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Soybean responses to foliar amino acid application and high plant densities

Respuesta de la soya a la aplicación foliar de aminoácidos y altas densidades de plantas

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ABSTRACT

The combination of plant densities and foliar application of amino acids may be a viable strategy to increase sustainable soybean crop production. Therefore, the objective of this study was to evaluate the effects of high plant densities and the foliar application of amino acids (VIUSID® Agro) on the growth and yield of soybeans [*Glycine max* (L.) Merr] in the rainy season. This research was conducted from May to September 2023, with treatments arranged in split plots, randomized blocks, and replicated three times. The main plots corresponded to the two plant densities (PD) (300,000 and 500,000 ha^{-1}), and the secondary plots were defined by the foliar application of amino acids (AA)-non-AA (A0), 0.25 L ha^{-1} (A1), and 0.50 L ha^{-1} (A2) -to improve soybean growth and yield responses. The results showed that plant densities increased growth indicators, with the most significant increase was observed at a density of 300,000 plants ha^{-1}. In contrast, production parameters and yield were higher at a density of 500,000 plants ha^{-1}. However, the 0.25 L ha^{-1} treatment showed superior effects on growth at the density of 300,000 plants ha^{-1}, while yield and yield components were higher at the 0.5 L ha^{-1} treatment. Additionally, at a density of 500,000 plants ha^{-1}, the 0.5 L ha^{-1} treatment stimulated the growth of soybean plants, while the 0.25 L ha^{-1} treatment exhibited higher yields.

Keywords: biostimulants; *Glycine max*; growth promoter; productivity; sowing density; VIUSID® Agro

RESUMEN

La combinación entre las densidades de plantas y la aplicación foliar de aminoácidos puede ser una estrategia viable para aumentar la producción sostenible del cultivo de la soya. Por lo tanto, el objetivo de este estudio fue evaluar los efectos de las altas densidades de plantas y la aplicación foliar de aminoácidos (VIUSID® Agro) en el crecimiento y rendimiento de la soya (*Glycine max* L. Merr.) en época lluviosa. Esta investigación se desarrolló de mayo a septiembre de 2023, y los tratamientos fueron ordenados en parcelas divididas en bloques al azar con tres réplicas. Las parcelas principales fueron las dos densidades de plantas (300000 y 500000 plantas ha^{-1}), y las parcelas secundarias por la aplicación foliar de aminoácidos (AA): no-AA (A0), 0,25 L ha^{-1} (A1), y 0,50 L ha^{-1} (A2), para mejorar las respuestas del crecimiento y rendimiento de la soya. Los resultados mostraron que las densidades de plantas incrementaron los indicadores del crecimiento, especialmente para la densidad de 300000 plantas ha^{-1}, mientras que los parámetros productivos y el rendimiento fueron superiores en la densidad de 500000 plantas ha^{-1}. Sin embargo, el tratamiento de 0,25 L ha^{-1} de AA mostró efectos superiores en el crecimiento en la densidad de 300000 plantas ha^{-1}, mientras que el rendimiento y sus componentes fueron superiores con el tratamiento de 0,50 L ha^{-1}. Adicionalmente, en la densidad de 500000 plantas ha^{-1}, el tratamiento de 0,50 L ha^{-1} estimuló el crecimiento de las plantas de soya, mientras que el tratamiento de 0,25 L ha^{-1} exhibió mayores rendimientos.

Palabras clave: bioestimulantes; densidad de siembra; *Glycine max*; productividad; promotor del crecimiento; VIUSID® Agro

INTRODUCTION

Soybean (*Glycine max* L. Merr.) is an important source of vegetable protein and high-quality edible oil. With its biological nitrogen fixation (BNF) capacity, soybean is an important crop to use for decreasing nitrogen (N) fertilizer application while maintaining high yields in crop rotation systems (Lin *et al.*, 2022). The increase in soybean production coincides with an evolution in land, water, and fertilizer use also with a shift in the main export destination in the 2000s from Europe to China (Peña-Calzada *et al.*, 2022).

In 2021, 371.7 million tons of soybeans were produced worldwide. This represented a growth of around 16 million over the production volume recorded the previous year, thus ending the declining trend that started in 2018, with an average yield of 2,782 kg ha^{-1} (FAO, 2021). However, this is still unable to meet the needs of a growing population. Yields can vary greatly depending on water availability, fertilization, and row spacing. Under non-irrigated conditions, good soybean yields range from 1.5 to 2.5 t ha^{-1}. High yields of improved varieties are between 2.5 and 3.5 tons of seeds per hectare under irrigated conditions (Sandrakirana & Arifin, 2021).

Factors influencing soybean yield include climatic conditions, soil characteristics, soybean varieties, nutrient management, cultivation practices, pests, and sowing density (Yanes-Simón *et al.*, 2023). Employing adequate plant densities increases soybean yields by improving light energy utilization in the leaves, thereby promoting nutrient uptake and increasing dry matter accumulation with the yield (Calero-Hurtado *et al.*, 2020).

Plant density depends on several factors, the most important of which are soil fertility, moisture (soil and air), germination rate, agronomic characteristics of varieties or cultivars, crop competitiveness against weeds, and fertilization (Andrade *et al.*, 2023). It is widely reported that a uniform sowing pattern increases spatial uniformity and the leaf area index, reduces mutual shading, and accelerates leaf closure (Pérez-Díaz *et al.*, 2024; Acevedo-González *et al.*, 2025; Yanes-Simón *et al.*, 2023). These changes result in increased radiation interception by the leaves (Chețan *et al.*, 2021) as well as increased plant growth and productivity (Calero-Hurtado *et al.*, 2021).

Fertilization of the soybean crop is another key factor for increasing productivity, although it is carried out without a prior comprehensive diagnosis, and it relies on the empirical application of solid fertilizers based on chemical synthesis and common visual characteristics, as is the case in several regions (Lin *et al.*, 2022). It has become popular for many producers to use pre-set chemical fertilizer rates, regardless of the fertility of their soils (Dai *et al.*, 2021). It is important to highlight that the inadequate management of synthetic fertilization not only causes negative alterations in plant physiological activities and yields but also degrades the soil, so it is necessary to integrate sustainable management methods for soil preservation (Calero *et al.*, 2019; Sandrakirana & Arifin, 2021).

Many bioproducts, including biostimulants, have been used to enhance ecological management and crop productivity. In recent years, a large number of biostimulants have been developed that allow plants to overcome stressful situations and nutrient deficiencies, thus favoring plant growth, development, and yield, with a reduction in the use of chemicals (Abbas *et al.*, 2021).

