

## Vigor of seeds and use of biostimulant: effect on resulting seedling and soybean performance in the field

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**ABSTRACT.** In order to increase the productive potential of the soybean crop, it is necessary to use, in addition to fertilizers and soil improvers, seeds with high germination rates and vigor, as these are capable of generating plants with greater physiological performance. Two experiments were installed corresponding to the 2019/20 and 2020/21 harvests; carried out at laboratory, greenhouse and field levels. In each experiment and at all levels, the design used was complete randomized blocks, with four replications and arranged in a 2x4 factorial scheme, with two levels of physiological seed quality and four biostimulant managements in the treatment of seeds. Superiority of root volume, vigor and productivity was seen using seeds of higher physiological quality. For the main management effects, superiority was inferred for the 2019/20 harvest. Similar behavior was identified for the main effect of PQL of seeds. The clustering techniques showed that the dissimilarity was highly influenced by both the crop and the physiological quality level of the seeds (PQL), where the biostimulants were responsible for the differentiation of the sublevels. The positive effects of biostimulants in increasing the agronomic characteristics of soybeans are higher the higher the physiological quality of the seeds, regardless of the agricultural harvest and the genotype used, resulting in greater crop productivity.

**Key words:** *Glycine Max* (L.) Merr.; Seed quality; Biostimulants; Adjustment of cultural practices.

## INTRODUCTION

In recent decades, the production of agricultural commodities, mainly soybeans, maize and wheat, are among the economic activities that have shown the greatest increases in Brazil and Paraguay. The agribusiness chain has an important share in the Gross Domestic Product of these countries, representing more than 20% of its composition. Due to this, these countries have well-structured production chains, which are of fundamental importance for economic and social advancement and development. Soybean [*Glycine max* (L.) Merr.] is the most cultivated oilseed in Brazil and in the world and its importance is due to its chemical composition, which has a high protein content and provides a varied application in industry and human and animal nutrition. High crop productivity is directly related to the adequate establishment of plants at field level, which is the main foundation. In order to increase the productive potential of the crop, it is necessary to use, in addition to fertilizers and soil improvers, seeds with high germination rates and vigor, as these are capable of generating plants with greater physiological performance, capable of tolerating abiotic stresses and better utilizing environmental resources (Bagateli, et al., 2022).

Several biotic and abiotic factors exist, such as light, water, temperature, nutrients, diseases, insect pests and stresses that affect soybean productivity (Silva, et al., 2023), which can also directly influence the establishment of crops. According to Teixeira, et al. (2020), one of the strategies to maintain soybean productivity at adequate levels, even in stressful environments, consists of maximizing the plants' ability to exploit soil water, by increasing the depth of the root system and by stimulating the antioxidant metabolism. And in this sense, the use of new technologies, as in the case of the use of biostimulant products, becomes important as several studies have demonstrated their effectiveness, making them a focus of agronomic interest as they have the ability to stimulate the natural processes of plants, such as nutrient absorption and tolerance to abiotic stresses (Nardi, et al., 2016; Heap, et al., 2020; Barrera-Ortiz, et al., 2023). These products become more economical alternatives with less environmental impact, both as a source of nutrients and as a source of growth-promoting substances.

Biostimulants are products that normally use organic carbon, free amino acids, micronutrients and algae extracts in their formulation. Among these compounds, humic substances have the role of stimulating the rapid initial growth of the root system and the general development of the seedling, in addition to contributing to an increase in the photosynthetic rate and the plants' resistance to stress (Xu, et al., 2021). Furthermore, these substances can also interfere with the physiology and morphology of plants, acting on the processes of germination, vegetative growth, flowering, fruiting, senescence and abscission. This interference can occur through the application of these substances via seeds, soil or foliar, being absorbed by each corresponding organ so that they can carry out their activity.

It is also worth highlighting that the use of micronutrients in seed treatment is a way to quickly make minerals available for the germination process and initial seedling development (Alves, et al., 2023). Cobalt (Co) and molybdenum (Mo), even required in small quantities, are essential for plant development (Ul Hassan, et al. 2023), as they act in biological nitrogen fixation (BNF) in legumes. Co is a constituent of vitamin B12 (cobalamin) and thus participates in the synthesis of leghemoglobin,

responsible for preventing the oxidation of nodules (Lange, et al., 2017). Mo plays a role in N metabolism in plants, as it is a component of the enzymes nitrate reductase (catalyzes the reduction of  $\text{NO}_3^-$  to  $\text{NO}_2^-$ ), xanthine, aldehyde and sulfate oxidase and nitrogenase (catalyzes the reduction of atmospheric  $\text{N}_2$  to form  $\text{NH}_3$ ) (Taiz, et al., 2017).

