

1 Diagnosis and Recommendation Integrated System 2 (DRIS) in Determining Mineral Status of Cotton in the 3 Cotton Zones of Benin

8 ABSTRACT

This study aimed at developing parameters of the Diagnosis and Recommendation Integrated System (DRIS) model for the assessment nutrient status for cotton grown in Benin. 150 plant samples were gathered from farmers' fields in 2018. Leaves nutrient concentration (N, P, K, Ca, Mg) and cotton fiber yield. Nutrient indices were computed using standard DRIS procedures. Results showed that phosphorus was in excess in the petiole and the whole leaves but in deficit in the limb. Potassium content was adequate according in the petiole and leaves but deficient in the limb. Ca content was limiting in the limb or the whole leaves and adequate in the petiole. Based on the diagnosis made in the petioles, Mg was deficient while adequate in the limbs and leaves. In the limb, the order of the macronutrients is as follows: $K > P > N$. On the other side, in the petiole and the whole leaves, the order of the macronutrients becomes: $P > K > N$. In the whole leaves and limb, the order of the secondary elements is as follows: $Mg > Ca$, whereas in the petiole the order of the secondary elements becomes: $Ca > Mg$.

Comment [H1]: ?????

Key words: DRIS, nutrient balance, leaf organs, soil fertility, cotton, yield.

14 1. INTRODUCTION

In Benin and as many other countries of Africa, cotton constitutes an essential element of the economic activity. Its production in West and Central Africa reaches up to 1,100,000 tons and represents 5% of the world production and 12 to 13% of the cotton fiber on the world market. In Benin, cotton represents 40% of slogan entrances, 12 to 13% of the Gross Domestic Product (GDP) and income source for more than a third of the population [1]. Cotton is an important path for the socioeconomic development and, therefore, contributes to the struggle against poverty. However, stagnant or continuous poor yields has been observed due to the decrease in soil organic carbon content which has a negative impact on nutrient uptake [2; 3, 4]. On the sandy-loam soil of the Huanghuai-Hai Plain in China, Yang et al. [5] reported that the use of NPK fertilizer over a period of 14 years has maintained a good yield level of wheat and maize compared to manure, even though the soil had a significantly higher organic matter content than the normal. Other authors as Koulibaly et al. [6], Bationo et al. [7], Bado [8] and Fey et al. [9] reported that the continuous use of mineral fertilizer over a long period reduces soil organic matter content and leads to soil degradation. In this context, soil fertility diagnosis is needed for a rational fertilization. The Diagnosis and Recommendation Integrated System (DRIS) has been used to establish nutrient ratios that could be used to diagnose the cotton plant nutrition. This method uses a comparison of leaf tissue concentration ratios of pairs nutrient with the norms developed from high-yielding populations to diagnose nutrient status. DRIS has been reliable than any other method and successfully used to interpret the results of foliar analyses for a wide range of crops such as vegetables, potatoes, wheat [10, 11]; rubber and sugarcane [12]; forage grass [13, 14]; mango [15]; pineapple [16; 17; 18], ananas [19], yam [20] and cotton [21]. The DRIS approach has been designed to provide a valid diagnosis irrespective of the age of the plant, organ harvested [22, 23], cultivars, local conditions [24], or changes in tissue sampling method or sampling time [25]. However, Agbangba et al. [26] claimed that DRIS norms can vary according to pineapple varieties. In this work we hypothesize that the results of nutrient diagnosis

40 based on petiole, limb or on the whole leaf gives the same result. This study aimed to develop
41 parameters for cotton crop nutrition status using DRIS model in Benin.
42

43 2. MATERIAL AND METHODS

44 2.1. Site location and characteristics

45 The study was conducted in six (6) high cotton producing communes in Benin. They included :
46 Banikoara (11° 18' 00" N, 2° 26' 00" E), Kandi (11° 07' 43" N, 2° 56' 13" E), Ouassa-Péhunco (10° 13'
47 42" N, 2° 00' 07" E), Sinendé (10° 20' 41" N, 2° 22' 45" E), Savalou (7° 55' 50" N, 1° 58' 31" E), Djidja
48 (7° 20' 40" N, 1° 56' 00" E).

49 The communes of Banikoara, Kandi, Ouassa-Péhunco and Sinendé are characterized by a
50 Sudano-Sahelian climate [27]. The rainfall pattern is unimodal characterized by the succession of a
51 rainy season from April-May to October and a dry season from November to March with the
52 harmattan. The average annual temperature is 27 ° C. The average annual rainfall is 900 mm.

53 The communes of Savalou and Djidja benefit of a subequatorial climate [27]. It is
54 characterized by two rainy seasons (a long one from March-April to July then a short one from
55 September to November) and two dry seasons (a short one that occurs July-August and a long one
56 from December to March). The average annual temperature is 24°C. The average annual rainfall is
57 1000 mm.
58
59

60 2.2. Sampling design and chemical analyses

61 The fully mature leaves on the main stem of youngest plants were sampled in a (5 x 4) m² plot
62 at the first bloom as recommended by FAO [28]. The leaves petioles and limbs were obtained by hand
63 from 150 producers. After air drying, material was further dried at 70°C for 72 hours to a constant
64 weight, pre-ground by a Brabender mill and stored in plastic bags. Soil samples, were also collected
65 from 150 producers at 0-20 cm depth. Cotton fiber was harvested in a (5 x 4) m² area and repeated
66 thrice per farmer's field.

67 Soil samples and leaves have been analyzed in the Soil Science, Waters and Environment
68 Laboratory based in Agonkanmey in Benin. Soil texture (5 fractions) have been determined according
69 to the international method modified by the use of ROBINSON pipette Tran and Boko (1978); the
70 organic carbon by of Walkey and Black method, the total nitrogen by Kjeldahl method, the pH (1/2.5
71 ratio soil-water), the phosphorus according to Bray1 method, the exchangeable cations by the acetate
72 of ammonium method (pH7). The potassium was measured with a Flame Photometer. The
73 phosphorus has been determined in leaves by the spectrophotometer 1,100. Calcium and Magnesium
74 was determined by Atomic Absorption Spectrophotometer.
75
76

77 2.3. Diagnosis and Recommendation Integrated System Methodology

78 The population was divided into high and low yielding subpopulations using the mean +
79 interval of confidence as criteria for cut-off. The nutrient ratio was calculated for both of the high and
80 low yielding populations so that each of the nutrients determined in the tissue appeared in the
81 denominator and again in the numerator in the ratio with each of the other elements (for example N/P
82 and P/N). For each form of expression, the variance for both high and low yielding populations was
83 calculated. A variance ratio for each nutrient ratio wasie also determined by dividing the variance of
84 the low yielding population by the variance of the high yielding population [29, 30]. For each pair of
85 nutrients, the form of expression, which gave the highest variance ratio, was selected as the
86 parameter to be used for DRIS-evaluation. The mean of the selected parameters for the high yielding
87 population became the foliar diagnostic norms were then used, along with the standard deviation, to
88 calculate DRIS indices for diagnostic purposes.
89

