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EVALUATION OF PERFORMANCE OF SUGARCANE GENOTYPES INOCULATED WITH ENDOPHYTIC DIAZOTROPHIC BACTERIA

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Abstract

The present experiment was aimed to evaluate the response of sugarcane varieties inoculated with endophytic diazotrophic bacteria. Sixteen sugarcane varieties were used for three kind of treatments (no inoculation and inoculation with 120 kg N/hectare). The inoculant was a mixture of five strains of bacterial species isolated from sugarcane. There were significant differences between genotypes (G) and genotype x treatment interaction (GxT) for all the traits. The variety SP 71-6949, SP 78-4764 and RB 867515 were found responsive to biological nitrogen fixation and can be used in breeding programs which are directed to study and selection of promising genotypes responsible for the biological nitrogen fixation.

Key words: Biological nitrogen fixation; sugarcane; genotypic performance.

INTRODUCTION

Brazil produces about 624.99 million tons of sugarcane, of which 336.2 million tons (53.8%) utilized in the production of 27.7 billion of liters of ethanol and the remaining, 288.7 million tons (46.2%) was used to produce sugar (CONAB 2010). The sugarcane is the main source of energy (biofuel). The great advantage of sugarcane for the production of biofuel is the positive energetic balance. In Brazil this balance is approximately +9, which means ,for every unit of energy consumed in the production process, nine units are generated (Macedo and Koller 1997). This highly positive balance can be attributed to the cultural yields with low nitrogen fertilization (Urquiaga et al. 1992). In other productive countries like USA, Australia, Cuba, Venezuela and Peru, the nitrogen fertilization varies between 200 to 400 kg/ha (Xavier 2006; Boddey et al. 1995), resulting in an energetic balance that rarely achieves + 1 (Azeredo et al. 1986).

The sugarcane improvement in Brazil have resulted in low nitrogen fertilizer inputs of about 60 kg/ha because of genotypes that rarely respond significantly to nitrogen nutrient (Azeredo et al. 1986). These types of studies help to observe that there might be some genotypes which express their potential for the biological nitrogen fixation (BNF) associated with diazothrophic bacteria. Many authors have studied different genotypes in the presence of diazothrophic bacteria which are responsible for the biological nitrogen fixation in sugarcane (Boddey et al. 2001; Xavier 2002; Baldani et al. 2002; Boddey et al. 2003; Baldani and Baldani 2005, Nogueira et al. 2005; Oliveira et al. 2006). However, various factors can be involved in the efficiency of the biological nitrogen fixation in the sugarcane culture. Because its a biological process which depends on the interaction between the microorganisms, plants and environment. To express the fixation potential, it is necessary that all these three components be in good conditions, or the microorganisms involved be genetically favorable for this interaction and the genotypes be responsive to the inoculation and the environment. The objective of this work was to evaluate the growth characteristics of different sugarcane varieties subjected to the inoculation with endophytic diazotrophic bacteria.

MATERIAL AND METHODS

The experiment was conducted during the cropping year of 2010/2011 in the farm area of Triunfo Mill, Atalaia, State of Alagoas (Brazil). The experiment was conducted in randomized block design with three replications in a factorial scheme (16 x 3) comprized of total 48 treatments. The treatments were composed of 16 cultivar in combination with inoculant, without inoculant and with nitrogenous fertilization (120 kg of Nitrogen). Every plot had six rows of 5 m separated by 1.40 m row to row space. The fertilizer, excepting nitrogen, was applied according to soil analysis. The mixture of endophytic diazotrophic bacteria (inoculants) supplied by Embrapa Agrobiology, was used before the planting. The inoculants contained a mixture of bacteria, *viz.*,

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Gluconacetobacter diazotrophicus, Herbaspirillum seropedicae, H. rubrisubalbicans, Azospirillum amazonense e *Burkholderia tropica*. Microbial inoculants was mixed with 100 liters of water and the setts used in planting were immersed in this mixture for a period of 1 h and planted immediately. After seven and nine months of planting, the field evaluations were made to record the following data: the average stalk height; average stalk diameter (at the height of 1/3 from the base with the help of a paquímeter); leaf length; leaf width; number of dried leaves; The number green leaves and Chlorophyll content (determined by indirect method using SPAD 502 Chlorophyll meter machine, Minolta Corporation, Ramsey, Japan). The averages values were obtained from 10 replicates per leaf (+3 leaf). The statistical analyses were performed by using Genes Program.

