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Zn application does not alleviate plant stress Cr in wild passion fruit seedlings

Aplicação de Zn não alivia o estresse de Cr em mudas de maracujá silvestre

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ABSTRACT

Heavy metals (HMs) can be classified as essential (e.g., zinc, Zn) or non-essential mineral elements (e.g., chromium, Cr) for plant development. Several wild species show environmental survival potential in soil contaminated with HMs, but the initial plant growth (seedling) stage has not been elucidated. The hypothesis tested whether Zn supply alleviates Cr stress during seedling growth and initial establishment of *Passiflora cincinnata* Mast. cv. 'BRS Sertão Forte' (wild passion fruit). The factorial experiment 2x2 considering Zn and Cr dosages was carried out in a nursery production system with a nutrient solution of Hoagland and Arnon (1950) nº2 (50% of ionic strength), applied via roots to supply 10 plant replicates completely randomly distributed. We quantified plant height (cm), leaf area (dm^2), plant length (cm), total number of leaves (units), and photosynthetic pigments, such as chlorophyll *a* ($\mu mol.L^{-1}$), chlorophyll *b* ($\mu mol.L^{-1}$), and carotenoids ($\mu mol.L^{-1}$). The data were analyzed using Mixed Analysis of Variance (MIXED ANOVA) and the differences between means were tested using Student's test with a significance level of 5%. Our results showed that Cr has a harmful effect on plant growth parameters, but it also led to an increase in the expression of plant physiology parameters. However, Zn had no significant effects, except for the leaf area, or interactions with Cr. Therefore, in conclusion, we reject the hypothesis that Zn supply alleviates Cr stress during the initial establishment of *P. cincinnata* seedlings.

Keywords: competition-inhibition, heavy metals, plant growth, passiflora, seedling-establishment, stress tolerance.

RESUMO

Metais pesados (MPs) podem ser classificados como elementos minerais essenciais (ex.: zinco, Zn) ou não essenciais (ex.: cromo, Cr) para o desenvolvimento das plantas. Diversas espécies selvagens apresentam potencial de sobrevivência ambiental em solo contaminado com MPs, todavia o estádio inicial de crescimento da planta (plantas jovens) não foi elucidado. Nossa hipótese testou se o suprimento de Zn alivia o estresse de Cr durante o crescimento das mudas e o estabelecimento inicial de *Passiflora cincinnata* Mast. cv. 'BRS Sertão Forte' (maracujá-silvestre). O experimento fatorial 2x2 utilizou dosagens de Zn e Cr em um sistema de produção em viveiro com solução nutritiva de Hoagland e Arnon (1950) nº2 (50% da força-iônica), aplicada via raízes, para suprir 10 repetições de plantas distribuídas de forma completamente aleatória. Quantificamos a análise de crescimento da planta, tais como área foliar (dm^2), altura da planta (cm), comprimento da planta (cm), número de folhas e pigmentos fotossintetizantes da planta, como clorofila *a* ($\mu mol.L^{-1}$), clorofila *b* ($\mu mol.L^{-1}$) e carotenoides ($\mu mol.L^{-1}$). Os dados foram analisados utilizando a Análise de Variância Mista

(MIXED ANOVA) e a diferença entre médias foi verificada por meio do teste *t* de *Student* com nível de significância de 5%. Nossos resultados mostraram que o Cr tem um efeito prejudicial nos parâmetros de crescimento da planta, todavia também promoveu aumento na expressão dos parâmetros de fisiologia da planta. No entanto, o Zn não teve efeitos significativos - exceto para a área foliar - ou interações com o Cr. Logo, rejeitamos a hipótese de que o fornecimento de Zn alivia o estresse de Cr durante o estabelecimento inicial de plantas jovens de *P. cincinnata*.

Palavras-chave: inibição competitiva, metais pesados, crescimento vegetal, passiflora, estabelecimento de mudas, tolerância ao estresse.

