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Performance of Some Sugarcane Genotypes under N-Stressed Field Condition

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ABSTRACT

A field experiment was conducted at Bangladesh Sugarcane Research Institute farm, Ishurdi during 2007-08 to identify and screen out sugarcane clones endowed with biological nitrogen fixation (BNF) in absence of nitrogenous fertilizer. Ten promising sugarcane genotypes viz., B 34-104 , Isd 28, Isd 32, Isd 35, Isd 37, CO 846, I 95-01, I 112-01, I 53-94 and I 486-99 were used as treatments in the study. Results revealed that all the growth parameters except pol per cent cane were influenced significantly in different genotypes grown without N-fertilizer. In respect of yield, they followed the trend: Clone B 34-104> CO 846> Isd 37> Isd 32> I 95-01> I 486-99>I 112-01>Isd 28> I 53-94> Isd 35. Among the genotypes, Clone B 34-104 showed higher yield of 75.91 TCH having no urea fertilizer, which might have the ability to fix atmospheric nitrogen through BNF with that gram negative bacteria. However, further comprehensive microbiological studies are required to identify those bacteria and results.

Key words: Performance, sugarcane genotype, N-stressed, field condition

INTRODUCTION

Sugarcane is a long duration exhaustive crop. It depletes considerable amount of nutrients from soil causing soil degradation. Sustainable sugarcane production involves the successful management of resources by maintaining the quality of the environment and conserving natural resources. Plant nutrients in soil, whether naturally endowed or artificially maintained, are a major determinant of the success or failure of a crop production system. Sugarcane is grown well in the soils having sufficient level of organic matter and available nutrients (Islam *et al.*,1998). But the organic matter content in Bangladesh soil is far below (~1-2%) than crop requirement and it is depleting day by day. Parthasarathy (1972) reported that if a field receives adequate or optimum quantities of organic matter, such fields rarely deteriorate in crop productivity. The uses with soil conditioner of blended organic and inorganic mineral nutrients, and integrated nutrient management of organic and inorganic fertilizers demonstrated sustainable sugarcane yields in Old Himalayan Piedmont Plain soils of Bangladesh (Paul *et al.*, 1999, 2004, and 2005a,b). Nitrogen is the most limiting nutrient for increasing sugarcane productivity. Biological nitrogen fixation (BNF) helps in improving soil fertility by using nitrogen, which is in abundance in the atmosphere (~ 79%). It is reported that several Brazilian sugarcane varieties are capable of obtaining over 60% of their nitrogen (>150 kg N ha^{-1} $year^{-1}$) from BNF through free-living and associative bacteria in sugarcane (Boddey *et al.*, 1987). Therefore, an attempt was necessary to undertake a comprehensive study on the contribution of BNF in appropriate sugarcane genotypes that can make an ecological and economic break through in sustainable sugarcane production in Bangladesh. Thus, the study was undertaken to identify and screen out suitable sugarcane genotypes favored with biological N_2-fixing system with the bacteria under N-stressed field condition.

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MATERIALS AND METHODS

A field experiment was conducted at Bangladesh Sugarcane Research Institute farm, Ishurdi, Pabna during 2007-08 cropping season with a view to screen out promising sugarcane clones that might have biological nitrogen fixation (BNF) capacity under N-stressed field condition without N-fertilizer. It was laid out in RCBD with three replications. Ten promising sugarcane genotypes viz., B 34-104 , Isd 28, Isd 32, Isd 35, Isd 37, CO 846, I 95-01, I 112-01, I 53-94 and I 486-99 were taken under observation. The plot size was 6m x 8m. Three-budded sugarcane setts were planted during November' 2007 in traditional method and harvested in December' 2008 after full maturity of the crop. All other recommended intercultural practices were followed as scheduled. All amounts of P from triple super phosphate, S from gypsum and Zn from zinc sulphate were applied as basal. One third of K from muriate of potash was also applied as basal. The rest two splits of K were applied at 120 and 160 days after planting as top dressing. Culture, isolation and characterization of the diazotrophic bacteria sampled from the stem and root of sugarcane genotypes, and rhizosphere soils of the experiment were carried out at soil microbiological laboratory, Bangladesh Institute of Nuclear Agriculture, Mymensingh by using appropriate selective growing media.

Isolation of microorganism from sugarcane rhizosphere soils

Rhizosphere soil around the sugarcane clones of the experiment was collected. All impurities were removed from those soil samples and smashed the soil particles. 10 g soils were put into a 250 ml conical flask with 90 ml sterile water. Mixture was vortex for 2 to 3 minutes. It was kept 1ml suspension in a vial containing 9 ml water. Then 1ml dilution was taken from the vial and it was transferred to the next vial containing 9 ml water and the mixture was vortex. It was continued up to 10^{-7} dilution following serial dilution procedure. From that dilution 0.1ml solution was taken in different LB plate. It was spread with the help of spreader.