RESULTS AND DISCUSSION

Significant differences ($p < 0.001$) were observed amongst genotypes and treatments which indicated the presence and genetic variability for all traits. No significant difference observed between seasons (Table 1). This variability reflected the heterogeneity of the genetic material, and indicated the possibility of identifying promising genotypes responsible for the biological nitrogen fixation. The variation coefficient (VC) ranged from from 6.33 to 14.66, and showed good experimental precision in the determination of growth characteristics as in plant height (PH), diameter of stem (DS), leaf length (LL), width of leaf (WL), number of dried leaves (NDL), number of green leaves (NGL), and chlorophyll content (CC). Table 2 showed the estimated average of genotypes in each treatment. The varieties 1, 2 and 7 inoculated with endophytic diazotrophic bacteria showed a higher average when compared to the same variety with other treatments (non-inoculated and nitrogen) for these characteristics, this reflected the responsive behavior of these genotypes to the biological fixation of nitrogen. Varieties 10, 14 and 15 showed the best performance and the highest values for the same characteristics, and varieties 15 and 16 showed good development in growth when they were not subjected to treatment of inoculation and nitrogen application. According to the reports of Ferreira at al. (2007), DS and PH features are positively correlated with productivity and tons per hectare (TCH). Plants with high values of height and diameter can be suggested as productive plants. The varieties 1, 2 and 7 subjected to inoculation showed high mean values of plant height and diameter of the stem. LL, DS and PH features were linked to the growth, and the development of the plants was directly related to the stimulus provided by plant hormones. Several diazotrophic bacteria are able to produce phytohormones responsible for the observed stimulatory effect on plant growth. Bastian et al. (1998) detected the presence of IAA and gibberellins A1 and A3 in cultures of *Herbaspirillum seropedicae*. Similar results were obtained by Fuentes-Ramirez et al. (1993) evaluating the IAA production by *Gluconacetobacter diazotrophicus* strains. Differences between sugarcane varieties and the potential for nitrogen fixation were studied by Coelho et al. (2003), who reported that the BNF was observed in all commercial sugarcane genotypes evaluated (RB739735, SP79-2313, RB72454, RB758540, RB835089, RB825336 and SP79-2312), and also in wild accessions, Krakatau (*S . spontaneum*) and Chunnee (*S. barberi*). In this study, the greatest contributions of BNF were observed in varieties RB739735, RB758540, RB835089 and SP79-2312 varieties. Boddey et al. (2003) stated that the capacity for N_2 fixation greatly depend on the sugarcane variety. In the study conducted by Polidoro et al. (2001), the RB72454 and SP80-1842 commercial sugarcane varieties showed high potential for BNF. Therefore, accounting for the N from biological nitrogen fixation and the differential response in each particular genotype in plant breeding programs is still an issue which should be thoroughly studied especially with regards to the sugarcane culture. Such studies are important to understand the genotype, environmental and microorganism interaction, because bacteria present

Table 1. Analysis of variance for seven characteristics evaluated [Plant height (PH); Diameter of the stem (DS); Length of the leaf (LL); Width of the leaf (WL); Number of dead leaves (NDL); Number of live leaves (NLL) and Chlorophyll content (CC)] at the seventh and ninth months of age.

		MS						
VF	DF	PH (cm)	DS (dm)	LL (cm)	WL (cm)	NDL	NLL	CT
Genotype (G)	15	2361.79**	34.49**	1272.41**	1.61**	6.12**	1.78**	94.20**
Season (S)	1	226499.55**	30.65NS	272.87NS	56.94**	932.13**	84.95**	17732.43**
Treatment (T)	2	224.03NS	5.65NS	268.55NS	1.02NS	3.04NS	0.47NS	5.32NS
G x S	15	134.43NS	2.14NS	141.84**	0.33**	3.31*	0.77*	66.86**
G x T	30	244.41*	4.87*	134.12**	0.14NS	2.27NS	0.45NS	20.77*
S x T	2	111.58NS	0.28NS	14.14NS	0.01NS	1.32NS	0.14NS	44.69NS
G x S x T	30	49.87NS	1.72NS	34.53NS	0.13NS	0.81NS	0.23NS	16.45NS
Resíduo	180	139.71	3.14	58.49	0.13	1.68	0.38	13.53
Average		119.95	26.22	120.75	3.33	8.86	5.16	34.46
Coefficient of Variation (%)		9.85	6.76	6.33	11.15	14.66	12.01	10.67

Table 2. Estimated measurements of 16 sugarcane genotypes referring to the characteristics of Plant height (PH); Diameter of the stem (DS); Length of the leaf (LL); Width of the leaf (WL); Number of dead leaves (NDL); Number of live leaves (NLL) and Chlorophyll content (CC) evaluated at the seventh and ninth months of age

