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Mineral composition and visual symptoms of nutrients deficiencies in Curauá plants (*Ananas comosus* var. *erectifolius*).

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Abstract

Curauá plant (*Ananas comosus* var. *erectifolius*) is a producer of high strength fibers and because of this quality, it is used by textile, automotive and pulp factories. However, despite the promising scenario, researches have been incipient, particularly in the area of mineral nutrition, in relation to their nutritional requirements. The aim of this study was to evaluate nutritional and biochemical responses in curauá plants exposed to the - macronutrients deficiency. The experimental design was a randomized block design with seven treatments (complete and individual omissions N, P, K, Ca, Mg and S) and four replications. The omission of individual macronutrient showed deficiency symptoms well defined and promoted reduction in plant growth, wherein the omission of potassium was the most limiting factor to the development of the plant. The foliar contents with complete treatment and control (omission of macronutrients) were: N: 11.8 – 25.2 g kg^{-1}; P: 0.3 – 5.8 g kg^{-1}; K: 8.2 – 22.2 g kg^{-1}; Ca: 1.9 – 3.4 g kg^{-1}; Mg: 0.5 – 2.9 g kg^{-1}; S: 0.6 – 2.8 g kg^{-1}. The omission of N resulted in decreased amounts of amino acids (34.6 – 54.1 µmol g^{-1}), proteins (3.4 – 22.5 mg g^{-1}), carbohydrates (0.5 – 0.6 mmol g^{-1}), nitrate (0.5 – 65.3 µmol g^{-1}) and ammonia (25.6 – 75.2 mmol kg^{-1}). Meanwhile, the omissions of other macronutrients promoted increases in the percentages of carbohydrates (lack of P), ammonia, amino acids (lack of K), starch (lack of Ca) and nitrate (lack of of S).

Keywords: Macronutrient content, mineral nutrition, nutritional deficiency.
Abbreviations: N_nitrogen, P_phosphorus, K_potassium, Ca_calcium, Mg_magnesium, S_sulfur.

Introduction

Curauá (*Ananas comosus* var. *erectifolius* L. B. Smith.) is a plant of the family Bromeliaceae which presents as a characteristic mechanical resistance, lightness, absence of odor and softness to the touch, which the attributes result in the production of fiber of excellent quality. The curauá fiber is extracted from the leaves and has excellent chemical properties, which are appreciated by the automotive industry for the manufacture of car seats and paper, being currently listed as a replacement for fiberglass in automobile parts and as a compound of beams resistant to earthquakes. Thus, it appears that the species has different uses, which makes it of great importance in the world economy. The demand for fiber contributes to the expansion of the planted area, however neither the volume of production nor the supplies of seedlings are enough. The state of Pará has a planted area of 800 hectares with a production of 20 tons per month, insufficient to meet the automobile and textile market demand, of thousand tons of fiber per month (Lameira, 2007). The researches with Curauá culture are scarce, particularly those related to mineral nutrition, which are non-existent. In addition, adequate concentrations of essential nutrients to plant must be investigated considering requirement for an optimal growth rate (Epstein and Bloom, 2006). The definition of nutritional deficiency in plants is characterized by lower element concentration in substrate or when the chemical element is present in unavailable form for plant absorption. When deficiencies are sufficiently severe, the symptoms are manifested by more intense form, but in field or light deficiency this work are difficult. Therefore, description of visual symptoms can help farmers and experts in this species to identify nutritional deficiencies (Viégas et al., 2012). The visual diagnosis is based on the fact that plants with deficiencies or excesses of certain nutrient usually have different characteristics, and for that diagnosis becomes more efficient, it indispensable to describe the visual symptoms of deficiency of each nutrient for a specific culture (Frazão et al., 2013). In addition, the diagnosis of nutritional problems through observation of visual symptoms has great practical importance, because it allows making quick decisions in the field on correct deficiencies and consequent action of fertilization. However, it is necessary to develop controlled research in greenhouse using nutritive solution and applying the missing element technique thus enabling the advancement of knowledge in plat mineral nutrition (Viégas et al., 2012). In general, deficiency or restriction of nutrients negatively affects the growth of higher plants, because several reactions are depending on essential nutrients (Nayyar and Kaushal, 2002). On the other hand, growth parameters like number of leaves, plant height and plant dry matter and yield are normally influenced by the availability of nutrients (Cruz et al., 2011; Marques et al., 2011).

