

Amino acids in morpho-agronomic traits of common bean varieties under water deficit in a greenhouse

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ABSTRACT

Objective: To evaluate the effect of an amino acid-based biostimulant on the growth and yield parameters of bean varieties under irrigated and drought conditions in a greenhouse.

Design/methodology/approach: A randomized complete block design with a 2×2×3 factorial arrangement was used, consisting of two moisture levels (irrigation and drought), two levels of amino acid-based biostimulant application (with and without application), and three bean varieties (Azufrado Regional 87, Azufrado Reyna, and Pinto Saltillo), with four replicates. Seed yield and its components, accumulated dry weight, and phenology were evaluated.

Results: Yield and its components, as well as dry biomass, were higher under irrigation than under drought conditions. Differences were observed among varieties in interaction with moisture levels and amino acid application levels. Regarding amino acids, no influence was detected on the traits evaluated under the experimental conditions of this study.

Limitations on study/implications: Physiological variables related to plant responses to water deficit and amino acid application were not evaluated. In addition, agronomic management should not have included the foliar application of a fertilizer containing amino acids.

Findings/conclusions: According to the analysis, amino acids had no effect on growth or yield parameters. However, drought exerted a negative effect by reducing these parameters and shortening the crop cycle. Azufrado Regional 87 showed greater stability in yield components, as did Pinto Saltillo, which outperformed the other varieties for these parameters in interaction with moisture and amino acid application levels.

Keywords: *Phaseolus vulgaris* L., biostimulant, drought, amino acids.

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the most relevant agricultural species worldwide due to its nutritional quality, its contribution to farmers' income generation, and its benefits to soil health, climate, and the resilience of agricultural systems (Abera *et al.*, 2020). In Mexico, during 2024, a total area of 1,091,125.32 ha was planted, with a



production of 723,642.32 t and an average yield of 0.66 t ha⁻¹ under rainfed conditions and 1.8 t ha⁻¹ under irrigation. Of this total area, 82.4% was cultivated under rainfed conditions, contributing only 52.3% of total production, whereas 17.9% was established under irrigation, accounting for 47.7% of production (SIAP, 2025). These data clearly demonstrate that common bean is strongly affected by abiotic factors, particularly drought, which limits crop growth and yield. Moreover, drought directly affects morphological and agronomic traits such as biomass, number of flowers, pods, and seeds per pod, variables widely used as indicators of drought tolerance (De Oliveira *et al.*, 2022). Under these conditions, reductions of up to 47% in flower number have been reported, as well as losses ranging from 21 to 65% in pods and seeds, associated with floral abscission and pod abortion (Romero-Félix *et al.*, 2019). Under these circumstances, the use of biostimulants has emerged as a strategy to mitigate the effects of abiotic stress through the activation of plant defense mechanisms. These products are defined as preparations that favorably influence crop growth, development, and yield (Kocira *et al.*, 2020). Among biostimulants, amino acids (AAs) have shown positive effects when applied foliarly under both field and greenhouse conditions, improving bean quality and productivity by promoting growth, biomass accumulation, and yield (El-Hay *et al.*, 2022). Likewise, a 13% increase in the number of seeds and pods under water stress has been reported (Villa *et al.*, 2016), as well as improved shoot and root growth in bean plants treated with AAs (Romero-Félix *et al.*, 2023). However, despite these favorable results, some reports indicate that AAs do not always induce significant effects on morphological and agronomic parameters when drought is severe, which has generated controversy regarding their effectiveness (Chahine *et al.*, 2020; Decsi *et al.*, 2024). These discrepancies may be related to factors such as crop agronomic management, the inappropriate use of biostimulants, particularly dosage, which may cause negative effects or no effect at all, as well as the application method, which must be adjusted to each crop and region in order to maximize efficiency (Li *et al.*, 2022). Despite these advances, there is still limited information on the effect of AA-based biostimulants on common bean under drought conditions, especially in studies comparing different varieties and water regimes under controlled conditions. Therefore, the effect of an amino acid-based biostimulant on the growth and yield parameters of bean varieties under irrigation and drought conditions in a greenhouse was evaluated, with the aim of providing evidence that may help clarify its impact and reduce the existing uncertainty.

MATERIALS AND METHODS

Experimental site

This research was conducted during the autumn-winter 2024-2025 growing cycle in a greenhouse at the Facultad de Agricultura del Valle del Fuerte, Universidad Autonoma de Sinaloa, located in Juan José Rios, Sinaloa (25° 45' 20.88" N, 108° 50' 22.16" W). The experimental area is located at 14 m above sea level.