In view of the above, understanding the effect of using biostimulant products in association with the vigor level of seeds from different soybean genotypes is of great importance in the current scenario, with the aim of understanding the dynamics of agronomic traits and their possible effects on the potential of crop productivity. Given the considerations, the purpose of the study was to evaluate the agronomic performance of seeds, resulting seedlings and crops at field level, when under the use of different managements with biostimulant products, used via seed treatment from lots with levels of physiological quality contrasting.

## MATERIAL AND METHODS

The work took place in the municipality of Los Cedrales, Alto Paraná – Paraguay ( $25^{\circ}39'07.2''\text{S}$ ;  $54^{\circ}43'00.6''\text{W}$  and altitude of 250 m). The predominant climate in this region is classified as mesothermal (Koppen, 1936), where rain occurs more frequently in spring and summer, with no defined dry season. The site's soil has a clayey texture, being classified as Oxisol according to the American classification (Soil Taxonomy) adopted in Paraguay (López, et al., 1995).

Two experiments were installed corresponding to the 2019/20 and 2020/21 harvests; carried out at laboratory, greenhouse and field levels. In each experiment and at all levels, the design used was complete randomized blocks, with four replications and arranged in a 2x4 factorial scheme, with two levels of physiological seed quality (high and low) and four biostimulant managements in the treatment of seeds (three products and a control control).

The genotypes evaluated were the cultivars 63i64 Ipro in the 2019/20 harvest and M5947 Ipro in the 2020/21 harvest (relative maturity group of 6.3 and 5.9; respectively). Both are registered and recommended for cultivation in the southern region of Brazil and Paraguay, they have an indeterminate growth type, with physiological maturation estimated between 120 and 130 days after emergence.

To determine the physiological quality level factor (PQL), the lots of each genotype had their seeds evaluated by the germination test according to the methodology indicated by the Rules for Seed Analysis (Regras para Análises de Sementes - RAS) (Brazil, 2009), with four replications of 50 seeds and with assessments being carried out on the seventh day after sowing (DAS) of the test, using the percentage of normal seedlings. Vigor was determined using the accelerated aging test according to the methodology described by Marcos Filho (2020), at a temperature of  $41^{\circ}\text{C}$  ( $\pm 0.5^{\circ}\text{C}$ ), for a period of 48 hours. At the end of the aging period, the germination test procedure as described previously. With these results in hand, we proceeded to determine the factor related to the level of physiological quality of the seeds, being: genotype 63i64 Ipro (2019/20 Harvest), **high PQL** 98 and 91% **and low PQL** 83 and 75% of germination and vigor, respectively; and for the M5947 Ipro genotype (2020/21 harvest), **high PQL** 96 and 90% and **low PQL** 84 and 78% of germination and vigor, respectively.

Each experimental plot was composed of two kilograms of seeds that received fungicide based on thiabendazole ( $150 \text{ g L}^{-1}$ ), metalaxyl-M ( $20 \text{ g L}^{-1}$ ) and fludioxonil ( $25 \text{ g L}^{-1}$ ) and insecticide based on fipronil. ( $250 \text{ g L}^{-1}$ ). For the product factor, the seeds (with the exception of the control

– **AU**, without application) received the biostimulant products **P1**: Wuxal Como Amino (15; 150 and 46 g l<sup>-1</sup> of Co, Mo and free amino acids; respectively) at a dosage of 150 ml 100 kg seeds<sup>-1</sup>; **P2**: Fertiactyl Legumes (0.5% Co and 2.7% Mo) at a dosage of 200 ml 100 kg seeds<sup>-1</sup> and **P3**: Germinate (0.4 Co; 6.0% Mo; 0.4 % Ni and 2.0% Zn and 10.0% humic substances) in a dosage of 120 ml 100 kg seeds<sup>-1</sup>.

A seed sample from each plot was allocated for the laboratory-level experiment in each agricultural season. The variables germination (**GER**) and vigor were determined using the accelerated aging test (**AA**) methodology, as previously described for determining the PQL; seedbed emergence (**SE**) with four replications of 50 seeds, sown in a substrate of 30% sand and 70% clay soil, distributed equidistantly (2cm apart) at a depth of 3cm, receiving 20mm irrigation daily, with results expressed by percentage of normal seedlings emerged at ten DAS. To determine root volume (**ROOTV**), five seeds were sown in plastic columns (10cm in diameter and 100cm in length) containing a mixture of 30% sand and 70% clay soil, kept in a greenhouse with light, humidity and air temperature controlled, receiving irrigation (soil at field capacity). At 10 DAS, three plants were left per column and at 30 DAS the plants were carefully removed and separated from the roots, which were washed in running water and immersed in a graduated cylinder with a subdivision of one milliliter (mL). The reading was made by difference in the displacement of the water column, results expressed in mL plant root<sup>-1</sup>. The dry matter weight (**DMW**) was determined on these roots and subjected to drying in an oven with air circulation (80°C until reaching a constant weight), weighed on an analytical balance; results expressed in g plant<sup>-1</sup>.

