including post harvest residues remaining in the fields[9]. Inoculation methods of biological nitrogen fixing bacteria have also been investigated. In addition, it is widely accepted that some biological nitrogen fixing bacteria produce plant growth promoters such as auxins, gibberellins and cytokinins[10].

The findings of this preliminary study indicate the presence of BNF in sugarcane under Sri Lankan conditions. It is therefore necessary to conduct detailed studies to utilize BNF in sugarcane growing areas of Sri Lanka.

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References

1. Perchase B.S. (1980). Nitrogen fixation associated with sugarcane. *Proceedings of the South African Sugar Technologists' Association* June: 173-176.
2. Boddey R.M., Urquiaga S., Reis V. & Dobereiner J. (1991). Biological nitrogen fixation with sugarcane. *Plant and Soil* **137**(1): 111-117.
3. Ando S., Meunchang S., Thippayarugs S., Prasertsak P., Matsumoto N. & Yoneyama T. (2004). Role of nitrogen fixation for sustainable sugarcane production. *Proceedings of the International Symposium on Sustainable Sugarcane and Sugar Production Technology*, Nanning, China, 29 November –2 December, 2004, 156 -159.
4. Yoneyama T., Muraoka T., Kim T.H., Dacanay E.V. & Nakanishi Y. (1997). The natural ^{15}N abundance of sugarcane and neighboring plants in Brazil, the Philippines and Miyako(Japan). *Plant and Soil* **189(**2): 239-244.
5. Boddey R.M., Peoples M.B., Palmer B. & Dart P.J. (2000). Use of the ^{15}N natural abundance technique to quantify biological nitrogen fixation by woody perennials. *Nutrient Cycling in Agro Ecosystems* **57(**3): 235-270.
6. Boddey R.M., Polidoro J.C., Alexander S.R., Bruno J.R.A. & Urquiaga S. (2001). Use of the ^{15}N natural abundance technique for the quantification of the contribution of N_2 fixation to sugarcane and other grasses. *Australian Journal of Plant Physiology* **28(**9): 889-895.
7. Sugarcane Research Institute of Sri Lanka (2000, 2005 and 2006). *Annual Reports.* Sugarcane Research Institute of Sri Lanka, Uda Walawe.
8. Ovidio P., Ovalle W. & Urquiaga S. (2005). Update on biological nitrogen fixation research on sugarcane in Guatemala. *Proceedings of the International Society of Sugercane Technologists* **25**: 105-109.
9. Fuentes L.E., Jimenez-salgado T., Abarca I.R. & Mellado J.C. (1993). *Acetobacter diazotrophicus*, an indoleacetic acid prodcing bacterium isolated from sugarcane cultivars of Mexico. *Plant and Soil* **154**(2): 145-150.
10. Moutia J.F.Y., Umrit G., Saumtally A.S. & Ngkee kwong K.F. (2005). Occurrence of diazotrophic and growth promoting rhizobacteria associated with sugarcane in Mauritius. *Proceedings of the International Society of Sugercane Technologyists* **25**: 111 -116.