Article **ECORFAN Journal-Bolivia**
December 2023, Vol.10 No.19 15-32

Effect of exogenous application of L-glutamic acid on agronomic values and seed quality of maize (*Zea mays* L.)

Efecto de la aplicación exógena de ácido L-glutámico sobre valores agronómicos y calidad de semillas de maíz (*Zea mays* L.)

SENDA-NÚÑEZ, Adrián Alejandro†, CASTELLANOS-HERNÁNDEZ, Osvaldo Adrián, ACEVEDO-HERNÁNDEZ, Gustavo Javier and RODRÍGUEZ-SAHAGÚN, Araceli*

Laboratorio de Biología Molecular Vegetal, Centro Universitario de la Ciénega, Universidad de Guadalajara. Avenida Universidad, No.1115, Col. Lindavista, CP. 47810, Ocotlán, Jalisco. México.

ID 1st Author: *Adrián Alejandro, Senda-Núñez* / **ORC ID:** 0009-0000-8121-3988, **CVU CONAHCYT ID:** 1178464

ID 1st Co-author: *Osvaldo Adrián, Castellanos-Hernández* / **ORC ID:** 0000-0002-1453-8149, **CVU CONAHCYT ID:** 39920

ID 2nd Co-author: *Gustavo Javier, Acevedo-Hernández* / **ORC ID:** 0000-0002-3959-9220, **CVU CONAHCYT ID:** 123260

ID 3rd Co-author: *Araceli, Rodríguez-Sahagún* / **ORC ID:** 0000-0002-1964-6675 **CVU CONAHCYT ID:** 39871

DOI: 10.35429/EJB.2023.19.10.15.32 Received: July 20, 2023; Accepted: December 30, 2023

Abstract

The application of amino acids as biostimulants in agriculture has allowed for the improvement of performance parameters in a wide variety of crops. The objective of this study was to evaluate the effects of four concentrations of L-glutamic acid in mg·L-1 (L-Glu0, L-Glu200, L-Glu400, and L-Glu800) following seed treatment and foliar sprays in rainfed maize. Both agronomic and biochemical parameters were determined, and the data obtained were evaluated through analysis of variance (ANOVA) and Duncan's mean comparison test ($P \leq 0.05$). The application of L-Glu800 significantly increased chlorophyll b content, fresh weight, dry weight, grain yield, 100-seed weight, and crude fat content of the grains. L-Glu applications also increased plant height, germination rate, kernel hardness, soluble protein content, and decreased ash content in the grain.

Plant biostimulation, Amino acid, Maize

Resumen

La aplicación de aminoácidos como bioestimulates en agricultura ha permitido la mejora de parámetros de rendimiento en una amplia variedad de cultivos. El objetivo de este trabajo consistió en evaluar los efectos de cuatro concentraciones de ácido L-glutámico en mg·L-1 (L-Glu0, L-Glu200, L-Glu400 y L-Glu800) luego del tratamiento de semillas y aspersiones foliares en maíz de temporal de lluvia. Se determinaron tanto parámetros agronómicos como bioquímicos, y los datos obtenidos fueron evaluados mediante un análisis de varianza (ANOVA) y la prueba de comparación de medias de Duncan ($P \leq 0.05$). La aplicación de L-Glu800 aumentó significativamente el contenido de clorofila b, el peso fresco, el peso seco, el rendimiento de grano, el peso de 100 semillas y el contenido de grasa cruda de los granos. Las aplicaciones de L-Glu también incrementaron la altura de la planta, la tasa de germinación, la dureza y el contenido de proteína soluble de la semilla, y disminuyeron el contenido de cenizas en el grano

Bioestimulación vegetal, Aminoácido, Maíz

Citation: SENDA-NÚÑEZ, Adrián Alejandro, CASTELLANOS-HERNÁNDEZ, Osvaldo Adrián, ACEVEDO-HERNÁNDEZ, Gustavo Javier and RODRÍGUEZ-SAHAGÚN, Araceli. Effect of exogenous application of L-glutamic acid on agronomic values and seed quality of maize (*Zea mays* L.). ECORFAN Journal-Bolivia. 2023. 10-18:15-32.

* Correspondence to Author (E-mail: araceli.rsahagun@academicos.udg.mx)
† Researcher contributing as first author.

December 2023, Vol.10 No.19 15-32

Introduction

The term "biostimulant," when referring to plant nutrition, pertains to substances or microorganisms that promote plant growth, enhance tolerance to abiotic stress, and/or improve crop quality characteristics, regardless of their nutrient content (Calvo *et al.*, 2014; Du Jardin, 2015, Iqbal *et al.*, 2021). Some of these biostimulants have the capacity to influence metabolic, physiological, and morphological processes, as well as the interaction of the plant with the ecosystem (Woo and Pepe, 2018). A biostimulant can be a molecule in its pure state or complex mixtures of substances with variable compositions. According to Du Jardin (2015), substances such as humic compounds, algae and/or plant extracts, beneficial fungi and bacteria, chitosans, biopolymers, inorganic compounds, protein hydrolysates, or nitrogenous compounds like peptides and free amino acids can be included in the category of biostimulants. Particularly, the benefits of exogenous application of free amino acids in plants have been extensively studied, with a focus on supply through foliar solutions as a method of rapid absorption (Haghighi *et al*., 2022). Raposo-Junior *et al*. (2013) reported an increase in the yield of sugarcane (*Saccharum officinarum*) when a biostimulant formulation with amino acids was applied, while Al-Karakia and Othman (2023) found that amino acid application influences biomass increase in lettuce (*Lactuca sativa*). In the same species, Noroozlo *et al.* (2019) increased vitamin and mineral content through foliar sprays with glycine and glutamine. In tomato (*Solanum lycopersicum*), the application of tyrosine, lysine, and methionine has the capacity to raise sugar content (Alfosea-Simón *et al*., 2020).

L-Glutamic acid (L-Glu) is an essential amino acid that plays various roles in plants. Beyond protein synthesis, in its free form it acts as a chelating agent, growth stimulator, and inducer of resistance to biotic and abiotic stress. It also serves as an organic nitrogen reservoir for the synthesis of other amino acids such as proline and γ-aminobutyric acid (GABA). Additionally, it functions as an inhibitor of abscisic acid in seed germination (Kong *et al*., 2015; Qiu *et al.*, 2020). L-Glu acts as a signaling molecule in stress situations (Toyota *et al.*, 2018) and can restore beneficial microbiota in flowers and the rhizosphere (Kim *et al.*, 2021).

Furthermore, foliar application of L-Glu can increase oil levels, proline content (Ahmed *et al.*, 2017), stimulate growth (Soares *et al.*, 2016), induce resistance to pathogens (Goto *et al.*, 2020), and enhance biomass, photosynthetic pigment content, protein, and nitrogen levels (Yu *et al.*, 2010).

Maize (*Zea mays*) is one of the most significant crops globally in terms of production volume and one of the crucial grains for human consumption on an international scale (Ureta *et al.*, 2020). The fruits can be harvested in their tender state for consumption as a vegetable, while the dried grain is used for direct human consumption or as a raw material for the production of oils, syrups, alcoholic beverages, and biofuels (Cooter *et al.*, 2017) (Figure 1).

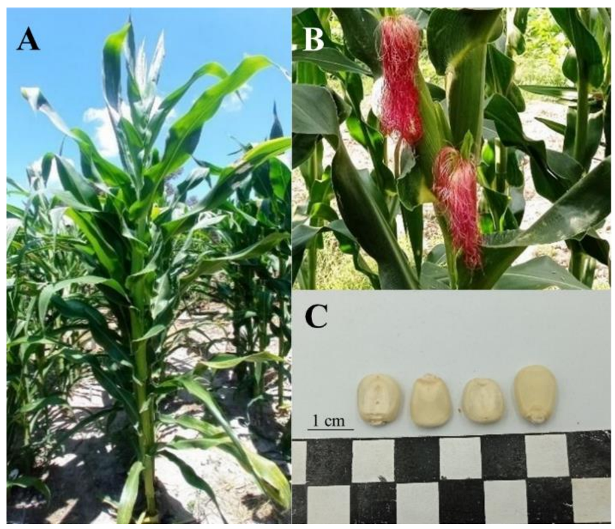


Figure 1 Zea. *mays*: A. plant; B. ears and C. dry kernels

A s Mexico is the center of origin and diversification of maize (Kato *et al.*, 2009), it stands as one of the largest repositories of genetic diversity worldwide, harboring approximately 50% of the known diversity in the American continent. Furthermore, maize is a staple food in Mexico, constituting over 50% of the caloric intake in many population sectors (SADER, 2021). This country is the eighth-largest maize producer globally, generating around 27.5 million tons of grain and 17.25 million tons of forage in the year 2021. Sinaloa, Jalisco, and the State of Mexico are the leading entities in maize production in the country (SIAP, 2022).

ISSN-On line: 2410-4191

While the benefits of L-Glu on phytochemical content and plant growth under stress conditions have been extensively studied, there is less information regarding its effects on cereal yield. The objective of the present study was to assess the effects of different concentrations of L-glutamic acid on the production of white maize under rainfed conditions.

Materials and Methods

Field Conditions

The trials were conducted under rainfed or seasonal rain conditions between June and November 2022 in the locality of Zalamea, La Barca municipality, Jalisco, Mexico, located at coordinates 20°18'42.6"N 102°30'29.7"W, at an altitude of 1533 meters above sea level. Prior to sowing, a soil chemical analysis was performed (Table 1) using the methods for the HI83325 multiparameter photometer (HANNA®). Specifically, the following reagents were employed: HI93715-03 (ammonium nitrogen), HI93717-01 (phosphate), HI93750-01 (potassium), HI937521-01 (calcium), HI937520-01 (magnesium), and HI937501-0 (sulfate). The pH was determined using a Bante920 Benchtop pH Meter (Bante Instruments®).

