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Soil and plant nutrition

Determination of the standard leaf for nutritional diagnosis of assai palm plants

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Abstract – Assai palm is the main palm tree grown in the Brazilian Amazon. Nutritional diagnosis has become an essential tool in management of the crop. The aim of this study was to determine the standard leaf for nutritional diagnosis of the assai palm tree through evaluation of leaf nutrient content and use of two methods of statistical analyses: traditional (univariate) and multivariate. The study was conducted in the municipality of Belém, with eight treatments: the phyllotaxic positions of the leaves (number 2 to 9), in a completely randomized design. The leaf concentration of nutrients (N, P, K, Ca, Mg, S, B, Cu, Fe, Mn and Zn) was determined for performing descriptive analyses, analyses of variance, correlation analysis, and principal component analysis (PCA). Young leaves had greater concentrations of N, P, K, Mg, S, Cu, and Zn, whereas in the older leaves, there were greater concentrations of Ca, B, Fe, and Mn. The leaves that exhibited less variation in nutrient concentrations were from 2 to 5. PCA analysis indicated interaction of positive loads of N-P and Cu-Mg and interaction of negative loads of Ca-Mn. The index of nutrient distribution indicated leaf number 5 as having the lowest factor loading value. Thus, leaf number 5 is most recommended for nutritional diagnosis of assai palm plants through sampling of 25 plants.
Index Terms: diagnostic leaf, *Euterpe oleracea* Mart., leaf sampling, nutritional state, plant nutrition.

Determinação da folha padrão para diagnóstico nutricional do Açaizeiro

Resumo - O açaizeiro é a principal palmeira cultivada na Amazônia brasileira, e a diagnose nutricional torna-se uma ferramenta essencial ao manejo da cultura. O objetivo deste trabalho foi determinar a folha padrão para a diagnose nutricional do açaizeiro por meio da avaliação dos teores foliares e da utilização de dois métodos de análises estatísticas, tradicional (univariada) e multivariada. O estudo foi conduzido no munícipio de Belém - PA, com oito tratamentos: posições filotáxicas das folhas (folhas 2 a 9), em delineamento inteiramente casualizado. A concentração foliar dos nutrientes (N, P, K, Ca, Mg, S, B, Cu, Fe, Mn e Zn) foi determinada para a realização das análises descritivas, de variância, correlação e análise de componentes principais. As folhas novas apresentaram maiores concentrações de N, P, K, Mg, S, Cu e Zn, ao passo que, nas folhas mais velhas, houve maiores concentrações de Ca, B, Fe e Mn. As folhas que apresentaram menor variação nas concentrações dos nutrientes foram de 2 a 5. A análise de PCA indicou interação de cargas positivas de N-P e Cu-Mg e interação de cargas negativas de Ca-Mn. O índice de distribuição dos nutrientes indicou a folha 5 com o menor valor de carga fatorial. Nesse sentido, a folha número 5 é a mais indicada para a diagnose nutricional de plantas de açaizeiro por meio de amostragem de 25 plantas.
Termos para indexação: folha diagnóstica, *Euterpe oleracea* Mart., amostragem foliar, estado nutricional, nutrição de plantas.

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Introduction

The assai palm (*Euterpe oleracea* Mart.) originated in the Amazon and is notable for its rich characteristics and production of fruit used as a food by the local population (ARAÚJO et al., 2016). The assai palm develops in many different soil types and is naturally found on solid ground and in areas subject to flooding, though it does not tolerate permanently swamped areas (VIÉGAS et al., 2004).

In the Amazon region, the assai palm is commonly grown on Yellow Oxisol of low natural chemical fertility, making nutritional management essential for achieving maximum yield potential (LINDOLFO et al., 2020). Viégas et al. (2009) report that to attain the yield potential of assai palm, it is necessary to understand the nutrients that most limit its development and, consequently, provide for these nutrients. Therefore, an understanding of adequate nutrition of the assai palm is essential, since poorly managed nutrition can limit production in the species.

For better management of cultivated plants, leaf diagnosis has become a nutritional evaluation tool, allowing comparison with pre-established standards of adequate ranges of nutrients (CANTARUTTI et al., 2007; PRADO, 2020). The use of leaf analysis as a criterion of nutritional analysis is based on the premise of there being a relationship between plant nutrient concentrations and yield (PRADO, 2020), with the expectation that leaf concentrations are capable of representing the nutritional state of plants (PRADO; NATALE, 2003). Nevertheless, information regarding assai palm nutrition is still quite limited. Consistent results that allow precise evaluation of the nutritional state of this species of palm trees are lacking.

Leaves are the plant tissues most commonly used for chemical analysis of nutrients; they are the central organ of plant metabolism, and they have the greatest concentration of nutrients (MALAVOLTA et al., 1997; FERNANDES et al., 2018). In adult assai palm trees (cultivar BRS - Pará), the newly opened leaf was the organ that showed the least variation in nutrient concentrations (BRASIL et al., 2008). There are recommendations for the leaf most suitable for nutritional diagnosis of species grown in the Amazon, e.g., the coconut palm and oil palm (VELOSO et al., 2020), though not yet for assai palm. Studies have used leaves number 4 and 5 (RIBEIRO, 2017) or leaf number 6 (LINDOLFO et al., 2020) for nutritional diagnosis of the assai palm, though without any specific rationale. Marschner (2012) emphasizes that for fruit-bearing plants, leaf analysis is more recommended than soil analysis.

For the main palm trees grown in the world, such as coconut palm and oil palm, the leaf was already defined as the plant material recommended for nutritional diagnosis. For oil palm (*Elaeis guineensis* Jacq.), leaflets from leaf number 17 (standard leaf) are collected in adult plants and from leaf number 9 in young plants (up to the 3rd year after planting) (JACQUEMARD, 1995; VELOSO et al., 2020). For coconut palm (*Cocos nucifera* L.), the standard leaf is leaf number 14 (TAFFIN, 1993). In the case of peach palm (*Bactris gasipaes* H.B.K.), the position of leaf number 5 is considered the most adequate for nutritional analyses (VELOSO et al., 2020).

In this regard, the aim of the present study was to determine the standard leaf for nutritional diagnosis of assai palm through evaluation of the leaf contents and use of two methods of statistical analyses (univariate and multivariate) and thus contribute to sustainable management of this palm tree in the Amazon region.

Materials and methods

The study was conducted with plants (4 years and 8 months of age) of assai palm (*Euterpe Oleracea* Mart.; cultivar BRS – Pará) in the experimental field of Embrapa Amazônia Oriental (1º21'10" S and 48º9'15" W), municipality of Belém, PA, in the month of November. In the period in which the study was conducted, the region had an average monthly rainfall of 82.5 mm and an average monthly temperature of 27.5 ºC (Figure 1). The soil was classified as a Yellow Oxisol of medium texture (GAMA et al., 2020). The chemical attributes of the soil were determined at two depths (0-0.2 and 0.2-0.4 m) (Table 1), according to the methodologies of Donagema et al. (2011).

