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Methods of inoculation of plant growth-promoting rhizobacteria in specialty maize genotypes under organic agriculture system

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ABSTRACT. Organic agriculture systems have the nutrients supplied by plant or animal by-products, bioinoculants, and compost-based products as earthworm composts and green manures. However, the quantitative and qualitative parameters of soil amendments depend on their sources, and soil amendments are generally not sufficient to supply the nutritional requirements of maize crops. Moreover, specialty maize requires high levels of N. Thus, the aim of this study was to investigate specialty maize varieties supplied with two microbial inoculants applied in two inoculation methods. These factorial treatments were compared with their checks (varieties without inoculation), and the interaction among these factors was also investigated. The trials were carried out during the growing season in 2017–2018 in the State University of Maringá. The popcorn trial followed the randomized complete block design where the factorial 3 × 2 × 2 + 3 had five replications. The trial with white grits maize followed the same experimental design but the factorial scheme was 2 × 2 × 2 + 2 with three replications. Both trials had maize varieties and two species of microbial inoculants (*Azospirillum brasilense* and *Methylobacterium* sp.) applied in two inoculation methods, in the seeds and the foliar spray at V4 stage of plant development. The response traits were grain yield and the components of crop production. In both trials, we verified that the majority of the interactions among the factors was non-significant ($p > 0.05$), indicating the independence of these factors. Furthermore, the microbial inoculants had no beneficial effects on the traits. The possibility of a higher crop yield did not confirm the application of the inoculant in the stage V4. The organic compost may be the key point in mitigating the treatments with microbial inoculants due to the availability of N in the first stages of plant development. The traits also suggest the necessity of more trials about the influence of microbial inoculants on specialty maize production.

Keywords: green agriculture; popcorn; white grits maize; bioinoculants.

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Introduction

Brazil has been one of the largest organic food producers, with a hectarage of 1.13 million and a 19 % increase in the number of organic farmers in the last decade (Lima, Galiza, Valadares, & Alves, 2019). This growth has been the result of an increased "costumers" preference for healthy food, producing quality and fair trade. In this scenario, maize crops have been important players due to their versatile roles in rural estates producing plant and animal food, applying rotational and plant succession systems, and allowing the consortium of crops (Cruz et al., 2006). Furthermore, specialty maize production such as for white grits corn, sweet corn, baby corn, and popcorn could increase the profits from organic agriculture systems because they aggregate significant values from the produce.

The conventional farming system is considered highly dependent on external inputs such as chemical fertilizers that can, when used improperly, cause contamination of the soil, water, and air. High dependence on fertilizers, for example, could cause increased energy costs for the conversion of atmospheric N (Macdonald, Bennett, Potter, & Ramankutty, 2011).

The excess nutrients applied in conventional agriculture could cause environmental problems. The environmental costs of all N losses in Europe have recently been estimated at 70 to 320 billion euros per year, which outweighs the direct economic benefits of the use of N in agriculture (Foley et al., 2011). Conventional agriculture is considered one of the main polluters of water resources, with salinity and nitrate contamination being the main pollution indicators (Diaz & Rosenberg, 2008). In agriculture, high grain yield demand high N levels, and maize has the highest absorption capacity of nutrients from the soil (Setiyono, Walters, Cassman, Witt, & Dobermann, 2010).

The input of N into organic agriculture systems is through plant or animal by-products, organic composts, green manures, earthworm composts, and biofertilizers (Shennan et al., 2017). However, the quality of these inputs depends on their sources, and their nutritional composition may be insufficient to meet the crop's nutritional needs. Among the alternatives used to complement the supply of nutrients in the organic system is the use of inoculants containing plant growth-promoting rhizobacteria (PGPR) (Larsen et al., 2017). This group of bacteria has an important effect on the uptake of nutrients by plants, as they work as bio-fertilizers and phyto-stimulants and they mitigate the biotic and abiotic stresses (Pii et al., 2015; Zeffa et al., 2019). The genera of PGPR that are associated to crops are the *Arthrobacter*, *Azobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Clostridium*, *Gluconacetobacter*, *Herbaspirillum*, *Metyilobacterium*, and *Pseudomonas* (Chandra, Pallavi, Barh, & Sharma, 2018).

