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Influence of the relationship among nutrients on yield of cocoa (*Theobroma cacao* L.) clones

Influencia de las relaciones entre nutrientes sobre el rendimiento en clones de cacao (*Theobroma cacao* L.)

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Abstract

Relations among nutrients affect the plant nutrition, promoting growth and yield of crops. Therefore, this study aimed to determine the influence of the relationships among foliar nutrients on the yield of four clones of fine cocoa (*Theobroma cacao* L.) two clones self-incompatible, TSH-565-ICS-39, and two self-compatible, CCN-51-ICS-95. Different doses of fertilization was applied to determine which of this ratio nutrients more affect the yield, for that, was used a randomized complete block design with five treatments (TC (control), T1, T2, T3, T4 were successive increments of NPK respectively 25%, 50%, 75% and 100% of the TC) and four replications was used. Results show differences a level leaf in nutrient concentration under the influence of clone, treatment and interaction clone*treatment; the effect clone in P, Mg^{2+}, B, Mn^{2+} and Zn^{2+}; the effect treatment in all nutrients and effect interaction clone*treatment in N, P, Mg^{2+}, B, Fe^{2+}, Mn^{2+} and Zn^{2+}; this indicates that the applied fertilizer levels influencing the availability and absorption of nutrients. Overall all clones have affinity for Mg^{2+}, S, P; in different order of preference for N, K^{+}, Ca^{2+} and the minor elements for Mn^{2+}, Fe^{2+}, Zn^{2+}, Na^{+}, B and Cu^{2+}, except TSH-565. From the sixty six relationship among nutrients studied at the foliar level, it was found that the relationships are associated with more performance are: N/Mg, N/P, N/S, N/K, the first two are positive and the remainder negative yield.

Keywords: Clone self-incompatible, clone self-compatible, fertilization, plant nutrition, *Theobroma cacao*.

Resumen

Las relaciones entre nutrientes afectan la nutrición de las plantas, favoreciendo el crecimiento y el rendimiento. Esta investigación tuvo como objetivo determinar la influencia de las relaciones entre nutrientes a nivel foliar, sobre el rendimiento de cuatro clones de cacao fino (*Theobroma cacao* L.), dos clones autoincompatibles, TSH-565–ICS-39, y dos autocompatibles, CCN-51–ICS-95. Se aplicaron diferentes dosis de fertilización para determinar cuál de estas relaciones afecta más el rendimiento, para ello se utilizó un diseño en bloques completos al azar con cinco tratamientos (TC (control), T1, T2, T3, y T4) consistentes en incrementos sucesivos de NPK, respectivamente de 25%, 50%, 75% y 100% sobre el tratamiento control (TC) y cuatro repeticiones. Los resultados muestran diferencias en la concentración de nutrientes a nivel foliar por influencia del clon, tratamiento e interacción clon*tratamiento; por efecto del clon en P, Mg^{2+}, B, Mn^{2+} y Zn^{2+}; por efecto del tratamiento en todos los nutrientes y por efecto de la interacción clon*tratamiento en N, P, Mg^{2+}, B, $Fe^{2,}$ Mn^{2+} y Zn^{2+}; lo cual indica que los niveles de fertilización aplicados influyen en la disponibilidad y absorción de nutrientes. En general todos los clones presentan afinidad por Mg^{2+}, S, P; en diferente orden de preferencia por N, K^{+}, Ca^{2+} y en los elementos menores, por Mn^{2+}, Fe^{2+}, Zn^{2+}, Na^{+}, B y Cu^{2+}. De las sesenta y seis relaciones entre nutrientes estudiadas a nivel foliar, se encontró que las relaciones que más se asocian con el rendimiento son: N/Mg, N/P, N/S, N/K; las primeras dos tienen efecto positivo y el resto efecto negativo sobre el rendimiento.

Palabras clave: Clon autoincompatible, clon autocompatible, fertilización, nutrición de plantas, *Theobroma cacao*.

Introduction

The cocoa has its geographical and cultural roots in Mesoamerica, but its use extended to Europe in the 16th and 17th centuries and from there to African countries (Forrest and Glick, 2007). Currently, the largest producers of cocoa are the Ivory Coast and Ghana in Africa, followed by Indonesia in Asia and Brazil, Ecuador, the Dominican Republic and Colombia in the Americas (FEDECACAO, 2010).

