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USE OF THE PRACTICE OF COINOCULATION VIA SEED TREATMENT IN UP TO 60 DAYS BEFORE SOY SOWING

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Abstract: Inoculation of seeds before commercialization, that is, inoculated or coinoculated a few days before sowing, are technologies used for different crops, in addition to soybeans, consolidating themselves as a practice that tends to be increasingly carried out in soybean crops in Brazil. Thus, the objective was to evaluate agronomic traits in soybean cultivated in the field in Pindorama-SP, 2021/22 season, submitted to different treatments via seeds with commercial inoculant for coinoculation at different times before soybean sowing. The treatments tested were: T1 = Control (without addition of biological inputs), T2 =. Nitrogen fertilization (200 kg of N/ha); T3= Inoculum based on Bradyrhizobium applied at planting and T4, T5, T6, T7, T8 and T9 = Commercial inoculant for co-inoculation based on Bradyrhizobium and Azospirillum bacteria applied via seed treatment respectively at 0, 7, 15, 30, 45 and 60 days before soybean planting. At the R8 stage, the following traits were evaluated: plant height at maturation (APM) and insertion of the first pod (AIV) in cm, average number of grains per pod (NMGV) and grain yield (PG) in kg.ha-1. From the results obtained, it was verified for the APM and AIV characters that the tested treatments did not differ statistically from each other, with, respectively, general average of 81.04 and 18.38 cm. As for the NMGV, T2 was the highlight with the highest average of grains per pod of 2.62, although it did not differ significantly from T4 and T5 with respectively average values of 2.58. In terms of PG, treatments T5, T3, T4, T2 and T6 did not differ statistically from each other and stood out providing higher values of respectively 3056.1, 2971.3, 2940.1, 2841.1 and 2789.20 kg.ha-1. Thus, it can be concluded that the practice of coinoculation can be applied via commercial inoculant in the treatment of seeds up to 15 days before the soybean

sowing date, without compromising grain productivity.

Keywords: *Glycine max., Bradyrhizobium, Azospirillum; early seed treatment.*

INTRODUCTION

Considering the benefits verified in several cultures with the use of inoculation with plant growth promoting bacteria (BPCPs), especially due to the phytohormonal effect acting directly in the promotion of plant growth, development of the root system, and consequently greater absorption of water and nutrients (HUNGRIA et al., 2013; GALINDO et al., 2018), biological control of plants, production of natural antibiotics and protective effect against secondary phytopathogens in the soil (SANTOYO et al., 2012; MAZZUCHELLI et al., 2014; SIVASAKTHI et al., 2012; MAZZUCHELLI et al., 2014; SIVASAKTHI et al. al., 2014), in addition to the potential to increase biological nitrogen fixation and the efficiency of N use (PANKIEWICZ et al., 2015; GALINDO et al., 2016), it can be deduced that coinoculation of Bradyrhizobium sp. together with BPCPs, such as Azospirillum brasilense, they can favor the development of the soybean crop, and consequently the production of grains.

On the other hand, few studies have been elucidated with the purpose of evaluating the association between pre-inoculation and treatment of industrial seeds (ANGHINONI et al., 2017; MACHINESKI et al., 2018), and little information is known about the period maximum time before sowing that the seed could be treated without compromising the viability of the bacteria contained in the inoculants, plant nodulation and soybean yield (PEREIRA et al., 2010; ZILLI et al., 2010). While authors such as Machineski et al. (2018) report that if associated with a protector, it can be extended to 60 days.

Bárbaro-Torneli et al. (2021) in order to evaluate the performance of three soybean cultivars, regarding physiological and biological nitrogen fixation and agronomic characters when subjected to pre-coinoculation three days before sowing, comparing with the response to standard inoculation carried out on the day of sowing and control without addition of bacteria observed that in terms of grain yield and for most of the characteristics and parameters evaluated, early coinoculation can be recommended for use up to three days before sowing.

In view of the above, the objective of this study was to evaluate agronomic traits in soybean grown in Pindorama-SP, 2021/22 harvest, submitted to different treatments via seeds with commercial inoculant for coinoculation at 0, 7, 15, 30, 45 and 60 days prior to sowing.

MATERIAL AND METHODS

PLACE WHERE THE EXPERIMENT WAS CONDUCTED

The experiment was installed under field conditions, on November 29, 2021 at the Regional Pole for Technological Development of Agribusiness in the North Center, linked to the Paulista Agency for Agribusiness Technology -APTA, located in the municipality of Pindorama -SP. The region's relief is undulating with altitudes ranging from 498 to 594 m, whose geographic coordinates are 21º 13' south latitude and 48º 55' west longitude.

