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EVALUATION OF SOYBEAN PRODUCTION COMPONENTS SUBMITTED TO THE USE OF LONG-LIFE COMMERCIAL INOCULANT

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Abstract: Long-life inoculants come into existence as an innovative technology, enabling early inoculation in order to facilitate the sowing operation. The objective was to evaluate production components in soybean grown in Pindorama-SP, 2021/22 season, submitted to different treatments via seeds with long-life commercial inoculant applied at 0, 15, 30, 45 and 60 days prior to sowing, associated with the package Basf's chemical technology. The experiment was installed in the field, and consisted of a randomized block design with 8 treatments and 5 replications. The treatments tested were: T1 = Control (without addition of biological inputs), T2 =. Nitrogen fertilization (200 kg of N/ha); T3= commercial inoculant A applied at 0 DAS (days before sowing); T4, T5, T6, T7 and T8 = long-life commercial inoculant B applied to seeds at respectively 0, 15, 30, 45 and 60 DAS. In R8, the number of plants per linear meter (EF) was counted; plant height at maturation (APM) and first pod insertion (AIV). And when the experimental plots were harvested, the average number of grains per pod (NMGV) was evaluated; number of pods per m2 (NV); thousand-grain mass (MMG); grain yield (PG), and total nitrogen accumulated in grains (NTG). The variables were submitted to analysis of variance using the F test, and when significant, the means were compared with each other using the Tukey test at 5%. The results showed that for EF, the treatments did not differ statistically with an overall average of 14.30 plants/m. The tested treatments were also statistically similar for APM, AIV and NMGV, where the general average of 81.55, 16.05 cm and 2.31 grains per pod were observed, respectively. As for NV, T3, T4, T5, T6, T7 and T8, as well as the application of chemical nitrogen fertilization (T2) stood out and statistically differed only from T1. For MMG, T2 stood out with the highest mass of a thousand grains of 136.80 g, differing from the

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treatment (T7) inoculation at 45 DAS, despite having statistical equivalence with treatments T1, T3, T4, T5, T6 and T8. As for NTG, it was found that T2, T4, T5 and T6 provided the highest increments in relation to this parameter with means of respectively 55.68, 53.51, 53.73 and 54.42 g.kg-1. Treatments T3, T4, T5 and T2 did not differ statistically from each other and stood out with higher PG values of 2961.5, 2996.8, 2883.2, and 2863.9 kg. ha-1, respectively. It can be concluded that the pre-inoculation with the long-life inoculant B applied in up to 15 DAS promoted increases for most of the production components and in terms of PG of 773 kg ha-1, when compared to the non-inoculated control, which can be recommended for use.
Keywords: long life inoculants, *Bradyrhizobium*, *Glycine max* L; fungicides and insecticides.

INTRODUCTION

For a good response in terms of productivity, the soybean crop requires the absorption of several nutrients, among which nitrogen (N) is the most required (EMBRAPA, 2013). Nitrogen is responsible for increasing productivity and protein content in soybean seeds, in addition to its fundamental role in plant metabolism, which requires a high amount of N in the biosynthesis processes of amino acids, chlorophyll, nucleic acids and nitrogenous bases (HUNGRIA et al, 2013). Nitrogenous chemical fertilizers, such as ammonium sulfate and urea, are N supply options for soybeans, both of which are commonly used in agriculture due to the rapid assimilation of N by plants. However, although they have the advantage of being absorbed more quickly, they have a high cost, which is often not an advantage for the soybean farmer (CHEBOTAREV et al., 2017; CHAE et al., 2018).

However, there is another way to make N available to crops, in addition to chemical fertilization, with atmospheric N that can be assimilated by bacteria, in symbiosis with leguminous plants, through the process of biological nitrogen fixation (BNF). The most common associations between legumes, such as soybeans and bacteria, are with those of the *Bradyrhizobium* genera (MEENA et al., 2018). The inoculation process consists of using nitrogen-fixing bacteria, selected by research, added to plant seeds before sowing. Inoculation is done with a product called inoculant (MENDES; REIS JUNIOR; CUNHA, 2010). Although this practice is used in most soybean crops in the country, the inoculation process has the obstacle that once the seed has been inoculated, it must be sown immediately, otherwise the low bacterial survival will compromise the efficiency of the inoculation. Inoculation is usually done at sowing and is often a limiting factor for sowing efficiency, due to the time spent for its operation (CAMPO; HUNGRIA, 2007).

This way, the demands of the agricultural market led to the creation of new pre-inoculants, products that allow the pre-treatment of seeds. These biological inputs must become increasingly common in the market, as they facilitate and make seed treatment and sowing processes more flexible (SEI, 2013). Allowing the early treatment of soybean seeds, long-life inoculants appear as an innovative technology, aimed at the industrial treatment of seeds, associated with phytosanitary treatments, conferring prolonged useful life to bacteria of the genus Bradyrhizobium (RIZOBACTER, 2017).