Concentration in parts per million (ppm)						
pH	N (NH_3)	K (K_2O)	P (PO_4^{3-})	Ca (Ca^{2+})	Mg (Mg^{2+})	S (SO_4^{2-})
6.8	0.49	22	3.05	112.5	8.5	29
6.0-7.2*	25-40**	131-175**	36-50**	>400**	>30**	>10**
*Desirable pH range for mineral soils; **Optimal ranges of nutrient concentrations (Espinoza et al., 2012; MSU, 2023).						

Table 1 Chemical soil characteristics prior to maize sowing

Inorganic fertilization with a triple 17 physical mixture (N-P-K) was applied during sowing, followed by two subsequent fertilizations using urea after the crops were established. Seed sowing was done manually on June 12th and 13th, 2022, in rows oriented from east to west, placing 9 seeds per linear meter at a depth of 5 cm and with a spacing of 75 cm between rows. To manage pests, tefluthrin was employed against the fall armyworm (*Helicoverpa armigera*) and blind chicken (*Phyllophaga* spp.).

Experimental design

The design was completely randomized with one single factor and three replications per treatment, with experimental units consisting of rows of 10 plants.

Seed treatment

Commercial white maize seeds were obtained from a local market, selecting those with healthy and uniform morphology. The seeds were subjected to a triple wash with sterile distilled water and then immersed for 1 hour in 10 mL of aqueous solutions of L-glutamic acid (Sigma-Aldrich®) at three concentrations: 200 $mg \cdot L^{-1}$, 400 $mg \cdot L^{-1}$, and 800 $mg \cdot L^{-1}$, along with a control or reference treatment (L-Glu0).

Foliar sprays

The first application of L-Glu took place 30 days after sowing (DAS) when most of the plants were in the V6-V7 stages, using a manual sprayer until reaching the dew point. The same solutions used during pre-sowing (L-Glu0, L-Glu200, L-Glu400, and L-Glu800) were applied. The second spray was conducted at 60 DAS, coinciding with the appearance of the flag leaf (stages V12-V14). These applications were carried out between 8-9 A.M.

Agronomic parameters

Plant height

The height of the plants was determined at 100 DAS, using a measuring tape. For this parameter, only the aboveground part was measured, from the root collar to the tip of the panicle (cm).

Plant weight

Fresh weight was assessed by randomly selecting three plants per treatment at 100 DAS, including their roots. The soil was carefully removed with running water, and the plants were then weighed using a digital scale (Torrey L-PRC®). For dry weight measurement, the plants were dried in partial shade for two weeks before being re-evaluated on the scale. Measurements were expressed in kilograms (kg).

Ear size

At 140 DAS, ears from each treatment were harvested, and their length and diameter were measured (cm) using a measuring tape.

Grain Yield per Plant

The harvested ears were placed in partial shade at room temperature for two weeks to reduce moisture content. Kernels of each ear were manually removed, and their total weight was determined in grams (g) using a precision analytical balance (A&D Company, Limited®).

Weight of 100 Seeds

This test involves randomly selecting and weighing 100 seeds per treatment, aiding in the estimation of seed size according to the methodology proposed by Palacios Rojas (2018). One hundred seeds were randomly chosen, and their combined weight was determined in grams (g).

Seed flotation index

Flotation index is an indirect parameter of grain hardness. The test was conducted in accordance with the specifications of Mexican Standard NMX-FF-034/1-SCFI-2002, wherein the seeds undergo flotation in a solution with a specific density (ρ) of 1.25 $g \cdot mL^{-1}$. One hundred seeds in good physical condition were selected, immersed in 500 mL of a 67% sucrose solution, stirred with a stainless-steel spatula, and allowed to rest for 1 minute (Palacios Rojas, 2018). The floating seeds were removed from the solution and counted. The flotation index was determined using the following formula:

$$Flotationindex = \frac{Floatingseeds}{Totalseeds} * 100 \quad (1)$$

Seed germination rate

Germination tests were conducted based on the methodologies proposed by Akinyosoye *et al*. (2014) and Odje *et al*. (2022) with some modifications. The seeds underwent a wash with running water and sodium dodecyl sulfate (SDS) as a detergent, followed by disinfection in 70% ethanol for 3 minutes, then immersion in a 50% commercial solution of sodium hypochlorite (NaClO) for 5 minutes, and were rinsed three times with sterile distilled water.

Finally, the seeds were germinated on autoclave-sterilized paper towels (121 °C/15 psi/15 min), moistened with sterile running water. Twenty seeds were placed on each paper towel, wrapped in transparent plastic bags, and kept in semi-darkness for germination over 7 days. Germination rate was determined using the following formula:

$$Germinationrate = \frac{Germinatedseeds}{Totalseeds} * 100 \quad (2)$$

Seedling size

Randomly selected maize seedlings from the germination test at 7 DAS were measured using a millimeter ruler, evaluating the total length of the shoot, cotyledon, and primary root in centimeters (cm).

Biochemical parameters

Photosynthetic pigments

The extraction of photosynthetic pigments was carried out following the 80% acetone method described by Ghosh *et al*. (2018) with some modifications. One hundred milligrams of fresh leaves were taken and macerated in a cold mortar with 5 mL of 80% acetone previously cooled to 4°C. Subsequently, the mixture was centrifuged at 8,000 rpm for 8 minutes, and the supernatant was poured into Falcon tubes, repeating this step until the tissue was depleted, and the supernatant became colorless. The volume was brought up to 15 mL. The absorbance of the extract was measured at 470 nm, 645 nm, and 663 nm against the solvent as a blank using a UV-Vis spectrophotometer GENESYS™ 150. Arnon's equations (1949) were used for quantifying chlorophyll a, b, and total chlorophyll, while Lichtenthaler and Wellburn's equation (1983) was employed to estimate total carotenoids in milligrams per gram of fresh tissue ($mg \cdot gft^{-1}$):

$$Cla = \frac{12.7(A663) - 2.69(A645) * V}{1000 * W} \quad (3)$$

$$Clb = \frac{22.9(A645) - 4.69(A645) * V}{1000 * W} \quad (4)$$

$$Clt = \frac{20.9(A645) + 8.02(A663) * V}{1000 * W} \quad (5)$$

$$Ctd = \frac{1000(A470) - 1.82(Cla) - 82.02(Clb)}{198} \quad (6)$$

Where:

Cla: Chlorophyll a

Clb: Chlorophyll b

Clt: Total chlorophylls

Ctd: Total carotenoids

A: Absorbance for the given wavelength

V: Final volume of chlorophyll extract in 80% acetone (mL)

W: Fresh weight of the tissue (g)

Ash content

The method 08-01 (AACC, 1995) was employed for the quantification of total ash. Whole grain flour was obtained from 10 g of harvested maize seeds, which underwent grinding using a mill (Hamilton Beach® 80393). Porcelain crucibles were used and placed in an electric muffle furnace (Terlab™) at 600 °C for 1 hour, followed by placement in a desiccator with silica gel beads for 20 minutes, until reaching room temperature. The weight of the dried crucibles was determined, and 2 g of corn flour from each treatment was added to each one. The crucibles with the samples were maintained at 600 °C for 6 hours. After the allotted time, the crucibles were returned to the desiccator for 20 minutes and allowed to cool. The residue of ashes was weighed, and the data were recorded, with the weight of the empty crucibles subtracted to obtain the ash weight. To determine the percentage of ash in each sample, the following formula was applied:

$$\%Ash = \frac{Weightofresidue(g)}{Weightofloursample(g)} * 100 \tag{7}$$

Quantification of soluble proteins

The colorimetric method by Bradford (1976) was employed to estimate the soluble protein content in the seeds. A calibration curve was constructed using bovine serum albumin (BSA, Sigma-Aldrich®) from a 0.5 mg·mL^{-1} stock solution. Aliquots of 25, 50, 75, and 100 µL were taken, diluted to a volume of 100 µL with distilled water, representing dilutions containing 12.5, 25, 37.5, and 50 µg of BSA, respectively.

Subsequently, 1 mL of Bradford reagent (Sigma-Aldrich®) was added to each dilution, and they were incubated at room temperature for 2 minutes. Absorbances were then determined using a UV-Vis spectrophotometer GENESYS™ 150 at 595 nm.

For protein extraction, 500 mg of whole grain flour was macerated in 5 mL of phosphate-buffered saline (PBS) extraction buffer in cold mortars. The mixture was centrifuged at 10,000 rpm for 10 minutes. The supernatant containing the extracted proteins was separated, and 100 µL aliquots were taken, to which 1 mL of Bradford reagent was added and incubated at room temperature for 2 minutes. Absorbances were read at 595 nm using a spectrophotometer. The results were compared with the BSA calibration curve, and the protein content was expressed as milligrams equivalent to albumin per gram of dry weight (mgEA·gdw^{-1}).