According to various results reported in the literature on trials evaluating foliar fertilizers, this technique seems to be influenced by factors related to the crop, including the technological and environmental conditions of its growth. They also reported on the products used in terms of their compositions and nutrient concentrations, the phenological stages at which they are applied, and their dosage (Bărdaş *et al.*, 2023).

One of these products is the growth promoter VIUSID® Agro, which has been subjected to a biocatalytic molecular activation process that improves its biological activity and the biochemical reactivity of all its molecules (Peña-Calzada *et al.*, 2025). This biostimulant enhances the vegetative phase of crops, increasing the length of stems, the number of leaves and leaf area, and also accelerates the reproductive phase and increases the number of flowers and fruits, which positively influences yield increases (Peña-Calzada *et al.*, 2022). Similar effects were produced by VIUSID® Agro exogenous supplementation recently in different plant species, such as beets (Peña-Calzada *et al.*, 2024), peanut (Acevedo-González *et al.*, 2025), tomato (Calero-Hurtado, Meléndrez-Rodríguez *et al.*, 2025), and sunflower (Calero-Hurtado, Peña-Calzada *et al.*, 2025).

Based on the above assumptions and on insufficient knowledge in soybean crop management, the effects of using high plant densities and amino acid application on soybean growth and yield responses when sowing in the rainy season are still unknown. Therefore, the objective of this research was to evaluate the effects of high plant densities and the foliar application of amino acids (VIUSID® Agro) on soybean growth and yield during the rainy season.

MATERIAL AND METHODS

Location

The experiment was conducted on the areas of the Las Aromas farm, belonging to the collective farms “Manuel Ascunce” in the Taguasco municipality, Sancti Spíritus province, Cuba. The soybean seeds (variety SOYIG-22) were obtained from the agro-industrial grain company Sur del Jíbaro, with a 95% germination rate. Phytotechnical practices (pest control, cleaning, etc.) were carried out according to the Guide for Soybean Cultivation (MINAG, 2020), with modifications in fertilization (no chemical or organic fertilizers).

Plant material

Sowing was carried out manually on 27 May 2023, during the rainy season. Row spacing was 0.50 m, both for single- and double-sowing spacing, to obtain plant densities of 300,000 and 500,000 plants ha^{-1}, respectively. The predominant soil type in the study area was Cambisol (Schad, 2023).

Experimental design

Treatments were arranged in split plots, in randomized blocks, with three replications. The main plots were divided into two plant densities (D): 300,000 plants ha^{-1} (plot 1, single-spread seeder, with 0.5 m between furrows, D1) and 500,000 plants ha^{-1} (plot 2, double-spread seeder, with 0.5 m between furrows, D2). The secondary plots were characterized by the foliar application of three different doses of amino acids (AA): 0 (A0), 0.25 L ha^{-1} (A1), and 0.5 L ha^{-1} (A2), resulting in six different combined treatments as follows: D1A0—300,000 plants ha^{-1} without AA; D1A1—300,000 plants ha^{-1} with 0.25 L ha^{-1}; D1A2—300,000 plants ha^{-1} with 0.5 L ha^{-1}; D2A0—500,000 plants ha^{-1} without AA; D2A1—500,000 plants ha^{-1} with 0.25 L ha^{-1}; and D2A2—500,000 plants ha^{-1} with 0.5 L ha^{-1}. The experimental plots consisted of five furrows. The plot size was 16 m^2 (8 m long by 2 m wide), and the effective area for sampling and observations was 6 m^2.

Foliar application of amino acids

Amino acid application was carried out between 9:00 and 10:00 hours every seven days at the vegetative (V3, V4, and V5) and reproductive (R5 and R6) stages, according to Fehr *et al.* (1971) (see Table 1). The source of AA was the growth promoter VIUSID® Agro (Catalisys, Spain), and the applications were carried out with the support of a backpack pressure sprayer (Matabi, 16 L, Goizper Group, Spain). The amino acid doses used were A0 (water only), 0.25, and 0.5 L ha^{-1}, following the results reported previously for the bean crop (Peña-Calzada *et al.*, 2022).

Table 1. Phenological stage of the soybean plant, date, and dose of amino acid applications (VIUSID® Agro, L ha^{-1})

Treatments	Phenological stage, date, and dose of AA applications (L ha^{-1})				
	V2 15/06/23	**V3** 22/06/23	**V4** 29/06/23	**R5** 06/07/23	**R6** 13/07/23
D1A0			A0 (water only)		
D1A1	0.25	0.25	0.25	0.25	0.25
D1A2	0.5	0.5	0.5	0.5	0.5
D2A0			A0 (water only)		
D2A1	0.25	0.25	0.25	0.25	0.25
D2A2	0.5	0.5	0.5	0.5	0.5

Determination of growth and yield parameters

Growth parameters. At 20 and 35 days after emergence (DAE), 30 plants were randomly sampled in the useful area of each plot to observe the following parameters. Plant height (PH, cm): This is measured from the base of the stem to the apex, using a ruler. Number of trifoliate leaves (NLP): This was done by direct counting. Leaf area index (LAI, cm^2): This was calculated by Kemp's formula (Kemp, 1960) with some modifications on 30 plants per treatment according to the formula below.

$$LAI = \sum(l \cdot w) * f \ (1) \qquad (1)$$

Where l is the length of each leaflet, **w** is the width of each leaflet, and **f** is the constant factor equal to 0.75 previously determined in soybean plants (Tagliapietra *et al.*, 2018).

The leaf dry mass (LDM), stem dry mass (SDM), and aboveground biomass (AB) were determined at 30 and 50 DAE. On each date, 30 plants per treatment were collected from the effective area of the plots, separated into leaves and stems, and placed in paper bags. These were then transferred to a forced ventilation oven and dried at 60 °C until a constant mass was obtained. A precision scale was used to obtain LDM (g) and SDM (g). Aboveground biomass (AB, g) was determined by adding LDM and SDM together.

Productive parameters. At harvest time (R9, 102 DAE), the following yield parameters were assessed on 30 plants per treatment. Number of branches per plant (NBP): All branches on the plants assessed were counted. Number of pods per plant (NPP): All pods on the plants were assessed by direct count. Number of pods with seeds (PWS) and without seeds (PWG) and their percent as %PWS and %PWG: PWS and PWG were carried out by direct count, and the %PWS and %PWG were obtained by dividing the average of PWS and PWG, respectively, by the NPP. Number of grains per plant (NSP): All seeds of each plant were counted. Number of seeds per pod (NSL): All grains of the plants were counted and divided by the NPP. Mass of 100 grains (M100, g): Different samples of 100 grains were collected and weighed on a precision scale (12% dry matter). Grain mass per plant (GMP, g): Grains of each plant were collected and weighed on a precision scale (12% dry matter). Yield (YD, t ha-^{1}): Determined from the production of the number of plants per m^2 (kg) and converted to t ha-^{1} (12% dry matter).

