

BEHAVIOR OF SOYBEAN PLANTATIONS SEED IN A SURFACE REFORM AREA AND SUBMITTED TO COINOCULATION AND INOCULATION IN THE FURROW

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Abstract: With the objective of evaluating biological nitrogen fixation parameters and production components in soybean plantations submitted to different Treatments with biological inoculants, an experiment was installed in the 2018-19 harvest in Tanabi-SP, in an area of sugarcane reform. The same was carried out in the scheme of demonstrative strips or parcels being implemented as a 7 x 3 factorial, in the experimental design of randomized blocks with three replications. The sources of variance in the analysis of variance were composed of seven Plantations BRS 7380 RR, TMG 7063 IPRO, TMG 7062 IPRO, SYN 13610 IPRO, SYN 15640 IPRO, M 6410 IPRO and NS 7007 IPRO and three Treatments: coinoculation and furrow inoculation of sowing, and control without inoculation. In R1, the total number of nodules (NNODT), nitrogen content in the shoot (TNPA) and total dry mass of nodules (MSNODT) were evaluated and at harvest, in R8, grain yield (PG) and mass thousand grains (MMG). Based on the results obtained, there was a different behavior between the tested Plantations, and the cv. BRS 7380RR stood out with the highest mean of NNODT (27.81 nodules plant⁻¹); SYN 15640 IPRO had the highest mean TNPA (42.03 g.kg⁻¹) and the two TMG Plantations stood out, showing higher mean values for MSNNOT and MMG. As for PG, the Plantations TMG 7063 IPRO and M 6410 IPRO did not differ statistically from each other and stood out, obtaining the best productive performances of, respectively, 2370.40 and 2264.80 kg ha⁻¹. The practice of co-inoculation in the sulcus promoted increments in TNPA and PG; and had statistical equivalence with the inoculation for the other parameters and production components evaluated. It can be concluded that 71.43% of the Plantations tested in the present work can be used in conjunction with the practice of co-inoculation and/or inoculation in the sowing

furrow; the plantation M 6420 IPRO to obtain high PG must be used in conjunction with the practice of inoculation in the sowing furrow; on the other hand, it is recommended to use the plantation BRS 7380 RR in association with the practice of co-inoculation to obtain higher PG.

Keywords: Varieties; soybean-cane succession; grain yield, *Azospirillum*.

INTRODUCTION

For soybean, there is a strong future trend towards expansion of sugarcane reform areas, mainly in the State of São Paulo (CANASAT, 2016). Its cultivation can contribute to raising the average productivity of sugarcane fields, and the sowing of soybeans in the off-season tends to reduce the costs of renewing sugarcane fields.

On the other hand, it is known that soybeans require around 78 kg of nitrogen (N) for each ton of grain production (PRADO, 2008), and annually due to the fact that multinationals develop many new plantations that have been made available to soybean growers, which have a higher production ceiling, have been demanding larger amounts of this nutrient.

The main contribution of nitrogen in Brazil to soybeans is due to the action of nitrogen-fixing bacteria collectively called rhizobia (HUNGRIA et al., 2007). The symbiotic process called biological nitrogen fixation (BNF) that occurs in the nodules of the plant roots manages to convert atmospheric nitrogen (N₂) into assimilates that the plant absorbs easily, and presents itself as advantageous for the soybean grower in relation to minimization of their production costs, given the total independence in the use of chemical nitrogen fertilizers (DELAMUTA et al., 2013). Thus, the practice of inoculation with bacteria has been used, either in the treatment of soybean seeds, or in the sowing furrow.

For a better understanding of the practice of co-inoculation or mixed inoculation based on bacteria of the genus *Bradyrhizobium* and *Azospirillum brasilense*, it is emphasized that *Azospirillum brasilense*, because it is a free-living diazotrophic bacterium, can be associated with the root system of plants and has been used mainly for inoculation in grasses such as: corn and wheat always accompanied by reduced doses of synthetic nitrogen fertilizers (FERLINI, 2006; BÁRBARO et al., 2011). *A. brasilense* are plant growth promoting bacteria (PGPB) found to colonize the rhizosphere (KLOPPER AND SCHROTH, 1978). PGPB induces plants to generate growth hormones, such as auxins, gibberellins, cytokinins and ethylene (TIEN et al., 1979). In turn, it activates shoot growth and lateral root emission (FERLINI, 2006), in addition to increasing plant resistance to diseases (WANG et al., 2009) and environmental nitrogen fixation (ASHRAF et al., 2011).