The experimental design used was completely randomized, with 40 replications and 8 treatments of phyllotaxic position of the leaf (leaf number 2 to 9; Figure 2), totaling 40 plants. The plants were grown at a spacing of 5.0 × 5.0 m and were healthy, without signs of pest attack or injury from pathogens. It was performed a single leaf sampling: the leaflets were collected on the largest stipe of the clump, between 7:00 and 11:00 a.m. and never less than 36 hours after a rain (> 20 mm). Leaves were sampled with eight leaflets on each leaf on the two sides of the rachis in the middle portion. The leaf samples were dried in a laboratory oven (70 ºC) and ground in a Wiley type mill equipped with a fine sieve. The samples were identified, stored in plastic bags, and sent to the Foliar Analyses Laboratory of the Universidade Federal de Lavras (UFLA), where the N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn total concentrations were determined according to the methodologies of Malavolta (1997).

Table 1. Chemical characteristics of the soil (0.0 - 0.2 and 0.2 - 0.4 m) of the experimental area of assai palm cultivation determined in the leaf sampling period

Depth	pH	SOM	P	K	Ca^{2+}	Mg^{2+}	Al^{3+}	Cu^{2+}	Fe^{2+}	Mn^{2+}	Zn^{2+}
(m)	(H_2O)	g kg^{-1}	mg dm^{-3}		------cmol$_c$ dm^{-3}------			---------mg dm^{-3}--------------			
0 - 0.2	4.3	20.2	26	30	0.4	0.4	1.0	1.2	377	3.4	1.9
0.2 - 0.4	4.5	16.9	42	23	0.5	0.6	1.0	1.4	353	2.8	1.6

SOM - Soil organic matter. P, K, Cu, Fe, Mn and Zn extracted by Mehlich-1 solution. Ca, Mg an Al extracted by KCl solution (1 mol/L). Analyses performed according to the methodologies of Donagema et al. (2011).

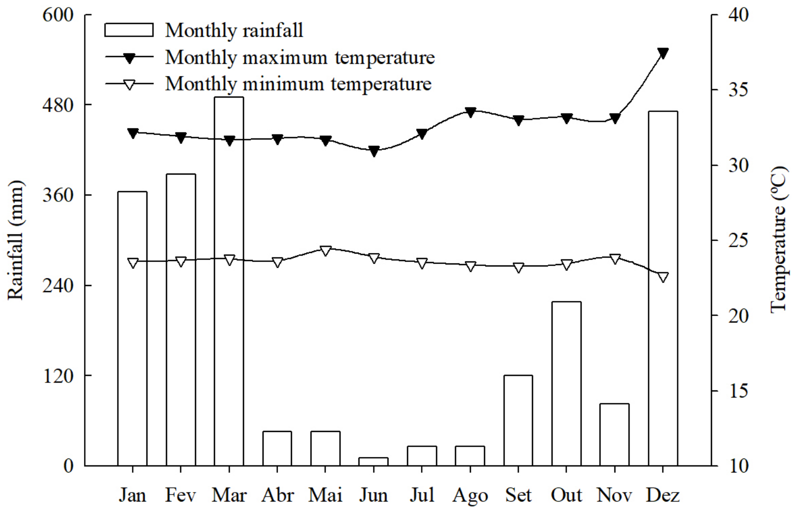

Figure 1. Rainfall and maximum and minimum temperature of the experimental area of assai palm cultivation during the study year (INMET, 2021).

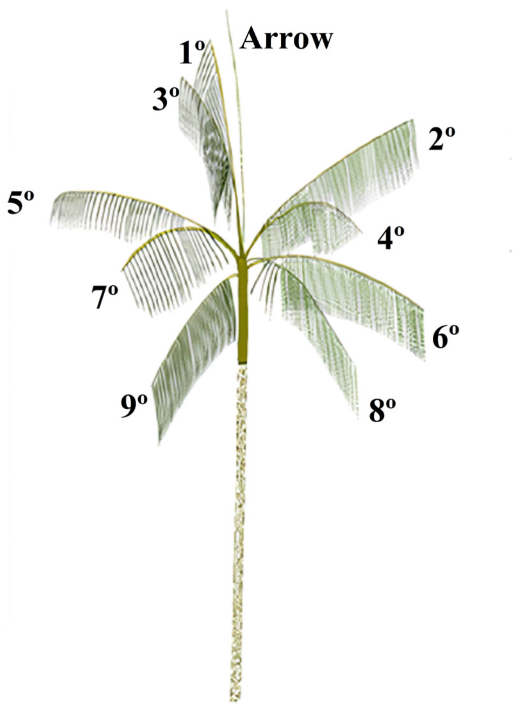

Figure 2. Illustration of the phyllotaxic position of the leaf (number 1 to 9) of assai palm plants.

Descriptive analyses (mean, standard deviation, and coefficient of variation) for each nutrient according to the phyllotaxic position of the leaf, analysis of variance and, when significant, Scott & Knott's Test ($p < 0.05$), and regression (LANZA, 2007) were performed. Pearson correlation analyses were performed between pairs of nutrients for the phyllotaxis positions of the leaves, determining regression equations for each nutrient and phyllotaxic position, as well as the F test ($p < 0.05$). After checking the dependence structure in the set of variables, the multivariate factor analysis technique was carried out, using the KMO (Kaiser-Meyer-Olkin Measure of Sampling Adequacy) and Bartlett sphericity tests, which tests the null hypothesis that the variables analyzed are not correlated (HAIR JÚNIOR et al., 2009).

In multivariate factor analysis, for extraction of the factors, the principal component method was used with biquartimax normalized rotation for the purpose of analyzing the structure of correlations among the variables (nutrient contents), thus generating a set of common latent dimensions called factors. The factors extracted were chosen according to the Kaiser criterion, in which factors with eigenvalues higher than the value 1.0 are chosen. After that procedure, the factor loading matrix of the factors extracted was obtained, as well as the rotated factor scores, which represent the estimates of the contributions of the various factors to each original observation and are used in classification of samples (HAIR JÚNIOR et al., 2009). The Statistica 7.0 program (STATSOFT, 2005) was used in statistical analyses.

An index was constructed as described in Andrews et al. (2002), Santana (2007), and Cherubin et al. (2016) for the purpose of integrating the latent variables in a single index containing the linear combination of the factor scores and the proportion of the variance explained in each factor in relation to the common variance. A ranking can be created in this index, with values ranging from zero to one, allowing identification and evaluation of the variations in the nutrient concentrations according to the phyllotaxic position of the leaf in an integrated manner. The factor scores of each factor were extracted (latent variables) by multivariate factor analysis, and statistical analysis was performed on the integrated index of distribution of the nutrients in the leaves with the procedure of the general linear model (GLM).

