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Chlorophyll and macronutrients content in leaf tissue of *Musa* sp 'Prata-Anã' under fertigation

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The banana (*Musa* sp.) crop is notable for its social and economic importance in tropical and subtropical regions of world. In Brazil, despite being a main banana producer, its production is considered low, mainly due to water and nutrient shortages in soils. This fact motivated this study; so, it aimed to evaluate chlorophyll and macronutrients content in leaf tissue of banana 'Prata-Anã' (AAB) under fertigation with doses of nitrogen and potassium. It were evaluated chlorophyll *a*, *b* and total and leaf contents of nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg) and calcium (Ca) in banana trees grown in an Ultisol on the coastal tablelands of Sergipe State, Brazil. The experiment was conducted in the field, in a 4^2 factorial with four randomized blocks. Two factors with four levels were tested: nitrogen (0; 350; 700 and 1050, in kg ha^{-1} of N, as urea) and potassium (0; 400; 800 and 1200, in kg ha^{-1} of K_2O, as potassium chloride). Increasing N applied through irrigation enhances leaf nitrogen until approximately 631.08 kg ha^{-1} of N, while chlorophyll total remains constant after 250 kg ha^{-1}. Phosphorus and calcium are increased due to high doses of N, however, magnesium and potassium in the leaves are reduced. High doses of K increase leaf content of K until estimated dose of 698.07 kg ha^{-1} and reduce calcium and magnesium of banana 'Prata-Anã'.

Key words: *Musa* sp., mineral fertilizer, nutrition, photosynthetic pigments, nutrient splitting.

INTRODUCTION

Banana crop (*Musa* sp.) is notable for its social and economic importance and it is a strategic crop for global food security. It is cultivated in tropical and subtropical regions, in an area of 4.8 million ha, with an average fruit

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yield of 19 t ha^{-1}, and world production reaches 91.2 million metric ton (Furlaneto et al., 2011). Banana cultivation has a strategic role in the world food production, which is justified by its peculiar nutritional characteristics and yield potential, which can reach 100 t ha^{-1}. However, Santos et al. (2009) pointed out that the Brazilian yield is very low, due to water and nutrient shortages, mainly in the northeast of the country, where the largest production of the country is located (Fernandes et al., 2008).

Low yields are related to environmental limitations such as climate and soil fertility, and it is due to low input of technology in most of the plantations. In some plantations, however, improvements in fertilizer use, by applying the fertilizers through the irrigation water, have increased the yield. This technology has been consolidated by Teixeira et al. (2001); Duenhas et al. (2002); Guerra et al. (2004); Sousa et al. (2004) and Pinto et al. (2005), however there is a shortage of experimental data in order to improve fertigation in soils of the coastal tablelands. Monitoring nutrient dynamics is vital because nutrients act in plant growth, fruit ripening and plant senescence (Moreira and Fageria, 2009). Leaf analysis is an important tool to evaluate plant nutritional status, and is a technique that complements visual diagnosis in studying nutrient dynamics (Donato et al., 2010). Among nutrients applied through irrigation water, N and K are the most used due to their chemical and physical characteristics such as high water solubility, when urea and potassium chloride are used as their source. According to Epstein and Bloom (2006),

nitrogen is associated to organic compounds, such as amino acids, proteins, coenzymes, nucleic acids, vitamins and chlorophyll, as a constituent of their molecules. All these compounds are related to enzyme reactions and to the photosynthesis process. Similarly, potassium is a very required macronutrient for the nutrition of banana tree, because its accumulation in the plant creates an osmotic gradient that facilitates the water movement, regulating the opening and closing of stomata, playing a key role in saving water and turgidity of the cells, in carbohydrate transport and in respiration (Epstein and Bloom, 2006). Due to scarcity of studies on the nutritional effects of N and K splitting in banana, the objective of this paper was to evaluate the chlorophyll and macronutrient contents in the leaf tissue of banana ‘Prata-Anã’ (AAB) under fertigation with nitrogen and potassium applied through irrigation water in an Ultisol on the coastal tablelands of the northeast of Brazil.

MATERIALS AND METHODS

The experiment was established in an Ultisol on the coastal tablelands located in the Sergipe Federal University Experiment Station, in São Cristóvão County, Brazil, latitude 10°19’S; longitude 36°39’O of Greenwich, twenty meters above sea level. According to the Köppen climate classification, it is tropical with a dry season, rainfall of around 1200 mm $year^{-1}$, and a rainy season from April to September.