Cocoa production not only depends on the fertility level of the soil but also on interactions with ambient factors, crop management, and the ratios present between nutrients. It is known that the absorption of ions by plants depends on the soil solution, whose composition is modified by the manner and quantity of applied nutrients. The same author suggested that each time nutrients are added on an individual basis, the behavior of the ions and the relationship between them in the soil solution are changed and the absorption of cations and the relationship between them in the solution are altered, as a consequence of changes at the exchange sites. Knowledge on this nutrient dynamic in soils helps to improve crop nutrition.

Studies on the effect of ratios between nutrients as a mechanism that involves some of the factors that affect nutrient absorption and transport that, in the end, generate disbalances that affect yield have been carried out by Torres *et al.* (2009) in orange orchards, who affirmed that binary relationships in nutrient concentrations have demonstrated effectiveness in the diagnosis of nutritional problems. With Eucalipto (*Eucalyptus grandis*), Battie-Lacau *et al.* (2014) studied photosynthetic and anatomical responses and the influence of supplying Potassium and Sodium on the net assimilation rate of CO_2 and on stomatal conductance, which, in the end, influences other physiological processes of plants and yields. In the same vein, Balkos *et al.* (2010) demonstrated with rice that an increase in ammoniacal Nitrogen applications affects the absorption and transport of Potassium, generating a disbalance in the plant. Gattward *et al.* (2012), over the course of 180 days under greenhouse conditions with six-month-old cocoa plantlets, studied the synergistic Na/K relationship and its effect on photosynthesis stimulation, on water use efficiency and on mineral nutrition, but did not evaluate the possible impact on the yield of the crop. Individual nutrient applications with average crop doses often lack satisfactory responses in the yield because it is not considered necessary to maintain an appropriate ratio between certain nutrients, resulting in metabolic alterations. In this sense, Salisbury & Ross (1992) demonstrated that the N/P ratio affects maturity, with excess Nitrogen impeding it and excess Phosphorus accelerating it or otherwise stimulating the development of roots with regards to the growth of the aerial zone.

Little is known on the effect of different fertilizer doses produced on nutrient ratios at the foliar level in cocoa and the repercussion on crop yield. In this sense, the reviewed literature facilitated this study, with the aim of determining the influence of nutrient ratios at the foliar level on the yield of four fine cocoa clones with variance in fertilization doses in the soil.

Materials and methods

The present study was carried out at the Center of Investigation of the *Federación Nacional de Cacaoteros*, (3°15′ North and 76°15′ West in Colombia) at an altitude of 1120 m and with an average annual precipitation of 1379 mm and an average temperature of 25°C (IGAC, 1993). The fine cocoa clones used in the present study were: ICS-95 and CCN-51 (self-compatibles) and TSH-565 and ICS-39 (self-incompatibles) (Puentes *et al.*, 2014b)

Soil samples were taken at a depth of 30 cm (Muñoz, 2001); the following were evaluated: pH, soil–water ratio 1:2.5, with a potentiometer; Organic material by Walkley Black, Phosphorus by Bray II; cationic exchange capacity (CEC) and cation changes (Ca^{2+}, Mg^{2+}, k^{+}, Na^{+}) with ammonium acetate 1M at a pH of 7, quantified by atomic absorption; lesser elements (Cu^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+}) by modified Olsen; S and B with monocalcium phosphate; texture by the pipette method and apparent density by the nucleus method.

The foliar sampling was carried out in accordance with the INIAP methodology (Carrillo.,2006), for which, the fourth leaf of branches in the middle part of the cocoa tree was taken, with twenty five leaves per sample; over the course of three years, (2010-2012), the following elements were analyzed: Nitrogen, Phosphorus, Potassium, Calcium, Magnesium, Iron, Manganese, Copper, Zinc, Boron, and Sulphur; afterwards, the nutrient ratios were determined, which were obtained by division of the concentration of each analyzed major element (NPK) in the leaf by the rest of the determined nutrients, for which it was assured that all were in equal units.

In order to standarized the production, at the start of the experiment, fruits that were three or more months-old were eliminated from the cocoa trees; the fertilizer doses were applied in two periods, one in May and one in September of the years: 2010, 2011, and 2012. The yield was determined by the product of: the numbers of seeds per pod, the number of pods per tree and the seeds per density of plants per hectare index (952 plant ha^{-1}).