Oil content

The measurement of oil content relied on the 30-25 method (AACC 1995), involving extraction using a Soxhlet apparatus. Samples of 5 g of dehydrated whole grain flour were placed in cellulose thimbles and inserted into condenser tubes. Subsequently, 150 mL of petroleum ether (Golden Bell™) was added to the tubes, and the setups were placed on preheated magnetic stirrers with hotplate at ~70 °C. A cooling system, pumping water at 20 °C, was connected, and extraction took place for 6 hours. The recovered etheric extracts were stored at 4 °C until use. They were then double-filtered and concentrated by rotary evaporation at 60 °C/120 rpm until reaching a volume of ~5-10 mL, which was then deposited into pre-weighed glass vials. The extracts remained open at room temperature for 48 hours to evaporate the remaining solvent. The weight of the oil in milligrams (mg) per gram of dry weight (gdw) was determined using the following formula:

$$Oilcontent = \frac{Vialw/oil(mg) - Emptyvial(mg)}{Weightofloursample(g)} \tag{8}$$

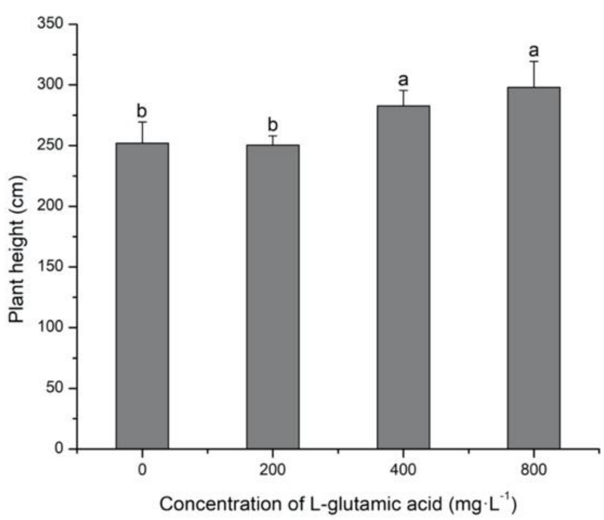
Statistical analysis

The statistical analysis was conducted using Analysis of Variance (ANOVA) and the Duncan Multiple Range Test (95.0%) to determine significant differences, employing Statgraphics Centurion XVI software. All tests were performed in triplicate.

Results and discussion

Height: The use of L-glutamic acid at concentrations of 400 and 800 mg·L^{-1} demonstrated a significant increase in the height of maize plants at 100 DAS, with an increment of 12.16% and 18.25%, respectively, compared to the control (Graphic 1).

The height of maize plants typically depends on the variety but can reach up to 4 meters (CONAHCYT, 2019). The increase in maize height usually does not exhibit significant effects when subjected to biostimulation (Blanco-Valdes *et al*., 2022), especially in hybrids, as size uniformity is a sought-after characteristic in plant breeding (Ibañez *et al.*, 2004). Nevertheless, the doses of L-glutamic acid used in this work proved to enhance the height of plants of the specific variety used. Maize plant height is also influenced by climatic and geological conditions during sowing (Gyenes-Hegyi *et al.*, 2002; Boomsma *et al.*, 2010; Liu *et al.*, 2021), water availability (Sari-Gorla *et al*., 1999), macronutrients (Iqbal *et al.*, 2015; Pedersen *et al.*, 2022), and the expression of certain genes (Pereira and Lee, 1995; Wu *et al.*, 2014).



Graphic 1 Height of maize plants (100 DAS). Mean ± SD. Different letters indicate statistically significant differences (Duncan, P ≤ 0.05)

Weight

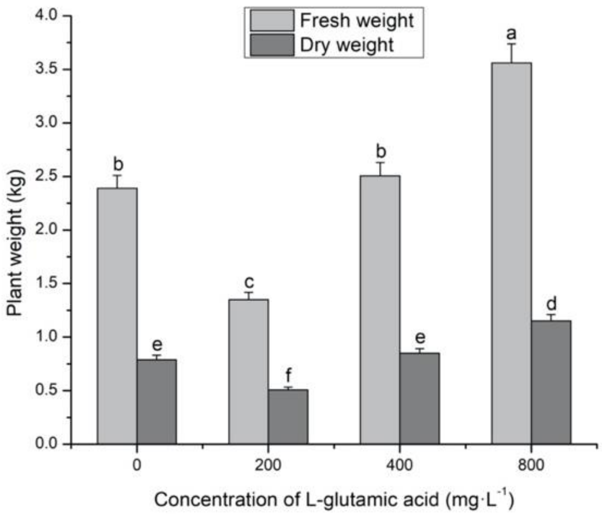
For the accumulation of plant biomass, the L-Glu 800 treatment had a significant effect increasing both fresh and dry weight values of the plants (Graphic 2).

The increase in plant weight has previously been linked to the exogenous application of amino acids. According to various studies, this can be associated with their effects on increased enzymatic activity, carbohydrate and nutrient content in leaves, and tolerance to adverse climatic conditions (Shehata *et al.*, 2011; Ragheb, 2016; Lee *et al.*, 2017; Noroozlo *et al*., 2019; Farahmandi *et al*., 2022). The shoot biomass of maize, whether fresh or dry, is utilized as forage in the livestock sector (Hanif and Akhtar, 2020), for cellulose production, ethanol, and biofuels (Manmai *et al*., 2021; Fu *et al*., 2022), or reintegrated into the soil for organic matter utilization in the no-till technique (Martínez-Gamiño and Jasso-Chaverría, 2005).

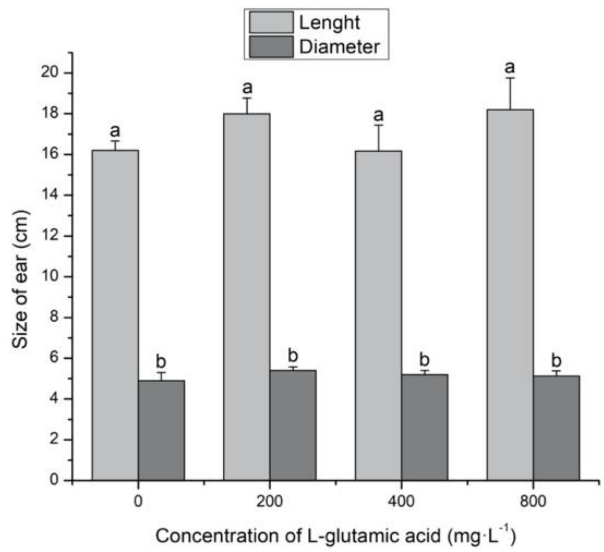
Ear size

The exogenous application of L-Glu did not significantly affect the size of maize ears in any of the three parameters evaluated in this study. Although the L-Glu800 treatment increased ear size by 12.34%, and L-Glu200 elevated values in diameter (10.2%) and the number of kernel rows (9.08%), no statistically significant differences were found in these variables (Graphic 3).

In comparison to results published in other studies involving exogenous amino acid applications (Rahgeb, 2016; Abdo *et al*., 2022), this study did not reveal a significant increase in ear dimensions. Similar results were obtained in the length and diameter of ears subjected to each treatment.



Graphic 2 Fresh and dry weight of maize plants (100 DAS). Mean ± SD. Different letters indicate statistically significant differences (Duncan, P ≤ 0.05).



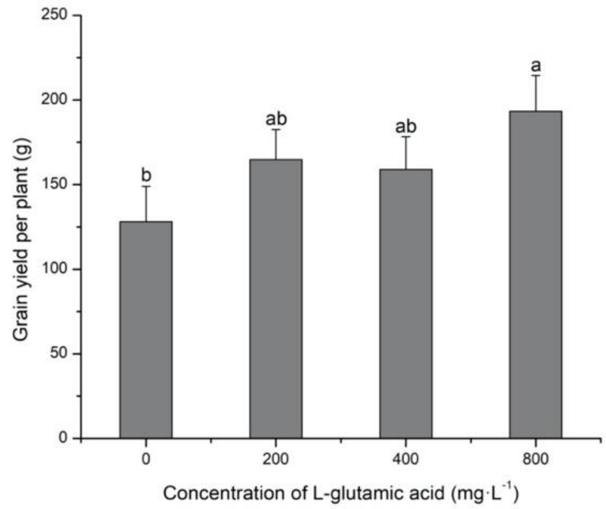
Graphic 3 Length and diameter of ears (140 DAS). Mean ± SD. Different letters indicate statistically significant differences (Duncan, P ≤ 0.05)

Yield

Although there was no significant difference between the applied levels of L-Glu (Graph 4), an increase in yields was observed for ears from plants exposed to the amino acid compared to the control, especially with the L-Glu800 treatment, which rose by up to 49.98%.

One possible explanation for the crop yield increments is the relationship between L-Glu and factors such as increased photosynthetic rate and protein synthesis (Khan *et al.*, 2012; Alfonsea-Simón *et al.*, 2021; Báez-Pérez *et al.*, 2022).

The results presented here are similar to those reported by Abdo *et al*. (2022), who achieved similar grain yields through biostimulation with humic acids and amino acids. The increase in agronomic crop yields due to amino acid application has been previously reported in various studies (Lee *et al*., 2017, Souri *et al*., 2017; Basanth and Mahesh, 2018).



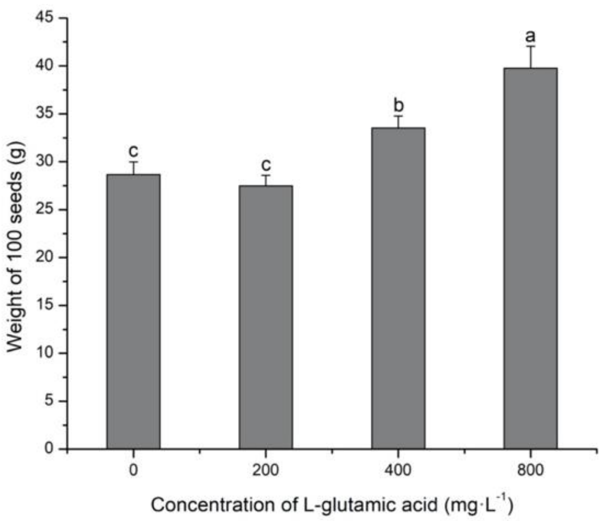
Graph 4. Grain yield per plant (140 DAS). Mean ± SD. Different letters indicate statistically significant differences (Duncan, P ≤ 0.05)

Weight of 100 seeds

The test for the weight of 100 seeds to determine grain size revealed that the application of 800 mg·L^{-1} of L-Glu acid produced the heaviest grains, and therefore the largest, with an average weight of 39.769 g, while the grains from the application of 400 mg·L^{-1} resulted in medium-sized grains, with an average weight of 33.512 g. On the other hand, the treatments of 200 mg·L^{-1} and the control produced small grains, with 27.482 g and 28.648 g, respectively, and there was no statistically significant difference between these two treatments (Graph 5).

