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Evaluation of Sugarcane Clones with Biological Nitrogen Fixation Endowment

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Abstract: The study was conducted with 45 Sugarcane clones grown at zero-N and 120 kg N ha^{-1} levels in order to evaluate their possible BNF (biological nitrogen fixation) capability under field conditions. Field evaluation led to identification of some clones viz., I 153/94, Co 846, B 34-104 and Isd 28 that demonstrated considerable BNF capability. The yields of these clones at zero-N were 54.30, 61.48, 53.69 and 63.97 t ha^{-1} but at 120 kg N ha^{-1} it was 59.72, 65.75, 57.15 and 70.59 t ha^{-1}, respectively. The non BNF clones Isd 2/54 and Isd 18 showed poor yield at zero-N (31.38 and 28.17 t ha^{-1}) while at 120 kg N ha^{-1} it was 54.61 and 61.58 t ha^{-1}. The BNF capable clones performed almost equally well under both zero-N and 120kg N ha^{-1} as demonstrated through number of tiller (000 ha^{-1}), number of millable cane (000 ha^{-1}) and leaf nitrogen content at 120 and 200 days after plantation (DAP). Under aseptic culture condition, root extract and cane juice from the BNF- endowed clones showed the presence of gram negative bacterium that have been subjected to further studies.

Key words: Sugarcane, biological nitrogen fixation (BNF), gram negative bacterium, zero-N input, clone, fertilizer, field evaluation

Introduction

In Bangladesh the average yield of Sugarcane staggers around 50 t ha^{-1} in the mill-zone and about 36 t ha^{-1} in non mill-zone areas (Anonymous, 1996). Among the various causes of this poor management, which is in normal practice by the resource-poor farming community is considered responsible. Large scale diversion of fertilizers, supplied on credit to cane growers, often take place because of the obvious socio-economic condition of the farmer. Under such a situation, cultivation of BNF (biological nitrogen fixation)-endowed Sugarcane varieties, as developed in Brazil, appear to be highly remunerative.

Contrary to our common believe that the legumes can only fix nitrogen in their root nodules, it has lately been well documented that non legume like Sugarcane and rice can also benefit from the atmospheric nitrogen fixed through some bacteria of the genera *Acetobacter* and *Herbaspirillum*. Two endophytic bacteria viz. *Acetobacter diazotrophicus* and *Herbaspirillum* spp. are major contributors of nitrogen fixation in Sugarcane. Such has been found within the roots and arial tissue of Sugarcane (Boddey *et al*., 1995; Ruschel and Vose, 1977; Dobereiner *et al*., 1993).

Recent discovery that some Sugarcane varieties can obtain very large contribution of BNF under field conditions and the existence of aboundant population of endophytic diazotrops (*Acetobacter diazotrophicus* and *Herbaspirillum* spp.) in this crop has opened a new and potential avenues of research that may greatly benefit the resources-poor cane growers in Bangladesh.

Since the experiment was aimed at substainly reducing/minimizing usage of N-bearing commercial fertilizers. It has negative impact on the environment, rather it should receive priority attention as an environment-friendly approach for increasing Sugarcane productivity. Development of Sugarcane variety with BNF-capability has great economic significance in a country like Bangladesh, where poor cane growers hardly apply the recommended dose of urea to Sugarcane.

Materials and Methods

The field testing was carried out at Bangladesh Sugarcane Research Institute (BSRI) farm, Ishurdi, Pabna, Bangladesh, in the cropping seasons 1999-2000 and 2000-2001. During the maturity stage any retention of green foliage indicates a plant's availability of nitrogen either from soil or other sources. Based on the above consideration, field evaluation was done for selection of 45 clones from different test stages and BSRI germplasm bank. These clones were planted in the field under zero-N input condition. Other inputs such as P and K were applied as TSP, MP @ 277 kg ha^{-1} and S as gypsum @ 185 kg ha^{-1}. Standard cultural practices were followed as and when required. Data were recorded on different growth parameters viz., germination (%) at 45 days after plantation (DAP), tiller number at 120 DAP, stalk elongation rate at June-July, visual foliage grading, number of millable cane and yield. Six clones viz., I 153/94, I 87/93, CO 846, I 197/93, Isd 28 and B 34-104 out of these 45 clones, showed better performance in first year (1999-2000) field testing.

So these 6 clones were selected as BNF-endowed clones for next year (2000-2001) field trail and planted under two

field conditions such as zero-N and 120 kg N ha^{-1}. In the second year, data were collected, as like as 1st year trial, but leaf nitrogen (N_2) content was determined at 120 and 200 DAP by using modified Kjeldahl method (Jackson, 1973). By this trail only four clones (I 153/94, Co846, B 34-104 and Isd 28) were selected as BNF-endowed clones.

