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Growth analysis of sugarcane inoculated with diazotrophic bacteria and nitrogen fertilization

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The use of inoculants containing growth-promoting diazotrophic bacteria can stimulate mass and nutrient accumulation in sugarcane. The purpose of this study was to evaluate plant growth and accumulation of macroelements in sugarcane, variety RB92579, under bacterial inoculation with or without N fertilization. The field experiment was carried out in a Red-Yellow Podzolic soil in Seropédica, RJ, in a randomized block design with four replications. The treatments consisted of 50 kg N ha^{-1}; 50 kg N ha^{-1} + inoculation; inoculation; and an absolute control. The following bacteria were inoculated: *Gluconacetobacter diazotrophicus*, *Herbaspirillum seropedicae*, *Herbaspirillum rubrisubalbicans*, *Azospirillum amazonense,* and *Burkholderia tropica.* The plants were sampled at 100, 130, 168, 212, 261, and 295 days after planting (DAP), and growth and nutrient accumulation rates were estimated by functional analysis of plant growth. Nutrient accumulation rates were highest around 180 DAP for N and P, and around 160 DAP for K, in the different treatments, preceding the maximal crop growth rate (between 210 and 220 DAP). The accumulation of biomass, N, P and K was greater and crop growth rates were higher in the treatments with bacterial inoculation fertilized or not with 50 kg N ha^{-1}, compared with the control.

Key words: *Saccharum* species, inoculum, growth promotion.

INTRODUCTION

The release of new sugarcane varieties contributed to this increase in cultivated area and productivity. The selection of varieties was based on various parameters related to growth patterns, e.g., shoot dry matter accumulation throughout the cycle. In the case of sugarcane, this pattern is represented by a sigmoid curve which shows an initial phase of slow growth followed by an exponential growth phase and finally maturation, when growth becomes slow and eventually stagnant (Oliveira et al., 2010).

Nitrogen is one of the most required nutrients for sugarcane growth, along with potassium. The N fertilizer rates used on Brazilian fields are on average 40 kg ha^{-1} N for plant cane and 80 kg ha^{-1} N for ratoon crops (Nunes
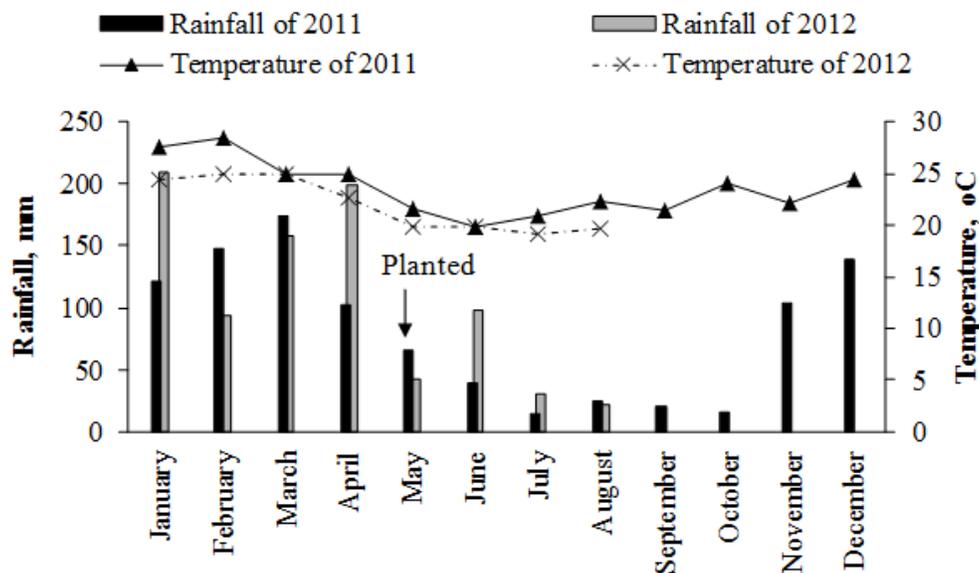
Figure 1. Accumulated precipitation and average monthly temperature from January 2011 to August 2012. Source: Instituto Nacional de Meteorologia. Estação Pesagro, RJ.

et al., 2005). However, the low use efficiency of N fertilizer in commercial sugarcane crops (Trivelin et al., 2002), calling for an increase in the efficiency of these fertilizers is a challenge to improve the profitability and sustainability of this crop.

The use of organic inputs in agriculture has increased in recent decades. Among the microorganisms that can stimulate plant growth, the growth-promoting diazotrophic bacteria have been the subject of several studies. These bacteria can contribute to plant N metabolism in different ways: aside from the proper fixation of atmospheric N_2, they can modify nutrient uptake from the soil, indirectly, by increasing the root system, or directly, by stimulating the N transport system of plants (Spaepen et al., 2007). Bacteria isolated from the rhizosphere have the particular capacity of producing growth regulators that stimulate the root development, resulting in greater soil exploitation and nutrient uptake (Dobbelaere et al., 1999). Studies indicate that sugarcane plants inoculated with bacteria of the genus *Azospirillum* may have a greater drought tolerance (Moutia et al., 2010), higher matric potential and leaf water content, as well as a lower canopy temperature than non-inoculated plants (Dobbelaere et al., 2003).

