Sugar Tech 7(2&3) (2005) : 28-31

Response of Sugarcane Varieties to Application of Nitrogen Fixing Bacteria under Different Nitrogen Levels

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ABSTRACT

A field study was undertaken to evaluate the response of sugarcane varieties to application of *Azotobacter, Azospirillum* and *Gluconacetobacter* under different levels of fertilizer nitrogen. *Azospirillum* significantly improved the cane and sugar yield compared to *Gluconacetobacter, Azotobacter* and uninoculated control. *Gluconacetobacter* and *Azotobacter* were on par. *Gluconacetobacter* was better than uninoculated control. There was differential response of varieties to Biofertilizers for cane yield and CCS per cent. Co 8014, Co 8122, Co 8021 and Co 6304 responded in terms of cane yield to *Azospirillum* while Co 8021 responded to *Gluconacetobacter* and Co 6304 responded to *Azotobacter.* The response to *Azospirillum* in terms of cane yield was more under lower nitrogen level (200 kg ha^{-1}) compared to normal nitrogen level (300 kg ha^{-1}). Co 8122 showed a significant improvement in CCS per cent for *Azotobacter, Azospirillum* and *Gluconacetobacter*, while CoC 85061 only to *Azospirillum.* Varieties Co 8014, Co 8021 and Co 6304 did not show any improvement in CCS percent for biofertilizers. Biofertilizers significantly improved the nitrogen content of stem at 200 and 300 kg N ha^{-1} compared to control. Biofertilizers did not influence the soil available nitrogen status.

Key Words: sugarcane, biofertilizer, *Azotobacter, Azospirillum* and *Gluconacetobacter,* nitrogen fixing bacteria

INTRODUCTION

Integrated nutrient supply system is the need of the hour, involving a judicious combination of organic, inorganic and biofertilizers for sustainable crop production. Biofertilizers play an important role in achieving this goal in an ecofriendly manner by fixing nitrogen, improving the crop growth by production of growth promoting chemicals and improving the nutrient uptake of the crops. Association of several bacterial genera and high nitrogenase activity in sugarcane crop has been reported (Boddey and Dobereiner, 1995). Among several beneficial bacterial genera reported with sugarcane, *Azotobacter* and *Azospirillum* have been widely used as biofertilizers for sugarcane. Association of endophytic nitrogen fixing bacteria *Gluconacetobacter diazotrophicus* (formally known as *Acetobacter diazotrophicus*) with sugarcane has also been reported by Cavalcante and Dobereiner (1988).

To get better benefit from biofertilizer application, it is essential that the bacterial culture be used in combination with suitable level of fertilizer. Yield improvement and N fertilizer economy for application of different biofertilizers *viz. Azotobacter, Azospirillum* or *Gluconacetobacter* have been reported by many workers (Patil and Hapase, 1981; Srinivasan and Naidu, 1987; Muthukumarasamy *et al.*, 1994). These organisms were evaluated independently in different environments. Because of this, it is not possible to grade their efficacy for making valid recommendation to sugarcane production. Hence, the present field study was undertaken to compare them in the same environment.

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MATERIALS AND METHODS

Field experiment was conducted at the Sugarcane Breeding Institute, Coimbatore during 1995 - 98 to study the response of sugarcane varieties to biofertilizer application. The experiment was conducted in a sandy loam soil of medium fertility (initial available N, P and K of 86, 5.3 and 189 mg kg^{-1}, respectively). The area normally receives about 650 mm of rainfall in about 50 rainy days (maximum during the North East monsoon period, Oct-Dec) and the temperature conditions are moderate with monthly mean maximum temperature ranging from 28.2° to 36.8°C and minimum temperature from 16.5° to 26.5° C with average sunshine of 8.6 hours.

The trial was laid out in factorial randomized blocks design. The plot size was 5.4 m x 6.0 m (6 rows of 6.0 m spaced 0.9 m apart) for the plant crop. The treatments consisted of five sugarcane varieties - Co 8021, Co 8122, Co 8014, Co 6304 and CoC 85061, three levels of N (0, 200 and 300 kg N ha^{-1}) and

