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Effect of phosphate-solubilizing bacteria on soybean yield and enzymatic activity in sandy soil

Alexandre de Oliveira[1], Augusto Rodrigues[1], Adriana Smanhotto[1], Félix Garcia[1], Rafael da Silva[1], Raphael Campos[2], Gustavo Moretti[1], Gustavo Vieira[1]

[1] Instituto Federal de Educação, Ciência e Tecnologia do Mato Grosso do Sul, câmpus Nova Andradina, Nova Andradina, Mato Grosso do Sul, Brasil. E-mail: alexandre.oliveira@ifms.edu.br , augusto.rodrigues@ifms.edu.br, adriana.smanhotto@ifms.edu.br, rafael.silva@ifms.edu.br, gustavo.moretti@estudante.ifms.edu.br, gustavo.vieira4@estudante.ifms.edu.br

[2] Instituto Federal de Educação, Ciência e Tecnologia do Paraná, câmpus Paranaguá, Paranaguá, Paraná, Brasil. E-mail: serradomaragro@gmail.com

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ABSTRACT

Soybean [*Glycine max* (L.) Merrill] is Brazil leading agricultural commodity, positioning the country as the world largest crop producer. Soybean yield is determined by the combined contribution of several primary and secondary components, all directly influenced by adequate plant nutrition. Among essential nutrients, phosphorus (P) stands out as one of the most limiting for plant development, and its deficiency compromises plant physiology, negatively affecting yield performance. In this context, research has shown promising results regarding phosphate solubilization by microorganisms such as *Bacillus subtilis*, *B. megaterium*, and *Pseudomonas fluorescens*, applied individually or in combination. This study evaluated soil enzymatic activity and soybean yield in response to seed-applied microbial inoculants. The experiment was conducted during the 2023/2024 growing season at IFMS Nova Andradina, using two soybean cultivars in a randomized complete block design (RCBD), arranged in a 2×6 factorial scheme with five replications. The seed treatments included: a control (no inoculation); inoculation with 100 mL ha^{-1} of Bioma Brady; 100 mL h^{-1} of BiomaPhos®; 100 mL ha^{-1} of Nod Phos®; 300 mL h^{-1} of Biomaphos®; and 300 mL ha^{-1} of Nod Phos®. The results confirmed that seed inoculation and/or co-inoculation with phosphate-solubilizing microorganisms (PSM) and biological nitrogen-fixing (BNF) agents enhanced grain yield in both soybean genotypes compared to the non-inoculated control. Additionally, the use of BiomaPhos® and Nod Phos® increased soil enzymatic activity.

Keywords: Soil enzymatic activity, β-glucosidase, *Glycine max* (L.) Merrill, Grain yield, Soil health.

Atuação de bactérias solubilizadoras de fosfato na produtividade da soja e atividade enzimática em solo arenoso

RESUMO

A soja [*Glycine max* (L.) Merrill] é a principal commodity agrícola do Brasil, destacando-se como o maior produtor mundial do grão. A produtividade, ou rendimento de grãos na cultura da soja é determinada pela soma de diversos componentes primários e secundários, os quais dependem diretamente da nutrição adequada da planta. Entre os nutrientes essenciais, o fósforo (P) destaca-se como um dos mais limitantes no desenvolvimento vegetal, e sua deficiência interfere na fisiologia da planta, afetando negativamente o desempenho produtivo. Neste contexto, pesquisas têm demonstrado resultados promissores na solubilização de fosfatos, a partir de microrganismos como *Bacillus subtilis*, *B. megaterium* e *Pseudomomonas fluorescens* aplicados isolados e/ou em misturas. Este ensaio teve como objetivo avaliar a atividade enzimática do solo e o rendimento da soja em função da aplicação de diferentes microrganismos via tratamento de sementes. O experimento foi conduzido na safra 2023/2024 no IFMS Nova Andradina, em Nova Andradina, utilizando dois cultivares de soja no delineamento de blocos completos casualizados (DBC) em esquema fatorial 2x6 com cinco repetições. As inoculações via sementes consistiram em: Controle (sem inoculação); inoculação com 100 mL ha^{-1} de Bioma Brady; inoculação com 100 mL ha^{-1} de BiomaPhos®; inoculação via semente com 100 mL ha^{-1} de Nod Phos®, inoculação com 300 mL ha^{-1} de BiomaPhos® e inoculação de 300 mL ha^{-1} de Nod Phos®. As análises comprovaram que a inoculação e/ou coinoculação via sementes com microrganismos solubilizadores de fosfato (MSP) e promotores de fixação biológica de nitrogênio (FBN) elevaram o rendimento grãos em ambos genótipos de soja, em comparação ao controle sem inoculação. Além disso, o uso de BiomaPhos® e NOD PHOS® promoveu o aumento enzimático do solo.

Palavras-chave: Atividade enzimática do solo, β-glicosidase, *Glycine max* (L.) Merril, Rendimento de grãos, Saúde do solo.

1. Introduction

Soybean [*Glycine max* (L.) Merrill] is Brazil leading agricultural commodity and the world largest crop producer. In the 2022/2023 growing season, according to the twelfth crop report - grains (CONAB, 2023), Brazil harvested an estimated 322.8 million tons. This figure represents an 18.4% increase, equivalent to 50.1 million more tons than in the previous growing season. This growth was driven by an expansion in planted area reaching 78.5 million hectares and improved average grain yield, which rose from 3,656 kg ha^{-1} in the previous season to 4,111 kg ha^{-1}.

Combining several primary and secondary components determines soybean grain yield. Among the primary components, plant population per unit area is the factor with the greatest potential for management-based control, and the optimal number is established through technical trials (Bertolino et al., 2023).