The quantitative analysis of plant growth is based on the evaluation of data obtained from sequential samplings to describe changes in dry matter production in function of time, by calculating growth rates. Its application has been extended to studies on nutrient uptake and utilization based on data from nutrient accumulation at different crop stages (Araújo and Rossiello, 2013). Growth analysis in sugarcane is a tool for assessing the sequential dry matter accumulation and

nutrients and relate them to environmental parameters such as irradiance, water availability, soil fertility, among others (Santos et al., 2009; Silva et al., 2012; Batista, 2013).

However, studies on the effect of diazotrophic inoculation using growth analysis in sugarcane are scarce. In order to evaluate the effect of nitrogen and diazotrophic inoculation, growth rate parameters were tested using the Brazilian variety RB92579. This genotype was selected for all production evironments since yield levels are good under the different soil-climate conditions of Brazil (RIDESA 2003). It is an important variety nowadays and is been planted in most of the sugarcane regions.

Based on these potential beneficial effects of the application of organic inputs in sugarcane, this study aimed to evaluate biomass and macroelements accumulation in sugarcane variety RB92579 throughout the early growth stages plant cane cycle under field conditions, when subjected to application of microbial inoculants in the presence or absence of nitrogen fertilization.

MATERIALS AND METHODS

Site location and characterization

The experiment was carried out on an experimental field of Embrapa Agrobiologia in Seropédica-RJ, in May 2011, in a Red-Yellow Podzolic soil. The climate is Aw, according to the Köppen classification, with hot, dry summers and wet winters, and an average annual temperature of 23.7°C. Climatic data of the experimental period were provided by the National Institute of Meteorology (INMET) (Figure 1). Prior to soil tilling, the soil

chemical properties in the 0 to 20 cm layer were analyzed with the following results: pH (H_2O) 5.7; 0.1 $cmol_c$ dm^{-3} Al; 2.7 $cmol_c$ dm^{-3} Ca; 1.3 $cmol_c$ dm^{-3} Mg; 45 mg L^{-1} K and 18 mg L^{-1} P. In the 20 to 40 cm layer: pH (H_2O) 5.3; 0.1 cmolc dm^{-3} Al; 2.0 $cmol_c$ dm^{-3} Ca; 0.8 $cmol_c$ dm^{-3} Mg; 26 mg L^{-1} K, and 5.5 mg L^{-1} P. Soil tillage consisted of plowing, harrowing and liming with 1 Mg ha^{-1} dolomitic limestone, incorporated by disking, followed by planting after 45 days. Planting fertilization was based on soil chemical analysis and crop nutrient requirements, as described by Rossetto et al. (2013). At the bottom of the furrow, 120 kg ha^{-1} P_2O_5 was applied as single superphosphate, 40 kg ha^{-1} of micronutrients as Fritted Trace Elements (FTE BR-12) and 0.4 kg ha^{-1} of ammonium molybdate at planting, and 160 kg ha^{-1} K_2O as potassium chloride, 50% at the bottom of the furrow at planting and 50% after 60 days. Nitrogen was fertilized at planting in a single application at the bottom of the planting furrow, in the form of urea.

The variety RB92579 used in the study has an upright growth habit, leaves with curved ends and wide limb, low flowering, a slow growth rate, average maturation, high sucrose content, as well as good sprouting, high tillering in plant cane, good canopy closure, and is equally suited for all production environments (RIDESA, 2003).

The experiment was arranged in a randomized block design with four replications, with four treatments: fertilization with 50 kg N ha^{-1} as urea, 50 kg N ha^{-1} + inoculation, inoculation, and a control with no N fertilization or inoculation. The experimental plot consisted of four 4 m long rows spaced 1.2 m apart. The seedlings for planting (cuttings with three buds) were inoculated in the field at planting, as described by Schultz et al. (2012).

Inoculation procedure

The inoculated diazotrophic bacteria species were *Gluconacetobacter diazotrophicus* (PAL5[T] = BR11281), *Azospirillum amazonense* (Cbamc = BR11145), *Herbaspirillum seropedicae* (HRC54 = BR11335), *Herbaspirillum rubrisubalbicans* (HCC103 = BR11504) and *Burkholderia tropica* (PPe8[T] = BR 11366), previously selected by Oliveira et al. (2003, 2006). To obtain the inoculum, bacteria from the collection of diazotrophic bacteria of Embrapa Agrobiologia (with initials BR) were subcultured on DYGS medium (Baldani et al., 2014). The inoculum solution was prepared by diluting 100 ml of medium containing 10^9 cells ml^{-1} in 100 L of clean water. All strains with this bacterial density were mixed at planting. Cuttings (plantlets) with three buds were placed in raffia bags according to the quantity required per crop row (15 buds per meter), immersed for 30 min in the inoculum suspension in 200 L containers and left to stand under natural shade for 60 min after inoculation.

Data analysis of growth rate

Six biomass samples were taken monthly from August 2011 to February 2012 (100, 130, 168, 212, 261, and 295 days after planting). At each evaluation, the plants growing along 1 m of the two center rows per plot were sampled. Within each evaluated plant row, an internal boundary of 0.5 m between each sampled meter was established, resulting in three samplings per row.

