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BASIC AREAS - Article

Agronomic performance and sweet corn quality as a function of inoculant doses (*Azospirillum brasilense*) and nitrogen fertilization management in summer harvest

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ABSTRACT: The inoculation of sweet corn seeds with *Azospirillum brasilense* in association to nitrogen fertilizer may be an agronomic alternative for increasing the crop yield and net income of plant growers. Therefore, the aim of this study was to investigate the effect of the different doses of inoculant (*Azospirillum brasilense*) associated to the nitrogen fertilization management on the phenotypic traits of one sweet corn hybrid in summer growing periods, under supplemental irrigation, in the Northwestern Paraná state, Brazil. The experiment followed the complete randomized block design with four replications. The treatments were: i) five inoculant doses (0.0, 50, 100, 150 and 200 mL·ha^{-1}) containing *Azospirillum brasilense*; ii) two N doses (0.0 and 30.0 kg·ha^{-1}) applied at sowing time; and iii) two topdressing doses of N (0.0 and 110.0 kg·ha^{-1}) applied at the V_4 stage. The sweet corn hybrid RB 6324 was evaluated in 2012/2013, 2013/2014 and 2014/2015. The traits plant height (ranged from 2.11 to 2.26 m), leaf area index (3.33 to 4.32), crop yield (7.21 to 10.43 Mg.ha^{-1}), and the sugar kernel contents (38.46 to 43.31%) and protein (12 to 12.81%) were positively influenced by the seed inoculation with *A. brasilense*, and the nitrogen fertilizer increased all the traits except the kernel total sugars. The dose of inoculant that provided the best agronomic result was 100 mL·ha^{-1} in conjunction with the application of N either at sowing or topdressing.

Key words: *Zea mays*, shrunken-2, diazotrophic bacteria, ear yield.

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INTRODUCTION

In Brazil, sweet corn plantations are stretching from Rio Grande do Sul to Goiás through São Paulo and Minas Gerais, where Goiás has the largest hectarage. Currently, the national production has supplied the industry of processed food (Barbieri et al. 2005). In contrast with the common maize, sweet corn has been enlarging in the national market because of its higher net return to plant growers (Canianto et al. 2007). The highest added value is the natural sweet flavor from the kernels because of the expressive quantity of sugar stored into the seed endosperm (Tracy 2010). This characteristic in the super sweet group is the effect from the mutant recessive gene Shrunken-2 (*Sh*2) that impedes the conversion of sugar into starch. Sweet corn is also considered a vegetable (Tracy 2001) for consuming *in natura* or canned (Luz et al. 2014). Other crop possibilities are the production of baby corn (Santos et al. 2014) or silage (Idikut et al. 2009).

Despite many technologies and technical information available in the maize literature, little attention has been paid to the management of sweet corn under cropping systems. This fact may result in improper management of nitrogen fertilizers (Okumura et al. 2014), thereby reducing the potential of crop production. Otherwise, the application of *Azospirillum brasilense* has been useful for promoting plant growth (Piccinin et al. 2015; Spolaor et al. 2016). Many studies indicate the seed inoculation with *A. brasilense* in conjunction with nitrogen fertilizer for improving the vegetative traits of sweet corn, thereby increasing the crop yield (Hungria et al. 2010; Braccini et al. 2012; Brum et al. 2016). The method stimulates the seed germination and accelerates the seedling growth under field conditions (Cassán et al. 2009). Furthermore, the *A. brasilense* can increase tolerance of sweet corn to water deficits and increase the biomass production as reported by Rodríguez-Salazar et al. (2009).

Nitrogen use efficiency (NUE) in cropping systems has been affected by the levels of soil moisture, organic matter, texture and fertility. Nitrogen losses by lixiviation (Aita et al. 2006), volatilization (Carvalho et al. 2006), denitrification and immobilization by microorganisms (Silva et al. 2006) justify the efforts for improving the crop management. We know that N is absorbed in great quantities by sweet corn plants (Silva et al. 2005), thereby affecting significantly their physiological activities (Fernandes et al. 2005). Nitrogen application represents 75% of the costs with sweet corn fertilization, which means about 40% of the inputs along growing periods (Machado et al. 1998). Thus, seed inoculation with diazotrophic bacteria can reduce these costs, increasing the sweet corn yield as verified with soybean crops (Alves et al. 2006) and common maize that had increases of 29% (Ferreira et al. 2013), 11% (Piccinin et al. 2015) and 4.3% (Brum et al. 2016) when the *A. brasilense* was applied to the seeds.

Currently, studies related to N management involving diazotrophic bacteria, specifically from the genus *Azospirillum* and sweet corn plants are scarce, and scientific studies at regional level for making correct recommendations to plant growers have been necessary. Therefore, the purpose of the current experiment was to evaluate the effect of the different doses of inoculant (*Azospirillum brasilense*) associated to the nitrogen fertilization management on the phenotypic traits of one sweet corn hybrid in summer growing periods applying supplementary irrigation.

