

Effects of citrulline and humic acids on germination, vigor and biomass in *Cajanus cajan* (L.) Millsp.

Efraín Méndez-Gaona ¹

Sergio Iban Mendoza-Pedroza ^{1, §}

Eusebio Ortega-Jiménez ²

Pablo Alfredo Domínguez-Martínez ³

Patricio Sánchez-Guzmán ⁴

1 1 Posgrado en Recursos Genéticos y Productividad-Ganadería. Colegio de Postgraduados. Carretera México-Texcoco km 36.5, Montecillo, Texcoco, Estado de México, México. CP. 56264.

2 Programa de Agroecosistemas Tropicales-Campus Veracruz-Colegio de Postgraduados. Carretera Federal Xalapa-Veracruz km 88.5, Veracruz, México.

3 Campo Experimental Valle del Guadiana-INIFAP. Carretera Durango-El Mezquital km 4.5, Durango, México. CP. 34170.

4 Posgrado en Edafología-Colegio de Postgraduados. Carretera México-Texcoco km 36.5, Montecillo, Texcoco, Estado de México, México. CP. 56264.

Autor para correspondencia: sergiomp@colpos.mx

Abstract

Pigeon peas, *Cajanus cajan* (L.) Millsp., are a legume of great nutritional importance due to their nutritional quality and ability to adapt to adverse conditions. The study was conducted in 2024 at the College of Postgraduates, Montecillo, Texcoco, State of Mexico, with the aim of evaluating the effects of citrulline and humic acids, applied as treatments, on germination, vigor and biomass accumulation in *Cajanus cajan* (L.) Millsp., under greenhouse conditions. Four treatments were evaluated: control without treatment, citrulline (1 g L^{-1}), humic acids (1 ml L^{-1}), and the combination of citrulline + humic acids, using a completely randomized design. The germination variables evaluated, such as the number of seeds germinated, germination percentage, mean germination time, mean germination rate, germination uncertainty index, and germination synchrony index, did not show significant differences between treatments, indicating that the seed had high viability. At 120 days after sowing, significant differences were observed in leaf dry matter accumulation, with greater values in treatments with citrulline and citrulline + humic acids. The slenderness index did not differ between treatments; however, the plant vigor index was higher in the combination of citrulline + humic acids compared to the control. The results indicate that, although pre-germination treatments did not affect germination, citrulline promoted the accumulation of leaf biomass and, when combined with humic acids, promoted greater plant vigor.

Keywords:

biostimulant, feasibility, fodder, germplasm.

Introduction

Pigeon peas, *Cajanus cajan* (L.) Millsp., are a shrub legume with diverse uses in agricultural and food systems, valued for its tolerance to semi-arid conditions and its potential as a multipurpose crop (grain, fodder and green manure), with biomass production of up to 24 t ha⁻¹. In the literature, *Cajanus cajan* (L.) Millsp. is described as a relevant agronomic option for marginal areas due to its edaphoclimatic adaptation and its protein contribution of up to 22% in human and animal diets (Atuna *et al.*, 2023; Bopape *et al.*, 2023).

In addition, this crop participates in the biological fixation of nitrogen, with rates of 80 to 230 kg ha⁻¹, which improves soil fertility and contributes to the sustainability of cultivation systems by reducing the use of synthetic nitrogen fertilizers (Bopape *et al.*, 2023).

Germination and seedling vigor are critical factors in the establishment and final yield of crops; the speed of implantation, uniformity of emergence, and the ability to tolerate abiotic stress in the initial stage directly influence productivity (Reed *et al.*, 2022). In many legumes, dormancy due to impermeability of the seed testa limits the imbibition process, with the consequent decrease in the speed and synchrony of germination.

In some plant species, the structure and composition of the seed integument cause impermeability; nevertheless, there are field and laboratory methods that accelerate emergence and germination, including mechanical and chemical scarification, heat or osmotic treatments, and the use of biostimulants (Wen *et al.*, 2024).

Among biostimulants, citrulline has been described as a non-protein amino acid involved in nitrogen metabolism, osmotic functions and redox regulation, and it also participates in repair pathways and in nitrogen transport to growing tissues in *Cucurbitaceae* (Joshi and Fernie, 2017; Song *et al.*, 2020).

