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Cristhian J. Yarce, Giovanni Rojas

Near infrared spectroscopy for the analysis of macro and micro nutrients in sugarcane leaves*

Nahinfrarot-Spektroskopie zur Analyse von Makro-und Mikronährstoffen in Zuckerrohrblättern

About ten years ago, NIR technology was implemented at the Colombian Sugarcane Research Center Cenicaña for the analysis of sugarcane quality, followed by the development of methodologies for the quantification of some elements in soils and sugarcane leaves. Methodologies were based on the presence of high content materials, such nitrogen, potassium, calcium, and magnesium; however, determination of micronutrients which are found in soils and leaves in the range of parts per million has been challenging.
Development of a NIR methodology for quantification of macro (N, P, K, Ca, Mg) and micronutrients (Cu, Zn, Mn, Fe) in a single experiment is here reported. Calibration curves were constructed using approximately 500 samples that were previously analyzed by methods of reference such atomic absorption. Statistical analysis of the data showed that there are not significant differences between the methods of reference and NIR, suggesting that NIR is a very fast, economical and convenient methodology for the daily analysis of sugarcane leaves.

Key words: near infrared spectroscopy, sugarcane leaves, macronutrients, micronutrients

Vor etwa zehn Jahren wurde die Nahinfrarot-Spektroskopie (NIR) beim kolumbianischen Zuckerrohr-Forschungsinstitut Cenicaña zur Analyse der Zuckerrohrqualität eingeführt. Es folgte die Entwicklung von Methoden zur Quantifizierung einiger Elemente in Böden und Zuckerrohrblättern. Die Methoden basierten auf dem Vorhandensein hoher Gehalte an Stickstoff, Kalium, Calcium und Magnesium. Die Bestimmung von Mikronährstoffen in Böden und Blättern im mg/kg-Bereich ist jedoch eine Herausforderung gewesen. Die Entwicklung einer NIR-Methode zur Quantifizierung von Makro- (N, P, K, Ca, Mg) und Mikronährstoffen (Cu, Zn, Mn, Fe) mit einer einzigen Messung wird vorgestellt. Die Eichkurven wurden unter Verwendung von etwa 500 Proben erstellt, die zuvor mit Referenzmethoden wie der Atomabsorption analysiert wurden. Die statistische Analyse der Daten zeigte, dass es keine signifikanten Unterschiede zwischen Referenz- und NIR-Methode gibt. Das deutet darauf hin, dass die NIR eine sehr schnelle, preiswerte und bequeme Methode zur täglichen Analyse der Zuckerrohrblätter ist.

Schlagwörter: Nahinfrarot-Spektroskopie, Zuckerrohrblätter, Makronährstoffe, Mikronährstoffe

1 Introduction

Quantification of the macro and micronutrients in the sugarcane leaves is an important tool for farmers and agronomists, elements concentration are used to describe the nutritional status, requirements, and limitations of plants, which at the end will impact the final production of sucrose during the harvest period [1]. The leaf chemical composition conveys information about numerous aspects of plant ecophysiology such as growth capacity [2], photosynthetic and respiration rates [3], and nutrient use efficiency [4–6]. In general terms for agriculture, studies require chemical analysis of a large number of samples that sometimes are available in small amounts and require expensive sample preparation.
Normally the reference methods for macro and micronutrients chemical analysis include inductively coupled plasma (ICP), atomic absorption spectroscopy (AA), and X-ray fluorescence spectroscopy (XRF) [7]. Because current methods are expensive and time consuming, leaf chemical analysis of sugarcane needs a rapid, simplified, and cost-effective method for routine analysis. Near-infrared reflectance spectroscopy (NIR) is accepted worldwide as a tool for the analysis of several chemical constituents in different fields such as pharmaceuticals, food industries, quality control of process and raw materials, etc. [8]. The technique is extremely rapid, nondestructive, involves no sample preparation, and it is less expensive than conventional methods. NIR combines applied spectroscopy and statistics for the analysis of absorption of light in the near infrared wavelength range (780 to 2500 nm) by organic materials composed of carbon, hydrogen, oxygen, nitrogen, sulfur and phosphorus [8].
Since NIR measures absorption of molecular bonds such as C-H, N-H, O-H, its use for pure minerals may seems to have no sense, simple because theoretically there are no absorption bonds in the mineral species of macro and micronutrients of sugarcane leaves [7]. However, prediction of some minerals at

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certain concentrations in forages by NIR it is possible through their association with the organic matrix [6, 9–13]. Only a few papers related to the use of NIR for macro and trace minerals are reported, mostly in the fields of grasses and hay samples [10], natural grasses [14], and botanical fractions of semi-arid grasslands [15]. It has been found that magnesium is associated with chlorophyll, and potassium is associated with organic acids, especially malate [10]. Based on those principles, since 1994 the Colombian Sugarcane Research Center established the analysis of sugarcane juice and macronutrients in leaves by NIR as routine methods [16, 17].

