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SOIL SCIENCE

Detection of nutritional stress in sugarcane by VIS-NIR-SWIR reflectance spectroscopy

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ABSTRACT: This study applied spectroradiometry techniques with hyperspectral data to identify the correlations between sugarcane leaf reflectance and the contents of Nitrogen (N), phosphorus (P), Potassium (K), Sulfur (S), Calcium (Ca) and Magnesium (Mg). During the harvests 2019/20 and 2020/21, sugarcane was introduced to nutritional stress by the application of limestone doses. Liming was applied in a fractional way and, at the end of five years, the amounts corresponded to 0, 9, 15 and 21 t ha^{-1} of dolomitic limestone. The leaf hyperspectral reflectance data and the state of nutrients in the exponential growth phase of the culture were registered. The wavelengths correlated with N, P, K, S, Ca and Mg were identified using the Spearman's correlation analysis. The test of similarity (ANOSIM) and the Principal Component Analysis (PCA) were applied to evaluate data variability, as well as the Partial Least Squares Regression (PLSR) for the prediction of the nutritional contents. The order of the degree of correlation in the region of visible was: P > K > N > Ca > S > Mg and for the region of the near infrared: P > K > Ca > N > S > Mg. P presented peaks with high correlations in the wavelengths 706-717 nm (-0.78) and 522-543 nm (-0.76). The values of the PLSR registered the best spectral responses in the region of VIS and red-edge, regions that are more sensitive to the deficiency of sulfur, potassium and phosphorus.
Key words: liming, leaf reflectance, spectroradiometry.

Detecção de estresse nutricional em cana-de-açúcar por espectroscopia de reflectância VIS-NIR-SWIR

RESUMO: Este estudo aplicou técnicas de espectrorradiometria com dados hiperespectrais para identificar as relações da reflectância foliar da cana-de-açúcar com os teores de Nitrogênio (N), Fósforo (P), Potássio (K), Enxofre (S), Cálcio (Ca) e Magnésio (Mg). Durante as safras 2019/20 e 2020/21 a cana foi induzida ao estresse nutricional a partir da aplicação de doses de calcário. A calagem foi aplicada de forma fracionada e ao final de cinco anos as quantidades corresponderam a 0, 9, 15 e 21 t ha^{-1} de calcário do tipo dolomítico. Foram registrados os dados de reflectância hiperespectral da folha e o estado de nutrientes na fase de exponencial crescimento da cultura. Os comprimentos de onda correlacionados ao N, P, K, S, Ca e Mg foram identificados usando análise de correlação de Spearman. Aplicou-se o teste de similaridade (ANOSIM) e Análise de Componentes Principais (ACP) para avaliar a variabilidade dos dados, assim como, a Regressão por Mínimos Quadrados Parciais (PLRS) para a predição dos teores nutricionais. A ordem do grau de correlação na região do visível foi: P > K > N > Ca > S > Mg e para região do infravermelho próximo: P > K > Ca > N > S > Mg. O P teve picos com alta correlação nos comprimentos de onda 706-717 nm (-0,78) e 522-543 nm (-0,76). Os valores do PLRS registraram melhores respostas espectrais na região do VIS e red-edge, regiões mais sensíveis a deficiência do enxofre, potássio e fósforo.
Palavras-chave: calagem, reflectância foliar, espectrorradiometria.

INTRODUCTION

Studies regarding the application of limestone to correct soil acidity considering harvest systems without burning and with sugarcane residue in the soil are still scarce (CRUSCIOL et al., 2017). The recommendations of lime application in sugarcane have been developed for the harvesting system of burnt sugarcane, with the possibility of influencing the applications of mineral nutrients in sugarcane crops.

In this sense, it is known that nutrient availability is an important factor for sugarcane growth and productivity (GOPALASUNDARAM et al., 2012). In areas with sugarcane cultivation, the application of limestone improves nutrient availability in the soil, microbial quantity and diversity, as well as the plant physiological parameters and productivity (PANG et al., 2019). Additionally, the use of chemical fertilizers is the main source of nutrients for the cultures of agricultural production. Despite the benefits, excessive applications of chemical fertilizers (nitrogen, for instance) in an agricultural system harm human health and well-being, both directly and indirectly, besides negatively affecting

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surface waters and groundwater (GALLOWAY et al., 2008; HAMMAD et al., 2020).

In this context, it is essential to develop new techniques to quickly and precisely monitor plant nutritional status, maximizing economic return and reducing the adverse impact to environment. Techniques for detection by reflectance spectroscopy play a fundamental role in the identification of functional relationships among spectral features, physiological and chemical processes, especially information related to chlorophyll content in the leaf (HOUBORG et al., 2015).

In this case, it is known that leaf pigments such as chlorophyll and carotenoids are directly linked to the nutrients nitrogen (N), phosphorus (P), potassium (K) and sulfur (S), besides playing important roles in the nutritional status of the plants, also contributing to a good performance of photosynthesis (HOU et al., 2019; CROFT & CHEN, 2017; CROFT et al., 2017). The changes caused in the photosynthetic activities, cell structure and stretch alter plant spectral reflectance in the region of the Visible - VIS spectrum, Near-Infrared - NIR and Shortwave Infrared - SWIR (POZONI et al., 2012). Therefore, hyperspectral remote sensing becomes a potential tool in the prediction of the leaf biochemical concentration of nutrients. Besides being promising, these techniques offer faster and non-destructive estimates in comparison with laboratory analyses (MAHAJAN et al., 2014).

Some studies reported that hyperspectral remote sensing has a potential in predicting the concentration of essential nutrients, such as N, P, K, Ca and Mg. Among the most studied cultures are: lettuce (PACUMBABA et al., 2011); wheat (MAHAJAN et al., 2014; ANSARI et al., 2016); corn (OSBORNE et al., 2002); beet and strawberry (SIEDLISKA et al., 2021); rice (MAHAJAN et al., 2017); citrus (LIU et al., 2015) and woody plants: willow, mopane and olive tree (FERWERDA & SKIDMORE, 2007). Few studies have been reported for sugarcane; furthermore, the researches have been limited to the prediction of nitrogen and biomass (BARROS et al., 2021; ROSA et al., 2015; MOKHELE & AHMED, 2010).

It is suggested, from the cited studies, that it is possible to monitor the nutritional stress in different cultures using hyperspectral remote sensing. Nevertheless, as far as we know, there are still few works dedicated to the identification of nutritional stress in sugarcane from the spectrum in the region of VIS-NIR-SWIR. In this case, the possibility of identifying the deficiency in the nutrients N, P, K, S, Mg and Ca from the hyperspectral data obtained by sugarcane leaf reflectance deserves to be investigated.

Therefore, this study analyzed, in two consecutive harvests (2019/20 and 2020/21), from spectral data obtained in the laboratory, the correlations of sugarcane leaf reflectance with the contents of nitrogen, phosphorus, potassium, sulfur, calcium and magnesium under conditions of nutritional stress caused by applications of limestone doses in different periods.

MATERIALS AND METHODS

The experiment was installed in an experimental area of the São Paulo's Agency for Agribusiness Technology – APTA, located in the municipality of Piracicaba, São Paulo, Brazil (Figure 1). The climate in the region according to the Koppen classification is called humid subtropical (CWa), with an average annual rainfall below 1400 mm and two well-defined seasons, with dry winter and rainy summer.

