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Preliminary Diagnosis and Recommendation Integrated System (DRIS) Norms for *Hevea brasiliensis* **Grown in the Humid Forest Zone of Cameroon**

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Authors' contributions

All the authors participated in preparing the protocol for sample collection. The samples were collected by author NJN who equally drafted the manuscript which was corrected by authors EEE and EGN. Author ES managed the analysis of soil samples in the laboratory. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

The main source of natural rubber is the *Hevea brasiliensis,* an important crop to the Cameroonian economy. In order to improve yields in a sustainable manner, nutrient norms must be derived for proper nutrient and sustainable soil fertility management. The aim of this study was to derive Diagnosis and Recommendation Integrated System (DRIS) norms for *Hevea brasiliensis* grown under the ecological conditions of Cameroon. A survey of 130 *Hevea brasiliensis* fields was carried out to collect leaf samples and yield data. The leaves were analysed for N, P, K, Ca, Mg, S, Fe, Mn, Mo, Na and Zn. Using the yield data, the sampled population was divided into high- and low–yielding sub populations. The DRIS norms were derived from a high-yielding sub-population (fields yielding >1, 486kg/ha) following standard procedures. Some of the DRIS norms obtained as ratios of nutrients presented significant differences between low- and high- yielding

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sub-populations. The DRIS norms were made up of ratios of macro nutrients, ratios of micro nutrients as well as ratios of macro and micro nutrients. Some of the obtained norms were different from literature reports that locally derived norms can be more accurate for nutrient diagnostic purposes. The DRIS indices were calculated and used for the assessment of the nutrient status of a low- yielding sub-population of *Hevea*. The results showed that most macro nutrients were deficient. The obtained nutrient norms will be used for nutrient assessment in *Hevea* plantations in Cameroon.

Keywords: Hevea brasiliensis; DRIS norm; macronutrients; micronutrients; yields.

1. INTRODUCTION

Natural rubber is an important crop to the economy of Cameroon. It is obtain from *Hevea brasiliensis*. In order to improve and obtain sustainable yields, proper management of all essential nutrients is required. This leads to minimizing waste, economic losses and environmental impacts [1,2]. Proper nutrient management begins with adequate and correct nutrient diagnoses where the nutrient status is accurately determined. The use of plant chemical analysis for nutrient diagnosis is based on the assumption that causal relationships exist between growth rates (yield) and nutrient content in the plant part tested [3]. For the full benefit of the use of foliar or tissue analysis for assessing plant nutrient status to be obtained, adequate procedures for the interpretation of analytical data must be available [4].

Critical nutrient concentrations have frequently been used to diagnose nutritional status of plants [5,6]. However, the critical nutrient approach has some limitations based on the fact that the 'critical nutrient concentrations' can vary in magnitude as the background concentrations of other nutrients vary in crop tissue [7]. In order to circumvent this difficulty, Beaufils in 1973, and Walworth and Sumner (1987), proposed the Diagnosis and Recommendation Integrated [3] System (DRIS) method for nutrient evaluation. This method considers nutrient ratios in order to avoid the effect of leaf age and physiological interactions [8,9], which limits the applicability of leaf tissue analysis for nutrient diagnosis [4]. It is designed to assess relative nutrient imbalances or deficiencies or both, in plant tissue [10,11]. Nutrient balance is an inherent part of the DRIS system. It is suggested that the DRIS method might be more accurate in the detection of nutritional deficiencies and/or excesses because it considers relationships among nutrients [9]. Once the ratios have been established, the data is divided into low- and high-yielding sub-populations. Following the DRIS evaluation procedure, the ratios of nutrients in a representative sample are compared with the mean ratios of these elements in high-yielding populations [8].