Therefore, studies on morphological responses and accumulation of nutrients are fundamental aiming to define the mineral requirements of Curauá plants. In this way, the aim of this study was to evaluate the effect of macronutrient omission in growth, symptoms of nutritional deficiencies as well as on the mineral composition and biochemical properties of curauá plants.

Results and Discussion

Dry matter production

The leaves and total dry matter production were reduced with the omissions of macronutrients in relation to complete treatment (Table 1), this occurred because the plant nutritional status may influence the plant growth (Malézieux and Bartholomew, 2003). Macronutrient absences caused limitation in the production of dry matter in "D" leaves and root treatments (Table 1). Except for the omission of Ca, which did not differ from complete, which coincides with the data obtained by Veloso et al. (2001) for the pineapple culture, demonstrating that curauá has good tolerance to soil acidity. The treatment with potassium omission was the most limiting in the production of total dry matter, once reduced by 514% when compared to complete, which demonstrates high potassium requirement by curauá culture. Batista et al. (2003) point out that the limitation promoted by the omission of potassium is combined with the speed in which the symptom occurs.

Symptomatology

The symptoms express nutritional deficiency of an element, which is found in small amounts in the plant, below the desired, characterizing symptoms that are specific for certain nutrients. Visual deficiency symptoms are the final result of series of biochemical events that begin with a molecular lesion, followed by changes in subcellular organelles, and then they establish themselves and ultimately affect a group of cells or tissues (Marschener, 1995; Silva et al., 2009).

The symptom manifestation, through visual diagnosis linked to analyzes of the plant tissue, conditions the levels of nutrients in their respective amounts in plant organ, thereby it express levels that can affect its metabolism, unconfigure its physiological process and cause biochemical changes, such as the contents of amino acids, soluble proteins, carbohydrates, starch, nitrate and ammonia on "D" leaves of curauá. In a general way, Fontes (2001) reports that each nutrient has specific functions in plants and the different elements produce different symptoms of deficiencies or toxicity and, sometimes, the deficiency of a particular nutrient does not necessarily affect the same metabolic process in all species.

Visual symptoms promoted by nitrogen deficiency

The symptoms of N deficiency in curauá plants were the first to manifest at 30 days after the beginning of treatment, resulting in the appearance of reddish color in the center of the leaf blade, evolving to a pale green along the banks of limbo in mature leaves and in the course of disability becoming yellowish leaves (Fig. 1a, 2a and 2b). The yellow coloration is related to the production of chlorophyll, since nutritional disturbance of N cause changes in the formation of chloroplasts (Malavolta, 2006), more specifically, chlorosis is associated with decreased chlorophyll content and activity of Rubisco, which provides low rates of photosynthesis in plants (Hermans et al., 2006).

Consequences of phosphorus deficiency in the plant

Symptoms of phosphorus deficiency emerged 104 days after the treatment beginning, showing dark brown color in the center of the blade and dark green at the edge of the old leaves (Fig. 1b) and, in some reddish and purplish hues, due to the accumulation of the pigment anthocyanin in the vacuoles (Epstein and Bloom, 2006; Gautam et al., 2011).

Regarding curauá plant it was observed a reduction in plant height and length and the number of leaves (unpublished data), common to many species with P deficiency, due to the reduction in the cell division (Chiera et al., 2002; Lavres Júnior et al., 2005).

Beginning and consequences of the symptoms of potassium deficiency

With 36 days after the start of treatment it was possible to visualize the symptoms of potassium deficiency in plants. Characterized by the manifestation of the loss of purple color on older leaves, changing to a light green which extended along the limb and, with the passage of days the leaf edges necrosed (Fig. 1c).

Symptoms of calcium deficiency

At 160th days of the beginning of treatments, the first symptoms of calcium deficiency were manifested. Initially the new leaves have developed light green color in the center of the blade and in the edge of the leaves (Fig. 1d), as well as reduction in plant height, length and width of leaves (unpublished data). Plants with calcium deficiency presented polyploidy nuclei, binucleate cells, amitotic divisions that cause cell death and paralyze plant growth, especially root, with subsequent vegetable tissue necrosis (Prado et al., 2008), which justifies the results obtained in the present study.