Soil samples were collected at 0.0 to 0.2 m depth and the following attributes were found: pH = 5.2; P^{3+} = 2.4 mg dm^{-3}; K^+ = 0.08 $cmol_c$ dm^{-3}; $Ca^{2+} + Mg^{2+}$ = 0.89 $cmol_c$ dm^{-3}; Al^{3+} = 0.2 $cmol_c$ dm^{-3}; $H^+ + Al^{3+}$ = 2.56 $cmol_c$ dm^{-3}; Na^+ = 0.055 $cmol_c$ dm^{-3}; V = 42.47%; CTC = 4.45 $cmol_c$ dm^{-3}; M.O. = 2.1 dag dm^{-3}; clay, silt and sand were 632, 296 and 72 g kg^{-1} respectively; soil density was 1.59 kg dm^{-3}; *in situ* field capacity 0.199 m^3 m^{-3} and wilting point (1500 kPa) = 0.033 m^3 m^{-3}.

The experiment was set up in a factorial 4^2 and treatments were N (0, 350, 700 and 1050 kg ha^{-1} $year^{-1}$, as urea) and K (0, 400, 800 and 1200 kg K_2O ha^{-1} $year^{-1}$, as potassium chloride) in a randomized blocks with four replications. In each plot, eight micro propagated seedlings of ‘Prata-Anã’ (*Musa* sp. AAB) were planted, in double rows and a spacing of 3 x 2 x 2 m. Soil was plowed and limed to reach 70% bases saturation. Two and half ton of dolomite lime was applied per ha^{-1} and incorporated to the soil at approximately 0.2 m. Planting holes with diameter and depth of 0.5 m were made by a mechanical drill.

Planting holes were fertilized with 300 g of single superphosphate, 60 g of a mixture of micronutrients FTE-BR12 (9% Zn; 1.8% B; 0.85% Cu; 3% Fe; 2.1% Mn e 0.10% Mo), 200 g of dolomite lime and 10 L of cow manure, mixed all together and applied 45 days before planting. 10 g of zinc sulphate were applied every 60 days through irrigation water (Maia et al., 2003).

In each hole, the plants in excess were cut, leaving only three of them, the mother plant, a daughter and a granddaughter plants in order to avoid competition for nutrients and water. Cutting took place up to six months when a daughter plant was selected. Plant remains were left between rows in order to act as a mulching. Weeds were controlled by Glyphosate plus 2.4-D every three months. When cutting of the leaves was performed, the bunch heart was also cut at approximately 0.15 m from the last completely formed bananas fruits. Management of pests and diseases were eliminated according to Moreira (1999).

Irrigation was implemented by compensating sprinkling with nominal flow of 94 x 10^{-5} m^3 s^{-1} (70 L h^{-1}) and irrigation management was based on monitoring of climatic data and the reference evapotranspiration was calculated by Penman-Monteith model and standardized by Allen et al. (1998). Meteorological variables were obtained from an automatic weather station, installed next to the experiment. Crop coefficient (Kc) was obtained in Maia et al. (2003). Leaf sampling for chlorophyll and macronutrients N, P, K, Mg and Ca analysis was carried out according to Martin-Prével (1980). Chlorophyll *a* and *b* (mg dm^{-2}) were obtained according to Arnon (1949), by using twenty five foliar discs. From the extracts, chlorophyll total concentration (Clt) readings were achieved at 645 and 663 nm. Afterwards, chlorophyll total concentration (µg cm^{-3}) was transformed into mg of chlorophyll dm^{-2} of leaf area (Arnon, 1949). Leaf samples were dried, ground and contents of N, P, K, Mg and Ca were obtained according to Malavolta et al. (1997).

Statistical analysis

Data were submitted to variance analysis using the F test at ($p<0.05$). Regression equations were adjusted and their coefficients were tested by Student ($p<0.1$). Pearson linear correlations also were carried out by using SAEG 9.1.

