Research Article

Nutritional diagnosis of banana (*Musa* AAA Simmonds subgroup Cavendish) with root sap analysis[1]

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ABSTRACT

The status of mineral nutrients in the banana crop is commonly determined by foliar and soil analyses, which often do not present a significant relation with its production performance. This study aimed to evaluate whether the root sap analysis determines the nutritional status of plants more accurately in response to fertilization. The experiment was carried out in a completely randomized design, with three treatments (complete fertilization, traditional fertilization and no fertilization), three replicates and four plants per replicate. The contents of macro (N, P, K, Ca and Mg) and micronutrients (B, Zn, Mn, Fe and Cu) were analyzed in the root sap, leaves and soil at the base of the plant. Potassium was the macronutrient found in the highest quantity in the root sap of the fertilized and unfertilized plants, while the predominant micronutrients were Mn in the fertilized plants and Fe in the unfertilized ones. The concentrations of N, P, K, Ca and Mg in the root sap were significantly lower for no fertilization than for complete and traditional fertilization, but did not show significant differences between the foliar and soil analyses. The root sap analysis was more sensitive than leaf analysis to diagnose the nutritional status of the banana plants.

KEYWORDS: Plant nutrition, plant-soil relations, foliar analysis, soil analysis.

RESUMO

Diagnóstico nutricional de banana (*Musa* AAA Simmonds subgrupo Cavendish) com análise de seiva da raiz

O estado nutricional mineral da cultura da banana é geralmente determinado por análise foliar e de solo, que, com frequência, não apresentam relação significativa com seu desempenho produtivo. Objetivou-se avaliar se a análise da seiva da raiz determina com maior precisão o estado nutricional da planta em resposta à fertilização. O experimento foi conduzido em delineamento inteiramente casualizado, com três tratamentos (fertilização completa, fertilização tradicional e sem fertilização), três repetições e quatro plantas por repetição. Foram analisados os conteúdos de macro (N, P, K, Ca e Mg) e micronutrientes (B, Zn, Mn, Fe e Cu) na seiva da raiz, folhas e solo na base da planta. Potássio foi o macronutriente encontrado em maior quantidade na seiva da raiz das plantas fertilizadas e não fertilizadas, enquanto os micronutrientes predominantes foram Mn nas plantas fertilizadas e Fe nas não fertilizadas. As concentrações de N, P, K, Ca e Mg na seiva da raiz foram significativamente menores para a não fertilização do que para a fertilização completa e tradicional, mas não mostraram diferenças significativas entre as análises foliar e do solo. A análise de seiva foi mais sensível do que a análise foliar para diagnosticar o estado nutricional da bananeira.

PALAVRAS-CHAVE: Nutrição de plantas, relações planta-solo, análise foliar, análise de solo.

INTRODUCTION

Bananas (*Musa* AAA) and plantains (*Musa* AAB) are grown in more than 120 developing countries, representing the basic food for nearly 400 million people and, in terms of total production value, are considered the fourth most important food worldwide after rice, wheat and milk (Perea & Tirado 2010, FAO 2022).

Generally, big differences can be observed between the concentrations of mineral nutrients in the soil and the requirements of mineral nutrients by plants, although the concentrations of nutrients in soils are commonly higher than the plant

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physiological requirements (White 2012). However, to estimate the production of the banana crop, the plant nutrient status is commonly determined based on foliar and soil samples.

There has always been a controversy if the soil or foliar analysis is more appropriate to provide fertilizer recommendations; the consensus is that the nutrient status of a plant is better shown by the element concentration in leaves than in other plant organs (Römheld 2012).