Results and discussion

The leaf N concentrations were higher in the younger leaves (numbers 2, 3, and 4) and the intermediate values of leaf N concentrations occurred in leaves number 6 and 7 (Table 2). Leaf N concentrations had lower coefficients of variation (CV) in leaves number 5 and 6 and the smallest standard deviation was observed on leaf 5 and 8 (Table 2). Nitrogen is a mobile nutrient in plants and it accumulates in younger tissues (FERNANDES et al., 2018). A study performed with B doses in assai palm under field conditions in the Eastern Amazon found variation in the N concentration in the leaf of position 6 from 16.8 to 18.8 g kg^{-1} (LINDOLFO et al., 2020). In adult assai palm plants in the Eastern Amazon, leaf concentrations of 23 g kg^{-1} of N were found (RIBEIRO, 2017). Native populations of adult assai palm of the Marajoara region in Pará had mean leaf concentration of 21.9 g kg^{-1} of N (BRASIL et al., 2009). In an improved assai palm cultivar (BRS Pará), leaf concentration was 27.3 g kg^{-1} of N (BRASIL et al., 2008). In young plants, 2 years of age, leaf N concentration was 13.1 g kg^{-1} (CORDEIRO, 2011). Araújo et al. (2016), evaluating withholding of nutrients in assai palm seedlings, found leaf concentration of 24.14 g kg^{-1} of N in the treatments that received full fertilization. The assai palm seedlings had mean leaf concentrations of 15.7 g kg^{-1} of N (VIÉGAS et al., 2009).

For other palm trees grown in the Amazon region, the critical level of 22 g kg^{-1} of N was determined for coconut palm (SALDANHA et al., 2017) and 24.3 g kg^{-1} of N for oil palm (MATOS et al., 2016). The leaf N concentrations obtained in leaves number 2 to 6 are above the critical level established for coconut palm and oil palm. It should be noted that there is not yet a recommendation of adequate leaf nutrient concentration for assai palm grown in the state of Pará (VELOSO et al., 2020).

For P and K, higher leaf concentrations were also in the younger leaves, leaves number 2 and 3 for P and leaf number 2 for K, with the decline proportional to the position of the leaves (Table 2). Intermediate values of leaf concentrations occurred in leaves 6 and 7 for P and, for K, in leaves 3 to 8 (Table 2). In the plants, P and K are considered nutrients of greater mobility in the tissues and, thus, they accumulate in the younger organs (FERNANDES et al., 2018). For the leaf P and K concentrations, coefficients of variation (CV) less than 10 % were found in all the phyllotaxic positions evaluated, except for K in the leaf number 9 position (Table 2). The smallest standard deviations were observed in leaves 3, 5 and 7 for P, while for K it was observed in leaf number 2 and 7 (Table 2). For Cantarutti et al. (2007), the position of the ideal sampling leaf is that which is able to show the least fluctuation in the nutrient concentrations. In addition, very low CV values are signs of greater sensitivity of the nutrient in indicating nutritional deficiency (ROCHA et al., 2007; SANTANA et al., 2008; SALDANHA et al, 2017).

Table 2. Mean, standard deviation (SD), and coefficient of variation (CV) of the leaf concentrations of N, P, K, Ca, Mg, and S according to the positions of the leaves on assai palm plants

Phyllotaxic position	------------N-----------			------------P------------			------------K------------		
	Mean (g kg^{-1})	SD	CV (%)	Mean (g kg^{-1})	SD	CV (%)	Mean (g kg^{-1})	SD	CV (%)
2	26.78 a	1.76	6.57	1.77 a	0.12	6.78	7.86 a	0.42	5.34
3	26.44 a	1.72	6.51	1.74 a	0.10	5.75	6.55 c	0.56	8.55
4	26.21 a	1.91	7.28	1.69 b	0.13	7.69	6.88 b	0.49	7.12
5	25.63 b	1.48	5.77	1.62 c	0.10	6.17	6.76 b	0.48	7.1
6	24.44 c	1.52	6.21	1.55 d	0.12	7.74	6.73 b	0.48	7.13
7	23.41 d	1.56	6.66	1.51 d	0.09	5.96	6.69 b	0.42	6.28
8	21.79 e	1.47	6.74	1.48 e	0.11	7.43	6.50 c	0.63	9.69
9	20.47 f	1.70	8.30	1.43 e	0.13	9.09	5.90 d	0.65	11.02
Phyllotaxic position	-----------Ca------------			----------Mg-----------			----------S-------------		
	Mean (g kg^{-1})	SD	CV (%)	Mean (g kg^{-1})	SD	CV (%)	Mean (g kg^{-1})	SD	CV (%)
2	3.75 f	0.53	14.13	0.81 a	0.11	13.58	2.87 a	0.46	16.1
3	4.23 e	0.45	10.64	0.68 b	0.11	16.18	2.88 a	0.48	17.07
4	4.55 d	0.62	13.63	0.58 c	0.12	20.69	2.65 b	0.45	16.47
5	4.79 d	0.93	19.42	0.46 d	0.09	19.57	2.72 a	0.44	16.39
6	5.22 c	1.17	22.41	0.38 e	0.10	26.32	2.47 b	0.44	17.3
7	5.59 b	0.75	13.42	0.33 f	0.10	30.3	2.54 b	0.47	18.32
8	5.97 b	0.86	14.41	0.32 f	0.12	37.5	2.60 b	0.48	19.37
9	7.54 a	1.67	22.15	0.23 g	0.12	52.17	2.53 b	0.49	21.04

Mean values followed by the same letters in the column are not statistically different from each other by Scott & Knott's Test ($p > 0.05$).

Evaluations of concentrations in leaf number 6 in assai palm in the Eastern Amazon indicated variation from 1.4 to 1.5 g kg^{-1} of P and from 8.8 to 9.9 g kg^{-1} of K (LINDOLFO et al., 2020). A study performed with assai palm seedlings under controlled conditions indicated variations from 0.9 to 1.3 g kg^{-1} of P and from 4.8 to 14.5 g kg^{-1} of K (ARAÚJO et al., 2016). In plantings of assai palm in the Eastern Amazon, there were leaf concentrations of 2.3 g kg^{-1} of P and 12 g kg^{-1} of K in seven-year-old plants (RIBEIRO, 2017) and, in native adult plants, leaf concentrations of 1.2 g kg^{-1} of P and 7.4 g kg^{-1} of K (BRASIL et al., 2009). For adult plants of the cultivar BRS Pará, leaf concentrations were 1.9 g kg^{-1} of P and 7.1 g kg^{-1} of K (BRASIL et al., 2008). In young assai palm plants, leaf concentrations of 1.0 g kg^{-1} of P and 4.6 g kg^{-1} of K were observed (CORDEIRO, 2011). Viégas et al. (2009), evaluating leaf concentrations in assai palm seedlings, obtained values of 0.7 g kg^{-1} of P and 3.5 g kg^{-1} of K. For coconut palm, the critical level of 1.4 g kg^{-1} of P and 14 g kg^{-1} of K was determined for the Amazon region (SALDANHA et al., 2017). For oil palm, the critical level of 1.7 g kg^{-1} of P and 7.5 g kg^{-1} of K was determined (MATOS et al., 2016). For P, leaves number 2 and 3 had concentrations above the critical level determined for coconut palm and oil palm. However, all the mean values of K concentrations are below the critical level for coconut palm, and only leaf number 2 had a nutrient concentration above the critical level for oil palm.

