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# Response of Sugarcane Varieties to Nitrogen and Phosphorus as Inoculated by *Gluconacetobacter diazotrophicus* and PSB

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***Authors' contributions***

*This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.*

***Original Research Article***

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## ABSTRACT

**Aims:** To study the growth, yield and quality in two sugarcane varieties inoculated with *Gluconacetobacter diazotrophicus* and phosphate solubilizing bacteria in normal, sodic and saline-sodic soils.
**Study Design:** 6 x 2 x 2 factorial completely randomized design (FCRD).
**Place and Duration of Study:** Central Sugarcane Research Station, Padegaon - 415 521, Tal.- Phaltan, Dist.- Satara, Maharashtra State, India between 2012 and 2013.
**Methodology:** A pot experiment was conducted in different problematic soils. Two sugarcane varieties *viz*., Co 86032 and Phule 265 were used. Six soil samples (1 normal, 3 sodic and 2 saline-sodic soils) were selected for the study. The single eye bud sets were inoculated with *G. diazotrophicus* 10 kg + PSB 1.25 kg in 100 lit water/ha for 30 min before planting and the pots planted with such treated sets received 50% recommended N (125 kg/ha) and 75% recommended $P_2O_5$ (86.25 kg/ha). The corresponding control pots planted with the untreated bud sets received 100% recommended N (250 kg/ha) and $P_2O_5$ (115 kg/ha). The initial and final soil properties and the effect on growth, yield and quality of sugarcane were studied. The population of *G. diazotrophicus* in cane juice and that of PSB in rhizosphere soil was determined at 9 months and maturity.
**Results:** The results indicated that the treatment of sugarcane bud sets with *G. diazotrophicus* 10 kg + PSB 1.25 kg in 100 lit water/ha for 30 min. coupled with 50% recommended N and 75% recommended P significantly improved the growth, yield and

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quality. The overall performance of the varieties was best in the normal soil (S-6) followed by S-4 (Sodic) and S-1 (Sodic). The inoculation of sugarcane sets showed the best results in normal soil (S-6) closely followed by S-4 (Sodic) and S-1 (Sodic) indicating better performance of inoculation in these problematic soils. The higher population and activity of *G. diazotrophicus* and PSB was observed at 50% recommended N and 75% recommended P in both the varieties.

**Conclusion:** The pre-planting bud set treatment in sugarcane with *G. diazotrophicus* + PSB coupled with 50% recommended N and 75% recommended P significantly improved the growth, yield and quality of cane juice. Bud set treatment showed the best results in normal soil closely followed by sodic soils indicating better performance of inoculants in these problematic soils.

*Keywords: Sugarcane; Gluconacetobacter diazotrophicus; PSB; nitrogen; phosphorus.*

## 1. INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is an exhaustive crop that can uptake great amount of soil nutrients for its biomass production. In addition to micronutrients exportation, about 65 kg N, 90 kg $P_2O_5$ and 170 kg $K_2O$ are taken up for a target yield of 50 t $ha^{-1}$ [1]. The use efficiency of applied N fertilizers in sugarcane applied with recommended dose of N in the range of 250 to 400 kg $ha^{-1}$ is only 20-30% and hence at every harvest of the crop, soil suffers a net loss of 50-100 kg N/ha. Similarly, out of the total phosphorus fertilizers applied to the crop, only 15-20% can be used, the rest is fixed in the soil as phosphates of Ca, Al or Fe depending on the soil reaction.

A permanent manurial trial, conducted for 33 years at Anakpalle (Andhra Pradesh), revealed that sugarcane crop without addition of fertilizers yielded about 40 t $ha^{-1}$ of cane annually. The soil nitrogen reserve under this crop, however, increased by 50% of the initial value which clearly indicated that the root associated diazotrophs contribute significant quantity of nitrogen for sustaining the production of sugarcane [2].