While the soil analysis indicates the quantity and potential availability of the nutrients that the roots can absorb in favorable conditions, the foliar analysis shows the concentration of these elements accumulated in the leaves at a given phase of the developmental stage (Farneselli et al. 2014, Nadezhdina et al. 2020). However, these analyses are often uncertain, because they do not necessarily correlate with the productive performance of the crop. The nutrient concentration in the leaves has been considered a reliable indicator of the long-term nutrient status of plants, although some researchers reported poor relationships between the soil and foliar analyses and the yield in banana (Twyford & Walmsley 1974, Turner et al. 1988). Hernández (2004) found no relationship between the nutrient content in the soil and the productivity of bananas, even though the nutrients were available in the soil solution. In banana plantations of Costa Rica, the foliar concentrations of mineral nutrients were similar in areas of good growth as in areas of poor growth, indicating that there was no direct relationship between the soil and foliar analyses and the nutrient status of the plantations (López & Espinosa 1995).

Plant sap analysis is an option for determining the plant nutrient status and provides the opportunity for growers to adjust fertilization and apply the specific amount of nutrients needed (Esteves et al. 2021), because it can assess the nutrient uptake more precisely and increase the fertilizer use efficiency (Cadahía et al. 2008, Goffart et al. 2008, Incrocci et al. 2017).

The sap extracted from roots is mainly composed of fluids of the vascular bundles of xylem and phloem and the vacuolar content (Cadahía et al. 2008, Gangaiah et al. 2016). Plant sap analysis is an early determination of plant nutrient status, since it comes from real-time information (Goffart et al. 2008, Incrocci et al. 2017). The root sap analysis could be an effective tool for quick and economic diagnosis of the deficiencies or excesses of mineral nutrients (Errebhi et al. 1998, Aguilera et al. 2014), thus allowing the conducting of programs and timely adjustments of fertilization in horticultural crops (Gómez et al. 2017).

Since the fluid collected with root excision is a mixture of xylem and phloem saps, its analytical separation in the laboratory is difficult (Frutos et al. 2009). Additionally, it is difficult to obtain pure sap from phloem or xylem for analysis, due to the possibility of contamination of other injured tissues at the time of sample collection and the immediate sealing of vascular cells at the time of cutting (Shakya & Lal 2018).

Thus, this research aimed to analyze and compare the concentrations of nutrients in the sap of roots, leaves and soil at the base of the plant pseudostem, in order to determine the nutrient status of banana plants.

MATERIAL AND METHODS

The study was carried out at the Cenibanano banana research center (Carepa, Antioquia, Colombia) (7º46'46"N, 76º40'20"W and 20 m a.s.l.), where the maximum, minimum and mean temperatures are 32.3 ºC, 23.2 ºC and 26.7 ºC, respectively, with 1,700 hours of sunshine/year, 2,896 mm of mean annual rainfall (Ideam 2015) and climatic characteristics corresponding to tropical humid forest (bh-T) (Holdridge 1967). The soils are classified as Fluventic fine Eutrudepts, Fluvaquentic fine Eutrudepts loamy on clay and Vertic fine loamy Endoaquepts, with 51 % of silt, 40 % of clay, 9 % of sand, pH 5.9 and low K and P contents in the first horizon (Gutiérrez 2007). A plantation of the 'Williams' banana (*Musa* AAA Simmonds subgroup Cavendish) was used, with plants established in a triangle, at distances of 2.5 m between rows and 2.5 m between plants, for a density of 1,600 plants ha^{-1}.

The experiment was conducted in three lots managed with different levels of fertilization: complete fertilization, established based on soil and foliar analyses in the lot; traditional fertilization, established without soil and foliar analysis in the lot; and no fertilization (lot unfertilized for five years) (Table 1).

The fertilizers used in the study were urea (46 % of N), diammonium phosphate (46 % of P_2O_5),

Table 1. Fertilization treatments.

Type of fertilization	N	P_2O_5	K_2O	CaO	MgO	S	B	Zn
	kg ha^{-1} $year^{-1}$							
Complete	312.2	43.8	467	31.3	22.4	68.3	1	0.3
Traditional	317.4	30.5	468	0	0	0	0	0
No fertilization	0	0	0	0	0	0	0	0

KCl (60 % of K_2O), Nitromag (21 % of N; 11 % of CaO; 7.5 % of MgO), Nitrabor (15.45 % of N; 25.5 % of CaO; 0.3 % of B) and Sulpomag (22 % of K_2O; 18 % of MgO; 22 % of S), and the fertilization was done every 21 days, for 13 weeks.

