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Performance of maize hybrids from a partial diallel in association with *Azospirillum*

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One of the most prominent strategies to increase maize grain yield with a higher benefit/cost ratio and a lower environmental impact is the inoculation of plant growth-promoting bacteria. Among other factors, the success of the interaction plant-microorganism depends on genetic traits, therefore, selection of plant genotypes compatible with this association is extremely important to the viability of this technology. This article presents an innovative study that investigates the interactions between *Azospirillum brasilense* Ab-V5 and 27 genotypes of maize, including 24 experimental hybrids from a partial diallel (denotated H_{ij} as a result of the crosses among the parental inbred lines L_i and the tester breeding lines T_j), the variety ST0509 from UEL and the commercial hybrids DKB390 and DKB390H from Monsanto. The plots consisted of treatments with or without inoculation in three replicates and the 27 maize genotypes were randomly distributed in the sub-plots. The inbred lines L_2 , L_3 , L_6 , L_{11} , T_2 and T_3 present the highest general combining ability, producing the best hybrid combinations. The additive effects of genes are more important than the non-additive effects for all traits evaluated. The most promising experimental hybrids are $H_{2\ 3'}$, $H_{3\ 2'}$, $H_{11\ 2'}$, $H_{11\ 3'}$ and $H_{12\ 3'}$. Significant effect for inoculum was not verified when performed at the seedling stage in the experimental conditions of this study.

Key words: *Zea mays* L., *Azospirillum brasilense*, inoculation, biological nitrogen fixation, combining ability.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal crops for mankind due to the type and quantity of reserve substances of its grains, being used for human food and

animal feed, consumed *in natura* and in industrial forms (Pereira et al., 2009). This grass presents high productive potential as well as high demand for nutrients, especially

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Table 1. Incomplete partial diallel formed by simple hybrids (H) derived from the crosses among 12 elite breeding lines (L₁ and L₁₂) and three tester lines (T₁, T₂ and T₃).

Lines	T ₁	T ₂	T ₃
L ₁	-	H _{1 2'}	H _{1 3'}
L ₂	H _{2 1'}	-	H _{2 3'}
L ₃	H _{3 1'}	H _{3 2'}	-
L ₄	H _{4 1'}	H _{4 2'}	-
L ₅	-	H _{5 2'}	H _{5 3'}
L ₆	H _{6 1'}	-	H _{6 3'}
L ₇	H _{7 1'}	H _{7 2'}	-
L ₈	H _{8 1'}	-	H _{8 3'}
L ₉	H _{9 1'}	H _{9 2'}	-
L ₁₀	H _{10 1'}	-	H _{10 3'}
L ₁₁	-	H _{11 2'}	H _{11 3'}
L ₁₂	H _{12 1'}	-	H _{12 3'}

(N), which directly influences grain yield components such as photosynthesis rate, ear size, mass, sanity, number and protein content of grains (Dechorgnat et al., 2011).

Despite the benefits of the use of nitrogen fertilizer, it represents up to 40% of the total cost of maize production, due to the facts that it is largely required to reach high yields and that the use efficiency of this nutrient by the crop is low (Rambo et al., 2007). In addition to the high cost of this input, it presents risks of environmental pollution associated with leaching, denitrification and volatilization (Vitousek et al., 2009), which may lead to acidification of soils, eutrophication and increase of greenhouse gases in the atmosphere (Galloway et al., 2008). Therefore, the importance of developing strategies to increase the nitrogen use efficiency (NUE) of crops and consequently decrease the economic and environmental impact on agricultural systems is evident.

One of the strategies to increase yield, with the best benefit/cost ratio considering the environmental impact, is the use of inoculation of plant growth-promoting bacteria (diazotrophic PGPB), representing a technology of low cost and simple implementation. The mechanisms of plant growth-promotion manifested by diazotrophic PGPB encompass direct processes such as biological nitrogen fixation (BNF), production of plant growth regulators, nutrient mineralization, inorganic phosphate solubilisation and increased uptake by roots; as well as indirect effects including biological control of phytopathogens, production of siderophores and induction of systemic resistance in plants (Oliveira et al., 2014).

A great number of studies have shown that plant growth-promoting bacteria (PGPB), including *Azospirillum*,

are able to promote growth and increase yield of numerous plant species (Fallik and Okon, 1996), such as wheat, rice, maize and sorghum, where the average increase in productivity was around 20 to 30 % (Kennedy et al., 2004, Morrissey et al., 2004, Andreotti et al., 2008).

Commercial inoculants formulated with the diazotrophic plant growth-promoting bacteria (PGPB) *Azospirillum brasilense* are available for use in Brazil; however, its application is not yet adopted as a routine practice for partial substitution of synthetic nitrogen fertilizers. Inconsistencies in the performance of the inoculation with diazotrophic PGPB in field studies are a major obstacle to its wide spread, resulting mainly from limitations in the process of plant colonization. Among the factors that hinder the establishment of the inoculated microorganisms are: the use of low quality formulations and/or improper practices during transport, storage and field application, and the occurrence of unfavorable edaphoclimatic conditions for the maintenance of a high population size of the inoculated bacteria within the plant (Bashan et al., 2014).

In addition, specific molecular interactions between the associative pair are crucial for plant colonization by PGPB, which depends on genetic factors (Drogue et al., 2012; Jha et al., 2013). In this regard, the identification of highly compatible plant genotypes for association with PGPB may enhance plant colonization, enabling a higher level of expression of genes related to the compatibility of the interaction and consequently maximizing growth promotion (Meneses et al., 2011; Alquéres et al., 2013; Beauregard et al., 2013).

Therefore, the selection of genotypes favorable to this association is a field of research to be explored in order to consolidate the inoculation technology with diazotrophic PGPB as a viable alternative to synthetic nitrogen fertilizers for maize production. In this context, diallel analysis is an essential tool to identify superior parents for hybrid or cultivar development related to several traits of interest (Patel et al., 1998). Thus, the objectives of this work were to determine, using partial diallel crossing, the general and specific combining ability of twelve elite inbred lines of maize with three tester lines and to verify their possible interactions with *A. brasilense* strain Ab-V5.

MATERIALS AND METHODS

The experimental hybrids used in these experiments were developed by the Maize Breeding Programme at the Department of Biology from the State University of Londrina (UEL), derived from partial diallel crosses among three tester lines (T₁, T₂ and T₃) and twelve elite breeding lines (L₁ to L₁₂) obtained from the synthetic cultivars (improved varieties) ST06 and ST20, respectively (Table 1).

