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Selection of index leaf for foliar diagnosis of critical nutrient levels in physic nut (*Jatropha curcas*)

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Abstract

Basic information about mineral nutrition of physic nut is scarce. Especially, determination of index leaf to evaluate nutritional status is very rare. Therefore, objective of this study is to find the index leaf, the type and leaf position in the floral branch and propose critical levels of nutrients for nutritional diagnosis of physic nut. The nutrient concentrations in leaf limb, petiole and complete leaf and shoot dry weight of plant were recorded from the experiment under nutrient omissions in greenhouse. The complete leaf samples were also collected from two types of branches (main and lateral) and in three positions (apical, medial and basal of the branch), corresponding to the branches between the first and fourth, between the fifth and eighth and between the ninth and twelfth leaves, respectively, at flowering and seed productivity stages. These data were obtained from an experimental plot of NPK fertilization to define the position of the complete leaf to be sampled and to propose values of critical nutrient levels for physic nut. Critical levels of nutrients were proposed by means of reduced normal distribution criterion with field experiment data. The complete leaf (petiole and leaf limb) in the median position between the fifth and eighth leaves in the main and lateral branches in the full flowering of crop was indicated as sampling standard for the diagnosis of nutritional state of physic nut. In the present work, the critical levels of and macronutrients in leaf sampling were proposed as following (g kg^{-1}): 27.8 for N, 1.4 for P, 11.8 for K, 12.8 for Ca, 6.7 for Mg and 1.3 for S and, micronutrients (mg kg^{-1}): 78.8 for B, 14.2 for Cu, 139.7 for Fe, 129.3 for Mn and 20.4 for Zn.

Keywords: biodiesel; critical levels; leaf sampling; nutrients; nutritional status.
Abbreviations: n_i_ nutrient concentration, r_ simple linear correlation, R^2_ determination coefficient, s_1 and s_2_standard deviation, SDW_ shoot dry weight, X_1 and X_2_arithmetic mean, Y_ relationship between productivity and n_i.

Introduction

Physic nut (*Jatropha curcas* L.) is a species with great potential for the production of biodiesel. Its oil-rich seed can be easily converted into biodiesel, matching international standards (Mahanta et al., 2008). The species is a shrubby plant belonging to the Euphorbiaceae family and widely distributed in tropical and subtropical regions (Parawira, 2010). Physic nut is still an untamed plant. Only in recent years, the agronomic studies on this species have been stated. The efficient production of large-scale physic nut depends on greater investments in research to improve the cultivation techniques, especially nutrition and fertilization (Silva et al., 2009).

Soil chemical analysis is the routine form to evaluate soil fertility and fertilization of different crops (Fageria et al., 2009). Normally, nutritional diagnosis of plants is performed by evaluation of results of foliar chemical analysis, among others, constituting a tool that allows planning, calibrating fertilization for crops, being complementary to the soil analysis (Fageria, 2007, Prado and Caione, 2012). Foliar analysis has been widely used for diagnosis of plant's nutritional status. It is based on the fact that there is a direct correlation between growth or yield and nutrient concentration in plants tissues (Marschner, 2012). From this correlation, values are established for nutrient concentrations corresponding to changes in terms of yield. These points represent critical levels and delimit ranges of concentrations related to nutrient deficiencies and appropriate levels or toxicity of nutrients (Mourão Filho, 2004, Prado and Caione, 2012). There are some other factors that can determine the variations in nutrient concentrations in leaves, such as leaf age and its position in the branch, fruiting performance, cultivar, genotype, soil type and cultural practices and position of leaves on plant (Lima et al., 2007).

