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Inoculating Sugarcane Micropropagated with Gluconacetobacter Diazotrophicus and Herbaspirillum Sp., We Were Able to Save N-Fertilizer

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ABSTRACT

Introduction: A key component of integrated nutrient management, sustainable agricultural productivity, and environmental preservation is the use of biological nitrogen fixation (BNF).
Aim of the study: the main aim of the study is to inoculating sugarcane micropropagated with gluconacetobacterdiazotrophicus and herbaspirillum sp., we were able to save n-fertilizer
Material and method: When these two strains of bacteria were mixed together, the short-term experiment showed a significant increase in biomass under Nlimited conditions. A decrease in the population of G. diazotrophicus was seen throughout the same time period, but the population of inoculated Herbaspirillum sp. remained consistent during the long-term experiment. When plants were infected with G. diazotrophicus and Herbaspirillum sp. and then fertilised with 50 percent of the prescribed N (140 kg ha-1), the total biomass and leaf N content were greater than when the recommended quantity of inorganic N was used on the plants that weren't (280 kg ha-1).
Conclusion:it is concluded that theinoculating sugarcane varieties Co 86032 and 86033 with these bacteria reduced fertiliser N application significantly, according to the results of this experiment.
Keywords : Inoculating, Sugarcane, Micropropagated, Gluconacetobacter, Diazotrophicus, Herbaspirillum Sp

I. INTRODUCTION

1.1 OVERVIEW

Nowadays, inoculating plants with beneficial microorganisms is a popular practise in agriculture. It offers crops with a variety of advantages, including enhanced plant growth and disease prevention. The development and production of plant growth-promoting rhizobacteria (PGPR) may be influenced by root exudates from plants, which can have both immediate and long-term consequences. Apart from the above stated strains, PGPR may be found in Azospirillumbrasilense, Bacillus subtilis, and Enterobacter cloacae, Gluconacetobacterdiazotrophicus, Pantoeaagglomerans, and P. fluorescens, as well as Rhizobium leguminosarum, Sinorhizobiummeliloti, and P. fluorescens, and Pseu

Plant growth might be aided by PGPR in a variety of ways, including biochemical N2 fixation, phosphate solubilization, and the production of phytohormones. The production of antimicrobial compounds or the establishment of induced systemic resistance may also be exploited by PGPR to indirectly increase plant

growth as an extra advantage, as previously mentioned (ISR). Sustainability in agriculture cannot be ignored any longer, and microbial inoculants may prove to be a cost-effective method of maintaining crop output over the long run.

It is possible to improve plant growth promotion and biological control by inoculating seeds with a large number of helpful bacteria rather than a single strain. Because different species of microorganisms may work together to give nutrients, remove inhibitory chemicals, and stimulate one another via physical or biochemical activities, using bacterial consortiums as inoculants may provide more advantages to plants. Comparing inoculation with individual bacterial strains with Serratia marcescens (SF3) and Mesorhizobiumciceri (ST9), it was discovered that the combination of the two bacteria increased the number of nodules per plant (SF3), the nodule dry weight (ST9), and the number of pods per plant (ST9) under irrigated and rainfed conditions. Inoculated sugarcane plants produced more stems than mono-inoculated plants in two soils with low- to medium-level chemical fertiliser, according to the results of the study. The diazotrophic bacteria Gluconacetobacterdiazotrophicus, Herbaspirillumseropedicae, Herbaspirillumrubrisubalbicans, Azospirillumamazonense, and Paraburkholderiatropica were among the five diazotrophic bacteria

1.2 INOCULANT CARRIERS

Since the beginning of the inoculant manufacturing sector, the industry has been focused on developing more efficient products at a lower cost that satisfy the needs and expectations of farmers everywhere. As an important aspect, microorganisms must be transported by a carrier that maintains cell viability for an extended period of time while also being easy to employ. The first commercially produced inoculant, "Nitragin" (Fig. 1.1), was made from gelatin, and later on, gelatin was utilised as a transporter for bacteria in nutritional media. In their stead came peat, which was less deadly but retained its status as the "gold" transporter until the late 1990s, when things began to change (Fig. 1.1)

