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EFFECTS OF INDIVIDUAL PLANT GROWTH REGULATORS AND A COMMERCIAL REGULATOR MIXTURE ON SOYBEAN GERMINATION, ROOT MORPHOLOGY, AND YIELD COMPONENTS

EFEITOS DE REGULADORES DE CRESCIMENTO VEGETAL INDIVIDUAIS E DE UMA MISTURA COMERCIAL DE REGULADORES SOBRE A GERMINAÇÃO, MORFOLOGIA RADICULAR E COMPONENTES DE RENDIMENTO DA SOJA

EFECTOS DE REGULADORES DE CRECIMIENTO VEGETAL INDIVIDUALES Y DE UNA MEZCLA COMERCIAL DE REGULADORES SOBRE LA GERMINACIÓN, MORFOLOGÍA RADICULAR Y COMPONENTES DEL RENDIMIENTO DE LA SOJA

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Abstract

The use of plant growth regulators has been widely investigated as an alternative to improve soybean agronomic performance. This study aimed to evaluate the effects of the individual application of indole-3-

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butyric acid, kinetin, and gibberellic acid, as well as a commercial product containing these compounds, on soybean germination, root morphology, and yield components. The field experiment was conducted in a randomized complete block design with four replications. In parallel, a laboratory experiment was performed on Germitest® paper to evaluate seed germination and root morphological traits. No statistically significant differences were detected among treatments for yield components, grain yield, or final germination. Final germination ranged numerically from 91.00% to 94.50%, but these differences were not significant. Root morphological responses were more evident: gibberellic acid treatments were associated with reduced root length, whereas indole-3-butyric acid showed a descriptive tendency toward a higher relative proportion of finer roots. Overall, the results indicate that the plant growth regulators evaluated did not significantly affect final germination or soybean grain yield under the conditions tested, although they were associated with changes in early root morphology.

Keywords: Auxin, Cytokinin, Gibberellin, Root architecture.

Resumo

O uso de reguladores de crescimento vegetal tem sido amplamente investigado como uma alternativa para melhorar o desempenho agronômico da soja. Este estudo teve como objetivo avaliar os efeitos da aplicação isolada de ácido indol-butírico, cinetina e ácido giberélico, bem como de um produto comercial contendo esses compostos, sobre a germinação, a morfologia radicular e os componentes de produção da soja. O experimento de campo foi conduzido em delineamento de blocos casualizados, com quatro repetições. Paralelamente, realizou-se um experimento em laboratório, utilizando papel Germitest®, para avaliar a germinação das sementes e as características morfológicas das raízes. Não foram detectadas diferenças estatisticamente significativas entre os tratamentos quanto aos componentes de produção, à produtividade de grãos ou à germinação final. A germinação final variou numericamente de 91,00% a 94,50%, mas essas diferenças não foram significativas. As respostas morfológicas radiculares foram mais evidentes: os tratamentos com ácido giberélico associaram-se à redução do comprimento da raiz, enquanto o ácido indol-butírico apresentou uma tendência descritiva para uma maior proporção relativa de raízes mais finas. De modo geral, os resultados indicam que os reguladores de crescimento vegetal avaliados não afetaram significativamente a germinação final ou a produtividade de grãos de soja nas condições testadas, embora tenham estado associados a alterações na morfologia radicular inicial.

Palavras-Chave: Auxina, Citocinina, Giberelina, Arquitetura radicular.