MATERIAL AND METHODS

The experiments were conducted in the summer growing seasons 2012/2013, 2013/2014 and 2014/2015, on Fazenda Experimental de Iguatemi (23°20'48" SL, 52°04'17" WL, and altitude of 550 m), Universidade Estadual de Maringá, Northwestern Paraná, Brazil. The soil in the experimental area is classified as Distroferric Red Nitosol with clay texture (clay: 520 $g\cdot kg^{-1}$; silt: 140 $g\cdot kg^{-1}$; sand: 340 $g\cdot kg^{-1}$) (Embrapa 2013). The chemical characteristics in uppermost soil layer from 0.0 to 0. 20 m, collected in three growing seasons were: in 2012/2013, pH ($CaCl_2$) = 4.10, $H^+ + Al^{3+}$ = 3.97 $cmol_c\cdot dm^{-3}$, K^+ = 0.29 $cmol_c\cdot dm^{-3}$, Ca^{2+} = 4.05 $cmol_c\cdot dm^{-3}$, Mg^{2+} = 1.50 $cmol_c\cdot dm^{-3}$, V = 59.53 %, P = 22.4 $mg\cdot dm^{-3}$, C = 13.81 $g\cdot kg^{-1}$; in 2013/2014, pH ($CaCl_2$) = 4.68, $H^+ + Al^{3+}$ = 2.32 $cmol_c\cdot dm^{-3}$, K^+ = 0.44 $cmol_c\cdot dm^{-3}$, Ca^{2+} = 4.75 $cmol_c\cdot dm^{-3}$, Mg^{2+} = 1.95 $cmol_c\cdot dm^{-3}$, V = 77.82 %, P = 24.2 $mg\cdot dm^{-3}$, C = 13.63 $g\cdot kg^{-1}$; 2014/2015, pH ($CaCl_2$) = 4.39, $H^+ + Al^{3+}$ = 2.42 $cmol_c\cdot dm^{-3}$, K^+ = 0.58 $cmol_c\cdot dm^{-3}$, Ca^{2+} = 4.20 $cmol_c\cdot dm^{-3}$, Mg^{2+} = 2.95 $cmol_c\cdot dm^{-3}$, V = 78.30 %, P = 18.25 $mg\cdot dm^{-3}$, C = 17.92 $g\cdot kg^{-1}$. The regional climate is the Cfa based on the Köppen classification (IAPAR 1994). Data set of rainfall, maximum and minimum temperatures collected daily by Seed Science and Technology Laboratory are in Fig. 1 for the three summer growing seasons.

The treatments follow the combination of five doses (0.0, 50, 100, 150 and 200 $mL\cdot ha^{-1}$) of inoculant containing

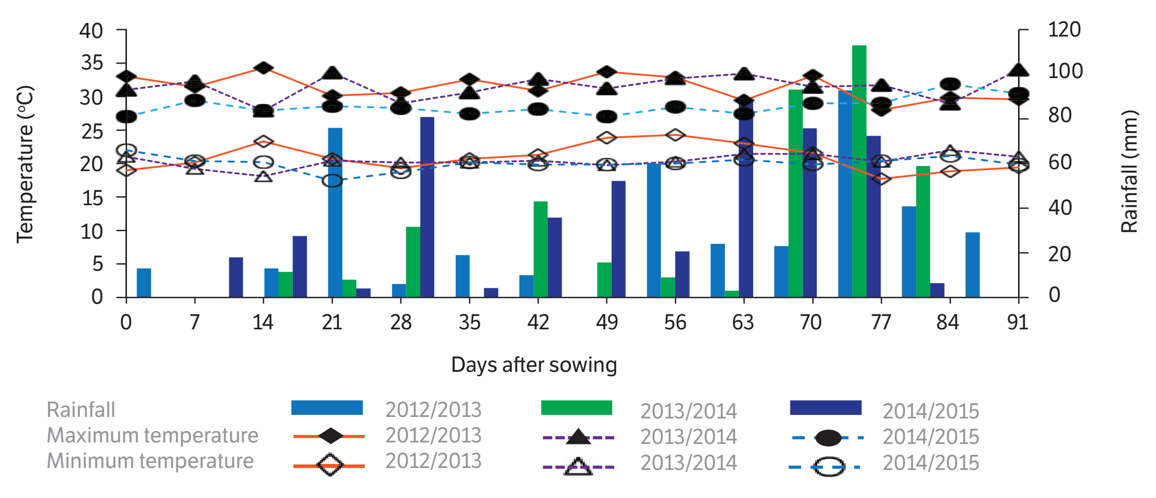

Figure 1. Rainfall, maximum and minimum temperatures during the summer growing seasons 2012/2013, 2013/2014 and 2014/2015, in Maringá, Northwestern Paraná, Brazil.

Azospirillum brasilense (strains AbV5 and AbV6) with minimum 2×10^8 colony forming unit (CFU) applied to the seeds; two N doses applied at the sowing time (0.0 and 30.0 kg·ha^{-1}); two topdressing doses of N applied at the V_4 stage (0.0 and 110.0 kg·ha^{-1}) (Ritchie et al. 1993), to fertilize the soil for growing the single hybrid RB 6324 from the super sweet group. Each plot had five rows with 6 m in length and 0.9 m apart where the total area had 27.0 m^2 and the useful area had 13.5 m^2.