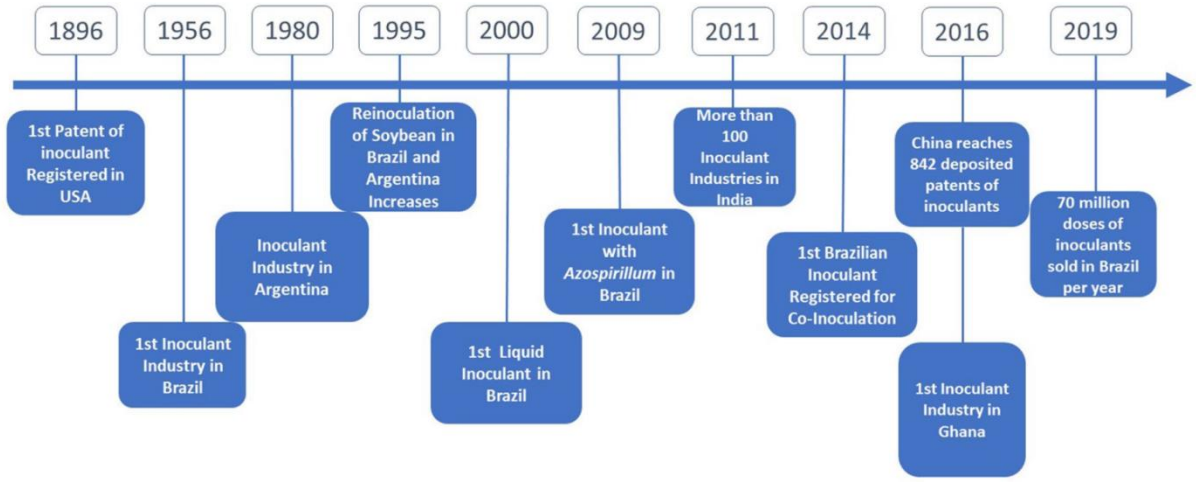

Fig. 1 Inoculant development timeline, including some key milestones

1.3 MICROBIAL INOCULANTS MAY BE AFFECTED BY THESE ACTUAL THREATS

There are several dangers that arise from the rising scientific and economic interest in microbial inoculants, which should be taken into consideration. A number of studies have shown that bacteria that may be

detrimental to plants, animals, and people may have positive effects on plant development. It is clear from these investigations that soil-borne strains of Enterobacter, Burkholderiacepacia complex, and other pathogens may be identified that are capable of enhancing the development of plants. But they cannot be used as vaccines. Priority should be given to identifying the taxonomic status of such isolates before conducting experiments to validate the plant's performance. Pre-inoculated seeds that have been kept in pesticides for extended periods of time are making the situation worse because of their incompatibility with the agrochemicals often used in seed treatments, particularly insecticides. Finding appropriate agrochemicals and cell protectors, or alternate methods of application such as the use of inoculants in-furrow to prevent direct contact with items used for seed treatment should be given first priority.