For Ca, greater concentration was observed in the oldest leaf (number 9), decreasing in proportion to the phyllotaxic position of the leaves and intermediate values of leaf Ca concentrations occurred in leaves 4 to 7 (Table 2). The lowest concentration of Ca in the younger leaves of assai palm is expected, since the nutrient has a restricted mobility in plants. This fact is explained by the low permeability of Ca through the cytoplasmic membranes, resulting in a low concentration of the nutrient in the cytosol and, in addition, Ca forms complexes of insoluble salts (oxalate and phosphate) in the phloem (PRADO, 2021).

The CV of the leaf Ca concentrations had higher values, though less variation in leaf number 3, and the smallest standard deviation was also observed in leaf number 3 (Table 2). In a study performed with B fertilization in assai palm, Lindolfo et al. (2020) found variation in the Ca concentration in leaf 6 from 5.9 to 7.8 g kg^{-1}. In adult plants of assai palm grown in the Eastern Amazon, the leaf Ca concentration was 9.8 g kg^{-1} (RIBEIRO, 2017). In Marajó, PA, in evaluation of two native populations of assai palm, the mean leaf Ca concentration was 4.4 g kg^{-1} (BRASIL et al., 2009). In contrast, an improved assai palm cultivar (BRS Pará) had leaf Ca concentration of 8.9 g kg^{-1} (BRASIL et al., 2008). In young plants, research developed in the Eastern Amazon indicated mean leaf Ca concentration of 1.9 g kg^{-1} (CORDEIRO, 2011). For assai palm seedlings, the leaf Ca concentration was 6.4 g kg^{-1} (VIÉGAS et al., 2009). Additionally, in assai palm seedlings, in evaluation of withholding nutrients, leaf Ca concentrations from 2.6 to 7.3 g kg^{-1} were observed (ARAÚJO et al., 2016). For other palm trees, the critical level of 3 g kg^{-1} of Ca was determined for coconut palm (SALDANHA et al., 2017), and for oil palm, 8.6 g kg^{-1} of Ca (MATOS et al., 2016).

For the leaf Mg concentrations, there was a higher value in the youngest leaf (number 2) and reduction proportional to the position of the leaves, while the intermediate values of leaf Mg concentrations occurred in leaf 5 (Table 2). The higher leaf concentration in younger leaves can be explained by the high mobility of Mg. The highest concentration of the nutrient in new tissues is expected, since 80 % of the Mg is found in the ionic form in intrathylakoid spaces and, under chloroplast lighting conditions, the nutrient is secreted into the stroma, activating several enzymes (PRADO, 2021). In this sense, it is expected that Mg is redistributed to younger leaves for the formation of new tissues, indicating that very young leaves are not good indicators of the nutrient status in plants.

The CV of the leaf Mg concentrations was higher, especially in the older leaves, at 52.17 %, however the smallest standard deviation was observed in leaf number 5 (Table 2). In a study evaluating assai palm nutrition in the Eastern Amazon, leaf Mg concentrations from 0.9 to 2.6 g kg^{-1} were obtained (LINDOLFO et al., 2020). Ribeiro (2017), evaluating fertigated assai palm plants, obtained leaf Mg concentration of 2.2 g kg^{-1}. In native assai palm, leaf Mg concentrations of 0.99 and 0.89 g kg^{-1} were found in populations of São Sebastião da Boa Vista and Breves, respectively (BRASIL et al., 2009). In an assai palm planting of the cultivar BRS Pará, mean leaf Mg concentration in adult plants was 1.46 g kg^{-1} (BRASIL et al., 2008). In contrast, two-year-old assai palm had Mg leaf concentration of 0.5 g kg^{-1} (CORDEIRO, 2011). Additionally, in assai palm seedlings, another study indicated variation in leaf Mg concentrations from 0.8 to 3.2 g kg^{-1} (ARAÚJO et al., 2016). Viégas et al. (2009), working with assai palm seedlings, observed leaf Mg concentrations of 2.2 g kg^{-1}. Other studies conducted with palm trees in the Amazon region determined the critical levels of 2.2 and 2.4 g kg^{-1} of Mg for coconut palm and oil palm, respectively (MATOS et al., 2016; SALDANHA et al., 2017).

The leaf S concentrations had higher values and less variation in the younger leaves (2, 3, 4, and 5), while intermediate values of leaf S concentrations occurred in leaf 5 (Table 2). The smallest standard deviations of leaf S concentrations were observed in leaf number 5 and 6 (Table 2). Despite higher concentrations in younger leaves, it appears that leaf S concentrations do not differ at very high values, indicating the low mobility of this nutrient and low capacity to redistribute to younger tissues. For this reason, it is indicated that very young and very old leaves are not able to indicate the nutritional status for S of the assai palm plant. A study conducted in the Eastern Amazon on assai palm found variation in S concentrations in leaf number 6 from 0.1 to 1.4 g kg^{-1} (LINDOLFO et al., 2020). Adult plants of fertigated assai palm had leaf S concentration of 3.7 g kg^{-1} (RIBEIRO, 2017), whereas young assai plants had leaf S concentration of 1.9 g kg^{-1} (CORDEIRO, 2011). In assai palm seedlings, Viégas et al. (2009) found leaf S concentration of 1.4 g kg^{-1}. Araújo et al. (2016), evaluating withholding of nutrients from assai palm seedlings, found variation in leaf S concentrations from 3.0 to 7.4 g kg^{-1}. For other species of the palm family, the S critical level has already been determined at 1.5 g kg^{-1} for coconut palm and 1.2 g kg^{-1} for oil palm (MATOS et al., 2016; SALDANHA et al., 2017).

Considering the mean leaf concentrations of the macronutrients of the present study, the decreasing order for assai palm was N > K > Ca > S > P > Mg. The decreasing order of demand for coconut palm was N > K > Ca > Mg > S > P (SALDANHA et al., 2017), and for oil palm, it was N > Ca > K > Mg > P > S (MATOS et al., 2016).

The leaf B concentrations had higher values in the leaves of positions number 7, 8, and 9, and the intermediate values of B concentrations occurred in leaves 2 and 9 (Table 3). The leaf B concentration behaves similarly to that of Ca, also indicating its low mobility; the nutritional demand of the B in new tissues is almost totally dependent on the amount of nutrient absorbed by the plant at the time of tissue formation (PRADO, 2021). The lowest variation and the smallest standard deviation of leaf B concentrations were observed in leaf number 2 (Table 3).

