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Morphometry and production of maize inoculated with *Azospirillum brasilense*

Tauane Santos Brito, Vandeir Francisco Guimarães, Elisiane Inês Dall'Oglio Chaves, Renan Pan, Alexandre Wegner Lerner, Victor Matheus Roske, Roberto Cecatto Junior

Universidade Estadual do Oeste do Paraná- UNIOESTE, PR. E-mail: tauane-brito@hotmail.com

Abstract
This study aimed to analyze the influence of inoculation methods with *Azospirillum brasilense* in morphometric and nutritional parameters of the maize crop. Maize plants, hybrid Formula VT®, were grown under different forms of inoculation: absence of inoculation; seed inoculation; leaf inoculation; seed inoculation associated to leaf inoculation. In the phenological stages V8 and VT the parameters number of leaves, aerial height, root volume, stem diameter, besides the leaves, stem, sheath, root and total dry mass and the leaf content of nitrogen, phosphorus and potassium were determined. In the R6 stage, in addition to the previous evaluations the following parameters were determined: ear diameter and length, number of kernel rows, number of kernels per row, the reproductive structure and thousand grains dry mass, and total number of grains, besides the grain's content of nitrogen, phosphorus, and potassium. The SPAD index from the leave's apex, medial and basal thirds was measured fortnightly. The inoculation methods with *A. brasilense* had little influence in maize's morphometric and nutritional parameters, also not influencing in production. Maize plants inoculated with *A. brasilense*, via seed and via seed associated to leaf spraying, positively stood out for the stem diameter, leaves dry mas, root volume, and for the nitrogen, phosphorus, and potassium leaf content.
Keywords: chlorophyll relative content; plant growth promotion; *Zea mays* L.

Morfometria e produção de milho inoculado com *Azospirillum brasilense*

Resumo
O objetivo do estudo foi analisar a influência de métodos de inoculação com *Azospirillum brasilense* em parâmetros morfometricos e nutricionais da cultura do milho. Foram cultivadas plantas de milho híbrido Formula VT® sob diferentes formas de inoculação: ausência de inoculação; inoculação via semente; inoculação via foliar; inoculação via semente associada a foliar. Nos estádios fenológicos V8 e VT foram mensurados número de folhas, altura de parte aérea, volume de raiz, diâmetro de colmo, massa seca de folha, colmo e bainha, raiz e total, além dos teores de nitrogênio, fósforo e potássio foliar. No estádio R6, junto as análises anteriores, analisou-se diâmetro e comprimento de espiga, número de fileiras, número de grãos por fileira, massa de estrutura reprodutiva e de mil grãos e número total de grãos, e os teores de nitrogênio, fósforo e potássio dos grãos. Quinzenalmente, foi mensurado o índice SPAD dos terços apical, mediano e basal foliar. Os métodos de inoculação pouco influenciaram em parâmetros morfométricos e nutricionais do desenvolvimento do milho, não influenciando na produção. A inoculação via semente e via foliar associado a semente influenciou positivamente nas plantas quanto ao diâmetro de colmo, massa seca foliar, volume de raiz, e aos teores de nitrogênio, fósforo e potássio.
Palavras-chave: promoção de crescimento vegetal; teor relativo de clorofila; *Zea mays* L.

Introduction

The plant development cycle covers production, consumption, and energy accumulation stages, seeking to obtain descendants at its end, which will propagate the specie. These processes start at the beginning within the absorption of water, light, and nutrients, reflecting directly on one product, the mass.

Maize has high adaptability in various environments to reach the apex of its potential, that is greater mass, the crop is subsidized by soil preparation methods, fertilization, irrigation, pest, pathogen and weeds control, genetic improving methods, among other techniques that have been improved over years of research (GUIMARÃES *et al.*, 2017; SANGOI *et al.*, 2015).

Mass represents how much was absorbed and converted of metabolites, resulting in the descendant reserves. In maize (*Zea mays* L.), the monitoring of mass accumulation along the cycle is important to define hydric, nutritional, and luminous demands according to the phenological stage. Due to the evapotranspirative demand imposed by the atmosphere, the processes of absorption, transport and transpiration processes of plant metabolisms occur, aiming to supply the crop physiological and nutritional needs, being closely linked to photosynthesis, limiting the carbon assimilation and the conversion of metabolites into dry mass (TAIZ *et al.*, 2017).

