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REGULAR ARTICLE

Glutamic acid seed priming enhances seedling growth through metabolic and antioxidant modulation in maize (*Zea mays* L.)

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Author contribution (CRediT)

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Abstract

Glutamic acid (Glu) is a central metabolite involved in nitrogen assimilation and cellular signaling in plants; however, its effects as a seed-priming agent in maize remain poorly understood. This study investigated the influence of Glu seed priming on germination, growth, photosynthetic pigments, antioxidant metabolism, primary and secondary metabolism, and endogenous phytohormones in the maize hybrid FS615PWU. Seeds were primed with 0, 0.25, 0.5, or 1 mmol L^{-1} Glu for 10 min and evaluated under controlled laboratory conditions. Germination percentage was not affected by the treatments. However, seed priming promoted greater seedling vigor, resulting in increased epicotyl and root growth, total seedling length, and fresh biomass, particularly at 0.5 mmol L^{-1}. In addition, treated seedlings exhibited higher chlorophyll *a* and total chlorophyll contents, along with lower accumulation of hydrogen peroxide and malondialdehyde, indicating reduced oxidative damage. The improved redox status was associated with increased superoxide dismutase and peroxidase activities. Glu also enhanced the accumulation of soluble sugars, starch, phenolic compounds, and flavonoids, while increasing antioxidant capacity. Furthermore, higher endogenous levels of indole-3-acetic acid and gibberellic acid were observed in primed seedlings, suggesting stimulation of growth-related hormonal pathways. Overall, Glu seed priming improved the physiological and metabolic performance of maize seedlings during early establishment, with 0.5 mmol L^{-1} providing the most consistent responses under the experimental conditions.

Keywords

Amino acid; Seed treatment; Plant Metabolism; Antioxidant activity; Growth.

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1. Introduction

Maize (*Zea mays L.*) occupies a strategic position in world agriculture, not only because of its significant cultivated area, but also because of its versatility in human food, animal nutrition, and various agro-industrial chains. Studies indicate that losses in maize production due to poor initial establishment can reach 20%, severely impacting global supply. Its domestication from teosinte, which occurred millennia ago, gave the species a high capacity for adaptation, enabling its cultivation across contrasting environments and under diverse management systems (De-la-Vega-Camarillo et al., 2023). However, this productive potential is strongly conditioned by the crop's initial establishment, a stage susceptible to environmental variations driven by current climate change scenarios (Gopalakrishna et al., 2023; Hu et al., 2023).

The germination and emergence phases represent critical periods in the maize cycle, during which minor physiological limitations can compromise subsequent growth and reduce final yield (Vilas-Boas et al., 2025). In this context, seed treatment has been established as an efficient strategy to enhance seedling performance at the outset, promoting greater emergence uniformity, increased vigor, and greater responsiveness to abiotic stresses, such as water deficit, extreme temperatures, and salinity (Paulikienė et al., 2025).

In recent years, there has been a clear transition from the exclusive use of chemical inputs to more integrated approaches that combine bioregulators with more precise application technologies. As climate variability increases, compromising climate resilience and leading to seedling stress, these integrated strategies become essential. They aim not only to maximize production efficiency but also to reduce environmental impacts and increase crop resilience under adverse conditions (Pereira & Simonetti, 2021; Gorni & Polimeno, 2023). In this scenario, compounds that directly act on plant metabolism and signaling have attracted particular interest, as they are fundamental to addressing the challenges posed by climate change and ensuring the future success of agriculture.

Bioregulators can modulate fundamental physiological processes, influencing everything from germination to initial plant growth. These plant growth-promoting compounds, for example, have been associated with stimulating root development, enhancing nutrient absorption, and inducing stress-tolerance mechanisms in maize (Pereira & Simonetti, 2021; Mohy-Ud-Din et al., 2025; Gorni et al., 2025). In a complementary manner, bioregulators modulate hormonal pathways and antioxidant responses, thereby favoring more efficient metabolism during the early stages of plant development (Silveira et al., 2025). Among compounds with

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potential for agricultural applications, glutamic acid (Glu) stands out for its functions that go beyond its structural role in protein synthesis. In plants, glutamate acts as a signaling molecule, regulating root growth, germination, and adaptive responses to various stresses (Qiu et al., 2020; Yu et al., 2022). This effect is associated with the activation of glutamate-like receptors, which promote calcium-mediated signaling, thereby triggering physiological and metabolic adjustments that support cell growth and redox homeostasis (Qiu et al., 2020). Recent evidence indicates that exogenous application of Glu can enhance seedling vigor, improve physiological efficiency, and mitigate environmental stresses, reinforcing its potential as an agricultural bioregulator (Rosa et al., 2023; Saleem et al., 2024). When applied through seed treatment, this compound can act early, influencing key processes of early plant development (Qiu et al., 2020; Yu et al., 2022).

Despite these advances, the physiological and metabolic responses of maize to Glu, when applied as a seed-priming agent, remain poorly understood. In particular, little information is available on how this treatment influences seedling growth, antioxidant metabolism, carbon allocation, and hormonal regulation during early plant establishment. We hypothesized that Glu seed priming enhances early maize development by coordinating these physiological and metabolic processes. Therefore, the present study aimed to evaluate the effects of Glu seed priming on germination, seedling growth, photosynthetic pigments, antioxidant metabolism, primary and secondary metabolism, and endogenous phytohormone levels in maize.