The DRIS method provides the relative order of nutrient need [12] as well as the overall status of nutrient balance in the plant [9]. It would be possible to have all low nutrient levels in a plant, and still have the nutrient ratios within the optimal range because DRIS is based on nutrient ratios and balance [9]. One of the most important advantages of using DRIS diagnosis is that it is applicable irrespective of varietal or geographic origin or both [13]. However, other workers working on maize suggested that locally calibrated norms may be more accurate in diagnosing nutrient deficiencies than norms developed from plant materials gathered in other geographic regions [14]. DRIS norms for *Hevea* applied in Cameroon were derived in other rubber producing countries thus the objective of this study was to derive DRIS norms for *Hevea brasiliensis* under the ecological conditions of Cameroon.

2. MATERIALS AND METHODS

2.1 The Study Area

A total of 130 leaf samples were collected from rubber estates belonging to the Cameroon Development Corporation of Cameroon and Socièté Forestiere Agricole du Cameroon (SAFACAM) (Fig. 1). The climate of the study site is characterized by high temperatures and seasonal rainfall. The mean temperature ranges from 25 - 28ºC and rainfall ranging from 700 - 1250 mm. The soils depth ranged from shallow to deep and the texture from sandy loam through clay loam to sandy clay. Following the FAO classification the soils ranged from Ferric/Humic Acrisols to orthic/xanthic Ferrasols.

2.2 Leaf Sampling and Analysis

2.2.1 Leaf sampling

The *Hevea* trees sampled were all matured trees in tapping (exploitation) and the leaves sampled were at least 100 days old. Leaves from the shaded canopy were sampled from 12 – 15 trees/site and all samples were mixed together to make a composite sample. Upon arrival in the laboratory, the samples were washed in running water to remove dirt, followed by the removal of the leaves stalk. The leaves were cut into smaller pieces and placed in paper bags for oven drying at 70 to 75ºC for 48 hours. After drying, the leaves were crushed into a powder using a home use blender (Mulinex) and sealed in polythene bags for latter analysis.

Fig. 1. The map of Cameroon showing the sampling sites

2.2.2 Leaf analysis

Leaf N was analysed using the Elemental analyser, vario MAX CNS and the macro nutrients (P, K, Ca, S and Mg) by Inductively coupled plasma optical emission-spectrometry (ICP- OES) after dry combustion followed by dilution in nitric acid + Hydrogen peroxide. The other leaf nutrients (Fe, Mn, B, Na, Mo and Zn) were microwave digested then, diluted in nitric acid and hydrogen peroxide. The extract was read using the Inductively coupled plasma optical emission-spectrometry (ICP-OES) method.

2.3 Yield Recording

Harvested latex from the various blocks/sites was subjected to various durations of maturation to obtain field coagula (cuplumps). The dry rubber from each block was collected by their respective managements, weighed and the annual yields recorded in kilogram per hectare (kg/ha).

2.4 DRIS Methodology and Data Analysis

Using *Hevea* yield and leaf nutrient concentration data, DRIS norms and coefficients of variation (CVs) were derived according to the procedure by Walworth and Sumner (1987) [4]. The yield cut off point was determined following the Cate and Nelson statistical procedure [15]. The yield data was arranged in a decreasing order together with the corresponding nutrient concentrations and an iteration process was applied. The data was divided into two groups with the first yield data forming group A and the rest of the data forming group B. The coefficient of determination (R^2) between the two population sets was determined and recorded. The next highest yield data was added so that the two highest yields formed set A and the rest of the data formed set B, the R^2 was determined and recorded. This procedure was continued till when all but one data set formed set A and the lowest data value formed set B. Thus a set of R^2 values were obtained and the yield cut off point was the yield value whose addition into set A data, gave the highest R^2 value.

The nutrient concentrations were expressed into as many ratios as possible (N/P, P/N, N/Ca etc.). For each nutrient pair the choice of considering N/P or P/N (for instance) for DRIS norms derivation depended on which of the ratios presented the highest ratio of variances between low and high-yielding sub-populations (Sl/Sh). The selected nutrient ratios were later used for the calculation of DRIS indices. The population was divided into a high- and a low-yielding sub-population. The average, the skewness and the coefficient of variation of nutrient ratios were obtained for both the low- and the high-yielding sub-populations. The variance of low yielding population (Sl) on variance of high-yielding sub-population (Sh) was also calculated. The nutrient ratio that was considered as DRIS norm was that which had a skewness value less than 1 in the high yielding sub-population and a variance ratio (Sl/Sh) ≥1 thereby maximizing the potential for such expressions to differentiate between 'healthy' and 'unhealthy plants' [4].