Endophytic diazotrophs like *Acetobacter diazotrophicus* fix atmospheric N_2 in Sugarcane (Faente *et al*., 1993; Li and Macre, 1992). *Acetobacter diazotrophicus* was found to colonize extensively the exterior and interior of shoot and root (James *et al*., 1994). Based on these reports bacteria were tried to isolate in this research work, from the root extract and cane juice of these clones. For this work, root tip extract and cane juice were cultured aseptically on the bacterial culture medium and incubated at 26°C for 72 h. Both root extract and cane juice showed bacterial growth on the medium. The nature of the growing bacteria was tested as gram negative.

Data on different parameters were subjected to statistical analysis and mean values were compared using LSD at 5% level of significant (Gomez and Gomez, 1984).

Results and Discussion

In the first year field trail six clones, I 153/94, I 87/93, Co 846, I 197/93, Isd 28 and B 34-104 at zero-N input condition showed better performances (Table 1). After 45 days of plantation the highest germination (70.83%) was recorded from the clone Isd 28 followed by clone I 197/93 (61%), while the lowest germination (48.66%) was recorded from B 34-104. At 120 DAP the highest number of tiller ($87.30x10^3$ ha^{-1}) was recorded from clone I 153/94 while the lowest ($65.00x10^3$ ha^{-1}) from clone I 87/93. From the foliage grading, it was observed that clones which were better in tillering, showed deep green in foliage colour. Stalk elongation rate was higher (2.02 cm day^{-1}) in Co 846 followed by I 153/94 (1.76 cm day^{-1}) at June-July. The highest number ($75.22x10^3$ ha^{-1}) of millable cane was recorded from Isd 28 followed by I 153/94 ($72.00x10^3$ ha^{-1}) and the lowest ($62.30x10^3$ ha^{-1}) was recorded from I 87/93. After harvest, the highest cane yield (63.50 t ha^{-1}) was recorded in Isd 28 followed by Co 846 (62.70 t ha^{-1}) and the lowest yield (49.24 t ha^{-1}) showed by I 87/94. On the basis of these results Co 846, Isd 28, I 153/94 and B 34-104 were selected as possible BNF-endowed clones.

From the 2nd year field trail possible BNF-endowed clones (I 153/94, Co 846, B 34-104 and Isd 28) at zero-N and 120 kg N ha^{-1} input conditions showed that highest tiller number, $85.80x10^3$ ha^{-1} was recorded at zero-N by the clone Isd 28 while at 120 kg N ha^{-1}, it was $88.98x10^3$ ha^{-1}; followed by I 153/94 ($84.60x10^3$ ha^{-1} at zero-N and $85.50x10^3$ ha^{-1} at 120 kg N ha^{-1}). The lowest number of tiller, $78.90x10^3$ ha^{-1} at zero-N and $80.57x10^3$ ha^{-1} at 120 kg N ha^{-1} was recorded by the clone Co 846. Table 2 further indicated that the non BNF clone Isd 18 produced less ($50.35x10^3$ ha^{-1}) tiller, it was the lowest of all the test clones at zero-N, but at 120 kg N ha^{-1} it was the highest ($90.82x10^3$ ha^{-1}) obtained from the clone Isd 2/54 (non BNF). The possible BNF-endowed clones respond similarly in tiller production and showed less significant difference, but possible non BNF clones showed significant difference in tiller production at zero-N and 120 kg N ha^{-1} input conditions. (Table 2)

Millable cane indicated that the clone Isd 28 gave the highest number of millable cane of $69.20x10^3$ ha^{-1} at zero-

Table 1: Performance of BNF possible clones (1st field trail) at zero-N input condition

Varieties/ Clones	Germination (%)	Tiller '000' ha^{-1}	Millable cane 000 ha^{-1}	Elongation rate cm day^{-1} (June-July)	Visual grading	Yield (t ha^{-1})
I 87/93	51.25e	65.00e	62.30de	1.79b	**	49.24cd
Co 846	56.94cd	74.00c	63.80cd	2.02a	***	62.70a
I 197/93	61.00b	69.30cd	65.50c	1.48d	**	50.04c
Isd 28	70.83a	84.40ab	75.22a	1.68c	***	63.50a
B 34-104	48.66ef	68.00cd	62.40de	1.69c	***	53.44b
I 153/94	59.50bc	87.30a	72.00ab	1.76b	***	54.02b

1: Visual grading was clone on the basis of foliage colour, **: Normal green, ***: Deep green

Table 2: Performance of BNF-endowed clones at Zero-N input and 120 kg N ha^{-1} input condition