For the field-level experiment, sowing occurred mechanized, in a direct planting system on maize straw residue, in the second half of September of each agricultural harvest (recommended time for the genotypes). The plot had a total area of 16.2 m<sup>2</sup> and a useful area of 11.2 m<sup>2</sup> (5 lines x 5.0 m x 0.45 m). A sowing density of 14 and 16 seeds m was used for genotypes with high and low PQL, respectively.

The chemical analysis of the soil (carried out in August/2019) revealed a pH (CaCl<sub>2</sub>): 6.49; Al content: 0.0; Ca: 7.97; Mg: 1.89; K: 0.59; CTC: 13.91 cmolc dm<sup>3</sup>; P: 20.9; S: 4.06; Fe: 36.32; Mn: 83.62; Cu: 12.95; Zn: 10.27 and B:0.67 mg dm<sup>3</sup>; and still 3.94; 25.27 and 70.79% sand, silt and clay, respectively; with V(%) of 75.15% and M.O: 3.05%. Chemical fertilization took place fifteen days before sowing, with 10-60-30 kg ha<sup>-1</sup> of N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O; respectively.

Cultural treatments followed technical recommendations, keeping the plants free from the presence of weeds, and protected against attack by pests and diseases that could interfere with the good development of the crop.

At the phenological stage of full flowering, 10 plants were demarcated within the useful area of the plot, and the plant height was determined (**PHR2**, cm). At harvest maturity, the height (**PHR8**, cm) was determined on these same plants; productive nodes per plant (**NPN**, number); legumes per plant (**NLP**, number), seeds per plant (**NSP**, number) and productivity per plant (**PP**, g). After harvesting the AUP, the plants were mechanically threshed, measuring the weight and degree of humidity (13%, wet basis) of the seeds, in order to determine the thousand seed weight (**TSW**, g) and productivity (**PROD**, kg ha<sup>-1</sup>).

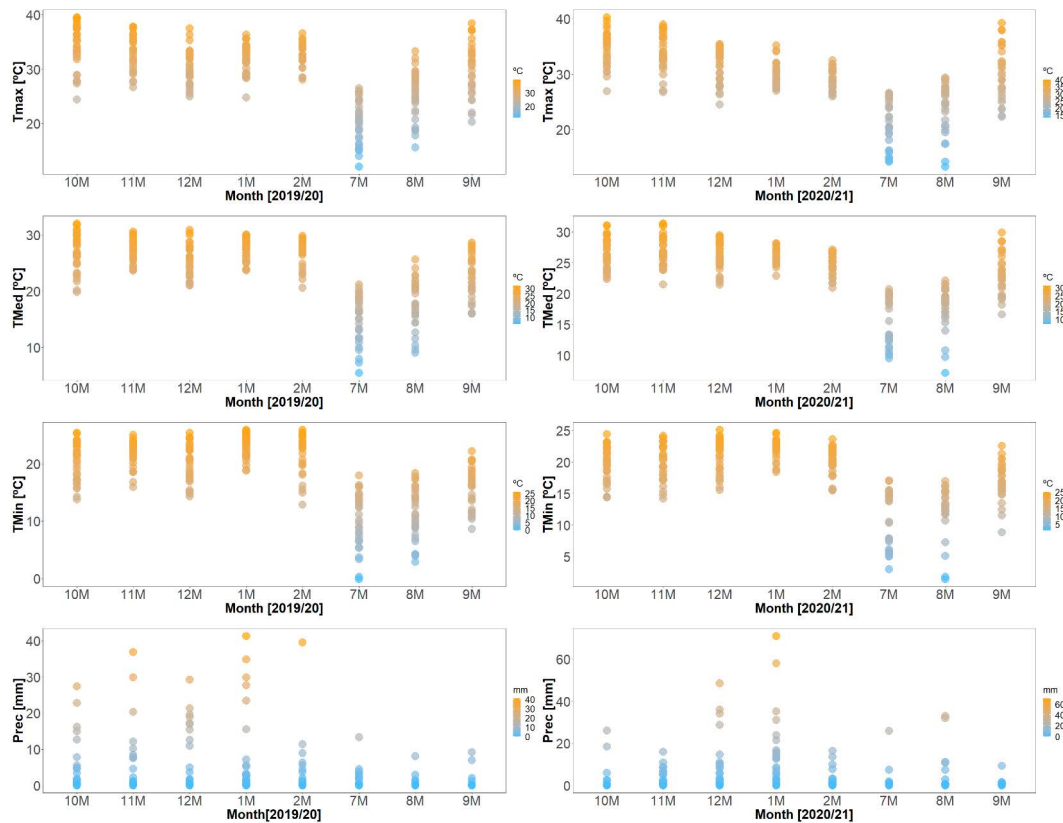
In order to better explain the results obtained, the climatological variables maximum air temperature (Tmax, °C) and mean air temperature (Tmean, °C) were estimated. minimum air temperature (Tmin) and precipitation (mm) using satellite data (NASA POWER, 2023).