A Randomized Complete Block (RCB) design was used with five treatments and four repetitions; the experimental unit consisted of four five-year-old cocoa trees planted at a distance of 3m x 3m between plants and 4m between rows. In order to determine the effect of the applied treatments on the nutrient ratios at the foliar level and their influence on yield, the treatments were designed from the initial concentrations of NPK of the soil, which was dubbed TC, the rest of the treatments (T1, T2, T3, T4) were successive increments of the NPK at the respective levels of 25%, 50%, 75% and 100% over the TC (Table 1).

Table 1. Description of the treatments treated with kg ha^{-1} of each nutrient.

Treatment	N	P_2O_5	K_2O
TC	49	23.5	146.5
T1	61	29.3	183
T2	73	35.2	219.7
T3	86	41	256.4
T4	98	47	293

The results were subjected to an analysis of variance (ANOVA) and a mean comparison Duncan test was used in those cases where a significant difference was detected. In order to determine which nutrient ratios at the foliar level had an effect on yield, an Analysis of Principal Components (APC) was used. Also, using a KMO test (Kaiser-Meyer-Olkin Measure of Sampling Adequacy) and Bartlett´s sphericity, the suitability of the nutrient ratio information for analysis was verified. The KMO contrasted the partial correlations between the variables, indicating that the factorial analysis was suitable with higher values. Those ratios of high value extraction in components where the yield variable was located and with large contributions to the matrix of correlations of the same were selected; afterwards, in order to predict the behavior of the yield, an automated linear regression was carried out between the obtained ratios in the APC and the yield, using the statistical program: *SPSS* 20.

Results and discussion

The chemical analysis demonstrated that the soil presented good conditions for the development of the crop (Puentes *et al.*, 2014a), possessing a suitable pH (6.4), a high organic material content (7.4%), and normal mean contents of Potassium (0.26 $Cmol^+kg^{-1}$), Phosphorus (8.6 mg kg^{-1}) and CEC (19.35 $Cmol^+kg^{-1}$). There were good Ca/Mg and K/Mg ratios and a high concentration of lesser elements.

In the physical aspect, there were no limitations; the soil was suitable for agriculture with good porosity (55%), apparent density (1.2 g cm^{-3}) and texture (silt-loam). In the mineralogy of the clay, high proportions of Illite were observed with interstratifications of Illite/Vermiculite.

Absorption and concentration of the nutrients at the foliar level. There were highly significant ($p<0.01$) differences in the foliar content of the nutrients related to the clone, treatment and the clone*treatment interaction with effects from the clones on P, Mg^{2+}, B, Mn^{2+} and Zn^{2+}, effects from the treatments on all the nutrients and effects from the clone*treatment interaction on N, P, Mg^{2+}, B, $Fe^{2,}$ Mn^{2+} and Zn^{2+}.

At the foliar level, the extraction and concentration of the nutrients were different. The results show that the applied fertilization levels influenced the absorption of clones CCN-51, TSH-565, ICS-95, and ICS-39, as seen in Figure 1, having

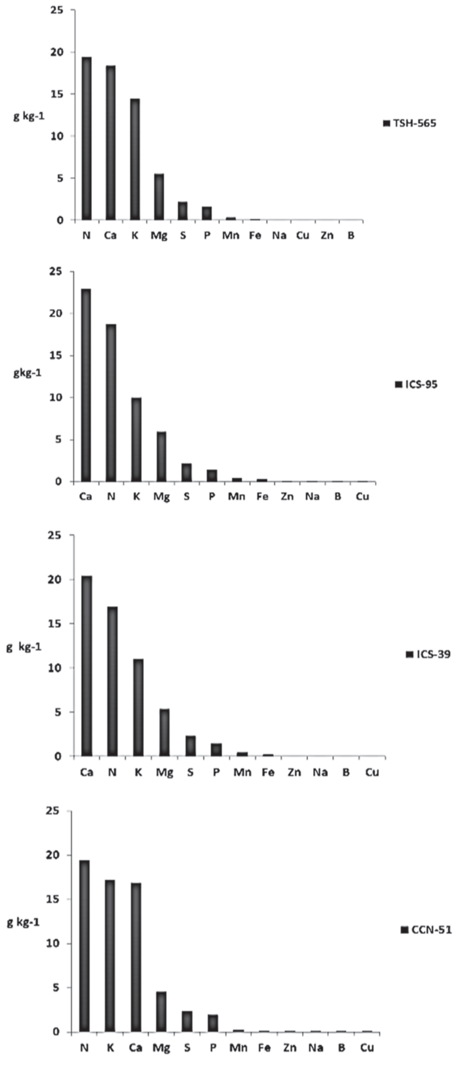

Figure 1. Extraction and preference order of the nutrients at the foliar level for the four clones.

