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**Micronutrient deficiencies and nickel toxicity differentially alter growth,
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Abstract

Micronutrient homeostasis is essential for maintaining plant metabolism and cellular integrity, whereas micronutrient deficiency and excess nickel (Ni) disrupt fundamental physiological processes. However, the cellular responses associated with individual micronutrient deficiencies and Ni exposure remain poorly understood in passion fruit (*Passiflora edulis* Sims). This study characterized the morphophysiological and ultrastructural responses of passion fruit seedlings subjected to individual omissions of boron (B), manganese (Mn), iron (Fe), zinc (Zn), copper (Cu), and molybdenum (Mo) or to Ni addition under hydroponic conditions. Seedlings were grown in a complete nutrient solution, Ni-supplemented solution, or solutions lacking individual micronutrients. Visual symptoms, nutrient concentrations, chlorophyll content, gas exchange, root morphology, biomass accumulation, and leaf mesophyll ultrastructure were evaluated. The order of symptom appearance was Fe > Mn > Zn > Cu > Mo > B, indicating a greater sensitivity of seedlings to Fe and Mn deprivation. Each treatment induced a distinct phenotype. Zn and Cu deficiencies caused the greatest reductions in shoot and root biomass, whereas Fe and Mn deficiencies produced the strongest declines in chlorophyll content and photosynthetic performance. Ni-toxicity induced chlorosis, growth inhibition, and pronounced alterations in chloroplast organization. Transmission electron microscopy revealed nutrient-specific ultrastructural changes, including modifications in chloroplast morphology, thylakoid organization, starch granules, plastoglobules, and middle lamella integrity. Overall, micronutrient imbalance and Ni addition differentially affected plant growth, physiology, and mesophyll ultrastructure, providing new insights into the cellular consequences of disrupted micronutrient homeostasis in *P. edulis* and contributing to the diagnosis of nutritional disorders associated with micronutrient deficiency and Ni toxicity.

Keywords: chloroplast organization; micronutrient homeostasis; nickel toxicity; *Passiflora edulis*; root morphology; photosynthetic performance; transmission electron microscopy

1. Introduction

Passion fruit is the most commercially important species of the *Passifloraceae* family and is widely cultivated throughout tropical regions (Cárdenas-Pira et al. 2021). As with other perennial fruit crops, the growth, productivity, and establishment of passion fruit seedlings depend on an adequate nutritional balance. Therefore, understanding the physiological and structural consequences of micronutrient deficiencies is essential for accurate nutritional diagnosis and the development of efficient fertilization strategies (Mendonça Freitas et al. 2008; Lalmuanpuii et al. 2025). Despite its economic relevance, yellow passion fruit has been comparatively underexplored with respect to micronutrient deficiency symptoms, and only a few studies have examined its responses to nutrient omission under controlled conditions (da Cunha et al. 2024; Lizcano Toledo et al. 2025).

Micronutrients regulate fundamental processes associated with plant growth and development because they participate as structural components or enzymatic cofactors in photosynthesis, respiration, antioxidant metabolism, nitrogen assimilation, and cell-wall biosynthesis (Hänsch and Mendel 2009; Tripathi et al. 2015). Micronutrients are required in relatively small amounts but are indispensable for plant growth because each fulfills unique biochemical and physiological functions. Fe is essential for chlorophyll biosynthesis, photosynthetic electron transport, and respiration. Mn participates in the oxygen-evolving complex of photosystem II and contributes to antioxidant defense. B is primarily involved in cell-wall formation, membrane stability, and carbohydrate transport. Zn acts as a structural and catalytic component of numerous enzymes and transcription factors regulating protein synthesis and growth. Cu participates in photosynthetic and respiratory electron transport, lignification, and reactive oxygen species detoxification, whereas Mo is required for nitrate reductase

and other molybdoenzymes involved in nitrogen metabolism. Furthermore, Ni, although required only in trace amounts, is indispensable for urease activity and nitrogen recycling; however, the narrow range between deficiency, sufficiency, and toxicity makes it unique among plant micronutrients. Consequently, disturbances in the availability of these elements rapidly compromise chloroplast function, carbon assimilation, and plant development.

Because these micronutrients regulate interconnected metabolic pathways, their deficiency rapidly disrupts fundamental physiological processes and commonly produces characteristic visual, physiological, and structural symptoms (Gupta et al. 2024). Reduced nutrient availability impairs chlorophyll biosynthesis, photosynthetic electron transport, antioxidant metabolism, membrane stability, and cell-wall organization, leading to alterations in leaf pigmentation, morphology, gas exchange, and biomass accumulation (Lizcano Toledo et al. 2025). Although each micronutrient performs specific biochemical functions, deficiencies often induce overlapping responses because multiple metabolic pathways depend on their coordinated activity (Tripathi et al. 2015). These physiological disturbances precede visible deficiency symptoms and are frequently accompanied by ultrastructural alterations, particularly in chloroplasts, before culminating in reduced plant growth.

Because chloroplasts are among the first organelles affected by nutritional imbalance, transmission electron microscopy (TEM) has become an indispensable tool for identifying the earliest structural alterations associated with micronutrient deficiencies and metal toxicity. (Khoshravesh et al. 2022). Changes in chloroplast morphology, grana organization, thylakoid membranes, plastoglobules, and starch accumulation frequently precede measurable reductions in photosynthetic performance and biomass production (Li et al. 2021). Consequently, integrating ultrastructural observations with physiological measurements, such as chlorophyll content and leaf gas exchange, provides a mechanistic framework for understanding how nutritional disorders impair plant development (Yu et al. 2024).

94 Among plant micronutrients, Ni deserves particular attention because, unlike the other essential
95 elements, its physiological window is extremely narrow. Although low concentrations are required
96 for urease activity and nitrogen metabolism, a slight increase in tissue Ni rapidly promotes oxidative
97 stress, chloroplast dysfunction, membrane destabilization, and inhibition of photosynthesis
98 (Alibakhshi and Khoshgoftarmanesh 2014; Kumar et al. 2025). While low concentrations are
99 necessary for urease activity and nitrogen metabolism, excessive Ni rapidly induces oxidative stress,
100 disrupts chloroplast organization, inhibits photosynthesis, and restricts plant growth (Hänsch and
101 Mendel 2009; Lizcano Toledo et al. 2025). Consequently, Ni addition may produce physiological and
102 ultrastructural alterations comparable to those induced by micronutrient deficiencies, despite arising
103 through different biochemical mechanisms. Understanding these similarities and differences is
104 essential for accurately diagnosing nutritional disorders and metal stress in crop plants.

105 Despite the recognized importance of micronutrients for plant development, information
106 regarding the integrated responses of passion fruit to individual micronutrient deficiencies remains
107 scarce. Existing studies have primarily focused on visual deficiency symptoms or growth responses,
108 whereas the physiological and ultrastructural mechanisms underlying these nutritional disorders are
109 still poorly understood. Likewise, the effects of Ni addition on chlorophyll accumulation,
110 photosynthetic performance, and chloroplast organization in passion fruit have received little
111 attention, despite the increasing concern over Ni contamination in agricultural systems. Integrating
112 morphometric, physiological, and ultrastructural analyses is therefore essential to identify early
113 indicators of nutritional imbalance, improve the diagnosis of micronutrient disorders, and provide
114 mechanistic insights into how nutritional stress affects plant development. Such information may
115 contribute to more efficient nutrient management strategies and enhance seedling quality in
116 commercial passion fruit production.

Based on the central role of micronutrients in chloroplast function and photosynthetic metabolism, we hypothesized that Ni addition and individual micronutrient deficiencies would induce distinct but convergent alterations in leaf morphology, chlorophyll accumulation, gas exchange, and chloroplast ultrastructure, ultimately limiting biomass accumulation in passion fruit seedlings. Therefore, the objective of this study was to characterized the growth, physiological and ultrastructural responses of passion fruit seedlings subjected to individual micronutrients (B, Mn, Fe, Zn, Cu, and Mo deficiencies or to Ni addition under hydroponic conditions.

2. Materials and methods

2.1 Plant material and growth conditions

A pot experiment was conducted at São Paulo State University - UNESP, Campus de Jaboticabal, SP, Brazil (21°15'22" S and 48°18'58" W, 610 m altitude) in greenhouse conditions. The greenhouse temperature and relative humidity were monitored throughout the experiment and averaged at 25 ± 2 °C and 70 % under natural daylight conditions (approximately 12 h day length during the experimental period). Seeds of the hybrid BRS Yellow Giant of passion fruit were sown in vermiculite-filled containers and was irrigation every day with deionized water to maintained the moisture. The experimental unit consisted of polypropylene pots (8 dm³) filled with 6 dm³ of medium-textured vermiculite (90 to 100% of particles with a diameter between 0.30 and 2.40 mm), bulk density: 80-150 kg m⁻³, solution retention capacity: 495.6 mL L⁻¹.

The vermiculite was subsequently rinsed several times with deionized water until chloride ions were no longer detected using 1 mol L⁻¹ AgNO₃ solution (absence of AgCl precipitate). After the treatment with HCl, vermiculite underwent repeated washes with deionized water until the washing water gave a negative test with 1 mol L⁻¹ silver nitrate (AgNO₃) solution (absence of white precipitate formation of the silver chloride-AgCl (Lizcano Toledo et al. 2025). Seeding was done in

polypropylene trays with 50 cells filled with vermiculite at a depth of 1.0 cm. The substrate was moistened daily with deionized water twice.

2.2 Nutrient solution preparation and treatment application

The seedlings were transplanted to pots 40 days after sowing, when they had two pairs of fully formed leaves. Immediately after transplantation, nutrient solution was prepared using the methodology previously proposed by Hua and Meyerowitz (1998) following composition: 0.1 mM KH_2PO_4 , 2.0 mM KNO_3 , 2.0 mM $\text{Ca}(\text{NO}_3)_2$, 0.2 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.7 mM KCl , 10 μM H_3BO_3 , 0.2 μM MnCl_2 , 0.5 μM ZnSO_4 , 0.2 μM CuSO_4 , 0.05 μM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$, and 60 μM Fe-EDTA. Nutrient solution was supplied daily at 0.35, 0.40 and 0.45 L plant⁻¹ during 0–15, 15–35 and 35–60 DAT, respectively, using solutions with 25%, 50% and 100% ionic strength. The nutritional solution was maintained at pH 6.0 ± 0.1 using 1 mol L⁻¹ HCl; the electrical conductivity was maintained in the range of 1-2 dS m⁻¹.

