



Aluminum Stress Responses in Quinoa Suggest Adaptive Potential in Acidic Environments

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Abstract

Aluminum (Al) toxicity is a major limiting factor for crop production in acidic soils, especially in tropical regions. Although quinoa (*Chenopodium quinoa* Willd.) is widely recognized for its resilience to environmental stresses, its responses specifically to Al exposure remain insufficiently characterized, particularly for Brazilian cultivars such as BRS Piabiru. This study evaluated the physiological and biochemical adjustments of quinoa under increasing Al concentrations, addressing this knowledge gap by identifying dose-dependent stress thresholds under conditions of high Al availability. Quinoa plants were grown hydroponically at pH 4.0, a condition under which Al³⁺ availability is high, and exposed to six Al concentrations (0, 0.2, 1, 5, 25, and 125 mg L⁻¹). The lowest concentration (0.2 mg L⁻¹) was selected because it corresponds to the maximum Al level permitted in drinking water in Brazil and is consistent with the World Health Organization guideline. The subsequent concentrations followed a five-fold geometric progression (0.2 → 1 → 5 → 25 → 125 mg L⁻¹), allowing a controlled and proportional increase in Al supply. The highest concentration (125 mg L⁻¹) was chosen based on previous preliminary trials, in which it induced visible leaf chlorosis during the vegetative stage, thus representing a clearly inhibitory level. Fifty-four days after planting, growth parameters, biomass, oxidative markers (MDA and H₂O₂), antioxidant enzyme activities, and non-enzymatic indicators (proline, soluble sugars, carotenoids) were quantified. High Al concentrations (125 mg L⁻¹) severely inhibited growth, reducing root length by 92%, root volume by 95%, and total dry mass by 54%, while increasing MDA and H₂O₂ by 82% and 105%, respectively. Antioxidant enzymes, particularly GSH-Px, increased by 220%. In contrast, low Al exposure (0.2 mg L⁻¹) produced a mild stimulatory response, with root volume increasing by 22%, total dry mass by 5%, and proline by 296%, consistent with hormetic adjustment. Quinoa cv. BRS Piabiru exhibits a concentration-dependent response to Al, with low doses inducing mild metabolic stimulation and high doses causing pronounced oxidative damage and growth suppression. These hydroponic results clarify physiological tolerance limits and biochemical responses under conditions of high Al³⁺ availability; however, extrapolation to field soils should be made cautiously due to the complexity of soil chemical interactions not reproduced in hydroponics.

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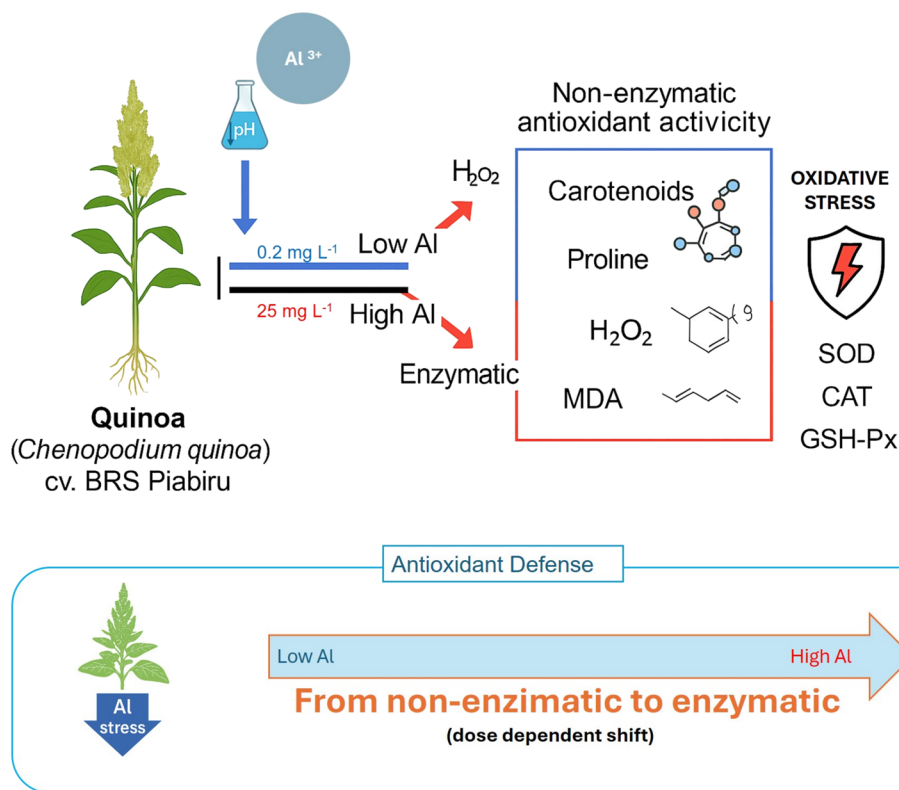
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Graphical Abstract

Antioxidant Defense Responses in Quinoa under Aluminium Stress: Dose-Dependent Shifts between Enzymatic and Non-Enzymatic Mechanisms



Keywords *Chenopodium quinoa* · Aluminum stress · Oxidative stress · Antioxidant enzymes · Proline · Carotenoids · Reactive oxygen species (ROS) · Soil acidity

1 Introduction

Aluminum (Al) is the most abundant metal in the Earth's crust, and its free ionic form (Al³⁺) is toxic to most cultivated species even at low concentrations (Famoso et al. 2010; Cárcamo et al. 2019). The deleterious power of Al is associated with triggering the overproduction of reactive oxygen species (ROS) which, in excess, promote disruption of cellular homeostasis, oxidation of biological macromolecules (lipids, sugars, proteins and nucleic acids), lipid peroxidation and changes in enzymatic activity (Giannakoula et al. 2010; Guo et al. 2018; Yu et al. 2019).

This toxicity is particularly relevant in tropical and subtropical regions, where acid soils with high Al availability limit crop productivity. The high affinity of Al for cell walls, membranes, and metabolites contributes to its toxicity, posing a significant challenge to agricultural production, particularly in tropical and subtropical regions where quinoa cultivation is expanding (Amjad et al. 2022; Phukunkamkaew et al. 2021). Understanding the physiological and

biochemical mechanisms underlying Al tolerance in crops is crucial for enhancing agricultural productivity and ensuring food security in these regions (Lata-Tenesaca et al. 2021).

While organic acid exudation from roots has been widely documented as a key tolerance strategy, recent studies point to the antioxidant defense system—both enzymatic and non-enzymatic—as a crucial mechanism for mitigating Al-induced oxidative damage (Miyasaka et al. 1991; Hinojosa et al. 2019). However, the roles of specific enzymes such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPOX), and glutathione peroxidase (GSH-Px) in Al detoxification remain underexplored, particularly in underutilized but promising crops (Du et al. 2020; Carcamo-Fincheira et al. 2021; Angeli et al. 2020; Sun et al. 2022).

Although Al is traditionally regarded as a toxic element, recent evidence indicates that at low concentrations it may stimulate root growth and antioxidant capacity in certain tolerant species (e.g., tea, rye, maize). Whether such hormetic behavior also occurs in quinoa, a stress-resilient crop, remains unclear.

Quinoa (*Chenopodium quinoa* Willd.), a facultative halophyte and highly nutritious pseudocereal (Grenfell-Shaw & Tester 2021), has gained global attention due to its remarkable adaptability to harsh environments (UNITED NATIONS (UN) 2020). Despite being promoted by FAO as a crop for future food security, there is limited knowledge regarding its physiological and biochemical responses to Al toxicity. Given that quinoa is being introduced into acid soils in Brazil and elsewhere, it is critical to elucidate its tolerance mechanisms to Al stress.

Therefore, this study aimed to investigate the dose-dependent physiological and biochemical responses of quinoa plants to Al exposure, in order to elucidate their adaptive potential to acidic, Al-rich environments.