Biological indicators, or bioindicators, of soil quality deserve special attention, as microorganisms play a key role in mediating the release of nutrients from soil organic matter processes regulated by soil enzymatic activity (Kreling et al., 2022).

Enzymes involved in nutrient cycling have been proposed as potential indicators of soil quality, as they reflect changes in the soil microbiota and tend to be more sensitive than chemical or physical attributes when detecting alterations in soil quality caused by land use practices. According to Mendes et al. (2021), soil enzymatic activity represents the cumulative activity of enzymes produced by plants, macroorganisms, and microorganisms. In addition to their role in intracellular metabolic reactions within living organisms, extracellular enzymes play a fundamental role in the soil environment. They participate in a range of reactions, including the decomposition of organic residues (ligninases, cellulases, proteases, glucosidases, and galactosidases), nutrient cycling (phosphatases, amidases, urease, and sulfatase), and the formation of soil organic matter (SOM).

Organic phosphorus (P) accounts for approximately 4%-90% of the total soil P, and organic matter (OM) represents the second largest reservoir of phosphorus in the soil, primarily in the form of inositol phosphate, phosphate monoesters, and phosphate diesters (Xie et al., 2023). The slow and gradual mineralization of organic residues enables the release and redistribution of organic P forms, which are more mobile in the soil and less prone to adsorption reactions. When soil P availability is limited, microorganisms play a crucial role in the mineralization of organic P and the solubilization of inorganic P, increasing the amount of available phosphorus (AP) in the soil.

On the other hand, Carvalho and Castro (2023) emphasize that the low clay and organic matter (OM) content of sandy soils gives them a distinctive character marked by high fragility and strong susceptibility to degradation from various processes. In addition to the limited phosphorus availability, the high concentrations of Fe^{2+} and Al^{3+} in these soils increase phosphorus adsorption, further restricting this nutrient. Nevertheless, when proper management practices are implemented, it is often possible to achieve yields comparable to or even higher than those obtained in medium-textured and sandy Cerrado soils compared to clayey soils.

Regarding the microorganisms used in the study, the inoculant BiomaPhos® contains the strains *Bacillus subtilis* and *B. megaterium* phosphate-solubilizing bacteria (PSB) capable of increasing phosphorus use efficiency in plants, which may lead to higher yields and, in the future, reduced need for phosphate fertilizers. This genus of Gram-positive bacteria, widely distributed in the environment, has stood out for its influence on the physiological, metabolic, and phenotypic traits of crops. It promotes plant growth through phosphate solubilization, indole-3-acetic acid (IAA) production, and other phytohormones.

Santos et al. (2019) also discuss the other phosphate-solubilizing inoculant used in the study. Nod Phos® is composed of a proprietary blend of three beneficial microbial strains: it includes strains of *Pseudomonas fluorescens* and *Azospirillum brasilense*, functioning both as a phosphate solubilizer and a plant growth-promoting agent.

In light of the above, this study aimed to evaluate the influence of seed inoculation with different phosphate-solubilizing microorganisms on the yield of two commercial soybean cultivars and soil enzymatic activity under sandy soil conditions.

2. Material and Methods

The experiment was conducted in the Plant Production area of the Federal Institute of Education, Science and Technology of Mato Grosso do Sul (IFMS) - Nova Andradina Campus, located at 22°04'58.0"S and 53°28'12.3"W. The experimental area features flat to gently undulating terrain, with an average slope of 3% and an average elevation of 380 meters. The region experiences average annual temperatures ranging from 20 to 22 °C and annual precipitation between 1,500 and 1,700 mm (Mato Grosso do Sul, 2015), and the climate is classified as Tropical Savanna (Aw-type) according to the Köppen-Geiger system.

The area was originally covered by typical Cerrado vegetation and had been cultivated for six years with annual crops during the growing season and cover crops during the off-season. It has been maintained under a no-tillage system (NTS), with the soil classified as a sandy-textured Latossolo Vermelho Distrófico, containing 810 g kg^{-1} of sand, 145 g kg^{-1} of clay, and 45 g kg^{-1} of silt (Santos, 2018). Base saturation (V%) was corrected to levels above 60% through the surface application of dolomitic lime at a rate of 1.0 t ha^{-1}. Both base fertilization and potassium topdressing were conducted to supply the nutrients required for crop development.

During the agricultural year, the highest rainfall volumes occurred in the third ten-day period of November, exceeding 110 mm, with average temperatures around 25.5 °C considered favorable for soybean growth. In the first ten-day period of January, average temperatures reached nearly 30 °C, a condition that promotes the abortion of reproductive structures, coinciding with phenological stages R3-R4. Total rainfall recorded during the crop cycle was approximately 430 mm, with 200 mm occurring during the vegetative stages and 130 mm during the reproductive stages, which was insufficient for the crop full development. To achieve maximum yield, soybeans require between 450 and 800 mm of water throughout the growing season, depending on climate conditions, management practices, and varietal cycle length (Sartori et al., 2015).

The experimental design used was a randomized complete block design (RCBD) in a 2×6 factorial scheme, with five replications. The first factor consisted of two soybean cultivars: 1 - Nidera® NS 6446 I2X and 2 - Brasmax® BMX Nexus I2X, both featuring Intacta Xtend technology and a physiological maturity group of 6.4 (microregion 2002). The second factor consisted of five seed-applied inoculation treatments using two species of phosphate-solubilizing bacteria, along with a non-inoculated control: 1 - Control (no inoculation); 2 - Inoculation with 100 mL ha^{-1} (single dose) of Bioma Brady®, composed of Bradyrhizobium japonicum, containing strains SEMIA 5079 and 5080, at a concentration of 7.2×10^9 CFU mL^{-1} (Bioma®); 3 - Inoculation with 100 mL ha^{-1} (single dose) of Biomaphos®, containing strains of Bacillus subtilis (CNPMS B2084 - BRM034840) and Bacillus megaterium (CNPMS B119 - BRM033112), with a viable cell concentration of 4×10^9 CFU mL^{-1} (Bioma®); 4 - Seed inoculation with a single dose of Nod Phos®, composed of Pseudomonas fluorescens (strain ATCC 13525, 2×10^8 CFU mL^{-1}) and Azospirillum brasilense (strains Ab-V5 and Ab-V6), at a concentration of 2×10^8 CFU mL^{-1} (Agrocete®); 5 - Inoculation with 300 mL ha^{-1} (three doses) of Biomaphos®; and 6 - Seed inoculation with three doses of Nod Phos®.

