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Leaf sampling to assess mineral nutrient composition of physic nut plants (*Jatropha curcas* L.)

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Abstract

The physic nut is an important plant for biodiesel production. However, research is still lacking as to the best leaves to be sampled in determining ideal mineral content. It is important that all factors causing variations in the mineral nutrient concentrations in leaves are identified and evaluated to determine the most efficient sampling technique. The positions of the leaves on the branches and on the plant shoot are critical variables affecting leaf composition. Thus, this study aimed to determine the best leaf position for sampling to evaluate the health status of adult physic nut plants. The experiment was conducted in the field, using a 5 × 3 factorial design, with leaves sampled at five positions on the branch (1st, 2nd, 3rd, 4th, and 5th node, from the apex), and three sections of the plant crown (apical, middle, and basal), in a completely randomized design with four replications, and four plants per plot. The concentration of macronutrients (N, P, Ca, Mg, and S) and micronutrients (Cu, Fe, Mn, and Zn) were determined for all samples. The results indicated that the sampling of leaves at the 3rd and 4th node of the flower-bearing branches, located in the middle section of the plant canopy, was the most indicative of the mineral composition of adult jatropha plants.

Keywords: *Jatropha curcas*, mineral composition, leaf sampling.
Abbreviations: PC_ position of the leaves on plant crown section; PB_position of the leaf on the branch; N_ nitrogen; K_ potassium; P_phosphorus; Ca_calcium; Mg_magnesium; S_sulfur; Mn_manganese; Fe_iron; Zn_zinc; Cu_copper; SB_sum of bases; T_cation exchange capacity; V_base saturation; OM_organic matter; pH_hydrogen potential; Na_sodium; H_hydrogen; Al_aluminum.

Introduction

The seeds of jatropha (*Jatropha curcas* L.) are thought to be a highly promising alternative raw material for the production of biodiesel (Maes et al., 2009; Young et al., 2010; Bang-Zhen and Zengfu, 2011; Sousa et al., 2011; Simón et al., 2013; Chaudhary et al., 2014; Patel and Saraf, 2014). It is a perennial plant, which attains commercial productivity during the second year, but stabilizes only from the fourth or fifth year onward (Silva et al., 2012). Although there is much research on the genetic aspects (Biabani et al., 2012; Jiang et al., 2012; Wu et al., 2015), fertilization (Young et al., 2010; Chaudary et al., 2014; Patel and Saraf, 2014), and heavy metal uptake (Majid et al., 2012) by this plant, studies on leaf diagnosis to assess mineral composition are still scarce.

Leaf diagnosis is a key tool for determining the health of perennial crops. It is also a complementary criterion, to be used in parallel with soil analysis, as tools to determine initial or corrective fertilization of crops (Lima et al., 2007; Souza et al., 2010). Early leaf diagnosis may identify plant nutritional deficiencies, which are very difficult to distinguish by visual analysis. The application of further advanced diagnostic methods for jatropha plant health would require a consensus regarding which part of the plant should be the focus of foliar analysis.

Several factors affect the variations in nutrient concentration of plant leaves, such as age (Mickelbart, 2010; Lima et al., 2011b), sampling period (Chaudhary et al., 2009), position of the leaf on the branch (Lima et al., 2011a), leaf position within the tree canopy (Wright et al., 2006; Mickelbart, 2010), and number of leaves and sampled plants (Rozane et al., 2007). In the case of lychee (*Litchi chinensis* Sonn.), Kotur and Spingh (1993) reported that the 2nd pair of leaves from the top of the branch, for all directions of the crown, and at mid-height of the trees, were the most sensitive, and therefore, the most useful for the accurate prediction of the health of that tree. Natale et al. (1996) observed that the 3rd pair of recently mature leaves of guava plants, on the 3rd mid-section of the top of the tree, during full flowering, is the most indicative tissue for nutritional analysis of that species.