RESULTS AND DISCUSSION

Chlorophyll concentration

Chlorophylls (*a* and *b*) were reduced in the absence of nitrogen or when doses were low (Figure 1A and B). In

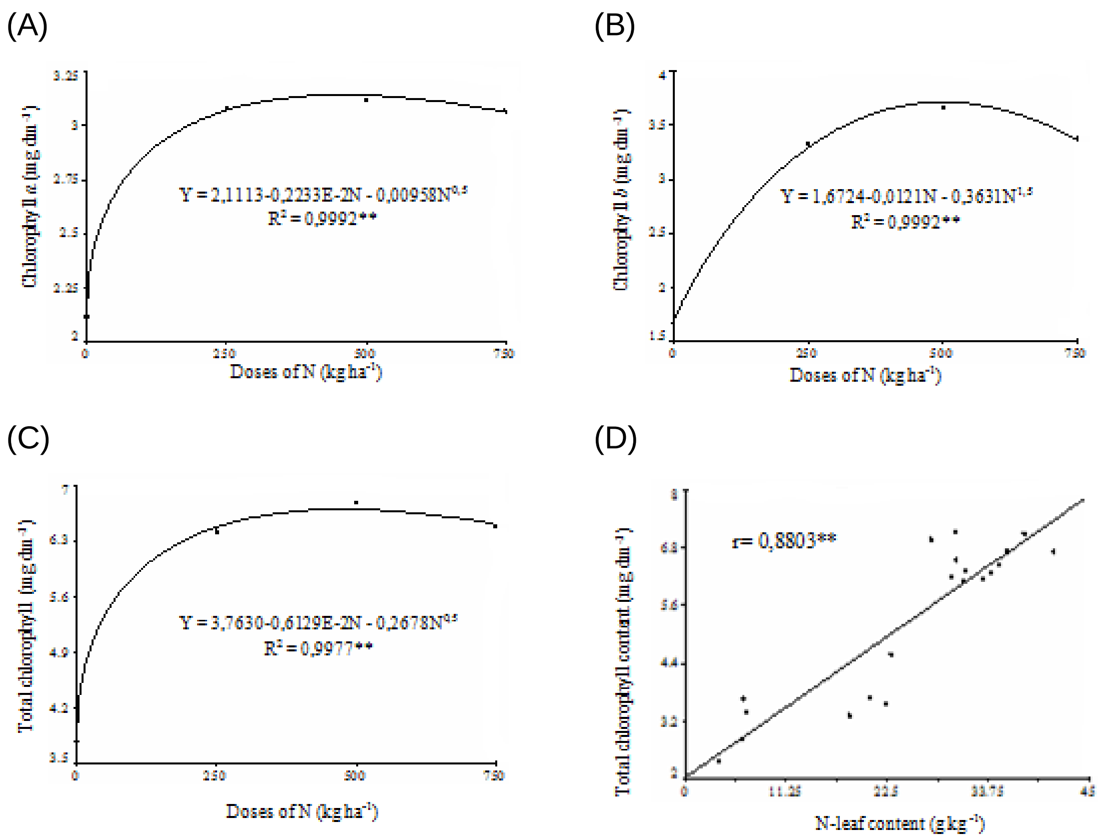

Figure 1. Effects of nitrogen applied through irrigation water on chlorophyll *a* (A), chlorophyll *b* (B), total chlorophyll (C) and correlation between N-leaf and chlorophyll (D) in banana tree leaves cv. 'Prata-Anã'.

the absence of N, chlorophyll *a* content (2.11 mg dm^{-2}) was greater than chlorophyll *b* (1.67 mg dm^{-2}), increasing the ratio *a*/*b*. According to Evans (1989), this behavior indicates greater amounts of reaction centers in the photosystem II and lower capacity in the capture of solar energy incident, through the protein complex II of light gathering (LHCII). Similar results were obtained by Cruz et al. (2007) on photosynthetic rate on papaya as influenced by N. On the other hand, at higher levels of N the chlorophyll *b* content (3.69 mg dm^{-2}) was greater than chlorophyll *a* (3.13 mg dm^{-2}), decreasing the ratio *a/b*. This increases photochemical capacity, assuming higher electron transport speed (Evans, 1989). Chlorophyll *b* plays an important role in increasing the spectrum of light that can be used in the photosynthetical process (Raven et al., 2001).