2.3 Experimental design

The experimental design was completely randomized, with eight treatments and with five repetitions. Passion fruit seedlings were grown hydroponically under a complete nutrient solution (FS), Ni addition, or individual omission of B, Mn, Fe, Zn, Cu, or Mo. Each pot was considered one experimental unit.

2.4 Nickel addition and micronutrient deficiency treatment application

Treatments involving micronutrient (B, Mn, Fe, Zn, Cu, and Mo) omission were prepared using the complete nutrient solution, except that the respective micronutrient was omitted. The Ni treatment consisted of supplementing the complete nutrient solution with 0.03 mg L⁻¹ Ni supplied as $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$. The treatments were conducted until the symptoms of visible deficiency symptoms developed, except for the treatment with complete nutrient solution, which was conducted until the

emission of the first tendril, which occurred 60 DAT, the period considered suitable for the formation of yellow passion fruit seedlings (Lizcano Toledo et al. 2025).

2.5 Shoot and Root Growth analysis

Seedlings were harvested immediately after visible deficiency symptoms had developed. Except where otherwise indicated, shoot and root growth parameters were evaluated at the end of the experiment. Plant height (cm) and internode length (cm) were quantified using a millimeter ruler; the stem diameter (mm) was measured using a digital caliper. Relative chlorophyll content (RCC) was measured weekly using a portable chlorophyll meter (SPAD-502, Konica Minolta Sensing, TECNAL, Piracicaba, Brazil), beginning at week 0 (immediately before the imposition of Ni addition and micronutrient omission treatments) and continuing throughout the experimental period. Immediately after harvest, leaf area was measured using a leaf area meter (L-3100, Li-Cor, USA). Following harvest, plants were separated into roots and shoots, placed in paper bags, and dried in a forced-air circulation oven (M214Ai, BEL Analytical Equipment Ltd., Brazil) at 60 °C until constant dry mass was obtained. The dry mass of roots and

After harvest, roots were carefully removed from the vermiculite and washed with running deionized water to eliminate all substrate particles without damaging fine roots. The cleaned root systems were blotted dry with absorbent paper and spread uniformly in a transparent acrylic tray containing a thin layer of water to minimize root overlap during image acquisition. Root images were obtained using a Delta-T Scan root analysis system (Delta-T Devices Ltd., Cambridge, UK) coupled to a flatbed scanner at a resolution of 400 dpi. The software automatically quantified root length (mm), root surface area (cm²), root diameter (mm), and root density (cm cm⁻²), calculated as the ratio between root length and the scanned image area. All images were analyzed using the same calibration settings and threshold parameters to ensure consistency among samples.

2.6 Determination of leaf gas exchange parameters

Measurements were performed at the end of each treatment immediately before harvest, when the seedlings had 8 to 10 vigorous true leaves and the first tendril was emerging, evaluations were carried out on the fourth leaf, fully developed, from the apex, in one plant per pot. The variables evaluated were: net photosynthetic rate (A ; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), transpiration (E , $\text{mmol m}^{-2} \text{ s}^{-1}$), stomatal conductance (g_s ; $\text{mol m}^{-2} \text{ s}^{-1}$), and the intercellular-to-ambient CO_2 concentration ratio (C_i/C_a) using an infrared gas analyzer (LICOR, Inc., LI-6400), under ambient CO_2 concentrations ($372 \pm 10 \mu\text{mol mol}^{-1}$), with a photosynthetically active photon flux of $1,200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, between 7 and 8 am.

2.7 Ultrastructural analysis of leaf mesophyll in passion fruit

When symptoms of nutrient deficiency appeared, ten fully expanded leaves were collected per treatment with and without B, Mn, Fe, Zn, Cu, Mo, the addition of Ni, and complete treatment to assess the changes in leaf mesophyll ultrastructure. Leaf segments ($1\text{--}2 \text{ mm}^2$) were excised to determine the cellular structures in the leaves using TEM. Samples were dehydrated and fixed according to the method of Wullschleger and Oosterhuis (1987), after which ultrastructural features were visualized and documented using a Jeol JEM-1010 transmission electron microscope.

2.8 Statistical analysis

Data obtained for biometric parameters and leaf gas exchange were tested for normality and homogeneity of variances using the Shapiro–Wilk and Levene's tests, respectively. After confirming that these assumptions were satisfied, the data were subjected to one-way analysis of variance (ANOVA). When a significant treatment effect was detected ($p < 0.05$), means were separated using Tukey's honestly significant difference (HSD) test. The RCC were analyzed separately for each treatment by fitting linear and quadratic regression models using ordinary least squares (OLS). The most appropriate model was selected based on the Akaike Information Criterion (AIC), with the model

presenting the lowest AIC considered the best fit. Model performance was evaluated using the adjusted coefficient of determination (Adjusted R^2), and the significance of each regression was assessed by ANOVA (F-test). Because quadratic models consistently provided the best fit across all treatments, only the selected quadratic models are presented. All analyses were performed using R software (version 4.6.1).

3 Results

3.1 Impact of nickel addition and micronutrient deficiencies on relative chlorophyll content

Ni addition and micronutrient deficiencies differentially affected the temporal dynamics of relative chlorophyll content (RCC), resulting in distinct patterns of chlorophyll accumulation throughout plant development (Fig. 3). The selected models explained a high proportion of the temporal variation in RCC, with adjusted coefficients of determination (Adjusted R^2) ranging from 0.77 to 0.99. The strongest model fits were obtained for the CK, Fe, and Mn treatments (Adjusted $R^2 = 0.99$), followed by B (Adjusted $R^2 = 0.98$), Mo (Adjusted $R^2 = 0.95$), and Ni (Adjusted $R^2 = 0.91$), whereas Cu (Adjusted $R^2 = 0.77$) and Zn (Adjusted $R^2 = 0.78$) exhibited comparatively greater temporal variability while maintaining significant quadratic responses. All fitted models were highly significant ($p < 0.001$), confirming that sampling time exerted a marked influence on RCC irrespective of micronutrient treatment.

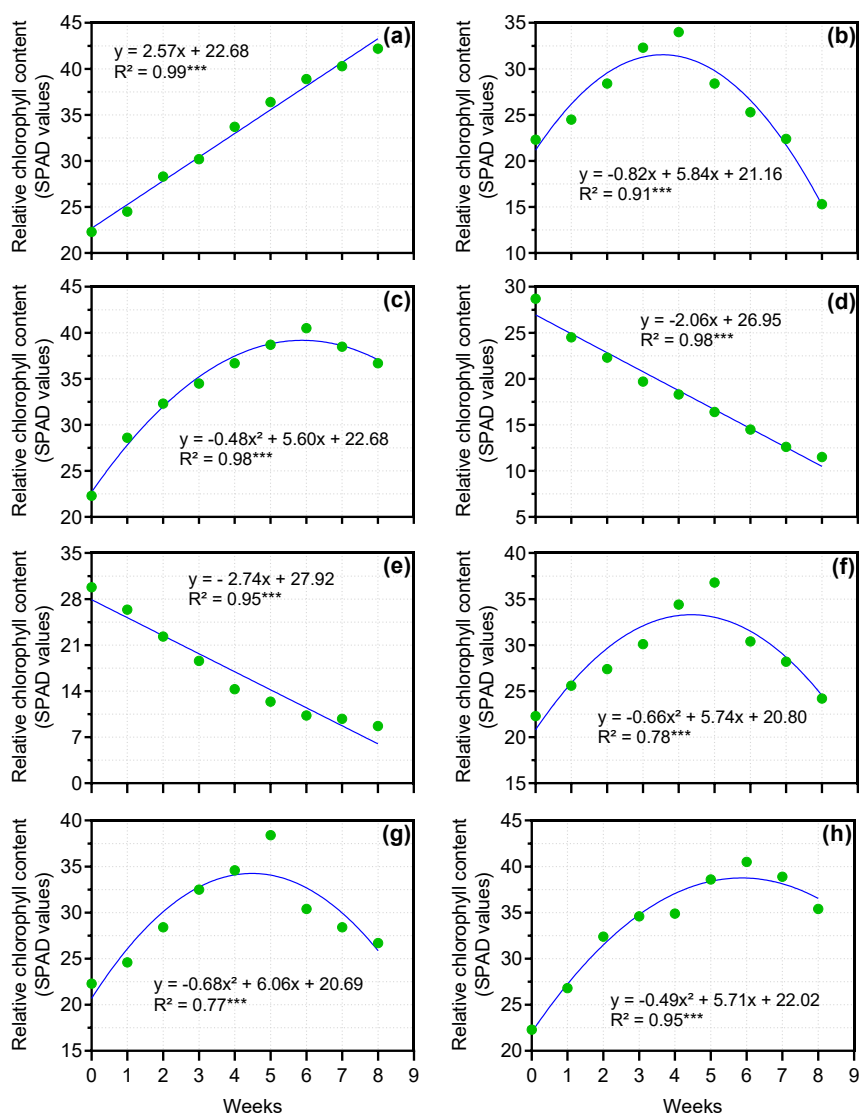


Fig. 1 Temporal changes in relative chlorophyll content (RCC) as a function of week after different treatment application: full treatment (a), nickel addition (b), Boron deficiency (c), Manganese deficiency (d), Iron deficiency (e), Zinc deficiency (f), Copper deficiency (g), Molybdenum deficiency (h). Green circles represent the observed values and blue lines indicate the best-fitting linear or quadratic regression models selected according to the Akaike Information Criterion (AIC). Each panel includes the regression equation, the adjusted coefficient of determination (R^2), and $^{***}(p < 0.001)$.

3.2 Impact of Ni addition and micronutrient deficiencies on leaf gas exchange

Ni addition and micronutrient deficiencies differentially modulated leaf gas exchange in passion fruit plants, resulting in distinct physiological responses in net CO₂ assimilation (A), stomatal conductance (g_s), transpiration rate (E), intercellular-to-ambient CO₂ concentration ratio (C_i/C_a), and instantaneous water-use efficiency (WUE) (Fig. 4). Net CO₂ assimilation (A) was reduced by all micronutrient treatments relative to the complete nutrient solution (FS), with Zn deficiency producing the greatest reduction, followed by Cu deficiency, Ni addition, Fe deficiency, B deficiency, Mo deficiency, and Mn deficiency, the latter showing the smallest decrease in photosynthetic carbon assimilation (Fig. 2a).

Stomatal conductance (g_s) also declined under all treatments compared with FS (Fig. 2b). The greatest reduction was observed under Zn deficiency, followed by Ni addition, Cu deficiency, Fe deficiency, Mn deficiency, Mo deficiency, and B deficiency, indicating distinct degrees of stomatal limitation among micronutrient treatments. Transpiration rate (E) was similarly affected by micronutrient availability (Fig. 2c). Compared with FS, transpiration was most strongly reduced by Ni addition, followed by Zn deficiency, Cu deficiency, B deficiency, Mo deficiency, Fe deficiency, and Mn deficiency, with the latter exhibiting the smallest reduction.