Although there is information on leaf sampling for most common crops, little is known for less popular species, such as adult jatropha trees. An initial study using jatropha plants determined the optimal sampling period for young plants (Chaudhary et al., 2009). In addition, in 2009 a few studies using adult jatropha trees attempted to define the best canopy section (Lima et al., 2011b) and branch position for jatropha

leaf sampling (Lima et al., 2011a). These studies gave some insight; however, climatic differences among the study years may influence the results. Therefore, there is still not enough data to precisely conclude as to which position on the branch and crown section will provide the best material to accurately predict the mineral nutrient status of adult jatropha plants. Considering the importance of *J. curcas* in the biodiesel production industry, and the fact that research in leaf diagnostics for this species is still lacking, this study endeavored to determine the optimal canopy section and branch position for leaf sampling that could accurately evaluate the health of adult jatropha plants.

Results

Macronutrients

The results obtained from the soil analysis (Table 1) revealed that plants used in this study were cultivated on fertile soil, under rainfed conditions (Fig. 1). Under these conditions, the N content of the leaves was neither influenced by the position of the leaf on the branch nor by their positions on distinct sections of the plant crown (Table 2).Values ranged between 26.21 and 27.18 g kg^{-1} for leaves harvested at the 4^{th} and 5^{th} node of the branch located on the apical part of the plant (Table 3). Furthermore, since the N content in leaf tissues was not significantly affected by harvesting from the middle or basal sections of the trees (Table 3), it is possible that there are no specific sections of the crown that are the best for tissue collection in evaluating the N status of jatropha plants.
The P content was not affected by the position of the leaves on the branches or by the plant crown segment (Table 3). In general, the P content was lower for leaves collected in the apical region when compared to that of leaves in the apexes of the branches. This indicates that P content is higher in younger tissues. Conversely, P concentration was more stable in the middle and basal regions of the tree crown. There was only a slight variation with increased leaf age. In other words, the position of the leaf on a particular branch node in the two regions of the crown did not affect the P concentration in the leaf tissue. The standardization of a diagnostic leaf is necessary, however the data suggests any one of the studied positions would be suitable for tissue collection.

The leaf K content did not differ significantly, when leaves were collected from any of the positions between the 1^{st} and 5^{th} node of the branches (Table 3). However, there were significant differences in K concentration with regard to the position of the branch in a particular section of the crown. The highest concentrations were found in leaves collected from the basal (43.49 g kg^{-1}) and middle part (34.53 g kg^{-1}) of the plant crown. According to the literature (Mickelbart, 2010), the crown region is more suitable for leaf sampling, and it is where the concentrations of the minerals in the leaves are most stable. For K, the apical and middle regions are the most indicative, so the branches with the most suitable leaves are those between the 1^{st} and the 5^{th} node from the branch tip.

According to data shown in the Table 3, the Ca and Mg content differed statistically with leaf position and location on the plant crown. The lowest Ca and Mg content in the apical region occurred in leaf tissues collected from the 1^{st} node (Ca = 14.76 g kg^{-1} and Mg = 4.61 g kg^{-1}), whereas the highest concentrations were obtained in leaf tissues from the 5^{th} node of the apical branches (Ca = 23.38 g kg^{-1} and Mg = 7.31 g kg^{-1}). The same trend was also found in tissues collected from the middle and basal sections of the plant crown. This indicates that Ca and Mg have low mobility in the phloem, with slow redistribution rates, leading to increased accumulation in older plant tissues. Further significant variations generally occurred with Ca and Mg concentrations in the leaves from the basal part of the plant crown, and were observed especially in leaves collected from the 4^{th} node. This is in accordance with the results by Lima et al. (2011b), who showed that Ca and Mg are nutrients that accumulate in leaf tissues as the leaves age. Consequently, the highest concentration occurred in the basal region of the plant crown, mainly in leaves of the 5^{th} node (26.34 g kg^{-1}–7.66 g kg^{-1}).

In contrast to other macronutrients, the lowest concentrations of S were observed in the basal leaves, although no differences were noted with regard to leaf position on the branch (Table 3). This suggests the intense translocation of S from the basal canopy toward the middle and apical plant parts, and that S is easily distributed among the leaves in the same branch.