90 Means and standard deviation (SD) of DRIS reference parameters in the high yielding
91 subpopulation were then programmed for diagnostic purposes using the following general calibration
92 formula [31, 32].
93
94

Formatted: Indent: First line: 0.49"

Formatted: Indent: First line: 0.49"

95 $X \text{ indices} = \left[f\left(\frac{X}{A}\right) + f\left(\frac{X}{B}\right) + \dots - f\left(\frac{M}{X}\right) - f\left(\frac{N}{X}\right) - \dots \right],$

96 - where $f\left(\frac{X}{A}\right) = 100 \left[\left(\frac{X}{A}\right) / \left(\frac{x}{a}\right) - 1 \right] / CV$

97 - when $\frac{X}{A} > \frac{x}{a} + SD$

98 - and $f\left(\frac{X}{A}\right) = 100 \left(1 - \left(\frac{x}{a}\right) / \left(\frac{X}{A}\right) \right) / CV$

99 - when $\frac{X}{A} < \frac{x}{a} - SD$, where:

100 $\frac{X}{A}$ is the ratio of concentration of nutrient X and A in the sample while $\frac{x}{a}$, CV, SD represent the
 101 mean, coefficient of variation, and standard deviation for the parameter $\frac{X}{A}$ in the high-yielding

102 population, respectively. Similarly, other nutrient ratios $\frac{X}{B}$, $\frac{M}{x}$ and $\frac{N}{x}$ etc. are calibrated against the

103 corresponding DRIS reference parameters, $\frac{x}{b}$, $\frac{m}{b}$ and $\frac{n}{x}$, etc. Nutrient indices calculated by this

104 formula could range from negative to positive values depending on whether a nutrient is relatively
 105 insufficient or excessive with respect to all other nutrients considered. The more negative is the index
 106 value for a nutrient, the more limiting is that nutrient. According to Kelling and Shulte [33], a DRIS
 107 index from -15 to +15 indicates good nutrient balance in the plant; values from -25 to -15 indicate a
 108 possible deficiency and values lower than -25 show a likely nutrient deficiency of plant. Wadt (1996)
 109 proposed an interpretation method which has an advantage to detect excess of nutrients. This method
 110 compares nutrient index or its absolute value with the nutritional balance index (NBI). The nutritional
 111 balance index is the average of the distance to zero of all nutrient's indices. For N indices, NBI = (|
 112 Index A | + | Index B | + ... + | Index N |) / N. According to Wadt (1996), these conclusions could be
 113 formulated for each nutrient (Nut) as,

- 114 • Deficiency = $I_{Nut.} < 0$ (and) $|I_{Nut.}| > NBI$
- 115 • Adequate = $|I_{Nut.}| < NBI$
- 116 • Excess = $I_{Nut.} > 0$ (and) $|I_{Nut.}| > NBI$

117 Descriptive statistics were performed for fiber yield, leaf nutrient concentration and nutrient ratio
 118 expression data using R statistical software. They included, means, medians, minimum and maximum
 119 values, variances, CV's and skewness values, where a skewness value of zero indicates perfect
 120 symmetry, and values greater than 1.0 indicate marked asymmetry.

121

122 3. RESULTS

123

124 3.1. Soil physico-chemical characteristics

125

126 | Three types of soils (table-Table 1) have been identified according to their texture: (i) soil with loamy
 127 sand texture; (ii) soil with a sandy clay loam texture and (iii) and sandy loam texture. The clay contents
 128 range from 4.74 to 24.15% and sand between 55.96 and 84.53% for all textures. Nitrogen content
 129 (0.056 and 0.06 g kg⁻¹) with pH ranging from 6.0 to 6.1 (medium to low acid) are average. The
 130 potassium, the sum of exchangeable cations and the cationic exchangeable capacity on sandy clay
 131 loam textured soils were globally low on other soil types. All soils have low potassium levels. The

132 phosphorus content is low on soils with a sandy clay loam texture but high on the other soil types. So,
 133 soil used for this study presented more than three moderate limitations associated with one severe
 134 limitation.

135
 136
 137

Table 1. Physico-chemical characteristics of the soils of the study area

Parameters	Clay	Loam	Sand	N	OM	V	C/N	pH _{eau}	pH _{KCl}	Ca	Mg	K	SC	CEC	Pass
				[%]							[Cmol kg ⁻¹]				[mgkg ⁻¹]
Soil with loamy Sand texture [N=51]															
Mean	4.74	10.73	84.53	0.056	0.99	68.3	12.1	6.1	5.7	3.00	0.59	0.18	4.00	6.02	21
SD	1.54	2.33	2.83	0.024	0.35	17.2	6.2	0.4	0.5	1.26	0.26	0.09	1.53	2.53	13
Soil with sandy clay loam texture [N=12]															
Mean	24.15	19.89	55.96	0.060	1.04	61.9	10.3	6.0	5.4	4.89	1.39	0.17	6.67	10.35	8
SD	4.70	5.72	3.28	0.021	0.45	21.8	2.6	1.0	1.0	3.02	0.72	0.03	3.53	2.95	2
Soil with Sandy loam texture [N =87]															
Mean	11.86	16.53	71.61	0.061	1.14	62.8	12.1	6.1	5.5	3.50	0.82	0.19	4.77	7.28	14
SD	4.77	4.19	6.59	0.029	0.62	18.0	6.8	0.6	0.7	2.10	0.57	0.10	2.73	3.02	10

138

139 OM = organic matter; V = base saturation SC = sum of exchangeable cations; ECC = Exchangeable
 140 cations capacity; N = number of analyzed soil samples.