Vis*ual symptoms promoted by magnesium deficiency*

Symptoms of magnesium deficiency in plants curauá initiated 44 days after the beginning of treatments, by means of a light brown color in midrib of older leaves and in the edges, a light Green color, progressing to a yellowish tinge throughout the length of the foliar edge according to the intensity of nutritional deficiency (Fig. 1e and 2b).

Beginning and consequences of the symptoms of sulfur deficiency

With 154 days after starting the treatment it was possible to identify the symptoms of sulfur deficiency by the occurrence

Table 1. Production dry matter of leaves, "D" leaf, roots and total, and relative growth (RG%) of curauá plants according to complete and individual nutrient omission treatments.

Treatments	Leaves	"D" leaf	Roots	Total	RG[**]
	------------------------------- g plant^{-1}			-------------------------------	%
Complete	132.80a[*]	5.48a	27.37a	166.65a	100.00a
Omission of N	41.19e	2.34de	7.96c	51.50e	30.96e
Omission of P	78.76d	3.21c	17.52b	99.50d	59.93d
Omission of K	27.21f	1.64e	3.51c	32.37f	19.47f
Omission of Ca	115.50b	5.12a	24.31ab	144.94b	87.23b
Omission of Mg	49.95e	2.58cd	5.86c	58.40e	34.80e
Omission of S	96.31c	4.28b	16.80b	117.39c	70.62c
L.S.D. (5%)	8.83	0.72	7.64	11.30	10.30
C.V. (%)	4.96	8.98	22.32	5.13	7.79

[*] Means followed by the same letter in columns do not differ at the 5% level of probability by Tukey's test. [**] Percentage of relative growth for total dry matter.

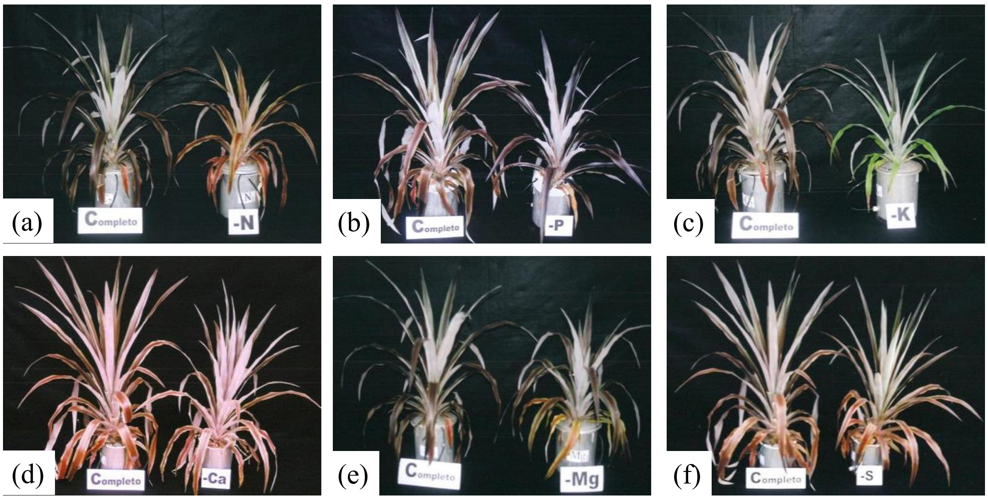

Fig 1. Curauá (*Ananas comosus* var. *erectifolius* L. B. Smith.) plants developed in complete treatment (Complete) and deficiency symptoms individual omission of nitrogen (a), phosphorus (b), potassium (c), calcium (d), magnesium (e) and sulfur (f) cultivated in nutrient solution lacking nutrients.

of a light brown color in the center of the leaf blade and by the appearance of a light green color along the banks of the upper leaves (Fig. 1f). Furthermore, it was found that the absence of S provided a reduction in plant height and length and width of leaves (unpublished data).