In this study, an increase in the weight of 100 seeds was found as the L-glutamic acid dose increased, with a better response compared to other studies involving amino acid biostimulation in maize hybrids (Rahgeb, 2016; Abdo *et al*., 2022; Blanco-Valdes *et al*., 2022). According to Batistella *et al*. (2002), the weight of a thousand seeds based on the weight of a hundred seeds is the most convenient size classification method, even surpassing screening, regardless of the variety.

However, Peña-Betancourt *et al*. (2013) reported that, for example, some native maize varieties in Mexico can have an average weight of 100 seeds of 43.5 g, compared to the 29.8 g average weight in hybrid varieties.

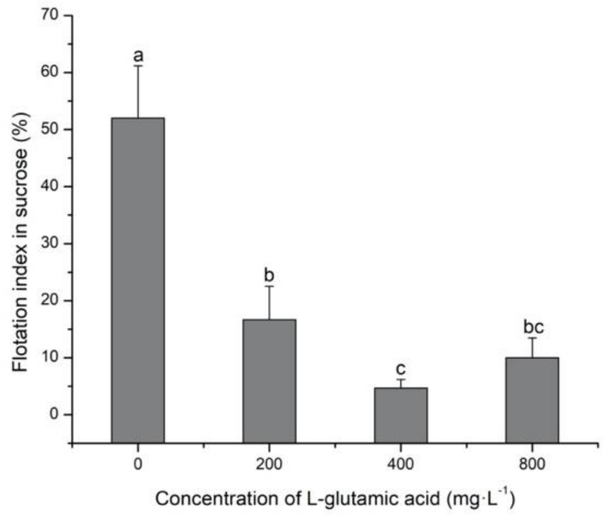


Graphic 5 Weight of 100 seeds (140 DAS). Mean ± SD. Different letters indicate statistically significant differences (Duncan, P ≤ 0.05)

Flotation index

L-Glu applications significantly decreased the flotation percentage of seeds in a 67% sucrose solution. On average, seeds from plants treated with the increasing concentrations of L-Glu had flotation percentages of 16.6%, 4.6%, and 10%, respectively, well below the 52% of control seeds (Graph 6). From this, it can be suggested that exogenous L-Glu could be involved in increasing grain density, and consequently, its hardness.

In Mexico, flotation tests are a practical and indirect way to measure the hardness of maize grain, especially when it is destined for nixtamalization, as grains with high flotation indices denote lower yields for tortilla manufacturing. Generally, this parameter increases over time due to grain senescence or storage conditions (Odjo *et al*., 2022). When the flotation index is above 63%, it is considered a soft grain, between 38-62% is a medium-hard grain, and if it is below 37%, it is classified as a hard grain (Palacios-Rojas, 2018).

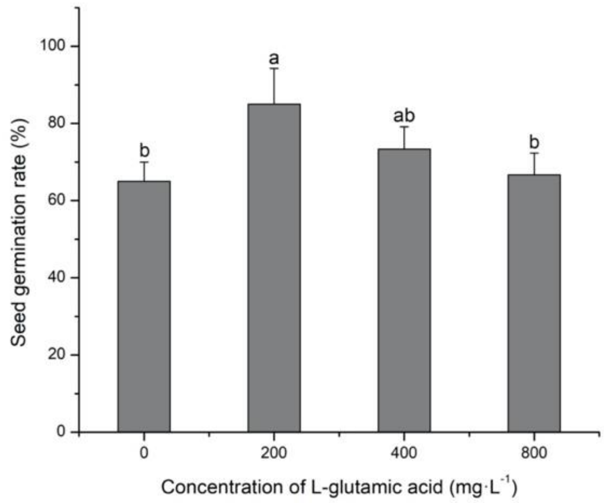


Graphic 6 Flotation index of seeds in 67% sucrose solution (ρ = 1.25 g·mL^{-1}). Mean ± SD. Different letters indicate statistically significant differences (Duncan, P ≤ 0.05)

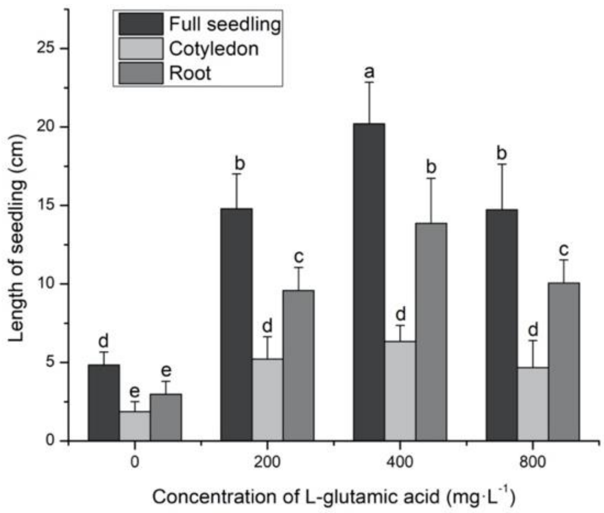
Germination rate and seedling size

Seed germination increased with two of the treatments. The application of L-Glu200 increased the germination rate by 30% compared to the control treatment, while the application of L-Glu400 increased it by 12.82%. On the other hand, L-Glu800 showed no significant difference (Graph 7). These results show a decrease in the germination rate as the administered dose of L-Glu increased.

Meanwhile, all three levels of L-Glu application significantly increased the size of the seedlings compared to the control (Graph 8). The highest averages were observed with the L-Glu400 treatment, where the complete seedlings reached an average length of 20.2 cm, the average cotyledon length was 6.34 cm, and the root length was 13.86 cm, values that were 4, 3.4, and 4.6 times higher than in the control (Figure 2).



Graphic 7 Seed germination rate (7 DAS). Mean ± SD. Different letters indicate statistically significant differences (Duncan, P ≤ 0.05)



Graphic 8 Length of seedlings (7 DAS). Mean ± SD. Different letters indicate statistically significant differences (Duncan, P ≤ 0.05)

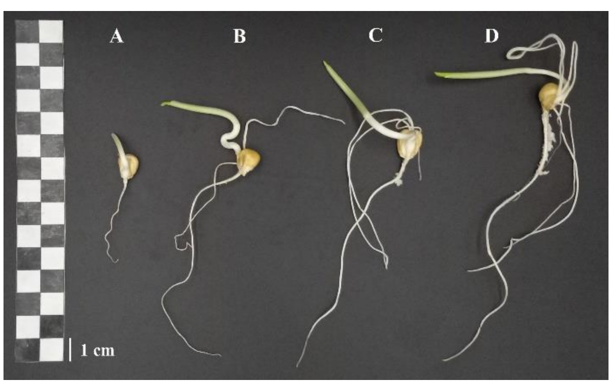


Figure 2 Comparison of maize seedlings with different treatments (7 DAS). A. L-Glu0; B. L-Glu200; C. L-Glu400; D. L-Glu800.

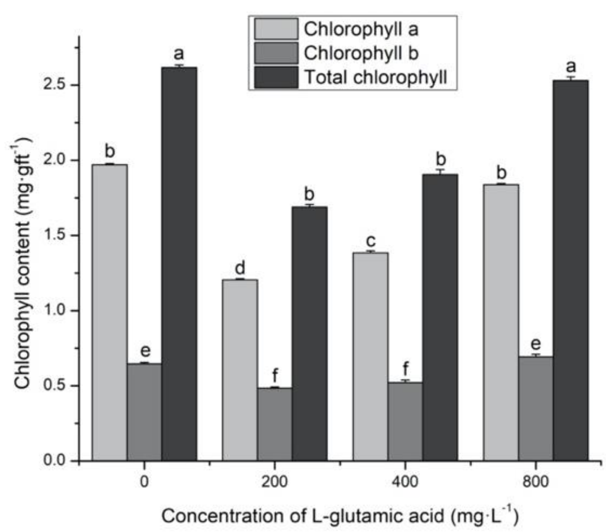
The direct effects of biostimulants on increasing seed vigor had been previously studied in other species, especially those biostimulants based on growth-promoting microorganisms (Colla *et al*., 2014; Cardarelli *et al*., 2022; Dong *et al*., 2020). Currently, there is limited information on the effects of amino acid biostimulation on seed germination (Makhaye *et al*., 2021), although some protein hydrolysates, such as collagen extract or bovine -hide gelatin, have been studied and found to promote germination and growth (Gaidau *et al*., 2013; Wilson *et al*., 2015; Niculescu *et al*., 2019).

Biochemical parameters

Chlorophylls

Evaluations of photosynthetic pigments (chlorophylls and carotenoids) revealed a decreasing trend in content in fresh leaves compared to the control, which showed the highest values for chlorophyll a (1.969 $mg \cdot g^{-1}$), total chlorophylls (2.616 $mg \cdot g^{-1}$), and total carotenoids (4.739 $mg \cdot g^{-1}$).

Chlorophyll an exhibited values below the control treatment in plants subjected to different concentrations of L-glutamic acid: 1.204 $mg \cdot g^{-1}$ with L-Glu200, 1.384 $mg \cdot g^{-1}$ in L-Glu400, 1.837 $mg \cdot g^{-1}$ in L-Glu800, representing a decrease of 38.85%, 29.71%, and 6.7%, respectively (Graphic 9). In the case of chlorophyll b, L-Glu800 showed the highest content (0.692 $mg \cdot g^{-1}$).



