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Agronomic efficiency of liquid inoculant based on *Bradyrhizobium japonicum* and *Azospirillum brasilense* for soybean crop

Eficiência agronômica de inoculante líquido a base de Bradyrhizobium japonicum e Azospirillum brasilense para a cultura da soja

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ABSTRACT
This research was conducted with the objective of evaluating the agronomic efficiency of different doses of the product formulated with two nitrogen-fixing bacteria (Bradyrhizobium japonicum and Azospirillum brasilense), in the development of soybean crop, as well as evaluating the possible pathogenic effect of crop treatments. To this end, four studies were installed in the cities of Ponta Grossa – PR, Paranapanema – SP, Bandeirantes – PR and Paranavaí – PR, in the 2020/2021 Harvest. The statistical design used was in randomized blocks, with six treatments and four replications, using the cultivar 58I60 RSF IPRO – BMX LANÇA IPRO. The results obtained in the four soil-climatic locations showed that product inoculation via seed positively influenced the growth of soybean plants and did not cause any symptoms of phytotoxicity. The dose of 300 mL 50 kg seeds^{-1} of Inoculant tested showed greater stability in the response in the different locations where the studies were conducted. Therefore, it provided an increase in the germination and final stand of plants, an increase in the number of nodules, an increase in the biomass of the shoot and root and a positive increase in the mass of a thousand grains and productivity in relation to the control.

Palavras-Chave
soybean
germination
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RESUMO
Esta pesquisa foi conduzida com o objetivo de avaliar a eficiência agronômica de diferentes doses do produto formulado com duas bactérias fixadoras de nitrogênio (Bradyrhizobium japonicum e Azospirillum brasilense), no desenvolvimento da cultura da soja, bem como o possível efeito patogênico dos tratamentos da cultura. Para tanto, foram instalados quatro estudos nos municípios de Ponta Grossa – PR, Paranapanema – SP, Bandeirantes – PR e Paranavaí – PR, na Safra 2020/2021. O delineamento estatístico utilizado foi em blocos casualizados, com seis tratamentos e quatro repetições, utilizando-se a cultivar 58I60 RSF IPRO – BMX LANÇA IPRO. Os resultados obtidos nos quatro locais edoclimáticos mostraram que a inoculação do produto via semente influenciou positivamente o crescimento das plantas de soja, e não causou nenhum sintoma de fitotoxicidade. A dose de 300 mL 50 kg sementes^{-1} do Inoculante testado apresentou maior estabilidade na resposta nos diferentes locais onde os estudos foram conduzidos. Dessa forma, proporcionou aumento na germinação e estande final das plantas, aumento no número de nódulos, aumento na biomassa da parte aérea e da raiz e aumento positivo na massa de mil grãos e produtividade em relação à testemunha.

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Introduction

The correct management practices can be decisive for the proper conduct of soybean farming to achieve high productivity. Among the practices, the following stand out: biotic elements (pests, diseases and weeds), climate, plant, soil, as well as the interaction between these factors (DOURADO NETO et al., 1998).

Increases in soybean crop productivity are also dependent on plant nutrition (MOREIRA, 1991) and in this sense microorganisms have played an important role, since studies demonstrate that efficient root infection by nitrogen-fixing bacteria has promoted an increase in production (HUNGRIA, 2011).

The formulation of inoculants and/or biofertilizers containing bacteria that promote plant growth and protection, which have shown satisfactory results for various crops (HALLMANN et al., 1997; BALDOTTO et al., 2010). The use of this technology has had a very positive impact on soybean production and others crops, as it considerably reduces the need for nitrogen fertilization, greenhouse gas emission, as well as reducing groundwater contamination by leaching and runoff of nitrite and nitrate (HUNGRIA; MENDES, 2015).

Hungria et al. (2010), showed that strains of *Azospirillum brasilense* and *Azospirillum lipoferum* promote increased productivity of corn and wheat, with *A. brasilense being* the species most used in agriculture for formulating inoculants, and in Latin America there are more than 100 inoculants containing the species as an active ingredient (CASSÁN; DIAZ-ZORITA, 2016). *Azospirillum* species promote plant growth due to nitrogen fixation, phosphate solubilization, production of phytohormones, among other characteristics, thus, their ability to promote growth is not explained by a single mechanism (BASHAN; DE BASHAN, 2010).

Given the ability of rhizobia to form nodules on plant roots and fix nitrogen, the inoculation of both types of bacteria results in a great alternative for increasing crop productivity. Hungria et al. (2013), observed that the Coinoculation of *Azospirillum* and *Bradyrhizobium species* resulted in an increase of 420 $kg.ha^{-1}$ in soybean production, when compared to plots without co-inoculation. In addition to the increase in productivity, studies have shown an increase in nodulation in soybeans when co-inoculated with *Azospirillum* spp. and *Bradyrhizobium* spp. (BARBOSA et al., 2021).

Based on the literature, new research evaluating the growth promotion of inoculants formulated with *A. brasilense* and *B. japonicum* is a viable alternative for the development of new products. In this sense, the objective of the present study was to evaluate the agronomic efficiency of different doses of the product formulated with two nitrogen-fixing bacteria (*Bradyrhizobium* japonicum e *Azospirillum brasilense*), in the development of the soybean crop, as well as the possible pathogenic effect of the different treatments on the crop.