Resumen

El uso de reguladores del crecimiento vegetal ha sido ampliamente investigado como una alternativa para mejorar el desempeño agronómico de la soja. Este estudio tuvo como objetivo evaluar los efectos de la aplicación individual de ácido indol-3-butírico, kinetina y ácido giberélico, así como de un producto comercial que contiene estos compuestos, sobre la germinación, la morfología radicular y los componentes del rendimiento de la soja. El experimento de campo se llevó a cabo bajo un diseño de bloques completos al azar con cuatro repeticiones. Paralelamente, se realizó un experimento de laboratorio utilizando papel Germitest® para evaluar la germinación de las semillas y los rasgos morfológicos de la raíz. No se detectaron diferencias estadísticamente significativas entre los tratamientos en cuanto a los componentes del rendimiento, el rendimiento de grano o la germinación final. La germinación final osciló numéricamente entre el 91,00 % y el 94,50 %, pero dichas diferencias no fueron significativas. Las respuestas morfológicas radiculares fueron más evidentes: los tratamientos con ácido giberélico se asociaron con una reducción de la longitud de la raíz, mientras que el ácido indol-3-butírico mostró una tendencia descriptiva hacia una mayor proporción relativa de raíces más finas. En conjunto, los resultados indican que los reguladores del crecimiento vegetal evaluados no afectaron significativamente la germinación final ni el rendimiento de grano de la soja bajo las condiciones probadas, aunque sí se asociaron con cambios en la morfología radicular temprana.

Palabras-clave: Auxina, Citoquinina, Giberelina, Arquitectura de la raíz.

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Introduction

Soybean (*Glycine max* (L.) Merrill) is one of the most important oilseeds crops worldwide, serving as a major source of protein and vegetable oil for human and animal consumption (Fang et al., 2024). In this context, the use of plant growth regulators has emerged as a management tool to modify plant architecture and metabolism, potentially affecting crop productivity (Cannon et al., 2025). The effects of plant growth regulators are strongly influenced by the compound, application rate, developmental stage and environmental conditions (Cannon et al., 2025, Cheng et al., 2025).

Auxins, cytokinins, and gibberellins stand out for regulating processes such as germination, rooting, flowering, fruiting and plant branching, acting as regulators of plant growth and development (Jan et al., 2024, Sosnowski et al., 2023, Ritonga et al., 2023). These phytohormones also play a role in defining root architecture, influencing water and nutrient uptake. The interaction between them regulates root development and plant adaptation to environmental conditions, making it essential to understand their isolated effects to optimize the agronomic use and increase soybean productivity (Jan et al., 2024).

In soybeans, auxins are essential for growth and cell elongation (Kotov; Kotova, 2023, Jan et al., 2024, Sosnowski et al., 2023). Furthermore, they influence apical dominance, stimulating the growth of the main stem and regulating the development of lateral roots (Ljung 2013). Under suitable conditions, auxins contribute to improved crop establishment, greater nutrient absorption and increased yield potential of soybean crops (Sosnowski et al., 2023, Jan et al., 2024).

Cytokinins are phytohormones that stimulate plant branching by promoting cell division and development of lateral buds (Jan et al., 2024, Sosnowski et al., 2023, Ritonga et al., 2023, Schaller et al., 2015). They act by reducing apical dominance, favoring the growth of secondary branches and altering plant architecture (Schaller et al., 2015). In contrast, auxins maintain apical dominance, suppressing axillary bud outgrowth (Kotov; Kotova, 2023, Jan et al., 2024). Therefore, the interaction between cytokinins and auxins is antagonistic, regulating the balance between vertical and lateral growth according to the physiological context, which directly influences the productivity and adaptation of cultivated plants (Kotov; Kotova, 2023).

Gibberellins are phytohormones that regulate soybean growth and development,

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promoting stem elongation, leaf expansion, and reserve mobilization during germination (Sanada; Agehara, 2023, Jan et al., 2024). They stimulate cell division and elongation, contributing to greater leaf area and light interception (Ritonga et al., 2023). Furthermore, they can influence reproductive processes, such as flowering and the development of reproductive structures, depending on physiological and environmental conditions (Kotov; Kotova, 2023, Jan et al., 2024). In production systems, the use of growth regulators associated with gibberellins can increase the initial plant vigor and development, resulting in improvements in crop yield performance (Bertolin et al., 2010, Albrecht et al., 2011).

Bertolin et al. (2010) found that the application of 0.009% kinetin, 0.005% gibberellic acid, and 0.005% indole-3-butyric acid, using a commercial product, increased the number of pods per plant and grain yield. Therefore, this study aimed to evaluate the effects of applying indole-3-butyric acid, kinetin, and gibberellic acid separately, comparing them with a mixture of these regulators in soybean.