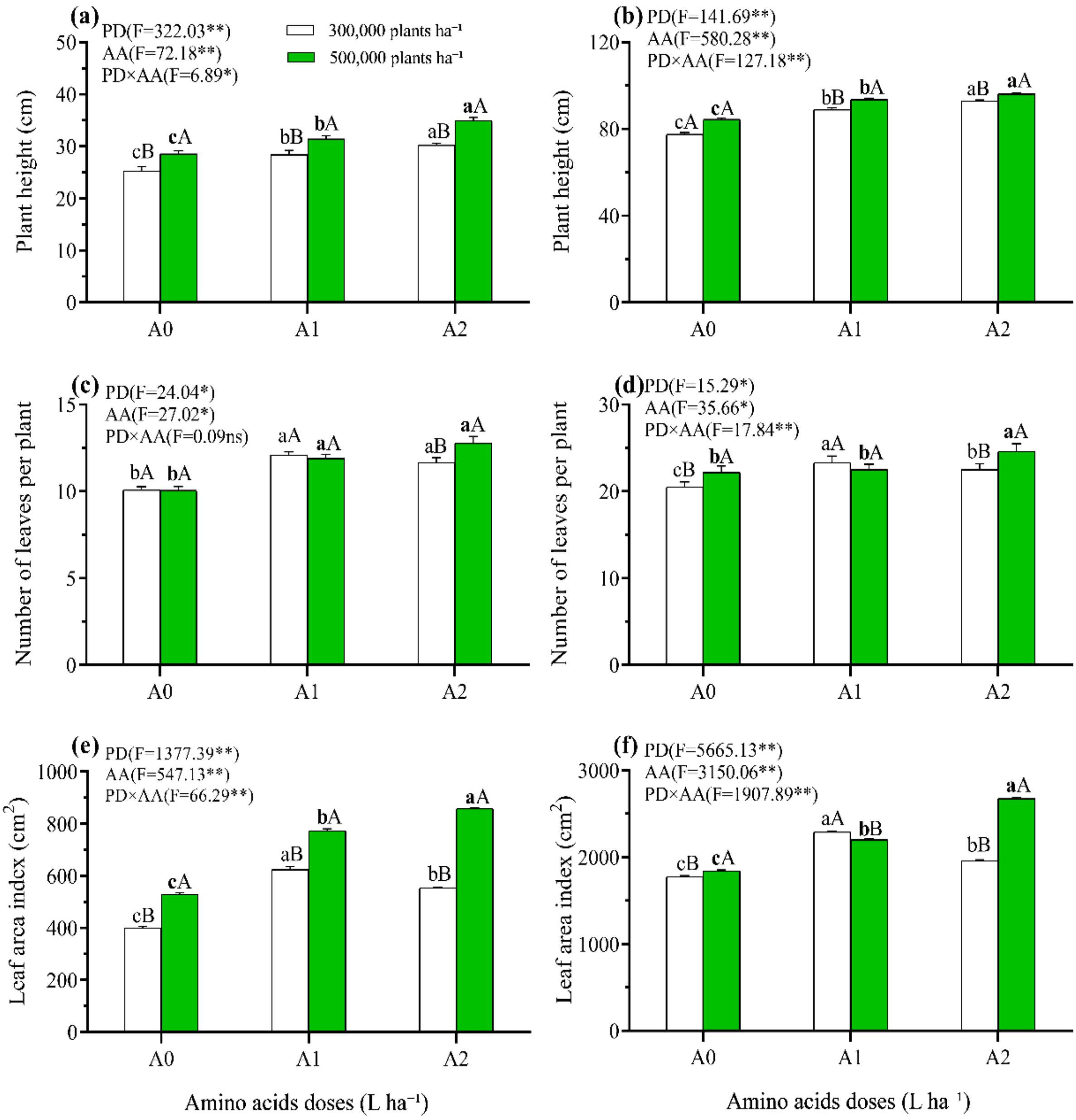
Data analysis

Dates obtained were subjected to the assumptions of normality and homogeneity of variance using the Shapiro-Wilk and Levene's tests, respectively. Data were analyzed through a multivariate analysis of variance (ANOVA), and when Fisher's (F) test was significant ($p < 0.05$), Tukey's test was used for comparison of means ($p < 0.05$). All statistical analyses and comparisons were performed using IBM SPSS Statistics v.19.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

Effect of plant densities and amino acid application on the growth of soybean plants

ANOVA showed significant interaction between plant density (PD) and amino acid (AA) factors on plant height (PH) at 20 DAE ($p < 0.04$) and 35 DAE ($p < 0.01$) in soybean plants (Figure 1a, b). The PH under the 500,000 plants ha-^{1} (D2 treatment) was higher by 17% and 22% at 20 and 35 DAE, respectively, than the 300,000 plants ha-^{1} (D1 treatment). However, under the D2 treatment, the PH was greater in the 0.25 L ha-^{1} (A1) treatment, with significant ($p < 0.001$) increases of 20% and 10% compared to the non-AA (A0) and 0.5 L ha-^{1} (A2) treatments, respectively. Additionally, the D2 treatment was significantly ($p < 0.01$) higher by 13% than the A0 treatment (Figure 1a). Similarly, under the D1 treatment, the PH was higher in the A2 treatment by 23% and 10% compared with the A0 and A1 treatments ($p < 0.01$), respectively, although the latter A1 treatment was 13% higher than the A0 treatment (Figure 1a). However, at 35 DAE and in the D1 treatment, the PH was increased in the A1 treatment concerning the A0 and A2 treatments, although the A2 treatment was significantly ($p < 0.01$) higher than the A0 treatment. Moreover, under the D2 treatment, the PH increased in the A2 treatment compared to the A0 and A1 treatments, but the A1 treatment reached greater PH averages compared to the A0 treatment (Figure 1b).



Plant height at 20 DAE (a) and at 35 DAE (b), number of leaves per plant at 20 DAE (c) and at 35 DAE (d), and leaf area index at 20 DAE (e) and at 35 DAE (f). Normal (e.g., a, b, c) or bold (e.g., **a, b, c**) lower-case letters indicate significant differences among AA treatments in the densities of 300,000 and 500,000 plants ha-1, respectively. Capital letters (e.g., A, B) indicate significant differences between both densities (300,000 and 500,000 plants ha-1) in the same AA treatment, according to the Tukey HSD test ($p < 0.05$).