The association of Poaceae with plant growth promoting bacteria (PGPB) stood out in researches due to these microorganisms potential for biological nitrogen fixation, but nowadays it has been aimed to find out and comprehend other potentials they have, specially related to their capacity to produce phytohormones, influences in the tolerance to deficit situations, mineral phosphates solubilization, biological control, among others (FUKAMI *et al.*, 2018; GUIMARÃES *et al.,* 2017).

The genus *Azospirillum* is widely used in researches with maize (SANGOI *et al.*, 2015; BULEGON *et al.*, 2017; MORAIS *et al.*, 2017). Some studies aimed to understand how the growth promotion stimulated by these microorganisms can reduce production costs. However, PGPB have a wide action spectrum resulting in different effects in the plant development. This versatility influences even the area of the plant that is inoculated with the organism, influencing the responses obtained.

Studies carried with the maize crop highlight that inoculated plants show increases in characteristics of agronomic interest, such as stem diameter, aerial height, dry mass, root mass, yield (DARTORA *et al.*, 2013; QUADROS *et al.*, 2014; KAPPES *et al.*, 2013; NOVAKOWISKI *et al.*, 2011), increased nutrient use efficiency (MORAIS *et al.*, 2016), and higher leaf chlorophyll indices (DARTORA *et al.*, 2013; QUADROS *et al.*, 2014; KAPPES *et al.*, 2013).

Studies of Morais *et al.* (2016), aiming to evaluate the effect of *A. brasilense*-based inoculant doses, in the sowing groove combined with doses of nitrogen fertilization, reveal that the use of inoculation influences the grain production, and that the grooving application technique is more efficient in the Brazilian "Cerrado" conditions.

Aiming to reduce production costs, by associating microorganisms and reducing the doses of inorganic fertilizer, Garcia *et al.* (2017) using *A. brasilense* with variations, to more or less, of the nitrogen ideal dose, found that when using half dose of inoculant it causes a superior agronomic performance in maize, especially in the grain yield, thousand grain mass and root and aerial dry matter.

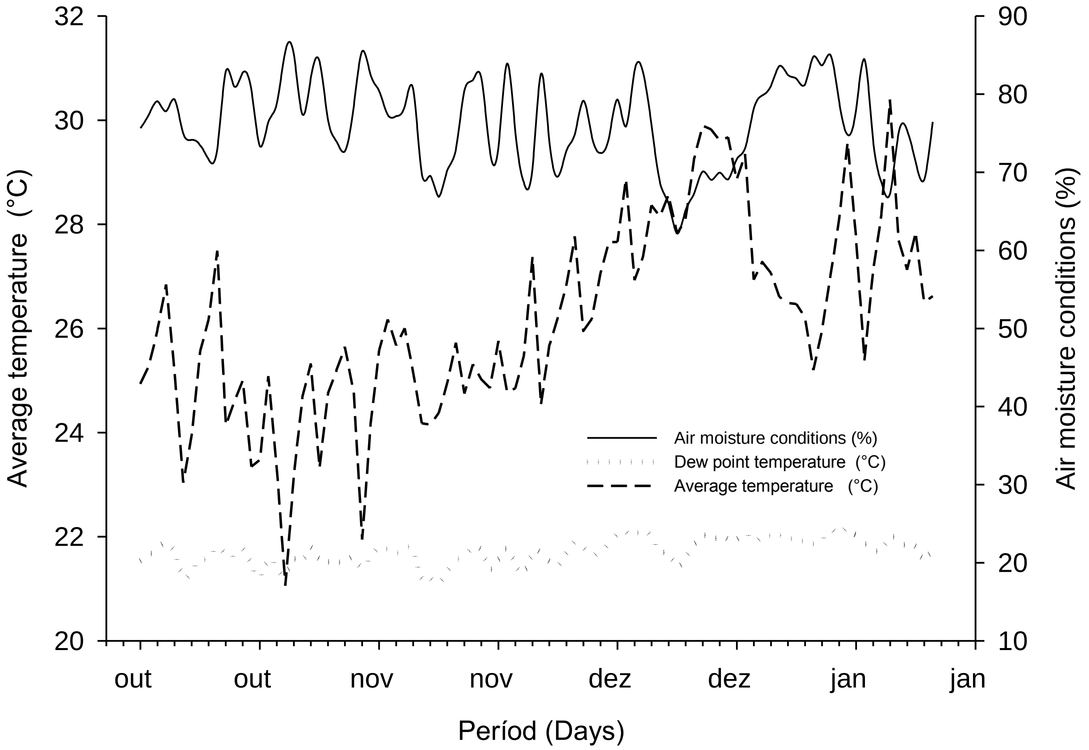
Evaluating the inoculation with *A. brasilense* in maize second crop, Costa *et al.* (2015) testing three inoculation methods (absence of inoculant, seed inoculation and leaf inoculation) associated to doses of fertilizer, report that inoculated plants presented superior performance for plant's height, biomass accumulation, ear length, chlorophyll content and grain production, when compared with the non-inoculated control, independently of the method used.