Varieties/Clones	Treatments	Number of tiller '000' ha^{-1}	Number of millable Cane '000' ha^{-1}	Yield (t ha^{-1})
I 153/94	Zero-N	84.60cd	67.75de	54.30fg
	120 kg N ha^{-1}	85.50bcd	66.00ef	59.72de
Co 846	Zero-N	78.90f	61.75g	61.48cd
	120 kg N ha^{-1}	80.57ef	62.10g	65.75b
B 34-104	Zero-N	83.70de	63.17g	53.69g
	120 kg N ha^{-1}	80.16ef	64.33fg	57.15ef
Isd 28	Zero-N	85.80bcd	69.20cd	63.97bc
	120 kg N ha^{-1}	88.98ab	71.22bc	70.59a
non BNF clone Isd 2/54	Zero-N	56.75g	47.50h	31.38h
	120kg N ha^{-1}	90.82a	77.19a	54.61fg
Isd 18	Zero-N	50.35h	39.50i	28.17i
	120kg N ha^{-1}	88.32abc	73.35b	61.58cd
Sem±		1.28	0.89	0.99
LSD (5%)		3.75	2.62	2.92

Table 3: Leaf nitrogen contents of Sugarcane clones grown under zero-N and 120 kg ha^{ha-1} N level

Clones	Sampling stage (DAP)	Leaf nitrogen content (%)	
		Zero-N	120 kg N ha^{-1}
I 153/94	120	1.85	1.91
	200	1.36	1.41
Co 846	120	1.61	1.68
	200	1.25	1.29
B 34-104	120	1.66	1.66
	200	1.31	1.26
Isd 28	120	1.54	1.57
	200	1.19	1.21
Isd 18	120	1.20	1.73
	200	0.68	1.32

N and 71.22x10^3 ha^{-1} at 120 kg N ha^{-1} input condition followed by the clone I 153/94 (67.7x10^3 ha^{-1} at zero-N and 66.00x10^3 ha^{-1} at 120 kg N ha^{-1}).

The lowest number of millable cane, 61.75x10^3 ha^{-1} at zero-N and 62.10x10^3 ha^{-1} at 120 kg N ha^{-1} shown by the clone Co 846, another BNF capable clone B 34-104 produced 63.17x10^3 ha^{-1} at zero-N and 64.33x10^3 ha^{-1} at 120 kg N ha^{-1} condition. The results showed less significant difference in millable stalks production at zero-N and 120 kg N ha^{-1} condition of BNF possible clones. In the same conditions Isd 2/54 (47.50x10^3 ha^{-1} at zero-N and 77.19x10^3 ha^{-1}at 120 kg N ha^{-1}) and Isd 18 (39.50x10^3 ha^{-1} at zero-N and 73.35x10^3 ha^{-1} at 120 kg N ha^{-1}), the non BNF clones showed highly significant difference to produce millable cane.

The clone Isd 28 gave the highest cane yield 63.97 t ha^{-1} at zero-N and 70.59 t ha^{-1} at 120 kg N ha^{-1}, while the clone B 34-104 produced the lowest cane yield 53.69 t ha^{-1} at zero-N and 57.15 t ha^{-1} at 120 kg N ha^{-1} input condition. (Table 2)

Second highest yield 61.48 t ha^{-1} at zero-N and 65.75 t ha^{-1} at 120 kg N ha^{-1} input condition from the clone Co 846. BNF possible another clone I 153/94 showed 54.30 t ha^{-1} cane yield at zero-N and 59.72 t ha^{-1} at 120 kg N ha^{-1} condition, respectively. The yield results of all the test clones showed significantly different at zero-N and at 120 kg N ha^{-1} condition. But these clones produced higher cane yield (53.56 – 63.97 t ha^{-1}) at zero-N input condition than the average cane yield (36 t ha^{-1} at non mill zone and 50 t ha^{-1} at mill zone area) of Bangladesh. From these, we assume that selected clones can fix/earn 120 kg N ha^{-1}. Boddey *et al*, (1991) reported that some of the Brazilian Sugarcane varieties could fix 150 kg ha^{-1} N fertilizer. Stanford and Ayres (1964) reported that a Sugarcane crop yielding 100 t cane ha^{-1} accumulates between 180 and 250 kg N ha^{-1}.

At two times (120 and 200 DAP) leaf nitrogen contents of these clones which grown under zero-N and 120 kg N ha^{ha-1} level showed that highest (1.85%) leaf nitrogen content at 120 DAP of clone I 153/94 but nitrogen content at 120 kg N ha^{ha-1} condition at the same times was 1.91%. But at 200 DAP, leaf nitrogen content was decreased, (1.36%) under zero-N and 1.41% at 120 kg N ha^{-1} condition. At 120 DAP of clone B 34-104 showed similar (1.66%) leaf nitrogen content at zero-N and 120 kg N ha^{-1} condition. It was 1.31% at zero-N and 1.26% at 120 kg N ha^{-1} at 200 DAP. Leaf nitrogen of all the clones were decreased from 120 DAP to 200 DAP. A non BNF possible clone Isd 18 at 120 DAP showed 1.20% leaf nitrogen at zero-N and 1.73% at 120 kg N ha^{-1}, here nitrogen percentage was highly different at zero-N to 120 kg N ha^{-1} condition than BNF possible clones. Leaf nitrogen of Isd 18 at 200 DAP was very low (0.68%) at zero-N but at 120 kg N ha^{-1}, it was 1.32% (Table 3).