The database was subjected to the Shapiro-Wilk, Bartlett and Durbin-Watson tests in order to meet the assumptions of normality, homogeneity of variances and homoscedasticity of errors. After checking the assumptions, analysis of variance took place to identify the effect of the treatments and their interaction on the analyzed variables, using the F test ( $p < 0.05$ ). For significant effects, Scott-Knott clustering with a 95% confidence interval was used in order to classify the treatment means. Statistical analyzes were performed using the R software (R CORE TEAM, 2023). In a complementary way, machine learning techniques were applied to cluster information, for which a **dissimilarity dendrogram** was used applying the Unweighted Pair Group Method using Arithmetic Averages (UPGMA), based on Euclidean distance, **principal component analysis**, to identify the joint influence of factors on the study variables and **regression tree** as a way of stratifying the results and identifying strategies with the potential to increase the productive potential of soybeans. The dissimilarity dendrogram, principal component analysis and regression tree were obtained using the Orange Data Mining software (Demsar, et al., 2013).

## RESULTS AND DISCUSSION

Satellite climatological data on air temperature and precipitation for the 2019/20 and 2020/21 harvests are presented in figure 1. The average values for maximum, mean and minimum air temperatures for the 2019/20 harvest were 29.73, 23.56 and 17.98°C, while in the 2020/21 harvest the averages were 29.31, 23.37 and 17.85°C, respectively. A greater temperature range was noted during sowing and crop establishment in the 2019/20 harvest, with mean air temperatures below 10°C on some occasions. Alsajri, et al. (2020), evaluating the relationship between temperature and productivity and quality of soybean seeds, obtained an optimal temperature to reach high production potentials of 25.5°C, which promoted greater retention of flowers and, subsequently, legumes. Accumulated precipitation was 830.29mm in 19/20 and 985.24mm in 20/21, higher than the water demand of 450 to 800mm described by Farias, et al. (2007), making it necessary to evaluate the distribution of precipitation throughout the cycle. Precipitation volumes were higher in the first three months of the soybean cycle in the 2020/21 harvest, which promoted ideal conditions for the establishment and vegetative development of the crop. On the other hand, there was a period of water restriction during flowering and the beginning of grain filling, which may have penalized productivity. Water deficiencies in these periods limit the supply of photoassimilates, which can accentuate flower abortion and vegetable abscission in the plant's attempt to maintain physiological balance (Rodrigues, et al., 2017). In the 2019/20 harvest, even with a reduced initial rainfall, the greater concentrations of rain from October onwards, together with satisfactory air temperature conditions, were the difference in obtaining higher yields. According to Li, et al. (2020), water use efficiency under optimal soil moisture conditions for grain productivity is 9.36kg ha<sup>-1</sup> mm<sup>-1</sup> (Figure 1).

The summary of the analysis of variance (Table 1) demonstrates the significance ( $p < 0.05$ ) of the effects of crops (C), seed physiological quality level (PQL) and biostimulant application protocols (B), in addition to their interactions, on the variables analyzed. A significant effect of the C x PQL interaction was identified for germination (GER), root volume (ROOTV), vigor due to accelerated aging (AA) and plant height at harvest maturity (PHR8). GER was also influenced by the C x B interaction, with the other interactions not being significant. All treatments significantly influenced plant height at the phenological stage of full flowering (PHR2). A significant effect of C and PQL treatments was observed for seedbed emergence (SE), number of productive nodes (NPN), number of legumes per plant (NLP) and significant influence of PQL and B on SWP and C and B on TSW and PP. For NSP, a significant effect of the harvest was inferred.



**Figure 1.** Maximum temperature (Tmax, °C), Mean temperature (Tmean, °C), minimum temperature (Tmin, °C) and precipitation (mm) for the 2019/20 and 2020/21 harvests.