141

142 **3.2. Leaf nutrients concentration variation**

143
 144 The cotton yield ranged from 562.5 kg ha⁻¹ to 1690.0kg ha⁻¹ with an average of 1255.9 kg ha⁻¹ in the
 145 full population. Sixty-seven (67) out of one hundred and fourteen (140) data points were assigned to
 146 the high-yielding subpopulation (≥ 1725.7 kg ha⁻¹). The mean values, range, coefficient of variation,
 147 skewness and kurtosis for the concentration of different nutrients in the different leaf organs are listed
 148 in Table 2. The concentration of N, P, K, Ca and Mg in the petiole samples of cotton grown by
 149 commune varied from 0.7 to 2.3, 0.2 to 2.5, 1.0 to 5.9, 0.5 to 2.1 and 0.1 to 1.4 g kg⁻¹, respectively.
 150 The concentration of N, P, K, Ca and Mg in the leaf limbs ranged from 2.3 to 4.7, 0.5 to 2.3, 0.7 to 3.6,
 151 0.2 to 4.2, 0.2 to 1.4 g kg⁻¹, respectively. In the whole leaf, the concentration of N, P, K, Ca and Mg
 152 varied from 2.3 to 4.7, 0.5 to 2.1, 1 to 5.9, 0.5 to 2.1, 0.1 to 1.4 g kg⁻¹, respectively. Considering the
 153 average concentration, the content of potassium ~~is-was~~ higher in Petiole (3.4 g kg⁻¹) and the whole leaf
 154 (3.4 g kg⁻¹) than in limb (1.7 g kg⁻¹). In contrast, limb presented higher content in N than petiole. The
 155 nutrients P and Mg had relatively similar contents in petiole and whole leaf. Calcium content ~~is-was~~
 156 higher in limbs than in petiole and the whole leaf. Nitrogen content in all the leaf (petiole plus limb) was
 157 higher and similar to that in limb.

158 **Table 2.** Summary statistics for Cotton yield and leaf nutrient concentration data for total (n=150) and
 160 high-yielding (n=68) sub-populations
 161

Parameters	Low yielding sub population [n=82]						High yielding sub population [n=68]					
	Mean	CV	Min	Max	Skew	Kurt	Mean	CV	Min	Max	Skew	Kurt
Yield [kg⁻¹]	1255.9	22.6	562.5	1690.0	-0.4	-0.6	2054.4	16.7	1725.7	3187.5	1.6	2.7
Nutrients [g kg⁻¹]												
Petioles and limbs												
N	3.5	16.5	2.3	4.7	0.2	-0.5	3.5	18.3	1.7	4.6	-0.1	-0.5
P	0.8	32.5	0.5	2.1	2.2	7.5	0.9	77.4	0.5	6.3	7.0	54.0
K	3.4	27.3	1.0	5.9	-0.3	0.9	3.6	23.9	1.5	7.2	0.7	3.6
Ca	1.4	23.1	0.5	2.1	0.0	0.1	1.3	26.8	0.2	2.4	0.4	1.7
Mg	0.4	68.0	0.1	1.4	3.6	13.3	0.4	62.0	0.1	1.3	2.1	4.7
Limbes												
N	3.4	15.7	2.3	4.7	0.3	-0.1	3.5	17.9	1.7	4.6	-0.2	-0.5
P	0.8	36.7	0.5	2.3	2.5	8.9	0.9	76.9	0.5	6.3	7.0	53.9
K	1.7	32.7	0.7	3.6	1.1	2.1	2.1	40.5	1.0	4.3	1.0	-0.1
Ca	2.0	32.2	0.2	4.2	0.6	1.4	2.0	34.7	1.0	3.8	0.6	0.1
Mg	0.3	57.6	0.2	1.4	4.4	21.1	0.4	70.2	0.2	1.4	2.7	7.0
Petioles												
N	1.4	20.2	0.7	2.3	0.5	0.9	1.3	19.1	0.6	1.8	-0.4	-0.2
P	0.8	60.1	0.2	2.5	1.9	3.5	1.2	54.8	0.2	3.4	1.2	1.4
K	3.4	27.3	1.0	5.9	-0.3	0.9	3.6	22.6	1.5	5.4	-0.4	0.6
Ca	1.4	23.1	0.5	2.1	0.0	0.1	1.3	24.7	0.7	2.4	1.0	1.0
Mg	0.4	67.9	0.1	1.4	3.6	13.3	0.4	62.1	0.1	1.3	2.2	5.4

162 Max=maximum Min=Minimum Skew= Skewness Kurt= Kurtosis

163

164 **3.3. Binary nutrients ratio and nutrient diagnosis**

165
166 The mean values, range, coefficient of variation, skewness, kurtosis and the variance ratios
167 (Vlow/Vhigh) were presented in Table 3, 4 and 5. The selected nutrient ratios had relatively large
168 variance ratios (Vlow/Vhigh) and, therefore, these nutrient ratios got the maximum potential to
169 differentiate between “healthy” and “unhealthy” plants (Walworth and Sumner, 1987) (Table 6). Equal
170 number of ratios for each of the five elements (N, P, K, Ca and Mg) were selected in this study to meet
171 an orthogonal requirement of the mathematical model. The mean values for these five nutrient ratios
172 were taken as the reference value for calculation of DRIS indices for the different part of collected leaf
173 samples. The comparison of the developed norms to the previous developed in Benin revealed highly
174 significant difference even when the whole leaf was used as diagnosis material.

175 DRIS indices for each part of the cotton leaf and the whole leaf were computed using the DRIS norms
176 established from the high yield population of nutrient indexing survey of cotton were presented in
177 figure 1. The indices of nutrient N for petiole and the whole leaf were adequate ($|I_{Nut}| < NBI$) revealing
178 normal nutrition in this element. The P is in excess nutrient as diagnosed based on petiole and the
179 whole leaf and diagnosed as limiting based on limb. Potassium content was adequate according to
180 petiole and leaf diagnosis but deficient based on limb. The Ca was limiting based on limb or whole leaf
181 and adequate based on petiole diagnosis (Table 7). Based on petiole, Mg is deficient while adequate
182 based on limb and leaf. The order of macro-element was $K > P > N$ remained the same for diagnoses
183 based on limb and $P > K > N$ for petiole and for the whole leaf. The order of secondary element ($Mg > Ca$)
184 were the same for both limb and the whole leaf and the order was $Ca > Mg$ for petiole.

Comment [H2]: But were all these symptoms found in plants?

187

188

189

190

191

192

193

194

195

196

197

198

199 | **Table 3.** Mean values of nutrient ratios for high and low-yielding sub-populations together with their
 200 | respective coefficients of variance (CV's) and variances (low and high), skewness values for the high-
 201 | yielding sub-population, and the variance ratios (Vlow/Vhigh) for petiole and limb
 202