A total of 27 genotypes were evaluated: 24 experimental hybrids from the partial diallel, the variety ST0509 developed at UEL and

the commercial hybrids DKB390 and DKB390H from Monsanto. The commercial hybrids were used as a performance standard for the comparison of the experimental hybrids. The variety was used for inoculation purposes, to test whether its rustic genotype would favor the association with the rhizobacteria.

The experiments were conducted at the State University of Londrina, located in the Northern region of the State of Paraná (23° 19'19 "S and 51° 12'04" W, 580 m of altitude) during the first and second growing season of 2011/2012, in a randomized block design with treatments arranged in split-plots with or without inoculation, with three replicates. Each plot with or without inoculum contained one representative of each genotype in a row of 4 m containing 30 plants per row, with 0.8 m between rows and 0.2 m between plants within the row. Soil preparation for sowing was done by harrowing and applying 300 kg ha⁻¹ of the formulated 08-28-16 (N-P-K). Weed was controlled by manual weeding and pest control (for example, *Spodoptera frugiperda*) was carried out according to technical recommendations for the crop.

The inoculum was prepared with *A. brasilense* strain Ab-V5 from isolated colonies grown in the solid medium Dygs (2 g glucose, 1.5 g peptone, 2 g yeast extract, 0.5 g K₂HPO₄, 0.5 MgSO₄, 1 L distilled water, pH 6.0) and further multiplied in the liquid medium M15 for 48 h on orbital shaker at 30 ± 2 °C. The cell concentration of the bacterial culture was estimated by reading its absorbance in a spectrophotometer at 560 nm and diluting it in water to a final concentration of 3 × 10⁷ cells mL⁻¹. The inoculation was performed on the seventh day after the seedlings emergence (V2), in the afternoon (after 16 h), using a portable spray to apply a dose of 30 mL per meter of culture directed at the seedlings.

The characteristics evaluated were: grain yield (GY, t ha⁻¹); ear length (EL, cm); ear diameter (ED, cm); cob diameter (CD, cm); number of grain rows per ear (RE); percentage of damaged ear (% DAE); percentage of diseased ear (% DIE); days to male flowering (DF); plant height (PH, cm) and ear height (EH, cm). Grain yield was estimated based on the mass of grains harvested in each experimental subplot, with moisture corrected to 13.5 % and an ideal stand of 20 plants per row, and it was extrapolated to tons per hectare. Corrections of grain weight to ideal stand (STI) were performed using the covariance methodology, modified by Miranda Filho (Vencovsky and Barriga, 1992).

Individualized and combined analysis of variance was made for the first and second harvest for the evaluation of hybrids. The individual analyses of variance were performed with the effects of genotypes decomposed on effects of controls (C), experimental hybrids (Hy) and the contrast C vs Hy. The degrees of freedom of the experimental hybrids were decomposed using diallel analysis, according to the model proposed by Griffing (1956): $Y_{ij} = m + \hat{g}_i + \hat{g}_j + \hat{s}_{ij} + \bar{e}_{ij}$, where: Y_{ij} is the mean value of the hybrid combination of the inbred line L_i with the tester line T_j ; m is the overall mean of the experimental hybrids; \hat{g}_i and \hat{g}_j are the effects of the general combining ability (GCA) of the i -th inbred line L_i and the j -th inbred line T_j , respectively; \hat{s}_{ij} is the effect of the specific combining ability (SCA) for crosses among the genitors i and j ; and \bar{e}_{ij} is the average experimental error.

The analyses of the diallels, for the first and second harvest, and their respective decomposition were made following the methodology proposed by Filho and Vencovsky (1995). For the analysis of variance of the diallel and the estimates of \hat{g}_i , \hat{g}_j and \hat{s}_{ij} , the matrix algebra model was used: $Y = X\beta + \varepsilon$ where: Y is the vector of observed data for experimental hybrids; X is the matrix of constants related to the parameters m , \hat{g}_i , \hat{g}_j , and \hat{s}_{ij} ; β is the vector of the parameters m , \hat{g}_i , \hat{g}_j , and \hat{s}_{ij} and ε is the vector representing the error associated with the values (\bar{e}_{ij}). The program used to perform the analysis of variance was the Statistical Analysis System

(SAS/STAT® software) and the groupings of means from the treatments of each experiment were done by the Scott-Knott test, at a significance level of 5 % of probability, using the program GENES (CRUZ, 2013).

RESULTS AND DISCUSSION

The data indicates significant effect for the majority of the traits investigated regarding growing season (harvest), except for percentage of diseased ear (Table 2). The second harvest presented a reduction of 3.45 t ha⁻¹, ears 3.6 cm smaller in length and 0.5 cm in diameter, cobs 0.5 cm smaller in diameter, 3 less grain rows per ear, 7.2% more damaged ears, 1.1% less diseased ears, 1 extra day to male flowering, and plant and ear height was 52 and 39 cm lower, respectively (Table 3). These findings are in accordance with the literature, since the climatic conditions of the second harvest are generally less favorable to the development of the plants compared to the spring-summer period (first harvest), mainly due to the decrease in light intensity and rainfall (Magalhaes et al., 2007).

The effect of inoculation was not significant for any of the traits evaluated, neither for the interactions inoculum x harvest and inoculum x cultivar x harvest (Table 2). Although the recommendation for most of the commercial inoculants based on *Azospirillum* is an application to the seeds before planting (Soja, 2011), in this study, the introduction of the inoculant was performed via spraying on V2 seedlings in order to avoid contact of the bacteria with chemicals commonly used in seed treatment, what would possibly reduce its efficiency.

These results indicate that the procedure of spraying the inoculant at the seedling stage in this study was probably not able to successfully carry the bacteria due to unfavorable environmental factors that affects the colonization and establishment of their population, such as extreme temperatures, water stress and competition with native bacteria (Figure 1) (IAPAR, 2012). Optimization of this methodology should be sought to elude climatic influence on bacterial survival on the soil and plant colonization.

Santos (2011) tested the efficiency of some inoculation methods: seedling spraying, via peat and liquid path in the seed, concluding they were all successful as vehicles, especially peat and liquid under seed. Thus, this methodology, as well as the inoculation in the plantation furrows or in the soil has demonstrated efficacy even though further studies are necessary for the fine adjustment of dose, volume applied by area and time of application (Fukami et al., 2016; Morais et al., 2016).