Materials and methods

To test the proposed hypotheses, two experiments were conducted. The first experiment was carried out under field conditions, whereas the second was conducted under controlled laboratory conditions to evaluate the effect of growth regulators on soybean seed germination.

Foliar application of plant growth regulators

The field experiment was conducted during the 2023/24 growing season, in the experimental area of the Department of Crop Science at the Federal University of Santa Maria (UFSM - Main Campus), at an elevation of 118 m above sea level. The region has a humid subtropical climate (Cfa), characterized by hot summers, no defined dry season, and an average annual rainfall of approximately 2100 mm (Alvares et al., 2013).

The experimental design was a randomized complete block design (RCBD), with four replications. The experimental units were represented by plots composed of five rows spaced 0.45 m apart and 4 m in length. The soybean cultivar used was BMX FIBRA 64I61RSF IPRO, at a target plant population of 14 plants per linear meter, sown on December 5, 2023, in a no-till system without irrigation. NPK (05-20-20) fertilizer was applied as the basal fertilizer at a rate of 200 kg ha^{-1}. Weed control was carried out with herbicides registered and recommended for soybean, and pest and disease control was

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performed according to the needs and intervals recommended for the crop (Martin et al., 2023).

The phytohormones were selected based on the composition of a commercially available and widely used plant growth regulator. They were applied at the V5 phenological stage, defined by Albrecht et al. (2011) as the application stage associated with the greatest yield response. The plant growth regulators were applied using an electric backpack sprayer equipped with a four-nozzle boom and flat-fan spray nozzles with a nominal flow rate of 0.2 gal min^{-1} per nozzle. The sprayer was calibrated to deliver a spray volume of 120 L ha^{-1}. During application, a constant walking speed and boom height were maintained to ensure uniform spray deposition over the crop canopy. The treatments described in Table 1 consisted of an untreated control, the commercial product and, separately, each of its active ingredients: kinetin, gibberellic acid, and indole-3-butyric acid.

Table 1. Treatments and active ingredient doses of plant growth regulators used in the field experiment.

Treatments	Active ingredient dose
Control	-
Commercial product	0.0225 g ha^{-1} + 0.0125 g ha^{-1} + 0.0125 g ha^{-1}
Kinetin	0.0225 g ha^{-1}
Gibberellic acid	0.0125 g ha^{-1}
indole-3-butyric acid	0.0125 g ha^{-1}

At the R8 stage (maturity), three plants per replicate were randomly collected. These samples were used to evaluate plant height (PH), number of productive nodes (NPN), number of pods (NP), number of grains (NG), number of grains per pod (NGP), thousand-seed weight (TSW) and yield (YLD). Grain yield was determined by harvesting the three central rows of each plot (0.45 m spacing by 3.0 m in length, corresponding to a harvested area of 4.05 m^2). The moisture-corrected grain mass was subsequently converted to kg ha^{-1} based on the harvested area. Plant height was determined by measuring from the base to the top of the main stem. The number of productive nodes, number of pods per plant, number of grains, and number of grains per pod were determined by manual counting. The number of grains per plant was determined by manually threshing the pods and counting the grains using an electronic grain counter.

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The thousand-seed weight was estimated by weighing 500 grains using a precision analytical balance and multiplying the resulting weight by two.

The plots were harvested manually and threshed using a thresher. The harvested grain was combined with the grains obtained from three plants sampled for yield component assessments to determine the thousand-seed weight and yield. Grain moisture was adjusted to 13% before the determination of final grain mass and yield calculation.

Seed treatments with plant growth regulators

In the second experiment, to evaluate the influence of phytohormones on soybean germination, the treatments described in Table 2 and their respective compositions listed in Table 3 were used.

Table 2. Germination experiment treatments and their compositions.

