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Silicion supplementation increases the *in vitro* regeneration of oil palm (*Elaeis guineensis* Jacq.) somatic embryos

Suplementação do silício aumenta a regeneração *in vitro* de embriões somáticos de palma de óleo (*Elaeis guineensis* Jacq.)

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ABSTRACT

The use of silicates in plant tissue culture has resulted in positive effects regarding the morphological, anatomical and physiological characteristics of *in vitro* plants. However, biological responses to this mineral are dose and genotype dependent. The objective of the present study was to evaluate the influence of silicon supplementation on the somatic embryogenesis of oil palm (*Elaeis guineensis* Jacq.). The study was carried out in three stages of the cloning process, namely: Regeneration (i) and germination (ii) of somatic embryos and during *ex vitro* acclimatization (iii) of regenerated plantlets. The culture media were supplemented with four doses (0.0, 0.5, 1.0 and 1.5 g L^{-1}) of calcium silicate ($CaSiO_3$). The resulting plantlets were transplanted and acclimatized in a greenhouse. The regression analysis showed that the dose of 0.63g L^{-1} of $CaSiO_3$ induced a higher rate of regeneration, which would allow a 58% increase in the number of somatic embryos formed. However, the germination of the plantlets, as well as their morphometric characters, were not affected by the different doses of $CaSiO_3$, although the presence of this compound promoted an increase in the thickness of the epidermis and a reduction in the amount of epicuticular wax. Overall, silicon does contribute to the cloning of *E. guineensis.*

Index terms: Arecaceae; micropropagation; oil palm tree; silicate; somatic embryogenesis.

RESUMO

O uso de silicatos em cultura de tecidos tem resultado em efeitos positivos no que tange os caracteres morfológicos, anatômicos e fisiológicos das plantas *in vitro*. Contudo as respostas biológicas a este mineral são dose e genótipo-dependente. Nesse sentido, objetivou-se avaliar a influência da suplementação de silício no processo de embriogênese somática da *Elaeis guineensis* Jacq. O estudo foi realizado em três etapas do processo de clonagem, sendo elas: Regeneração (i) e germinação (ii) dos embriões somáticos e durante a aclimatação *ex vitro* (iii) das plântulas. Os meios de cultivo foram suplementados com quatro doses (0.0, 0.5, 1.0 e 1.5 g L^{-1}) de silicato de cálcio ($CaSiO_3$). As plântulas resultantes foram transplantadas e aclimatizadas em casa de vegetação. A análise de regressão evidenciou que a dose de 0.63g L^{-1} de $CaSiO_3$ induziu maior taxa de regeneração, o que possibilitaria um aumento de 58% no número de embriões somáticos formados. Contudo, a germinação das plântulas, bem como, seus caracteres morfométricos não foram afetados pelas diferentes doses de $CaSiO_3$, embora a presença deste composto tenha promovido aumento da espessura da epiderme e redução na quantidade de cera epicuticular. No geral, o silício contribui para a clonagem de *E. guineensis*.

Termos para indexação: Arecaceae; micropropagação; palmeira oleaginosa; silicato; embriogênese somática.

Introduction

Elaeis guineensis (Jacq.), is a palm tree popularly known as oil palm with a strict tropical distribution (Okolo et al., 2019). The species is characterized by high yield, reaching around 5 to 7 t ha^{-1} $year^{-1}$ of vegetable oil (Hansen et al., 2015). Worldwide, its oils are used in food, pharmaceutical and cosmetics preparations and, in recent years, for biofuel production (United States Department of Agriculture - USDA, 2023).

Currently, the world production of palm oil is 79.4 million t, mainly from crops in Southeast Asia, Equatorial Africa and Tropical-Equatorial America (USDA, 2023). Given the global agro-economic importance of this palm tree and the growing demand for its oils, genetic breeding programs for the species are an ongoing effort (Silva & Miranda, 2020). However, elite genotypes may produce a limited number of seeds, which may have a low germination rate or yield poor quality plantlets. This is partially due to the high heterozygosity manifested by parents

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that are allogamous plants (Dickson et al., 2021). Furthermore, *E. guineensis* is a species with monopodial growth, which makes propagation by tillers unfeasible. Therefore, vegetative multiplication by conventional means becomes impractical (Gomes et al., 2015).

The *in vitro* cultivation of superior genetic materials is a viable alternative to produce, on a large scale, plants with the same characteristics as the target donor (Borém, Miranda, & Fritsche-Neto, 2021; Vilela et al., 2021). Regeneration protocols, via somatic embryogenesis, have been established for *E. guineensis* (Corrêa et al., 2015, Gomes et al., 2015, Corrêa et al., 2016, Almeida et al., 2020). The regeneration of somatic embryos of the species occurs indirectly, from calluses that, after cellular transdifferentiation, will give rise to somatic embryos (Corrêa et al., 2016). The regeneration of somatic embryos is an important step for the subsequent germination and development of the plantlets. The success of mass production of plantlets, via somatic embryogenesis, is dependent on the number of adequately regenerated embryos, which also must present good morphological quality, and sufficient accumulation of reserves to germinate and nourish the plantlets in formation (Rai et al., 2022).

Although not essential, silicon (Si) is a mineral capable of stimulating somatic embryogenesis, through increased callus production, growth and longevity (Sivanesan & Jeong, 2014). In the early stages of development, Si can improve the morphological, anatomical and physiological characteristics of the plantlet, increasing its tolerance to low temperatures and salinity and reducing the incidence of hyperhydricity (Sivanesan & Park, 2014). For example, the use of Si in culture medium stimulated an increase in chlorophyll content, biomass gain and shoot length in banana (Asmar et al., 2011). In date palm the presence of Si provided greater formation of callus and somatic embryos, increased fresh and dry weight of embryos, as well as reduced oxidation rates and hyperhydricity levels (Abo & Reda, 2014). Despite the biostimulatory capacity of Si (Pavlovic et al., 2021; Trejo-Téllez Gómez-Trejo, & Gómez-Merino, 2023) its effects on *E. guineensis* somatic embryogenesis are unknown. The beneficial influence of the element on the entire process, or part of it, could help to attain commercial plantlets on a larger scale.

In the present study, it was hypothesized that Si added to *in vitro* cultivation could lead to greater formation of somatic embryos of *E. guineensis* and reflect on the successful plantlets establishment in a greenhouse. Therefore, the objective was to evaluate the influence of Si supplementation in culture medium on the regeneration and germination phases of somatic embryos and on the *ex vitro* acclimatization of *E. guineensis* plantlets. The gathered results indicate that Si have a positive impact on the regeneration of oil palm somatic embryo.

