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# COMBINED EFFECT OF *GLUCONOACETOBACTER DIAZOTROPHICUS* AND AM FUNGI IN RAISING SUGARCANE SEEDLINGS UNDER SSI TECHNOLOGY

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## Abstract

The experiment was conducted at Sugarcane Research Station, Cuddalore during January 2012, with the view to study the effect of bioinoculants *viz.*, *Gluconoacetobacter diazotrophicus,* and AM fungi on the growth of chip bud seedlings and also to standardize the dose of bioinoculants to be added to the rooting medium under Sustainable Sugarcane Initiative (SSI) technology. The treatment comprises of different combinations of cocopeat, vermicompost and micronutrient mixture were studied as rooting medium for raising the chip bud seedlings along with the different concentrations of bioinoculants. The results revealed that the seedlings raised with cocopeat, vermicompost and 2% micronutrient mixture in combination with 1% AM fungi + 0.1% *G. diazotrophicus* as the rooting medium recorded the highest germination percentage of 98% and the biometric observations *viz.,* root length (25.12 cm), shoot length (63.67 cm), stem girth (1.2 cm) and number of leaves (5.53) are also highly significant. This is the pioneer work in studying the performance of nitrogen fixers and phosphate mobilizers on the growth of chip bud seedlings that provides the evidence that the bioinoculants has encouraging effect on the growth of chip bud seedlings.

***Key words :*** SSI – Chip bud seedling – Endophytes - *G. diazotrophicus*- – Phosphorous mobilizing – AM fungi.

## Introduction

'Sustainable Sugarcane Initiative' (SSI) is one method that has caught the imagination of all the stake holders, especially the farmers, because of its proven ability to increase the productivity at reduced inputs. SSI addresses the most important aspect of the sugar industry, the supply and the quality of raw material. It also addresses the bigger picture of improved natural resource exploitation, reduced environmental footprints and improved livelihoods of farmers by means of technologies that are as appropriate and effective at household farm level. All better management practices are packaged in SSI around six basic principles for easy understanding and promotion for field adoption, raising nursery using single budded chips, transplanting young seedlings (25-35 days old), maintaining wider spacing in the main field, providing sufficient moisture to plants and avoiding flooding of fields, encouraging application of organic manures, better cultural and plant protection measures, practicing intercropping to utilize land effectively.

The most important aspect of SSI methodology is the practice of transplanting raised chip bud seedlings, instead of the normal sett planting. Van Dillewijn was the first to suggest in 1952 that a small volume of tissue and a single root primordium adhering to the bud are enough to ensure germination in sugarcane. Narasimha Rao and Satyanarayana (1974) reported that drenching of three budded setts in fungicide solution before planting did not control diseases owing to ineffective permeation of the solution whereas in chip bud the permeation was complete and there was disease control in the seedling raised crop. Ramaiah, *et al.,* (1977) reported that with chip bud raised seedlings and the resultant crop with high and synchronous tillering with heavier canes led to higher yields and better recovery.

Also sugarcane is a very demanding crop as for a cane yield of 100 t/ha it removes about 205 kg N, 55 kg $P_2O_5$, 275 kg $K_2O$ and a large amount of micronutrients from soil (Yaduvanshi and Yadav, 1990). Since its fertilizer

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consumption is higher than that of other crops it has negative effect on soil health in the long term. In order to sustain productivity major nutrients are provided each year at the recommended application rates of 150 kg/ha of N and 60 kg each of $P_2O_5$ and $K_2O$ for sugarcane. The efficiency of sugarcane to utilize N range between 16 and 45% as large quantities of applied N leach down through soil layer due to irrigation (Yadav and Prasad, 1992).

Endophytes play major role in sugarcane cultivation and in broader term endophytes includes fungal, actinomycetal and bacterial forms. They reside with in the interior of plants without causing disease or forming symbiotic structure and inhabitat various tissues of seeds, roots, stems and leaves (Dobereiner *et al.,* 1995). Field trials conducted in sugarcane with *Glucanoacetobacter diazotrophicus* with other diazotrophs can match yield level equal to 275kg N/ha application (Sevilla *et al.,* 1998; Muthukumarasway *et al.,* 2002; Oliveria *et al.,* 2002).

Prevalence of endophytic PGPR strain in sugarcane has been recently established and their antagonistic activity against red rot pathogen was identified (Viswanathan and Samiappan, 2002). Glick (1995) studied that mycorrhiza plays major role in terms of resistance to bacterial and fungal pathogens, increased photosynthetic rate and enhanced stomatal regulation under water stressed condition in sugarcane. The general recommendation for AM fungi is 100 g per $m^2$, hence application of VAM in main field is a problem because of its heavy requirements of nearly 1 tonnes per hectare, hence this approach is tried to inoculate the AM fungi in the bud stage in the nursery level by adding it through the rooting medium. Since the bioinoculants are endophytes once applied in the nursery can be transferred to main field along with root of the plants, thereby reduces the application of bioinoculants in the main field. Concerning the above problems, the study is proposed to develop the nutrient mixture for chip bud seedling nursery and also to standardize the dosage of bioinoculants at nursery level and thereby reducing the large quantity of biofertilizer to the main field.