In view of the above, the objective of this study was to evaluate agronomic traits and production components in soybean grown in Pindorama-SP, 2021/22 harvest, submitted to different treatments via seeds with commercial inoculant in 0, 15, 30, 45 and 60 days prior to sowing, associated with Basf's chemical technological package.

MATERIAL AND METHODS

PLACE WHERE THE EXPERIMENT WAS CONDUCTED

The experiment was installed under field conditions, in an area with conventional soil preparation on November 29, 2021 at the URPD Regional Research and Development Unit, linked to the Paulista Agency for Agribusiness Technology -APTA, located in the municipality of Pindorama -SP. It is located at latitude 21º13'28.16" S and longitude 48º54'20.86" W, with an altitude of 554 meters.

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. The climatic data from the installation period to the harvest of the experiment is described in figure 1 (CIIAGRO, 2022).

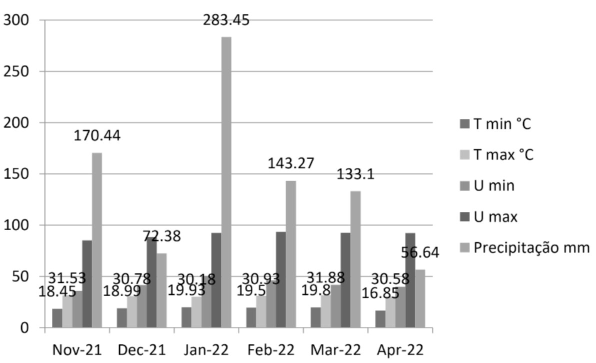

Figure 1. Climatic data from the period in which the experiment was conducted at the Regional Research and Development Unit of Pindorama-SP. Crop 2021/22.

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.

Trat.	Description
T1	Control (without inoculant and without nitrogen fertilization)
T2	Nitrogen fertilization (200 kg of N/ha) [1] (without inoculant)
T3	Commercial inoculant A2 (2 mL/kg of seed) - 0 DAS[3] (days before sowing
T4	Commercial inoculant B[4] (2 mL/kg) - 0 DAS[3]
T5	Commercial inoculant B[4] (2 mL/kg) – 15 DAS
T6	Commercial inoculant B[4] (2 mL/kg) – 30 DAS
T7	Commercial inoculant B[4] (2 mL/kg) – 45 DAS
T8	Commercial inoculant B[4] (2 mL/kg) – 60 DAS

Table 1. Treatments and doses of commercial inoculants used in the trial to evaluate agronomic efficiency via seed treatment, at different times before soybean sowing. Agricultural Year 2021/22. URPD of Pindorama-SP.

[1] In treatment 2, 50% of nitrogen fertilization was applied at sowing and 50% at flowering or 35 days after emergence;[2] inoculant A = MASTERFIX L SOJA ®;[3] DAS = days before sowing;[4] inoculant B = MASTERFIX L PREMIER® *Treatments 3 to 8 applied via seed treatment.

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 m2) will be considered as useful area. Thus, the experimental design was randomized blocks composed of the eight treatments mentioned above with 5 replications, in a total of 40 experimental plots.

The description of the 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 109 CFU mL-1 of *Bradyrhizobium elkanii* (Cepa Semia 5019) and *Bradyrhizobium japonicum* (Cepa Semia 5079).

B) MASTERFIX L PREMIER®: is an inoculant that represents a major evolution in formulation technology, enhancing the survival of bacteria, even in the face of the most adverse situations, which guarantees superiority in relation to the inoculants on the market.

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 carried out with fertilizer formulated 0-20-20, at a dose of (350 kg. ha-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 was performed in order to obtain an average final population of 320,000 plants ha-1.

The seeds have already been treated with Standak ® Top, which offers protection of 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 increased tolerance to water stress and nematode occurrence.

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

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

Fertilizer containing the micronutrients cobalt and molybdenum was applied via foliar spraying with the product CoMo Platinum (150 mLha-1); at the phenological stage V4 in all treatments including the control. Diseases and pests were also controlled 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).

ASSESSMENTS

EVALUATIONS AT THE REPRODUCTIVE STAGE R8

At the time of maturation (R8), before harvesting the plots useful for estimating grain productivity, which was carried out on April 5, 2022, the following characters of agronomic interest were also measured in the field by experimental plot:

- final stand (EF) = count of the number of plants obtained in 5 linear meters, in m-1 plants;

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

- height of insertion 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).

-number of pods per square meter (NV) – count of the number of full pods obtained in a square meter.