DRIS indices were calculated for each nutrient using the general formula, for A to N nutrient [9].

Index A = $[f(A/B) + f(A/C) + f(A/D)$ ……..+ $f(A/N)$] / Z Index $B = [f(A/B) + f(B/C) + f(B/D)$ ……..+ $f(B/N)] / Z$ Index $N = [-f(A/N) - f(B/N) - f(C/N)$ ……...- $f(M/N)] / Z$ When $A/B \ge a/b$, $f(A/B) = (A/B - 1)1000$ a/bCV

When $A/B \le a/b$, $f(A/B) = (1-a/b)1000$ A/BCV

Where, A/B is the tissue nutrient ratio of the plant to be diagnosed; a/b is the optimum value or norm for that given ratio; CV is the coefficient of variation associated with the norm; and Z is the number of functions in the nutrient index composition. Values of other functions such as f(A/C) and f(A/D) were calculated in the same way using appropriate norms and CV.

The index value for each nutrient represents an integrated measure of its sufficiency as compared to all other nutrients. The more negative the index value for a nutrient, the more limiting is that nutrient. The descriptive statistics for *Hevea* yield, leaf nutrient concentration and nutrient ratio expressions were carried out using the Excel 2010 Microsoft package.

3. RESULTS AND DISCUSSION

3.1 Leaf Nutrient Concentration Data

The descriptive statistics for *Hevea* natural rubber yields and leaf nutrient concentrations are presented in Table 1. The yield data ranged between 369 kg/ha and 2,550 kg/ha with a mean of 1,869 kg/ha. The yield cut off point used for the development of preliminary DRIS norms was 1,486 kg/ha. The highest R^2 value was obtained at this yield and 41 % of the total population fell under the high-yielding sub-population. Data for the high-yielding sub population was relatively less skewed with most nutrients having skewness values less than 1. This is an indication that most of the data was normally distributed and therefore suitable for the derivation of DRIS norms. This is because symmetrical data provides realistic approximations of the likely range of interactive influences of different nutrients on crop productivity [16].

3.2 Diagnosis and Recommendation Integrated System – DRIS Norms

The selected nutrient ratios are presented in Table 2 together with their corresponding variance ratios. Seventy eight nutrient ratios were selected by considering the pair that has the highest variance ratio of low to high-yielding sub-population. Nineteen nutrient ratios were further selected that presented the highest variance ratio of low/high (Sl/Sh) (variance ratio ≥1) and were less skewed (skewness <1) in the high-yielding sub-population as DRIS norms. The choice of nutrient ratios that present maximum variance ratio between the low and high-yielding sub-populations was to maximize the potential for such expressions to differentiate between 'healthy' and 'unhealthy plants' [4]. The variance ratio provides an indication of the importance of a particular nutrient ratio to the yield parameter [17] with very high ratios showing that the nutrients involve are very important to the plant. The overall aim of the DRIS procedure is to determine highly precise norms [18].