Formatted: Justified

Parameters	Low yielding sub population [n=82]						High yielding sub population [n=68]						V
	Mean	CV	Min	Max	Skew	Kurt	Mean	CV	Min	Max	Skew	Kurt	low/high
N/P	4.4	25.0	1.3	6.7	-0.1	-0.3	4.5	33.7	0.5	7.6	0.2	-0.4	0.5
P/N	0.2	35.7	0.1	0.8	3.4	19.0	0.3	79.5	0.1	1.9	6.6	50.0	0.2
N/K	1.2	50.8	0.5	3.7	2.3	6.0	1.0	32.5	0.4	2.1	1.0	1.3	3.1
K/N	1.0	32.8	0.3	1.8	-0.2	-0.3	1.1	32.5	0.5	2.3	1.2	2.6	0.9
P/K	0.3	56.9	0.1	1.1	3.0	11.2	0.3	74.4	0.1	1.7	6.4	47.0	0.6
K/P	4.4	34.5	0.9	8.3	-0.1	-0.3	4.6	36.4	0.6	11.2	1.2	3.4	0.8
N/Ca	2.6	36.9	1.5	7.4	2.7	10.2	3.0	82.4	1.5	22.1	7.2	56.0	0.2
Ca/N	0.4	26.6	0.1	0.7	0.1	0.3	0.4	29.9	0.0	0.6	0.2	0.1	0.9
N/Mg	11.6	34.7	3.2	23.4	0.6	0.9	11.7	39.5	3.3	24.6	0.4	0.1	0.8
Mg/N	0.1	50.5	0.0	0.3	2.9	9.7	0.1	51.0	0.0	0.3	1.7	2.9	0.9
P/Ca	0.6	36.9	0.3	1.4	1.3	2.0	0.8	103.1	0.3	6.0	5.6	33.8	0.1
Ca/P	1.8	33.6	0.7	3.7	0.7	0.5	1.7	34.2	0.2	3.1	0.2	0.7	1.1
P/Mg	2.8	44.8	0.6	8.6	1.2	4.0	3.4	121.3	0.5	34.1	6.4	47.3	0.1
Mg/P	0.4	61.9	0.1	1.7	2.7	9.0	0.5	74.4	0.0	1.8	1.8	3.0	0.6
K/Ca	2.6	42.6	0.8	8.1	1.9	7.2	3.1	66.6	1.5	17.9	6.1	44.2	0.3
Ca/K	0.5	45.4	0.1	1.3	1.8	4.2	0.4	31.8	0.1	0.7	0.4	0.4	2.9
K/Mg	11.8	44.8	1.6	23.5	0.1	-0.6	12.8	49.1	1.9	35.3	0.7	1.3	0.7
Mg/K	0.1	92.8	0.0	0.6	3.1	10.1	0.1	73.5	0.0	0.5	2.8	11.0	2.0
Ca/Mg	4.7	33.3	1.1	10.5	0.1	2.3	4.5	42.1	0.3	10.2	0.2	0.5	0.7
Mg/Ca	0.3	65.7	0.1	1.0	2.9	8.3	0.3	145.1	0.1	3.9	6.6	49.2	0.1

203 | Max=maximum Min=Minimum Skew= Skewness Kurt= Kurtosis

204
205
206
207
208
209
210
211
212
213
214
215
216

217 | **Table 4.** Mean values of nutrient ratios for high and low-yielding sub-populations together with their
 218 | respective coefficients of variance (CV's) and variances (low and high), skewness values for the high-
 219 | yielding sub-population, and the variance ratios (Vlow/Vhigh) for limb.
 220

Formatted: Justified

Parameters	Low yielding sub population [n=82]						High yielding sub population [n=68]						V low/high
	Mean	CV	Min	Max	Skew	Kurt	Mean	CV	Min	Max	Skew	Kurt	
N/P	4.4	26.4	1.3	6.4	-0.2	-0.1	4.5	33.1	0.	7.6	0.2	-0.4	0.6
P/N	0.3	41.0	0.2	0.8	3.3	14.8	0.3	79.8	0.	1.9	6.7	50.8	0.2
N/K	2.2	34.2	1.0	5.7	1.7	5.3	1.8	35.5	0.	4.2	1.1	2.2	1.3
K/N	0.5	30.5	0.2	1.0	0.7	1.0	0.6	37.4	0.	1.4	1.3	1.8	0.5
N/Ca	2.1	110.4	0.9	21.9	8.1	70.1	2.0	44.8	1.	4.2	1.3	0.7	6.5
Ca/N	0.6	30.9	0.0	1.1	0.1	0.8	0.6	34.5	0.	1.0	-0.1	-0.8	0.8
N/Mg	11.4	25.6	3.2	18.7	-0.1	1.4	11.4	40.6	2.	23.6	0.5	0.6	0.4
Mg/N	0.1	44.2	0.1	0.3	3.6	15.2	0.1	68.3	0.	0.4	2.7	7.4	0.3
P/K	0.5	40.7	0.2	1.2	1.3	1.8	0.5	49.7	0.	1.7	2.4	11.5	0.9
K/P	2.2	39.8	0.8	5.3	1.3	2.3	2.7	51.0	0.	6.4	1.3	0.8	0.4
P/Ca	0.5	75.5	0.2	3.4	6.5	51.1	0.5	130.1	0.	6.0	7.6	60.5	0.3
Ca/P	2.5	34.9	0.3	4.7	0.2	-0.3	2.5	39.5	0.	5.0	0.7	0.4	0.8
P/Mg	2.8	39.3	0.6	7.7	1.2	4.4	3.2	124.9	0.	34.1	7.0	54.3	0.1
Mg/P	0.4	57.8	0.1	1.6	2.9	10.1	0.5	86.6	0.	2.1	2.3	4.9	0.3
K/Ca	1.2	169.0	0.3	17.9	8.1	70.1	1.3	73.9	0.	3.6	1.4	0.3	4.0
Ca/K	1.3	43.8	0.1	3.3	0.8	1.8	1.1	50.	0.	2.	0.4	0.4	1.0
K/Mg	5.7	39.9	1.6	13.3	0.8	0.9	7.1	64.	1.	20	1.6	1.9	0.3
Mg/K	0.2	47.1	0.1	0.6	1.9	4.9	0.2	60.	0.	0.	1.7	3.4	0.7
Ca/Mg	6.8	39.8	0.3	16.3	0.7	2.0	6.3	43.	1.	14	0.4	1.3	0.9
Mg/Ca	0.2	192.2	0.1	3.9	7.8	65.7	0.2	92.	0.	1.	2.6	5.4	4.4

221 | Max=maximum Min=Minimum Skew= Skewness Kurt= Kurtosis

222

223

224

225

226

227

228

229 | **Table 5.** Mean values of nutrient ratios for high and low-yielding sub-populations together with their
 230 | respective coefficients of variance (CV's) and variances (low and high), skewness values for the high-
 231 | yielding sub-population, and the variance ratios (Vlow/Vhigh) for petiole.
 232