Physiological and transport studies detected the presence of citrulline in sap and young tissues, suggesting its role in mobilizing nitrogenous compounds during germination and early growth in *Juglans regia* L. (Mapelli *et al.*, 2001; Song *et al.*, 2020).

On the other hand, humic acids obtained from organic matter have promoting effects on root physiology, nutrient absorption, and enzymatic activity related to the antioxidant protection of seeds and seedlings. Recent experimental studies show increased germination rates and seedling vigor after applications of humic acids across different crops and stress conditions (Basahi, 2021; Yang *et al.*, 2023).

Although there is evidence supporting the use of citrulline and humic acids as establishment promoters in various species, such as cucurbits and grasses, the number of studies focused on legumes, particularly *Cajanus cajan* (L.) Millsp., remains limited.

Based on the above, this study aimed to evaluate the effects of applying citrulline and a commercial humic acid on the parameters of germination, emergence uniformity, and initial development of *Cajanus cajan* (L.) Millsp. seedlings under greenhouse conditions.



Materials and methods

The research was conducted in a greenhouse of the Center for Training and Technological Innovation (CCIT, by its Spanish initialism) of the College of Postgraduates-Montecillo Campus, located in Montecillo, Texcoco, State of Mexico.

The present study used seeds harvested five months before the research was established, collected from plants previously established on the Campus, which were subjected to different pre-germination treatments.

Pre-germination treatments were evaluated: T1= seed without pretreatment; C treatment= 1 h of immersion in 1 L of water, added with 1 g of citrulline powder (L-citrulline, Evolution®); HA treatment= 1 h of immersion in 1 L of water, added with 1 ml of Agrobeta® humic acids; and C+HA treatment= 1 h of immersion in 1 L of water, added with 1 g of citrulline + 1 ml of humic acids.

After treating the seeds, sowing was carried out. To this end, 200-300-gauge nursery bags (27 cm x 27 cm) were used and filled with soil from the 'Predio Nuevo' plot (sandy clay loam texture, pH of 7.9 and an organic matter content of 2%) on the same Montecillo Campus.

A seed was placed in each bag at a depth of two centimeters and 25 bags were assigned to each treatment. Finally, saturation irrigation was applied to promote germination and repeated every third day until the conclusion of the work.

During the evaluation period, the temperature inside the greenhouse was recorded using an RC-5+ data logger (Elitech Technologies, Milpitas, CA, USA).

Seedling emergence was monitored for 30 days after sowing, in the morning between 8 and 9 am, throughout the assessment. The above was done in order to calculate the speed of emergence (Maguire, 1962), number of germinated seeds (ngs), percentage of germination (pg), mean germination time (mgt), mean germination rate (mgr), germination uncertainty index (unc), and germination synchrony index (syn) on the platform (Kim *et al.*, 1987; Ranal and Santana, 2006; Martínez *et al.*, 2010; ISTA, 2018).

Seedlings were considered to have emerged once the plumule hook was observed at the soil surface and the process of photosynthetic autotrophism initiated (Forcella *et al.*, 2000).

On day 120 after sowing (DAS), a destructive sampling was carried out, in which five plants were randomly selected per treatment. In this sampling, plant height (cm), number of trifoliate leaves and diameter of the root neck (mm) were measured.

This information was used to calculate the slenderness index (SI) employing the following equation:

$$SI = \frac{\text{Plant height (cm)}}{\text{Diameter of the root neck (mm)}}$$

In addition, the plant vigor index (PVI) was determined with the equation proposed by Kim *et al.* (1987):

$$PVI = (\% \text{ germination}) * (\text{plant height}) \text{ in cm.}$$

On the other hand, the dry matter of the morphological components (roots, stems, and leaves) was also obtained. Dry matter was obtained by recording the fresh weight (g) of each component on a KMT® Style analytical balance, with a margin of error of ± 0.1 g and ± 0.01 g.

The samples were placed in paper bags identified by the morphological component and dried in a forced-air drying oven at 55 °C for 72 h until a constant weight was reached; thereafter, only the difference between the fresh and dry weights was calculated.

The analysis of variance of the data considered a completely randomized experimental design with five replications per treatment. The comparison of means was made using Tukey's test ($p < 0.05$). Statistical analyses were performed with SigmaPlot 15 (Systat Software, San Jose, California, USA).

Results and discussion

The results (Table 1) indicate that none of the germinative variables presented significant differences ($p > 0.05$) between treatments.