Description of the experiment

The experimental area had been previously cultivated with sugarcane for three crop cycles, and the analyses were performed on the 4th and 5th production cycles of the culture. The area is characterized as flat terrain, Red Latosol with clay texture. Soil correction was performed manually with the application of dolomitic limestone doses, indicated for soil correction with low Ca and Mg contents. The application occurred in the straw after sugarcane cutting, according to the doses pre-determined for each treatment.

The experiment was installed on October 6th, 2016, and the applications of the limestone doses were distributed among the years 2016 (deployment), 2017, 2018, 2019 and 2020. The adopted design was in randomized blocks with 4 doses and 6 replications, totalizing 24 plots. On the fourth cycle or third sugarcane ratoon, the sums of the doses were 0, 6, 12 and 18 t ha^{-1}, with the addition of 3 tons in the last cycle (harvest 2020/21) for all doses, except for the control (Figure 1).

The variety used in the study was IACSP95-5000, known by its high productivity, good upright stature, excellent ratoon sprouting, good tillering and closing between rows, low concentration of fallen plants and with little flowering, resistant to diseases and presenting excellent results in situations of water deficit (CHAVES et al., 2015).

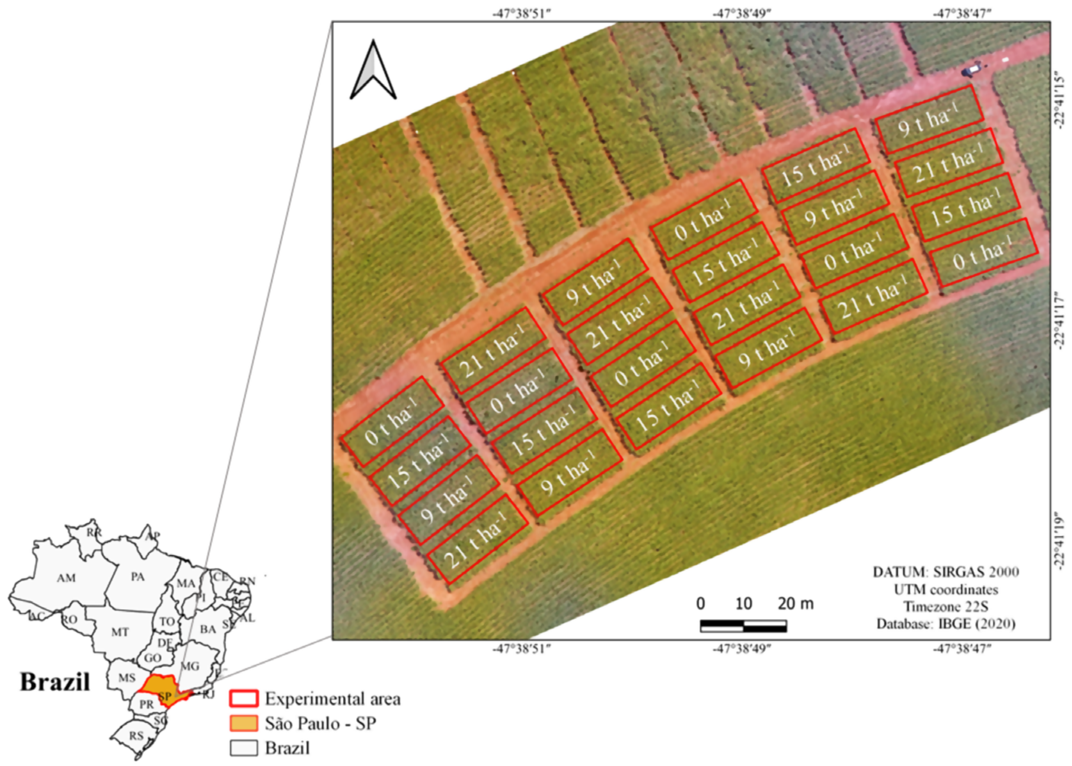

Figure 1 - Location map of the experimental area and the sketch with the doses of harvest 2020/21.

Spectral analyses and leaf macronutrients

In all plots in the two harvests (2019/20 and 2020/21), 10 sugarcane leaves were collected when the culture was in the exponential vegetative growth. The collected leaf is characterized as the first completely expanded in the apex of the plant, called "leaf+1". After field collection, the leaves were stored in properly identified plastic bags and transported in coolers with ice, in order to maintain leaf turgidity. They were sent to the laboratory of geoprocessing.

In the harvest 2019/20, there was only one measurement of leaf spectroscopy, which was performed in March 2020, a period in which the culture was in the exponential vegetative growth. In the subsequent harvest (2020/21), three spectral measurements were performed, in the months March, May and in the end of June 2021 (Figure 2).

Given the water deficit that occurred in the region in the last harvest (2020/21), the plants retarded their growth and moved to the maturation stage earlier. Thus, the exponential growth of the culture occurred only in may, two months after the expected. As a means of evaluating the influence of water content on spectral reading, the monthly distribution of the rainfall in the area of the experiment was observed.

Figure 2 shows the monthly spectral measurements and the distribution of rainfall (mm) in the period from August 2019 to August 2021. The station used to obtain the data stays at less than 500 m of distance from the experimental area.

The spectral reads referred to the reflectance of all 240 sugarcane leaves from each harvest, obtained using the spectroradiometer ASD FieldSpec FR Spectroradiometer® (ASD – Analytical Spectral Devices Inc., Boulder, CO, USA). The spectroradiometer collects data in the spectrum between the wavelengths from 350 to 2500 nm, thus covering the regions of Visible/VIS, Red-edge, Near-Infrared/NIR and shortwave-infrared/SWIR, with a spectral resolution of 3 nm in the range of 350-1000 nm and of 10 nm between 1000 and 2500 nm (ASD, 2010).

The spectral curves were obtained in terms of reflectance using the software ViewSpec Pro (ASD – Analytical Spectral Devices Inc., Boulder, CO, USA) and exported to the software Microsoft Excel. Subsequently, a pre-treatment of the data was performed with the exclusion of the responses caused

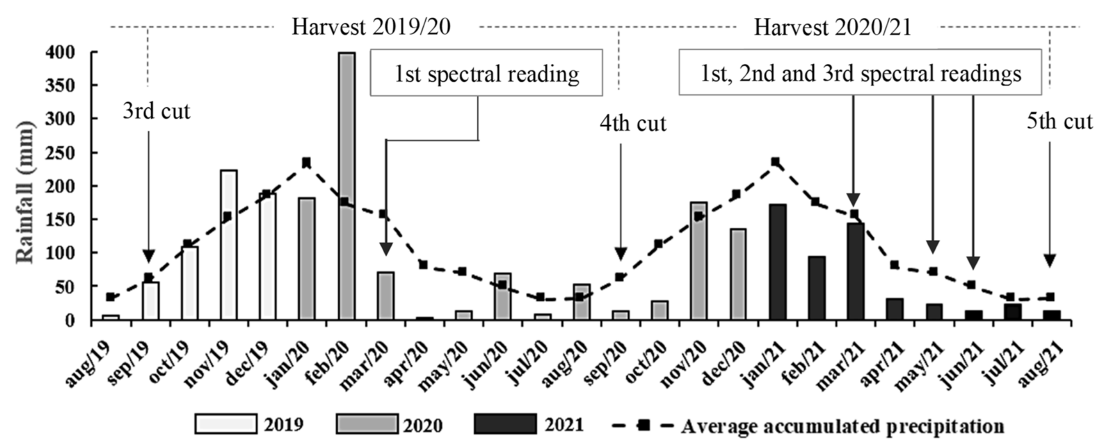

Figure 2 - Graph of the monthly rainfalls in the period from August 2019 to September 2021 and average accumulated precipitation in the period from 1980 to 2021.

by noise at the 350-400 nm end, thus resulting in a spectral curve from 400 to 2500 nm.