A completely randomized design was used, with three treatments, three replicates and four plants per replicate. The irrigation, weed control and phytosanitary management of the experimental lots were uniform and followed the management of commercial plantations.

The samples were obtained at the initial phase of bunch development, when the first hand of fruits become visible, after the unfolding of their floral bracts (Kuhne et al. 1973, Robinson & Galán-Saúco 2012), which is the most appropriate moment to determine the nutrient requirements of the plant (Belalcázar et al. 1976). In four plants per replicate, sap samples were collected from the primary (first order adventitious) roots. The leaf samples were obtained from the youngest fully expanded leaf (leaf 3), which was separated from the plant with a knife, and then a central sector of 10 cm leaf blade was taken on both sides of the leaf midrib. Soil samples were collected at 30 cm from the base of the plants and at a depth of 30 cm.

For the root sap samples, part of the soil around the plant pseudostem was removed at a depth of 20 cm, and mature and healthy primary roots with white color, typical firmness and flexibility, and 5-8 mm diameter were chosen (Soto 1992, Robinson & Galán-Saúco 2012); these did not present physical damage that could interrupt the sap flow (Figure 1A). Each selected root was washed with distilled water and then cut with a clean blade at a distance of 10 cm from the rhizome and immediately placed in a 20 mL plastic vial. The vials were covered and hermetically sealed with a polyethylene bag, to prevent the collected sap from being contaminated with soil particles, rainwater or living organisms (Figure 1B).

The vial was left for 24 hours until about 80 mL of the sap sample were collected, and then one drop of 1 % HCl was added to stabilize the pH and prevent sap denaturation by microorganisms. The sample was sent to the laboratory to determine the

Photos: Manfred Ricardo Palacio

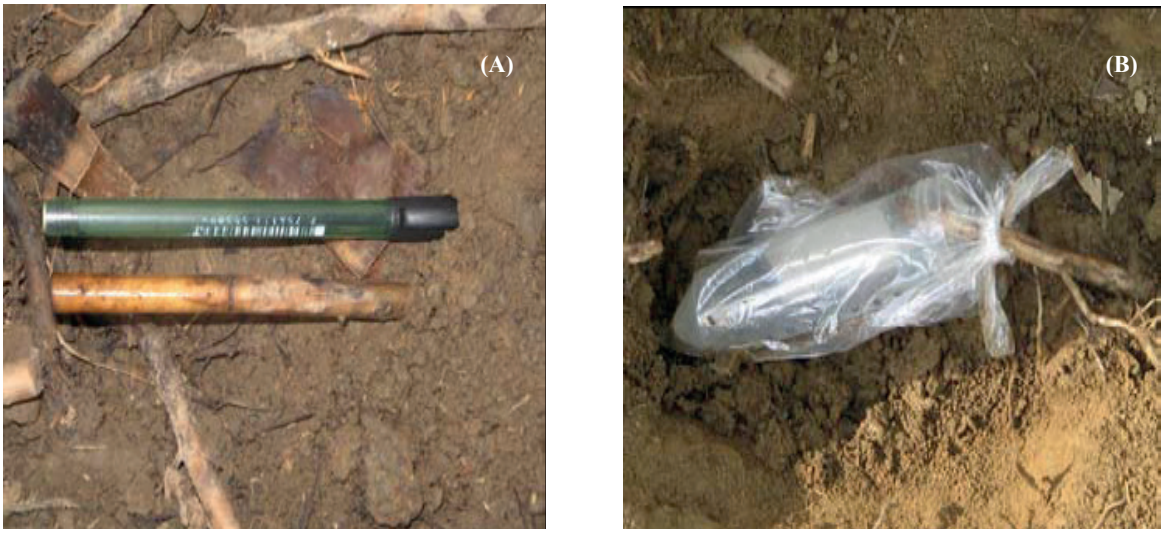

Figure 1. Selection of the primary root (A) and root sap collection (B).