INTRODUCTION

Heavy metals (HMs) are classified as essential or non-essential for plant survival (Rasheed et al., 2021), and HM toxicity concentration critical levels for plants vary by species, but it is not clear for wild plant species (Kirkby, 2012). HMs toxicity on plant development includes cell damage, such as chlorosis, root damage, and interference with cellular osmoregulation (Hunt et al., 2003; Nascimento et al., 2018; Farouk and Al-Amri, 2019), plant growth capacity, and biomass production (Berilli et al., 2016; Baron et al., 2018). While the effects of HMs on plant growth can be quantified by calculating the relative accumulation of organic matter about preexisting biomass to indirectly calculate the carbonic assimilation rate of leaf expansion (Radford, 1967), few studies have quantified plant growth rates in HMs environments contaminated. Among the potentially toxic chemical elements, zinc (Zn) and chromium (Cr) are the most relevant pollutant cations to plant growth and plant development.

Zn cations (Zn^{2+}) are essential for plant biochemical metabolism (Hänsch and Mendel, 2009; Cristaldi et al., 2020), playing fundamental roles in protein synthesis and activator RNA polymerase enzymes, carbonic anhydrase enzymes, and as substrates for carbonic acid (HCO_3^-) (Humayan Kabir et al., 2014; Thapa et al., 2019). Regarding Cr cations, there is no conclusive evidence of the essentiality of the role in plant metabolism; this HM contamination, particularly, is increasingly posing a serious threat to the environment and emerging as a major health hazard to the biota and is considered non-essential and toxic to plants because they have no function in enzymatic activities and can cause physiological damage to plants depends of oxidation form (Kushwaha et al., 2018). For example, Cr cations, which have several forms of oxidation, mainly in three oxidative states metallic chromium [Cr(0)], trivalent [Cr(III)], and hexavalent [Cr(VI)], which are the most stable forms of Cr (Shanker et al. 2005). The Cr(III) and Cr(VI) ionic forms are found in lower concentrations in the environment (Tsybulskaya et al., 2019).

The literature indicates that the HMs absorption is primarily facilitated by membrane transporters found on the plasma membrane. These proteins not only regulate metal ion homeostasis but also play a vital role in responding to metal stress. Basit et al. (2022) suggest that zinc oxide (ZnO) mitigates chromium (Cr) stress in plants by reducing oxidative stress and enhancing antioxidant levels. Nevertheless, our limited understanding of the systematic role of zinc in alleviating chromium stress, particularly in wild passion fruit, underscores the necessity for more in-depth research. This need is further emphasized by the scarcity of publications addressing the relationship between zinc and chromium in native plants as research subjects (Sharma et al., 2025).

Plant native species belonging to the family Passifloraceae, including *Passiflora cincinnata* Mast. cv. ‘BRS Sertão Forte’ (wild passion fruit), are potentially financially (Kidd et al., 2007). This species has great commercial potential, including in the international market, highlighting its productivity and tolerance to water stress, being very important for family farmers, especially in semiarid regions, because it can be cultivated in dryland conditions with low consumption of technological inputs, thus reducing the environmental impact by reducing the extractivism of the species. In addition, wild passion fruit has a wide geographic distribution in Brazil and occurs spontaneously in practically the entire region of the Brazilian semiarid region and has the potential to survive in soil amended with biosolids (sewage sludge) that contain Zn and Cr, according to limited studies (Andrade et al., 2018). Besides, it can survive in soils amended with biosolids (sewage sludge) containing concentrations of up to 80 mg.kg^{-1} of Zn and 30 mg.kg^{-1} of Cr (Romeo et al., 2013).

In the present study, our hypothesis tested whether Zn supply alleviates Cr stress during the initial establishment of wild passion fruit seedlings.