Table 3. Mean, standard deviation (SD), and coefficient of variation (CV) of the leaf concentrations of B, Cu, Fe, Mn, and Zn according to position of the leaves on assai palm plants

Phyllotaxic position	-----------B------------			----------Cu------------			------------Fe-----------		
	Mean (g kg^{-1})	SD	CV (%)	Mean (g kg^{-1})	SD	CV (%)	Mean (g kg^{-1})	SD	CV (%)
2	22.20 b	1.61	7.25	11.37 a	1.91	16.8	81.05 e	7.710	9.51
3	21.53 b	4.54	21.09	9.74 b	1.46	14.99	90.80 d	16.70	18.39
4	22.07 b	3.50	15.86	7.98 c	0.99	12.41	109.39 c	24.08	22.01
5	22.17 b	3.42	15.43	7.95 c	2.69	33.84	118.01 b	14.72	12.47
6	22.02 b	3.11	14.12	6.50 d	1.2	18.46	122.26 b	17.66	14.44
7	25.34 a	5.91	23.32	6.31 d	0.91	14.42	121.96 b	16.22	13.3
8	26.28 a	4.95	18.84	6.15 d	0.95	15.45	130.49 a	17.16	13.15
9	23.63 b	2.47	10.45	5.64 d	1.16	20.57	122.46 b	20.51	16.75

Phyllotaxic position	-------------Mn-------------			-----------Zn------------		
	Mean (g kg^{-1})	SD	CV (%)	Mean (g kg^{-1})	SD	CV (%)
2	201.51 d	49.63	24.63	25.52 a	2.55	9.99
3	233.94 d	43.02	18.39	25.96 a	3.05	11.75
4	277.61 c	50.49	18.19	25.29 a	2.59	10.24
5	280.89 c	60.92	21.69	24.87 a	2.88	11.58
6	284.08 c	73.86	26.00	23.97 b	2.06	8.59
7	379.13 b	59.86	15.79	23.94 b	1.76	7.35
8	411.53 b	82.86	20.13	24.07 b	2.09	8.68
9	453.59 a	172.11	37.94	24.15 b	2.22	9.19

Mean values followed by the same letters in the column are not statistically different from each other by Scott & Knott's Test ($p > 0.05$).

A study on B fertigation in assai palm indicated leaf B concentrations from 12 to 18 mg kg^{-1} (LINDOLFO et al., 2020) and in adult fertigated assai palm plants in the Eastern Amazon, there was leaf B concentration of 72 mg kg^{-1} (RIBEIRO, 2017). For two-year-old assai palm, mean leaf B concentration of 17 mg kg^{-1} was observed (CORDEIRO, 2011); and Viégas et al. (2009), evaluating withholding of nutrients in assai palm seedlings, found leaf B concentration of 31.3 mg kg^{-1}. For other palm trees grown in the Amazon region, the critical B level of 20 mg kg^{-1} for coconut palm (SALDANHA et al., 2017) and 24.3 mg kg^{-1} for oil palm (MATOS et al, 2016) was determined. In this sense, the leaf B concentrations observed in the present study are below (VIÉGAS et al. 2009; RIBEIRO, 2017) or above (CORDEIRO, 2011; LINDOLFO et al., 2020) the values recommended in the literature for assai palm. It should be considered that the B concentrations presented by Viégas et al. (2009) and Cordeiro (2011) are of assai palm in the seedling and young plant (2 year) phases, respectively, unlike the plants evaluated (4 years and 8 months) in the current study. Lindolfo et al. (2020) evaluated the leaf concentrations in irrigated orchards, which, together with the high rainfall in the region, can lead to greater leaching of B and lower uptake of the nutrient. In tropical soils, under high rainfall conditions, B leaching is common (ROSELEM; BÍSCARO, 2007).

For the leaf Cu concentrations, a higher mean value was found in the phyllotaxic position of number 2 and the intermediate values of Cu concentrations occurred in leaves 3 to 5 (Table 3). However, the lowest variation of Cu concentration was found in leaf number 4 and the smallest standard deviation was found in leaf number 7 (Table 3). In growing assai palm in the Eastern Amazon, there was variation in the leaf Cu concentration from 13 to 16.8 mg kg^{-1} (LINDOLFO et al., 2020). Ribeiro (2017), evaluating adult assai palm plants, found leaf Cu concentration of 12 mg kg^{-1}, and, in young plants, a mean leaf Cu concentration of 7.0 mg kg^{-1} was observed (CORDEIRO, 2011). For other palm trees, the critical level of 10 mg kg^{-1} of Cu for coconut palm (SALDANHA et al., 2017) and 5.2 mg kg^{-1} for oil palm (MATOS et al., 2016) was determined. The leaf Cu concentrations in the present study (leaf 2) were lower than those obtained in irrigated assai palm (LINDOLFO et al., 2020); however, they were superior to those observed in young assai palm under dryland conditions (CORDEIRO, 2011). The leaf Cu concentrations of the current study were found to be similar to coconut palm grown in the Amazon region (SALDANHA et al., 2017).

For the leaf Fe concentrations, there were higher values in the older leaves (number 6 to 9) and intermediate value of Fe concentration was found in leaf 4 (Table 3) It is expected that older leaves have higher Fe concentrations, since the nutrient is poorly redistributed. These results indicate that the youngest leaves and the oldest leaves are not good indicators for evaluating the Fe status of the assai palm. The lowest variation and the smallest standard deviation of leaf Fe concentrations were observed in leaf number 2 (Table 3). Lindolfo et al. (2020), evaluating assai palm nutrition in a fertigated planting, obtained variation in leaf Fe concentration from 137 to 158 mg kg^{-1}, and in adult assai plants grown in the Eastern Amazon, leaf Fe concentration was 647 mg kg^{-1} (RIBEIRO, 2017). For Cordeiro (2011), evaluating 2-year-old assai palm, mean leaf Fe concentration was 319 mg kg^{-1}. For other palm trees, the critical level of 40 mg kg^{-1} of Fe for coconut palm and 87 mg kg^{-1} of Fe for oil palm was determined (MATOS et al., 2016; SALDANHA et al., 2017). Thus, the leaf Fe concentrations of the present study were lower than those obtained by Cordeiro (2011), Ribeiro (2017), and Lindolfo et al. (2020). These different leaf concentrations are explained by various factors, such as genetic material, ages, climate, soils, and crop treatments, among others (MALAVOLTA et al., 1997). As the critical nutritional levels may be altered due to drastic changes in biotic and abiotic factors (PRADO, 2021), thus there is a need to establish them for each region.