Material and Methods

Experiments were carried out in the field to validate the agronomic efficiency and viability of the inoculant based on *Bradyrhizobium japonicum* and *Azospirillum brasilense* in promoting the growth and development of soybean (*Glycine max*) in four locations with different soil and climate conditions. The product is registered under number 21000.018572/2022-39. To develop the experiment, the protocols proposed by MAPA (Ministry of Agriculture, Livestock and Food Supply) for the registration of products from microorganisms that promote plant growth, according to IN 53 (BRASIL, 2013), were applied.

The experiments were carried out during the 2020/21 harvest and in four experimental areas: Ponta Grossa – PR, Paranapanema – SP, Bandeirantes – PR and Paranavaí – PR. All experimental tests were installed in areas of the partner company 3M Experimentação Agrícola. Information about sowing and harvest dates is described below.

Ponta Grossa – PR: The experimental test was installed on December 8, 2020 and the harvest was carried out on April 12, 2021. The soil in the area according to the Brazilian National Soil Program – Pronasolos, is classified as Cambissolo Haplico Aluminum with clay texture (PRONASOLOS, 2021). It has a pH of 6.43, at the time the study was set up, the soil was covered by wheat straw. The area was sown with soybeans and received fertilization of 350 kg ha -1 of formula 10-15-15. The city's climate is classified as humid subtropical (Cfb, according to the Köppen classification), with an average temperature of 17.5°C and average annual rainfall of 1495 mm. Considered edaphoclimatic region 103 by Normative Instruction No. 1 of February 2, 2012 of the Ministry of Agriculture, Livestock and Supply.

Paranapanema – SP: The research was installed on December 9, 2020, and the harvest was carried out on April 13, 2021, The soil in the area is classified as Dystrophic Red Oxisol with a clayey texture (PRONASOLOS, 2021). It has a pH of 6.18, at the time of installation of the study covered by corn straw. Regarding soybeans, it received fertilization of 400 kg ha -1 of formula 04-14-08. The municipality's climate is classified as subtropical (Cfa, according to the Köppen classification), with temperatures ranging from 14 °C to 30 °C with an average annual rainfall of 1210 mm. Considered edaphoclimatic region 203 by the Normative Instruction.

Bandeirantes – PR: The experiment was installed on December 10, 2020, and the harvest was carried out on April 1, 2021. The soil in the area is classified as a Eutroferric Red Oxisol with a very clayey texture. It has a pH of 5.70 and, at the time of implementing the experiment, it was covered by corn straw. Regarding soybeans, it received fertilization of 285 kg ha -1 of the 10-15-15 formula. The municipality's climate is classified as tropical (Aw, according to the Köppen classification), with an average temperature of 22.2°C and average annual rainfall of 1339 mm. Considered edaphoclimatic region 201 by the Normative Instruction.

Paranavaí – PR: The research was installed on December 11, 2020, and the harvest was carried out on April 15, 2021. The soil in the area is classified as Dystrophic Red Oxisol of medium texture. It has a pH of 4.50 and, at the time of implementing the experiment, it was covered by corn straw. Regarding soybean, it received fertilization of 400 kg ha -1 of formula 04-30-16. The municipality's climate is classified as high-altitude tropical (Cwb, according to the Köppen classification), with temperatures ranging between 14 °C and 32 °C, with an average annual rainfall of 1279 mm. Considered edaphoclimatic region 202 by the Normative Instruction.

The research was conducted in the four locations using a randomized block experimental design with six treatments and four replications. Each plot had a total area of 24.0 m2 (3.0 mx 8.0 m), with a useful area of 5.0 m2.

Soybean was sown with a spacing of 0.50 m between rows and a population density of 300,000 plants per hectare. A base fertilizer was applied with 380 kg ha-1 of formula 02-20-20. Phytosanitary maintenance and cultural treatments for soybeans were carried out in accordance with the recommendations for the crop for each region and following the premise of good agricultural practices.

The experiments were conducted using the soybean cultivar 58I60 RSF IPRO - BMX LANÇA IPRO. This cultivar has medium size, indeterminate growth and maturity group 5.8. It requires high soil fertility, which must be adjusted according to the soil characteristics of each region.

The treatment with nitrogen fertilizer consisted of the application of urea, 50% at sowing and 50% at flowering, with 222 $kg.ha^{-1}$ of urea being applied in each application, which represented 100 $kg.ha^{-1}$ of N at sowing and 100 kg ha^{-1} of N at flowering. The liquid inoculant Rhizonit (*Bradyrhizobium japonicum* SEMIA 5079/SEMIA5080 - Registration MAPA/PR nº 94581/10000-8) and the liquid inoculant Azonit (*Azospirillum brasilense* - AbV5/AbV6 – Registration MAPA/PR nº 94581/10011-3), manufactured by Nitro1000 Ind. and Com. Prod. Agricultural and Textile. Ltd., were applied as a positive co-inoculation control, following the manufacturer's recommendations. The tested inoculant based on *Bradyrhizobium japonicum* and *Azospirillum brasilense* 1 x 10^{9} and 1 x 10^{8} UFC mL^{-1}) was applied via seed using three different doses, specified in Table 1.