However, in the literature there is still great variability of response regarding research about this practice, also justified by the different histories of the predecessor cultures, as well as differences in modes and doses of application and other active biotic and abiotic factors. Thus, some research carried out by Bárbaro et al. (2009), Hungary et al. (2013), Embrapa, 2014 and Bárbaro et al. (2017) and Bárbaro-Torneli et al. (2018 a and b) found positive responses of co-inoculation on soybean agronomic characteristics, however, Gitti et al. (2012), Zuffo et al. (2016) and Finoto et al. (2017) did not notice significant differences in agronomic characters with the use of this practice in soybean.

In relation to the State of São Paulo, the fact that it is positioned in a transition region is also highlighted, and therefore, recommendations for positioning the Plantations are still quite incipient, especially

regarding the cycle, adaptability and stability of the genotypes, which has further hampered their recommendations, as well as their symbiotic associations with bacteria for both inoculation and co-inoculation. On the other hand, the lack of research on the productive performance of genotypes in direct sowing on raw cane makes it difficult to choose Plantations and therefore, the lack of accurate information can contribute to the minimization of grain yields and consequent decrease in activity.

That said, the present work aimed to evaluate the behavior in terms of biological nitrogen fixation, agronomic characters and production components of soybean plantations sown in a sugarcane renewal area and subjected to inoculation and co-inoculation practices in the sowing furrow and non-inoculated control.

MATERIAL AND METHODS

The experiment was carried out under field conditions, on a private property on November 1, 2018, in an area of sugarcane renewal, located in the municipality of Tanabi-SP, at 20° 37' 35" S, 49° 38' 57" W and altitude of 518 m. According to the climate classification proposed by Köppen (2001), based on temperature and precipitation, the municipality of Tanabi is located in a region characterized by a humid tropical climate type with dry winter, designated Cwa, with average temperatures in the hottest month above 22°C. and in the coldest month above 18°C, with two well-defined seasons, one rainy and hot, from September to March, and another dry and less hot, from April to August.

The same was carried out in the scheme of demonstrative strips or parcels being implemented as a 7 x 3 factorial, in the experimental design of randomized blocks with three replications. The sources of variance in the analysis of variance were composed

of seven Plantations BRS 7380 RR, TMG 7063 IPRO, TMG 7062 IPRO, SYN 13610 IPRO, SYN 15640 IPRO, M 6410 IPRO and NS 7007 IPRO, and three Treatments being: coinoculation and inoculation in the sowing furrow, and control without inoculation. It is important to point out that the seeding density was adjusted for the different Plantations according to the germination percentage provided by the manufacturer.

For the implementation of the experiment, a 9-row seeder-fertilizer was used. At every 50 m from the seeder-fertilizer shot, the Treatments of: co-inoculation in the furrow, inoculation in the furrow and non-inoculated control were distributed.

As for the practice of co-inoculation, a formulation from the company Stoller do Brasil Ltd was used, which contains both genera of bacteria and which is in the registration phase in MAPA, and it has

Bradyrhizobium japonicum SEMIA 5079 in the concentration of 1×10^9 UFC mL⁻¹ and *Azospirillum brasilense* (strains AbV5 and AbV6) at a concentration of 1×10^7 CFU mL⁻¹, at the recommended dose of 0.5 L ha⁻¹. The inoculant for inoculation with *Bradyrhizobium* in the sowing furrow used was Masterfix L[®] Soy at a dose of 0.8 L ha⁻¹, that is, six doses more than the seed treatment whose indication is from 0.1 to 0.2 L ha⁻¹. At the development stage V5, the micronutrients cobalt and molybdenum were applied via foliar spray, in all Treatments, including the control, at a dose of 0.1 L ha⁻¹.

The application of inoculants for inoculation and co-inoculation was carried out using a sprayer coupled to a seeder-fertilizer. Some precautions were taken to ensure greater efficiency in the Treatments, such as preparing the solution in the shade, checking the pH of the solution and adequate calibration of the spray nozzles for uniform distribution of the inoculants, that is, the

adoption of good inoculation practices and co-inoculation for the success of the process and aiming to guarantee the survival of the bacteria contained in the inoculants.

At the beginning of flowering, approximately at the R1 phenological stage, 3 plants were collected per experimental plot and taken properly and properly identified to the laboratory of APTA Regional - Polo Alta Mogiana, Colina-SP for evaluation of the following physiological parameters and related to biological fixation of nitrogen: total nodule number (NNODT) in plant-1 units; total dry nodule mass (MSNODT) in mg plant-1, and shoot nitrogen content (TNPA) in g.kg-1 which was measured in the soil laboratory of the Centro Universitário da Fundação Educacional de Barretos (UNIFEB).