There is no consensus in interpretation of data from several application of methods for the diagnosis of nutritional status in physic nut. Especially, there is problem to indicate the best part or tissue of plant to evaluate the nutritional state. Therefore, regardless of correlation of the yield or growth of the plant with the foliar concentrations of nutrients, Lima et al. (2011b) could not define a standard leaf sampling for physic nut, where the position of leaf collection on the branch varies widely according to the nutrient. It is presumed that the leaves harvested from the second and third positions are more adequate for diagnose of the nutritional status of N, P, K, S, Cu, Fe, Mn and Zn in plants. It has also been recommended that samples from the basal leaves, those located in the fifth or tenth position are more appropriate for detection of Ca and Mg, because in this phenological stage, the vegetative or floriferous branch does not influence the nutrient concentrations, except Cu and Fe. Also without correlation, Kurihara and Silva (2015) indicated the collection of sixth and tenth position leaves in floral branches of the upper third of the plant because they were more sensitive to variations in availability of macronutrients in the soil for nutritional diagnosis of physic nut. Without the objective of obtaining the index leaf for physic nut, Laviola and Dias (2008) obtained nutrient concentrations of crops by sampling leaves with expanded limbs, located between the sixth and eighth leaves from the apex of the branch below the inflorescence, without specifying type of main or lateral branch.

Several factors may cause variations in nutrient concentrations in leaves, such as leaf age and its position in the branch, fruiting performance, cultivar, genotype, the soil type and cultural practices (Fageria, 2007; Fageria et al., 2009, Prado and Caione, 2012). Therefore, there is no information on the index leaf for foliar diagnosis in physic nut within the premise of a well-defined relationship between growth and crop yield and the nutrient concentration in the tissues (Fageria et al., 2009; Marschner, 2012, Prado and Caione, 2012). Given the above, the study aimed to define the index leaf, the type and leaf position in the floral branches and to propose the critical level of nutrients for nutritional diagnosis of physic nut.

Results and Discussion

Definition of leaf index for physic nut

The multiple linear regressions between the nutrient concentrations in leaf limb, petiole and complete leaf and shoot dry weight of physic nut were significant (F-test) with better adjustment index results (R^2 and R^2 adjusted) (Table 1). The complete leaf presented higher value of R^2 adjusted between nutrient concentrations and shoot dry weight of physic nut, which did not occur for the petiole and leaf limb (Table 1). The estimated parameters of the regression (intercept and nutrient concentrations) were all significant at the 1% level by t-test for leaf limb, petiole and complete leaf (Table 1) showing the relationship between nutrients and the plant growth (Fageria et al., 2009, Marschner, 2012, Prado and Caione, 2012).

The statistical study of the standardized residual was adequate for this type of procedure (Seber and Lee, 2003), since the points are random and homogeneous around the horizontal axis with value equal to zero. This characterizes a better result for the linear regression between the shoot dry weight of physic nut and the nutrient concentrations for leaf limb, petiole and complete leaf (Fig. 1). In addition, simple linear correlations (r) were elevated between the estimated and observed shoot dry weight of physic nut (Fig. 1). In this way, it was possible to use leaf limb, petiole and complete leaf. However, the complete leaf sampling and leaf indexing would be ideal for standardization of the nutritional status and recommendation to physic nut farmers.

The field experiment was used to define the type (main and lateral) and position (apical, median and basal) of the complete leaf in the floral branch of physic nut. The multiple linear regressions adjusted between the seeds productivity of physic nut and all the nutrient concentrations in the complete leaf at full flowering of the plants can verify that the sampling of the complete leaf from the median position of main and lateral branch presented statistical significance (F test) and the highest adjustment indexes (R^2 and R^2 adjusted) (Table 2).

The estimated parameters of the regression (intercept and nutrient concentrations) were all significant at the 1% level by t test (Table 2) for sampling the complete leaf in the median position (between the fifth and eighth leaves) in main and lateral branch showing the relation that the nutrient concentrations have with the plant productivity (Fageria et al., 2009; Marschner, 2012, Prado and Caione, 2012). The statistical study of the standardized residues showed a better result for the regression, when sampling of the complete leaf was done from the median position in main and lateral branch. It showed greater simple linear correlation (r) between the estimated and observed values for seeds productivity in physic nut (Fig. 2).