To evaluate the nutritional state of the plants, the macronutrient (N, Ca, Mg, K, P and S) contents in the plant tissues of the leaves collected in the two harvests studied (2019/20 and 2020/21) were analyzed, when the culture was in the exponential vegetative growth. The criterion adopted was based on the spectral reading of 10 leaves from each plot. The leaves presenting reflectance values higher than twice the standard deviation in relation to the median of the plot were excluded, and the others were placed in paper bags for drying in a forced ventilation oven at 65 °C, until constant weight was reached. Subsequently, the samples were ground and sent to the plant tissue laboratory for nutrient determination, according to the methodology of MALAVOLTA et al. (1997).

Statistical analyses

The chemical analyses of the plant tissues for both production cycles were evaluated according to the analysis of variance by the F test. For the source of variation of the doses, the regression test was applied and the selection of models was based on the significance of betas and the highest coefficient of determination (R^2), using the computational program SISVAR.

For statistical purposes, the data underwent the normality test by the method of SHAPIRO & WILK (1965) at $p < 95\%$. The data regarding reflectances did not fit the normal distribution; therefore, the Spearman's correlation analysis was employed to evaluate the correlation of reflectance between all wavelengths and the leaf nutrients. The Spearman correlation evaluates the monotonic correlation relationships whether the data are linear or non-linear, with correlation intervals from +1 to -1 (WANG et al., 2021).

Multivariate techniques were used to compare the variability among the doses, as well as their spectral similarity. To evaluate whether the reflectance groups for the doses were different, an analysis of similarity was performed among the groups. A matrix of association was generated for leaf reflectances using the Euclidean distance, using the test of analysis of similarity (ANOSIM).

The ANOSIM of 9999 permutations was used to compare classifications of similarities among the limestone doses, and it can be interpreted by the value of R, where a value close to zero indicates little or no separation, whereas the value 1 indicates the complete separation of the groups (CASAL et al., 2013).

To investigate the possibility of clustering, the spectral data were subjected to the principal component analysis (PCA). PCA is an exploratory method of multivariate statistical analysis which was used to analyze the possibility of spectral separation by the reflectance of the sugarcane leaves subjected to the limestone doses. The PCA score chart helps in the identification of the tendency of grouping, whereas the loading showed the main wavelengths associated to a certain component (JUNGES et al., 2020).

The partial least squares regression (PLSR) technique was used for the prediction of nutritional contents, from the spectral behavior of the leaves. PLSR is a technique of multivariate analysis, greatly used in prediction analyses using spectral data. During the calibration step, PLSR uses information from the independent variables (spectra) and dependent variables (contents), to generate new variables named latent variables (or factors). When a model is adjusted by PLSR, the aim is to find the smallest number possible of PLS factors necessary to explain the dependent variables.

During cross validation (leave-one-out), both the model with an optimal number of factors and its respective performance were defined. The best model presented the highest R^2 and the lowest RMSE (root mean square error). To define which spectral bands are in fact relevant for the prediction, the Variable Importance in the Projection (VIP) was calculated. The VIP indices are calculated for each spectral band, and only values above 0.8 are considered relevant. The whole processing described was performed using the program ParLeS 3.1 (VISCARRA ROSSEL, 2008).

RESULTS AND DISCUSSION

Leaf macronutrient contents

Table 1 shows the mean values and the statistical regression analyses of the sugarcane leaf nutrients as a function of the limestone doses applied in the harvests 2019/20 (0, 6, 12 and 18 t ha^{-1}) and 2020/21 (0, 9, 15 and 21 t ha^{-1}). It was verified that for harvest 2019/20 there was no significant effect in the interaction among almost all nutrients and limestone doses, except for Mg and K at 1 and 5% of probability, respectively. In the harvest 2020/21, only Ca and Mg had a significant effect at 1%, and P was significant at 5% (Table 1).

In harvest 2019/20, the dose of 6 t ha^{-1} concentrated the highest levels of N (12.1 $g.kg^{-1}$) and K (4.06 $g.kg^{-1}$) in the leaves, whereas 0 and 18 t ha^{-1} had the lowest concentrations of both N and K. Similarly to the previous harvest, in 2020/21 the lowest dose of limestone (9 t ha^{-1}) was the one that generated the best concentrations of nitrogen and potassium, with contents of 11.2 $g.kg^{-1}$ and 8.98 $g.kg^{-1}$, respectively, with a more accentuated reduction in the control and in the treatment 21 t ha^{-1}. The contents of Ca, Mg and S had contrary effects to N and K, since their contents increased linearly as a function in the limestone contents (Table 1).

Regardless of the limestone doses and the production cycle, the leaf nutrients did not present major oscillations, especially between doses 6 and 12 t ha^{-1} (harvest 2019/20) and 9 and 15 t ha^{-1} (harvest 2020/21). Nevertheless, on the 4th production cycle the contents of N, P, K and S stayed below the ideal range for sugarcane (MALAVOLTA et al., 1997; RAIJ et al., 1996); Ca and Mg maintained the concentrations inside the recommended, with values between 3.26 and 3.82 g kg^{-1} and 2.23 and 2.69 g kg^{-1}, respectively. In the last harvest (2020/21), almost all

Table 1 - Statistical regression analyses of the mean leaf contents of the macronutrients (N, Ca, Mg, K, P and S) in sugarcane as a function of the application of limestone doses for the harvests 2019/20 and 2020/21.

------Harvest 2019/20------								
Nutr.($g.kg^{-1}$)	------Doses------				C.V.	------Regression function------		
	0	6	12	18	(%)	------F------	------Model------	------R^2------
N	10.8	12.1	11.6	11.3	8.8	0.08ns	-	-
Ca	3.26	3.36	3.39	3.82	19.6	0.19ns	-	-
Mg	2.23	2.30	2.51	2.69	10.5	0.004*	y = 0.0266x + 2.1944	0.96
K	3.14	4.06	3.89	3.75	17.8	0.04**	y = -0.0074x² + 0.160x + 3.19	0.87
P	1.28	1.32	1.37	1.36	6.92	0.09ns	-	-
S	0.87	0.90	0.91	0.93	9.77	0.24ns	-	-
------Harvest 2020/21------								
$g.kg^{-1}$	0	9	15	21	C.V.	------F------	------Model------	------R^2------
N	10.7	11.2	11.2	11.1	6.04	0.38ns	-	-
Ca	3.60	3.84	4.08	4.28	7.91	0.001*	y = 0.0327x + 3.5808	0.99
Mg	2.18	2.42	2.66	2.58	12.4	0.007*	y = 0.0255x + 2.1957	0.94
K	7.81	8.98	8.89	8.18	30.9	0.40ns	-	-
P	1.30	1.34	1.33	1.40	4.55	0.13**	y = 0.0045x + 1.2907	0.84
S	1.84	2.08	2.10	2.03	9.70	0.07ns	-	-

Nutr. = Leaf nutrients; CV = Coefficient of Variation (%); ns = not significant; ** = significant at 5% of probability and * = significant at 1% of probability.

macronutrients were inside the ideal range, except for nitrogen (RAIJ et al., 1996).