Cane juice of 2 BNF possible clones (I 153/94 and Co 846) showed the bacterial growth on the culture medium, other 2 (Isd 28 and B 34-104) clones juice did not show bacterial growth (Table 4). Root tip extract of all BNF possible test clones showed bacterial activities on the culture medium. This findings agreed with the report of James *et al.* (1994). *Acetobacter diazotrophicus*, the nitrogen fixing bacterium of Sugarcane is a gram negative . In this study, the nature of growing bacterium was tested that these were gram

Table 4: Screening bacterial activities in the cultured medium by the culture of aseptic cane juice and root tip extract

Clones	Cane juice			Root tip extract		
	Presence of bacteria	Nature of bacteria		Presence of bacteria	Nature of bacteria	
		Gram positive	Gram negative		Gram positive	Gram negative
I 153/94	+	N	Y	+	N	Y
CO 846	+	N	Y	+	N	Y
B 34/104	-	-	-	+	N	Y
Isd 28	-	-	-	+	N	Y
Isd 2/54	-	-	-	-	-	-
Isd 18	-	-	-	-	-	-

+: Indicate presence of bacteria, -: Indicate absence of bacteria; N: no, Y: yes

1: Visual grading was done on the basis of foliage colour;

: Normal green; *: Deep green

negative but the bacterium was not identified. Both cane juice and root tip extract of non BNF clones (Isd 2/54 and Isd 18) did not show any bacterial activities on the medium indicated that these 2 clones were unable to fix atmospheric nitrogen.

It could be concluded that the selected clones have some characteristics to fix nitrogen from the atmosphere. It may be alternative strategies for nitrogen supplementation of Sugarcane cultivation and can reduce the cost of input for N_2 use.

References

Anonymous, 1996. Benchmark survey of Sugarcane, final report. Department of agriculture extension, cash crop wing, Khamarbari, Dhaka, Bangladesh, pp: 5-41.

Boddey, R.M., S. Urquiaga, V.M. Reis and J. Dobereiner, 1991. Biological Nitrogen Fixation associated with Sugarcane. Pl. Soil, 137: 111-117.

Boddey, R.M., O.C. de Oliveira, S. Urquiaga, V.M. Reis, F.L.de Oliveres, V.L.D. Baldani and J. Dobereiner, 1995. Biological nitrogen fixation associated with Sugarcane and rice: Contribution and prospects for improvement. Pl. Soil, 174: 195-209.

Dobereiner, J., V.M. Reis, F.L. de Olivares, F.B.D.R. Junios and R. M. Boddey, 1993 Elimination of N fertilizer for Sugarcane in Brazillian N_2-fixing cane genotypes: The key to a High Energy Balance for Biofuel Production. Proc. Inter. Am. Sug. Cane Sem. Soc., pp: 209.

Faente R.L. E., J.T. Salgado, I.R.A. Ocampo and J.C. Mellado, 1993. *Acetobacter diazotrophicus*, an idolacetic acid-producing bacterium isolated from Sugarcane cultivars in Mexico. Pl. Soil, 154: 145-150.

Gomez, K.A. and A.A. Gomez, 1984. Statical procedures for agricultural research (2nd ed), A wiley interscience publication, John eiley and sons, NY, USA.

Jackson, M.L., 1973. Soil chemical analysis. Prentice Hall of India, P.R. Ltd., New Delhi, India.

James, E.K., V.M. Reis, F.L. Olivares, J.I. Baladani and J. Dobereiner, 1994. Infection of Sugarcane by the nitrogen-fixing bacterium *Acetobacter diazotrophicus*. J. Exp. Bot., 45: 757-766.

Li, R. and I.C. Macre, 1992. Specific identification and enumeration of *Acetobacter diazotrophicus* in Sugarcane Soil Biol. Biochem., 24: 413-419.

Ruschel, A.P. and P.B. Vose, 1977. Present situation coucesning studies on associative nitrogen fixation in Sugarcane, CENA Boletin Cinetifico BC-045, Piracicab, Brasil, pp: 27.

Stanford, G. and A. Ayres, 1964. The internal nitrogen requirement of Sugarcane. Soil Sci., 98: 338-344.