Genotype[1/]	Treatament	PH (cm)	DS (dm)	LL (cm)	WL (cm)	NDL	NLL	CT
1	Not-inoculation	127.25	25.11	117.62	3.64	9.16	5.25	33.07
1	Inoculation	143.25	26.11	117.58	3.81	10.45	5.58	33.36
1	Nitrogen	127.33	25.14	104.58	4.16	8.04	5.25	32.71
2	Not-inoculation	137.83	24.75	120.00	3.24	8.91	5.29	35.33
2	Inoculation	150.37	25.11	126.08	3.31	8.91	5.79	37.22
2	Nitrogen	126.33	25.00	116.87	3.38	9.08	4.87	33.78
3	Not-inoculation	118.91	29.43	127.61	3.89	9.36	4.65	34.24
3	Inoculation	112.87	27.81	128.21	3.32	7.91	5.37	35.03
3	Nitrogen	101.54	27.28	120.16	3.67	8.37	4.62	37.79
4	Not-inoculation	137.41	24.82	127.44	3.20	9.72	5.11	36.52
4	Inoculation	125.87	26.48	128.37	3.26	8.83	5.12	37.09
4	Nitrogen	132.79	24.94	125.50	3.28	8.87	4.91	35.96
5	Not-inoculation	104.41	24.32	119.08	2.68	9.20	4.91	36.94
5	Inoculation	100.47	25.40	109.87	2.66	9.77	5.04	35.77
5	Nitrogen	114.21	25.64	121.50	3.14	8.54	4.58	33.94
6	Not-inoculation	107.83	24.70	111.08	3.24	9.33	4.83	36.55
6	Inoculation	99.95	23.71	110.41	3.33	9.20	5.37	38.82
6	Nitrogen	105.91	25.46	106.50	3.73	9.95	4.71	35.86
7	Not-inoculation	129.58	27.05	127.79	3.52	9.37	5.04	32.07
7	Inoculation	141.12	25.36	134.08	3.42	9.54	5.04	33.75
7	Nitrogen	139.21	25.90	128.29	3.77	8.87	4.87	32.13
8	Not-inoculation	125.33	27.91	117.04	3.32	9.54	6.15	34.22
8	Inoculation	124.33	29.47	115.37	3.44	9.41	5.91	32.65
8	Nitrogen	128.41	27.95	114.33	3.47	8.91	5.62	32.83
9	Not-inoculation	130.58	23.57	121.16	3.74	8.87	5.20	30.71
9	Inoculation	123.54	25.03	110.91	3.40	9.16	4.87	33.21
9	Nitrogen	122.33	24.81	111.62	3.52	9.00	5.37	30.25
10	Not-inoculation	117.83	25.63	135.58	3.27	10.25	5.37	38.42
10	Inoculation	109.51	24.66	133.76	3.17	9.50	4.70	37.14
10	Nitrogen	111.00	26.40	130.12	3.53	9.16	5.29	41.28
11	Not-inoculation	109.33	26.49	114.00	2.61	7.83	5.58	38.42
11	Inoculation	103.87	28.65	111.12	2.73	8.45	5.34	33.75
11	Nitrogen	112.62	26.77	120.50	2.77	8.21	5.87	35.88
12	Not-inoculation	124.91	26.69	116.58	3.05	8.66	5.66	31.84
12	Inoculation	119.70	28.37	105.37	2.80	7.50	5.16	29.62
12	Nitrogen	120.83	26.99	110.16	2.95	7.87	5.08	28.72
13	Not-inoculation	114.45	23.15	133.87	2.98	8.71	4.62	31.72
13	Inoculation	111.33	25.47	119.25	3.29	8.37	5.04	29.12
13	Nitrogen	124.16	25.58	134.08	3.49	8.83	5.08	36.61
14	Not-inoculation	115.75	25.46	142.12	3.31	8.08	4.87	35.23
14	Inoculation	105.45	27.19	137.25	3.31	5.91	5.16	32.83
14	Nitrogen	114.95	26.07	131.46	3.49	8.37	4.91	34.20
15	Not-inoculation	133.37	29.01	117.70	3.67	8.87	5.62	34.18
15	Inoculation	120.54	26.89	114.29	3.35	9.12	5.25	35.29
15	Nitrogen	128.41	27.04	120.33	3.46	9.29	5.55	31.66
16	Not-inoculation	109.21	28.34	114.29	3.39	9.21	4.79	35.73
16	Inoculation	102.91	28.19	114.12	3.38	7.91	4.62	33.02
16	Nitrogen	108.41	27.45	121.21	3.33	8.95	4.66	37.54