total concentration of macro (N, P, K, Ca and Mg) and micronutrients (B, Zn, Mn, Fe and Cu) in the root sap. The total nitrogen content was determined by the micro-Kjeldahl method and volumetric titration, and P was determined by colorimetric titration with ammonium vanadate and molybdate. Ca, K, Mg, Cu, Fe, Mn and Zn were quantified with titration by atomic absorption spectrophotometry, and B was determined by colorimetric titration with Azomethine-H (IGAC 2006).

The foliar and soil analyses were carried out according to the international reference method for sampling of fertilizer experiments in banana (López & Espinosa 1995, Espinosa & Mite 2002). Leaf samples were obtained from the central sector of the third leaf blade and dried in a forced ventilation oven at 65 ºC, until constant weight. Soil sampling was done at the base of the pseudostem of each sampled plant and three sites around the roots selected for sap extraction. Nitrogen was quantified in a Leco TruSpec CN Elemental Analyzer, and P was determined by calcination at 475 ºC and colorimetric titration with molybdate and ammonium vanadate. K, Ca, Mg, B, Zn, Mn, Fe and Cu were quantified by atomic absorption spectrophotometry (IGAC 2006).

The data that did not present homogeneity of variance were transformed with $(x + 0.5)^{0.5}$ and subjected to analysis of variance (Anova) for the evaluated nutrients to verify the significant differences among the types of fertilization. A multivariate analysis of variance (Manova) was also performed to contrast the differences, considering the three types of analysis (root sap, foliar and soil) simultaneously. The means were compared using the Tukey test ($p \leq 0.05$). The analyses were carried out with the SAS 9.4® statistical software (SAS Institute, Inc., NC, USA).

RESULTS AND DISCUSSION

The sap collected from plant roots is a mixture of xylem and phloem fluids composed of water, mineral nutrients, hormones and other mostly organic substances (Cadahía et al. 2008, Gangaiah et al. 2016). The concentration of the macronutrients in the root sap was highly variable among the fertilizer treatments (Table 2). Potassium was the element found in the highest quantity in the root sap and in the soil where the plants were grown. The concentrations of N, P, K, Ca and Mg in the root sap were significantly lower in the treatment with no fertilization than in the complete and traditional fertilization treatments, but did not show significant differences between the foliar and soil analyses. The N content in the sap was significantly higher in the complete (40.8 mg L^{-1}) than in the traditional fertilization (25.4 mg L^{-1}) and no fertilization (9.8 mg L^{-1}) treatments (Table 2). The contents of N, K, Ca and Mg were comparatively higher in the root sap than in the leaves and soil, while the content of P was higher in the soil.

The foliar N concentration was lower in the non-fertilized plants, while P, K and Ca did not show differences among the fertilization treatments. The concentrations of nutrients in the root sap might differ from those in the leaves, because, after being absorbed, the plant distributes the nutrients among the organs depending on its functional needs at that time. The composition of the root sap changes during the ontogenesis of plants and its concentrations of elements and organic solutes vary according to the plant species, type of fertilization, age of the organ, climatic conditions, time of the year and time of sampling (Pino et al. 2012, White 2012).

The N in the soil did not show differences among the fertilizer treatments, while those of P, Ca and Mg were lower in the non-fertilized plants. Phosphorus was found in a greater quantity in the soil of the traditional fertilization plants, while Ca and Mg were the major elements in soils of the complete fertilization plants, and K had the highest concentrations in soils of the non-fertilization plants (Table 2). The contents of N and P in the soil samples were considered low, those of K were medium and those of Ca were very high (ICA 1992, López & Espinosa 1995).