In general, there were no significant ($P > 0.05$) variations on N and P concentrations in the studied samples. The K, Ca, Mg, and S concentrations varied with plant crown section. Only the Ca and Mg content varied with the leaf position on the branch. In addition, no significant effects were observed regarding the macronutrient content for the PC × PB interaction.

Micronutrients

The Fe, Mn, and Cu content in leaves varied significantly with plant crown section, and independently from the position on the branch. However, Mn content was significantly affected by interactions between these factors (Table 4).

The Cu content in the leaves varied significantly in the crown section, and such variations were observed mainly in the 3^{rd} leaf from the branch tip (Table 5). Leaves of the same branch showed similar values for Cu concentration. The Cu levels clearly tended to decrease in leaves from basal to apical regions of the plant crown (Table 5) in young and mature leaves.

The Fe concentrations in leaves only differed significantly among leaves from distinct positions within the plant crown (Table 4). The Fe concentration was higher in leaves from the basal part of the plant crown, compared to leaves from the apical part of the plants. Leaves from the middle section of plants showed intermediate mean concentrations (Table 5). This trend was clearly indicated by the Tukey test results, but only for the 4^{th} leaf of the branches. The same trend seems to occur in leaves on other branch positions, but the relatively high coefficient of variation for these data (24%) hindered statistical verification. These results suggest that the ideal leaves for Fe evaluation are between the 3^{rd} and 4^{th} nodes of the branches, on the middle part of the plant crown, where the leaves are less sensitive to short term variations or long-term accumulation.

The results of the Mn evaluation presented a trend similar to those observed for Fe (Table 4, 5). The leaf of the 5^{th} node in the branch was the most sensitive for Mn detection, so this leaf was clearly distinguished when sampled in a distinct section of the plant crown. In the middle and apical sections of the plants, no differences were observed among leaves of the same branch. However, in the basal part of the crown, the Mn concentration tended to increase with the leaf number on the branch. Therefore, in this part of the plant the Mn concentration exhibits a clear decrease with leaf age.

The analysis of variance for Zn data revealed no effect of the studied factors, or their interaction, on this variable (Table 4). In spite of a relatively low variance coefficient (14.64%), no difference was detected by the Tukey test (5%) among the

Table 1. Chemical attributes of the soil in the *Jatropha* crop area.

pH	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	SB	H + Al	T	V	Al^{3+}	P	OM
	Exchangeable complex of the soil ($mmol_c\ dm^{-3}$)							%	$mmol_c\ dm^{-3}$	$mg\ dm^{-3}$	$g\ kg^{-1}$
6.2	25.7	14.3	1.47	2.3	43.7	14.0	57.7	76	0.5	11.4	14.7

SB_Sum of bases; T_Total cation exchange capacity at pH = 7.0; V_Base saturation; OM_organic matter.

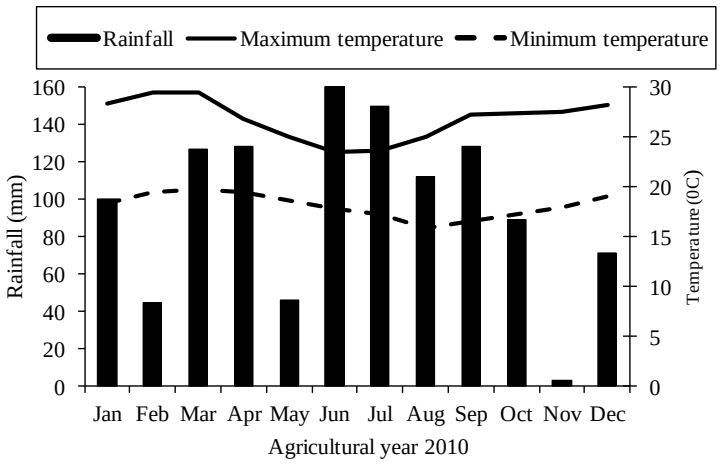

Fig 1. Mean rainfall and temperature between January and December 2010.

Table 2. Summary of the analysis of variance (F test) for N, P, K, Ca, Mg, and S content in jatropha leaf samples collected from three sections of the plant crown (PC) and five branch positions (PB).