Using the index leaf for foliar diagnosis of crops is based on the direct correlation between growth rate or yield and the nutrient concentrations in tissues (Fageria et al., 2009; Marschner, 2012, Prado and Caione, 2012). Therefore, failure to correlate all nutrients with crop growth and yield may provide misleading recommendation of the sampled leaves and leaf index to diagnose the nutritional status of plants. Without correlating the yield or growth of the plant with the nutrient concentrations, a wide variation was observed for physic nut as a function of the nutrient to be diagnosed (Lima et al., 2011b). For N, P, K, S, Cu, Fe, Mn and Zn, the leaves harvested from the apical position are more appropriate. For the Ca and Mg, sampling from the basal leaves is more appropriate and phenological stage of the vegetative or floriferous branch does not influence the nutrient concentrations, except Cu and Fe (Lima et al., 2011b). Similarly, Kurihara and Silva (2015) recommended sampling of leaves from those located between the sixth and fifteenth of the floral branches for nutritional diagnosis of physic nut, because they are more sensitive and reflective to variations in nutrient availability in the soil.

Foliar diagnosis is recommended to collect physiologically mature leaves, i.e. those with photosynthetic activity at its maximum and fully forms (Marschner, 2012, Prado and Caione, 2012). These leaves behave as a source of photoassimilates (Koch, 2004). Young leaves collected at the apex of the branch are physiologically immature with low photosynthetic activity. At this stage, they function as a drain, with older leaves presenting a reduction of physiological activity with the onset of senescence (Kitajima et al., 2002). The optimum physiological functioning on the leaf limb of the physic nut defends on the perfect mineral nutrition of the plant. These nutrients are directly linked to the electron transport complex in the chloroplasts, related to the oxidation reactions of elements such as Fe and Cu, Mg. For example, is constituent of the chlorophyll molecule and N is present in all protein complexes (Lima et al., 2011a). In the senescence process leaf, translocation of mobile nutrients towards the younger leaves and reproductive organs of the plant are occurred (Watanabe et al., 2007).

Table 1. Parameters of multiple linear regression equations between nutrient concentrations in leaf limb, petiole and complete leaf as independent variables and shoot dry weight (g plot^{-1}) of physic nut as dependent variable.

Independent variable	Leaf limb	Petiole	Complete leaf
	....	Estimated parameter	....
Intercept	31.0414**	24.0400**	39.5593**
N	-0.6727**	-2.3688**	-1.1750**
P	0.6006**	38.1255**	2.4453**
K	-2.0890**	-0.6963**	-2.8064**
Ca	5.8060**	4.5690**	5.4642**
Mg	-6.3568**	-101.1976**	-9.5838**
S	-14.8901**	87.8634**	-1.2591**
B	-0.0211**	-0.3388**	-0.0685**
Cu	-0.0416*	0.3135**	-0.0428**
Fe	-0.0237*	0.0269**	-0.0345**
Mn	-0.0615**	-0.4190**	-0.0551**
Zn	0.0851**	-0.2720**	-0.0832**
F test	4.3743**	4.5867**	5.9286**
R^2	0.9855	0.9721	0.9962
R^2 adjusted	0.9723	0.9502	0.9926