1/ Sugarcane varieties: 1- SP 71-6949; 2-SP 78-4764; 3-SP 79-1011; 4-SP 81-3250; 5-RB 72 454; 6-RB 845210; 7-RB 867515; 8-RB863129; 9-RB 92579; 10-RB 93509; 11-RB 931003; 12-RB 951541; 13-RB 98710; 14-RB 98395; 15-VAT 90-186; 16-VAT 90-212.

in the inoculant have a direct influence on the growth components and are directly related to sugarcane productivity.

CONCLUSIONS

The sugarcane varieties SP 71-6949, SP 78-4764 and RB 867 515were responsible for the biological nitrogen fixation and may be utilized in breeding programs made to the selection of promising genotypes fixing biological nitrogen.

REFERENCES

Azerdo DF, Bolsanello J, Weber H, Vieira JR (1986) Nitrogênio em cana-planta - doses e fracionamento. STAB, Piracicaba, 6(5): 26-33

Baladani JI, Reis VM, Baladani VLD, Dobereiner JA (2002). A brief story of nitrogen fixation in sugarcane – reasons for sucess in Brasil. Funct. Plant. Biol., 29: 417-423.

Baladani JI, B Baladani VLD (2005). History on the biological nitrogen fixation research in graminaceous plants: special emphasis on the brazilian experience. An Acad. ras. Cienc., 77, (3): 549-579.

Bastian F, Cohen A, Piccioli C, Luna V, Baraldi R, Bottini R (1998). Producion of indol-3-acetic acid and gibberellins A1 and A3 by Acetobacter diazotroficus and Herbaspirillum seropedicae in chemically defined culture media, Plant Growth Regul, 24: 7-11

Boddley RM, PolidoroJC, Resende AS, Alves BJR, Urquiaga S (2001). Use of the 15N natural abundance technique for the quantification of the contribution of N2 fixation to sugarcane and other grasses. Aust. J. Plant Physiol., (28): 889-895.

Boddey RM, Urquiaga S, Alves JRA, Reis VM (2003). Endophytic nitrogen fixation in sugarcane: present knowledge and future applications. Plant and Soil, 252: 139-149

CONAB (2010). Acompanhamento da safra brasileira de cana-de-açúcar safra 2010/terceiro levantamento, dezembro/2010. Brasília: Companhia Nacional de Abastecimento. 18 p.

Fuentes-Ramirez LE, Jimenez-Salgado T, Abarca-Ocampo IR, Cabalero-Mellado J (1993). Acetobacter diazotrophicus, an indole-acetic producing bacterium isolated from sugarcane cultivars of Mexico. Plant and Soil, 154: 145-150

Ferreira FM, Barros WS, Silva FL, Barbosa MHP; Cruz CD, Bastos IT (2007). Relações fenotípicas e genotípicas entre componentes de produção em cana-de-açúcar. Bragantia, 66(4): 605-610

Macedo IC, Koller HW (1997). Balanço de energia na produção de cana-de-açúcar e álcool nas usinas cooperadas em 1996. International Report, Centro Tecnológico da Copersucar Piracicaba, pp23

Nogueira EM, Olivares FL, Japiassu JC, Vilar C, Vinagre F, Balsani JI, Henemy AS (2005). Plant Science, 169: 819-832.

Polidoro JC, Resende S, Quesada DM, Xavier RP, Coelho CHM, Alves BJR, Boddey RM, Urquiaga S (2001). Levantamento da contribuição da fixação biológica de nitrogênio para a cultura da cana-de-açúcar no Brasil. Seropédica: Embrapa Agrobiologia, dez. (Embrapa Agrobiologia. Documentos, 144), pp8

Scott A, Knott M (1974). Cluster-analysis method for grouping means in analysis of variance. Biometrics, Washington D.C., 30 (3): 507-512

Urquiga S, Cruz KHS, Boddey RM (1992). Contribution of nitrogen fixation to sugar cane: Nitrogen-15 and nitrogen balance estimates. Soil Science Society America Journal, 56(1): 105-114

Xavier RP (2006). Contribuição da fixação biológica de nitrogênio na produção sustentável da cultura de cana-de-açúcar. Tese (Doutorado em Ciência do Solo) - Universidade Federal Rural do Rio de Janeiro, Seropédica, 2006, pp75.

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