three biofertilizers, *Azotobacter chroococcum, Azospirillum brasilense* and *Gluconacetobacter diazotrophicus,* with uninoculated control. Nitrogen was applied as urea in two equal splits as per the treatments at 45 and 90 days after planting as urea. *Azospirillum* and *Azotobacter* were obtained from Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore and *Gluconacetobacter diazotrophicus* from Late Dr. Dobereiner, EMBRAPA, Brazil. The bacterial cultures were mass multiplied in a suitable medium and lignite based biofertilizer containing bacterial population above 10^8 colony forming units g^{-1} were prepared. Ten kg of biofertilizer per hectare was used for field application. Biofertilizer was applied in two equal splits, at 30 and 60 days after planting. The biofertilizer was thoroughly mixed with 500 kg powdered farmyard manure and applied uniformly in the furrow near the base of the sugarcane clumps. Same quantity of farmyard manure was applied in the control treatments. The biofertilizer was covered with soil by light earthing up followed by irrigation. Crop was managed by adopting the standard package of practices with a seed rate of 60,000 two budded setts ha^{-1}, single super phosphate to supply 75 kg P_2O_5 ha^{-1} and muriate of potash 112 kg K_2O ha^{-1} applied as a basal dose at the time of planting.

Cane yield data was recorded at the age of twelve months from the plots and were converted to yield per ha. For juice analysis, juice samples were drawn from 10 canes cut at random in each plot. Brix and sucrose per cent were determined by following standard procedures (Meade and Chen, 1977). From the values of Brix and sucrose, commercial cane sugar per cent (CCS %) was calculated as CCS (%) = 1.022 S - 0.292 B, where S and B are sucrose and brix per cent respectively in the juice. From cane yield and CCS per cent data, sugar yield was calculated. Soil samples (0-30 cm) were collected from ten spots at random from each experimental plot and a composite sample of each plot was used for the estimation of soil available nitrogen (Piper, 1950). Plant samples were collected at the time of harvest by randomly collecting 10 canes per plot. The samples were processed separately for leaf, stem and sheath. The plant samples were dried in oven at 80° C powdered and used for estimation of total nitrogen content (Piper, 1950).

RESULTS AND DISCUSSION

Biofertilizer application on cane yield and quality of sugarcane varieties

Improvement of mean cane yield over N levels was observed due to biofertilizer application. *Azospirillum* significantly improved the cane and sugar yield followed by *Gluconacetobacter* compared to uninoculated control (Table 1). *Gluconacetobacter* improved the cane yield significantly than control but was on par with *Azotobacter.* Michaelraj *et al.,* (1984) compared the effect of *Azospirillum* and *Azotobacter* and reported that soil application of *Azospirillum* was better in improving the cane yield than *Azotobacter.* But Muthukumarasamy *et al.,* (1994) has reported that the response of sugarcane in terms of cane yield was better for *Gluconacetobacter* application than *Azospirillum.* Present study indicated that *Azospirillum* improved the cane yield more efficiently than *Gluconacetobacter* and *Azotobacter* and over the uninoculated control. *Gluconacetobacter* has been reported as an efficient N_2 fixing bacterium for sugarcane, but there were problems associated with this bacterium for colonization under traditional inoculation methods (James *et al.,* 1994), poor survival in soil (Boddey and Dobereiner, 1995) and has pH optimum of 4.5 to 5.5 (Cavalcante and Dobereiner, 1988). These reasons could have contributed to the poor response of sugarcane varieties to *Gluconacetobacter* application. Requirement of organic matter has been reported for better survival of *Azotobacter* (Gaur and Mathur, 1966). Singh *et al.,* (1985) reported that cane yield improvement due to application of *Azotobacter* was better when applied with compost. The better results for *Azospirillum* may be attributed to the associative symbiotic nature of *Azospirillum,* better survival and efficient colonization in wide environmental conditions.

The possibility that varieties respond differently to biofertilizer inoculation has been demonstrated in sugarcane (Ruschel and Ruschel, 1977 and Srinivasan and Naidu 1987). Present study also revealed the presence of interaction between sugarcane varieties and the type of biofertilizer.