Due to its location, Köeppen (1948) classified the climate of the region of the municipality of Pindorama as a Warm climate with dry winter (Cwa) with maximum and minimum temperatures between 37º and 10 C, with the normal rainy season being the months of September to March, with an average annual rainfall of 1,255 mm.

Pindorama's predominant soil is eutrophic Argisol, considered deep, with sandy A horizon and textural B horizon with high fertility and flat topography.

TREATMENTS AND EXPERIMENTAL DESIGN

The treatments tested in the present work are described in Table 1.

Treatment	Description
1	Control (without inoculant and without nitrogen fertilization)
2	Nitrogen fertilization (200 kg of N/ha) [1] (without inoculant)
3	Masterfix L Soja® (2 mL/kg) – 0 DAS[2]
4	Masterfix L Dual Force® (4 mL/kg) – 0 DAS[2]
5	Masterfix L Dual Force (4 mL/kg) – 7 DAS
6	Masterfix L Dual Force® (4 mL/kg) – 15 DAS
7	Masterfix L Dual Force® (4 mL/kg) – 30 DAS
8	Masterfix L Dual Force® (4 mL/kg) – 45 DAS
9	Masterfix L Dual Force® (4 mL/kg) – 60 DAS

[1] Do not treat. 2, 50% of nitrogen fertilization was applied at sowing and 50% at flowering or 35 days after emergence;

[2] DAS = days before sowing;

* Treatments 3 to 9 applied via seed treatment

Table 1. Treatments and doses of commercial inoculants used in the co-inoculation efficiency evaluation test, applied via seed treatment, at different times before soybean planting. Agricultural Year 2021/22. North Center Regional Pole. Pindorama-SP.

The experimental plot consisted of 4 rows of 15 m in length, and the two central rows of 15 m in length and spacing between rows of 0.5 m (15 m^2) will be considered as useful area. Thus, the experimental design was randomized blocks composed of the nine treatments mentioned above with 5 replications, in a total of 45 experimental plots.

The description of the commercial inoculants used in this experiment follows below:

a) Masterfix® L Soja (standard inoculant): liquid inoculant for soybean registered and produced by Stoller do Brasil Ltda, with a guarantee of a minimum concentration of 5 x 10^9 UFC ml^{-1} of *Bradyrhizobium elkanii* (Strain Semia 5019) and *Bradyrhizobium japonicum* (Strain Semia 5079).

b) Masterfix L Dual Force®: liquid inoculant for soybean cultivation, registered (MAPA Registration - No. SP 003718-4.000002) and produced by Stoller do Brasil Ltda, guaranteed by the presence of two species of bacteria, which are: *Bradyrhizobium japonicum* (Semia strain 5079), in the concentration of 1 x 10^9 UFC/mL and *Azospirillum brasilense* (cepas AbV5 e AbV6), in the concentration of 1 x 10^7 UFC/mL.

CONDUCTING THE EXPERIMENT

Before installation, soil samples were collected from the experimental area for further chemical and granulometric analysis, in addition to counting Bradyrhizobium bacteria and associative diazotrophic bacteria in the soil before sowing. Bacterial counts were performed at the FCAV/UNESP Agricultural Microbiology Laboratory, Jaboticabal/SP campus, in accordance with the recommendations by Dobereiner et al. (1995).

Soil samples for chemical (RAIJ et al., 2001) and granulometric (DAY, 1965) characterization were collected in October 2021, in the 0-0.20 m depth layer, and the results obtained were: pH (CaCl2) = 5.80; M.O. = 10.00 g dm^{-3}; CO = 5.8 g dm^{-3}; P = 36.00 mg dm^{-3}; K = 3.1 mmolc dm^{-3}; Ca = 26.00 mmolc dm-3; Mg = 11.00 mmolc dm-3; H + Al = 16.00mmolc dm^{-3}; V = 71%, Total Sand = 892 g kg-1 of soil; Clay = 72 g kg-1 of soil and Silt = 36 g kg^{-1} of soil. Sowing fertilization was

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carried out with fertilizer formulated 0-20-20, at a dose of (350 kg. Há$^{-1}$).

Only in Treatment T2 (200 kg ha^{-1} of Nitrogen) were the rest of the N dose manually applied, half at the base and half at the top using the urea source, 35 days after emergence.

The soybean cultivar used was RESULT I2X, which has maturation group 6.3, indeterminate growth, gray pubescence color, purple flower color, imperfect black hilum color, trait: Intacta 2 X tend. It shows resistance to stem canker, stem necrosis and frog eye spot and moderate resistance to phytophthora root rot (CREDENZ RESULT I2X, 2022).

20 seeds m^{-1} were sown, using an experimental plot seeder, in order to obtain 14 to 15 plants per linear meter. For this purpose, thinning will be carried out in order to obtain an average final population of 320,000 plants ha^{-1}.