In maize, the genus *Azospirillum* has been the focus of investigations (Hungria, Campo, Souza, & Pedrosa, 2010; Fukami, Nogueira, Araujo, & Hungria, 2016; Spolaor et al., 2016; Zeffa et al., 2018; Zeffa et al., 2019), as it has been used as an inoculant in Brazil. *Azospirillum* promotes plant growth through the biosynthesis and release of amino acids, indoleacetic acid, cytokinins, and other polyamines that promote root growth (Vejan, Abdullah, Khadiran, Ismail, & Nasrulhaq Boyce, 2016). Furthermore, this genus fixes N from the air, with a direct contribution to the available N in non-leguminous species (De-Bashan et al., 2016). In Brazil, the standard inoculation of maize with *A. brasilense* is carried out by mixing microbial strains with the seeds. However, seed treatment with fungicides may be toxic to these microorganisms and might affect the efficiency of the *Azospirillum* as well as other PGPR (Yang, Hamel, Vujanovic, & Gan, 2011), which is not a part of the organic agriculture scope. Thus, post-emergence inoculation may be the alternative (Fukami et al., 2016; Galindo et al., 2019; Omara et al., 2020). Furthermore, Andrade, Zoz, Zoz, Oliveira, and Witt (2019) applied *A. brasilense* in the seeds and verified lower percentage and index of seedling emergence in some genotypes. Moreover, the application of *A. brasilense* in seeds or into furrows also reduced the percentage of emergence and increased the average time of emergence in some genotypes of sorghum.

Genus *Methylobacterium*, also called pink-pigmented facultative methylotrophic (PPFM) bacteria, has also been widely studied as a plant growth-promoting bacteria in several agricultural crops (Chanratana et al., 2017; Grossi et al., 2020; Krug et al., 2020). Species of this genus benefit plants by the production of indole acetic acid, cytokinins, and vitamin B12 or through the production of growth-modulating enzyme 1-aminocyclopropane-1-carboxylate (ACC) desaminase (Joe, Saravanan, Islam, & Sá, 2013; Dourado, Neves, Santos, & Araújo, 2015).

In this study, we hypothesized that spraying other strains of bacteria on maize leaves at stage V4, as an additional treatment to the seed application, could increase the grain yield of some genotypes. The foliar spray at V4 stage may also be dependent on the maize genotype and bacterial strains. Thus, we aimed to analyze the interaction of two inoculants with varieties of popcorn and white grits maize applied under two inoculation methods and verify the possibility of increasing the crop yield under the organic system of crop production.

Material and methods

Experimental conditions

The trials were carried out in the growing season from 2017 to 2018 in the Iguatemi Research Farm, State University of Maringá (UEM), northwestern Paraná State, Brazil (23°11" S, 52°03" W, and altitude of 550 m). The soil is the *Latossolo Vermelho eutroférrico*, having a sandy-clay-silt texture based on the Brazilian classification (Santos et al., 2018). The physical structure contains sand (75%), clay (21 %), and silt (4%). The experimental site was certified as an organic agriculture system of crop production by the ECOCERT. We collected the maximum and minimum daily temperatures and rainfalls along the experimental period, as shown in Figure 1.