We hypothesized that low Al concentrations may elicit adaptive, hormetic responses in quinoa, enhancing antioxidant defenses and osmotic adjustment, whereas high aluminum levels would induce oxidative stress and growth inhibition.

2 Materials and Methods

The experiment was conducted in a hydroponic growing system in a controlled conditioned room (temperature, light and humidity) located at Department of Biology, UNESP-FCAV (Faculty of Agrarian and Veterinary Sciences ‘Júlio de Mesquita Filho’) at the São Paulo State University, in Jaboticabal, Brazil. Seeds of quinoa cv. BRS Piabiru (Spehar 2006) were obtained from EMBRAPA, Brazilian Agricultural Research Corporation of the Ministry of Agriculture, Livestock and Food Supply, Brazil.

2.1 Growth Conditions and Experimental Conditions

Seeds of quinoa (*Chenopodium quinoa* Willd.) cv. BRS Piabiru were surface-sterilized in 5% sodium hypochlorite for 5 min, rinsed three times with distilled water, and sown in plastic trays containing a 1:1 (v/v) mixture of BioPlant® commercial substrate (sphagnum peat, vermiculite, and coconut fiber) and vermiculite. The trays were maintained in a controlled-environment room.

Seven-day-old seedlings were transplanted into 2-L pots containing aerated Clark nutrient solution (Clark 1975). Plants remained for seven days in 25% ionic strength for acclimatization and for fifteen additional days in 50% ionic strength prior to the imposition of treatments. The nutrient solution was renewed every three days, and pH was monitored and maintained at 4.0 ± 0.1 using HCl or NaOH.

Al treatments were initiated at 20 days after emergence (DAE). Aluminum was supplied as analytical-grade

aluminum chloride (AlCl_3), following Cárcamo et al. (2019). Six Al concentrations were established: 0 mg L^{-1} (control), 0.2 mg L^{-1} , 1 mg L^{-1} , 5 mg L^{-1} , 25 mg L^{-1} , and 125 mg L^{-1} (equivalent to 4625 $\mu\text{M Al}^{3+}$; Shaff et al. 2010). These concentrations were selected to represent sub-toxic, moderate, and severe Al exposure, enabling the evaluation of dose-dependent changes in growth and stress-related responses.

Clark solution was chosen because its low phosphate concentration minimizes Al precipitation (e.g., AlPO_4 formation) and provides high nutrient stability under acidic conditions. Aluminum speciation was estimated using Geochem-EZ (Shaff et al. 2010), indicating that $\approx 86\%$ of total Al remained as free Al^{3+} at pH 4.0. The pH of all treatment solutions was checked daily to maintain consistent Al^{3+} activity.

The experimental design was completely randomized, with six treatments and twenty replicates per treatment (ten used for growth measurements and ten for biochemical analyses). Plants were harvested at 54 DAE, corresponding to the end of the vegetative stage of cv. BRS Piabiru. Roots and shoots were rinsed with distilled water; part of the material was oven-dried at 60 °C until constant mass for dry-weight and nutrient analyses, and another portion was immediately frozen in liquid nitrogen and stored at -80 °C for biochemical assays (H_2O_2 , MDA, total sugars, proline, and antioxidant enzymes).

2.2 Plant Growth Parameters (Shoot and Root)

Biometric measurements were performed at the end of the experiment, measuring the length of the root and shoot of the plant using a ruler graduated in mm, and for foliar area, using the Image J® software (Orsini et al. 2010).

The plant roots were placed in a graduated beaker with a known volume of water, following the methodology proposed in Siemens and Zwiazek (2008). After the total immersion of the roots, the volume of roots was obtained by the difference between the previous and the current volume. The volume of roots was expressed in cm^3 .

To quantify the accumulation of dry mass, the plants were divided into two parts (shoot and root) and all samples were then placed in paper bags and placed in a hot air oven at 65 °C until constant weight. The final weight was recorded using a scale with a precision of 0.001 g, and the average of each plant in each pot, and was used to determine the dry mass.

2.3 Chlorophyll and Carotenoids Determination

Chlorophyll and carotenoid content were analyzed spectrophotometrically. Fresh leaf samples (0.50 mg) were mixed with 2 mL of cold 80% acetone and shaken for 72 h at 60 g

and 4 °C. Absorbance was measured at 470 nm for carotenoids, 645 nm for chlorophyll a, and 662 nm for chlorophyll b (Lichtenthaler 1987).

Chlorophyll *a*, chlorophyll *b*, and carotenoid concentrations were calculated using the following equations:

$$\begin{aligned}\text{Chlorophyll } \alpha \text{ (mg g}^{-1}\text{FW)} &= 12.7 \times A_{663} - 2.69 \times A_{645} \\ \text{Chlorophyll } \beta \text{ (mg g}^{-1}\text{FW)} &= 22.9 \times A_{645} - 4.68 \times A_{663} \\ \text{Total Chlorophyll (mg g}^{-1}\text{FW)} &= 20.2 \times A_{645} - 8.02 \times A_{663} \\ \text{Carotenoids (mg g}^{-1}\text{FW)} &= \frac{(1000 \times A_{470} - 1.82 \times \text{Chl } a - 85.02 \times \text{Chl } b)}{198}\end{aligned}$$

2.4 Hydrogen Peroxide Measurement

Hydrogen peroxide (H₂O₂) concentration was determined following Alexieva et al. (2001). Frozen plant tissue (200 mg) was ground in liquid nitrogen and mixed with 2 mL of 0.1% TCA. The homogenate was centrifuged (10,000 rpm, 15 min, 4 °C), and 200 µL of supernatant was combined with 200 µL potassium phosphate buffer (pH 7.5) and 800 µL potassium iodide. Absorbance was measured at 390 nm after the mixture was left on ice for 1 h. Results were expressed as µmol g⁻¹ fresh weight (FW) using a standard curve.

2.5 Lipid Peroxidation Analysis

Lipid peroxidation, assessed by thiobarbituric acid-reactive substances (TBARS), followed Heath and Packer (1968). Ground root and leaf samples were mixed with polyvinylpyrrolidone (PVPP) and TCA, centrifuged (11,000×g, 15 min, 4 °C), and reacted with a solution of TCA and TBA. The mixture was heated at 95 °C for 30 min, cooled, and centrifuged. MDA equivalents were measured spectrophotometrically at 535 and 600 nm, using an extinction coefficient of 1.55 × 10⁻⁵ mol⁻¹ cm⁻¹, and expressed as µmol mg⁻¹ FW (Gratão et al. 2012).

2.6 Proline Content Determination

Proline content was measured based on Bates et al. (1973). The leaf and root samples (0.5 g) were homogenized in 3% sulfosalicylic acid, filtered, and reacted with acid ninhydrin and acetic acid. The mixture was heated for 60 min, then extracted with toluene. Absorbance was read at 520 nm, and proline concentration was calculated as mmol g⁻¹ FW.

2.7 Total Sugar

For extraction, 0.5 g of plant material was grinded in porcelain mortar and pestle, and mixed with 5 mL of MCW solution (60% methanol, 25% chloroform and 15% water) and kept inside refrigerator for 24 h at 4 °C. The material

was vortexed and centrifuged at 10,000 rpm for 10 min at 4 °C. In another tube, 2.0 mL of MCW extract supernatant was transferred and mixed with 0.5 mL of chloroform and 0.75 mL of deionized water. After phase separation, the concentration of total sugars was determined using the water-soluble phase.

To quantify total sugars, 10 µL of supernatant was mixed with 250 µL of 5% (m/v) phenol and 1 mL of concentrated sulfuric acid (DuBois et al. 1956). The mixture was vortexed and after cooling down to room temperature, the absorbance of samples was read at 490 nm. The results were expressed as mg g⁻¹ FW.