Formatted: Justified

Parameters	Low yielding sub population [n=82]						High yielding sub population [n=68]						V low/high
	Mean	CV	Min	Max	Skew	Kurt	Mean	CV	Min	Max	Skew	Kurt	
N/P	2.3	58.7	0.5	6.8	1.5	2.3	1.5	68.6	0.4	6.3	2.4	7.5	1.7
P/N	0.6	63.9	0.1	1.9	1.7	2.9	0.9	50.5	0.2	2.4	0.8	0.6	0.7
N/K	0.5	54.1	0.2	1.5	2.4	6.7	0.4	38.8	0.2	1.0	1.5	3.6	2.7
K/N	2.6	37.2	0.7	5.9	0.6	1.5	2.8	35.6	1.0	6.4	0.9	1.3	0.9
N/Ca	1.1	36.1	0.6	2.9	2.1	6.6	1.0	23.9	0.4	1.5	0.0	-0.4	2.5
Ca/N	1.0	28.8	0.3	1.8	0.2	-0.1	1.0	28.9	0.7	2.2	1.7	4.5	1.0
N/Mg	4.7	38.2	0.9	10.5	0.7	1.6	4.5	39.4	1.1	9.1	0.3	-0.1	1.1
Mg/N	0.3	69.3	0.1	1.1	3.5	12.9	0.3	56.5	0.1	0.9	2.1	5.1	1.4
P/K	0.3	78.9	0.1	1.2	2.4	5.8	0.4	61.2	0.1	1.2	1.4	2.8	0.9
K/P	5.4	55.2	0.9	18.0	1.4	3.7	4.0	64.9	0.8	14.0	1.6	3.3	1.3
P/Ca	0.6	55.6	0.1	1.9	1.7	3.6	0.9	53.8	0.1	2.4	0.9	0.6	0.4
Ca/P	2.2	51.2	0.5	6.9	1.3	2.8	1.5	79.7	0.4	7.6	2.8	9.7	0.8
P/Mg	2.7	60.6	0.4	8.5	1.5	2.6	4.0	67.1	0.4	14.6	1.7	4.2	0.4
Mg/P	0.5	69.8	0.1	2.3	2.3	6.8	0.4	94.6	0.1	2.6	3.8	19.4	1.0
K/Ca	2.6	42.6	0.8	8.1	1.9	7.3	2.8	26.9	1.5	4.7	0.1	-0.5	2.2
Ca/K	0.5	45.3	0.1	1.3	1.8	4.2	0.4	29.9	0.2	0.7	0.9	-0.1	3.1
K/Mg	11.8	44.9	1.6	23.5	0.1	-0.6	12.8	50.2	1.9	35.3	0.7	1.2	0.7
Mg/K	0.1	92.7	0.0	0.6	3.1	10.1	0.1	84.0	0.0	0.5	2.9	10.0	1.4
Ca/Mg	4.7	33.3	1.1	10.5	0.1	2.3	4.6	40.5	1.1	10.2	0.3	0.5	0.7
Mg/Ca	0.3	65.7	0.1	1.0	2.9	8.3	0.3	64.7	0.1	0.9	2.1	3.4	0.9

233 | Max=maximum Min=Minimum Skew= Skewness Kurt= Kurtosis

234

235

236

237

238

239

240

241

242

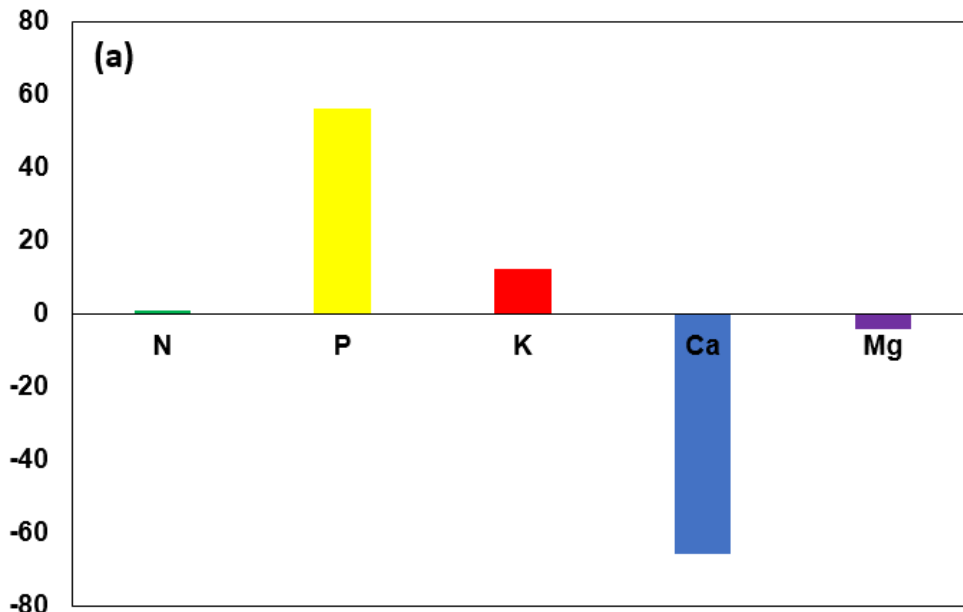
243

244 **Table 6.** DRIS norms, CV's and skewness values for the high-yielding sub-population, and variance
 245 ratios (Vlow/Vhigh) of nutrient ratio expressions selected for inclusion in the DRIS model for **Cotton**
 246 **cotton** and comparison to norms developed by Dagbenonbakin et al., 2010.
 247

Parameters	High yielding sub population [n=68]						V low/high	Norms developed from Dagbenonbakin et al. 2010
	Mean	CV	Min	Max	Skew	Kurt		
Petioles and limbes								
N/P	4.5	33.7	0.5	7.6	0.2	-0.4	0.5	9.65***
N/K	1.0	32.5	0.4	2.1	1.0	1.3	3.1	1.69***
K/P	4.6	36.4	0.6	11.2	1.2	3.4	0.8	5.26***
Ca/N	0.4	29.9	0.0	0.6	0.2	0.1	0.9	NA
Mg/N	0.1	51.0	0.0	0.3	1.7	2.9	0.9	0.09 ns
Ca/P	1.7	34.2	0.2	3.1	0.2	0.7	1.1	5.79***
Mg/P	0.5	74.4	0.0	1.8	1.8	3.0	0.6	0.96***
Ca/K	0.4	31.8	0.1	0.7	0.4	0.4	2.9	1.08***
Mg/K	0.1	73.5	0.0	0.5	2.8	11.0	2.0	0.18***
Ca/Mg	4.5	42.1	0.3	10.2	0.2	0.5	0.7	5.77***
Limbes								
N/P	4.5	33.1	0.5	7.6	0.2	-0.4	0.6	9.65***
N/K	1.8	35.5	0.7	4.2	1.1	2.2	1.3	1.69 ns
N/Ca	2.0	44.8	1.0	4.2	1.3	0.7	6.5	NA
N/Mg	11.4	40.6	2.4	23.6	0.5	0.6	0.4	10.55 ns
P/K	0.5	49.7	0.2	1.7	2.4	11.	0.9	0.19 ns
Ca/P	2.5	39.5	0.2	5.0	0.7	0.4	0.8	5.79 ns
P/Mg	3.2	124.9	0.5	34.1	7.0	54.	0.1	1.04***
Ca/K	1.1	50.2	0.3	2.7	0.4	0.4	1.0	1.08***
Mg/K	0.2	60.0	0.0	0.6	1.7	3.4	0.7	0.18***
Ca/Mg	6.3	43.5	1.1	14.8	0.4	1.3	0.9	5.77 ns
Petioles								
N/P	1.5	68.6	0.4	6.3	2.4	7.5	1.7	9.65***
N/K	0.4	38.8	0.2	1.0	1.5	3.6	2.7	1.69***
N/Ca	1.0	23.9	0.4	1.5	0.0	-0.4	2.5	NA
Mg/N	0.3	56.5	0.1	0.9	2.1	5.1	1.4	0.09***
K/P	4.0	64.9	0.8	14.0	1.6	3.3	1.3	5.26***
Ca/P	1.5	79.7	0.4	7.6	2.8	9.7	0.8	5.79***
Mg/P	0.4	94.6	0.1	2.6	3.8	19.4	1.0	0.96***
Ca/K	0.4	29.9	0.2	0.7	0.9	-0.1	3.1	1.08***
Mg/K	0.1	84.0	0.0	0.5	2.9	10.0	1.4	0.18***
Mg/Ca	0.3	64.7	0.1	0.9	2.1	3.4	0.9	0.17***