Inoculation of N fixing microbes to sugarcane have increased the cane yield by 5-15%, saved 25 kg fertilizer N $ha^{-1}$ and also improved the juice quality parameter *viz*; sucrose and purity [3-5]. *Gluconacetobacter diazotrophicus* is a nitrogen fixing bacterium highly specific to sugar-rich crops like sugarcane, sweet potato, pineapple, sugarbeet, etc. It was found to occur in the roots, stems, leaves [6-8], rhizosphere soil and even in cane juice [9] in appreciable number in the intercellular spaces of parenchyma and is considered as an obligate entophyte [10]. It can excrete about half of its fixed nitrogen in a form that plants can use; excess nitrogen fertilization decreases the population of *G. diazotrophicus* associated with sugarcane [11]. It has also been reported that besides N fixation, all the strains of *G. diazotrophicus* produced indole acetic acid in a culture medium supplemented with tryptophan in the range of 0.14 to 2.42 µg $ml^{-1}$ [12]. Furthermore, it has been reported regarding its ability to solubilize insoluble inorganic phosphates from the soil and make available P for the inoculated crops. Hence, *Gluconacetobacter* inoculation to sugarcane significantly increased the plant height, chlorophyll content, total nitrogen, cane length, number of millable canes resulting the cane yield increase by 42% over control [13].

Thus, a pot culture experiment was conducted during 2012-13 to study the effect of *G. diazotrophicus* and PSB inoculation on two sugarcane varieties in different problematic soils and possibility of saving of chemical fertilizer nitrogen and phosphorus.

## 2. MATERIALS AND METHODS

A pot culture experiment was conducted under natural conditions in a 6 x 2 x 2 factorial completely randomized design (FCRD) with two replications during *Suru* season (January planting) of 2012-13 at Central Sugarcane Research Station, Padegaon, Tal. Phaltan, Dist. Satara (Maharashtra state, India). Six different problematic soil samples were studied as Factor A, two sugarcane varieties as Factor B and two inoculation treatments as Factor C in this experiment. The treatment details are given below:

### A. Problematic soils

| Sr.No. | Soil type | ECe | pHe | OC (%) | Available N (kg/ha) | Available P (kg/ha) | Exch. K (kg/ha) | CaCO3 (%) |
|---|---|---|---|---|---|---|---|---|
| 1. | S-1 (Sodic) | 0.54 | 9.54 | 0.31 | 165 | 9.38 | 247 | 18 |
| 2. | S-2 (Sal-Sod) | 4.61 | 9.70 | 0.08 | 143 | 4.75 | 213 | 12 |
| 3. | S-3 (Sal-Sod) | 11.03 | 12.59 | 0.06 | 118 | 4.02 | 186 | 12 |
| 4. | S-4 (Sodic) | 3.57 | 8.52 | 1.48 | 173 | 12.57 | 263 | 6 |
| 5. | S-5 (Sodic) | 3.30 | 8.57 | 1.52 | 155 | 5.81 | 226 | 11 |
| 6. | S-6 (Normal) | 0.31 | 7.60 | 1.29 | 185 | 15.75 | 297 | 4 |

*ECe- Electrical conductivity of 1 : 2.5 soil: water extract, pHe- pH of 1 : 2.5 soil : water soil extract.*

### B. Varieties

a) **$V_1$** Co 86032
b) **$V_2$** Phule 265

### C. Inoculation treatments

**$I_1$** Uninoculated control with recommended N and P (250 kg N and 115 kg $P_2O_5$/ha)

**$I_2$** Treatment of sugarcane sets with *G. diazotrophicus* 10 kg + PSB 1.25 kg in 100 lit water/ha for 30 min before planting with 50 % recommended N (125 kg/ha) and 75 % recommended $P_2O_5$ (86.25 kg/ha).

**Note:** Recommended $K_2O$ (115 kg/ha), FYM Compost (20 t/ha) and pretreatment of sets with dimethoate + carbendazim were common to all the treatments.