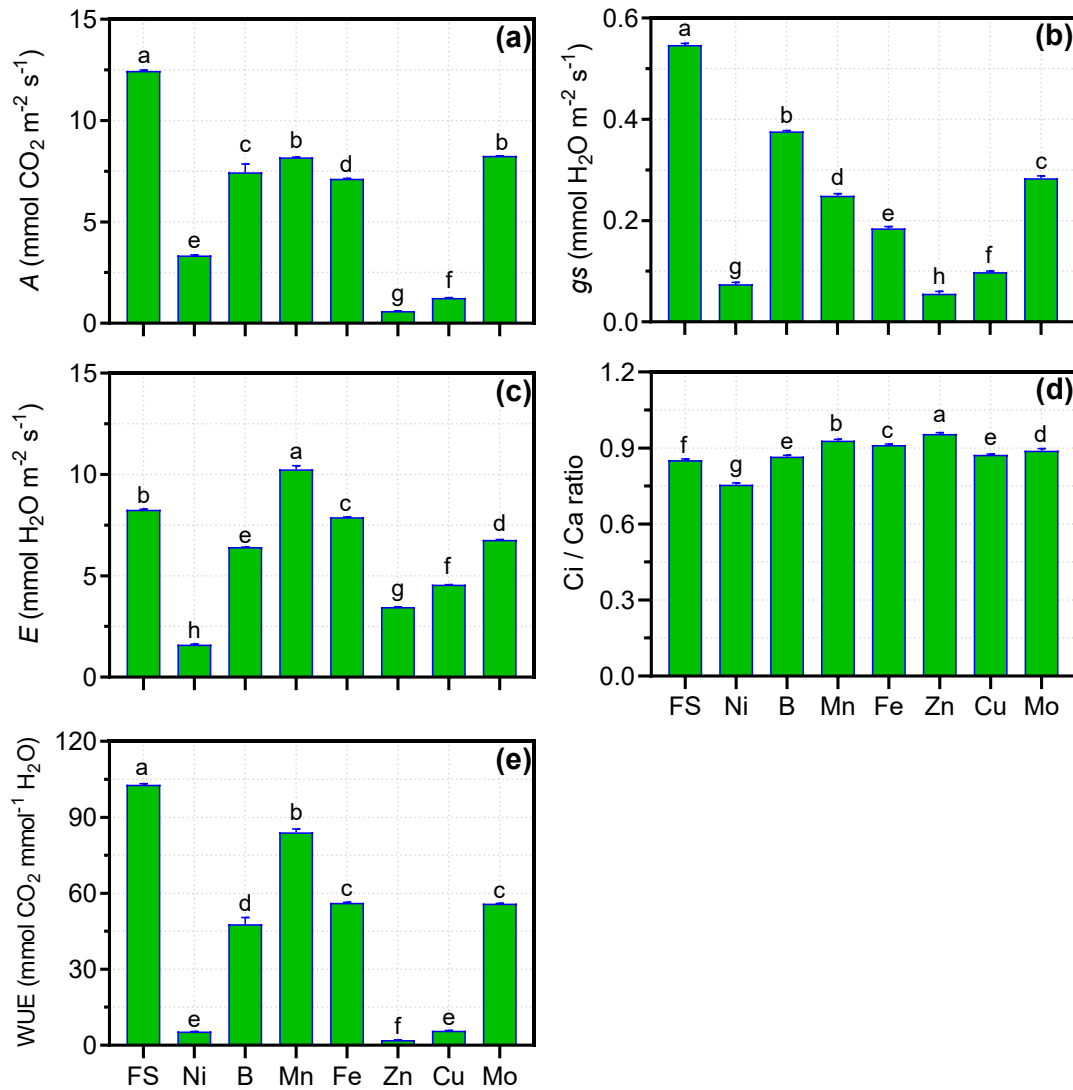


Fig. 2 Effects of micronutrient treatments on leaf gas exchange parameters. (a) Net CO₂ assimilation rate (A), (b) stomatal conductance (g_s), (c) transpiration rate (E), (d) intercellular-to-ambient CO₂ concentration ratio (C_i / C_a ratio), and (e) instantaneous water-use efficiency (WUE). Bars represent the mean $\pm$ standard error ($n = 5$). Different lowercase letters indicate significant differences among treatments according to Tukey's multiple comparison test ($p < 0.05$). Treatments: full treatment (FS), nickel addition (Ni), Boron deficiency (B), Manganese deficiency (Mn), Iron deficiency (Fe), Zinc deficiency (Zn), Copper deficiency (Cu), Molybdenum deficiency (Mo)

The C_i/C_a ratio exhibited a different response pattern (Fig. 2d). Relative to FS, the ratio increased under all micronutrient treatments, with the greatest increase observed under Zn deficiency, followed by Mn deficiency, Fe deficiency, Mo deficiency, B deficiency, Cu deficiency, and Ni addition, which showed the smallest increase. Additionally, instantaneous water-use efficiency (WUE) decreased under all micronutrient treatments relative to FS (Fig. 2e). The greatest reduction occurred under Zn deficiency, followed by Ni addition, Cu deficiency, B deficiency, Fe deficiency, Mo deficiency, and Mn deficiency, indicating that Mn deficiency had the least detrimental effect on the balance between carbon gain and water loss.

3.3 Impact of Ni addition and Micronutrient Deficiencies on Leaf ultrastructure

Ni toxicity markedly altered the ultrastructure of leaf mesophyll cells relative to the FS treatment (Fig. 3). At the tissue level, mesophyll cells from control plants exhibited well-organized cellular architecture, with intact cell walls, clearly distinguishable nuclei, and chloroplasts uniformly distributed along the cell periphery (Fig. 3b). In contrast, Ni-treated plants maintained the overall cellular organization but displayed conspicuous alterations in chloroplast morphology and intracellular organization (Fig. 3a). Higher-magnification observations confirmed that chloroplasts from control plants possessed compact grana, homogeneous stroma, and well-preserved plastoglobules (Fig. 3d, f), whereas those from Ni-treated plants exhibited irregular morphology, less organized thylakoid membranes, and altered plastoglobule distribution, indicating substantial disruption of chloroplast ultrastructure relative to the complete treatment (Fig. 3c, e).

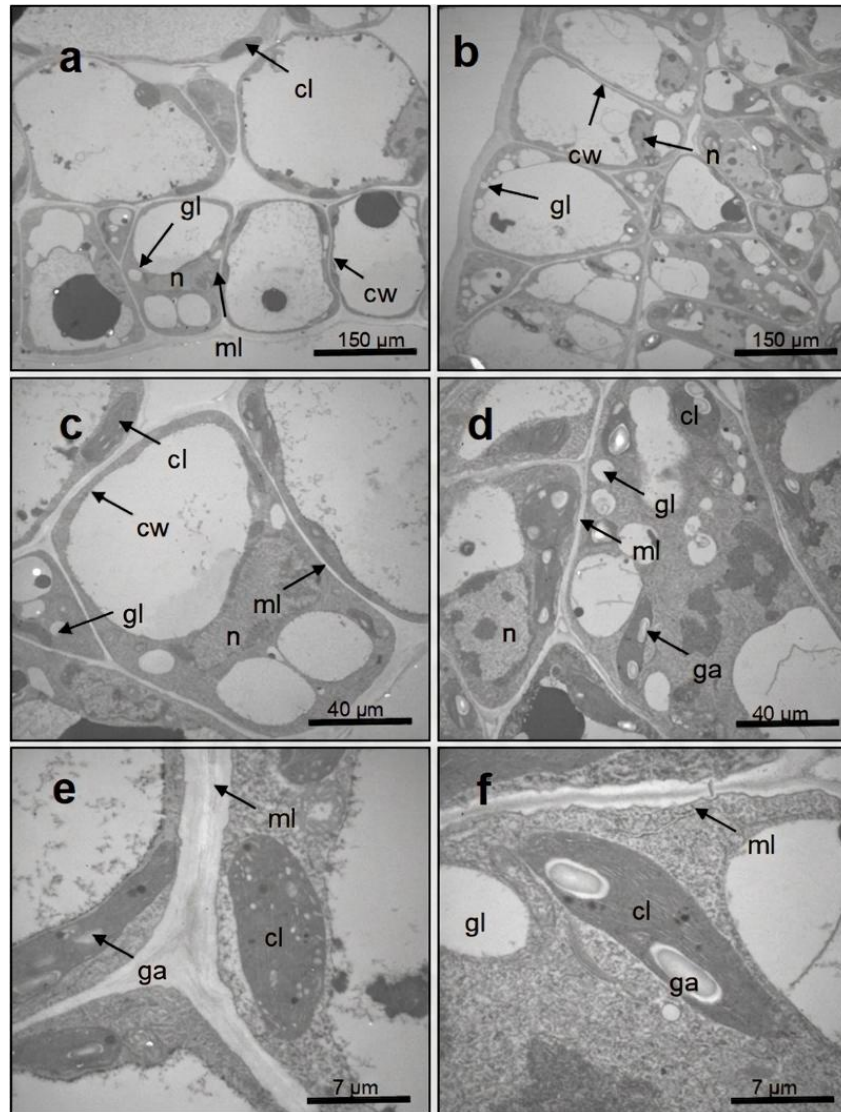


Fig. 3 Transmission electron micrographs showing the ultrastructure of leaf mesophyll cells of *Passiflora edulis* subjected to Ni addition and FS treatment. General ultrastructural organization of mesophyll cells under nickel addition (a) and FS treatment (b). Higher-magnification views highlighting chloroplast ultrastructure in leaves exposed to Ni addition (c, e) and FS treatment (d, f). Arrows indicate the position of chloroplasts (cl), cell wall (cw), middle lamella (ml), nucleus (n), grana (ga), and plastoglobules (gl). Scale bars = 150 μm (a, b), 40 μm (c, d), and 7 μm (e, f).

B deficiency altered the ultrastructure of leaf mesophyll cells compared with the FS treatment, primarily affecting chloroplast organization while preserving the overall cellular architecture (Fig. 4).