As expected, higher germination rates (GER) were evaluated when using seeds with superior physiological quality (Table 2), where, when comparing the effect of biostimulants on this trait, emphasis was given to the use of P3 (Germinate), with an average of 90.6%. According to Alves, et al. (2023), superior quality seeds result in high-performance seedlings, with easier establishment in environments with adverse biotic and abiotic conditions and greater speed of emergence. For the simple effect of the interaction between C x PQL, superiority of ROOTV, AA and PROD was seen when using seeds of higher physiological quality. In this sense, averages of 13.62 and 12.56 mL root<sup>-1</sup> were obtained for ROOTV, 87.0 and 85.8% for AA and 4301 and 4220 kg ha<sup>-1</sup> for PROD, in 2019/20 and 2020/21, respectively. The results corroborate the study by Sousa et al. (2023), in which it was inferred that plant height and grain productivity are closely related to the root development potential of soybeans. Seed quality also significantly influenced PHR8 in the 2019/20 management, where values of 110.1 and 106.2 cm were obtained for plant height at harvest maturity when using seeds of higher and lower physiological quality, respectively.

For the main management effects (Table 3), superiority was inferred for the 2019/20 harvest, for all variables, with averages of 51 cm for PHR2, 89.34% for SE, 162.97g for TSW, 117.25 vegetables for NLP, 18.63 knots for NPN, 139.75 seeds for NSP and 13.39 g for PP. Similar behavior was identified for the main effect of PQL of seeds on PHR2, SE, NLP and NPN, where, when using

**Table 1.** Summary of the analysis of variance for two crops (C), two levels of seed physiological quality (PQL) and four biostimulant application protocols (B). GER: germination (%); ROOTV: root volume (mL root<sup>-1</sup>); AA: vigor due to accelerated aging (%); SE: seedbed emergence (%); PHR2: plant height in R2 (cm); PHR8: plant height in R8 (cm); NPN: number of productive nodes (units); NLP: number of legumes per plant (units); NSP: number of seeds per plant (units); SDW: seedling dry weight (g); TSW: thousand seed weight (g); PP: productivity per plant (g plant<sup>-1</sup>); PROD: productivity (kg ha<sup>-1</sup>); SV: source of variation; DF: degrees of freedom; MS: mean square; CV: coefficient of variation; \*: significant at 5% probability using the F test.

SV	DF	GER	ROOTV	AA	SE	PHR2	PHR8	NPN
		MS	MS	MS	MS	MS	MS	MS
C	1	19.1406*	0.1406	97.5156*	30.2500*	1287.0156*	2475.0625*	228.7656*
PQL	1	2104.5156*	221.2656*	3859.5156*	2304.0000*	141.0156*	90.2500*	8.2656*
B	3	10.2656*	33.7239*	20.8072*	13.5208	37.3906*	91.2292*	3.0156
Block	3	3.3906	3.3073	4.0572	6.2292	11.1406	2.0625	1.7656
C x PQL	1	47.2656*	21.3906*	26.2656*	7.5625	0.0156	36.0000*	0.3906
C x B	3	12.6823*	6.5156	2.0573	2.4583	18.7240	5.5625	0.6823
C x B	3	4.474	1.8073	0.3906	4.2083	3.8906	2.0833	1.0990
C x PQL x B	3	0.724	0.4323	0.8073	9.3542	5.3906	4.6667	0.1406
Residuals	45	3.2351	4.2073	3.7128	5.6736	8.4073	7.9514	1.0878
CV (%)	-	2.02	18.26	2.45	2.69	6.23	2.77	6.23
Mean	-	88.83	11.23	78.64	88.66	46.52	101.97	16.73
SV	DF	NLP	NSP	SDW	TSW	PP	PROD	
		MS	MS	MS	MS	MS	MS	
C	1	5662.5625*	826.5625*	1.0060	3011.2656*	41.1202*	162106.89	
PQL	1	870.2500*	484.0000	16.4714*	0.0156	0.3164	475237.89	
B	3	160.5208	155.1042	4.5507*	83.3073*	24.5160*	378467.81*	
Block	3	48.7292	92.0625	0.4843	10.0990	15.7418	299388.81	
C x PQL	1	9.0000	16.0000	0.1584	9.7656	0.4727	530530.14*	
C x B	3	21.2708	109.1875	0.4682	2.3073	0.3352	20802.89	
C x B	3	7.7917	113.2917	0.2797	5.0573	0.2439	79788.7200	
C x PQL x B	3	6.6250	107.8750	0.3804	2.8073	0.3602	98286.14	
Residuals	45	64.8847	178.8736	0.3328	7.6101	4.7626	74092.37	
CV (%)	-	7.47	9.82	18.82	1.77	17.34	6.23	
Mean	-	107.84	136.16	3.07	156.11	12.59	4174.05	

**Table 2.** Breakdown of the simple effects of the interaction between crops (C) and seed physiological quality level (PQL) and the interaction between crops (C) and biostimulant application protocols (B) on soybean traits. GER: germination (%); ROOTV: root volume (mL root<sup>-1</sup>); AA: vigor due to accelerated aging (%); PHR8: plant height in R8 (cm); PROD: productivity (kg ha<sup>-1</sup>). HIG: seeds with a higher level of physiological quality; LOW: seeds with a lower level of physiological quality; P1: Wuxal; P2: Fertiactyl Leg; P3: Germinate; AU: control without application. Means followed by the same lowercase letter in the column and capital letter in the row do not differ from each other by Scott-Knott grouping (p<0.05).