MATERIAL AND METHODS

Plant Material and Treatments

Passiflora cincinnata Mast. cv. 'BRS Sertão Forte' (wild passion fruit) seedlings were transplanted into pots (1 dm^3) filled with medium-texture vermiculite substrate and acclimatized in the greenhouse belonging to the Universidade Federal de São Carlos Lagoa do Sino *campus*. The plant replicates selected were in the juvenile phenological stage and appeared healthy (e.g., leaf color and plant size). Irrigation control was carried out by monitoring the pots and plants daily, and nutrient solution was applied using a Hoagland and Arnon nº2 nutrient solution (Hoagland and Arnon, 1950) at 50% of ionic strength (Table 1). The treatments were applied according to Freitas (2013) applied on roots to supply Zn essential metal ($ZnSO_4 \cdot 7\ H_2O$, 5.75 mg.dm^{-3}) (Samreen et al., 2017), non-essential metal Cr(VI) ($K_2Cr_2O_7$, 0.15 mg.dm^{-3}) (Santana et al., 2012), and a combination of Zn (5.75 mg.dm^{-3}) and Cr(VI) (0.15 mg.dm^{-3}). The elements will be supplied to the plants from 10 days after transplanting (DAT) and applied every two weeks to 10 plants per treatment, randomly distributed in 4 blocks.

Plant Growth and Photosynthetic Pigments

We measured the plant growth, such as plant height (cm), plant length (cm), collected leaves ('destructive analysis') and measured the total number of leaves (units) and leaf area using an graph paper (dm^2) according to Benincasa (2003) at 22, 29, 36, and 43 days after transplant (DAT). The contents of photosynthetic pigments, such as chlorophyll *a* (µmol.L^{-1}), chlorophyll *b* (µmol.L^{-1}), and carotenoids (µmol.L^{-1}) were measured at 663 nm, 647 nm, and 470 nm, respectively, according to Sims and Gamon (2002).

Statistical Analysis

A 2x2 factorial experiment was conducted in which the experimental factors were Zn and Cr. The levels of the factors considered were absence (0 mg.L^{-1}) and presence (5.75 mg.L^{-1} for Zn and 0.15 mg.L^{-1} for Cr), levels considered high for species of agronomic interest, according to Freitas et al. 2013. The experiment was conducted in a completely randomized design, with 10 plants replicated per treatment, evaluated at four distinct time points (22, 29, 36, and 43 DAT) to plant growth parameters. Meanwhile, plant physiology data were collected only at the last evaluation time, at 43 DAT. First, data cleaning was performed. Regarding the plant growth variables, two measurements were found to be outliers and were replaced with the average value of the measurements obtained between the previous and subsequent times. The data on plant physiology parameters (photosynthetic pigments) also presented issues, as many observations had missing values. Consequently, only seven or eight plants were used in the evaluation of the pigments. Plant growth parameters measured over time were analyzed using linear models with fixed and random effects (Pinheiro and Bates, 2000). The experimental factors Zn and Cr and their interactions, along with temporal variations, were considered as fixed effects in the models, while the random effect of the plant was considered to account for heterogeneity among the seedlings that composed the experiment plots.

In total, fourteen (14) linear models were studied. They differed by including or not interactions between treatments and evaluation times, having or not a random plant effect (intercept), and different variance structures were tested. Correlations between repeated measures on the same plant or conducted at the same evaluation time were tested using three covariance structures: compound symmetry (CompSymm), where all measurements on the same plant have equal correlation regardless of measurement times, suggesting a constant covariance structure; first-order autoregressive (AR1), which assumes a fixed correlation for successive measurements on the same plant, with the correlation decaying exponentially as the distance between measurement times increases; and unstructured correlation matrix (UnStruct), which allows the correlations between a plant's measurements to differ, providing greater flexibility in the fit but at the cost of additional parameters.

Table 1. Hoagland and Arnon's (1950) no. 2 nutrient solution at 50% of ionic strength (*I*).