Descriptive analysis of the spectral curves

As observed in figure 3, limestone doses 0 and 18 t ha^{-1} registered the highest reflectance rates, especially in the region of the near-infrared (720-1100 nm) and visible (400-680 nm). The range of the NIR spectrum was the one which best responded to nutritional stress in the plants. In this case, the curves relative to doses 6 and 12 t ha^{-1} indicated healthier leaves, registering a lower reflectance factor at the wavelengths of visible, especially at 550 nm; this shows a higher absorbance of incident energy in the leaf to perform the physiological processes of the plant, such as photosynthesis (AYALA-SILVA & BEYL, 2005).

According to HOU et al. (2019), the photosynthetic rate and the morphological and physiological characteristics of the leaves, including mass, dry leaf area and chlorophyll content are significantly influenced by N, K and their interactions with the leaf. The chlorophyll molecules have an important role on plant photosynthesis, performing the absorption of solar radiation for the conversion into chemical energy (CROFT & CHEN, 2017). Therefore, the spectral behavior of the leaf is a great indicator of photosynthetic capacity (CROFT et al., 2017), besides presenting high correlation with the nitrogen content (SCHLEMMER et al., 2013; CHENG et al., 2018).

AYALA-SILVA & BEYL (2005) mentioned that macronutrient deficiency in wheat reduced chlorophyll content in the leaves and increased reflectance in the ranges of 400–700 nm (VIS) and 700–1100 nm, NIR ranges. Results corroborated those reported in this study, since the N and K contents stayed far below the ideal for the culture, especially nitrogen (Table 1). According to RAIJ et al. (1996), the range of the appropriate N contents in the sugarcane leaf is from 18 to 25 g.kg^{-1}, whereas the contents reported in this study stayed below 12 g.kg^{-1}. This stress caused in the plant can be observed by leaf reflectance, especially in the ranges of visible (400-680 nm), red-edge (680-740 nm) and near-infrared (740-1300 nm) (Figure 3).

Another important characteristic observed regarding the negative effect of doses 0 and 18 t ha^{-1} for the culture was through the range of red-edge. In a severely degraded vegetation, there is a reduction in the capacity of absorption in the visible and red-edge regions, together with the decrease in leaf nutritional status, which shifts reflectance towards the blue end and away from the red in a slightly degraded vegetation (PENG et al., 2014; PENG et al., 2020).

Leaf reflectance in the subsequent harvest (2020/21) was measured in March, the same date of the previous cycle (harvest 2019/20). Nevertheless, the culture still had not reached exponential vegetative growth. This delay in crop growth derived from the low rainfall that occurred in the region in the last harvest (2020/21). In this case, leaf reflectance for this date registered the greatest variations in the range that is sensitive to the water content in the leaf, specifically in the wavelengths 1450 and 1950 nm (Figure 4A). Since

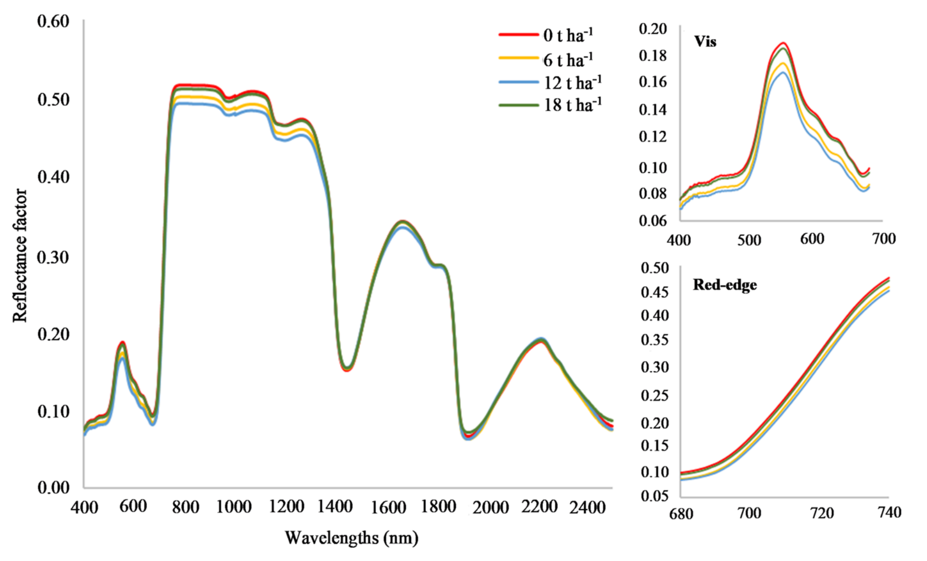

Figure 3 - Sugarcane leaf spectral signatures under the effect of different limestone doses, harvest 2019/20.

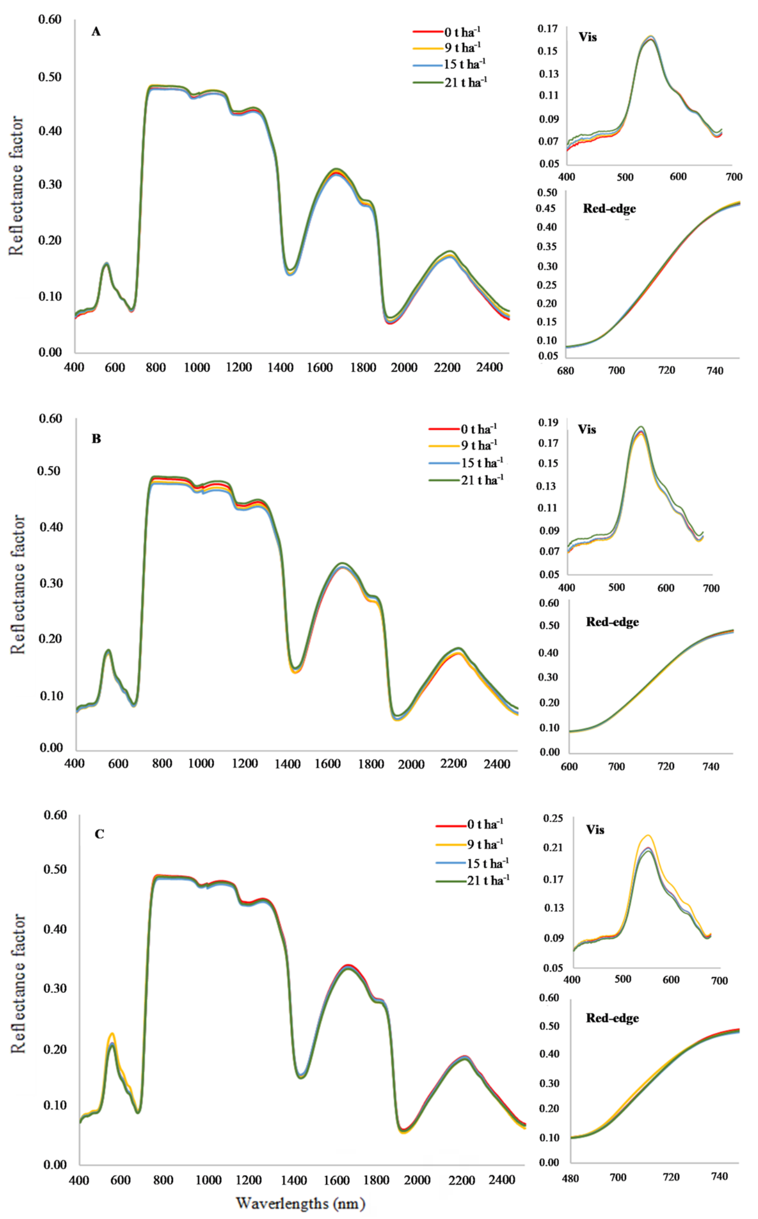

Figure 4 - Spectral signatures of the sugarcane leaf under the effect of different limestone doses when the culture was in the initial growth phase (A), exponential vegetative growth (B) and beginning of maturation (C), harvest 2020/21.

water absorbs electromagnetic radiation, the plants with the highest water content in the leaf registered the smallest reflectance factor at the wavelengths 1450 and 1950 nm (PONZONI et al., 2012).