Table 1. Response of sugarcane varieties to application of biofertilizers on cane yield

Sl. No.	Varieties	Cane yield (t ha^{-1})				
		Control	*Azotobacter*	*Azospirillum*	*Glucon-acetobacter*	Mean
1.	Co 8014	91.6	97.1	116.8	91.1	99.2
2.	Co 8122	85.5	84.4	101.2	86.8	89.5
3.	Co 8021	94.1	106.8	112.1	101.2	103.6
4.	Co 6304	100.8	99.8	112.7	115.2	107.2
5.	CoC 85061	110.9	104.4	115.4	113.3	111.0
Mean		96.6	98.5	111.7	101.5	102.1

CD at 5% level of significance Biofertilizer: 4.7, Variety : 5.3, Biofertilizer xVariety : 10.6

Table 2. Response of sugarcane varieties to application of biofertilizers on juice CCS per cent

Sl. No.	Varieties	CCS per cent				
		Control	*Azotobacter*	*Azospirillum*	*Gluconacetobacter*	Mean
1.	Co 8014	11.85	11.59	11.22	11.14	11.45
2.	Co 8122	11.81	12.38	12.30	12.54	12.26
3.	Co 8021	11.82	12.06	11.88	11.89	11.91
4.	Co 6304	11.24	11.54	11.05	10.64	11.12
5.	CoC 85061	11.64	12.15	12.45	12.01	11.06
	Mean	11.67	11.94	11.78	11.64	11.76

CD at 5% level of significance Biofertilizer: NS, Variety: 0.29, Biofertilizer x Variety: 0.57

Table 3 Response of sugarcane varieties to application of biofertilizers on sugar yield

Sl. No.	Varieties	Biofertilizers				
		Control	*Azotobacter*	*Azospirillum*	*Gluconacetobacter*	Mean
1.	Co 8014	10.8	11.2	13.1	10.1	11.3
2.	Co 8122	10.0	10.4	12.5	10.9	11.0
3.	Co 8021	11.1	12.8	13.3	12.2	12.3
4.	Co 6304	11.3	11.4	12.4	12.2	11.8
5.	CoC 85061	12.9	12.6	14.3	13.5	13.3
	Mean	11.2	11.7	13.1	11.8	11.9

CD at 5% level of significance Biofertilizer: 0.6 Variety: 0.6 Biofertilizer x Variety: NS

Varieties Co 8014, Co 8122, Co 8021 and Co 6304 recorded higher cane yield response to *Azospirillum*, while Co 8021 also responded to *Gluconacetobacter.*

Misra and Naidu, (1990) reported that soil application of *Azospirillum* and *Azotobacter,* has no influence on juice quality, while Muthukumarasamy and Revathi (1999) reported improvement in juice quality due to biofertilizers. But, the results of the present experiment indicate that *Azotobacter, Azospirillum* and *Gluconacetobacter* showed significant interaction between varieties and the type of biofertilizer over N levels for CCS per cent (Table 2). Co 8122 showed a significant improvement in CCS per cent for *Azotobacter, Azospirillum* and *Gluconacetobacter*, while CoC 85061 only to *Azospirillum.* Varieties Co 8014, Co 8021 and Co 6304 did not show any improvement in CCS percent for biofertilizers. Sugar yield was significantly improved by the *Azospirillum* compared to *Azotobacter, Gluconacetobacter* and uninoculated control (Table 3). Though significant interaction between biofertilizers and varieties was not observed for sugar yield, all the varieties have recorded higher sugar yield for *Azospirillum.* This is due to the higher cane yield response of varieties to *Azospirillum.*

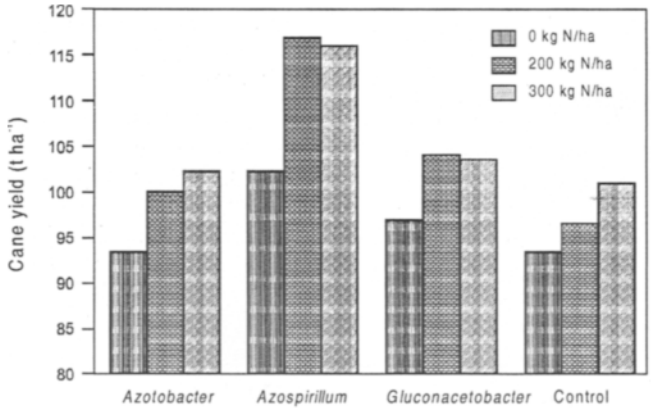

Fig. 1. Effect of different doses of fertilizer nitrogen and biofertilizers on cane yield

Fertilizer level and biofertilizer on sugarcane yield and quality

It has been reported that the cane yield response of sugarcane varieties was more pronounced at lower fertilizer N-doses (Patil and Hapase, 1981; Misra and Naidu, 1990). Present study also indicated a better cane yield response to biofertilizer with fertilizer application than without fertilizer application. Highest cane yield (116.7 t ha^{-1}) was recorded to *Azospirillum* at 200 kg N ha^{-1}, which was on par to the cane yield (116.0 kg t ha^{-1}) at 300 kg N ha^{-1} (Fig 1). The cane yield response to biofertilizers at 200 kg N ha^{-1} and 300 kg N ha^{-1} were on par.