Generally, the ion concentration in the root sap is higher than in the soil solution, with those of K, NO_3^- and P being the most evident nutrients (White 2012). In the root sap of rice, the Mg concentration exceeds that of the external nutrient solution when the Mg concentration in the nutrient solution is less than 3 mM (Tanoi et al. 2011). The requirement of P by banana plants is not as high as that of N and K (Castillo González et al. 2011); however, banana plants could absorb more P than required for their physiological processes (Robinson & Galán-Saúco 2012).

Soil analysis is frequently considered an imprecise indicator of N availability for banana cultivation, since it does not allow the examination

Table 2. Macronutrient contents in the banana root sap (mg L^{-1}), leaf samples (%) and soil samples (mg kg^{-1}) of 'Williams' banana (*Musa* AAA Simmonds subgroup Cavendish) under three levels of fertilization.

Fertilizer treatments	N			P			K		
	Root sap	Leaves	Soil	Root sap	Leaves	Soil	Root sap	Leaves	Soil
Complete	40.8 a	2.7 a	26.9	4.71 a	0.15	5.5 b	233.0 a	3.2	0.26 b
Traditional	25.4 ab	2.8 a	31.5	1.55 b	0.17	17.6 a	189.6 a	3.5	0.26 b
No fertilizer	9.8 b	2.3 b	23.8	0.32 b	0.17	3.4 b	21.2 b	3.5	0.65 a
Mean	25.3	2.58	27.0	2.13	0.16	7.7	152.1	3.43	0.38
CV (%)	22.5	3.9	14.0	39.2	11.1	16.8	24.0	11.4	22.0
Significance	*	**	ns	**	ns	**	**	ns	**
Fertilizer treatments	Ca			Mg		
	Root sap	Leaves	Soil	Root sap	Leaves	Soil
Complete	21.7 a	0.66	13.0 a	24.1 a	0.33 ab	5.6 a
Traditional	26.3 a	0.78	11.4 ab	15.5 ab	0.36 a	4.7 ab
No fertilizer	10.5 b	0.56	8.2 b	5.7 b	0.24 b	2.9 b
Mean	19.7	0.66	11.1	14.2	0.31	4.4
CV (%)	16.4	16.9	14.0	14.5	18.3	24.2
Significance	**	ns	*	**	*	*

Averages followed by the same letter do not differ significantly according to the Tukey test. * $p \leq 0.05$; ** $p \leq 0.01$; ns: $p > 0.05$.

of the close relationship between the soil N and the physiological response of the plants (Haifa 2009). On the contrary, Rodrigo et al. (2006) found a direct relationship between soil nitrate levels and nitrate concentration in the root sap of artichoke (*Cynara cardunculus*).

The micronutrient concentration in the root sap also varied significantly among the fertilizer treatments (Table 3). Manganese was the microelement found in the highest proportion in the root sap. Although the Anova did not show significant differences among the treatments, the contents of Mn in the sap (0.5 mg L^{-1}) of the no fertilization treatment were lower. The concentrations of B and Fe were also lower in the sap of the non-fertilized plants. The higher concentration of B present in the sap of the plants under complete fertilization could be because this element was applied only in the complete fertilization treatment (Table 1). Although reliable data on micronutrient concentrations in the root sap

Table 3. Micronutrient contents in the banana root sap (mg L^{-1}), leaf samples (mg kg^{-1}) and soil samples (mg kg^{-1}) of 'Williams' banana (*Musa* AAA Simmonds subgroup Cavendish) under three levels of fertilization.

