Chapter 4

Nutritional Status of Cotton Plant Assessed by Compositional Nutrient Diagnosis (CND)

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Abstract

The use of compositional nutrient diagnosis (CND) to assess the nutritional status of cotton crop is quite important to improve knowledge on plant nutritional requirement and assist the fertilizer recommendation. The aim of this chapter is to introduce the possibility of using CND for cotton crop. This method has scarcely been used to assess the nutritional status of cotton plant although a few results have indicated that it can be promising. In fact, CND methodology seems to be better in the nutritional diagnosis than traditional methods such as sufficient range (SR) and critical value approach (CVA). Its efficiency has increased with the possibility of applying multivariate analysis, principal component analysis (PCA), canonical correlation, and so on. The application of PCA possibility to note some interactions among the nutrients is important for understanding the dynamics of nutrients in plants.

Keywords: multivariate analysis, nutritional balance, nutrient concentration, PCA, plant nutrition, soil science, *Gossypium hirsutum* L

1. Introduction

The knowledge of nutritional status of cotton is essential to understand plant nutritional requirement and to help the fertilizer recommendations to improve crop production. Without understanding cotton nutrient requirement, it is difficult to know about plant response to fertilizers. The traditional methods to assess plant nutrient status are the sufficiency range (SR) and critical value approach (CVA); however, these methods do not allow the diagnosis of the nutritional balance of the plant, and their usage is dependent on sampling time and plant development stage [1]. Additionally, in SR and CVA, it is not possible to know which nutrient is more or less required by plant, because there is no interaction among nutrients in these traditional diagnoses.

In order to improve nutritional diagnosis by conventional methods (CVA and SR), Beaufils [2] developed the diagnosis and recommendation integrated system (DRIS), which was based on "minimum law." Through the DRIS index, it is possible to observe the plant nutritional requirement, and the most deficient nutrient is limiting the plant development and production. It is possible to consider DRIS as an early attempt to analyze the compositional space of leaf analysis results [3]. Parent and Dafir [4] developed the compositional nutrient diagnosis (CND) that is conformed to be a multivariate analysis and can combine other refined analysis to improve the nutritional diagnosis. CND uses the row-centered log ratio instead of dual ratios between nutrients [5]. CND consists in the relation between each individual nutrient concentration with the geometric mean of the other nutrients in composition. Thus, CND comprises multiple interactions among all nutrients in diagnosis. Therefore, CND method has more advantages than DRIS method due to the interaction among all nutrients instead of dual ratios mentioned above [4].

For cotton crop, Serra et al. [6, 7] have compared CVA and SR diagnosis with CND method and observed that CND shows more precise diagnosis results than CVA and SR, and the authors have reported that the use of SR may generate wide nutrient range that can disturb the accurate diagnosis.

The aim of this chapter is to introduce the CND applications to assess the nutritional status of cotton plant as a more precise method of plant diagnosis than the widely applied traditional CVA and SR methods.

2. Nutritional diagnosis in cotton plant

CVA or SR method has been defined as an interventionist approach because the production variability is explained by the variation on nutrient supply or availability of the nutrients under analysis. In CVA and SR, the other production factors are remained under unlimited levels. The understanding of the principles, considered by different diagnosis methods, and the comparison of their results are important to the application of these diagnosis tools.

In cotton and most grain crops, usually, the nutritional diagnoses are conducted using SR and CVA, which measure only individual leaf nutrient concentration of a nutrient in diagnosis without any relationship with other nutrients in the sample of leaf tissue. These traditional methods do not report the nutritional balance in plant tissue. Bates [1] noted the nutritional diagnosis through CVA was affected by the interaction of nutrients in plant tissue and other factors related to plant growth depending on the age of plant.

In the last decade, the interests for bivariate and multivariate methods of nutritional diagnosis, as DRIS and CND, increased. DRIS is based on the dual ratio between nutrients, as N/P, N/K, etc., which is considered to have a bivariate relation. DRIS method allows the assessment of the nutritional equilibrium of a plant, ranking the nutrient contents in relative order of nutritional requirement, from the most deficient nutrient to the most excessive [2].

