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EFFECTS OF SILICON ON *Theobroma cacao* L. GROWING IN DEGRADED SUBSTRATES: AN ANALYSIS OF MORPHOPHYSIOLOGICAL RESPONSES AND CHLOROPHYLL A FLUORESCENCE

EFEITOS DO SILÍCIO EM *Theobroma cacao L.* CRESCENDO EM SUBSTRATOS DEGRADADOS: UMA ANÁLISE DAS RESPOSTAS MORFOFISIOLÓGICAS E DA FLUORESCÊNCIA DA CLOROFILA A

EFECTOS DEL SILICIO EN *Theobroma cacao* L. QUE CRECE EN SUSTRATOS DEGRADADOS: UN ANÁLISIS DE LAS RESPUESTAS MORFOFISIOLÓGICAS Y LA FLUORESCENCIA DE LA CLOROFILA A

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ABSTRACT

Silicon (Si) is considered a beneficial element due to its positive effects in mitigating several biotic and abiotic stresses. Despite the promising results reported for different crops, studies involving Si application in *Theobroma cacao* are still incipient and are more been commonly associated with the mitigation of biotic stress. This study aimed to evaluate the mitigating potential of Si in cacao seedlings subjected to aluminum (Al) and iron (Fe) toxicity through morphophysiological responses. The seedlings were cultivated in three substrates: organic substrate (Sor), substrate with high aluminum saturation (S_{Al}), and substrate with high iron saturation (S_{Fe}), with or without soil application of 2 mM potassium silicate. Growth variables, chlorophyll *a* fluorescence parameters, JIP-test, and SPAD index were evaluated. Si application reduced Fv/Fm and PIabs values in S_{Al} and S_{Fe}, as well as PI_{total} in S_{Fe}, compared with S_{or}. Regardless of the substrate, plants treated with Si showed inferior responses compared with plants without Si in terms of energy absorption, capture, and transfer along

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the electron transport chain. In addition, metal toxicity promoted reductions in growth and biomass allocation. In *T. cacao*, Si did not mitigate the effects of Al and Fe toxicity on the photochemical performance and growth of seedlings; however, further studies are needed to better understand the response of Si under different soil conditions and cacao genotypes.

Keywords: Growth. Aluminum. Iron. Cocoa. JIP Test.

RESUMO

O silício (Si) é considerado um elemento benéfico devido aos seus efeitos positivos na mitigação de diversos estresses bióticos e abióticos. Apesar dos resultados promissores relatados para diferentes culturas, os estudos envolvendo a aplicação de Si em *Theobroma cacao* ainda são incipientes e têm sido mais comumente associados à mitigação de estresses bióticos. Este estudo teve como objetivo avaliar o potencial mitigador do Si em mudas de cacau submetidas à toxicidade por alumínio (Al) e ferro (Fe), por meio de respostas morfofisiológicas. As mudas foram cultivadas em três substratos: substrato orgânico (S_{or}), substrato com alta saturação por alumínio (S_{Al}) e substrato com alta saturação por ferro (S_{Fe}), com ou sem aplicação ao solo de 2 mM de silicato de potássio. Foram avaliadas variáveis de crescimento, parâmetros de fluorescência da clorofila *a*, teste JIP e índice SPAD. A aplicação de Si reduziu os valores de Fv/Fm e PIabs em SAl e S_{Fe}, bem como o PItotal em SFe, em comparação com S_{or}. Independentemente do substrato, as plantas tratadas com Si apresentaram respostas inferiores às das plantas sem Si em termos de absorção, captura e transferência de energia ao longo da cadeia de transporte de elétrons. Além disso, a toxicidade metálica promoveu reduções no crescimento e na alocação de biomassa. Em *T. cacao*, o Si não mitigou os efeitos da toxicidade por Al e Fe sobre o desempenho fotoquímico e o crescimento das mudas; entretanto, estudos adicionais são necessários para melhor compreender a resposta do Si sob diferentes condições de solo e genótipos de cacau.

Palavras-chave: Crescimento. Alumínio. Ferro. Cacau. Teste JIP.