2.8 Enzyme Extraction and Analysis

Enzyme extraction from leaves and roots followed Bradford (1976) with modifications by Boaretto et al. (2014). Tissues were homogenized in a chilled mortar with pestle using an extraction buffer (100 mM potassium phosphate buffer, pH 7.5, 1 mM EDTA, 3 mM DL-dithiothreitol, and 5% w/v insoluble polyvinylpyrrolidone) at a 3:1 ratio for leaves and fruits, and 2:1 for roots. The homogenate was centrifuged at 10,000 ×g for 30 min, and the supernatant stored at -80 °C for further assays of superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), guaiacol peroxidase (GPOX, EC 1.11.1.7), and glutathione peroxidase (GSH-Px, EC 1.11.1.9). Protein concentration was determined using the Bradford (1976) method with bovine serum albumin as a standard.

SOD activity was measured using the Giannopolitis and Ries (1977) protocol, with an assay mixture of 50 mM sodium phosphate buffer (pH 7.8), 50 mM methionine, 10 mM EDTA, 1 mM nitro blue tetrazolium chloride, and 0.1 mM riboflavin, incubated under a fluorescent lamp at 25 °C for 5 min. Absorbance was recorded at 560 nm, and results were expressed in U mg⁻¹ protein.

CAT activity was determined by H₂O₂ degradation at 240 nm over 60 s using a reaction mixture of protein extract, 1 mL 100 mM potassium phosphate buffer (pH 7.5), and 25 µL H₂O₂ (30%) at 25 °C. Results were expressed as mmol min⁻¹ mg⁻¹ protein (Nogueirol et al. 2015).

GSH-Px activity, following Monteiro et al. (2011) with modifications, involved incubating plant extract with 100 mM potassium phosphate buffer (pH 7.0), 3 mM EDTA, 0.24 U GR/mL, 10 mM GSH, and 1 mM sodium azide at 37 °C for 10 min. After adding 1.5 mM NADPH and 1.5 mM H₂O₂, NADPH oxidation was monitored for 5 min at 340 nm. GSH-Px activity was calculated using a molar extinction coefficient of 6.22 mM⁻¹ cm⁻¹ and expressed as µmol min⁻¹ mg⁻¹ protein.

GPOX activity was assessed according to Monteiro et al. (2011) using a phosphate-citrate buffer (pH 5.0), plant

extract, and 0.5% guaiacol. After incubating at 30 °C for 15 min, the reaction was stopped with 2% sodium metabisulfite. GPOX activity was monitored at 450 nm, with one unit corresponding to a 0.001 increase in absorbance per minute per mg^{-1} protein.

2.9 Statistical Analysis

The data were submitted to the normality test and Levene's homoscedasticity, both at 0.05 probability ($p < 0.05$) using software R (R Development Core Team 2015). Subsequently, normal data were subjected to analysis of variance by the F test ($p < 0.05$) and, when significant, the means were compared using the Tukey test ($p < 0.05$).

Results were expressed as the mean and standard error of the mean (SEM) of four independent replicates for plant growth, chlorophyll and carotenoids contents, proline content, total sugars and total soluble proteins and for each plant extract MDA, H_2O_2 , SOD, CAT, GPOX and GSH-Px activities. Statistical analysis was performed using the statistical software AgroEstat®. Analysis of variance (ANOVA) was calculated for each parameter measured, and when significant, means were compared using Tukey's post hoc test at 5% probability ($p < 0.05$).

Pearson's correlation analysis ($p < 0.05$) was also performed to verify the dependent variables that were directly correlated to the treatments. The "corrplot" package was accessed to create the heatmap, using the "color" and "cor.mtest" functions to create coefficient and p-value matrices, respectively. To facilitate the visualization of significant correlations, asterisks were inserted in the heatmap cells.

3 Results

3.1 Effects of Aluminum on Shoot and Root Growth

The highest Al concentration (125 mg L^{-1}) was responsible for decreasing length of shoot (Fig. 1A), foliar area (Fig. 1B), root length (Fig. 1C) and root volume (Fig. 1D). However, there was tolerance and a trend towards a beneficial effect of Al at the lowest experimental concentration (0.2 mg L^{-1}). As for the length of the shoot, the concentration of 0.2 mg L^{-1} of Al obtained greater shoot height (Fig. 2A), with an increase of 2% in relation to the control treatment, however it did not differ from this one (Fig. 2A). The smallest shoot lengths were found in the highest Al concentrations, with a decrease of 12 and 13%, respectively in 25 and 125 mg Al L^{-1} of Al. The foliar area (Fig. 1B) was reduced as the Al concentration increased, and the largest foliar areas were obtained in the control treatment and 0.2 mg of Al L^{-1} , in which a slight increase of 2.5% in the foliar area was observed, compared to the control treatment. Subsequent treatments were responsible for decreases of 31%, 34%, 46% and 62% of foliar area in relation to the control treatment (Fig. 1B).

3.2 Root Length and Root Volume Responses to Aluminum

Interestingly, from the concentration of 1 mg of Al L^{-1} there was a sudden decrease in the length of roots (43%, 52%, 60% and 92%); however, there was no statistical difference between concentrations 1, 5, 25 and 125 mg of Al L^{-1} (Fig. 1C). However, they were lower compared to the control treatment and 0.2 mg L^{-1} .

Fig. 1 Effects of aluminum on growth parameters of quinoa. Shoot length (A), leaf area (B), root length (C), and root volume (D) of fifty-four-day-old quinoa plants grown for twenty-four days under six aluminum concentrations (0, 0.2, 1, 5, 25, and 125 mg L^{-1}). Values represent the mean $\pm$ standard error of the mean (SEM, $n = 10$). Different letters above the columns indicate significant differences among treatments at $P < 0.05$ according to Tukey's test