(1)Nutrients/ compounds	Solution Stock ($g.L^{-1}$)	(3) Solution Stock ($mL.L^{-1}$)	Nutrient solution at 50% I ($mM.L^{-1}$)
$NH_4H_2PO_4$ (M)	115.0	0.5	0.237
KNO_3 (M)	101.1	3.0	1.680
$Ca(NO_3)_2.4H_2O$ (M)	236.1	2.0	1.948
$MgSO_4.7H_2O$ (M)	246.5	1.0	1.017
(1) Nutrients/ compounds	**Solution Stock 'Unique' ($mg.L^{-1}$)**	**(3) Solution Stock ($mL.L^{-1}$)**	**Nutrient solution at 50% I ($mM.L^{-1}$)**
H_3BO_3	2.86	0.5	5.899
$MnCl_2.4H_2O$	1.81		3.713
$ZnSO_4.7H_2O$	0.22		0.454
$CuSO_4.5H_2O$	0.08		0.165
$H_2MoO_4.H_2O$	0.02		0.041
(2) Fe solution	**Solution Stock 'Unique' ($mg.L^{-1}$)**	**(3) Solution Stock ($mL.L^{-1}$)**	**Nutrient solution at 50% I ($mM.L^{-1}$)**
Fe-E.D.T.A.	26.1	0.5	0.054
$FeSO_4.7H_2O$	24.9		0.051
			Nutrient solution at 50% I ($mM.L^{-1}$)
pH	-	-	5.5 to 6.5
EC	-	-	1.00 ± 0.2 mS cm^{-1}

(M) = Molar; pH = hydrogen potential; EC = electrical conductivity. (1)Dilution in distilled water and volume completed to 1 L. (2)Dilution in 700 mL distilled water containing 268 mL of NaOH (40 $g.L^{-1}$) and volume completed to 1 L. (3)Volume of the stock solution used for the preparation of nutrient solution in mL L^{-1} for 100% *I*.

Let $Y_{\{ijkl\}}$ be the observation on the l-th plant (l = 1, …, 10) grown under the i-th zinc level (i = 0, 1; 0 = absence, 1 = 5.75 mg L^{-1}), the j-th chromium level (j = 0, 1; 0 = absence, 1 = 0.15 mg L^{-1}), and recorded at the k-th time point (k = 1, …, 4; 22, 29, 36 and 43 DAT). The maximal mixed model for the growth traits was described as follows:

$$\begin{aligned} Y_{\{ijkl\}} &= \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{\{ij\}} \\ &+ (\alpha\gamma)_{\{ik\}} + (\beta\gamma)_{\{jk\}} + (\alpha\beta\gamma)_{\{ijk\}} + u_{\{l(ij)\}} + \varepsilon_{\{ijkl\}} \end{aligned} \tag{1}$$

where μ is the overall mean; the Greek letters represent fixed effects (main and interaction terms); $u_{\{(ij)\}} \sim N\left(0, \sigma_u{}^2\right)$ is the random intercept accounting for plant-to-plant heterogeneity within each Zn × Cr combination; and the residual vector $\varepsilon_{\{ij \cdot l\}} = \left(\varepsilon_{\{ij1l\}}, \ldots, \varepsilon_{\{ij4l\}}\right)^T$ follows a multivariate normal distribution with mean 0 and covariance matrix Σ that may assume compound symmetry, first-order autoregressive (AR(1)), or an unstructured form, thereby modelling alternative correlation patterns among the repeated measures on the same plant across time.

Fixed-effect linear models that did not consider random effects for the plants were fitted using the generalized least squares (GLS) method, while models with random effects were fitted using the restricted maximum likelihood (REML) method. The fittings were done using the nlme package in R, which offers a wide range of methods for constructing mixed linear and nonlinear models, such as defining random effects, varied covariance structures, and estimation methods like GLS, ML, and REML (R Core Team, 2021).

Models were selected based on information theory criteria (Akaike Information Criterion - AIC, Bayesian Information Criterion - BIC), which penalize model complexity and allow choosing models that provide good fits while being parsimonious. Model selection also considered diagnostics through standardized residuals – that is, after removing the influence of treatments, random effects, and correlation structure – such that cases violating the normality assumption of the residuals were disregarded. At this stage, the Shapiro-Wilk test at a 5% significance level was used.

The best model selection was performed iteratively, according to the following steps: (i) firstly, all the models were fitted using REML or GLS and the valid models (with normal residuals) were ranked according to AIC and BIC; (ii) Considering the best covariance structure, models with and without interaction between treatments and times were refitted using the maximum likelihood method, and a likelihood ratio test was applied to verify if the interactions provided improvements to the model or if their inclusions were insignificant; (iii) Finally, after determining whether the interactions with time should be part of the model, it was refitted using the REML method to reduce biases during variance component estimation.