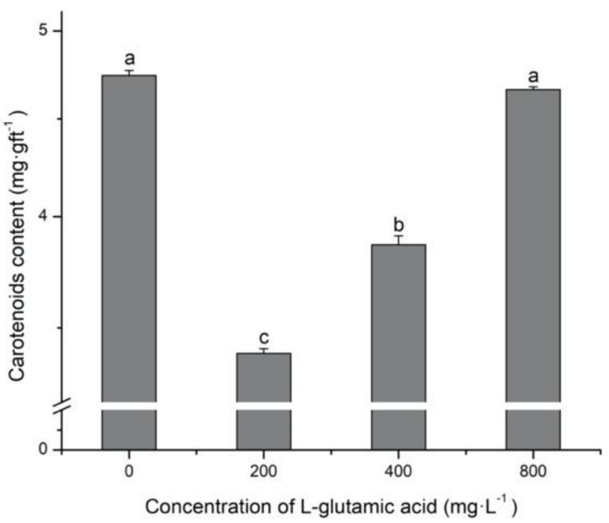
Graphic 9 Content of chlorophylls (100 DAS). Mean ± SD. Different letters indicate statistically significant differences (Duncan, P ≤ 0.05)

The total chlorophylls content (Graph 9) of the leaves revealed reductions in treatments with L-glutamic acid compared to the control (2.616 mg·g^{-1}), with values of 1.689 mg·g^{-1} in the application of 200 mg·L^{-1}, 1.906 mg·g^{-1} applying 400 mg·L^{-1}, and 2.530 mg·g^{-1} in the treatment with 800 mg·L^{-1}.

It is noteworthy that the content of chlorophyll a in plants is always higher than that of chlorophyll b, and a distinct trend can be observed in their values compared to other photosynthetic pigments. According to Serna-Rodríguez *et al*. (2011), the application of L-glutamic acid in plants increases the synthesis of chlorophyll b compared to chlorophyll a, resulting in greater photon capture, as chlorophyll b is part of the antennae responsible for light absorption.

Carotenoids

The quantification of total carotenoids (Graph 10) yielded information very similar to that obtained in the evaluation of total chlorophylls, where compared to the control, the L-Glu200, L-Glu400, and L-Glu800 treatments showed reductions of 28.4%, 18.4%, and 1.67%, respectively.

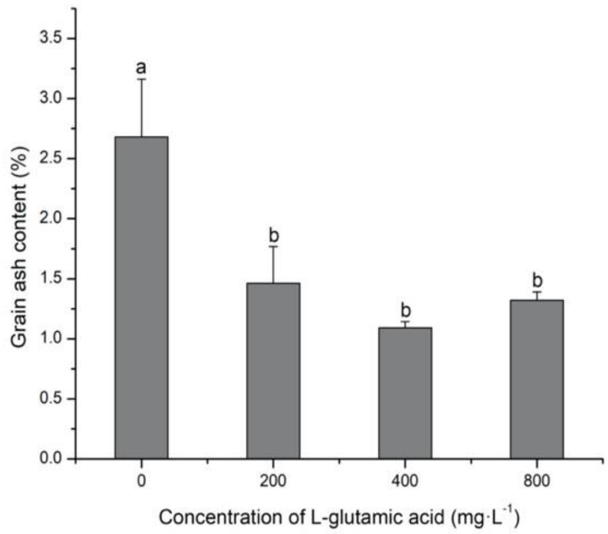


Graphic 10 Content of total carotenoids (100 DAS). Mean ± SD. Different letters indicate statistically significant differences (Duncan, P ≤ 0.05)

Ash content

The results of this test showed a significant decrease in ash content in kernels across all three doses of L-Glu. For instance, the control had an ash content of 2.86%, which was 45.43% higher compared to the treatment with the closest content, in this case, L-Glu200, with 1.46%.

The other two doses yielded very similar percentages; for instance, L-Glu400 with 1.09%, and L-Glu800 with 1.32%, showing decreases of 59.3% and 50.7%, respectively (Graphic 11).



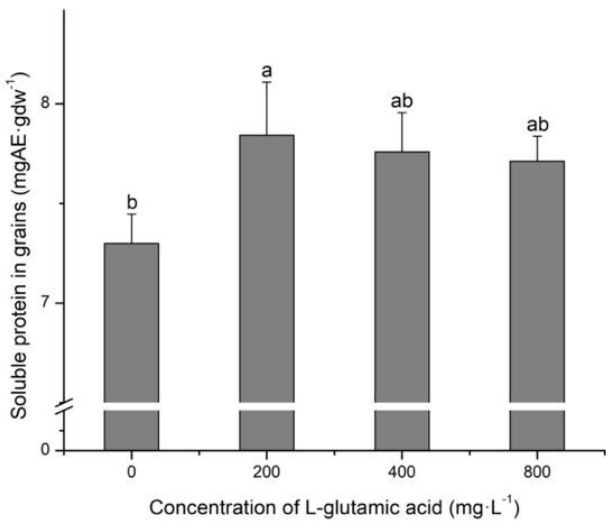
Graphic 11 Ash content in whole grain flour. Mean ± SD. Different letters indicate statistically significant differences (Duncan, P ≤ 0.05)

Ash determination is very useful for assessing the total mineral content in a food item, which is not susceptible to evaporation, as is the case with water, or oxidation in the case of organic matter (Park, 1996). The ash content in plants can be primarily affected by the mineral characteristics of the soil or the use of agrochemicals (Rashid and Iqbal, 2012; Aslam *et al*., 2023). These ashes may contain substances such as heavy metals, silicates, sulfates, or phosphates. A low ash content would indicate a higher percentage of assimilable material as food and a lower amount of potentially toxic substances for living organisms (Marshall, 2010). Besides the food industry, this property is also desirable for biofuel production, as low inorganic matter content represents higher energy efficiency during combustion (Zając *et al*., 2020; Kukuruzović *et al*., 2023). In corn, ash content in seeds typically concentrates in the germ, and its values range between 1-3%, depending on the variety and other environmental conditions (FAO, 1985; Cázares-Sánchez *et al*., 2015; Bello-Pérez *et al*., 2016; Sinay and Harijati, 2021).

Protein content

L-Glu applications increased the amount of protein in the seeds of treated plants, especially with the 200 mg·L^{-1} dose, where an increment of 7.4% was observed.

Likewise, the protein content showed a gradual decrease as the L-Glu doses of 400 and 800 mg·L^{-1} increased, with no significant difference compared to the control (Graphic 12).

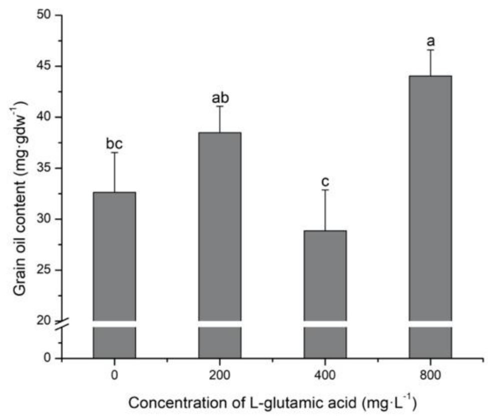


Graphic 12 Content of soluble protein in whole grain flour. Mean ± SD. Different letters indicate statistically significant differences (Duncan, P ≤ 0.05)

Given that it is a precursor for the synthesis of other amino acids and essential for polypeptide synthesis, the exogenous application of L-Glu can increase the protein content in plants. Previously, the effect of exogenous amino acid application on protein content in other plant species has been studied with positive results (Zhou *et al*., 2007; Haghighi *et al*., 2020). In crops such as Chinese hawthorn, lentil, tomato, and rice, L-Glu increased the soluble protein content when administered exogenously (Yu *et al*., 2010; Fardus *et al*., 2021; Lee *et al*., 2021; Luo *et al*., 2023). In maize, previous research had studied the effects of other biostimulants, such as valine, leucine, isoleucine, silicon, or salicylic acid, on protein content (Shaner and Reider, 1986; Moussa, 2006; Feng *et al*., 2022). Other factors influencing protein production in cereals may include planting density, soil conditions, fertilization, humidity, and temperature (Fowler *et al*., 1990; Casagrande *et al*., 2009; Chen *et al*., 2012; Széles *et al*., 2018).

Oil content

The lipid concentration in seeds increased when plants were subjected to applications of L-Glu200 and L-Glu800, although with significance only in the latter treatment, where the oil content was 35% higher than the control (Graph 13). On the other hand, L-Glu400 showed contents below the control treatment by up to 11.54%.



Graphic 13 Oil content in whole grain flour. Mean ± SD. Different letters indicate statistically significant differences (Duncan, P ≤ 0.05)

Hybrid corn seeds contain between 3-4% of oil, which is mainly concentrated in the germ and is rich in polyunsaturated fats and tocopherols (vitamin E), depending on whether it is white or sweet corn (Sanjeev *et al*., 2014; Ray *et al*., 2019). In Mexico, there are native varieties with a high oil content, where the percentage can rise to 5-6% (Torres-Morales *et al*., 2010; Guzmán-Maldonado *et al*., 2015). The lipid production in maize can be affected by variables such as temperature, planting location, humidity, or the expression of specific genes (Jellum and Marion, 1966; Shen *et al*., 2010; Veljković *et al*., 2018).

Conclusion

In general, it was observed that the application of L-Glu800, through seed treatment and foliar application in maize, increased the content of chlorophyll b, plant height, plant weight, grain yield per ear, and the weight of one hundred grains. On the other hand, no significant differences were observed in the effect of L-Glu on ear size. These studies on the biostimulating activity of L-glutamic acid allow us to refine existing alternatives that make it possible to increase the yield and quality of crops of agronomic interest. It is necessary to continue research efforts aimed at expanding the understanding of the application of free amino acids as plant biostimulants.

Funding

This work has been funded by CONAHCYT [Grant number 1178464].

Acknowledgment

The research team would like to express gratitude to the National Council of Humanities, Sciences, and Technologies (CONAHCYT), as this work was fully financed through grant 1178464.