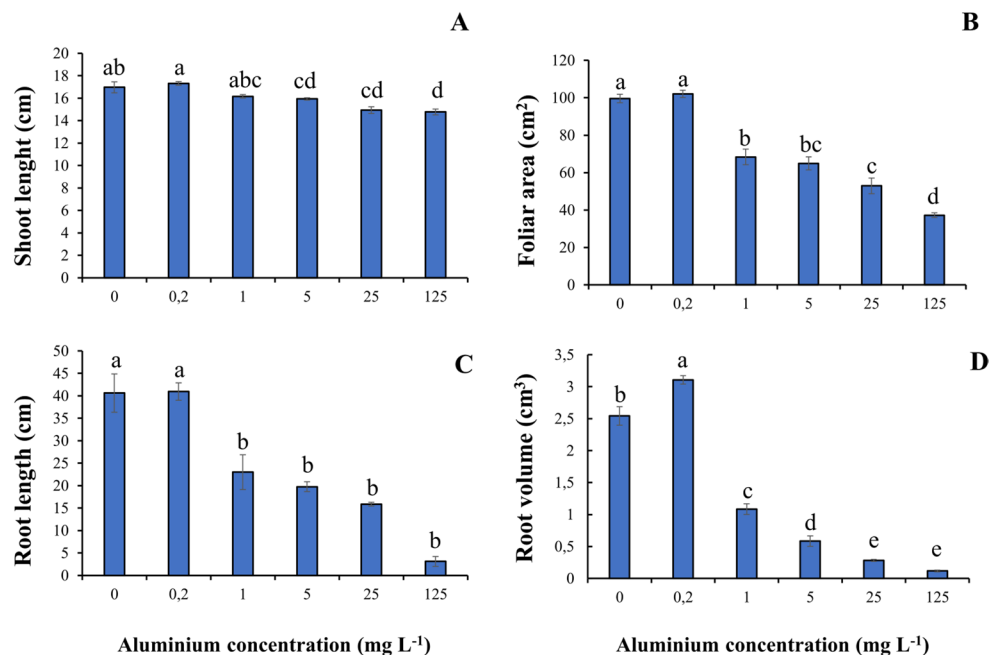
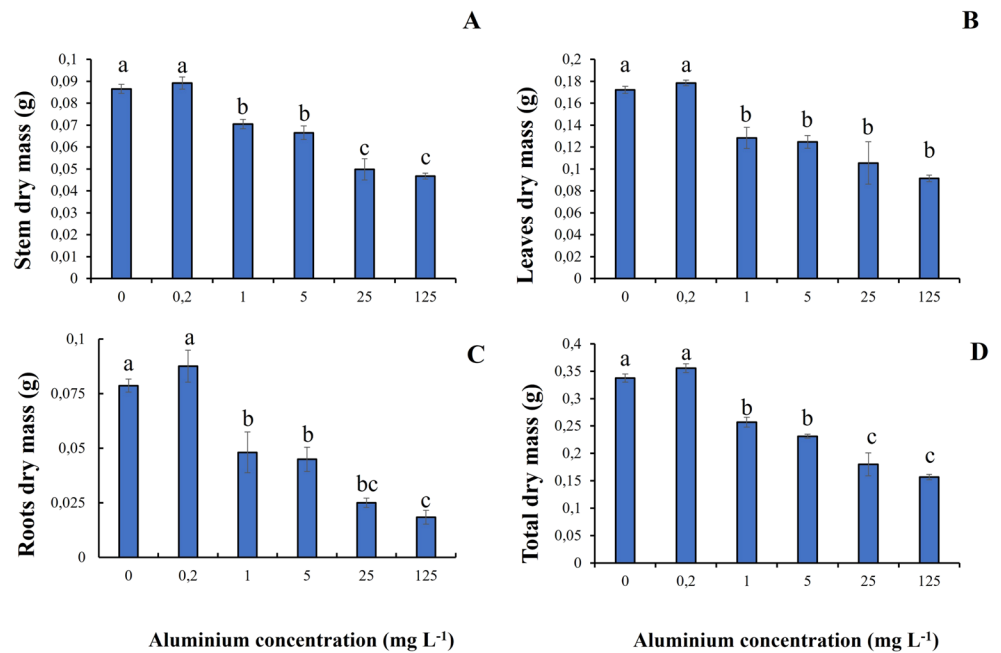


Fig. 2 Effects of aluminum on biomass accumulation in quinoa. Stem dry mass (A), leaf dry mass (B), root dry mass (C), and total dry mass (D) of fifty-four-day-old quinoa plants grown for twenty-four days under six aluminum concentrations (0, 0.2, 1, 5, 25, and 125 mg L⁻¹). Values represent the mean $\pm$ standard error of the mean (SEM, $n=10$). Different letters above the columns indicate significant differences among treatments at $P<0.05$ according to Tukey's test



The largest volumes of roots were seen in the control treatment and in the concentration of 0.2 mg of Al L⁻¹, and the lowest concentration of Al (0.2 mg) had an increase of 3.12% in the volume of roots in relation to the control treatment (Fig. 1D). The decrease in root volume in treatments 1, 5, 25 and 125 mg of Al L⁻¹ was 59%, 78%, 89% and 95% in relation to the control treatment (Fig. 1D).

3.3 Dry Mass Accumulation of Shoots and Roots

The dry mass of quinoa plants was gradually reduced as Al concentrations increased. The highest dry mass of plants was obtained in control treatment and the lowest Al concentration (0.2 mg Al L⁻¹), and there was no difference between these treatments for roots and shoots. The dry mass of the shoot had an increase of 3.5% in the concentration of 0.2 mg Al L⁻¹ and a decline in the highest concentrations of Al. Such reductions were 19%, 27%, 40% and 46% at concentrations of 1, 5, 25 and 125 mg of Al, respectively (Fig. 2A).

The dry mass of plant roots increased by 11.34% at the concentration of 0.2 mg Al L⁻¹ in relation to the control treatment. At concentrations 1, 5, 15 and 125 mg of Al L⁻¹ there was a decrease in the dry mass roots of 39%, 43%, 68% and 76%, respectively (Fig. 2C). The total dry mass was increased by 5% at the concentration of 0.2 mg of Al in relation to the control treatment. In the subsequent concentrations (1, 5, 25 and 125 mg L⁻¹) there was a decline of 24%, 31%, 46% and 53% of the dry mass of plants (Fig. 2D).

3.4 Soluble Protein and Total Sugar Contents

The soluble protein content was lower in the highest Al concentrations and higher in the plants of the control treatment, 0.2 mg L⁻¹ and 1 mg L⁻¹ (Fig. 3A). At the highest concentrations of Al (25 and 125 mg of Al) there was a decrease in total protein content of 46% and 61%, respectively. There was no statistical difference between treatments with higher protein content (control, 0.2 and 1 mg L⁻¹).

Total plant sugars were higher at concentrations of 0.2 and 5 mg L⁻¹, being 28% and 37% higher than the control treatment. The highest concentration of Al (125 mg L⁻¹) was responsible for a 17% decrease in relation to the control treatment (Fig. 3B).

3.5 Photosynthetic Pigment Contents

The plants that received the control treatment, 0.2 and 1 mg of Al L⁻¹ did not differ from each other in terms of total chlorophyll content, being superior to the others. The lowest total chlorophyll content was observed in plants treated with 25 and 125 mg of Al, with decreases of 43% and 75%, respectively (Fig. 4A).

The plants that received the treatment with 0.2 mg of Al L⁻¹ resulted in the highest carotenoid content, with an increase of 13% in relation to the control treatment. Al concentrations of 25 and 125 mg were responsible for a 36% and 71% decline in carotenoid content (Fig. 4B).

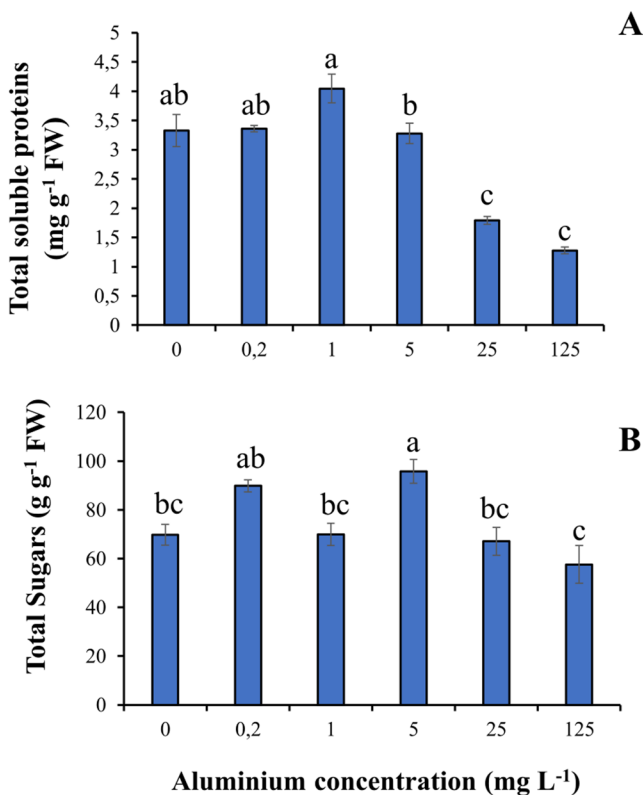


Fig. 3 Total soluble proteins and sugars in quinoa under aluminum stress. Total soluble proteins (**A**) and total sugars (**B**) measured in leaves of fifty-four-day-old quinoa plants grown for twenty-four days under six aluminum concentrations (0, 0.2, 1, 5, 25, and 125 mg L⁻¹). Values represent the mean $\pm$ standard error of the mean (SEM, $n=10$). Different letters above the columns indicate significant differences among treatments at $P<0.05$ according to Tukey's test

3.6 Proline Accumulation and Hydrogen Peroxide Content

Proline content in plants was increased with the addition of Al concentrations in plants, except for the concentration of 0.2 mg L⁻¹, which was higher than the control treatment and lower than the concentration of 125 mg L⁻¹. The content of proline in plants that received the treatment 125 mg of Al L⁻¹ was increased 501% in relation to the control treatment. Surprisingly, the 0.2 mg Al concentration also resulted in a 295% increase in proline content in quinoa plants, compared to the control treatment. There was no statistical difference between the control and 1 mg Al treatments (Fig. 5A).