After selecting the best model, the effects of Cr, Zn, and Time and their interactions were examined using the F-test from the Analysis of Variance (MIXED ANOVA). Simple effects and interactions were studied through post-hoc marginal mean comparisons, performed with the emmeans package in R (Lenth, 2016). Plant physiology parameters (photosynthetic pigments) were analyzed similarly using two-way ANOVA, and post-hoc mean comparisons were conducted using Student's t-test. In both cases, the validity of the adjustments was checked through residual plots and the Shapiro-Wilk normality test. All analyses were conducted in the R program, using the RStudio Desktop IDE (open source version) (R Core Team, 2021; RStudio Team, 2020).

RESULTS

Our data reveals that chromium (Cr) emerges as a significant factor affecting several physiological parameters. Specifically, Cr significantly influences plant height (P = 0.0417), leaf area (P = 0.0088), number of leaves (P = 0.0416), chlorophyll *a* (P = 0.0195), chlorophyll *b* (P = 0.0391), and carotenoid content (P = 0.0348) (see Tables 2 and 3).

The analysis reveals a significant negative impact of Cr on plant height. The estimated marginal mean height of

Table 2. Analysis of Variance (ANOVA) results for growth traits over time.

Source of Variation*	Plant height	Leaf area[a]	Plant length
Time	**<0.0001**	**0.0060**	**<0.0001**
Zn	0.5451	0.1906	0.4176
Cr	**0.0417**	**0.0088**	0.1132
Time:Zn	0.2941	**0.0042**	0.1140
Time:Cr	**0.0001**	0.6136	0.0659 (.)
Zn:Cr	0.4724	0.4736	0.8385
Time:Zn:Cr	0.0839 (.)	0.1891	0.1507 (.)

*: P-values < 0.05 are shown in bold; (.) indicates trend (0.05 < P < 0.10).
[a]: square root transformed.

Table 3. Analysis of Variance (ANOVA) results for the number of leaves and photosynthetic pigments.

Source of Variation*	Number of leaves	Cla	Clb	Carotenoid
Time	**<0.001**	–	–	–
Zn	0.6668	0.2851	0.1095	0.2502
Cr	**0.0416**	**0.0195**	**0.0391**	**0.0348**
Time:Zn	–	–	–	–
Time:Cr	–	–	–	–
Zn:Cr	0.2666	0.1198	0.1224	0.0683 (.)
Time:Zn:Cr	–	–	–	–

*: P-values < 0.05 are shown in bold; (.) indicates trend (0.05 < P < 0.10).

plants without Cr is 11.20 cm, while with Cr, it drops to 9.78 cm. The 1.42 cm reduction in plant height due to Cr is statistically significant (t = 2.112; P = 0.0417) at the 5% significance level. This result quantitatively confirms that Cr exposure significantly affects plant growth, reducing overall plant height by approximately 12.7% (Figure 1).

Cr also presented significant interactions with evaluation times for the plant height (P<0.0001). The application of Cr exhibited a consistent inhibitory effect on plant height across all four evaluation times. At the first evaluation time, the reduction in plant height was marginally significant (P = 0.0700), with plants without Cr (Cr-) having a mean height of 10.35 compared to 9.07 for plants with Cr (Cr+). Similarly, at the second time point, the reduction was also marginally significant (P = 0.0873), with mean heights of 11.07 for Cr- and 9.88 for Cr+. The effect of Cr became statistically significant at the third evaluation time (P = 0.0440), where the mean height was 11.53 for Cr- and 10.10 for Cr+, indicating a significant inhibitory effect. This trend continued and strengthened at the fourth time point, where the reduction in plant height was significant (P = 0.0140), with mean heights of 11.84 for Cr- and 10.07 for Cr+. These results indicate that the negative impact of Cr on plant height increases over time, becoming more pronounced and statistically significant at later evaluation times.