Although quantification of fairly high concentration of elements has shown to be accurate [17], in the past research papers concerned with the prediction of trace elements (mircronutrients) have indicated that NIR does not estimate these elements accurately [9, 10, 14, 18, 19]. The aim of this research was to explore the potential and accuracy of NIR for predicting all macro (Ca, Mg, N, P) and micronutrients (Cu, Zn, Mn, Fe) present in Colombian sugarcane leaves in only one run of analysis with virtually none sample preparation.

2 Materials and methods

2.1 Samples and reference analysis

All samples of sugarcane leaves came from different varieties from the geographic valley of the Cauca River in Colombia, dried samples used for developing the model were collected between the years 2008 and early 2011. Every sample was oven dried for 48 h at 60 °C, grounded and sieved (1 mm). In order to determine micro and macronutrients, samples were digested and extracted accordingly pre-established primary methodologies [20, 21] Analysis were developed using among others the following equipment: atomic absorption Perkin-Elmer Aanalyst 400, spectrophotometer UV 1800 Shimadzu and a multi-burette E 485 Tecator.

2.2 Spectral and chemometric analysis

Around 4000 samples were analyzed in total; while 580 samples were used to build the calibration curves, 304 samples were used to verify the models, and 16 samples were used to cross validate every each element model, the rest of the samples were analyzed using the calibration curves here found and reported as NIR results. Samples were analyzed using a NIR Master from Büchi. The software used to operate the NIR, collect samples, and developing the calibration curves were NIR Ware Management console, NIR Ware operator and NIR Cal, respectively.

The raw data were subjected to mathematical treatments, first and second derivative (1D, 2D) of log 1/*R*. For all samples, the chemical data determined by the reference methods were added to the corresponding NIR spectral files, and multiple linear regression (MLR) and partial-least squares regression (PLSR) were used to develop the calibration equations. Validation of the calibration curves was assessed by simple regression between NIR predicted values and those obtained by the reference method.

The accuracy of the prediction process (external validation) was assessed by the coefficient of determination (r^2), standard error of prediction (SEP), and BIAS (difference between NIR predicted values and the values determined by the reference chemistry). The SEP is a true measure of the performance of the equation on unknown samples and is the preferred statistic for the comparison of regression equations [6]. Two other statistics parameters used to evaluate calibrations were the relative percent difference (RPD) and the range error ratio (RER). The RPD is the ratio of the standard deviation of the constituent reference values in the prediction set to the SEP. The RER is the ratio of the range of the constituent reference values in the prediction set to the SEP. In agricultural applications, a RPD > 3 is considered acceptable and an RPD > 5 excellent. RER should be above 10 to be considered acceptable, and if RER > 15 it is considered excellent. In environmental applications, where samples are much more variable, "acceptable" values for RPD and RER have not been established [22].

3 Results and considerations

After incorporation of the all primary values obtained by the classical chemical analyses, calibration curves for each and every element were developed. Calibration curves for macro and micronutrients (Ca, Mg, N, P, K, Cu, Zn, Mn, and Fe) showed high correlations (CV > 0.9) and low deviation errors (Deviation < 0.15), as shown in Table 1.

Calibration curves were built by plotting the NIR predicted values versus the reference values obtained from the classical analyses. Figure 1 shows an example of a calibration curve

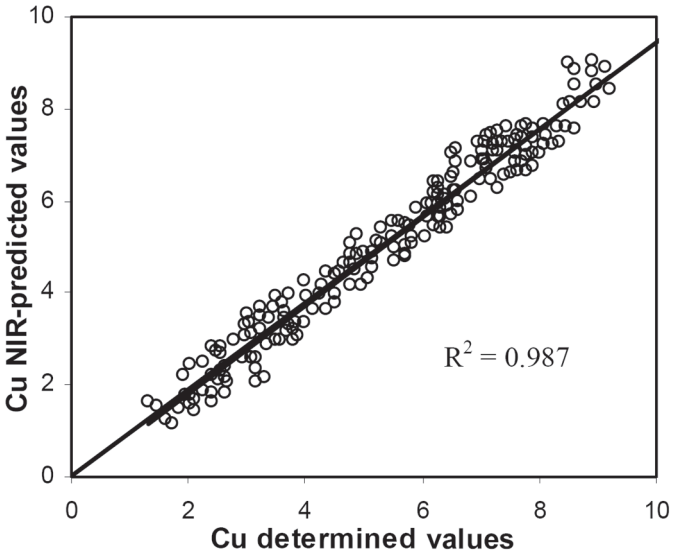

Fig. 1: NIR predicted values versus reference values for the copper content in sugarcane leaves samples

