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Biochar improves growth and physiology of *Swietenia macrophylla* king in contaminated soil by copper

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The production of açaí seed waste from the commercial and extractive exploitation of the *Euterpe oleraceae* palm tree is a serious problem that contributes to environmental contamination and production of greenhouse gases, a fact that suggests the need for an environmentally correct destination for this waste produced on a large scale. To this end, this study was conducted to evaluate the potential of acaí seed biochar (BCA) in mitigating the toxic effects of copper in Brazilian mahogany plants, analyzing biometrics and gas exchange. The experimental design was in randomized blocks, with five blocks, in a 4 × 3 factorial scheme, corresponding to the control (without Cu) and three concentration of Cu (200, 400, and 600 mg Cu kg^{-1}) and three levels of BCA (0%, 5% and 10%) proportional to the amount of soil in the pots, totaling sixty experimental units. The use of 5% BCA in soils contaminated with up to 200 mg kg^{-1} Cu promoted biometric increase (height, diameter, number of leaves), maintaining gas exchange (photosynthesis, stomatal conductance, transpiration, internal carbon and internal/external carbon), and consequently, maintaining water use efficiency in plants under abiotic stress, resulting in plant growth. The findings of this study allow us to indicate the use of biochar in remediating and improving the growth of plants grown in copper-contaminated soils. The production of biochar from açaí seeds is an ecologically sustainable alternative, because it reduces its accumulation on public roads and contributes to reducing soil pollution. In the context of public policies, biochar production could be a source of income in the context of the bioeconomy and circular economy practiced in the Amazon, because it is produced in large quantities.

Keywords Charcoal, Abiotic stress, Heavy metals, Brazilian mahogany, Gas exchange

Heavy metals (HM) is a term widely used to describe groups of metals and metalloids with atomic densities greater than 4 g/cm3[17,28]. They occur naturally and are widely found in the Earth's crust. They originate from rocks of volcanic, sedimentary or metamorphic origin[4].

Soil contamination by the presence of HM is a global problem that is intensifying due to the increase in industrialization and agricultural activities[54]. Excess HM promotes soil degradation, becoming an environmental risk that affects plants, animals and humans, as they are highly toxic, non-biodegradable and have a half-life of over 20 years in nature[6].

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Among the HM, copper (Cu) is an important essential micronutrient because it plays important roles in plant metabolism, such as in redox reactions during electron transport in the photochemical stage of photosynthesis in the thylakoid membranes present in chloroplasts[34], phosphorylation chain of mitochondrial cristae, iron-sulfur protein structure[33], oxidative metabolism, cell wall metabolism and hormonal signaling[44].

Soil contamination by Cu is caused, above all, by human action represented by industrial, mining and agricultural activities. In agricultural soils, the intensive use of agrochemicals containing copper or swine manure are the main sources of copper entry into the soil[3]. This scenario is worrying because the world population is expected to reach nine billion inhabitants in 2050[21], which suggests an increase in the planted area to meet the global demand for food, intensifying the use of agrochemicals containing Cu for phytosanitary purposes[3].

The solubility of Cu in soil depends on soil texture, pH, and the presence of organic matter. Soils with a pH below 6 increase Cu availability due to the increase in variable positive charges in the soil. Organic matter, in turn, increases Cu adsorption as soil pH increases due to the increase in the number of negative charges in the soil. Organic binding sites can be humic acids and dissolved organic fractions that have a strong influence on the adsorption efficiency of Cu. This is due to varying degrees of decomposition of different fractions of organic matter[7,23,62]. Therefore, the addition of organic material with the potential to raise pH and that has adsorption sites to make HM unavailable is a way to minimize the impact of HM toxicity on soil and plants.

One strategy to minimize the harmful effects of HM on soil and plants is the conversion of waste from agricultural activities (e.g., industrial effluents, municipal solid waste, sewage sludge, food waste, animal manure and agricultural waste) into biochar through the pyrolysis process[26,50]. Biochar is a carbon-rich solid produced from biomass pyrolysis and is indicated as an efficient tool in the remediation of contaminated soils due to its stability and ability to improve the physical, chemical and biological properties of the soil, in addition to reducing the availability of HM due to the presence of adsorption sites on its surface and its alkalizing nature[2,8,12,59,61,65].

Biochar (BC) production is an environmentally friendly way to dispose of agricultural waste, because agricultural activity generates several byproducts that can have a negative impact on the environment. For example, in the Amazon, the commercial and extractive exploitation of the *Euterpe oleracea*Mart. palm tree generates large quantities of seeds that accumulate near establishments that process the fruit. This fact is reinforced by data from the agricultural census[30], which show that in 2022, the amount of açai fruit produced in Brazil was 1,699,588 tons, and the state of Pará was responsible for 94% of Brazilian production (1.595.455 tons). Considering that only 20% of the fruit is processed for drink, 80% of the production is seeds that are discarded daily, generating a high volume of waste without adequate disposal, presenting serious risks to the environment[20].

The use of açaí seeds for biochar production is an interesting alternative because of the alkalizing nature of some types of biochar, which raises soil pH, allowing Cu adsorption[23,37,65]. In this sense, the use of techniques such as the application of açaí seed biochar to recover contaminated soils is a viable alternative that can present gains such as environmental recovery and an increase in crop and forestry productivity in contaminated areas due to maintenance of their biometric and physiological parameters.

The species S. macrophylla is found throughout much of the tropical forest in the Americas, starting in Mexico and ending in Bolivia and part of the extension that runs through the southern Brazilian Amazon[9,49]. Due to its reddish-brown color, good workability and moderate resistance to weathering and biological action[15], mahogany is used in several areas such as musical instruments, carpentry, furniture, interior lining, among others[57]. It is one of the most valuable timber species in the tropics, but it is at risk of extinction due to its predatory exploitation[60].

In addition to the excellent properties of its wood, Swietenia macrophylla has been used in the recovery of degraded areas due to its rapid growth[39]. However, in degraded areas, soil fertility is an important factor that limits the growth and establishment of plant species. Therefore, the use of biochar can improve soil quality and the response of plants to stress factors such as the presence of HM in the soil, which is quite common in areas degraded by mineral exploration or agricultural activity that makes intense use of Cu-rich pesticides.