Material and Methods

Plant material and induction of embryogenic lineages

The process of *E. guineensis* somatic embryogenesis is represented in Figure 1. Pre-established embryogenic callus of *E. guineensis*, maintained for 37 months in multiplication medium, were transferred to polystyrene Petri dishes (90 x 15 mm) containing the regeneration medium composed of salts and vitamins Y3 (Eeuwens, 1978) (Table 1), in addition to the components listed in Table 2. The original callus was from the elite palm tree AM37 - obtained from the commercial plantation belonging to Agropalma S.A., located in the municipality of Thailand/PA, Brazil. For that, immature leaves from the palm heart region were excised and subjected to the somatic embryogenesis protocol described by Corrêa et al. (2015).

Calcium silicate ($CaSiO_3$) was added to the regeneration medium, in four different doses (0.0, 0.5, 1.0 and 1.5 g L^{-1}). The culture medium was autoclaved for 20 min at 121 °C at 1.5 atm. Each treatment consisted of six replications, each experimental unit consisting of a Petri dish containing three pro-embryogenic masses (~2mm³ each). The cultures were maintained in a growth room for 45 days at 28º ± 2ºC, in the absence of light. At the end of this period, the number of somatic embryos was quantified. For this evaluation, only somatic embryos were considered that presented translucent granular structures, with a well-defined embryonic axis, measuring 4mm³.

Germination of somatic embryos and plantlets formation

The somatic embryos obtained in the previous stage were used in a germination test in which they were transferred to 300 ml glass flasks containing 40 ml of germination medium (GM1) composed of salts and vitamins from the C4 medium (Table 1), supplemented with the components in table 2 and the same $CaSiO_3$ doses previously described. In this experiment, each treatment consisted of seven replications, each one corresponding to a glass vial containing nine somatic embryos (4mm³). After inoculation, the flasks were kept for 20 days in a growth room with a photoperiod of 16 hours/day and irradiance of ± 30 μmol $(m^2)^{-1}$. S^{-1} provided by LED tube lamps (18 W, Arapeva Iluminação LED).

Once this step was complete, the germinated embryos were transferred to flasks containing C4 culture medium, as described in the previous step, except for the doses of myoinositol and methionine (GM2; Table 2), to obtain the plantlets. $CaSiO_3$ doses were also maintained. The flasks were kept in a growth room for 40 days, under the same conditions as previously described.

The resulting plantlets were transferred to test tubes (25x150 mm) containing 10 mL of elongation medium, composed of salts and vitamins from the M4 medium (Table 1), supplemented with

the other components (Table 2). The same doses of $CaSiO_3$ were added to the culture medium. The different culture media were autoclaved for 20 min at 121 ºC at 1.5 atm and poured into the respective containers. The test tubes were kept in a growth room for 30 days under the same conditions as previously described.

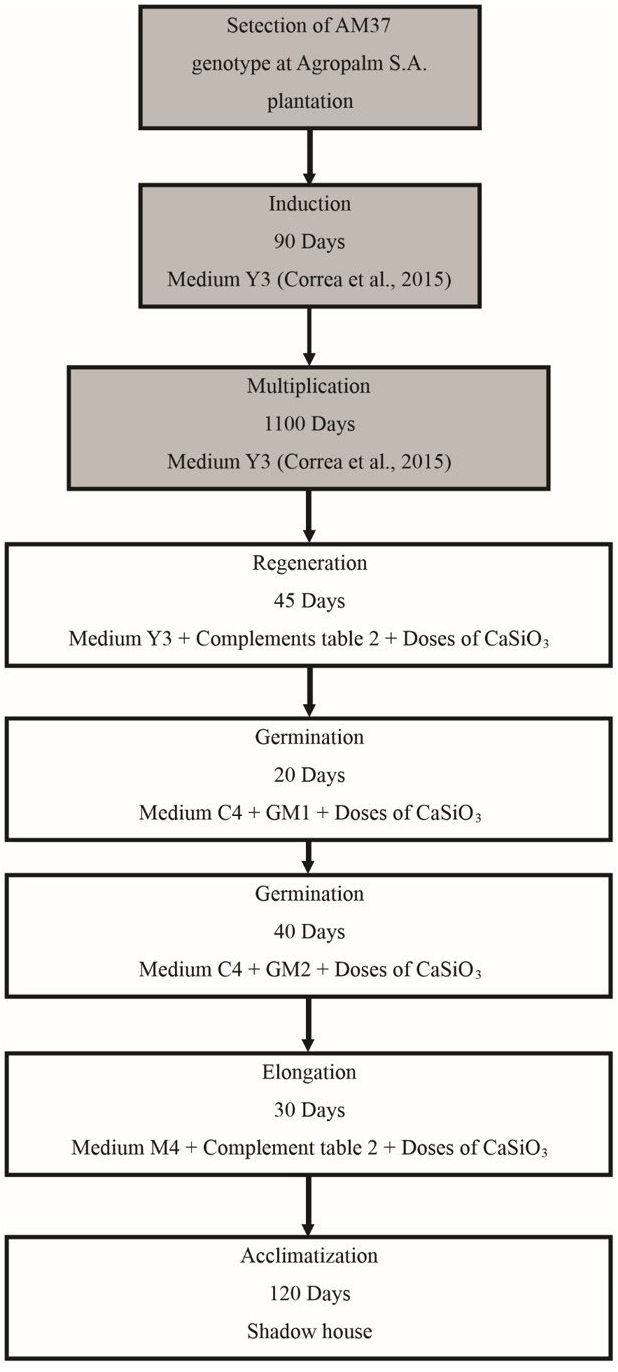

Figure 1: Stages of the *E. guineensis* somatic embryogenesis. The gray boxes refer to steps performed in previous studies and the white boxes to the steps carried out in the present study.

Table 1: Formulation of different *in vitro* culture media used in the regeneration and germination stages of *E. guineensis* somatic embryos.