In DRIS results, in order to express the relative nutrient balance into the plant tissue, the DRIS index is calculated through the comparison of dual nutrient ratios (N/P, P/K, K/Ca, Ca/Mg, etc.) in the sample with the DRIS norms from a reference population (N/P′, P/K′, K/Ca′, Ca/Mg′, etc.). Besides, in the DRIS, there are other bivariate methods such as modified DRIS (M-DRIS) [8] and multivariate method like the CND, which was developed by Parent and Dafir [4].

3. Steps to develop CND norms

3.1. Obtaining leaf nutrient concentration and cotton crop yield to compose the database

The first step to develop CND norms is to obtain leaf nutrient concentration and cotton yield (database). The leaf sampling may be conducted in commercial or experimental cotton field, but it is recommended that the number of samples cannot be under 30 samples of complete leaves (blade + petiole) per plot, which must be combined to form just 1 composed sample [9].

One leaf sample per plant should be taken from the fifth leaf on the main stem, during flowering of the cotton crop (stages F1–F4), according to classification of Marur and Ruano [10]. The cotton yield is assessed at the end of plant cycle, which is usually accomplished with a combine harvester in commercial field or manually in experimental plots.

In the leaf samples, the total concentrations of N, P, K, Ca, Mg, S, B, Zn, Cu, Mn, and Fe are usually determined. Nevertheless, there are cases where authors used just five nutrients (N, P, K, Ca, and Mg) [4, 11] or more, as the case of Anjaneyulu and Raghupathi [12], who used ten nutrients (N, P, K, Ca, Mg, S, Fe, Mn, Zn, and Cu).

The leaf nutrient concentration must follow normal distribution. That is why, it is necessary to transform the data of nutrient concentration from leaf tissue. However, with CND method, the calculation of row-centered log ratio corrects the non-normal distribution. As observed by Serra et al. [6], the multinutrient variable (Zi) showed 100% normal distribution in data of leaf nutrient concentration of cotton.

The size of the database (leaf nutrient concentration and yield) is not well defined in the literature. Serra et al. [6, 7] used 65 sampling from commercial plots to develop CND norms in the Western region of Bahia State, in Brazil, whereas Serra et al. [13] used 108 sampling in the database for DRIS. Khiari et al. [11] used 240 observations of commercial plots of sweet corn and five nutrient determinations, and Parent et al. [5] collected 1117 samples in potato crop to establish the database. More than quantity, the quality of the database might be more far reaching to obtain profitable CND norms. In fact, the essential to have an effective database is to acquire plots with high-yield and healthy leaves with no damage to make possible the development of the CND or DRIS norms.

3.2. Selecting the high-yielding subpopulation to develop CND norms

In order to develop the CND norms, it is necessary to define the high-yielding subpopulation into the database. The database might be divided into two subpopulations, using the mean + 0.5 standard deviation (SD) as criterion to separate the populations into a high-yielding group and low-yielding group [6].

Serra et al. [13] published a paper about the criterion of population selection for cotton crops to separate low-yielding from high-yielding population to develop DRIS norms; nevertheless, it is possible to apply these criteria for CND norms. In this criterion, they used the mean and standard deviation of the yield in the database to separate the high-yielding from low-yielding subpopulation.