One pot was used for each treatment in each replication. The pot size used was 30 cm dia. x 30 cm ht. which accommodated 22 kg soil sampled to a depth of 45 cm from each problematic soil described as above. The experiment was planted on 19-02-2012 and harvested on 01-02-2013. The single eye bud setts were prepared from the canes brought from seed nursery before planting. The biofertilizers *viz.*, *G. diazotrophicus* and PSB were procured from Biological Nitrogen Fixation Scheme, College of Agriculture, Pune. Pots were applied with fertilizers (250 kg N, 115 kg $P_2O_5$ and 115 kg $K_2O$ $ha^{-1}$) by placement as per the treatments. The fertilizer doses were applied in four splits; nitrogen was applied @ 10% at planting, 40% at 6 weeks, 10% at 14 weeks and 40% at 18 weeks whereas 50% each of $P_2O_5$ and $K_2O$ were applied at planting and remaining 50% were applied at 18 weeks. For treatment of sugarcane bud sets, the single eye bud sets were dipped in the biofertilizer suspension prepared by mixing 10 kg *G. diazotrophicus* and 1.25 kg PSB in 100 lit. water per hectare as per treatments for 30 minutes prior to planting. After planting, a light irrigation (8 cm) was applied and the subsequent irrigations (total 250 ha cm) were applied at the interval of 3-4 days.

The initial soil properties like ECe and pHe were studied by saturated paste method [14]. The organic carbon, available N, $P_2O_5$ and $K_2O$ and total $CaCO_3$ content were determined as per the standard procedures described [15]. The observations on days required for germination were recorded. The data on cane height, number of leaves, internode length, cane girth and number of millable canes were recorded before harvest and the commercial cane sugar content (%) and fresh cane weight were recorded after harvest. The population of *G. diazotrophicus* in cane juice and that of PSB in rhizosphere soil was determined at 9 months and maturity by using serial dilution and plating technique. The soil samples were also analyzed for the available N and P after harvest. The data were subjected to statistical analyses by employing the standard methods of analysis of variance using MSTATC package.

## 3. RESULTS AND DISCUSSION

The data on various growth, yield and quality parameters of sugarcane as influenced by the soil types, varieties and the bud-set inoculation treatments are presented in Table 1 and 2 and described as follows.