In banana tree by presenting self-shading characteristic of its leaves, a greater relative proportion of chlorophyll *b* is an important factor as it enables the capture of energy from other wavelengths and its transfer to the specific molecule of chlorophyll *a*, which then transforms it into chemical energy in photosynthesis.

Increasing N doses enhanced the total chlorophyll, reaching a value of 6.69 mg dm^{-2} at 478 kg N ha^{-1} (R^2=0.99**) (Figure 1C), greater than 5.23 mg dm^{-2} found in the 'Grand Naine' banana trees by Thomas and Turner (2001). A higher concentration of chlorophyll in the middle of the foliar surface maximizes CO_2 assimilation. There was an increase of 77.92% in chlorophyll content from 0 to 478 kg N ha^{-1}. The relationship between the N applied and the chlorophyll content (Figure 1C) can provide a prediction of the amount of N necessary to increase total chlorophyll, as it has already done in other crops (Guimarães et al., 1999; Torres Netto et al., 2002).

Concentration of N leaf

The relationship between N leaf content and chlorophyll concentration (r=0.88**) (Figure 1D), provides additional information in predicting the amount of chlorophyll in banana tree leaf, from N leaf data. This is important, because 50 to 70% of N in the leaves is component of enzymes that are associated to chloroplasts (Evans, 1989). The relationship between the N applied through irrigation water and leaf N was significant (R^2=0.96**) (Figure 2A). Based on the equation shown on Figure 1, it was found that the N dose which maximize N leaf was

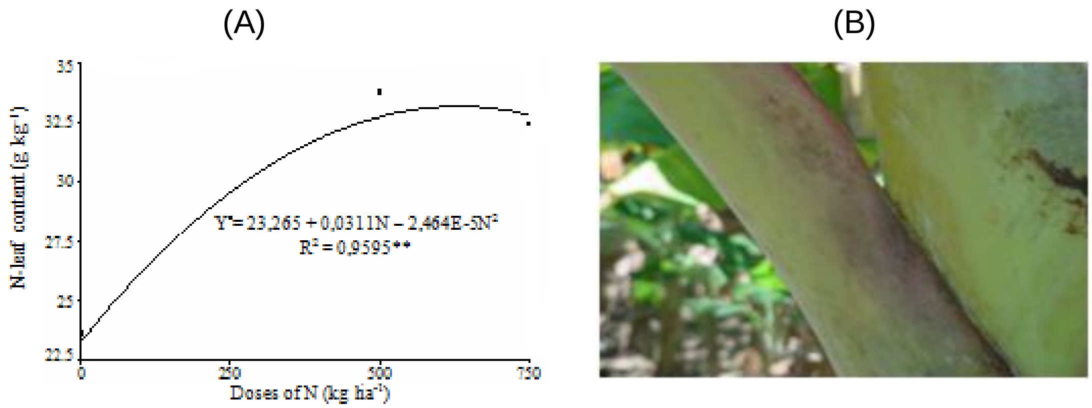

Figure 2. Effects of nitrogen applied through irrigation water on N-leaf content (A) and N deficiency symptom, showing antocianin accumulation at petiole margins (B) in banana tree cv. ‘Prata-Anã’.

632.39 kg ha^{-1}. At this dose, the leaf N concentration was 33.11g kg^{-1} at the flowering stage, and was higher than the sufficiency range (25 to 29 g kg^{-1}) proposed by Silva et al. (2002). The leaf N was also higher than 30 g kg^{-1}, found by Damatto Junior et al. (2006), and between the range (27 to 36 g kg^{-1}) found by Malavolta et al. (1997). In the absence of N, the leaf N content was 23.26g kg^{-1}, which is below from the critical level proposed by Gallo et al. (1972); Martin-Prével (1984); Moreira (1999); Silva et al. (2002); Teixeira et al. (2002); Borges and Caldas (2004). In banana trees lacking nitrogen, besides plant growth decreasing, a pink color was observed at petiole margins due to antocianin accumulation (Figure 2B). Carbohydrates not used in N metabolism can be utilized for antocianins biosynthesis. The accumulation of this pigment is also an indication of nitrogen deficiency (Marschner, 1995; Taiz and Zeiger, 2009).