II. LITERATURE REVIEW

Ryan L. Sebring (2022) Leaf lettuce (Lactuca sativa L.) cultivars "Black Seeded Simpson" and "Bibb/Limestone" were infected with Gluconacetobacterdiazotrophicus, which is a bacterium that causes bacterial blight. Three or four replications of a randomised factorial design with three or four cultivars each were carried out in a growth chamber-controlled environment, with hydroponically grown plants in Kratky jars in each of the three or four replications. There was just one run of each experiment. If nitrogen (N) fertilisation is concerned, there are two options: (1) with or without an inoculant, and (2) seven levels ranging from inadequate to excessive (172.5 mg L1 N). For each change, the biomass accumulations, nitrogen densities, and carbon/nitrogen (C/N) ratios of the different species were analysed in detail. The shoot output of Black Seeded Simpson was much greater than the root production, suggesting a shift in the plant's production away from the root system and toward aerial tissues. At a N rate of 105 mg L1 N, inoculation plants produced 14.8 percent more shoot biomass than uninoculated plants, despite the fact that the N rate was insufficient. Shoot tissues from inoculated plants with lower N density and greater C/N ratios imply that the plants are more efficient in their utilisation of nitrogen. Shoot production and root biomass rose by an average of 10.9 percent in Bibb/Limestone after inoculation, according to the results of the study. When the N density of inoculated shoot tissues was measured, the Bibb/Limestone combination was found to have a lower value and a higher C/N ratio. Gardeners who desire to increase lettuce harvests by increasing leaf yields without increasing their reliance on nitrogen fertiliser may find G. diaz inoculation to be a useful supplement to their growing system.

Rosa, Poliana Aparecida & Galindo (2022) Due to its adsorption to soil colloids, phosphate (P) is a crucial nutrient for excellent sugarcane yields throughout its cultivation cycles. In certain cases, plant growth-promoting bacteria (PGPBs) may be capable of enhancing the availability of P to plants and producing phytohormones that contribute to crop growth, quality, and yield. As a result, the goal of this research was to examine the effects of phosphate fertilisation and PGPB inoculation on sugarcane leaf nitrogen (N) and phosphorus (P) contents, yield, and technical quality. So Paulo, Brazil's IlhaSolteira was the site of the experiment. Three replications of the randomised block design, which included five phosphorus rates (0, 25, 50, 75 and 100 percent of the recommended P2O5 rate) and eight inoculations of three species of PGPBs (Azospirillumbrasiliense, Bacillus subtilis, and Pseudomonas fluorescence) were used in this study. Sugarcane had a greater level of leaf P after being inoculated with B. subtilis and P. fluorescens. Sugarcane stalk yield is affected by the P2O5 rates and the bacteria inoculation. Regardless of the usage of growth-promoting bacteria, excess or lack of phosphate fertiliser is detrimental to sugarcane farming. We recommend the inoculation with

A. brasilense + B. subtilis associated with 45 kg ha□1 of P2O5 aiming at greater stalk yield. In addition to increasing sugar output, this treatment also reduces sugarcane crop production costs by 75% of the acceptable P2O5 rate, making it a more effective and long-term solution.

Mora-Poblete, Freddy & Zhao (2022) As plant-microbe interactions have become increasingly complex, understanding the diversity of root-associated microbiomes in wild progenitors and closely related crossable species may aid in the development of improved cultivars. This is because genetic background plays an important role in plant-microbe interactions. Sugarcane, the world's second-largest biofuel crop, is reliant on the rhizosphere and diazotroph bacterial variety of its wild progenitors to grow and produce ethanol. The goal of this study is to fill a critical knowledge gap in this field. The 16S rRNA and nifH genes of Saccharum officinarum (BRS), Saccharum barberi (S. barberi), Jesw. cv Pansahi (PRS), and Saccharum robust (RRS), as well as the rhizosphere and diazotroph bacterial communities of Saccharum officinarum, Saccharum spontaneum, and Saccharum robust, were sequenced using a high-throughput sequencing (HTS According to the results of the HTS, a total of 6,202 bacteria-specific operational taxonomic units (OTUs) were found, which were subsequently grouped into 107 separate bacterial groups based on their characteristics. All five species are related to one another via the rhizobacterial family, which has 31 members. Among the OTUs for the nifH gene, S. barberi had the highest number while S. spontaneum had the lowest number of OTUs. The results of quantitative PCR analyses of both genes corroborated these conclusions. A total of 1,099 OTUs were identified for diazotrophs, with a core microbiome consisting of nine families that was spread throughout all sugarcane species. In the core microbiome, microbes from 20 distinct genera were found to be present. The increase in rhizosphere microbial diversity was mostly attributed to changes in soil physiochemical properties, according to the study. For the great majority of the species investigated, it was discovered that rhizobacteria and diazotrophs had a positive relationship with soil properties. Rhizospheric diversity is shown to be prevalent among the progenitors and close relatives of the species under investigation, according to the research. The rhizosphere microbial abundance of the progenitors of current sugarcane, on the other hand, was at the lower end of the range, indicating that the potential of Saccharum species introgression breeding could further increase the nutrient usage and disease and stress tolerance of commercial sugarcane in the future. As a result of the variety of rhizosphere microbiomes seen in Saccharum species, researchers may be able to utilise this diversity as a knowledge base and an experimental system to better understand how rhizobacteria and host plants developed together during the history of agricultural domestication.