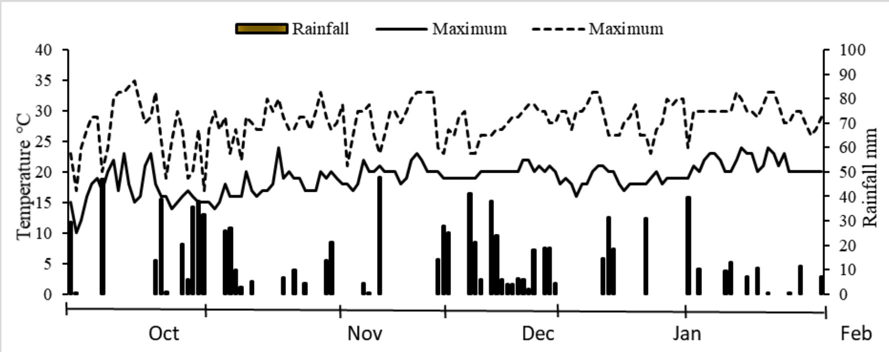

Figure 1. Maximum and minimum daily temperatures and rainfalls along the experimental period; Maringá, Paraná State, Brazil, 20017-20018.

The soil chemical analysis (0–20 cm) is reported in Table 1.

Table 1. Results from the soil chemical analysis.

pH				cmol$_c$ dm^{-3}						%			
CaCl$_2$	H$_2$O	Al^{+3}	H$^+$+Al^{+3}	Ca^{+2}	Mg^{+2}	K$^+$	SB	T	V	Ca		Mg	K
5.2	5.9	0.09	3.60	1.42	0.87	0.19	2.48	7.45	57.33	23.36		14.31	2.82
				g dm^{-3}					mg dm^{-3}				
Ca/Mg	Ca/K	Mg/K	(Ca+Mg) K	OM	C	P	S	Cu	Zn	Fe	Mn	Na	B
1.63	7.47	4.58	19.25	16.29	5.31	8.71	6.81	264.	1.62	42	71	NA	0.19

SB = total of bases; OM = organic matter; T = CEC total; V = base saturation; and NA = not available.

The open pollination varieties used in this study were "IAC 125" (Instituto Agronômico de Campinas, IAC), "Composto Angela" (Empresa Brasileira de Pesquisa Agropecuária, Embrapa), and "Composto Gaúcha" (State University of Maringá, UEM). In the original genetic background, the genotype "IAC 125" is one top-cross of popcorn, and "IAC Nelore" and "IPR 119" (Instituto de Desenvolvimento Rural do Paraná, IDR-Paraná) are hybrids from the inbred lines of white grits maize. The "Composto Gaúcha" is the result of crossing American hybrids with national varieties. Furthermore, many generations were obtained at random crosses by the plants of each hybrid; therefore, all of them may be considered open pollination varieties.

Two seeds were sowed into planting holes, but 40 d later, we thinned them to maintain just one plant in every planting hole. The plots had eight lines of 6 m in length, 0.9 m apart, and the useful area was of 9 m^2. Organic compost from laying hen manure following the composition in Table 2 was applied into the soil at a dose of 4.5 t ha^{-1}.

Table 2. Chemical analysis of the laying hen manure.

Nutrients and humidity determination	Unity	Sample
Nitrogen (N)	g kg^{-1}	19.53
Calcium (Ca^{2+})	g kg^{-1}	26.25
Magnesium (Mg^{2+})	g kg^{-1}	7.63
Phosphorus (P)	g kg^{-1}	10.57
Potassium (K$^+$)	g kg^{-1}	32.00
Humidity	%	12.86

We controlled the insects (*Spodoptera frugiperda*) by applying *Azadiractina*, 300 mL ha^{-1}), following the recommendation from the company, and we controlled the weeds in the plots by handy-hoeing.

Bacterial growth and inoculation

The inoculants from the bacterial collection of the Laboratory of Molecular Biochemistry in the State University of Londrina (LBM-UEL) were applied at a dose of 1 × 10^8 cells. We cultivated the colonies of *A. brasilense* strain Ab-V5 and *Metyilobacterium* sp. strain 40 GRM1 in liquid medium Dygs (Rodrigues Neto, Malavolta Jr., & Victor, 1986), 2.0 g of glucose, 1.5 g of peptone, 2.0 g of yeast extract, 0.5 g of K_2HPO_4, 0.5 g of $MgSO_4$, and 1 L of distilled water at pH 6.0 for 24h. Thereafter, we multiplied them in 250 mL of liquid medium M15 (Oliveira et al., 2017). This composition was formulated by the LBM-UEL, a patent presented to the National Institute of Industrial Property (INPI, deposit in the BR 1020140171746), and we cultivated them under shaking using an orbital incubator for