2. Materials and methods

The experiment was conducted at the Seed Analysis Laboratory of the Escola Superior de Agronomia de Paraguaçu Paulista (ESAPP), Paraguaçu Paulista, São Paulo, Brazil. Seeds of the commercial maize hybrid FS615PWU were used throughout the study. Prior to the experiment, seeds were visually inspected to remove damaged or malformed units, ensuring uniformity among treatments. Glu (molecular weight 147.13 g mol^{-1}) was dissolved in distilled water to prepare solutions containing 0 (control), 0.25, 0.5, and 1 mmol L^{-1}. Seeds were immersed in the respective solutions for 10 min at room temperature. After priming, excess solution was removed with absorbent paper, and the seeds were immediately subjected to the germination test.

The experiment followed a completely randomized design with four treatments and three biological replicates of 25 seeds each, totaling 75 seeds per treatment and 300 seeds in the entire experiment. For the biochemical and phytohormone analyses, three independent biological replicates were used, corresponding to the experimental replicates established in the study. Each biological replicate consisted of pooled shoot tissues collected from normal seedlings within the same experimental unit to obtain sufficient plant material for the analysis. All laboratory determinations were performed according to the respective analytical protocols, and each sample was analyzed using analytical replicates when required by the methodology.

Seed germination was evaluated in accordance with the Rules for Seed Testing (Brasil, 2009). Twenty-five seeds from each replicate were placed on germination paper previously moistened with distilled water to a volume equivalent to 2.5 times the paper's dry weight. The sheets were rolled and maintained in a germination chamber at 23 ± 3 °C throughout the experimental period. The first germination count was performed five days after sowing, whereas the final germination percentage was determined on the seventh day. Seedlings were classified as normal according to the official criteria established by the Brazilian Rules for Seed Testing. Germination speed index (GSI) was calculated using the method proposed by Maguire (1962), based on the daily number of germinated seeds throughout the evaluation period. Seven days after sowing, ten normal seedlings were randomly selected from each replicate for biometric evaluations. Epicotyl length and primary root length were measured using a digital caliper, and total seedling length was obtained by summing both measurements. Fresh biomass was determined immediately after sampling using an analytical balance with 0.001 g precision.

Photosynthetic pigments were determined following the method of Lichtenthaler (1987). Approximately 0.2 g of fresh shoot tissue was homogenized in 80% (v/v) acetone under low-light conditions. Samples were maintained at 4 °C for 24 h to ensure complete pigment extraction, then centrifuged. The absorbance of the supernatant was measured at 663, 647, and 470 nm using a UV–Vis spectrophotometer (BEL-V-M5). Chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid contents were calculated using the equations proposed by Lichtenthaler (1987) and expressed on a fresh weight basis. Oxidative stress was evaluated by determining hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents in the shoots of maize seedlings. Fresh shoot tissue was immediately frozen in liquid nitrogen and homogenized in 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 × g for 15 min at 4 °C, and the supernatant was used for all analyses.

Hydrogen peroxide concentration was determined according to Alexieva et al. (2001). Briefly, the extract was mixed with 0.1 M potassium phosphate buffer (pH 7.5) and 1 M potassium iodide solution. The reaction mixture was incubated on ice for 1 h in the dark, and absorbance was measured at 390 nm using a UV–Vis spectrophotometer. H_2O_2 concentration was calculated from a standard curve and expressed as μmol g^{-1} fresh weight. Lipid peroxidation was estimated by determining malondialdehyde (MDA) content according to Heath and Packer (1968). Aliquots of the extract were mixed with 0.5% (w/v) thiobarbituric acid prepared in 20% TCA and incubated at 95 °C for 30 min. The reaction was stopped in an ice bath, and absorbance was recorded at 535 and 600 nm. MDA concentration was calculated using the extinction coefficient of the MDA–TBA complex and expressed as nmol g^{-1} fresh weight.

Fresh shoot samples (1 g) were homogenized in ice-cold 0.1 M potassium phosphate buffer (pH 7.5). The homogenate was centrifuged at 10,000 × g for 30 min at 4 °C, and the supernatant was used as the crude enzyme extract.

Superoxide dismutase (SOD; EC 1.15.1.1) activity was determined according to Giannopolitis and Ries (1977) based on the inhibition of nitro blue tetrazolium (NBT) photoreduction. Reaction mixtures were exposed to fluorescent light at room temperature for 20 min, and absorbance was measured at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to inhibit NBT photoreduction by 50%. Peroxidase (POD; EC 1.11.1.7) activity was determined according to Matsuno and Uritani

(1972) by monitoring guaiacol oxidation in the presence of hydrogen peroxide. The increase in absorbance was recorded at 470 nm, and enzyme activity was expressed on a protein basis. Total soluble protein content was quantified according to Bradford (1976).