Another point of greater difference in reflectance among the doses was in the range from 400 to 500 nm, region of visible. According to PENG et al. (2020), the bands sensitive to the K contents in the leaves, in their studies, were mainly located in the bands which vary between 360 nm and 450 nm. Potassium, in this study, stayed below the recommended and had several variations in the leaf contents in the limestone doses, which can be an indicative of the sensibility to potassium in this region of the spectrum (400-470 nm).

In the stage of exponential vegetative growth of harvest 2020/21 (Figure 4B), the spectral curve presented similar characteristics to the reading of the previous harvest (2019/20), since the plants subjected to the highest limestone dose (21 t ha^{-1}), followed by dose 0 t ha^{-1}, presented the highest reflectance in the leaves. Again, these ranges in the spectrum differed among the doses, being more characteristic in the region of green (553 nm) and NIR (720 to 1100 nm).

This similarity in sugarcane spectral response between the highest doses 18 and 21 t ha^{-1} in the harvests 2019/20 and 2020/21, respectively, and the control (0 t ha-1) derives from the nutritional stress to which the plants of these treatments were subjected. The N and K contents in these doses were considerably below the recommended (RAIJ et al. 1996), especially in harvest 2019/20. This nutritional stress was observed by spectral reflectance and confirmed with the leaf chemical analysis (Table 1). Even with the curves presenting some similarity in the spectral behavior in the two harvests studied, in 2020/21 the variation among the curves was smaller, since nutrient deficiency had already been overcome, except for potassium (Table 1). In this case, K, among other functions, is responsible for activating more than 60 enzymatic systems, besides acting in plant photosynthesis, favoring a high energy state necessary for ATP production, regulating the opening and closing of stomata, promoting water absorption and regulating nutrient translocation in the plants (MEURER et al., 2018).

When the culture was in the beginning of the maturation stage (Figure 4C), leaf reflectance was similar in almost all wavelengths; nevertheless, there was a reflectance peak in the range of visible in the leaves subjected to dose 9 t ha^{-1}, specifically among the wavelengths of green, yellow and red (550 to 680 nm). On the date of the spectral reading, the culture presented a more yellow coloration of the leaves, and this was even more evident in some plots of the experiment. According to AUDE (1993), when sugarcane completes the cycle, it decreases assimilation, stops growth and initiates sugar concentration, the leaves become yellowish and the lower ones dry.

For maturation to occur, the nutrients N, P, K and Mg present in the aerial part must translocate to the stems and root system of the culture, being a physiological reflex of this phenological stage (FRANCO et al., 2007). In this case, it is believed that the plants subjected to 9 t ha^{-1} of limestone entered the maturation stage first, with climate as one of the main factors for the change in the growth stage for sugarcane maturation (AUDE, 1993). In this study, the month of June had the longest water drought of the whole 5th production cycle (Figure 2), being the ideal period for sugar accumulation in the stem (FRANCO et al., 2007).

Spearman's Correlation
Harvest 2019/20

Figure 5 refers to the results of the Spearman's coefficient of correlation between the nutrients and the reflectances. The analyses occurred when the culture was in its exponential vegetative growth of harvest 2019/20.

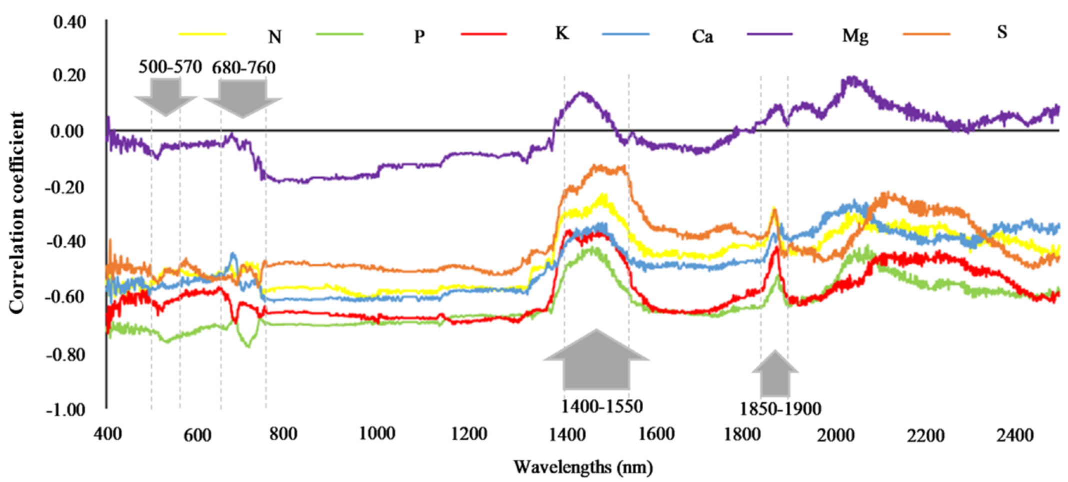

Figure 5 - Spearman's coefficient of correlation between Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg) and Sulfur (S) in the leaf and reflectance in the wavelengths between 400 and 2500 nm for the harvest 2019/20. Spectral regions with the greatest correlation variations in the ranges of Visible (500-570 nm), Red-Edge (680-760 nm) and Mid-Infrared (1400-1550 nm and 1850-1900 nm) were also identified.

In this case, the order of the degree of correlation in the region of visible was: P > K > N > Ca > S > Mg. In almost the entire range of near-infrared, the correlation started to present the following order: P > K > Ca > N > S > Mg, with nitrogen starting to have a smaller correlation in comparison with calcium. MAHAJAN et al. (2014) reported that, for the culture of wheat, the order of the degree of correlation of the different nutrients in the region of VIS was N > P > K.

Phosphorus was the nutrient with the highest degree of correlation, with negative sharp peaks and with high correlation in the wavelengths 706-717 nm (red-edge), with the correlation of -0.78; another peak occurred in the range of 522-543 nm (green), with -0.76. Another important nutrient was K, since it had a high correlation (-0.73) in the wavelengths 404-407 nm, with a second peak between the wavelengths 678-689 nm (red-edge), with -0.68. The results corroborate those found by PENG et al. (2020), since they highlighted the bands sensitive to the contents of N and P in the regions green, green-yellow, red and NIR; for K, the short wavelengths, varying from 360 nm to 450 nm and covering the regions violet, blue, cyan, green and yellow of the spectrum were the most sensitive.