-mass of a thousand grains (MMG) = determined by weighing three subsamples of 100 grains, per repetition (BRASIL, 2009).

- accumulated nitrogen content in grains (NTG) = in g.kg-1 determined by the methodology described by Bataglia et al. (1978).

STATISTICAL ANALYSIS OF RESULTS

When significant differences were detected in the analysis of variance using the F test, the means were compared using the Tukey test at 5% probability. Statistical analyzes were 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 EF, APM, AIV and NMGV where statistical significance was not observed.

For EF, the treatments were not differentiated by the average test, and therefore, the general average of the experiment was 14.30 plants per linear meter, a value close to that recommended by breeders of the cultivar used in this work. This way, it is noted that the test was very well installed considering the cultivar used in question, and this statistical equality between treatments is a preponderant factor for the viability of the experiment, since treatments with plots that present heterogeneity in terms of stand could compromise the results., overestimating or underestimating data such as grain yield, as well as production components.

Considering the APM, it is noted that the general average of the experiment was above 60 cm (81.55 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. However, in a study carried out by Fipke (2015) when measuring the final height of soybean plants submitted to different forms of inoculation, as well as pre-inoculation in up to 10 days, significant differences were obtained, with values of 124 cm for standard inoculation, higher than height for early inoculation at 7 and 10 days before sowing, with 122 and 121 cm, respectively.

For AIV, the treatments also did not differ by the mean test applied, with the overall mean of the experiment being 16.05 cm. This is considered a characteristic of each cultivar, which may or may not be influenced by inoculation (HUNGRIA, 2011).

When analyzing the NV, treatments T2, T3, T4, T5, T6, T7 and T8 did not differ statistically from each other, and showed the highest mean values of respectively 2574.4, 2359.6, 2404.8, 2296.4 2286,4, 2503.6 and 2381.2 pods per m2. With a smaller increment in relation to NV, T1 was positioned, which showed the worst average value of 2151.6 pods. m2. Based on this production component, it can be inferred that the use of commercial inoculant B, regardless of the date of application, produced more pods per square meter when compared to the control without the use of inoculant. Schweig et al. (2017) aiming to study inoculation times associated with seed treatment, being carried out at 39, 32, 25, 18, 11 days before sowing (DAS) and standard inoculation on the sowing day, as well as a non-inoculated treatment, verified that for the number of pods per plant there was an increase for the standard inoculation and for the anticipated inoculation at 11, 18 and 25 DAS.

Regarding the NMGV, the results showed that the general average of the experiment was 2.31 grains per pod. And for the treatments tested, it is noted that they did not differ statistically from each other. Despite the non-statistical significance, it is noted that treatments T4 and T5 that used inoculant B applied on the day and 15 days before sowing were the ones that showed numerically the highest average number of grains per pod of 2.40. In turn, the use of the same inoculant applied to the seeds 60 days before sowing (T8) and non-inoculated control (T1) numerically showed the lowest mean NMGV values of 2.24 and 2.16, respectively. In turn, Schweig et al. (2017) in a study conducted using a long-life inoculant applied at different times before planting, observed very close values obtained for the variable number of grains per pod (NGV), which ranged from 2.35 to 2.47 grains per pod, without statistical differences. For Bulegon et al. (2015), the NGV also showed no significant difference when evaluating inoculation in two cultivars, but found values

TRAT	EF	APM	AIV	NV	NTG	NMGV	MMG	PG
	plantas/m	-----------cm-----------		m^2		unidades	g	kg ha^{-1}
T1	13,800 a	79,200 a	15,200 a	2151,6 b	48,51 d	2,16 a	132,40 ab	2110,2 d
T2	15,200 a	80,600 a	15,400 a	2574,4 a	55,68 a	2,30 a	136,80 a	2863,9 a b
T3	15,000 a	81,800 a	18,000 a	2359,6 a b	51,32 bcd	2,30 a	133,40 ab	2961,5 a
T4	15,000 a	81,400 a	15,800 a	2404,8 a b	53,51 abc	2,40 a	132,80 ab	2996,8 a
T5	13,800 a	80,800 a	15,800 a	2296,4 a b	53,73 ab	2,40 a	134,20 ab	2883,2 a b
T6	14,200 a	81,400 a	16,000 a	2286,4 a b	54,42 ab	2,36 a	133,80 ab	2742,4 b
T7	14,000 a	83,000 a	16,600 a	2503,6 a	52,94 bc	2,30 a	131,60 b	2722,4 b
T8	13,400 a	84,200 a	15,600 a	2381,2 a b	50,35 cd	2,24 a	132,80 ab	2300,0 c
F	0,6524NS	1,4873NS	0,2220NS	3,9944**	10,420**	1,9036NS	2,622*	61,652**
CV (%)	13,480	0,7768	9,4086	1,9017	3,09	3,5669	1,63	0,4591
Average	14,300	81,550	16,050	2369,8	52,56	2,3075	133,48	2697,6