presented, in general terms, affinity, in all the clones, for the extraction order of Mg^{2+}, S, and P; but, a different order of preference for N, K^+, and Ca^{2+}. For the lesser elements, the preference order, rather than the concentration, was Mn^{2+}, Fe^{2+}, Zn^{2+}, Na^+, B and Cu^{2+} for the clones, except TSH-565.

The values reported for Nitrogen were considered low for ICS-39 and TSH-565 and normal for ICS- 95 and CCN-51, consistent with Sodré (2002). In general terms, the self-compatible clones presented a higher tendency to accumulate N, P and K than the self-incompatible clones. The concentration of the major elements produced similar evidence as that of the lesser elements: influence from the applied treatments on the clones. These results show that the variance in the fertilization levels in the soil changed the soil solution, which influenced the absorption and concentration of the nutrients at the foliar level of the clones. Corroborating, that the concentration of nutrients in the plant depends of the availability of these plant nutrients in soil (Mengel & Kirkby, 2001).

Nutrient ratios and their effect on yield. In regards to yield, there were highly significant ($p<0.01$) differences due to the effects of the clones and treatments. The highest yield was seen in T1 of the CCN-51 clone (2300 kg ha^{-1}), followed by T2 in the ICS-39, ICS-95 and TSH-565 clones (1660, 1359 and 1352 kg ha^{-1} respectively). These values are higher than those reported for CCN-51, ICS-95, TSH-565 previously. The lower yields were seen in the TC with the following order: ICS-95, ICS-39, TSH-565 and, finally, CCN-51 with a normal presentation, due to the fact that the TC did not have fertilizer applications.

66 nutrient ratios were analyzed at the foliar level, including the combinations of the major elements with the rest of the analyzed elements. In the initial commonalities phase of the APC, 17 variables remained due to their good and/or excellent measure of sampling or extraction value (0.8<KMO≤0.1); with these, four components were identified that represented 87.95% of the variance.

With the 17 ratios selected by APC, the yield modeling proceeded with automated linear regression. The ratios that were more associated with the yield were: N/Mg, N/P, N/S, and N/K. The first two had a positive effect while the rest had a negative effect.

Figure 2 shows that the N/Mg ratio most favored the yield at an order of 8:1, that is to say eight parts Nitrogen per one part Magnesium. The clones presented different responses, for example the ICS-39 and TSH-565 self-incompatible clones achieved this ratio in the leaves with the lowest fertilizer level, that is to say in T1. The ICS-95 and CCN-51 self-compatible clones achieved the same in T3, thereby favoring yields that can reach values of 2300 kg ha^{-1}. Values below 2:1 in ratios of the leaves were seen in the ICS-39, ICS-95 and CCN-51 clones with the T4 fertilization level. The TSH-565 clone presented ratios below two in T3. This ratio level for N/Mg produced yields that are not very attractive for cacao producers, that is to say less than 500 kg ha^{-1}.

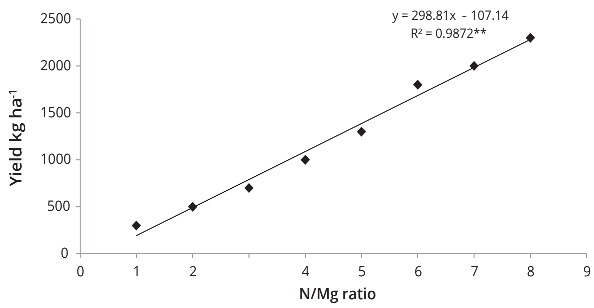

Figure 2. Influence of the N/Mg ratio on cacao yield.