	GER			ROOTV			AA	
(SxPQL)	LOW	HIG	(SxPQL)	LOW	HIG	(SxPQL)	LOW	HIG
2019/20	84.5aB	94.2 aA	2019/20	8.75 aB	13.62 aA	2019/20	72.8 aB	87.0 aA
2020/21	81.7 bB	94.9 aA	2020/21	10.00 aB	12.56 aA	2020/21	69.0 bB	85.8 aA
	GER			PHR8			PROD	
(SxB)	2019/20	2020/21	(SxPQL)	LOW	HIG	(SxPQL)	LOW	HIG
AU	89.1 aA	87.0 bB	2019/20	106.2 aB	110.1 aA	2019/20	3946 bB	4301 aA
P1	89.0 aA	88.0 bA	2020/21	95.3 bA	96.2 bA	2020/21	4229 aA	4220 aA
P2	90.1 aA	87.5 bB						
P3	89.2 aA	90.6 aA						

**Table 3.** Main effects of crops (C), seed physiological quality level (PQL) and biostimulant application protocols (B) on the PHR2 variables: plant height in R2 (cm); SE: seedbed emergence (%); TSW: thousand seed weight (g); NLP: number of legumes per plant (units); NPN: number of productive nodes (units); NSP: number of seeds per plant (units); PP: productivity per plant (g plant<sup>-1</sup>); SDW: seedling dry weight (g); PROD: productivity (kg ha<sup>-1</sup>); ROOTV: root volume (mL root<sup>-1</sup>); AA: vigor due to accelerated aging (%); HIG: seeds with a higher level of physiological quality; LOW: seeds with a lower level of physiological quality; P1: Wuxal; P2: Fertiactyl Leg; P3: Germinate; AU: control with lack of application. Means followed by the same lowercase letter in the column do not differ from each other by Skott-Knott grouping, at 5% probability.

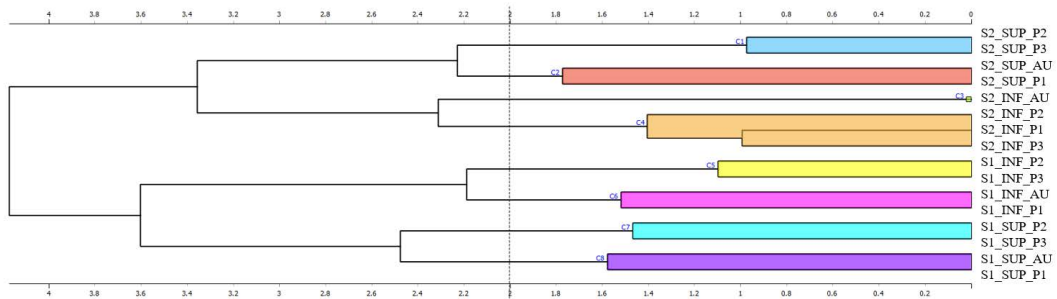
(C)	PHR2	SE	TSW	NLP	NPN	NSP	PP	
2019/20	51.00 a	89.34 a	162.97 a	117.25 a	18.63 a	139.75 a	13.39 a	
2020/21	42.03 b	87.97 b	149.26 b	98.44 b	14.84 b	132.56 b	11.78 b	
(PQL)	PHR2	SE	NLP	NPN	SDW			
HIG	48.00 a	94.66 a	111.53 a	17.09 a	3.57 a			
LOW	45.03 b	82.66 b	104.16 b	16.38 b	2.56 b			
(B)	PHR2	PHR8	TSW	PROD	ROOTV	AA	PP	SDW
P1	46.94 a	101.88 a	155.44 b	4111.75 b	11.06 a	79.25 a	12.42 a	3.00 b
P2	47.44 a	103.63 a	155.31 b	4259.94 a	12.44 a	77.56 b	13.89 a	3.46 a
P3	47.44 a	103.75 a	159.44 a	4334.63 a	12.19 a	79.94 a	13.08 a	3.46 a
AU	44.25 b	98.63 b	154.25 b	3989.88 b	9.25 b	77.81 b	10.96 b	2.33 c

higher quality seeds, the averages evaluated fall into the superiority group, with values of 48 cm for PHR2, 94.66% for SE, 111.53 vegetables for NLP and 17.09 knots for NPN. Considering the main effect of biostimulants on PROD, P2 (Fertiactyl Leg) and P3 (Germinate) promoted the largest increases (4259.94 kg ha<sup>-1</sup> for P2 and 4334.63 kg ha<sup>-1</sup> for P3), statistically differing from P1 (Wuxal) and AU (control without application). For the TSW variable, superiority is attributed to the biostimulant P3 (Germinate). These results corroborate those found by Galindo, et al., (2017), who, when evaluating methods of applying Co and Mo to soybeans, obtained a hundred grain weight and a grain yield of 15.88g and 6083 kg ha<sup>-1</sup> for nutrient application in seed treatment and 14.60g and 5685 kg ha<sup>-1</sup> via foliar, respectively.