In vitro culture media (mg L^{-1})			
Macronutrients			
Components	Y3	C4	M4
KNO_3	2020	2581	2020
NH_4CL	535	-	-
$NaH_2PO_4.2H_2O$	312	713	312
$CaCl_2.2H_2O$	294	-	-
KCl	1492	786	1492
$MgCl_2.6H_2O$	247	1275	247
$MgSO_4.7H_2O$	-	387	-
NH_4NO_3	-	1021	800
$CaNO_3.4H_2O$	-	-	472
Micronutrients (mg L^{-1})			
$Fe_2SO_4.7H_2O$	13.9	24.3	24.3
Na_2EDTA	37.2	33.5	33.5
$MnSO_4.4H_2O$	11.2	2.3	11.2
KI	8.3	1.7	8.3
$ZnSO_4.7H_2O$	7.2	2.8	7.2
H_3BO_3	3.1	5.0	3.1
$CoCl_2.6H_2O$	0.24	0.24	0.24
$Na_2MoO_4.2H_2O$	0.24	0.24	0.24
$CuSO_4.5H_2O$	0.25	1.2	0.25
$NiCl_2.6H_2O$	0.024	0.24	0.024
Vitamins (mg L^{-1})			
Nicotinic acid	100	100	100
Pyridoxine	100	100	100
Thiamine	100	1000	100
Silicon treatments (g L^{-1})			
$CaSiO_3$	0.0, 0.5, 1.0 and 1.5		

At the end of this period, the number of plantlets developed in each treatment was evaluated. A portion of the plantlets was used to evaluate i) total length of the aerial part (cm) and root system (cm), measured with the aid of a ruler (cm); ii) collection diameter (mm) with the aid of a digital caliper (mm); iii) fresh mass (g) and dry mass (g) - after drying for 72 h in an oven at 65 ºC - on a precision scale; iv) plantlet quality index (SQI), estimated following Dickson (1960) (Equation 1).

$$SQI = \frac{MST(g)}{\frac{H(cm)}{D(mm)} + \frac{MSPA(g)}{MSR(g)}} \quad (1)$$

where: MST- Total dry mass (g), H- Height of the shoot (cm), D- Diameter of the base (mm), MSPA- Dry mass of the shoot (g) and MSR- Dry mass of the root (g).

Epicuticular wax quantification

Twelve plantlets from each $CaSiO_3$ treatment, intended for acclimatization, were subjected to the epicuticular wax quantification methodology, adapted from Viana et al. (2010). The leaves were cut at the petiole and scanned on an HP DeskJet Ink Advantage 5520 series multifunctional printer, with the images used to obtain the leaf area using a leaf area meter implemented by the Windias 2.0 software. The leaves were distributed in samples composed of three units comprising four replications per treatment.

The samples were kept 30 ml of chloroform at room temperature for 30 seconds, followed by filtering through filter paper. Then, the resulting solution (wax + chloroform), kept in a 50 ml beaker, was evaporated on a hotplate at 80 ºC until they reached a volume of 10 ml. This volume was transferred to a 25 ml test tube with a known weigh. In a water bath at 85 ºC, the remaining of the chloroform was evaporated, leaving only the solid residue (wax). The amount of wax was expressed as the amount of wax per unit of leaf area (μg cm^{-2}).

Light microscopy and micromorphometric analysis

The longest leaf was chosen from each individual to have its middle portion collected for histological purpose. A total of three plantlets per treatment were sampled. Samples were fixed in FAA (formaldehyde, acetic acid, 50% ethanol, 1:1:18, volume: volume) for 48 h, dehydrated in an increasing ethanolic series and embedded in 2-hydroxyethyl methacrylate (Historesin, Leica, Heidelberg, Germany). Transverse sections 5-μm thickness, obtained on an automatic advancement rotary microtome (RM2155, Leica Microsystems Inc., Deerfield, USA), were stained with 0.05% toluidine blue pH 4.4 (O'Brien, Feder, & Mccully, 1964) and mounted with synthetic resin (Permount, Fisher Scientific, Pittsburgh, USA). The images of the histological analyzes were obtained with a digital camera (AxioCam HRc, Zeiss, Göttinger, Germany) and a microcomputer with the Axion Vision image capture program coupled to a light microscope (AX-70 TRF, Olympus Optical, Tokyo, Japan).

For the micromorphometric analysis, six images were obtained for each treatment, in which the thickness of the epidermis on the adaxial and abaxial sides, the thickness of the cell wall of epidermal cells and the thickness of the mesophyll were measured. For each image, ten measurements of each parameter were taken using the Image J software, totaling 960 measurements.

Acclimatization in a greenhouse

Twenty plantlets per treatment were transferred to plastic tubes (180 cm^3) filled with substrate consisting of 50% coconut shell fiber and 50% sand. They were kept in a greenhouse covered with a thermo-reflective blanket (Aluminet®) with 50% light restriction, controlled temperature ranging between 30 to 35ºC and humidity of 90%. After the thirtieth day of transplanting, each plantlet was supplied weekly with 10 mL of nutrient solution composed of 0.5g L^{-1} potassium chloride, 0.3g L^{-1} magnesium sulfate and 0.3g L^{-1} urea.

After 120 days, the following traits were measured: the number of surviving plants, the total length of the aerial part (cm) and the root system (cm) measured with the aid of a ruler; the neck diameter (mm) with the aid of a digital caliper; the fresh mass (g) and dry mass of the aerial part and root system, (g) on a precision scale; and plantlet quality using the Dickson index (Equation 1).

Table 2: Components supplemented in the medium at the respective stages.

Components	Regeneration/Maturation	Germination		Elongation
		GM1	GM2	
Sucrose (g L^{-1})	30	30	30	30
Arginine (mg L^{-1})	100	139	139	574
Myo-inositol (mg L^{-1})	100	300	200	566
Glutamine (mg L^{-1})	100	186	186	685
Casein (mg L^{-1})	1	-	-	-
Proline (mg L^{-1})	-	294	294	100
Methionine (mg L^{-1})	-	169	-	100
Activated charcoal (g L^{-1})	3	-	-	-
Phytagel (g L^{-1})	2.5	2.5	2.5	2.5
Putrescine (μM)	1000	1000	1000	1000
Naphthaleneacetic acid (μM)	-	0.54	0.54	0.54
Potential of hydrogen (pH)	5.7 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	5.7 ± 0.1

Statistical analysis

Statistical analyzes were performed using the R software (R Development Core Team, 2021). The assumptions of normality of residuals, homoscedasticity of variances and independence of errors were verified. After performing the analysis of variance, the variables that showed variation were analyzed using regression analysis, the data that showed quadratic behavior had the ideal dose determined by the first derivative of the adjusted function. Data that did not vary depending on the different treatments were described using bar graphs containing their respective confidence intervals.