Macronutrient content in "D" leaves of curauá

The full treatment presented N content in "D" leaf of 25.21 g kg^{-1}, g kg^{-1}, while the omission treatment resulted in 11.80 g kg^{-1} (Table 2). Siebeneichler et al. (2002), Coelho et al. (2007) and Ramos et al. (2009) studied the pineapple culture, obtaining in the complete treatment values between 18:33 to 13.4 g kg^{-1}, which are lower than those obtained in this study, which may indicate a lower requirement of N by curauá, although being the same family. Plants subjected to the omission of phosphorus showed a significant reduction in content in comparison with the full treatment, with values obtained from 5.87 g kg^{-1} without manifestation and 0.39 g kg^{-1} with symptoms of nutritional deficiency (Table 2). In pineapple plants, Coelho et al. (2007) and Ramos et al. (2009), reported levels ranging from 0.32 to 0.78 g kg^{-1} with P omission and from 1.04 to 1.32 g kg^{-1} in the complete treatment. Regarding potassium, it was observed values of 8.26 g kg^{-1} for treatment with the nutrient omission, while the complete treatment was 22.25 g kg^{-1} (Table 2). In pineapple plants of the Jupi cultivar potassium foliar content with the omission treatment was 14.75 g kg^{-1} and without disabilities resulted in 32.77 g kg^{-1} (Coelho et al., 2007), thus being higher than those presented in this study. The omission of calcium in curauá plants provided nutritional content in curauá "D" leaves of 1.95 g kg^{-1} and those submitted to complete nutrient solution showed a value of 3.47 g kg^{-1} (Table 2). The calcium foliar content obtained in this study are higher than those determined by Ramos et al. (2009) in imperial pineapple plants, with values with and without omission of 1.30 and 4.40 g kg^{-1}, respectively. The magnesium foliar contents with the omission showed the same tendency of other cations analyzed (K$^+$ and Ca^{2+}), resulting in higher values for the treatments compared to complete treatment without Mg (Table 2). Finally, the levels of sulfur in plants subjected to omission and the complete treatment differed significantly, providing the S omission the lowest content 0.62 g kg^{-1}, comparatively to the full treatment which resulted in the content of 2.80 g kg^{-1}(Table 2), possibly it occurred due to the synthesis of glutathione polypeptide, which acts as a signaling, coordinating the root uptake of sulfate and its assimilation in the aerial part, which have been limited by N deficiency (Epstein and Bloom, 2006). Ramos et al. (2009) have found that foliar concentration of 0.54 g kg^{-1} resulted in symptoms of sulfur deficiency in plants pineapple, Imperial cultivar.

Table 2. Macronutrient content of "D" leaves in curauá plants under complete treatment and omissions of individual nutrients.

Treatments	N	P	K	Ca	Mg	S
	--	---------	---- $g\ kg^{-1}$ ----	---------	---------	--
Complete	25.21a[*]	5.87a	22.25a	3.47a	2.94a	2.80a
Omission of N	11.80c	3.00cd	20.54ab	2.85ab	1.33c	1.38c
Omission of P	14.08bc	0.39e	15.00c	2.32bc	1.51bc	1.46b
Omission of K	12.63c	5.86a	8.26d	2.08bc	2.07b	1.34c
Omission of Ca	13.32bc	2.80d	19.29ab	1.95c	1.88bc	1.10e
Omission of Mg	17.42b	3.87bc	20.83ab	2.44bc	0.51d	1.20d
Omission of S	12.50c	4.14b	17.87bc	3.38a	1.67bc	0.62f
L.S.D. (5%)	4.54	0.89	3.23	0.05	0.57	0.04
C.V. (%)	12.72	10.22	7.00	14.35	14.43	1.31

[*] Means followed by the same letter in columns do not differ at the 5% level of probability by Tukey's test.