The plants treated with 125 mg L⁻¹ had the highest levels of H₂O₂. The control treatment and the concentration of 5 mg L⁻¹ were responsible for the lowest levels of H₂O₂ in quinoa plants. The treatment 1 mg of Al and 25 mg of Al obtained an increase of 36% and 24% in the hydrogen peroxide content compared to the control. Interestingly, in the 5 mg Al treatment there was a 12% decrease in the peroxide content, compared to the control treatment (Fig. 5B).

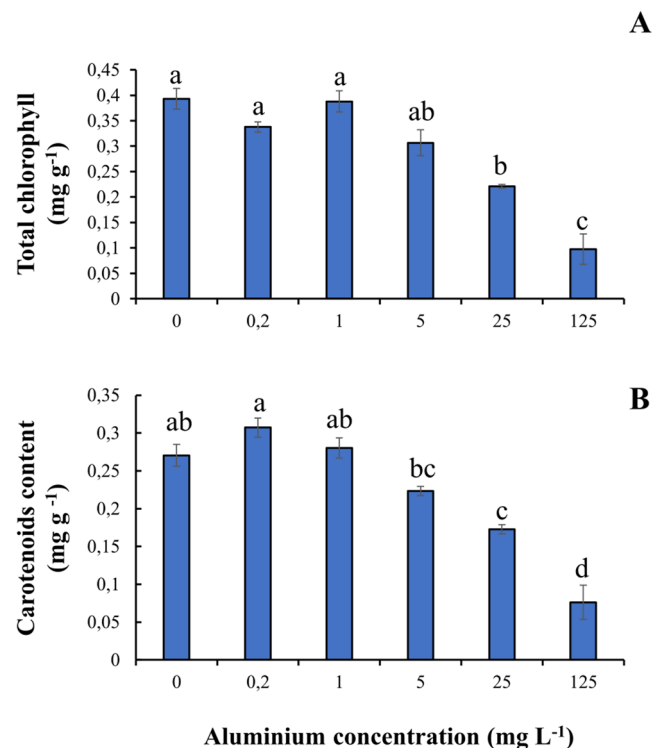


Fig. 4 Photosynthetic pigments of quinoa exposed to aluminum. Total chlorophyll (**A**) and carotenoid content (**B**) measured in leaves of fifty-four-day-old quinoa plants grown for twenty-four days under six aluminum concentrations (0, 0.2, 1, 5, 25, and 125 mg L⁻¹). Values represent the mean $\pm$ standard error of the mean (SEM, $n=10$). Different letters above the columns indicate significant differences among treatments at $P<0.05$ according to Tukey's test

3.7 Lipid Peroxidation (MDA content)

The MDA content increased as Al concentrations increased; however, there was no difference between the lowest Al concentration (0.2 mg Al) and the control treatment. The increase in MDA content of plants was 9.45%, 24%, 45%, 61% and 82% in treatments 0.2, 1, 5, 25 and 125 mg L⁻¹, respectively, compared to the control treatment (Fig. 5C). The highest concentration of MDA was observed in plants that received the highest concentration of Al (125 mg L⁻¹).

3.8 Antioxidant Enzyme Activities

In plants that received the treatments 25 mg L⁻¹ and 125 mg L⁻¹, higher activity of the SOD enzyme was observed (78% and 149%, respectively), and there was no difference between the control treatments, 0.2, 1 and 5 mg L⁻¹ of Al (Fig. 6A). In plants treated with 5 mg L⁻¹ and 125 mg of Al, an increase of 62% and 56% in the activity of the catalase enzyme was observed, and there was no difference between the control treatments, 0.2 and 1 mg L⁻¹ (Fig. 6B).

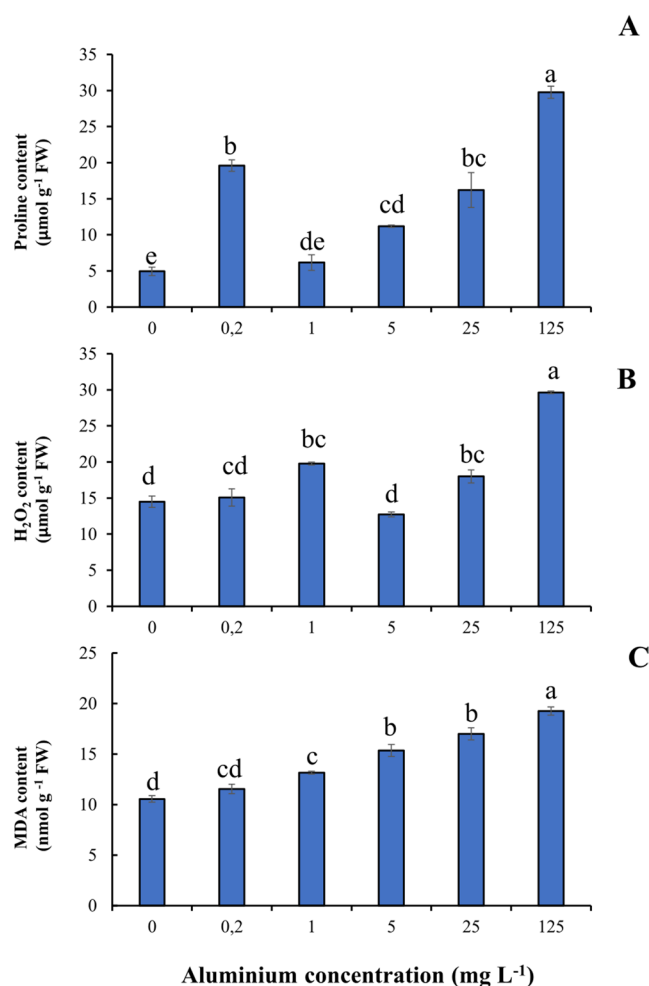


Fig. 5 Oxidative stress markers in quinoa under aluminum exposure. Proline (A), H₂O₂ content (B), and MDA content (C) measured in leaves of fifty-four-day-old quinoa plants grown for twenty-four days under six aluminum concentrations (0, 0.2, 1, 5, 25, and 125 mg L⁻¹). Values represent the mean $\pm$ standard error of the mean (SEM, $n=10$). Different letters above the columns indicate significant differences among treatments at $P<0.05$ according to Tukey's test

The treatment 0.2 mg of Al L⁻¹ was responsible for higher activity of the GPOX enzyme in quinoa plants, with an increase of 220.55% in relation to the control treatment (Fig. 6C), and the lowest activities were observed in the control treatment and 25 mg L⁻¹.

Al stimulated the enzymatic activity of GSH-Px, such that the highest activities of the enzyme GSH-Px were obtained in plants with higher concentrations of Al (25 and 125 mg of Al), being 135% and 219% higher than the control treatment. Except for the concentration of 1 mg L⁻¹, there was a tendency for a progressive and linear increase in GSH-Px activity as the Al concentration in the medium increased by 57%, 14%, 51%, 135% and 219%, respectively, compared to the control treatment (Fig. 6D).