Treatments	Composition (Usage solutions)	Active ingredient dose (mg kg^{-1} seed)
Water	10 mL (10 mL water)	0
Kinetin + Water	10 mL (5 mL Solution 1 + 5 mL water)	0.4
Gibberellic acid + Water	10 mL (5 mL Solution 2 + 5 mL water)	0.4
indole-3-butyric acid + Water	10 mL (5 mL Solution 3 + 5 mL water)	0.4
Kinetin (isolated)	10 mL (10 mL Solution 1)	0.8
Gibberellic acid (isolated)	10 mL (10 mL Solution 2)	0.8
indole-3-butyric acid (isolated)	10 mL (10 mL Solution 3)	0.8

Table 3. Composition and concentration of working solutions for dosing.

Working solution	A.I.*	[Concentration] (g L^{-1})	Reagent weight (g kg^{-1} seed)
Solution 1	Kinetin	0.2	0.0008
Solution 2	Gibberellic acid	0.2	0.0008
Solution 3	indole-3-butyric acid	0.2	0.0008

*A.I.: Active ingredient

The working solutions of kinetin, gibberellic acid (GA_3), and indole-3-butyric acid (IBA) were prepared separately at a final concentration of 0.2 g L^{-1}. GA_3 and IBA were initially solubilized by adding 95% analytical-grade ethanol dropwise until complete

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dissolution, whereas kinetin was solubilized by adding 1 M NaOH dropwise until complete dissolution. After initial solubilization, each solution was quantitatively transferred to a volumetric flask and brought to the final volume with distilled water to obtain the desired concentration. The solutions were then homogenized before preparation of the seed-treatment solutions described in Table 2.

The soybean cultivar used was BMX FIBRA 64I61RSF IPRO. To characterize the effects of phytohormones on germination, the tests were conducted in a BOD (Biochemical Oxygen Demand) incubator at the Department of Biology. For each replicate, 50 soybean seeds were sown on Germitest® paper. Sterile and disposable syringes were used to measure each solution. A total volume of 1.0 mL of treatment solution per 250 g of seeds was applied, calculated based on the thousand-seed weight, with the seeds placed in plastic bags and manually shaken for three minutes to ensure homogenization.

The Germitest® paper sheets were weighed and moistened with distilled water at a ratio of 2.5 times the paper's dry weight. A total of 35 replicates were prepared, 5 per treatment, with 50 seeds sown on three sheets of Germitest® paper. These were rolled up and taken to the Seed Laboratory of the Department of Biology (CCNE/UFSM) for incubation in a BOD incubator in a horizontal position, following the protocols of the Brazilian Rules for Seed Analysis (Brasil 2009). According to RAS, this method is the most recommended for seeds of large-scale crops that are not sensitive to light exposure. Vigor counts (first count) and germination counts (second count) were performed as follows:

$$\mathbf{G\ (\%) = (N/A) \times 100}$$

Where G is the germination percentage, N is the number of germinated seeds, and A is the total number of seeds placed for germination.

For the analysis of root morphological variables, the seedlings were stored in a freezer at -20 °C after counting, to be scanned later. Six seedlings per replicate were used, and their roots were spread in a 0.5 cm deep layer of water in a transparent 30 x 40 cm acrylic tray and then scanned using an EPSON® Expression 11000XL scanner at 600 dpi resolution in grayscale, utilizing a transparency unit (TPU). Following scanning, the images were processed using WinRhizo® Pro 2013 software (Regent Instruments Canada Inc., Quebec, Canada) in accordance with the equipment manual, to obtain data on total root length (cm) and mean total root diameter (mm). Based on these data, it is possible to

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classify the roots according to their length and diameter.