RESUMEN

El silicio (Si) es considerado un elemento beneficioso debido a sus efectos positivos en la mitigación de diversos estreses bióticos y abióticos. A pesar de los resultados prometedores reportados para diferentes cultivos, los estudios relacionados con la aplicación de Si en Theobroma cacao aún son incipientes y se han asociado con mayor frecuencia a la mitigación de estreses bióticos. Este estudio tuvo como objetivo evaluar el potencial mitigador del Si en plántulas de cacao sometidas a toxicidad por aluminio (Al) y hierro (Fe), mediante respuestas morfofisiológicas. Las plántulas fueron cultivadas en tres sustratos: sustrato orgánico (Sor), sustrato con alta saturación de aluminio (SAl) y sustrato con alta saturación de hierro (SFe), con o sin aplicación al suelo de 2 mM de silicato de potasio. Se evaluaron variables de crecimiento, parámetros de fluorescencia de la clorofila a, prueba JIP e índice SPAD. La aplicación de Si redujo los valores de Fv/Fm y PIabs en SAl y SFe, así como el PItotal en SFe, en comparación con Sor. Independientemente del sustrato, las plantas tratadas con Si presentaron respuestas inferiores a las de las plantas sin Si en términos de absorción, captura y transferencia de energía a lo largo de la cadena de transporte de electrones. Además, la toxicidad metálica promovió reducciones en el crecimiento y en la asignación de biomasa. En Theobroma cacao, el Si no mitigó los efectos de la toxicidad por Al y Fe sobre el desempeño fotoquímico y el crecimiento de las plántulas;

sin embargo, se requieren estudios adicionales para comprender mejor la respuesta del Si bajo diferentes condiciones de suelo y genotipos de cacao.

Palabras clave: Crecimiento. Aluminio. Hierro. Cacao. Prueba JIP.

1 INTRODUCTION

Silicon (Si) is the second most abundant element in the Earth's crust, surpassed only by oxygen (Exley, 1998). In soils, it occurs in the form of silicates (aluminum, calcium, iron, etc.), with concentrations ranging from 1 to 45%, depending on physicochemical characteristics (Sommer et al., 2006). Silicon content in plants varies among species from 0.1 to 10% of dry mass (Ma and Takahashi, 2002). According to these concentrations, plants may be classified as excluders (less than 0.5% Si), intermediates (more than 1% Si), and accumulators (more than 4% Si) (Mitani and Ma, 2005; Guerriero et al., 2016). Although Si is not considered an essential nutrient according to the criteria established by Arnon and Stout (1939), it is regarded as beneficial due to its promising effects in mitigating several biotic and abiotic stresses (Ahmad et al., 2023; Du et al., 2026).

The mitigation of metal toxicity by silicon has been investigated in several crops and for various elements, including aluminum (Fang et al., 2025; Deus et al., 2026), iron (Santos et al., 2019; Santos et al., 2020), arsenic (Faisal et al., 2025), and cadmium (Anwart et al., 2025). The strategies involved in mitigation are diverse and depend on both the species and the chemical element interacting with Si. Among the morphological strategies, the following stand out: compartmentalization of excess heavy metals in cell walls (Zhang et al., 2008) and thickening of the root endodermis (Santos, 2017; Ali et al., 2025). Among the physiological strategies, the following are noteworthy: increased leaf gas exchange through enhanced stomatal (gs) (Lavinsky et al., 2016) and mesophyll conductance (gm) (Detmann et al., 2012; Sanglard et al., 2014; Santos et al., 2019; Santos et al., 2020), as well as increased activity of oxidative stress enzymes (Sanglard et al., 2014; Yusuf et al., 2025). In roots, silicon may inhibit cadmium transporters from roots to shoots in peanut plants (Shi et al., 2010) and promote the formation of hydroxyaluminosilicates (HAS) in the apoplast of root apex cells, thereby reducing metal mobility and concentration in leaves and roots, as observed in maize and rice (Wang et al., 2004; Santos et al., 2019). Although these mechanisms have been verified in several crops, information regarding tropical species, such as cacao, remains scarce.