3.9 Correlation Analysis

A positive correlation was observed between biometrics parameters, dry mass, photosynthetic pigments and total protein. A negative correlation was obtained between MDA and biometrics parameters, dry mass, photosynthetic pigments and total proteins. SOD correlated positively with MDA, H₂O₂ and proline. CAT negatively correlated with chlorophyll a, carotenoids and proline. Sugars were positively correlated with proline and negatively correlated with MDA. GPOX was positively correlated with total sugars. A positive correlation was observed between GSH-Px and proline, SOD, MDA and H₂O₂, and a negative correlation was observed between GSH-Px and all biometrics parameters, photosynthetic pigments, dry mass and total soluble proteins (Fig. 7).

4 Discussion

The high availability of Al³⁺ in the growing environment of plants limits the growth of most cultivated agricultural species. Although plant breeding programs have selected cultivars with greater tolerance to Al³⁺, the biochemical and physiological mechanisms involved in plant protection against this element still need to be clarified. Quinoa is the only species among cereals and pseudo-cereals, which, in addition to having all the essential amino acids for the human diet (Oliveira Vergara et al. 2019), was selected as a food for global food security (UNITED NATIONS, 2020) and has been considered tolerant to various abiotic stresses (Lata-Tenesaca et al. 2021). Thus, we consider the hypothesis that the BRS Piabiru quinoa cultivar presents detoxification mechanisms to the deleterious effects of Al³⁺, given that the cultivar was developed for a savana environment, notably with acidic pH with high Al³⁺ contents (Spehar et al. 2006).

Our results showed that quinoa plants grown in the presence of 0.2 mg L⁻¹ Al showed an increase in root volume (Fig. 1D) and root dry mass (Fig. 2C), maybe because of the intensification of secondary roots, stimulated by Al in non-toxic concentrations. This phenomenon may be related to the already reported effect of the metal observed in other species, especially in cultures of *Camellia sinensis*, Indian rhododendron (*Melastoma malabathricum*) and rice (*Oryza sativa*), in which Al is able to induce root elongation, in addition to the development of secondary roots, which may explain the increase in root volume in such plants (Ghanati et al. 2005; Watanabe et al. 2005; Matsumoto & Motoda 2012), but the mechanisms involved in this induction are still not well understood.

Fig. 6 Antioxidant enzyme activities in quinoa under aluminum stress. Superoxide dismutase (SOD) (A), catalase (CAT) (B), guaiacol peroxidase (GPOX) (C), and glutathione peroxidase (GSH-Px) (D) measured in leaves of fifty-four-day-old quinoa plants grown for twenty-four days under six aluminum concentrations (0, 0.2, 1, 5, 25, and 125 mg L⁻¹). Values represent the mean $\pm$ standard error of the mean (SEM, $n=10$). Different letters above the columns indicate significant differences among treatments at $P<0.05$ according to Tukey's test

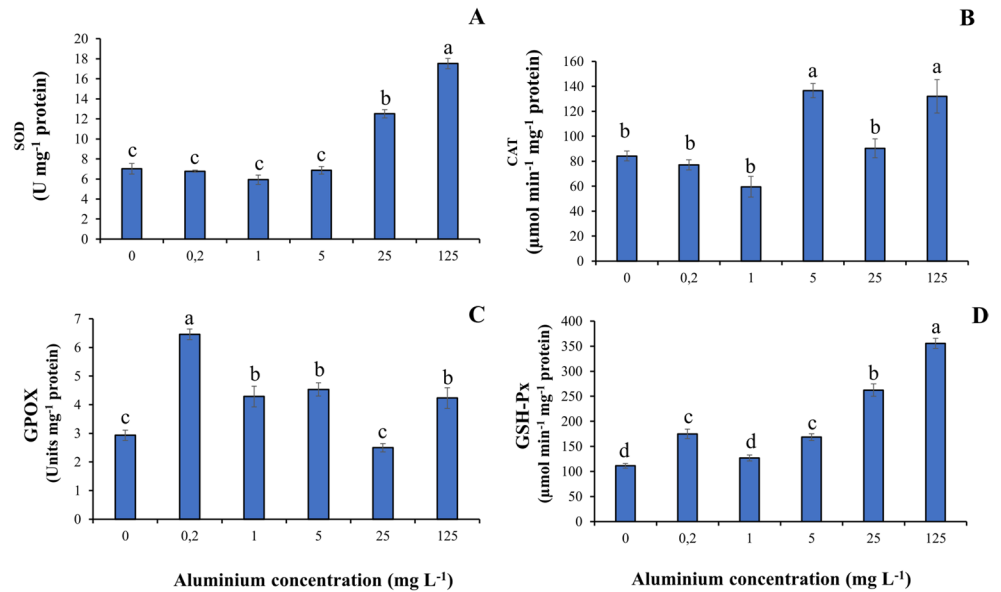
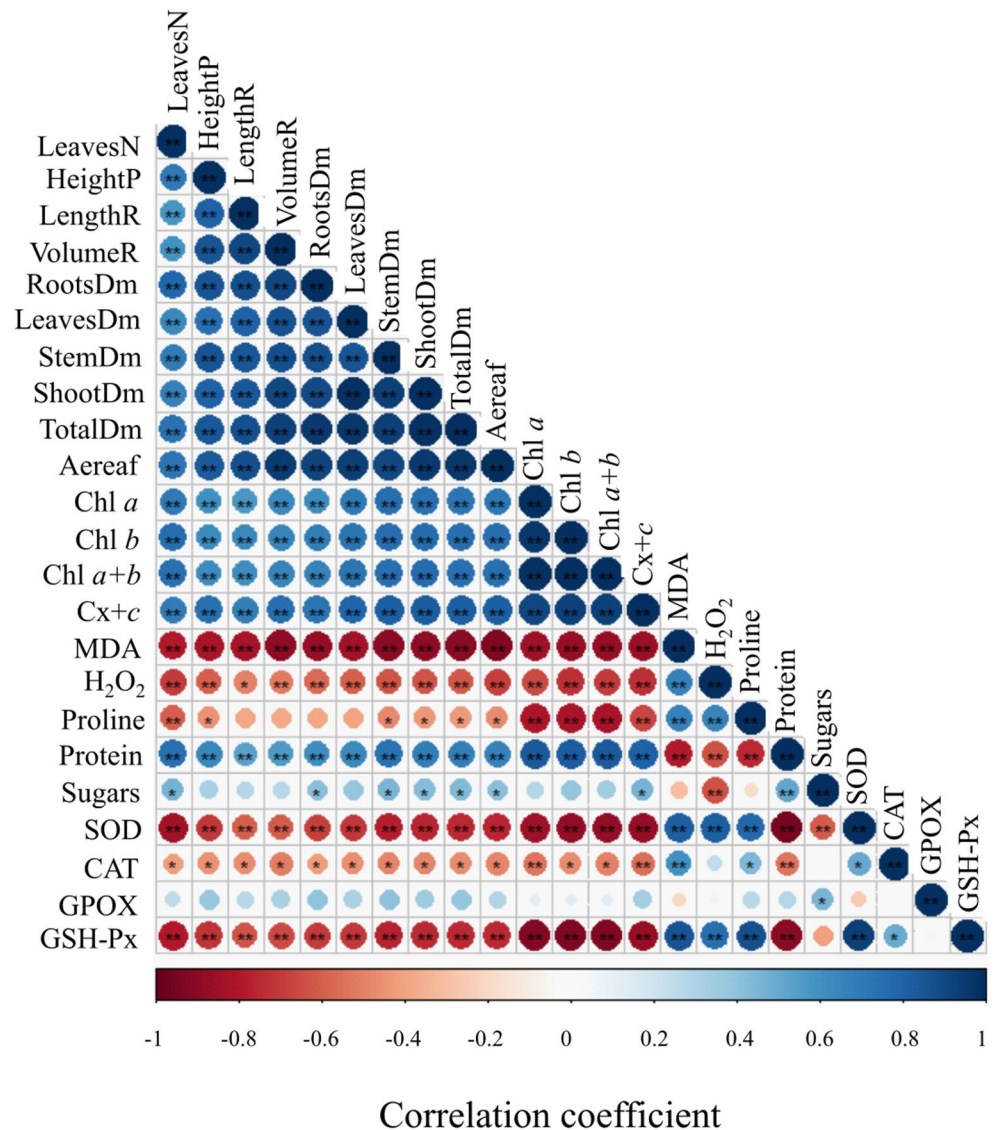