48h (180 rpm at 28ºC). Next, we determined the cell concentration in the Neubauer chamber and normalized the cell suspension culture with dilution in the inoculant UEL liquid. We applied two inoculation methods—in the seeds (30 mL kg^{-1}) and in the stage V4 by spraying it on the growing plants (1.0 L ha^{-1}).

Traits

The harvesting of the plants in the stage R6 from the two central lines of the useful area was a handy work. We also evaluated the plant height (m), ear height (m), ear number per plot (n), ear length (cm), stalk and ear diameter (cm), yield (kg ha^{-1}), and biomass of 100 grains (g).

Experimental design and statistical analysis

The experimental design in both trials was randomized complete blocks with the treatments following the triple factorial with additional checks (varieties without the application of microorganisms). The popcorn trial followed the factorial 3 × 2 × 2 + 3 (varieties × inoculants × inoculation methods + checks) with five replications. The white grits maize had the trial following the factorial 2 × 2 × 2 + 2 (varieties × inoculants × inoculation methods + checks) with three replications.

After verifying the homogeneity of variance and the normality of residues using the tests de Bartlett and Shapiro-Wilk, respectively, we analyzed the data by analysis of variance (ANOVA) ($\alpha = 0.05$). We applied t-test (Fisher's least significant difference) to discriminate the varieties of popcorn, but we compared the white grits maize varieties by the F test. The Dunnett test compared the checks with the treatments in the factorial. The software SISVAR (Ferreira, 2011) and SAS (2013) were used for the respective analysis.

Results and discussion

The main factor varieties in the trial with popcorn had significant effects ($p < 0.05$) for all the traits, indicating genetic differences (Table 3). Otherwise, the responses from both inoculants (*A. brasilense* and *Methylobacterium* sp.) were non-significant ($p > 0.05$) (Table 3) as well as from all the interactions among the factors. These results for the interactions indicated the independence of factors. We also found significant differences for the inoculation methods, seeds or V4, ear height, ear number per plot, stalk diameter, and biomass of 100 grains (Table 3).The coefficients of variation ranged from 6.03% for the ear diameter (ED) to 17.92 % for the grain yield (Y), which are low to average values, indicating accuracy in the environmental control by the experimental design and the outcome of the reliable data (Fritsche-Neto, Vieira, Scapim, Miranda, & Rezende, 2012).

Table 3. Analysis of variance of eleven traits in the popcorn crops: plant height (PH), ear height (EH), ear number per plot (EN); stalk diameter (SD), ear diameter (ED), ear length (EL), grain yield (Y), and biomass of 100 grain (B100) from the trial in the Iguatemi Research Farm, Maringá, Paraná State, Brazil, 2017–2018.