The effect of the Inoculant formulated (*Bradyrhizobium japonicum* and *Azospirillum brasilense*) on soybean crop with the evaluation of variables carried out at different phenological stages of the crop, coded according to the BBCH scale (2001).

Plant stand: The number of plants emerged in one linear meter, in three central lines. The assessments were carried out in the emergence room (BBCH – 09); 07 days after emergence (7 DAE BBCH – 11); 10 days after emergence (10 DAE BBCH – 12) and at harvest (BBCH – 96).

Plant vigor: For the vigor analysis, all plants present per plot at 10 DAE were considered. The evaluation was carried out visually, assigning a grade from 1 to 7, where: Grade 1: vigor much lower than the control; Grade 2: lower vigor than the control; Grade 3: vigor slightly lower than the control; Grade 4: vigor close to the control; Grade 5: vigor equal to control; Grade 6: vigor greater than the control and Grade 7: vigor much greater than the control.

Nodulation and biomass of the shoot and root: Five complete plants were collected in the central area of the second row of each plot, at the beginning of flowering or in the immediately preceding stage, but always between 35 – 40 days after emergence. The roots were collected using a straight shovel, taking care not to damage them and detach the nodules. Then, the roots were washed and the number of nodules in the first two cm depth of the main root was counted. Subsequently, they were placed to dry in an oven at 65 °C. After drying, the dry mass of the nodules was evaluated. The fresh mass of the aerial part and roots was evaluated and then placed to dry in an oven at 65 °C, until the weight stabilized, then evaluate the dry weight of the aerial part and roots.

Leaf analysis: For leaf analysis, each location was considered as a replication, taking a sample composed of four plants from each plot, at full soybean flowering (BBCH 65). The samples were sent for analysis to Laborsolo Laboratórios.

Productivity: It was evaluated in central plants totaling 5 m2 per plot. The grains were cleaned and weighed, correcting the humidity to 13% and transforming the values into kg ha-1. At the time of harvest, the mass of one thousand grains (MMG) was also evaluated.

Phytotoxicity: The possible pathogenic effect caused to plants by the inoculant formulation under test (*Bradyrhizobium japonicum* and *Azospirillum brasilense* 1 x 10^{9} and 1 x 10^{8} UFC/mL^{-1}), was evaluated by assigning notes to anomalies that were observed, using as a basis the phytotoxicity scale proposed by the EWRC (1964). Assessments were carried out 7 days after emergence (7 DAE BBCH – 11) and 15 days after emergence (15 DAE BBCH – 14).

Statistical analysis: The data obtained were subjected to analysis of variance and, when significant at 5% or 10%, the means of the treatments were compared using the Duncan test, also at the 5% or 10% level of significance, using the statistical program SASM-Agri® [System for analysis and separation of means in agricultural experiments - Canteri et al. (2001).

Climatic Data: Climatic data throughout the study at each location were collected from the first date of the month planted to the last date of the month harvested, with data from the weather station at the location and/or location of the nearest weather station.

Results and Discussion

In Brazil, bacterial inoculants formulated mainly with *Bradyrhizobium japonicum strains* are sold., recommended for soybean cultivation as it meets up to 100% of the crop's nitrogen demand (HUNGRIA; MENDES, 2015), as well as with *Azospirillum brasilense*, recommended for different grass crops (HUNGIRA, 2011) and for use in soy co-inoculation (HUNGRIA; NOGUEIRA; ARAUJO, 2015).

Nowadays the country is a leader in both, food production and the use of inoculants (ANPII, 2019), and with the high adherence to the use of bioinputs, the development of new inoculants continues to expand.

However, co-inoculation of soybean using *A. brasilense* and *Bradyrhizobium* spp is a relatively new technology in soybean cultivation and has been recommended by Embrapa Soja since 2015 (HUNGRIA; NOGUEIRA; ARAUJO, 2015). Compared to isolated *Bradyrhizobium inoculation*, co-inoculation promotes an increase in grain yield and quality (BARBOSA et al., 2021).

The beneficial effects of co-inoculation with *A. brasilense* and *Bradyrhizobium* are caused by several mechanisms, including acceleration of biological nitrogen fixation in nodules, increase in nodule dry matter, promotion of the occurrence of heterologous nodulation through increased formation of root hairs and secondary roots, due to the increase in infection sites, inhibition of pathogens (BÁRBARO et al., 2009), in addition to the production of phytohormones by *Azospirillum* , *such as* indoleacetic acid (BARBOSA et al., 2021).

The application of the Inoculant via seed, in doses of 100 – 300 mL.50 kg seeds^{-1}, had a positive impact on the germination and final stand of soybean plants, in the four locations where the studies were carried out (Table 2). In Ponta Grossa and Paranavaí, it was observed that the dose of 300 mL.50 kg seeds^{-1} of the inoculant under test, presented the highest number of plants and statistically differed from the control at the 5% probability level. Compared to the treatment that received nitrogen fertilizer, the treatment was statistically superior to this one (Table 2).