In October, 20 days before seed sowing, we controlled the weeds applying glyphosate (480 g·L^{-1} a.i.) at 2.5 L·ha^{-1}. The crops before the sweet corn were black oats (*Avena strigosa* Schreb.) and fodder turnip (*Raphanus sativus* L.) in 2012/2013, sweet corn (*Zea mays* L.) in 2013/2014, and millet (*Pennisetum glaucum* L.) in 2014/2015. At the sowing time, we applied 50 kg·ha^{-1} of P_2O_5 using triple superphosphate and 40 kg·ha^{-1} of K_2O using potassium chloride at 0.10 m into the soil for the treatments in the three agricultural years. The N dose of 30.0 kg·ha^{-1} was applied only onto the plots. Nitrogen fertilization at 110.0 kg·ha^{-1} using urea (46% N) as the topdressing N source was carried out at the V_4 stage (Ritchie et al. 1993) and applied by handy workers (Okumura et al. 2014). Supplementary spray irrigation was provided during critical periods of the plant development as the flowering stage and beginning of the grain filling.

The experiments were carried out under no-till system for achieving the population of 55.500 plants·ha^{-1} or 5.0 plants·m^{-1}. After the crop establishment, the weeds were controlled by atrazine at 3.0 L·ha^{-1} associated to the tembotrione herbicide at 0.24 L·ha^{-1} both amended with 1.0 L·ha^{-1} of methylated soybean oil as adjuvant before the V_4 stage. The insects *Dichelops melacanthus*, *Agrotis ípsilon* and *Elasmopalpus lignosellus* were controlled by seed treatment with imidacloprid + thiodicarb at 0.3 L·ha^{-1}. The fall armyworm (*Spodoptera frugiperda*) and the corn earworm (*Helicoverpa zea*) were controlled by methomyl at 0.5 L·ha^{-1} and lufenuron at 0.3 L·ha^{-1} when the insects reached the threshold level for sweet corn crops tolerance (Gallo et al. 2002).

At full flowering (V_T) (Ritchie et al. 1993) we evaluated the vegetative traits plant height and leaf area index. Plant height (PH) was taken by the stem length (m) from the ground up to the base of the panicle by choosing ten randomly plants from each plot (Lana et al. 2009). The leaf area index (LAI) was first calculated by the expression $LA = C \times L \times 0.74$, where C and L are the length and width taken from leaves with green area larger than 50% by choosing five random plants in each plot. Next, the leaf area index was calculated by the equation $LAI = LA / (e_1 \times e_2)$, where e_1 and e_2 are the space in meters between plants in the row and sowing rows, respectively (Francis et al. 1969). After harvesting the ears at the phenotypic stage R_3 (Ritchie et al. 1993), we assessed the following traits: yield of marketable ear (YME) (diameter larger than 3 cm and longer than 15 cm, free from insect damage and disease symptoms following the recommendations from Albuquerque et al. (2008) and Rocha et al. (2011)); protein content (PROT); and total sugars (TS) in the kernels (Brasil 2005).

First, the experimental data from every growing season were submitted to the Shapiro-Wilk to verify the error normality and the Levene tests (p > 0.05) for the homocedasticity of variances. Thus, data were submitted to individual analysis to verify whether the ratios between the residual mean squares were lower than 7:1 (Banzatto and Kronka 2006). Next, the data sets were submitted to joint analysis of variance with the pertinent partitions when necessary (Perecin and Cargnelutti Filho 2008; Barbin 2013). The effects of seed inoculation were evaluated by polynomial regression. The effects of the N doses applied at sowing and topdressing were studied by the F test (p < 0.05) and the effects of growing seasons were studied by the t test (LSD) (p < 0.05). All these statistical analyses were carried out using the Sisvar Software (Ferreira 2011).

RESULTS AND DISCUSSION

The joint analysis of variance indicated the seed inoculation as the significant factor (p < 0.05) affecting the LAI and the kernel PROT (Table 1).

The N factor at sowing time was significant for all the traits except TS, similarly to the factor growing season (p > 0.05). The N factor at topdressing was non-significant (p > 0.05) only for the LAI (Table 1). The partition of the first order interaction was significant (p < 0.05) for PH, LAI and YME. Finally, the results of the joint analysis did not show significant effects from second or third order interaction for all the phenotypic traits (Table 1). The partitions of the first (I × GS) and second order (NS × NT × GS) interactions were significant (p < 0.05) for PH. We found that in the first growing season the seed inoculation in conjunction with the mean of N doses applied at sowing time and topdressing stage had positive influence on the vegetative development of the plants (Fig. 2a).