Biofertilizer application on plant and soil available nitrogen content

Biofertilizer and N levels has shown significant interaction for leaf, sheath and stem nitrogen content. But the influence was more pronounced for biofertilizer application in stem than sheath and leaf at 200 and 300 kg N ha^{-1} compared to 0 kg N ha^{-1} (Fig. 2.). Maximum leaf nitrogen content was observed in sugarcane applied with *Azospirillum* at 200 kg N ha^{-1}. *Azospirillum* is known for its growth promoting effect on root in terms of profuse root hairs and higher root biomass

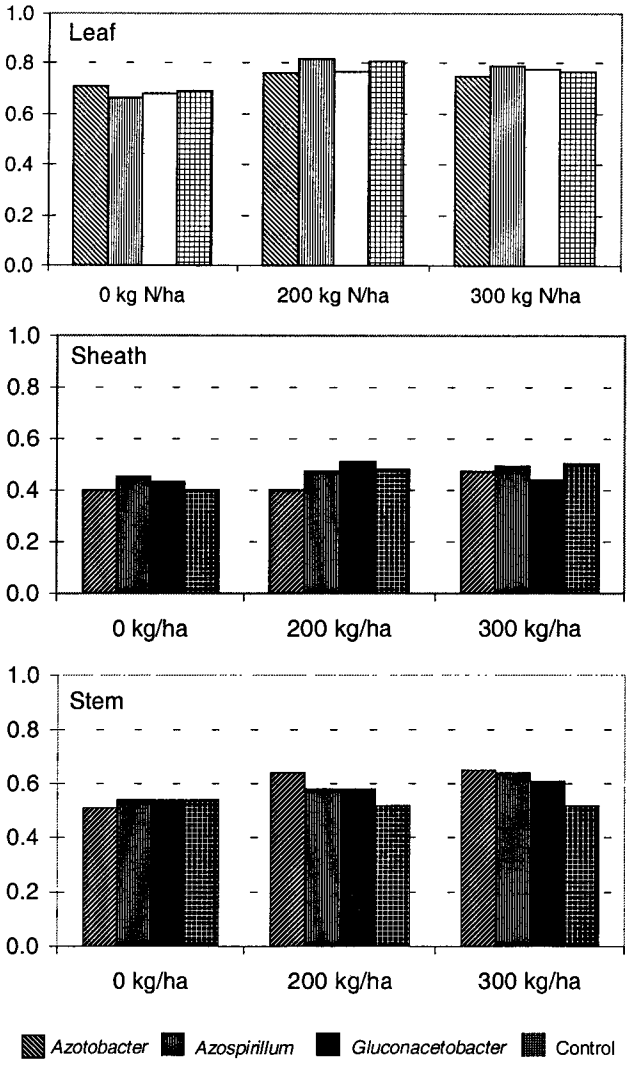

Fig. 2. Nitrogen content of sugarcane in biofertilizer and nitrogen treatments (CD at 5% : Leaf: 0.06, Sheath: 0.05, Stem: 0.03)

(Dobbelaere *et al.*, 2003). This would have enhanced higher uptake of applied nitrogen. Muthukumarasamy and Revathi (1999) reported that application of *Gluconacetobacter* individually and in combination with VAM significantly increased the leaf N content. Presence of differences among sugarcane varieties in their capacity to utilize the biologically fixed nitrogen has been reported by Lima *et al.*, (1987)

Misra and Naidu (1990) reported that the soil available nitrogen content was not significantly improved by biofertilizer application. Similar results were also observed in the present study (data not given).

CONCLUSION

Among the three biofertilizers *viz.*, *Azotobacter*, *Azospirillum* and *Gluconacetobacter* evaluated, soil application of *Azospirillum* at the rate of 10 kg ha^{-1} in two equal splits at 30 and 60 days after planting with 200 kg ha^{-1} of nitrogenous fertilizer was found advantageous in terms of higher cane yield and reduction in fertilizer nitrogen by 100 kg ha^{-1}. Soil available nitrogen content was not improved by biofertilizer application.

ACKNOWLEDGEMENT

The authors are thankful to the Director, Sugarcane Breeding Institute, Coimbatore for providing facilities to conduct this study.

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