Fertilizer treatments	B			Zn			Mn		
	Root sap	Leaves	Soil	Root sap	Leaves	Soil	Root sap	Leaves	Soil
Complete	1.6 a	18.0 ab	0.2 a	0.58	17.25	0.8 b	2.9	445.0 ab	39.5 b
Traditional	0.5 b	19.3 a	0.2 a	0.38	18.5	1.2 a	1.9	522.8 a	42.8 a
No fertilizer	0.3 b	13.0 b	0.1 b	0.34	16.8	0.9 b	0.5	293.0 b	14.2 c
Mean	0.72	16.8	0.17	0.42	17.5	1.0	1.77	431.8	31.5
DMS	0.61	6.1	0.03	0.59	5.53	0.12	3.19	195.7	0.39
CV (%)	40.2	18.2	5.9	60.1	16.0	5.9	71.8	21.3	0.58
Significance	**	*	ns	ns	ns	**	ns	*	**
Fertilizer treatments	Fe			Cu		
	Root sap	Leaves	Soil	Root sap	Leaves	Soil
Complete	0.14 b	90.0	119.5	0.09	9.0 ab	6.8 b
Traditional	0.17 b	108.3	215.3	0.08	10.5 a	7.3 a
No fertilizer	6.82 a	95.0	111.1	0.05	6.8 b	3.1 c
Mean	2.38	97.8	152.1	0.07	8.75	6.0
DMS	4.5	69.0	1.1	0.05	2.93	0.27
CV (%)	74.8	28.2	0.33	30.2	16.9	2.14
Significance	**	ns	**	ns	*	**

Averages followed by the same letter do not differ significantly according to the Tukey test. * $p \leq 0.05$; ** $p \leq 0.01$; ns: $p > 0.05$.

are very scarce (White 2012), B concentrations of 200-500 μM have been reported (Huang et al. 2008). The contents of Cu and Zn in the root sap did not vary among the fertilizer treatments (Table 1).

The concentrations of B, Zn, Mn and Cu in the leaves were lower in the non-fertilized plants than for the complete and traditional fertilization treatments, except for Fe, which had similar concentrations among the treatments. In the soil samples, no differences were found in the contents of B, Zn and Fe among the complete fertilization, traditional fertilization and no fertilization treatments (Table 3).

The significant differences found among the fertilizer treatments for the contents of macronutrients and micronutrients in the root sap, leaves and soil indicate that the root sap more feasibly reflected changes in the levels of mineral nutrients in response to fertilization, as compared with foliar and soil analyses. The foliar analysis does not allow for an early diagnosis of the plant nutrient requirements, because it expresses average values from the beginning of the crop cycle until the moment of taking the sample, when different phenological phases have already passed. The element contents in the root sap may differ from their amounts in the leaves, since plants re-distribute the nutrients, depending on the nutrient requirements in each phase of growth and development. Additionally, the re-translocation of certain elements, such as Ca, Mg or Fe, from leaves could be limited (Fageria 2015).

The multivariate analysis of variance (Manova) showed significant differences (Table 4). The Tukey test ($p \leq 0.05$) of the first canonical correlation to establish the differences among the three treatments in the joint analysis of all variables indicated that the most accurate analysis of the nutrient content was that of the root sap, if compared to the soil and foliar analyses. These results confirmed the data observed in the univariate analyses of variance (Tables 2 and 3).

Concerning the proportion of mineral nutrients in the root sap in the three fertilization treatments, K was the predominant macronutrient and P was the minor one (Figure 2). This coincides with what was observed in other studies that reported K as the element that the banana plants absorb in the highest quantity (López & Espinosa 1995, Martínez Acosta & Cayón Salinas 2011). Potassium is the major mineral

Table 4. Multivariate analysis of variance (Manova) and Tukey test ($p \leq 0.05$).