Causes of variation	DF	N	P	K	Ca	Mg	S
		---------- Mean squared ----------					
PC	2	5.55^{ns}	0.28^{ns}	213.58^{**}	73.29^{**}	5.88^{**}	0.90^{**}
PB	4	0.19^{ns}	0.08^{ns}	12.27^{ns}	121.60^{**}	10.38^{**}	0.08^{ns}
PC x PB	8	2.01^{ns}	0.08^{ns}	18.19^{ns}	5.36^{ns}	0.82^{ns}	0.02^{ns}
SDM	45	1.41	0.14	21.14	13.56	0.58	0.09

Results of ANOVAs, with the F test significance, are shown: **_P ≤ 0.01, *_P ≤ 0.05, ns_P > 0.05; DF_Degrees of freedom; SDM_Standard deviation mean.

means of distinct treatments (Table 5). This fact suggests that Zn is easily distributed throughout the plant. In general, the micronutrient content displayed few oscillations among the leaves of the same branch, but tended to vary within plant crown sections (Table 4, 5).

Discussion

According to the literature, several factors may affect variations in the concentration of nutrients in plant leaves, such as their age (Mickelbart, 2010; Lima et al., 2011b), the sampling period (Chaudhary et al., 2009), the position of the leaf on the branch (Lima et al., 2011a), or the leaf position within the tree canopy (Wright et al., 2006; Mickelbart, 2010). However, in this study, no difference was observed among distinct leaves in terms of N concentration (Table 2, 3). This result may be due to the low N content in leaves obtained for the study, compared to the range (between 31.4 and 42.4 $g\ kg^{-1}$) considered to be nutritionally optimal by Laviola and Dias (2008) and Lima et al. (2011a). Notwithstanding, in previous reports, no difference was found among mature, young, or developing leaves, except for senescent leaves which had lower N levels (Lima et al., 2011a, b). Thus, although differences in N levels are expected due to plant age (Mickelbart, 2010) and sampling time (Chaudhary et al., 2009), it seems clear that there is no limitation as to the type of leaf or plant crown section that can be used to sample photosynthetically active tissue to evaluate the N concentration. The P concentration in the leaves ranged between 2.90 and 3.46 $g\ kg^{-1}$, in other words, within the range found by Laviola and Dias (2008) and Lima et al. (2011a), who considered nutritionally sufficient P concentrations to be between 2.8 and 2.9 $g\ kg^{-1}$ for leaf tissues of adult jatropha plants. The results obtained in this work suggested that, similar to N, the plant and branch position are not limiting factors for leaf sampling in relation to P. The K content varied between 30.68 and 43.49 $g\ kg^{-1}$ in leaves collected from the 5th and 3rd node of flowering branches; intermediate concentrations varied between 32.99 and 34.53 $g\ kg^{-1}$ in leaf tissues harvested from the middle portion of the trees. The K content of leaves in this study are within the sufficient range (between 13.7 and 34.5 $g\ kg^{-1}$), according to Laviola and Dias (2008) and Lima et al. (2011a), as determined by the completely expanded leaves of approximately two year old plants. By analyzing the results of the leaves collected at the 3rd branch node, it was clear that K levels tend to decrease from the basal to apical plant parts. Data for other node positions showed a similar tendency, but no statistical difference was noted (Table 3). The Ca and Mg content in leaves varied between 14.76 and 26.34 $g\ kg^{-1}$, and between 4.61 and 7.69 $g\ kg^{-1}$, respectively (Table 3). Comparison of these concentrations with those reported as reference values for adult jatropha plants show that they are within the range considered adequate by Laviola and Dias (2008) and Lima et al. (2011a). These nutrients tended to be more concentrated in basal leaves and increased with distance from the branch tip. This tendency was clearly observed in the leaf of the 4th node of the branch and basal part of the plant (Table 3). Lima et al. (2011a) also observed an increase of both Ca and Mg in leaves with increased distance from the branch tip, and the Ca was more concentrated in leaves of flowering branches in comparison to vegetative ones. Lima et al. (2011b) found no statistical difference in Mg concentration among very young, somewhat young, mature,

Table 3. Mean of the N, P, K, Ca, Mg, and S content in the leaves of four-year-old jatropha plants; the leaves were collected from three sections of the plant crown and five branch positions.