At the time of harvest, the following components of production grain yield (PG) in kg ha-1 were evaluated at the reproductive stage R8 (values corrected to 13% moisture), and the experimental plots were harvested and properly identified and transferred to the Regional Center Centro Norte, Pindorama-SP, where they were threshed, their moisture content was sampled and then the mass of a thousand grains (MMG) in grams was measured, according to BRASIL (2011). All soybean cultivation techniques, such as sowing time, fertilization, plant population, weed control, insects and diseases, followed the technical recommendations for soybean cultivation by EMBRAPA (2013).

For the parameters and characters, the estimated and applied Box-Cox transformations were performed as proposed by Hawkins and Weisberg (2017), with the average values maintained in the original scale. The variances, standard deviations, coefficients of variation, DMS, analysis of variance and mean comparisons were calculated with the transformed data. Subsequently, the normality of the residues

was verified by the Shapiro-Wilk test at 5% probability (ROYSTON, 1995). And also Homoscedasticity through the homogeneity of variances by Levene's test at 5% probability (GASTWIRTH et al., 2009). When significant differences were detected in the analysis of variance, the means were compared by Tukey's test at 5% probability. The analyzes were performed with the aid of the AgroEstat Software, online version (MALDONADO JUNIOR, 2019).

RESULTS AND DISCUSSION

Table 1 shows the summary of the analysis of variance and general means obtained in the parameters evaluated at the beginning of flowering considering seven soybean plantations, in relation to the use of three Treatments: practice of co-inoculation and inoculation in the sowing furrow and non-inoculated control, in experiment installed in an area of sugarcane renewal located in the city of Tanabi-SP.

There are highly significant effects of the factors Plantations (C), Treatments (T) and CxT interaction for all variables analyzed (Table 1).

For the effect of Plantations (Table 1), with the highest general average in terms of the number of total nodules (NNODT), the plantation BRS 7380 RR stood out, showing respectively, 27.81 nodules plant-1. And with the lowest number of nodules, the plantation NS 7007 IPRO was positioned with 15.93 nodules plant-1. Some studies have shown differences between Plantations in terms of nodulation parameters (MERCANTE, 2006; DE SOUZA et al., 2008; BULEGON et al., 2016, BÁRBARO et al., 2018a), corroborating the results found in the present work. When analyzing the TNPA, it is noted that the plantation SYN 15640 IPRO had the highest accumulation of foliar nitrogen in relation to the other Plantations tested, with an average

PARAMETERS	NNODT ¹	TNPA	MSNODT ¹
PLANTATIONS (C)			
BRS 7380 RR	27,81 a	35,16 c	278,40 bc
TMG 7063 IPRO	18,48 cd	38,06 b	342,10 a
TMG 7062 IPRO	20,47 b	37,51 b	316,20 ab
SYN 13610 IPRO	20,04 bc	37,21 b	209,70 e
SYN 15640 IPRO	18,25 cd	42,03 a	224,90 de
M 6410 IPRO	17,58 de	34,71 c	228,10de
NS 7007 IPRO	15,93 e	38,33 b	250,80 cd
F (C)	81,88**	25,39**	33,05**
TREATMENT (T)			
Co-inoculation in the sulcus	22,17 a	40,03a	282,70 a
Inoculation in the furrow	21,36 a	37,91 b	299,80a
Control	15,85 b	34,78 c	210,50 b
F(T)	150,23**	74,83**	74,10**
F Interactions			
C x T	6,05**	45,36**	4,82**
General average	19,79	37,57	264,30
CV (%)	7,09	1,60	4,95

¹Means of three repetitions followed by the same lowercase letter in the column do not differ statistically from each other by Tukey's test at 5% probability; NNODT = total number of nodules (sum of the number of nodules in the main root and secondary roots) in units. plant-1; TNPA = nitrogen accumulated in the shoot in g.kg-1; MSNODT= total dry mass of nodules in mg.plant-1; 1 Average respectively of three plants per repetition.