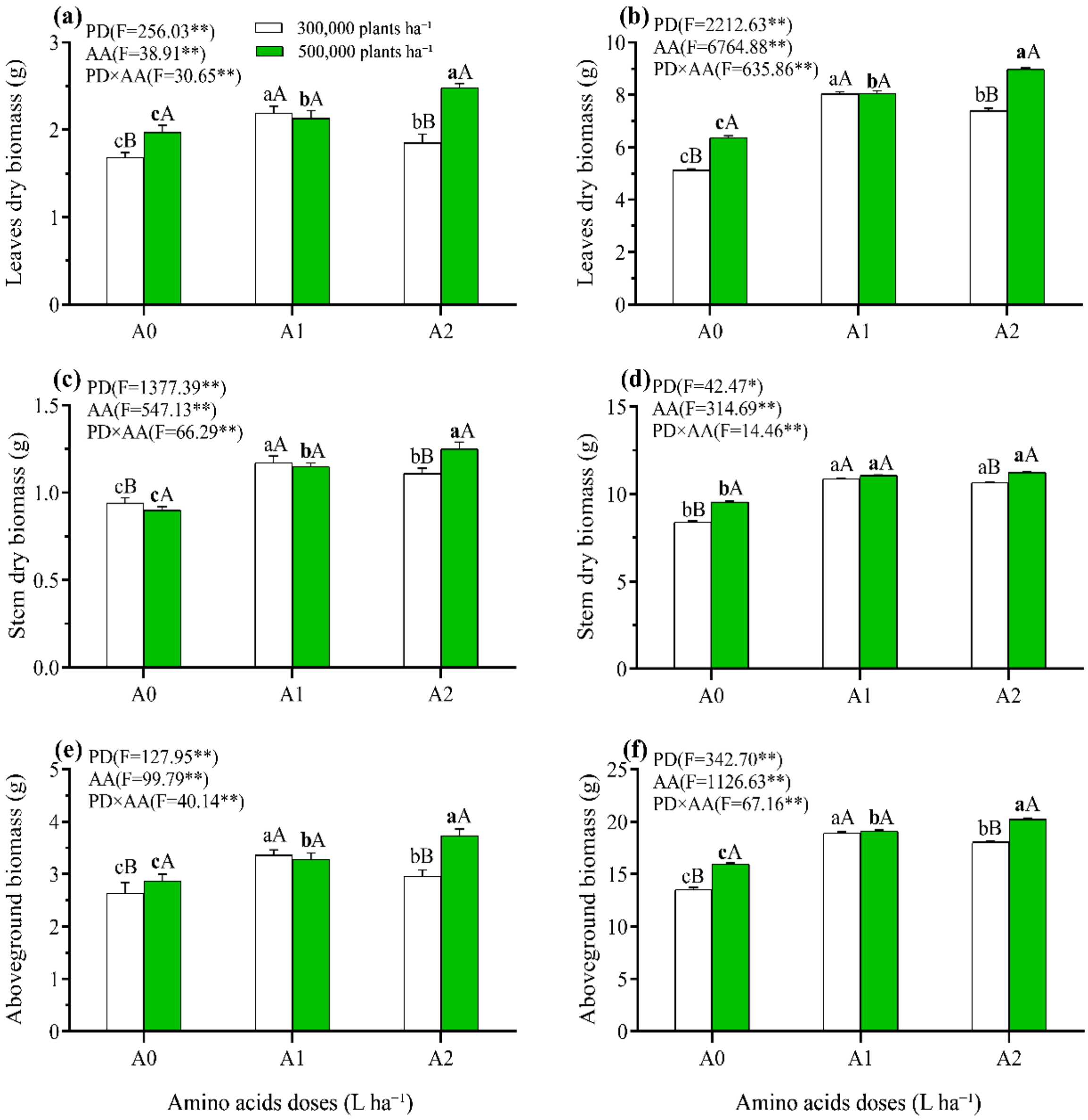
Figure 1. *Effect of plant densities and amino acid doses on soybean growth at 20 and 35 DAE.*

A significant interaction between PD and AA factors on the number of leaves per plant (NLP) was observed only at 35 DAE ($p > 0.04$). In contrast, at 20 DAE, no significant interaction was detected ($p > 0.32$) in soybean plants (Figure 1c, d). At 20 DAE, the D2 treatment exhibited a slight increase (8%) in NLP compared to the D1 treatment (Figure 2c). In addition, at 20 DAE, the NLP under the D1 treatment showed similar effects ($p = 0.07$) between A1 and A2 treatments, but both increased NLP by 20% compared to the A0 treatment. Similarly, at D2 treatment, applying A1 and A2 treatments showed equal effects on NLP and increased NL by 23% compared to the A0 treatment (Figure 1c). Additionally, at 35 DAE, the NLP under the D1 treatment showed similar effects in the A1 and A2 treatments but were significantly higher (19%) than the A0 treatment (Figure 2d). However, under D2 treatment, the NL increased significantly (27%) in the A2 treatment with respect to the A0 and A1 treatments, but at the same time, these last treatments (A0 and A1) exhibited similar effects on NLP (Figure 2d).

The two-way ANOVA showed significant interaction between the PD and AA factors on leaf area index (LAI) at 20 DAE ($p < 0.001$) and 35 DAE ($p < 0.001$) (Figure 1e-f). The D2 treatment showed greater increases in LAI by 29% and 36% compared to the D1 treatment at 20 and 35 DAE, respectively (Figure 1e-f). Supplying AA under D1 treatment significantly increased the LAI, especially under the A2 treatment; the LA increased by 56% and 30% compared with the A0 and A1 treatments, respectively, but at the same time, the A1 treatment increased the LAI by 38% more than the A0 treatment (Figure 1e). Similarly, under D2 treatment, the LA increased in the A2 treatment by 62% and 11% in comparison with the A0 and A1 treatments; however, at the same time, the A1 treatment increased the LAI by 46% more than the A0 treatment (Figure 1e). In addition, under D1 treatment, the LAI was increased in the A1 treatment by 26% compared to the A0 and A2 treatments, although at the same time, the A2 treatment was significantly higher (13%) than the A0 treatment. Nevertheless, under D2 treatment, the LAI was higher in the A2 treatment by 33% and 21% compared to the A0 and A1 treatments, but the A1 treatment increased LA by 16% compared with the A0 treatment (Figure 1f).