**Table 1. Effect of *Gluconacetobacter diazotrophicus* and PSB on sugarcane varieties in different problematic soils**

| Soil | Varieties | Treat. | Days for germin. | Cane height (cm) | No. of leaves | Internode length (cm) | Cane girth (cm) | NMC/ Pot | Cane yield (kg/ Pot) |
|---|---|---|---|---|---|---|---|---|---|
| S-1 | Co 86032 | $I_1$ | 16.50 | 96.00 | 6.00 | 5.75 | 6.53 | 2.00 | 0.83 |
| | Co 86032 | $I_2$ | 15.00 | 106.50 | 6.92 | 7.34 | 7.50 | 3.00 | 1.19 |
| | Phule 265 | $I_1$ | 16.00 | 100.00 | 6.38 | 6.13 | 6.88 | 2.50 | 1.15 |
| | Phule 265 | $I_2$ | 14.50 | 111.00 | 6.90 | 7.38 | 7.75 | 3.50 | 1.30 |
| S-2 | Co 86032 | $I_1$ | 19.00 | 78.50 | 5.13 | 4.42 | 5.50 | 1.50 | 0.43 |
| | Co 86032 | $I_2$ | 18.00 | 84.00 | 6.00 | 5.40 | 6.08 | 2.00 | 0.48 |
| | Phule 265 | $I_1$ | 18.50 | 81.50 | 5.50 | 4.50 | 6.00 | 2.00 | 0.48 |
| | Phule 265 | $I_2$ | 17.00 | 94.00 | 6.25 | 6.17 | 6.50 | 2.00 | 0.76 |
| S-3 | Co 86032 | $I_1$ | 21.50 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Co 86032 | $I_2$ | 20.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Phule 265 | $I_1$ | 21.50 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Phule 265 | $I_2$ | 20.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| S-4 | Co 86032 | $I_1$ | 15.50 | 104.00 | 6.50 | 6.50 | 7.47 | 3.00 | 1.02 |
| | Co 86032 | $I_2$ | 13.50 | 123.00 | 7.25 | 7.84 | 9.00 | 4.50 | 1.32 |
| | Phule 265 | $I_1$ | 15.50 | 102.50 | 6.38 | 6.50 | 7.10 | 2.50 | 1.09 |
| | Phule 265 | $I_2$ | 14.00 | 120.00 | 7.20 | 7.50 | 8.00 | 4.00 | 1.34 |
| S-5 | Co 86032 | $I_1$ | 17.50 | 91.50 | 6.00 | 5.63 | 6.46 | 1.50 | 0.40 |
| | Co 86032 | $I_2$ | 16.00 | 99.00 | 6.33 | 6.25 | 6.75 | 2.50 | 0.84 |
| | Phule 265 | $I_1$ | 17.50 | 88.00 | 5.25 | 5.44 | 6.15 | 1.50 | 0.65 |
| | Phule 265 | $I_2$ | 16.00 | 97.00 | 7.00 | 6.20 | 6.67 | 2.50 | 0.96 |
| S-6 | Co 86032 | $I_1$ | 14.50 | 109.00 | 6.38 | 7.00 | 7.58 | 3.50 | 1.27 |
| | Co 86032 | $I_2$ | 13.00 | 125.00 | 7.50 | 8.18 | 9.00 | 4.50 | 1.50 |
| | Phule 265 | $I_1$ | 14.00 | 113.50 | 6.90 | 7.25 | 7.84 | 4.00 | 1.31 |
| | Phule 265 | $I_2$ | 12.50 | 126.25 | 7.58 | 10.25 | 9.25 | 4.50 | 1.94 |
| | C.D. P = 0.01 | | | | | | | | |
| | Soils (S) | | 1.94 | 3.80 | 0.92 | 0.89 | 0.81 | 0.70 | 0.14 |
| | Varieties (V) | | NS | NS | NS | NS | NS | NS | 0.08 |
| | Inocul. Treats. (I) | | 1.12 | 2.19 | 0.53 | 0.51 | 0.47 | 0.40 | 0.08 |
| | S x V | | NS | NS | NS | NS | NS | NS | NS |
| | S x I | | NS | 5.37 | NS | NS | NS | NS | 0.19 |
| | V x I | | NS | NS | NS | NS | NS | NS | NS |
| | S x V x I | | NS | NS | NS | NS | NS | NS | NS |