Zhang, Jinlian& Wei, Beilei& Wen (2021) Intercropping with legumes and strategies using the drought stress control gene family DREB (dehydration-responsive element binding) are emerging as viable solutions for boosting long-term sugarcane agriculture. Intercropping systems including transgenic crops are becoming the subject of research to better understand and so harness beneficial soil bacteria for plant development. These studies concentrate on root interactions in intercropping systems. The rhizosphere microbiota's reaction to two alternative intercropping patterns, one with soybean and wild-type (WT) sugarcane and the other with soybean and genetically modified (GM) Ea-DREB2B-overexpressing sugarcane, was examined using trials we constructed. Both patterns of intercropping had different levels of bacterial diversity in the rhizosphere microbial population. While the biomass of GM sugarcane intercropped with soybean was greatly enhanced, the aboveground biomass and root biomass of GM soybean intercropping sugarcane grew by 49.15 and 46.03 percent, respectively, compared to monoculture sugarcane. The systems intercropped with GM sugarcane also generated a favourable rhizosphere environment for Actinobacteria development. Crop genetic modification

gives new avenues to study the impacts of intercropping on plant roots and soil microbiota, which is an important method for increasing crop yields. Thus, this work offers a theoretical foundation for sustainable sugarcane production and a rationale for identifying optimum sugarcane–soybean intercropping patterns.

Chukwuneme, Chinenyewa& Uzoh (2021) Warmer temperatures and more evaporation from soils have resulted from the constant shift in the global climate. This results in a lack of water because of the unpredictable rainfall volume and distribution. Drought has devastating effects on human health and well-being, as well as the lives of those who rely on agriculture for a living, including farmers and whole communities. In addition, increasing temperatures generate more wildfires, resulting in lower agricultural yields, higher food prices, and food shortages, as well as a rise in the number of wildfires. When it came to dealing with drought stress, genetic engineering of transgenic or basic plant resistant genotypes was used as a substitute. However, these strategies have shown to be effective, albeit at a high price and requiring a significant amount of time and effort. The use of helpful microorganisms has emerged as a better and more effective approach to this issue. Bacteria that promote plant development have developed a variety of drought-tolerance tactics. These include alterations to phytohormones, antioxidant defence, and exopolysaccharides and osmolytes synthesis. Improved crop output, more food availability, and lower food prices may all be achieved via a better understanding of how beneficial bacteria fight drought stress in plants. Agriculture can benefit from this knowledge as well.

III. OBJECTIVES OF THE STUDY

- To studyG. diazotrophicus and Herbaspirillum sp. immunodetection in micropropagated plants
- To analysis the Without N fertilisation, G. diazotrophicus and Herbaspirillum sp. flourished, as did plants that received the authorised quantity of inorganic N (140 kg ha-1) (280 kg ha-1).