Cacao (*Theobroma cacao* L.) is a tropical woody species belonging to the Malvaceae family. Its high productivity and adaptability to different edaphoclimatic conditions have favored its broad distribution across cacao-producing regions in several Latin American countries (Jaimez et al., 2022). In Brazil, the state of Bahia accounted for 57.04% of cocoa and cocoa-derived product exports in 2022 (Agrostast, 2022). Given the global trend of increasing cocoa consumption and the observed decline in production in many countries, there is a promising outlook for the expansion of cocoa production in Bahia. Silicon application in CCN51 hybrid seedlings has shown positive effects on photosynthesis and chlorophyll a fluorescence even under aphid infestation (Pinto et al., 2012; Pinto et al., 2014) and Moniliophthora perniciosa infection (Fantinato et al., 2018). Although the authors did not report effects on leaf gas exchange, Si likely promoted increased photosynthesis through enhanced stomatal conductance (gs), as observed by Fantinato et al. (2018) in T. cacao infected with M. perniciosa. Regarding growth, Si reduced stem height and stem dry mass without compromising other growth variables, which was associated with the redirection of photoassimilates toward resistance strategies against aphid attack (Toxoptera aurantii) (Pinto et al., 2014). The reported positive responses to Si fertilization suggest that similar benefits may also occur under abiotic stress conditions, such as metal toxicity.

In southern Bahia, cacao is predominantly cultivated in clayey soils and Oxisols with favorable physical characteristics; however, these soils may be acidic and exhibit high levels of exchangeable Al (Ribeiro et al., 2013). High aluminum saturation in substrates directly affects cacao seedlings by influencing growth parameters such as biomass allocation, relative growth rate (RGR), and net assimilation rate (NAR) (Baligar and Fageria, 2005). In addition, photosynthetic parameters are also reduced in seedlings exposed to high Al saturation in the substrate (Ribeiro et al., 2013). Regarding iron, studies have evaluated the effects of different Fe sources on cacao physiology (Baligar et al., 2015); however, information concerning the effects of Fe toxicity on this crop remains limited. Research on iron toxicity has mainly focused on species such as rice (Zhang et al., 2011; Santos et al., 2020).

In rice, high Fe concentrations in the substrate may cause reduced photosynthesis through stomatal and non-stomatal limitations (Santos et al., 2019; Santos et al., 2020), increased reactive oxygen species (ROS) production (Pinto et al., 2016), and reduced grain yield (Santos et al., 2020). Other negative effects of iron toxicity include leaf bronzing and reduced leaf number in Eugenia uniflora (Jucoski et al., 2016), reduction of photosynthetic pigments in Glycine max (Lapaz et al., 2020) and Elodea nuttallii (Xing et al., 2010), decreased net assimilation rate due to impairment of the photochemical reactions of photosynthesis in Ipomoea batatas (Adamski et al., 2011), and negative effects on growth, biomass allocation, and photosynthesis in Genipa americana (Sousa-Santos et al., 2022).

Silicon has proven to be a viable alternative for mitigating biotic stress in T. cacao (Pinto et al., 2012; Pinto et al., 2014; Fantinato et al., 2018) and may potentially improve the performance of cacao seedlings in the field under unfavorable conditions of metal toxicity, such as aluminum toxicity in acidic substrates and Fe toxicity in waterlogged substrates. Despite promising results in numerous crops, studies on silicon in T. cacao L. are still incipient and are more strongly associated with the attenuation of biotic stress through induced resistance to pests (Pinto et al., 2012; Pinto et al., 2014) and fungi (Fantinato et al., 2018) than to abiotic stress (Zanetti et al., 2016).

In light of the above, the present study aimed to evaluate how CCN51 cacao seedlings exposed to substrates with high levels of aluminum and iron toxicity respond to Si application. It was hypothesized that Si fertilization mitigates the toxicity of these metals through adjustments in biomass allocation, growth, and chlorophyll a fluorescence.

2 METHODOLOGY

2.1 PLANT MATERIAL AND EXPERIMENTAL SETUP

The experiment was conducted at State University of Santa Cruz, located in Ilhéus, Bahia, Brazil (39°13′59″ W; 14°45′15″ S). Seedlings of *Theobroma cacao* genotype CCN51 were purchased from Instituto Biofábrica de Cacau and transplanted into 5-L pots containing three different substrate types. The substrates were collected from a cocoa/cabruca agroforestry system in the district of Salobrinho, municipality of Ilhéus (39°10′0″ W; 14°48′0″ S), in the municipality of Uruçuca (39°17′29″ W; 14°35′12″ S), and at the Almada Experimental Station in the municipality of Ilhéus (39°10′00″ W; 14°38′00″ S). These substrates were characterized as organic substrate (SOr), substrate with high aluminum saturation (SAl), and substrate with high iron saturation (SFe), respectively, according to Sousa-Santos et al. (2022). Half of the plants from each treatment received weekly applications of 2 mM silicon (Si) in the form of potassium silicate diluted in water. A total of 30 plants were used, with half of them treated weekly for 170 days with 2 mM Si supplied as potassium silicate (K_2SiO_3) diluted in water.