248 Max=maximum,Min=Minimum,Skew= Skewness,Kurt= Kurtosis, NA: not available

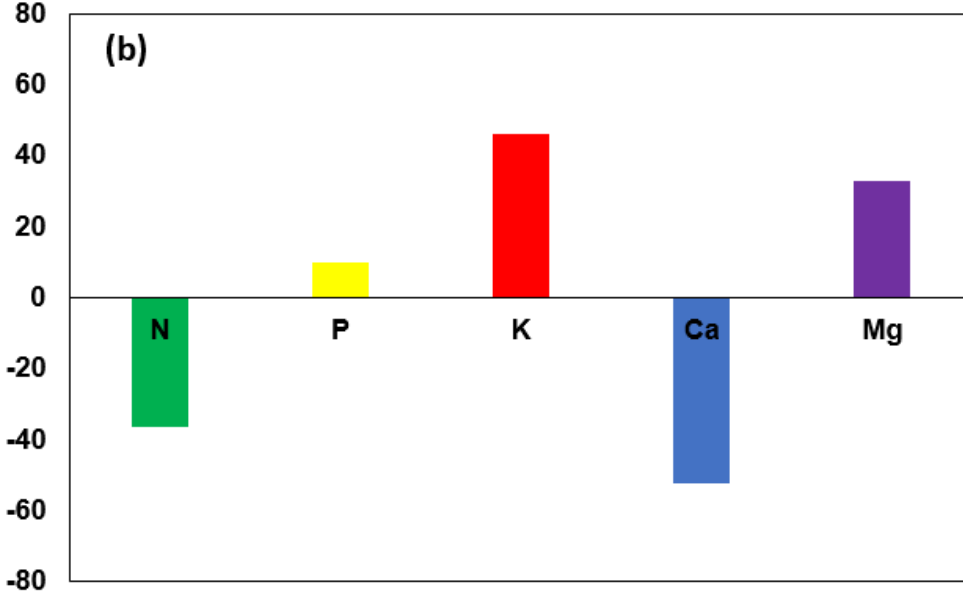
249

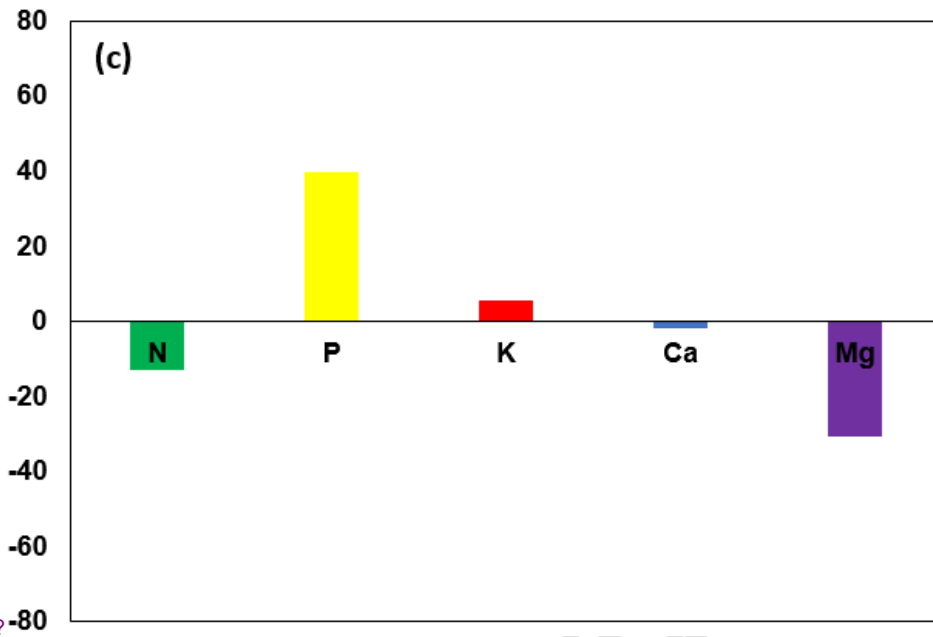


250

251

252





253

?

254 **Figure 1.** Nutrient indices for petiole + limbe (a), limbe (b) and petiole (c).

255

Comment [H3]: When is the figure presented in text ?

256 **Table 7.** Nutrient diagnosis status

257

Leaf Part	Nutrients					Nutrient balance Index
	N	P	K	Ca	Mg	
Petiole	A	E	A	A	D	18.1
Limbe	D	A	E	D	A	35.5
Leaf	A	E	A	D	A	27.9

A = adequat; D= Deficient; E= excess

258

259

260 **4. DISCUSSION**

261

262

263

264

265

266

267

268

269

270

271

272

273

274

275

276

277

278

279

280

281

282

283

284

285

286

287

DRIS norms established in this study can prove useful diagnostic tool to predict deficiencies or imbalances in macro, secondary and micronutrient supply to cotton plants. The results suggested that DRIS norms developed in this study is different from those developed by Dagbenonbakin *et al.* [21]. In fact, in this work the yield of the reference population varies from 1725.7 to 3187.5 kg ha⁻¹ while it ranged from 658.5 to 1240.9 kg ha⁻¹ in the report of Dagbenonbakin *et al.* [21]. DRIS indices for specific leaf part and the whole leave were obtained. According to Kelling and Schulte [33] an index from -15 to +15 indicates good nutrient balance in the plants. Indices from -15 to -25 indicate possible deficiency, and indices lower than -25 are likely to be deficient. The diagnosis result using DRIS approach depends on the leaf part used. The result of diagnosis is not consistent regardless the leaf organ used. Thus, calcium is the most limiting nutrient in the whole leave and in limb. Magnesium is the most limiting nutrient in the petiole. According to their texture, soils are poor in calcium and magnesium. This is confirmed by the pH which was medium to low acid and the poor soil Ca and Mg content.