Table 1. Parameters of biological nitrogen fixation, evaluated at the R1 phenological stage in a "Strip Test" experiment, carried out in an area of sugarcane reform in Tanabi-SP. 2018/19 crop, involving different soybean plantations in response to co-inoculation and inoculation via sowing furrow and non-inoculated control.

value of 42.03 g.kg⁻¹. Still in relation to this parameter, it was found that the Plantations TMG 7063 IPRO, TMG 7062 IPRO, SYN 13610 IPRO and NS 7007 IPRO, did not differ from each other and positioned themselves as intermediaries in the accumulation of nitrogen in the aerial part, being the average of these Plantations of 37.78 g.kg⁻¹ and with the lowest mean value of TNPA were the plantation M 6410 IPRO with 34.71 g.kg⁻¹. Regarding MSNOT, it was found that the plantation TMG 7063 IPRO stood out and was statistically superior to the other Plantations, with an average value of 342.10 mg plant⁻¹, respectively. It is important to emphasize that characters with direct and indirect relation to FBN have not been covered by soybean breeding programs, which implies the need for research that addresses the correlation between FBN and productivity in a group of representative genotypes of the species. The next step to increase FBN activity is the selection of soybean plantations, aiming at possible indications of strategies for improvement (TORRES, et al., 2015; SCICLAIR AND NOGUEIRA, 2018). Analyzing the effect of Treatments, in the general average of the experiment (Table 1), it is noted that the practice of co-inoculation in the sowing furrow in soybean provided the best response for the nitrogen content in the aerial part (TNPA), being higher than inoculation, which in turn, was statistically superior to the non-inoculated control; however, it was equivalent to inoculation and both were superior to the control for most parameters evaluated at the beginning of flowering (NNODT and MSNODT). According to Reis et al. (2000) limited responses to the use of inoculants may be associated with genotypes of low symbiotic capacity. Another important fact is that these practices that make use of biological inputs depend on other factors, as highlighted by Deak et al. (2019), co-

inoculation causes soybean roots to increase in length, volume, surface area and diameter, mainly in the temperature range of 20 to 30°C. It also significantly induces the dry mass and the number of nodules to increase as long as the water holding capacity in the soil is between 56 and 96%.

Table 2 shows the general results obtained for MMG and PG of seven soybean plantations submitted to three Treatments involving co-inoculation and inoculation in the sowing furrow and non-inoculated control. Significant effects of the factors Plantations (C), Treatments (T) and CxT interaction can be observed.

As for MMG, the two TMG Plantations (7062 IPRO and 7063 IPRO) obtained statistical equality and showed the highest average values of MMG, with respectively, 131.50 and 135.86 g, statistically differing from the other Plantations tested. Also in relation to this parameter, it is noted that again the SYN 15640 IPRO had the lowest mass of a thousand grains, which was 116.70 grams. The others obtained intermediate mean values for MMG. In terms of grain yield, two Plantations did not differ from each other and stood out, obtaining the best productive performances, being: TMG 7063 IPRO and M 6410 IPRO with average values, respectively, of 2370.40 and 2264.80 kg ha⁻¹, being superior to the other plantations tested. The plantation that had the lowest performance in terms of PG was BRS 7380 RR with only 1538.90 kg ha⁻¹. Thus, when analyzing the most productive plantation with the one with the lowest productivity, considering the average of the three Treatments tested, there is a significant increase of 832 kg ha⁻¹, or 13.87 bags per hectare more if to choose this plantation with the highest PG for this sugarcane reform environment. These results reflect, therefore, the importance of choosing the correct soybean plantation destined for sugarcane

Characters	MMG	PG
Factors	G	kg ha ⁻¹
PLANTATIONS (C)		
BRS 7380 RR	121,93 cd	1538,90 f
TMG 7063 IPRO	131,50 ab	2370,40 a
TMG 7062 IPRO	135,86 a	2198,10 bc
SYN 13610 IPRO	127,21 bc	2088,90 c
SYN 15640 IPRO	116,70 d	1709,30 e
M 6410 IPRO	124,00 bcd	2264,80 ab
NS 7007 IPRO	120,48 cd	1927,80 d
F (C)	12,38**	129,31**
TREATMENT (T)		
Coinoculation	126,18 a	2118,10 a
Inoculation	128,82 a	2071,00 b
Control	121,15 b	1853,00 c
F(T)	10,00**	59,13**
F Interactions		
C x T	2,44*	9,16**
General average	125,38	2014,00
CV (%)	1,99	0,09

Means of three repetitions followed by the same lowercase letter in the column do not differ statistically from each other by Tukey's test at 5% probability; MMG = mass of one thousand grains determined according to Brasil (2011) in g and PG = grain yield in kg ha⁻¹.