2.2 GROWTH EVALUATION

At the time of transplanting, root dry mass (RDM), stem dry mass (SDM), leaf dry mass (LDM), total dry mass (TDM), and total leaf area (LA) were evaluated in ten seedlings. For these analyses, the seedlings were harvested, washed, and separated into roots, stems, and leaves. The plant material was individually placed into properly labeled paper bags and dried in a forced-air oven at 60 °C until constant mass was achieved.

The total leaf area of each plant was determined from leaf images obtained using an HP DeskJet Ink Advantage 2676 printer. The images were properly labeled and saved, and leaf area measurements were performed using ImageJ software. The same procedure was repeated at the end of the experiment. Stem diameter (D) was measured using a caliper, and plant height (H) was measured using a ruler. Based on dry mass data, the root mass ratio (RMR = RDM/TDM), stem mass ratio (SMR = SDM/TDM), and leaf mass ratio (LMR = LDM/TDM) were calculated. Relative growth rate (RGR) and net assimilation rate (NAR) were then calculated from dry mass and leaf area (LA) data, according to Hunt et al. (2002).

2.3 CHLOROPHYLL *A* FLUORESCENCE ANALYSIS

Chlorophyll fluorescence parameters were obtained using a portable Pocket PEA fluorometer between 08:00 and 12:00 h. Selected leaves were dark-adapted for 30 min using leaf clips. After this period, the leaves were exposed to a saturating light pulse (3000 $\mu mol\ m^{-2}\ s^{-1}$, wavelength of 650 nm, for 1 s).

From the JIP-test parameters, the following were evaluated: (1) specific energy fluxes (per reaction center) for absorption (ABS/RC), trapping (TR_0/RC), dissipation (DI_0/RC), and electron transport (ET_0/RC) at the antenna chlorophyll level (Strasser et al., 2001); and (2) flux ratios or yields, including the maximum quantum yield of primary PSII photochemistry ($\phi Po = TR_0/ABS$), maximum efficiency of non-photochemical de-excitation ($\phi Do = DI_0/ABS$), the probability that a trapped electron ($\psi o = ET_0/TR_0$), or an absorbed photon ($\phi Eo = ET_0/ABS$), moves an electron beyond QA; maximum quantum efficiency of photosystem II (Fv/Fm); performance index on absorption basis (PI_{ABS}); and total performance index (PI_{total}).

2.4 SPAD INDEX

Chlorophyll index (Chl) evaluation was performed using a portable chlorophyll meter, model SPAD-502 (SPAD-502, Minolta Corp., Ramsey, Japan), on the same leaves used for chlorophyll *a* fluorescence measurements.

2.5 STATISTICAL ANALYSIS

The experiment was arranged in a completely randomized design (CRD) in a 3 × 2 factorial scheme, consisting of two silicon (Si) concentrations and three substrate types (S_{Or},

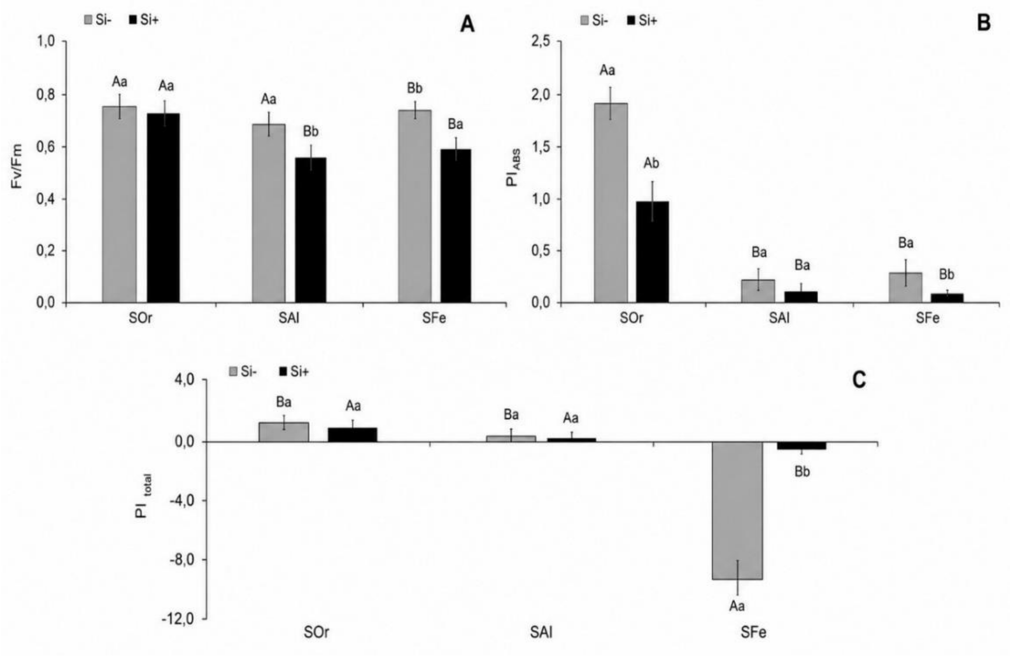
S_{Al}, and S_{Fe}), with five replicates per treatment, totaling 30 plants. Before analysis, assumptions of error normality (Shapiro–Wilk test) and homogeneity of variances (Levene's test) were verified. Data that met these assumptions were subjected to analysis of variance (ANOVA), and means were compared using Tukey's test at a 5% probability level. Statistical analyses were performed using Sisvar software.