References

Abdo, A.I., El-Sobky, E.S.E., Zhang, J. 2022. Optimizing maize yields using growth stimulants under the strategy of replacing chemicals with biological fertilizers. *Frontiers in Plant Science*, 13. DOI: 10.3389/fpls.2022.1069624

Ahmed, A.M.A., Talaat, I.M., Khalid, K.A. 2017. Soil moisture and glutamic acid affect yield, volatile oil and proline contents of oregano herb (*Origanum vulgare* L.). *International Journal of Botany,* 13: 43-51. DOI: 10.3923/ijb.2017.43.51

American Association of Cereal Chemists (AACC). 1995. Approved methods of the AACC, St. Paul, MN.

Akinyosoye, S.T.; Adetumbi, J.A.; Amusa, O.D.; Olowolafe, M.O.; Olasoji, J.O. 2014. Effect of seed size on *in vitro* seed germination, seedling growth, embryogenic callus induction and plantlet regeneration from embryo of maize (*Zea mays* L.) seed. *Nigerian Journal of Genetics,* 28 (2): 1-7. DOI: 10.1016/j.nigjg.2015.06.001

Alfosea-Simón, M., Simón-Grao, S., Zavala-Gonzalez, E.A., Cámara-Zapata, J.M., Simón, I., Martínez-Nicolás, J.J., Lidón, V., García-Sánchez, F. 2021. Physiological, nutritional and metabolomic responses of tomato plants after the foliar application of amino acids aspartic acid, glutamic acid and alanine. *Frontiers in Plant Science,* 11: 581234. DOI: 10.3389/fpls.2020.581234

Alfosea-Simón, M., Zavala-Gonzalez, E.A., Camara-Zapata, J.M., Martínez-Nicolás, J.J., Simón, I., Simón-Grao, S., García-Sánchez, F. 2020. Effect of foliar application of amino acids on the salinity tolerance of tomato plants cultivated under hydroponic system. *Scientia Horticulturae*, 272: 2-9. DOI: 10.1016/j.scienta.2020.109509

Al-Karakia, G.N., Othman, Y. 2023. Effect of foliar application of amino acids biostimulants on growth, macronutrient, total phenols contents and antioxidant activity of soilless grown lettuce cultivars. *South African Journal of Botany.* 154: 225-231. DOI:10.1016/j.sajb.2023.01.034.

Arnon, D. 1949. Copper enzymes isolated chloroplasts, polyphenoloxidase in *Beta vulgaris*. *Plant Physiology,* 24 (1): 1-15. DOI: 10.1104/pp.24.1.1

Aslam, A., Nawaz, H., Khan, A., Ghaffar, R., Abbas, G. 2023. Effect of exogenous application of citric acid on growth of maize (*Zea mays* L.) under sodium fluoride stress. *Fluoride,* 56: 329-350.

Báez-Pérez, A., Bautista-Cruz, A., Morales, I., García-Sánchez, E., Aquino-Bolaños, T., Ramírez-Cruz, M. Á. 2022. La aplicación foliar de ácido glutámico mejora el rendimiento y algunos parámetros físicos y químicos de la calidad del fruto de tomate (*Solanum lycopersicum* L.). *Interciencia*, 47(1/2): 31-38. ISSN: 2244-7776

Basanth, N., Mahesh, G. 2018. Bioefficacy of nova Nutri Boost for yield and yield components in paddy (*Oryza sativa* L.). *International Journal of Current Microbiology and Applied Sciences,* 7 (10): 2250-2253. ISSN: 2319-7706

Batistella, F. F., Moro, F. V., De Carvalho, N. M. 2002. Relationships between physical, morphological, and physiological characteristics of seeds developed at different positions of the ear of two maize (*Zea mays* L.) hybrids. *Seed Science and Technology,* 30 (1): 97-106.

Bello-Pérez, L.A., Camelo-Mendez, G.A., Agama-Acevedo, E., Utrilla-Coello, R.G. 2016. Aspecto nutracéuticos de los maíces pigmentados: digestibilidad de los carbohidratos y antocianinas. *Agrociencia,* 50 (8): 1041-1063. ISSN 1405-3195

Blanco-Valdes, Y., Cartaya-Rubio, O. E., Espina-Nápoles, M. 2022. Efecto de diferentes formas de aplicación del Quitomax® en el crecimiento del maíz. *Agronomy Mesoamerican,* 33 (3): 47246-47246. DOI: 10.15517/am.v33i3.47246

Boomsma, C. R., Santini, J. B., West, T. D., Brewer, J. C., McIntyre, L. M., Vyn, T. J. 2010. Maize grain yield responses to plant height variability resulting from crop rotation and tillage system in a long-term experiment. *Soil and Tillage Research*, 106 (2): 227-240. DOI: 10.1016/j.still.2009.12.006

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 72 (1-2): 248-254. DOI: 10.1006/abio.1976.9999

Calvo, P., Nelson, L., Kloepper, J.W. 2014. Agricultural uses of plant biostimulants. *Plant Soil*. 383, 3–41. DOI:10.1007/s11104-014-2131-

Cardarelli, M., Woo, S. L., Rouphael, Y., Colla, G. 2022. Seed treatments with microorganisms can have a biostimulant effect by influencing germination and seedling growth of crops. *Plants*, 11 (3), 259. DOI: 10.3390/plants11030259

Casagrande, M., David, C., Valantin-Morison, M., Makowski, D., Jeuffroy, M.H. 2009. Factors limiting the grain protein content of organic winter wheat in south-eastern France: a mixed-model approach. *Agronomy for Sustainable Development*, 29: 565–574. DOI: 10.1051/agro/2009015

Cázares-Sánchez, E., Chávez-Servia, J.L., Salinas-Moreno, Y., Castillo-González, F., Ramírez-Vallejo, P. 2015. Variación en la composición del grano entre poblaciones de maíz (*Zea mays* L.) nativas de Yucatán, México. *Agrociencia*, 49 (1): 15-30. ISSN 1405-3195.

Chen, Y., Wang, M., Ouwerkerk, P.B.F. 2012. Molecular and environmental factors determining grain quality in rice. *Food and Energy Security*, 1 (2): 111-132. DOI: 10.1002/fes3.11

Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCYT). 2019. Maíz. [Consultado el 13 de octubre de 2023]. Disponible en https://conahcyt.mx/cibiogem/index.php/maiz

Colla, G., Rouphael, Y., Canaguier, R., Svecova, E., Cardarelli, M. 2014. Biostimulant action of a plant-derived protein hydrolysate produced through enzymatic hydrolysis. Frontiers in Plant Science, 5: 448. DOI: 10.3389/fpls.2014.00448

Cooter, E.J., Dodder, R., Bash, J., Elobeid, A., Ran, L., Benson, V., Yang, D. 2017. Exploring a United States maize cellulose biofuel scenario using an integrated energy and agricultural markets solution approach. *Annals of Agricultural & Crop Sciences*, 2 (2): 1031.

Dong, C., Wang, G., Du, M., Niu, C., Zhang, P., Zhang, X., Ma, D., Ma, F., Bao, Z. 2020. Biostimulants promote plant vigor of tomato and strawberry after transplanting. *Scientia Horticulturae*, 267: 109355. DOI: 10.1016/j.scienta.2020.109355

Du-Jardin, P. 2015. Plant biostimulants: Definition, concept, main categories and regulation. *Scientia Horticulturae*. 196: 3-14. DOI:10.1016/j.scienta.2015.09.021

Espinoza, L., Slaton, N., Mazaffari, M. 2012. Understanding your numbers in your soil test report. Agriculture and Natural Resources, Publication FSA2118-PD-1-12RV. University of Arkansas Extension Service, Little Rock, Arkansas.

Farahmandi, S. R., Samavat, S., Mostafavi, M., Mohammadi-Torkashvand, A., Kalate-Jari, S. 2022. Combined foliar-applied L-glutamic acid, nitrogen, and potassium improve plant growth, physio-chemical attributes, minerals, and longevity of gerbera (*Gerbera jamesonii*). *Journal of Plant Nutrition*, 45 (7): 951-962. DOI: 10.1080/01904167.2021.1998526.

Fardus, J., Hossain, M.S., Fujita, M. 2021. Modulation of the antioxidant defense system by exogenous L-glutamic acid application enhances salt tolerance in lentil (*Lens culinaris* Medik.). *Biomolecules*, 11, 587. DOI: 10.3390/biom11040587

Feng, Y., Wang, X., Du, T., Shu, Y., Tan, F., Wang, J. 2022. Effects of exogenous salicylic acid application to aboveground part on the defense responses in Bt (*Bacillus thuringiensis*) and Non-Bt Corn (*Zea mays* L.) seedlings. *Plants*. 1 (16): 2162. DOI: 10.3390/plants11162162

Food and Agriculture Organization (FAO). 1985. STANDARD FOR WHOLE MAIZE (CORN) MEAL. CXS 154-1985. Revised in 1995. Amended in 2019.