The highest values in Mn concentration were observed in older leaves (number 8 and 9) and the intermediate values of Mn concentrations were found in leaves 4 to 8 (Table 3). Mn is also expected to show higher concentrations in older leaves, indicating its low mobility, excluding younger leaves as good indicators of its nutritional status. Lower variations of Mn concentrations were found in leaves number 3, 4, and 7, and for the standard deviation, the smallest value was verified in leaf number 3 (Table 3). In a study conducted in an assai palm planting, variation in leaf Mn concentrations from 44.6 to 61.2 mg kg^{-1} of Mn was observed (LINDOLFO et al., 2020). In adult plants of fertigated assai palm, the leaf Mn concentration was 557 mg kg^{-1} (RIBEIRO, 2017) and, in young plants of the species, a mean leaf Mn concentration of 180 mg kg^{-1} de Mn was observed (CORDEIRO, 2011). The critical leaf Mn level in the Amazon region for other palm trees that are grown was determined at 70 mg kg^{-1} for coconut palm and 258 mg kg^{-1} for oil palm (MATOS et al., 2016; SALDANHA et al., 2017). The leaf Mn concentrations found in the current study are below (RIBEIRO, 2017) or above (CORDEIRO, 2011; LINDOLFO et al., 2020) those observed in the literature, indicating the influence of factors such as genotype, climate and plants age and also indicates the need to establish the critical level of the nutrient since it has not yet been determined for assai palm in the region (VELOSO et al., 2020).

For the Zn concentrations, higher values were found in the younger leaves (number 2 to 5) and the lower values of Zn concentrations were observed in leaves 6 to 9 (Table 3). The lowest variations of Zn concentrations were observed in the older leaves (number 6 to 9) and the smallest standard deviation was observed in leaf number 7 (Table 3). A study evaluating B fertilization in assai palm under field conditions observed leaf Zn concentrations from 22.6 to 30.6 mg kg^{-1} (LINDOLFO et al., 2020). Ribeiro (2017), evaluating adult assai palm plants, found leaf Zn concentration of 42 mg kg^{-1}, and in young plants, mean leaf Zn concentration was 29 mg kg^{-1} (CORDEIRO, 2011). In leaves of assai palm seedlings, Araújo et al. (2016) found 93 mg kg^{-1} of Zn as the mean concentration. In general, the leaf Zn concentrations are similar to those obtained in the literature for assai palm plants (CORDEIRO, 2011; LINDOLFO et al., 2020). For other palm trees that are grown, the critical level of leaf Zn of 8 mg kg^{-1} for coconut palm (SALDANHA et al., 2017) and 15.4 mg kg^{-1} for oil palm (MATOS et al., 2016) was determined.

Thus, for the micronutrients evaluated in the assai palm plants in the present study, leaf concentrations were observed in the descending order Mn > Fe > Zn > B > Cu. In oil palm plants, the order of micronutrients required was Mn > Fe > B > Zn > Cu (MATOS et al., 2016); and in coconut palm plants, the order was Mn > Fe > B > Cu > Zn (SALDANHA et al., 2017). The cationic micronutrients (Cu, Fe, Mn, and Zn) have greater availability in more acidic soils, the condition of the present study; and Fe and Mn are present in large amounts in weathered tropical soils in the form of oxides and hydroxides (ABREU et al., 2007).

Using analysis of variance for the macronutrients, the minimum number of assai plants required for each nutrient and phyllotaxic position was estimated (Table 4). Thus, with the exception of the phyllotaxic positions of the leaves for Mg and for Ca, the minimum number of plants (2 to 11 plants ha^{-1}) is in accordance with the recommendations for nutritional sampling in the literature for the estimate of the mean concentrations of the nutrients. Leaf sampling is responsible for 50 % of the variability observed in the evaluations, since it is subject to interferences of diverse factors in its composition, making it necessary to perform sampling in homogeneous plots, in an appropriate time period, and in the correct phyllotaxic position (CANTARUTTI et al., 2007). For other palm trees, Malavolta et al. (1997) recommend leaf sampling in 25 plants ha^{-1} for oil palm and 30 plants ha^{-1} for peach palm, whereas Raij (1991) recommends leaf sampling with 15 plants ha^{-1} for coconut palm, 20 plants ha^{-1} for oil palm, and 30 plants ha^{-1} for peach palm.

Table 4. Estimate of the number of palm trees required to estimate the mean level of the nutrients according to position of the leaf, with a degree of accuracy of 10 % and degree of significance of 5 %

Phyllotaxic position	Nutrient						Mean
	N	P	K	Ca	Mg	S	
2	2	3	9	49	36	11	18
3	3	3	10	44	42	12	19
4	4	3	6	37	58	11	20
5	2	3	6	37	90	11	25
6	8	4	6	29	127	12	31
7	4	2	5	29	138	14	32
8	7	3	7	28	217	15	46
9	6	3	9	23	204	18	44

In general, 50 to 100 leaves are recommended for sufficiently reliable sampling (CANTARUTTI et al., 2007). For Fontes (2016), a sample composed of 10 to 30 leaves is sufficient for species with small leaves, whereas for species that have leaves with greater areas, a larger number of replications is necessary in order to increase accuracy. It should be emphasized that each species grown has its own particularities or variabilities (FONTES, 2016). Except for Ca, P (did not differ) and K (leaves 4 to 7), in assai palm, the younger leaves (2 to 5) had estimates of a smaller number of plants for sampling that would be suitable with the aim of determination of macronutrient concentrations (Table 4).

Observing the trends of the horizontal fluctuations in the macronutrient contents, it can be determined where the difference in mean values between contiguous positions is least, and this can be associated with the vertical fluctuation, which indicates the data dispersion or the coefficient of variation of each position. The leaves most representative of the nutritional state of the plants are believed to be those in which this difference is minimal (FONTES, 2016). The regression equations obtained for each leaf sampling zone (Table 5) give a general idea of the variation in the concentrations in relation to a group of positions.

The leaves most recommended would be located in the straight-line segments, which represent the mean concentrations of the nutrients with a lower coefficient of variation. For the sampling zone, smaller variation for N, Mg, and S was found in leaves 2 to 5, and for P and Ca, in leaves 7 to 9 (Table 5). Comparison between the current results and the results reported in the literature is limited because there are no trials or indication of the phyllotaxic position for leaf sampling for assai palm plants. A study performed on physic nut (*Jatropha curcas* L.) indicated that the determination of the phyllotaxic position varies according to the nutrient (LIMA et al., 2011), as was also observed in the present study (Table 5).

The correlations between the N and P concentrations were significant and positive in all the phyllotaxic positions, except for leaf number 6 (Table 6). The positive correlation between N and P in palm trees is recognized, indicating synergistic effects between these nutrients, and this is reported for oil palm plants (MATOS et al., 2016) and coconut plants (SALDANHA et al., 2017). The correlations between N and K, as well as Ca and Mg (except for leaf number 8), were not significant; and for N and S, they were only significant in leaves number 3, 7, 8, and 9 (Table 6). The positive correlation between N and S was expected, since the two nutrients are directly involved in protein synthesis; unbalanced conditions may lead to reduced yield of the cultivated plants (PRADO, 2020). S is also involved in N assimilation in plants, since it acts in nitrite reductase (VITTI et al., 2018). These significant correlations between N and P and between N and S indicate that there is the possibility of using these nutrients to support determination of the diagnostic leaf for nutritional analysis, especially when the correlations are positive.