Concentration of K leaf

Potassium leaf content was influenced ($p<0.01$) by potassium applied through irrigation water (Figure 3A). Making the second derivative of the equation equal to zero, the K dose that maximizes K leaf is 700 kg ha^{-1}, associated to 22.13 g kg^{-1} of potassium leaf content at the flowering stage, which it is lower than the sufficiency level of 27 g kg^{-1} along with Silva et al. (2002). According to Teixeira et al. (2002), 90% of the maximum yield was achieved with 23.2 g K kg^{-1} in the leaf of banana tree ‘Nanicão’. Gomes (2004) did not find any response to K fertilization applied through irrigation water on banana tree ‘Prata-Anã’ leaf K content. Similar results were obtained by Guerra et al. (2004). In his work the author noticed that K leaf was lower than 32 g kg^{-1} (Prezotti, 1992) without influencing the fruit yield. According to Marschner (1995), between 20 and 50 g K kg^{-1} of dry matter are sufficient for plant growth.

Increasing applied N in the absence of K decreased leaf K by 23.78% (Figure 3B). Similar results were obtained by Teixeira et al. (2002) and Silva et al. (2002). However, it was higher than 16.77% observed by Teixeira et al. (2002) at 800 kg N ha^{-1}. The decrease in leaf K can be explained by K remobilization by sugar translocation from old leaves to young organs, or due to less K absorption caused by high concentration of NH_4^+ in the soil (Marschner, 1995; Epstein and Bloom, 2006). Potassium deficiency symptom came up due to low leaf K (Figure 3C). Nitrogen soluble compounds such ammines, amides and putrescines accumulate in the apoplast and in the leaf surface, causing necrotic spots on the leaf margins, and making the plant susceptible to fungal diseases (Marschner, 1995). The symptom was higher severity at flower emission. Afterwards, leaves became old more quickly. Thereafter, the leaves entered into early senescence (Figure 3D).

Concentration of P, Ca and Mg leaf

Leaf P increased from 1.5 to 1.7 g kg^{-1} (R^2=0.9987**) (Figure 4A) as influenced by N applied through irrigation water. These values are very close to the ones found by Silva and Rodrigues (2001) and Silva et al. (2002). 16.60% of this increase may be due to an increase in root growth; that, probably, could have been an effect on phosphorus absorption. The root growth has a positive correlation with leaf area expansion, plant height and false stem circumference. By their relationship with N, P is fixed by organic compounds and used together with N in protein synthesis and in activation of enzymes needed for dry matter yield (Marschner, 1995; Taiz and Zeiger, 2009).

Leaf calcium increased with applied N (Figure 4B). The amount of N that maximized leaf calcium (7.8 g Ca kg^{-1}) was 750 kg N ha^{-1}. Increasing calcium leaf content by N