287 Mesophyll cells from the complete treatment exhibited well-defined cell walls, distinguishable nuclei,
288 and chloroplasts uniformly distributed along the cell periphery (Fig. 4b, d, f). In contrast, B-deficient
289 cells maintained their structural integrity but showed evident changes in chloroplast distribution and
290 organization (Fig. 4a, c, e). At higher magnification, chloroplasts from the complete treatment
291 displayed well-defined grana, homogeneous stromal organization, and clearly distinguishable lipid
292 globules, whereas chloroplasts from B-deficient plants appeared more elongated and exhibited
293 alterations in plastid morphology, grana arrangement, and the internal membrane system (Fig. 4c–f).
294 Overall, B deficiency induced pronounced ultrastructural changes in chloroplasts without disrupting
295 the integrity of mesophyll cells.

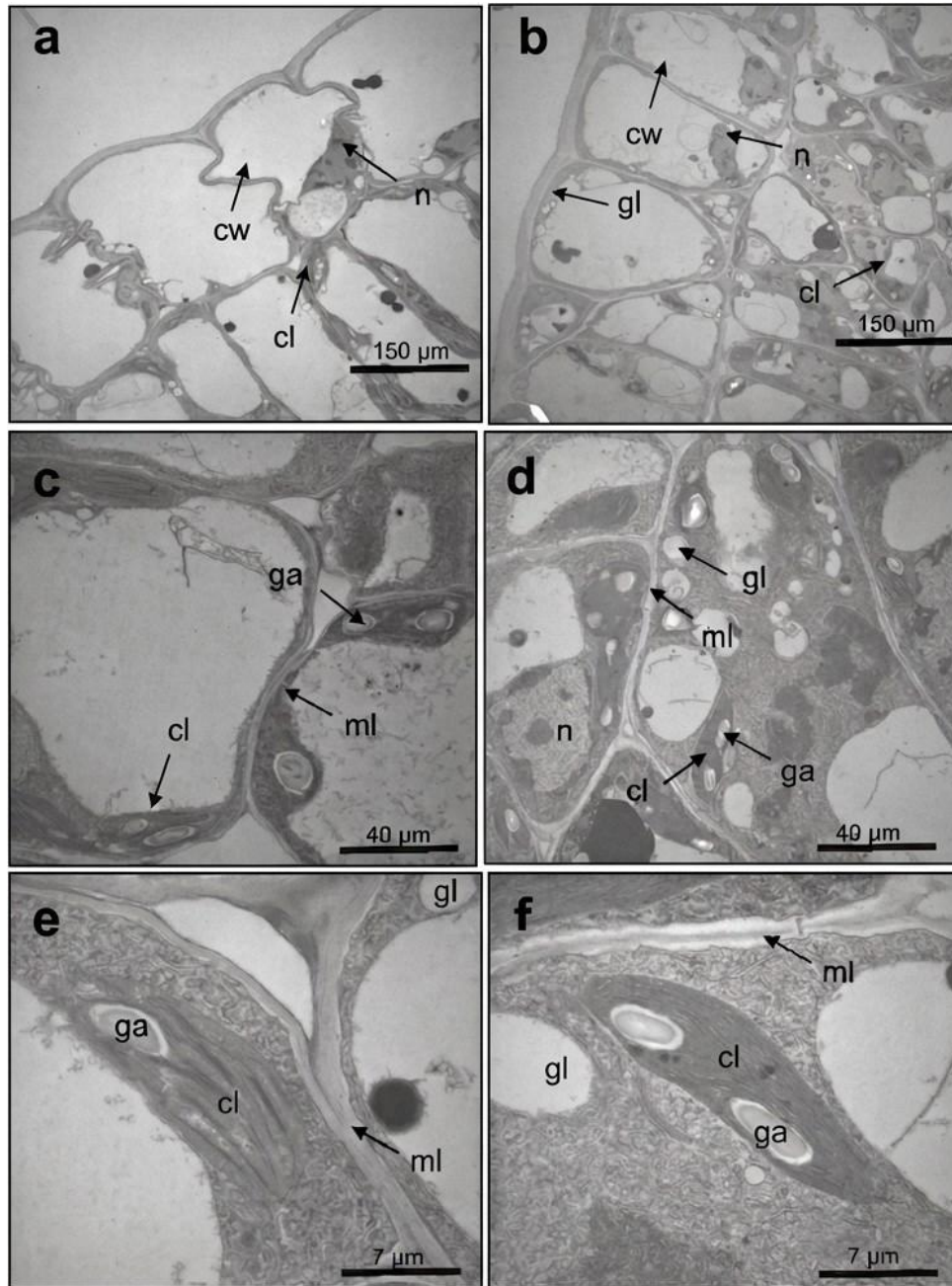


Fig. 4 Transmission electron micrographs showing the ultrastructure of leaf mesophyll cells of *P. edulis* grown under B deficiency and FS treatment. General organization of mesophyll cells under B deficiency (a) and FS treatment (b). Higher-magnification views highlighting chloroplast ultrastructure in leaves subjected to B deficiency (c, e) and complete nutrient solution (d, f). Arrows indicate the position of chloroplasts (cl), middle lamella (ml), nucleus (n), lipid globules (gl), grana (ga), and cell wall (cw). Scale bars = 150 μm (a, b), 40 μm (c, d), and 7 μm (e, f).

Mn deficiency altered the ultrastructure of leaf mesophyll cells compared with the FS treatment, primarily affecting chloroplast organization while preserving the overall cellular architecture (Fig. 5). Mesophyll cells from the complete treatment exhibited intact cell walls, well-defined nuclei, and chloroplasts regularly distributed along the cell periphery, with a well-organized internal structure characterized by clearly distinguishable grana and lipid globules (Fig. 5b, d, f). In contrast, mesophyll cells subjected to manganese deficiency maintained their structural integrity but displayed noticeable changes in chloroplast distribution and morphology, accompanied by alterations in the organization of internal membranes, grana, and lipid globules relative to the complete treatment (Fig. 5a, c, e).

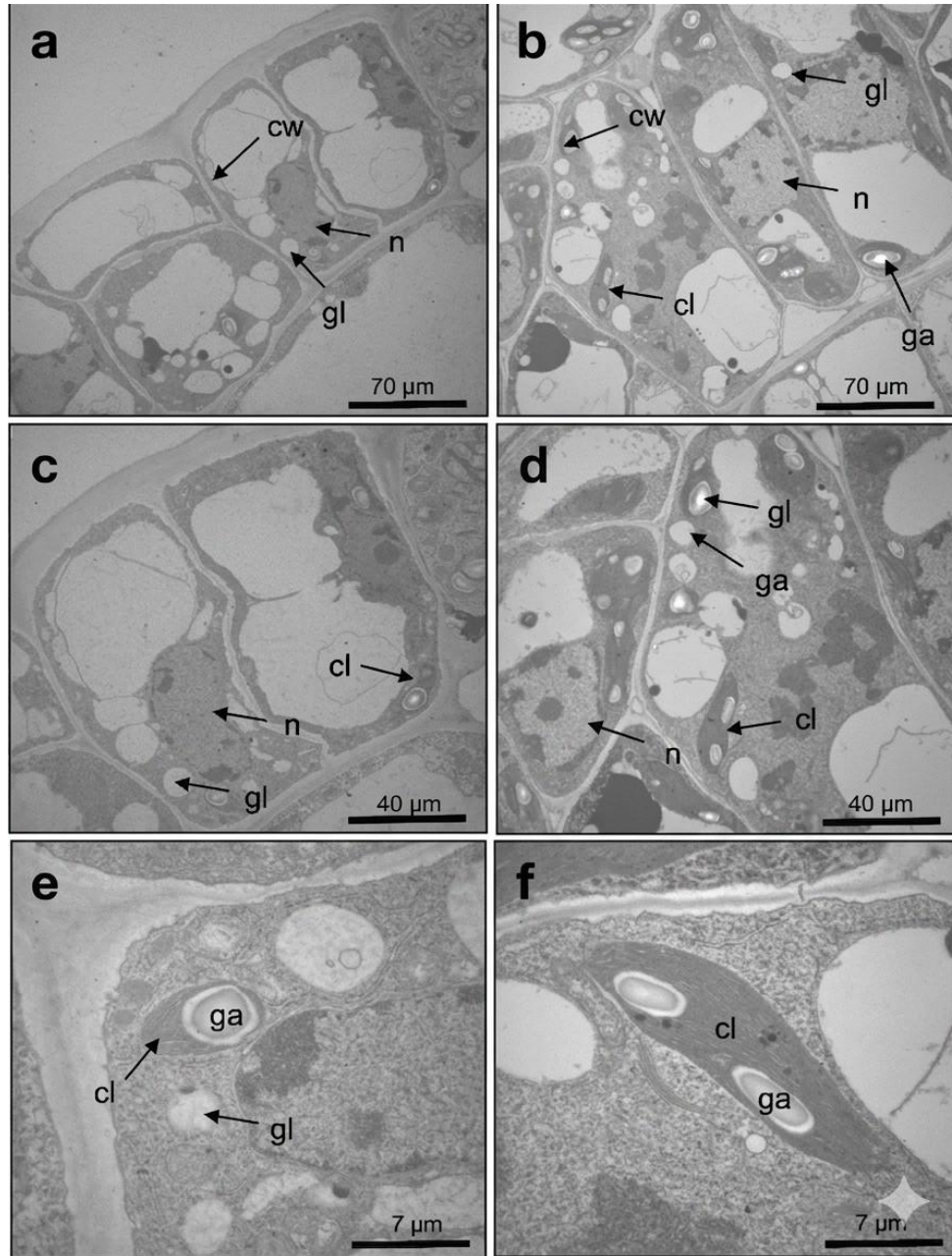


Fig. 5 Transmission electron micrographs showing the ultrastructure of leaf mesophyll cells of *P. edulis* grown under Mn deficiency and FS treatment. General organization of mesophyll cells under manganese deficiency (a) and FS treatment (b). Higher-magnification views highlighting chloroplast ultrastructure in leaves subjected to Mn deficiency (c, e) and complete nutrient solution (d, f). Arrows indicate the position of chloroplasts (cl), nucleus (n), lipid globules (gl), grana (ga), and cell wall (cw). Scale bars = 70 μm (a, b), 40 μm (c, d), and 7 μm (e, f)

A clear ultrastructural difference between Fe-deficient plants and those supplied with the FS treatment was observed (Fig. 6). Mesophyll cells from the complete treatment exhibited well-defined cellular organization, characterized by intact cell walls, clearly distinguishable nuclei, and chloroplasts regularly distributed along the cell periphery (Fig. 6b). In contrast, Fe-deficient plants maintained the overall integrity of mesophyll cells but displayed conspicuous alterations in chloroplast ultrastructure (Fig. 6a). At higher magnification, chloroplasts from the complete treatment showed well-preserved internal organization, with clearly distinguishable grana, organized thylakoid membranes, and visible lipid globules (Fig. 6d, f). Conversely, chloroplasts from Fe-deficient plants exhibited altered morphology, accompanied by disorganization of grana and thylakoid membranes and changes in the distribution of lipid globules (Fig. 6c, e).