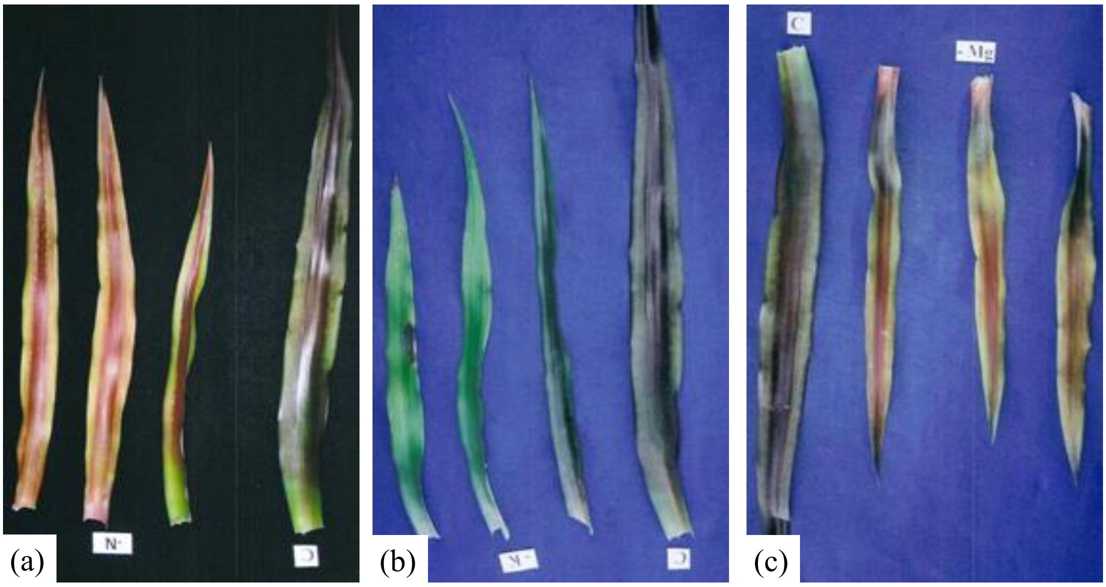

Fig 2. Curauá "D" leaves subjected to the omission of macronutrients nitrogen (a, b) and magnesium (c).

Aminoacids content in "D" leaves of curauá

The biochemical content of amino acids (aa) obtained in the complete treatment was 54.09 µmol g^{-1}, whereas in relation to other treatments using omission of nutrients, it is found that the higher amino acid content was obtained in the treatment with K omission (125.35 µmol g^{-1}) (Table 3), one possible explanation is that increased amino acid content in the treatment of potassium omission results from the reduction in protein synthesis (Kawaguchi et al., 2004), due to the accumulation of amino acids cause an osmotic adjustment in order to minimize the harmful impacts of low solution input in the plant. The lower content of amino acids was observed in the treatment with N omission (34.62 µmol g^{-1}) (Table 3), which was expected since the N absorbed by plants combines with carbon skeletons for the production of amino acids, in the N absence the plant could not synthesize amino acids. According to Prado et al. (2008) plants which treatment was the omission of N shows a decrease in RNA synthesis, starch and lipids, reducing the activation of amino acids and therefore causing protein deficiency which is responsible for the depression in the vegetative development of the plant.

Soluble proteins in curauá plants

The complete treatment had a higher content of soluble proteins (24.49 mg g^{-1}) in curauá "D" leaves, while the N omission caused the lowest value (3.43 mg g^{-1}) (Table 3), which is justified by being the protein resulting from the synthesis of amino acids and, therefore, reinforces the results obtained in amino acid contents. The treatment in which the nutrient omitted was N showed the lowest protein content, for being structural element (Kerbauy, 2012) and be low in concentration in the leaf cells, probably did not facilitate the joining of the protein which is poorly formed, binding to amino acids, in order to form enzymes and thereby alter the speed of reaction, not allowing equilibrium, and thus the way it acts as a cofactor binding to other macronutrients and even micronutrients, it resulted in physiological problems, such as leaves fall and reduced plant growth, because the enzyme at low speed will have difficulty carrying element into the cell.

Biochemical content of carbohydrates

In Table 3, complete treatment showed carbohydrate content of 0.64 mmol g^{-1}. Comparatively with other treatments with nutrient omission, it is found that the higher carbohydrate content in curauá "D" leaves was the P omission treatment (1.06 mmol g^{-1}), while with N omission treatment (0.54 mmol g^{-1}) resulted in lower carbohydrate content. The low values provided by the N omission is justified by increased content of carbohydrates in plants under stress that is associated with decreased starch content of the cell, besides the decrease in photosynthetic capacity which will paralyze

Table 3. Biochemical contents in "D" leaves of amino acids, soluble proteins, carbohydrates, starch, nitrate and ammonia in curauá plants according to complete treatment and the omission of individual nutrients.