Studies indicate biochar doses in the range of 0–100 kg ha^{-1} and 0–50 kg ha^{-1}in the evaluation of the growth of Raphanus sativus plants[13]. However, the raw material used to produce biochar, the soil texture and the plant species are factors that direct the best dose of biochar for the cultivation of plants under field or pot conditions. However, crops in pot conditions recommend a dose of 7.5% in eucalyptus plants[36]and 10% biochar for the growth of soybean plants[22].

The hypothesis tested in this study is based on the possibility of biochar mitigating the damage caused by copper toxicity on the growth and gas exchange of Swietenia macrophylla plants. Some studies indicate that biochar improves gas exchange and plant growth because it remediates contaminated soils, making it possible to grow plants[55,64]. However, the behavior of Swietenia macrophylla in soils contaminated with copper and treated with biochar has not yet been reported in the literature. The objective of this study was to evaluate the potential of biochar in mitigating the toxic effects of copper by evaluating the growth and gas exchange of Brazilian mahogany plants.

Materials and methods

Experimental design, sampling and soil description

The experiment was conducted in a greenhouse, located in the experimental area of the Federal Rural University of the Amazon (UFRA), Belém Campus, Pará, Brazil (1°27'17.3"S 48°26'18.0"W). The soil used was collected in a native forest area at UFRA, in the 0–20 cm deep layer e classified as a dystrophic yellow Latosol[19]. The average temperature, humidity and photosynthetically active radiation inside the greenhouse throughout the experimental period were 36 ± 4 °C, $88 \pm 6\%$ and 342 ± 53 µmol photons mol m^{-2} s^{-1}.

The experimental design used was in randomized blocks with five blocks, in a 4×3 factorial scheme, corresponding to the control treatment (without Cu) and three doses of copper (200, 400, and 600 mg Cu kg^{-1})

in the form of copper sulfate pentahydrate ($CuSO_4.5H_2O$) and three levels of BCA (0%, 5% and 10% proportional to 0, 100 and 200 t biochar ha^{-1}) proportional to the amount of soil used in the pots, totaling sixty experimental units. The biochar (BC) was produced in a model NT 380 muffle furnace (Marca NovaTécnica).

The collected soil was air-dried, crushed, homogenized and passed through sieves with a 2 mm mesh, and a composite sample was taken for chemical characterization[18]and granulometric analysis[25]. The results of the chemical and granulometric analysis are described in Tables 1 and 2, respectively.

Based on the analysis of soil chemistry (Table 1), acidity correction was carried out using the base saturation method (V%) using 9.7 g of dolomitic limestone per pot (PRNT 97.4%), with the objective of reaching 50% base saturation and maintain soil pH between 5.5 and 6.5. Throughout the experiment, soil moisture at field capacity was maintained at 60% of the total pore volume, through daily watering in the late afternoon, starting at 5 pm.

After a period of 30 days for the limestone reaction, the soil was contaminated with copper (Cu) in 3 dm^3 pots (0.8, 1.6 and 2.4 g pot^{-1}), corresponding to doses of 200, 400 and 600 mg Cu kg^{-1}, the Cu doses used in the experiment are in accordance with the values established[14] for agricultural, residential and mining areas, respectively.

After contamination of the soil with copper, the pots were moistened and incubated for 40 days in hermetically sealed plastic bags, a period sufficient for the metal to react with the substrate and stabilize. Furthermore, during this period, the soil was turned over every two days, so that the reaction between them was homogeneous[38].

Production, characterization and application of biochar.

To produce biochar from açaí seeds (BCA), residues from the açaí agroindustry (seeds with fibers) were collected in commercial establishments in the metropolitan region of Belém, Pará. The collected residues were washed in running water and dried in an oven at 70 °C for 24 h, just to dry the material before being subjected to high temperatures.

After drying, the material was packaged in aluminum foil and placed in a muffle furnace with a heating time of 10 °C min^{-1}, and after reaching the ideal temperature for heat treatment under pyrolysis conditions, the carbonization of the material followed the methodology proposed[56], being one hour at 600 °C. With the BCA produced, the material was ground and passed through a 2 mm sieve.

To characterize the biochar, all analyses were performed in triplicate, calculating the mean and standard deviation of the results obtained, determining the hydrogen potential (pH), specific surface area (SSA) and water retention capacity (WRC)[40].

From the immediate analysis of the biochar produced, the following characteristics of the material were determined: moisture (M), volatile material (VM), ash and fixed carbon (FC), following the methodology proposed in the ASTM D1762-84 standard[5] Table 3.

The application of BCA (150 and 300 g) corresponds to treatments of 5 and 10% of the total amount of soil in the pot (3 kg), respectively, remaining incubated for 60 days for complete stabilization with the soil. At this stage, disturbance was disregarded, as improvements in soil quality from biochar are affected by the soil disintegration process[63].

After the BCA incubation period, the soil was fertilized with macronutrients (0.075 g of N, 0.053 g of P and 0.116 g of K pot^{-1}) in the form of urea, triple superphosphate and potassium chloride; For micronutrients, fertilization was carried out for copper and manganese (0.008 g of Cu and 0.046 g of Mn), as they presented low levels in the soil analysis, in the form of copper sulfate and manganese sulfate, respectively, according to the recommendation proposed in the fertilization manual[31].

Growth conditions

Parallel to the previous steps, Brazilian mahogany seedlings aged 30 days after sowing were made available by Embrapa Amazônia Oriental, being transferred to polystyrene bags with soil and plant residues, where they remained for 3 months until the formation of seedlings. Subsequently, 60 healthy seedlings with homogeneous height and sanity were visually selected, and definitive planting was carried out in 3 kg pots to compose the experimental units.

Brazilian mahogany plants were cultivated for 90 days after definitive planting, a period sufficient for changes in metabolism to result in reductions in the growth of forest species in soils contaminated by metals[58].

Biometrics and photosynthetic pigments

After the end of the experiment, plant height (H) was determined by measuring the base to the shoot apex, root length (RL) with a measuring tape, stem diameter (SD) with a digital caliper, and the number of leaves (NL)[42].

Photosynthetic pigments (chlorophyll a, b and total) were determined in the leaf of the middle third of the plants in each experimental unit, with the aid of a digital chlorophyllometer (ClorofiLOG model CFL 2060, Falker), in the morning between 09:00 and 10:00 am, the results obtained with the equipment are expressed as Falker Index (FI).