**Significant at p = 0.01. Concentration values for macronutrients are in g kg^{-1} and micronutrients in mg kg^{-1}.

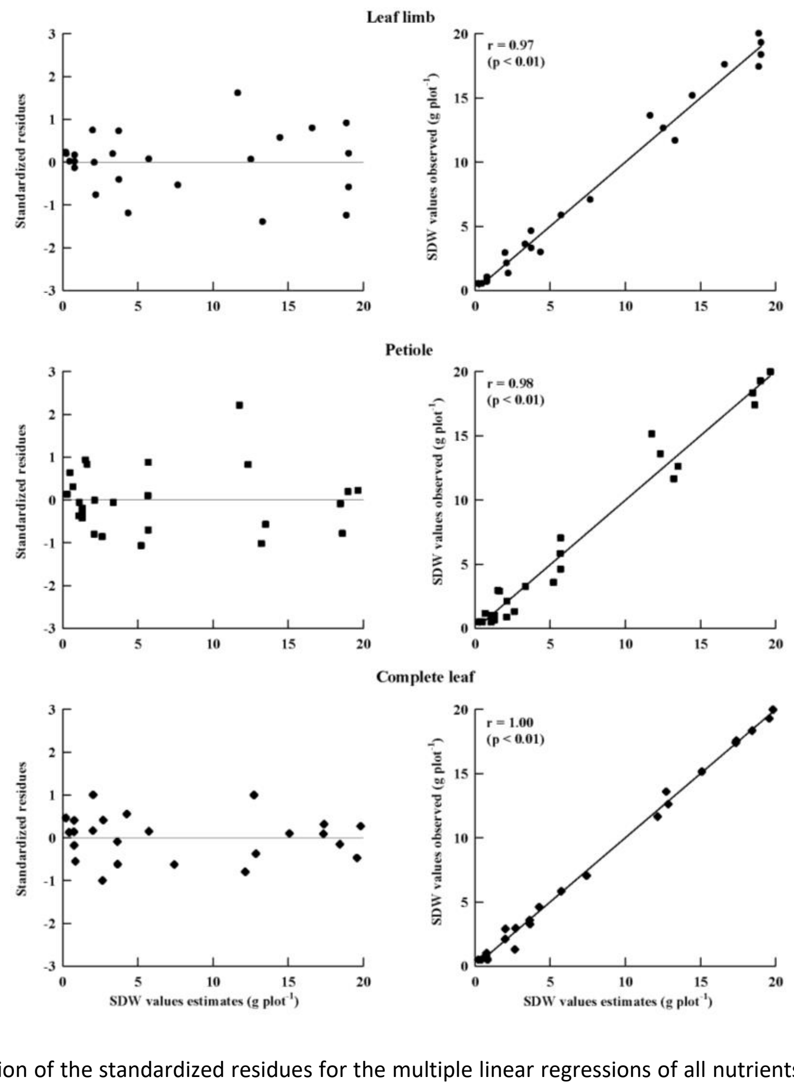

Fig 1. Graphical distribution of the standardized residues for the multiple linear regressions of all nutrients in leaf limb, petiole and completed leaf with the estimated shoot dry weight (SDW) of physic nut and the simple linear correlation (r) between SDW values observed and estimates.

Table 2. Parameters of multiple linear regression equations between complete leaf nutrient concentrations in two types of branches (main and lateral) and in three positions (basal, median and apical of the branch) as independent variable and seed productivity (kg ha^{-1}) of physic nut as dependent variable.

Independent variable	Main branch			Lateral branch		
	Basal	Median	Apical	Basal	Median	Apical
	.... Estimated parameter............					
Intercept	-56320.01	-54136.13**	12163.21	-65013.23	105142.21**	58698.24
N	643.84	600.96**	-126.12	737.87	-1.199.95**	-666.15
P	9.934.45	9.497.57**	-2.034.65	11.442.05	-18.618.04**	-10.396.04
K	-524.02	-491.82**	110.87	-596.46	969.06**	543.41
Ca	315.53	312.08**	-69.24	371.74	-599.64**	-327.78
Mg	-983.14	-962.75**	87.28	-1.189.63	1.989.97**	1.097.94
S	961.97	1.072.63**	-212.92	1.086.25	-1.688.85**	-975.45
B	1.509.67	1.463.84**	-311.97	1.751.16	-2.841.47**	-1.587.26
Cu	-156.23	-156.85**	34.95	-172.36	291.68**	162.37
Fe	-559.24	-542.56**	115.13	-648.48	1.053.53**	587.44
Mn	-240.91	-234.03**	53.07	-278.81	457.34**	252.65
Zn	-911.09	-881.91**	190.91	-1.060.62	1.720.85**	964.16
F test	1.35	3.96**	1.17	1.88	3.67**	0.88
R^2	0.53	0.96	0.57	0.37	0.94	0.41
R^2 adjusted	0.14	0.72	0.24	-0.13	0.70	-0.05