Treatments	Amino acids	Protein	Carbohydrate	Starch	Nitrate	Ammonia
	μmol g^{-1}	mg g^{-1}	---------- mmol g^{-1}	---------	μmol g^{-1}	mmol kg^{-1}
Complete	54.08bc*	22.49a	0.64b	0.024c	65.27bc	75.19d
Omission of N	34.62c	3.43e	0.54c	0.020d	0.50e	25.62e
Omission of P	66.96bc	6.79d	1.06a	0.027bc	55.90e	87.70b
Omission of K	125.35a	8.82c	0.66b	0.029b	64.31bc	109.06a
Omission of Ca	76.12b	10.61bc	0.67b	0.036a	70.00ab	75.55cd
Omission of Mg	75.41b	12.08b	0.64b	0.027b	63.24c	82.13bcd
Omission of S	82.93b	6.35d	0.68b	0.026bc	72.50a	83.08bc
LSD (5%)	34.40	1.88	0.05	0.004	6.30	7.71
CV (%)	19.14	8.10	3.33	6.00	4.83	4.36

* Means followed by the same letter in columns do not differ at the 5% level of probability by Tukey's test.

the cellular growth, which reduces the synthesis of sucrose export (Martinez et al., 2007). In this research, we note that complete treatment, the one that presents all macro and micronutrients needed for plant development was the treatment with lower levels of carbohydrates, when compared to treatments which omit macronutrients, probably due to the formation of new leaves. This fact was confirmed by Cruz et al. (2007), by studying carbohydrate consumption in Tahiti lime, in which it was verified that the low levels was due to the formation of new leaves, associated with the initial growth period of the branches (Rodrigues et al., 2006).

Starch content in curauá "D" leaves

The complete treatment provided 0.024 mmol g^{-1} starch content. Regarding the omission of nutrients, it was observed that the highest content was obtained by treatment with omission of Ca (0.036 mmol g^{-1}), while N omission treatment (0.020 mmol g^{-1}) had lower starch content (Table 3). It occurs due to the fact that most of the assimilates to move to other vegetative organs, in which sugars are converted to starch, causing the fall of new leaves, that promotes an increase in the concentration of starch in the plant. It is noteworthy that the starch in the plant depends on their developmental stage, as well as the sampled site (Corsato et al., 2008), since the highest levels are obtained in the leaves (Souza et al., 2011).

Levels of nitrate and ammonia in curauá plants

For the nitrate content, complete treatment provided the greatest value (65.27 μmol g^{-1}), and among the treatments with nutrient omission, the one which presented higher levels was the S absence (72.50 μmol g^{-1}), in turn, the lowest level of nitrate in the curauá "D" leaves was obtained by treatment with omission of N (0.50 μmol g^{-1}) (Table 3). In Table 3, we observe that complete treatment showed ammonia content of 75.19 mmol kg^{-1}, however the value was lower than the verified in the treatment with K omission (109.06 mmol kg^{-1}), being the lower content resulting from an absence of N (25.62 mmol kg^{-1}). The low concentration of nitrate and ammonia in curauá "D" leaves provided by the absence of N, probably occurred because the low concentrations of these elements in solution result in less efficient capture by carriers (protein). When it occurs, the plant shows common symptoms of N deficiency, as well as severe loss of vigor of the plant to form new leaves, as well as reduced growth and tissue chlorophyllous (Kerbauy, 2012).

Material and Methods

Location of the experiment

The experiment was conducted in a greenhouse at Embrapa, Eastern Amazon, in Belém, State of Pará, located in the geographic coordinates 1° 28' 0'' S e 48° 27' 0'' W, with air temperatures ranging from 24°C to 30°C for 200 days. According to Köppen, the climate of Belém can be classified as the type Afi, tropical rainy climate with small annual thermal amplitude and average monthly rainfall exceeding 60 mm.