Table 1. Parameters of seed germination of *Cajanus cajan* (L.) Millsp.

Treatment	ngs	pg	mgt	mgr	unc	syn
T1	3.2 ±0.58	64 ±11.6	5.4 ±1.32	0.32 ±0.16	1.1 ±0.38	0.3 ±0.18
C	3.8 ±0.73	76 ±14.6	3.2 ±0.91	0.46 ±0.15	1.2 ±0.36	0.1 ±0.08
HA	4.4 ±0.4	88 ±8	5.3 ±0.5	0.19 ±0.01	1.8 ±0.14	0.07 ±0.03
C + HA	4.6 ±0.24	92 ±4.89	4.1 ±0.24	0.24 ±0.01	1.7 ±0.1	0.1 ±0.01
P > F	0.264	0.264	0.238	0.399	0.221	0.478

Number of germinated seeds (ngs); percentage of germination (pg); mean germination time (mgt); mean germination rate (mgr); germination uncertainty index (unc), and germination synchrony index (syn) in *Cajanus cajan* (L.) Millsp. seeds subjected to different pre-germination treatments.

The lack of effect of pre-germination treatments on germination could be explained by the high initial viability of the seed, which was used five months after harvest and because the experimental conditions favored germination, thereby narrowing the response window to biostimulants applied to the seed, a phenomenon that is similar to that observed in other studies with germplasm of high physiological quality in corn and sorghum (Nicchio *et al.*, 2013; Vendruscolo *et al.*, 2014; Rodrigues *et al.*, 2017).

In the analysis of growth and biomass accumulation at 120 days after sowing (Table 2), only the proportion of dry matter in leaves (DM-l) showed significant differences among treatments ($p = 0.002$). Treatments with citrulline and the combination of citrulline + humic acids showed greater accumulation of leaf mass compared to the control. This suggests a physiological effect of citrulline on the partitioning of biomass towards leaves in *Cajanus cajan* (L.) Millsp.

Table 2. Morphological parameters.

Treatment	H	NL	SD	DM-l	DM-s	DM-r
T1	71.4 ±4.8	22 ±5.7	0.59 ±0.04	32 ±1.3 bc	33 ±2.5	34.2 ±1.2
C	80.4 ±4.9	31 ±3.4	0.74 ±0.04	38.3 ±1.9 a	28.4 ±1.2	31 ±1.8
HA	80.6 ±4.5	18.6 ±3.1	0.6 ±0.02	30.3 ±0.8 c	26.5 ±1.1	32.4 ±1.2
C + HA	82.4 ±3.1	25 ±3.3	0.72 ±0.04	35.8 ±0.7 ab	42 ±6.9	32.6 ±2
P > F	0.328	0.214	0.055	0.002	0.051	0.596

Plant height (H, cm); number of leaves (NL); root neck diameter (SD, mm); dry matter in leaves (DM-l, %); dry matter in stem (DM-s, %); dry matter in root (DM-r, %) in *Cajanus cajan* (L.) Millsp. plants obtained from seeds subjected to different pre-germination treatments 120 days after sowing. The values presented are the mean of five observations and their standard error.

Citrulline acts as a compatible osmolyte, a chelator of reactive oxygen species and a modulator of amino acid metabolism and other nitrogen-related pathways; these mechanisms can increase biomass under limited nitrogen conditions (Joshi and Fernie, 2017; Song *et al.*, 2020).

In addition, recent research shows that citrulline priming and exogenous citrulline application improve growth parameters and tolerance to abiotic stress (Song *et al.*, 2020; Farooq *et al.*, 2025). Nonetheless, under non-stressful conditions, the effect on germination is usually small (Song *et al.*, 2020; Farooq *et al.*, 2025), which is consistent with the results observed in this research.