Gas exchange

Gas exchange measurements were carried out one day before withdrawing from the experiment between 09:00 and 11:00 am, a period that favors maximum and almost constant photosynthesis rates. A fully expanded leaf from the middle third was selected in all experimental units of each treatment, using one block of plants at a time. To this end, a photosynthesis meter, model LI-6400XT from LI-COR, Inc. Lincoln, was used, coupled with a 2 × 3 cm leaf chamber (Li6400-40) at a CO_2 concentration of 400 μmol^{-1} with an artificial light source. flow rate of 1000 μmol photons $m^{-2}s^{-1}$.

The net CO_2 assimilation rate (A) (μmol CO_2 $m^{-2}s^{-1}$), stomatal conductance (gs) (mol H_2O $m^{-2}s^{-1}$), transpiration (E) (mmol H_2O $m^{-2}s^{-1}$) was quantified, intercellular CO_2 concentration (Ci) (μmol CO_2 $molar^{-1}$),

layer	pH	SOM	*P*	K^+	Ca^{2+}+Mg^{2+}	Ca^{2+}	Al^{3+}	H^++ Al^{3+}	SB	CTC (pH7)	V*	m**
	$CaCl_2$	g kg^{-1}	mg dm^{-3}	$mmol_c$ dm^{-3}							%	
0–20	3.5	29	6	0.6	6	4	18	133	7	140	5	72

Table 1. Soil chemical analysis of the 0–20 cm deep layer. *pH* = hydrogen potential; *SOM* = Soil organic matter; *P* = phosphorus; *K* = potassium; *Ca* = calcium; *Mg* = magnesium; *Al* = aluminum; *H* = hydrogen; *CTC* = cation exchange capacity; *SB* = sum of bases; *base saturation, **aluminum saturation.

Layer	clay	sand	silt	Textural classification
cm	g kg^{-1}			BSSC*
0–20	212	693	95	Sandy Clay Loam

Table 2. Particle size analysis of the soil in the 0–20 cm deep layer. * Brazilian System of Soil Classification.

Material	pH	SSA	WRC	M	VM	Ash	FC
-	H_2O	$m^2 g^{-1}$	g g^{-1}	%			
BCA	11	340	2.5	9.2	0.5	98.4	1.1

Table 3. Characterization of biochar (BCA) from açaí seeds. pH = hydrogen potential; SSA = specific surface area; WRC = water retention capacity; M = moisture; VM = volatile material; Ash = ashes; FC = fixed carbon.

		Mean square						
Source of variation	D.L	H	SD	NL	RL	Chl a	Chl b	Chl total
Copper (Cu)	3	309**	6.53**	1444.3**	4.4^{ns}	3.4^{ns}	6^{ns}	14.8^{ns}
Biochar (BCA)	2	416**	6.57**	191^{ns}	23.8**	153**	258**	797.7**
Cu x BCA	6	202.8**	1.35^{ns}	563.5*	6.7**	29.4**	42.8**	132.8**
CV (%)	-	11.3	11.4	19	5.4	8.4	12	8.5

Table 4. Analysis of variance for height (H), stem diameter (SD), number of leaves (NL), root length (RL), chlorophyll a (Chl a), b (chl b) and total (Chl total) in plants of Brazilian mahogany (*Swietenia macrophylla* King) depending on different concentrations of copper (Cu) and açaí seed biochar (BCA). CV = coefficient of variation; ns = not significant; * = significant ($p < 0.05$); ** = significant ($p < 0.01$) by F test.

internal/external carbon ratio (*Ci/Ca*) and instantaneous water use efficiency (WUE) were obtained by the ratio between *A* and *E*.

Statistical analysis
The data were subjected to analysis of variance using the F test, and when significant, the Tukey test was applied to compare treatment means at a 5% probability level. The collected data were analyzed in the AgroEstat statistical program[32].

Results
Biometrics and phosynthetic pigments.

According to the analysis of variance (Table 4), there was a significant interaction between the concentration of copper (Cu) and the concentrations of biochar from the açaí seed (BCA) for the variables plant height (H), number of leaves (NL), root length (RL), chlorophyll a (Chl a), chlorophyll b (Chl b) and total (Chl total).

For the variable stem diameter (SD), there was a significant isolated effect for Cu concentrations and BCA concentrations.

For stem diameter as a function of the Cu concentrations applied (Fig. 1A), there was an increase of 22.1% at the dose 400 mg kg^{-1} (8.56 mm) in relation to the control (7.01 mm). Considering the BCA concentrations (Fig. 1B), a reduction of 14.4% in SD was observed at the 10% concentration (7.1 mm) compared to plants at the 5% BCA concentration (8.3 mm).

Observing plant height (H) at the same concentration of biochar (5%) and at different Cu concentrations, it is observed (Fig. 2A) that the addition of BCA promoted better performance up to a concentration of 400 mg kg^{-1} Cu (57.5 cm), with an increase of 54.1%, compared to control plants (37.3 cm). When analyzing the concentration of 600 mg kg^{-1} Cu, at different concentrations of BCA, the application of 5% (47.6 cm) or 10% (46.7 cm) of biochar increased by 26% and 23.5% the height, respectively, compared to plants without added BCA (37.8 cm).

When analyzing H at a concentration of 10% biochar at a concentration of 600 mg Cu, it was observed that there was a 35.7% increase in plant height in relation to plants without metal and with 10% biochar.

At concentration of 200 mg and 400 mg of Cu in different concentrations of BCA, it is noted that the addition of 10% BCA reduced and maintained H, respectively in relation to plants without BCA.

The number of leaves (NL) (Fig. 2B) obtained a higher result with the application of 5% biochar at a concentration of 200 mg kg^{-1} of Cu (92), with an increase of 35% in relation to the control (68), however, the beneficial effect of BCA occurred up to 400 mg kg^{-1} Cu (86). At a concentration of 600 mg kg^{-1} Cu, plants that received 5% (59) and 10% (69) of BCA had a reduction of 28.1% and 16.3%, respectively, in relation to the control (82).