The leaf area was determined for the leaf blades on the representative stems of the clump of stalks (main stems), by counting the number of green leaves (fully expanded leaves with a minimum of 20% green area, counted from leaf zero and the measurements made on leaf blade +3, which is the first leaf blade with open ligule (Casagrande, 1991). The length and width of the middle portion of leaf blade +3 was measured according to the methodology described by Hermann and Hall (1999). The leaf area was estimated by the expression LA = L × W × 0.75 × (N+2), where

LA is the leaf area per stem, L is the length of leaf blade +3, W is the width of leaf blade +3, 0.75 - form factor, and N is the number of open leaves with a green area of at least 20% (leaf blade 0 to +7). The leaf area index (LAI) was obtained (in m^2 m^{-2}) based on the leaf area per tiller and number of tillers per m^2 area, according to the methodology used by Larcher (2000).

After each sampling, the plants were separated into stem, straw and flag leaf (green leaves). The samples were dried in a circulating-air oven at 65°C during 2 to 3 days until it reached constant weight. The material was ground and sent to a laboratory to determine the N, K, P, Ca and Mg concentrations (Nogueira and Souza, 2005). Nutrient accumulation in biomass was calculated as the product of the nutrient concentration by the dry weight of the material per unit of land area.

To compute the rates of growth and nutrient accumulation, the method of functional plant growth analysis was applied (Araújo and Rossiello, 2013). The data of biomass, leaf area index and nutrient accumulation, with replications, were adjusted by linear regression to a second degree exponential polynomial, $W = \exp(a + bT + cT^2)$, which was linearized by $\ln W = a + bT + cT^2$, where W corresponds to the observed data, T to the time in days after planting, and a, b and c to the coefficients obtained by regression. The second degree exponential polynomial model was chosen due to the observed R^2 (greater than 70%), the simplicity of the model and the appropriate biological significance of the curves obtained.

From the functions fitted to the primary data, the functions corresponding to the instantaneous growth rates were derived, e.g. crop growth rate, net assimilation rate and absolute nutrient accumulation rate (Hunt, 1982; Araújo and Rossiello, 2013). The crop growth rate (CGR) represents the variation in dry matter accumulation by the crop per unit land area in a time interval, the net assimilation rate (NAR) is the increase in dry matter per unit leaf area and time and the absolute rates of nutrient accumulation represent the variation in the nutrient amount accumulated by the crop per unit land area and time.

Analysis of variance of primary data was performed as a factorial design between N sources and sampling dates, considering the evaluation times as subplots (Araújo, 2003). Due to the heteroscedasticity, the original data were transformed into natural logarithms prior to analysis of variance (Araújo, 2003).

RESULTS

The analysis of variance of natural-log transformed data indicated significant effects of interactions between N sources and sampling dates for data of accumulation of biomass, N, P, K, Ca, and Mg in the shoots. This indicates that the evaluated N sources modified the patterns of accumulation of biomass and nutrients by sugarcane plants and therefore the growth and nutrient accumulation rates (Araújo, 2003). The coefficients of the second degree exponential polynomial models fitted to the primary data in the four treatments are presented in Table 1. The R^2 values of the model ranged from 0.91 to 0.97 for shoot biomass, from 0.69 to 0.80 for leaf area index, and were consistently above 0.80 for shoot nutrient accumulation, indicating an adequate fit to the primary data.

The curve of shoot biomass accumulation of variety RB92579 indicated that the months of the highest vegetative growth (Figure 2a) coincided with the summer months (between 200 and 250 DAP). This intensified growth was accompanied by greater leaf production, as

Table 1. Coefficients of the polynomial exponential second order models (ln W = a + bt + ct2) fitted to the data of shoot dry matter, leaf area index, and accumulation of nitrogen, phosphorus, potassium, calcium and magnesium in shoots in sugarcane under four treatments of inoculation and nitrogen fertilization.

Coefficent	50 kg ha^{-1} N	Inoculation	50 kg ha^{-1} N + Inoculation	Control
Shoot dry matter				
A	3.01616	2.08679	2.64622	1.99155
B	0.03155	0.042002	0.036924	0.039941
C	-4.60783 e-5	-7.07358 e-5	-5.85386 e-5	-6.36908 e-5
R^2	0.97655	0.97183	0.97497	0.91577
Leaf area index				
A	-2.66629	-2.62893	-3.45236	-2.53861
B	0.032358	0.031927	0.044601	0.030176
C	-6.3187 e-5	-6.0486 e-5	-9.7061 e-5	-5.7165 e-5
R^2	0.75678	0.83963	0.69290	0.80139
Nitrogen accumulation				
A	-1.87628	-2.92432	-2.07834	-2.74933
B	0.039227	0.050440	0.043102	0.046095
C	- 7.89332 e-5	-10.2512 e-5	-8.75183 e-5	-9.1912 e-5
R^2	0.91139	0.87896	0.93669	0.88511
Phosphorus accumulation				
A	-4.26377	-4.78127	-3.75275	-5.14993
B	0.043359	0.047418	0.038768	0.050305
C	-8.88574 e-5	-9.32955 e-5	-7.43086 e-5	-10.1752 e-5
R^2	0.92062	0.87054	0.92697	0.86217
Potassium accumulation				
A	-0.8776	-2.335770244	-2.10233	-3.10984
B	0.029572	0.045617291	0.045493	0.050165
C	-5.7044E-05	-9.65883E-05	-9.71342E-05	-0.000104814
R^2	0.89415	0.89820	0.96418	0.87242
Calcium accumulation				
A	-2.05901	-3.38103	-2.26855	-3.07062
B	0.027623	0.042133	0.031635	0.035030
C	-4.81566 e-5	-8.19558 e-5	-5.82413 e-5	-6.17385 e-5
R^2	0.80420	0.87436	0.829910	0.85461
Magnesium accumulation				
A	-4.43944	-5.44985	-4.74746	-4.13817
B	0.041572	0.053564	0.047306	0.036513
C	-87.53068 e-5	-105428 e-5	-9.18091 e-5	-6.19901 e-5
R^2	0.92317	0.95817	0.94965	0.89755