IV. MATERIALS AND METHODS

4.1 Inoculation of micropropagated plants

According to Sreenivasan and Sreenivasan's findings, the apical meristems of cv. Co 86032 were used in the process of producing micropropagated sugarcane seedlings (1984). To facilitate the development of shoots from apical meristems and subsequent shoot and root multiplication, an adjustment was made to the concentration of growth hormones present in the liquid MS medium. Inoculations were carried out in flasks that showed no signs of having been contaminated visually. Maceration of materials at a ratio of 1:9 weight-to-volume was used to test the sterility of callus and plantlets by plating them on nutritional agar (NA), potato dextrose agar (PDA), and LGI P agar, respectively (100 mg l-1 yeast extract). The discarded plant debris was removed from the premises. The multiplication process was carried out in test tubes with a volume of 50 ml, each of which contained 20 ml of MS liquid media that had been diluted by one tenth and included plants that were 75 days old. Before inoculation, strains of Herbaspirillum sp. and G. diazotrophicus were grown in dilute MS liquid medium for 48 hours. This produced 0.1 ml of suspension that contained 108 cells per millilitre. The infected plants flourished in an environment with a temperature of 287.1 degrees Celsius and a light intensity of 60 mmol m-2 s-2 for a period of 15 days.

4.2 G. diazotrophicus and Herbaspirillum sp. immunodetection in plants

In line with the procedures that James et al. described, a polyclonal anti-G. diazotrophicus antibody was generated and used to test it against the microorganisms that were used in this study (1994). After being grown in log-phase cultures in a centrifuge and rinsed in normal saline, both strains of SYP were resuspended and killed with heat before being injected into rabbits. The antibody was developed by Bangalore Genei, which is located in Bangalore, India. The antisera of G. diazotrophicus and Herbaspirillum sp. were examined using ELISA against Azospirillum spp. and Pseudomonas spp., which generated no reaction but displayed reactivity against these two bacteria, as stated by Li and MacRae (1992). Both the serial dilution method and the heat death method were used in order to eradicate the cultures that were utilised in the inoculation research, as well as the plant roots and leaves that were either infected or not inoculated. Nitrocellulose membranes were stained with different dilutions of anti-G. diazotrophicus and anti-Herbaspirillum sp. antibodies. These antibodies were employed to stain the membranes.

After first blocking the membranes with a solution containing 3 percent milk, the membranes were then treated with primary antisera against G. diazotrophicus and Herbaspirillum sp., followed by a goat anti-rabbit IgG (secondary antibody) alkaline phosphatase conjugate. In order to see the specific antigen–antibody combination on the membrane, the salts of 5-bromo-4-chloro-3-indolylphosphate (BCIP) and nitroblue tetrazolium chloride (NBT) were added to the reactions as substrates. This allowed for the visualisation of the specific antigen–antibody pairing. As was mentioned before, collected samples were also put through an immunodetection process. This was done in order to confirm the results of the preceding statement. After establishing by immunodetection that the micropropagated sugarcane seedlings had been colonised, we began the process of hardening them by providing them with sterile composted agricultural wastes and vermiculite. These two components made up the majority of the medium. Non-sterile soil (loamy, pH-7.5, EC-0.07 m mhos cm-1; accessible nutrients in mg kg-1; N–31, P–12.5, K–100, and organic carbon–0.18 percent) was transferred to plastic containers (L B H 14 60 30 30 cm) with a total of 40 kilogrammes of non-sterile soil. The dimensions of the plastic containers were as follows: In the first set of experiments that were carried out, G. diazotrophicus and Herbaspir illum sp were examined both on their own and in conjunction with one another. Before the plants were harvested, they had reached up to 45 days of growth without the use of fertiliser. In the first experiment, G. diazotrophicus and Herbaspirillum sp. were infected separately and in combination without N-fertilization. In the second experiment, they were also injected with 50 percent of the needed quantity of N. (140 kg N ha-1).