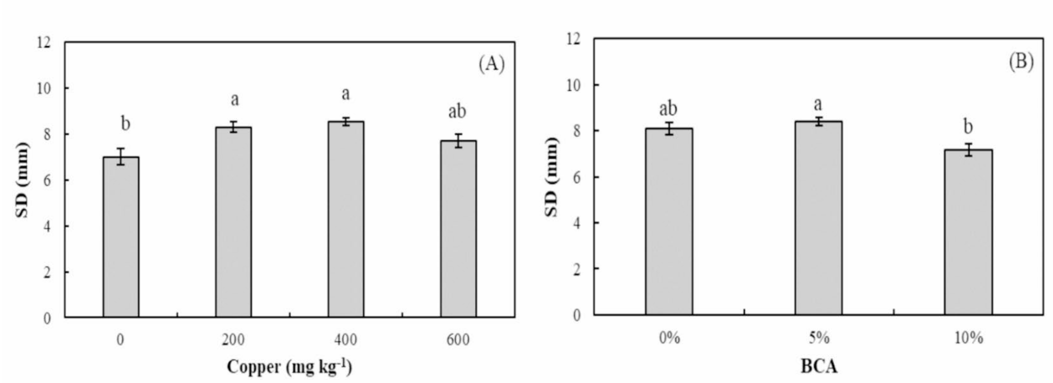

Fig. 1. Stem diameter (SD) in Brazilian mahogany plants subjected to different concentrations of Cu (**A**) and BCA (**B**). Means followed by the same letter do not differ statistically using the Tukey test ($p < 0.05$). Bars indicate the standard error of the mean ($n = 5$).

In root length (RL) (Fig. 2C), the use of biochar, regardless of concentration, did not differ from the control, in the different concentration of Cu applied to the soil. However, when observed at the same concentration of Cu, at 200 mg kg^{-1} Cu there was a reduction of 8.6% with the addition of 10% BCA (25.75 cm) compared to control (28.2 cm). At 400 mg kg^{-1} Cu concentration, plants that received 5% (24.4 cm) and 10% (26 cm) of BCA had a reduction of 15.1% and 9.53%, respectively, in relation to the control (28.74 cm).

The use of biochar at a concentration of 5% (27.87 FI) reduced the chlorophyll *a* (Chl *a*) index by 21.8% compared to control plants (35.65 FI) at a concentration of 200 mg kg^{-1} Cu (Fig. 3A). In the other Cu concentrations, there was no significant difference. When observing the concentration 400 mg kg^{-1} Cu, there was a 24.4% decrease in this pigment, when 10% (27.92 FI) of biochar was used, in contrast to the plants that did not receive BCA (36.95 FI).

For soils contaminated with Cu concentrations (200, 400 and 600 mg kg^{-1}), the addition of BCA at all concentrations (5 or 10%) reduced chlorophyll *b* (Chl *b*) levels (Fig. 3B). At 200 mg kg^{-1} Cu concentration, plants that received 5% (11 FI) and 10% (12.6 FI) of BCA had a reduction of 33.3% and 23.63%, respectively, in relation to the control (16.5 FI). At a concentration of 400 mg kg^{-1} Cu, the use of biochar with 5% (14.1 FI) and 10% (8.85 FI) decreased the Chl *b* value by 33.7% and 58.39%, compared to control (21.27%). At 600 mg kg^{-1} Cu concentration, the Chl *b* index reduced by 17.67% and 32.56% with the application of BCA by 5% (13.27 FI) and 10% (10.87 FI), in relation to control (16.12 FI).

In the variable total chlorophyll (Chl total), at 0 mg kg^{-1} Cu concentration, plants that received 10% (37.95 FI) of BCA had a reduction of 23.9% in relation to the control (49.91 FI) (Fig. 3C). At a concentration of 200 mg kg^{-1} Cu, the use of biochar with 5% (38.9 FI) and 10% (44.8 FI) decreased the total Chl value by 25.5% and 14.2%, respectively, compared to the control (52.23%). At 400 mg kg^{-1} Cu concentration, the total Chl index reduced 36.8% with the application of 10% BCA (36.77 FI) in relation to the control (58.22 FI).

Gas exchange

According to the analysis of variance for physiological variables (Table 5), there was an interaction between Cu and BCA concentrations for photosynthesis (*A*), stomatal conductance (*gs*), transpiration (*E*), water use efficiency (WUE), internal carbon (*Ci*) and internal/external carbon ratio (*Ci/Ca*).

Considering photosynthesis (*A*) (Fig. 4A), the use of 5% BCA promoted a higher photosynthetic rate at a concentration of 200 mg kg^{-1} Cu (10.8 μmol.m^{-2}.S^{-1}), with an increase of 38.1% compared to control plants (7.82 μmol.m^{-2}.S^{-1}). At a concentration of 400 mg kg^{-1} Cu, regardless of the BCA concentration, there were no statistical differences between treatments. At the highest concentration of Cu (600 mg kg^{-1}), the photosynthetic rate reduced by 37% and 18.3% with the application of BCA by 5% (7.59 μmol.m^{-2}.S^{-1}) and 10% (9.85 7.82 μmol.m^{-2}.S^{-1}), in relation to the control (12 μmol.m^{-2}.S^{-1}).

For stomatal conductance (*gs*) (Fig. 4B), the addition of biochar (5 or 10%) did not differ statistically from control plants, in soil contaminated with 200 and 400 mg kg^{-1} of Cu concentration. At a concentration of 600 mg kg^{-1} Cu, plants that received 5% (0.045 mmol.m^{-2}.S^{-1}) and 10% (0.05 mmol.m^{-2}.S^{-1}) of BCA had a reduction of 30 0.7% and 23%, respectively, in relation to the control (0.065 mmol.m^{-2}.S^{-1}).

For transpiration of brazilian mahogany plants (*E*) (Fig. 4C), the use of 5% biochar obtained a higher transpiration rate at a concentration of 200 mg kg^{-1} Cu (1.67 mmol.m^{-2}.S^{-1}), with an increase of 30% compared to control plants (1.28 mmol.m^{-2}.S^{-1}). In soil contaminated with 400 mg kg^{-1} Cu concentration, 10% Bc showed higher *E* (1.46 mmol.m^{-2}.S^{-1}) with an increase of 33.9% compared to plants without biochar addition (1.09 mmol.m^{-2}.S^{-1}).

The WUE (Fig. 4D) at different concentrations of biochar (5% and 10%) evaluated within the same concentrations of Cu in the soil (200 and 600 mg kg^{-1}), did not present statistical differences between the averages in relation to control plants. However, at a concentration of 400 mg kg^{-1} Cu, the incorporation of 10% of BCA (5.27 mol CO_2mol H_2O^{-1}) reduced 36.2% of US compared to plants without BCA (8.26 mol CO_2mol H_2O^{-1}).