Table 1: Correlation coefficients for all elements and deviation errors obtained for the calibration curves

	Ca	Mg	N	P	K	Cu	Zn	Mn	Fe
R^2	0.974	0.976	0.989	0.988	0.979	0.987	0.979	0.976	0.989
Deviation error	0.009	0.005	0.012	0.008	0.013	0.015	0.002	0.034	0.150

obtained, it is depicted how well correlated the copper content predicted by NIR versus the reference values.

Since well correlated calibration curves do not mean necessary highly predictive results during routine analysis, a statistical study using a set of 16 samples were carried out. The accuracy of the prediction was evaluated by calculation of the following parameters: standard error of prediction (SEP), BIAS (difference between NIR predicted values and the values determined by the reference chemistry), Table 2.

Table 3 summarizes the accuracy of the prediction using each and every elements calibration curve. SEP shows how the model performs with unknown samples, the lower the value the better. However, more important the RPD (SD/SEP) for application in agricultural practices must be bigger than 5, and RER or the ratio of the range of the constituent reference values in the prediction set must be bigger than 15. Based on those results and what is considered "excellent" results for the agricultural practices, the NIR models herein reported falls into the category of excellent results and demonstrates the applicability of NIR as a predictive tool for analysis of micro and macronutrients in sugarcane leaves, which gives an alternative to process many data in less time without any expensive preparation and few operative training.

4 Conclusions

For the laboratory capacity of the Colombian Sugarcane Research Center the preparation and analysis of the 4000 samples would take about ten months, analysis via NIR reduced the time into only two months of work. Also no chemicals were involved during the NIR analysis, which was a saving of important magnitude and more importantly an environmental friendly technique.

Although these results are very promising, it is necessary to emphasize that these models are exclusively applicable to routine samples similar in conditions to the samples used to calibrate the models, in other words, as normal as any NIR studies [6, 11–13, 23, 24], new calibration curves or enrichment of the current ones need to be perform if samples from other characteristics want to be promptly analyzed by NIR.

Table 2: Accuracy of prediction for the implemented model, difference between NIR predicted values and the values determined by the reference chemistry (BIAS) for every each macro and micronutrient

BIAS								
Ca	Mg	N	P	K	Cu	Zn	Mn	Fe
0.004	0.010	0.018	0.012	0.001	0.181	0.122	0.274	2.114
0.021	0.006	0.003	0.008	0.002	0.090	0.083	0.803	1.578
0.010	0.003	0.006	0.024	0.018	0.091	0.080	0.926	1.121
0.009	0.003	0.003	0.015	0.024	0.033	0.005	0.183	1.677
0.002	0.003	0.007	0.004	0.004	0.027	0.005	0.366	0.630
0.004	0.010	0.012	0.007	0.028	0.079	0.095	0.847	0.436
0.008	0.009	0.000	0.000	0.001	0.068	0.067	0.786	0.862
0.009	0.001	0.020	0.025	0.006	0.046	0.071	1.164	0.001
0.008	0.003	0.020	0.008	0.010	0.083	0.039	1.985	1.875
0.015	0.006	0.006	0.008	0.007	0.088	0.067	0.441	1.138
0.017	0.002	0.002	0.004	0.030	0.002	0.017	0.374	1.434
0.014	0.001	0.024	0.012	0.019	0.012	0.071	0.587	1.657
0.005	0.010	0.026	0.012	0.017	0.005	0.071	0.649	0.127
0.000	0.013	0.021	0.000	0.016	0.068	0.005	1.657	1.581
0.006	0.003	0.008	0.001	0.014	0.068	0.095	1.634	0.739
0.012	0.000	0.004	0.001	0.017	0.006	0.067	0.091	1.767

Table 3: Accuracy of prediction for calibration for macro and micronutrients using cross validation procedure of 16 samples

	Ca	Mg	N	P	K	Cu	Zn	Mn	Fe
r^2	0.974	0.976	0.989	0.988	0.979	0.987	0.979	0.976	0.976
SEP	0.006	0.004	0.009	0.007	0.009	0.045	0.035	0.543	0.623
RPD	5.336	7.033	55.950	8.256	6.621	54.304	140.637	35.268	83.616
RER	16.330	23.121	202.091	34.852	17.617	159.391	505.082	121.049	230.273

SEP standard error of prediction. RPD relative percent difference. RER range error ratio.