3 RESULTS

For chlorophyll *a* fluorescence, it was observed that, for both soils (Al and Fe), Si application significantly reduced Fv/Fm values by 26% and 20%, respectively (Figure 1A). For the performance index (PI_{ABS}), metal toxicity drastically reduced values by 91% and 85% under Al and Fe saturation, respectively, compared with the control treatment (Figure 1B). Under high Fe saturation, Si application reduced PI_{ABS} by 49%. The total performance index (PI_{total}) showed a significant difference only in plants grown in substrate with high Fe saturation. In this treatment, plants grown without Si exhibited a 96% reduction compared with plants supplied with Si (Figure 1C).

Figure 1

A – Maximum quantum efficiency of photosystem II (Fv/Fm); B – Performance index (PI_{ABS}); C – Total performance index (PI_{total}) of T. cacao grown in different substrates and fertilized or not with silicon



By dissecting the JIP-test parameters that reflect electron transport along the photosynthetic electron transport chain (ETC), it is possible to verify that plants grown in substrates with high metal saturation exhibited greater energy trapping per reaction center (TR_0/RC) compared with the control treatment, with increases of 60.4% and 73.9% for S_{Al} and S_{Fe}, respectively. However, this greater energy trapping was not reflected in higher energy transfer efficiency (Ψo and ϕEo) in substrates with high Al and Fe saturation. This lower efficiency was directly associated with greater dissipation of energy absorbed by the antenna complex in the form of heat (DI_0/ABS), which increased on average by 87% under Al and Fe saturation compared with the control (Table 1).

Table 1

Mean values of the effects of substrates containing organic matter (S_{Or}) and high aluminum (S_{Al}) and iron (S_{Fe}) saturation on maximum trapping rate per reaction center (TR_0/RC); probability that a trapped electron moves an electron further than QA ($\psi o = ET_0/TR_0$); probability that an absorbed photon moves an electron further than QA ($\phi Eo = ET_0/ABS$); and maximum efficiency of non-photochemical de-excitation ($\phi Do = DI_0/ABS$) in T. cacao seedlings

	Substrates		
Variable	S_{Or}	S_{Al}	S_{Fe}
TRo/RC	1.34 ± 0.04 b	2.15 ± 0.18 a	2.33 ± 0.11 a
ETo/TRo (Ψo)	0.41 ± 0.02 a	0.13 ± 0.02 b	0.22 ± 0.05 b
ETo/ABS (φEo)	0.31 ± 0.02 a	0.09 ± 0.01 b	0.14 ± 0.04 b
DIo/ABS (φDo)	0.05 ± 0.07 b	0.67 ± 0.03 a	0.64 ± 0.03 a

Means followed by the same letter in the rows do not differ statistically from each other by Tukey's test ($P<0.05$), n=5.

The application of Si (+Si) in cacao plants grown, regardless of the substrate, resulted in lower responses for energy absorption, trapping, and transfer along the ETC when compared with plants without Si (-Si) (Table 2). Regarding the electron flow along the ETC, the main inefficiency is associated with electron transfer, since the greatest decrease was observed for the parameter ETo/RC (specific electron transport flux per reaction center), with a 43% reduction in -Si plants compared with +Si plants (Table 2). This response is associated with a 37% reduction in trapping (ETo/TRo) and a 33% reduction in absorption (ETo/ABS).