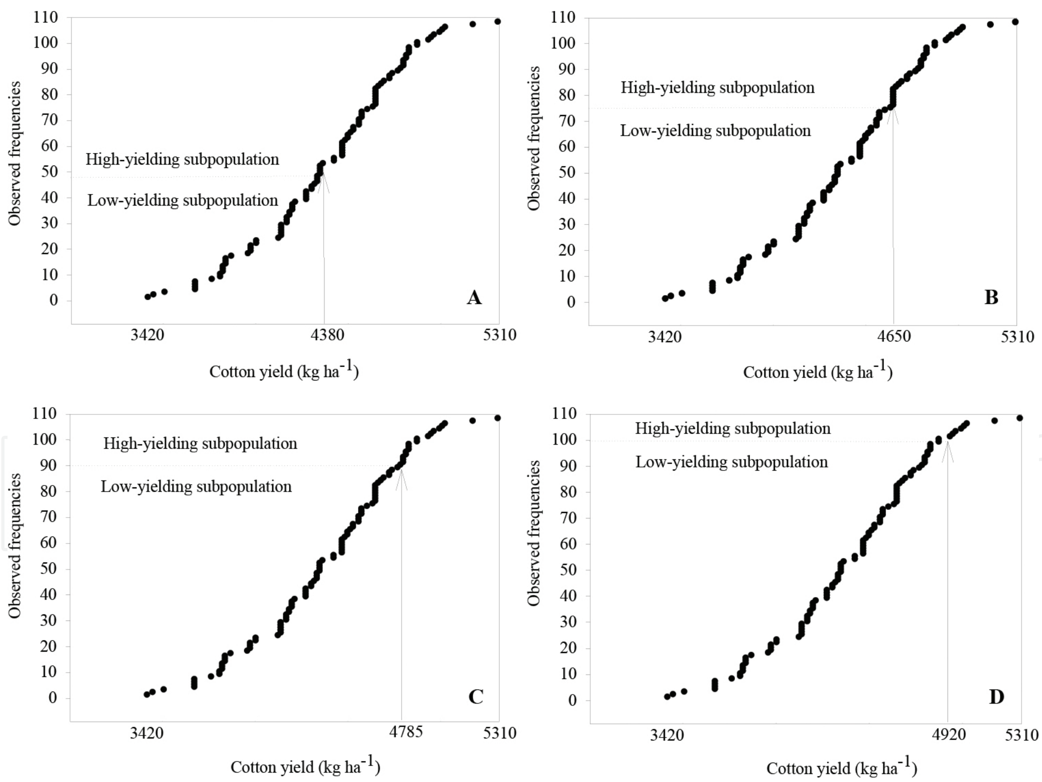

Figure 1. Graphic representation of the observed frequency of yields. The criteria for establishing the high-yield subpopulations were plots with (A) yield above average (4380 kg ha^{-1}), (B) yield above average + 2/3 standard deviation (above 4650 kg ha^{-1}), (C) yield above average + 1 standard deviation (above 4785 kg ha^{-1}), and (D) yield above average + 4/3 standard deviation (above 4920 kg ha^{-1}).

As shown in **Figure 1**, Serra et al. [13] used as criteria for determining the high-cotton-yield subpopulation plots with yields above the population mean (**Figure 1A**), yield above mean + 2/3 standard deviation (SD) (**Figure 1B**), yield above mean + 1 SD (**Figure 1C**), and yield above mean + 4/3 SD (**Figure 1D**). They concluded that the increasing of rigor to select the high-yielding subpopulation increases the capacity to discriminate low yielding from high-yielding subpopulation.

For other crops as well as for cotton crop, the selection of the high-yielding reference subpopulation may be carried across multiple ratios using a cumulative variance function fit to cubic [11] or Boltzmann [14] equations.

3.3. Calculation of the CND norms

The nutrient leaf tissue composition is defined by the simplex S where the sum of all components is constrained to 100%, which forms a d-dimensional nutrient arrangement, i.e., simplex (S^d) made of $d + 1$ nutrient proportions including d nutrients and a filling value (R_d) [4]:

$$S^d = [(N, P, K, Ca, Mg, S, B, Zn, Cu, Fe, Mn, \ldots, R_d): N > 0, P > 0, K > 0, \ldots R_d > 0; N + P + K + \cdots + R_d = 100]$$

where 100 is the dry matter concentration (%); N, P, K, etc., are nutrient proportions (%); and R_d is the filling value computed as follows:

$$R_d = 100 - (N + P + K + Ca + Mg + S + B + Zn + Cu + Fe + Mn + \cdots)$$

The geometric mean (G) is computed as follows [4]:

$$G = (NxPxKx \ldots xR_d)^{\frac{1}{d+1}}$$

After calculation of the geometric mean (G), the new expression for the multinutrient is log-transformed to generate a row-centered log ratio as follows [4]:

$$V_N = log\left(\frac{N}{G}\right), V_P = log\left(\frac{P}{G}\right), V_K = log\left(\frac{K}{G}\right), \ldots, V_{R_d} = log\left(\frac{R_d}{G}\right)$$