Results and Discussion

Calcium silicate influences the quality and quantity of somatic embryos

In the present study, supplementation of small doses of Si (0.5 – 1.0 g L^{-1} $CaSiO_3$) provoked visible effects in the quality and quantity of the resulting somatic embryos, compared to 0.0 g L^{-1} treatment (Figure 2). The somatic embryos formed in the 0.0 g L^{-1} and 0.5 g L^{-1} $CaSiO_3$ treatments (Figure 2A and B) showed translucent color, adhered to each other, with an elongated hypocotyl-radicle axis and well defined. In contrast, the embryos exposed to 1.0 g L^{-1} (Figure 2C) and 1.5 g L^{-1} $CaSiO_3$ (Figure 2D) showed a yellowish color and globular embryos. They also presented larger globular structures, with a spongy appearance, with no differentiation into somatic embryos.

A quadratic effect of the $CaSiO_3$ doses on the number of somatic embryos formed was observed (Figure 2E). According to the adjusted equation, the dose that would provide the greatest formation of somatic embryos, providing an average of 138 embryos per pro-embryogenic mass, would be 0.63 g L^{-1} of $CaSiO_3$. Similar results were reported for embryogenic callus of date palm, in which 0.004 g L^{-1} of K_2SiO_3 provided an increase in the number of somatic embryos formed (Abo & Reda, 2014). The growth and development of embryogenic callus of *Phragmites australis* was also benefited by the supplementation of 0.031– 0.062 g L^{-1} of Na_2SiO_3 in the culture medium (Máthé et al., 2012).

The inclusion of Si sources in the culture medium stimulated somatic embryogenesis and increased callus growth, but its mechanism of action is still unknown (Iyyakkannu & Jeong, 2014). We postulate that, Si may attenuates the extravasation of osmolytes and lipid peroxidation (Kadyampakeni & Souza Júnior, 2023), preventing structural and functional disruptions of cell membranes. Once the cellular integrity is preserved, the growth of *E. guineensis* callus is possible. On the other hand, the reduction in the number of embryos formed, caused by the higher doses of Si can be attributed to a deleterious action, direct or indirect, of the element. It is reported that Si can cause inhibition of the absorption, leading to nutritional deficiency, or excessive absorption of essential metals, such as iron, which can be phytotoxic (Cooke & Leishman, 2011).

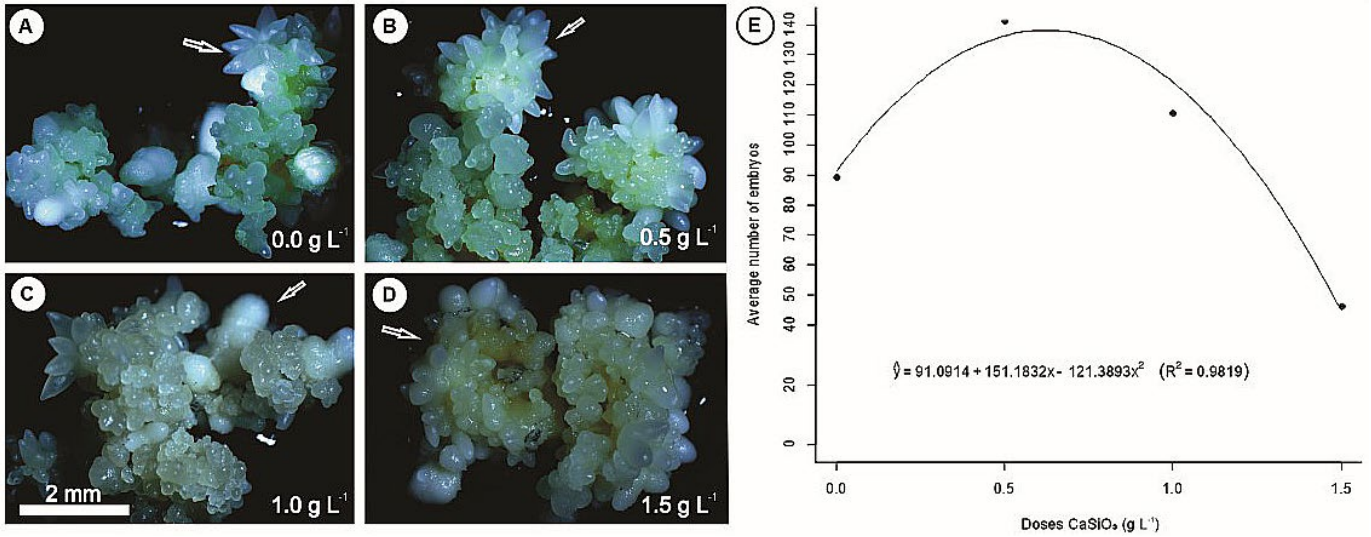

Figure 2: Effect of calcium silicate ($CaSiO_3$) supplementation on the formation of somatic embryos of *E. guineensis*. A-D: Embryogenic masses obtained after 45 days in Y3 medium, in the absence (A) and with supplementation of 0.5 (B), 1.0 (C) and 1.5 g L^{-1} (D) $CaSiO_3$. E: Average number of somatic embryos formed in each $CaSiO_3$ treatment. Arrows A-B = somatic embryos with a granular appearance and translucent; C-D = globular and spongy structures that have not differentiated.

In vitro development of plants is not affected by calcium silicate

The germination of somatic embryos began seven days after their transfer to the germination medium (GM1). At the end of the process, the plantlets formed had a well-developed root system and aerial part, regardless of the $CaSiO_3$ treatment (Figure 3A-H). Furthermore, there was no difference in the number of plantlets achieved (Figure 4A).