Seeds from all treatments except the control also received the standard inoculation with Bioma Brady® at a rate of 100 mL ha^{-1}. Each dose of inoculant applied to the seeds corresponded to approximately 2 mL per kg of seed, based on a seeding density of 50 kg ha^{-1}.

Each experimental plot consisted of four rows, 20 meters long, with 0.45 m row spacing, and the two central rows were considered the useful area. The target final population was 280,000 plants ha^{-1}, with a density of 13 plants per linear meter. Sowing was conducted on October 26, 2023. The fertilization in the sowing furrow consisted of applying 300 kg ha^{-1} of the 11-52-00 formulation (33 kg ha^{-1} of N, 156 kg ha^{-1} of P_2O_5), while potassium fertilization was applied as a topdressing in two split applications, using 135 kg ha^{-1} of KCl (80 kg ha^{-1} of K_2O), with half applied at the V2 stage and the remainder at the V4 stage.

Pest and disease management followed the technical recommendations for the crop. According to each manufacturer specifications, the seeds used had already undergone industrial treatment with fungicides and insecticides to ensure seedling health and uniform emergence.

The primary yield components evaluated were: Plant density (plants m²): determined by counting the number of plants within six meters of row length using a measuring tape; Number of pods: counted for each plant; Total number of seeds: counted per plant (Bertolino et al., 2023); Total grain mass: determined by weighing all the seeds produced by each analyzed plant using a semi-analytical electronic balance with two-decimal precision; and 100-grain weight: calculated using the equation (Eq.

1) described by Sartori et al. (2015). Based on the total number of pods and seeds per plant, the average number of seeds per pod was also calculated.

$$100 - \text{grain weight} = \left(\frac{\text{total grain mass}}{\text{total number of grains}}\right) x\ 100$$

(eq. 01 - Sartori et al., 2015).

For the secondary yield components, the following were measured: Number of fertile branches: determined by counting the number of primary branches bearing at least one pod on each harvested plant; Number of fertile nodes: counted as the number of nodes with at least one pod, present on all branches of each harvested plant (Bertolino et al., 2023). Final grain yield (productivity) of the evaluated cultivars was determined and estimated in kg ha^{-1} (Eq. 2), according to Sartori et al. (2015).

Grain yield = plants per hectare×pods per plant×grains per pod×100-grain weight/1000

(eq. 02 - Sartori et al., 2015).

Still in the field, at the R8 phenological stage, measurements were taken for plant height and the height of the first pod insertion using a measuring tape, with a random sampling of 15 plants per plot. The first pod insertion height was measured from the plant base to the first pod located on the primary branch, while plant height was measured from the base to the plant vertical apex. The averages were then calculated, following the methodology proposed by Sartori et al. (2015).

Also at the R8 phenological stage, evaluations were conducted for soil-dependent variables. Soil samples were collected at a 0-10 cm depth for biological analysis, following the BioAS protocol described by Mendes et al. (2021). Enzymatic activity levels were determined using the method proposed by Tabatabai (1994).

The data obtained were tested for normality and homogeneity of variances using the maximum F-test. As the data met the required assumptions, they were analyzed by analysis of variance (ANOVA). Treatment means were compared using the Tukey test at a 5% probability level ($p \leq 0.05$), with the aid of R software (R Statistic).

3. Results and Discussion

In the analyses performed on soybean, no significant interaction was observed between the factors soybean cultivars NS 6446 I2X (Nidera®) and BMX Nexus I2X (Brasmax®) and inoculation with different doses of phosphate-solubilizing microorganisms for the following variables: plant stand (STA), number of fertile nodes per plant (NFN), number of pods per plant (NP), number of grains per plant (NG), number of grains per pod (NGP), total grain mass per plant in grams (GM), as well as the enzymes measured in soil enzymatic activity (β-glucosidase, arylsulfatase, and acid phosphatase). These results indicate that the factors behaved independently in the trial (Tables 1 and 4).

The target plant population in the experiment was 13 plants m^{-1} (28 plants m²), adjusted according to genotype germination rates and supplemented with an additional 10% of seeds (as a safety margin) to ensure a uniform stand. The results for this variable showed no significant differences for the independent factors cultivar and inoculation. The observed plant population in the trial was 22.1 plants m² for the NS 6446 I2X cultivar, and 22.8 plants m² across both cultivars within the inoculation treatment with 100 mL Brad + 300 mL Bio (Table 1).

The results revealed unsatisfactory plant stands (STA), likely due to unfavorable edaphoclimatic conditions during the initial seedling establishment phase (Table 1). The achieved stands 221,000 and 210,000 plants ha^{-1} for the NS 6446 I2X and BMX Nexus I2X cultivars, respectively, representing approximately 15% and 20% reductions compared to the recommended populations.

These values were lower than those recommended by the respective seed companies, which define the ideal plant population for NS 6446 I2X as 260,000 to 300,000 plants ha^{-1} (Nidera®), and 220,000 to 240,000 plants ha^{-1} for the BMX Nexus I2X cultivar (Brasmax®).