In 2012/2013, the quadratic model had the best goodness of fit, and the maximum PH was 2.26 m, achieved after applying the inoculant at 116.90 $mL\cdot ha^{-1}$. The explanation may rest on the fact that *A. brasilense* makes the N fixed from the atmosphere partially available to the plants. Furthermore, these diazotrophic bacteria can influence the growth and

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Table 1. Summary of the variance analysis for the phenotypic traits: plant height (PH), leaf area index (LAI), yield of marketable ear (YME), protein content (PROT) and total sugar (TS) in the kernels of sweet corn cultivate in three summer growing seasons.

Source of variation	DF^{1}	Mean squares				
		PH (m)	LAI ($m^2\cdot m^{-2}$)	YME ($Mg\cdot ha^{-1}$)	PROT (%)	TS (%)
Inoculant (I)	4	0.005^{ns}	0.546*	0.403^{ns}	6.157*	8.174^{ns}
N Sowing (NS)	1	0.292*	2.765*	8.303*	17.822*	0.048^{ns}
N Topdressing (NT)	1	0.185*	8.543*	96.698*	29.260*	191.602*
Growing Season (GS)	2	0.215*	6.916*	153.223*	4.174*	65.182^{ns}
NS × NT	1	0.053*	1.837*	3.626^{ns}	0.265^{ns}	17.281^{ns}
I × NS	4	0.004^{ns}	0.271^{ns}	1.290^{ns}	0.441^{ns}	9.395^{ns}
I × NT	4	0.010^{ns}	0.090^{ns}	0.109^{ns}	0.332^{ns}	16.068^{ns}
I × NS × NT	4	0.000^{ns}	0.134^{ns}	0.833^{ns}	1.382^{ns}	64.892^{ns}
I × GS	8	0.010^{ns}	0.446*	2.145*	0.897^{ns}	47.441^{ns}
NS × GS	2	0.042*	0.447^{ns}	5.837*	0.964^{ns}	38.196^{ns}
NT × GS	2	0.039*	1.072*	45.480*	0.368^{ns}	72.210^{ns}
NS × NT × GS	2	0.011^{ns}	0.135^{ns}	0.381^{ns}	0.082^{ns}	43.877^{ns}
I × NS × GS	8	0.005^{ns}	0.131^{ns}	1.681^{ns}	0.591^{ns}	60.324^{ns}
I × NT × GS	8	0.011^{ns}	0.174^{ns}	1.278^{ns}	0.804^{ns}	70.565^{ns}
I × NS × NT × GS	8	0.005^{ns}	0.218^{ns}	1.143^{ns}	1.353^{ns}	36.497^{ns}
Blocks/GS	9	0.022	0.358	3.084	4.132	76.793
Residues	171	0.007	0.213	1.015	0.935	36.173
Averages		2.18	3.71	8.41	12.56	40.08
CVs (%)^{2}		3.81	12.45	11.98	7.70	15.00

* Significant (p < 0.05), and ^{ns} not significant (p > 0.05) by F test. ^{1}DF = Degrees of freedom, and ^{2}CV = Coefficient of variation.

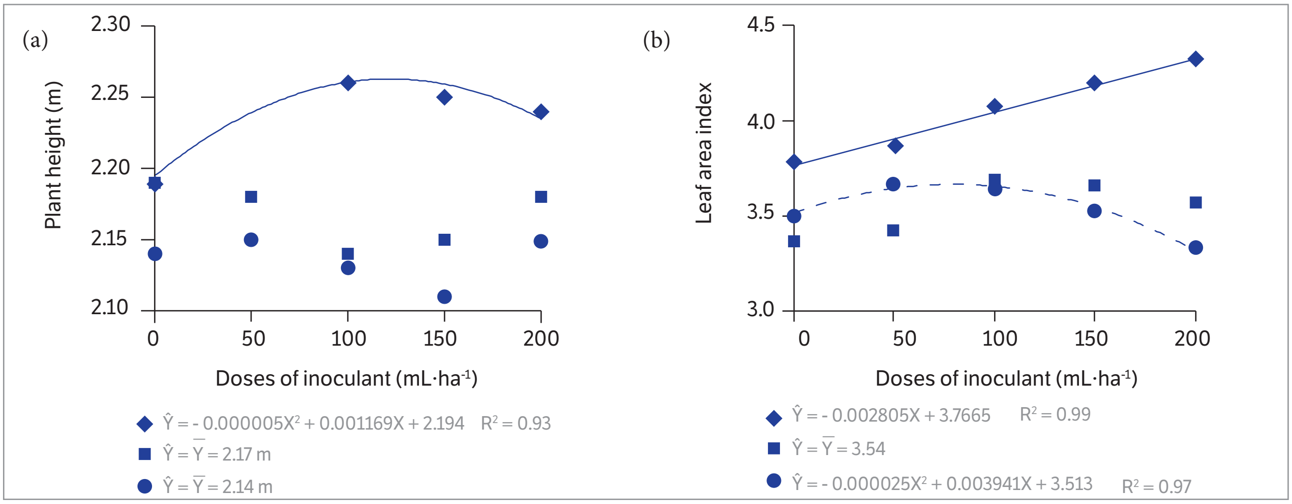

Figure 2. (a) Plant height and (b) leaf area index of sweet corn (RB 6324) based on the inoculant doses in the average of the nitrogen fertilizations in the summer growing seasons: 2012/2013, 2013/2014 and 2014/2015.