shown by the higher values of LAI during this period (Figure 2b). In the treatments 50 kg N ha^{-1} + inoculation and inoculation, shoot dry matter accumulation 240 DAP was around 3.4 and 3.3 kg m^{-2}, respectively, that is, 17.6 and 15.2% greater than in the treatments 50 kg N ha^{-1} and control, respectively (Figure 2a). In the treatments inoculation and control, this dry matter accumulation was only reached approximately 25 days later. These results suggest that inoculation with growth-promoting bacteria improved plant vigor, increasing sugarcane dry matter
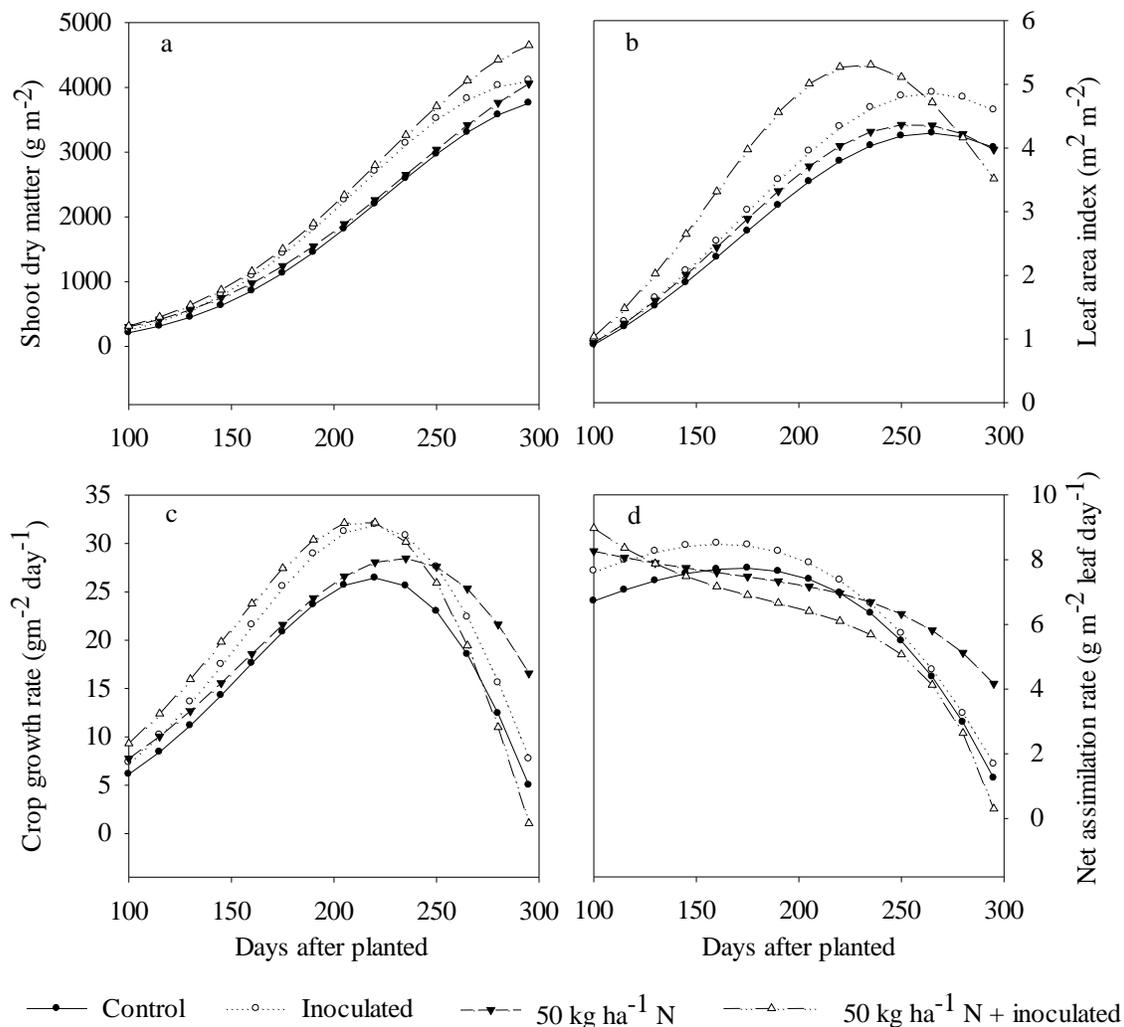
Figure 2. Aboveground part (a) leaf area index (b), crop growth rate (c) and net assimilation rate (d) of sugarcane, RB92579 variety, grown in a Red-Yellow Podzolic soil in Seropédica-RJ, Brazil. Values estimated from the 2nd order exponential polynomial model fitted to the primary data set.

accumulation.