The sum of all row-centered log ratios must be equal to zero ($V_N + V_P + V_K + \ldots + V_{R_d} = 0$. CND norms are the means and standard deviations (SD) of row-centered log ratios of high-yielding subpopulation from the database.

Following the procedure above, Serra et al. [6] developed the CND norms for cotton crop in the Western region of Bahia State, Brazil (**Table 1**).

Row-centered log ratios	Mean	SD
V_N	3.136	0.0696
V_P	0.456	0.1432
V_K	2.4282	0.2256
V_{Ca}	2.6399	0.1554
V_{Mg}	1.4709	0.1769
V_S	0.9033	0.2539
V_{Fe}	−2.0931	0.046
V_{Zn}	−2.8972	0.042
V_{Cu}	−5.0268	0.0812
V_{Mn}	−3.6224	0.1641
V_B	−3.8234	0.0464

[1]CND norms based on the high-yield subpopulation (>4250 kg ha^{-1}) of cotton crop in the Western region of Bahia state in Brazil [6].

Table 1. Compositional nutrient diagnosis (CND) norms [mean and standard deviation (SD) of row-centered log ratio][1].

4. CND index and interpretation

4.1. Procedure to calculate CND index in cotton crop

As reported in Section 3.3, from the leaf nutrient concentration of the high-yielding subpopulation (>4250 kg ha^{-1}), CND norms were defined (**Table 1**), which are the means, and standard deviations of row-centered log ratios of the nutrients concentration in leaf tissue, denoted as $'V_N + 'V_P + 'V_K + ... + 'V_{R_d}$ and $'SD_N$, $'SD_P$, $'SD_K$,.... $'SD_{Rd}$, respectively, were then calculated.

The CND indices, denoted as I_N, I_P, I_K,…, I_{Rd}, were calculated from the row-centered log ratios as follows [4]:

$$I_N = \frac{V_N - 'V_N}{'SD_N}, I_P = \frac{V_P - 'V_P}{'SD_P}, I_K = \frac{V_K - 'V_K}{'SD_K}, \dots, I_{R_d} = \frac{V_{R_d} - 'V_{R_d}}{'SD_{R_d}},$$

The nutrient imbalance index (NII) of a diagnosed specimen, which is its CND r^2, followed the recommendation of Parent and Dafir [4]:

$$r^2 = I_N^2 + I_P^2 + I_K^2 + \cdots + I_{R_d}^2$$

The interpretation of CND is based on CND index. CND index is defined as the distance of a given nutrient X_i from its geometric mean [4], which is relative to the distance of the same nutrient from the geometric mean of the target population (reference population with high yield).

From this point of view above, it is expected that when CND index is closer to zero, X_i nutrient is less imbalanced than others in analysis. Serra et al. [6, 7] observed CND index close to zero showed higher nutritional balance. This may be related to nutrient leaf concentration and result in high relationship in cotton crop.

4.2. Interpretation of CND index by nutrient application potential response (NAPR)

Wadt [15] developed the nutrient application potential response (NAPR), which was originally used to interpret the DRIS index. However, the use of this method was extended to interpret CND index for cotton crop [6, 7]. This method proved to be efficient to accomplish the interpretation of CND index. Besides the interpretation of CND index in cotton crop, the methodology has already been applied in other crops to interpret the DRIS index, such as *Eucalyptus grandis* [16], coffee [17], and "cupuaçu" trees [18].