*NS = Non-significant*

***Table 1 continued ……***

| Soil | Varieties | Treat. | CCS (%) | *Acetobacter* count ( x $10^3$) | | PSB count ( x $10^4$) | | Avail. Soil N (kg/ha) | Avail. Soil P (kg/ha) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 9 Mon. | Maturity | 9 Mon. | Maturity | | |
| S-1 | Co 86032 | $I_1$ | 12.61 | 3.00 | 3.75 | 2.50 | 4.25 | 161.0 | 7.14 |
| | Co 86032 | $I_2$ | 12.92 | 4.00 | 5.25 | 3.50 | 6.25 | 174.0 | 12.36 |
| | Phule 265 | $I_1$ | 12.48 | 3.25 | 3.75 | 2.75 | 4.25 | 168.5 | 7.38 |
| | Phule 265 | $I_2$ | 12.89 | 4.25 | 5.50 | 3.50 | 6.25 | 178.5 | 12.84 |
| S-2 | Co 86032 | $I_1$ | 11.98 | 2.25 | 2.75 | 1.50 | 3.25 | 142.0 | 5.14 |
| | Co 86032 | $I_2$ | 12.31 | 2.50 | 4.25 | 1.75 | 4.50 | 148.5 | 5.46 |
| | Phule 265 | $I_1$ | 11.88 | 2.50 | 3.00 | 1.75 | 3.75 | 143.0 | 5.06 |
| | Phule 265 | $I_2$ | 12.24 | 2.75 | 4.75 | 2.25 | 5.00 | 159.5 | 5.55 |
| S-3 | Co 86032 | $I_1$ | 0.00 | 0.00 | 0.00 | 1.00 | 2.00 | 120.5 | 4.55 |
| | Co 86032 | $I_2$ | 0.00 | 0.00 | 0.00 | 1.25 | 2.25 | 123.0 | 4.59 |
| | Phule 265 | $I_1$ | 0.00 | 0.00 | 0.00 | 1.25 | 2.50 | 122.0 | 4.54 |
| | Phule 265 | $I_2$ | 0.00 | 0.00 | 0.00 | 1.50 | 2.75 | 124.5 | 4.59 |
| S-4 | Co 86032 | $I_1$ | 12.71 | 3.75 | 4.25 | 3.00 | 4.75 | 172.5 | 8.68 |
| | Co 86032 | $I_2$ | 13.14 | 5.00 | 6.25 | 4.25 | 6.50 | 189.5 | 17.99 |
| | Phule 265 | $I_1$ | 12.73 | 3.50 | 4.00 | 2.75 | 4.50 | 169.5 | 8.50 |
| | Phule 265 | $I_2$ | 13.18 | 4.50 | 5.75 | 4.25 | 6.25 | 182.5 | 17.31 |
| S-5 | Co 86032 | $I_1$ | 12.25 | 2.75 | 3.75 | 2.25 | 4.25 | 158.0 | 5.55 |
| | Co 86032 | $I_2$ | 12.47 | 3.25 | 4.75 | 2.50 | 5.50 | 166.0 | 7.31 |
| | Phule 265 | $I_1$ | 12.29 | 2.50 | 3.75 | 2.00 | 4.00 | 152.5 | 5.43 |
| | Phule 265 | $I_2$ | 12.62 | 3.00 | 4.75 | 2.50 | 5.50 | 163.5 | 7.15 |
| S-6 | Co 86032 | $I_1$ | 13.10 | 4.25 | 4.50 | 3.50 | 6.00 | 177.5 | 12.88 |
| | Co 86032 | $I_2$ | 13.25 | 6.25 | 6.75 | 4.50 | 7.25 | 194.0 | 19.16 |
| | Phule 265 | $I_1$ | 12.85 | 4.25 | 4.75 | 3.75 | 6.25 | 180.0 | 13.39 |
| | Phule 265 | $I_2$ | 13.21 | 6.50 | 7.25 | 4.75 | 7.50 | 197.5 | 19.75 |
| | C.D. P = 0.01 | | | | | | | | |
| | Soils (S) | | 0.38 | 0.65 | 0.83 | 0.64 | 0.97 | 6.12 | 0.65 |
| | Varieties (V) | | NS | NS | NS | NS | NS | NS | NS |
| | Inocul. Treats. (I) | | 0.22 | 0.38 | 0.48 | 0.37 | 0.56 | 3.53 | 0.37 |
| | S x V | | NS | NS | NS | NS | NS | NS | NS |
| | S x I | | NS | 0.92 | NS | NS | NS | NS | 0.91 |
| | V x I | | NS | NS | NS | NS | NS | NS | NS |
| | S x V x I | | NS | NS | NS | NS | NS | NS | NS |

*NS = Non-significant*

## 3.1 Days Required for Seed Germination

The number of days required for germination was significantly influenced by soil types and inoculation treatments whereas the varieties and various interactions of treatments showed non-significant effect. The normal soil (S-6) recorded the earliest germination (13.5 days). However, it was at par with S-4 (14.6 days). The number of days for germination prolonged as long as 15.5 days in S-1 to 20.7 days in S-3. The set inoculated treatment ($I_2$) recorded significantly earlier germination (15.8 days) than the uninoculated one (17.3 days). The early germination due to set treatment with *G. diazotrophicus* and PSB may be attributed to the production of growth promoting substances. It has been reported that besides N fixation, all the strains of *G. diazotrophicus* produce indole acetic acid in a culture medium supplemented with tryptophan in the range of 0.14 to 2.42 µg $ml^{-1}$ [12].