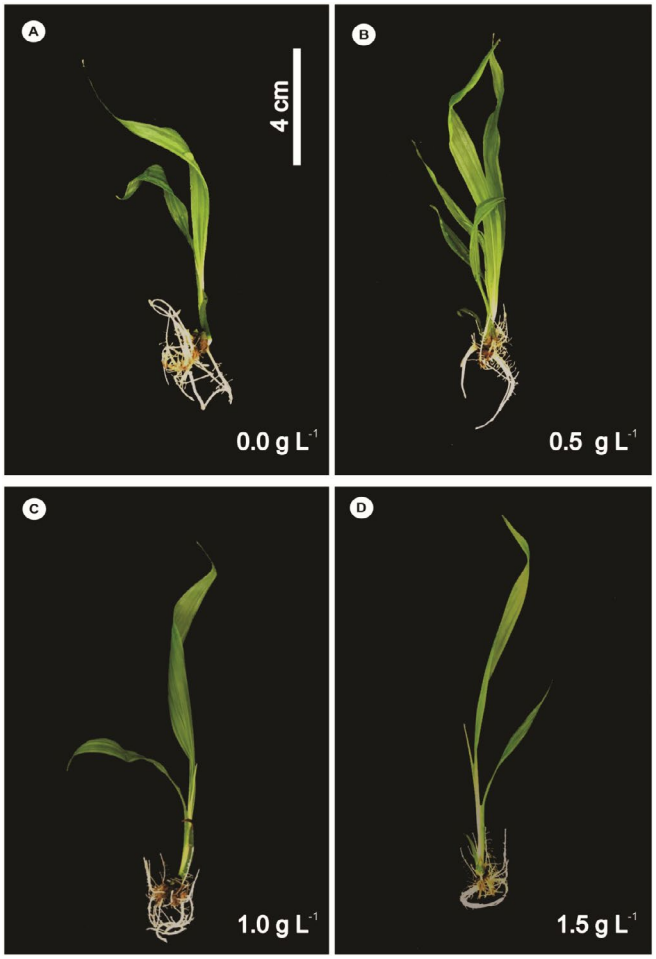

Figure 3: Plantlets formed at the end of the germination process from culture media without supplementation (A) and with supplementation of 0.5 (B), 1.0 (C) and 1.5 g L^{-1} (D) of $CaSiO_3$.

The $CaSiO_3$ treatments did not result in changes in the fresh and dry masses (Figure 4B, C) of the aerial part and root system, nor in the plantlet length (Figure 4D), showing that the applied evaluated did not cause significant effects on the development of plantlets. The Si-supplementation, using different silicic acid doses, also did not affect the *in vitro* development of *Physalis peruviana* L. (Lazzarini, 2020).

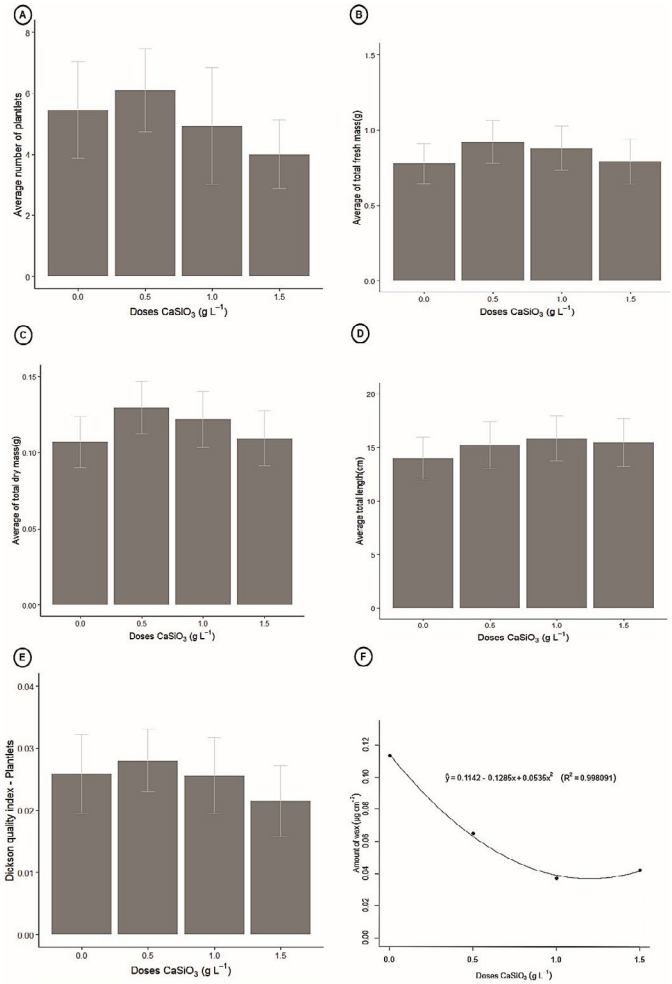

Figure 4: Effect of different doses of calcium silicate ($CaSiO_3$) on the development of *E. guineenses*. plantlets. Average number of plantlets formed at the end of the germination process (A). Average amount of fresh mass in plantlets at the end of the germination stage (B). Average amount of dry mass in plantlets at the end of the germination stage (C). Average total length of plantlets at the end of the germination stage (D). Dickson quality index (E). (Graphs with respective confidence intervals at 95% reliability). Amount of epicuticular wax in the leaves of *E. guineensis* plantlets at the end of the germination process (F).

The similarity between the characteristics of height, dry and fresh masses was reflected in the quality analysis of the plantlets. Although no significant differences were observed, there was a clear contrast in the SQI (Figure 4E) between treatments 0.0 and 0.5 $CaSiO_3$ g L^{-1} in relation to treatments 1.0 and 1 .5 $CaSiO_3$ g L^{-1}. The results indicate that those initial doses of $CaSiO_3$ g L^{-1} may favor the development of plantlets. The higher the SQI values, the higher the quality and the expected performance of the seedlings. However, SQI vary depending on the genetic material, growing conditions, age of the material

and timing of evaluation. Additional studies focusing on vigor and physiological parameters are recommended to examine the overall quality of the oil palm plantlets pre-exposed to Si.

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Epicuticular wax deposition on leaves is affected by calcium silicate

The deposition of epicuticular wax on the leaves, verified at the end of the elongation process, varied depending on the different $CaSiO_3$ treatment. According to the adjusted function, the greatest amount of wax was found in leaves of plantlets developed in a medium without $CaSiO_3$ (Figure 4F). The Si significantly interfered with the amount of wax per leaf area of *E. guineensis* plantlets. However, the amount of wax decreased as the $CaSiO_3$ doses increased. The opposite response was observed in strawberry leaf tissue grown *in vitro* in medium supplemented with silicon sources (Braga et al., 2009).