Statistical analysis

Data analysis was performed using the R statistical environment, utilizing the R language and RStudio® software. For the field experiment, data were analyzed according to a randomized complete block design using the statistical model $Y_{ij} = \mu + T_i + B_j + \varepsilon_{ij}$, where Y_{ij} is the observed value, μ is the overall mean, T_i is the effect of the i-th treatment, B_j is the effect of the j-th block, and ε_{ij} is the experimental error. Treatment was considered the factor of interest, whereas block was included in the model as a blocking factor to account for spatial variability within the experimental area. Initially, residual normality and homogeneity of variances were assessed using the Shapiro–Wilk and O'Neill–Mathews tests, respectively. When at least one assumption was not met, a Box–Cox power transformation was evaluated, with the transformation parameter (λ) estimated by profile likelihood. The number of grains (NG) and number of pods (NP) were transformed using λ values of −1.50 and −1.83, respectively. After transformation, residual normality and homogeneity of variances were satisfied for NG (Shapiro–Wilk: p = 0.135; O'Neill–Mathews: p = 0.656) and NP (Shapiro–Wilk: p = 0.138; O'Neill–Mathews: p = 0.591). Plant height (PH) showed heterogeneous variances on the original scale and remained heterogeneous after Box–Cox transformation (λ = 2.00); therefore, treatment effects for PH were evaluated using the Friedman test, considering blocks as the blocking factor. The remaining variables met the assumptions on the original scale. Analysis of variance (ANOVA) was performed at a 5% significance level (α = 0.05) for variables meeting the parametric assumptions.

Results and discussion

No significant differences among treatments were detected by the F-test ($p > 0.05$) for any of the yield components or grain yield evaluated in the field experiment (Table 4). However, some assumptions of the statistical analysis, particularly the normality and homogeneity of residuals, were not fully satisfied. Even after estimating the optimal Box-Cox transformation parameter (λ), the results remained statistically non-significant.

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Table 4. Yield components and grain yield of soybean as affected by foliar application of plant growth regulators (mean ± standard error; n = 4).

Treatment	NG	NPN	PH	NGP	NP	TSW	YLD
Control	219.8 ± 34.1	18.3 ± 0.4	115.8 ± 2.1	1.89 ± 0.09	117.8 ± 14.2	119.1 ± 3.6	3163 ± 239
Commercial product	234.3 ± 28.3	20.0 ± 1.1	119.6 ± 0.5	1.78 ± 0.07	131.7 ± 11.8	112.0 ± 1.3	3188 ± 254
Kinetin	238.7 ± 43.5	16.5 ± 1.2	108.8 ± 3.4	1.79 ± 0.03	133.3 ± 23.2	116.9 ± 2.9	3168 ± 263
Gibberellic acid	251.8 ± 30.7	20.1 ± 1.4	116.7 ± 1.7	1.87 ± 0.08	133.6 ± 13.2	114.4 ± 3.3	2646 ± 204
indole-3-butyric acid	239.3 ± 74.5	18.4 ± 1.1	110.9 ± 5.0	1.72 ± 0.12	137.8 ± 35.0	118.9 ± 6.0	2710 ± 252
Treatment p-value	0.871	0.136	0.267	0.661	0.931	0.586	0.162
	MeanB.O values						
	236.78	18.66	114.34	1.81	130.83	116.24	2975

Values are presented as means ± standard error (n = 4). NG: number of grains per plant; NPN: number of productive nodes per plant; PH: plant height (cm); NGP: number of grains per pod; NP: number of pods per plant; TSW: thousand-seed weight (g); YLD: grain yield (kg ha^{-1}). NG and NP were analyzed after Box–Cox transformation (λ = −1.50 and −1.83, respectively), although means are presented on the original scale. PH was analyzed using the Friedman test because homogeneity of variances remained unmet after Box–Cox transformation. The remaining variables were analyzed by analysis of variance according to a randomized complete block design.

The lack of a significant response to the plant growth regulator treatments may be associated with several factors, such as environmental conditions during the experiment, the phenological stage at the time of application, and the dosages used. Although these compounds are known to influence plant physiological processes, their use in isolation may have been insufficient to promote measurable changes in the crop's agronomic performance under the conditions evaluated. These findings are consistent with previous studies showing that soybean responses to plant growth regulators are dependent on the regulator, application rate, environmental conditions, and management practices (Cannon et al., 2025).