There was also a significant negative impact of Cr on leaf area (P = 0.0088) when it is transformed by the square root function. The estimated marginal mean transformed leaf area of plants without Cr is 11.01 cm^2, while with Cr it drops to 8.88 cm^2. This represents a reduction of about 19.3%, which is statistically significant at 5% level of significance (t = 2.700; P = 0.0105).

On the other hand, the presence of Cr significantly increases the content of chlorophyll a, chlorophyll b, and carotenoids in plants. The increases are 148 μmol.L^{-1} for chlorophyll *a* (42.2% increase), 64.5 μmol.L^{-1} for chlorophyll *b* (36.5% increase), and 49.9 μmol.L^{-1} for carotenoids (28.4% increase), with all differences being statistically significant at the 5% level (t-test; P<0.05). These findings suggest that Cr positively influences the biochemical pathways associated with these pigments in plants (Figure 2).

In contrast, Zn does not show significant main effects for all the parameters studied. However, there are significant interactions between Zn and evaluation times for leaf area (sqrt transformed). Zn does not show effects on leaf area at the first and second evaluation times, but its effect increasing the leaf area was significant at the third time and marginally significant at the fourth time. The increase related to the Zn in leaf area reached 18.7% at the first evaluation time.

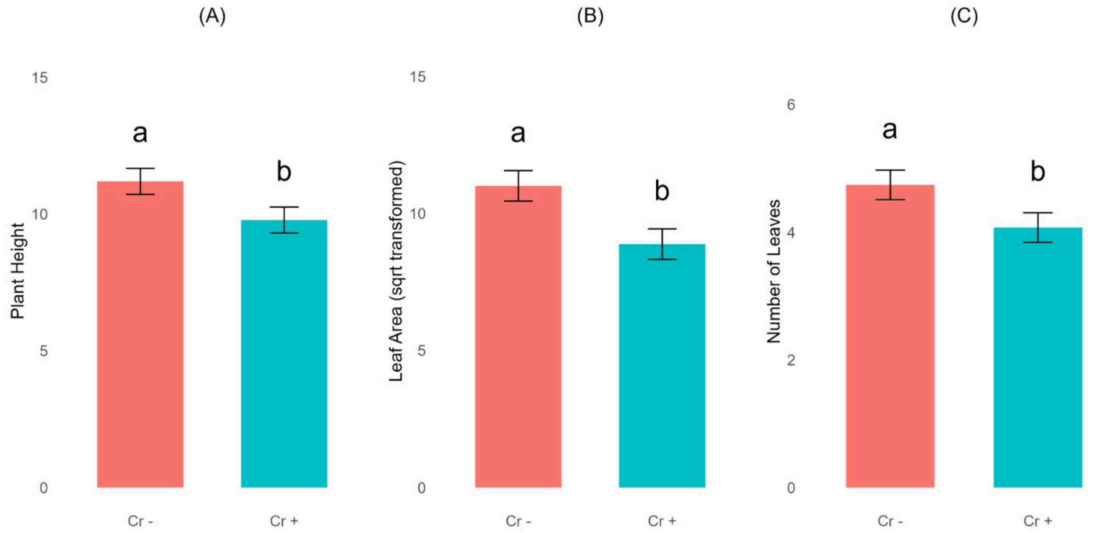

Figure 1. Simple effects of chromium on various plant growth parameters. Mean values, standard error bars, and comparisons of marginal means by the t-test at the level of 5% of significance. Responses: Plant height (A); Leaf area (sqrt transformed) (B); Number of leaves (C).