Variation in plant population can alter spatial arrangement, changing the area and the shape of the space available for each plant, which affects the intensity of intraspecific competition (Ferreira and da Silva, 2023). Taiz et al. (2017) state that greater light interception promotes increased cell division in vegetative and reproductive organs, contributing to more pods and improved seed filling. Therefore, it can be inferred that different genotypes respond differently to variations in plant population arrangement.

Regarding the number of fertile nodes per plant (NFN), the highest average was observed in the BMX Nexus cultivar, with 42.3 nodes per plant, significantly different from NS 6446, which recorded 29.3 nodes per plant. All treatments showed statistical similarity for the inoculation levels and were superior to the control, which had 27.4 fertile nodes per plant (Table 1), differing only from the treatment with Brad alone, resulting in an intermediate statistical mean. Tuppan et al. (2024) also reported that in the BMX Ultra and Desafio cultivars, increasing plant population leads to a more pronounced reduction in the number of nodes per plant.

No significant differences were observed within the cultivar factor for the variables NP, NG, and NGP. However, differences were found within the inoculation factor (Table 1).

Table 1. Plant stand (STA), number of fertile nodes (NFN), number of pods (NP), number of grains (NG), number of grains per pod (NGP), and grain mass (GM) in soybean cultivars NS 6446 I2X (Nidera®) and BMX Nexus I2X (Brasmax®), inoculated with different doses of phosphate-solubilizing microorganisms, grown in sandy soil in Nova Andradina/MS during the 2023/2024 growing season.

[1]FI	STA plant m^2	NFN	NP	NG	NGP	GM g $plant^{-1}$
		---------------- $plant^{-1}$	------------			
NS6446 I2X	22.1	29.3b	42.4	101.3	2.35	14.0
BMX Nexus I2X	21.0	42.3a	68.7	158.0	2.31	16.2
C.V.(%)	14.2	17.0	18.6	20.8	7.3	24.8
[2]FII	STA plant m^2	NFN	NP	NG	NGP	GM g $plant^{-1}$
		--------- $plant^{-1}$	------------------			
Control	20.7	27.4b	37.0c	80.4c	2.19b	6.9b
100mL Brad[3]	21.6	32.7ab	47.0bc	106.8bc	2.26ab	10.7b
100mL Brad + 100mL Bio[4]	21.5	38.3a	60.9a	144.0a	2.39a	17.9a
100mL Brad + 100mL Nod[5]	21.8	39.7a	64.8a	151.0a	2.32ab	18.4a
100mL Brad + 300mL Bio	22.8	40.3a	64.9a	156.7a	2.41a	16.9a
100mL Brad + 300mL Nod	20.7	36.6a	58.6ab	139.0ab	2.39a	17.0a
C.V.(%)	14.2	17.0	18.6	20.8	7.3	24.8

*Means followed by the same letter in the column do not differ statistically according to the Tukey test at 5% probability. ¹FI: Factor 1 - soybean cultivars; ²FII: Factor 2 - seed-applied inoculants and doses; [3]Brad - Bioma Brady®; [4]Bio - Biomaphos®; [5]Nod - Nod Phos®.

For NP and NG, the highest averages (64.9 pods and 156.7 grains per plant) were observed in the treatment inoculated with 100 mL Brad + 100 mL Nod, differing only from the treatments with 100 mL Brad alone and the control, which had the lowest values (47.0 pods and 106.8 grains per plant). The treatment with 100 mL Brad + 300 mL Nod did not differ from either the top statistical group or the Brad-only inoculation (Table 1), and showed intermediate averages (58.6 pods and 139.0 grains per plant).

It is worth noting that the Nod Phos® inoculant, in addition to containing the phosphate-solubilizing bacterium *Pseudomonas fluorescens*, also includes the plant growth-promoting bacterium *Azospirillum brasilense*, which is known for stimulating plant tissue development and is typically recommended at a maximum dose of 200 mL ha^{-1} (Hungria et al., 2017).

Hungria et al. (2017) point out several advantages of co-inoculation with *Azospirillum brasilense*, such as increased grain yield. Benefits have been observed with the application of *Bradyrhizobium* on seeds and *Azospirillum* in the planting furrow or directly on seeds. However, it is important to note that the maximum application rate in the furrow should be 2.5 times higher for *Bradyrhizobium* and 2 times higher for *Azospirillum* than the seed-applied dose. It is reported that, for *Azospirillum*, this should be considered the upper limit; exceeding it may inhibit soybean growth.

Thus, it can be inferred that the treatment with 100 mL Brad + 300 mL Nod may have shown lower performance in the evaluated variables due to the high concentration of *Azospirillum* in the applied dose. Rezende et al. (2021) also emphasize that *Azospirillum* dosage should not be increased indiscriminately, as excessive amounts may lead to overproduction of phytohormones (auxins, gibberellins, and cytokinins), ultimately impairing plant growth.

In correlating plant population with the crop yield components, Lima et al. (2017) define phenotypic plasticity as the ability of a living organism to exhibit different characteristics depending on environmental conditions. Many plant species display this trait, including soybean, which can compensate for reduced plant density by increasing per-plant yield, without showing significant differences in the number of pods per area. In this sense, the authors support the high values observed in the present trial, which resulted in elevated yield levels.

Within the inoculation factor, the variable NGP also showed the lowest value in the control treatment, with 2.19 seeds per pod, statistically similar to the treatments with 100 mL Brad and 100 mL Brad + 100 mL Nod. These did not differ from the other treatments, forming an intermediate group ($p \leq 0.05$). The highest NGP value was 2.41 grains per pod, observed in the treatment with 100 mL Brad + 300 mL Bio.