There was significant interaction between PD and AA factors with regard to LDM ($P < 0.03$), SDM ($p < 0.01$), and AB ($p < 0.01$) of the soybean plants at 20 and 35 DAE (Figure 2a-f). Overall, the D2 treatment showed higher values of LDM, SDM, and AB compared to the D1 treatment in all AA treatments ($p < 0.01$) (Figure 2a-f). The average of LDM under the D1 treatment was higher by 30% and 18% in the A2 treatment compared to the A0 and A1 treatments, but the A1 treatment was significantly higher (10%) than the A0 treatment. However, under the D2 treatment, applying A2 treatment significantly ($p < 0.001$) increased LDM by 26% and 16% compared with the A0 and A1 treatments; although, at the same time, the A1 treatment showed significant ($p < 0.01$) increases in LDM compared to the A0 treatment (Figure 2a).

The average LDM at 35 DAE and under the D1 treatment was significantly ($p < 0.01$) higher at the A1 treatment compared to the A0 and A2 treatments, but at the same time, the A2 treatment showed a superior mean compared to the A0 treatment ($p < 0.002$). In addition, at the D2 treatment, the A1 treatment showed significant increases in LDM compared to the A0 and A1 treatments, although this last treatment significantly ($p < 0.03$) increased the LDM more than the A0 treatment (Figure 2b).



Leaf dry biomass at 20 DAE (a) and at 35 DAE (b), stem dry biomass at 20 DAE (c) and 35 DAE (d), and aboveground biomass at 20 DAE (e) and 35 DAE (f). Treatments and statistics as in Fig. 1.

Figure 2. *Effect of plant densities and amino acid doses on biomass production at 20 and 35 DAE.*

Regardless of D1 treatment, the SDM production at 20 DAE increased by 25% under A1 and A2 treatments compared to the A0 treatment ($p < 0.01$; Figure 2c). However, under D2 treatment, the SDM was higher at A2 treatment by 39% and 10% compared to the A0 and A1 treatments, but this latter treatment, at the same time, revealed significant ($p < 0.01$) increases in SDM by 28% compared to the A0 treatment (Figure 2c). Additionally, at 35 DAE, the SDM accumulation at the D1 treatment showed similar effects ($p = 0.08$) between A1 and A2 treatments, but these treatments achieved higher SDM compared to the A0 treatment ($p < 0.01$; Figure 2d). However, the SDM at the D2 treatment was higher in the A1 and A2 treatments and showed significant ($p < 0.001$) increases relative to the A0 treatment (Figure 2d).

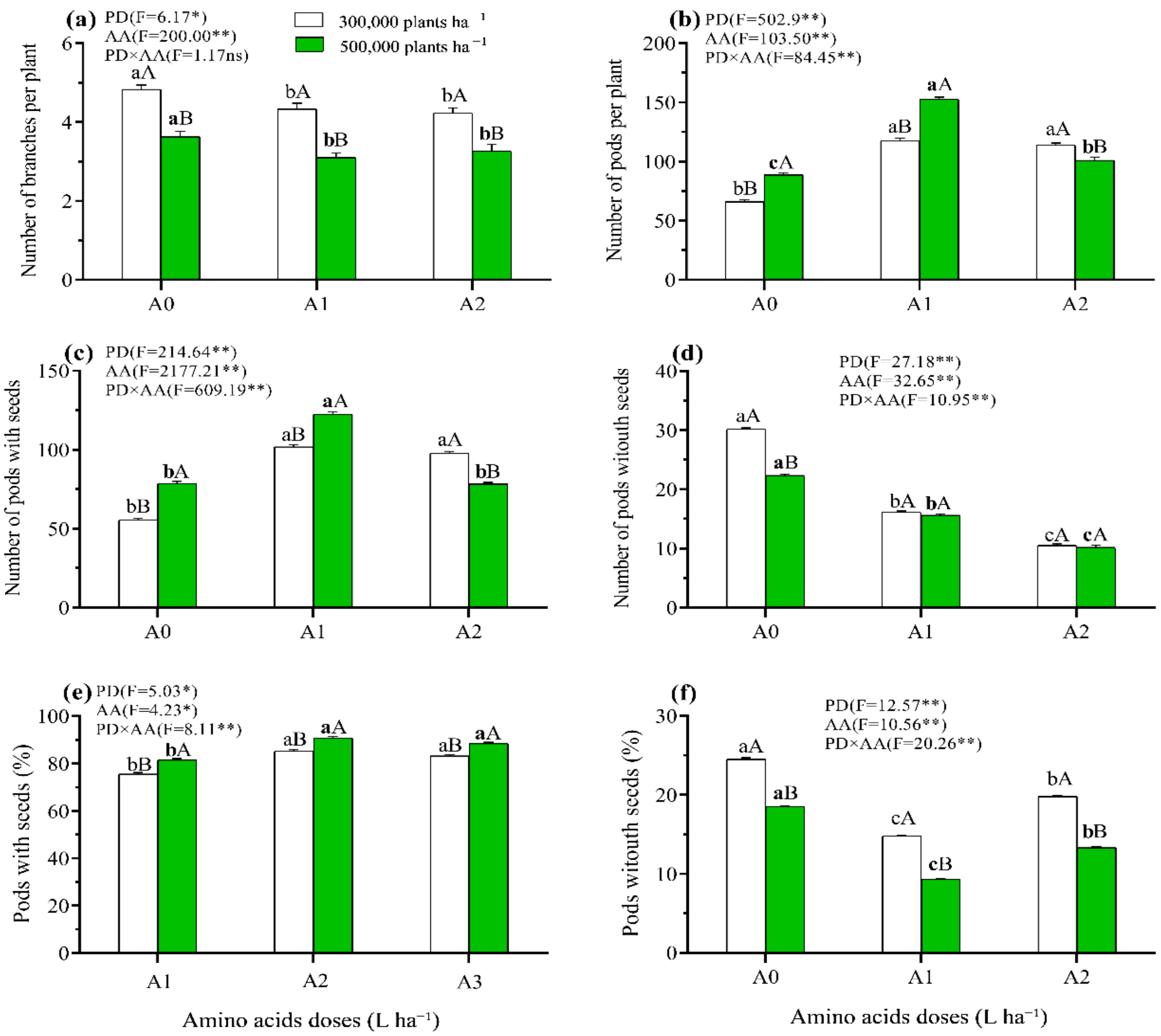
The aboveground biomass (AB) showed greater increases under the D2 treatment than the D1 treatment in all AA treatments (Figure 2e). Regardless of D1 treatment, the AB was higher in the A1 treatment by 28% and 14%, respectively, compared to the A0 and A2 treatments; but at the same time, the A2 treatment increases the AB production by 13% relative to the A0 treatment (Figure 2e). In addition, under the D1 treatment, the AB average was increased in the A2 treatment by 30% and 14% in comparison with the A0 and A1 treatments, respectively. However, supplying A1 treatment revealed significant (p < 0.001) increases in AB by 14% compared to the A0 treatment (Figure 2e). Similarly, the AB accumulation under D1 treatment was higher in the A1 treatment by 33% compared to the A0 and A2 treatments, but at the same time, the A2 treatment increased the AB by 19% concerning the A0 treatment (p < 0.003, Figure 2f). Meanwhile, under the D2 treatment, the A2 treatment significantly increased the AB by 38% and 17% compared to the A0 and A1 treatments, respectively; even so, the A1 treatment showed significant (p < 0.01) increases in AB by 22% compared to the A0 treatment (Figure 2f).