In Paranapanema, the dose of 200 mL.50 kg seeds^{-1} differed statistically from the control in all evaluations carried out for number of plants (Table 2). It was observed that from 7 DAE, this dose presented a greater number of plants, statistically differing from the treatment with nitrogen fertilizer. In Bandeirantes, it was observed that the treatments that received the inoculant formulated in doses of 100 mL.50 kg seeds^{-1} and 300 mL.50 kg seeds^{-1} showed greater stand in the four evaluations carried out, in relation to the control and the treatment with nitrogen fertilization, statistically differing from these (Table 2). It is important to highlight that in the final plant stand evaluated at the time of harvest, the three doses of inoculants tested showed a greater number of plants compared to the control and the treatment which received nitrogen fertilization, statistically differing from these (Table 2).

Compared to the standard co-inoculation treatment, (Rhizonit + Azonit), the stand evaluations at harvest, in the Ponta Grossa study, were statistically similar to the treatment that received the dose of 300 mL.50 kg seeds^{-1}. In Paranapanema, the dose of 200 mL 50 kg seeds^{-1} was statistically superior in the plant stand at the time of harvest. In Bandeirantes, the standard co-inoculation treatment was statistically similar to the treatments that received doses of 100 mL.50 kg seeds^{-1} and 300 mL.50 kg seeds^{-1}. In Paranavaí, the three doses of inoculant tested were statistically equal to the co-inoculation standard.

Regarding the effect of the tested inoculant on the vigor of soybean plants (Table 3), it was observed that the dose of 300 mL.50 kg seeds^{-1} was the one that had the greatest positive impact on vigor for the study conducted in Ponta Grossa (Table 3). In Paranapanema and Paranavaí, doses of 200 and 300 mL.50 kg seeds^{-1} showed greater vigor, statistically differing from the controls. These doses showed a variation of 10% greater in relation to the vigor of the control. In Bandeirantes, the dose of 100 mL.50 kg seeds-1 was the one that showed the greatest vigor, statistically differing from the control and the treatment with nitrogen fertilizer (Table 3). The dose of 100 mL.50 kg seeds-1 showed a 15% higher variation in relation to the vigor of the control.

The results obtained in the present research showed that the Inoculant tested promoted an increase in nodulation in the four locations where the studies were conducted (Table 4), observing a variation between 5.0% and 40.0% in relation to nodulation of the control plots. Increased nodulation due to *Bradyrhizobium* spp. and *A. brasilense* co-inoculation was previously found by Chibeba et al. (2015) and Hungria et al. (2015). This characteristic is linked to the fact that *Azospirillum* stimulates the formation of root hairs and root growth, thus increasing the soil exploration capacity of the root system (CHIBEBA et al., 2015), in addition to changing the morphology of the root system (SAIKIA et al., 2010).

Another factor which contributes to the increase in nodulation is that *Azospirillum bacteria,* when co-inoculated with *Bradyrhizobium*, stimulate the secretion of flavonoids by the plant (REGO et al., 2018), with daidzein and genistein being the main flavonoids secreted by soybeans (SUGIYAMA et al., 2016; MATSUDA et al., 2020), these flavonoids activate the expression of nodulation genes in rhizobia, which in turn synthesize and release lipo-chitooligosaccharides (Nod factors) (SUGIYAMA et al., 2017; MATSUDA et al., 2020). When the nodal factors are perceived by the roots, a root change is induced that will finally allow the infection of the rhizobia and consequently the morphogenesis of the nodule (SCHWEMBER et al., 2019).

The average nodulation per plant in the four trials was 32.6 nodules, a high value when compared to the average of 17.8 nodules per plant, obtained in the Technical Reference Units of the state of Paraná in the 2019/20 harvest (PRANDO et al., 2020). Prando et al. (2020) demonstrated that co-inoculation of *Bradyrhizobium* and *Azospirillum* contributed to an average 36% increase in the number of nodules.

Compared to soybeans inoculated with Bradyrhizobium alone, co-inoculation can promote an increase in root biomass by an average of 11% (BARBOSA et al., 2021). In the four studies carried out in this report, it was observed that the inoculant tested promoted gains in fresh weight and dry weight of the root (Table 5), observing a variation between 4% and 41.9% in relation to the control for the fresh pasta. For dry mass, a variation between 3.8% and 29.4% was observed in relation to the dry mass of the control plots. The effects of co-inoculation on soybean roots have been observed mainly in increasing root length, volume and branching (MOLLA et al., 2001; RONDINA et al., 2020).

Similar results were obtained regarding the effect of the inoculant under test on the biomass of the aerial part of the plants, observing that the application of the inoculant promoted a greater variation in relation to the control between 2.7% and 28.6% for the fresh weight and a variation between 4.2% and 27.7% for dry weight (Table 6).

Hungria et al. (2015), showed that co-inoculation of Bradyrhizobium and Azospirillum promoted an increase in the dry weight of the aerial part, being statistically comparable to the weight obtained in treatments that received nitrogen fertilization. The positive response to Bradyrhizobium and Azospirillum co-inoculation observed in the roots and aerial parts of soybean plants, is associated with the production of hormones (BARBOSA et al., 2021), mainly with mechanisms associated with the production of indolylacetic acid (PUENTE et al., 2018).