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

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= Commercial inoculant A (2 mL/kg of seed) – 0 DAS[2] (days before sowing); T4= Commercial inoculant B (2 mL/kg) – 0 DAS[2]; T5= Commercial inoculant B ((2 mL/kg) – 15 DAS; T6= Commercial inoculant B (2 mL/kg) – 30 DAS; T7= Commercial inoculant B (2 mL/kg) – 45 DAS; T8 = Commercial inoculant B (2 mL/kg) – 60 DAS;.EF = final stand; APM = plant height at maturation; AIV = first pod insertion height; NV = number of pods obtained in one square meter; NTG = Nitrogen content in grains; NMGV = average number of grains per pod; MMG = mass of one thousand grains; PG = grain yield.1 Average of three replications of 10 pods per experimental plot for NMGV; A = commercial inoculant Masterfix L Soja®; B = inoculant commercial Masterfix L Premier®

of 2.79 to 2.90 grains per pod, higher than those reported in this study. Similar results were found by Fipke (2015), who evaluated NGV and quantified from 2.3 to 2.4 grains per pod.

Regarding MMG, it is noted that the treatment that used chemical nitrogen fertilization (T2) stood out with the highest mass of a thousand grains of 136.80 g, differing from the treatment (T7) inoculation 45 days before sowing, despite having had statistical equivalence with treatments T1, T3, T4, T5, T6 and T8. Pardinho (2015) found when evaluating the MMG that the inoculation with *Bradyrhizobium* provided 184.60 g statistically higher than the control with 162 g, causing an increase of 12% for this variable. The MMG is a characteristic value of each cultivar, similar to the NMGV, but this does not prevent this value from changing depending on the environmental and management conditions to which the crop is subjected (SILVA et al., 2011).

For NTG, it was verified that treatments T2 (chemical nitrogen fertilization), T4, T5 and T6 (application of commercial inoculant B on the day, 15 and 30 days before sowing) provided the highest increments in relation to this parameter with averages of respectively 55.68, 53.51, 53.73 and 54.42 $g.kg^{-1}$. With the lowest average increment in total nitrogen in the grains was the non-inoculated control (T1) with an average value of 48.51 g.kg-1, which was statistically similar to the treatments that used inoculant B applied 60 days in advance (T8) and the standard inoculant (T3) applied at sowing with means of respectively 50.35 and 51.32 $g.kg^{-1}$.

As for grain yield (PG), (Table 2) an overall average of 2697.6 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 Conab estimates (2020), in the state of São Paulo, the oilseed was highly benefited by the weather 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 T3, T4, T5 and T2 did not differ statistically from each other and stood out with higher PG values of 2961.5, 2996.8, 2883.2, and 2863.9 kg.ha-1, respectively. It must be noted that these treatments corresponded to the use of biological inoculants applied on the day and at most 15 days before planting and T2 (chemical nitrogen fertilization). According to Anghinoni et al. (2016) the use of conventional nitrogen fertilization does not affect crop productivity when compared to inoculation and pre-inoculation treatments. In an intermediate position according to the average test applied, treatment T6 (inoculant B applied with 30 DAS) remained, which was statistically equivalent to T7 (inoculant B applied with 45 DAS) with an average of 2722.4 kg ha-1. average values were treatments T1 (non-inoculated control) with 2110.2 kg ha-1 and T8 (inoculant B applied with 60 DAS) with 2300 kg.ha-1. In terms of increment, it is noted that considering the average value of the four best treatments (T3, T4, T5 and T2) which was 2926.35kg ha-1, there was an increase in terms of PG, that is, 816.15kg ha-1 or 13.60 bags ha-1 when compared to the non-inoculated control (T1) and 626.35 kg ha-1 or 10.44 bags ha-1 compared to T8 which corresponded to the use of inoculant B with longer time until sowing. In this case, the results allow us to infer that, for the analyzed response variable, there is no difference between inoculating the seeds at the time of sowing or up to 15 days before.

Still in relation to PG, when analyzing the T6 treatment that made use of early inoculation in 30 days with commercial inoculant B, an increase of 632.2 and 442.4 kg ha-1, respectively, is observed in relation to the control treatments (T1) and application of the same inoculant but with a longer period of application (60) until the time of planting (T8). 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

Based on the conditions of this study, it can be concluded that it is possible to recommend the commercial inoculant B to be applied via seed a maximum of 15 days before sowing, aiming at increasing grain productivity and for most production components.

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