Similar results have been reported in previous studies. Binsfeld et al. (2014) and Moterle et al. (2008), who, using the same commercial biostimulant formulation at the same concentration, also reported limited soybean response, even under favorable environmental conditions. The use of higher concentrations may represent an alternative strategy for achieving positive results with these biostimulants. Buzzello et al. (2017),

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employing an application rate 50% higher than that used in the present study, observed increased grain yield and reduced plant height without affecting the number of pods, which may contribute to a potential reduction in crop lodging, associated with an increase in yield per cultivated hectare.

Differences among treatments were observed in the germination test (Table 5). The use of gibberellic acid combined with water resulted in the highest germination percentage, whereas the indole-3-butyric acid treatment showed the lowest. These results reinforce the role of gibberellins in promoting seed germination, contributing to greater seedling vigor and establishment (Jaybhaye et al., 2024). Conversely, the lower germination performance associated with indole-3-butyric acid may be related to hormonal imbalance, interfering with the initial cellular metabolic processes of the seeds.

Table 5. Final germination of soybean seeds subjected to plant growth regulator treatments.

Treatment	Final germination (%)
Water	94.25 ± 0.63
Kinetin + Water	91.00 ± 1.08
Gibberellic acid + Water	94.50 ± 0.65
indole-3-butyric acid + Water	91.25 ± 1.60
Kinetin	92.00 ± 0.41
Gibberellic acid	92.25 ± 0.63
indole-3-butyric acid	92.25 ± 1.03

Values are presented as means ± standard error. No significant treatment effect was detected by analysis of variance (p = 0.093; CV = 2.03%).

Considering the distribution of root diameter classes and seedling length (Figure 1 - A), descriptive trends suggested differences among the treatments, pointing to potential morphological changes in the seedlings in response to plant growth regulators. These patterns were descriptive during the early stages of development. The Gibberellic acid + Water treatment showed the highest numerical final germination value; however, this response did not differ statistically from the other treatments. Consistent with previous findings (Leite et al., 2003, Hedden; Sponsel, 2015, Borges et al., 2023), an effect of gibberellic acid on reducing the total root length of the evaluated plants was also observed

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(Figure 1 - B). This effect was evident in the treatments with isolated GA_3 (8.1 cm/plant) and GA_3 + water (8.4 cm/plant), characterizing a gibberellin-induced effect on this process.

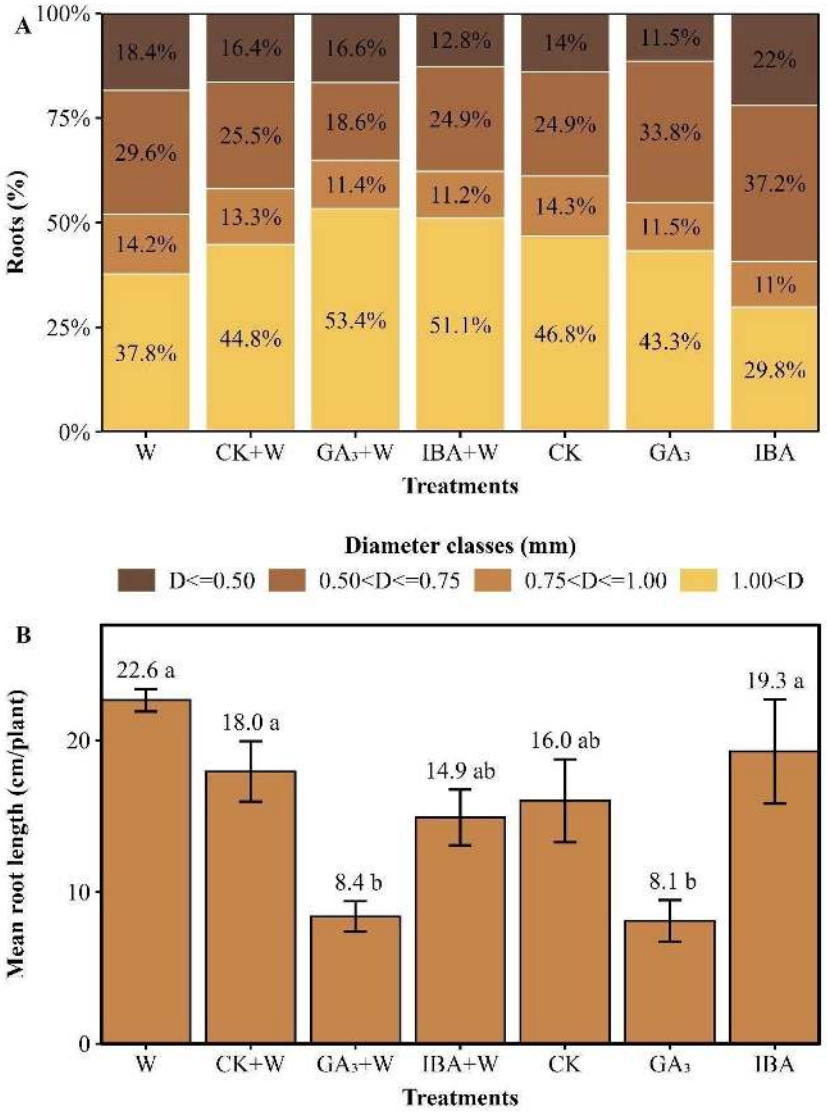
Indole-3-butyric acid was associated with changes in root morphology, inducing the formation of roots with smaller diameters when applied alone at a higher concentration, consistent with the role of auxins in root system development (Sanada; Agehara 2023, Jan et al., 2024, Sosnowski et al., 2023, Ritonga et al., 2023). However, this effect was not observed when applied at low concentrations (IBA + W), and the percentage of fine roots was lower than that in the control. Thinner roots are generally associated with greater soil exploration and resource acquisition, although the functional advantage of root diameter depends on environmental conditions (Bui et al., 2022, Jan et al., 2024).