Table 2. Production components evaluated at harvest in a "Strip Test" experiment, conducted in a sugarcane reform area in Tanabi-SP, 2018/19 harvest, involving different soybean plantations in response to co-inoculation and inoculation via furrow of sowing and non-inoculated control.

reform areas in the State of São Paulo, which is a transition region that needs to be better studied by the research. The results expressed in this work were below those found by CONAB (2019) which for the State of São Paulo estimated a PG of 3028 kg ha⁻¹ in the 2018/19 harvest.

When analyzing the Treatments factor, for MMG, there is statistical equality of the co-inoculation that showed 126.18 g with inoculation (128.82 g), being higher than the non-inoculated control that held 121.25 g.

Regarding PG, it is noted that the co-inoculation showed 2118.10 kg ha⁻¹ and provided an increase of 47.1 kg ha⁻¹ or 0.79 bags ha⁻¹ and 265.10 kg ha⁻¹ or, 4.42 bags ha⁻¹ when compared to inoculation and control, respectively. These general results reveal that co-inoculation in this sowing situation in an area of sugarcane renewal stood out in relation to inoculation based on *Bradyrhizobium*. An important fact to be highlighted is that environments characterized by low water availability and high temperatures, as occurred in this experiment conducted in Tanabi-SP, may have caused a decrease in biological nitrogen fixation (BNF) (ZULLU JUNIOR et al., 2008) and consequently a reduction in terms of grain productivity. This fact must be aggravated in the next 70 to 100 years, as the global temperature rises 4°C, as predicted (CLINE, 2007). Soil temperature also reduces the efficiency of the fixation processes, as it can cross 40°C at 5 cm depth, imposing limitations on FBN and viability of nodules in the soil (HUNGRIA AND VARGAS, 2000).

Furthermore, according to Pavanelli and Araújo (2009) there is an important relationship between inoculation and the history of previous crops in the area; both can influence the inoculation efficiency of soybeans since even inoculated, it will lose the efficiency in inoculation by nitrogen fixing bacteria if there is not an efficient crop rotation

system. In sugarcane cultivation, after the last cut, the area often remains fallow until the next planting. Thus, in this succession of sugarcane-soybeans, there is another complicating factor, since the sugarcane normally remains in the area until the 5th to the 7th harvest, to later be renewed with another crop, unlike what usually occurs annually in Brazil. with the off-season soybean-corn succession (PADOVAN et al., 2013). Although it is profitable, only the continuous succession system soybean corn off-season tends to cause the degradation of soil components and consequent decrease in productivity, in addition to providing favorable conditions for the incidence of diseases, pests and weeds.

In tables 3, 4, 5, 6 and 7, in the unfolding of the Treatments interaction within Plantations, it was verified that with the practice of co-inoculation in the sowing furrow, the plantation BRS 7380 RR was the highlight for NNODT with an average value of 30.63 g.plant⁻¹ ; the TMG 7063 IPRO presented, when associated with the practice of co-inoculation in the sowing furrow, greater accumulation of nitrogen in the aerial part (51.20 g.kg⁻¹), and greater MSNNOT (402.00 mg plant⁻¹), as well as exhibiting better productive performance within this practice, presenting 2555.60 kg ha⁻¹, being statistically superior to the other Plantations when it comes to these characters; for MMG the TMG 7063 IPRO with 140.33 g stood out despite not differing from the TMG 7062 IPRO which had 135.23 g. In an experiment carried out under field conditions, involving four locations and different soybean plantations, submitted or not to inoculation and co-inoculation via sowing furrow using the same formulation at a dose of 0.45 L ha⁻¹, containing: *Bradyrhizobium* and *Azospirillum brasilense*, expressive gains were obtained in terms of grain yield with the practice of co-inoculation (BÁRBARO et al.,

2018a), corroborating the results obtained in the present work when the association of the plantation TMG 7063 IPRO with the practice of co-inoculation in the furrow was mainly considered. of sowing.

With the practice of inoculation in the sowing furrow, statistical superiority of the plantation BRS 7380 RR was verified, which was superior to the other Plantations presenting a greater number of nodules (NNODT) with respectively, 31.96 nodules plant-1 ; the SYN 15640 IPRO was the highlight when in association with the practice of inoculation, in relation to the other six plantations analyzed, presenting the highest TNPA with an average value of 51.15 g.kg-1; on the other hand, TMG 7063 IPRO had the lowest accumulation of foliar nitrogen with an average value of 32.07 g.kg-1; the two Plantations of the TMG Company obtained, respectively, 368.70 and 415.30 mg plant-1, that is, they were the Plantations with statistical superiority in terms of MSNNOT; Plantations TMG 7062 IPRO (141.08 g), TMG 7063 IPRO (129.70 g), SYN 13610 IPRO (136.86 g) and NS 7007 IPRO (128.76 g) were statistically equivalent to each other and superior to the others for MMG; in relation to PG, the plantation M 6410 IPRO with 2555.60 kg ha-1 was the highlight within the Plantations under inoculation in the furrow despite not being statistically different from the Plantations TMG 7063 IPRO with 2366.70 kg ha-1 and TMG 7062 IPRO with 2305.60 kg ha-1. In turn, in this situation, the lowest productivity was the BRS 7380 RR which had an average value of 1380 kg ha-1, that is, the M 6410 IPRO promoted an increase of 1175.60 kg ha or 19.6 bags per hectare more when it was chosen because of the BRS 7380 RR, when both were submitted to the practice of inoculation in the furrow. This way, once again, the importance of the correct choice of plantation is highlighted, mainly for sugarcane