Fowler, D. B., Brydon, J., Darroch, B. A., Entz, M. H., Johnston, A. M. 1990. Environment and genotype influence on grain protein concentration of wheat and rye. *Agronomy Journal*, 82 (4): 655-664. DOI: 10.2134/agronj1990.00021962008200040002x
Fu, H., Zhang, H., Yao, X., Zhou, L., Pan, G. 2022. Can corn stove bioethanol production substantially contribute to China's carbon neutrality ambition?. *Resources, Conservation & Recycling Advances*, *15*, 200111. DOI: 10.1016/j.rcradv.2022.200111

Gaidau, C., Niculescu, M., Stepan, E., Epure, D.-G., Gidea, M. 2013. New mixes based on collagen extracts with bioactive properties, for treatment of seeds in sustainable agriculture. *Current Pharmaceutical Biotechnology,* 14 (9): 792–801. DOI: 10.2174/1389201014666131227112020

Ghosh, P., Das, P., Mukherjee, R., Banik, S., Karmakar, S., Chatterjee, S. 2018. Extraction and quantification of pigments from Indian traditional medicinal plants: A comparative study between tree, shrub, and herb. *International Journal of Pharmaceutical Sciences and Research*, 9 (7): 3052-3059. DOI: 10.13040/IJPSR.0975-8232

Goto, Y., Maki, N., Ichihashi, Y., Kitazawa, D., Igarashi, D., Kadota, Y., Shirasu, K. 2020. Exogenous treatment with glutamate induces immune responses in *Arabidopsis. Molecular Plant-Microbe Interactions.* 33 (3): 474-487. DOI: 10.1094/MPMI-09-19-0262-R

Guzmán-Maldonado, Salvador H., Vázquez-Carrillo, M.G., Aguirre-Gómez, J.A., Serrano-Fujarte, I. 2015. Contenido de ácidos grasos, compuestos fenólicos y calidad industrial de maíces nativos de Guanajuato. *Revista Fitotecnia Mexicana*, 38 (2): 213-222. ISSN 0187-7380

Gyenes-Hegyi, Z., Pók, I., Kizmus, L., Zsubori, Z., Nagy, E., Marton, L.C. 2002. Plant height and height of the main ear in maize (*Zea mays* L.) at different locations and different plant densities. *Acta Agronomica Hungarica,* 50 (1): 75–84. DOI: 10.1556/aagr.50.2002.1.9

Haghighi, M., Saadat, S., Abbey, L. 2020. Effect of exogenous amino acids application on growth and nutritional value of cabbage under drought stress. *Scientia Horticulturae*, 272: 109561. DOI: 10.1016/j.scienta.2020.109561

Haghighi, M., Barzegar Sadeghabad, A., Abolghasemi, R. 2022. Effect of exogenous amino acids application on the biochemical, antioxidant, and nutritional value of some leafy cabbage cultivars. *Scientific Reports*, 12 (1): 17720. DOI: 10.1038/s41598-022-21273-6

Hanif, N.Q., Akhtar, N. 2020. Nutritional evaluation of maize plant fodder grown in spring and autumn season in Punjab, Pakistan. *Journal of Bioresource Management,* 7 (1): 74-93. DOI: 10.35691/JBM.0202.0123

HANNA® Instruments. Manual de instrucciones. Serie HI833XX. pp. 19, 47-49, 58-60, 197-199, 132-133, 231-232.

Ibañez, M., Bonamico, N., Salerno, J., Aiassa, J., Di Renzo, M., Díaz, D. 2004. Caracterización y clasificación de híbridos simples de maíz con marcadores SSR. *Revista de Investigaciones Agropecuarias,* 33 (2): 129-144. ISSN: 0325-8718

Iqbal, M.A., Ahmad, Z., Maqsood, Q., Afzal, S., Ahmad, M.M. 2015. Optimizing nitrogen level to improve growth and grain yield of spring planted irrigated maize (*Zea mays* L.). *Journal of Advanced Botany and Zoology*, 2 (3): 1-4. ISSN: 2348–7313

Iqbal, P., Ghani, M. A., Ali, B., Shahid, M., Iqbal, Q., Ziaf, K., Azam, M., Noor, A., Cheema, K.L., Ahmad, J. 2021. Exogenous application of glutamic acid promotes cucumber (*Cucumis sativus* L.) growth under salt stress conditions. *Emirates Journal of Food and Agriculture*, 407-416. DOI: 10.9755/ejfa.2021.v33.i5.2699

Jellum, M.D., Marion, J.E. 1966. Factors affecting oil content and oil composition of corn (*Zea mays* L.) Grain1. *Crop Science*, 6: 41-42. DOI: 10.2135/cropsci1966.0011183X000600010012x

Kato, T. A., Mapes, C., Mera, L. M., Serratos, J. A., Bye, R. A. 2009. Origen y diversificación del maíz: una revisión analítica. Universidad Nacional Autónoma de México, Comisión Nacional para el Conocimiento y Uso de la Biodiversidad. México, DF, 166. p. 17.

Khan, A.S., Ahmad, B., Jaskani, M.J., Ahmad, R., Malik, A.U. 2012. Foliar application of mixture of amino acids and seaweed (*Ascophylum nodosum*) extract improve growth and physicochemical properties of grapes. *International Journal of Agriculture & Biology*, 14: 383-388. ISSN 1560–8530

Kim, D.R., Jeon, C.W., Cho, G., Thomashow, L.S., Weller, D.M., Paik, M.J., Lee, Y.B., Kwak, Y.S. 2021. Glutamic acid reshapes the plant microbiota to protect plants against pathogens. *Microbiome*, 9 (1): 1-18. DOI: 10.1186/s40168-021-01186-8

Kong, D., Ju, C., Parihar, A., Kim, S., Cho, D., Kwak, J. M. 2015. *Arabidopsis* glutamate receptor homolog3.5 modulates cytosolic Ca^{2+} level to counteract effect of abscisic acid in seed germination. *Plant Physiology*. 167 (4): 1630–1642. DOI: 10.1104/pp.114.251298

Kukuruzović, J., Matin, A., Kontek, M., Krička, T., Matin, B., Brandić, I., Antonović, A. 2023. The effects of demineralization on reducing ash content in corn and soy biomass with the goal of increasing biofuel quality. *Energies*, 16: 967. DOI: 10.3390/en16020967

Lee, H. J., Kim, J. S., Lee, S. G., Kim, S. K., Mun, B., Choi, C. S. 2017. Glutamic acid foliar application enhances antioxidant enzyme activities in kimchi cabbages leaves treated with low air temperature. *Horticultural Science and Technology*, 35 (6): 700-706. DOI: 10.12972/kjhst.20170074

Lee, H. J., Lee, J. H., Wi, S., Jang, Y., An, S., Choi, C. K., Jang, S. 2021. Exogenously applied glutamic acid confers improved yield through increased photosynthesis efficiency and antioxidant defense system under chilling stress condition in *Solanum lycopersicum* L. cv. Dotaerang Dia. *Scientia Horticulturae*, 277, 109817. DOI: 10.1016/j.scienta.2020.109817

Lichtenthaler, H.K., Wellburn, A.R. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents, *Biochemical Society Transactions*, 11: 591- 592. DOI: 10.1042/bst0110591.

Liu, W., Liu, G., Yang, Y., Guo, X., Ming, B., Xie, R., Liu, Y., Wang, K., Hou, P., Li, S. 2021. Spatial variation of maize height morphological traits for the same cultivars at a large agroecological scale. *European Journal of Agronomy*, 130: 126349. DOI: 10.1016/j.eja.2021.126349

Luo, H., Duan, M., Xing, P., Zhang, Y., Qi, J., Kong, L., Tang, X. 2023. Effects of L-glutamic acid application on yield, grain quality, photosynthetic pigments, 2-acetyl-1-pyrroline, and antioxidant system of aromatic rice. *Field Crops Research*, 303: 109134. DOI: 10.1016/j.fcr.2023.109134

Makhaye, G., Mofokeng, M. M., Tesfay, S., Aremu, A. O., Van Staden, J., Amoo, S. O. 2021. Influence of plant biostimulant application on seed germination. *Biostimulants for Crops from Seed Germination to Plant Development*: 109-135. DOI: 10.1016/B978-0-12-823048-0.00014-9

Manmai, N., Unpaprom, Y., Ramaraj, R., Wu, K. T. 2021. Transformation of lignocellulose from corn stove for bioethanol production. *Maejo International Journal of Energy and Environmental Communication*, 3 (1): 44-48. DOI: 10.54279/mijeec.v3i1.245155

Marshall, M. R. 2010. Ash analysis. En Nielsen, S. (Ed), Food analysis 4th Edition. Springer. pp. 105-116.

Martínez-Gamiño, M. A., Jasso-Chaverría, C. (2005). Rotación maíz-avena forrajera con labranza de conservación en el altiplano de San Luis Potosí, México. *Terra Latinoamericana*, 23 (2): 257-263. ISSN: 2395-8030

Michigan State University (MSU). 2023. Soil nitrate test for corn. Soil and Plant Nutrient Laboratory. [Consultado el 23 de junio de 2023]. Disponible en https://www.canr.msu.edu/spnl/soil-nitrate-test-for-corn

Moussa, H. R. 2006. Influence of exogenous application of silicon on physiological response of salt-stressed maize (*Zea mays* L.). *International Journal of Agriculture & Biology*, 8 (3): 293-297.

Niculescu, M.D., Epure, D.G., Lason-Rydel, M., Gaidau, C., Gidea, M., Enascuta, C. 2019. Biocomposites based on collagen and keratin with properties for agriculture and industry applications. *The Eurobiotech Journal,* 3 (3): 160-166. DOI: 10.2478/ebtj-2019-0019

NMX-FF-034/1-SCFI-. PRODUCTOS ALIMENTICIOS NO INDUSTRIALIZADOS PARA CONSUMO HUMANO - CEREALES – PARTE I: MAÍZ BLANCO PARA PROCESO ALCALINO PARA TORTILLAS DE MAÍZ Y PRODUCTOS DE MAÍZ NIXTAMALIZADO - ESPECIFICACIONES Y MÉTODOS DE PRUEBA; Secretaría de Economía: México, 2002: p. 18.

Noroozlo, Y., Souri, M., Delshad, M. 2019. Stimulation effects of foliar applied glycine and glutamine amino acids on lettuce growth. *Open Agriculture*, 4 (1): 164-172. DOI: 10.1515/opag-2019-0016

Odjo, S., Palacios, N., Burgueño, J., Corrado, M., Ortner, T., Verhulst, N. 2022. Hermetic storage technologies preserve maize seed quality and minimize grain quality loss in smallholder farming systems in Mexico. *Journal of Stored Products Research*, 96: 101954. DOI: 10.1016/j.jspr.2022.101954

Park, Y. W. 1996. Moisture and Ash Contents of Food. *Handbook of food analysis*. pp 43-92.