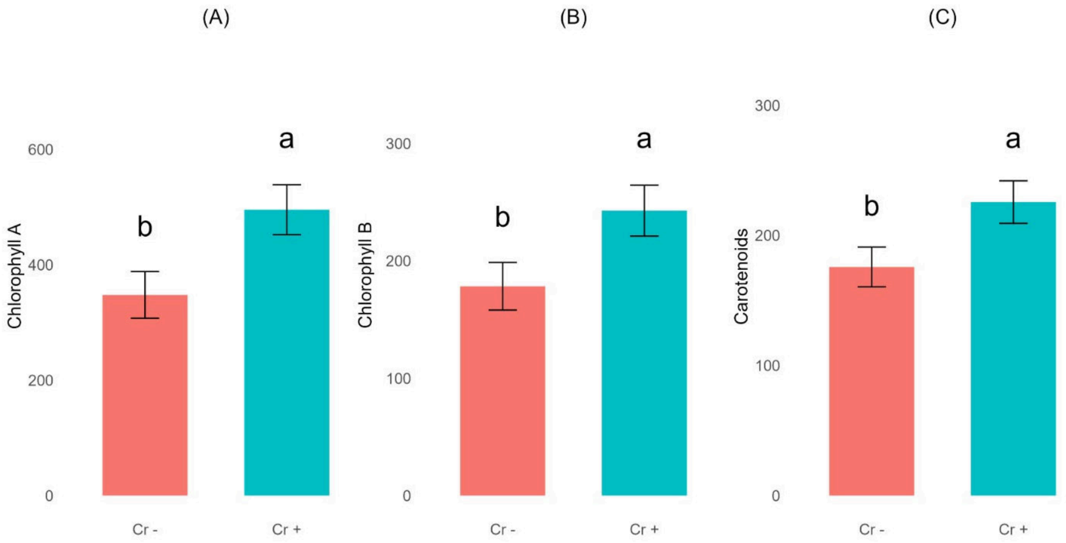

Figure 2. Simple effects of chromium on the physiological parameters. Mean values, standard error bars, and comparisons of marginal means by the t-test at the level of 5% of significance. Responses: Chlorophyll *a* (A); Chlorophyll *b* (B); Carotenoids (C).

The lack of Zn significant impact on plant height, plant length, number of leaves, and chlorophyll content (P-values ranging from 0.1977 to 0.5096) suggests that Zn, within the tested concentrations, does not play a critical role in growth and physiological aspects of plants. Additionally, the interaction between Zn and Cr does not significantly affect any of the parameters, indicating that these two elements do not have a synergistic or antagonistic relationship in influencing the measured plant traits under the conditions of this study. This lack of interaction implies that the effects of Zn and Cr on plant growth and physiology are independent of each other.

DISCUSSION

Our research aimed to investigate whether the supply of Zn can alleviate Cr stress during the initial establishment of wild passion fruit seedlings. However, our findings demonstrate that Zn does not mitigate the harmful effects of Cr on plant growth, such as reduced leaf area (excepting for 36 and 43 DAT). Existing literature generally suggests that the presence of Cr(VI) leads to a decrease in total leaf area, as well as impairment of leaf gas exchange and photosynthesis. Higher concentrations of Cr(VI) also result in leaf chlorosis, necrosis, wilting, and a reduction in leaf size, biomass, and the total number of leaves (up to 50% in *Oryza sativa* L.) (Sundaramoorthy et al., 2010). Additionally, when subjected to Cr(VI) concentrations, *Lolium perenne* L. exhibited significant wilting, along with the degradation of leaf pigments (Vernay et al., 2007). Concerning Cr's role in aerial plant tissues (shoot) spatially distant from root tissue uptakes, Shanker et al. (2005) report that the inefficient translocation of Cr to leaves is due to Cr sequestration into root cells vacuoles, to perform its non-toxic to the plant, thus becoming a defensive response that results in a lower leaf expansion rate.

Moreover, additional strategies are currently being developed to address the issue of Cr toxicity in plants. These strategies encompass biotechnological approaches, such as the creation of transgenic plant lines with enhanced Cr tolerance, as well as traditional methods like breeding plants with increased chromium tolerance. These approaches aim to improve phytoremediation capabilities for environmental purification through enzymatic activities (Ghuge et al., 2023). Plant nutrient uptake occurs through membrane protein carriers, located in cells membrane of shoot and root organs (Broadley et al., 2012). Nevertheless, about 98% of all the mineral elements required by plants are obtained through enzyme systems (membrane carriers) located in the root cells. Therefore, plant nutrient requirements must be significant via root supply. According to Kraemer (2009), plants face a "dilemma" as they cannot differentiate between essential and non-essential mineral elements, posing significant challenges to their survival. Essential HMs cannot be replaced by another element in their metabolic functions (Santa-María et al., 2023). Zn, an essential metal, remains in its ionic form within cells and plays a crucial role as a component of antioxidant enzymes, promoting plant growth (Stanton et al., 2022). Though the essentiality of Cr is still under investigation, research efforts are ongoing to uncover its uptake mechanism, accumulation, and cellular effects (Jobby et al., 2024).