This high correlation in the visible may derive from the characteristic of absorption of the pigments chlorophyll and carotenoids, since the deficiency in P and K reduced the concentration of chlorophyll in the plants (MAHAJAN et al., 2014). The deficiency or excessive application of P in pots caused very high drops in the chlorophyll and carotenoid concentrations in strawberry leaves (SIEDLISKA et al., 2021). AYALA-SILVA & BEYL (2005), also reported that the deficiency in mineral nutrients such as P and K reduced significantly the chlorophyll content in wheat plants under greenhouse conditions, being similar to the results of leaf contents in this study, since the levels of P, K and N stayed far below the ideal for the sugarcane culture.

When the wavelengths of water absorption are observed, nutrient correlation and reflectance decrease, a characteristic which was already expected, since in this range the water content in the cell is the main factor influencing reflectance (PONZONI et al., 2012). Another important characteristic refers to the near-infrared (720-1100 nm). In this range, the correlation with the nutrients was high and stayed stable, without great oscillations. However, the whole range of visible and red-edge had major oscillations among the correlations.

Harvest 2021

Phosphorus (P), similarly to the last harvest (2019/20), was the nutrient with the highest correlation with reflectance; nevertheless, this correlation was positive and with smaller coefficients (Figure 6). The range of 400-420 nm had the highest mean for the correlation coefficient, with 0.61, with a second and third peaks in the region of green (560-574 nm) and red-

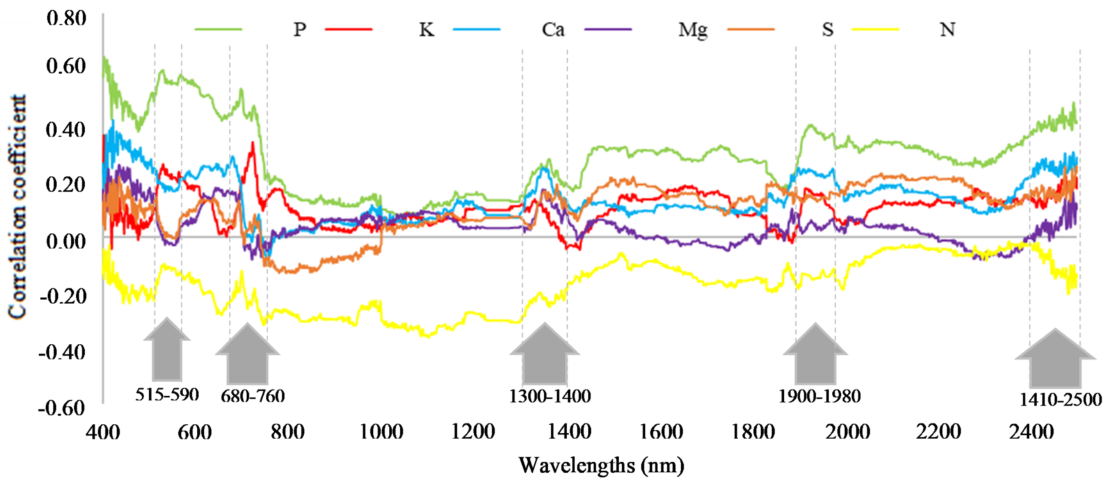

Figure 6 - Spearman's coefficient of correlation between Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg) and Sulfur (S) in the leaves and reflectance in the wavelengths between 400 and 2500 nm for the harvest 2020/21. Spectral regions with the greatest correlation variations in the ranges of Visible (515-590 nm), Red-Edge (680-760 nm) and Mid-Infrared (1300-1400 nm,1900-1980 nm and 1410-2500 nm) were also identified.

edge (690-730 nm), respectively. Differently from the last production cycle, almost all nutrients, except for N, presented positive values and of smaller correlation.

It is worth highlighting that in the first harvest (2019/20), the P, K and N contents were below the appropriate for the culture; consequently, they presented high and negative coefficients, especially in the region of VIS (400-680 nm) and red-edge (680-760 nm), since these nutrients present a high correlation with chlorophyll and carotenoids (SIEDLISKA et al., 2021; AYALA-SILVA & BEYL, 2005).

Conversely, on harvest 2020/21, the concentrations of P and K went to appropriate levels, but with a lower and positive correlation with reflectance. In this case, N was the only nutrient with contents below the recommended, presenting a similarity in behavior with the correlation of harvest 2019/20, remaining negative. The results showed that in this study, reflectance was directly related to nutrient deficiency in the culture and this relation is inversely proportional.

In the two cycles studied, the region of red-edge (680-760 nm) demonstrated it is very sensitive to the variations in the nutrients P, K and N, in this order in degree of correlation. This happens because the red-edge (680-760 nm) is greatly correlated with the variations in the chlorophyll contents in the leaves (LIN et al., 2019). According to PENG et al. (2020), the bands which are indicative of P in the leaf are also in the region of 580-710 nm; although, this varies among the different case studies. The N contents and their correlation with red-edge are already greatly discussed in studies with hyperspectral data (BARROS et al., 2021; WEN et al., 2021). Conversely, few studies address spectral characteristics caused by the nutritional stress of K.

Furthermore, the variation in reflectance among the limestone doses evidenced the correlation with the nutritional status of the cane, since, when the culture was under nutritional stress, the correlation factor was higher and negative; on the other hand, when the conditions went to appropriate patterns, the correlation started to be smaller and positive (Figures 5 and 6).

Analysis of similarity (ANOSIM) and Principal Component Analysis (PCA)

The analysis of similarity (ANOSIM) regarding sugarcane leaf reflectance in the harvest of 2019/20 denotes the test rejected the null hypothesis, indicating at least one group was formed ($P = 0.0051$). Nevertheless, a very low global value of R (R = 0.01) indicates overlap and low possibility of difference among the groups.

In the PCA analysis (Figure 7), PC1 and PC2 explained 97% of the variance in reflectance, forming four groups referring to the doses 0, 6, 12 and 18 t ha^{-1}. It is worth highlighting that, among the doses, the one which best grouped was of 6 t ha^{-1}. Nonetheless, the groups did not differ from each other, confirming the results of ANOSIM.

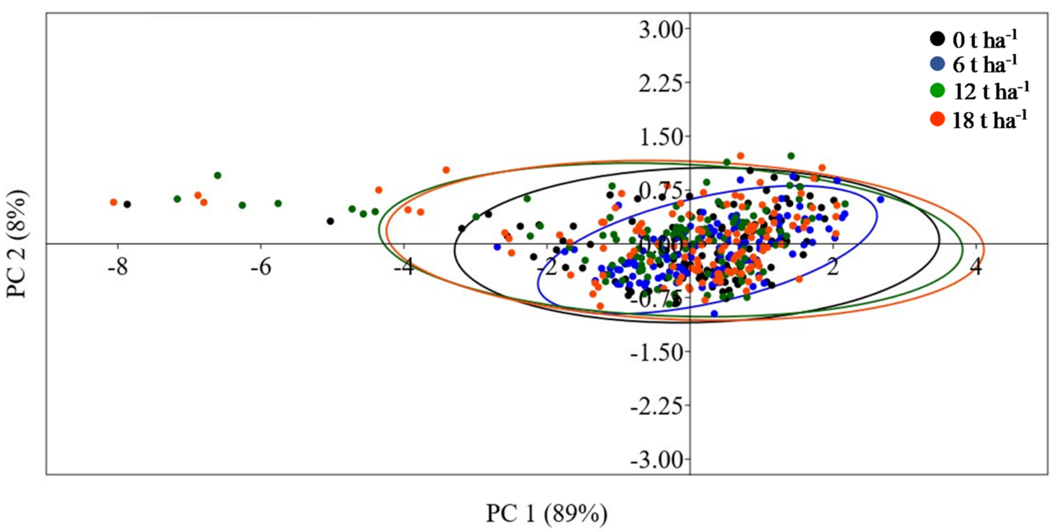

Figure 7 - Distribution of the Principal Components (PC) 1 and 2 according to the leaf reflectance of the sugarcane subjected to the doses 0, 6, 12 and 18 t ha^{-1}, referring to the harvest 2019/20.