The NAPR consists in defining five groups of crop response (**Table 2**). The use of this methodology enables to obtain the most responsive nutrient in the case of fertilizer recommendation; however, this recommendation might be carried with the soil physical and chemical analysis to guide the decisions.

Nutritional status	Criteria	Type of nutrient application potential response[1]
Deficiency	IA < 0, |IA| > NIIa[2], and IA is the index of lower value	Positive, with higher probability (p)
Deficiency prone	IA < 0 and |IA| > NIIa	Positive, with low probability (pz)
Sufficient	|IA| ≤ NIIa	Null (z)
Excess prone	IA > 0 and |IA| > NIIa	Negative, with a low probability (nz)
Excess	IA > 0, |IA| > NIIa, and IA is the index of higher value	Negative, with a higher probability (n)

[1]The NAPR was calculated based on Wadt [17].
NIIa = nutritional imbalance index average. IA = CND index for nutrient A.
Adapted with permission from Wadt [17].

Table 2. Criteria to interpret the CND index by nutrient application potential response (NAPR).

The practical interpretation for the nutritional status of the plant in **Table 2** is that "deficiency" means this nutrient shows high probability to positive response if applied in soil, which expects higher cotton yields. On the other hand, "excess" means the application of the nutrient in soil may result in luxury consumption and no response ought to be shown in terms of yield or food quality [15].

Nutrients	Method of diagnosis	Nutrient application potential response (NAPR)[1] (%)				
		p	**pz**	**z**	**nz**	**n**
N	DRIS	0	16.92	64.62	13.85	4.62
	CND	1.54	24.62	47.69	9.23	16.92
P	DRIS	7.69	12.31	49.23	6.15	24.62
	CND	6.15	9.23	58.46	4.62	21.54
K	DRIS	23.08	13.85	40	7.69	15.38
	CND	20	7.69	53.85	9.23	9.23
Ca	DRIS	23.08	10.77	50.77	9.23	6.15
	CND	21.54	7.69	56.92	9.23	4.62
Mg	DRIS	6.15	6.15	41.54	32.31	13.85
	CND	3.08	4.62	60	23.08	9.23
S	DRIS	9.23	12.31	50.77	12.31	15.38
	CND	7.69	10.77	60	13.85	7.69
Fe	DRIS	3.08	4.62	86.15	1.54	4.62
	CND	6.15	10.77	61.54	16.92	4.62
Zn	DRIS	0	4.62	89.23	6.15	0
	CND	0	7.69	75.38	12.31	4.62
Cu	DRIS	7.69	18.46	61.54	9.23	3.08
	CND	10.77	16.92	56.92	10.77	4.62
Mn	DRIS	18.46	15.38	46.15	9.23	10.77
	CND	13.85	15.38	50.77	10.77	9.23
B	DRIS	1.54	10.77	76.92	6.15	4.62
	CND	6.15	13.85	58.46	13.85	7.69

[1](p) positive, with higher probability; (pz) positive, with low probability; (z) null; (nz) negative, with a low probability; (n) negative, with a higher probability.

Table 3. Percentage of nutrient application potential response (NAPR) in cotton crop in Western region of Bahia State, Brazil [6].

The use of CND index interpretation for cotton crop can be a useful tool in association with soil analysis and experience of the agronomist to recommend fertilizer for cotton crop. Furthermore, the absence of nutritional diagnosis can result in excess of fertilizer as the case of P fertilizer observed by Serra et al. [6] in cotton crop in Western region of Bahia State, Brazil. In some specific plots where 180 kg P_2O_5 ha^{-1} was applied, Serra et al. [6] noted that by using NAPR the P In some specific leaf tissue was higher than plant requirement and it could be reduced by the P fertilizer doses (**Table 3**).

5. Nutrient interactions through principal component analysis on CND multivariable

In order to calculate the principal components analysis (PCA) of the row-centered log ratio in the high-yielding subpopulation, it was used the database (leaf nutrient content and crop yield) from Serra [19]. As reported by Parent and Dafir [4], the row-centered log ratios are compatible with PCA; based on it, this section aims at observing some interaction among the nutrients in PCA.