Table 2

Mean values of the effects of the absence (-Si) or presence (+Si) of silicon on T. cacao seedlings for electron transport rate per reaction center (ETo/RC); maximum quantum yield of primary photochemistry (TRo/ABS); probability that a trapped electron moves an electron further than QA (ψo = ETo/TRo); and probability that an absorbed photon moves an electron further than QA (ϕEo = ETo/ABS).

Variable	-Si	+Si
ETo/RC	0.63 ± 0.16 a	0.36 ± 0.03 b
TRo/ABS (φPo)	0.72 ± 0.13 a	0.63 ± 0.03 b
ETo/TRo (Ψo)	0.30 ± 0.05 a	0.19 ± 0.03 b
ETo/ABS (φEo)	0.21 ± 0.03 a	0.14 ± 0.02 b

Means followed by the same letter in the rows do not differ statistically from each other by Tukey's test ($P<0.05$), n=5.

Among the growth variables analyzed, ten were significantly affected by the different substrate types, and three were affected by the presence or absence of silicon application, regardless of substrate type. The variables height (H), diameter (D), leaf area (LA), specific leaf mass (SLM), leaf mass ratio (LMR), stem mass ratio (SMR), root mass ratio (RMR), relative growth rate (RGR), net assimilation rate (NAR), and SPAD index were affected by substrate type (Table 3). Silicon fertilization affected root mass ratio (RMR), leaf mass ratio (LMR), and SPAD index (Table 4).

The values of H, D, LA, LMR, and SPAD index were higher in cacao seedlings grown in the organic substrate (S_{Or}) compared with those grown in the other two substrate types. Seedlings grown in aluminum-rich (S_{Al}) or iron-rich (S_{Fe}) substrates did not differ significantly from each other for these same variables. The opposite result was observed for RMR, with the highest values recorded in the S_{Al} and S_{Fe} substrates, without statistical differences between them, whereas the lowest value was observed in S_{Or} (Table 3). Notably, leaf area was approximately seven times greater in cacao seedlings grown in S_{Or} compared with those grown in S_{Al}, and ten times greater than in those grown in S_{Fe}. No statistical differences were observed between seedlings grown in aluminum-rich and iron-rich substrates (Table 3).

Regarding SLM, the highest value was obtained for seedlings grown in the S_{Al} substrate, whereas the lowest value was observed in seedlings grown in S_{Or}. The value obtained for seedlings grown in S_{Fe} was statistically similar to those observed for both S_{Or} and S_{Al} seedlings. For SMR, the highest value was observed in seedlings grown in SFe, being 63.64% higher than that observed in S_{Or} seedlings, with no statistical differences between S_{Al} and S_{Or}. The opposite pattern was observed for NAR, with the lowest value recorded in seedlings grown in the S_{Fe} substrate, approximately four times lower than that observed in seedlings grown in S_{Or}. No differences in NAR were observed between seedlings grown in S_{Al} and S_{Or}. The RGR of the seedlings differed among the three substrate types. The highest value was obtained for seedlings grown in the S_{Or} substrate, followed by those grown in S_{Al} and then S_{Fe} (Table 3).

Table 3

Mean values of the effects of substrates containing organic matter (S_{Or}) and high aluminum (S_{Al}) or iron (S_{Fe}) saturation on height (H), diameter (D), leaf area (LA), specific leaf mass (SLM), leaf mass ratio (LMR), stem mass ratio (SMR), root mass ratio (RMR), relative growth rate (RGR), net assimilation rate (NAR), and SPAD index in T. cacao seedlings

	Substrates		
Variable	S_{Or}	S_{Al}	S_{Fe}
H (cm)	32.95 ± 1.08 a	23.91 ± 1.24 b	21.22 ± 1.39 b
D (mm)	4.11 ± 0.26 a	2.76 ± 0.31 b	3.08 ± 0.12 b
LA (cm^{-2})	1421.89 ± 98.92 a	191.155 ± 22.47 b	130.94 ± 18.24 b
SLM ($g\ m^{-2}$)	4.78 ± 0.25 b	9.33 ± 2.06 a	6.14 ± 0.64 ab
LMR ($g\ g^{-1}$)	0.49 ± 0.01 a	0.26 ± 0.03 b	0.22 ± 0.02 b
SMR ($g\ g^{-1}$)	0.22 ± 0.01 b	0.26 ± 0.02 b	0.36 ± 0.02 a
RMR ($g\ g^{-1}$)	0.29 ± 0.01 b	0.47 ± 0.02 a	0.44 ± 0.03 a
RGR ($mg\ g^{-1}\ dia^{-1}$)	9.79 ± 0.36 a	4.43 ± 0.34 b	1.46 ± 0.32 c
NAR ($mg\ cm^{-2}\ dia^{-1}$)	0.12 ± 0.01 a	0.10 ± 0.01 a	0.03 ± 0.01 b
SPAD	35.04 ± 1.27 a	14.75 ± 1.61 b	14.53 ± 0.86 b