development of the sweet corn roots through the synthesis of plant hormones such as the auxins (indol-3-acetic acid and indol-3-butiric acid), gibberellins (GA_1, GA_3, GA_9, GA_{19} and GA_{20}) and cytokinin (Cassán et al. 2014) that justify greater absorption of mineral nutrients and water from the soil. Probably, *Avena strigosa* Schreb (with high C/N ratio in the straw) and *Raphanus sativus* L. (with low C/N ratio) that preceded the sweet corn have collaborated to make the N from other mineral nutrients significantly available during the sweet corn sub-stages. This possibility may explain the detrimental responses from last two growing seasons 2013/2014 with $\bar{Y} = 2.17$ m and 2014/2015 with $\bar{Y} = 2.14$ m. Furthermore, high temperatures (> 30 °C) observed in the vegetative period probably favored the physiological plant mechanisms.

Kappes et al. (2013) reported higher plant height and ear insertion when sweet corn seeds were inoculated with *A. brasilense* (strains AbV5 and AbV6). They based on the averages of the topdressing N doses and the urea applied onto the leaves. These authors suggested that their results were the responses from growth substances from the bacteria. In contrast, Lana et al. (2012) and Piccinin et al. (2015) did not find significant increases in plant height of common corn when they inoculated the seeds with the *A. brasilense*. This response could be attributed to the genetic characteristics of the hybrids assessed in their experiments. The LAI of the sweet corn plants varied with inoculant doses and growing seasons (Fig. 2b), and the responses were positive to the nitrogen fertilizer. In 2012/2013, the responses were linear. The increase estimated by the angular coefficient was 0.028 for each 10 mL of inoculant applied to the seeds. In 2014/2015, the best fit was achieved with the quadratic model from which the maximum LAI was estimated at 3.67 for the dose 78.82 mL·ha^{-1}. In 2013/2014, no significant effect was observed from the regression model ($\bar{Y} = 3.54$). Marini et al. (2015) also reported significant responses ($p < 0.05$) in the leaf area of hybrids of common sweet corn treated with *A. brasilense* and the increase was 11.0 % higher than estimates from non-inoculated plants.

The crops *Avena strigosa* Schreb and *Raphanus sativus* L. cultivated before the sweet corn in 2012/2013, as verified in the plant height characteristic, probably made large quantities of N (mainly) available through the mineralization process during the vegetative stages (Fontoura and Bayer 2009). This association with the N biologically fixed by *A. brasilense* justifies the better leaf area in the growing seasons 2013/2014 and 2014/2015. Plants well supplied with N have better leaf areas and root systems because this nutrient participates directly in the cell division and expansion along the photosynthesis process (Efthimiadou et al. 2009). In plants, the N available either through nitrogen fertilizer or associated to biological fixation tends to increase the synthesis of hormones. This allows the cell division and expansion in the leaves based on leaf lengthening and expansion rate (Sivasankar et al. 1993; Taiz and Zeiger 2014).

No matter the growing season, non-significant differences were found when we did not inoculate the seeds, but the plants from 2012/2013 were taller than plants from the treatment with seed inoculation (Table 2). These responses are likely associated to environmental factors that have

Table 2. Plant height (m) in relation to the N doses applied at sowing and topdressing, in three summer growing seasons.

N at sowing[1]	N at topdressing					
	2012/2013		2013/2014		2014/2015	
	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.
Abs.	2.22Aa	2.23Aa	2.09Bb	2.18Aa	2.00Bb	2.15Ba
Pres.	2.25Aa	2.26Aa	2.19Aa	2.21Aa	2.17Aa	2.22Aa

Growing seasons[2]	NS Abs. NT Abs.	NS Abs. NT Pres.	NS Pres. NT Abs.	NS Pres. NT Pres.
2012/2013	2.22a	2.23a	2.25a	2.26a
2013/2014	2.09b	2.19ab	2.19b	2.22a
2014/2015	2.00c	2.15b	2.17b	2.21a

Means followed by distinct letters in the column (upper case) and in the line (lower case) are different at a 5% probability level by [1]F test and by [2]t-test (LSD), LSD = 0.052 m. NS = N applied at sowing and NT = N applied at topdressing. Abs. = Absence and Pres. = Presence.

direct influence on the performance of the diazotrophic bacteria during the fixation of the atmospheric N. These environmental factors are high temperature and high soil moisture (Bashan et al. 2004; Malhotra and Srivastava 2009) usually found in subtropical and tropical regions. Concerning the LAI, the responses from the growing season 2012/2013 was better than the responses from 2014/2015 ($p < 0.05$) at all the doses of inoculant; in 2013/2014 the LAI estimates under no inoculation and at 50 mL·ha^{-1} did not differ from the growing season 2012/2013 ($p > 0.05$). The *A. brasilense* provided better leaf area development and likely a higher number of leaves photosynthetically active per plant (Müller et al. 2016) taking into account the average of the N doses applied at sowing and topdressing. The enzymes associated to photosynthesis (phosphoenolpyruvate carboxylase and ribulose 1.5-biphosphate carboxylase/oxidase) generally increase their activities in plants inoculated with *A. brasilense* (Stancheva et al. 1992).