From the exposed, this study aimed to analyze the influence of inoculation methods with *Azospirillum brasilense* over the morphometric and nutritional parameters of the maize crop.

Material and Methods

The experiment was conducted in a gable type greenhouse covered with 10.00 mm alveolar polycarbonate plates treated for ultraviolet rays. This unity contains evaporative panels for cooling and a datalogger Easy Log (model EL-USB-2-LCD) for monitoring the temperature, air moisture and dew point temperature hourly (Figure 1).

Figure 1. Conditions of air relative moisture, dewpoint temperature and average temperature in protected environment, in Marechal Cândido Rondon, Paraná, in the years 2017 and 2018.



The structure is located in the horticulture and protected environment station Prof. Dr. Mário César Lopes, belonging to the experimental nucleus station of the "Universidade Estadual do Oeste do Paraná – Unioeste" campus Marechal Cândido Rondon city, located in the western region of Paraná, which climate is classified as Cfa mesothermic humid (PEEL *et al.*, 2007) with mean altitude of 420 m and geographic coordinates 54° 22' W and 24° 46' S.

The experimental design used was of randomized blocks, with four treatments and six replicates. The treatments consisted in four inoculation methods: absence of inoculation, seed inoculation at sowing; leaf inoculation in the V4 stage (50% of all plants with four completely expanded leaves); and seed inoculation associated to leaf inoculation (at sowing and at the V4 stage, respectively).

The experimental unities consisted of 25 dm^3 polyethylene pots (35 cm high, 34 cm diameter), filled with soil from the experimental area. The soil, classified as an Oxisol, removed from the horizon A, presented the following chemical characteristics: pH ($CaCl_2$): 5.32; organic matter: 10.25 g dm^{-3}; P available ($Mehlich^{-1}$): 112.06 mg dm^{-3}; K ($Mehlich^{-1}$): 0.45 $cmol_c$ dm^{-3}; Ca^{2+} (KCl 1 mol L^{-1}): 5.51 $cmol_c$ dm^{-3}; Mg^{2+} (KCl 1 mol L^{-1}): 1.03 $cmol_c$ dm^{-3}; Al^{3+} (KCl 1 mol L^{-1}): 0.00 $cmol_c$ dm^{-3}; H+Al (pH SMP 7.5): 3.73 $cmol_c$ dm^{-3}; bases sum (BS): 7.00 $cmol_c$ dm^{-3}; bases saturation (V): 65.23%. Being fertilized with 1900 mg dm^{-3} of N as urea, 590 mg dm^{-3} of K as KCl and also, 162 mg dm^{-3} of Zn as zinc sulphate, as recommended by Novaes *et al.* (1991) for pot cultivations.

Maize seeds from the "Fórmula Viptera®" hybrid, classified as of very early cycle and high yield, were inoculated with the Abv5 and Abv6 strains of the plant growth promoting bacteria *Azospirillum brasilense*, 30 minutes before sowing (the hybrid was chosen due to its popularity among local farmers besides its good adaptability to the region climate conditions). The inoculant "Nitro 1000 Gramíneas" (commercial brand) from the Nitro 1000 company was used in the concentration of 2.0 x 10^8 CFU mL^{-1}, in the dose of 100 mL of inoculant to each 60,000 seeds. For the leaf application, the same inoculant was used in the dose of 600 mL ha^{-1}, being diluted in water and applied with aid of a backpack sprayer, regulated for constant pressure of 40 kgf cm^{-2}, kept by CO_2.