**Significant at p = 0.01. Concentration values for macronutrients are in g kg^{-1} and micronutrients in mg kg^{-1}.

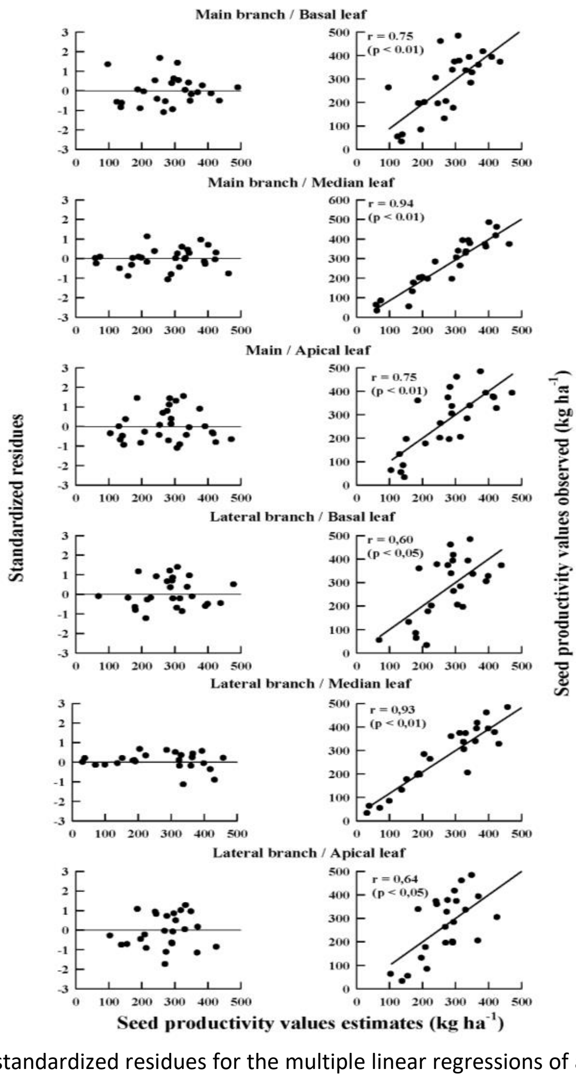

Fig 2. Graphical distribution of the standardized residues for the multiple linear regressions of all nutrients in two types of branches (main and lateral) and in three positions (basal, median and apical of the branch) with the estimated seed productivity of physic nut and simple linear correlation (r) between the seed productivity values observed and estimates.

Table 3. Mean, standard deviation (s) and Lilliefors test for seed productivity and relation between productivity and nutrient concentration in the complete leaf in the median position of main and lateral branches of physic nut to obtain the critical levels of nutrients by reduced normal distribution criterion.

Variable	Complete leaf median					
	Main branch			Lateral branch		
	Mean	s	Lilliefors test	Mean	s	Lilliefors test
Seed productivity	280.5	137.2	0.098**	280.5	137.2	0.098**
Seed productivity / N	11.2	5.9	0.098**	7.9	5.2	0.016**
Seed productivity / P	226.2	156.2	0.098**	164.0	87.6	0.091**
Seed productivity / K	19.2	9.4	0.081**	20.9	22.9	0.029**
Seed productivity / Ca	17.4	11.2	0.024**	22.6	14.3	0.015**
Seed productivity / Mg	41.7	19.8	0.086**	43.1	20.4	0.101**
Seed productivity / S	218.2	106.7	0.097**	203.1	106.3	0.078**
Seed productivity / B	2.9	1.6	0.187**	4.0	2.3	0.114**
Seed productivity / Cu	11.9	6.6	0.104**	10.4	5.6	0.125**
Seed productivity / Fe	6.0	4.3	0.117**	6.3	4.1	0.135**
Seed productivity / Mn	6.8	7.5	0.131**	8.1	5.2	0.158**
Seed productivity / Zn	6.9	10.7	0.142**	8.8	12.2	0.139**

**Significant at p = 0.01. Productivity is in kg ha^{-1}. Concentration values for macronutrients are in g kg^{-1} and micronutrients in mg kg^{-1}.

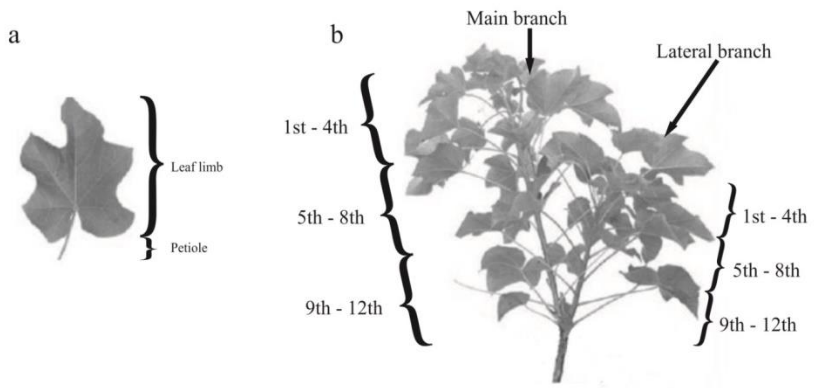

Fig 3. Sampling scheme of leaf type to be sampled (leaf limb, petiole and, complete leaf) (a) two types of branches (main and lateral) and in three positions (apical, median and basal of the branch) that correspond to the branch between the first (1st) and fourth (4th), the fifth (5th) and eighth (8th) and the ninth (9th) and twelfth (12th) (b) physiologically mature leaves in flowering of physic nut.