Table 2 has the results from the partition NS × NT × GS. In 2013/2014 and 2014/2015 we find significant differences when the N was applied only at sowing time. The average increase in 2013/2014 was 0.10 m (4.8%), and 0.17 m (8.5%) in 2014/2015. The combination of the N doses applied at sowing (30.0 kg·ha^{-1}) and topdressing (110.0 kg·ha^{-1}) were different and the estimates were higher than the dose of 110.0 kg·ha^{-1} applied only one time when the increase was only 0.07 m. The topdressing nitrogen fertilizer significantly increased the PH when N was not applied at sowing time in 2013/2014 (0.09 m) and 2014/2015 (0.15 m). Gomes et al. (2007) also found linear increase in the plant height of common corn with the estimate 2.22 m at 150 kg·ha^{-1}. In 2012/2013, the plants were taller (Table 2), but the estimates from the combination no application of N at sowing with the topdressing application did not differ ($p > 0.05$) from 2013/2014. Finally, when the nitrogen fertilizer was applied at both times, no difference for growing season was found ($p > 0.05$). Piccinin et al. (2015) also found significant increases with N doses applied at topdressing in two growing seasons, in Northwestern Paraná state, Brazil.

Nitrogen fertilizer significantly increased the LAI regardless the application period. N application in both periods did not differ from just one application ($p > 0.05$) either at sowing or topdressing (Table 3) based on the average from the growing seasons. Veloso et al. (2009) found positive LAI responses when they increased the N doses at topdressing application in the R_5 stage of the common maize growing in marsh drained soil. We highlighted LAI dependency on crop development stages as reported by França et al. (2011) when the maximum LAI (4.41) was observed at the R_1 stage with the highest N available. The LAI decreased after this period because of the leaf senescence.

The partition of the first order interaction (I × GS) for YME indicated different performances based on the linear (2012/2013) and quadratic model (2014/2015), in the mean of the N doses applied at sowing and topdressing (Fig. 3).

Table 3. Leaf area index in relation to the N doses applied at sowing and topdressing.

N at sowing	N at topdressing	
	Absence	Presence
Absence	3.32Bb	3.88Aa
Presence	3.71Ab	3.92Aa

Means followed by distinct letter in the line (lower case) and in the column (upper case) are different ($p < 0.05$) by F test.

In 2013/2014, the regression models were non-significant ($p > 0.05$).

The increase in the YME estimated by the angular coefficient was approximately 39.04 kg·ha^{-1} for each 10.0 mL of inoculant. We found that the maximum yield estimated by the model was 10.38 Mg·ha^{-1} for 99.48 mL·ha^{-1}, and this increase was 740 kg·ha^{-1} higher than the control. We highlight that the commercial ears should have the length higher than 14.0 cm, diameter larger than 3.0 cm, and free from insect damages and diseases symptoms (Albuquerque et al. 2008; Rocha et al. 2011). High production of commercial ears in the field is also desirable because they will be commercialized (Okumura et al. 2014). We inferred that the biological activity of *A. brasilense* in conjunction with the nitrogen fertilizer did not only result in gain of kernel mass but improved the chemical/physical quality of the ears. The different YME responses from growing seasons (Fig. 3) may be associated to weather and environmental effects. Furthermore, yield is also influenced by participation and supply of carbohydrates and organic matter acids produced by plants to attend the requirements from the diazotrophic bacteria (Hartman and Baldani 2006) and carbohydrates accumulation into the kernels (Magalhães and Jones 1990).

Economically, based on the quote from the green sweet corn (US\$0.32 = R\$1.15·kg^{-1} green ears) in the wholesale national market (CEASA/PR; Curitiba County) in April 20, 2017, the price of inoculant, and the hybrid seeds of the RB 6324, the gain with the seed inoculation in the 2014/2015 was about R\$920.00·ha^{-1} for the maximum crop yield of 10.38 Mg·ha^{-1} based on the model $\hat{Y} = -0.000075X^2 + 0.014922X + 9.636$. Therefore, cropping sweet corn on small farms is more profitable than common corn (Canianto et al. 2007) because of the high unitary price of the ear (Camilo et al. 2015).