However, there was a significant difference for percentage of damaged ear with a decrease of 2.83% in this trait for inoculated plants cultivated in the first growing season (Table 4). Although the factors that determine associative efficiency between *Azospirillum*

Table 2. Mean squares based on treatment totals, significance levels of F test, means of inoculated and non-inoculated plots, general means and the coefficients of variation for grain yield (GY, t ha⁻¹), ear length (EL, cm), ear diameter (ED, cm), cob diameter (CD), number of grain rows per ear (RE), percentage of damaged ear (% DAE), percentage of diseased ear (% DIE), days to male flowering (FL), plant height (PH, cm) and ear height (EH, cm), evaluated in Londrina in the first and second harvest of 2011/2012.

Source of variation	DF	GY	EL	ED	CD	RE	% DAE [□]	% DIE [□]	FL	PH	EH
Block/Harvest	4	4.2102*	0.6991	0.1023*	0.0161*	1.6815	356.68*	419.40*	0.8781	414.01*	204.95*
Harvest (Ha)	1	968.05*	1060.6*	17.700*	4.5986*	598.62*	4148.1*	90.798	61.797*	217342*	125450*
Inoculum	1	0.5262	2.8900	0.0378	0.0474	6.7600	294.94	2.6039	8.5069	29.642	115.68
Inoculum x Ha	1	0.4170	1.5211	0.1304	0.0465	0.2612	69.843	82.318	1.4267	307.03	27.040
Error (a)	4	1.8918	2.8381	0.0411	0.0699	2.2128	67.739	174.98	5.3210	616.63	591.02
Cultivar	26	6.3059*	6.8935*	0.2237*	0.3033*	9.0875*	140.96	187.00*	17.117*	1199.6*	766.93*
Control (C)	2	37.749*	5.0544*	0.7811*	0.5426*	15.453*	326.78*	382.87*	56.694*	995.68*	181.88*
Exp Hybrid (Hy)	23	3.8400*	7.2386*	0.1828*	0.2957*	8.8638*	127.10	169.64	8.1476*	1223.4*	842.55*
GCA-L	11	3.6721*	8.4926*	0.2651*	0.5032*	9.8843*	73.225	134.53	14.657*	2113.3*	1377.0*
GCA-T	2	20.194*	23.075*	0.2905*	0.2891*	39.428*	111.72	28.323	2.4345	1687.0*	1580.2*
SCA	10	0.7534	2.6886*	0.0711*	0.0694*	1.6259*	189.45	236.52*	2.1296	151.73*	107.09*
C vs Hy	1	0.1332	2.6322	0.0493	0.0000	1.5022	88.024	194.60	144.26*	1060.3*	197.78*
Cultivar x Ha	26	1.8685*	1.9827*	0.0367*	0.0195*	0.8947	133.71	276.20*	3.0310*	189.76*	83.103*
Control x Ha	2	11.584*	4.9478*	0.0033*	0.0100	0.6711	134.62	210.11	3.5833	425.92*	102.45
Hy x Ha	23	1.1044	1.8059	0.0407	0.0211*	0.9063	137.73	263.72*	1.5697	126.24*	78.142*
GCA-L x Ha	11	1,0659	2.3496*	0.0310*	0.0213	1.0466	120.17	220.92*	1.0645	214.56*	103.77*
GCA-T x Ha	2	3.0627*	4.7056*	0.1226*	0.0746*	0.9234	345.20*	737.41*	6.7446*	16.193	108.90
SCA x Ha	10	0.7560	0.6218	0.0343	0.0094	0.7690	115.63	216.07*	1.0977	51.102	43.845
(C vs Hy) x Ha	1	0.0108	0.1168	0.0117	0.0016	1.0756	39.269	695.23*	35.537*	1178.6*	158.52*
Inoculum x Cultivar	26	0.4992	1.0115	0.0255	0.0140	0.9918	115.28	109.79	1.2986	75.310	50.376
Inoculum x Culti x Ha	26	0.8949	0.8662	0.0327	0.0236	0.6469	103.58	84.471	1.8402	98.306	54.953
Error (b)	208	0.7565	1.1494	0.0242	0.0120	0.7882	104.77	109.24	1.8239	75.319	40.508
Inoculated	-	6.66	15.96	4.71	2.89	15.50	13.77	24.18	65.98	163.05	93.02
Non-inoculated	-	6.58	15.77	4.69	2.87	15.21	15.68	24.35	66.30	162.45	91.82
General Mean	-	6.62	15.90	4.70	2.90	15.40	14.70	24.30	66.10	162.80	92.40
CV% (a)	-	8.5	4.3	1.8	3.7	3.9	22.8	22.3	1.4	6.2	10.7
CV% (b)	-	13.1	6.8	3.3	3.8	5.8	69.5	43.1	2.0	5.3	6.9

*Significance level of 5 %, [□] = Variance analysis with data transformed to arc sine of (% DAE or DIE /100)^{0.5}.

and maize are unknown, several studies demonstrate significant increases in grain yield components in response to inoculation, even

though a large number of trials are required to eliminate spatiotemporal variations that may mask such effects (Díaz-Zorita et al., 2015).

The absence of significance for the interaction between *A. brasilense* and the different maize genotypes used in the present study indicates the

Table 3. Means of experimental hybrids (H_{ij}), resulting from the crosses of the inbred lines $L_i \times T_j$, and genotype controls for grain yield (GY, in $t\ ha^{-1}$), ear length (EL, cm), ear diameter (ED, cm), cob diameter (CD, cm), number of grain rows per ear (RE), percentage of damaged ear (% DAE), percentage of diseased ear (% DIE), days to flowering (FL), plant height (PH, cm) and ear height (EH cm), evaluated in Londrina in the first and second harvest of 2011/2012.