Epicuticular wax is a complex polymer with important functions in epidermal cells, including protection against water loss and, as a physical barrier on the leaf surface. It also acts to mitigate excess light damage and microorganisms attack (Bhanot, Fadanavis, & Panwar, 2021). Therefore, its presence can be an advantage for *in vitro* plantlets during their initial stages in a greenhouse. However, despite the decrease in the amount of wax, the plantlets establishment in the acclimatization stage was similar among the $CaSiO_3$ treatments. This may have happened due to the possibility of structural changes occurring in the plantlets during the acclimatization stage, such as the correction of wax deficiency. Asmar et al. (2011) reported that the wax layer gradually became thicker and better distributed in banana leaves formed *ex vitro*, contrary to what occurs *in vitro* leaves.

Although the amount of leaf wax was inversely to the dose of $CaSiO_3$, the plantlets – once transferred to the greenhouse – showed similar development and quality. Since the cuticle protects against climate elements it is possible that the conditions inside the greenhouse were suitable enough for the development of all plantlets, regardless of the treatment and the initial amount of wax deposited on the leaves. In fact, the aluminized shade-cloths, such those used in present experiment, provide uniform shade, better diffused light transmission and moderates temperatures variations, enabling an efficient microclimate control in greenhouses (Cohen & Fuchs, 1999).

Calcium silicate affects the characteristics of the leaf epidermis

The leaves of *E. guineensis* obtained in all $CaSiO_3$ treatments showed the same anatomical characteristics, such as uniseriate amphistomatic epidermis and homogeneous mesophyll composed of 5 to 6 layers of parenchyma cells, with idioblasts dispersed throughout the mesophyll (Figure 5A- D).

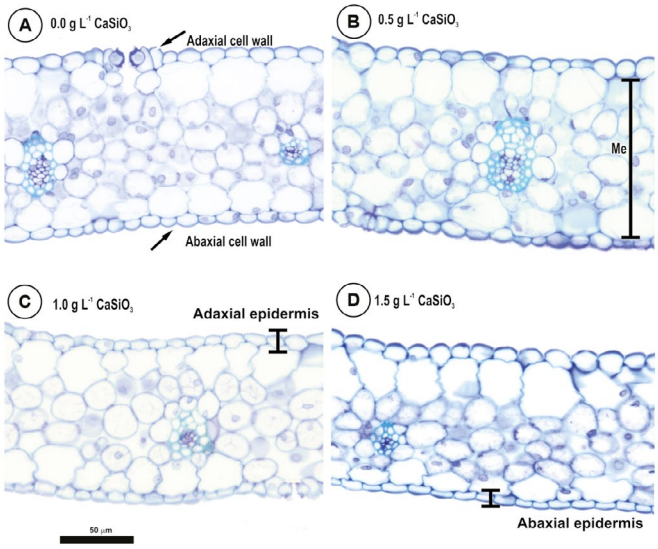

Figure 5: Transverse anatomical sections of *E. guineensis* leaves showing the adaxial and abaxial cell wall (A), the mesophyll (B), adaxial epidermis (C) and abaxial epidermis (D).

The micromorphometric analysis revealed that the thickness of the cell wall on the adaxial side of the epidermis was similar between the different treatments (Figure 6A). However, the thickness of the cell wall on the abaxial side of the leaves was influenced by the different doses of $CaSiO_3$ used. A quadratic behavior of the adjusted function was observed, in which the dose that determines the greatest wall thickness is 0.91 g L^{-1} $CaSiO_3$, providing a maximum average thickness of 1.91µm (Figure 6B). Commonly, the absorption of $CaSiO_3$ and deposition of silica result in anatomical changes in plant tissues, such as epidermal cells with thicker cell walls, resulting in better architecture for plants, increased photosynthetic capacity and resistance to pathogens, increasing the survival rate during the acclimatization process (Zambolim, Zanão Júnior, & Ventura, 2012). These advantages can certainly have a positive impact on plants and therefore corroborate the results of this study. An analogous result was found in gerbera, cultivated *in vitro*, where the thickness of the epidermis did not change depending on the different sources and doses of Si (Silva et al., 2020).

The thickness of the mesophyll did not differ between treatments (Figure 6C), as did the thickness of the adaxial face of the epidermis (Figure 6D), indicating that the different doses of $CaSiO_3$ presented similar effect in its development. This result

can be linked to the evenness in leaf mass (dry and fresh) of the plants in all treatments. In cucumber, the addition of silicon sources in the cultivation medium resulted in greater leaf mass, both dry and fresh, and changed the thickness of the mesophyll of its leaves (Adatia & Besford, 1986). The abaxial epidermis was influenced by the different doses of $CaSiO_3$, since the adjusted function demonstrated quadratic behavior, with the ideal dose being 0.47 g L^{-1} of $CaSiO_3$, which provided a maximum average abaxial face epidermis thickness of 9.13 µm (Figure 6E).

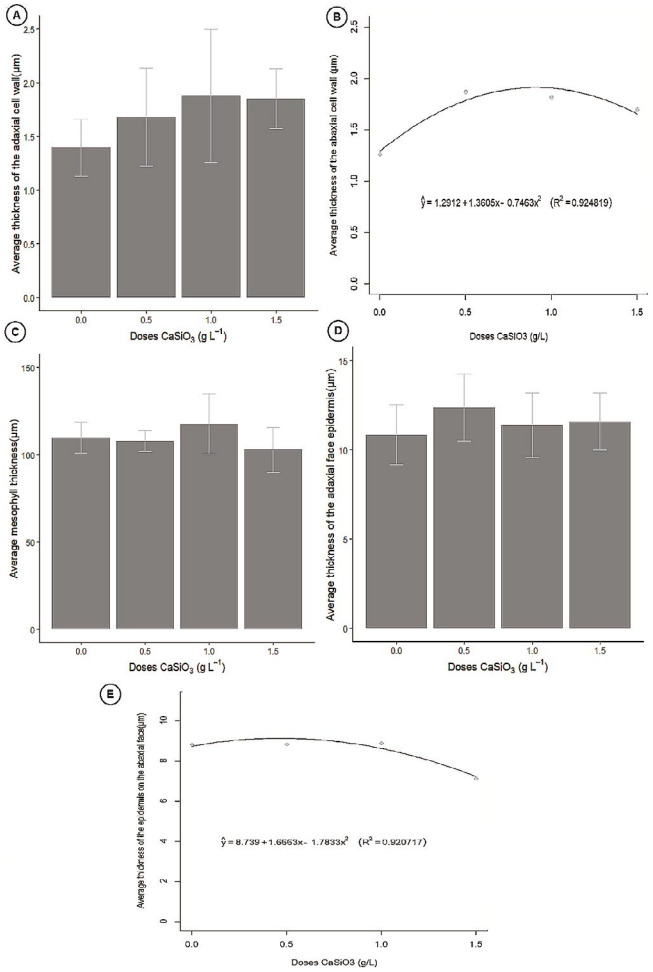

Figure 6: Effect of different doses of calcium silicate ($CaSiO_3$) on *E. guineensis* leaf anthomycete structures. Average thickness of the adaxial cell wall (A). Average thickness of the abaxial cell wall (B). Average mesophyll thickness (C). Average thickness of the adaxial face epidermis (D). Average thickness of the abaxial epidermis(E). (Bar graph with respective confidence intervals at 95% reliability).