Table 5. Regression equations and coefficient of variation (CV) obtained for four sampling zones of assai palm leaves (2 to 9, 2 to 5, 5 to 7, and 7 to 9) for the macronutrient concentrations

Nutrient	Leaves	Linear equations	CV	Nutrient	Leaves	Linear equations	CV
N	2 – 9	y = 30.326 – 1.100x	10.45	Ca	2 – 9	y = 2.260 + 0.542x	28.31
	2 – 5	y = 28.572 – 0.554x	8.56		2 – 5	y = 2.731 + 0.412x	31.49
	5 – 7	y = 32.828 – 1.469x	10.72		5 – 7	y = 2.095 + 0.536x	27.57
	7 – 9	y = 30.634 – 1.160x	11.70		7 – 9	y =-0.079 + 0.838x	25.45
P	2 – 9	y = 1.908 – 0.054x	8.59	Mg	2 – 9	y = 0.935 – 0.072x	45.79
	2 – 5	y = 1.900 – 0.051x	8.42		2 – 5	y = 1.030 – 0.099x	35.42
	5 – 7	y = 1.930 – 0.057x	8.90		5 – 7	y = 0.863 – 0.065x	52.97
	7 – 9	y = 1.896 – 0.053x	8.35		7 – 9	y = 0.694 – 0.041x	66.73
K	2 – 9	y = 7.976 – 0.022x	13.39	S	2 – 9	y = 3.021 – 0.071x	17.55
	2 – 5	y = 8.422 – 0.360x	13.70		2 – 5	y = 3.042 – 0.078x	16.43
	5 – 7	y = 7.197 – 0.096x	11.71		5 – 7	y = 2.852 – 0.043x	17.30
	7 – 9	y = 8.547 – 0.284x	13.12		7 – 9	y = 3.383 – 0.115x	19.44

Table 6. Pearson correlation coefficient (r) between pairs of macronutrients according to the phyllotaxic position of the assai palm leaves

Pairs	Phyllotaxic position of the leaves							
	2	3	4	5	6	7	8	9
N–P	0.64^{**}	0.71^{**}	0.44^{**}	0.60^{**}	0.31^{NS}	0.71^{**}	0.40^{*}	0.51^{**}
N–K	-0.05^{NS}	0.12^{NS}	-0.15^{NS}	-0.20^{NS}	0.20^{NS}	-0.09^{NS}	-0.07^{NS}	-0.04^{NS}
N–Ca	0.08^{NS}	0.18^{NS}	0.05^{NS}	0.10^{NS}	-0.23^{NS}	-0.12^{NS}	-0.23^{NS}	-0.10^{NS}
N–Mg	-0.31^{NS}	-0.13^{NS}	0.27^{NS}	0.25^{NS}	0.06^{NS}	0.14^{NS}	0.37^{*}	0.31^{NS}
N–S	0.15^{NS}	0.33^{*}	0.24^{NS}	0.31^{NS}	0.14^{NS}	0.51^{**}	0.55^{**}	0.50^{**}
P–K	0.30^{NS}	0.22^{NS}	0.11^{NS}	0.08^{NS}	0.02^{NS}	-0.06^{NS}	0.07^{NS}	-0.06^{NS}
P–Ca	0.10^{NS}	0.16^{NS}	0.27^{NS}	0.20^{NS}	0.24^{NS}	0.04^{NS}	0.02^{NS}	0.24^{NS}
P–Mg	-0.08^{NS}	-0.15^{NS}	-0.09^{NS}	-0.06^{NS}	0.08^{NS}	-0.11^{NS}	0.04^{NS}	0.03^{NS}
P–S	0.11^{NS}	0.09^{NS}	0.24^{NS}	0.37^{*}	0.34^{*}	0.33^{NS}	0.27^{NS}	0.39^{*}
K–Ca	-0.38^{*}	-0.49^{**}	-0.54^{**}	-0.57^{**}	-0.58^{**}	-0.60^{**}	-0.57^{**}	-0.57^{**}
K–Mg	-0.08^{NS}	-0.25^{NS}	-0.43^{**}	-0.30^{NS}	-0.32^{*}	-0.30^{NS}	-0.30^{NS}	-0.12^{NS}
K–S	0.002^{NS}	0.01^{NS}	0.08^{NS}	-0.07^{NS}	-0.05^{NS}	-0.06^{NS}	-0.004^{NS}	0.04^{NS}
Ca–Mg	0.19^{NS}	0.18^{NS}	0.23^{NS}	0.03^{NS}	0.14^{NS}	-0.01^{NS}	-0.13^{NS}	-0.29^{NS}
Ca–S	0.38^{*}	0.26^{NS}	0.22^{NS}	0.19^{NS}	0.13^{NS}	0.04^{NS}	-0.17^{NS}	-0.21^{NS}
Mg–S	0.04^{NS}	0.10^{NS}	0.10^{NS}	0.12^{NS}	0.19^{NS}	0.18^{NS}	0.33^{*}	0.28^{NS}

* and ** - correlation significant at $p < 0.05$ and $p < 0.01$, respectively. ^{ns} – correlation not significant ($p > 0.05$).

The leaf P concentration also showed positive correlation with S in the phyllotaxic positions of leaves number 5, 6, and 9 (Table 6); however, it did not show significant correlation with the other macronutrients evaluated. The correlation of the leaf concentrations between K and Ca was significant and negative for all the phyllotaxic positions, and for K and Mg, there was negative correlation only in leaves number 4 and 6 (Table 6). The antagonistic interactions between K and Ca and between K and Mg were expected, since the interaction among the cations K, Ca, and Mg is known in the literature. Lima et al. (2018) affirm that there is an antagonistic relationship among K, Ca, and Mg, explained by the competition among these cations for the same absorption sites. For the other possibilities of correlation between the macronutrients, there was significance only between Ca and S (leaf number 2) and between Mg and S (leaf number 8) (Table 6).

Dependency relationships and/or interactions between the concentrations of nutrients in the leaves can be observed through principal component analysis (PCA) (Figure 3a). An interaction of positive loads is found between N and P, as well as Cu and Mg, and interactions of negative load of Ca and Mn for the F1 and F2 components (Figure 3a). PCA indicated a dependence relationship or interactions among the nutrients in the assai palm plants. Studies on other palm trees in the Eastern Amazon have already indicated the importance of the N and P relationship in nutrition of the plants, as also observed in the present study (Table 6), recommending a N and P ratio of 16 for oil palm (MATOS et al., 2016) and near 14 for coconut palm (SALDANHA et al., 2017).