renewal environments, in terms of greater yield to the soybean grower and viability of the culture even in atypical periods such as the summer season that occurred in January and affected soybean productivity in the State of São Paulo.

In the specific case of non-addition of bacteria in the different Plantations tested, it is noted in the present work that all Plantations tested presented formation of nodules, and the BRS 7380 RR produced more NNODT than the other Plantations tested with an average of respectively, 20, 85 nodules plant-1; still in control; also in the non-inoculated control, the situation was obtained that, with the exception of TMG 7063 IPRO with 31.73 g.kg-1, the other plantations analyzed did not differentiate between themselves and were superior in terms of TNPA; the BRS 7380 RR, TMG 7063 IPRO, TMG 7062 IPRO, M 6410 IPRO and NS 7007 IPRO were statistically equivalent in the control and superior in relation to the other Plantations in terms of MSNODT; the NS 7007 IPRO also in the control obtained a lower average of MMG of 110.57 g, differing statistically from the Plantations of the TMG company; Plantations TMG 7063 IPRO with 2188.90 kg ha-1, TMG 7062 IPRO (2094.40 kg ha-1) and M 6410 IPRO (2033.30 kg ha-1) stood out and were statistically superior by the tukey test in the control in relation to other plantations. The plantation BRS 7380 RR had the worst productive performance, which produced only 1410 kg ha-1.

And also in Tables 3, 4, 5, 6 and 7, when considering the behavior of each plantation in response to the three treatments tested, it is observed that in the BRS 7380RR plantation, the co-inoculation in the sowing furrow did not statistically differ from the inoculation practice and both were superior to the control without addition of inoculants for NNODT and MSNNOT; the three treatments tested

Treatments(T)	NNODT ----- plant unit ¹ -----			
	COIN	I	C	F
BRS 7380 RR	30,63 a A	31,96 a A	20,85 a B	68,80**
TMG 7063 IPRO	20,67 bcd A	18,30 b AB	16,48 b B	8,00**
TMG 7062 IPRO	23,67 b A	21,19 b A	16,55 b B	23,74**
SYN 13610 IPRO	22,48 bc A	21,00 b A	16,63 b B	16,85**
SYN 15640 IPRO	19,81 cd A	18,15 b AB	16,78 b B	4,18*
M 6410 IPRO	19,67 cd A	20,52 b A	12,55 c B	34,18**
NS 7007 IPRO	18,26 d A	18,41 b A	11,11 c B	30,77**
F	31,98**	43,98**	18,01**	-

Means of three repetitions followed by the same lowercase letters in the column and uppercase letters in the row do not differ from each other by Tukey's test at 5% probability; I = Inoculation in the sowing furrow with an inoculant based on Bradyrhizobium; C = Control without inoculation; COI = Co-inoculation using an inoculant containing bacteria of the genus Bradyrhizobium and Azospirillum in the sowing furrow; NNODT = total number of nodes.

Table 3. Interaction breakdown for NNOT evaluated at the R1 phenological stage in different soybean plantations submitted to three Treatments involving inoculation and co-inoculation in the sowing furrow and control. Tanabi - SP. 2018/19 harvest.