Sowing was manually made at the end of 10/10/2017 under average temperature conditions of 25 ºC and air relative moisture around 55% (Figure 1). Each pot received six seeds which were thinned to two seeds per pot at 10 days after sowing. The leaf inoculation was carried 16 days after sowing (DAS), in the end of the day, with mean temperature of 25 °C and air relative moisture near to 50% (Figure 1). The crop managements and watering were made according to the crop need.

At 15, 30, 45, 60, 75 and 90 days after emergence (DAE), plants were analyzed for chlorophyll relative content (SPAD index) from the apex, medial and lower thirds (three, two and one third the leaf length, respectively from the base) using the portable meter SPAD 502 plus Konica Minolta.

Maize plants were morphometrically evaluated for destructive and non-destructive parameters in the phenological stages V8, VT and R6, being measured the number of leaves; aerial length, with aid of a tape, expressed in cm; stem diameter, with digital caliper, expressed in mm.

For the determination of leaf area, it was used the methodology from Benincasa (2003), where sections of known size (cm²) were removed from the leaf blade, avoiding the central nervure, and considered as leaf sample area (LS area). These were kept in paper bags and dried in an air forced circulation oven at 65 ± 3 °C for 72 hours. Then, it was measured the sample dry mass in analytical precision scale, obtaining the mass in g (DM sample). In the following, the total leaf area was measured according to the following formula

$$\text{LA Total} = \frac{\text{LS area} \times \text{TDM}}{\text{DM sample}}$$

where TDM represents the plant total dry mass in g. LA Total was expressed in cm².

Root volume was determined through the volume shifting method. Roots were washed and the leftover moisture was removed with paper towel. Next, proceeded the measuring by liquid displacement method in a 500 mL graduated cylinder, containing a 250 mL solution made by 90% water and 10% ethylic alcohol (70%), used to break the water surface tension, aiming to prevent the formation of air bubbles. Roots were completely submerged into the solution for subsequent reading, determining the volume in cm³.

Plants were sectioned into leaves, stem and sheath, root and reproductive structure (evaluated only at the R6 stage), stored in paper bags and put to dry in a forced circulation oven at 65 ± 2 °C for 72 hours. After this process, it was obtained the values for leaves dry mass, stem and sheath dry mass, root dry mass, reproductive structure dry mass and total dry mass, expressed in g.

In the R6 stage were determined the production components: ear diameter, with aid of a digital caliper, expressed in mm; ear length, with aid of a graduated ruler, expressed in cm; number of kernel rows; number of kernels per row; and, after drying util all mass reached 13% of moisture, it was determined the reproductive structure dry mass, 100 grain mass and total grain mass, expressed in g.

The material from the drying process, leaves and grains, was mowed in a blade mower type, containing a 20-mesh sieve. Next the material passed through a sulfuric digestion in heating blocks up to 375 °C (LANA *et al.*, 2016). From the resulting sample, were determined the contents of nitrogen, via drag of cases Kejldahl; phosphorus, via colorimetry spectrophotometry; and potassium, via atomic absorption spectrophotometry (LANA *et al.,* 2016). The determined value of each nutrient was expressed in g kg^{-1} of dry mass.

Data were tabulated and submitted to variance analysis by the F test of Fisher-Snedecor and, if meaningful, compared by the Tukey test at 5% probability of error, with aid of statistical software SISVAR (FERREIRA, 2014).

Results and Discussion

In figure 2 we present the data for chlorophyll relative content in the leaf's apex, medial and basal thirds, measured fortnightly along the crop cycle, in response to the inoculation methods in greenhouse.

Only at 45 days after emergence (DAE) there was a significant difference ($p \leq 0.05$) in the leaf apex third (Figure 2A) between the application methods. In the medial third (Figure 2B), there was no statistic difference between the inoculation methods in none of the evaluated periods. At 15 DAE, in the basal third (Figure 2C), there was a statistic difference ($p \leq 0.05$) between the methods tested, where the SPAD index in control plants was superior to plants that received seed inoculation.

At 45 DAE (Figure 2A), the leaf apex third presented a high chlorophyll relative content. Plants submitted to the seed inoculation method presented averages higher than plants submitted to the other inoculation methods. However, in the basal third (Figure 2C), there was a drastic reduction in the chlorophyll relative content. In this period of evaluation, it was observed high temperatures, ranging between 24 °C and 30 °C, and relative moisture content between 60 and 85%. Posteriorly to these highlighted periods, the evaluated thirds did not present meaningful differences for the SPAD index in relation to the inoculation methods tested.