**Table 2. Interaction of Soils (S) x Inoculation treatments (I) for cane height, cane yield, *Acetobacter* count at 9 months and available soil P at harvest**

| **Cane height (cm)** | | | |
|---|---|---|---|
| **Soils** | **Inoculation treatments** | | **Mean** |
| | **$I_1$** | **$I_2$** | |
| S-1 | 98.00 | 108.75 | 103.38 |
| S-2 | 80.00 | 89.00 | 84.50 |
| S-3 | 0.00 | 0.00 | 0.00 |
| S-4 | 103.25 | 121.50 | 112.38 |
| S-5 | 89.75 | 98.00 | 93.88 |
| S-6 | 111.25 | 125.63 | 118.44 |
| Mean | 80.38 | 90.48 | |
| | | **C.D. P=0.01** | 5.37 |
| **Cane yield (kg/pot)** | | | |
| **Soils** | **Inoculation treatments** | | **Mean** |
| | **$I_1$** | **$I_2$** | |
| S-1 | 0.99 | 1.24 | 1.12 |
| S-2 | 0.45 | 0.62 | 0.53 |
| S-3 | 0.00 | 0.00 | 0.00 |
| S-4 | 1.05 | 1.33 | 1.19 |
| S-5 | 0.53 | 0.90 | 0.71 |
| S-6 | 1.29 | 1.72 | 1.51 |
| Mean | 0.72 | 0.97 | |
| | | **C.D. P=0.01** | 0.19 |
| ***Acetobacter* count at 9 months ( x $10^4$)** | | | |
| **Soils** | **Inoculation treatments** | | **Mean** |
| | **$I_1$** | **$I_2$** | |
| S-1 | 3.13 | 4.13 | 3.63 |
| S-2 | 2.38 | 2.63 | 2.50 |
| S-3 | 0.00 | 0.00 | 0.00 |
| S-4 | 3.63 | 4.75 | 4.19 |
| S-5 | 2.63 | 3.13 | 2.88 |
| S-6 | 4.25 | 6.38 | 5.31 |
| Mean | 2.67 | 3.50 | |
| | | **C.D. P=0.01** | 0.92 |
| **Available soil P at harvest (kg/ha)** | | | |
| **Soils** | **Inoculation treatments** | | **Mean** |
| | **$I_1$** | **$I_2$** | |
| S-1 | 7.26 | 12.60 | 9.93 |
| S-2 | 5.10 | 5.50 | 5.30 |
| S-3 | 4.54 | 4.59 | 4.57 |
| S-4 | 8.59 | 17.65 | 13.12 |
| S-5 | 5.49 | 7.23 | 6.36 |
| S-6 | 13.14 | 19.46 | 16.30 |
| Mean | 7.35 | 11.17 | |
| | | **C.D. P=0.01** | 0.91 |

## 3.2 Cane Height and Internode Length

The cane height and internode length at harvest were significantly influenced by the soil types and inoculation treatments whereas the varieties and various interactions except that of soil types x inoculation treatments for cane height showed non-significant effect. The

normal soil (S-6) recorded significantly higher cane height (114.64 cm) and internode length (8.17 cm) than the other soil types. It was followed by S-4 (112.38 cm, 7.08 cm) and S-1 (103.38 cm, 6.65 cm). Between the inoculation treatments, the pretreatment of sugarcane bud sets ($I_2$) recorded significantly higher cane height (90.48 cm) and internode length (6.04 cm) than the uninoculated one (80.38 cm, 4.93 cm). The cane height was significantly influenced by interaction of soil types and inoculation treatments. Treatment of bud sets in normal soil (S-6) recorded the highest cane height (125.63 cm). However, it was at par with inoculation in S-4 soil (121.50 cm) indicating better performance of inoculation in S-4 soil. *Gluconacetobacter* inoculation to sugarcane has been reported to significantly increase the plant height and cane length over control [13].