Isolation of microorganism from the roots of sugarcane

Roots from the sugarcane clones were collected. 1g healthy roots were weighed and washed with distilled water to remove the surface particles. Then it was washed by 3% H_2O_2 for 3 minutes for surface sterilization. Then the roots were washed up to 6-7 times with sterile water. The sample was at maceration by using motor and pestle with 99 ml sterile water. Mixture was vortex for 2 to 3 minutes. Then, it was followed the steps for the isolation of microorganism like as rhizosphere soils. It was allowed that incubations at $25^0C \pm 1^0C$, except when mentioned otherwise. The shaker was used for broth culture.

Purification of Culture and Cloning to Single Colony Type

A loopful of inoculums was suspended in dispersion buffer and it was vortexes. The suspension was streaked on LB plate to obtain discrete colonies. In another plate, 0.1 ml suspension of inoculums was placed and was spread all over the surface of the medium using a sterile glass spatula. The plate was incubated for 5 to 14 days. It was observed the colonies and recorded the different form of colonies. For getting typical colony, which were majority, repeated the steps of 1 to till 4 for consistent morphology. The growth from a discrete type of colony was transferred for showing its consistency and its morphology. It was incubated till sufficient growth and stored in a refrigerator for characterization of microbial culture.

Growth in broth culture

The broth medium of LB was made for growth of microbial culture. A loopful of growth from young culture was mixed with liquid LB media of 100 ml in 250 ml conical flask covered with cotton plug. For successful growth, it was kept on shaker for gently shaking.

Cultural and biochemical test of microorganism Cultural test

The strains were streaked and were spread on CRYEMA plates. When single colony was formed a typical colony was re-streaked on CRYEMA plates again and again, so that the single colony was isolated as contamination free pure culture.

Biochemical test

a) BTB- YEMA test was carried out in the laboratory

By this test, acid production and the rate of acid production was observed. The strain was being tested whether it produced acid or alkali.

b) Salinity tolerance test

For this test, different LB media were prepared to change the rate of NaCl at 0.1% NaCl/l, 0.5% NaCl/l, 1% NaCl/l, 3% NaCl/l, 5% NaCl/l, 8% NaCl/l and were recorded.

Microscopic observations of pure culture

To know the cell shape, size, arrangement and color reactions, simple staining and gram staining were done. Characterization of microbial culture is presented in Table 4.
Data on number of tillers, millable canes, height, girth and weight of cane, and brix per cent in cane were recorded. All these data interpretations were done on the basis of statistical analysis and were presented in Table 1. Initial and post harvest soil nutrient statuses and nutrient contents in leaf were shown in Table 2 and 3, respectively. Means of treatments were compared by LSD and DNMR test statistically using 'Analysis of Variance' technique (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Data on different growth parameter viz., germination, tiller, millable canes, yield, height, girth and pol per cent cane are presented in Table 1. It was found that all the growth parameters responded significantly in N-stressed condition. The highest germination of 47.74 % was recorded from lsd 37 followed by B 34-104 (45.93 %). The highest tiller and millable cane of 214.20 x 10^3 and 112.00 $\times 10^3$ ha^{-1} were recorded from B 34-104, respectively. The highest yield of 75.91 TCH was also recorded from B 34-104 followed by Co 846 (69. TCH) receiving no N-fertilizer. All the varieties and clones in N-stressed condition showed no significant effect on pol per cent in cane. It revealed that some sugarcane genotypes are capable of obtaining over 150 kg N ha^{-1} $year^{-1}$ from biological nitrogen fixation (BNF) through free-living and associative bacteria (Boddey *et al.*, 1991). The present results were supported by many researchers in their studies where some sugarcane clones had BNF capacity due to the presence of free living and associative bacteria like *Acetobacter diazotrophicus*, *Azospirillum spp*, *Azotobacter spp*, *Agrobacterium diazotrophicus*, *Herbaspirillum seropedica*, *Bacillus spp*, *Derxia spp*, etc. and improve soil fertility workers (Dobereiner, 1988; Boddey *et al.*, 2003; Wang *et al.*, 2006; Xing *et al.*, 2006). It was observed from Table 2 that a little of soil N % at 120 DAP and at post harvest were decreased in all treatments under N-stressed condition. Table 3

further shows that the highest leaf-N of 0.78% was found in Isd 35 followed by Isd 37 with 0.74% N content in leaf of sugarcane at maturity. From the in vitro observation and study results shown in Table 4, the associative bacteria were found as gram negative.

From the study results in field, it may be concluded that the clone B 34-104 showed superiority in respect of cane yield over all other clones in absence of nitrogenous fertilizer. Again from the observation and study results in laboratory the associative microorganisms were found as gram negative bacteria. Thus it could be inferred that the said clone may have the capacity to fix atmospheric nitrogen with bacteria belongs to gram negative world i.e. may be *Azotobacter, Acetobacter, Azosperillum* and others, however, that needs further specific microbiological studies for the confirmation.