The absorption of certain nutrients is conditioned by their availability in the soil solution, whose composition changes depending on many factors and on any applications. In this sense, Hansen (1972) carried out a study on the effect of the quantity and form of Nitrogen on the soil solution and the absorption of various nutrients, including Magnesium, demonstrating that both the concentrations and the proportions of the cations on an individual basis change when nitrate or ammonium are applied. The availability of Magnesium increased when the Nitrogen application took the form of NH_4^+ because it displaces Mg in the exchange sites of the soil solution to where the plants can take it up. According to Marschner (2003), when Magnesium is hydrated it presents the lowest attraction force for the cellular wall and the plasmatic membrane; and so, it is important to maintain the balance of the N/Mg ratio because Magnesium participates in the chlorophyll molecule and in many enzymes that are necessary in the photosynthesis process (Salisbury and Ross, 1992).

In regards to the N/P ratio, values of 9:1, that is to say nine parts of Nitrogen per one part of Phosphorus, produced good yields. With ratios below 5:1, the yields fell to below 500 kg ha^{-1}, which are not profitable (Figure 3). All of the clones presented the best foliar level ratio and yield in T2, with the exception of CCN-51 which did so in T1. Even clearer than these ratios, the genetic potential plays an important role in yield along with the management practices.

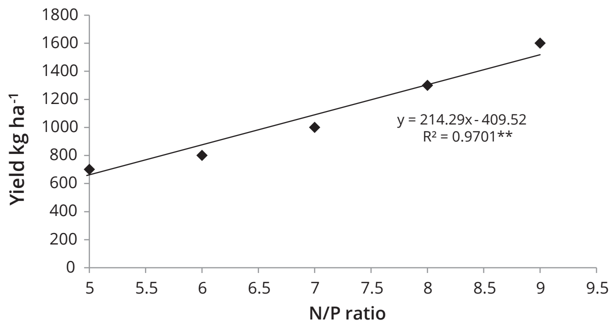

Figure 3. Influence of the N/P ratio on cacao yield.

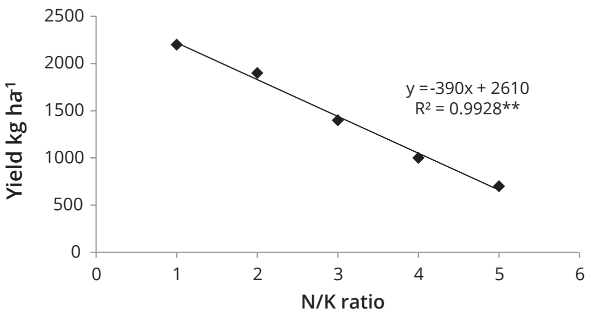

Figure 4. Behavior of the N/K ratio and its relationship with cacao yield.

The results of the study on the effects of the N/P ratio demonstrate that the phosphate content changes in plants due to the dose and form of the Nitrogen application. When nitrates are applied, a similar situation occurs with the concentration of phosphates in the phloem, which is favored by the application of ammoniacal Nitrogen. This situation must be considered in the search for a suitable ratio between these nutrients.

Bot *et al.* (1998) suggested the existence of a narrow ratio between the content of Nitrogen and Phosphorus and the metabolism of Carbon and also suggested that a lack of Nitrogen in plants induces changes in the synthesis of carbohydrates due to the accumulation of starch and a decrease in specific leaf area. These authors put forth that a deficiency in Phosphorus affects the growth of biosynthetic tissues and decreases the concentration of Phosphorus in the biomass and, therefore, the maximum photosynthesis rate. Recalling that the absorption of Phosphorus depends on and is related to the development of the root system in plants. Pellerin *et al.* (2000) found a delay in the appearance of adventitious roots in plants in conditions of low phosphorus contents, in addition, the foliar area index and net photosynthesis rate were affected. Perhaps the increase in Nitrogen levels per Phosphorus unit has a favorable effect on the development of the foliar area, on the roots and on the photosynthesis rate, creating a balance between Nitrogen and Phosphorus, which favors the metabolism of Carbon, which translates into good yields.

In regards to the N/K ratio, values below 1:1, that is to say one part Nitrogen per one part Potassium, favored yield with production over 2200 kg ha^{-1}, when this ratio passed values over 5:1, five parts Nitrogen per one part Potassium, they appeared to affect cacao yield with values under 500 kg ha^{-1} (Figure 4). Similar results were obtained by Hernandez *et al.* (2009), who showed that the best N/K ratio combination of yield and fruit quality was obtained with the 1:0.75 N/K ratio, however, when this ratio changes to 1:0.90 the yield decreases.