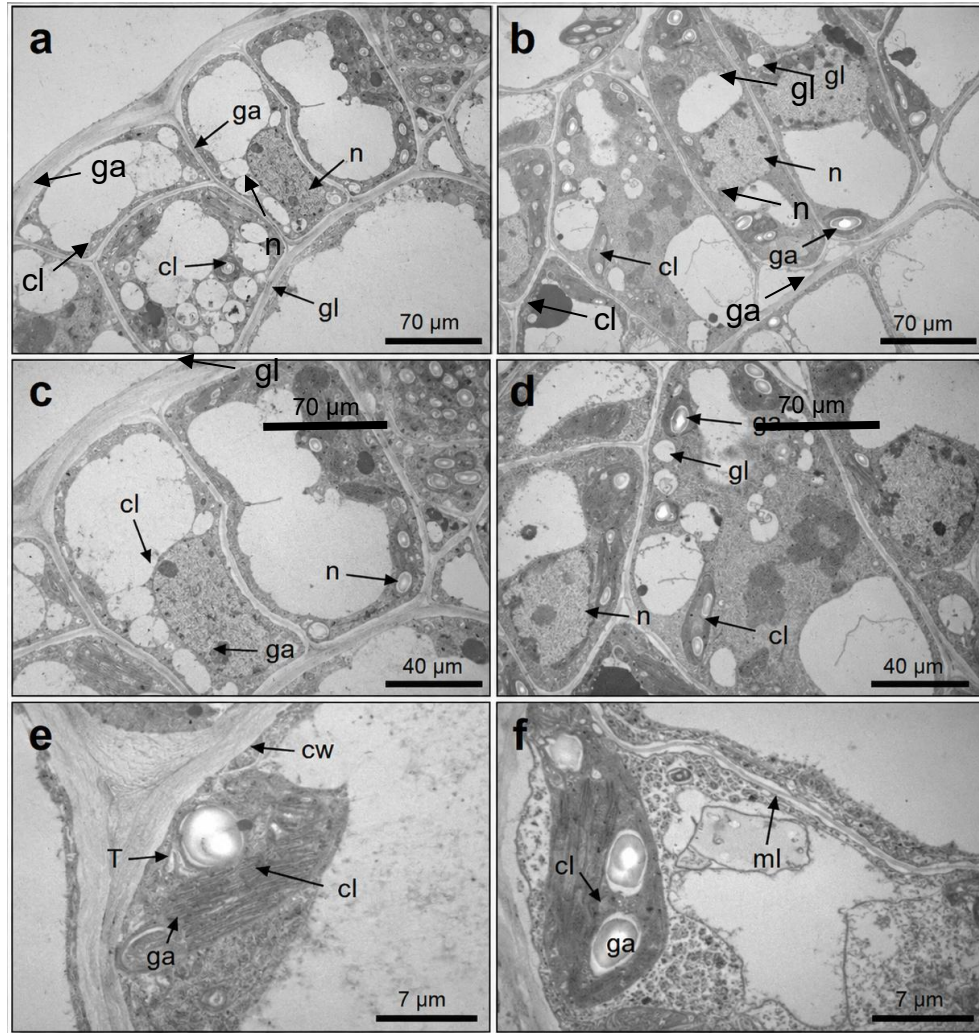


Fig. 6 Transmission electron micrographs showing the ultrastructure of leaf mesophyll cells of *P. edulis* grown under Fe deficiency and complete nutrient solution (control). General organization of mesophyll cells under Fe deficiency (a) and complete nutrient solution (b). Higher-magnification views highlighting chloroplast ultrastructure in leaves subjected to Fe deficiency (c, e) and complete nutrient solution (d, f). Arrows indicate chloroplasts (cl), middle lamella (ml), nucleus (n), lipid globules (gl), grana (ga), thylakoids (T), and cell wall (cw). Scale bars = 70 μm (a, b), 40 μm (c, d), and 7 μm (e, f)

Zn-deficient plant revealed marked ultrastructural differences compared to the those grown under the FS treatment (Fig. 7). Mesophyll cells from the complete treatment exhibited well-organized

chloroplasts with preserved morphology, abundant starch granules, intact nuclei, and a well-defined middle lamella (Fig. 7b, d, f). In contrast, Zn deficiency induced evident alterations in cellular organization, characterized by fewer and smaller chloroplasts, reduced starch granules, increased lipid droplets, and a thinner cell wall (Fig. 7a, c, e). In addition, Zn-deficient cells displayed a less organized mesophyll structure, with enlarged intercellular spaces and chloroplasts exhibiting irregular morphology compared with the complete treatment.

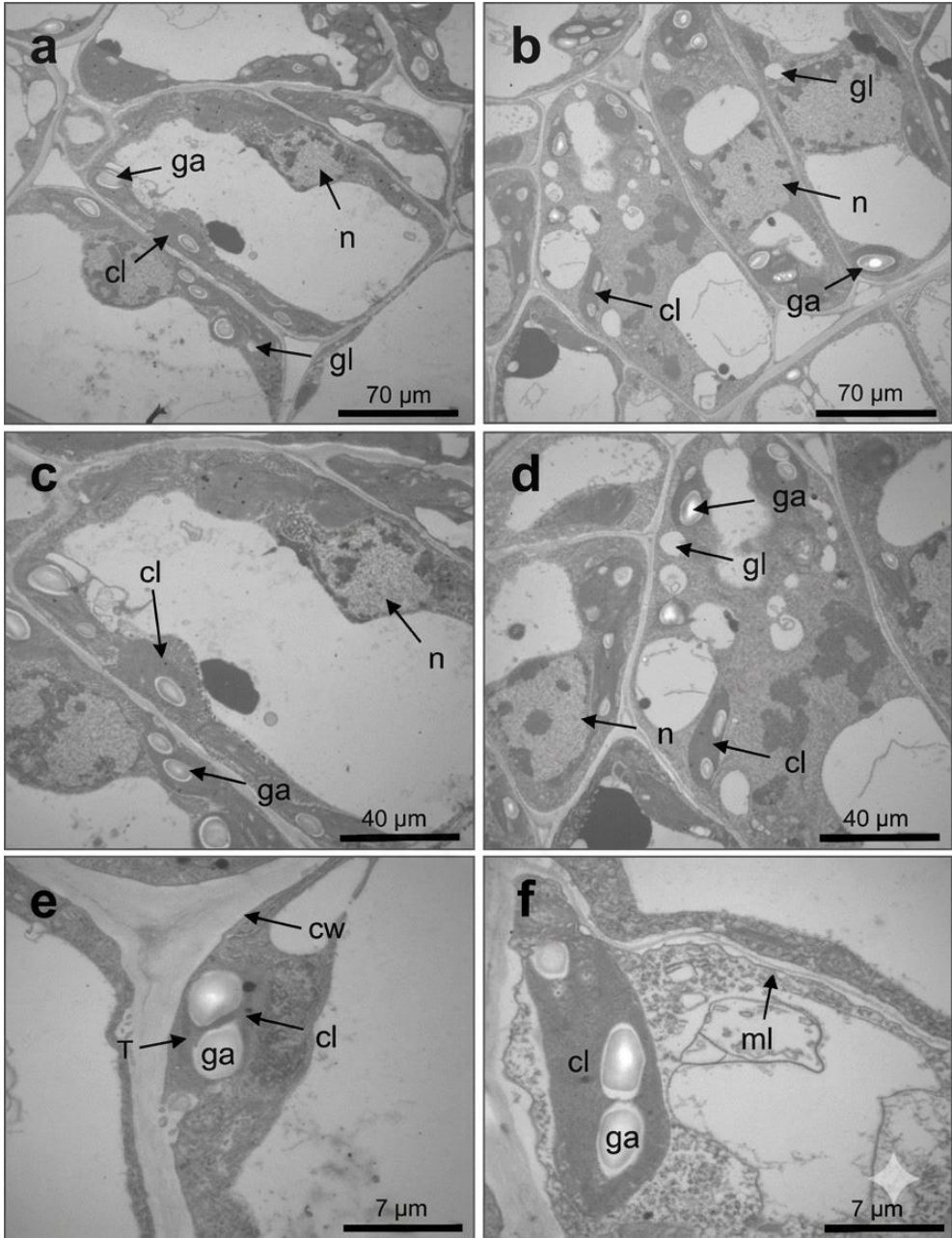


Fig. 7 Transmission electron micrographs showing the ultrastructure of leaf mesophyll cells of *P. edulis* grown under Zn deficiency and FS treatment. General organization of mesophyll cells under Zn deficiency (a) and FS (b). Higher-magnification views highlighting chloroplast ultrastructure in leaves subjected to Zn deficiency (c, e) and FS treatment (d, f). Arrows indicate chloroplasts (cl), middle lamella (ml), nucleus (n), lipid globules (gl), grana (ga), thylakoids (T), and cell wall (cw). Scale bars = 70 μm (a, b), 40 μm (c, d), and 7 μm (e, f)

Compared with TS plants (Fig. 8a, c, e), Zn-deficient leaves (Fig. 8b, d, f) exhibited pronounced alterations in mesophyll cell ultrastructure. At the tissue level (Fig. 8a, b), mesophyll cells from Zn-deficient plants appeared more compact, with reduced intercellular spaces and increased cytoplasmic electron density, whereas TS leaves maintained a well-preserved cellular organization with large vacuolar compartments. At higher magnification (Fig. 8c, d), chloroplasts from TS leaves displayed a regular shape and a well-organized internal membrane system, while Zn deficiency resulted in irregular chloroplast morphology, disorganization of the thylakoid membrane system, and increased accumulation of lipid globules. Ultrastructural details of the chloroplasts (Fig. 8e, f) further revealed that TS plants possessed densely stacked grana connected by well-defined thylakoids, whereas Zn-deficient chloroplasts exhibited disrupted grana architecture, less organized thylakoid membranes, and a higher abundance of plastoglobules, indicating a marked loss of chloroplast structural integrity under Zn deficiency.