Sowing and plant material

Three common bean varieties were used: Azufrado Regional 87, Azufrado Reyna, and Pinto Saltillo. Azufrado Regional 87 is an early-maturing variety with a semi-erect growth

habit (Rosales *et al.*, 2004). Azufrado Reyna has a determinate Type I growth habit, tall stature, and abundant branching; physiological maturity regularly occurs at 115 days after planting (DAP), making it the latest-maturing variety (Navarro-Sandoval, 2018). Pinto Saltillo exhibits an indeterminate prostrate Type III growth habit (Sánchez *et al.*, 2006).

Planting was carried out on November 21, 2024, in 242-cell trays, using a substrate composed of 60% soil and 40% sand. Seedlings were transplanted 14 days after transplanting (DAT) into 9-kg polyethylene bags. The bags were filled with a sandy loam soil (67% sand, 14% silt, and 17% clay), with 0.48% organic matter (OM), a bulk density (BD) of 1.5 g cm⁻³, electrical conductivity (EC) of 2.42 dS m⁻¹, pH 8.94, field capacity (FC) of 19%, and permanent wilting point (PWP) of 11%. It should be noted that neither the substrate nor the seeds were previously disinfected.

Experimental design and treatments

A randomized complete block design with a 2×2×3 factorial arrangement was used, consisting of two moisture levels (M; irrigation and drought), two levels of application of a commercial biostimulant (with and without foliar application), formulated on the basis of the amino acids glycine betaine (GB) and proline (99.59%), N (0.10%), P₂O₅ (0.05%), and K₂O (0.26%), and three common bean varieties (Azufrado Regional 87, Azufrado Reyna, and Pinto Saltillo). Four replicates were used, and the experimental unit consisted of three bags, each containing one individual plant. The biostimulant was applied foliarly beginning at the flowering stage (from 40 DAT onward), for a total of four applications at 63, 70, 77, and 83 DAT, at a rate of 2.5 g L⁻¹. The application method and the rate used were those recommended by the manufacturer.

Soil moisture treatments also began at the flowering stage and were imposed once per week. For this purpose, the bags were brought to field capacity (FC), and the bag weight at FC was taken as the initial reference weight (PIWC; 9 kg) for subsequent irrigations throughout the crop growth cycle, as well as for monitoring bag weight and the amount of water to be applied.

Each week, the amount of water lost was determined by weighing the bags, in order to add the required amount of water and restore the soil in each bag to PIWC, thereby maintaining soil moisture close to FC from planting to physiological maturity. This procedure was applied only to the irrigation treatment; under drought, irrigation was suspended from the onset of flowering (40 DAT) until physiological maturity, and only bag weight was recorded without water application.

Agronomic crop management

A foliar fertilizer (Lifer-Plus[®]), formulated on the basis of amino acids (2%), N (11.2%), P₂O₅ (9.84%), K₂O (2.55%), Fe (9100 mg L⁻¹), Mn (678 mg L⁻¹), Cu (75 mg L⁻¹), Zn (6400 mg L⁻¹), and B (240 mg L⁻¹), was applied three times at 15-day intervals (25, 40 and 55 DAT) at a rate of 2.5 mL L⁻¹, to both irrigated and drought-stressed plants in order to prevent nutrient deficiencies. Pest control for thrips (*Caliothrips phaseoli*) and whitefly (*Bemisia tabaci*) was carried out at 45 and 76 DAT with two applications of Capsikron (garlic extract, 5 mL L⁻¹) and EPA 90 (soybean seed vegetable oil, 5 mL L⁻¹).

Evaluated variables

Seed yield and its components, and phenology: Seed yield per plant (SY, g) was determined by weighing all normal seeds produced by each plant; total biomass (TB, g) was calculated as the sum of stem dry weight, leaf dry weight, root dry weight, and seed weight within the pod; harvest index (HI, %) was calculated by dividing SY by TB [$HI = (SY/TB) \times 100$]; normal pods per plant (NPP) were quantified as the total number of normal pods per individual plant, considering as a normal pod any pod containing at least one normal seed; normal seeds per plant (NSP) were quantified as the number of normal seeds produced in the normal pods of each individual plant; seeds per pod (SP) were determined by counting the number of seeds in each pod; empty pods (EP) were counted as pods that did not form any seed; aborted seeds (AS) were counted as seeds within each pod that did not reach maturity; days to flowering (F) were recorded when each individual plant presented open flowers; and days to physiological maturity (PM) were determined when 80% of the pods on each individual plant turned straw-colored.