Sources of variation	DF	Mean Square(1)							
		PH (m)	EH (m)	EM (-)	SD (cm)	ED (cm)	EL (cm)	Y(2) (kg ha^{-1})	B100 (g)
Variety (V)	2	0.492*	0.523*	482.017*	26.365*	217.113*	11.137*	7.062*	126.842*
Methods (M)	1	0.043ns	0.044*	160.067*	19.574*	0.0008ns	0.308ns	0.004ns	8.050*
Inoculant (I)	1	0.022ns	0.006ns	8.067ns	11.731ns	0.015ns	0.628ns	0.010ns	0.712ns
V × M	2	0.003ns	0.004ns	59.617ns	1.128ns	0.811ns	0.559ns	0.006ns	0.177ns
V × I	2	0.006ns	0.001ns	25.017ns	0.833ns	3.209ns	0.476ns	0.111ns	0.099ns
M × I	1	0.002ns	0.006ns	32.267ns	1.270ns	0.687ns	0.032ns	0.013ns	3.208ns
V × M × I	2	0.000ns	0.004ns	57.817ns	0.082ns	3.566ns	0.680ns	0.002ns	0.022ns
Factorial vs Checks	1	0.008ns	0.00ns	38.881ns	16.442*	0.707ns	0.559ns	0.384*	1.617ns
Checks	2	0.068*	0.075*	114.867*	9.213ns	34.29*	1.196ns	1.220*	22.947*
(Treatments)	(14)	0.087*	0.091*	122.711*	8.876*	37.099*	2.116ns	1.230*	22.411*
Blocks	4	0.142	0.043	106.353	5.410	9.243	2.339	0.413	3.812
Residual	56	0.011	0.008	35.075	3.580	4.052	1.386	0.086	0.883
Total	74	-	-	-	-	-	-	-	
CV (%)		6.55	10.55	15.47	11.20	6.03	8.61	17.92	6.37
Overall average		1.630	0.851	38.293	16.898	33.355	116.574	1.639	14.737
Check average		1.608	0.831	39.733	15.962	33.549	13.846	1.496	14.443
Basic assumptions(3)									
Shapiro-Wilk – W		0.9886ns	0.9856ns	0.9839ns	0.9840ns	0.9270*	0.9799ns	0.5117*	0.9884ns
Bartlett – χ^2		12.1134ns	27.1458*	13.5732ns	13.3626ns	41.8949*	12.5792ns	645.3000*	9.3245ns

DF = Degree of freedom. CV = Coefficients of variation; (1)*:significant effect at 5 % by the F test; ns, non-significant effect at 5 % by the F test; (2):productivity correction according to the grain humidity; (3):Basic assumptions were normal residues by Shapiro-Wilk test, calculated value of W; homogeneity of variance by Bartlett; and χ^2, chi-square value.

In Table 3, the contrast "Factorial vs Check" was significant ($p < 0.05$) for stalk diameter (SD) and Y, indicating differences in the means of the factorial treatment scheme and the means of the additional checks (varieties without inoculation). Thus, the Dunnett test (Table 4) compared the means from the factorial with every mean from the additional checks (varieties without inoculants). In fact, we found no difference between varieties under inoculation and the respective checks for both responses. According to Matsumura et al. (2015), the response to inoculation depends on plant genotypes, bacterial strains, environmental conditions, agricultural practices, and quantity and quality of the bacterial cells inoculated. In this sense, it is interesting to point out that when these bacteria were inoculated in soils with a large amount of nutrients, they generated energy costs for the plants, thus not improving the production components in the first harvest that was used (Oliveira et al., 2020).

Table 4. Estimates of contrasts from stalk diameter (SD) and grain yield (Y).

	Treatments	Checks		
		P_2SI	P_3SI	P_1SI
SD	P_2AS	1.112	0.732	3.250
	P_2MS	1.910	1.530	4.048*+
	P_2AV_4	0.666	0.286	2.804
	P_2MV_4	1.098	0.718	3.236
	P_3AS	1.274	0.894	3.412
	P_3MS	2.886	2.506	5.024*+
	P_3AV_4	0.034	0.414	2.104
	P_3MV_4	1.062	0.682	3.200
	P_1AS	0.682	1.062	1.456
	P_1MS	0.434	0.054	2.572
	P_1AV_4	1.482	1.862	0.656
	P_1MV_4	1.230	1.610	0.908
Y	P_2AS	0.2612	0.1254	0.8552*+
	P_2MS	0.1162	0.2704	0.7102*+
	P_2AV_4	0.2888	0.0978	0.8828*+
	P_2MV_4	0.1570	0.2296	0.7510*+
	P_3AS	0.6104*+	0.2238	1.2044*+
	P_3MS	0.7144*+	0.3278	1.3084*+
	P_3AV_4	0.5470*+	0.1604	1.1410*+
	P_3MV_4	0.7304*+	0.3438	1.3244*+
	P_1AS	0.4834	0.8700*-	0.1106
	P_1MS	0.6064*-	0.9930*-	0.0124
	P_1AV_4	0.4878	0.8744*-	0.1062
	P_1MV_4	0.5276	0.9142*-	0.0664