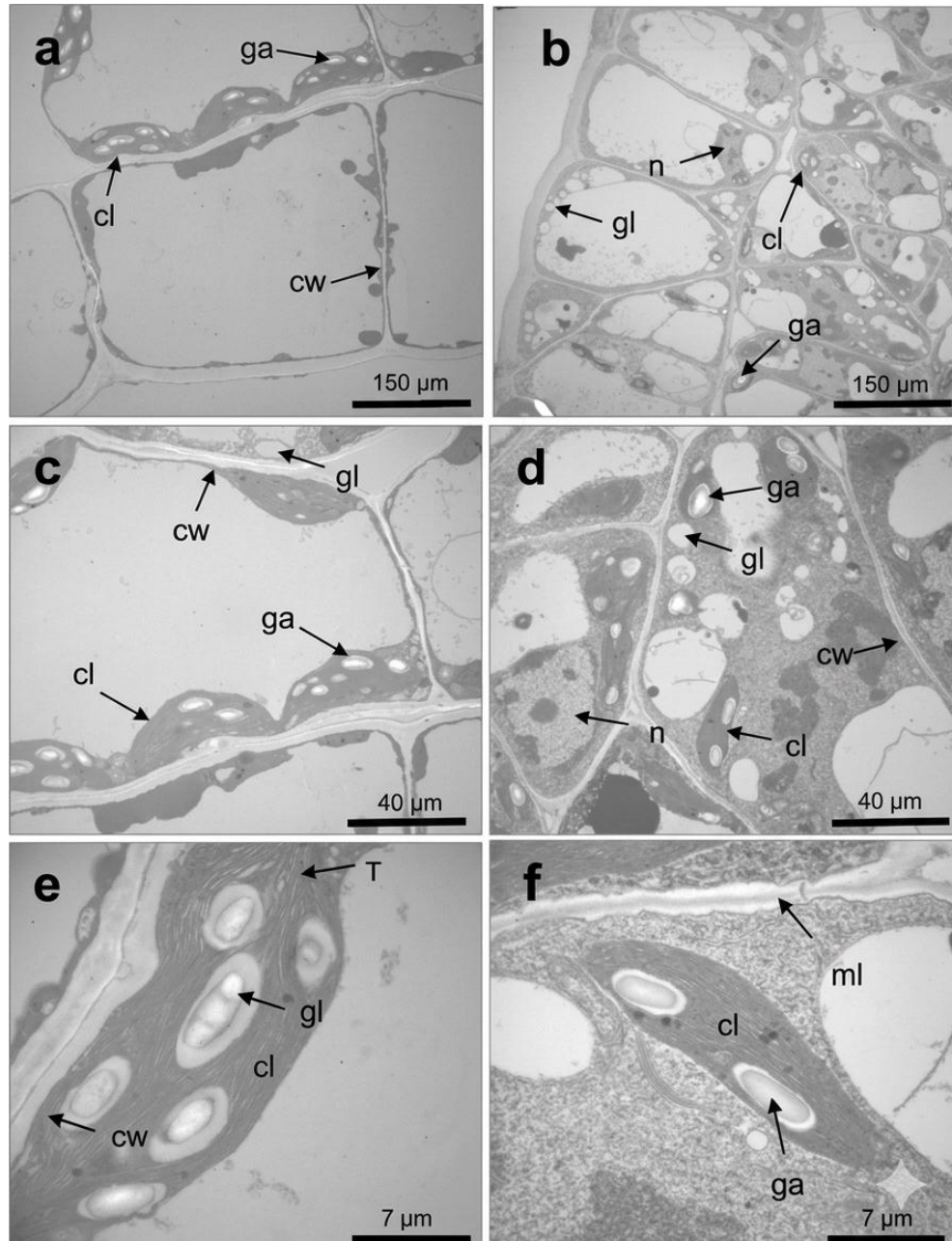


Fig. 8 Transmission electron micrographs showing the ultrastructure of leaf mesophyll cells of *P. edulis* grown under Cu deficiency and FS treatment. General organization of mesophyll cells under Zn deficiency (a) and FS (b). Higher-magnification views highlighting chloroplast ultrastructure in leaves subjected to Cu deficiency (c, e) and FS treatment (d, f). Arrows indicate chloroplasts (cl), middle lamella (ml), nucleus (n), lipid globules (gl), grana (ga), thylakoids (T), and cell wall (cw). Scale bars = 70 μ m (a, b), 40 μ m (c, d), and 7 μ m (e, f)

Transmission electron microscopy revealed that mesophyll cells under molybdenum (Mo) deficiency exhibited noticeable ultrastructural alterations compared with the FS treatment. Under Mo deficiency (Fig. 9a, c, e), chloroplasts displayed a less organized internal structure, increased accumulation of lipid globules, and grana with reduced stacking and disorganized thylakoid membranes, indicating damage to the photosynthetic apparatus. In contrast, leaves subjected to the FS treatment (Fig. 9b, d, f) showed better-preserved cellular ultrastructure, with chloroplasts maintaining a more compact organization, well-defined grana, and orderly thylakoid membranes, together with a lower apparent accumulation of lipid globules. These observations suggest that the FS treatment alleviated the structural damage caused by Mo deficiency and contributed to maintaining chloroplast integrity and cellular organization.

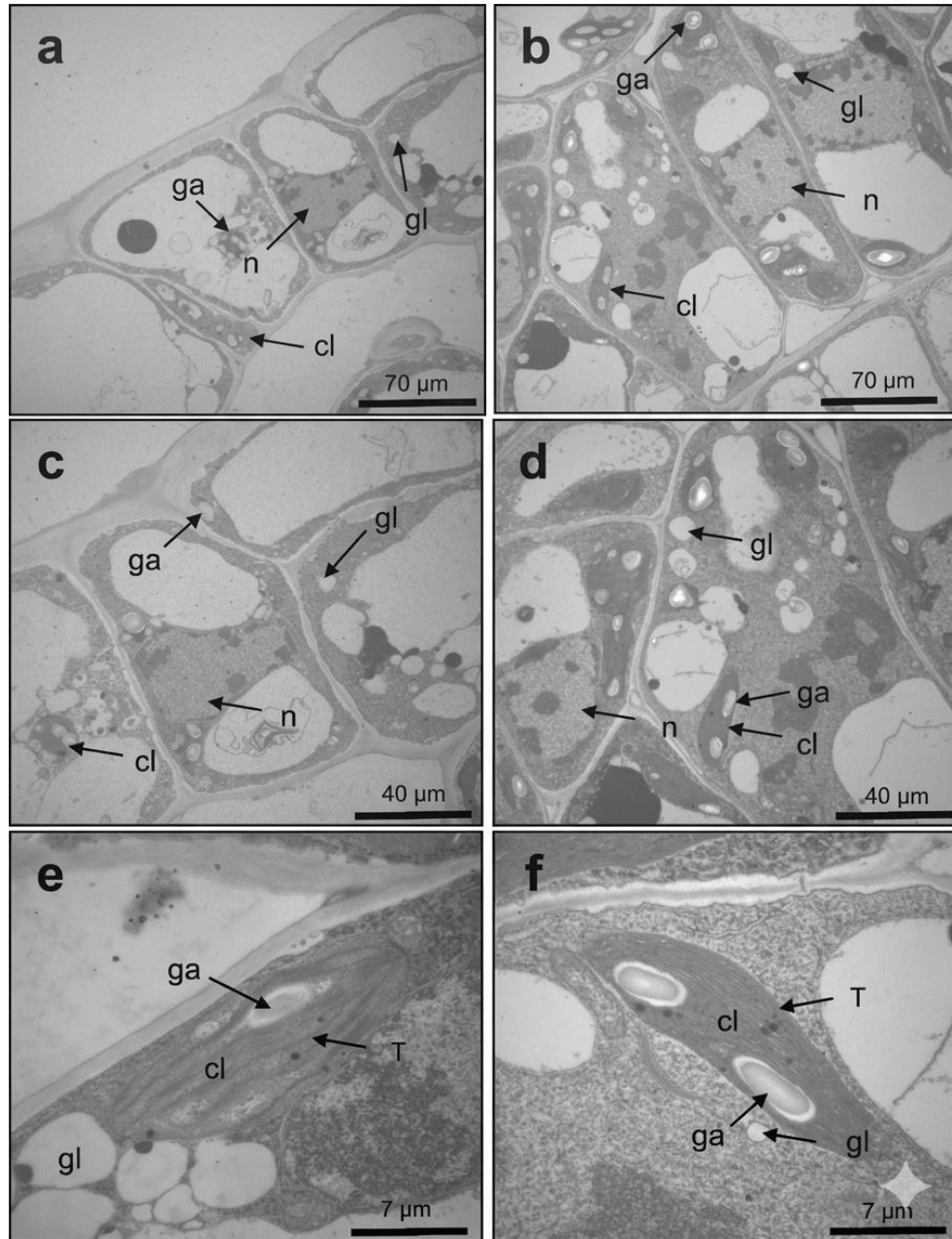


Fig. 9 Transmission electron micrographs showing the ultrastructure of leaf mesophyll cells of *P. edulis* grown under Mo deficiency and FS treatment. General organization of mesophyll cells under Mo deficiency (a) and FS (b). Higher-magnification views highlighting chloroplast ultrastructure in leaves subjected to Mo deficiency (c, e) and FS treatment (d, f). Arrows indicate chloroplasts (cl), middle lamella (ml), nucleus (n), lipid globules (gl), grana (ga), thylakoids (T), and cell wall (cw). Scale bars = 70 μm (a, b), 40 μm (c, d), and 7 μm (e, f)

3.4 Impact on nickel toxicity and micronutrient deficiencies on plant growth responses

Ni addition and micronutrient deficiencies significantly affected root morphological characteristics (Fig. 10). Compared with the complete nutrient solution (FS), the greatest reduction in root length was observed under Zn deficiency, although it did not differ significantly from Cu deficiency (Fig. 10a). In turn, Cu deficiency showed similar values to Mn and Fe deficiencies, which were also statistically similar to B and Mo deficiencies. Likewise, B and Mo deficiencies did not differ from Ni addition, although all treatments significantly reduced root length compared with FS.

A similar pattern was observed for root area (Fig. 10b). Zinc deficiency caused the greatest reduction, although its effect was statistically similar to Cu deficiency. Likewise, Cu deficiency did not differ from Mn and Fe deficiencies, which in turn overlapped with B and Mo deficiencies. Ni addition produced the smallest reduction among the stress treatments but remained significantly lower than the complete nutrient solution. Root diameter responded differently (Fig. 10c). Zinc deficiency resulted in the greatest reduction relative to FS, followed by Cu deficiency. Copper deficiency differed significantly from the intermediate group formed by Ni addition and B, Mn, and Fe deficiencies, which did not differ from the complete nutrient solution. In contrast, Mo deficiency significantly increased root diameter compared with FS.