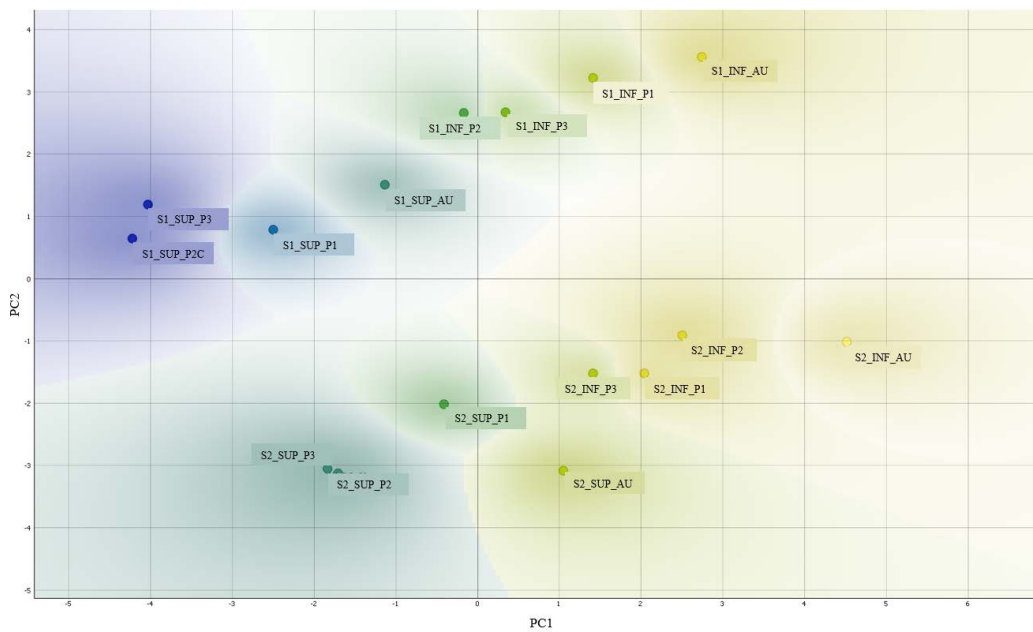
In the case of PHR2, PHR8, ROOTV and PP, all biostimulants differed from AU. When evaluating the averages for AA and SWP, it was observed that the percentage of vigor (measured by the accelerated aging test) was higher for P3 and P1 (79.94 and 79.25%), while the seedling dry weight was higher for P2 and P3, with an average of 3.46g (Table 3).

The clustering regarding the dissimilarity dendrogram for the treatments (Figure 2) was highly influenced by both the crop (C) and the physiological quality level of the seeds (PQL), where the biostimulants (B) were responsible, to a large extent, for the differentiation of the sublevels. The 2020/21 crop was classified in its entirety in clusters C1:C4, while the 2019/20 crop fell into clusters C5:C8, where a contrasting effect was observed for this factor, where the quality of the seeds caused dissimilarities second order. This was possibly due to the fact that high vigor seeds have greater water absorption and early protrusion of the radicle compared to low vigor seeds, in addition to a higher concentration of nutrients (Alves, et al., 2023), and thus a high ability to generate seedlings with high physiological performance (Bagateli, et al., 2019). For the effect of biostimulants on clustering, similarity was observed between protocols P2 and P3 and between P1 and AU, both for seeds of higher and lower quality, with the exception of cluster C3, where all protocols differed from AU.