The seeds are already treated with Standak ® Top, which protects the genetic potential of the soybean seeds. The product has multiple and complementary functions in its insecticidal and fungicidal effect, shielding the seeds against the attack of pests and soil diseases that interfere with the germination process and seedling development in the soybean crop. The solution has three distinct active principles, and they provide high efficiency for the management of pests such as caterpillar, grub and soybean anteater. Standak® Top also offers greater tolerance to water stress and the occurrence of nematodes (BASF, 2019).

Subsequently, to compose the different treatments when using biological inputs in the laboratory, only treatments T3, T4, T5, T6, T7, T8 and T9 were prepared according to the procedures described in Table 1, and treatments T1 and T2 did not have the addition of inoculants.

Some precautions were adopted to ensure greater efficiency of the inoculants, such as storing the seeds treated with the biological inputs in periods that preceded sowing, in a place with air conditioning at 16°C, until the moment of planting, and at the time of sowing, seed inoculation involving treatments T3 and T4 were carried out in the shade. For all treatments with application of inoculants in the seeds, their uniform distribution was carried out.

Fertilizer containing the micronutrients cobalt and molybdenum was applied via foliar spraying with the product CoMo Platinum (150 mL/ha); at the phenological stage V4 (Fehr and Caviness, 1977), in all treatments including the control. Disease and pest control will also be carried out using fungicides and insecticides when necessary.

All soybean cultivation techniques, such as cultivar choice, sowing time, plant population, weed, insect and disease control, followed the technical recommendations for soybean cultivation by EMBRAPA (2013).

AGRONOMIC CHARACTERS

At the time of maturation (R8), before harvesting the plots useful for estimating grain productivity, the following traits of agronomic interest were also measured in the field by experimental plot:

- plant height at maturation (APM) = given by the distance from the base of the plant to the end of the main stem, in cm;
- insertion height of the first pod (AIV) = given by the distance from the base of the plant to the lower end of the first pod in cm;

And at the time of harvesting the experimental plots, the following production components were measured:

- Average number of grains per pod (NMGV) = determined by the sum of the average number of grains obtained in three repetitions of 10 pods per experimental plot divided by the average number of these ten pods per experimental plot;

- Grain productivity (PG) = harvested in two 15 m long central rows with 0.5 m spacing between rows. From the average values referring to the production of the plots of each treatment, the productivity was calculated, being expressed in kg ha-1 (values corrected for 13% of humidity).

STATISTICAL ANALYSIS OF RESULTS

For the parameters and characters, the estimated and applied Box-Cox transformations were performed as proposed by Hawkins and Weisberg (2017), with the mean values maintained in the original scale. The variances, standard deviations, coefficients of variation, DMS, analysis of variance and comparisons of means will be 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 the Homoscedasticity through the homogeneity of variances by the Levene 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. Statistical analyzes will be performed using the AgroEstat Software online version (MALDONADO JUNIOR, 2019).

RESULTS AND DISCUSSION

Table 2 describes the average results obtained for agronomic traits and production components. The F test detected highly significant statistical significance ($p \leq 0.01$) for some characters with the exception of APM and AIV where no statistical significance was observed.

Considering the APM, it is noted that the general average of the experiment was above 60 cm (81.04 cm), which is within the range recommended by Sediyama et al. (2005). The tested treatments were not differentiated by the mean test applied. According to Torres et al. (2015), a greater plant height is an important characteristic in the soybean crop, as it allows the plant to have a greater number of nodes, which directly influences the production of the crop. In the conception of Hungary (2011), the height of the plant can be directly influenced by the amount of nitrogen and other nutrients absorbed through the root system, where it allows greater photosynthetic capacity for the crop, however, the same author reports that the highly developed plant can bed.

For AIV, the treatments were also not differentiated by the mean test, with the overall mean of the experiment being 18.38 cm. This is considered a characteristic of each cultivar, which may or may not be influenced by inoculation and coinoculation (HUNGRIA, 2011).

Regarding the NMGV, the results showed that the general average of the experiment was 2.45 grains per pod. The T2 treatment was the highlight with the highest mean of grains per pod of 2.62, although it did not differ significantly from T4 and T5 with mean values of 2.58 and 2.56 NMGV respectively. Then the treatments T6 and T7 were positioned with respectively 2.44 and 2.42 NMGV, and they did not differ among themselves and also from T3 that used the inoculant for inoculation on the day of sowing of the experiment. The treatments whose coinoculation was performed 45 (T8) and 60 (T9) days before planting, produced lower mean values for NMGV and were similar to the non-inoculated control (T1) with respectively 2.38.