Primary metabolites were extracted from 1 g of fresh shoot tissue using 10 mL of 70% (v/v) ethanol. Samples were maintained at room temperature for 24 h and then centrifuged at 10,000 × g for 10 min. The resulting supernatant was used for carbohydrate and amino acid analyses. Total soluble sugars were quantified by the phenol–sulfuric acid method described by Dubois et al. (1956). Reducing sugars were determined according to Bezerra Neto and Barreto (2004). Starch content was measured in the residual pellet after acid hydrolysis, following Sadasivam and Manickam (1996). Total free amino acids were determined using the ninhydrin method described by Yemm et al. (1955). All results were expressed on a fresh weight basis. Phenolic compounds and flavonoids were quantified using ethanolic extracts obtained from fresh shoot tissue. Total phenolic content was determined by the Folin–Ciocalteu method (Singleton and Rossi, 1965), using gallic acid as the calibration standard. Absorbance was measured spectrophotometrically, and results were expressed as gallic acid equivalents. Total flavonoid content was determined according to Yao et al. (2013), using rutin as the reference compound. Absorbance was measured after color development, and flavonoid concentration was expressed as rutin equivalents. The antioxidant capacity of the extracts was evaluated using the DPPH free-radical scavenging assay (Blois, 1958). The decrease in absorbance after reaction with the DPPH solution was monitored spectrophotometrically, and antioxidant activity was expressed as the percentage of radical scavenging.

Endogenous indole-3-acetic acid (IAA) and gibberellic acid (GA_3) were quantified in fresh shoot tissue. Approximately 1 g of tissue was homogenized in 10 mL of 1 M sodium phosphate buffer (pH 7.4) and centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant was used for hormone determination. IAA concentration was determined according to Gordon and Weber (1951) using the Salkowski colorimetric reagent. Briefly, 500 µL of the extract was mixed with 1 mL of Salkowski reagent and incubated in the dark for 25 min. Absorbance was measured at 530 nm, and IAA concentration was calculated using a standard calibration curve. GA_3 concentration was determined following Graham and Thomas (1961). 100 µL aliquots of the extract were mixed with 900 µL of 3.75 M hydrochloric acid and incubated for 1 h. Absorbance was measured at 550 nm, and hormone concentration was calculated from a standard curve prepared with analytical-grade GA_3. The experiment was conducted in a completely randomized design with four treatments and three biological replicates. Data were initially tested for normality and homogeneity of variances before statistical analyses. Subsequently, the data were analyzed using analysis of variance (ANOVA), and treatment means were compared using Tukey's multiple comparisons test at the 5% significance level ($p \leq 0.05$). When appropriate, regression analyses were performed to describe the responses of the evaluated variables to increasing Glu concentrations. To complement the univariate analyses, Cohen's effect size (d) and percentage variation relative to the control treatment were calculated to estimate the magnitude of treatment effects. Pearson's correlation analysis, principal component analysis (PCA), and hierarchical clustering were performed using the MetaboAnalyst 6.0 platform (Pang et al., 2024) to investigate relationships among morphological, physiological, biochemical, and hormonal variables and to identify the metabolic responses associated with Glu seed priming.

3. Results and discussion

Figure 1 shows the effects of Glu concentrations on germination and initial growth of maize seedlings. Germination remained high at all concentrations, with no significant response to Glu (Figure 1a). Epicotyl length (Figure 1b) increased by 23, 11, and 18%, respectively, at concentrations of 0.25, 0.5, and 1 mmol L^{-1} in Glu-treated seeds. However, in root length (Figure 1c), there were significant increases in 0.5 and 1 mmol L^{-1} concentrations of 3% compared to untreated plants. The total seedling length (Figure 1d) increased linearly with increasing doses, by 5, 6, and 10% compared to the control plants. On the other hand, the total fresh mass (Figure 1e) increased by 10% and 12% at concentrations of 0.25 and 0.5 mmol L^{-1}, respectively. In general, intermediate concentrations favored initial development, while higher concentrations supported satisfactory growth, with a relatively minor relative increase. Among the evaluated concentrations, 0.5 mmol L^{-1} produced the most consistent physiological and metabolic responses under the experimental conditions adopted in this study.

Our results show that applying 0.25 and 0.5 mmol L^{-1} Glu increased chlorophyll concentrations by 8% and 4%, respectively, compared to the control (Figure 2a). In contrast, chlorophyll *b* (Figure 2b) and carotenoids (Figure 2d) did not show significant responses to the doses applied. The total chlorophyll content (Figure 2c) increased by 2% compared to the control. Glu application favored the accumulation of photosynthetic pigments, especially at a concentration of 0.25 mmol L^{-1}. However, the high dose (1 mmol L^{-1}) consistently reduced chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids.