Silicon did not influence the quality of plantlets established *ex vitro*

After 120 days in a greenhouse the average survival rate of 86% was similar to those found for gerbera and orchid plantlet treated with Si *in vitro* (Silva et al., 2020; Mantovani et al., 2020). Plantlet survival rates for treatments of 0.0, 0.5, 1.0 and 1.5 g L^{-1} $CaSiO_3$ were 84, 92, 80 and 88%, respectively. This equivalence shows that, even though the transition from an *in vitro* environment to a greenhouse condition often causes high mortality rates (Silva et al., 2020), the plantlets in our study did not experience such impact. Furthermore, success in the acclimatization stage is strongly linked to the elongation and development of the root system while the plantlets are still *in vitro*, as pointed out by Pádua et al. (2014) in a study that evaluates the success of growth regulators in rooting of *E. guineensis* plantlets pre-obtained *in vitro*.

The residual effect of Si used in the germination stagedid not significantly affect the length, fresh and dry mass of the aerial part and root system of the plantlets (Figure 7). As their quality is linked to these variables, there was no significant difference in the results obtained with the application of $CaSiO_3$, as was observed in the evaluation of the SQI.

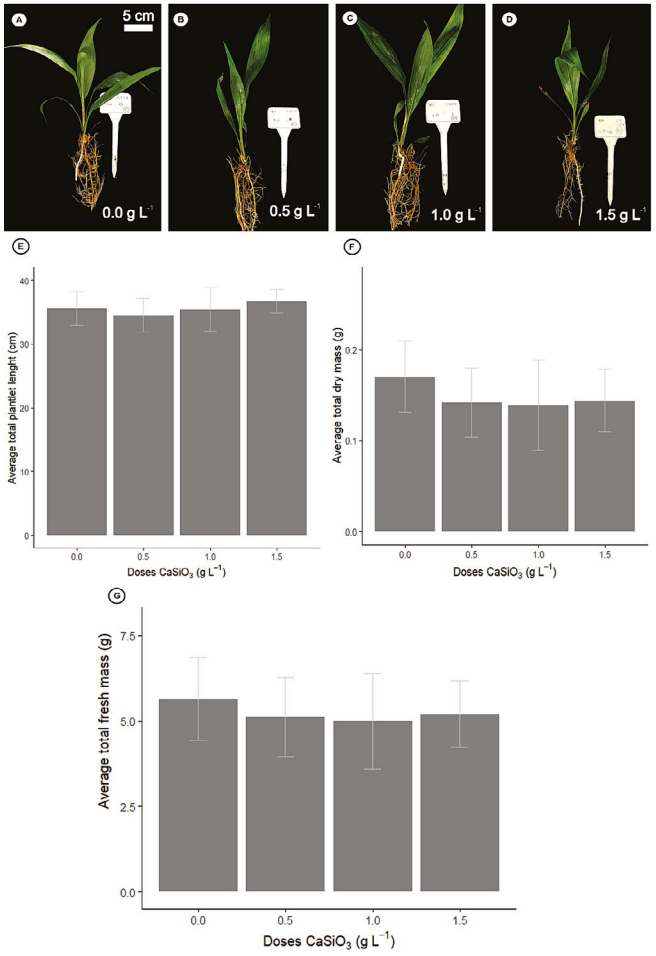

Figure 7: Effect of calcium silicate ($CaSiO_3$) supplementation on the acclimatization of *E. guineenses* plantlets. *E. guineensis* plantlets at the end of the acclimatization process (A-D). Average amount of fresh mass (E). Average amount of dry mass (F). Average total length (G). Graphs with respective 95% confidence intervals).

Conclusions

The present study proves the beneficial role of silicon in the production of somatic embryos, which allows large-scale output of clones, positively impacting the oil palm culture. The $CaSiO_3$ supplementation increased the number of somatic embryos regenerated without affecting germination, plantlet quality index and acclimatization in a greenhouse. The dose of 0.63g L^{-1} is recommended due to its positive interference with *in vitro* cultivation, especially in the yield of embryos.

Author Contribution

Conceptual idea: Motoike, SY; Methodology Design: Oliveira, LB, Filho SM; Data collection: Oliveira, LB, Rocha, DE; Data analysis and interpretation: Oliveira, LB, Filho SM, Motoike, SY, Rocha, DE; Writing and Editing: Oliveira, LB, Motoike, SY, Kuki, KN, Rocha, DE, Melo, LA.

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References

Abo, E. F., & Reda, E. (2014). Effect of silicon on somatic embryogenesis and shoot regeneration of dry date palm (*Phoenix dactylifera* l.) Cv bartamuda. *Egyptian Journal of Desert Research*, *64*:65-82.

Adatia, M. H., & Besford, R. T. (1986). The effects of silicon on cucumber plants grown in recirculating nutrient solution. *Annals of Botany*, *58*(3):343-351.

Almeida, R. F. et al. (2020). Capacity for somatic embryogenesis of adult oil palm genitors (*Elaeis guineensis*, var. *pisifera*) from immature leaf tissues. *South African Journal of Botany*, *131*:229-239.

Asmar, S. A. et al. (2011). Fontes de silício no desenvolvimento de mudas micropropagadas de bananeira 'Maçã'. *Ciência Rural*, *41*:1127-113.

Avelino, N. R. et al. (2022). Alocação de biomassa e indicadores de crescimento para a avaliação da qualidade de mudas de espécies florestais nativas. *Ciência Florestal*, *31*:1733-1750.

Bhanot, V., Fadanavis, S. V., & Panwar, J. (2021). Revisiting the architecture, biosynthesis and functional aspects of the plant cuticle: There is more scope. *Environmental and Experimental Botany*, *183*:104364.