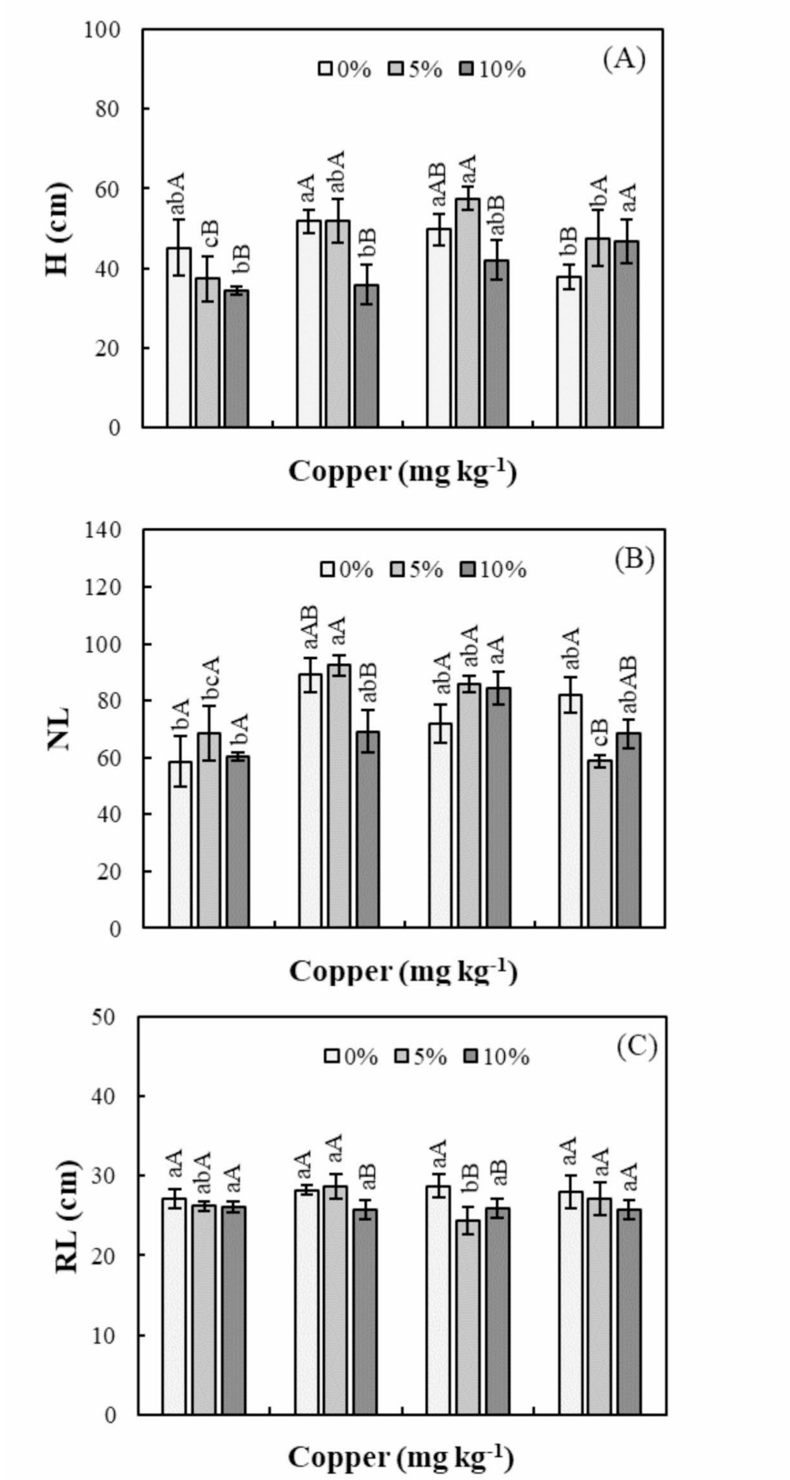

Fig. 2. Height (H), number of leaves (NL) and root length (RL) in Brazilian mahogany plants, exposed to different Cu doses, considering BCA concentrations. Means followed by the same lowercase letter do not differ by the Tukey test ($p < 0.05$) between Cu doses at the same concentration of BCA, and means followed by the same capital letter do not differ by the Tukey test ($p < 0.05$) between Cu concentrations. BCA in the same dose as Cu. Bars indicate the standard error of the mean ($n = 5$).