## 3.3 Number of Leaves/Plant

Various soil types and inoculation treatments showed significant effect on number of leaves whereas the effect of varieties and various interactions was not significant. The highest number of leaves (7.09) was recorded in the normal soil (S-6). However, S-1 (6.65) and S-4 (6.83) were statistically similar with normal soil (S-6) in this respect. Bud sets treated with *G. diazotrophicus* + PSB ($I_2$) recorded significantly higher number of leaves (5.74) than the uninoculated ones (5.03). The improvement in number of leaves in sugarcane crop due to diazotrophic bacterial inoculation has been reported earlier [4,5] and the results of this investigation are also in agreement with these reports.

## 3.4 Cane Girth

The effect of soil types and the inoculation treatments on cane girth was significant. However, the varieties and the interactions of different factors showed non-significant effect. The normal soil (S-6) was the most superior in respect of cane girth (8.42 cm). The sodic soil S-4 (7.89 cm) was almost similar to the normal soil S-6. As regards the effect of inoculation treatments, the treatment ($I_2$) recorded significantly higher cane girth (6.38 cm) than the uninoculated one (5.62 cm). The highest cane girth in the normal soil may be attributed to the maximum crop growth in this soil as compared to the corresponding growth in the problematic soils. The nitrogen fixing bacterial inoculations to sugarcane have also been able to improve the cane girth [4,5].

## 3.5 Number of Millable Canes (NMC)

The number of millable canes is a major yield contributing character which determines the cane yield. In the present study, various soil types used and the inoculation treatments employed resulted in significant influence on NMC. The NMC was, however not significantly affected by the varieties and interactions of any of the factors with each other. The highest NMC could be recorded in the normal soil i.e. S-6 (4.13) which was statistically similar to that recorded by the sodic soil i.e. S-4 (3.50). The effect of inoculation treatments was conspicuous. The inoculation of sugarcane bud sets with *G. diazotrophicus* + PSB ($I_2$) recorded statistically higher NMC/pot (2.75) than the uninoculated one (2.00). Significant improvement in NMC in sugarcane due to *Gluconacetobacter* inoculation resulting in the increased cane yield has been reported [13].

### 3.6 Cane Yield and Commercial Cane Sugar (CCS) Content

The soil types studied exhibited marked effect on the cane yield and CCS content. The highest cane yield of 1.51 kg/pot and CCS content of 13.10% was recorded in the normal soil (S-6). However, the soil types S-4 (1.19 kg, 12.94%) and S-1 (1.12 kg, 12.72%) did not differ significantly from the normal soil. The inoculation treatments also imparted significant effect on both cane yield and CCS %. Sugarcane seed inoculation treatment ($I_2$) recorded significantly higher cane yield (0.97 kg) and CCS (10.68%) than the uninoculated one (0.72 kg, 10.40%). The effect of varieties was statistically significant only on the cane yield. Averaged across the other factors, the sugarcane variety Phule 265 registered significantly higher cane yield (0.91 kg) than Co 86032 (0.77 kg). All the factorial interactions except that of soil types x inoculation treatments for the cane yield were non-significant. The sugarcane set inoculation recorded the highest cane yield (1.72 kg) in normal soil (S-6) followed by sodic soils S-4 (1.33 kg) and S-1 (1.24 kg) indicating better performance of inoculation in these soils.

The improvement in sugarcane yield to the tune of 7 to 10 t $acre^{-1}$ and in sugar recovery by 0.5 to 1.0% with 50% reduction in the recommended dose of chemical nitrogen by use of the nitrogen fixing *A. diazotrophicus* has been reported [16]. Over 40% of atmospheric nitrogen fixed was contributed by *A. diazotrophicus* besides increased cane yield by 10 to 25% [17]. The N fixing microbial inoculations to sugarcane have increased the cane yield by 5-15% and also improved the juice sucrose and purity [3-5] besides saving 25 kg fertilizer N $ha^{-1}$. *Gluconacetobacter* inoculation to sugarcane has been reported to increase the cane yield by 42% over control [13].

