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EVALUATION OF THE BIOLOGICAL NITROGEN FIXATION CONTRIBUTION IN SUGARCANE PLANTS ORIGINATED FROM SEEDS AND INOCULATED WITH NITROGEN-FIXING ENDOPHYTES

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ABSTRACT

The inoculation technique with endophytic diazotrophic bacteria in sugarcane has been shown as an alternative practice to plant growth promotion. The aim of this work was to evaluate the biological nitrogen fixation (BNF) contribution by different strains of *Herbaspirillum seropedicae* and *Gluconacetobacter diazotrophicus* in sugarcane plant inoculated from seeds. The seeds were planted in pots filled with non-sterile soil, inoculated with the bacterial strains and grown 10 months outside of the greenhouse. The BNF contribution of the inoculated bacteria varied depending on the plant species used as a control. The highest BNF contribution as well as the highest populations of reisolated bacteria was observed with inoculation of *H. seropedicae* strains. The roots appeared to be the preferential tissues for the establishment of the inoculated species.

Key words: endophytic diazotrophic bacteria, sugarcane, biological nitrogen fixation, *Herbaspirillum seropedicae*, *Gluconacetobacter diazotrophicus.*

INTRODUCTION

Nitrogen balance studies showed contributions of the biological nitrogen fixation (BNF) around 60% for some sugarcane varieties grown in large concrete tank planted with material originated from sets (5). On the other hand, studies with micropropagated sugarcane plants grown in smaller pots showed that the inoculation of endophytic diazotrophic bacteria could contributed with up to 30% of the total N present in the plants (3). Endophytic diazotrophic bacteria inoculated in seed born sugarcane contributed significantly to increase the root system (2). In this work, the BNF contribution by different strains of endophytic diazotrophic bacteria was tested, envisaging a fast and accurate methodology to select plant growth promoting bacteria to be used in sugarcane crop.

MATERIALS AND METHODS

Five different strains from Diazotrophic Bacterial Collection (DBC) were used to inoculate seeds of sugarcane (Table 1). The bacterial strains were grown overnight in Dyg´s liquid media, and approximately 10^8 cells mL^{-1} were introduced directly over the sugarcane seeds (SP 701143 x Co 421 breeding cross), sown in the pots filled with 50 kg of unsterile soil (A horizon of an Ultisol). The experimental design utilized was randomized complete with 4 replicates. The plant species *Sorghum bicolor* and a *Pennisetum* hybrid were used as control plants to BNF quantification, using the $\delta^{15}N$-isotope analysis with the following formula:

$$\%BNF = 100 \times \frac{(\delta^{15}N \text{ control plant} - \delta^{15}N \text{ test plant})}{(\delta^{15}N \text{ control plant})}$$

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Table 1. Bacterial strains used in this study.

Strain	Specie	DBC code	Isolation source	Origin
3R-2	*G. diazotrophicus*	BR 11509	RB 739359 roots	CNPAB/RJ
PAL5	*G. diazotrophicus*	BR 11281	*Saccharum* spp. roots	Alagoas
HRC54	*H. seropedicae*	BR 11335	CB 45-3 roots	CNPAB/RJ
HRC50	*H. seropedicae*	BR 11380	SP 701143 roots	CNPAB/RJ
HCC101	*H. seropedicae*	BR 11510	SP 701284 stems	CNPAB/RJ

The bacterial counting were carried out after 9 month using the Most Probable Number (MPN) technique, using the semi-solid JNFb and LGI-Pcaldo media to reisolate the inoculated *H. seropedicae* and *G. diazotrophicus*, respectively. Statistical analysis was runned with the SisVar 4.3 variance analysis software.

RESULTS

The highest total dry matter was observed in plants inoculated with the HRC50, 3R-2 and HRC54 strains (Fig. 1). These values were significantly higher than that presented in uninoculated plants, but significantly lower than the total dry matter accumulated in nitrogen fertilised plants.

The highest bacteria population was also observed in the plants inoculated with strains of the *Herbaspirillum* genus (1.0 10^6 cell g^{-1} fresh tissue). In addition, the highest populations were related to the root tissues as compared to stem tissues for all the inoculated strains (Table 2). The only exception was 3R-2 strain which presented higher population in the stem as compared to the root tissues. Endophytic bacteria of the genus *Herbaspirillum* were also observed in the uninoculated plants, but in lower numbers than the inoculated ones.

The BNF contribution to sugarcane plants varied accordingly to the plant species used as a control. The values varied from 7.95 to 28.28% when the *Pennisetum* hybrid was used as a control (Table 3). A much higher BNF contribution (39.05 to 52.51%) was observed using the *S. bicolor*. The highest BNF contribution was observed with the inoculation of the *H. seropedicae* HCC101, while the lowest efficient strain was the *G. diazotrophicus* PAL5.