Table 1 contains de data for the morphometric parameters evaluated in three phenological stages of maize. In the V8 stage, the stem basal diameter and the aerial dry mass differed in function of the inoculation methods. For the stem basal diameter, it is highlighted that plants inoculated via leaf may had a higher metabolic need, wasting energy with other development parameters, reducing the storage of photoassimilates in the stem and, consequently, reducing its diameter.

The response for the leaves dry mass accumulation in the phenological stage V8 was similar to the basal stem diameter. Leaf inoculated plants converted smaller amounts of dry biomass without differing from control plants and from those that received seed inoculation. Plants inoculated in the association seed+leaf presented higher averages of dry mass accumulation.

In the VT stage (Table 1) the parameters leave's dry mass, total dry mass and leaf content of nitrogen and potassium differed statistically in function of inoculation methods. The leaf dry mass and the total dry mass presented the same response observed in the V8 stage where plants inoculated with the methods associating seed and leaf presented higher averages, approximately 30% superior to the obtained for plants with leaf inoculation alone.

As for the nitrogen, phosphorus and potassium contents obtained in leaves from maize plants in the phenological stage VT (Table 1), plants inoculated plants via seed presented higher values for leaf nitrogen, with averages that were statistically similar to plants inoculated via leaf. However, for the potassium content, the highest averages were obtained in plants from the control, which did not differ from plants inoculated with the association seed+leaf but were statistically superior to plants submitted to the other inoculation methods tested.

In the phenological stage R6 (Table 1), the parameters root volume and leaf content of phosphorus and potassium differed statistically in function of the inoculation methods. The root volume of control plants was statistically superior to plants inoculated via seed and via leaf, without differing from the association of inoculation via seed and leaf. This difference was not significant in the previous phenological stages.

Figure 2. Fortnightly analysis of the chlorophyll relative content from the leaf apex (A), medial (B) and basal (C) thirds, of maize plants hybrid Formula VT® submitted to different inoculation methods with *Azospirillum brasilense*. *different lowercase letters differ between inoculation methods at 5% probability of error according to the Tukey test, comparing the treatments alone on the day of analysis. ns - not significant at 5% probability of error according to the Tukey test.

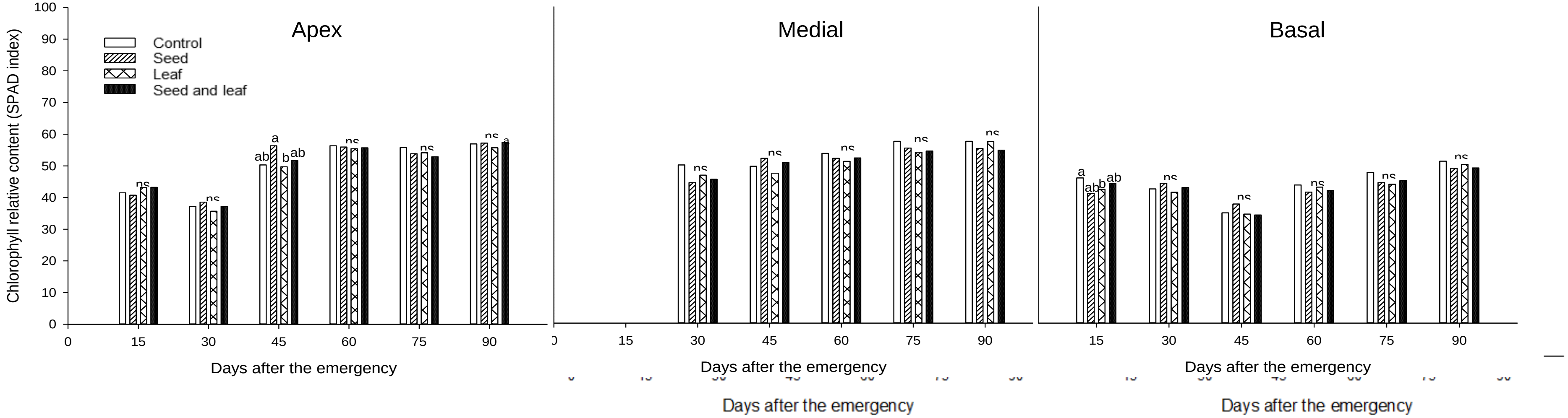


Table 1. Analysis of the morphometric components number of leaves (NL), aerial height (AH), root volume (RV), stem diameter (SD), leaves dry mass (LDM), stem and sheath dry mass (SSDM), total dry mass (TDM), leaf area (LA), leaf nitrogen content (N_l), leaf phosphorus content (P_l) and leaf potassium content (K_l) in maize plants hybrid Formula VT® submitted to different inoculation methods with *Azospirillum brasilense* evaluated in the phenological stages V8, VT and R6.