Effects of plant densities and amino acid applications on soybean yields and their components

A two-way ANOVA showed significant interaction (p < 0.01) between PD and AA on NBP, NPP, PWS, PWG, %PWS, and %PWG (Figure 3a-e). The average NBP showed similar effects (p = 0.09) between both PD (Figure 3a). In addition, under the D1 treatment, applying A1 and A2 treatments showed similar effects (p = 0.22) on NBP and were significantly superior compared to the A0 treatment. However, at the D2 treatment, exogenous application of A1 treatment significantly (p < 0.01) increased the NBP compared with the other A2 and A0 treatments, but the A2 treatment showed higher increases in NBP as compared to the A0 treatment (Figure 3a).

The NPP average was significantly higher under the D2 treatment compared to the D1 treatment in all AA treatments (Figure 3b). In addition, under D1 treatment, the NPP showed equal effects (p = 0.11) between A1 and A2 treatments and were significantly (p < 0.01) higher by 75% compared to the A0 treatment (Figure 3B). Whereas, under the D2 treatment, the A2 treatment increased the AB by 78% and 51% in comparison with the A0 and A1 treatments; nonetheless, at the same time, the A1 treatment enhanced the NPP mean by 14% more than the A0 treatment (p < 0.01; Figure 3b).

Regardless of PD and AA factors, the average PWG was higher under D2 treatment and showed a significant difference (p < 0.01) compared to the D1 treatment in all AA treatments (Figure 3c). In addition, under D1 treatment, the PWS showed equal effects (p = 0.14) between A1 and A2 treatments and revealed higher PWS by 80% with respect to the A0 treatments. Meanwhile, under D2 treatment, applying A2 treatment increased the PWS by 56% compared to the A0 and A1 treatments (Figure 3c). Likewise, the average PWG in the D1 treatment was higher (35%) than in the D2 treatment (Figure 3d). Further, under A1 and A2 treatments, the PWG showed similar effects under D1 and D2 treatments and were significantly higher than the A0 treatment (Figure 3d).



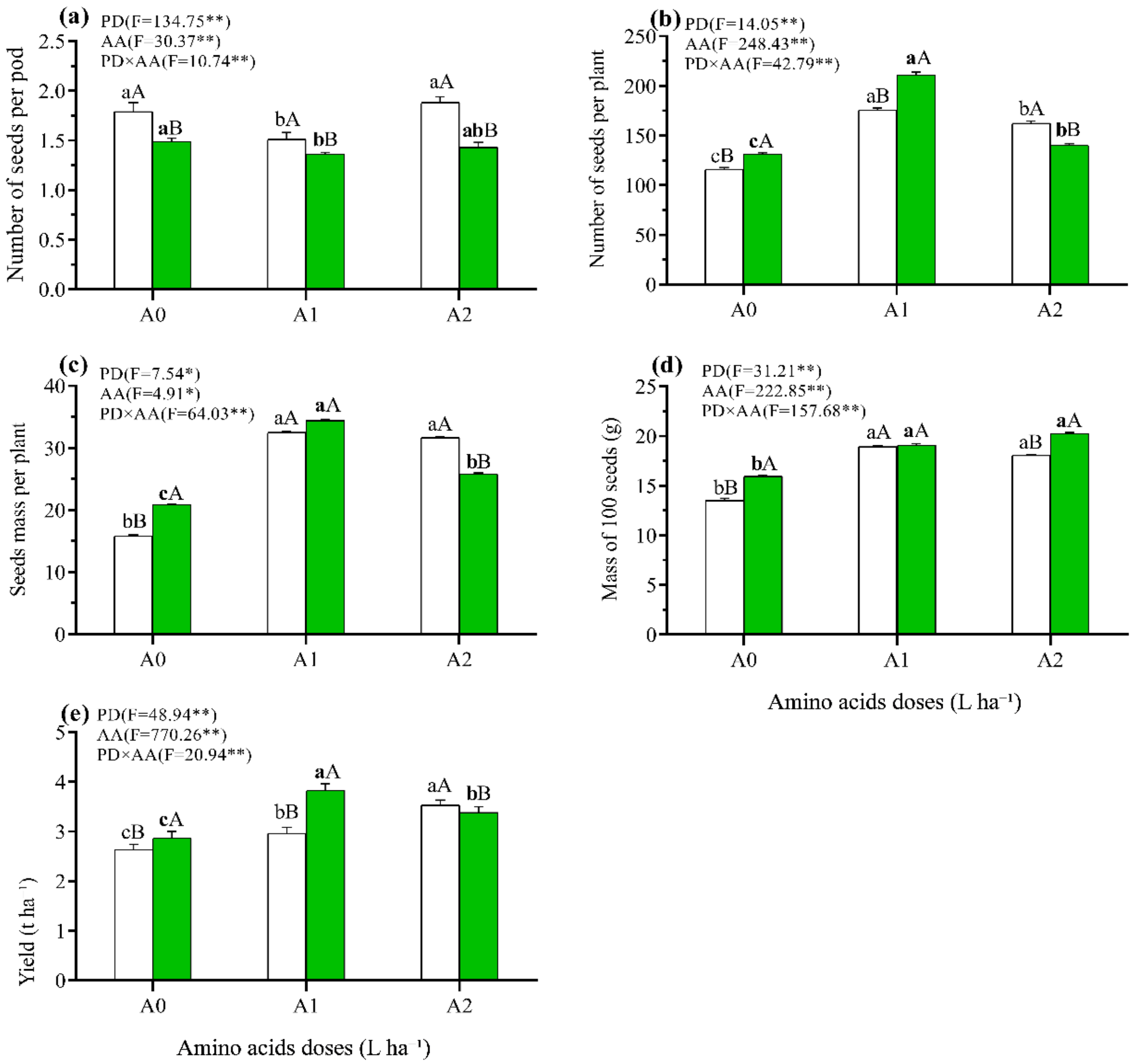
Number of branches per plant (a), number of pods per plant (b), pods with seeds (c), pods without seeds (d), percent of pods with seeds (e), and percent of pods without seeds (f). Treatments and statistics as in Fig. 1.