Plant materials

The plants were derived from the Laboratory of Biotechnology in the city of Benevides, Pará State. The seedlings were selected in order to obtain uniform plants, having as parameter the development of aerial portion and root system.

Implementation of the experiment

The material used as substrate was crushed washed silica, type thick zero (inert substrate). The seedlings were transplanted to plastic pots with 5 L capacity, with a perforated base in order to facilitate the disposal and replacement of the nutrient solution being used in the experiment. The nutrient solution was the Bolle-Jones (1954), initially diluted at the proportion 1:10 in order to adapt the plants, and subsequently diluted from 1:5 to the final ratio of a 1:1 total load. Treatments were initiated when the plants demonstrated to be established after transplanting. During the conduct of research, the pH of the nutrient solution Bolle-Jones was maintained at 5.5 ± 0.5 by additions of dilute solutions of HCl or NaOH. The nutritive solutions were changed every 15 days, and checked daily for the volume of the solutions and, when necessary it was supplemented with deionized water.

Experimental design

The experimental design was randomized blocks, with four replications including seven treatments, as specified: complete (C): (macronutrients + micronutrients) as described in Viégas et al. (2013), and the individual omissions, zero levels, of nitrogen (-N); phosphorus (-P); potassium (-K); calcium (-Ca); magnesium (-Mg) e sulfur (-S), where each plant was considered an experimental unit. The nutrient solutions were supplied by percolation in pots, and drained daily in the afternoon and irrigated in the morning, getting 9 hours irrigated and 15 hours drained (Frazão et al., 2013).

Collection of vegetal material

The experiment had its final with the definition of visual symptoms caused by deficiencies of macronutrients. Then, the plant material was collected and separated into leaves, "D" leaves and root according to treatments and then stored in paper bags, previously labeled and sent to the greenhouse at a temperature of 65˚C until constant weight. After drying, the plant material was weighed on a digital scale in order to obtain the dry weight and then triturated in mill type Willey to determine the analysis of vegetal tissues (Cruz et al., 2011).

Determination of macronutrient content

The plant tissue analyzes were conducted at the Laboratory of Soil and Plant Tissue of Embrapa Eastern Amazon, only with "D" leaves of curauá plant. The collection of "D" leaf was defined based on the indication for the pineapple culture (*Ananas cosmofolium*), from the same family (Siebeneichler et al., 2002), since there is no survey for the standard leaf to curauá. The determinations of foliar levels of macronutrients were performed according to the methodology of Embrapa (1999).

Determination of biochemical levels

The determination of biochemical contents contained in the leaves "D" of curauá plant was conducted at the Laboratory of Plant Physiology and Biochemistry, in Capitão Poço Campus of Federal Rural University of Amazonia (UFRA), State of Pará, in which were carried out the amino acid analyzes (Peoples et al., 1989), soluble proteins (Bradford, 1976), carbohydrates, starch (Dubois et al., 1956), nitrate (Cataldo et al., 1975) and ammonia (Weatherburn, 1967).

Statistical analyzes

The results obtained for plant growth (dry matter production of leaves, "D" leaves, roots and total), as well as the macronutrient (N, P, K, Ca, Mg and S) and biochemical (amino acids, soluble proteins, carbohydrates, starch, nitrate and ammonia) were subjected to Tukey's test at 5% probability by using the statistical software Assistat (Silva and Azevedo, 2002).

Conclusions

The diagnostic method for subtraction of macronutrients proved to be efficient to visually evaluate deficiency symptoms in curauá culture.

The higher curauá dry matter production followed in ascending order in leaves Ca > S > P > Mg > N > K; “D” leaf Ca > S > P > Mg > N >K; in the roots Ca > P > S > N > Mg > K. The P, Ca, Mg and S were the treatments that more limited the development of and leaves curauá "D" leaves;

Deficiencies of macronutrients have affected plant development, and the omission of N resulted in decreased amounts of amino acids, proteins, carbohydrates, nitrate and ammonia. While the omission of other macronutrients promoted increases in the percentages of carbohydrates (P omission), ammonia, amino acids (K omission), starch (Ca omission) and nitrate (S omission).

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