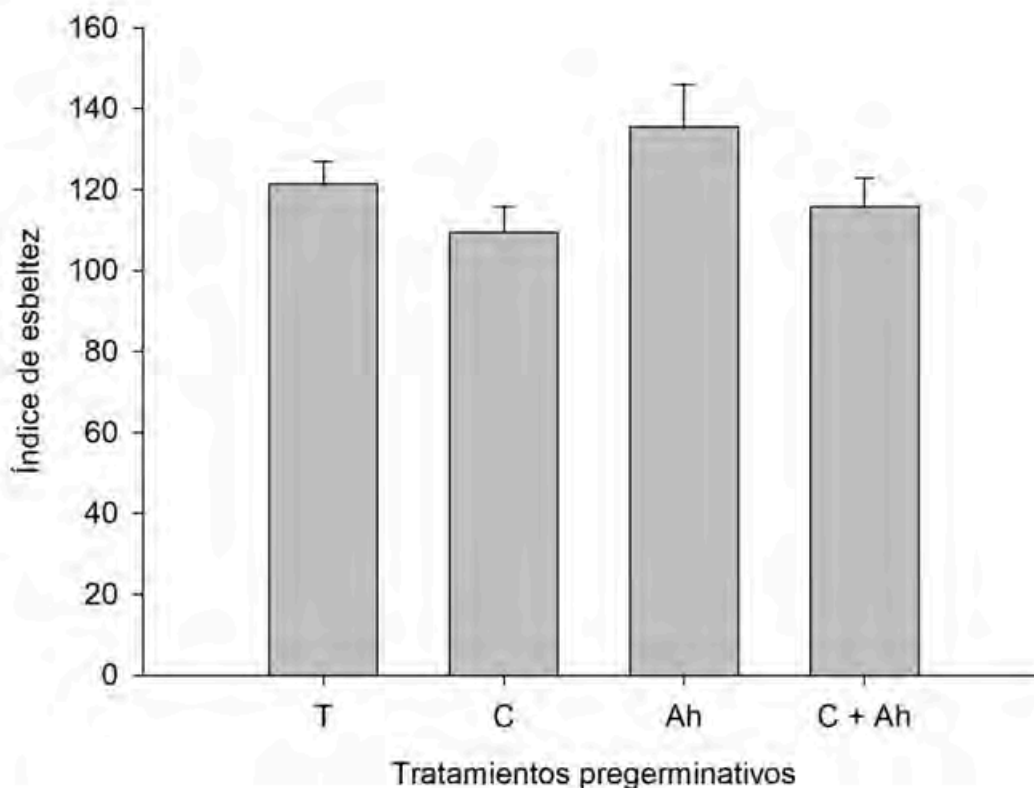
Regarding the effects of humic acid, its effectiveness has been shown to depend on the source, molecular structure, dose and crop conditions. In addition, positive effects on growth, biomass, root architecture and nutrient use efficiency tend to manifest in seedling development rather than in the germination of seeds with high viability (Nunes *et al.*, 2019; Nardi *et al.*, 2021; Yang *et al.*, 2023; Vitti *et al.*, 2024).

Recent work shows that certain humic acid fractions regulate plasma membrane H^+ -ATPase activity, Ca^{2+} signaling, and TOR kinase activation (target of rapamycin), all of which stimulate initial root growth (Canellas *et al.*, 2022; Nabi *et al.*, 2025; Santos *et al.*, 2025).

Such mechanisms explain why, even without an effect on germination, the presence of humic acids can indirectly increase seedling development and biomass accumulation observed in some of the treatments used in the present study (Nardi *et al.*, 2021; Yang *et al.*, 2023).