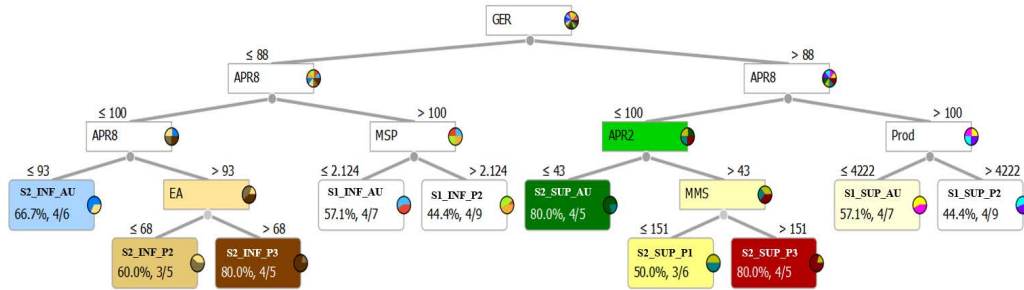


**Figure 2.** Dissimilarity dendrogram using clustering with the Unweighted Pair Group Method using Arithmetic Averages (UPGMA) for two crops (C), two levels of physiological seed quality (L) and four biostimulant application protocols (B). A1: 2019/20; A2: 2020/21; HIG: seeds with a higher level of physiological quality; LOW: seeds with a lower level of physiological quality; P1: Wuxal; P2: Fertiactyl Leg; P3: Germinate; AU: control without application.



**Figure 3.** Analysis of main components for two crops (C), two levels of physiological seed quality (PQL) and four biostimulant application protocols (B). C1: 2019/20; C2: 2020/21; HIG: seeds with a higher level of physiological quality; LOW: seeds with a lower level of physiological quality; P1: Wuxal; P2: Fertiactyl Leg; P3: Germinate; AU: control without application.

According to principal components analysis (PCA), similar to that reported for the dissimilarity dendrogram, it was again verified that the managements were contrasting with each other (Figure 3), where the vectors presented opposite directions, which implies the absence of a relationship between crops. C1 (2019/20) and C2 (2020/21). Regarding biostimulant application protocols, P1 (Wuxal) was located intermediately to protocols P2 (Fertiactyl Leg) and P3 (Germinate), between which proximity was identified, as well as to the AU protocol (control without application).



**Figure 4.** Regression tree for two crops (C), two physiological seed quality levels (PQL) and four biostimulant application protocols (B). M1: 2019/20; M2: 2020/21; HIG: seeds with a higher level of physiological quality; LOW: seeds with a lower level of physiological quality; P1: Wuxal; P2: Fertiactyl Leg; P3: Germinate; AU: control without application.

In the context of evaluating agronomic traits, regression trees are a way of identifying, based on predictions, the factors that are related to the variations presented by a given variable of interest. For the study, it is noted that the germination percentage was the most important predictor in stratifying the results (Figure 4), a situation in which the superior extracts were attributed to germination levels greater than 88%. Another important character was plant height at harvest maturity (PHR8), in which plants greater than 100 cm reflected greater potential productivity. When evaluating the extracts attributed to high productive potential, it was observed that it was assertive to use the C1\_HIG\_P2 strategy (44.44%) to achieve high productivity (PROD>4222 kg ha<sup>-1</sup>) and the C2\_HIG\_P3 strategy (80%) to achieve high productivity values of a thousand seed weight (TSW >151g). On the other hand, lower quality seeds can be attributed to extracts with lower productive potential, mainly related to vigor due to accelerated aging (AA). In this case, the strategies C2\_LOW\_P3 (80%) and C1\_LOW\_P2 (44.44%), with the use of P3 (Germinate) and P2 (Fertiactyl Leg), were able to compensate for the negative effect of seed inferiority, mainly with regard to seed weight per plant (SWP >2,124g). When studying physiological attributes of soybean seeds treated with micronutrients, Werner, et al., (2020) obtained average germination values of 95.50, 94.75 and 91.50% for the application of molybdenum, boron and zinc to the seeds. It can be inferred, therefore, that the greater variety of micronutrients present in P3 favored the germination and emergence process for both levels of seed quality.

Nickel (Ni) is effective in promoting growth and biomass accumulation, depending on the type of soil (Levy, et al., 2019). Martínez Cuesta, et al., (2023), states that zinc (Zn), when applied to seed treatment, can result in an increase in the number of seeds per plant, which corroborates what was described by Ortez, et al., (2019), where differences in soybean productivity are explained by the number of seeds per area rather than individual grain weight. Furthermore, the organic carbon load present in the Germinate product may have caused an increase in soybean productivity components. These organic acids are a source of carbon and other substances normally exuded by roots, altering the diversity, structure and microbial activity in the rhizosphere, which indirectly affects the availability of nutrients for absorption by plants (Lian, et al., 2019).

## CONCLUSION

The use of seeds of high physiological quality is a key element in achieving high production potential.

The positive effects of biostimulants in increasing the agronomic characteristics of soybeans are higher the higher the physiological quality of the seeds, regardless of the agricultural harvest and the genotype used, resulting in greater crop productivity.

There was a difference in productivity of 355 kg ha<sup>-1</sup> of grains between physiological quality levels of seeds in the harvest with unfavorable soil and climatic conditions.

Productivity gains of 8.26% can be attributed to the use of superior quality seeds and biostimulants.

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Not applicable.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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