Values followed by *+ are different and higher than that of the check, Dunnett test at 5 % of probability; Values followed by *- are different and similar to that of the check, Dunnett test at 5 % of probability; P_1, IAC 125; P_2, "Composto Angela"; P_3, "Composto Gaúcha"; A, *Azospirillium*; M, *Metylobacterium*; S, seed application; V_4, application at the plant stage V4; and SI, checks.

In the current trial, the effect of the organic fertilizer may be the key factor that affected the absence of response from the biofertilizer because of the high availability of N in the initial stages of development. Rozier, Hamzaoui, Lemoine, Czarnes, and Legendre (2017), who studied the effect of the inoculation with *Azospirillum lipoferum* associated with different levels of N fertilizer, verified that the application of the chemical fertilizer increased the grain yield. However, there was no influence of the inoculation with *A. lipof*erum on the increase. This result suggests that both technologies are non-additive. Similarly, a meta-analysis study about the effect of the *Azospirillum* sp. on the crop yield of maize by Zeffa et al. (2018) also found a non-additive effect of the inoculant with the application of N fertilizer, and the increase was observed just in the absence of the side-dressing chemical fertilizer.

The popcorn varieties "Composto Angela" and "Composto Gaúcha," which had the seeds inoculated with *Methylobacterium* sp. (P2MS and P3MS, respectively), had better performance than that of the check "IAC 125" (P1SI) for the trait SD (Table 4). As for the grain yield, the variety "Composto Angela," which had the seeds and V4 stages inoculated with *Methylobacterium* sp. or *Azospirillum* sp. (P_2AS, P_2MS, P_2AV_4, and P_2MV_4), differed and had a higher grain yield than that of the check "IAC 125" (P_1SI). Otherwise, "Composto Gaúcha" was different and higher than the checks "Composto Angela" and "IAC 125" (P2SI and P1S1, respectively) when inoculated with microorganisms both in the seeds and stages of development (P_3AS, P_3MS, P_3AV_4, P_3MV_4). These results corroborated those of the other trials in literature about the genotypes and their interactions with the inoculants (Matsumura et al. 2015; Vidotti et al., 2019; Zeffa et al., 2019). A study by

Zeffa et al. (2019), which assessed the effect of *A. brasilense* on 27 maize genotypes from three trials, also reported different responses from the genotypes after the inoculation.

In Table 5, we report the results from the t test (LSD) for the different varieties of popcorn. We observed that "Composto Angela" and "Composto Gaúcha" had similar responses for the majority of the traits. The variety "IAC 125" had the lowest estimate for plant height (PH) with 1.46 m, ear height (EH) with 0.68 m, and ED with 29.60 cm, but the "Composto Gaúcha" had higher values than those of the other varieties for Y (2.22 kg plot^{-1}) and biomass of 100 grains (B100) with 17.64 g. All these results could be explained by inbreeding or losses in the hybrid vigor from several traits of the "IAC 125."

Table 5. Means from the t-test (LSD) discriminating eight traits of popcorn: plant height (PH), ear height (EH), ear number per plot (EN), stalk diameter (SD), ear diameter (ED), ear length (EL), grain yield (Y), and biomass of 100 grains (B100) from the trial in the Iguatemi Research Farm, Maringá, Paraná State, Brazil, 2017–2018.

Varieties	PH (m)	EH (m)	EM (-)	SD (cm)	ED (cm)	EL (cm)	Y(1) (kg ha^{-1})	B100 (g)
"Composto Angela"	1.771 a	0.993 a	39.250 ab	17.745 a	34.416 a	13.987 a	1.771 b	13.956 b
"Composto Gaúcha"	1.673 a	0.899 a	42.050 a	17.845 a	35.904 a	14.133 a	2.216 a	17.644 a
IAC 125	1.464 b	0.678 b	32.500 b	15.808 a	29.601 b	12.774 a	1.039 c	12.830 c

(1)Means followed by the same letter in the column do not differ from one another at 5 % of probability by the t-test (LSD). Crop yield was corrected by the humidity.