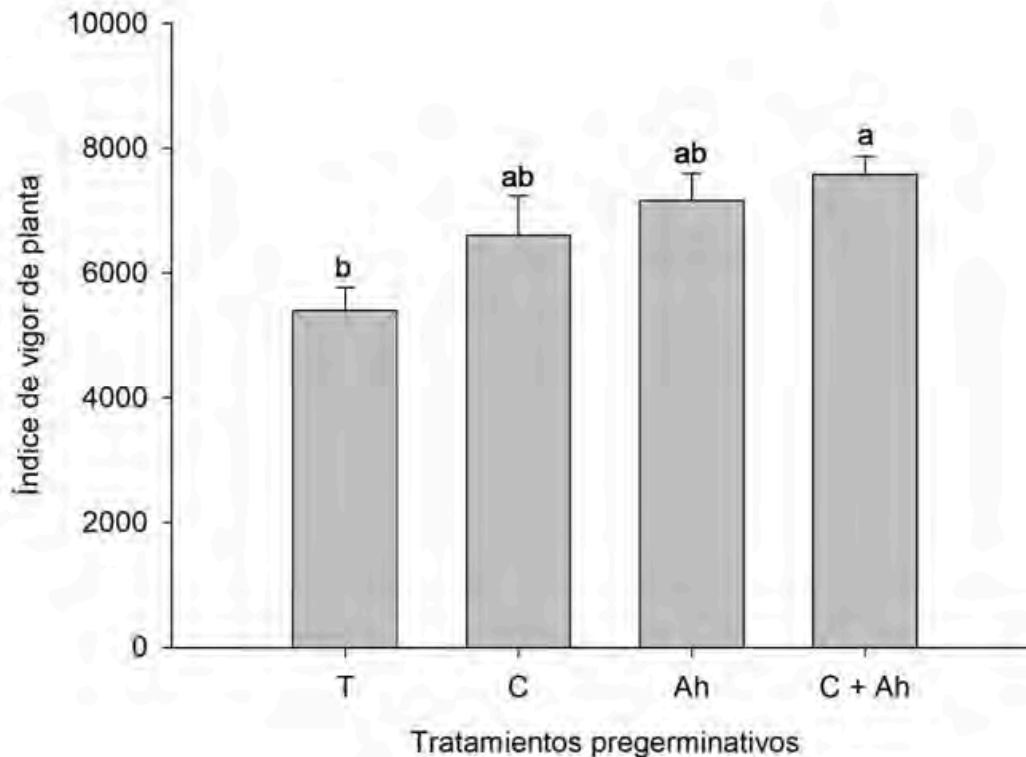
The slenderness index showed no significant differences ($p > 0.05$) among seedlings obtained from seeds subjected to different pre-germination treatments (Figure 1). This indicates that the root neck diameter/height ratio remained stable in all treatments evaluated.

Figure 1. Slenderness index in *Cajanus cajan* (L.) Millsp. plants obtained from seeds with different pre-germination treatments (control= without pre-germination treatment; C= 1 h of immersion in 1 L of water added with 1 g of citrulline powder (L-citrulline, Evolutionv); Ah= 1 h of immersion in 1 L of water added with 1 ml of Agrobeta® humic acids; and C+Ah= 1 h of immersion in 1 L of water added with 1 g of citrulline + 1 ml of humic acids, at 120 days after sowing.



In contrast, the plant vigor index showed significant differences ($p = 0.018$). Plants originating from seeds treated with the C + HA combination exhibited superior vigor compared with the control (Figure 2).

Figure 2. Plant vigor index in *Cajanus cajan* (L.) Millsp. plants obtained from seeds with different pre-germination treatments (control= without pre-germination treatment; C= 1 h of immersion in 1 L of water added with 1 g of citrulline powder (L-citrulline, Evolution®); Ah= 1 h of immersion in 1 L of water added with 1 ml of Agrobeta® humic acids; and C+Ah= 1 h of immersion in 1 L of water added with 1 g of citrulline + 1 ml of humic acids), at 120 days after sowing.



This result suggests a synergistic interaction between citrulline and humic acids that favors vigor attributes in the medium term, possibly through an improvement in antioxidant protection (by citrulline) and stimulation of nutrient acquisition and root remodeling (by humic acids).

Recent observations support the hypothesis of synergy between biostimulant amino acids and humic substances, as they enhance plant response under stress conditions and improve nutritional efficiency (Canellas *et al.*, 2022; Nabi *et al.*, 2025).

Conclusions

The application of citrulline favored the accumulation of leaf dry matter, and humic acids are more relevant during the seedling development phase and in root remodeling during germination; however, when combined with citrulline, they showed positive effects on plant vigor.

This offers the possibility of applying citrulline and humic acids as a biostimulation strategy, with a greater probability of observing positive effects when the seed has low viability or when seedlings are under stress or nutritional limitations.