Cultivars	GY		EL		ED		CD		RE		% DAE		% DIE		FL		PH		EH		
	Harvest		Harvest		Harvest		Harvest		Harvest		Harvest		Harvest		Harvest		Harvest		Harvest		
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	
H1 ₂	8.53 ^b	4.34 ^b	17.60 ^a	12.8 ^b	5.0 ^a	4.6 ^b	3.2 ^a	2.9 ^a	15.1 ^c	13.4 ^b	1.0 ^b	22.9 ^a	15.0 ^b	24.1 ^a	64 ^c	65 ^c	186 ^c	128 ^c	102 ^d	64 ^c	-
H1 ₃	8.08 ^b	5.03 ^a	18.10 ^a	14.3 ^a	4.9 ^a	4.5 ^c	3.1 ^b	2.8 ^b	15.5 ^c	13.6 ^b	3.2 ^b	10.6 ^a	22.5 ^b	13.9 ^a	65 ^c	66 ^c	189 ^c	131 ^c	107 ^d	65 ^c	-
H2 ₁	7.89 ^b	4.43 ^b	16.60 ^b	13.5 ^b	5.0 ^a	4.4 ^c	3.0 ^c	2.7 ^c	18.6 ^a	14.9 ^a	7.5 ^a	9.2 ^a	27.1 ^b	18.4 ^a	65 ^c	66 ^c	185 ^c	135 ^c	111 ^c	74 ^b	-
H2 ₃	8.84 ^b	5.65 ^a	16.90 ^b	13.8 ^b	5.0 ^a	4.5 ^b	3.0 ^c	2.7 ^c	16.5 ^b	13.5 ^b	9.5 ^a	12.3 ^a	23.4 ^b	13.0 ^a	64 ^c	66 ^c	190 ^c	141 ^b	124 ^b	78 ^b	-
H3 ₁	8.56 ^b	4.80 ^a	17.73 ^a	15.0 ^a	4.9 ^b	4.5 ^c	3.1 ^b	2.9 ^b	17.9 ^a	15.1 ^a	10.8 ^a	15.5 ^a	13.2 ^b	28.3 ^a	65 ^c	67 ^b	202 ^b	143 ^b	111 ^c	70 ^c	-
H3 ₂	9.89 ^b	5.35 ^a	18.83 ^a	14.9 ^a	5.0 ^a	4.6 ^b	3.1 ^b	3.0 ^a	17.1 ^a	14.7 ^a	2.4 ^b	11.3 ^a	7.7 ^b	14.2 ^a	67 ^b	66 ^c	191 ^c	140 ^b	105 ^d	68 ^c	-
H4 ₁	6.95 ^c	4.17 ^b	15.80 ^b	12.6 ^b	5.0 ^a	4.5 ^b	3.2 ^a	2.9 ^b	17.8 ^a	15.0 ^a	5.5 ^b	11.6 ^a	13.6 ^b	16.0 ^a	65 ^c	66 ^c	166 ^d	123 ^d	94 ^e	62 ^c	-
H4 ₂	8.46 ^b	3.75 ^b	17.73 ^a	13.0 ^b	5.0 ^a	4.4 ^c	3.1 ^b	2.8 ^b	16.4 ^b	13.4 ^b	8.8 ^a	18.9 ^a	24.0 ^b	27.8 ^a	65 ^c	66 ^c	162 ^d	119 ^d	94 ^e	61 ^c	-
H5 ₂	8.33 ^b	4.30 ^b	17.73 ^a	12.6 ^b	5.1 ^a	4.6 ^b	3.3 ^a	2.9 ^a	16.9 ^a	13.5 ^b	4.4 ^b	22.3 ^a	19.2 ^b	19.4 ^a	67 ^b	68 ^b	190 ^c	133 ^c	116 ^c	77 ^b	-
H5 ₃	8.50 ^b	5.32 ^a	18.50 ^a	15.6 ^a	4.9 ^b	4.6 ^b	3.0 ^c	2.8 ^b	16.5 ^b	14.3 ^a	4.9 ^b	5.9 ^a	19.1 ^b	12.1 ^a	67 ^b	68 ^b	201 ^b	153 ^a	128 ^b	92 ^a	-
H6 ₁	7.94 ^b	4.95 ^a	16.87 ^b	14.2 ^a	5.1 ^a	4.5 ^b	3.2 ^a	2.8 ^b	18.4 ^a	15.5 ^a	2.8 ^b	11.0 ^a	18.8 ^b	17.6 ^a	67 ^b	67 ^b	191 ^c	141 ^b	114 ^c	77 ^b	-
H6 ₃	9.41 ^b	5.17 ^a	18.17 ^a	15.3 ^a	5.0 ^a	4.5 ^b	3.0 ^c	2.8 ^b	17.1 ^a	14.3 ^a	7.5 ^a	10.7 ^a	16.1 ^b	18.9 ^a	67 ^b	67 ^b	213 ^a	153 ^a	134 ^a	90 ^a	-
H7 ₁	7.82 ^b	4.50 ^b	17.77 ^a	13.1 ^b	4.9 ^a	4.4 ^c	3.1 ^b	2.7 ^c	16.5 ^b	14.0 ^b	4.7 ^b	17.4 ^a	19.7 ^b	20.5 ^a	65 ^c	68 ^b	191 ^c	137 ^b	121 ^b	73 ^b	-
H7 ₂	8.68 ^b	5.07 ^a	18.00 ^a	13.1 ^b	4.7 ^c	4.4 ^c	2.8 ^d	2.6 ^c	15.1 ^c	12.7 ^c	4.7 ^b	13.6 ^a	14.6 ^b	17.9 ^a	67 ^b	67 ^c	193 ^c	137 ^b	120 ^b	73 ^b	-
H8 ₁	7.95 ^b	4.20 ^b	18.10 ^a	14.1 ^a	4.8 ^b	4.1 ^d	2.8 ^d	2.5 ^d	17.3 ^a	14.7 ^a	9.7 ^a	19.8 ^a	21.5 ^b	26.1 ^a	65 ^c	67 ^b	200 ^b	142 ^b	108 ^d	71 ^c	-
H8 ₃	8.86 ^b	4.80 ^a	19.03 ^a	14.6 ^a	4.7 ^c	4.3 ^c	2.7 ^d	2.6 ^d	15.4 ^c	13.7 ^b	9.5 ^a	15.3 ^a	44.1 ^a	12.5 ^a	65 ^c	66 ^c	203 ^b	149 ^a	116 ^c	70 ^c	-
H9 ₁	6.74 ^c	4.33 ^b	16.37 ^b	13.5 ^b	4.9 ^b	4.4 ^c	3.1 ^b	2.9 ^b	17.8 ^a	14.7 ^a	9.8 ^a	19.4 ^a	17.8 ^b	18.3 ^a	66 ^b	68 ^b	173 ^d	125 ^d	100 ^e	66 ^c	-
H9 ₂	8.67 ^b	5.64 ^a	18.13 ^a	14.8 ^a	5.0 ^a	4.8 ^a	3.2 ^a	3.0 ^a	17.6 ^a	14.4 ^a	6.6 ^b	5.7 ^a	22.3 ^b	13.1 ^a	65 ^c	66 ^c	169 ^d	131 ^c	105 ^d	68 ^c	-
H10 ₁	7.46 ^c	4.60 ^b	16.87 ^b	13.7 ^b	5.1 ^a	4.4 ^c	3.0 ^c	2.8 ^b	17.6 ^a	14.6 ^a	13.1 ^a	10.4 ^a	31.1 ^a	15.1 ^a	65 ^c	66 ^c	171 ^d	132 ^c	106 ^d	74 ^b	-
H10 ₃	8.30 ^b	5.29 ^a	17.67 ^a	14.1 ^a	4.9 ^b	4.4 ^c	2.7 ^d	2.7 ^c	15.7 ^c	12.9 ^c	5.0 ^b	9.2 ^a	36.2 ^a	15.6 ^a	65 ^c	66 ^c	182 ^c	138 ^b	114 ^c	79 ^b	-
H11 ₂	8.90 ^b	5.78 ^a	18.30 ^a	14.6 ^a	5.1 ^a	4.7 ^a	3.2 ^a	3.0 ^a	16.2 ^b	14.3 ^a	3.8 ^b	12.8 ^a	17.9 ^b	18.9 ^a	64 ^c	65 ^c	183 ^c	141 ^b	107 ^d	76 ^b	-
H11 ₃	9.18 ^b	5.51 ^a	18.83 ^a	15.2 ^a	4.9 ^b	4.4 ^c	2.8 ^d	2.7 ^c	16.1 ^b	12.7 ^c	8.7 ^a	7.7 ^a	16.0 ^b	17.2 ^a	64 ^c	66 ^c	188 ^c	142 ^b	119 ^c	77 ^b	-
H12 ₁	8.10 ^b	4.92 ^a	17.37 ^b	14.2 ^a	4.7 ^c	4.3 ^d	2.8 ^d	2.5 ^d	16.8 ^a	13.8 ^b	6.6 ^b	9.1 ^a	11.5 ^b	22.1 ^a	64 ^c	66 ^c	185 ^c	30 ^c	109 ^c	65 ^c	-