Based on the inoculation methods, popcorn plants had significant differences ($p < 0.05$) for EH, EN, SD, and B100 (Table 6). The application of inoculants on the seed promoted higher values for EH, SD, and B100 than that of EN, from which the highest value was caused by the inoculant spray in the stage V4, which was investigated and reported for the first time (Table 6). The possibility of a higher crop yield did not confirm the application of the inoculant in the stage V4, as the number of ears per plot is just a component of the grain yield.

Table 6. Means from the inoculation methods of the following traits of popcorn: ear height (EH), ear number per plot (EN), stalk diameter (SD), and biomass of 100 grains (B100) from the trial in the Iguatemi Research Farm, Maringá, Paraná State, Brazil, 2017–2018.

Inoculation methods(1)	EH (m)	EM	SD (cm)	B100 (g)
Seed	0.884 a	36.300 b	17.704 a	15.176 a
V4	0.829 b	39.567 a	16.561 b	14.444 b

(1)Means followed by the same letter in the column do not differ from one another at 5 % of probability by the F test.

In the white grits maize, we did not find significant differences ($p > 0.05$) for all the interactions among the factors for all traits, except for the interaction of varieties (V) × inoculation methods (M) for ear length (EL) and B100 (Table 7). We did not carry out the partition of this interaction, V × M, for the two traits, as both are components of the grain yield, and these principal traits had no significant difference. The contrast "Factorial vs Check" did not have significant differences for all the traits. All these inferences are valuable because the CVs from all the traits were low or average, indicating high experimental accuracy. The varieties of white grits maize had significant differences ($p < 0.05$) in the traits PH, EH, ED, and B100 (Table 7), and the "IAC Nelore" had means higher than that of the "IPR119" (Table 8). Similarly, we did not find efficiency of the inoculation applied in the V4 stage in comparison to the application to the seeds (Table 8).

Mumbach et al. (2017) studied the effect of seed inoculation of commercial maize with *A. brasilense* and did not find significant responses for the stalk diameter, ear and plant height, and foliar index. Some researchers have reported that seed inoculation is an important alternative for sustainable agriculture systems (Fukami et al., 2016). However, several factors such as weather conditions, soil classification, soil microbiology, cultivars, and fertilizers can affect the responses from the inoculation due to their influence on the bacterial survivorship. All these factors must be a motive of concern as well as the inoculation due to their influence on bacterial survivorship (James, 2000). Portugal et al. (2016) investigated simple hybrids under various doses of N (0, 30, 60, and 90 kg ha^{-1}) together with foliar spraying of *A. brasilense* in the summer growing season and found increases of 14.75% in the grain yield in seed inoculation. Thus, we suggest more trials under the organic agriculture systems applying new doses of inoculants, with partition of N doses using other organic sources as well as without N application, and with application of organic manures in lines in the soil to better investigate the interaction of the different varieties under organic agriculture systems.

Table 7. Analysis of variance for ten traits of white grits maize: plant height (PH), ear height (EH), ear number per plot (EN), stalk diameter (SD), ear diameter (ED), ear length (EL), grain yield (Y), and biomass of 100 grains (B100) from the trail in the Iguatemi Research Farm, Maringá, Paraná State, Brazil, 2017–2018.