The application of Glu induced significant changes in the redox state of maize seedlings, reducing oxidative damage and strengthening the antioxidant system in a concentration-dependent manner. Malondialdehyde (MDA) levels showed a sharp decrease with the increase in Glu concentrations, with a reduction of 42%, 37%, and 76%, respectively, in relation to the control, indicating lower lipid peroxidation and greater stability of cell membranes (Fig. 3a). Similarly, hydrogen peroxide (H_2O_2) levels were significantly reduced, showing a decrease of 80%, 47% and 74%, respectively, compared to the control, followed by a slight increase in the highest concentrations, suggesting a physiological adjustment of the redox balance (Fig. 3b). On the other hand, the activity of superoxide dismutase (SOD) increased consistently over the applied doses, with an increase of 110%, 95% and 267%, respectively, in the concentrations of 0.25, 0.5 and 1 mmol L^{-1} of Glu in relation to the control, showing an intensification of the first level of antioxidant defense (Fig. 3c). Peroxidase activity (POD) was also stimulated, showing increases of 160%, 249%, and 65% compared to untreated treatments. Taken together, these results demonstrate that Glu effectively mitigates oxidative stress in maize by reducing ROS production and stimulating the antioxidant system, especially at intermediate doses.

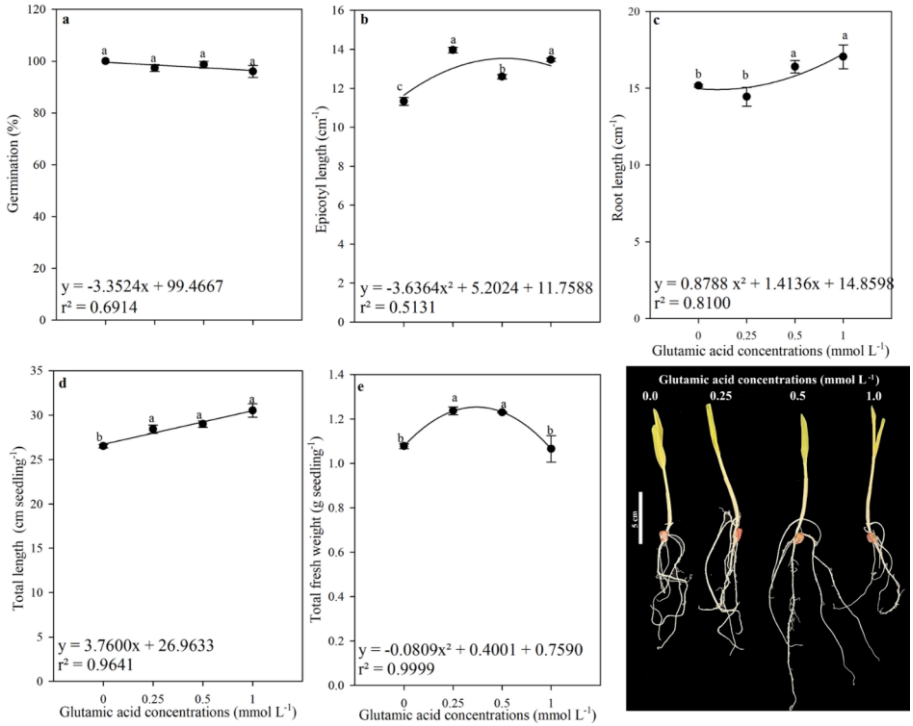

Figure 1. Effect of different concentrations of glutamic acid (0, 0.25, 0.5, and 1 mmol L^{-1}) on germinative and morphological parameters of maize seedlings. (a) Germination (%); (b) length of the epicotyl; (c) root length; (d) total length; (e) total fresh weight per seedling. The lowercase letters indicate significant differences between the means, according to Tukey's test ($p \leq 0.05$). The equations presented represent the regression models adjusted for each variable, with their respective coefficients of determination (r^2).

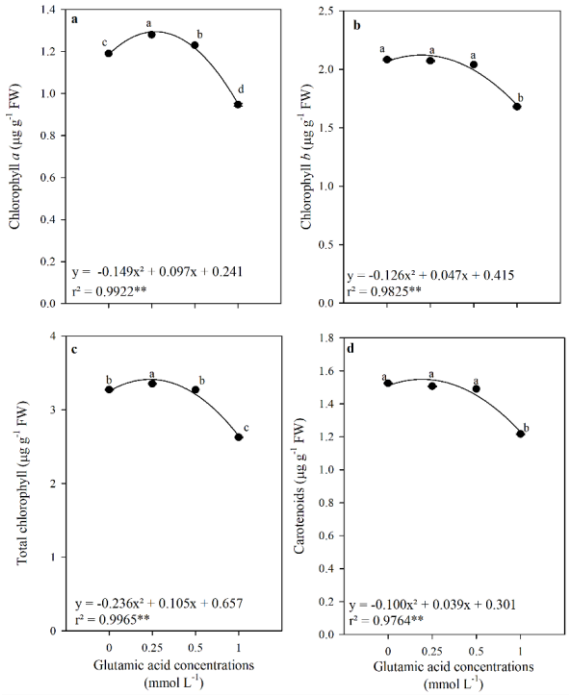

Figure 2. Effect of different concentrations of glutamic acid (0, 0.25, 0.5, and 1 mmol L^{-1}) on the levels of photosynthetic pigments in maize seedlings. (a) Chlorophyll *a*; (b) Chlorophyll *b*; (c) Total chlorophyll; (d) Carotenoids. The lowercase letters indicate significant differences between the means, according to Tukey's test ($p \leq 0.05$). The equations presented represent the regression models adjusted for each variable, with their respective coefficients of determination (r^2).