Table 4. Critical levels of nutrients in complete leaf in the median position of main and lateral branches of physic nut by reduced normal distribution criterion.

Nutrient	Complete leaf median			Nutrient concentrations			
	Main branch	Lateral branch	Mean	Raviv and Lieth (2008)	Laviola and Dias (2008)	Lima et al. (2011b)	Kurihara and Silva (2015)
N	24.3	31.4	27.8	10-56	31.4	43.0	25.5
P	1.1	1.7	1.4	1.2-5.0	2.8	2.7	2.4
K	14.6	9.1	11.8	14-64	13.7	32.8	20.8
Ca	14.4	11.2	12.8	2.0-9.4	19.0	14.8	10.2
Mg	6.8	6.6	6.7	1.4-2.1	4.8	10.7	9.8
S	1.3	1.3	1.3	2.8-9.8	1.1	2.1	1.1
B	92.4	65.3	78.8	1-35	29.2	-	-
Cu	12.5	15.9	14.2	2.3-7.0	10.0	10.6	-
Fe	139.7	139.8	139.7	53-550	150.5	77.0	-
Mn	127.8	130.8	129.3	50-250	314.5	120.3	-
Zn	22.2	18.7	20.4	10-100	22.7	26.7	-

Concentration values for macronutrients are in g kg^{-1} and micronutrients in mg kg^{-1}.

Thus, it is understandable that the leaves with the highest degree of maturity are ideal samples for leaf diagnosis of the physic nut crop.

Critical nutrient level

The data of complete-leaf obtained from the median position of main and lateral branches in full flowering was submitted to the methodology of the critical levels of nutrients by the criterion of the reduced normal distribution (Maia et al., 2001). This has been characterized as efficient and its use is fully feasible for nutrient standards of this crop (Table 3). Although the data are obtained from a crop yield cycle, these new data may eventually be used to evaluate the nutritional status of the physic nut as a first approximation.

The critical levels of nutrients from leaves of physic nut obtained from median position of main and lateral branches (Table 4) were high for Ca, Mg, B and Cu, adequate for N, P, Fe, Mn and Zn and low for K and S, when compared to the established range proposed by Raviv and Lieth (2008). For average concentrations in leaves of branches with inflorescence, Laviola and Dias (2008) found concentrations above the critical levels of this work for N, P, K, Ca, Fe, Mn and Zn and below for Mg, S, B and Cu in physic nut. In medium nutrient concentrations, Lima et al. (2011b) reported that in physic nut plants the critical levels were low for the macronutrients and high for the micronutrients except to Zn, with low concentrations, when sampled leaves from the basal section of floral branches from fifth to tenth position from the apex without mentioning the position. For the average macronutrient concentrations in leaf samples located from the sixth to fifteenth position from the apex of floral branches in the middle third of physic nut, Kurihara and Silva (2015) found that, the critical levels were high for Ca, adequate for N and S and low for P, K and Mg.

In this study, changes in the classification of critical levels of nutrients can be observed compared with other reports (Raviv and Lieth, 2008, Laviola and Dias, 2008, Lima et al., 2011b and Kurihara and Silva, 2015). The differences in recommendation of plant parts to be sampled caused misunderstandings in the diagnosis of the nutritional state of physic nut. In this work, the definition of index leaf and critical levels of nutrients for physic nut may reduce the occurrence of inadequate diagnosis of nutritional deficiencies, excesses or imbalances, which may reduce increases in crop production cost.

Materials and Methods

Physic nut and growing conditions

The experiments were carried out in Diamantina, state Minas Gerais, Brazil (18º15'S, 43°36'W, 1,250 m a.s.l.) in a greenhouse in a nutrient solution, and in Governador Valadares, state Minas Gerais, Brazil (18º42'S, 42°03'W, 170 m a.s.l.) in field condition.