For root density (Fig. 10d), the greatest reduction was observed under Zn deficiency, although its values did not differ significantly from those of Cu and Mn deficiencies. Likewise, Mn and Cu deficiencies overlapped statistically with Fe deficiency, whereas Fe deficiency showed similar values to B deficiency. In turn, B deficiency did not differ significantly from Ni addition, which produced the smallest reduction among the stress treatments, although remaining significantly lower than FS.

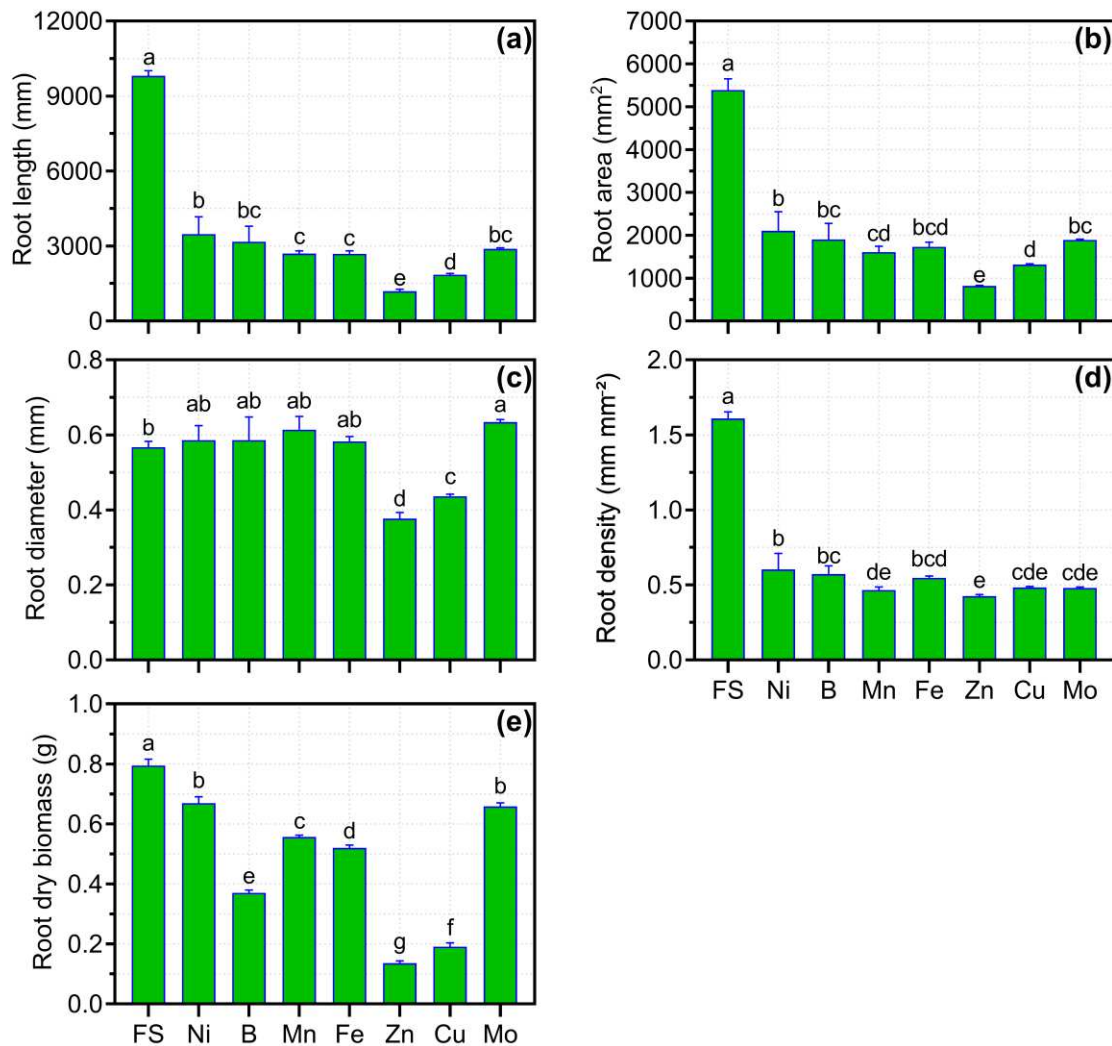


Fig. 10 Effects of micronutrient treatments on root development: (a) root length, (b) root area, (c) root diameter, and (e) root dry biomass. Bars represent the mean $\pm$ standard error ($n = 5$). Different lowercase letters indicate significant differences among treatments according to Tukey's multiple comparison test ($p < 0.05$). Treatments: full treatment (FS), nickel addition (Ni), Boron deficiency (B), Manganese deficiency (Mn), Iron deficiency (Fe), Zinc deficiency (Zn), Copper deficiency (Cu), Molybdenum deficiency (Mo)

Root dry biomass (Fig. 10e) exhibited the greatest reduction under Zn deficiency (84%) followed by Cu (76%), B (53%), Fe (34%), Mn (29%) and Ni and Mo (16%) deficiencies compared

to the FS treatment. Nevertheless, all treatments significantly reduced root dry biomass compared with the complete nutrient solution.

Ni addition and micronutrient deficiencies significantly affected leaf area, stem diameter, internode length, plant height, and shoot dry biomass (Fig. 11). Compared with the complete nutrient solution (FS), the greatest reduction in leaf area was observed under Zn deficiency, although it did not differ significantly from Cu deficiency (Fig. 11a). In turn, Cu deficiency showed similar values to Ni addition, B, Mn, and Fe deficiencies. Likewise, Mn and Fe deficiencies did not differ significantly from Mo deficiency, whereas Mo deficiency exhibited the smallest reduction among the stress treatments.

Stem diameter also responded significantly to the treatments (Fig. 11b). Compared with FS, the greatest reduction was observed under Zn deficiency, followed by B and Cu deficiencies. Copper deficiency showed values similar to B deficiency, whereas B deficiency did not differ significantly from Ni addition and Mo deficiency. Likewise, Ni addition and Mo deficiency exhibited similar values to Fe deficiency, while Mn deficiency did not differ from the complete nutrient solution.

Internode length (Fig. 11c) was most strongly reduced by Zn deficiency, although it did not differ significantly from Cu deficiency. In turn, Cu deficiency showed similar values to Ni addition and B deficiency. Likewise, Mn, Fe, and Mo deficiencies did not differ significantly from one another and produced intermediate reductions, whereas all treatments remained significantly lower than FS.

Plant height (Fig. 4d) showed a greatest reduction under Zn deficiency, although it did not differ significantly from Cu deficiency. Cu deficiency, in turn, exhibited values similar to B and Mo deficiencies, whereas B, Mo, Ni, Mn, and Fe deficiencies did not differ significantly from each other, respectively, in comparison with the FS treatment.

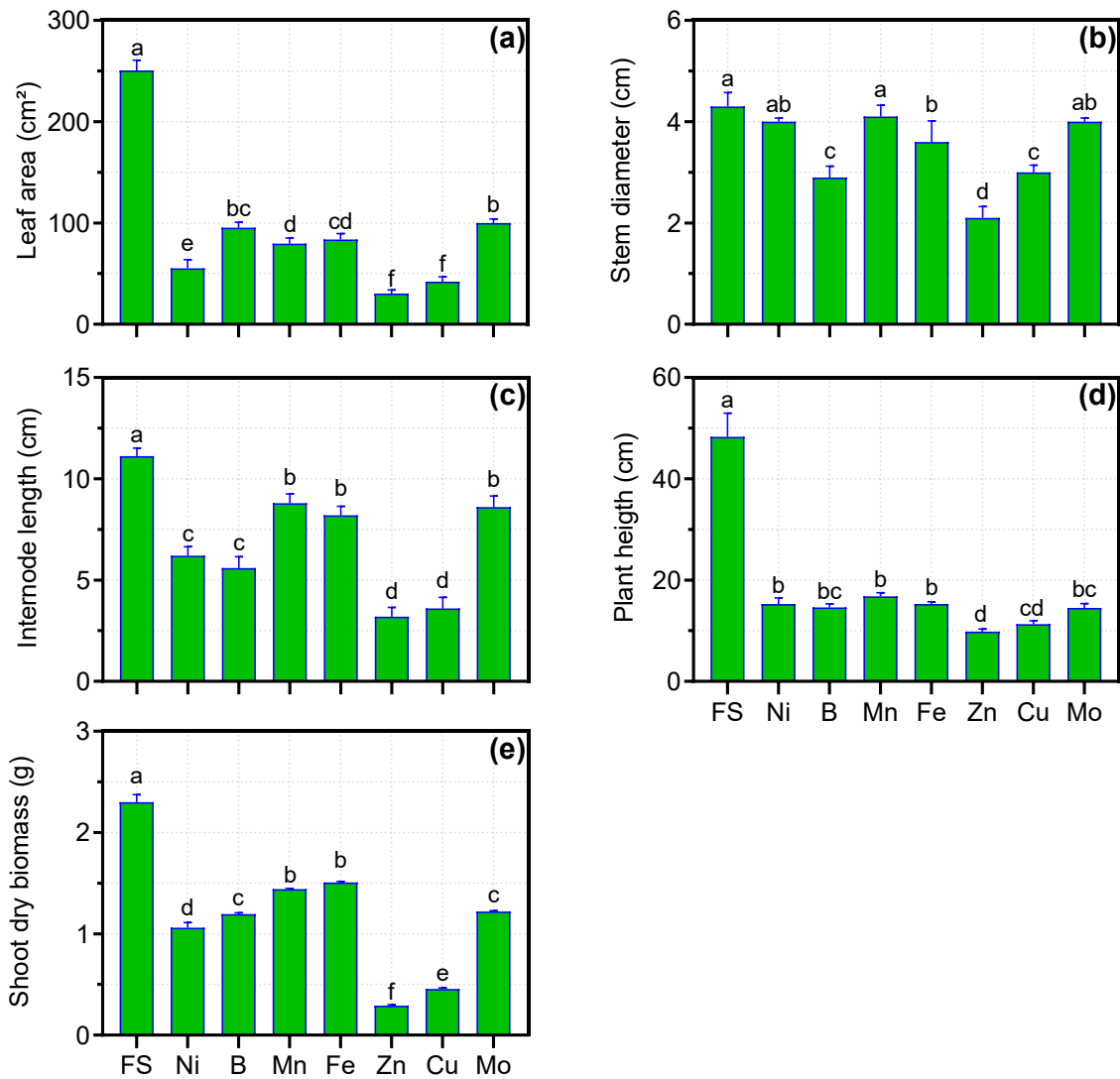


Fig. 11 Effects of micronutrient treatments on shoot growth: (a) leaf area, (b) stem diameter, (c) internode , and (e) root dry biomass. Bars represent the mean $\pm$ standard error ($n = 5$). Different lowercase letters indicate significant differences among treatments according to Tukey's multiple comparison test ($p < 0.05$). Treatments: full treatment (FS), nickel addition (Ni), Boron deficiency (B), Manganese deficiency (Mn), Iron deficiency (Fe), Zinc deficiency (Zn), Copper deficiency (Cu), Molybdenum deficiency (Mo)

Compared with the complete nutrient solution the shoot dry biomass (Fig. 4e) was most severely reduced under Zn deficiency (87%), followed by Cu (80%), Ni (54%), B deficiency (48%). Likewise, B deficiency did not differ significantly from Mn, Fe, and Mo deficiencies, whereas Mn (37%) and Fe (35%) also exhibited similar effects.