Figure 3. *Effect of plant densities and amino acid doses on pod characteristics of the soybean crop*

The average %PWS showed higher values under D2 treatment than D1 treatment in all AA treatments (Figure 3e). In addition, the A1 and A2 treatments showed similar effects (p = 0.21) on %PWS under D1 and D2 treatments and increased the %PWS by 12% and 10%, respectively, compared to the A0 treatment (Figure 3e). Likewise, the mean of %PWG was decreased by the D2 treatments compared to the D1 treatments in all AA treatments ($p < 0.01$). Additionally, under both D1 and D2 treatments, the A1 treatment showed the lowest %PWG compared with the A0 and A2 treatments; nevertheless, the A2 treatment decreased the %PWG on both D1 and D2 treatments compared to the A0 treatment (Figure 3f).

A two-way ANOVA showed significant interaction between PD and AA factors on NSP ($p < 0.01$), GP ($p < 0.01$), MG ($p < 0.01$), M100 ($p < 0.01$), and YD ($p < 0.01$) (Figure 4a-e). The NSP mean was higher at the D1 treatments concerning the D2 treatment in all AA treatments (Figure 4a). Under the D1 treatment, the NSP was significantly ($p < 0.001$) increased in the A1 treatment compared to the A0 and A2 treatments, but the A2 treatment showed higher NSP ($p < 0.001$) than the A0 treatment. However, under D2 treatment, the highest average of NSP was achieved in the A1 and A2 treatments compared to the A0 treatment ($p < 0.012$; Figure 4a).

The number of seeds per pod (NSL) was higher under D2 treatment and showed a significant difference ($p < 0.01$) compared to the D1 treatment (Figure 4b). Similarly, the NSL in the A1 and A2 treatments under D1 treatment showed similar effects ($p = 0.24$) and was significantly higher than in the A0 treatment; meanwhile, under D2 treatment, the NSL exhibited significantly ($p < 0.01$) superior effects in the A1 treatment relative to the A0 and A2 treatments, but the A2 treatment was significantly ($p < 0.03$) higher compared to the A0 treatment (Figure 4b).



Number of seeds per pod (a), number of seeds per plant (b), seed mass per plant (c), 100-seed mass (d), and yield (e). Treatments and statistics as in Fig. 1.

Figure 4. *Effect of plant densities and amino acid doses on yield and its components in soybean crops.*

The higher SMP was observed in the D2 treatment compared to the D1 treatment in all AA treatments (Figure 4c). Under D1 treatment, the A1 treatment increased the SMP compared to the A0 and A2 treatments ($p < 0.01$), although the A2 treatment showed higher SMP averages than in the A0 treatment ($p < 0.02$). However, the A2 treatment under the D2 treatment revealed higher SMP relative to the A0 and A1 treatments ($p < 0.001$), but the A1 treatment showed a significant difference with respect to the A0 treatment (Figure 4c).

The M100 was increased significantly ($p < 0.01$) under D2 treatment in relation to the D1 treatment (Figure 4d). Similarly, the A1 and A2 treatments under D1 and D2 treatment showed equal effects ($p = 0.31$) on M100 and significant differences ($p < 0.001$) compared to the A0 treatment (Figure 4d). Likewise, the yield (YD) average was higher under the D2 treatment compared to the D1 treatment in all AA treatments (Figure 4e). In addition, the A1 treatment under the D1 treatment increased the YD by 28% and 14% compared to the A1 and A2 treatments ($p < 0.01$), respectively; although, the A2 treatment significantly increased ($p < 0.03$) the YD by 13% as compared to the A0 treatment. Additionally, the A2 treatment under D2 treatment increased the YD by 32% and 14% in comparison with the A0 and A1 treatments ($p < 0.01$), respectively; however, the A1 treatment, at the same time, showed higher YD (14%) as compared to the A0 treatment (Figure 4e).

DISCUSSION

The results of this research proved that plant densities have a major impact on the growth of soybeans. According to the current study, the plant density of 300,000 plants ha^{-1} showed more structural development in the above-ground part of the soybean plants in relation to the density of 500,000 plants ha^{-1}, but the latter density exhibited a greater PH with the lower density ones. This suggests that the soybean plants responded very well to high plant density. These effects may have been due to the fact that there was better spatial uniformity and the competition for intercepting radiation, water, and nutrients was eliminated, which corroborates the above criteria (Ríos-Hilario *et al.*, 2023). Additionally, recent studies suggest that the response of soybeans to high plant density may be due to peak light interception occurring faster (Andrade, *et al.*, 2019; Andrade *et al.*, 2023). Also, current research indicates that there are positive phenotypic correlations between plant densities and soybean growth and yield (Yanes-Simón *et al.*, 2023). Similar results to those observed in the current study were recently reported in Brazil using a seeding rate of 290,000 to 345,000 plants ha^{-1} (Andrade *et al.*, 2023; Werner *et al.*, 2021).

Understanding how amino acids contribute to plant growth is essential, especially in crops like soybeans that hold significant nutritional and commercial value. In this study, we found that foliar application of a mixture of amino acids positively affected soybean plant growth. These findings align with previous research on soybean plants (Peña-Calzada et al., 2022). This can be explained because amino acids are vital for plant growth, functioning as key components of proteins, enzymes, photosynthesis, stress responses, nutrient absorption, and other essential processes (Peña-Calzada et al., 2025; Zhang et al., 2025). Our results show that the right amount of foliar amino acids is crucial for maximizing soybean growth and yield, consistent with recent studies in other plant species like sesame (Pérez-Díaz et al., 2024), peanut (Acevedo-González et al., 2025), tomato (Calero-Hurtado, Meléndrez-Rodríguez et al., 2025), and sunflower (Calero-Hurtado, Peña-Calzada et al., 2025).

Considering local and sustainable alternatives that enhance plant production can serve as an eco-friendly strategy for agricultural systems. Therefore, the management of plant density, along with the exogenous application of a mixture of amino acids, can effectively enhance soybean productivity. In this study, we observed significant modifications in soybean plants when combining plant density with the foliar application of amino acids. For example, supplying 0.50 L ha^{-1} of amino acids at the density of 300,000 plants ha^{-1} to soybean increased the PH, NLP, LAI, LDM, SDM, and AB, which indicated higher growth. These findings suggest that moderate doses of amino acids applied to under 300,000 plants ha^{-1} improve soybean growth. A possible explanation for this phenomenon is that under high density, the plants can obtain light early, and amino acid supplementation probably enhances nitrogen assimilation and metabolism, likely contributing to enhanced protein synthesis, a fundamental process for cell division and elongation in both shoot and root tissues (Calero-Hurtado, Peña-Calzada *et al.*, 2025; Zhang *et al.*, 2025).