Accumulated biomass: Leaf dry weight (LDW, g), stem dry weight (SDW, g), and root dry weight (RDW, g) were determined by collecting each plant organ from every experimental unit separately in kraft paper bags and drying them in an ECOSHEL model 9025H oven for 72 h at 70 °C.

Statistical analysis

The data were analyzed using InfoStat[®] statistical software, version 2020, in a combined analysis to determine differences among soil moisture levels (SM), amino acid application levels (AA), variety (V), and their interactions. Tukey's test ($p \leq 0.05$) was used for mean comparisons.

RESULTS AND DISCUSSION

Drought began at 40 DAT, under which soil moisture started to decline from 56 DAS onward, reducing the weight of the soil contained in the bags by 16.4% at 98 DAS relative to PIWC (Figure 1).

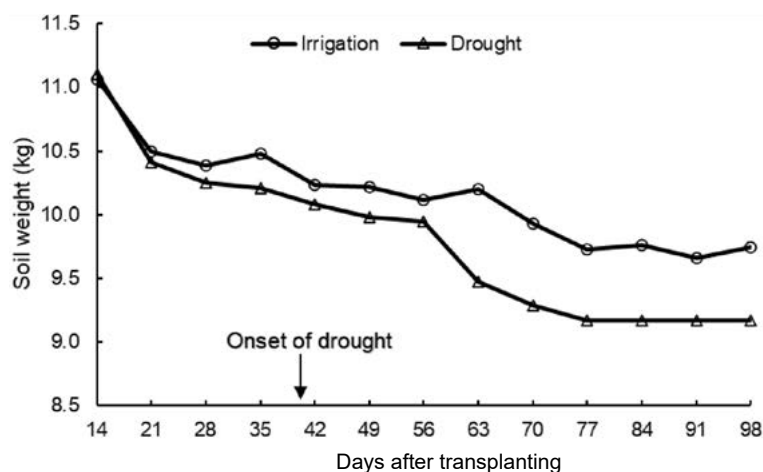


Figure 1. Behavior of the soil weight contained in the bags under irrigation and drought conditions.

Seed yield and its components, and phenology

Moisture levels significantly affected yield and its components when drought was imposed during the reproductive stage of the crop. Under irrigation, seed yield (SY, 15.56%), total biomass (TB, 81.24%), harvest index (HI, 81.34%), normal pods per plant (NPP, 75.64%), normal seeds per plant (NSP, 89.76%), and seeds per pod (SP, 68.67%) were higher than under drought conditions (Table 1). A similar trend has been reported in other studies, in which yield and its components tend to be higher under irrigation than under drought during flowering (Sandoval-Bojórquez *et al.*, 2025). Tay *et al.* (2025) found that drought significantly reduced seed yield (22.7%), shoot biomass (37%), harvest index (19.5%), number of seeds per pod (61.3%), and seed weight (10.1%) in 20 bean genotypes under field conditions. It has been observed that drought during the reproductive stage (flowering and grain filling) can reduce grain yield by 35% to 50%, whereas during the vegetative stage the reduction is lower (18-26%) (El-Gedwy, 2020; Hütsch *et al.*, 2024).

The lower yield of bean under drought conditions is associated with the increase in the number of empty pods (EP, 14.7%) and aborted seeds (AS, 33.6%) relative to irrigation. Sousa *et al.* (2022) reported that seed yield in common bean depends primarily on the number of normal seeds per plant. When the number of aborted seeds increases, seed yield declines, since these do not contribute to either seed weight or the number of harvested seeds. This effect becomes even more pronounced when bean crops are exposed to abiotic factors such as drought or to inadequate crop management, both of which increase the proportion of empty and aborted seeds, thereby reducing final yield (De Oliveira *et al.*, 2022). Drought induced an earlier reproductive period, in which the flowering and physiological maturity stages occurred three and 20 days earlier, respectively, compared with the irrigation treatment (Table 1). This, in turn, translated into a more limited yield potential (Tapia *et al.*, 2022).

Table 1. Effect of moisture levels, amino acid application levels, and bean varieties on yield and its components, and plant phenology under greenhouse conditions.