Unlike dry matter accumulation (Figure 2a), the LAI peaked around 220 DAP in the treatment 50 kg N ha^{-1} + inoculation, whereas this peak occurred around 270 DAP in the other treatments (Figure 2b), with a reduction in leaf area at the end of the growth cycle due to leaf senescence.

The initial crop growth rate (CGR, Figure 2c) of variety RB92579 100 DAP was lowest in the control treatment (6 g m^{-2} day^{-1}) and about 9 g m^{-2} day^{-1} in the treatment 50 kg N ha^{-1} + inoculation. The CGR still differed between the two treatments at 220 DAP, 50 kg N ha^{-1} + inoculation peaked with 33 g m^{-2} day^{-1}, while the peak in the control treatment was 26 g m^{-2} day^{-1} (Figure 2c). The pattern of the net assimilation rate (NAR) differed from that of CGR (Figure 2d), because of the tendency of reduction in photosynthetic activity with increasing plant age (Hunt,

1982). In the control treatment, the NAR was highest at 190 DAP (9 g m^{-2} day^{-1}) and in treatment 50 kg N ha^{-1} 195 DAP (7 g m^{-2} day^{-1}). Although, the treatment fertilized with N + inoculation had a lower NAR curve than the other treatments, the larger LAI in this treatment may have offset these lower NAR values (Figure 2b).

The treatments inoculation and 50 kg N ha^{-1} + inoculation promoted the highest values of shoot N accumulation, observed around 240 DAP (Figure 3a), indicating that inoculation promoted an increase in N accumulation in relation to the treatments fertilization with 50 kg N ha^{-1} and control. The N accumulation rate was highest at 170 DAP, and decreased continuously thereafter until 250 DAP in all treatments (Figure 3b). The control and fertilization with 50 kg N ha^{-1} had lower rates over the evaluation period (maximum values of 0.15 and 0.16 g m^{-2} day^{-1}, respectively). Inoculation resulted in the
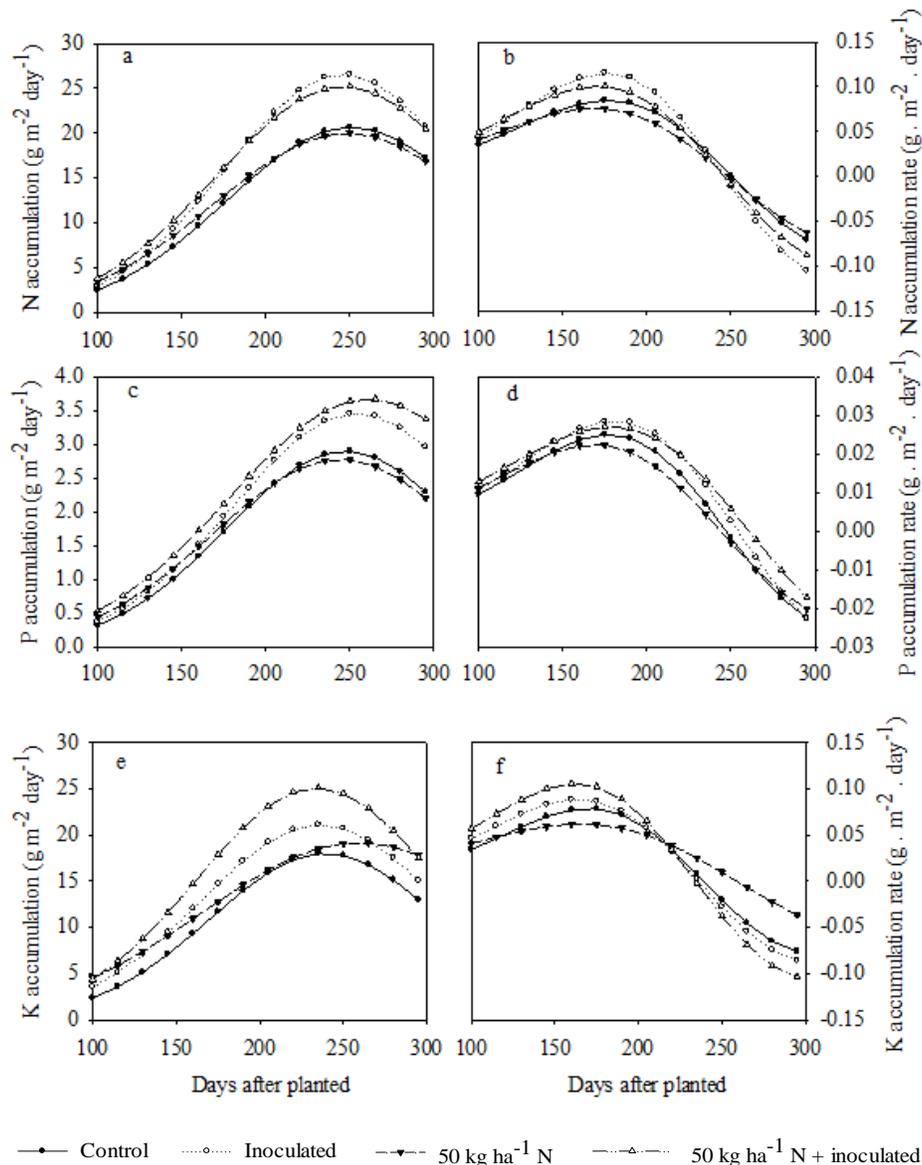
Figure 3. N accumulation (a), N accumulation rate (b), P accumulation (c), the accumulation rate of P (d), accumulation of K (e) and rate of accumulation of K (f) of sugarcane variety RB92579 grown in a Red-Yellow Podzolic soil in Seropédica-RJ, Brazil. Estimated values from the exponential polynomial model school set to primary data.