Plantations (C)	TNPA ¹ ----- g.kg ⁻¹ -----			
	COIN	I	C	F
BRS 7380 RR	35,93 c A	35,00 cd A	34,53 ab A	0,88 ns
TMG 7063 IPRO	51,20 a A	32,07 d B	31,73 b B	183,50 **
TMG 7062 IPRO	35,93 c B	41,60 b A	35,00 a B	20,68**
SYN 13610 IPRO	42,87 b A	34,07 cd B	34,70 ab B	38,82**
SYN 15640 IPRO	39,47 b B	51,15 a A	35,47 a C	90,13**
M 6410 IPRO	33,67 c A	35,00 cd A	35,47 a A	1,65ns
NS 7007 IPRO	41,13 b A	36,47 c B	36,59 a B	11,32**
F	49,56**	62,20**	4,34**	-

Means of three repetitions followed by the same lowercase letters in the column and uppercase letters in the row do not differ from each other by Tukey's test at 5% probability; I = Inoculation in the sowing furrow with an inoculant based on Bradyrhizobium; C = Control without inoculation; COI = Co-inoculation using an inoculant containing bacteria of the genus Bradyrhizobium and Azospirillum in the sowing furrow; TNPA = nitrogen content accumulated in the shoot.

Table 4. Interaction breakdown for TNPA evaluated at the R1 phenological stage in different soybean plantations submitted to three Treatments involving inoculation and co-inoculation in the sowing furrow and control. Tanabi-SP. Agricultural year 2018/19.

MSNNOT ¹				
mg. plant ⁻¹				
Treatments(T)	COIN	I	C	
BRS 7380 RR	311,00 b A	313,30 bc A	211,00 abc B	16,29**
TMG 7063 IPRO	402,00 a A	368,70 ab A	255,70 a B	23,70**
TMG 7062 IPRO	297,70 bc B	415,30 a A	235,70 a C	32,48**
SYN 13610 IPRO	235,70 cd A	222,30 e A	171,00 c B	6,93**
SYN 15640 IPRO	249,00 bcd A	250,00 cde A	175,70 bc B	10,34**
M 6410 IPRO	222,30 d A	233,00 de A	229,00 ab A	0,16 ns
NS 7007 IPRO	261,00 bcd A	295,70 cd A	195,70 abc B	13,14**
F	15,67**	21,31**	5,72**	

Means of three repetitions followed by the same lowercase letters in the column and uppercase letters in the row do not differ from each other by Tukey's test at 5% probability; I = Inoculation in the sowing furrow with an inoculant based on Bradyrhizobium; C = Control without inoculation; COI = Co-inoculation using an inoculant containing bacteria of the genus Bradyrhizobium and Azospirillum in the sowing furrow; MSNNOT = total dry mass of nodules.

Table 5. Breakdown of the interaction for total dry nodule mass, evaluated at the R1 phenological stage in different soybean plantations submitted to three Treatments involving inoculation and co-inoculation in the sowing furrow and control. Tanabi-SP. Agricultural year 2018/19.

Plantations (C)	MMG ¹			F
	-----grams-----			
Treatments (T)	COIN	I	C	-
BRS 7380 RR	124,31 bc A	123,36 bc A	118,13 ab A	1,12 ns
TMG 7063 IPRO	140,33 a A	129,70 abc B	124,46 a B	5,86**
TMG 7062 IPRO	135,23 ab A	141,08 a A	131,27 a A	2,12 ns
SYN 13610 IPRO	123,17 bc B	136,86 ab A	121,62 ab B	6,41**
SYN 15640 IPRO	113,77 c A	117,89 c A	118,43ab A	0,65 ns
M 6410 IPRO	124,33 bc A	124,10 bc A	123,55 ab A	0,02 ns
NS 7007 IPRO	122,11bc A	128,76 abc A	110,57 b B	8,44 **
F	7,26**	5,92**	4,07**	-

Means of three repetitions followed by the same lowercase letters in the column and uppercase letters in the row do not differ from each other by Tukey's test at 5% probability; I = Inoculation in the sowing furrow with an inoculant based on Bradyrhizobium; C = Control without inoculation; COI = Co-inoculation using an inoculant containing bacteria of the genus Bradyrhizobium and Azospirillum in the sowing furrow; MMG = mass of one thousand grains = mass of one hundred grains x 10.

Table 6. Breakdown of the interaction for MMG evaluated at the reproductive stage R8 in different soybean plantations submitted to three treatments involving inoculation and co-inoculation in the sowing furrow and control. Tanabi-SP. Agricultural year 2018/19.