## Materials and Methods

The pot culture experiment was conducted with thirteen treatments with three replications in Randominised Block Design. The sugarcane variety taken for the study was CoC24. The standardization of rooting medium for raising chip bud seedlings with different combinations of cocopeat, vermicompost and TN micronutrient mixture along with different concentrations of bioinoculants were done to study the effect of bioinoculants *viz.*, *Gluconoacetobacter diazotrophicus,* and AM fungi on the growth of chip bud seedlings and also to standardize the dose of bioinoculants to be added to the rooting medium under SSI technology. The treatment details are furnished below.

**Preparation of sugarcane chip buds**

Healthy seed cane of 7 to 9 months old was taken and the bud were chipped using a bud chipper. The buds were then treated with malathion (20 ml) and carbendazim (5g) (this is for the buds for 1 acre).

**Sowing of chip buds in portrays**

The portrays were half filled with different rooting medium as per the treatment details and over that the treated buds were placed with the bud facing upside and then the buds were covered completely with the same rooting medium.

**Treatment details**

**$T_1$** - Cocopeat + 2 % TN Micronutrient Mixture + 0.1%AM fungi + 0.0001 % *G. diazotrophicus*

**$T_2$** - Cocopeat + 2 % TN Micronutrient Mixture + 0.1%AM fungi + 0.1% *G. diazotrophicus*

**$T_3$** - Cocopeat + 2 % TN Micronutrient Mixture + 1%AM fungi + 0.0001 % *G. diazotrophicus*

**$T_4$** - Cocopeat + 2 % TN Micronutrient Mixture + 1%AM fungi + 0.1% *G. diazotrophicus*

**$T_5$** - Vermicompost + 2%TN Micronutrient Mixture + 0.1%AM fungi + 0.0001 % *G. diazotrophicus*

**$T_6$** - Vermicompost + 2%TN Micronutrient Mixture + 0.1%AM fungi + 0.1% *G. diazotrophicus*

**$T_7$** - Vermicompost + 2%TN Micronutrient Mixture + 1%AM fungi + 0.0001 % *G. diazotrophicus*

**$T_8$** - Vermicompost + 2%TN Micronutrient Mixture + 1%AM fungi + 0.1% *G. diazotrophicus*

**$T_9$** - Cocopeat + Vermicompost + 2% TN Micronutrient Mixture + 0.1%AM fungi + 0.0001 % *G. diazotrophicus*

**$T_{10}$**- Cocopeat + Vermicompost + 2% TN Micronutrient Mixture + 1%AM fungi + 0.1 % *G. diazotrophicus*

**$T_{11}$**- Cocopeat + Vermicompost + 2% TN Micronutrient Mixture + 0.1%AM fungi + 0.0001% *G. diazotrophicus*

**$T_{12}$**- Cocopeat + Vermicompost + 2% TN Micronutrient Mixture + 1%AM fungi + 0.1% *G. diazotrophicus*

**$T_{13}$** - Control with cocopeat alone

*Cocopeat and vermicompost in the ratio of 1:1

The data on germination, root length, shoot length, stem girth and no of leaves were recorded at 25th day after sowing the chip buds in the protray. The data were subjected to analysis of variance in factorial completely randomized design according to standard statistical method (Panse and Sukhatme, 1961).

## Results and Discussion

The germination percentage of the chip buds sown in the portrays with different rooting medium was recorded and the seedlings were observed for their biometric observations like root length, shoot length, stem girth and no of leaves were recorded at 25th day after sowing. The germination percentage was recorded significantly maximum of 98 per cent (Fig. 1) in the medium composed of cocopeat, vermicompost and 2% micronutrient mixture in combination with 1% AM fungi + 0.1% *G. diazotrophicus* and the same treatment recorded the maximum root length of 25.12 cm, shoot length of 63.67 cm, stem girth of 1.2 cm and the number of leaves of 5.53 Table 1 followed by seedlings raised with medium comprising of vermicompost + 2%TN Micronutrient Mixture + 1%AM fungi + 0.1% *G. diazotrophicus* which recorded the germination percentage of 92 percent and root length (22.91), shoot length (61.27) and stem girth (1.04).

The enhanced growth of the chip bud seedlings grown in the medium with cocopeat, vermicompost and 2% micronutrient mixture in combination with 1% AM fungi + 0.1% *G. diazotrophicus* is due to the combined effect of the bioinoculants along with the organic manure (vermicompost and cocpeat). The applied bacterial and fungal sources are endophytes and help in continuous production of growth promoting nutrients and secondary metabolites which might be the root cause for the enhanced growth of the seedlings. As they are endophytes after the transplanting of the seedlings, there will be continuous persistence and the colonization of these bioinoculatns in the crop and it also enhances the soil microbial population and mainly reduces the main field application of biofertilizers and inturn reduces the cost of cultivation. The general recommendation for AM fungi is 100 g per $m^2$, hence application of VAM in main field is a problem because of its heavy requirements of nearly 1 tonnes per hectare, hence this approach is tried to inoculate the AM fungi in the bud stage in the nursery level by adding it through the rooting medium. Since the bioinoculants are endophytes once applied in the nursery can be transferred to main field along with root of the plants, thereby reduces the application of bioinoculants in the main field.