However, this is not consistent with some previous studies claiming that DRIS approach has been designed to provide a valid diagnosis irrespective of the plant age, harvested organ [22, 10, 13, 35, 22]. According to diagnosis method of Braud [36], anions such as N, P, S, Cl and micronutrients are diagnosed in limbs whereas cations K, Ca and Mg are checked in petioles. This method of diagnosis is time consuming because it requires the separation of petiole and limbs.

The nutritional balance index (NBI) is a measure of balance among fields. It is calculated by adding the absolute values of indices generated for the sample [12]: the larger the value of the NBI, the greater the intensity of imbalances among nutrients at the time of sampling. Amongst the leaf organs, the NBI using limb is higher. Hence, the imbalance amongst the nutrients in cotton plants as diagnosed using limb is relatively higher than the others. The diagnosis of fertility has revealed a nutritional imbalance of the soil which necessitates the implementation of a balanced manure plan for the crop production.

Comment [H4]: How does texture interfere with Ca and Mg contents?

Comment [H5]: Why ?

Comment [H6]: So was this analysis method efficient?

288 **5. CONCLUSION**

289

290

291

292

293

294

295

296

297

298

299

300

301

302

303

304

305

306

307

This study aimed to assess the nutritional status of cotton in Benin cotton zones. The results showed a deficiency of magnesium, and especially calcium in cotton nutrition. In the whole leaf, nitrogen nutrition is hardly satisfied, whereas it is deficient in limb and petiole. However, the nutrition in potassium and especially in phosphorus was satisfied. In the different cotton zones, apart from assimilable phosphorus, the other fertility parameters are very low to low. This cannot facilitate proper mineral nutrition of the cotton plant. The improvement of the soil quality of the cotton zones through organic and / or mineral amendments, and the development of a balanced cotton fertilizer formula containing calcium and magnesium, is necessary to ensure the sustainable management of soils and soil optimum mineral nutrition of the cotton plant. Thus, the perspective of the study would be to look for optimal doses of calcium and magnesium in cotton growing in Benin.

Comment [H7]: Contradictory.

309 REFERENCES

- 310 1. World Bank (2016). Notes de politiques pour la nouvelle administration béninoise, 145p.
- 311
- 312 2. Bhandari AL, Ladha JK, Pathak H, Padre AT, Dawe D, Gupta RK (2002). Yield and soil nutrient
- 313 changes in a long-term rice-wheat rotation in India. *Soil Science Society of America Journal*
- 314 66(1), 162-170. doi:10.2136/sssaj2002.1620
- 315
- 316 3. Ladha JK, Pathak H, Tirol-Padre A, Dawe D, Gupta RK (2003). Productivity trends in intensive
- 317 rice-wheat cropping systems in Asia. Improving the Productivity and Sustainability of Rice-
- 318 Wheat Systems: Issues and Impacts, (improvingthepro), 45-76. doi:10.2134/asaspecpub65.c3
- 319
- 320 4. Dawe D, Dobermann A, Moya P, Abdulrachman S, Singh B, Plal S, YLi Blin, Panaullah G,
- 321 Sariam O, Singh Y, Swarup A, Tan S, Xzhen Q (2000). How widespread are yield declines
- 322 in long-term rice experiments in Asia? *Field Crops Research*, 66, 175-193.
- 323 [https://doi.org/10.1016/S0378-4290\(00\)00075-7](https://doi.org/10.1016/S0378-4290(00)00075-7)
- 324
- 325 5. Yang X, Li L (2011). miRDeep-P: a computational tool for analyzing the microRNA transcriptome
- 326 in plants. *Bioinformatics*, 27(18), 2614-2615. <https://doi.org/10.1093/bioinformatics/btr430>
- 327
- 328 6. Koulibaly B, Traoré O, Dakuo D, Zombré PN, Bondé D (2010). Effets de la gestion des résidus
- 329 de récolte sur les rendements et les bilans culturaux d'une rotation cotonnier- maïs-sorgho au
- 330 Burkina Faso. *Tropicultura*, 2010;28(3):184-189.
- 331
- 332 7. Bationo A, Waswa B, Kihara J, Adolwa I, Vanlauwe B, Saidou K (2012). Lessons learned from
- 333 long-term soil fertility management experiments in Africa. Springer Science & Business Media.
- 334
- 335 8. Bado BV (2002). Rôle des légumineuses sur la fertilité des sols ferrugineux tropicaux des
- 336 zones guinéennes et soudanienne du Burkina Faso. PhD Thèse, Université de Laval
- 337 Québec, 184p.
- 338
- 339 9. Fey MV, Mills AJ (2003). Declining soil quality in South Africa: effects of land use on soil
- 340 organic matter and surface crusting. *South African Journal of Science*, 99(9), 429-436.
- 341
- 342 10. Meldal-Johnson A, Sumner ME (1980). Foliar diagnostic norms for potatoes. *Journal of plant*
- 343 *nutrition*, 2(5), 569-576. <https://doi.org/10.1080/01904168009362799>
- 344
- 345 11. Amundson RL, Koehler FE (1987). Utilization of DRIS for diagnosis of nutrient deficiencies in
- 346 winter wheat. *Agronomy Journal* 79 (3): 472-476.
- 347 doi:10.2134/agronj1987.00021962007900030013x
- 348
- 349 12. Elwali AMO, Gascho GJ (1984). Soil testing, foliar analysis and DRIS as guides for sugarcane
- 350 fertilization. *Agronomy Journal*, 76(3), 466-470.
- 351 doi:10.2134/agronj1984.00021962007600030024x
- 352
- 353 13. Bailey JS, Beattie JAM, Kilpatrick DJ (1997a). The diagnosis and recommendation integrated
- 354 system (DRIS) for diagnosing the nutrient status of grassland swards: I. Model establishment.
- 355 *Plant Soil* 197(1): 127-135. <https://doi.org/10.1023/A:1004236521744>
- 356
- 357 14. Bailey JS, Cushnahan A, Beattie JAM (1997b). The diagnosis and recommendation integrated
- 358 system (DRIS) for diagnosing the nutrient status of grassland swards: II. Model calibration and
- 359 validation. *Plant Soil* 197 (1): 137-147. <https://doi.org/10.1023/A:1004288505814>
- 360
- 361 15. Hundal HS, Singh D, Brar JS (2005). Diagnosis and recommendation integrated system for
- 362 monitoring nutrient status of mango trees in submountainous area of Punjab, India.
- 363 *Communications in soil science and plant analysis* 36(15-16), 2085-2099.
- 364 <http://dx.doi.org/10.1080/00103620500194460>
- 365
- 366