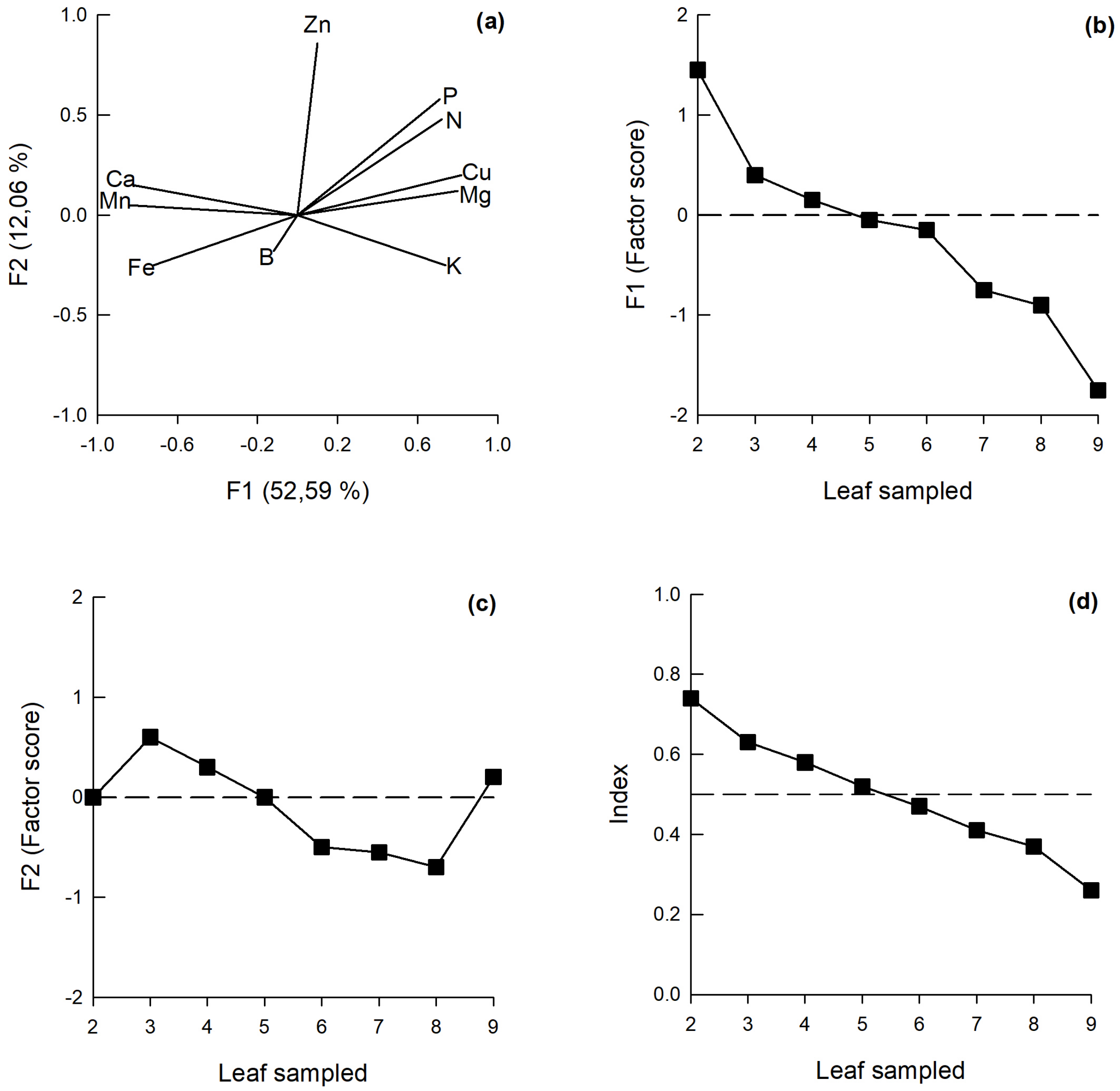

Figure 3. Factor loadings extracted by principal components of Factor 1 (F1; 52.59 %) and Factor 2 (F2; 12.06 %) for the leaf concentrations of the nutrients (a), values of factor score in the factors F1 (b) and F2 (c) according to position of the sampled leaf, distribution index (Index) of the nutrients in the leaves (d).

In nutrient uptake, competitive inhibitions may also occur between Cu and Zn, and K and N (PRADO, 2020). In addition, the interaction among K and Ca is known in the literature; increase in K supply may lead to reduction in the concentrations of Ca and, at high doses, is even able to decrease crop production (LIMA et al., 2018). Furthermore, the antagonistic interaction between Ca and Mg is known, in which the excess of one impedes uptake of the other (PRADO, 2020). On the other hand, there is an interaction of positive loads between Cu and Mg in assai palm leaves, indicating a positive result of the indirect relationship of Cu with K and Ca, promoting greater uptake of Mg and Cu, since Cu is able to competitively inhibit K uptake and, consequently, reducing competition between K and Mg in the uptake sites.

In the current study, the assai palm plants showed a positive interaction (synergism) between the leaf concentrations of Ca and Mn (Figure 3a), although the negative interaction between Ca and Mn is also cited in the literature, since the presence of other ions in high concentrations is able to reduce uptake of Ca, as well as N, K, Mg, Al and Mn (PRADO, 2020).

The F1 factor indicates the existence of the process of interaction of the N, P, K, Mg, and Cu nutrients within the plant tissues of the leaves sampled, in which the nutrients that have positive factor loading are inversely correlated with those of negative factor loading (Figures 3b and 3c). In multivariate factor analysis, the factors generated represent condensation of the original variables in only one latent variable (HONGYU et al., 2016). Thus, in Factor 1, when the values are nearer the factor loading equal to +2.0, the greater the correlation between the concentrations of N, P, K, Mg, and Cu, and the lower the concentrations of Ca, Mn, and Fe (Figure 3a). When the values of the factor loading draw near -2.0, the inverse process occurs in the interaction of the nutrients present in Factor 1 (Figure 3a).

The values of factor loadings nearer or equal to zero indicate that there is equilibrium among the nutrient concentrations contained in the leaves. Thus, those leaves that have this response in the multivariate factor analysis indicate equilibrium among the nutrient concentrations. In the present study, leaf number 5 had a factor loading value near zero (-0.03±0.07), indicating it to be the leaf that has the least variation in the concentrations of the nutrients N, P, K, Ca, Mg, Cu, and Fe (Figure 3d). In the analysis using the index integrated with the three factors extracted that represent the variation among the nutrient concentrations in the leaves of assai palm, leaf number 5 exhibited the value of the index near 0.50 (Figures 3a and 3b). The nearer to the mean value of the index, which ranges from 0 to 1, the lower the variation in the nutrient concentrations analyzed in the sampled leaf, indicating equilibrium among the concentrations. Thus, under the conditions of the present study, leaf number 5 is most recommended for nutritional diagnosis of assai palm grown in the northeast of Pará.

Conclusions

The highest leaf nutrient concentrations are in the young leaves (N, P, K, Mg, S, Cu, and Zn) and were in the following order: N > K > Ca > S > P > Mg > Mn > Fe > Zn > B > Cu;

Sampling of leaves 2 to 5 shows lower variation in the leaf nutrient concentrations in assai palm plants;

From the index of distribution of the nutrients in the assai palm leaves, leaf number 5 is recommended for sampling for nutritional diagnosis of the crop in the region;

The statistical analysis methods of analysis of variance and of multivariate analysis showed similar results, both indicating leaf number 5 as the most appropriate for representing the nutritional status of assai palm;

The estimated number of assai palm plants for foliar sampling for nutritional diagnosis is 25 plants.

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