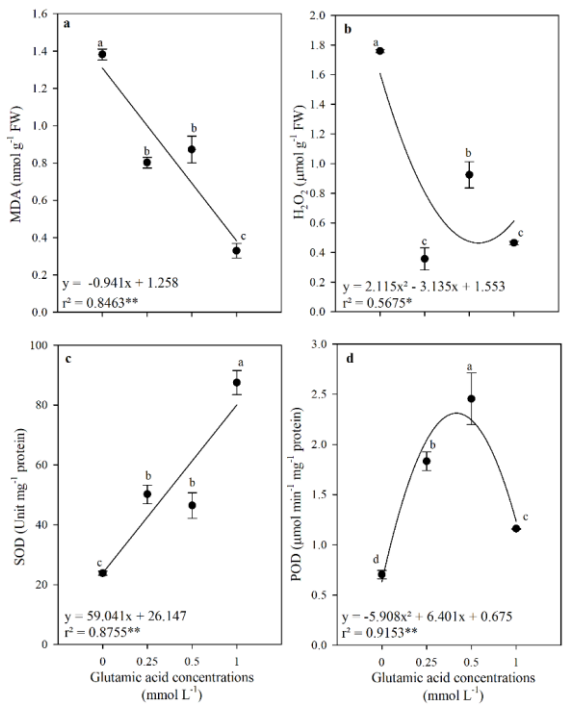

Figure 3. Effect of different concentrations of glutamic acid (0, 0.25, 0.5, and 1 mmol L^{-1}) on parameters associated with oxidative stress in maize seedling. (a) MDA; (b) H_2O_2; (c) SOD enzyme activity; (d) POD enzyme activity. The lowercase letters indicate significant differences between the means, according to Tukey's test ($p \leq 0.05$). The equations presented represent the regression models adjusted for each variable, with their respective coefficients of determination (r^2).

The total sugar content increased linearly with the increase in Glu doses, showing increases of 35%, 75% and 131%, respectively, in the concentrations of Glu in relation to the control, showing greater accumulation of carbon reserves (Fig. 4a). Similarly, starch content was also significantly increased (Fig. 4c), with increases of 34%, 74%, and 130%, respectively, indicating greater carbon targeting to reserve compounds. In contrast, reducing sugars showed a 10% increase in concentration at 0.25 mmol L^{-1} compared to the control (Fig. 4b); however, significant reductions were observed at 0.5 and 1 mmol L^{-1}, with decreases of 49% and 36%, respectively. On the other hand, the total amino acid contents (Fig. 4d) remained relatively stable between the treatments, with a variation of less than 11% in the concentration of 0.25 mmol L^{-1} compared to the control, which indicates that the application of Glu preferentially modulated carbon metabolism, without promoting significant accumulation of free amino acids. Taken together, these results demonstrate that Glu promotes the accumulation of energy reserves and the redirection of carbon metabolism in maize seedlings, thereby enhancing physiological efficiency during early development.

The total polyphenol content (Fig. 5a) increased progressively with increasing Glu dose, reaching 28%, 37%, and 41% relative to the control, indicating greater activation of phenolic biosynthetic pathways. Similarly, the flavonoid levels (Fig. 5b) exhibited a quadratic response, with a maximum increase in concentration of 0.25 mmol L^{-1}, corresponding to a 38% increase over untreated levels. In parallel, the antioxidant capacity evaluated by the DPPH method increased consistently with the addition of Glu, reaching 19% and 15% increases at concentrations of 0.5 and 1 mmol L^{-1}, respectively, compared to the control, indicating greater efficiency in neutralizing free radicals. Taken together, these results demonstrate that Glu stimulates the synthesis of secondary metabolites with antioxidant activity and strengthens non-enzymatic defense mechanisms, thereby contributing to a more balanced redox state and cell protection during the initial development of maize.

The application of Glu promoted significant changes in the hormonal balance of maize seedlings, showing stimulation of hormones associated with plant growth; however, the results show similarity in the increases of the hormones IAA (70% and 131%) and GA_3 (70% and 39%), at concentrations of 0.5 and 1 mmol L^{-1}, in relation to the control (Figs 6a and 6b). However, the results indicate greater activation of hormonal pathways involved in cell elongation and early growth. Taken together, these results demonstrate that Glu acts as a modulator of hormonal metabolism in maize, favoring the synthesis or maintenance of growth regulators essential to initial vegetative development, especially at intermediate concentrations.