Grain mass per plant (GM) showed a positive correlation with the variables NP, NG, and NGP, since a phenotype with higher values of reproductive structures will consequently produce higher values for this variable as well (Table 1).

The highest grain masses were observed in the treatments inoculated with phosphate-solubilizing bacteria at the following doses: 100 mL Brad + 100 mL Nod (18.4 g $plant^{-1}$), 100 mL Brad + 300 mL Nod (17.0 g $plant^{-1}$), and 100 mL Brad + 300 mL Bio (16.9 g $plant^{-1}$), differing only from the treatments with Brad alone (10.7 g $plant^{-1}$) and the control (6.9 g $plant^{-1}$).

Regarding the cultivar factor, no statistical difference was found between the genotypes used in the trial, confirming that this factor did not influence this variable (Table 1).

Supporting the results obtained in this study, Bononi et al. (2020) emphasized the positive effects of single or combined inoculation with *Bradyrhizobium* and *Pseudomonas* on soybean yield. The bacterial strains could produce the phytohormones indole-3-acetic acid (IAA) and gibberellic acid. In the trial, inoculation and co-inoculation with *Bradyrhizobium* and *Pseudomonas* strains resulted in grain yield increases of 38% in pot experiments and 12% in field trials compared to the non-inoculated control.

In a trial using the phosphate-solubilizing inoculant BiomaPhos®, Santos et al. (2019) reported a linear response to increasing inoculant dosages, with higher doses promoting an increase in the number of stems per plant. At a dosage of 6 mL BiomaPhos® per kg of seed, an average of 2.93 stems per plant was observed, a value lower than those recorded in all treatments of the present study.

However, the number of fertile branches (NFB) increased with higher doses of both BiomaPhos® and Nod Phos®, based on the interaction of the evaluated factors (Tables 2 and 3), except for the cultivar factor. The BMX Nexus I2X cultivar showed no response to inoculation with the different species of phosphate-solubilizing bacteria (PSB), nor to the different doses of these microorganisms, which are also classified as plant growth-promoting bacteria (PGPB), with no interaction observed between the evaluated factors.

The highest values for NFB were observed in the interaction between the NS 6446 I2X cultivar and the 100 mL Brad + 300 mL Nod treatment, with 4.7 fertile branches per plant, differing only from the control treatment, which showed 3.3 fertile branches per plant (Table 3). Table 2 presents the NFB means for the interaction between cultivar factor (F1) and inoculation levels (F2), the BMX Nexus I2X genotype outperformed NS 6446 I2X at all inoculation levels.

Tables 2 and 3 also present the results for the variables PH (plant height), FPH (first pod height), 100GW (100-grain weight), and GY (grain yield). The analyses performed on soybean showed a significant interaction between the factors soybean cultivars NS 6446 I2X (Nidera®) and BMX Nexus I2X (Brasmax®) combined with the inoculation of different doses of phosphate-solubilizing microorganisms.

For the variable plant height (PH), when evaluating the cultivar factor within the inoculation levels (Table 2), the highest values were recorded for the BMX Nexus I2X cultivar inoculated with 100 mL Brad + 300 mL Bio, with an average height of 99 cm, which was statistically different from NS 6446 I2X, which reached 82.6 cm.

Except for the treatment with 100 mL Brad + 100 mL Bio, where both genotypes showed statistically similar values, BMX Nexus I2X was superior in PH compared to NS 6446 I2X at all other inoculation levels.

Table 2. Plant height (PH), first pod insertion height (FPH), number of fertile branches (NFB), 100-grain weight (100GW), and grain yield (GY) of the soybean cultivars NS 6446 I2X (Nidera®) and BMX Nexus I2X (Brasmax®) inoculated with different doses of phosphate-solubilizing microorganisms, grown in sandy soil in Nova Andradina/MS during the 2023/2024 growing season.

[1]FI	[2]FII	PH	FPH	NFB	100GW	GY
		---cm plant^{-1}---		plant^{-1}	g	kg ha^{-1}
NS6446 I2X	Control	53.6	18.6	3.3b	10.2a	884.4b
BMX Nexus I2X		57.5	17.0	5.6a	8.4b	2,033.8a
NS6446 I2X	100mL Brad[3]	58.3b	16.5b	3.5b	12.6a	1,785.5b
BMX Nexus I2X		71.1a	20.2a	6.1a	9.6b	3,197.4a
NS6446 I2X	100mL Brad + 100mL Bio[4]	88.4	32.3a	4.4b	15.4a	3,917.8
BMX Nexus I2X		88.5	14.0b	5.9a	11.0b	3,958.1
NS6446 I2X	100mL Brad + 100mL Nod[5]	83.0b	29.4a	4.5b	14.6a	4,834.9a
BMX Nexus I2X		93.7a	15.5b	5.9a	11.1b	3,574.2b
NS6446 I2X	100mL Brad + 300mL Bio	82.6b	28.8a	4.6b	15.7a	4,664.6
BMX Nexus I2X		99.0a	19.9b	5.8a	11.2b	4,640.1
NS6446 I2X	100mL Brad + 300mL Nod	90.2b	15.8b	4.7b	14.3a	3,553.0
BMX Nexus I2X		97.3a	20.9a	5.6a	11.1b	3,707.0
C.V.(%)		5.5	10.4	13.1	4.9	25.6

*Means followed by the same letter in the column do not differ statistically according to the Tukey test at the 5% probability level. [1]F1: Factor 1 - soybean cultivars; [2]F2: Factor 2 - seed-applied inoculants and doses; [3]Brad - Bioma Brady®; [4]Bio - Biomaphos®; [5]Nod - Nod Phos®.