The greenhouse data in nutrient solution referred to an experiment that utilized the missing element technique. The experimental was designed in completely randomized with three replications and twelve treatments: complete nutrient solution and the individual omissions of N, P, K, Ca, Mg, S, B, Cu, Fe, Mn and Zn, totally 36 experimental plots including one plant in each pot. The experimental plot was black-colored pots with 4.0 L capacity, with addition of 3.0 L of nutrient solution.

The solutions were prepared with analytical reagents, and the complete nutrient solution was prepared in accordance with Hoagland and Arnon (1950) as following: 210.1 mg N, 31 mg P, 234.6 mg K, 200.4 mg Ca, 48.6 mg Mg, 64.2 mg S, 500 µg B, 20 µg Cu, 648 µg Cl, 5,022 µg Fe, 502 µg Mn, 11 µg Mo and 50 µg Zn per liter of nutrient solution. For the other treatments, the nutrients concentrations were identical to those of the complete solution, except for the omitted nutrient.

Physic nut plants were grown from seeds of a population provided by Company of Agricultural Research of Minas Gerais, from the center in the North of the State Minas Gerais, in Nova Porteirinha (15º48'S, 43°18'W, 500 m a.s.l.). Seeds were sown in sand trays and irrigated daily with deionized water, until transplanting to the pots. On this occasion, the seedlings had an average diameter of 5.0 mm had an average height of 0.09 m, 30 days after emergence. After the transplanting of the seedlings, 3.0 L of nutrient solution with half-concentration and the rest after the third day of the transplant of the seedlings were given. They were renewed every 10 days, with continuous artificial aeration system using air compressor. The pH of the nutrient solution was maintained about 6.0 ± 0.1; applying HCl 0.1 mol L^{-1} or NaOH 1.0 mol L^{-1}, with daily control as needed, using a portable pH meter. At 100 days after the start of the experimental period, seedlings were evaluated for their shoot dry weight, dry weight of stem, petiole and leaf limb. The last two parts were used to define the index leaf of physic nut.

To study NPK doses the physic nut plant were cultivated in Typic Hapludox classified according to Soil Taxonomy (Soil Survey Staff, 2014). Soil samples were air-dried, sieved (2.0 mm) and characterized according to Claessen et al. (1997) with the following results: pH_{water}=6.9; $P_{\text{Mehlich-1 extractor}}$ =10 mg kg^{-1}; $K_{\text{Mehlich-1 extractor}}$ =1.8; $Ca_{\text{KCl 1 mol L-1 extractor}}$ =21; $Mg_{\text{KCl 1 mol L-1 extractor}}$ =9; $Al_{\text{KCl 1 mol L-1 extractor}}$ =1; Cation-exchange capacity =58 $mmol_c$ kg^1; Bases saturation =55%, Organic carbon = 7 and Clay = 360 g kg^{-1}. The average annual precipitation was 1,478 mm and the average temperature 24.2 °C. The experiment was arranged in a randomized blocks, fractional factorial design $(4 \times 4 \times 4)^{½}$, with 32 treatments, totalizing 32 experimental plots. The following nutrient rates were applied: 0, 25, 50 and 100 kg ha^{-1} N, as urea; 0, 75, 150 and 300 kg ha^{-1} P, as triple superphosphate, and 0, 50, 100 and 200 kg ha^{-1} K, as potassium chloride. The experimental plot consisted of 18 plants, spaced 2.5 x 2 m (density of 2,000 plants ha^{-1}) being the plot useful to the four central plants. Soil preparation of the experimental area was conducted in conventional manner (plowing and harrowing). The fertilization with micronutrients was 1 kg of B (boric acid) and 4 kg of Zn (zinc sulfate) per ha.

At 12 months after planting the seedlings, leaf samples with complete leaf (petiole and leaf limb) were collected in the four useful plants of the experimental plots, in two types of branches (main and lateral) and in three positions (apical, median and basal of the branch), corresponding to the branches between the first and fourth, between the fifth and eighth and between the ninth and twelfth with physiologically mature leaves, respectively, in the flowering stage of crop. Each sample consisted of 15 leaves, counted in the apex direction for the base of the branches. Seed productivity of physic nut was evaluated by a useful plot in the year 2008, with seed moisture corrected to 12%.