The biofertilizers application enhanced the yield and quality parameters and also essential to maintain soil microflora population and protect soil fertility from deterioration. Significant changes in various plant growth parameters have been shown by the inoculation of various nitrogen fixing and plant growth promoting bacteria (Nayak *et al.,* 1986; Murthy and Ladh, 1988). In addition to nitrogen fixation the beneficial effects has been attributed to the production of plant growth hormones also (Sevilla and Kennedy, 2000).

**Table 1:** Effect of different rooting medium on the biometrics of the chip buds seedlings.

| Treatment | Root length (cm) | Shoot length (cm) | Stem girth (cm) | No. of leaves |
|---|---|---|---|---|
| $T_1$ | 18.83 | 33.13 | 0.85 | 3.60 |
| $T_2$ | 20.90 | 43.27 | 0.89 | 4.00 |
| $T_3$ | 22.03 | 45.60 | 0.92 | 4.60 |
| $T_4$ | 22.70 | 55.20 | 0.95 | 4.73 |
| $T_5$ | 16.58 | 53.60 | 0.88 | 4.60 |
| $T_6$ | 19.45 | 56.13 | 0.95 | 5.00 |
| $T_7$ | 20.46 | 58.27 | 0.97 | 5.00 |
| $T_8$ | 22.91 | 61.27 | 1.04 | 5.00 |
| $T_9$ | 19.34 | 57.27 | 0.92 | 5.07 |
| $T_{10}$ | 22.70 | 58.60 | 0.97 | 5.47 |
| $T_{11}$ | 22.91 | 60.60 | 1.03 | 5.53 |
| $T_{12}$ | 25.12 | 63.67 | 1.2 | 5.53 |
| $T_{13}$ | 14.23 | 29.65 | 0.75 | 3.15 |
| Mean | 33.67 | 53.88 | 0.95 | 4.84 |
| SED | 0.87 | 2.18 | 0.03 | 0.20 |
| CD(0.5) | 1.73* | 4.36* | 0.05** | 0.41* |

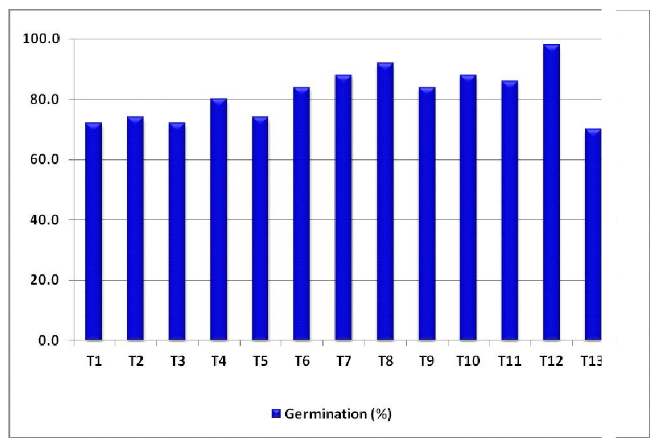


**Fig. 1:** Effect of different rooting medium on the germination of the chip buds seedlings.

Application of phosphorous from different sources *ie.* from inorganic and as bioinoculants (AM fungi and phosphobacteria) was found to be effective in sugarcane. Continuous availability of the valuable nutrients and their persistence and colonization in soil makes the soil more fertile and healthy. The mobilization of P from soil to the plants is mediated by hairy root systems of the mycorrhizal fungi through plant roots. It commonly infect plant roots, including those of sugarcane forming beneficial symbiotic relationships (Kelly *et al.,* 1997). The improvement in plant growth was attributed to an enhanced access of mycorrhizal root to soil phosphorous located beyond the rhizosphere (Sanders and Tinker. 1973) and infection by mycorrhizal fungi is significantly reduced at high soil phosphorous levels (Amijee *et al.,* 1989). Mycorrhiza was found to be compatible with nitrogen fixers *viz*., *Rhizobium* (Hayman, 1986), *Acetobacter* and phosphate solubilising bacteria (Singh and Kapoor, 1999). It also holds good for sugarcane.

This is the pioneer work in studying the performance of nitrogen fixers and phosphate mobilizers in the growth of chip bud seedlings that provides the evidence that the bioinoculants has encouraging effect on the growth of seedlings.

## Conclusion

The inoculation of bioinoculants is beneficial for seedling growth and for increasing the plant vigour at nursery levels, consequently the amount of fertilizer application in the main field could be reduced. AM fungi and phosphobacteria are very much essential to convert the unavailable form of the phosphorous source to available source and providing to the plants. The usage of these bioinoculants in the nursery level could inturn reduces its main field application and thereby reduces the cost of cultivation. With this references these bioinoculants can be recommended for their use in rooting medium along with organics for enhanced sugarcane productivity.

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