Evaluating FII within FI, plant height (PH) showed the highest means at all inoculation levels, differing only from the treatments with sole inoculation with Brad and from the control, which presented PH values of 58.3 cm and 53.6 cm, respectively, within NS6446 I2X. This suggests that this genotype is highly responsive to inoculation with phosphate-solubilizing microorganisms (PSM), as evidenced in nearly all variables measured in the trial (Table 3).

For first pod insertion height (FPH), NS6446 I2X showed the highest values at the inoculation levels of 100 mL Brad + 100 mL Bio, 100 mL Brad + 100 mL

Nod, and 100 mL Brad + 300 mL Bio, differing from BMX Nexus I2X only at the control level. All values recorded for NS6446 I2X were greater than 15 cm plant^{-1}, which is an undesirable trait for high-yielding cultivars, as it was inferred that, from 12 cm in height (the ideal cutting height for harvest) relative to the plant crown, there was a loss of trifoliolate leaves and reproductive structures, which negatively contribute to grain yield.

This fact can be explained by the extreme climatic conditions between stages R3 and R5, which caused pod and trifoliolate leaf abortion in the lower third of both genotypes. Regarding the inoculation factor with phosphate-solubilizing bacteria (PSB), almost all levels of this factor negatively affected FPH (Tables 2 and 3), except for 100 mL Brad + 100 mL Bio in interaction with BMX Nexus I2X and 100 mL Brad + 300 mL Nod in interaction with NS6446 I2X, which showed the lowest means for this variable (14.0 and 16.5 cm plant^{-1}, respectively), meeting the optimal values for mechanical harvesting. FPH is important because it determines the combined harvester cutting bar height adjustment to achieve maximum efficiency during harvesting. According to Taiz et al. (2017), to avoid harvest losses caused by the cutting bar, the minimum first pod height should be 10-12 cm in flat terrains and 15 cm in more sloped areas.

For the results obtained for 100GW (Table 3), the highest values recorded for NS 6446 I2X were 15.7 g and 15.4 g per 100 grains at the inoculation levels of 100 mL Brad + 300 mL Bio and 100 mL Brad + 100 mL Bio, respectively, forming the top statistical group. These did not differ statistically from seed inoculation with 100 mL Brad + 100 mL Nod. Still within this genotype, the lowest mean was observed in the control, which differed from all other levels of the inoculation factor.

A similar pattern was observed for the BMX Nexus I2X genotype (Table 3), which showed the highest means at all inoculation levels with PSM, differing from the treatment with Brad alone, which also differed from the control. The control recorded 8.4 g per 100 grains, the lowest value observed for both genotypes used in the experiment.

Table 3. Plant height (PH), first pod insertion height (FPH), number of fertile branches (NFB), 100-grain weight (100GW), and grain yield (GY) of the soybean cultivars NS 6446 I2X (Nidera®) and BMX Nexus I2X (Brasmax®) inoculated with different doses of phosphate-solubilizing microorganisms, grown in sandy soil in Nova Andradina/MS during the 2023/2024 growing season.

[2]FII	[1]FI	PH	FPH	NFB	100GW	GY
		-----cm -----		plant^{-1}	g	Kg ha^{-1}
Control	NS6446 I2X	53.6b	18.6b	3.3b	10.2d	884.4b
100mL Brad[3]		58.3b	16.5b	3.5ab	12.6c	1,785.5b
100mL Brad + 100mL de Bio[4]		88.4a	32.3a	4.4ab	15.4a	3,917.8a
100mL Brad + 100mL de Nod[5]		83.0a	29.4a	4.5ab	14.6ab	4,834.9a
100mL Brad + 300mL Bio		82.6a	28.8a	4.6a	15.7a	4,664.6a
100mL Brad + 300mL Nod		90.2a	15.8b	4.7a	14.3b	3,553.0a
Control	BMX Nexus I2X	57.5d	17.0ab	5.6	8.4c	2,033.8b
100mL Brad		71.1c	20.2a	6.1	9.6b	3,197.4ab
100mL Brad + 100mL de Bio		88.5b	14.0b	5.9	11.0a	3,958.1a
100mL Brad + 100mL de Nod		93.7ab	15.5b	5.9	11.1a	3,574.2ab
100mL Brad + 300mL Bio		99.0a	19.9a	5.8	11.2a	4,640.1a
100mL Brad + 300mL Nod		97.3a	20.9a	5.6	11.1a	3,707.0a
C.V.(%)		5.5	10.4	13.1	4.9	25.6

*Means followed by the same letter in the column do not differ statistically according to the Tukey test at the 5% probability level. ¹F1: Factor 1 - soybean cultivars; ²F2: Factor 2 - seed-applied inoculants and doses; [3]Brad - Bioma Brady®; [4]Bio - Biomaphos®; [5]Nod - Nod Phos®.

It is important to note that when evaluating the cultivar factor (F1) within the different levels of FII, the NS 6446 I2X cultivar showed outstanding results for 100-seed weight (Table 2), outperforming the other genotype. The 100SW is a trait directly linked to the genetic potential of each genotype, although proper adjustment of growing conditions can also influence this variable (Doná et al., 2024). However, under the same management conditions applied to both genotypes, the genetic characteristics of this cultivar stood out, resulting in the highest values.

In an experiment conducted by Doná et al. (2024) evaluating the adaptability and agronomic performance of soybean cultivars, the authors reported average values of 16.6 g and 12.7 g per 100 grains for NS 6446 I2X and BMX Nexus I2X, respectively, values higher than those found for the same genotypes subjected to different inoculation levels with PSM in the present study. Both the values reported by the authors and those obtained in this experiment were lower than the parameters established in the portfolios of the companies holding these cultivars, which are 15.4 g and 18.2 g per 100 grains for BMX Nexus I2X and NS 6446 I2X, respectively.