TRAT	APM	AIV	NMGV	PG
	----------cm----------		units	kg ha^{-1}
T1	80,200 a	17,200 a	2,3800 d	2359,6 d
T2	82,200 a	18,200 a	2,6200 a	2841,1 a b c
T3	80,400 a	18,000 a	2,4000 c d	2971,3 a b
T4	81,400 a	18,800 a	2,5800 a b	2940,1 a b c
T5	79,600 a	18,000 a	2,5600 a b c	3056,1 a
T6	82,800 a	18,400 a	2,4400 b c d	2789,2 a b c
T7	81,200 a	18,600 a	2,4200 b c d	2670,7 b c d
T8	80,600 a	19,800 a	2,3400 d	2611,6 c d
T9	81,000 a	18,400 a	2,3400 d	2356,0 d
F	0,5924NS	1,3926 NS	8,9159**	12,638**
CV (%)	1,1198	1,6008	8,4011	3,3460
Average	81,044	18,378	2,4533	2732,9

Mean of five repetitions followed by the same lowercase letters in the column do not differ by Tukey's test at 5%; T1 = Control (without addition of biological inputs), T2 =. Nitrogen fertilization (200 kg of N/ha) [1] (without inoculant); T3= Masterfix L Soja® (2 mL/kg of seed) – 0 DAS[2] (time of planting); T4= Masterfix L Dual Force® (4 mL/kg) – 0 DAS[2] (time of planting); T5= Masterfix L Dual Force ® (4 mL/kg) – 7 DAS; T6= Masterfix L Dual Force ® (4 mL/kg) – 15 DAS; T7= Masterfix L Dual Force ® (4 mL/kg) – 30 DAS; T8 = Masterfix L Dual Force ® (4 mL/kg) – 45 DAS; T9 = Masterfix L Dual Force ®(4 mL/kg) – 60 DAS ; APM = plant height at maturation; AIV = first pod insertion height NMGV = average number of grains per pod; PG = grain yield.[1]Average of three replications of 10 pods per experimental plot for NMGV. [2]DAS = Days After Sowing

Table 2. Agronomic characters and production components measured in an experiment to evaluate the agronomic efficiency of coinoculation applied via seed treatment, at different times before soybean planting. Agricultural Year 2021/22. Pindorama-SP.

As for grain yield (PG), (Table 2) an overall average of 2732.9 kg.ha^{-1} of treatments was observed, inferring that it was low. This lower grain yield was possibly associated with late sowing of the cultivar, that is, on November 29, 2021. According to estimates by Conab (2020), in the state of São Paulo, the oilseed was highly benefited by climatic conditions throughout the vegetative development and productivity was estimated at 3,650 kg ha^{-1} appearing as a state record, and an increase of 20.5% in relation to the past season.

Treatments T5, T3, T4, T2 and T6 did not differ statistically from each other and stood out providing higher PG values of respectively 3056.1, 2971.3, 2940.1, 2841.1 and 2789.20 kg.ha$^{- 1}$. With an intermediate position by the average test applied, treatment T7 was positioned, which was statistically equivalent to each other with T8, with an average of 2670.7 kg ha-1. With the lowest average values were treatments T1 (non-inoculated control) with 2359.6 kg ha^{-1} and T9 with 2356 kg.ha^{-1}. In terms of increment, it is noted that considering the average value of the five best treatments (T5, T3, T4, T2 and T6) which was 2919.56 kg ha^{-1}, there was an increase in terms of PG, that is, of 559.96 kg ha^{-1} or 9.33 bags ha^{-1} when compared to the non-inoculated control (T1) and 563.56 kg ha^{-1} or 9.39 bags ha^{-1} compared to T9.

Still in relation to PG, when analyzing the T6 treatment that made use of early co-inoculation in 15 days with Masterfix L Dual Force®, an increase of 429.6 and 433.2 kg ha^{-1}, respectively, in relation to the control treatments (T1) and application of the same inoculant but with a longer period of application (60) until the moment of planting (T9). These results obtained in the present work corroborate with Pereira et al. (2021) in a study to determine the ideal time between inoculation and sowing in the soybean crop, found that although there were no statistical differences between the production of the control and the other early inoculation treatments, there were gains of up to 700 kg ha^{-1} with the inoculant, showing that pre-inoculation can bring economic gains if performed correctly.

Bárbaro-Torneli et al. (2021) in order to evaluate the performance of three soybean cultivars, regarding physiological and biological nitrogen fixation and agronomic characters when subjected to pre-coinoculation three days before sowing, comparing with the response to standard inoculation carried out on the day of sowing and control without addition of bacteria observed that in terms of grain yield and for most of the characteristics and parameters evaluated, early coinoculation can be recommended for use up to three days before sowing.

CONCLUSION

In terms of grain yield in soybeans, the use of co-inoculation is recommended as long as the period of application of the commercial inoculant to the seeds does not exceed 15 days before sowing.

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