Cultivars	GY		EL		ED		CD		NR		% DAE		% DIE		FL		PH		EH		
	Harvest		Harvest		Harvest		Harvest		Harvest		Harvest		Harvest		Harvest		Harvest		Harvest		
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	
H12 ₃	8.34 ^b	5.66 ^a	18.13 ^a	15.4 ^a	4.7 ^c	4.3 ^d	2.7 ^d	2.6 ^d	15.6 ^c	13.4 ^b	5.8 ^b	10.1 ^a	47.3 ^a	18.9 ^a	65 ^c	66 ^c	194 ^c	140 ^b	113 ^c	75 ^b	-
DKB390	8.25 ^b	5.07 ^a	17.13 ^b	13.6 ^b	5.1 ^a	4.6 ^b	3.2 ^a	2.9 ^b	17.1 ^a	13.7 ^b	3.5 ^b	13.7 ^a	18.3 ^b	16.6 ^a	67 ^b	67 ^b	191 ^c	138 ^b	109 ^c	72 ^b	-
DKB390H	11.08 ^a	5.49 ^a	18.73 ^a	14.0 ^a	5.1 ^a	4.7 ^a	3.1 ^b	2.9 ^b	17.7 ^a	14.6 ^a	0.6 ^b	9.5 ^a	2.9 ^b	16.6 ^a	67 ^b	66 ^c	191 ^c	131 ^c	117 ^c	73 ^b	-
ST0509	5.59 ^d	3.89 ^b	16.23 ^b	14.0 ^a	4.7 ^c	4.2 ^d	2.7 ^d	2.6 ^d	15.2 ^c	12.7 ^c	12.7 ^a	16.5 ^a	17.8 ^b	26.3 ^a	72 ^a	69 ^a	216 ^a	140 ^b	123 ^b	74 ^b	-
Mean of hybrids	8.35	4.90	17.71	14.08	4.46	4.93	3.00	2.78	16.72	14.05	6.50	13.03	21.65	18.33	65.35	66.46	187.34	136.83	111.57	72.71	-
Mean of Control	8.30	4.82	17.36	13.87	4.50	4.96	2.99	2.80	16.69	13.67	5.57	13.23	13.00	19.83	68.53	67.33	199.16	136.33	116.28	73.00	-

Means followed by the same letter belong to the same group by the Scott-Knott test at a significance level of 5%.

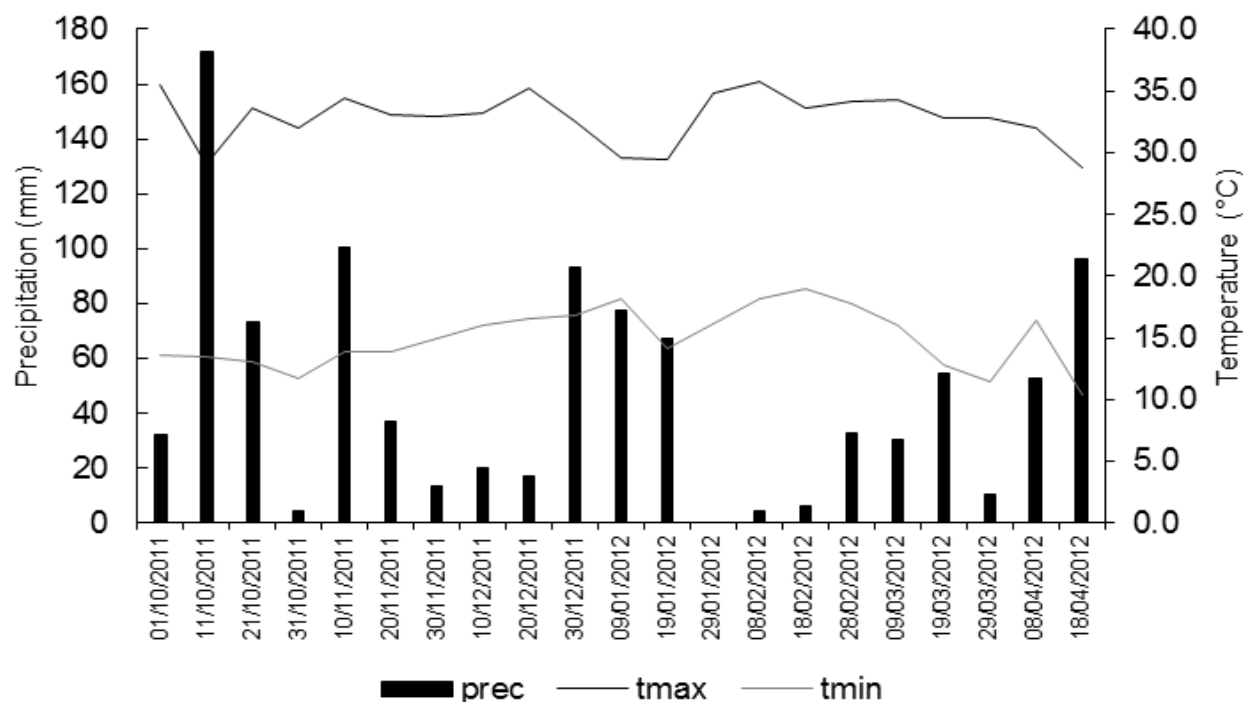


Figure 1. Maximum (Max) and minimum (Min) temperatures and precipitation (mm) in Londrina, from October 2011 to April 2012.