Row-centered log ratio	**High-yield subpopulation (*n* = 40)**		
	PC1	**PC2**	**PC3**
V_N	**0.6288**	**0.4875**	−0.0093
V_P	**−0.5588**	**−0.5935**	−0.2575
V_K	−0.2150	−0.1672	**−0.7761**
V_{Ca}	−0.1868	**0.7223**	−0.0605
V_{Mg}	0.2401	0.0017	**0.6842**
V_S	**−0.4352**	−0.2937	**0.5691**
V_{Fe}	**0.8159**	**−0.4529**	−0.0919
V_{Zn}	**0.8078**	**−0.4037**	−0.0469
V_{Mn}	**0.3879**	**0.6686**	−0.1689
V_B	**0.2892**	−0.0871	0.0494
V_{Cu}	−0.2150	0.1026	−0.1148
Eigenvalues	3.11	2.06	1.5192
Explained variance (%)	28.25	18.72	13.81
Accumulated variance (%)	28.25	46.97	60.78
Selection criterion (SC)	0.2836	0.3484	0.4056

Values in boldface are the dominant in the PC loadings by setting the level of significance defined according the selection criterion (SC).
Database from Serra [19].

Table 4. Correlations between the row-centered log ratio and the first three principal components (PC) for the high-yield subpopulation (40 observations).

One of the major objectives of the PCA is to reduce the number of interdependent variables into smaller numbers of independent principal component (PC), which are linear combinations of original variables. According to Parent and Dafir [4], CND is the multivariate expansion of CVA and DRIS and fully compatible to PCA.

Based on the PCA, it was obtained three PCs with eigenvalues above 1 and accumulated variance equal 60.78% (**Table 4**). The definition of the loading significance was defined according to Ovalles and Collins [20]. PC loadings or correlations between row-centered log ratios and the first three principal components must have values greater than the selection criterion (SC) for significant acceptation. SC proposed by Ovalles and Collins [20] was defined as follows:

$$SC = \frac{0.50}{\sqrt{PCeigenvalues}}$$

The three PCs combined explained 60.78 % of the total variance (**Table 4**). The first principal component was positively correlated with V_N, V_{Fe}, V_{Zn}, V_{Mn}, and V_B and negatively correlated to V_P, S, and V_{Cu} (**Table 4**). The second principal component was positively correlated with V_N, V_{Ca}, and V_{Mn} and negatively correlated with V_P, V_{Fe}, and V_{Zn} (**Table 4**). Finally, the third principal component showed positively correlated with V_{Mg} and V_S and negatively correlated with V_K (**Table 4**).