Section of the plant crown	Position of leaf on the branch, from the tip				
	Leaf 1	Leaf 2	Leaf 3	Leaf 4	Leaf 5
N (g kg^{-1})					
Apical	A 26.50 a	A 26.73 a	A 26.52 a	A 26.21 a	A 27.18 a
Middle	A 28.39 a	A 27.63 a	A 26.88 a	A 28.11 a	A 27.25 a
Basal	A 26.39 a	A 27.03 a	A 28.30 a	A 27.24 a	A 27.83 a
VC = 13.77%					
P (g kg^{-1})					
Apical	A 3.46 a	A 3.21a	A 3.06 a	A 3.02 a	A 2.96 a
Middle	A 2.95 a	A 3.17 a	A 2.90 a	A 2.96 a	A 2.99 a
Basal	A 3.18 a	A 3.24 a	A 3.13 a	A 3.35 a	A 3.23 a
CV = 11.91%					
K (g kg^{-1})					
Apical	A 31.77 a	A 31.16 a	B 31.77 a	A 31.71 a	A 30.68 a
Middle	A 33.93 a	A 34.53 a	B 32.99 a	A 34.09 a	A 33.99 a
Basal	A 35.03 a	A 35.29 a	A 43.49 a	A 37.62 a	A 38.06 a
CV = 13.36%					
Ca (g kg^{-1})					
Apical	A 14.76 b	A 16.62 ab	A 19.06 ab	B 18.42 ab	A 23.38 a
Middle	A 15.93 a	A 17.70 a	A 19.46 a	AB 21.29 a	A 22.07 a
Basal	A 16.30 b	A 19.97 ab	A 22.95 ab	A 24.95 a	A 26.34 a
CV = 18.46%					
Mg (g kg^{-1})					
Apical	A 4.61 b	A 5.07 b	A 5.44 b	B 5.11 b	A 7.31 a
Middle	A 4.89 b	A 5.43 b	A 5.82 ab	B 6.38 ab	A 7.08 a
Basal	A 5.14 c	A 6.14 bc	A 6.27 abc	A 7.69 a	A 7.66 ab
CV = 12.66%					
S (g kg^{-1})					
Apical	A 2.19 a	A 2.08 a	A 2.00 a	A 1.91 a	A 2.14 a
Middle	AB 1.96 a	A 1.98 a	A 1.92 a	A 1.78 a	AB 1.77 a
Basal	B 1.67 a	A 1.79 a	A 1.57 a	A 1.60 a	B 1.57 a
VC = 15.77%					

CV = Coefficient of Variation; for each variable data, means followed by same letter do not differ by Tukey test at P = 0.05 (uppercase letters compare means within lines, and lowercase letters, within columns).

Table 4. Summary of the analysis of variance (test F) for Fe, Mn, Zn, and Cu content in jatropha leaf samples collected from three sections of the plant crown (PC) and five branch positions (PB).

		Cu	Fe	Mn	Zn
Causes of variation	DF	---------- Mean squared ----------			
PC	2	18.60**	4367.77**	736.31**	3.09^{ns}
PB	4	1.29^{ns}	494.21^{ns}	49.22^{ns}	8.62^{ns}
PC × PB	8	0.58^{ns}	307.48^{ns}	57.11*	6.02^{ns}
EDM	45	0.83	457.65	22.28	6.32

Results of ANOVAs, with the F test significance, are shown: **_P ≤ 0.01, *_P ≤ 0.05, ns_P > 0.05; DF_Degrees of freedom; SDM_Standard deviation mean.

Table 5. Mean of the Cu, Fe, Mn, and Zn content in the leaves of four-year-old Jatropha plants collected at three sections of the plant crown, and in five branch positions.