The grain yield (GY) results in kg ha^{-1}, presented in Tables 2 and 3, indicated that the two independent factors showed a significant interaction for this variable. In Table 2, the highest grain yields were achieved by the BMX Nexus I2X cultivar within the control inoculation level (2,033.8 kg ha^{-1}), whereas within this factor, the NS 6446 I2X genotype had the lowest value for this variable, with a grain yield of 884.4 kg ha^{-1}, the lowest mean among the PSB inoculation treatments. This yield differed from BMX Nexus I2X and almost all other PSB inoculation forms. When evaluating each genotype individually, the control factor for NS 6446 I2X was similar only to the 100 mL Brad inoculation (1,785.5 kg ha^{-1}), representing the two inoculation levels with the lowest means for this cultivar.

The inoculation levels containing PSM showed the highest means in interaction with NS 6446 I2X: 4,834.9 kg ha^{-1} - 100 mL Brad + 100 mL Nod; 4,664.6 kg ha^{-1} - 100 mL Brad + 300 mL Bio; 3,917.8 kg ha^{-1} - 100 mL Brad + 100 mL Bio; and 3,553.0 kg ha^{-1} - 100 mL Brad + 300 mL Nod, all differing from the control and the Brad-only inoculation. This suggests that this genotype showed affinity for, and responded positively to, inoculation with bacteria of the genera *Bacillus* sp. and *Pseudomonas* sp. (Table 3).

For BMX Nexus I2X, the highest grain yield (GY) value was obtained with 100 mL Brad + 300 mL Bio (4,640.1 kg ha^{-1}), differing only from the control, which in turn showed no statistical difference from the Brad-only inoculation. Even without significant differences in GY, the BMX Nexus I2X genotype showed greater affinity for the BiomaPhos® inoculant, indicating that each genotype has a specific interaction profile with different PSM genera.

Based on the commercial doses of the bioproducts BiomaPhos® (100 mL Brad + 100 mL Bio) and Nod Phos® (100 mL Brad + 100 mL Nod), the grain yield gains relative to the control were 3,033.4 kg ha^{-1} with BiomaPhos® and 3,950.5 kg ha^{-1} with Nod Phos® for the NS 6446 I2X cultivar. For BMX Nexus I2X, the yield gains relative to the control were 1,924.3 kg ha^{-1} with BiomaPhos® and 1,540.4 kg ha^{-1} with Nod Phos®.

This strong response to PSM inoculation can be explained by the marginal conditions of the experimental area, which has soil with organic matter below 1%, a highly sandy texture, and consequently low chemical reactivity and reduced water-holding capacity. It can be inferred that, even under unfavorable climatic conditions during the 2023/2024 season, soil fertility combined with the PSB inoculated in the treatments (Rezende et al., 2021) promoted good root system development, which mitigated the damage caused by water stress and still resulted in yields above the national average for this period (CONAB, 2023) exceeding 4800 kg ha^{-1} for NS 6446 I2X and 4500kg ha^{-1} for BMX Nexus I2X.

Table 4 presents the mean values for the activity of the enzymes β-glucosidase, arylsulfatase, and acid phosphatase, obtained from assessing soil enzymatic activity in the research area, including the parameters for determining soil enzymatic activity.

Soil functioning is determined by the interaction between its physical, chemical, and biological properties; however, the biological component drives this complex system. Mendes et al. (2021) emphasize that, as the living and most active fraction of soil organic matter, certain biological indicators (bioindicators) can be used to assess soil biofunctioning and, in turn, support decision-making regarding soil management on agricultural properties. Among the parameters used to characterize the biological component of soils and evaluate their health and quality, microbial biomass and enzymatic activity assessments are particularly important.

In addition to participating in intracellular metabolic reactions in living organisms, extracellular enzymes in the soil also play a fundamental role, taking part in various reactions that lead to the decomposition of organic residues, nutrient cycling (phosphatases, amidases, urease, sulfatase), and the formation of soil organic matter (SOM). β-glucosidase and arylsulfatase are enzymatic soil health indicators (Mendes et al., 2021).

No significant difference was found for the cultivar factor (Table 4), with both genotypes showing values above 50.0 μg p-nitrophenol h^{-1} g^{-1} soil for β-glucosidase, above 10.0 μg p-nitrophenol h^{-1} g^{-1} soil for arylsulfatase, and greater than 60 μg p-nitrophenol h^{-1} g^{-1} soil for acid phosphatase. Mendes et al. (2021) reported enzymatic activity data obtained in a *Neossolo Quartzarênico* under no-tillage soybean cultivation, in different plots with similar SOM contents (averaging 6 g kg^{-1}), with reference values averaging around 40 μg p-nitrophenol h^{-1} g^{-1} soil for β-glucosidase and above 20 μg p-nitrophenol h^{-1} g^{-1} soil for arylsulfatase. These comparisons show that the β-glucosidase values obtained in the present trial were higher than the reference parameters, while the arylsulfatase averages were lower.

In Factor II, all treatments with PSB were superior ($p \leq 0.05$) to both the control level and the Brad-only inoculation for all three variables evaluated in the soil enzymatic activity analysis. The highest β-glucosidase value (66.7) was obtained with the 100 mL Brad + 100 mL Nod inoculation, which differed from the 100 mL Brad + 300 mL Bio dose. The latter also differed from the Brad-only inoculation and the control, which formed the lower statistical group in the analyses.

For arylsulfatase (Table 4), the highest and lowest values were obtained at the 100 mL Brad + 100 mL Nod (13.7) and control (9.1) levels, respectively, with these means differing statistically. The 100 mL Brad + 100 mL Nod inoculation also showed the highest mean for acid phosphatase (77.9), surpassing the same dose of BiomaPhos®.