Plant metabolic pathways for Cr uptake have not been elucidated. However, since Cr is a nonessential element, its uptake is not well understood. It has a specific and exclusive uptake mechanism and is also dependent on Cr speciation (Ali et al., 2023). The solubility of Cr (VI) is significantly higher than Cr (III), making it more toxic at lower concentrations and prone to forming more persistent complexes in the soil (Abdulmalik et al., 2023). Cr(VI) exhibits higher solubility, leading to increased bioavailability and greater toxicity at lower concentrations than Cr(III), which has a propensity to form stable complexes in the soil (López-Luna et al., 2009). Regarding to Cr(VI) uptake and translocation, some authors argue that Cr(VI) is reduced to Cr(III) in surface roots (Ramachandran et al., 1980; Zayed et al., 1998; Wani et al., 2022); other authors suggest that dissolved Cr(VI) is uptake by plants without reduction (Mishra et al., 1995; Malaviya and Singh, 2011). Cr(III) uptake occurs passively, requiring no energy expenditure by the plant (UdDin et al., 2015; Ao et al., 2023). The uptake of Cr(VI) is considered an active mechanism carried out by transporters responsible for uptakes of crucial elements like sulfate (Kim et al., 2006; Oliveira et al., 2016).

Plant species with recognized economic value show toxic responses to chromium (Cr) at concentrations of around 5–100 $mg.g^{-1}$ in the soil and 0.5–5.0 $mg.L^{-1}$ when applied via nutrient solution (Oliveira, 2012). It's important to note that the reported values have not been studied in wild plants, so caution is needed when applying these findings to another plant species. In this study, the application of Cr was below the reference values known to cause phytotoxicity. Furthermore, it has been observed that plant growth stimulation and other beneficial effects can occur in plants grown in soil or hydroponic solutions containing Cr (Barceló et al., 1993; Gomes et al., 2017). For example, the presence of 1 $\mu mol\ L^{-1}$ Cr was found to stimulate plant growth, photosynthetic pigments, and increase yield (Ghosh and Singh, 2005). When comparing the effects of Cr(III) and Cr(VI) on photosynthesis parameters of *Pontederia crassipes* (Mart.) Solms (water hyacinth), Paiva et al. (2009) found that Cr(III) was much less toxic than Cr(VI), and might eventually increase photosynthesis and chlorophyll content. In another study, low and moderate concentrations of Cr(III) (10^{-6} and 10^{-4} M) in irrigation solution increased pigment content in leaves, but higher Cr(III) concentrations (10^{-2} M) reduced the contents of chlorophyll *a*, chlorophyll *b*, and carotenoids in *Phaseolus vulgaris* L. (common bean) (Zeid, 2001).

We did not observe any significant differences in plant growth when combining the Zn and Cr metals. We believe that competitive and/or non-competitive inhibition may have occurred between these metals at their root uptake sites. Shanker et al. (2005) demonstrated that Cr competes with Fe, S, and P for carrier binding, suggesting that Cr follows the Michaelis-Menten kinetics at low concentrations due to the competitive effects of essential mineral metals. The precise mechanism of Cr uptake has not been fully understood, leading us to consider the possibility of non-competitive inhibition between metals. Non-competitive inhibition involves allosteric inhibition, where the inhibitor binds to the enzyme's allosteric site or in the presence of a specific metal (substrate), causing the absorption site of another substrate to deform. Our results showed that Cr has a harmful effect on plant growth parameters, but it also led to an increase in the expression of plant physiology parameters (photosynthetic pigments). Zn had no significant effects - except for the leaf area - or interactions with Cr.

CONCLUSION

We reject the hypothesis that Zn supply alleviates Cr stress during the initial establishment of passion fruit seedlings.

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