It is evident that high Nitrogen levels affect the absorption of Potassium, recalling that Potassium is the most abundant element in plant cells and participates in countless functions including the activation of many enzymes, its absorption depends on relationships with other nutrients, such as Sodium and Nitrogen (Britto and Kronsucker, 2008).

It is possible that the absorption of Potassium is affected by Nitrogen levels as confirmed by Mengel *et al.* (1976). In the same sense, the application of ammonium generates acidic conditions in cells, followed by an increase in cellular pH, forming transitory organic acids from starch and, when it dissipates, produces a decrease in the Hydrogen quantity, producing an increase in the absorption of Potassium (Salisbury and Ross, 1992).

And so, a decrease in cacao yield is seen with an increase in Nitrogen levels. According to Balkos *et al.* (2010), Potassium collaborates the metabolism and transport of Nitrogen; these authors suggested that a fertilizer regime that uses 10mM of NH_4^+ and 5mM of K^+ at a ratio of 2:1 could considerably improve and optimize the growth of field rice. This indicates that in addition to the increase in Nitrogen levels in the soil, it is also good for Potassium contents for the creation of a suitable balance such as a 3:1 ratio which produces good cacao yields. It is also possible that the clay mineralogy had an effect on the availability of Potassium because clay, like the Illite that was present in this soil, is very affinitive for this element, which produces a higher fixation and lower availability of the nutrient for the crop (Henríquez *et al.*, 1994).

In regards to the ratios that produced negative effects on the cacao yield, N/S is noteworthy as can be seen in Figure 5; values below 3:1, that is to say 3 parts Nitrogen per one part Sulphur, favored yield; values over 12:1 substantially affected yield in a negative manner to the point that the production was not profitable. Increasing the level of Nitrogen in soils without Sulphur in the fertilization plan creates a disbalance that affects yield.

The absorption of Sulphur and other anions is affected by the dose of and high levels of Nitrogen and also by the form of the Nitrogen, whether it is ammonium or nitrate. Beusichem *et al*. (1988) confirmed that the absorption of Sulphur and other anions and cations is affected by changes in the intercellular pH, suggesting an accumulation of sulphates in the xylem when Nitrogen is absorbed as NH_4^+. However, there is an increase in the concentration of sulphates in the phloem when the Nitrogen form taken by *Ricinus communis* is NO_3. Perhaps, for this reason, it is difficult to translocate it to the leaves, where the N/S ratio is disbalanced and negative effects are produced on the yield.

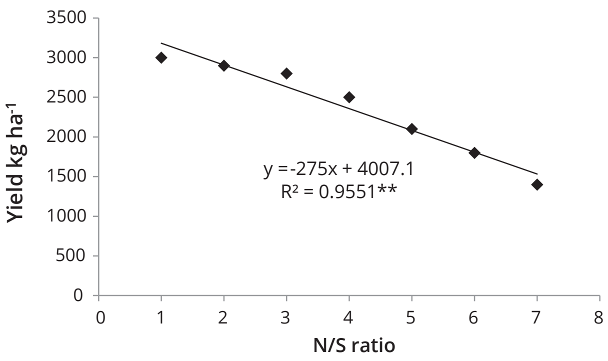

Figure 5. N/S ratio and its influence on cacao yield.

In effect, in the measurement that increased the fertilization levels in the soil, the ratios tended to change and the yield decreased; on the other hand, it is clear that the nutrient contents present in the TC were not sufficient for meeting the nutritional needs of the clones not for maintaining good ratios that would favor yield as suggested Monsuru *et al*. (2013) in the same direction for natural rubber (*Hevea brasiliensis*) clones.

Conclusions

When varying the fertilization levels, the concentrations of the nutrients were differential, which generated differences in the nutrient ratios, which in turn influenced the yield for each clone, with the CCN-51 clone being the one with the highest yield, followed by ICS-39, ICS-95, and, finally, TSH-565. So, the ratios that were more associated with yield were: N/Mg, N/P, N/S, and N/K, with the first two demonstrating a favorable effect on yield while the latter two demonstrated negative effects.

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