Treatment	SY	TB	HI	NPP	NSP	SP	EP	AS	F	PM
Moisture levels										
Irrigation	4.77 a	22.12 a	22.12 a	6.33 a	51.63 a	2.33 a	0.46 b	0.40 b	58 a	99 a
Drought	0.28 b	4.15 b	4.15 b	1.56 b	5.29 b	0.77 b	3.12 a	1.19 a	55 b	79 b
Tukey (p≤0.05)	0.56	1.7	1.51	0.76	6.92	0.34	0.61	0.66	2	1
Amino acid application levels										
With AA	2.51 a	13.59 a	12.55 a	3.86 a	27.50 a	1.54 a	1.79 a	0.90 a	57 a	88 b
Without AA	2.54 a	12.34 a	13.71 a	4.03 a	29.42 a	1.56 a	1.79 a	0.69 a	56 a	90 a
Tukey (p≤0.05)	0.5617	1.7	1.51	0.76	6.92	0.34	0.61	0.66	2	1
Varieties										
Pinto Saltillo	0.52 c	11.39 b	2.69 c	1.19 c	7.50 c	0.71 c	0.27 b	0.27 b	69 a	94 a
Azufrado Reyna	2.83 b	12.39 b	14.93 b	4.69 b	30.56 b	1.44 b	2.62 a	1.31 a	53 b	90 b
Azufrado Regional 87	4.23 a	15.12 a	21.78 a	5.96 a	47.31 a	2.50 a	2.48 a	0.81 ab	48 c	84 c
Tukey (p≤0.05)	0.83	2.52	2.24	1.13	10.22	0.49	0.9	0.98	3	2

SY: seed yield per plant (g); TB: total biomass (g); HI: harvest index (%); NPP: number of normal pods per plant; NSP: number of normal seeds per plant; SP: number of seeds per pod; EP: number of empty pods; AS: number of aborted seeds; F: flowering stage; PM: physiological maturity stage.

The application levels of the amino acid-based biostimulant did not generate significant differences in seed yield or its components (Table 1). Nevertheless, this lack of response does not necessarily indicate product inefficacy; rather, it may be associated with methodological and physiological factors inherent to the trial. In this regard, Al-Fahdawi and Al-Fahdawi (2024) noted that the effect of exogenous amino acid application, such as glycine betaine and proline, in bean may depend on the phenological stage, crop agronomic management, rates, and application timing, all of which directly influence their absorption and biostimulant effect. Under these conditions, experimental variability may attenuate or mask statistically detectable differences. Likewise, several studies have reported contrasting responses depending on environmental conditions and the formulation of commercial biostimulants. This suggests that the assimilation and effect of amino acids depend on the interaction among environment, agronomic management, and the type of compound applied; therefore, the lack of response observed in this study may be interpreted as a result confined to the specific experimental conditions (Decsi *et al.*, 2024). The physiological maturity stage was affected by the application of the biostimulant (glycine betaine and proline), which promoted earlier maturity by two days compared with the treatment without application (Table 1).

In other studies, amino acid application has likewise been documented to shorten the reproductive period of plants (Martínez-Lorente *et al.*, 2024; Brengi *et al.*, 2025). The varieties exhibited differential behavior in yield, its components, and phenology. Azufrado Regional 87 stood out by showing higher seed yield (SY), total biomass (TB), harvest index (HI), number of normal pods per plant (NPP), number of normal seeds per plant (NSP), and seeds per pod (SP) (Table 1). In this respect, Assefa *et al.* (2025) indicated that these differences are explained by genetic variability in the main yield components (NPP, SP, 100-seed weight, HI, and shoot biomass) and days to physiological maturity (PM). Azufrado Reyna showed the lowest SY and low values for its components, which was associated with a greater number of empty pods (EP) and aborted seeds (AS). Pekşen (2007) pointed out that there is a negative relationship between the number of AS, EP, and yield, since yield declines when the proportion of filled seeds is low. Conversely, Pinto Saltillo showed a greater number of days to flowering (F) and PM; however, this did not translate into higher yield. Although a prolonged reproductive period may increase the number of flowers and reproductive structures, its effect depends on genotype and environment (Chamorro *et al.*, 2024). In contrast, Azufrado Regional 87 exhibited a shorter reproductive period together with higher yield and productive components.