This similarity among the groups was already expected for the harvest 2019/20, since there was nutritional stress in the culture in all doses studied because of the decrease in the nutrients N, P, K and S (Table 1). Additionally, leaf reflectance of the doses 0, 6, 12 and 18 t ha^{-1} stayed very close, registering more accentuated differences only in the wavelengths of VIS and NIR.

The test of similarity (ANOSIM) in the last production cycle (2020/21), similarly to the previous harvest (2019/20), registered a low value of R (0.02), indicating an overlap among the classes and low possibility of difference among the groups; nevertheless, the value of p (0.01) was significant, demonstrating at least one group was formed.

Conversely, PCA responded 90% of the variability of the hyperspectral data, the first component (PC 1) with 68% and the second component (PC 2), 22%. In this harvest, the results demonstrated that the doses did not group very well and did not separate from each other (Figure 8), confirming the results of ANOSIM and the reflectance factors of Figure 4B, since they did not differ among the doses.

Analyzing the leaf nutrient contents in sugarcane in the harvest 2020/21, the concentrations of P, K, Mg, Ca and S were under appropriate conditions for the culture, without more accentuated differences in leaf reflectance in this period. It is believed to be one of the factors which influenced the similarity among the groups in PCA, as already demonstrated by the spectral curve itself.

Predictions of the nutrient contents with spectral data and PLSR

The analysis of the estimate of sugarcane nutrient content from the spectral response of the leaves show greater differences in the spectra from one harvest to the other; furthermore, the spectral responses suffered more significant influence from sulfur, potassium and phosphorus, with R^2 equal to 0.82, 0.67 and 0.35, respectively (Figure 9).

Although, reflectance is directly related to the N and P contents in the plant, especially because of the pigments chlorophyll and carotenoids (SIEDLISKA et al., 2021; MAHAJAN et al., 2014; BARROS et al., 2021; ROSA et al., 2015). In this study, the spectra had a good response for the S, K and P contents. The nutrients S and K act indirectly in the spectral response of the culture, hampering, in most cases, their correlation with leaf reflectance.

In this case, it was possible to identify, by the VIP test (Figure 10), the importance of each band in nutrient prediction. Even indirectly, in this study, the S and K contents, besides similar, had more expressive contributions in the spectrum of the region of VIS and red-edge, with accentuated peaks in the range 400-420, 480-600 and 700 nm.

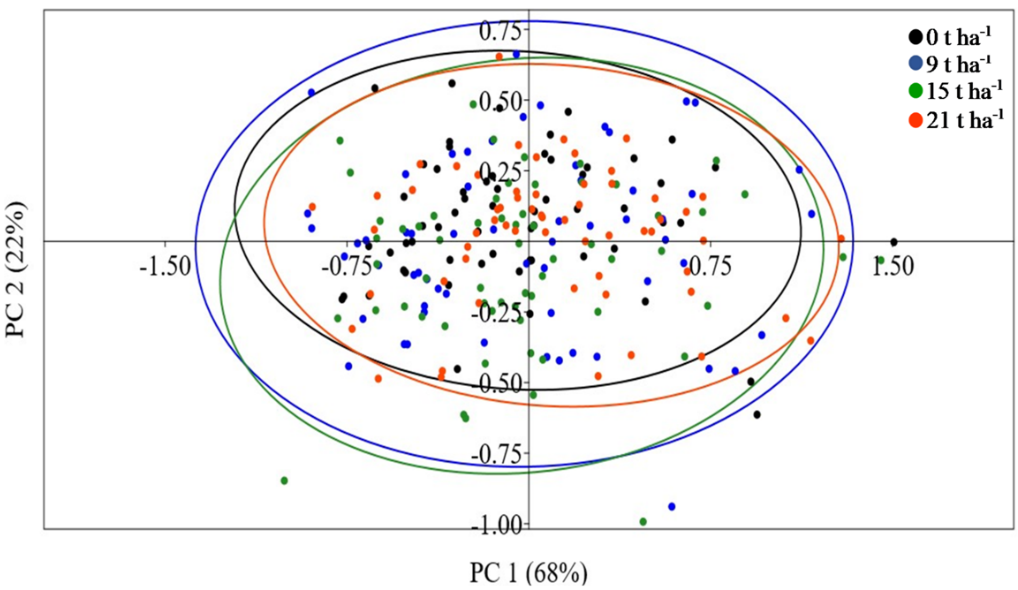

Figure 8 - Distribution of the Principal Components (PC) 1 and 2 according to the leaf reflectance of the sugarcane subjected to the doses 0, 9, 15 and 21 t ha^{-1}, referring to the harvest 2020/21.