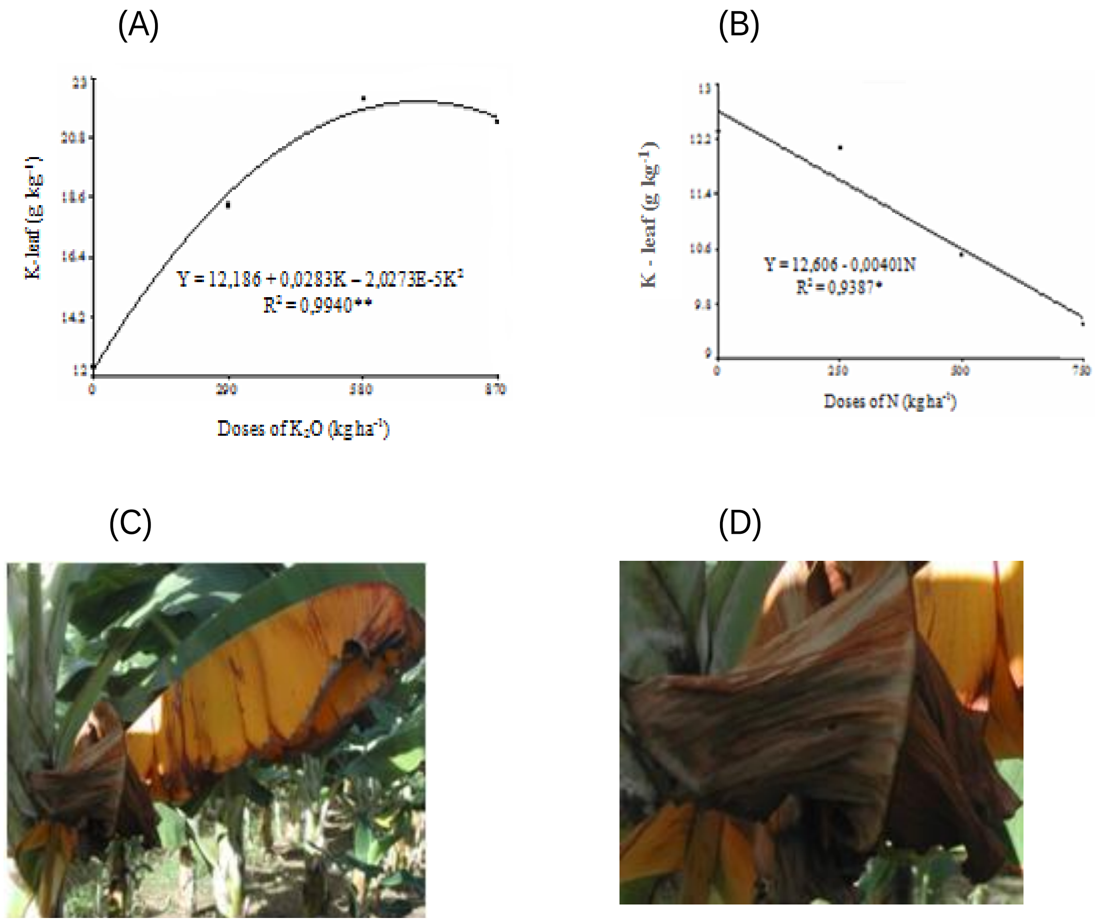

Figure 3. Effects of K (A) and N (B) on leaf and K deficiency symptoms observed in plots without K (C and D) in banana tree cv. 'Prata-Anã'.

application also was observed by Silva et al. (2002) in banana tree 'Prata-Anã'. According to the model found by those authors, 6.32 g of Ca kg^{-1} was reached with application of 750 kg N ha^{-1}. High calcium uptake was observed in plants that received N in comparison to those that did not receive N. Leaf calcium in plants that received N was 85.71% greater than in the ones that did not receive nitrogen.

Increasing K application decreased leaf calcium from 4.2 to 3.3 g kg^{-1} (Figure 4C). Though diminished by potassium application, calcium leaf content was still in the sufficiency range (2.5 a 12 g kg^{-1}) cited by Prezotti (1992); it was lower than the range of 4.5 to 7.5 g kg^{-1} according to Silva et al. (2002) and almost close to 4.1 g kg^{-1} found by Silva and Carvalho (2004). Leaf magnesium was reduced by N application from 7.3 to 5.8 g kg^{-1} (Figure 4D). The decrease in leaf Mg could be related to a greater conversion of the ammonium absorbed by amino acids avoiding magnesium fitotoxicity (Epstein and Bloom, 2006).

These authors mention that the primary pathway for this conversion involves the sequential action of glutamine synthetase and glutamate synthase. This process requires ATP hydrolysis involving a divalent cation such as Mg^{2+}. Furthermore, the increasing availability of K in soil increased the competitive effect on the absorption of magnesium, so that the amount of Mg in leaf varied from 7.2 to 6.3 g kg^{-1} when 870 kg ha^{-1} of K_2O were applied (Figure 4E). This confirms the antagonism related to the ionic balance in the absorption of Mg and K (Figure 4F), but there were no symptoms of blue-banana caused by the imbalance between K and Mg (Borges et al., 1999). It is necessary to point out that K contributes with 70.7% and Mg with 19.2% of the sum involving K+Mg+Ca found in banana tree leaves. The relative value of K is higher than 61% found by Borges et al. (1999) and the relative value of Mg is between 18 to 20%, as proposed by those authors.