As discussed above, the hypothesis assumes that applying 0.25 L ha^{-1} of amino acids at the density of 500,000 plants ha^{-1} showed more growth in soybean plants. These beneficial effects of the foliar application of amino acids in growing soybean plants are since by incorporating amino acids directly into the leaf tissues, the plant uses less energy and promotes the development of essential metabolic and physiological processes, with a consequent increase in plant structure (Zhang *et al.*, 2025). Similar results to those observed in this study were previously reported in soybeans under salt stress (Peña-Calzada *et al.*, 2022) and in other oilseed plants like sesame (Pérez-Díaz *et al.*, 2024), peanut (Acevedo-González *et al.*, 2025), and sunflower (Calero-Hurtado, Peña-Calzada *et al.*, 2025).

Moreover, amino acids are related to the mechanisms of plant growth and development regulation, which indicates that their application plays a significant role in promoting plant growth (Repke *et al.*, 2022). Another possible explanation for these facts is that the amino acids are rapidly metabolized to produce biologically useful substances that stimulate vegetative growth and are, therefore, of great interest in critical periods of the crop, such as the big growth period (Al-Karaki & Othman, 2023). Amino acids on plants may also be due to their hormonal effect, as they stimulate the formation of chlorophyll and indole acetic acid (IAA) and also assist in vitamin production, as well as synthesizing numerous enzyme systems (Peña-Calzada *et al.*, 2022).

On the other hand, the amino acids increased dry matter accumulation and production at both densities compared to no application, with the 0.50 L ha^{-1} dose at the density of 300,000 plants ha^{-1} and the 0.25 L ha^{-1} dose at the density of 500,000 plants ha^{-1} standing out. These positive effects of amino acids on dry matter accumulation were previously reported with the foliar application of amino acids on beet plants (Peña Calzada *et al.*, 2024) and on soybean plants (Peña-Calzada *et al.*, 2022). A possible explanation for these findings is that higher LAI increases the production of photoassimilates and other nutrients that promote nutrient accumulation (Calero-Hurtado, Meléndrez-Rodríguez *et al.*, 2025). The contribution of amino acids is one of the causes of these beneficial results in dry matter accumulation, as free amino acids are a growth-regulating factor thanks to their rapid absorption, transmission of micronutrients to the above-ground parts, and metabolization in the cell (Al-Karaki & Othman, 2023).

The results obtained in this study showed that there is a positive relationship between PD and soybean growth and productivity. Similar observations were recently reported in this crop (Ríos-Hilario *et al.*, 2023). Such increases in soybean productivity at higher PD (500,000 plants ha^{-1}) were probably caused by variations in the parameters evaluated as PH, NLP, LAI, LDM, SDM, AB, PWS, and PWG (Figures 1, 2, and 3). These effects of sowing densities on increasing soybean yield indicators have been observed previously (Andrade *et al.*, 2019). Similar studies reported that vegetative growth of soybeans was greater at higher PDs, stimulated faster LAI development and early canopy closure, and improved total biomass production (Chețan *et al.*, 2021; Ríos-Hilario *et al.*, 2023). The results obtained in this study are consistent with previous findings reported on soybean plants with increased PD (Seibert *et al.*, 2024; Yanes-Simón *et al.*, 2023).

However, the productivity achieved (1,850 kg ha^{-1}) by the highest density (24 plants/m^2) compared to the world average is relatively low (~2,782 kg ha^{-1}) (FAO, 2021) or compared to top-producing countries such as the United States, Brazil, and China, among others (Nair *et al.*, 2023). In contrast, better results than those obtained in this study at higher densities have been reported previously in soybean plants (Yanes-Simón *et al.*, 2023). These highest productivities were achieved by supplying 0.5 L ha^{-1} of this mixture of amino acid at the 300,000 plants ha^{-1} and the 0.25 L ha^{-1} dose at the 500,000 plants ha^{-1} density. These results can be explained by the fact that amino acids induce flowering, inhibit flower dropping, and increase the number of fruits per plant (Jiménez-Medina *et al.*, 2025). Similar studies in bean cultivation reported that amino acids can stimulate pod formation (Jacomassi *et al.*, 2024). A possible explanation for these results is that amino acids support the productive processes of plants, especially in germination and flowering, and are also important in the synthesis of nitrogenous bases required for the formation of new tissues (Zhang *et al.*, 2025).

Moreover, the application of amino acids has a hormonal effect on plants, stimulating the formation of chlorophyll, indole-3-acetic acid (IAA), and the production of vitamins, as well as the synthesis of numerous enzyme systems that promote flowering and fruit setting (Repke *et al.*, 2022). In addition, As are involved in mechanisms regulating plant growth and development (Al-Karaki & Othman, 2023). Further, amino acids also have a catalytic effect since they act on the fundamental enzymatic mechanisms; they carry micro-elements; and they improve fruit formation (Zhang *et al.*, 2025).

Plants can transport and absorb amino acids quickly due to their low molecular weight. They are utilized in protein synthesis, which saves a significant amount of energy that can be redirected toward increasing production (Peña-Calzada *et al.*, 2022). Finally, amino acids act by increasing certain metabolic and/or physiological expressions of plants, such as the development of different organs like roots and fruits, and they stimulate photosynthesis and reduce damage caused by stress (phytosanitary, diseases, cold, heat, toxicity, drought, etc.), which reduces growth and yield limitations. At the same time, they enhance the plants' natural defenses and improve the plants' nutritional status and hormonal balance, as well as the biological synthesis of hormones such as auxins, gibberellins, and cytokinins. All these benefits directly influence the increase in yields as a final expression of the crop cycle (Al-Karaki & Othman, 2023; Calero-Hurtado, Peña-Calzada, *et al.*, 2025; Peña-Calzada *et al.*, 2022).

The hypothesis of this study was ultimately tested and accepted, indicating that the foliar application of amino acids (VIUSID® Agro) can be an efficient alternative to modify the growth and yield parameters of soybeans sown at high plant densities during the rainy season.

CONCLUSIONS

The results of this research indicated that plant densities and the foliar application of amino acids showed significant interaction and beneficial effects on soybean growth and yield. Furthermore, plant densities revealed significant and positive effects on growth and productivity, whereas foliar fertilization with amino acids exhibited beneficial effects on soybean growth and yield. Therefore, it is suggested that applying 0.50 L ha-^{1} of amino acid under 300,000 plants ha-^{1} and supplying 0.25 L ha-^{1} of amino acid under 500,000 plants ha-^{1} is recommended. The results of this study suggest that the use of high plant densities combined with the low application of amino acids is an economical and viable strategy to increase sustainable soybean production when planting in the rainy season.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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