Source: Agronomic institute of Paraná- Technical Report N°77. July/2012 (IAPAR, 2012).

Table 4. Levels of significance from F (significance level of 5 %) test and means of plots inoculated and non-inoculated for grain yield, ear length, ear diameter, cob diameter, ear rows number, percentage of damaged ear, percentage of diseased ear, days to flowering, plant height and ear height, evaluated in Londrina in the first and second harvest of 2011/2012.

Traits	1 st Harvest			2 nd Harvest		
	F	Inoculated	Non-inoculated	F	Inoculated	Non-inoculated
Grain yield (t ha ⁻¹)	ns	8.35	8.34	ns	4.96	4.81
Ear length (cm)	ns	17.7	17.65	ns	14.22	13.89
Ear diameter (cm)	*	4.93	4.95	ns	4.5	4.44
Cob diameter (cm)	ns	3	3	ns	2.79	2.74
N° of grain rows per ear	ns	16.83	16.6	ns	14.17	13.83
Percentage of damaged ear (%)	*	9.73	12.56	ns	17.81	18.79
Percentage of diseased ear (%)	ns	25.21	24.38	ns	23.14	24.33
Days to flowering	ns	65.48	65.93	ns	66.48	66.67
Plant height (cm)	ns	187.98	189.32	ns	138.13	135.57
Ear height (cm)	ns	112.4	111.8	ns	73.63	73.85

need to furthering this approach since up to the present moment there is no knowledge built up on compatibility factors associated with the plant genotype that can be applied in genetic improvement programmes. However, it is evident that the proposed method of including interaction with PGPB as a desired trait in maize breeding programmes has great potential to select more

suitable genotypes to finally consolidate this technology. Furthermore, these findings suggest that this approach could be useful for selecting elite cultivars more adapted to different growing seasons.

Regarding cultivars, percentage of damaged ear was the only variable with no significant effect, proving the heterogeneity of the evaluated genotypes. By decomposing

Table 5. Estimates of general combining ability (GCA) of the inbred lines from the synthetic ST20 (g_L) and the tester lines (g_T) originated from the synthetic ST06 for grain yield (GY, in t ha⁻¹), ear length (EL, in cm), ear diameter (ED, cm), cob diameter (CD), number of grain rows per ear (RE), percentage of damaged ear (% DAE), percentage of diseased ear (% DIE), days to flowering (DF), plant height (PH, in cm) and ear height (EH, in cm), evaluated in Londrina in the first and second harvest of 2011/2012.

Estimates	GY		EL		ED		CD		RE		% DAE		% DIE		DF		PH		EH	
	Harvest		Harvest		Harvest		Harvest		Harvest		Harvest		Harvest		Harvest		Harvest		Harvest	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Means	8.40	4.91	17.8	14.1	4.9	4.5	3	2.8	17	14.1	6.4	13.1	21.8	18.3	65.4	66.4	187.3	136.9	111.6	72.7
Estimates of the GCA of the lines (\hat{g}_L) from the synthetic ST20																				
g_{L1}	-0.47	-0.43	-0.2	-0.7	0.1	0.0	0.2	0.1	-0.9	-0.2	-3.9	4.2	-5.2	1.8	-0.7	-0.3	-0.8	-9.0	-8.5	-9.3
g_{L2}	0.21	0.13	-0.9	-0.6	0.1	0.0	0.0	-0.1	0.7	0.1	1.3	-1.3	3.1	-2.1	-0.6	-0.8	-1.8	0.0	4.5	2.0
g_{L3}	0.96	0.35	0.7	1.3	0.0	0.1	0.0	0.2	0.5	0.7	0.5	-1.2	-8.9	1.4	0.7	0.2	11.9	7.4	-0.1	-1.8
g_{L4}	-0.56	-0.77	-0.8	-0.9	0.0	0.0	0.1	0.0	0.1	0.0	1.1	0.7	-0.6	2.0	-0.5	-0.2	-20.3	-12.9	-14.1	-9.1
g_{L5}	-0.37	-0.30	0.0	-0.2	0.1	0.1	0.2	0.1	0.5	0.3	-1.3	1.6	-4.8	-1.5	1.5	1.4	7.4	4.6	8.3	10.6
g_{L6}	0.52	0.15	-0.1	0.5	0.2	0.1	0.1	0.1	0.9	0.7	-2.0	-1.2	-4.6	0.5	1.6	0.5	12.8	8.7	10.6	10.0
g_{L7}	-0.02	0.06	0.3	-0.6	-0.2	-0.1	-0.1	-0.2	-1.2	-0.9	-1.4	0.9	-2.2	-0.7	0.7	0.7	7.2	2.9	12.1	2.3
g_{L8}	0.25	-0.41	0.9	0.1	-0.1	-0.2	-0.2	-0.2	-0.5	0.1	2.5	5.4	10.7	1.5	0.1	0.0	12.0	7.2	-1.3	-3.5
g_{L9}	-0.57	0.26	-0.3	0.4	0.0	0.2	0.1	0.1	0.7	0.3	2.1	-2.1	0.7	-4.2	0.0	0.4	-13.0	-6.1	-5.4	-3.5
g_{L10}	-0.28	0.04	-0.4	-0.4	0.1	0.0	-0.1	0.0	-0.2	-0.4	1.9	-2.3	11.6	-2.4	-0.3	-0.6	-12.6	-3.3	-3.2	3.1
g_{L11}	0.26	0.53	0.5	0.7	0.1	0.0	0.0	0.1	-0.1	-0.2	0.3	-2.3	-7.0	0.8	-1.5	-0.5	-2.7	3.4	-0.4	2.8
g_{L12}	0.06	0.38	0.1	0.5	-0.2	-0.1	-0.2	-0.2	-0.6	-0.5	-0.9	-2.4	7.3	2.8	-0.9	-0.8	0.0	-3.0	-2.6	-3.5
Estimates of the GCA of the lines originated from the synthetic ST06 used as testers (\hat{g}_T)																				
g_{T1}	-0.75	-0.39	-0.7	-0.3	0.0	-0.1	0.1	0.0	0.9	0.7	0.9	1.0	-4.3	2.1	-0.1	0.5	-2.0	-2.8	-3.5	-2.1
g_{T2}	0.49	0.02	0.3	-0.4	0.1	0.1	0.1	0.1	-0.3	-0.2	-1.5	2.0	-0.5	1.1	0.1	-0.4	-3.8	-2.7	-3.3	-1.9
g_{T3}	0.26	0.38	0.4	0.7	-0.1	0.0	-0.1	0.0	-0.6	-0.4	0.6	-3.1	4.9	-3.2	0.0	-0.1	5.8	5.5	6.8	4.0