The positive effects on nodulation and root development because of Bradyrhizobium and A. brasilense co -inoculation, are also generally associated with an increase in grain yield (BARBOSA et al., 2021), as a consequence of greater nitrogen availability (MORETTI et al., 2020). These records were corroborated in the present research, where in the four locations evaluated it was observed that the application via seed of the inoculant in test promoted an increase in relation to the control of up to 9.7% for the mass of a thousand grains and of up to 11.6 % for productivity values (Table 8). Hungria et al. (2013), proposed that co-inoculation can generate an increase in grain yield of up to 16%, however, the average increase varies between 3.2% (BARBOSA et a., 2021) and 8% as recorded by Prando et al. (2020) in the 2017/18 – 2019/20 harvests in the state of Paraná.

Co-inoculation can contribute to an average increase of 2.8% in the nitrogen content of the shoot (BARBOSA et al., 2021). Although no statistical differences were observed between the N content of the control and the N content of the three doses of test inoculant applied (Table 7), it was observed that the inoculation of test inoculant contributed to an increase of up to 13.8 % compared to the N content in the leaves of the control plots.

The active principle of the tested inoculant (*Bradyrhizobium japonicum* and *Azospirillum brasilense*) did not cause any symptoms of phytotoxicity in the evaluations carried out at 7 and 15 days after emergence, with the soybean plants showing normal development, reaching a score of 1 (no damage) on the proposed scale. by EWRC (1964).

The results obtained in the present research in the four studies conducted in different soil and climate regions corroborate the beneficial effects of co-inoculation with *Bradyrhizobium* and *A. brasilense* previously reported in the literature, indicating that the Inoculant under test (*Bradyrhizobium japonicum* and *Azospirillum brasilense*), is a promising product for use in soybean cultivation.

Conclusion

Based on the results obtained in the four studies conducted in different soil and climate regions, it can be concluded that:

• The inoculant formulated (*Bradyrhizobium japonicum* and *Azospirillum brasilense*) via seed, positively influenced the growth of soybean plants, and did not cause any symptoms of phytotoxicity.

• The dose of 300 mL 50 kg seeds -1 Inoculant under test showed greater stability in the response in the different locations where the studies were conducted.

Therefore, it provided an increase in germination and final plant stand, an increase in the number of nodules, as well as an increase in the biomass of the shoot and root.

• Inoculation with the inoculant formulated, at a dose of 300 mL.50 kg seeds^{-1}, showed a positive increase for the yield components, for thousand grain mass and productivity in relation to the control, with the average increase obtained being 11.60% and 9.70% respectively.

• It is recommended to use the Inoculant (*Bradyrhizobium japonicum* and *Azospirillum brasilense*) via seed in soybean crops, using the dose 300 mL 50 kg seeds^{-1}.

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Table 1- Description of treatments applied to evaluate the effect of the inoculant in promoting growth in soybean crop. 2020/21 harvest.

Treatment		Formulation		Dose
		Active ingredient	Concentration	
1	Negative control[1]	-	-	-
2	Positive control[2]	-	-	200 kg.ha^{-1}
3	Co-inoculation[3] [Rhizonit + Azonit]	*Bradyrhizobium japonicum* + *Azospirillum brasilense*	5 x 10^9 + 2 x 10^8 UFC mL^{-1}	100 + 100 mL.50 Kg seeds^{-1}
4	Inoculante testado (100)	*Bradyrhizobium japonicum* + *Azospirillum brasilense*	1 x 10^9 + 1 x 10^8 UFC mL^{-1}	100 mL.50 Kg seeds^{-1}
5	Inoculante testado (200)	*Bradyrhizobium japonicum* + *Azospirillum brasilense*	1 x 10^9 + 1 x 10^8 UFC mL^{-1}	200 mL.50 Kg seeds^{-1}
6	Inoculante testado (300)	*Bradyrhizobium japonicum* + *Azospirillum brasilense*	1 x 10^9 + 1 x 10^8 UFC mL^{-1}	300 mL.50 Kg seeds^{-1}

1: Absence of inoculation. 2: Application of N in the form of urea, 50% at sowing and 50% at flowering, representing 100 kg.ha-1 of N in each application. 3: Positive control (co-inoculation with standard products)
Source: the authors, (2024).

Tabela 2. Average plant stand per treatment (N°) and variation in values about the control V(%), in assessments carried out 7 and 10 days after emergence (7 DAE; 10 DAE) and at the time of harvest, in soybean cultivation. 2020/21 harvest.