4. Discussion

Micronutrient availability is essential for the coordinated development of passion fruit seedlings, and the present study demonstrates that both deficiencies of B, Mn, Fe, Zn, Cu, and Mo and Ni addition profoundly altered morphological, physiological, and ultrastructural traits of *P. edulis*. The distinct visual symptoms observed for each treatment demonstrate that passion fruit seedlings are highly sensitive to micronutrient imbalance, reinforcing the diagnostic value of deficiency-induced morphological traits (da Cunha et al. 2024; Lizcano Toledo et al. 2025). The sequence of symptom appearance (Fe > Mn > Zn > Cu > Mo > B) highlights the high physiological demand for Fe and Mn, which is consistent with their roles in redox regulation and photosynthetic electron transport (de Bang et al. 2021; Lilay et al. 2024). The severe growth inhibition under Zn and Cu deficiencies further supports their essential roles in auxin metabolism, chloroplast development and photosynthetic function (Kumar et al. 2021b; Stanton et al. 2022).

Exposure of passion fruit seedlings to individual micronutrient omissions for 60 days produced distinct patterns of growth reduction and leaf symptoms, confirming that each element has a characteristic role in vegetative development and morphogenesis. The strong reductions in relative growth rate under Zn and Mn omission, followed by Fe and Cu deficiency, agree with recent reports that passion fruit and other *Passiflora* spp. ((Mendonça Freitas et al. 2008; Cárdenas-Pira et al. 2021)) reinforce the high requirements for these cationic micronutrients to sustain shoot expansion and

canopy formation (Hänsch and Mendel 2009). The sequence of visual manifestation (Fe > Mn > Zn > Cu > Mo > B) is consistent with the relatively low mobility of Fe and Mn in the phloem and with their central roles in chlorophyll synthesis and redox metabolism, which make their deficiency rapidly apparent in young leaves.

Ni addition caused chlorosis in the youngest leaves followed by generalized yellowing and growth reduction, which is compatible with the narrow range between adequate and toxic Ni supply described for horticultural crops (Alibakhshi and Khoshgoftarmanesh 2014; Ahmad Yasin et al. 2022). The fact that Ni toxicity symptoms appeared only after 40 days suggests that passion fruit can initially buffer Ni in tissues, suggesting that prolonged Ni exposure exceeded vacuolar sequestration and antioxidant detoxification capacity, thereby promoting oxidative damage and impairing plant growth (Rizwan et al. 2022; Aguilar et al. 2023). Collectively, these results indicate that micronutrients with limited phloem mobility (Fe and Mn) induced symptoms rapidly in young tissues, whereas deficiencies affecting hormone regulation and cell wall formation (Zn and B) primarily altered organ development and leaf morphology.

Micronutrient omissions and Ni addition significantly altered chlorophyll status and gas exchange, indicating that passion fruit physiology is highly sensitive to imbalances in these elements. The marked decline in SPAD values under Fe and Mn deficiency, together with linear decreases over time, supports their direct participation in chlorophyll biosynthesis and in the protection of photosystems against oxidative stress, as shown in recent studies on micronutrient-deficient leaves in fruit crops (Sujetovienė and Bučytė 2021; Ammara et al. 2026). In contrast, the initial increase followed by decline in SPAD under Cu, Zn, Mo deficiency and Ni addition may reflect a transient compensation in pigment concentration before structural damage and impaired nutrient uptake reduce chlorophyll content (Carrillo-Niquete et al. 2022; Gupta et al. 2024; Lizcano Toledo et al. 2025).

These reductions in chlorophyll content were accompanied by lower photosynthetic rates, indicating that impairment of pigment biosynthesis was rapidly translated into reduced carbon assimilation.

All deficiency and Ni treatments reduced photosynthesis and stomatal conductance relative to the complete solution, with Zn omission and Ni addition producing the strongest inhibition, indicating both stomatal and non-stomatal limitations (Rizwan et al. 2022; Tripathi et al. 2022). The higher C_i/C_a ratios observed under Zn, Mn, Fe and Mo deficiencies indicate that photosynthesis was mainly limited by biochemical processes rather than CO_2 diffusion, reflecting impairment of PSII activity, electron transport, Rubisco activation and ATP generation (Rietra et al. 2017; Chen et al. 2022). In contrast, Ni addition reduced C_i/C_a , suggesting that stomatal closure represented the primary limitation to photosynthesis, probably due to Ni-induced membrane dysfunction and altered guard-cell regulation (Ahmad et al. 2011).

Each micronutrient deficiency produced a distinct ultrastructural signature that closely reflected its primary physiological function. B deficiency primarily affected cell wall organization, as evidenced by thickening of the middle lamella and disruption of cell wall continuity (Wimmer and Eichert 2013). These alterations are consistent with the structural role of B in the cross-linking of rhamnogalacturonan II (RG-II) pectic polysaccharides, which is essential for maintaining cell wall integrity and membrane stability (Mendonça Freitas et al. 2008; Bolaños et al. 2023).

In contrast, Fe and Mn deficiencies predominantly affected chloroplast organization, reducing chloroplast number, starch granule accumulation, and thylakoid integrity. These responses reflect the indispensable roles of Fe and Mn in photosynthetic electron transport, photosystem II function, chlorophyll biosynthesis, and the maintenance of redox homeostasis (Li et al. 2019; Gupta et al. 2024; Nair et al. 2025). Consequently, disruption of these processes' compromises ATP and NADPH production, limiting carbon assimilation and promoting chloroplast degradation (Briat et al. 2015; Kumar et al. 2021a). Furthermore, Zn and Cu deficiencies induced greater intercellular spaces, fewer

and smaller chloroplasts, and marked alterations in starch granules and plastoglobuli (Hänsch and Mendel 2009; Gupta et al. 2024). These changes are characteristic of oxidative stress, since both micronutrients are essential cofactors of antioxidant enzymes, particularly Cu/Zn-superoxide dismutase, which protects chloroplast membranes from reactive oxygen species (ROS) (Tripathi et al. 2015; Saquee et al. 2023). The loss of antioxidant protection accelerates lipid peroxidation, thylakoid disorganization, and impairment of photosynthetic metabolism (Fariduddin et al. 2021; Gupta et al. 2024).

Unlike the other micronutrients, Mo deficiency primarily altered chloroplast morphology through increased starch accumulation and thylakoid modifications. Because Mo is required for nitrate reductase and other molybdoenzymes involved in nitrogen metabolism, its deficiency restricts nitrate assimilation, leading to reduced protein synthesis and inefficient utilization of photoassimilates, which ultimately disrupts chloroplast function (Hänsch and Mendel 2009; Lizcano Toledo et al. 2025). Collectively, these ultrastructural responses demonstrate that each micronutrient deficiency affected a specific cellular process, yet all converged on reduced chloroplast functionality, thereby explaining the observed declines in chlorophyll content, photosynthesis, and biomass accumulation.

Reductions in shoot, root, and whole-plant dry matter under all deficiency treatments and Ni addition demonstrate that micronutrient balance is essential for early biomass accumulation in passion fruit. Zn and Cu deficiencies produced the largest decreases in total dry matter, followed by B and Ni, which is consistent with their strong impacts on photosynthesis, stomatal conductance, and ultrastructure (Saquee et al. 2023). The greater sensitivity of roots indicates that root growth constitutes one of the earliest responses to micronutrient imbalance, preceding severe reductions in shoot biomass (Yu et al. 2024). The greater sensitivity of root dry matter compared with shoot dry matter under Zn, Cu, B, Fe, and Mn omission underscores that root growth is an early and sensitive

indicator of micronutrient stress, with practical implications for seedling production and fertigation management in nurseries.

From an agronomic perspective, the combination of specific visual symptoms, physiological responses, and ultrastructural markers generated in this study offers a diagnostic framework for identifying early micronutrient disorders in passion fruit seedlings grown in nutrient solution or in controlled substrates. Given the increasing use of intensive fertigation and soilless cultivation, these results support the need for precise micronutrient supply, particularly for Fe, Mn, Zn, and Cu, and for careful monitoring of Ni contamination in water and inputs to avoid subclinical toxicity that can compromise photosynthesis and growth before overt symptoms appear. Therefore, integrating visual diagnosis with physiological indicators such as SPAD and gas exchange may improve the early detection of micronutrient disorders, allowing corrective fertilization before irreversible structural damage occurs.

Conclusions

This study clearly demonstrates that micronutrient imbalances and Ni addition induce marked morphophysiological and ultrastructural alterations in *P. edulis* seedlings. This study demonstrated that both micronutrient deficiencies and excess Ni disrupted the coordinated development of passion fruit seedlings by impairing plant growth, photosynthetic performance, root architecture, and mesophyll ultrastructure. These responses reflected the specific physiological functions of each micronutrient, while excess Ni induced toxicity through progressive disruption of chloroplast organization and cellular integrity.

The reductions in biomass accumulation were closely associated with alterations in chlorophyll content, gas exchange, and chloroplast organization, demonstrating that disruption of photosynthetic metabolism represented the primary mechanism linking micronutrient imbalance to growth inhibition. Among the evaluated nutrients, Fe and Mn deficiencies caused the earliest visible

symptoms, whereas Zn and Cu deficiencies produced the greatest reductions in plant growth, highlighting their critical roles during seedling establishment. Root growth proved to be highly sensitive to micronutrient imbalance, emphasizing its usefulness as an early indicator of nutritional disorders.

Collectively, these findings provide the first integrated characterization of the morphological, physiological, root architectural, and ultrastructural responses of *Passiflora edulis* seedlings to individual micronutrient deficiencies and Ni toxicity. This information improves the diagnosis of nutritional disorders and provides a physiological basis for optimizing micronutrient management during nursery production, thereby contributing to the production of vigorous seedlings with greater potential for successful field establishment.

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Declarations

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