References

1 *Güsewell, S.* (2004): N:P ratios in terrestrial plants: variation and functional significance. New Phytologist 164, 243–266

2 *Cornelissen, J.H.C.; Werger, M.J.A.; Castro-Díez, P.; Rheenen, J.W.A. van; Rowland, A.P.* (1997): Foliar nutrients in relation to growth, allocation and leaf traits in seedlings of a wide range of woody plant species and types. Oecologia 111, 460–469

3 *Reich, P.B.; Walters, M.B.; Tjoelker, M.G.; Vanderklein, D.; Buschena, C.* (1998): Photosynthesis and respiration rates depend on leaf and root morphology and nitrogen concentration in nine boreal tree species differing in relative growth rate. Functional Ecology 12, 395–405

4 *Aerts, R.; Chapin, F.S. III* (1999): The mineral nutrition of wild plants revisited: A re-evaluation of processes and patterns. 30, 1–67

5 *Mediavilla, S.; Escudero, A.; Heilmeier, H.* (2001): Internal leaf anatomy and photosynthetic resource-use efficiency: interspecific and intraspecific comparisons. Tree Physiology 21, 251–259

6 *Petisco, C., García-Criado, B.; Vázquez de Aldana, B.R.; García Ciudad, A.; Mediavilla, S.* (2008): Ash and mineral contents in leaves of woody species: Analysis by near infrared reflectance spectroscopy. Communications in Soil Science and Plant Analysis 39, 905–925

7 *Shenk, J.S.; Westerhaus, M.O.; Hoover, M.R.* (1979): Analysis of forages by infrared reflectance. Journal of Dairy Science 62, 807–812

8 Handbook of near-infrared analysis. 808 (CRC Press ET: Boca Raton, 2008): at <http://uh7qf6fd4h.search.serialssolutions.com/?V=1.0&L=UH7QF6FD4H&S=JCs&C=TC_008253539&T=marc&tab=BOOKS>

9 *Givens, D.I.; Deaville, E.R.* (1999): The current and future role of near infrared reflectance spectroscopy in animal nutrition: a review. Australian Journal of Agricultural Research 50, 1131–1145

10 *Clark, D.H.; Mayland, H.F.; Lamb, R.C.* (1987): Mineral analysis of forages with near infrared reflectance spectroscopy. Agronomy Journal 79, 485–490

11 *Fernandez-Cabanas, V.M.; Garrido-Varo, A.; Delgado-Pertinez, M.; Gomez-Cabrera, A.* (2008): Nutritive evaluation of olive tree leaves by near-infrared spectroscopy: Effect of soil contamination and correction with spectral pretreatments. Applied Spectroscopy 62, 51–58

12 *Huang, C.J.; Han, L.J.; Yang, Z.L.; Liu, M.* (2009): Exploring the use of near infrared reflectance spectroscopy to predict minerals in straw. Fuel 88, 163–168

13 *Cozzolino, D.; Cynkar, W.; Shah, N.; Smith, P.* (2011): Quantitative analysis of minerals and electric conductivity of red grape homogenates by near infrared reflectance spectroscopy. Computers and Electronics in Agriculture 77, 81–85