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Figure 1. Root morphological traits as affected by growth regulators. (A) Percentage distribution of root diameter classes (mm). (B) Mean root length per plant (cm/plant).



Different lowercase letters indicate significant differences among treatments based on the Scott-Knott clustering test ($p < 0.05$). Error bars indicate the standard error of the mean ($n = 5$).

The results reinforce the importance of hormonal balance during plant development and indicate that the effects of these regulators are more pronounced during the early stages of growth than at the end of the crop cycle. Therefore, the use of these substances should be planned by considering not only dosages but also the timing of application and potential interactions among the different phytohormones. Further studies are needed to

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explore the combined use of these compounds, as well as varying concentrations and environmental conditions, to provide a better understanding of their potential in soybean crop management.

Gibberellins are associated with cell elongation and can influence root tissue structure, contributing to variations in root diameter distribution (Jan et al., 2024, Sosnowski et al., 2023, Ritonga et al., 2023). Cytokinins, such as kinetin, regulate cell division and differentiation through interactions with auxins and gibberellins to regulate root development (Ritonga et al., 2023). Thus, the interaction between these phytohormones plays a significant role in modulating plant growth, particularly during the early stages of development. Although these results provide valuable insights regarding plant morphological and yield responses, certain limitations should be noted. The evaluation was restricted to specific microclimatic and soil conditions during a single growing season, which may not fully capture genotype-by-environment (GE) interactions across contrasting regions. Expanding this evaluation to multi-location and multi-year trials will further strengthen the broader applicability of these findings.

Conclusions

Under the environmental conditions, phenological stage and application rates evaluated in this study, the isolated application of plant growth regulators did not promote significant changes in yield components or soybean grain yield. The results suggest that, under the conditions evaluated, the treatments did not result in higher grain yield for the crop.

The effects of the plant growth regulators were more readily detectable in root morphological traits than in final germination or grain yield. No significant treatment effect was detected for final germination, although numerical variation among treatments was observed. Indole-3-butyric acid was associated with a higher relative proportion of roots in the smaller-diameter classes, whereas gibberellic acid was associated with reduced root length. However, these early morphological responses did not translate into increased grain yield. Therefore, future studies should consider different application rates and timings, as well as environmental conditions, when evaluating soybean responses to plant growth regulators.

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