Inoculation	NL	AL	RV	SD	LDM	SSDM	RDM	TDM	LA	N_l	P_l	K_l
		-- cm --	-- cm³ --	-- mm --	------------------- g -------------------				-- cm² --	----------- $g\ kg^{-1}$ ------------		
							V8					
Control	10.10	135.30	98.00	19.27 a	14.76 ab	2.11	5.15	22.11	2023.64	31.15	1.40	44.79
Seed	11.30	136.35	68.50	18.01 a	13.14 ab	1.62	4.58	17.69	1587.60	46.90	1.31	40.14
Leaf	9.10	118.40	48.00	14.75 b	8.94 b	1.36	2.05	12.43	1279.40	41.65	1.58	49.97
Seed and Leaf	9.40	140.20	69.00	18.37 a	15.98 a	2.22	3.10	23.10	2184.81	47.43	1.42	40.40
LSD	2.81	39.73	51.57	3.22	6.54	1.47	3.48	11.25	1077.77	16.71	0.30	14.91
CV (%)	15.00	15.96	38.74	9.75	26.35	42.98	49.76	31.81	32.44	21.30	11.25	18.12
							VT					
Control	12.00	250.83	200.00	15.54	23.93 ab	36.98	26.23	103.94 ab	9954.53	22.75 bc	1.18	23.64 a
Seed	12.33	242.67	197.50	17.02	24.21 ab	44.28	27.38	116.82 ab	10249.30	37.98 a	1.23	13.65 b
Leaf	12.42	245.08	144.17	15.29	21.27 b	33.30	16.95	87.05 b	9211.63	29.23 ab	1.21	15.10 b
Seed and Leaf	12.08	253.92	189.17	16.74	25.85 a	45.06	25.23	123.64 a	12259.84	17.85 c	1.36	19.37 ab
LSD	0.76	33.16	60.95	2.19	3.34	14.42	11.78	35.17	3867.83	11.07	0.25	6.09
CV (%)	3.76	8.03	20.04	8.14	8.420	21.66	29.55	19.59	22.30	21.86	10.62	18.08
							R6					
Control	11.42	262.00	283.75 a*	15.94	26.96	49.57	28.71	105.42	3303.83	12.25	0.75 b	7.09 c
Seed	11.42	268.75	183.33 b	15.03	26.84	50.58	33.65	111.25	3623.33	12.43	1.09 a	11.38 ab
Leaf	11.33	267.17	181.67 b	15.46	26.06	47.66	22.48	96.39	3059.38	16.45	0.88 b	13.04 a
Seed and Leaf	11.50	270.17	201.67 ab	15.28	25.97	46.82	21.67	94.64	2991.17	19.25	0.81 b	9.46 bc
LSD	0.84	22.39	99.21	2.74	3.31	10.31	13.92	20.19	671.83	11.25	0.15	2.60
CV (%)	4.40	5.04	8.04	10.67	7.53	12.72	31.41	11.90	12.44	39.68	9.23	13.51

* different lowercase letters between them present difference between inoculation methods at 5% of error probability according to the Tukey test.

The phosphorus leaf content was statistically superior in plants that received seed inoculation. The potassium content was statistically superior in plants that received leaf inoculation. As for the production components of maize (Table 2), there was a meaningful statistic difference ($p \leq 0,05$) only for ear diameter, where plants inoculated via seed presented averages superior to the control and to the ones inoculated with the other inoculation methods.