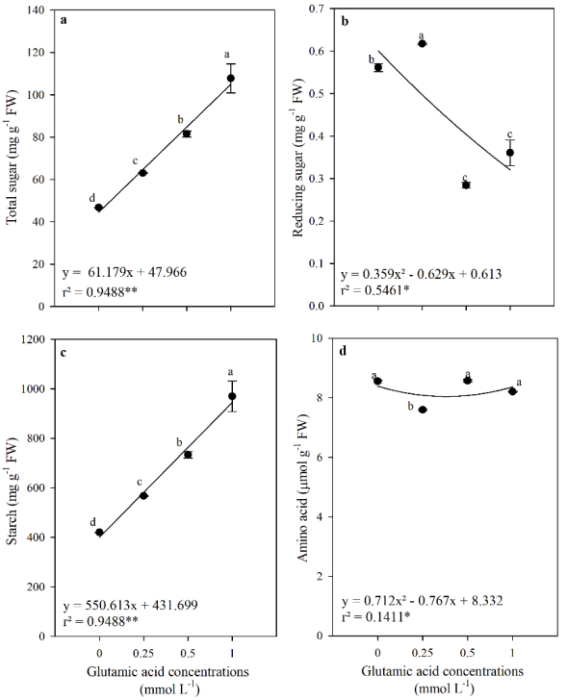

Figure 4. Effect of different concentrations of glutamic acid (0, 0.25, 0.5, and 1 mmol L^{-1}) on nitrogenous compounds in maize seedling. (a) Total sugars; (b) Reducing sugars; (c) Starch; (d) Amino acids. The lowercase letters indicate significant differences between the means, according to Tukey's test ($p \leq 0.05$). The equations presented represent the regression models adjusted for each variable, with their respective coefficients of determination (r^2).

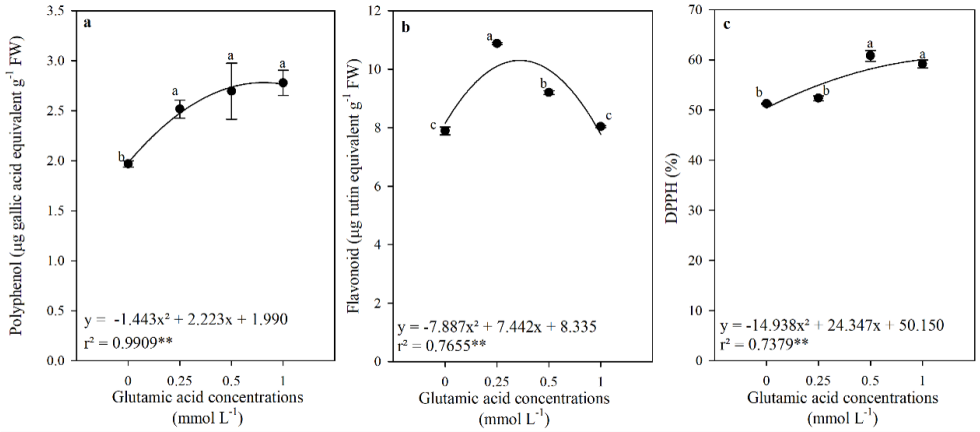

Figure 5. Effect of different concentrations of glutamic acid (0, 0.25, 0.5, and 1 mmol L^{-1}) on bioactive compounds and antioxidant capacity in maize seedlings. (a) Total phenols; (b) Flavonoids; (c) DPPH. The lowercase letters indicate significant differences between the means, according to Tukey's test ($p \leq 0.05$). The equations presented represent the regression models adjusted for each variable, with their respective coefficients of determination (r^2).

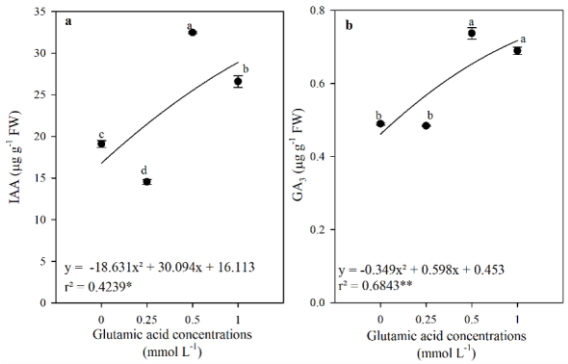

Figure 6. Effect of different concentrations of glutamic acid (0, 0.25, 0.5, and 1 mmol L^{-1}) on plant hormones in maize seedlings. (a) IAA and (b) GA_3. The lowercase letters indicate significant differences between the means, according to Tukey's test ($p \leq 0.05$). The equations presented represent the regression models adjusted for each variable, with their respective coefficients of determination (r^2).

The integrated multivariate analyses showed that treating seeds with Glu induced a coordinated reprogramming of metabolic and physiological processes during the initial establishment of maize seedlings (Figure 7). In simple terms, this figure shows how glutamate improves energy allocation and reduces oxidative stress in plants. The correlation heatmap (Figure 7a) showed strong positive associations between growth parameters, including epicotyl length (SL), root length (RL), total length (TL), and fresh mass (FW), and photosynthetic pigments (Chla, Chlb, and TChl). These variables were grouped with indicators of primary and secondary metabolism, such as soluble and reducing sugars (TS and RS), total polyphenols (TPP), indoleacetic acid (IAA), gibberellic acid (GA), and total flavonoids (TF), suggesting greater integration between carbon and nitrogen metabolisms, as well as intensification of anabolic activity in response to Glu.

In contrast, oxidative stress markers, especially malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), showed negative correlations with growth and photosynthesis parameters, indicating effective reduction of oxidative damage. The antioxidant enzymes, superoxide dismutase (SOD) and peroxidase (POD), showed positive correlations with phenolic compounds and antioxidant capacity (DPPH), evidencing the activation of an integrated antioxidant network, involving enzymatic and non-enzymatic components. This pattern indicates that treatment with Glu contributed to maintaining redox homeostasis, not only to stress-induced defensive responses.