	Total population ($n = 130$)					High-yielding population n= 54				
	Mean	Median	Min	Max	Skew	Mean	Median	Min	Max	Skew
Hevea yield (kg/ha)	1407.4	1343.5	369.0	2550.0	0.2	1869.2	1800.5	1486.0	2550.0	0.8
Nutrients										
N %	3.3	3.3	2.6	4.6	0.8	3.4	3.3	2.6	4.6	0.6
P (ppm)	2549.9	2424.2	1503.5	4521.1	1.2	2739.7	2511.6	1573.5	4521.1	0.8
K(ppm)	11451.7	11310.1	6194.0	21187.1	0.6	12030.3	11599.3	6596.6	21187.1	0.6
Mg(ppm)	3295.8	3332.8	1340.8	6049.9	0.6	3002.2	2879.4	1340.8	5654.4	1.2
Ca(ppm)	9386.2	9193.3	2151.9	16385.6	0.2	7997.2	7432.1	2151.9	16198.4	0.8
S(ppm)	2656.9	2653.5	1269.5	3674.8	-0.1	2713.5	2680.4	1269.5	3674.8	-0.5
B(ppm)	21.0	20.8	6.4	39.3	0.5	19.7	18.4	6.4	34.8	0.4
Cu(ppm)	17.6	15.2	11.3	41.5	1.9	19.7	17.9	11.5	41.5	1.4
Fe(ppm)	197.3	153.1	78.3	891.9	3.1	165.1	142.9	78.3	891.9	6.0
Mn(ppm)	317.6	327.1	35.6	832.2	0.4	259.8	153.3	40.8	832.2	0.9
Mo(ppm)	0.3	0.3	0.1	1.6	2.5	0.4	0.3	0.1	1.6	1.8
Na(ppm)	102.2	83.4	57.3	302.2	2.0	130.0	134.4	57.3	302.2	0.9
Zn(ppm)	55.2	54.1	19.7	96.9	0.4	53.6	52.5	19.7	96.9	0.6

Table 1. Descriptive statistics for *Hevea brasiliensis* **yields and nutrients for the total and high yielding population**

Mini: Minimum; Maxi: Maximum; Skew: Skewness.

Table 2. Mean, coefficient of variation (CV) and variance (S) of nutrient ratios of the low- and high-yielding populations, the variance ratio (Sl / Sh) and the selected ratios for *Hevea* **DRIS norms**

An analysis of variance (ANOVA) was performed to select ratios with a significant difference between the high and low-yielding sub-populations which will finally be considered as DRIS norms (Table 3). However, ratios that yielded non-significant variance relations between the low and high-yielding sub-populations were included in the analysis. This is because according to Beaufils and Sumner [6], it is important to retain ratios that present the highest variance relation in order to enable taken into consideration the interaction with other elements.

Most (58 %) of the mean nutrient ratios selected as DRIS norms showed significant differences between the low- and high-yielding sub-populations (Table 3). When there are no differences in nutritional balance between the low-and high-yielding groups, it is assumed that nutritional effects are not responsible for yield differences between the groups, and that the DRIS norms developed under this situation will not produce a reliable diagnostic tool [17]. However, for this study, the difference in nutritional norms between low-and high yielding groups indicates that the DRIS norms developed are reasonable. The presence of micro nutrients in most of the nutrient ratios suggests that they could be very important for *Hevea* production in Cameroon. Information on micro nutrient norms for *Hevea* is still very limited thus this study has provided some useful information on this area of research.

Nutrient ratio	Mean	$CV(\%)$
$N/K**$	3.01	28.08
N/P	12.98	21.92
P/K^*	0.24	31.51
$Ca/S^{\star\star}$	2.94	36.00
Zn/N^{**}	0.00	25.70
B/Fe	0.13	35.87
Mg/B	167.38	35.07
B/Mo**	66.90	61.45
B/P^{**}	66.90	61.45
B/Zn	0.37	28.30
Ca/Cu**	473.27	56.13
Ca/Mn	45.32	52.47
Ca/Mo**	27225.20	67.11
P/Cu	147.98	24.91
Zn/Cu**	3.07	38.42
Fe/Mn	1.03	70.23
Mg/Mn	18.27	58.36
Mg/Zn*	57.33	24.91
Zn/S^*	0.02	21.61

Table 3. Priliminary DRIS norms for *Hevea brasiliensis* **leaf grown in the humid forest zone of Cameroon showing the ratios that were significantly different in the high- and low-yielding sub-populations**

*Significant at 1 % (**), 5 % (*)*

3.3 A Comparison of Obtained DRIS Norms to Literature Values

The DRIS norms for micro nutrients were not available in literature whereas DRIS norms for macro nutrients are available. The DRIS norms obtained in this study and literature norms for *Hevea brasiliensis* are presented in Table 4. Some of the DRIS norms established in this

study are similar to those reported by other workers [19-21]. Among the norms found with values close to literature values were; N/P, N/K and P/K. However some of the optimum values were at the end of the optimum range in other studies. The ratio P/K was obtained for this study while the ratio K/P was found good in discriminating between the low and high yielding sub-populations by other workers [20,21]. However, calculating the inverse of the obtained norm gave a value close to the literature value.