Treatment	Ponta Grossa - PR								Paranapanema - SP							
	Emergence 15/12/2020 BBCH - 09		**7 DAE 22/12/2020 BBCH - 11**		**10 DAE 25/12/2020 BBCH - 12**		**Harvest 12/04/2021 BBCH - 96**		**Emergence 16/12/2020 BBCH - 09**		**7 DAE 23/12/2020 BBCH - 11**		**10 DAE 26/12/2020 BBCH - 12**		**Harvest 13/04/2021 BBCH - 96**	
	(N°) **	V(%)	(N°) **	V(%)	(N°) **	V(%)	(N°) **	V(%)	(N°) **	V(%)	(N°) **	V(%)	(N°) **	V(%)	(N°) **	V(%)
1	34,50 c	-	38,3 c	-	39,25 c	-	36,00 b	-	33,75 b	-	38,00 c	-	38,50 d	-	37,25 cd	-
2	35,50 bc	2,9	38,8 bc	1,3	39,75 bc	1,3	36,25 b	0,7	34,50 ab	2,2	38,25 bc	0,7	39,00 cd	1,3	37,00 d	-0,7
3	37,50 ab	8,7	39,8 ab	3,9	40,75 ab	3,8	37,00 ab	2,8	35,25 a	4,4	39,75 ab	4,6	40,25 ab	4,5	39,00 b	4,7
4	36,75 ab	6,5	39,5 ab	3,3	40,25 abc	2,5	36,50 ab	1,4	33,75 b	0,0	38,50 bc	1,3	39,50 bcd	2,6	38,50 bc	3,4
5	36,50 abc	5,8	39,8 ab	3,9	40,75 ab	3,8	37,00 ab	2,8	35,50 a	5,2	40,25 a	5,9	41,00 a	6,5	40,75 a	9,4
6	38,00 a	10,1	40,3 a	5,2	41,25 a	5,1	37,50 a	4,2	34,75 ab	3,0	39,50 abc	3,9	40,00 abc	3,9	39,25 b	5,4
Mean	36,46		39,38		40,33		36,71		34,58		39,04		39,71		38,63	
C.V.	3,74		2,00		1,97		1,76		2,60		2,40		1,91		2,39	

Treatment	Bandeirantes - PR								Paranavaí - PR							
	Emergence 17/12/2020 BBCH - 09		**7 DAE 24/12/2020 BBCH - 11**		**10 DAE 27/12/2020 BBCH - 12**		**Harvest 14/04/2021 BBCH - 96**		**Emergence 18/12/2020 BBCH - 09**		**7 DAE 25/12/2020 BBCH - 11**		**10 DAE 28/12/2020 BBCH - 12**		**Harvest 15/04/2021 BBCH - 96**	
	(N°) **	V(%)	(N°) **	V(%)	(N°) **	V(%)	(N°) **	V(%)	(N°) **	V(%)	(N°) **	V(%)	(N°) **	V(%)	(N°) **	V(%)
1	35,00 bc	-	40,00 b	-	40,25 b	-	39,00 c	-	33,25 b	-	37,75 c	-	39,00 c	-	37,50	-
2	34,50 c	-1,4	39,75 b	-0,6	40,25 b	0,0	39,25 bc	0,6	33,50 b	0,8	37,75 c	0,0	38,75 c	-0,6	37,25 b	-0,7
3	36,75 ab	5,0	41,75 ab	4,4	42,50 a	5,6	41,50 ab	6,4	34,00 b	2,3	39,00 bc	3,3	40,50 b	3,8	39,25 a	4,7
4	37,00 a	5,7	42,25 a	5,6	43,50 a	8,1	42,50 a	9,0	35,00 ab	5,3	39,25 b	4,0	40,75 b	4,5	39,50 a	5,3
5	36,75 ab	5,0	41,25 ab	3,1	42,00 ab	4,3	40,75 abc	4,5	34,25 ab	3,0	39,50 b	4,6	41,00 b	5,1	40,25 a	7,3
6	37,00 a	5,7	42,75 a	6,9	43,75 a	8,7	42,25 a	8,3	36,25 a	9,0	40,75 a	7,9	42,25 a	8,3	40,75 a	8,7
Mean	36,17		41,29		42,04		40,88		34,38		39,00		40,38		38,63	
C.V.	3,31		3,23		2,76		3,67		2,69		2,06		1,63		2,39	

CV = Coefficient of variation (%).
Means followed by the same letter in the column do not differ from each other using the Duncan test at the level of 5% (**) or 10% (*) of probability.
Source: the authors, (2024).

Table 3. Average vigor (grade) of plants per treatment and variation in values about the control V(%), in evaluations carried out 10 days after emergence (10 DAE) in soybean crops. 2020/21 harvest.

Treatment	Ponta Grossa - PR			Paranapanema – SP			Bandeirantes - PR			Paranavaí - PR		
	Grade	*	V(%)	Grade	*	V(%)	Grade	*	V	Grade	*	V(%)
1	5	b	-	5	b	-	5	b	-	5	a	-
2	5	b	0,0	5	b	0	5	b	0	5	a	0
3	5.5	a	10	5.5	ab	10	5.5	ab	10	5.25	a	5
4	5.25	b	5	5	b	0	5	b	0	5	a	0
5	5.25	ab	5	5.5	ab	5	5.25	b	5	5	a	0
6	5.5	ab	10	5.75	a	15	5.5	ab	10	5.5	a	10
Mean	5.25			5.29			5.32			5.18		
CV	6.35			6.42			6.8			6.99		

CV = Coefficient of variation (%).
Means followed by the same letter in the column do not differ from each other using the Duncan test at the level of 5% (**) or 10% (*) of probability.
Source: the authors, (2024).

Table 4. Average number and dry mass of nodules per treatment (N°) and variation in values about the control V(%), in evaluations carried out at the beginning of flowering (BBCH – 51) in soybean. 2020/21 harvest.