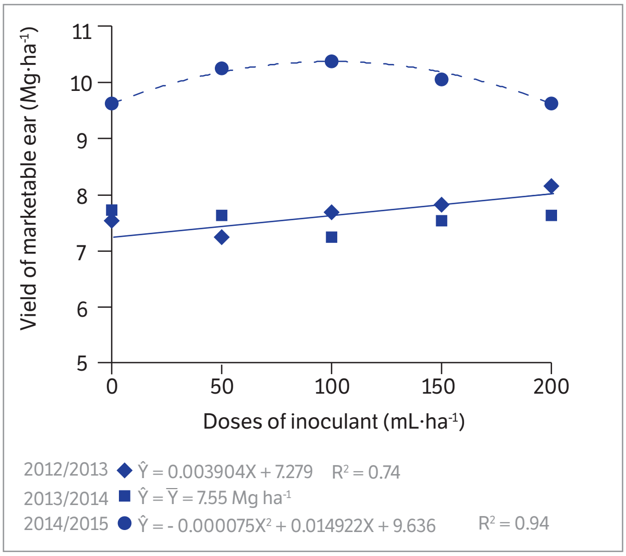

Figure 3. Yield of marketable ear of the RB 6324 sweet corn hybrid in relation to the inoculant doses in the summer growing seasons 2012/2013, 2013/2014 and 2014/2015.

Regardless the dose of the inoculant, the growing season 2014/2015 was better than 2012/2013 and 2013/2014 ($p < 0.05$). This result suggests that both the climatic conditions (temperature and rainfall) (Fig. 1) and soil fertility favored the agronomic performance of the sweet corn hybrid in the last growing season.

Despite the moment we applied the N, the fertilizer increased the YME, and the higher increment was verified after N applications either at seed sowing or topdressing (Table 4). The YME in 2014/2015 was higher than in 2012/2013 and 2013/2014 ($p < 0.05$) in all the combinations (NS + NT), except the first (with no N application). Okumura et al. (2014) also found positive results from sweet corn YME with the increased doses of topdressing N. The highest crop productivity of 10.41 Mg·ha^{-1} yields marketable ears at the N dose of 110.84 kg·ha^{-1}, in the Northwestern Paraná state, Brazil.

The joint analysis of variance for TS from sweet corn kernels had significant principal effect ($p < 0.05$) for the N factor topdressing (Table 1). Although the factors inoculant × growing seasons ($p > 0.05$) were independent after the partition, we found significant regression model (Fig. 4a). In this case, the first order interaction was chosen by the principal factor (N at topdressing). In 2014/2015, the quadratic model was the best (Fig. 4a) and the maximum TS in the kernels of the RB 6324 hybrid was 42.23% estimated after inoculating 106.38 mL·ha^{-1}. Thus, the sugar increase in the endosperm was 3.94% higher than the control (no inoculation). However, the increase in TS from the dose 200 mL·ha^{-1} may be related to decreases in photo-assimilates in the sweet corn plants. This happens when the carbohydrates reserves are used to supply the energy demand to maintain the association with the diazotrophic bacteria (Schubert and Evans 1976; Taiz and Zeiger 2014). Expressive sugar accumulation in the kernels is blocked by the sugar conversion into starch in the endosperm, specifically in the super sweet genotype group (*sh2*) (Tracy 2010). The small presence of the ADP-glucose pyrophosphorylase enzyme in the endosperm catalyzes the reaction between glycose-1-phosphate and adenosine

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Table 4. Yield of marketable ear (Mg·ha^{-1}) in relation to the N doses applied at sowing and topdressing, in three summer growing seasons.

N at sowing¹	2012/2013 N at topdressing Abs.	2012/2013 N at topdressing Pres.	2013/2014 N at topdressing Abs.	2013/2014 N at topdressing Pres.	2014/2015 N at topdressing Abs.	2014/2015 N at topdressing Pres.
Absence	7.65Aa	7.80Aa	6.81Bb	8.04Aa	7.94Bb	11.11Ba
Presence	7.67Aa	7.57Aa	7.46Aa	7.91Aa	9.12Ab	11.85Aa

Growing seasons²	NS Abs. / NT Abs.	NS Abs. / NT Pres.	NS Pres. / NT Abs.	NS Pres. / NT Pres.
2012/2013	7.65a	7.80b	7.67b	7.57b
2013/2014	6.81b	8.04b	7.46b	7.91b
2014/2015	7.94a	11.11a	9.12a	11.85a

Means followed by distinct letter in the column (upper case) and in the line (lower case) are different at the 5 % probability level by ¹F-test and by ²t-test (LSD), LSD = 0.225 Mg·ha^{-1}. NS = N applied at sowing and NT = N applied in topdressing. Abs. = Absence and Pres. = Presence.