Table1. Performances of sugarcane genotypes under N-stressed field condition without N-fertilizer.

Treatments	Germination (%)	Tiller ($\times 10^3$ ha^{-1})	Millable cane ($\times 10^3$ ha^{-1})	Yield (TCH)	Height (m)	Girth (cm)	Pol % Cane
V_1=B 34-104	45.93 a	214.20 a	112.00 a	75.91 a	2.83 ab	1.77 ef	10.95
V_2 = Isd 28	36.88 ab	119.10 b	71.25 cd	42.49 ef	2.35 cde	1.94 bcde	11.43
V_3= Isd 32	38.22 ab	103.50 bc	61.46 d	62.20 c	2.49 bcd	2.11 ab	11.06
V_4= Isd 35	32.51 bc	116.00 bc	84.03 b	36.42 f	2.19 de	1.60 f	10.59
V_5= Isd 37	47.78 a	97.29 bc	77.29 bc	63.79 bc	2.68 bc	2.21 a	11.10
V_6= CO 846	21.79 c	87.57 bc	61.74 d	69.72 ab	3.06 a	1.90 cdef	10.92
V_7= I 95-01	40.94 ab	85.91 c	71.32 cd	52.80 d	2.32 cde	1.74 f	11.02
V_8= I 112-01	40.79 ab	87.08 c	61.25 d	44.93 e	2.09 ef	2.07 abc	10.98
V_9= I 53-94	22.02 c	90.07 bc	64.79 d	36.98 f	1.75 f	1.98 bcd	11.82
V_{10}= I 486-99	36.83 ab	93.75 bc	68.95 cd	49.22 de	2.37 cde	1.83 def	11.83
LSD (0.05)	11.28	31.73	11.88	7.47	0.36	0.17	NS

In a column, the figures having same letter do not differ significantly as per DMRT at 5% level.

Table2. Initial and post harvest N-statuses in soils of the experiment.

Treatments	N %		
	Initial Soil	at 120 days	Post Harvest Soil
V_1=B 34-104	0.050	0.043	0.034
V_2 = Isd 28	0.060	0.050	0.043
V_3= Isd 32	0.054	0.050	0.047
V_4= Isd 35	0.070	0.060	0.043
V_5= Isd 37	0.050	0.045	0.043
V_6= CO 846	0.060	0.048	0.038
V_7= I 95-01	0.060	0.047	0.038
V_8= I 112-01	0.060	0.045	0.037
V_9= I 53-94	0.050	0.044	0.037
V_{10}= I 486-99	0.050	0.045	0.043

Table3. Per cent Nitrogen contents in the leaves of sugarcane at maturity phase.

Treatment	N (%)
V_1 = B 34-104	0.38
V_2 = Isd 28	0.52
V_3 = Isd 32	0.51
V_4 = Isd 35	0.78
V_5 = Isd 37	0.74
V_6 = CO 846	0.69
V_7 = I 95-01	0.57
V_8 = I 112-01	0.62
V_9 = I 53-94	0.71
V_{10} = I 486-99	0.49

Table4. Some morphological colony characters of the bacteria isolated from rhizosphere soils and roots of sugarcane.

Strains	Size (mm)	Shape	Color	Opacity	Surface	Margin	Odor	Consistency	Elevation
$V_{15}Rp_{5-3}$	2.50	Round	Off-white	Opaque	Smooth	Smooth	Odorless	Soft, less sticky	Raised
$V_{15}Rp_{5-5}$	2.00	Round	Off-white	Opaque	Smooth	Smooth	Odorless	Soft, less sticky	Raised
$V_{15}Rp_{5-2}$	2.70	Round	Off-white	Opaque	Smooth	Smooth	Odorless	Soft, less sticky	Raised
$V_{15}Rp_{5-1}$	2.50	Round	Off-white	Opaque	Smooth	Smooth	Odorless	Soft, less sticky	Raised
$V_{15}Rt_1$	3.00	Oval	Off-white	Opaque	Smooth	Rough	Odorless	Soft, less sticky	Flat
$V_{15}Rt_3$	2.50	Round	Yellowish	transparent	Smooth	Smooth	Odorless	Soft, less sticky	Flat
$V_{15}Rt_4$	4.00	Round to oval	Off-white	Opaque	Smooth	Rough	Odorless	Soft, less sticky	Flat
$V_{15}Rt_5$	3.50	Round	Yellowish	transparent	Smooth	Smooth	Odorless	Soft, less sticky	Flat
$V_{15}Rt_6$	3.50	Round	Yellowish	transparent	Smooth	Smooth	Odorless	Soft, less sticky	Flat

V_{15} = B 34-104, Rp_{5-3} =Rhizosphere soil, dilution 10^{-5}, colony 3, Rt_2 = Root, dilution 10^{-2}

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