Table 4. Soil enzymatic activity in the surface layer (0-10 cm layer) for soybean cultivars NS 6446 I2X (Nidera®) and BMX Nexus I2X (Brasmax®), inoculated with different doses of phosphate-solubilizing microorganisms, grown in sandy soil in Nova Andradina/MS during the 2023/2024 growing season.

[1]FI	β-Glucosidase	Arylsulfatase	Acid phosphatase
	-------------------(μg p-nitrophenol h^{-1} g^{-1} soil) ---------------------------		
NS6446 I2X	52.8	11.8	65.5
BMX Nexus I2X	51.1	11.8	64.6
C.V.(%)	12.3	10.7	13.5
[2]FII	β-Glucosidase	Arylsulfatase	Acid phosphatase
	---------------------(μg p-nitrophenol h^{-1} g^{-1} soil) ---------------------------		
Control	28.8d	9.1b	43.4c
100mL Brad[3]	39.3c	9.1b	50.9c
100mL Brad + 100mL Bio[4]	61.1ab	12.8a	65.8b
100mL Brad + 100mL Nod[5]	66.7a	13.7a	77.9a
100mL Brad + 300mL Bio	56.8b	12.5a	75.9ab
100mL Brad + 300mL Nod	59.2ab	13.5a	76.5ab
C.V.(%)	12.3	10.7	13.5

*Means followed by the same lowercase letter in the column compare treatments within the analyzed variable and do not differ statistically according to the Tukey test at the 5% probability level.
¹FI: Factor 1 - soybean cultivars; ²F2: Factor II - seed-applied inoculants and doses; [3]Brad - Bioma Brady®; [4]Bio - Biomaphos®; [5]Nod - Nod Phos®.

This suggests that the bacteria present in the Nod Phos® bioinput (*Pseudomonas fluorescens*) were more efficient at phosphate solubilization under the conditions of the present study. This result may be explained by the fact that Nod Phos® has a greater ability to solubilize phosphorus bound to iron (Fe^{2+}), in addition to the affinity of these microorganisms with the pedological formation of the soil under analysis.

In a study conducted by Santos et al. (2019), the authors inferred that, in general, acid phosphatase activity was higher in the 20-40 cm layer than in the surface layer (mean reference value for sandy-textured soils: 631.62 μg p-nitrophenol h^{-1} g^{-1} soil), which was not expected. At both depths, acid phosphatase activity in Cerrado soils was higher than the averages for pasture soils, indicating that liming in pasture areas may have reduced the biological activity of microorganisms involved in P cycling by increasing soil pH (Cerrado soil pH: 4.5; pasture soil pH: 5.9).

From this observation, it can be inferred that the low acid phosphatase values found in the trial (77.9 μg p-nitrophenol h^{-1} g^{-1} soil) with seeds inoculated at the 100 mL Brad + 100 mL Nod dose may have resulted from the adjustment of soil pH to 6.5 through surface liming.

Mendes et al. (2021) further contribute to understanding the results obtained in this trial, stating that a soil ability to stabilize and protect enzymes from the abiotic component is related to its texture and capacity to store and stabilize SOM. The lower the clay and SOM contents, the more the soil biological activity is reduced, such as in the case of the soil attribute conditions present in the experimental area.

Sun et al. (2023) support the results obtained in this trial by emphasizing that using phosphate-solubilizing microorganisms (PSM) promotes the conversion of insoluble phosphorus and the mineralization of organic phosphate compounds through enzyme hydrolysis. Mendonça et al. (2020) further state that the genera *Bacillus* and *Pseudomonas* bacteria can release organic acids, produce acid phosphatases, and employ various mechanisms that enable phosphate solubilization and mineralization.

Therefore, it is evident that the rhizobacteria employed in this trial increased the enzymatic activity of the measured variables, which are used to determine the soil biological quality index. Thus, it can be concluded that, besides increasing soil P availability and reducing dependence on synthetic fertilizers, using PSM in agricultural systems is a key factor for soil sustainability and ecosystem preservation (Sousa et al., 2021).

4. Conclusions

Seed inoculation and/or co-inoculation with beneficial microorganisms (PSM and BNF-promoting microorganisms) resulted in higher averages for the yield components of both soybean genotypes used in the trial, compared to the control without inoculation.

BMX Nexus I2X exhibited greater phenotypic stability than NS 6446 I2X; however, the latter proved more responsive to co-inoculation with PSB.

Phosphate-solubilizing bacteria can increase the availability of plant-accessible P forms in the soil for subsequent crop uptake. The application of PSM as soil bioinputs improved plant development and grain yield, attributed to their effective ability to convert insoluble P compounds into plant-available phosphorus.

The highest average grain yield across all inoculation levels was observed in the BMX Nexus I2X phenotype (3516 kg ha^{-1}), similar to the 3276 kg ha^{-1} obtained by the NS 6446 I2X genotype.

The use of BiomaPhos® and Nod Phos® inoculants at the doses applied in the experiment (commercial dose and three times the commercial dose) increased the averages of β-glucosidase, arylsulfatase, and acid phosphatase, resulting in enhanced soil enzymatic activity.

Authors' Contribution

All authors contributed to this manuscript. Alexandre Alonso de Oliveira and Augusto Manoel Rodrigues were responsible for conducting the experiment. Adriana Smanhotto Soncela was responsible for the physiological interpretation of the plants and for writing the results and discussion. Rafael Azevedo da Silva and Felix Placencia Garcia carried out the sanitary management and provided agrometeorological consulting. Raphael Pires de Campos served as a consultant for biological inputs. Gustavo Santos Vieira and Gustavo Moretti performed the field activities and data collection included in this work.

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