The first control had the correct quantity of nitrogen applied (280 kg N ha-1), the second control had nitrogen delivered at a rate that was 50 percent lower (140 kg N ha-1), and the third control had uninoculated plants with no nitrogen applied. The status quo was preserved for all three uninoculated controls. The requisite rates of phosphate and potassium, 65 and 115 kg ha-1, were applied across the board for all treatments. Following planting in soils contained in containers, the chemical fertilisers were applied in three distinct doses at 30, 60, and 90 days following the initial planting. The MPN method was used to count the number of Herbaspirillum species in duplicate vials containing N-free semisolid LGIP and JNFb medium. The total nitrogen content of the leaves was measured once every 30 days during the first 180 days after the plant was planted. After being sliced, dried in an oven at 80.1 degrees Celsius for 48 hours, digested, and analysed using Kjel-tech equipment for total nitrogen concentration, the fourth leaf from each treatment was examined. After a total of 180 days of growth, the plants were dug up and their weight was determined by weighing the individual roots. After doing an

ANOVA on the data, the Duncan's multiple range test was used in order to evaluate the significance of the differences between the means. For all of the statistical analyses that were performed for this study, a statistician's statistical package that was produced by FIPPAT was used.

V. RESULTS

Through the process of micropropagation, we were able to develop sugarcane plantlets in only 105 days after starting with a callus. None of the plantlets that were checked for the presence of bacteria contained bacteria. Sugarcane micropropagated plantlets of the cultivar Co 86032 that were grown in diluted (1/10) MS liquid medium for the purpose of inoculation were colonised by the fungi G. diazotrophicus and Herbaspirillum sp. Both G. diazotrophicus and Herbaspirillum sp. were found to be continuously present in the plant tissue samples that were analysed. Polyclonal antibodies produced against G. diazotrophicus and Herbaspirillumsp. sp. did not respond to any other bacteria that were tested, regardless of the dilution of the polyclonal antibodies that were used in the experiment. The pre-immune serum that was used as the primary antibody did not have any reactions with any of the microorganisms that were tested, and this included G. diazotrophicus and Herbaspirillum sp. In the tissues of the plants that were subjected to this test, inoculation bacteria of the species G. diazotrophicus and Herbaspirillum sp. were discovered. The increase in plant biomass that occurred after planting G. diazotrophicus and Herbaspirillum sp. in non-sterile soil for 45 days and then harvesting them was seen when the plants were later harvested (Fig. 2).

Even if they were administered one at a time, there was still a noticeable influence on the organism's capacity for growth (Table 1). In the short-term experiment, there were 106 cells of G. diazotrophicus (g-1 fresh tissue), as well as cells belonging to Herbaspirillum sp. (g-1 fresh tissue) (see Table 1). The second experiment, which lasted for a total of 180 days, indicated that the diazotrophic populations in the sugarcane roots were greater than those in the plant's aerial sections (Table 2). Herbaspirillum sp. counts in the crop were basically stable at around 105 cells per gramme during much of the harvest (g 1 fresh wt). There was a similar amount of G. diazotrophicus cells up to 90 days (104–105 cells g-1 fresh weight), but after that, their numbers started to decrease. It was discovered that the plant samples that had not been injected had more than 104 diazotrophic bacteria (g-1 fresh tissue). plants that had been infected by the bacteria G. diazotrophicus and Herbaspirillum sp. had a higher nitrogen content and a greater biomass than plants that had been fertilised with the required quantity of nitrogen (280 kg N ha-1) but had not been inoculated by these bacteria (there was no additional inorganic N) (Table 3).

Figure 2 Inoculation impact of G. diazotrophicus and Herbaspirillum sp. after 45 days of development on the micro-propagated sugarcane plants Herbaspirillum sp., G. diazotrophicus, G. diazotrophicus and an uninoculated plant (T4).

Table 1. When sugarcane plants are inoculated with G. diazotrophicus (Gd) and Herbaspirillum sp. (Hsp) 45 days after planting, the resulting biomass of sugarcane variety Co 86032 is studied.