Under irrigation, with and without biostimulant application, higher values of SY and its components were observed compared with drought, regardless of amino acid use. Under irrigation, a greater number of days to F was also recorded with biostimulant application, although without statistical differences relative to irrigation without application (Table 2). In contrast, under drought, SY and its components decreased both with and without amino acid application (Table 2). These results confirm that water availability was the main limiting factor. Although amino acids have been reported to be more effective under abiotic stress, their response depends on genotype, environment, rate, and application timing, which may explain the absence of a significant effect in this study (Arteaga *et al.*,

2020; Chahine *et al.*, 2020). Under irrigation conditions, the Pinto Saltillo variety showed higher values of SY, TB, HI, NPP, NSP, and SP than the Azufrado Regional 87 and Azufrado Reyna varieties. Under drought, Pinto Saltillo maintained relatively high values for these same parameters, suggesting a smaller reduction in productive performance across environments (Table 2). Likewise, this variety recorded lower values for F and PM under both irrigation and drought.

This behavior could be associated with the presence of agronomic and phenological traits that, taken together, may favor its performance under prolonged drought conditions. In addition, its response may be related to physiological mechanisms such as greater water-use efficiency, carbon assimilation, and a more efficient root system (Tay *et al.*, 2025; Prado-García *et al.*, 2025); however, these processes were not directly evaluated and therefore require confirmation through specific studies. As in the moisture × variety interaction, the amino acid × variety interaction also revealed a differential response among genotypes. Pinto Saltillo stood out by showing higher values of SY, TB, NPP, NSP, and SP, as well as fewer days to F and PM, both with and without biostimulant application (Table 2).

Table 2. Effect of interactions on yield parameters, its components, and phenology of three bean varieties under greenhouse conditions.

Treatment	SY	TB	HI	NPP	NSP	SP	EP	AS	F	PM
Moisture levels vs. amino acid application levels										
Irrigation–without	4.82 a	19.78 a	23.24 a	6.69 a	53.42 a	2.45 a	0.64 b	0.25 a	57 ab	100 a
Irrigation–with	4.72 a	21.67 a	20.99 a	5.97 a	49.83 a	2.22 a	0.29 b	0.56 a	59 a	99 a
Drought–without	0.26 b	4.91 b	4.18 b	1.36 b	5.42 b	0.68 b	2.94 a	1.14 a	55 b	80 b
Drought–with	0.29 b	5.50 b	4.11 b	1.75 b	5.17 b	0.86 b	3.31 a	1.25 a	55 b	78 c
Tukey (p≤0.05)	1.06	3.2	2.85	1.44	13.01	0.63	1.15	1.25	3.85	1.97
Moisture levels vs. variety										
Irrigation–Pinto Saltillo	7.75 a	22.97 a	33.63 a	8.79 a	79.50 a	2.93 a	0.42 b	0.13 b	49 cd	94 c
Drought–Pinto Saltillo	0.71 c	7.21 b	9.93 c	3.13 d	15.13 c	2.08 ab	4.53 a	1.50 ab	47 d	73 f
Irrigation–Azufrado Reyna	1.04 c	19.19 a	5.37 d	2.37 b	15 c	1.42 b	0.54 b	0.42 ab	72 a	104 a
Drought–Azufrado Reyna	0.00 c	3.58 b	0.00 e	0.00 c	0.00 c	0.00 c	0.00 b	0.13 b	65 b	85 d
Irrigation–Azufrado Regional 87	5.53 b	20.01 a	27.35 b	7.83 a	60.38 b	2.65 a	0.42 b	0.67 ab	52 c	100 b
Drought–Azufrado Regional 87	0.13 c	4.78 b	2.51 de	1.54 bc	0.75 c	0.25 c	4.83 a	1.96 a	54 c	79 e
Tukey (p≤0.05)	1.45	4.39	3.89	1.97	17.81	0.86	1.57	1.71	5.28	2.69
Amino acid application levels vs. varieties										
With–Pinto Saltillo	4.51 a	16.27 a	21.3 b	6.33 a	49.50 a	2.60 a	2.71 a	0.79 a	49 bc	83 d
Without–Pinto Saltillo	3.96 ab	13.97 ab	22.25 a	5.57 a	45.13 a	2.41 a	2.25 a	0.83 a	48 c	85 d
With–Azufrado Reyna	0.45 c	12.41 ab	2.01 c	0.83 b	7.13 c	0.67 b	0.08 b	0.38 a	70 a	93 b
Without–Azufrado Reyna	0.59 c	10.37 b	3.36 c	1.54 b	7.88 c	0.75 b	0.46 b	0.17 a	67 a	96 a
With–Azufrado Regional 87	2.57 b	12.09 ab	14.34	4.42 a	25.88 b	1.35 b	2.58 a	1.54 a	53 bc	90 c
Without–Azufrado Regional 87	3.08 ab	12.70 ab	15.53 b	4.96 a	35.25 ab	1.53 b	2.67 a	1.08 a	53 b	90 c
Tukey (p≤0.05)	1.45	4.39	3.9	1.97	17.81	0.87	1.57	1.71	5.28	2.7