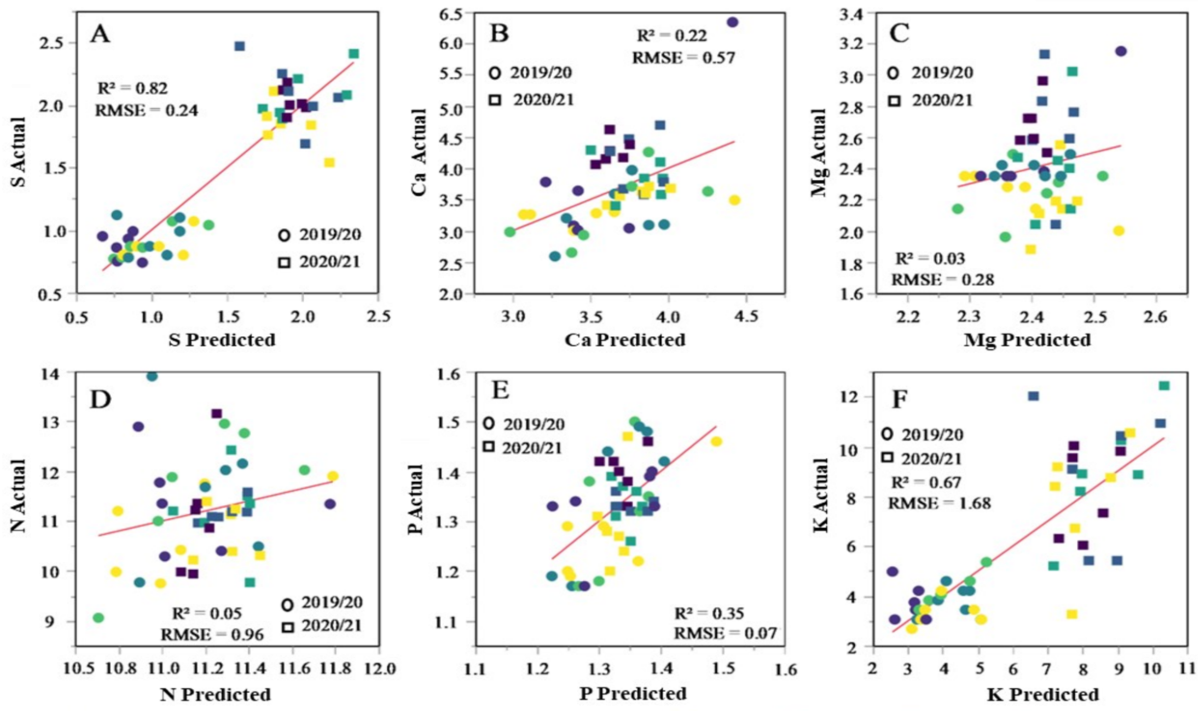

Figure 9 - Estimation of nutrient contents by observed versus predicted spectrum by the Partial Least Squares Regression - PLSR for the nutrients Sulfur (A), Calcium (B), Magnesium (C), Nitrogen (D), Phosphorus (E) and Potassium, harvests 2019/20 and 2020/21.

Studies with K demonstrate that the regions of VIS were the most sensitive, especially in the range from 360 to 450 nm (PENG et al., 2020). This correlation between the spectra and potassium can be explained by the fact that the deficiency in K reduces chlorophyll concentration in the plants (MAHAJAN et al., 2014; AYALA-SILVA & BEYL, 2005).

The bands in the range of VIS (400-680 nm) and red-edge (680-720 nm) had a great importance in the prediction of nitrogen, with a reduction in the region of NIR and more accentuated in the range of SWIR. The bands sensitive to N derive from the morphological and physiological characteristics of the plants (HOU et al., 2019), since the chlorophyll molecules play an important role in leaf photosynthesis, performing the absorption of solar radiation for the conversion into chemical energy (CROFT & CHEN, 2017), with the spectral behavior of the leaf in the range of VIS being a great indicator of the photosynthetic capacity (CROFT et al., 2017).

The contents of Ca and Mg had a higher importance of contribution in the region of NIR, which happened because calcium and magnesium are related to the cell walls of the leaves. Ca, for instance, increases the photosynthetic rate, besides playing a structural role in cell wall and in the membrane systems. Conversely, calcium deficiency can be observed as chlorotic leaf margins and may hamper leaf photosynthesis (ETICHA et al., 2017). In this case, and in a general way, calcium is directly related to reflectance, since the more spongy the internal leaf structure, the greater the internal scattering of incident radiation, and consequently, the higher will be the values of the reflectance factors (POZONI et al., 2012).

CONCLUSION

It was possible to identify nutritional stress in sugarcane from reflectance spectroscopy. The wavelengths of near-infrared, green and red-edge presented the best results in the identification of the plant nutritional stress. Nonetheless, it was not possible to differentiate the limestone doses by the spectral data.

In general, the results were promising and justify further studies using spectroradiometric data to determine nutritional deficiencies in the sugarcane crop. Therefore, new experiments are recommended involving other variables which influence nutrient absorption by the plants, such as: water deficit,

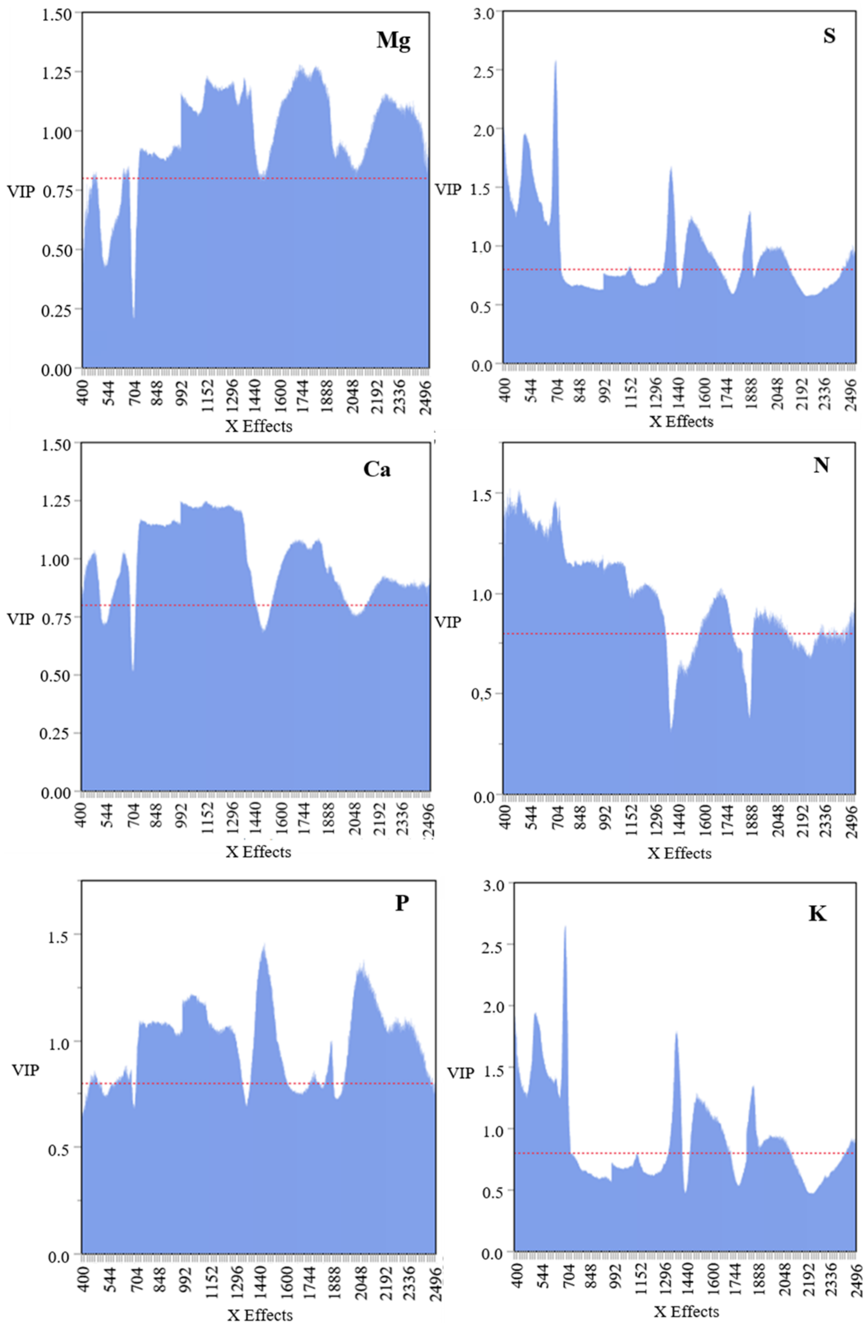

Figure 10 - Importance of each band for the prediction of the nutrients in the sugarcane leaves by the partial least squares regression (PLSR – VIP). Where: Mg (Magnesium), S (Sulfur), Ca (Calcium), N (Nitrogen), P (Phosphorus) and K (Potassium).

temperature and soil analysis. Furthermore, new studies in a controlled environment must be applied to investigate the interaction between leaf reflectance and nutritional stress for each nutrient (N, P, K, Ca, Mg and S).

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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