the effects of cultivar in the joint analysis, a significant effect of control (C) was observed for all traits evaluated and for the experimental hybrids (Hy), except for the percentage of damaged and diseased ear (Table 2). Thus, there are experimental hybrids with different agronomic performances, allowing for genetic selection among the genotypes. For the contrast control versus hybrids (C vs Hy), the overall mean of these groups of genotypes differed statistically for male flowering and plant and ear height; moreover, in the first harvest the hybrids showed a higher percentage of diseased ear compared to the control group (Table 3). However, interestingly, no significant difference was found for grain yield

between experimental and control hybrids.

As for the interaction of the control group and harvest (C x Ha), the joint analysis indicated significant differences for grain yield, ear length, ear diameter and plant height (Table 2), while experimental hybrids versus harvest (Hy x Ha) only showed significant values for cob diameter, percentage of diseased ear, and plant and ear height, demonstrating that these genotypes did not present a differentiated behavior between the different periods of cultivation, which means they suffered less with the unfavorable conditions of the second harvest, showing a more stable performance. From the data gathered, we can assume that the hybrids with the best average

yield between the 1st and the 2nd harvest are those that should be selected for grain yield, ear length, ear diameter, number of grain rows per ear, percentage of damaged ear and male flowering.

The decomposition of the experimental hybrids from the partial diallel reveals significant effects for the general combining ability of the inbred lines (GCA-L) and the tester lines (GCA-T) for the majority of the characteristics analyzed, except for percentage of damaged and diseased ear to GCA-L and percentage of damaged and diseased ear and male flowering to GCA-T (Table 5). The specific combining ability was significant for ear length and diameter, cob diameter, number of

grain rows per ear, percentage of diseased ear and plant and ear height.

The absence of significance for the other traits indicates that the parents do not present an appreciable degree of gene complementation in relation to the frequencies of the alleles in the loci of dominance (Vencovsky and Barriga, 1992). Experimental hybrids and period of cultivation (harvest) interaction showed significant GCA-L data for ear length and diameter, percentage of diseased ear, plant and ear height, and for GCA-T in almost all traits except number of grain rows per ear and plant and ear height.

In general, the inbred lines L₂, L₃, L₆, L₁₁, L₁₂ and testers T₂ and T₃ showed the best estimates of general combining ability for grain yield and other characteristics, producing the best hybrid combinations (Table 5). High estimates of GCA are associated with genotypes with high frequency of favorable alleles for agronomic traits of interest (Vencovsky, 1987). As can be seen from Table 2, the mean squares for general combining ability were, in general, higher than those of specific combining ability, indicating predominance of the additive effects of genes, which is in agreement with results obtained by Simon et al. (2004) and Júnior et al. (2006). Additionally, the greater contribution of effects of dominance to grain yield, found in this work, corroborates studies made by Simon et al. (2004) and Júnior et al. (2006).

Among the 24 experimental hybrids evaluated in the first harvest (Table 3), 13 did not differ statistically from the commercial hybrid DKB390 (control) for grain yield and showed similar performance for the other traits, especially the experimental hybrids H_{3 2'}, H_{6 3'} and H_{11 2'}. In the second harvest, 15 of the experimental hybrids did not differ statistically from the controls, and from this total, nine experimental hybrids showed a higher average grain yield than DKB390 and five surpassed its transgenic version DKB390H, which shows the excellent performance of the genetic material generated by this particular maize breeding programme that aims at outstanding varieties.

In general, the most promising hybrids in the second harvest were H_{2 3'}, H_{3 2'}, H_{9 2'}, H_{11 2'} and H_{11 3'} e H_{12 2'}, showing the highest means of the traits of interest and the smallest oscillations between the two growing seasons. Furthermore, 50% of the experimental hybrids out-yielded the commercial hybrid DKB390 when cultivated in conditions of high abiotic stress (2nd harvest) (data not shown).

Conclusions

From the research that has been carried out, it is possible to conclude that:

(1) The most promising experimental hybrids are H_{2 3'}, H_{3 2'}, H_{11 2'}, H_{11 3'} and H_{12 2'} and that

(2) The additive effects of genes are more important than the non-additive effects for all the traits evaluated.

Regarding the association with the diazotrophic bacteria.

(3) It is possible that the direct inoculation of *A. brasilense* on maize seedlings was not successful enough to allow significant effects of inoculum in the experimental conditions of this study.

Further research should be conducted to optimize the inoculation method in order to guarantee the evaluation for detection of maize genotypes more prone to PGPB colonization and its introduction in maize breeding programmes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