Conclusions

Increasing N applied through irrigation enhances the contents of nitrogen until dose of 631.08 kg ha^{-1} of N,

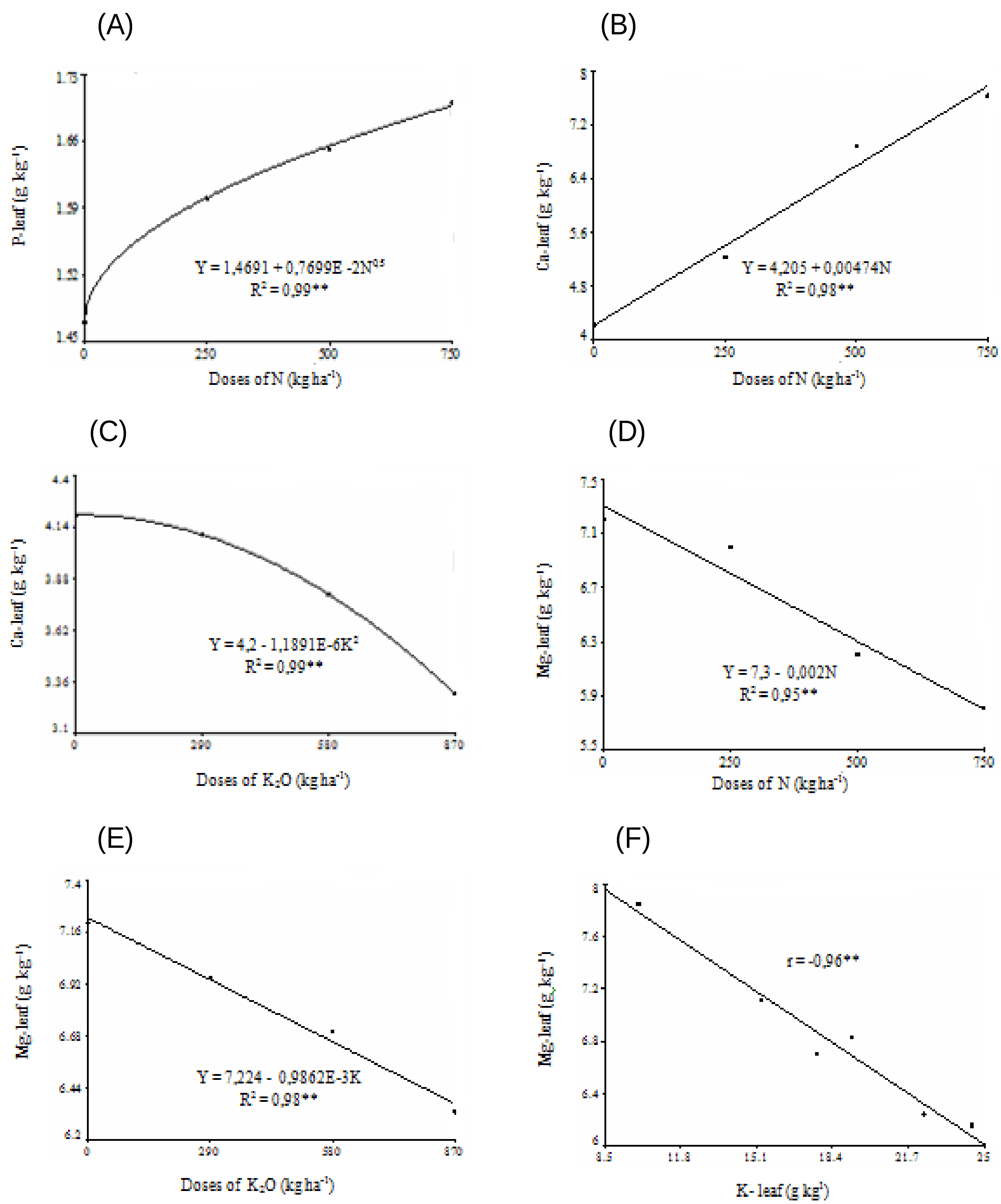

Figure 4. Effects of N on leaf P (A), Ca (B) and Mg (D) contents; effects of K on leaf Ca (C) and Mg (E) contents, and correlation between the content of Mg and K in banana tree leaf (F) cv. 'Prata-Anã'.

while chlorophyll total remains constant after 250 kg ha^{-1}. Phosphorus and calcium are increased due to high doses of N, however the contents of magnesium and potassium in the leaves of banana 'Prata-Anã' are reduced. Reference values for nitrogen, phosphorus, potassium calcium and magnesium were obtained. Increasing K applied through irrigation water increases leaf content of K until estimated dose of 698.07 kg ha^{-1} and reduces leaf contents of calcium and magnesium of banana tree.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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