SY: seed yield per plant (g); TB: total biomass (g); HI: harvest index (%); NPP: number of normal pods per plant; NSP: number of normal seeds per plant; SP: number of seeds per pod; EP: number of empty pods; AS: number of aborted seeds; F: flowering stage; PM: physiological maturity stage.

This variability confirms that the response to amino acids depends on the genotype \times environment \times agronomic management interaction, since each variety differs in its capacity to absorb, redistribute, and utilize these compounds in growth processes, osmotic adjustment, antioxidant defense, and stress tolerance. Furthermore, these effects are conditioned by rate, application timing, and stress intensity (Moreira and Moraes, 2016; Ulukapi *et al.*, 2022).

Accumulated biomass

Under drought conditions, leaf dry weight (LDW), stem dry weight (SDW), and root dry weight (RDW) decreased by 57%, 94%, and 55%, respectively, compared with irrigation (data not shown). This response is consistent with reports in bean, where shoot and root dry biomass decreased by 36% to 40% under water stress, depending on stress severity and genotype (Huaman *et al.*, 2024). Conversely, amino acid application showed no significant effect on LDW or SDW. However, RDW increased by 24% relative to the treatment without amino acids (data not shown). The absence of an effect on LDW and SDW, as well as on yield and its components, may be attributed to the agronomic management applied to the plants, which included foliar applications of a fertilizer formulated with macro- and micronutrients in addition to amino acids. These compounds may have masked the effect of the amino acid-based biostimulant, since plants have a limited capacity for absorption and transport, which may in turn alter vegetative growth (Liu *et al.*, 2024). Furthermore, previous studies have demonstrated that the amino acid glycine betaine promotes root growth in several crops (Baroi *et al.*, 2024; Dong *et al.*, 2024).

The Pinto Saltillo variety exhibited greater LDW and RDW, whereas Azufrado Regional 87 stood out in SDW. These differences can be explained by the interaction among genotype, physiological traits, environment, agronomic management, and growth habit (Wilker *et al.*, 2020; Merga, 2021; Mutari *et al.*, 2023). In the moisture \times amino acid interaction, LDW and SDW were higher under irrigation, regardless of biostimulant application.

RDW was higher when irrigation was combined with amino acid application than under drought or irrigation without application (data not shown). This suggests that the effect of amino acids is greater under conditions of adequate water availability, when metabolism is not constrained (Petropoulos *et al.*, 2020). Nevertheless, Repke *et al.* (2022) and Elshamly *et al.* (2023) reported that amino acids may partially mitigate the adverse effects of stress.

Among varieties, Pinto Saltillo showed greater SDW under irrigation, whereas Azufrado Reyna exhibited higher LDW and RDW under optimal conditions. All genotypes reduced their biomass under drought, since bean adaptation to this type of stress involves changes in photoassimilate partitioning, which depends largely on the availability of soil moisture (Polania *et al.*, 2022; Galeta *et al.*, 2024). Finally, in the amino acid \times variety interaction, Azufrado Reyna showed greater LDW both with and without application, as well as higher RDW with amino acids (data not shown). This confirms that the response to glycine betaine and proline depends on genotype, rate, phenological stage, and agronomic management, which explains the observed variability (Ashraf and Foolad, 2006; Henderson *et al.*, 2025).

CONCLUSIONS

Under the conditions in which this study was conducted, amino acids had no effect on yield and its components or on biomass accumulation, except for root dry weight. Drought reduced seed yield, its components, and the dry weight of both shoot and root tissues, as well as the periods to flowering and physiological maturity. The Azufrado Regional 87 variety showed the highest values for yield and its components. In the moisture level \times amino acid application interaction, the evaluated traits were higher under irrigation, with or without amino acids, and declined under drought, even when the biostimulant was applied. In the moisture level \times variety and amino acid level \times variety interactions, Pinto Saltillo exhibited greater stability in most yield components under irrigation, regardless of biostimulant application.

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