Bibliography

- 1 Atuna, R. A.; Mensah, M. A. S.; Koomson, G.; Akabanda, F.; Dorvlo, S. Y. and Amagloh, F. K. 2023. Physico-functional and nutritional characteristics of germinated pigeon pea (*Cajanus cajan*) flour as a functional food ingredient. Reino Unido. Scientific Reports. 13(1):16627. <https://doi.org/10.1038/s41598-023-43607-8>.
- 2 Basahi, M. 2021. Humic acid improved germination rate, seedling growth and antioxidant system of pea *Pisum sativum* L. var. Alicia grown in water polluted with CdCl₂; Estados Unidos. AIMS Environmental Science. 8(4):358-370.
- 3 Bopape, F. L.; Beukes, C. W.; Katlego, K.; Hassen, A. I.; Steenkamp, E. T. and Gwata, E. T. 2022. Symbiotic performance and characterization of pigeonpea *Cajanus cajan* L. Millsp. rhizobia occurring in South African Soils. Suiza. Agriculture. 13(1):30. <https://doi.org/10.3390/agriculture13010030>.
- 4 Canellas, L. P.; Canellas, N. O. A.; da Silva, R. M.; Spaccini, R.; Mota, G. P. and Olivares, F. L. 2022. Biostimulants using humic substances and plant-growth-promoting bacteria: effects on cassava *Manihot esculentus* and okra *Abelmoschus esculentus* yield. Suiza. Agronomy. 13(1):80- <https://doi.org/10.3390/agronomy13010080>.
- 5 Farcella, F.; Benech R. L.; Sánchez, R. A. and Ghera, C. M. 2000. Modeling seedling emergence. Países Bajos. Field Crops Research. 67(2):123-139. <https://www.sciencedirect.com/science/article/abs/pii/S0378429000000885>.
- 6 Farooq, U.; Ashraf, M. A.; Iqbal, M. and Rasheed, R. 2025. Effect of citrulline seed priming on growth, photosynthesis, redox equilibrium, secondary metabolic enzymes, metal sequestration and nutrient acquisition in sunflower *Helianthus annuus* L. under chromium toxicity. Suiza. BioMetals. 38(6):1991-2018. [https://www.semanticscholar.org/paper/Effect-of-citrulline-seed-priming-on-growth%2C-](https://www.semanticscholar.org/paper/Effect-of-citrulline-seed-priming-on-growth%2C-.).
- 7 ISTA. 2018. International Seed Testing Association International rules for seed testing. International Association Seed Testing Association Suiza. 300 p.
- 8 Joshi, V. and Fernie, A. R. 2017. Citrulline metabolism in plants. Estados Unidos. Amino Acids. 49(9):1543-1559. <https://link.springer.com/article/10.1007/s00726-017-2468-4>.
- 9 Kim, S. H.; Choe, Z. R. and Kang, J. H. 1987. Vigor determination in barley seeds by the multiple criteria. Corea del sur. Korean Journal of Crop Science. 32(4):417-424. <https://www.koreascience.or.kr/article/JAKO198711922896118.view>.
- 10 Maguire, J. D. 1962. Speed of germination: aid in selection and evaluation for seedling emergence and vigor. Estados Unidos. Crop Science. 2(1):176-177. <https://doi.org/10.2135/cropsci1962.0011183X000200020033xDigital>.
- 11 Mapelli, S.; Brambilla, I. y Bertani, A. 2001. Aminoácidos libres en nueces y plántulas jóvenes. Italia. Fisiología del Árbol. 21(17):1299-1302. <https://www.mendeley.com/catalogue/f116f090-d1be-3f0d-a2a6-3d2b0c453451/>.
- 12 Nabi, F.; Sarfaraz, A.; Kama, R.; Kanwal, R. and Li, H. 2025. Structure-based function of humic acid in abiotic stress alleviation in plants: a review. Suiza. Plants. 14(13):1916. <https://doi.org/10.3390/plants14131916>.
- 13 Nardi, S.; Schiavon, M. and Francioso, O. 2021. Chemical structure and biological activity of humic substances define their role as plant growth promoters. Suiza. Molecules. 26(8):2256. <https://www.mdpi.com/1420-3049/26/8/2256>.
- 14 Nicchio, B.; Boer, C. A.; Siqueira, T. P.; Vasconcelos, A. C. P.; Resende, W. S. y Lana, R. M. Q. 2013. Ácido húmico e bioativador no tratamento de sementes de milho. Brasil. Journal of Agronomic Sciences. Umuarama. 2(2): 61-73.
- 15 Nunes, R. O.; Domiciano, G. A.; Alves, W. S.; Melo, A. C. A.; Nogueira, F. C. S.; Canellas, L. P.; Olivares, F. L.; Zingali, R. B. and Soares, M. R. 2019. Evaluation of the effects of humic acids on maize root architecture by label-free proteomics analysis. Reino Unido. Scientific Reports. 9(1):12019. <https://doi.org/10.1038/s41598-019-48509-2>.