### 3.7 Population Dynamics of *G. diazotrophicus* and PSB

The *Acetobacter* count in cane at 9 months and at maturity was significantly influenced by soil types and inoculation treatments and likewise for their interactions at 9 months. The normal soil (S-6) recorded the highest *Acetobacter* count (5.31 and 5.81 x $10^3$). It was followed by S-4 (4.19 and 5.06 x $10^3$) and S-1 (3.63 and 4.56 x $10^3$) in that order. *Acetobacter* count (6.38 $10^3$) was further increased in normal soil with inoculation followed by the same treatments (S4 and S1). The treatment of sets ($I_2$) recorded significantly higher *Acetobacter* count (3.50 and 4.60 x $10^3$) than the uninoculated treatment (2.67 and 3.19 x $10^3$). The interaction of soil types and inoculation treatments was significant at 9 months. Sugarcane bud set inoculation in normal soil recorded the highest *Acetobacter* count (6.38 x $10^3$).

The PSB count in cane at 9 months and at maturity was significantly influenced by soil types and inoculation treatments whereas the effect of varieties and different interactions was not significant. The normal soil (S-6) recorded the highest PSB count (4.13 and 6.75 x $10^4$). It was followed by S-4 (3.56 and 5.50 x $10^4$) and S-1 (3.06 and 5.25 x $10^4$). Treatment of bud sets ($I_2$) recorded significantly higher PSB count (3.04 and 5.46 x $10^4$) than the uninoculated treatment (2.33 and 4.15 x $10^4$).

It has been reported that the number and activity of endophytic bacteria is reduced when the sugarcane crop is grown under high or optimal nitrogen input levels [11,16,2]. There are also reports that *G. diazotrophicus* tolerates salinity stress (1.0 to 1.5% NaCl), but its nitrogenase activity and carbon metabolism enzymes are affected by high NaCl dosage [10] and the

indigenous strains are more salt tolerant than the Brazilian strain [18]. The results of the present study are also in agreement with these findings.

### 3.8 Available N and P Status of Soil

The available N and P status of soil at harvest were significantly influenced by soil types and inoculation treatments whereas the effect of varieties was not significant. The normal soil (S-6) recorded the highest N (187.25 kg/ha) and P (16.30 kg/ha). It was followed by S-4 (178.5 kg/ha and 13.12 kg/ha) and S-1 (170.5 kg/ha and 9.93 kg/ha). The set inoculation ($I_2$) recorded significantly higher N (166.75 kg/ha) and P (11.17 kg/ha) than the uninoculated one (155.58 kg N and 7.35 kg P/ha). The interaction of soil types and inoculation treatments was significant only for the available P. Set inoculation in normal soil was the significantly superior (19.46 kg/ha) followed by set inoculation in S-4 soil (17.65 kg/ha) and S-1 soil (12.60 kg/ha) indicating better performance of inoculation in these soils. The increase in soil nitrogen reserve under sugarcane crop by 50 % of the initial value due to the nitrogen fixation by root associated diazotrophs helping sustained production of sugarcane has been reported [2].

## 4. CONCLUSIONS

The single eye bud set treatment of sugarcane varieties *viz.,* Co 86032 and Phule 0265 with *G. diazotrophicus* 10 kg + PSB 1.25 kg in 100 lit water/ha for 30 min. coupled with 50% recommended N and 75% recommended P significantly improved the growth, yield and quality of cane juice. The set inoculation showed the best results in normal soil (S-6) closely followed by S-4 (Sodic) and S-1 (Sodic) indicating better performance of inoculation in these problematic soils. The results also indicated the saving of 50% N and 25% $P_2O_5$ for sugarcane by employing pre-planting set treatment.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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