Table 2. Analysis of the production components ear diameter (ED). ear length (EL). number of kernel rows (NR). number of kernels per row (NKR). reproductive structure dry mass (RSDM). 100 grain mass (100M). grain total mass (GTM). grain content of nitrogen (N_g). grain content of phosphorus (P_g) and grain content of potassium (K_g) in maize plants hybrid Formula VT® submitted to different inoculation methods with *A. brasilense.*

Inoculation	**ED**		**EL**	**NR**	**NKR**	**RSDM**	**100M**	**GTM**	**N_g**	**P_g**	**K_g**
	-- mm --		**-- cm --**			**----------- g -----------**			**----------- g kg^{-1} ------------**		
Control	39.64	ab*	17.00	15.79	32.92	279.00	20.10	186.40	9.63	1.89	4.76
Seed	39.69	a	17.14	15.96	32.90	272.60	19.25	184.62	10.00	1.51	6.35
Leaf	39.39	ab	16.80	16.21	30.51	266.88	19.31	180.98	7.38	1.62	6.06
Seed and Leaf	36.90	b	17.11	15.70	32.56	266.07	19.12	165.25	9.63	1.69	5.95
LSD	2.79		1.40	1.31	5.45	34.04	1.30	32.44	3.79	0.39	1.61
CV (%)	4.30		4.96	4.93	10.17	7.54	4.01	10.87	27.42	15.55	18.46

* different lowercase letters between them present difference between inoculation methods at 5% of error probability according to the Tukey test.

The data obtained for the SPAD index (Figure 2) at the beginning of development (between 15 and 45 DAE) were inferior to the ones found in the literature in all the development stages of the crop (ARGENTA *et al.*, 2001; COSTA *et al.*, 2015; SANGOI *et al.*, 2015; MORTATE *et al.*, 2018), considering plants with the photosynthetic apparatus in full activity and with an adequate supply of nitrogen.

The studies of the supply of nitrogen via biologic fixation by plant growth promoting bacteria (PGPB) had its space reduced along the researches evolution when other functions attributed to them were discovered, especially the phytohormones production (GLICK, 2012). Phytohormones, especially auxin, act in the plant development, promoting growth (LEYSER, 2010; GUIMARÃES *et al.*, 2017). This way, in the initial development stage of maize, the high demand for nutrients and energy, stimulated by the growth promotion via bacterial colonization, may had influenced in the chlorophyll distribution in the plant, reducing the leaf content due to the redistribution.

In the leaf apex third (Figure 2A), the chlorophyll relative content for the phenological stage V8 were reduced, independent of the inoculation method tested. During this phenological stage (V8) the development rates are high, influencing in the distribution of the assimilated components to the organs in development, raising the nutritional demand.

The destructive parameters evaluated showed that plants inoculated via seed associated to leaf presented statistically superior basal stem diameter and leaf dry mass, with high leaf nitrogen content. This response of stem increase was already found in other studies, as well as the increase in the leaf dry mass. Guimarães *et al.* (2014), evaluating the development of maize in different types of soil, in function of inoculation with *A. brasilense* and *Herbaspirillum seropedicae*, highlight that inoculated plants present development superior to the non-inoculated ones in Oxisols and Ultisols, especially for the dry mass accumulation.

Calvo *et al.* (2016) evaluated how inoculants from *Bacillus* spp. affect the plant development and the nutritional contents, in different phenological stages of maize together with different nitrogen sources (urea, ammonium and calcium nitrate, and ammonium nitrate and urea), and highlighted that there was a growth promotion in inoculated plants and that the use of a microorganisms, together with nitrogen fertilization, resulted in a SPAD index statistically superior to control plants, as well as plant height, dry mass and stem diameter.

In this sense, Battistus *et al.* (2014) mention that the inoculation with growth promoting microorganisms may act positively in the physiologic development of plants. Testing how the result of inoculation is affected in seeds submitted to doses of thiamethoxam, the authors concluded that this compound is toxic for the population of *A. brasilense*, however, associating the microorganisms with the seeds treatment, gains might occur for the plants physiological quality, increasing morphometric parameters, as well as the germination velocity of seedlings.

During the phenological stage V12 (about 50 DAS), the high sensibility to drastic climatic changes may justify the reduction registered for the chlorophyll relative content in the leaves basal third (Figure 1; Figure 2B). When evaluating the inoculation with *Rhizobium phaseoli* and *Mesorhizobium ciceri* in maize in two places, Hussain *et al.* (2016) report increases in the leaf chlorophyll content in relation to the control for plants inoculated with *M. ciceri* RS-8 and RS-12 and *Rhizobium phaseoli* RS-3, in two places under field condition, in relation to the control. Used for diagnose in coffee, the methodology of the indirect content of leaf chlorophyll stands out as an advanced non-destructive method to study plant-specific processes, especially linked to photosynthesis (NETTO *et al.*, 2005).