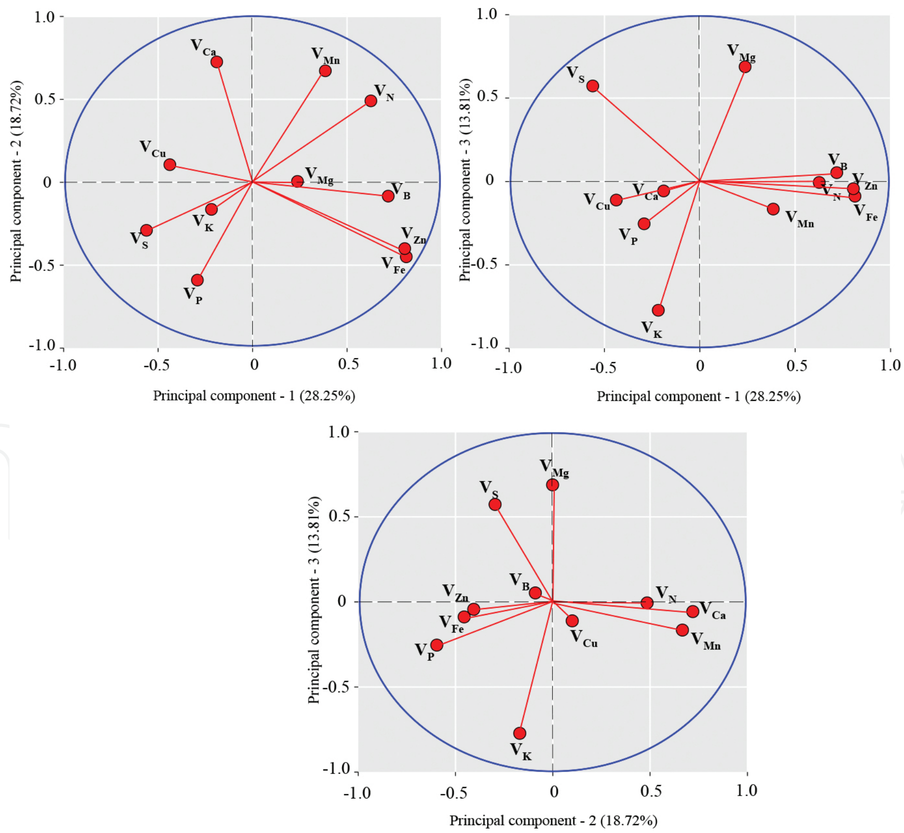

Figure 2. Distribution of principal component analysis (PCA) for row-centered log ratios. PC = principal components. Database from Serra [19].

The structures obtained by PC-1 (N+P-S-Fe+Zn+Cu-Mn+B+), PC-2 (N+P-Ca+Fe-Zn-Mn+), and PC-3 (K-Mg+S+) are supported by some rules of plant nutrition relative to interaction between nutrients, but other structures should be studied carefully for a better understanding (**Figure 2**).

In PC-1, it is feasible to observe positive interaction among N, Fe, and Zn; this result may be explained as reported by Marschner [21] that Fe, Zn, and protein are very highly correlated, which infer that protein is a sink for Fe and Zn. In PC-2, the inverse relation between P and Ca may be explained because of dilution and accumulation effects with plant age. The PC-3 showed the opposite direction for K and Mg inferring the antagonism effects between these nutrients (**Figure 2**).

N and S showed to be highly correlated with the first PC; however, S was negatively correlated with N (**Figure 2A**). It is important to have N availability in soil in balance with S, since this nutrient is a component of amino acids and proteins. Cysteine is the first stable compost of the assimilatory reduction of S that is the precursor of all compounds, in which S makes part in the plants [21]. Thus, S deficiency compromised the formation of amino acids. Furthermore, the N and S show essential functions in the activity of chlorophyll molecule. The S deficiency may deplete the action of chlorophyll and consequently reduction of photosynthesis [22].

The second PC was strongly influenced by positive and negative eigenvectors V_N, V_{Ca}, and V_{Mn} and V_P, V_{Fe}, and V_{Zn}, respectively (**Figure 2C**). In positive and negative quadrant, the nutrients are grouped according to the relationship among them in the plant metabolism, as example, the Zn affects the metabolism of Fe [23], or interactions among nutrients in the rhizosphere. V_{Mg} (positive) and V_K (negative) affected the third PC, which showed opposite directions between these variables inferring the excessive application of K source in soil, inhibit the uptake of Mg by plant roots [21].

6. Conclusion

Being an effective diagnosis tool for the nutritional status of cotton, CND may improve the diagnosis with the application of multivariate analysis. The development of CND norms including means and standard deviation of row-centered log ratios in high-yielding subpopulation can be accomplished based on field trials or experimental database. This information in database is comprised at least by the concentration of leaf nutrients and cotton yield in well-defined plots. CND, due to its interactions with multi-nutrients, can improve the nutritional diagnosis in cotton crop better than traditional methods as CVA and SR. In literature, there are not many results published about CND in cotton crop, but researches should be encouraged worldwide to use this method because of CND importance, to know the plant nutritional requirement, and to direct the decision on fertilizer management. Moreover, the row-centered log ratio is fully compatible with principal component analysis (PCA) that can expand the results of nutrient interaction in leaf tissue of cotton crops.

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