Means followed by the same letter in the rows do not differ statistically from each other by Tukey's test ($P<0.05$), n=5.

Considering only the effects of silicon fertilization, RMR, LMR, and SPAD index were affected. Both RMR and SPAD index showed higher values in cacao seedlings that did not receive silicon fertilization. Regarding biomass allocation, RMR was 14% higher in -Si plants compared with Si plants, whereas the opposite pattern was observed for LMR, with the highest value found in seedlings fertilized with Si, representing an increase of 20.69% compared with non-fertilized seedlings (Table 4).

Table 4

Mean values of the effects of the absence (-Si) or presence (+Si) of silicon on root mass ratio (RMR), leaf mass ratio (LMR), and chlorophyll SPAD index in T. cacao seedlings.

Variable	-Si	+Si
RMR (g g^{-1})	0.42 ± 0.03 a	0.37 ± 0.02 b
RMF (g g^{-1})	0.29 ± 0.04 b	0.35 ± 0.03 a
SPAD	22.94 ± 2.81 a	19.94 ± 2.66 b

Means followed by the same letter in the rows do not differ statistically from each other by Tukey's test ($P<0.05$), n=5.

4 DISCUSSION

Chlorophyll *a* fluorescence is a rapid, non-invasive, and non-destructive analysis that provides information regarding the efficiency and functionality of the photosynthetic electron transport chain (ETC) and how changes in electron flow may affect the production of reducing power (NADPH) and the biochemical stage of photosynthesis. Thus, it can indirectly integrate photosynthesis and growth by assessing the dynamics of electron transfer and the efficiency of electron flow in the ETC (Strasser and Govindjee, 1992).

The maximum quantum yield of photosystem II (F_v/F_m) is a sensitive indicator of environmental stress and reveals photoinhibition or indicates the downregulation of photosynthesis, in which values below 0.83 for the F_v/F_m ratio indicate damage to the photosynthetic apparatus (Krause and Weis, 1991; Larcher, 1995; Maxwell and Johnson, 2000). Under silicon fertilization, the reduction in F_v/F_m may be related to changes in photochemical energy flow, evidenced by lower electron transport efficiency and greater thermal dissipation under metal toxicity conditions. Pereira et al. (2013) reported a decrease of only 6% in F_v/F_m values for *T. cacao* seedlings under aluminum stress, whereas Akya and Takenaka (2001) observed no differences in this same variable for *Quercus glauca*, also under Al stress.

For CCN51, both metals and Si promoted reductions in the performance indices (PI_{ABS} and PI_{Total}), which were reflected in electron flow efficiency and light conversion efficiency. Regarding Si application in *T. cacao*, no differences were observed in PI_{ABS} and PI_{total} for the TSH1188 and Catongo genotypes, regardless of silicon fertilization (Pinto et al., 2012). In this context, the performance indices (PI_{ABS} and PI_{Total}) have been considered more sensitive parameters for detecting and quantifying the response of the maximum efficiency of photosystem II (F_v/F_m) (Christen et al., 2007; Oukarroum et al., 2007), because these variables relate the efficiency of absorption, capture, and transfer of excitation energy by photosystem II, thereby providing a broader assessment of the degree of environmental stress effects (Gonçalves and Santos Jr., 2005).