Plantations (C)	PG ¹ -----kg ha ⁻¹ -----			F
	Treatments (T)	COIN	I	
BRS 7380 RR	1826,70 cd A	1380,00 e B	1410,00 e B	47,22**
TMG 7063 IPRO	2555,60 a A	2366,70 ab AB	2188,90 a B	8,69**
TMG 7062 IPRO	2194,40 b AB	2305,60 ab A	2094,40 ab B	3,52*
SYN 13610 IPRO	2261,10 b A	2122,20 bc A	1883,30 bc B	13,88**
SYN 15640 IPRO	1761,10 d A	1705,60 d A	1661,10 d A	1,59ns
M 6410 IPRO	2205,60 c B	2555,60 a A	2033,30 ab B	19,65**
NS 7007 IPRO	2022,20 bc A	2061,10 c A	1700,00 cd B	19,53**
F	26,10**	78,38**	43,16**	

Means of three repetitions followed by the same lowercase letters in the column and uppercase letters in the row do not differ from each other by Tukey's test at 5% probability; I = Inoculation in the sowing furrow with an inoculant based on Bradyrhizobium; C = Control without inoculation; COI = Co-inoculation using an inoculant containing bacteria of the genus Bradyrhizobium and Azospirillum in the sowing furrow; PG = grain yield.

Table 7. Breakdown of the interaction for PG evaluated at the reproductive stage R8 in different soybean plantations submitted to three treatments involving inoculation and co-inoculation in the sowing furrow and control. Tanabi-SP. Agricultural year 2018/19.

were equivalent to each other for TNPA and MMG; and the co-inoculation in the sowing furrow was superior to the other treatments tested when considering the PG.

Regarding the plantation TMG 7063 IPRO, it was verified that the co-inoculation in the sowing furrow was statistically superior when compared to the other treatments analyzed for TNPA; this practice was also statistically equivalent to inoculation in the furrow and superior to the non-inoculated control for several parameters, among which are mentioned: NNODT, MSNNOT, MMG and PG.

Regarding the plantation TMG 7062 IPRO, it is noted that the treatments did not differ from each other for MMG; the co-inoculation in the sowing furrow was statistically equivalent to the inoculation also applied in the furrow and superior to the control for NNODT and PG; the

furrow inoculation was superior to the other practices used for TNPA and MSNNOT.

For the plantation SYN 13610 IPRO, it is noted that the practice of co-inoculation in the sowing furrow had statistical equality with inoculation and were superior to the control without addition of bacteria for NNODT, MSNNOT and PG; the co-inoculation stood out and was superior in relation to the other treatments for nitrogen accumulation in the shoot (TNPA); however the inoculation only outperformed the other treatments for MMG;

For the plantation SYN 15640 IPRO, there was variability when it comes to the different parameters and treatments analyzed.; co-inoculation was equivalent to inoculation and superior to control for NNODT and MSNNOT; the inoculation in the furrow, in turn, had better performance, being superior to the co-inoculation that was superior to

the control when considering the TNPA; treatments did not differ for MMG and PG.

When analyzing the plantation M 6410 IPRO, in general, there were three situations involving the different treatments tested when compared to each parameter or character: the co-inoculation in the furrow was equivalent to inoculation and both were superior to the control for NNODT; treatments did not differ statistically for TNPA, MSNODT and MMG; and the inoculation in the furrow stood out and was superior to the other treatments analyzed for PG in this plantation.

For the NS 7007 IPRO plantation, it is generally noted that in most of the analyzed parameters, co-inoculation was equivalent to inoculation. Thus, the results showed the above for: NNODT, MSNODT, MMG and PG; co-inoculation was superior to the other treatments tested for TNPA. In a research carried out by Flauzino et al. (2018) involving the soybean crop associated with inoculation and co-inoculation of *Bradyrhizobium* and *Azospirillum* after autumn-winter crops in Dourados in Mato Grosso do Sul, the authors noted increases in the productivity of inoculated and without inoculant when cultivated after single corn and corn consortium with *B. ruziense* inoculated with *Bradyrhizobium* and *Azospirillum* and, therefore, concluded that more research is

needed to relate previous cropping systems in succession with soybean, as well as with the co-inoculation technique.

FINAL CONSIDERATIONS

71.43% of the plantations tested in the present work can be used together with the practice of co-inoculation and/or inoculation in the sowing furrow;

The plantation M 6420 IPRO to obtain high grain yield must be used in conjunction with the practice of inoculation in the sowing furrow; on the other hand, it is recommended to use the plantation BRS 7380 RR in association with the practice of co-inoculation in the sowing furrow to obtain higher PG;

Despite the commercial plantations being mostly derived from related crosses, marked differences were verified, which shows the existence of genetic variability to be explored, mainly for the nodulation traits.

The concomitant selection of bacteria in the case: *Bradyrhizobium* and *Azospirillum* and host plants (genotypes) is the main challenge in improving the biological nitrogen fixation capacity in soybean. Soybean breeding programs must consider parameters related to FBN in their evaluations, focusing on obtaining new lines with greater symbiotic capacity.

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