highest N accumulation rate (0.23 g m^{-2} day^{-1}), while fertilization with 50 kg N ha^{-1} + inoculation in 0.20 g m^{-2} day^{-1}, indicating that inoculation stimulated N accumulation in sugarcane plants (Figure 3b).

In the treatments control and fertilization with 50 kg N ha^{-1}, P accumulation was similar in the evaluation period (Figure 3c). The association of inoculation with 50 kg N ha^{-1} fertilization resulted in increased P accumulation in the evaluation period, with maximal P accumulation 260 DAP (3.68 gm^{-2}). The P accumulation rate was highest around 180 DAP in all treatments. The rate of P accumulation was highest in the inoculation treatment

(around 0.029 g m^{-2} day^{-1}), followed by 50 kg N ha^{-1} + inoculation (maximum of 0.028 g m^{-2} day^{-1}) (Figure 3d). Fertilization with 50 kg N ha^{-1} and control treatment reached maximum P accumulation rates of the order of 0.025 to 0.022 g P m^{-2} day^{-1}, respectively. The accumulation curve suggests that inoculation favored P accumulation by sugarcane plants more than the control and fertilization with 50 kg N ha^{-1}.

In the inoculation treatment, K accumulation was 20 g m^{-2} 245 DAP, but the association of inoculation with fertilization with 50 kg N ha^{-1} had an additional effect on K accumulation (24 g m^{-2}) (Figure 3e). Thus, these results
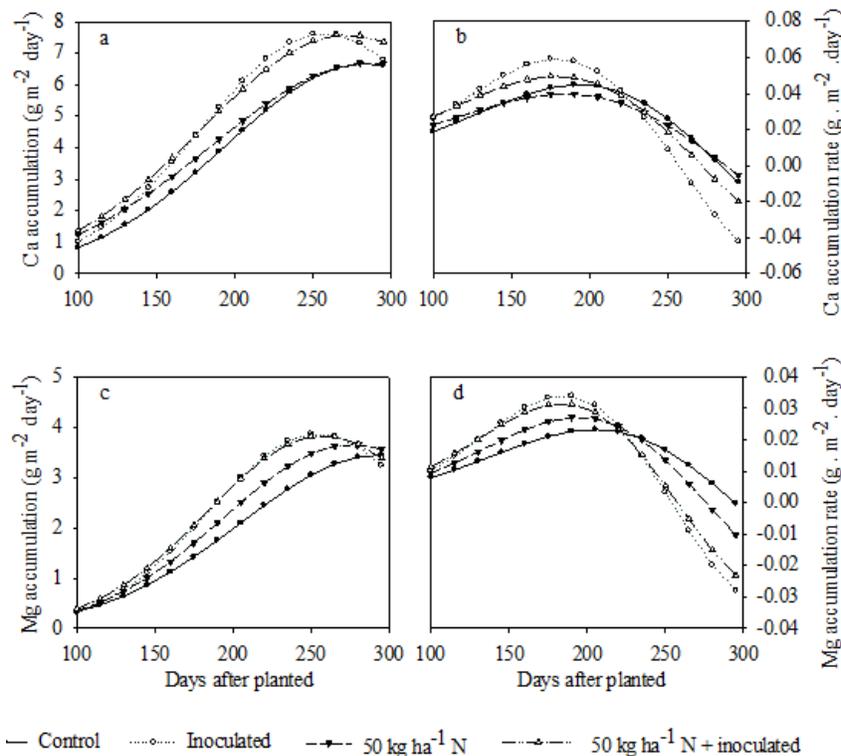
Figure 4. Ca accumulation (a), Ca accumulation rate (b), Mg accumulation (c) Mg accumulation rate (d) of sugarcane, RB92579 variety, grown in a Red-Yellow Podzolic soil in Seropédica-RJ, Brazil. Values estimated from the exponential polynomial model school set to primary data.

suggest that inoculation promoted greater K accumulation than the control treatment and 50 kg N ha^{-1}. The K accumulation rate was highest at 160 DAP in all treatments (Figure 3f). The K accumulation rate was the highest in the treatment fertilized with 50 kg N ha^{-1} + inoculation followed by the inoculation treatment (0.17 and 0.21 g m^{-2} day^{-1}, respectively), both higher than in the treatments with 50 kg N ha^{-1} and control, indicating a positive effect of the combination of 50 kg N ha^{-1} + inoculation.