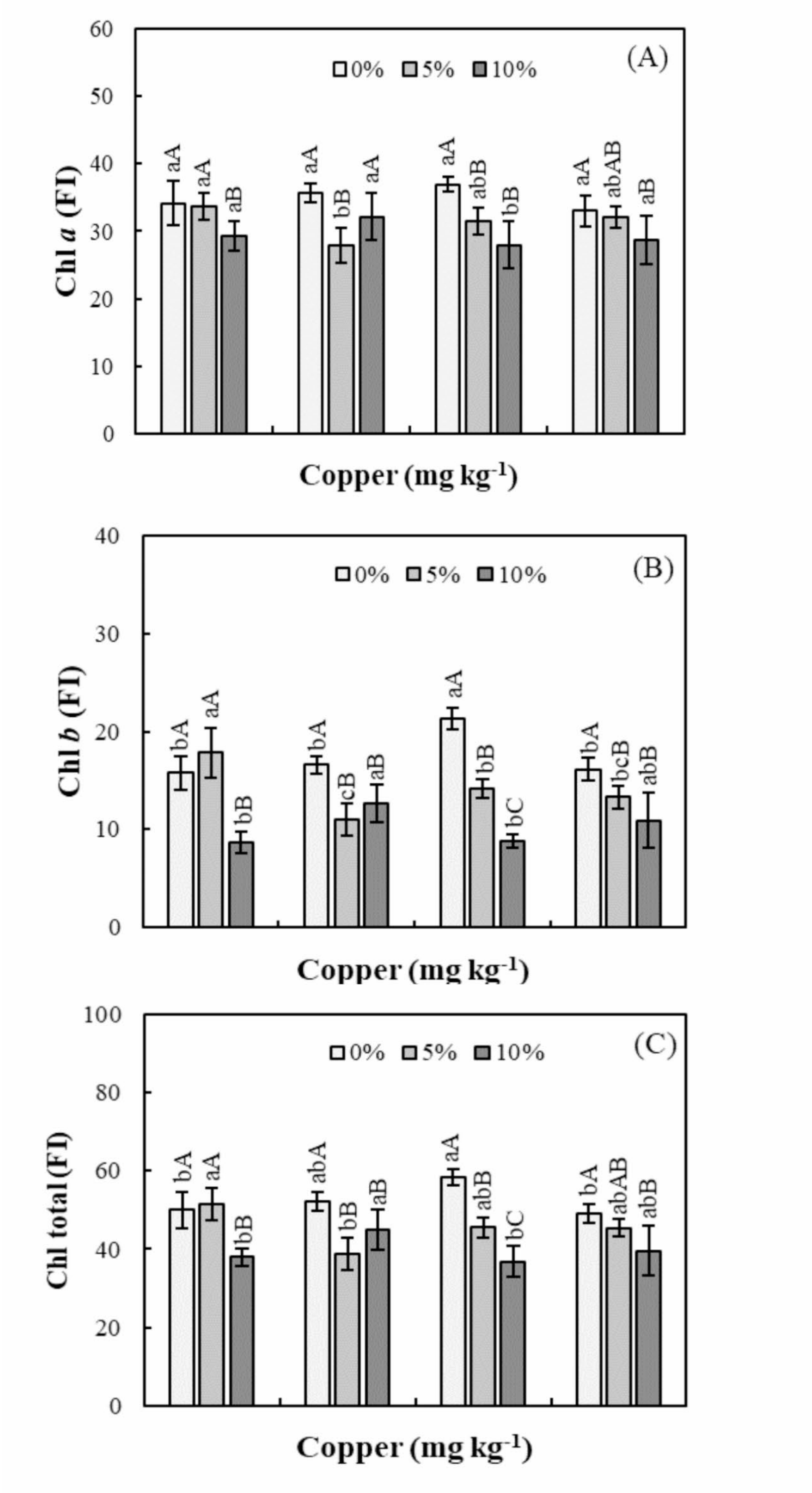

Fig. 3. Chlorophyll a (Chl *a*), b (Chl *b*) and total (Chl total) in Brazilian mahogany plants, exposed to different doses of Cu, considering BCA concentrations. Means followed by the same lowercase letter do not differ by the Tukey test ($p < 0.05$) between Cu doses at the same concentration of BCA, and means followed by the same capital letter do not differ by the Tukey test ($p < 0.05$) between Cu concentrations. BCA in the same dose as Cu. Bars indicate the standard error of the mean ($n = 5$).

Sources of variation	D.L	Mean square					
		A	*gs*	*E*	WUE	*Ci*	*Ci/Ca*
Copper (Cu)	3	31.8**	0.001**	0.23**	16.5**	1139.2^{ns}	0.0003^{ns}
Biochar (BCA)	2	13.5**	0.0009**	0.013^{ns}	9.6*	5689.2**	0.03**
Cu x BCA	6	17.1**	0.0009**	0.23**	9.3**	4123.6**	0.02**
CV (%)	-	16.5	22	18.2	23.5	14.6	16

Table 5. Analysis of variance for net photosynthesis (*A*), stomatal conductance (*gs*), transpiration (*E*), water use efficiency (WUE), internal carbon (ci) and internal/external carbon ratio (Ci/Ca) in mahogany plants Brazilian (*Swietenia macrophylla* King) depending on different concentrations of copper (Cu) and Biochar (BCA). CV = coeficiente of variation; ns = not significant; * = significant ($p < 0.05$); ** = significant ($p < 0.01$) by F test.