Treatment	Biomass(g^{-1} freshwt)[a]			BacterialpopulationsinLeaf[b](~10^6)	
	Root	Shoot	Biomass	Gd	Hsp
Gd	7.4b	3.56c	10.96b	1.21	—
Gd+Hsp	14.0a	11.76 a	25.76a	4.59	11.5
Hsp	7.6b	5.43b	13.13b	—	1.1 5
Uninoculated	5.1c	3.0c	8.10 c	—	—
$CD_{0.05}$	2.29	3.33	3.92		

Table 2. G. diazotrophicus (Gd) and Herbaspirillum sp. (Hsp) numbers in sugarcane varieties in long-term pot experiments for 180 days after planting in non-sterile soil

Treatment		Days after (bacterial numbers in leaves* 104 g-1 fresh wt)-1											
		60th		90th		120th		150th		180th			
		Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf		
N+		—	—	—	—	—	—	—	—	—	—		
Gd		11.5	2.0	11.5	4.59	7.35	2.12	2.12	1.15	2.12	2.12		
Hsp		11.5	4.59	11.5	7.35	11.5	11.5	11.5	11.5	11.5	11.5		
Gd+Hsp	Gd	11.5	4.59	11.5	11.5	11.5	7.35	4.59	4.59	4.59	2.12		
	Hsp	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5		
N/2		—	—	—	—	—	—	—	—	—	—		
N/2+Gd		11.5	4.59	11.5	4.59	7.35	7.35	4.59	2.12	2.12	2.12		
N/2 Hsp		11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5		
N/2+Gd+Hsp	Gd	11.5	7.35	11.5	11.5	7.35	7.35	4.59	4.59	4.59	2.12		
	Hsp	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5		
N—		—	—	—	—	—	—	—	—	—	—		

Table 3. G. diazotrophicus and Herbaspirillum sp. inoculation on sugarcane variety Co 86032 biomass and leaf N content after 180 days

Treatment	Biomass(kg/plant)[a]			LeafN[b]
	Shoot	Root	Total	(mgg^{-1}drywt)
N+	2.987a	0.276	3.263a	3.912a
Gd	2.290c	0.266	2.556b	3.623a
Hsp	1.913d	0.427	2.340c	3.384ab
Gd+Hsp	2.716a	0.630	3.346a	4.312a
N/2	1.983d	0.457	2.340c	3.460a
N/2+Gd	2.340b	0.560	2.900a	3.956a
N/2 Hsp	1.950d	0.140	2.090c	3.850a
N/2+Gd+Hsp	3.073a	0.322	3.395a	4.210a
N-	1.471e	0.105	1.576d	2.096c
CD0.05	3.2	NS	4.5	1.1

VI. CONCLUSION

The micropropagated sugarcane variety Co 86032 was found to have G. diazotrophicus and Herbaspirillum sp. established. Dot blot immunoassay verified this, as stated. For satisfactory inoculation and establishment of diazotrophs, diluted (1/10) MS liquid medium was adequate, as previously stated by Mun oz-Rojas and Caballer-Mellada (2003). In the presence of N2 fixing bacteria, sugarcane types exhibited improved growth. There was no evidence to support the theory that a single or group of diazotrophs directly transferred fixed nitrogen to their plant partner, resulting in sugarcane growth enhancement. G. diazotrophicus alone and with Herbaspirillum sp. resulted in increased biomass production in the first inoculation experiment, but there was no clear proof that these effects were related to BNF or the release of growth hormones by these bacteria. It has recently been observed that sugarcane plants inoculated with wild-type G. diazotrophicus grew better and contained more total N after 60 days under N-deficient circumstances than plants inoculated with nif– mutants or uninoculated plants. In the current study's short-term experiment, inoculation plants grew much faster than uninoculated plants. Probably due to N-supplementation and other hormonal effects of bacteria utilised in the research since these isolates were previously shown to release hormones considerably in laboratory circumstances.

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