Palacios-Rojas, N. 2018. Análisis físicos: Determinación del tamaño de grano a través del peso de cien granos (PCG). En Calidad nutricional e industrial de maíz: Laboratorio de Calidad Nutricional de Maíz "Evangelina Villegas": protocolos. Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT). p. 35, 39-40.

Pedersen, I. F., Christensen, J. T., Sørensen, P., Christensen, B. T., Holton Rubæk, G. 2022. Early plant height: A defining factor for yields of silage maize with contrasting phosphorus supply. *Soil Use and Management*, 38 (1): 537-548. DOI: 10.1111/sum.12697

Peña-Betancourt, S.D., Carmona-Medero, M.Á., Valladares-Carmona, B. 2013. Comparación de calidad física, contenido de fenoles y aflatoxinas en maíces híbridos y nativos. *Revista Mexicana de Ciencias Agrícolas,* 4 (5): 779-788. ISSN 2007-0934

Pereira, M. G., Lee, M. 1995. Identification of genomic regions affecting plant height in sorghum and maize. *Theoretical and Applied Genetics*, *90,* 380-388.

Qiu, X.M., Sun, Y.Y., Ye, X.Y., Li, Z.G. 2020. Signaling role of glutamate in plants. *Frontiers in Plant Science,* 10 (1): 1743. DOI: 10.3389/fpls.2019.01743

Ragheb, E. E. 2016. Sweet corn as affected by foliar application with amino–and humic acids under different fertilizer sources. *Egyptian Journal of Horticulture*, 43 (2): 441-456. DOI: 10.21608/EJOH.2016.3564

Raposo-Junior, J.L., Gomes-Neto, J.A., Sacramento, L. 2013. Evaluation of different foliar fertilizers on the crop production of sugarcane. *Journal of Plant Nutrition*, 36: 459-469. DOI: 10.1080/01904167.2012.748066

Rashid, M., Iqbal, M. 2012. Effect of phosphorus fertilizer on the yield and quality of maize (*Zea mays* L.) fodder on clay loam soil. *The Journal of Animal & Plant Sciences*, 22 (1): 199-203. ISSN: 1018-7081

Ray, K., Banerjee. H., Dutta, S., Hazra. A.K., Majumdar, K. 2019. Macronutrients influence yield and oil quality of hybrid maize (*Zea mays* L.). *PLoS ONE*, 14 (5): e0216939. DOI: 10.1371/ journal.pone.0216939

Sanjeev, P., Chaudhary, D. P., Sreevastava, P., Saha, S., Rajenderan, A., Sekhar, J. C., Chikkappa, G. K. 2014. Comparison of fatty acid profile of specialty maize to normal maize. *Journal of the American Oil Chemists' Society*, 91 (6): 1001–1005. DOI: 10.1007/s11746-014-2429-y

Sari-Gorla, M., Krajewski, P., Di Fonzo, N. Villa, M., Fonza, C. 1999. Genetic analysis of drought tolerance in maize by molecular markers. II. Plant height and flowering. *Theoretical and Applied Genetics*, 99: 289–295. DOI: 10.1007/s001220051234

Secretaría de Agricultura y Desarrollo Rural (SADER). 2021. Estima Agricultura crecimiento de 2.6 por ciento en la producción de maíz grano en año agrícola 2021. Gobierno de México. [Consultado el 8 de marzo de 2023]. Disponible en: https://www.gob.mx/agricultura/prensa/estima-agricultura-crecimiento-de-2-6-por-ciento-en-la-produccion-de-maiz-grano-en-ano-agricola-2021?idiom=es

Serna-Rodríguez, J.R., Castro-Brindis, R., Colinas-León, M.T., Sahagún-Castellanos, J., Rodríguez-Pérez, J.E. 2011. Aplicación foliar de ácido glutámico en plantas de jitomate (*Lycopersicon esculentum* Mill.). *Revista Chapingo Serie Horticultura*, 17 (1): 9-13. ISSN: 2007-4034

Servicio de Información Agroalimentaria y Pesquera (SIAP). 2022. Cierre de la producción agrícola 1980 a 2022. Gobierno de México. [Consultado el 8 de marzo de 2023]. Disponible en: https://nube.siap.gob.mx/cierreagricola/

Shaner, D. L., Reider, M. L. 1986. Physiological responses of corn (*Zea mays*) to AC 243,997 in combination with valine, leucine, and isoleucine. *Pesticide Biochemistry and Physiology*, 25 (2): 248–257. DOI: 10.1016/0048-3575(86)90051-9

Shehata, S. M., Abdel-Azem, H. S., Abou-El-Yazied, A., El-Gizawy, A. M. 2011. Effect of foliar spraying with amino acids and seaweed extract on growth chemical constitutes, yield and its quality of celeriac plant. *European Journal of Scientific Research,* 58 (2): 257-265. ISSN: 1450-216X

Shen, B., Allen, W.B., Zheng, P., Li, C., Glassman, K., Ranch, J., Nubel, D., Tarczynski, M.C. 2010. Expression of *ZmLEC1* and *ZmWRI1* increases seed oil production in maize. *Plant Physiology*, 153 (3): 980-987. DOI: 10.1104/pp.110.157537

Sinay, H., Harijati, N. 2021. Determination of proximate composition of local corn cultivar from Kisar Island, Southwest Maluku Regency. *Biosaintifika: Journal of Biology & Biology Education,* 13 (3): 258-266. DOI: 10.15294/biosaintifika.v13i3.30527

Soares, L. H., Neto, D. D., Fagan, E. B., Teixeira, W. F., dos Reis, M. R., Reichardt, K. 2016. Soybean seed treatment with micronutrients, hormones and amino acids on physiological characteristics of plants. *African Journal of Agricultural Research,* 11 (35): 3314-3319. DOI: 10.5897/AJAR2016.11229

Souri, M. K., Yaghoubi, F., Fahimi, F. 2017. Growth and development of tomato seedlings under foliar application of some aminochelates. *Horticulture, Environmental & Biotechnology,* 58: 530-536. DOI: 10.1007/s13580-017-0349-0

Széles, A., Horváth, É., Vad, A., Harsányi, E. 2018. The impact of environmental factors on the protein content and yield of maize grain at different nutrient supply levels. *Emirates Journal of Food and Agriculture*: 764-777. DOI: 10.9755/ejfa.2018.v30.i9.1800

Torres-Morales, B., Coutiño-Estrada, B., Muñoz-Orozco, A., Santacruz-Varela, A., Mejía-Contreras, A., Serna-Saldivar, S.O., García-Lara, S., & Palacios-Rojas, N. 2010. Selección para contenido de aceite en el grano de variedades de maíz de la raza comiteco de Chiapas, México. *Agrociencia*, 44 (6): 679-689. ISSN 1405-3195

Toyota, M., Spencer, D., Sawai-Toyota, S., Jiaqi, W., Zhang, T., Koo, A.J., Howe, G.A., Gilroy, S. 2018. Glutamate triggers long-distance, calcium-based plant defense signaling. *Science*, 361 (6407): 1112-1115. DOI: 10.1126/science.aat7744

Ureta, C., González, E.J., Espinosa, A., Trueba, A., Piñeyro-Nelson, A., Álvarez-Buylla, E.R. 2020. Maize yield in Mexico under climate change. *Agricultural Systems*, 177: 102697. DOI:10.1016/j.agsy.2019.102697.

Veljković, V. B., Biberdžić, M.O., Banković-Ilić, I.B., Djalović, I.G., Tasić, M.B., Nježić, Z.B., Stamenković, O.S. 2018. Biodiesel production from corn oil: A review. *Renewable and Sustainable Energy Reviews*, 91: 531–548. DOI: 10.1016/j.rser.2018.04.024

Wilson, H. T., Xu, K., and Taylor, A. G. 2015. Transcriptome analysis of gelatin seed treatment as a biostimulant of cucumber plant growth. *The Scientific World Journal*, 2015. DOI: 10.1155/2015/391234

Woo, S. L., Pepe, O. 2018. Microbial consortia: promising probiotics as plant biostimulants for sustainable agriculture. *Frontiers in plant science,* 9, 1801. DOI: 10.3389/fpls.2018.01801
Wu, L., Zhang, D., Xue, M., Qian, J., He, Y., Wang, S. 2014. Overexpression of the maize *GRF10*, an endogenous truncated growth-regulating factor protein, leads to reduction in leaf size and plant height. *Journal of integrative plant biology,* 56 (11): 1053-1063. DOI: 10.1111/jipb.12220

Yu, C., Lu, D.G., Qin, S.J., Yang, L., Ma, H.Y., Liu, G.C. 2010. Changes in photosynthesis, fluorescence, and nitrogen metabolism of hawthorn (*Crataegus pinnatifida*) in response to exogenous glutamic acid. *Photosynthetica* 48 (3): 339-347. DOI: 10.1007/s11099-010-0044-1
Zając, G., Maj, G., Szyszlak-Bargłowicz, J., Słowik, T., Krzaczek, P., Gołębiowski, W., Dębowski, M. 2020. Evaluation of the properties and usefulness of ashes from the corn grain drying process biomass. *Energies*, 13 (5): 1290. DOI: 10.3390/en13051290

Zhou, X.B., Chen, C., Li, Z.C., Zou, X.Y. 2007. Using Chou's amphiphilic pseudo-amino acid composition and support vector machine for prediction of enzyme subfamily classes. *Journal of Theoretical Biology,* 248: 546–551. DOI: 10.1016/j.jtbi.2007.06.001.