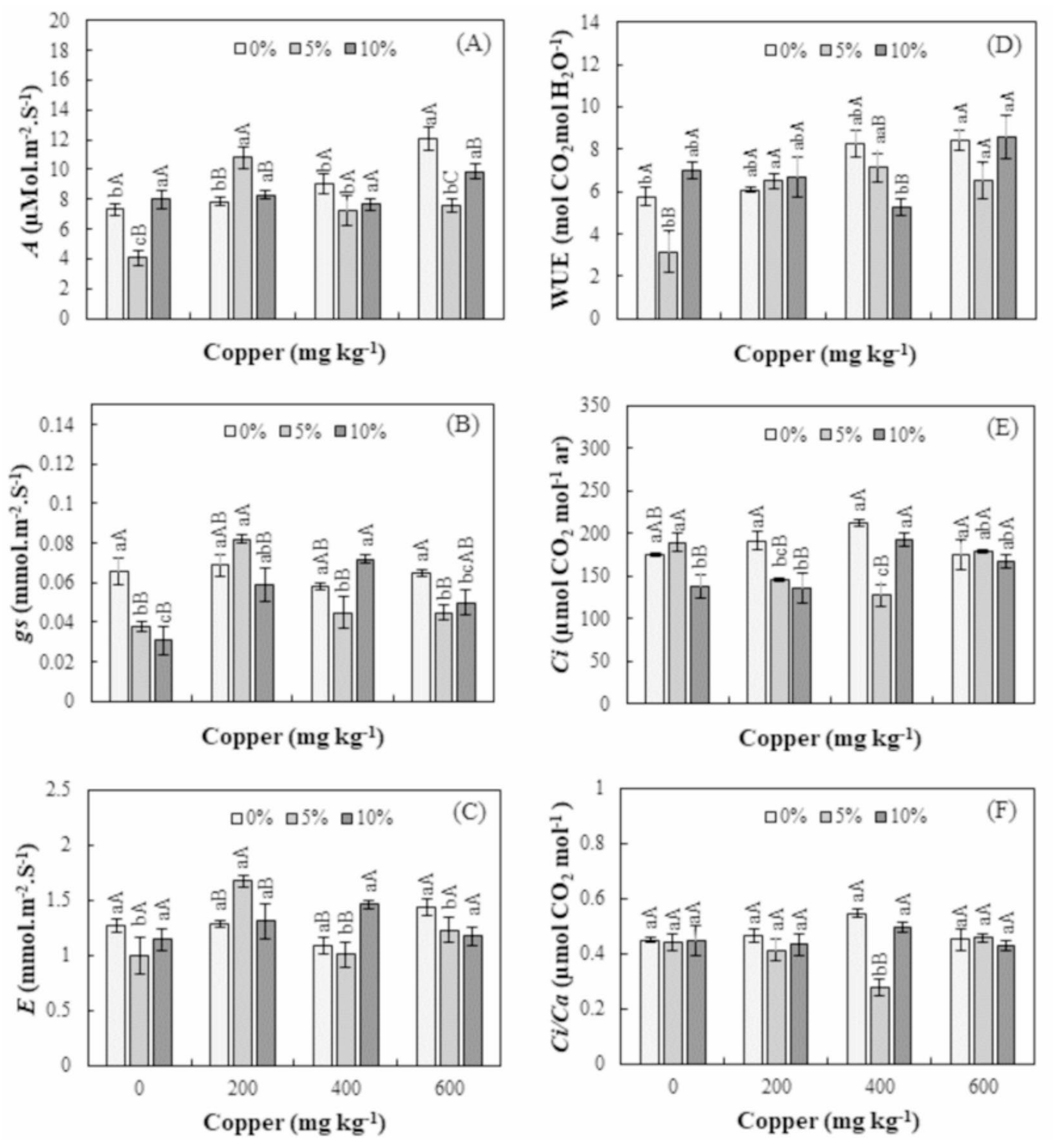

Fig. 4. Net photosynthesis (*A*), stomatal conductance (*gs*), transpiration (*E*), water use efficiency (WUE), internal carbon (*Ci*) and internal/external carbon ratio (*Ci/Ca*) in Brazilian mahogany plants (*Swietenia macrophylla* King) depending on different concentrations of copper (Cu) and biochar (BCA). Means followed by the same lowercase letter do not differ by the Tukey test ($p < 0.05$) between Cu doses at the same concentration of BCA, and means followed by the same capital letter do not differ by the Tukey test ($p < 0.05$) between Cu concentrations. BCA in the same dose as Cu. Bars indicate the standard error of the mean ($n = 5$).

In soils contaminated with 200 mg kg^{-1} Cu concentration, the use of BCA at concentrations of 5% (144.93 µmol CO_2 mol^{-1} air) and 10% (135.75 µmol CO_2 mol^{-1} air) reduced by 24.2% and 29%, respectively, the *Ci* (Fig. 4E) compared to plants without BCA addition. In soils with 400 mg kg^{-1} Cu concentration, the incorporation of 5% BCA (127 µmol CO_2 mol^{-1} air) reduced *Ci* by 40.1% compared to plants without BCA (212.8 µmol CO_2 mol^{-1} air). For higher Cu concentration (600 mg kg^{-1}), there were no statistical differences between treatments with and without biochar.

The *Ci/Ca* (Fig. 4F) showed significant differences only at the concentration 400 mg kg^{-1} Cu in plants that received 5% biochar (0.277 µmol CO_2 mol^{-1}), with a decrease of 49% in the *Ci/Ca* ratio when compared to Brazilian mahogany plants that were not incorporated with biochar (0.543 µmol CO_2 mol^{-1}).

Discussion

Biometric parameters evaluated in this study were influenced by high concentrations of Cu in the soil and the addition of biochar (BCA). The SD (Fig. 1) was not negatively affected by the concentrations of Cu and BCA, increasing the SD in soils contaminated with Cu, which demonstrates a certain resistance of the species to lodging and plant tipping[24]. However, a study evaluating the effect of Cu toxicity on the stem growth of Sena multijuga plants shows results contrary to those found in the present study[41]. Stem diameter has been considered the best predictor of field survival and growth. Therefore, a larger diameter also indicates a larger root system and stem volume[27]. Thus, it was suggested to use SD as an indicator of growth of young plants of tree species under stressful or non-stressful growth conditions. Plant growth is reduced by toxic levels of Cu, because under high concentrations Cu affects metabolism and inhibits plant growth, in addition to interfering with plant cell division mechanisms[33,51]. In addition, toxic levels of Cu affect the growth and functionality of the root system, reducing its ability to absorb water and mineral salts, affecting plant growth[48].

For height (H) (Fig. 2A) and number of leaves (NL) (Fig. 2B), the addition of 5% BCA helped maintain these variables, supporting high levels of Cu in the soil (400 mg kg^{-1}). The presence of biochar (BC) in the soil can retain the metal in several ways, such as: physical adsorption, ion exchange, complexation, precipitation and electrostatic interaction with biochar surface compounds[46]. Due to the presence of adsorption sites rich in hydroxyl and carboxyl radicals, and the large specific surface area, biochar adsorbs different metals, reducing their availability in the soil and, consequently, for plants[8,55,62]. These physicochemical properties of BC allow the remediation of contaminated soil, allowing plant growth. Plant height is an important morphological parameter in young plants of tree species because the higher its value, the greater the leaf area for gas exchange, in addition to allowing greater competitive capacity for capturing sunlight in the environment.

The root length (Fig. 2C) did not differ with the addition of BCA regardless of the Cu concentrations, but when we evaluated the BCA at the same dose of Cu, it was observed that only at 400 mg kg^{-1}Cu the biochar did not prevent the reduction of the system root. Although biochar helps retain the metal, it was not enough to prevent the absorption of Cu by the roots of Brazilian mahogany, which are voluminous and tubular, reaching up to five meters from the base[52], resulting in greater exploitation of the soil and metal absorption. The positive effect of BCA on plant growth occurred because Cu is adsorbed on BCA and its availability to plants is reduced. This set of physicochemical events creates a favorable environment for plant growth, mitigating the toxic effects of Cu on *S. macrophylla* plants.

Studies conducted with African mahogany and pink cedar in soils contaminated with copper showed similar results, showing in the two species evaluated that the increase in Cu concentrations (60, 200, 400 and 600 mg kg^{-1}) did not affect plant growth parameters, presenting characteristics of tolerant species[16], differing only for the height variable in our research, which reduced at the highest concentration of 600 mg kg^{-1}Cu. Similar to the results found in the present study, biochar has a positive effect on the root length of Salix alba plants treated with 2.5% BC in Cu-contaminated soil. These results coincided with lower Cu uptake by plants, due to the immobilization of Cu by BC[45].

The increase in Cu concentrations reduced the levels of chlorophyll *a*, *b* and total (Fig. 3), where biochar was added, not being sufficient to mitigate the effects of toxicity. However, plants that did not have BCA added to the soil maintained the concentration unchanged of its pigments in different doses of Cu. The decrease in the concentration of photosynthetic pigments occurs through the ion exchange mechanism, where surface functional groups in biochar have high binding affinity with essential nutrients of the same atomic radius as copper, such as calcium (Ca^{+2}) and magnesium (Mg^{+2})[46]. The unavailability of Mg^{+2}affects the production of pigments, due to Mg being a structural constituent in the porphyrin ring of chlorophyll molecules[53], this fact explains the reduction in chlorophyll levels in the present study. Corroborating these results, in studies with the grass *Phragmites karka* (Retz.) to evaluate the impact of biochar from wood waste on nutrient absorption and gas exchange, a reduction in Mg^{+2}content and chlorophyll content was found in treatments that received biochar[1].