Sources of variation	DF	Mean Square$^{(2)}$ PH (m)	EH (m)	EN	SD (cm)	ED (cm)	EL (cm)	Y$^{(2)}$ (kg ha^{-1})	B100 (g)
Varieties (V)	1	0.308*	0.123*	80.667^{ns}	1.675^{ns}	32.155*	0.015^{ns}	0.496^{ns}	71.553*
Methods (M)	1	0.009^{ns}	0.024^{ns}	0.167^{ns}	4.386^{ns}	12.995^{ns}	2.344^{ns}	0.021^{ns}	5.415^{ns}
Inoculant (I)	1	0.107^{ns}	0.089*	2.667^{ns}	0.150^{ns}	14.789^{ns}	0.448^{ns}	0.378^{ns}	16.138^{ns}
V × M	1	0.056^{ns}	0.034^{ns}	54.000^{ns}	3.713^{ns}	10.720^{ns}	11.152*	0.010^{ns}	45.706*
V × I	1	0.009^{ns}	0.007^{ns}	1.500^{ns}	0.564^{ns}	3.360^{ns}	0.400^{ns}	0.006^{ns}	1.540^{ns}
M × I	1	0.042^{ns}	0.015^{ns}	42.667^{ns}	3.168^{ns}	2.220^{ns}	1.075^{ns}	1.238^{ns}	2.136^{ns}
V × M× I	1	0.007^{ns}	0.010^{ns}	1.500^{ns}	2.124^{ns}	1.179^{ns}	0.400^{ns}	0.125^{ns}	0.960^{ns}
Factorial vs Checks	1	0.008^{ns}	0.001^{ns}	0.832^{ns}	6.130^{ns}	1.358^{ns}	1.912^{ns}	0.014^{ns}	0.304^{ns}
Checks	1	0.066^{ns}	0.045^{ns}	54.000^{ns}	2.761^{ns}	5.587^{ns}	0.595^{ns}	1.084^{ns}	26.250^{ns}
(Treatments)	(9)	0.068*	0.039*	26.444^{ns}	2.741^{ns}	9.374^{ns}	2.038^{ns}	0.375^{ns}	18.889*
Blocks	2	0.270	0.089	77.500	5.072	16.239	3.479	1.754	13.543
Residual	18	0.027	0.015	35.278	6.191	5.103	1.471	0.455	7.457
Total	29	-	-	-	-	-	-	-	-
CV (%)		8.53	12.76	12.64	12.41	4.77	7.08	17.59	8.64
Overall average		1.936	0.968	47.000	20.056	47.326	17.130	3.834	31.593
Check Average		1.902	0.957	47.333	19.152	47.752	17.635	3.878	31.392
Basic assumptions$^{(3)}$									
Shapiro-Wilk – W		0.974^{ns}	0.984^{ns}	0.965^{ns}	0.962^{ns}	0.963^{ns}	0.969^{ns}	0.965^{ns}	0.952^{ns}
Bartlett – χ^2		3.650^{ns}	7.106^{ns}	5.284^{ns}	5.863^{ns}	13.950^{ns}	11.271^{ns}	5.825^{ns}	7.819^{ns}

DF = Degree of freedom. CV = Coefficients of variation; $^{(1)*}$: significant effects at 5 % by the F test; ns, non-significant effect at 5 % by the F test; $^{(2)}$: crop yield corrected to grain humidity; $^{(3)}$: Basic assumptions were normal residues by the Shapiro-Wilk, value of the calculated W; homogeneity of variance by Bartlett; χ^2, chi-square value.

Table 8. Means of varieties for the following traits in crops of white grits maize: plant height (PH), ear height (EH), ear diameter (ED), and biomass of 100 grains (B100) from the trial in the Iguatemi Research Farm, Maringá, Paraná State, Brazil, 2017–2018.

Varieties$^{(1)}$	PH (m)	EH (m)	ED (cm)	B100 (g)
'IPR 119'	1.830 b	0.899 b	46.063 b	29.917 b
'IAC Nelore'	2.058 a	1.043 a	48.378 a	33.700 a

$^{(1)}$Means followed by the same letter in the column do not differ from one another at 5 % of probability by the F test.

Conclusion

The effects of applying inoculants on the varieties of white grits maize and popcorn were non-significant for the traits under evaluation. Moreover, possibility of higher crop yield did not confirm the application of the inoculant in the stage V4.

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