The hierarchical cluster analysis (Figure 7b) clearly separated treatments by Glu dose. The intermediate and higher doses were associated with greater growth and accumulation of photosynthetic pigments and antioxidant metabolites, whereas the control showed higher levels of MDA and H_2O_2. This grouping reinforces the dose-dependent effect of Glu in modulating the initial metabolic balance. Principal component analysis (PCA; Figure 7c) explained a substantial portion of the total variance, with PC1 (56.6%) strongly associated with growth parameters, photosynthetic pigments, carbohydrates, and antioxidant capacity. In comparison, PC2 (22.3%) primarily reflected variation in oxidative stress and antioxidant enzyme activity. Clear segregation was observed between control and Glu treatments throughout PC1, with treated seedlings being positioned in association with higher metabolic efficiency and growth. In contrast, controls remain associated with indicators of oxidative stress. Together, the results demonstrate that applying Glu via seed treatment promotes early maize development by increasing metabolic efficiency, strengthening the antioxidant system, and maintaining redox balance. These data support the role of Glu as a central modulator at the interface between nitrogen metabolism, carbon allocation, hormonal signaling, and oxidative stress control during initial seedling establishment.

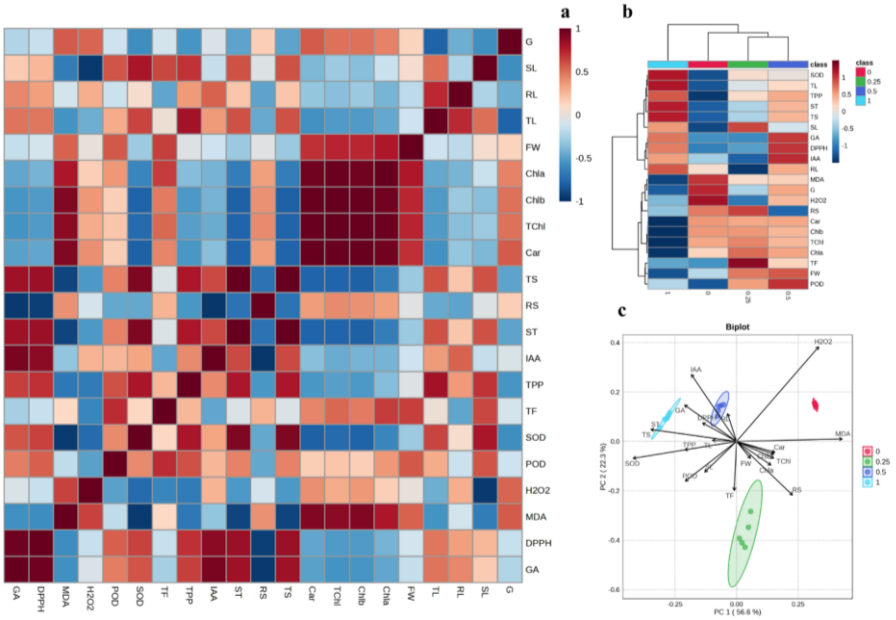

Figure 7. Integrated multivariate analyses of seed treatment with Glu on metabolism, growth, and redox state of maize seedlings. (a) Heatmap of correlation between morphological, physiological, and biochemical parameters, including vegetative growth, photosynthetic pigments, primary and secondary metabolites, antioxidant enzymes, and markers of oxidative stress. Colors indicate positive (red) and negative (blue) correlations. (b) Analysis of the hierarchical grouping of treatments according to the dose of Glu, showing the separation between the control and the doses applied based on the metabolic and physiological profile. (c) Principal component analysis (PCA) shows the distribution of the treatments and the contributions of the evaluated variables, with PC1 and PC2 explaining 56.6% and 22.3% of the total variance, respectively.

The present study aimed to evaluate the effects of Glu on physiological and metabolic responses during germination and early development of maize seedlings (Figure 1). Although the germination percentage did not show significant variation between treatments (Fig. 1a), the morphological parameters related to initial growth were clearly stimulated. This indicates that Glu mainly promoted vigor and cell expansion, rather than seed viability (Saleem et al., 2024). The increase in epicotyl, root, and total seedling length, especially at intermediate doses, indicates greater metabolic efficiency during initial establishment, a critical phase for crop success in the field (Rosa et al., 2023). The observed growth stimulus can be attributed to Glu's central role in nitrogen and carbon metabolism (Liao et al., 2022). This amino acid serves as an essential donor of amino groups and a precursor for the synthesis of other amino acids and crucial nitrogenous compounds, thereby favoring cell division and elongation (Hasanuzzaman et al., 2017). The longer root length observed at 0.5 and 1 mmol L^{-1} suggests that Glu contributed to a more efficient root architecture, thereby enhancing water and nutrient absorption in the early stages of development.