The Ca and Mg accumulation by sugarcane also reflect the data of other accumulated macro elements. Inoculation increased Ca accumulation, so that the maximum accumulation of the treatments inoculation and 50 kg N ha^{-1} + inoculation occurred at 255 DAP, that is, 25 days earlier than in the treatments control and fertilization with 50 kg N ha^{-1} (Figure 4a). The Ca accumulation rate was higher in the treatments inoculation and combination of N fertilizer with inoculant (Figure 4b). The maximum Ca accumulation rate in the treatments control and fertilization with 50 kg N ha^{-1} occurred at 185 DAP (0.039 and 0.044 g m^{-2} day^{-1}, respectively). Inoculation and the combination of 50 kg N ha^{-1} + inoculation accumulated 0.059 and 0.049 g Ca m^{-2} day^{-1}, 180 DAP, respectively. These results show that the inoculant increased Ca accumulation, both when applied separately or together with 50 kg N ha^{-1}, although the efficiency in the combined treatment was lower.

In the treatments control and 50 kg N ha^{-1}, Mg accumulation was lower than in the inoculation treatments and fertilization with 50 kg N ha^{-1} + inoculation until 275 DAP (Figure 4c). Thereafter, there was a decrease in Mg accumulation in the treatments inoculation and 50 kg N ha^{-1} + inoculation, with lower values than in the treatments control and fertilization with 50 kg N ha^{-1}. The maximum Mg accumulation in the control and 50 kg N ha^{-1} occurred 275 DAP (3.4 and 3.7 g m^{-2}, respectively). Magnesium accumulation was the same in the treatments inoculation and 50 kg N ha^{-1} + inoculation (maximum accumulation of 3.8 g m^{-2} 255 DAP). The Mg accumulation rate was improved by inoculation and by the combination of 50 kg N ha^{-1} + inoculation until 225 DAP (Figure 4d). The treatments control and 50 kg N ha^{-1} showed similar curves, with maximum values of the order of 0.026 g m^{-2} day^{-1} 205 DAP, indicating that fertilization with 50 kg N ha^{-1} had no additional effect on the Mg accumulation rate in comparison with the control. The Mg accumulation rate in the treatments inoculation and 50 kg N ha^{-1} + inoculation peaked 190 DAP (0.033 and 0.031 g m^{-2} day^{-1},

respectively). This shows that there was an additional effect of inoculum on Mg accumulation in relation to the treatment with 50 kg N ha⁻¹ fertilizer only, although in the treatment with inoculant only, Mg accumulation was greater than in the combination of the two treatments.

DISCUSSION

Inoculation using five selected diazotrophs showed positive effects on sugarcane growth parameters evaluated in the presence or not of N-fertilizer. These strains were previously selected by their functions based on plant growth regulators (Fuentes-Ramiréz et al., 1993; Muthukumarasamy et al., 2002; Radwan et al., 2002), phosphate solubilization as identified for *G. diazotrophicus* and *B. tropica* strains, antagonism to plant pathogens (Piñon et al., 2002; Muñoz-Rojas et al., 2005), among others. But endophytic colonization was also an important attribute for these elected PGPR strains tested (Cavalcante and Döbereiner, 1988; Caballero-Melado and Martinez-Romero, 1994; Olivares et al., 1997; Oliveira et al., 2004). All these attributes can partially explain the observed growth rate curves and macro elements accumulated as described in Figures 2, 3 and 4.

Based on these several features, this higher plant vigor was also a result of better exploitation of soil and applied nutrients, resulting in a higher mass accumulation (Figure 2). Although root growth was not assessed in this study, one can speculate that the treatments with diazotrophic bacterial inoculation producing growth regulators modified growth rates curves in response to the improved root development.

The parameters of sugarcane growth evaluated were compatible with other data describing varieties planted in other regions of Brazil. For example, the highest LAI was reached at 215 DAP in the treatment 50 kg N ha⁻¹ + inoculation (Figure 2b), which is different from the behavior observed by Gascho and Shih (1983) who reported a maximum LAI value in sugarcane 150 DAP. Similar growth pattern was described by Farias et al. (2007), who stated an increase in LAI until 210 days after sprouting in variety SP 79-1011 under different irrigation treatments in the first year of cultivation, reaching values of 4 m² m⁻², 150 days after sprouting in an irrigation treatment with 100% crop evapotranspiration replacement and of 5 m² m⁻², when combined with zinc fertilization. Oliveira et al. (2010) also observed the same growth for variety RB92579, divided into three phases also described by Machado et al. (1982), where in the first 200 days, growth was slow, from 200 to 400 days, the plants accumulated 70 to 80% of dry matter and in the following 100 days the remaining 10%. Averaged across all treatments, variety RB92579 accumulated about 14 Mg ha⁻¹ dry weight of shoot at 212 DAP, to 42.3 Mg ha⁻¹ at 293 DAP (Figure 2a), with a growth similar to that

observed by these authors. Based on the rates observed by Machado et al. (1982), at the end of the cycle the expected yield would be around 114 Mg ha⁻¹ dry weight, which would represent 257 Mg ha⁻¹ fresh shoots, based on the same proportions. Applying the same proportionality of dry matter/fresh matter of Machado et al. (1982), a final yield of 203 Mg ha⁻¹ of fresh stalks of variety RB92579 at the end of the cycle 500 DAP can be estimated.