Although there was a reduction in chlorophyll concentration in plants treated with BCA (mainly 5% BCA), these changes in chlorophyll pigments did not have a negative impact on the growth of S. macrophylla plants, because H, SD and RL were not reduced by the 5% BCA concentration at intermediate Cu concentrations (200 and 400 mg kg^{-1}). This result suggests that S. macrophylla plants present some mechanism that compensates for the adsorption of Mg by BCA, such as greater carboxylation of CO_2 by the rubisco enzyme, evidenced by the lower Ci accompanied by higher A in the 5% BCA treatment (Fig. 4A and E). In a study with biochar (2.5 and 5%) and soil containing Cu, Pb and Cd, cultivated with Salix alba plants, higher concentrations of chlorophyll pigments were found in the 2.5 and 5% BC treatments[45], indicating that the response of chlorophyll pigments is dependent on the species and type of BC, because some BCs can adsorb more exchangeable bases (Ca^{+2}, Mg^{+2}, for example) compared to other BCs.

Considering the variables photosynthesis (Fig. 4A), stomatal conductance (Fig. 4B) and transpiration (Fig. 4C) at the highest concentration of Cu (600 mg kg^{-1}), the BCA did not maintain satisfactory levels to alleviate the stress caused by Cu. Excess Cu affected the photosynthetic machinery by changing the composition of pigments and structure of chloroplasts, reducing the photosynthetic rate and inhibiting the electron transport chain[44], resulting in a impairs in the gas exchange in the highest dose of Cu.

Furthermore, the nutrient Cu has an important function in photosystem II (PSII), acting in the electron transport chain and water photolysis, however, in excess it is harmful as it affects photosynthesis and inhibits the activity in the PSII reaction center[11,33]. In studies conducted with *Limoniastrum monopetalum*, the effect of copper sulfate on growth and its physiological responses was evaluated, observing the reduction of physiological parameters (photosynthesis, stomatal conductance and the efficiency of photosystem II) with the increase in the doses of Cu applied[10].

Water use efficiency (WUE) (Fig. 4D) is defined as the relationship between photosynthesis (*A*) and transpiration (*E*), thus, when we analyzed this variable, we observed that the addition of biochar maintained WUE in different concentrations of copper, when compared to their respective control plants, in addition, the species itself maintained high levels. In the interaction between 5% BCA and intermediate Cu concentrations, there was a better response of *A* (Figure A4), because the lower *Ci* of the interaction between BCA and Cu mentioned above showed greater photosynthetic activity of *S. macrophylla* plants. These results allowed greater gain in the growth of *S. macrophylla* plants, suggesting that the BCA concentration mitigates the toxic effects of intermediate Cu concentrations, in addition to remediating the soil contaminated with Cu.

One of the physical characteristics of BC is the formation of porous structures on the surface[29]. This property of BCA contributed to reducing density and increasing soil porosity (macro and microporosity), the latter being responsible by soil water retention[35], in addition to the material having a high specific surface area (Table 3), increasing water availability to plants and contributing to maintaining WUE.

The internal carbon concentration (*Ci*) and the internal/external carbon ratio (*Ci/Ca*) were not severely affected, nor was the water availability provided by the biochar, which managed to retain up to 2.5 times its weight in water (Table 3), helped maintain exchanges with the atmosphere (*A*, *E* and *gs*), in this sense, there was no increase in *Ci* concentration and severe reduction in *Ci/Ca*.

Opposite results were found, where toxicity caused by Cu decreased *A*, *gs* and WUE, and increased *Ci*, being caused by changes in rubisco activity in response to Cu stress. of the Mg ion in its active site by the excess metal[43].

Although the combination of 200 mg Cu kg^{-1} and 5% BCA reduced the levels of chlorophylls a, b and total, this result did not affect *A*, *gs* and *E*, supposedly due to the fact that the dose of 200 mg kg^{-1} Cu did not impose stomatal limitation to net photosynthesis, and BCA does not limit *A* through the adsorption of essential plant nutrients such as Cu. Furthermore, the higher WUE, despite high averages of *gs* and *E*, highlighted the role of BCA in retaining water in the soil[23], improving WUE in Brazilian mahogany plants.

The positive effect of the above treatment combination on *A*, *gs*, *Ci* and WUE increased CO_2 carboxylation and, consequently, the growth of mahogany plants, as evidenced by the height and number of leaves (Fig. 2A and B). However, root growth was not impacted by Cu and BCA, probably because BCA creates a chemical and physical environment favorable to root growth[47].

Conclusion

The use of 5% BCA mitigates the effects of toxicity in soils contaminated with 200 mg kg^{-1} of copper, increasing water retention and improving gas exchange (*A*, *gs*, *E*, *Ci*, *Ci/Ca*), the water use efficiency (WUE), height (H) and number of leaves (NL) in Brazilian mahogany plants. These findings indicate the use of BCA at a concentration of 5% to remediate soils contaminated with copper up to a concentration of 200 mg kg^{-1}. In addition, the species *Swietenia macrophylla*, due to its fast growth, good response to BCA, and economic importance due to the properties of its wood, is a possible candidate for studies on the remediation of soils contaminated with copper. However, field studies are needed to prove the efficiency of BCA at a concentration of 5% and the role of *Swietenia macrophylla* in the remediation of areas degraded by copper. In this study, the use of biochar produced from açaí seeds reinforces the importance of this byproduct of the exploitation of the *Euterpe oleraceae* palm tree in the remediation of soils contaminated by heavy metals and its importance in the context of the circular economy and global climate change.

Data availability

The data generated in this research is in the hands of the person responsible for the research, Raphael Leone da Cruz Ferreira, who is available to provide any data that the evaluation process suggests, without any objection, the relevant data has not yet been placed on any platform.

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Author contributions

RF, CN and LS: Experimental design and developmentEC: Supplier of the plant materialCS, JC, AB and AC: Experimental conduction and laboratory analysisRF and DB: Experimental conduction and statistical analysisFC and GN: manuscript preparation and correctionsVN: Traduction of the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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