- 16 Ranal, M. A. and Santana, D. G. de. 2006. How and why to measure the germination process? Brasil. Revista Brasileira de Botânica. 29(1):1-11. <https://www.scielo.br/rbb/a/ZfHBJBGtpDcXzfknWfmNM4G/?format=html&lang=en>.
- 17 Reed, R. C.; Bradford, K. J. and Khanday, I. 2022. Seed germination and vigor: ensuring crop sustainability in a changing climate. Reino Unido. Heredity. 128(6): 450-459. <https://www.nature.com/articles/s41437-022-00497-2>.
- 18 Rodrigues, L. A.; Alves, C. Z.; Rego, C. H. Q.; Silva, T. R. B. D. and Silva, J. B. D. 2017. Humic acid on germination and vigor of corn seeds. Brasil. Revista Caatinga. 30(1): 149-154. <https://doi.org/10.1590/1983-21252017v30n116rc>.
- 19 Santos, M. P.; Zupunski, M.; Monda, H.; Gralian, J.; James, A.; Grossmann, G.; Lamar, R. T. and Zandonadi, D. B. 2025. Humic acids modify root architecture in *Arabidopsis* through H⁺-ATPase-dependent target of rapamycin activation in concert with a²⁺ and ROS signaling. Italia. Chemical and Biological Technologies in Agriculture. 12(1):1-15. <https://link.springer.com/article/10.1186/s40538-025-00764-4>.
- 20 Song, Q.; Joshi, M.; Di Piazza, J. and Joshi, V. 2020. Functional Relevance of citrulline in the vegetative tissues of watermelon during abiotic stresses. Suiza. Frontiers in Plant Science. 11(512):1-13. <https://doi.org/10.3389/fpls.2020.00512>.
- 21 Torres, A.; Cova, A. y Varela, D. 2008. Efecto del proceso de germinación de granos de *Cajanus cajan* en la composición nutricional, ácidos grasos, antioxidantes y bioaccesibilidad. Chile. Revista Chilena de Nutrición. 45(5):323-332. <https://www.scielo.cl/scielo.php?script=sci-arttext&pid=S0717-75182018000500323>.
- 22 Vendruscolo, E. P.; Santos, O. F. and Alves, C. Z. 2014. Substâncias húmicas na qualidade fisiológica de sementes de sorgo. Brasil. Journal of Agronomic Sciences. Umuarama. 3(2):169-177.
- 23 Vitti, A.; Coviello, L.; Nuzzaci, M.; Vinci, G.; Deligiannakis, Y.; Giannakopoulos, E.; Ronga, D.; Piccolo, A.; Scopa, A. and Drosos, M. 2024. Biostimulation of humic acids on *Lepidium sativum* L. regulated by their content of stable phenolic O# radicals. Suiza. Chemical and Biological Technologies in Agriculture. 11(2):92. <https://link.springer.com/article/10.1186/s40538-024-00613-w>.
- 24 Wen, Z.; Lu, X.; Wen, J.; Wang, Z. and Chai, M. 2024. Physical seed dormancy in legumes: molecular advances and perspectives. Suiza. Plants. 13(11):1473. <https://www.mdpi.com/2223-7747/13/11/1473>.
- 25 Yang, F.; Yuan, Y.; Liu, Q.; Zhang, X.; Gai, S.; Jin, Y. and Cheng, K. 2023. Artificial humic acid promotes growth of maize seedlings under alkali conditions. Países Bajos. Environmental Pollution. 327:121588. <https://doi.org/10.1016/j.envpol.2023.121588>.



Effects of citrulline and humic acids on germination, vigor and biomass in *Cajanus cajan* (L.) Millsp.

Journal Information
Journal ID (publisher-id): remexca
Title: Revista mexicana de ciencias agrícolas
Abbreviated Title: Rev. Mex. Cienc. Agríc
ISSN (print): 2007-0934
ISSN (electronic): 2007-9230
Publisher: Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (México)

Article/Issue Information
Date received: 01 March 2026
Date accepted: 01 June 2026
Publication date: 01 September 2026
Volume: 17
Issue: 6
Electronic Location Identifier: e4264
DOI: 10.29312/remexca.v17i6.4264

Categories

Subject: Article

Keywords:

Keywords:

biostimulant
feasibility
fodder
germplasm

Counts

Figures: 2
Tables: 2
Equations: 4
References: 25