	Ponta Grossa - PR						**Paranapanema – SP**					
Treatment	**Nodules**			**Nodules mass**			**Nodules**			**Nodules mass**		
	N°	**	**V(%)**	**(g)**	**	**V(%)**	**N°**	**	**V(%)**	**(g)**	**	**V(%)**
1	200,25	c	-	1,57	b	-	141,5	b	-	1,56	bc	-
2	218,75	bc	9,2	1,63	b	3,8	143,75	b	1,6	1,52	c	-2,4
3	245,25	abc	22,5	1,91	ab	21,5	159,75	ab	12,9	1,67	ab	7,1
4	245,25	bc	11,9	1,67	ab	6,6	150	ab	6	1,57	bc	0,6
5	224	ab	32,6	1,92	ab	22,5	168,25	a	18,9	1,77	A	13,3
6	263.5	a	40	2,06	a	31,4	158,5	ab	12	1,69	ab	8,5
Mean	280,25			1,79			153,63			1,63		
CV	238,67			11,31			7,42			5,13		
	Bandeirantes - PR						**Paranavaí – PR**					
Treatment	**Nodules**			**Nodules mass**			**Nodules**			**Nodules mass**		
	N°	**	**V(%)**	**(g)**	**	**V(%)**	**N°+**	**	**V(%)**	**(g)**	**	**V(%)**
1	184	b	-	1,38	b	-	159	cd	-	1,21	bc	-
2	173,75	b	-5,6	1,35	b	1.1	153,5	d	-3,5	1,15	c	-4,4
3	217,25	ab	18,01	1,54	b	15.1	168,25	abc	5,8	1,32	ab	9,1
4	231,5	a	25,8	1,61	b	3.4	167	bc	5	1,31	abc	8,3
5	205,75	ab	11,8	1,5	b	8,7	173,25	ab	9	1,29	abc	7,3
6	242,75	a	31,9	1,76	b	19.6	180	a	13,2	1,42	a	17,6
Mean	209,17			1,52			166,83			1,28		
CV	13,03			10,9			4,77			7,56		

CV = Coefficient of variation (%). Means followed by the same letter in the column do not differ from each other using the Duncan test at the level of 5% (**) or 10% (*) of probability.
Source: the authors, (2024).

Table 5. Average weight of fresh mass and dry mass of roots and variation in values about the control V(%), in evaluations carried out at the beginning of flowering (BBCH – 51) in soybean crops. 2020/21 harvest.

	Ponta Grossa - PR						**Paranapanema – SP**					
Treatment	**Fresh mass**			**Dry mass**			**Fresh mass**			**Dry mass**		
	(g)	*	**V(%)**	**(g)**	*	**V(%)**	**(g)**	*	**V(%)**	**(g)**	*	**V(%)**
1	99,75	b	-	52	b	-	80,75	c	-	31	bc	-
2	106	b	6,3	52,25	b	0,5	83	bc	2,8	31	c	0
3	121,25	ab	21,6	57	ab	9,6	88,25	abc	9,3	35,75	abc	15,3
4	108	b	8,3	54	b	3,8	84	bc	4	32,25	abc	4
5	125,5	ab	25,8	57,5	ab	10,6	92,75	a	14,9	40	abc	29
6	141	a	41,9	65,25	a	25,5	87,5	ab	8,4	34	a	9,7
Mean				56,33			85,04			34		
CV				9,81			7,43			11,4		
	Bandeirantes - PR						**Paranavaí – PR**					
Treatment	**Fresh mass**			**Dry mass**			**Fresh mass**			**Dry mass**		
	(g)	*	**V(%)**	**(g)**	*	**V(%)**	**(g)**	*	**V(%)**	**(g)**	*	**V(%)**
1	85,5	b	-	34	b	-	77,75	b	-	39,25	b	-
2	87,25	b	2	34,25	b	0,7	77,25	b	-0,6	39	b	-0,6
3	99,25	ab	16,1	37,5	ab	10,3	86,75	ab	12	42	b	7
4	109	a	27,5	44	a	29,4	84	ab	8	41	b	4,5
5	96,75	ab	13,2	40	ab	17,6	84,5	ab	8,7	43,25	ab	10,2
6	106	a	24	41,5	a	22,1	94,75	a	22	47,5	a	21
Mean	97,29			38,54			84,17			42		
CV	9,92			11,37			8,13			8,07		

Means followed by the same letter in the column do not differ from each other using the Duncan test at the level of 5% (**) or 10% (*) of probability.
CV = Coefficient of variation (%);
Source: the authors, (2024).

Table 6. The average weight of fresh mass and dry mass of the aerial part and variation in values about the control V(%), in evaluations carried out at the beginning of flowering (BBCH – 51) in the soybean crop. 2020/21 harvest.