Measurements

The petiole, leaf limb and complete leaf samples were dried in a forced-air oven at 70 C, ground and digested (nitric-perchloric acid) for nutrient determination. Concentrations of P, K, Ca, Mg, S, Cu, Fe, Mn and Zn (nitric-perchloric acid digestion) were determined by molecular absorption spectrometry (P), emission flame photometry (K), atomic absorption spectrophotometry (Ca, Mg, Cu, Fe, Mn and Zn), barium sulfate turbidimetry (S), N (sulphuric acid digestion) by the semi-micro-Kjedahl method and and B-incineration (muffle) by molecular absorption spectrometry.

Calculations and statistics

From greenhouse data, dry weight data of 36 shoots was measured. The dependent variable and the concentration of all nutrients in the leaf limb, petiole and complete leaf (petiole and leaf limb) were measured and sampled according to Fig. 3a. The independent variables were subjected to multiple linear regression study with the purpose to find the best index leaf of physic nut. Field experiment data of 32 experimental plots were subjected to multiple linear regression study between the concentrations of all nutrients in the two types of branches (main and lateral) and three leaf positions (apical, median and basal of the branch), sampled according to Fig. 3b, as independent variable and seeds productivity of physic as dependent variable.

The selection of index leaf in type and position in physic nut branch was considered: significance of the multiple linear regression, the adjustment index (R^2 and R^2 adjusted), the significance of each estimated nutrient parameter of the multiple linear regression with shoot dry weight part and seeds productivity of physic nut, study of the graphic distribution of standardized residues (Seber and Lee, 2003) and simple linear correlation (r) of observed and estimated shoot dry weight and seeds productivity of physic nut.

The critical levels of nutrients for the physic nut were obtained after the definition of the index leaf, using the criterion of the reduced normal distribution (Maia et al., 2001). The normality of the variables was tested by the Lilliefors test (Conover, 1971). The methodology consisted of verifying (i) if the productivity data have normal distribution, and (ii) if negative, can cause transformation of the data. When productivity has a normal distribution, the arithmetic mean (X_1) and the standard deviation (s_1) of the productivity were calculated. Next, productivity representing 90 % of the maximum was calculated by Equation 1.

$$\text{Productivity}_{90\%} = 1.281552{*}s_1 + X_1 \qquad (1)$$

The next step calculated the variable Y by Equation 2, where n_i is the nutrient concentration, desirable to find the critical level.

$$Y = \text{productivity} / n_i \qquad (2)$$

Subsequently, the Y values were verified for the normal distribution and the arithmetic mean (X_2) and the standard deviation (s_2) of the created variable calculated. Then, the value of Y referring to 90 % of the maximum was calculated by Equation 3.

$$Y_{90\%} = 1.281552{*}s_2 + X_2 \qquad (3)$$

Knowing that Y = productivity / n_i and replacing productivity with 90% of maximum (Equation 1), the critical level was calculated by Equation 4, for nutrient n_i.

$$n_i = 1.281552{*}s_1 + X_1 / 1.281552{*}s_2 + X_2 \qquad (4)$$

The critical levels of nutrients in the physic nut leaves were compared with those proposed by Raviv and Lieth (2008) in plant tissues and medium concentrations found by Laviola and Dias (2008), Lima et al. (2011b) and Kurihara and Silva (2015) on physic nut leaves.

Conclusion

Sampling of the complete leaf (petiole and leaf limb) in the median position between the fifth and eighth leaves in the main and lateral branches in the full flowering of crop can be used as sampling standard for diagnosis of nutritional state in physic nut. The critical levels of nutrients for the diagnosis of the nutritional state of physic nut were proposed as following for macronutrients (g kg^{-1}): 27.8 for N, 1.4 for P, 11.8 for K, 12.8 for Ca, 6.7 for Mg and 1.3 for S and, for micronutrients (mg kg^{-1}): 78.8 for B, 14.2 for Cu, 139.7 for Fe, 129.3 for Mn and 20.4 for Zn.

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