14 *Aldana, B.R.V. de; Criado, B.G.; Ciudad, A.G.; Corona, M.E.P.* (1995): Estimation of mineral content in natural grasslands by near infrared reflectance spectroscopy. Communications in Soil Science and Plant Analysis 26, 1383–1396
15 *Ruano-Ramos, A.; García-Ciudad, A.; García-Criado, B.* (1999): Near infrared spectroscopy prediction of mineral content in botanical fractions from semi-arid grasslands. Animal Feed Science and Technology 77, 331–343
16 *Palau G.F.*, et al. (1994): La espectroscopía infra-roja de reflectancia cercana (NIR) en evaluaciones analíticas de caña de azúcar. 8 (Cali)
17 *Larrahondo, J. E.; Palau, F.; Navarrete, A.; Ramírez, C.* (2001): Applications of near infrared spectroscopy in the sugarcane industry of Colombia. International Society of Sugar Cane Technologists. Proceedings of the XXIV Congress 1, 163–165
18 *Lavrenčič, A.; Stefanon, B.; Orešnik, A.* (2002): Estimation of mineral content in forages by near-infrared reflectance spectroscopy. Zb. Bioteh. Fak. Univ. Ljubl., Kmet. Zooteh 80, 41–49
19 *Garnsworthy, P.C.; Wiseman, J.; Fegeros, K.* (2000): Prediction of chemical, nutritive and agronomic characteristics of wheat by near infrared spectroscopy. The Journal of Agricultural Science 135, 409–417
20 IGAC Métodos analíticos del laboratorio de suelos. 647 (2006)
21 CENICAÑA Manual de métodos analíticos para el análisis de suelos y tejido foliar en caña de azúcar
22 *Malley, D.F.; Yesmin, L.; Wray, D.; Edwards, S.* (1999): Application of near-infrared spectroscopy in analysis of soil mineral nutrients. Communications in Soil Science and Plant Analysis 30, 999–1012
23 *Cozzolino, D.; Moron, A.* (2004): Exploring the use of near infrared reflectance spectroscopy (NIRS) to predict trace minerals in legumes. Animal Feed Science and Technology 111, 161–173
24 *Halgerson, J.L.; Sheaffer, C.C.; Martin, N.P.; Peterson, P.R.; Weston, S.J.* (2004): Near-infrared reflectance spectroscopy prediction of leaf and mineral concentrations in alfalfa. Agronomy Journal 96, 344–351

Spectroscopie dans le proche infrarouge pour l'analyse des macro- et micro-éléments nutritifs dans les feuilles de canne à sucre (Résumé)

La technologie dans le proche infrarouge (NIR) a fait l'objet d'un fort développement au cours de la dernière décennie entraînant l'utilisation d'appareils modernes dans de nombreux domaines de recherche pour le contrôle de la qualité. L'avantage le plus important de cette technique est le temps court de l'analyse ainsi que l'absence ou la réduction de la préparation de l'échantillon. Il y a environ dix ans que la technologie dans le proche infrarouge a été introduite au Centre de Recherche de la Canne à sucre de Colombie Cenicaña pour l'analyse de la qualité de la canne à sucre, suivie de la mise au point des méthodes pour quantifier la présence de certains éléments dans les sols et les feuilles de la canne à sucre. Les méthodes étaient basées sur la présence des éléments à teneur élevée tels que l'azote, le potassium, le calcium et le magnésium ; toutefois la détermination des micro-éléments trouvés dans les sols et les feuilles à raison de quelques parties par million a été mise en examen.

La mise au point de la méthodologie NIR pour mesurer la quantité des macro-éléments (N, P, K, Ca, Mg) et des micro-éléments (Cu, Zn, Mn, Fe) est décrite. Les courbes de référence ont été établies en utilisant environ 500 échantillons préalablement analysés par des méthodes de référence telles que l'absorption atomique. L'analyse statistique des données a montré qu'il n'y avait pas de différences significatives entre les méthodes de référence et l'analyse NIR, ce qui suggère que cette dernière est une méthode très rapide, économique et convenant pour l'analyse journalière des feuilles de canne à sucre.

Espectroscopia de Infrarrojo Cercano para el análisis de Macro y Micro nutrientes en tejido foliar de caña de azúcar (Resumen)

Desde hace aproximadamente diez años la tecnología NIR fue implementada en el Centro de Investigación de la Caña de Azúcar de Colombia, inicialmente para el análisis de calidad de caña de azúcar y posteriormente para la cuantificación de macroelementos en suelos y tejido foliar de caña. Dichas metodología fueron posibles gracias a la presencia de altos contenidos de nitrógeno, potasio, calcio y magnesio en la matriz, sin embargo la determinación de microelementos en el orden de partes por millón fue un reto. En este trabajo se reporta la cuantificación en un sola determinación vía NIR de macro (N, P, K, Ca, Mg) and micronutrientes (Cu, Zn, Mn, Fe) en hojas de caña de azúcar. Las curvas de calibración fueron construidas usando aproximadamente 500 muestras, las cuales fueron analizadas previamente por métodos de referencia tales como absorción atómica. El análisis estadístico de los datos mostró que no existen diferencias significativas entre los métodos de referencia y NIR, con lo cual se plantea que la tecnología NIR es una técnica rápida, económica y conveniente para el análisis rutinario de tejido foliar de caña de azúcar.

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