During the phenological stage VT the plant development rate is high, aiming at grain production and the production of viable pollen, thus, higher is its demand for water, nutrients and light (TAIZ *et al.*, 2017). Plants inoculated via seed associated to leaf, which presented higher mass accumulation, presented lower nutritional concentrations of nitrogen and potassium. While growing, plants consume the necessary nutrients and distribute the storable in different places in its organism, this way, these plants with higher mass may have distributed their nutrients in more places than the smaller ones, reducing then their concentration.

During the flowering (R1, about 70 DAS) the stability found for the SPAD readings may be related to the fact that the plant directs its metabolites aiming to obtain an adequate fecundation index, thus, maintaining production, without primarily redirecting it to mass accumulation.

When in reproductive phase, plants aim to guarantee the survival of their descendants in all situations (SOUZA *et al.*, 2010), therefore in fertilization, any biological need (hydric, nutritional or biologic) not directed to the production of a viable embryo, is considered secondary, without standing out.

The same happens during the rest of the period where the grains are being filled, where plants keep their basic development functions, directing a higher concentration of metabolites produced to the grain, guaranteeing that stressful situations will be contoured, as it occurs for the SPAD index, which stays constant, ensuring a photosynthetic production that is necessary for plants survival.

In the R6 stage (Table 1), the results found for root volume, where the control treatment was superior do the others, is adequate to the ones found in the literature using the ammonium nitrate as a fertilizer source (CALVO *et al.*, 2016). These authors report that, for different nitrogen sources, there was a variation in the behavior of the root development. Still, in the absence of nitrogen fertilization, the authors obtained averages 40% higher in inoculated plants than in the treatment control.

The accumulation of nutrients in the aerial part in the phenological stage R6 was superior in inoculated plants (Table 1). Besides the nitrogen biological fixation and phytohormones production, bacteria from the genus *Azospirillum* are mentioned as solubilizers of soil natural phosphate sources, increasing its availability in a usable way for plants (GLICK, 2012; CASTILLO *et al.*, 2015).

The following of the chlorophyll relative content along time is an indispensable tool for the comprehension of the plant evolution along its cycle. Directly related to the nitrogen content and, consequently to productivity (ARGENTA *et al.*, 2001), in this study it was possible to notice how the indexes stay constant, independent of the inoculation method, after the beginning of the reproduction phase of plants, where the growth becomes secondary due to productivity.

In this sense, inoculated plants always present smaller index SPAD from 60 DAS, gradually reducing in relation to the control until the final analysis at 90 DAS. Studies have affirmed that the process of plant senescence in maize starts at the VT stage (SADRAS *et al.*, 2000; LAFARGE; HAMMER, 2002; SANGOI *et al.*, 2014) basing itself in the equilibrium between the reduction in the auxin production and increase in the ethylene production, which stimulates the formation of breaking enzymes, which act to degrade walls, leading to leaf abscission (TAIZ *et al.,* 2017).

According to the affirmed, from the VT leaves should start their aging process, activating a series of biochemical processes. Between these processes there is a degradation of chlorophyll which results in a leaf yellowing at the end of the crops cycle (TAIZ *et al.*, 2017). However, the inoculated plants, different from the control, did not present small sings in the reduction of the leaf chlorophyll in the final analysis of the cycle, presenting even small increases, as in the superior third.

Between the bacterial actions related to the senescence process, the influence in the ethylene production is the main feature, where the bacteria use the hormone precursor ACA (1-aminociclopropane 1-carboxilic acid) as an energy source, blocking the ethylene production by the plant (GLICK, 2014). In addition, the bacteria also act in the auxin production, that, when in high concentrations, also inhibits the ethylene production (WOODWARD; BARTEL, 2005; SPAEPEN; VANDERLEYDEN, 2011).

The biggest nutritional concentration of inoculated plants may also be allied to this characteristic, where green plants still produce and assimilate nutrients to, then, move them to storage organs. This way, the inoculation might have influenced in the “green period’ of the plants, reflecting in the maize pre-senescence period.

The inoculation methods with *A. brasilense* had little influence in morphometric and nutritional parameters of the maize development, not influencing in production. Maize plants inoculated with *A. brasilense*, via seed and via seed associated to leaf spraying, positively stood out for the stem diameter, leaves dry mas, root volume, and for the leaf content of nitrogen, phosphorus and potassium.

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