Formatted: Portuguese (Brazil)

Formatted: Portuguese (Brazil)

Formatted: Portuguese (Brazil)

- 367 | 16. Angeles DE, Sumner ME, Lahav E (1993). Preliminary DRIS norms for banana. *Journal of Plant*
368 | *Nutrition* 16 : 1059-1070. <https://doi.org/10.1080/01904169309364594>
- 369 |
- 370 | 17. Agbangba CE (2008). Contribution à la formulation d'engrais spécifique pour la culture de
371 | l'ananas par le diagnostic foliaire dans la commune d'Allada. Thèse d'ingénieur agronome,
372 | Université de Parakou, Parakou, 159p.
- 373 |
- 374 | 18. Agbangba CE, Dagbenonbakin DG, Kindomihou V (2010). Etablissement des normes du
375 | système intégré de diagnostic et de recommandation de la culture d'ananas (*Ananas comosus*
376 | (L.) Merr) variété Pain de sucre en zone subéquatoriale du Bénin. *Annales de l'Université de*
377 | *Parakou, Série Sciences Naturelles et Agronomie* 1: 51-69.
- 378 |
- 379 | 19. Dagbenonbakin DG, Agbangba CE, Kindomihou V (2010). Comparaison du système intégré de
380 | diagnostic et de recommandation et de la méthode de la valeur critique pour la détermination du
381 | statut nutritionnel de l'ananas (*Ananas comosus* (L.) Merr) variété Cayenne Lisse au Bénin.
382 | *International Journal of Biological and Chemical Sciences*, 4(5).
383 | <http://dx.doi.org/10.4314/ijbcs.v4i5.65564>
- 384 |
- 385 | 20. Dagbenonbakin GD, Agbangba CE, JP, Bognikpe H Goldbach (2011). DRIS model
386 | parameterization to assess yam (*Dioscorea rotundata*) mineral nutrition in Benin (West Africa).
387 | *European Journal of Scientific Research* 49 (1): 142-151.
- 388 |
- 389 | 21. Dagbenonbakin GD, Agbangba EC, Glele Kakaï R (2010). Preliminary diagnosis of the nutrient
390 | status of cotton (*Gossypium hirsutum* L.) in Benin (West Africa). *Bulletin de la Recherche*
391 | *Agricole du Bénin* (BRAB) 67: 32-44.
- 392 |
- 393 | 22. Sumner M. E (1977 a). Application of Beaufil's Diagnostic Indices to Maize Data Published in the
394 | Literature Irrespective of Age and conditions". *Plant and Soil*. 46:359-369.
- 395 |
- 396 |
- 397 | 23. Sumner M. E (1977 b). Effect of Corn Leaf Sampled on N, P, K, Ca and Mg Content and
398 | Calculated DRIS Indices. *Commun. Soil Sci. Plant Anal.* 8:269-280.
- 399 |
- 400 | 24. Payne GG, Rechcigl JE, Stepherson RL (1990). Development of Diagnosis and
401 | Recommendation Integrated System Norms for Bahia grass. *Agron. J.* 82:930-934.
- 402 |
- 403 | 25. Moreno JJ, Lucena JJ, Carpena O (1996). Effect of the Iron Supply on the Nutrition of Different
404 | Citrus Variety/Rootstock Combinations Using DRIS. *J. Plant Nutr.*;19:698-704
- 405 |
- 406 | 26. Agbangba CE, Sossa EL, Dagbenonbakin DG, Diatta S, Akpo LE (2011). DRIS model
407 | parameterization to assess pineapple variety 'Smooth Cayenne' nutrient status in Benin (West
408 | Africa). *Journal of Asian Scientific Research* ;1(5) : 254- 264.
- 409 |
- 410 |
- 411 | 27. Hounkponou (2015). Rapport d'études sur les changements climatiques et avancées en matière
412 | d'adaptation : cas du bassin de Tèwi dans la Commune de Dassa-Zoumè. 32p
- 413 |
- 414 | 28. FAO (2000). Simple soil, water and plant testing techniques for soil resource management.
415 | Proceeding of a training course held in Ibadan, Nigeria, 6-27 September 1996. Edited by
416 | Adepetu J.A., Nabhan H. and Osinubi A. IITA/FAO. Land and water Development. Division. FAO
417 | : Rome. P. 2000 ;157.
- 418 |
- 419 | 29. Amundson RL, Koehler FE (1987). Utilization of DRIS for diagnosis of nutrient deficiencies in
420 | winter wheat. *Agron J.* 79 :472-476.
- 421 |
- 422 | 30. Payne GG, Rechcigl JE, Stepherson RL (1990). Development of Diagnosis and
423 | Recommendation Integrated System Norms for Bahia grass. *Agron. J.* ;82 :930-934.
- 424 |
- 425 | 31. Hallmark WB, deMooy CJ. and John Pesek (1987). Comparison of two DRIS methods for
426 | diagnosing nutrient deficiencies. *J. Fert. Issues* v. 4 Number 4. 151-158.

Formatted: Portuguese (Brazil)

Formatted: Portuguese (Brazil)

Formatted: Portuguese (Brazil)

Formatted: Portuguese (Brazil)

427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457

32. Rathfon, Joyce A., et al (1991). "Effects of Different Acceleration and Deceleration Rates on Isokinetic Performance of the Knee Extensors." *Journal of Orthopaedic & Sports Physical Therapy*, ; 14. (4):161-168.
33. Kelling K.A, Matocha JE (1990). Plant analysis as an aid in fertilising forage crops. In:Westerman RL (ed) Soil testing and plant analysis. 3rd edn. Soil Science Society of America, Madison, WI, USA ; 603–643
34. Wadt, P.G.S. Os métodos da chance matemática e do Sistema Integrado de Diagnose e Recomendação (DRIS) na avaliação nutricional de plantios de eucalipto. Viçosa: UFV, 1996;123. (Tese - Doutorado).
35. Jones JB, Eck HV, Voss R (1990). Plant analysis as an aid in fertilising corn and grain sorghum. In : Westerman RL (ed) Soil testing and plant analysis. 3rd edn. Soil Science Society of America, Madison, WI, USA, 521–547
36. Braud M (1987). La fertilisation d'un système de culture dans les zones cotonnières soudano-sahélienne. Coton et Fibres tropicales, Série et Document, Etudes et Synthèses, 8, 35p.

Formatted: French (France)

Formatted: French (France)

UNDER PEER REVIEW