In relation to the photosynthetic apparatus, the application of Glu promoted consistent increases in the levels of chlorophyll *a* and total chlorophyll, especially at the concentration of 0.25 mmol L^{-1} (Fig. 2). These results indicate that seed treatment with Glu favored the biosynthesis of photosynthetic pigments, possibly by stimulating nitrogen assimilation and the formation of tetrapyrroles, structural components of chlorophyll (Quan et al., 2022). The absence of a significant response to chlorophyll b and carotenoids suggests that Glu's effect was primarily directed toward optimizing the photosynthetic reaction center, without causing substantial changes in accessory pigments (Saleem et al., 2024). On the other hand, the reduction in pigments observed at the highest dose suggests that excessive concentrations can cause metabolic imbalance, reinforcing the importance of appropriate dosing. In contrast, studies such as Fard and Hassanpour (2023) have shown that, in some plants, including strawberries, the application of Glu did not significantly alter photosynthetic pigment levels, suggesting that its effectiveness may vary by species and experimental conditions. The introduction of these contrasting data strengthens the credibility of our findings, emphasizing that the impact of Glu on the photosynthetic apparatus depends on several factors.

The improvement in the redox state of seedlings treated with Glu was evident from the significant reduction in MDA and H_2O_2 levels (Figs 3a and 3b), indicating lower lipid peroxidation and greater stability of cell membranes. These results demonstrate that Glu helps maintain cell integrity, even under normal physiological conditions, acting as a modulator of redox balance (Iqbal et al., 2021). At the same time, a marked increase in the activities of the antioxidant enzymes SOD and POD was observed (Figs 3c and 3d), indicating strengthening of the enzymatic antioxidant system. This coordinated adjustment between ROS reduction and antioxidant activation suggests that Glu not only mitigates oxidative damage but also prepares seedlings for more efficient metabolic development (Lee et al., 2017; Iqbal et al., 2021). In primary metabolism, the application of Glu led to a transparent redirection of carbon toward reserve compounds. The linear increase in total sugars and starch contents (Fig. 4a and 4c) indicates greater efficiency in carbon assimilation and storage, providing energy support for initial seedling growth (Qiu et al., 2020; Yu et al., 2022). In contrast, the reduction of reducing sugars at higher doses suggests their rapid conversion into reserve compounds or their use in biosynthetic processes, such as the formation of cellular structures and secondary metabolites. The stability observed in total amino acid content indicates that Glu preferentially modulated carbon metabolism without promoting excessive accumulation of free amino acids, reflecting a balanced and efficient metabolism (Liao et al., 2022).

In addition to primary metabolism, Glu also stimulated the biosynthesis of secondary metabolites with antioxidant activity. The increase in total phenolic and flavonoid contents, along with the higher antioxidant capacity, as evaluated by the DPPH method (Fig. 5), indicates that seed treatment activated metabolic pathways associated with non-enzymatic antioxidant defense (Fard et al., 2023; Gregorio et al., 2023). These compounds play a key role in neutralizing free radicals and protecting cells, contributing to the maintenance of redox homeostasis during early development (Soleymani Aghdam et al., 2015). The hormonal balance of seedlings was also positively affected by Glu application. The significant increases in indoleacetic acid (IAA) and gibberellic acid (GA_3) contents (Fig. 6) indicate that Glu acted as an indirect modulator of hormonal signaling, favoring processes related to cell elongation and vegetative growth (Rosa et al., 2023). These hormones are directly associated with cell expansion, tissue differentiation, and early development, which, in part, explain the increases observed in seedling morphological parameters.

Integrated multivariate analyses reinforce this interpretation. The correlation heatmap showed strong positive associations between growth, photosynthetic pigments, carbohydrates, phenolic compounds, and plant hormones. In contrast, the oxidative stress markers showed negative correlations with these parameters (Fig. 7a). The hierarchical cluster analysis clearly separated the control from Glu treatments, highlighting the dose-dependent effect on initial seedling metabolism (Fig. 7b). In addition, PCA demonstrated that Glu treatments were associated with higher metabolic efficiency, growth, and antioxidant capacity. In comparison, the control remained associated with higher levels of MDA and H_2O_2 (Fig. 7c). Taken together, the results demonstrate that seed treatment with Glu promotes an integrated metabolic reprogramming during the initial establishment of maize, characterized by greater photosynthetic efficiency, strengthening of antioxidant systems, hormonal stimulation, and better carbon allocation (Hasanuzzaman et al., 2017; Qiu et al., 2020; Yu et al., 2022; Rosa et al., 2023). These effects explain the increased vigor and growth of seedlings and support Glu's potential as a promising tool to optimize the early development of maize under normal physiological conditions.

4. Conclusions

Seed priming with Glu improved the physiological and metabolic performance of maize seedlings during early establishment without affecting the final germination percentage. The most consistent responses were observed at

0.5 mmol L^{-1}, which promoted greater seedling growth, increased chlorophyll content, enhanced antioxidant enzyme activity, reduced oxidative damage, stimulated the accumulation of carbohydrates and phenolic compounds, and increased endogenous IAA and GA_3 levels. These responses indicate that Glu modulates interconnected metabolic pathways involved in growth, redox homeostasis, carbon metabolism, and hormonal regulation during the initial stages of seedling development. The present findings demonstrate the potential of Glu as a seed-priming agent to improve early seedling vigor in maize under controlled conditions. However, additional studies under greenhouse and field conditions are required to determine whether these physiological and metabolic responses are sustained throughout the crop cycle and lead to improvements in plant establishment, stress tolerance, and grain yield.

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