**Literature ratios that are in a reverse order compared to ratio in this study (e.g K/P instead of P/K in this study)*

The fact that some of the obtained DRIS norms were close to the literature values shows that they are reliable. However, the differences observed between the DRIS norms established for *Hevea brasiliensis* in this study and other studies could be attributed to differences in soil conditions, climate, and cultivar effects. The difference in norms derived in this study and literature norms is similar to the observation of [22] whose norms for pineapple were significantly different from the literature values. Though DRIS diagnoses are applicable irrespective of varietal or geographic variables or both [13], it was suggested that at least for maize, use of locally calibrated norms may be more accurate in diagnosing nutrient deficiencies than norms developed from plant materials gathered in other geographic regions [14]. The universal application of DRIS norms was also disputed by Elwali and others who stated that local calibration is necessary to improve the accuracy of DRIS diagnosis [23]. Thus in the absence of locally calibrated DRIS norms, norms developed under one set of conditions should only be applied if the nutrient concentrations of high-yielding plants from those different set of conditions are similar. This was supported by Elwali and Gascho [24] who, using a small data base (90) observations in each of the low-and high-yield sub-populations) concluded that local calibration is necessary to improve the accuracy of DRIS diagnosis, at least when based only on a small data set. The results of this study have also confirmed the fact that locally derived norms are important.

3.4 Diagnosis of the Nutrient Status of a Low-Yielding Sub-Population

DRIS indices were used to diagnose the nutrient status of a low-yielding sub-population. The low-yielding sub-population were constituted by fields that yielded less than 1,486 kg/ha. For the index values calculated, both positive and negative values of the indices were found in the entire dataset and the sum of all indices was zero. This shows the correct implementation of the DRIS method. The average index value for this sub-population is presented in Fig. 2. In this low-yielding sub-population, the indices for the N, P and K nutrients were all negative. This could imply that the low yields were due to macro nutrient deficiency. These macro nutrients 11 are usually supplied to *Hevea brasiliensis* plants by fertilization and the most commonly used fertilizer is N-P-K compound fertilizer. The low levels of the macronutrients indicate that there has been no fertilization or that the fertilizer dosage could not satisfy the nutrient requirements in *Hevea* in these fields. However the relative abundance of N compared to other macro nutrients could come from the leguminous

cover crops that are usually planted in *Hevea* fields as agronomic practice [25]. The application of mixed fertilizers to the sites with low yields could be beneficial. The most limiting macro nutrient was P (-2.5) followed by K (-1.2) and N (-0.84). For the micro nutrients, Mn, Fe, B and Zn were abundant with Mn being the most abundant (Fig. 2). They all had positive indices showing that they were sufficient and the fields do not require any fertilization. Their adequate values could be due to the overall good levels of the plant available forms of these nutrients in *Hevea* fields sampled. Nevertheless, two micro nutrients were also deficient namely; Cu and Mo.

Fig. 2. Nutrient index values for a low-yielding sub-population of *Hevea brasiliensis* **leaves harvested from plantations in Cameroon**

4. CONCLUSION

DRIS norms for *Hevea brasiliensis* in the humid forest zone of Cameroon have been derived. Some of the DRIS norms derived from this study were not exactly the same like those found in literature. This stresses the need for local derivation of norms to be use for nutrient diagnosis. The DRIS norms were made up of ratios of macro nutrients, micro nutrients and macro and micro nutrients. The DRIS indices showed that macro nutrient deficiency limited yield in the *Hevea brasiliensis* low-yielding sub population. The obtained norms will be used for the assessment of *Hevea* leaf nutrient in Cameroon.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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