Treatment	**Ponta Grossa - PR**						**Paranapanema – SP**					
	Fresh mass			**Dry mass**			**Fresh mass**			**Dry mass**		
	(g)	*	**V(%)**	**(g)**	*	**V(%)**	**(g)**	*	**V(%)**	**(g)**	*	**V(%)**
1	945,75	b	-	372,5	c	-	890,5	b	-	341,75	b	-
2	949,5	b	0,4	378,25	c	1,5	908,5	b	2	343	b	0,4
3	1038	ab	9,8	422,25	bc	13,4	998,75	ab	12,2	390	ab	14,1
4	971	b	2,7	391,	c	5	957,5	b	7,5	356	b	4,2
5	1039,75	ab	9,9	458	ab	23	1095	a	23	426,25	a	24,7
6	1120	a	18,4	475,75	a	27,7	995	ab	11,7	378,50	ab	10,8
Mean	1010,67			416,33			974,21			372,58		
CV	10,8			7,46			8,31			9,21		
Treatment	**Bandeirantes - PR**						**Paranavaí – PR**					
	Fresh mass			**Dry mass**			**Fresh mass**			**Dry mass**		
	(g)	*	**V(%)**	**(g)**	*	**V(%)**	**(g)**	*	**V(%)**	**(g)**	*	**V(%)**
1	900,25	b	-	374,25	bc	-	742,5	b	-	311	c	-
2	915,25	b	1,7	365,75	c	-2,3	753,75	b	1,5	316,25	c	1,7
3	1016,75	ab	12,9	411	abc	9,8	905	a	21,9	356,25	ab	14,5
4	1063,25	a	18,1	457,75	a	22,3	811,25	b	9,3	331	bc	6,4
5	974,5	ab	8,2	402,75	abc	7,6	931,25	a	25,4	345,75	b	11,2
6	1068,25	a	18,7	430,25	ab	15	955	a	28,6	377,25	a	21,3
Mean	989,71			406,96			849,79			339,58		
CV	8,16			9,34			6,69			5,46		

Means followed by the same letter in the column do not differ from each other using the Duncan test at the level of 5% (**) or 10% (*) of probability.
CV = Coefficient of variation (%);
Source: the authors, (2024).

Table 7. Comparison of average productivity, thousand-grain mass (MMG) and increase in production values about the control (V%) in soybean, cultivar 58I60 RSF IPRO – BMX LANÇA IPR. 2020/21 harvest.

Treatment	**Ponta Grossa - PR**						**Paranapanema – SP**					
	MMG			**Productivity**			**MMG**			**Productivity**		
	(g)	*	**V(%)**	**(kg.ha^{-1})**	*	**V(%)**	**(g)**	*	**V(%)**	**(kg.ha^{-1})**	*	**V**
1	161	b	-	2757,13	b	-	162,75	c	-	3165,43	b	-
2	160,25	b	-0,5	2775,79	b	0,7	160,75	c	-1,2	3201,20	ab	1,1
3	166,75	ab	3,6	2925,73	ab	6,2	168,25	bc	3,4	3388,64	ab	7,1
4	163,75	ab	1,7	2864,95	ab	3,9	163,75	c	0,6	3249,86	ab	2,7
5	168,5	a	4,7	2922,11	ab	6	172,75	ab	6,1	3494,96	a	10,4
6	170,25	a	5,8	3077,45	a	11,6	169,5	a	4,1	3429,13	ab	8,3
Mean	165,8			2887,19			166,29			3321,5		
CV	2,55			4,75			2,71			6,51		
Treatment	**Bandeirantes - PR**						**Paranavaí – PR**					
	MMG			**Productivity**			**MMG**			**Productivity**		
	(g)	*	**V(%)**	**(kg.ha^{-1})**	*	**V(%)**	**(g)**	*	**V(%)**	**(kg.ha^{-1})**	*	**V**
1	162,25	b	-	3353,03	c	-	152,5	c	-	3323,33	b	-
2	162,5	b	0,2	3572,37	bc	1,1	150,75	c	-1,1	3343,34	b	0,6
3	169,5	a	4,5	3733,81	abc	5,7	161	ab	5,6	3463,05	ab	4,2
4	174,25	a	7,4	3850,9	a	9	161,5	c	5,9	3476,71	ab	4,6
5	170	a	4,8	3747,18	abc	6,1	163,75	bc	7,4	3559,76	ab	7,1
6	173	a	6,6	3824,97	ab	8,3	167,25	ab	9,7	3671,98	a	10,5
Mean	168,58			3710,38			159,46			3473,03		
CV	2,15			4,4			3,01			4,42		

Means followed by the same letter in the column do not differ from each other using the Duncan test at the level of 5% (**) or 10% (*) of probability.
CV = Coefficient of variation (%). Source: the authors, (2024).

Table 8. Micronutrient content present in the plant tissue of the aerial part by treatment, in the soybean crop. 2020/21 harvest. The values represent the average of the results of the four locations: Ponta Grossa, Paranapanema, Bandeirantes and Paranavaí.

Treatment	**Cu**		**Co**		**Se**		**V**	
	----------------------------	**mg.kg^{-1}**					----------------------------	
		*		*		*		*
1	9,54	b	0,42	b	1,39	a	3,34	c
2	10,08	b	0,47	ab	0,69	b	4,04	bc
3	11,07	ab	0,39	b	0,94	ab	4,03	bc
4	9,90	b	0,5	ab	0,79	b	4,4	abc
5	12,74	a	0,58	a	0,62	b	5,44	a
6	9,97	b	0,54	a	0,49	b	4,96	ab
Mean	10,55		0,48		0,82		4,36	
CV	12,93		18,1		48,4		22,1	

Means followed by the same letter in the column do not differ from each other using the Duncan test at the level of 5% (**) or 10% (*) of probability.
CV= Coefficient of variation (%);
Source: the authors, (2024).