Statistics	W value	F value	DF	Pr > F
Wilks' lambda	0.00011	19.15	20	0.0055
Treatment	Average	-	-	-
Root sap	108.69 a	-	-	-
Soil	-91.96 b	-	-	-
Leaves	-483.41 c	-	-	-

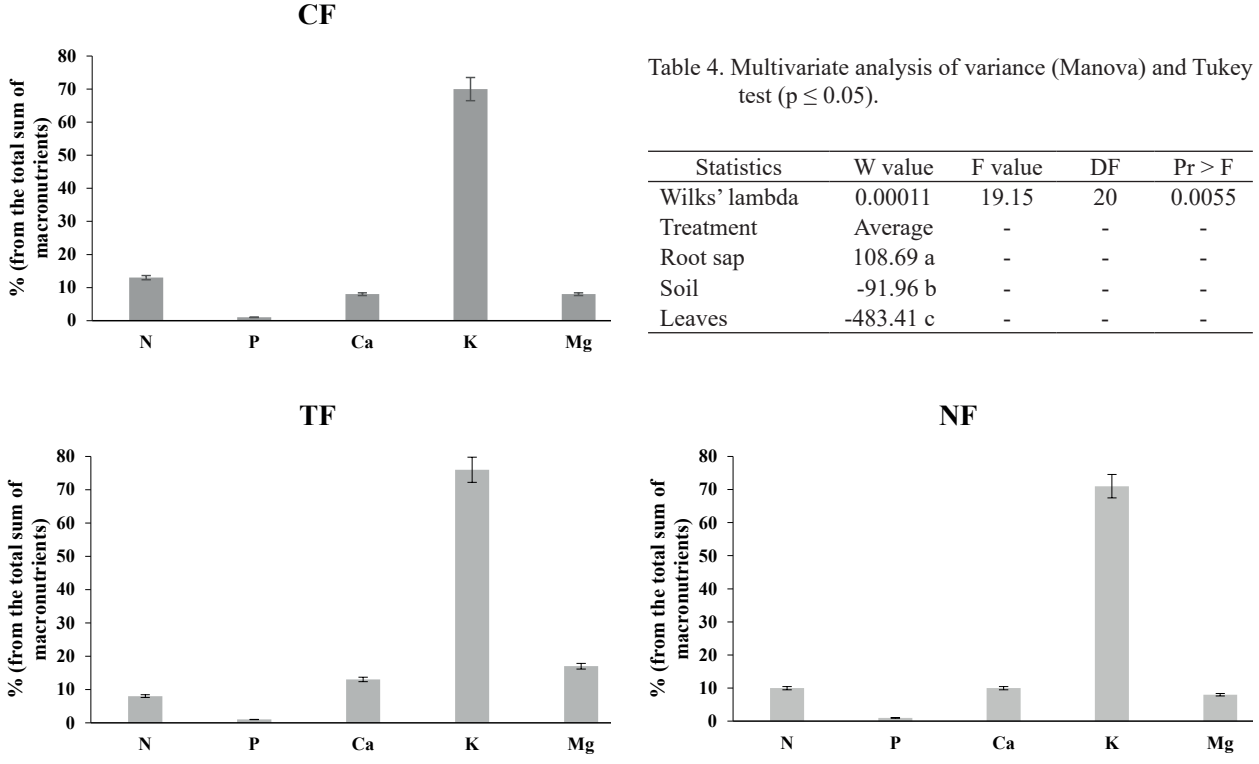

Figure 2. Macronutrients proportion in the banana root sap (% from the sum of N, P, Ca, K and Mg). CF: complete fertilization; TF: traditional fertilization; NF: without fertilization. The vertical bars represent the standard error of means for 16 replicates ($p \leq 0.05$).