Sections of the plant crown	Position of leaf on the branch, from the tip				
	Leaf 1	Leaf 2	Leaf 3	Leaf 4	Leaf 5
Cu (mg kg^{-1})					
Apical	A 4.98 a	A 4.74 a	B 4.22 a	B 3.74 a	B 3.72 a
Middle	A 5.96 a	A 5.74 a	AB 5.72 a	AB 5.24 a	A 6.23 a
Basal	A 6.49 a	A 6.24 a	A 6.22 a	A 6.00 a	A 5.47 a
CV = 16.92%					
Fe (mg kg^{-1})					
Apical	A 72.47 a	B 79.35 b	A 73.49 a	B 69.55 a	A 79.09 a
Middle	A 78.70 a	B 83.26 a	A 83.33 a	AB 79.03 ab	A 88.43 a
Basal	A 83.82 a	A 120.80 a	A 91.60 a	A 115.40 a	A 105.22 a
CV = 24.62%					
Mn (mg kg^{-1})					
Apical	A 31.62 a	A 30.19 a	B 29.79 a	C 26.18 a	B 33.95 a
Middle	A 35.74 a	AB 37.15 a	AB 35.83 a	B 37.39 a	B 37.61 a
Basal	A 34.68 b	A 40.18 ab	A 41.81ab	A 49.21 a	A 46.50 a
CV = 12.92%					
Zn (mg kg^{-1})					
Apical	A 19.92 a	A 17.22 a	A 18.38 a	A 15.21 a	A 16.35 a
Middle	A 17.13 a	A 17.45 a	A 18.16 a	A 15.21 a	A 15.70 a
Basal	A 17.47 a	A 17.47 a	A 16.68 a	A 18.22 a	A 17.15 a
CV = 14.64%					

CV_Coefficient of Variation; for each variable data, means followed by same letter do not differ by Tukey test at P = 0.05 (uppercase letters compare means within lines, and lowercase letters, within columns).

and senescent leaves, however, a significant increase was observed from young to mature, and from mature to senescent leaves. This is a typical distribution for mineral nutrients with low remobilization rates within the plant (Marschner, 2012). For these nutrients, the collection of young leaves may lead to a sub-estimate, and old leaves may provide a super-estimate, as to the plant nutrient status. So, in this case, leaves from the 3rd and 4th node of branches from the middle height of the plant crown should be sampled.

Contrary to the data for Ca and Mg, the highest S concentration was detected in the 1st leaf of branches located on the apical plant region (2.19 g kg^{-1}), and the lowest value (1.57 g kg^{-1}) was found in the 5th leaf of branches in the basal part of the plant canopy. However, no significant ($P > 0.05$) effect was observed for the position of leaves on the branches (Table 2, and 3). These results are in accordance with those of previous reports (Lima et al., 2011a, b), and are within the concentration range considered adequate for flowering jatropha plants cultivated under a rainfed agricultural system (Laviola and Dias, 2008).

In this study, Cu content ranged between 3.72 and 6.49 mg kg^{-1}. A comparison of the above concentrations with those found in previous studies showed that they are actually below the ranges reported by Laviola and Dias (2008) and Lima et al. (2011a), who considered optimal Cu content to be between 10.0 and 11.4 mg kg^{-1}. Cu is a micronutrient with moderate mobility in the phloem, so a greater concentration tends to be found in more mature tissues (Marschner, 2012). However, because of the high sensitivity of very young leaves to Cu concentration (Lima et al., 2007), the value may be significantly affected by low humidity or organic matter in the soil. Because of the low rate of Cu translocation in the plant, the leaves in the middle third of the crown are suggested for the assessment of the plants nutrient status, regarding Cu. Considering the non-significant effect of leaf position on the branch (Table 4), this variable may only be relevant for other nutrients.