Fig. 7 Correlation matrix of quinoa responses to aluminum. Heatmap showing the Pearson correlation among biometric, physiological, biochemical, and enzymatic parameters of quinoa plants exposed for twenty-four days to six aluminum concentrations (0, 0.2, 1, 5, 25, and 125 mg L⁻¹) supplied as aluminum chloride. Asterisks indicate significant correlations ($p\leq0.05$; ** $p\leq0.01$). Abbreviations: number of leaves (LeavesN), plant height (HeightP), root length (LengthR), root volume (VolumeR), root dry mass (RootsDm), leaf dry mass (LeavesDm), stem dry mass (StemDm), shoot dry mass (ShootDm), total dry mass (TotalDm), foliar area (AreaF), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl *a*+*b*), total carotenoids (Cx+c), malondialdehyde (MDA), hydrogen peroxide (H₂O₂), proline (Proline), total soluble proteins (Protein), total sugars (Sugars), superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPOX), and glutathione peroxidase (GSH-Px)



The stimulus caused by Al under low Al concentration (less than 0.5 mg L^{-1}), can exercise a positive influence on root growth and photosynthetic rate, in crops such as cereals (Famoso et al. 2010; Abubakar et al. 2023; Moustaka et al. 2018) and rice (Ma et al. 2012; Stein et al. 2019; Ashraf et al. 2015). In fact, our data showed an increase in carotenoid content (Fig. 4D) and root volume (Fig. 1D) at 0.2 mg L^{-1} concentration. At higher concentrations (1 to 125 mg L^{-1}), Al induced physiological damage both in roots and shoots, represented by reductions in the size and volume of roots and, in the foliar area, negatively impacting the accumulation of dry mass of these organs. (Figures 1 and 2). This suggests that Al damage to quinoa plants is systemic, affecting the entire plant.

In addition to plant root damage (Abd El-Moneim et al. 2014; Awasthi et al. 2019), Al also causes changes in the shoot, such as a decrease in foliar area (Gavassi et al. 2020) and alteration in leaf coloration, either by whitish dots (Rouphael et al. 2016) and/or yellowing (Blamey et al. 2015; Riaz et al. 2018;). The phytotoxic action of Al causes changes in leaf structures that can directly affect the activity of the photosynthetic system (Almeida Rodrigues et al. 2019). In fact, from 5 mg L^{-1} of Al, the quinoa plants showed a significant reduction in the levels of total chlorophyll and carotenoids (Fig. 4), indicating a lower capacity for photochemical efficiency. This directly affected cellular metabolic integrity reflecting decreases in total sugar and protein content (Fig. 3), important precursors of other essential plant metabolites (Wang et al. 2015). This mechanism contributes to elucidate the role of Al in reducing the growth of BRS Piabiru quinoa plants.

Oxidative stress in plants is characterized by the disruption of cellular homeostasis, generated from the overproduction of reactive oxygen species (ROS), with consequent damage or even collapse of cellular metabolism and numerous deleterious effects to the plant, if there is no activation of mechanisms plant defense antioxidants (detox), whether enzymatic or non-enzymatic (Sun et al. 2022).

Although there is evidence that the antioxidant system (enzymatic and non-enzymatic) is responsive to high concentrations of Al (Awasthi et al. 2019; Amjad et al. 2022; Sun et al. 2022), recent studies on these mechanisms of Al tolerance show divergences. For example, wheat plants exposed to 10 mM Al had a significant increase in the activity of catalase and ascorbate peroxidase enzymes (Yu et al. 2019). Whereas in rice, the same concentration of Al decreased the activity level of these enzymes (Awasthi et al. 2019).

Thus, when there is an overproduction of ROS, plants activate an enzymatic antioxidant defense system, which comprises, for example, the enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px),

guaiacol peroxidase (GPOX), and non-enzymatic mechanisms, such as proline (PRO) and carotenoids (CAR) that are also involved in the elimination of excess ROS in plant tissue (Serna et al. 2020; Amjad et al. 2022; Ashraf & Foolad 2007) and protect plant cells from oxidative damage, eliminating and neutralizing ROS, in order to restore the balance between their production and degradation.

In our results, stress and oxidative damage by Al was evidenced by the increase in MDA content (Fig. 5C), decline in the content of photosynthetic pigments (Fig. 4A and B), proteins (Fig. 3A) and total sugars (Fig. 3B) which negatively affected plant growth (Figs. 1) with a consequent decrease in biomass accumulation (Fig. 2D), with a decline in shoot dry mass (Fig. 2A and B) and root dry mass (Fig. 2C) from the concentration of 1 mg of Al L^{-1} , and the decrease being more pronounced from the concentration of 25 mg of Al^{-1} .

In this sense, our study showed that aluminum in concentrations above 5 mg L^{-1} caused an increase in malondialdehyde (MDA) concentration (Fig. 5C), capable of adversely affecting all biometric parameters, photosynthetic pigments, total soluble proteins and total sugars, with a consequent decrease in production of plant biomass (Figs. 1 and 2), with a more pronounced effect on the roots (Fig. 1D). Furthermore, concentrations above 5 mg L^{-1} led to a decrease in total proteins (Fig. 3A), although accompanied by an increase in the activity of enzymes, such as SOD (Fig. 6A) and GSH-Px (Fig. 6D).

The first defense response of plants to restore cellular homeostasis and alleviate Al toxicity are strategies of an enzymatic detox mechanism, with the participation of enzymes such as SOD, which dismutate superoxide (O_2^-) into hydrogen peroxide (H_2O_2). Then the H_2O_2 can be eliminated by catalase (CAT, EC 1.11.1.6), glutathione peroxidase (GPX, EC 1.11.1.9) (Noctor et al. 2018) and other peroxidases. The CAT and GPOX enzymes play an important role in the detoxification of H_2O_2 (hydrogen peroxide), as they use this substrate from the SOD enzyme and degrade it into H_2O and O_2 . Our results evidence such an event. In our work, the activity of the SOD enzyme was lower at the lowest Al concentrations (0.2 , 1 and 5 mg L^{-1}) and did not differ from the quinoa plants of the control treatment (Fig. 6A). Furthermore, there was no pronounced increase in MDA content at such concentrations (Fig. 5C), which can be deduced that there was no high enzymatic activity of this enzyme as there was no imbalance of cellular homeostasis with ROS overproduction at lower Al concentrations. However, the highest concentrations of Al (25 and 125 mg L^{-1}) caused an increase in the activity of the SOD enzyme (Fig. 5A), being even greater in the highest concentration of Al, which also corresponds to the highest content of H_2O_2 (Fig. 5B). Such a result is expected and indicates the high capacity of the enzyme in dismuting O_2^- into H_2O_2 .

These findings are consistent with the results of previous studies that showed that SOD activity was increased in other plants already considered Al tolerant, such as tea (Huang et al. 2021) and rice (Basit et al. 2022). This intensify of enzymes activities is also observed in in *ZmAT6* transgenic maize plants, which the activity of several enzymes of the antioxidant system, such as superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX) and catalase (CAT), and increased in *ZmAT6* transgenic maize plants, principally SOD (Du et al. 2020).