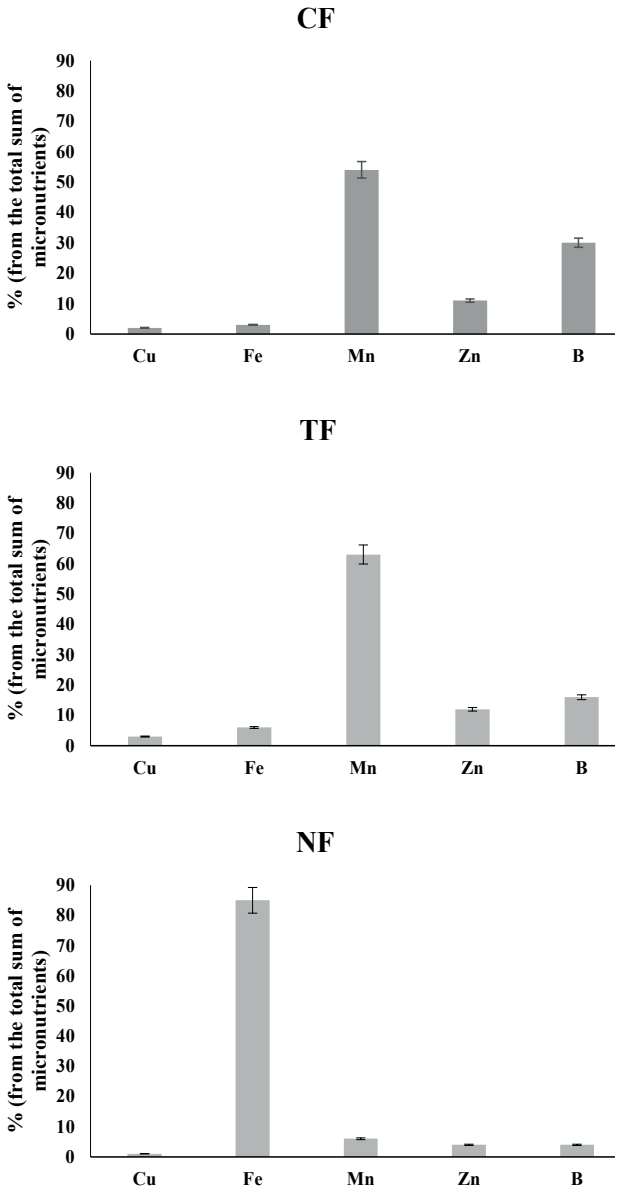

Figure 3. Micronutrients proportion in the banana root sap (% from the sum of Cu, Fe, Mn, Zn and B). CF: complete fertilization; TF: traditional fertilization; NF: without fertilization. The vertical bars represent the standard error of means for 16 replicates ($p \leq 0.05$).

element present in the phloem sap of the vascular plants, which plays a fundamental role in the loading and unloading of the phloem in the roots (Fageria 2015). The low proportion of P found in the root sap could be explained, in part, by the fact that its determination was made during the initial phase of the bunch development, and banana plants absorb most of the P required during the first 9 weeks of growth (Robinson & Galán-Saúco 2012).

The micronutrients Mn, Zn and B were predominant in the sap of the complete fertilization and traditional fertilization treatments, and Cu and Fe were the minor ones (Figure 3). In the root sap of the non-fertilized plants, Mn, Zn and B were also found in greater quantities, but the Fe content was significantly higher (6.82 mg L^{-1}), if compared to its contents in the complete and traditional fertilization treatments (Table 3). The increasing Fe contents in the root sap of the non-fertilized plants could be due to the higher metabolic demand of Fe (e.g., respiration) by the roots searching to increase their growth rate under conditions of no fertilizer application to the soil.

In general, the elemental composition of the root sap depends on different factors, including the species, its development stage, nutrient availability in the soil and agricultural practices (Alexou & Peuke 2013, Fageria 2015, Nadezhdina et al. 2020). In trees, the chemical composition in the xylem sap could be a potential marker of the tree's nutrient condition in different soil environments (Smith & Shortle 2001). However, almost no published records on this topic could be found for banana plants (Robinson & Galán-Saúco 2012). Therefore, the results of the present research could further serve for the development of a methodology that would employ the root sap analysis for the timely and accurate diagnosis of the nutrient status of Musaceae plants.

CONCLUSIONS

1. The methodology generated for the extraction and analysis of the banana root sap allows for the exact evaluation of the nutrients that the plant absorbs in a determined phase of development;
2. The root sap analysis is more sensitive than the foliar analysis for determining the nutrient status of banana plants.

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