- Alquères S, Meneses C, Rouws L, Rothballer M, Baldani I, Schmid M, Hartmann A (2013). The bacterial superoxide dismutase and glutathione reductase are crucial for endophytic colonization of rice roots by *Gluconacetobacter diazotrophicus* PAL5. *Molecular Plant-Microbe Interactions Journal* 26(8):937-945.
- Andreotti M, Araldi M, Guimarães V F, Furlani Júnior E, Buetti S (2008). Produtividade do milho safrinha e modificações químicas de um latossolo em sistema de plantio direto em função de espécies cobertura após calagem superficial. *Acta Scientiarum Agronomy* 30(1).
- Bashan Y, De-Bashan LE, Prabhu SR, Hernandez JP (2014). Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998-2013). *Plant and Soil* 378(1-2):1-33.
- Beaugregard PB, Chai Y, Vlamakis H, Losick R (2013). *Bacillus subtilis* biofilm induction by plant polysaccharides. *Proceedings of the National Academy of Sciences of the United States of America* 110(17):E1621-E1630.
- Cruz CD (2013). GENES – a software package for analysis in experimental statistics and quantitative genetics. *Acta Scientiarum Agronomy Maringá* 35(3):271-276.
- Dechorgnat J, Nguyen CT, Armengaud P, Jossier M, Diatloff E, Filleur S, Daniel-Dedele FX (2011). From the soil to the seeds: the long journey of nitrate in plants. *Journal of Experimental Botany* 62(4):1349-1359.
- Díaz-Zorita M, Canigia MVF, Bravo AO, Berger A, Satorre EH. (2015). Field evaluation of extensive crops inoculated with *Azospirillum* sp. In: Cassán, F.D.; Okon, Y.; Creus, C.M. (Eds.) *Handbook for Azospirillum*, Springer Int. Publishing, Switzerland pp. 435-445.
- Drogue B, Doré H, Borland S, Wisniewski-Dyé F, Prigent-Combaret C (2012). Which specificity in cooperation between phyto-stimulating rhizobacteria and plants? *Research in Microbiology* 163(8):500-510.
- Embrapa Soja (2011) *Inoculação com Azospirillum brasilense: inovação em rendimento a baixo custo*. Londrina: Empresa Brasileira de Pesquisa Agropecuária Embrapa Soja, 38. (Embrapa Soja. Documents, 325). Available on: <<http://www.cnpso.embrapa.br/download/doc325.pdf>>. Accessed in 1 jul. 2012.
- Fallik E, Okon Y (1996). The response of maize (*Zea mays*) to *Azospirillum* inoculation in various types of soils in the field. *World Journal of Microbiology Biotechnology* 12:511-515.
- Fukami J, Nogueira MA, Araujo M, Hungria M (2016). Accessing inoculation methods of maize and wheat with *Azospirillum brasilense*.

- AMB Express 6(1):3.
- Galloway JN, Townsend AR, Erismann JW, Bekunda M, Cai Z, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA (2008). Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* 320(5878):889-892.
- Griffing B (1956). Concept of general specific combining ability in relation to diallel crossing systems. *Australian Journal of Biological Sciences*, Melbourne 9(4):463-493.
- Instituto Agronômico Do Paraná (IAPAR) (2012). Avaliação estadual de cultivares de milho, Safra 2011/2012. Londrina: IAPAR, 121. (IAPAR.Thecnical Report 77). Available on: http://www.iapar.br/arquivos/File/zip_pdf/bt77_milhosafra2011_12.pdf. Accessed in: Sep 1, 2012.
- Jha PN, Gupta G, Jha P, Mehrota R (2013). Association of rhizospheric/endophytic bacteria with plants: A potential gateway to sustainable agriculture. *Greener Journal of Agricultural Sciences* 3(2):73-84.
- Júnior Freitas ATA, Pereira MG, Cruz CD, Scapim CA. (2006). Capacidade combinatória em milho-pipoca por meio de dialelo circulante. *Pesquisa Agropecuária Brasileira* 41(11):1599-1607.
- Kennedy IR, Choudhury ATMA, Kecskés ML (2004). Non-symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better explored. *Soil Biology and Biochemistry Oxford* 36:1229-1244.
- Magalhaes PC, Duarte AP, Guimaraes PE de O (2007). Tecnologias para desenvolvimento de milho em condições de safrinha. In: "9º Seminário Nacional de Milho Safrinha. Rumo à estabilidade, Dourados, MS. Annals. Dourados: Embrapa Agropecuária Oeste pp. 108-120.
- Meneses CH, Rouws LF, Simões-Araújo JL, Vidal MS, Baldani JI (2011). Exopolysaccharide production is required for biofilm formation and plant colonization by the nitrogen fixing endophyte *Gluconacetobacter diazotrophicus*. *Molecular Plant-Microbe Interactions Journal* 24:1448-1458.
- Miranda Filho JB, Vencovsky R (1995). Analysis of variance with interaction of effects. *Brazilian Journal of Genetics* 18(1):129-134.
- Morais T, Brito C, Brandão A, Resende W (2016). Inoculation of maize with *Azospirillum brasilense* in the seed furrow. *Revista Ciência Agronômica* 47(2):290-298.
- Morrissey JP, Dow JM, Mark GL, Fergal O'Gara Y (2004). Are microbes at the root of a solution to world food production? *Nature EMBO Reports*, London 5:922-926.
- Oliveira ALM, Costa KR, Ferreira DC, Milani KML, Santos OJAP, Silva MB, Zuluaga MYA (2014). Aplicações da biodiversidade bacteriana do solo para a sustentabilidade da agricultura. *Biochemistry and Biotechnology Reports* 3:56-77.
- Patel JA, Shukla MR, Doshi KM, Patel BR, Patel SA (1998). Combining ability analysis for green fruit yield and components in Chilli (*Capsicum annum*, L.). *Capsicum and Eggplant Newsletter* 17:34-37.
- Pereira AF, Melo PGS, Pereira JM, Assunção A, Nascimento AR, Ximenes PA (2009). Caracteres agrônômicos e nutricionais de genótipos de milho doce. *Bioscience Journal*, Uberlândia 25(1):104-112.
- Rambo LS, Silva PRF, Strieder ML, Sangoi L, Bayer C, Argente G. (2007). Monitoramento do nitrogênio na planta e no solo para predição da adubação nitrogenada em milho. *Pesquisa Agropecuária Brasileira* 42(3):407-417.
- Santos OJAP dos. (2011). Eficiência de metodologias para inoculação de bactérias promotoras de crescimento vegetal em milho, Trabalho de conclusão de curso, Universidade Estadual de Londrina.
- Simon GA, Scapim CA, Pacheco CAP, Pinto RJB, Braccini AL, Tonet A. (2004). Depressão por endogamia em populações de milho-pipoca. *Bragantia* 63(1):55-62.
- Vencovsky R (1987). Melhoramento de populações. In: Paterniani E, Viégas G P. Melhoramento e produção do milho. Campinas: Fundação Cargill 2:217-265.
- Vencovsky R, Barriga P (1992). Genética biométrica no fitomelhoramento. Riberão Preto; Sociedade Brasileira de Genética P 496.
- Vitousek PM, Naylor R, Crews T, David MB, Drinkwater LE, Holland E, Johnes PJ, Katzenberger J, Matinelli LAA, Matson PA, Nziguheba G, Ojima D, Palm CA, Robertson GP, Sanchez PA, Townsend, AR, Zhang FS (2009). Nutrient imbalances in agricultural development. *Science* 324(5934):1519-1520.