Our results indicate that there are fundamentally two mechanisms of Al tolerance in quinoa plants, depending on the concentration: at the lowest concentration (0.2 mg L^{-1}) there is an increase in the non-enzymatic antioxidant defense system, such as proline (Fig. 5A) and carotenoids (Fig. 4B), at the highest concentration (125 mg) there is a significant increase in the activity of antioxidant defense enzymes, such as SOD, CAT and GSH-Px (Fig. 6A, B and D). These results indicate the dynamism of the plant defense antioxidant system in maintaining the physiological activities of quinoa plants under different Al concentrations in acidic environments.

Furthermore, our data showed that the enzyme GSH-Px appears to be closely linked to promoting Al tolerance in quinoa plants. The increase in its activity is related to the improvement of biometric parameters, dry mass and photosynthetic pigments, negatively correlating with stress indicators, such as MDA and proline (Fig. 7).

Surprisingly, the 1 mg L^{-1} concentration stimulated the production of total soluble proteins in quinoa plants, however, at the highest concentrations, the decreases were linear. In a study by Tamman et al. (2018) on rice seedlings, the application of $500 \text{ }\mu\text{M}$ Al also increased the total soluble protein content by 20% compared to the control treatment. This addition is not clear in the literature; however, we speculate that it is also a mechanism of Al tolerance in quinoa plants.

The amino acid proline occurs widely in plants and normally accumulates in large amounts in response to abiotic stresses (Ashraf et al. 2015; Giannakoula et al. 2008), including Al stress (Galvez et al. 1991; Khan et al. 2000). However, our data reveal that the concentration of 0.2 mg L^{-1} did not represent a stressful factor for the tested quinoa cultivar, on the contrary, such concentration promotes root growth, with a stimulating effect on carotenoid, enzymatic and proline levels. Such responses are in line with studies by Moreno-Alvarado et al. (2017) in which $200 \text{ }\mu\text{M}$ of Al resulted in a beneficial effect on plant growth, amino acid and proline content. In this sense, our data show that the levels of proline in quinoa plants exposed to Al confirmed the role of this osmolyte in relieving Al toxicity, especially at the concentration of 0.2 mg L^{-1} (Fig. 5A). The role of

proline as a cell protector under stress conditions from the elimination and buffering of redox potential cells was also demonstrated in the work of (Ashraf et al. 2015) with rice culture. In addition to compatible osmolytes, the enzymatic antioxidant defense system also acts to minimize the negative effects of Al on plants (Lu et al. 2025; Kuo and Kao 2003). Recent studies show the contribution of enzymatic mechanisms in quinoa plants exposed to different types of stress such as salt (Parvez et al. 2020), water deficit (Serna et al. 2020), heat stress (Hinojosa et al. 2019) and heavy metals such as cadmium and lead (Guarino et al. 2020). In this study, increases in the activity of SOD, CAT, GPOX and GSH-Px enzymes were observed in plants subjected to aluminum (Fig. 6). However, such enzymatic activities showed different activation patterns: GPOX showed higher activity at the lowest Al concentrations (Fig. 6C), while SOD, CAT and GSH-Px were stimulated at the highest (Fig. 6A, B and D), suggesting that these antioxidant enzymes remained active, providing greater protection preventing plant collapse and death, although Al had negative effects on the growth and metabolism of quinoa plants, such enzymes prevented total metabolism collapse and plant death.

Although the antioxidant system was comprehensively analyzed, root organic-acid exudation—one of the classical external mechanisms of Al detoxification—was not quantified in this study. This omission was intentional, as our main objective was to characterize the internal physiological and enzymatic antioxidant responses that mitigate oxidative stress caused by Al exposure. Nonetheless, organic-acid efflux represents a complementary strategy that facilitates Al exclusion from the rhizosphere. Future studies should integrate root-exudate profiling and the expression analysis of transporters associated with citrate and malate release (e.g., ALMT and MATE genes) to achieve a more complete understanding of Al tolerance mechanisms in quinoa.

Since the experiments were conducted under hydroponic conditions, the extrapolation of the present results to field environments must be approached with caution. Hydroponic systems allow strict control of pH, ionic strength, and Al^{3+} activity, thus enabling clear identification of the physiological and biochemical responses to aluminum exposure. However, these systems do not replicate soil buffering capacity, mineral and organic matter interactions, or rhizosphere microbial processes that influence Al speciation and availability in natural acidic soils. Therefore, future studies under real soil conditions are required to validate whether the antioxidant adjustments and hormetic responses observed here are maintained when quinoa grows in Al-rich acidic environments.

These findings align with recent evidence highlighting the potential of soil amendments and nutrient interactions in mitigating Al toxicity under acidic conditions. For instance,

El-Desouki (2025) demonstrated that phosphorus-laden biochar can effectively reduce Al availability and promote plant growth in acid soils, emphasizing the importance of nutrient–Al interactions in improving crop tolerance. Such complementary strategies reinforce the physiological basis observed here for quinoa's resilience under Al exposure.

Furthermore, except for GPOX, there was a positive correlation between the enzymes SOD, CAT and GSH-Px with the main oxidative stress indicator MDA (Fig. 7), evidencing the important role of these enzymes in the protection of Al stress. Furthermore, the positive correlation between SOD, CAT and GSH-Px showed the synergism of action of these enzymes (Fig. 7).

Although Al³⁺ availability in the nutrient solution was estimated by Geochem-EZ (Shaff et al. 2010) to be approximately 86% of total Al, no direct quantification of soluble or tissue Al was performed. Future studies should therefore validate these results through ICP-OES or related analytical methods to determine both Al³⁺ activity in solution and Al accumulation in plant tissues. Such verification would provide a stronger basis for linking physiological responses to actual Al uptake and speciation.

Recent advances also indicate that epigenetic regulation plays an important role in plant adaptation to Al stress. Zhang (2024) reported that treatment with 5-azacytidine enhanced Al tolerance in grapevine by reducing DNA methylation levels, revealing a potential epigenetic component in Al response. In this context, future transcriptomic and epigenomic analyses in quinoa could help identify regulatory networks and signaling pathways associated with Al-induced stress tolerance.

Taken together, our results support the proposed hypothesis that low Al concentrations can stimulate adaptive physiological and biochemical responses in quinoa, whereas high concentrations induce oxidative damage and defense activation. This biphasic, concentration-dependent response reinforces the concept of hormesis in Al tolerance and confirms quinoa's remarkable capacity to adjust its antioxidant machinery under varying degrees of stress. Therefore, the findings provide experimental evidence validating the hormetic hypothesis and highlight the potential of quinoa as a strategic crop for cultivation in acidic and Al-rich environments.

5 Conclusion

This study elucidated the dose-dependent physiological and biochemical responses of quinoa to aluminum (Al) exposure under acidic conditions and assessed its adaptive potential to Al-rich environments. The results support the hypothesis that low Al availability triggers adaptive responses, whereas

high Al concentrations induce stress-related physiological alterations and growth inhibition.

Low Al exposure promoted slight growth stimulation associated with the activation of non-enzymatic antioxidant components, indicating a favorable physiological adjustment. In contrast, elevated Al levels caused marked growth reduction, increased oxidative stress markers, and enhanced antioxidant enzyme activities, reflecting a predominance of enzymatic defense responses under high Al availability.

Overall, quinoa exhibits concentration-dependent antioxidant strategies, demonstrating physiological plasticity under moderate Al availability in acidic conditions with high Al solubility. From an agronomic and ecophysiological perspective, these findings contribute to defining Al tolerance thresholds in quinoa. This work advances the understanding of plant–aluminum interactions and supports the strategic evaluation of quinoa as a stress-tolerant crop for sustainable production in marginal agroecosystems.

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Data Availability All data generated or analyzed during this study are included in this published article. Additional data is available from the corresponding author upon reasonable request.

Declarations

Conflict of Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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