The data of shoot biomass production observed in variety RB92579 are superior to those reported by Gava et al. (2001) in the third ratoon crop for var. SP80-1842 on a similar soil to that used in this study (Typic Haplustult). According to these authors, the relative growth rate (RGR) of sugarcane is high in the beginning, followed by a decline, which is related, among other factors, to the increased intraspecific competition for light and nutrients. Santos et al. (2009) also used growth data to estimate the RGR of var. RB75126, 120 DAP, observing a linear decrease in RGR, ending growth around 240 DAP, with initial values of around 0.05 g g⁻¹ day⁻¹ in the best treatment.

Unlike dry matter (Figure 2a), the LAI peaked around 220 DAP in the treatment 50 kg N ha⁻¹ + inoculation, whereas in the other treatments this maximum occurred around 270 DAP (Figure 2b). The maximum values of nutrient accumulation rates indicate the stage of greatest nutritional crop demand (Araújo and Rossiello, 2013). The highest accumulation rates of N and P were observed around 180 DAP, while K accumulation rate peaked around 160 DAP, in the different treatments (Figure 3). The crop growth rate peaked around 210 to 220 DAP (Figure 2), evidencing that the maximum nutrient demand precedes the maximum sugarcane growth rate. Santos et al. (2009) also observed P influence on sugarcane growth in Brazilian condition. Thus, in the later stages of the growth cycle, nutrient accumulation decreases and phenomena of internal remobilization and nutrient translocation predominate (Gava et al., 2001; Oliveira et al., 2010, 2013).

In general, the accumulation of macro elements was higher in the early growth stages (around 200 DAP), similar to dry matter accumulation, as also reported by Gava et al. (2001). Oliveira et al. (2013) observed critical limits of N in variety SP81-3250 in two growing seasons and three different soils and at all sites the N shoot concentration decreased during crop growth. Oliveira (2011) reported lower values of P accumulation rate, of 0.15 g m⁻² day⁻¹ in an Hapludox soil (distrophic Red Latossol), under climatic conditions that may have decreased plant growth. Values of K accumulation rate were higher than those reported in soils of lower fertility, such as the K accumulation rate of 0.15 g m⁻² day⁻¹ described by Leite (2011) in a treatment with ammonium nitrate application.

The data presented here are similar to those obtained by Oliveira (2008), where the N and K accumulation rates

in sugarcane were practically zero 300 DAP, denoting that at the beginning of the maturation stage the nutrient uptake ceases and sugarcane plant improves the internal remobilization of nutrients (Gava et al., 2001). In this particular case, the application of diazotrophs modified this natural behavior of sugarcane variety RB92579. This crop starts to mobilize the nutrients and accumulate sugars after the fast growth phase as expected, but the inoculation delays this behavior, elongating the growth period (Figure 2a) and still increasing the rates of leaf area index (Figure 2b). Limitation at this point is the space between plants reducing sunlight acquisition by the green leaves, that start to decrease rapidly after 200 to 250 DAP (Figure 2c). As the control and nitrogen treatments posses less leaf area at the beginning, they continue to accumulated biomass while the inoculated treatments were sunlight limited (Figure 2d). This particular movement of leaf area and crop growth rate will interfere on the final biomass at the end of this cane cycle, and also interfere on the crop yield at harvest as observed by other authors that used this same mixture of strains in different sugarcane locations using other varieties (Oliveira et al., 2006; Schultz et al., 2012). It can be easily observed in Figures 3 and 4. So, based on this growth rate curves, it can be expected that sugarcane growth period can be modified by the inoculation, independent on the N-fertilization. But significant differences on cane yield will be controlled by the environmental conditions.

Based on the data obtained, it is possible to conclude that inoculation with diazotrophic bacteria, in combination with N fertilization or not, increases the accumulation of shoot dry matter and leaf area index of the sugarcane variety RB92579 during the first crop year and can be used as an inoculant that increase nutrient accumulation in sugarcane RB92579, in particular of N, P and K.

ACKNOWLEDGEMENTS

Conflict of interests

The author has not declared any conflict of interests.

Abbreviations

CGR, Crop growth rate; **CONAB,** Companhia Nacional de Abastecimento; **UNICA,** União da Industria de Cana-de-açúcar; **RIDESA,** Rede Interuniversitária para o Desenvolvimento do Setor Sucroalcooleiro; **LAI,** leaf area index; **NAR,** net assimilation rate; **N,** nitrogen; **K,** potassium; **P,** phosphorus; **Ca,** calcium; **Mg,** magnesium; **DAP,** days after planting.

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