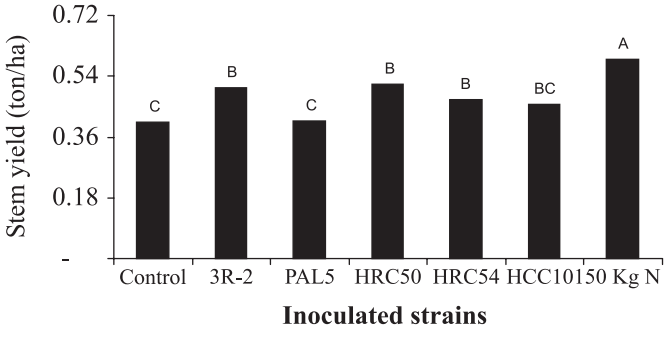

Figure 1. Total dry matter accumulated in sugarcane plants from seeds inoculated with endophytic diazotrophic bacteria. Same letters do not differ statistically by LSD test at 5% of confidence. Means of 4 pots. CV: 9.11%.

Table 2. MPN counting of endophytic bacteria present in plant tissues evaluated after 9 months of growth in pots outside greenhouse. Means of 4 replicates.

Strains	Culture	Log of the n. of cells/g fresh weight	
	media	Roots	Stems
Uninoculated	JNFb	$1.32\ 10^3$	$1.34\ 10^3$
Uninoculated +	JNFb	$3.63\ 10^3$	$9.77\ 10^2$
HRC54	JNFb	$1.00\ 10^6$	$8.51\ 10^4$
HRC50	JNFb	$9.33\ 10^5$	$7.09\ 10^4$
HCC101	JNFb	$7.94\ 10^5$	$3.38\ 10^4$
PAL5	LGI-Pcaldo	$6.45\ 10^4$	$2.81\ 10^4$
3R-2	LGI-Pcaldo	$7.24\ 10^4$	$1.38\ 10^5$

Table 3. Evaluation of the nitrogen fixing endophytes contributions to sugarcane plants originated from seeds using the $\delta^{15}N$-isotope technique.

Treatments	$\delta^{15}N$	BNF contribution (% of total N)* Control plants	
		Pennisetum	*S. bicolor*
Control	1,823	7.95	39.05
3R-2	1,770	10.61	40.80
PAL5	1,820	8.08	39.13
HRC50	1,718	13.26	42.56
HRC54	1,478	25.38	50.59
HCC101	1,420	28.28	52.51

* Significant at LSD test with 95% of confidence, obtained by comparison of $\delta^{15}N$ values of inoculated plants with $\delta^{15}N$ values of control plants.

DISCUSSION

In general, nitrogen fixing endophytic populations in grasses has been identified associated to roots (1). Our results also showed much higher population in the roots, suggesting that roots are the preferential sites for colonization and activity for such bacteria. In addition, the results showed that the BNF contribution varied with the endophytic bacteria strains inoculated and the plant used as a control to calculate the nitrogen derived from the nitrogen fixation. Studies on BNF contribution to sugarcane plants naturally grown in the field and evaluated with the same $\delta^{15}N$ technique also varied according to control plant used (4). The BNF contribution of the seed-borne inoculated sugarcane plants, calculated based on the *Pennisetum* $\delta^{15}N$ values, was quite similar to that observed for micropropagated sugarcane plants inoculated with a mixture of five diazotrophic bacteria species and measured with the ^{15}N-isotope dilution technique (3). Although it is still preliminary, the inoculation of sugarcane seeds with endophytic diazotrophic bacteria seems to be a good methodology to introduce selected strains envisaging growth promoting and nitrogen fixation in sugarcane plants during the initial steps of the breeding programs. Nevertheless, field grown experiments must be carried out to confirm these results.

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RESUMO

Avaliação da contribuição da fixação biológica de nitrogênio em cana-de-açúcar originada de sementes e inoculada com endófitos fixadores de nitrogênio

A técnica de inoculação com bactérias diazotróficas endofíticas na cana-de-açúcar apresenta-se como uma prática alternativa para promover o crescimento vegetal menos dependente da adubação nitrogenada. Este trabalho teve como objetivo avaliar a contribuição da fixação biológica de nitrogênio (FBN) por diferentes estirpes de *Herbaspirillum seropedicae* e *Gluconacetobacter diazotrophicus* inoculadas em plantas de cana-de-açúcar originadas de semente. As sementes foram plantadas em vasos com solo não estéril, inoculadas com as diferentes bactérias e mantidas por 10 meses ao ar livre. As maiores contribuições da FBN ocorreram com a inoculação de estirpes *Herbaspirillum seropedicae*, e dependeram da espécie vegetal utilizada como testemunha. As raízes apresentaram-se como o órgão vegetal preferencial para o estabelecimento das espécies inoculadas.

Palavras-chave: bactérias diazotróficas endofíticas, cana-de-açúcar, fixação biológica de nitrogênio, *Herbaspirillum seropedicae*, *Gluconacetobacter diazotrophicus.*

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