The concentration of Fe in the samples studied varied between 69.55 and 120.80 mg kg^{-1}. These values are lower than those obtained by Laviola and Dias (2008) and Lima et al. (2011a), who reported Fe content between 86.2 and 150.5 mg kg^{-1}. Taking into account that the Fe data showed the same trend observed for Cu data, the same reasoning is suggested for this case. The data for Mn content in the leaves exhibited behavior similar to that of Ca and Mg (Tables 3 and 5), and corresponds with the fact that this micronutrient has low mobility in the phloem of most plants (Marschner, 2012). The micronutrient Zn, in general, is considered to have low mobility in the phloem (Marschner, 2012). Therefore, it was expected to accumulate in leaves with age, as commonly occurs with Ca, for example. However, this research detected no effect on the position of leaves in the branch, or branch position on plant crown section (Table 4, and 5), while for other reports (Lima et al., 2011a, b) the Zn concentration tended to decrease with leaf age. This is an indication that in jatropha plants, Zn may have more mobility compared with other commonly known crops. Taking into account the low variation among leaf samples in this research, and considering that Zn concentration tends to stabilize from the 3rd leaf on the branch, which corresponds to the fully expanded and mature leaf (Lima et al., 2011a, b), this type of leaf is appropriate for Zn evaluation. Thus, leaves from the middle region of the plants' crown are recommended for foliar diagnosis because of the stable mineral concentrations in this region.

Materials and Methods

Location and plants used in this study

Healthy jatropha plants, in their full flowering stage, cultivated in a rainfed agricultural system, were selected within a homogeneous area. The plants were four years old, cultivated in a 3 × 2 m spacing in a commercial plantation located in Garanhuns-PE, Brazil (8°56′S; 36°27′W; 741 m altitude). The region has a mean monthly rainfall of 130 mm, and the climate is classified as BS (dry, semi-arid, megathermal, with four rainy months) according to the Köppen classification. Figure 1 shows the rainfall and temperature during the year in which the assay was conducted.

The soil in the experimental area was classified as Regolithic Neosol, with a sandy loam texture. Samples were collected for chemical analysis at a depth of 0–20 cm, homogenized, and analyzed at the Laboratory of Irrigation and Salinity of the Universidade Federal de Campina Grande (UFCG), following the methods described by EMBRAPA (1997). The soil was slightly acidic (pH 6.2), with a 76% base saturation (Table 1). Annual fertilization with 6 Mg ha^{-1} of organic manure (bovine) was applied in each of the four years of the plantation's operation.

Treatments

In the May of 2010, samples of jatropha leaves were collected at five leaf positions on the branches (at the 1st, 2nd, 3rd, 4th, and 5th node, beginning from the branch tip) and from three sections of the plant crown (apical, middle, and basal). Experimental design was completely randomized, with 4 replications each, and each experimental unit consisted of 4 plants, randomly selected within a 1 ha area of the plantation.

Measurements

Four leaves were collected from each plant and from each position on the branch within the three regions of the plant crown, with the appropriate replications.

After harvest and identification, the leaves were washed in deionized water to remove impurities, dried in a forced-air oven at 70°C, ground in a Willey-type mill, and taken to the Laboratory of Soil and Plant Nutrition at the University of São Paulo State, Jaboticabal-SP, Brazil, for chemical analysis. Measurements of all macronutrients and micronutrients (Cu, Fe, Mn, and Zn) in the plant tissue were performed according to the methods described by (Bataglia et al., 1983). Briefly: Part of each sample underwent nitric-perchloric acid digestion to determine the concentrations of P, K, Ca, Mg, S, and micronutrients, while another part underwent sulfuric acid digestion to determine N content. The N content was quantified using the Kjeldahl method; P by the phosphomolybdate-vanadate spectrophotometric method; the S by the barium-sulfate turbidimetric method, while K, Ca, Mg, Cu, Fe, Mn, and Zn measurements were assessed by atomic absorption spectroscopy.

Data analysis

Data were evaluated using an analysis of variance (F test), and the F-values were tested at 5% and 1% probability level ($P = 0.05$ and 0.01). The means were compared using a Tukey test ($P = 0.05$), according to the methods described by Santos and Gheyi (2003). The standard deviation mean, and

the coefficient of variance, were also calculated and presented with the respective data.

Conclusions

The leaves at the 3rd and 4th node of the flower-bearing branches located in the middle section of the plant canopy are the most informative for the evaluation of adult jatropha plant mineral nutrient status.

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