Borém, A., Miranda, G. V., & Fritsche-Neto, R. (2021). *Melhoramento de plantas*. São Paulo, SP, Brasil: Oficina de Textos, 371p.

Braga, F. T. et al. (2009). Anatomical characteristics of the strawberry plantlets micropropagated using different sources of silicon. *Pesquisa Agropecuária Brasileira*, *44*:128-132.

Cohen, S., & Fuchs, M. (1999). Measuring and predicting radiometric properties of reflective shade nets and thermal screens. *Journal of agricultural engineering research*, *73*(3):245-255.

Cooke, J., & Leishman, M. (2011). Silicon concentration and leaf longevity: Is silicon a player in the leaf dry mass spectrum? *Functional Ecology*, *25*(6):1181-1188.

Corrêa, T. R. et al. (2015). Estimation of genetic parameters for *in vitro* oil palm characteristics (*Elaeis guineensis* Jacq.) and selection of genotypes for cloning capacity and oil yield. *Industrial Crops and Products*, *77*:1033-1038.

Corrêa, T. R. et al. (2016). Accelerated *in vitro* propagation of elite oil palm genotypes (*Elaeis guineensis* Jacq.) by substituting cytokinin with putrescine. *African Journal of Biotechnology*, *15*:2767-2775.

Dickson, A. et al. (1960). Quality appraisal of white spruce and white pine seedling stock in nurseries. *The Forestry Chronicle*, *36*:10-13.

Dickson, D. O. et al. (2021). Germination of oil palm (*Elaeis guineensis* Jacq.) seeds-a function of heat treatment and progeny. *Plant Cell Biotechnology and Molecular Biology*, *22*(39-40):59-66.

Eeuwens, C. J. (1978). Mineral requirements for growth and callus initiation of tissue explants excised from mature coconut palms (*Cocos nucifera*) and cultured *in vitro*. *Physiologia Plantarum*, *36*:23-28.

Gomes, H. T., Bartos, P. M. C., & Scherwinski-Pereira, J. E. (2015). Optimizing rooting and survival of oil palm (*Elaeis guineensis*) plantlets derived from somatic embryos. *In Vitro Cellular and Developmental Biology - Plant*, *51*:111-117.

Hansen, S. B. et al. (2015). Trends in global palm oil sustainability research. *Journal of Cleaner Production*, *100*:140-149.

Iyyakkannu, S., & Jeong, B. R. (2014). Silicon promotes adventitious shoot regeneration and enhances salinity tolerance of *Ajuga multiflora* bunge by altering activity of antioxidant enzyme. *The Scientific World Journal*, *1*:521703.

Kadyampakeni, D., & De Souza Júnior, J. P. (2023). Silicon mitigates the effects of boron deficiency and toxicity in plants. In De Mello Prado, R. Benefits of Silicon in the Nutrition of Plants. Cham: Springer International Publishing, p. 149-165.

Lazzarini, L. (2020). Silício no desenvolvimento *in vitro* de fisális. *Revista Agrária Acadêmica*, *3*(5):36-43.

Mantovani, C. et al. (2020). Silicon toxicity induced by different doses and sources added to *in vitro* culture of epiphytic orchids. *Scientia Horticulturae*, 265:109272.

Máthé, C. et al. (2012). Genotype and explants-type dependent morphogenesis and silicon

response of common reed (*Phragmites australis*) tissue cultures. *Aquatic Botany*, *97*(1):57-63.

O'brien, T., Feder, N. M. E. M., & Mccully, M. E. (1964). Polychromatic staining of plant cell walls by toluidine blue O. *Protoplasma*, *59*:368-373.

Okolo, C. C. et al. (2019). The oil palm (*Elaeis guineensis* Jacq): Nature's ecological endowment to eastern Nigeria. *Agro-Science*, *18*(3):48-57.

Pádua, M. S. S. et al. (2014). *In vitro* development and acclimatization of dendezeiro (*Elaeis guineensis*). *Revista Árvore*, 38:1095-1102.

Pavlovic, J. et al. (2021). Interactions of silicon with essential and beneficial elements in plants. *Frontiers in Plant Science*, *12*:697592.

Rai, A. C. et al. (2022). *Advances in plant tissue culture: Current developments and future trends*. London, United Kingdom, United States: Academic Press, 407p.

R Development Core Team. (2021). *The R Project for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria, Version 4.1.24.2021. Available in: <https://www.R-project.org >.

Silva, A. J. B. D., & Miranda, I. P. D. A. (2020). Potencial bioeconômico das palmeiras e seus insumos como oportunidade de desenvolvimento sustentável para as comunidades locais. In Editora Poisson. (Org). *Meio Ambiente, Sustentabilidade e Tecnologia*. vol.3, Belo Horizonte - MG: Poisson, (pp. 9-17).

Silva, D. P. C. et al. (2020). Effectiveness of silicon sources for *in vitro* development of gerbera. *Plant Cell, Tissue and Organ Culture*, 141:77-85.

Sivanesan, I., & Jeong, B. R. (2014). Silicon promotes adventitious shoot regeneration and enhances salinity tolerance of *Ajuga multiflora* Bunge by altering activity of antioxidant enzyme. *The Scientific World Journal*. *1*:521703.

Sivanesan, I., & Park, S. W. (2014). The role of silicon in plant tissue culture. *Frontiers in Plant Science*, *5*:571.

Trejo-Téllez, L. I., Gómez-Trejo, L. F., & Gómez-Merino, F. C. (2023). Biostimulant effects and concentration patterns of beneficial elements in plants. In S. Pandey. et al. *Beneficial chemical elements of plants: Recent developments and future prospects*. Hoboken, NJ: Wiley, (pp.349-369).

United States Department of Agriculture - USDA. (2023). *National agricultural statistics service*. Available in: <https://ipad.fas.usda.gov/cropexplorer/cropview/commodityView.aspx?cropid=4243000&sel_year=2023&rankby=Production>.

Viana, R. G. et al. (2010). Quantification and chemical composition of epicuticular wax of *Eucalyptus* leaves. *Planta Daninha*, *28*(4):753-758.

Vilela, M. S. P. et al. (2021). Indução e manutenção das características embriogênicas de calo do dendezeiro híbrido manicoré. *Revista Árvore*, *45*:e4538.

Zambolim, L., Zanão Júnior, L. A., & Ventura, J. (2012). *Efeito da nutrição mineral no controle de doenças de plantas*. Viçosa, Minas Gerais, Brasil: Editora UFV, 322p.