In *T. cacao*, Si fertilization reduced the performance indices under both ideal cultivation conditions (S_{Or}) and stress conditions, associated with lower effective maximum quantum yield of PSII (φPo) and lower electron transport efficiency of PSII (Ψ0), which contributed to a lower photosynthetic performance index per absorbed energy (PI_{ABS}). This effect was evidenced by the low capture capacity (TRo/RC) and electron transfer efficiency (ETo/TRo) relative to the absorbed energy (ETo/ABS), indicating impairment in the probability of an electron moving toward PSI from quinone a (Qa−) (Strasser et al., 2000). Furthermore, for CCN51 seedlings grown under Al and Fe toxicity, the main form of energy dissipation absorbed by the antenna complex was heat dissipation (DIo/ABS), which indicates a photoprotective mechanism to avoid more severe damage to photosystem II (PSII). The results obtained in Si-fertilized plants differ from those reported in the literature, which demonstrate positive effects of Si on fluorescence in several cultivated species (Santos et al., 2020; Adamski et al., 2011; Sousa-Santos et al., 2022), including cacao (Pinto et al., 2012; Pinto et al., 2014). For CCN51, Pinto et al. (2012) observed an increase in the density of active PSII reaction centers (RC/ABS), higher φPo and Ψ0 values, which were responsible for higher PIABS and PITotal, indicating greater efficiency of the photosynthetic apparatus under biotic stress (aphids). Unlike biotic stress, metal-induced stress has indirect (Al) and direct (Fe) contributions to redox reactions that favor the formation of reactive oxygen species (ROS), which promote damage to the proteins composing the ETC and to pigments.

Indeed, all these responses in photochemical efficiency may be related to the lower SPAD chlorophyll index observed for both the substrate factor and Si treatment. The reduction in the SPAD index possibly occurred due to chlorophyll degradation as a result of metal concentration in leaf tissues and/or photodegradation (Taiz and Zeiger, 2013). Despite the decreases in the photochemical stage of photosynthesis and chlorophyll index, +Si plants showed greater biomass allocation to leaves (LMR) compared with roots (RMR), indicating positive growth aspects and changes in the source–sink relationship of photoassimilates. The higher LMR suggests a strategy to preserve photosynthesis for a longer period under stress conditions, since factors such as stress intensity and duration are important determinants of tolerance.

The values of H, D, LA, and LMR were lower in seedlings grown in substrates containing Al and Fe, demonstrating the negative effect of these metals on these parameters (Table 4). Pereira et al. (2013) also reported reductions in LA and H in cacao seedlings subjected to high Al concentrations in the substrate. In contrast, there are reports of reduced leaf mass and increased leaf area in cacao seedlings with increasing aluminum saturation in the soil, indicating increased leaf surface area and reduced thickness (Baligar and Fageria, 2005).

The LA observed in the S_{Al} and S_{Fe} substrates was 7- and 10-fold lower, respectively, than the LA observed in S_{Or}. This reduction in leaf area, together with the very low NAR and RGR values in seedlings grown under toxic substrates, demonstrates the negative effects of these metals on the growth and biomass allocation of *T. cacao* (Table 4). For NAR, the aluminum substrate was statistically similar to the organic substrate, indicating that high Al saturation had no influence on net assimilation rate, which differs from the findings of Baligar and Fageria (2005), who reported a reduction in this parameter.

When comparing the two toxic substrates, S_{Fe} showed even lower RGR values than S_{Al}. For NAR, the Fe substrate was the only one that negatively affected this parameter, demonstrating the low photosynthetic activity of these plants. Negative effects of excess iron in sweet potato have been reported through reductions in net assimilation rate (Adamski et al., 2011). Sousa-Santos et al. (2022) also demonstrated the negative effects of Fe toxicity on growth, biomass allocation, and photosynthesis in *Genipa americana*. Considering the effects of Si application on *T. cacao* seedlings, the only variable positively affected was leaf mass ratio (LMR), indicating positive aspects of growth. Growth reduction was also observed in cacao seedlings of different genotypes, including CCN51, when subjected to potassium silicate application; however, fertilization had positive effects by reducing the incidence of damage caused by insect pests (Pinto et al., 2014).

5 CONCLUSION

The application of silicon to leaves did not contribute significantly to mitigating the effects caused by metal toxicity (Al and Fe) in the CCN51 genotype under the evaluated conditions. However, silicon fertilization induced photochemical changes in which the reduction in performance indices suggests adjustments in electron transport efficiency and energy conversion aimed at photoprotection. The increase in biomass allocation to leaves (LMR) highlights adjustments in the source–sink relationship and may favor prolonged growth under stress conditions. The morphophysiological responses of *T. cacao* to Si and metal toxicity may depend on the intensity and duration of stress, the applied Si dose, and the genotype used. Therefore, further studies are required to better understand the mechanisms involved in the interaction between Si and metal toxicity in other genotypes of the species.

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