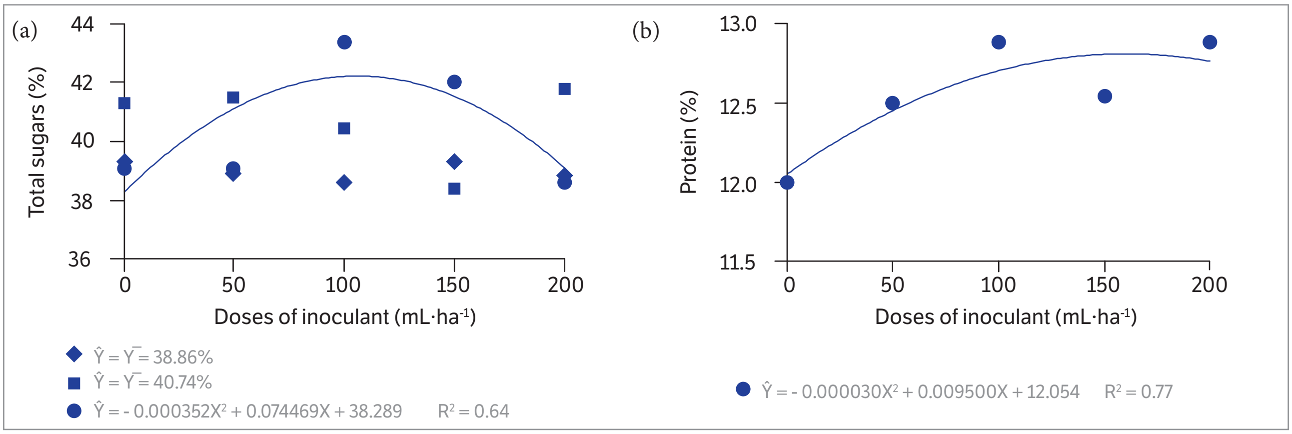

Figure 4. (a) Total sugars in kernels based on inoculant doses and summer growing seasons; (b) protein contents in the grain of sweet corn hybrid RB 6324.

triphosphate and drastically reduces the starch synthesis (Tracy 2001; Souza et al. 2013).

The PROT in the sweet corn kernels was described ($p < 0.05$) by the quadratic model for the inoculant main factor (Fig. 4b). Thus, the maximum PROT given by the model was 12.81% estimated after 158.33 mL·ha^{-1} or 4.9% higher than the control (no inoculation). These results are in agreement with reports from Naserirad et al. (2011) who found positive effects on the protein contents in kernels of three common maize genotypes inoculated with *Azospirillum* and *Azotobacter*.

The total sugars in the sweet corn grain after the seed inoculation with 100 mL·ha^{-1} was 43.31% in 2014/2015, and higher than 4.61% ($p < 0.05$) in 2012/2013. The values from the current study are similar to reports from Okumura et al. (2014) (an average of 34.0%). In 2012/2013, the PROT in the kernels was higher ($p < 0.05$) than in the other growing seasons, but this result was negatively correlated with the marketable ear (Uribelarrea et al. 2004; Khodarahmpour 2011). The topdressed N dose of 110.0 kg·ha^{-1} reduced the TS in the kernels by –1.79% as we reported in Table 5. In plants, increase in N concentration results in decreases of carbohydrates in the system because much of the nutrient is used for synthesizing amino acid, protein, and other nitrogen metabolites (Singletary and Below 1989; Kusano et al. 2011).

Although the joint analysis of variance showed interdependence between the N factors at sowing and topdressing, we verified after partitioning NS × NT significant effects on the PROT contents in the kernels. In this case we chose the first order interaction (Table 6) because we found that the N applied at sowing and topdressing resulted in a significant increase in the kernel PROT (Table 6). The greatest protein content was found when the N was applied at both times (13.15%), and also alone at topdressing (12.67%). Positive effects of nitrogen fertilizer on the kernel PROT were obtained by Bueno et al. (2009) and Okumura et al.

→

Table 5. Total sugar contents in sweet corn kernel in relation to the N doses applied at topdressing in summer growing seasons.

N at topdressing	Total sugar contents (%)
Absence	40.98a
Presence	39.19b

Means followed by distinct letter in the column are different at the 5% probability level by F test.

Table 6. Protein content (%) from the N doses applied at sowing time and topdressing, based on the mean dose of inoculant and growing seasons.

N at sowing	N at topdressing	
	Absence	Presence
Absence	11.90Bb	12.67Ba
Presence	12.52Ab	13.15Aa

Means followed by distinct letter in the line (lower case) and in the column (upper case) are different ($p < 0.05$) by F test.

(2014). Okumura et al. (2014) obtained significant increases in PROT with increasing doses of N applied at topdressing in three summer growing seasons, in Northwestern Paraná, Brazil. The N in the sweet corn plant is important not only for the vegetative and reproductive traits, but also for kernel chemical composition (Tables 5 and 6). Therefore, appropriate N management during the sweet corn cycle is fundamental to raise the nutritional quality of the kernel (Oikeh et al. 1998; Okumura et al. 2014).

CONCLUSION

Inoculation of the hybrid sweet corn seeds RB 6324 with *Azospirillum brasilense* affected positively the vegetative growth traits, ear yield, protein, and total sugars contents in kernels. The increase in ear yield was 39.04 kg·ha^{-1} for each 10.0 mL of inoculant and 740 kg·ha^{-1} from the dose 99.48 mL applied to the seeds in the summer growing seasons 2013/2014 and 2014/2015, respectively. The application of nitrogen fertilizer increased the vegetative growth traits, ear yield and kernel protein content. The dose of inoculant that provided the best agronomic result was 100 mL·ha^{-1} in conjunction with the application of N either at sowing or topdressing.

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