First attempt using Near Infrared Reflectance Spectroscopy to evaluate the element content of flag leaf rice in Malagasy farm fields

Fanjaniaina M. Lucia[1], Salgado Paulo[2,3], Tillard Emmanuel[2], Larvy Delarivière Jean[4], Ramahandry Fidiniaina[3], Razafimanantsoa Marie-Paule[1], Razafimahatratra Hery[1,5], Rabeharisoa Lilia[1], Becquer Thierry[4].

[1]Laboratoire des RadioIsotopes (LRI), Université d'Antananarivo- BP 3383, route d'Andraisoro, Antananarivo, Madagascar;
[2]CIRAD, UMR SELMET, F-97410 Saint-Pierre, Reunion Island, France;
[3]DP SCRID SRR Fofifa - B.P. 230 Antsirabe, Madagascar;
[4]Institut de Recherche pour le Développement (IRD), UMR Eco&Sols, Montpellier Cedex, France;
[5]Ecole Supérieure des Sciences Agronomiques, Département Agriculture, Université d'Antananarivo, BP 174- 101Antananarivo, Madagascar.
fanjalucia@yahoo.fr

INTRODUCTION

Agricultural is very important for the economy of Madagascar (FAO/PAM, 2009). Farmers have, generally, very small farms (# 1 ha) where crop and livestock were almost always associated.So, in a mixed farming agricultural and livestock, stocks and flows of biomass and nutrients must be quantified for a better management of mineral ressources in the farm (Rufino et al., 2006). Carbon(C), Nitrogen (N), Phosphorus (P), Calcium (Ca), Magnesium (Mg) and Potassium (K) are some of the most important biochemical components of plant organic matter. Therefore, the estimation of their contents can help monitor the metabolism processes and health of plants (Zhai, 2012). Traditionally, the contents of biochemical components can be estimated using chemical analyses in the laboratory. Although such estimations are accurate, the method is costly, time consuming, destructive, and complex. The use of NIRS (Near Infrared Spectroscopy) seems an interesting method for the characterization of constituents of plant biomass. It is rapid, timely, non-destructive, straightforward tool and sometimes more accurate than conventional analysis. However, NIRS was often used for the characterization of organic materials, but have rarely been used in tropical area, in particular for Madagascar. So there it is a strong interest to test these methodologies on plant content in Madagascar.

This study was initiated to test the potential of NIRS to predict element content in rice, the main Malagasy field crops, at first in this work in the flag leaf of rice.

MATERIALS AND METHODS

Chemical Analysis

A total of 130 flag leaf rice samples were collected in 2012 from seventy (70) mixed agriculture and livestock farms in Vakinankaratra region of Madagascar. Samples were oven dried at 60°C and ground before analyses. Reference values were determined from routine chemical analysis.

For total nitrogen content, two different methods were used: Kjeldahll method ($N_{Kjeldhall}$); total combustion with a CHN analyser (N_{CHN}). Total carbon content was also determined with a CHN analyser. Phosphorus, Ca, K, Mg were determined after mineralization with chlorhydric acid. Phosphorus was determined colorimetricaly, and Ca, K, Mg were determined by atomic absorption spectrophotometry.

NIRS Measurements and Spectral Treatments

The NIR spectra were recorded on NIRS spectrophotometric LabSpec®4 (Analytical Spectral Devices Inc.) with a spectral range of 350 to 2500 nm. Samples were scanned four times and the radiance of samples obtained by taking 50 consecutive scans. NIR absorbance data were recorded as mean log 1/reflectance values (log 1/R). Before calibration and validation, mathematical pretreatments were done: (1) standard normal variate and detrend transformation (SNDV), second derivate, detrend and smoothing. Principal Component Analisis (PCA) was then performed to check the spectral homogeneity of the database.

Model Calibration and cross -validation

The chemical data determined by the reference methods were added to the corresponding NIR spectral files. Calibration was performed by Partial Least Square (PLS) regression on transformed absorbance spectra with Unscrembler software 10.3 (Infrasoft International) to develop calibration equations). The accuracy of the calibration equations was evaluated using coefficient of multiple determination (R^2), standard error of calibration (SEC), and standard error of cross validation (SECV) (Rabetokotany, 2013). The SEC is an estimate of the best accuracy obtainable using the specific wavelengths of the calibration equation. Due to the small number of samples used, the method of cross- valisation was used. The cross validation is an internal process that splits the calibration set in several groups, and the model is repeated as independent variables for the calibration equation.

RESULTS AND DISCUSSION

Leaf Chemical Composition

The statistics of plant composition, including range, mean and standard deviation (SD) are presented in Table 1. The means ± SD were 26.20±2.57 g.kg-1 for $N_{Kjeldhall}$, 23.79±2.73 $g.kg^{-1}$for N_{CHN}; 1,10±0,16 $g.kg^{-}1$ for P; 42.22±1.80 % for C; 8.69±3.13g kg^{-1} for K; 0.11±0.03g kg^{-1} for Ca and 0.59±0.06 $g.kg^{-1}$ for Mg.

Table 1. Laboratory reference values of N, P, Ca, Mg, and K contents in flag leaf rice samples in Madagascar.

Content	Units	Mean	SD	CV	min	max
$N_{Kjeldahll}$	g.kg^{-1} dw	26,20	2,57	0,10	20,55	32,28
N_{CHN}	g.kg^{-1} dw	23,79	2,73	0,11	1,66	3,07
P	g.kg^{-1} dw	1,10	0,16	0,15	0,74	1,60
C	g. 100g^{-1} dw	42,22	1,80	0,04	31,84	45,74
Ca	g.kg^{-1} dw	0,11	0,03	0,26	0,04	0,20
Mg	g.kg^{-1} dw	0,59	0,06	0,10	0,44	0,72
K	g.kg^{-1} dw	8,69	3,13	0,36	2,15	17,84

SD: standard deviation, CV: coefficient of variation

Calibration and Validation

Table 2 summarizes the statistics, for calibration and cross- validation obtained for each constituent. The factor's number is seven for all samples. The calibration equations developed for the nitrogen content showed better results with low SEC (0.9-1.0 mg.kg^{-1}) and high R^2_{Cal} (0.86) and was produced a good effect of cross validation with R^2_{val} (0.75-0.82) and with less Standard Error of Validation SECV (1.11 g.kg^{-1}). Even if Kjeldhall method and CHN method give the same value of calibration coeffcient (≈0.85), the CHN method allows a higher R^2_{val}= 0.82 as compared to Kjeldhall method R^2_{val}= 0.75. For C, R^2_{Cal} (0.83) and R^2_{val} (0.78) are high. The calibration of P is slightly worse with R^2_{Cal} of 0.72 and R^2_{val} of 0. 63. For Ca, Mg, and K, the calibrations were in a medium range (R^2_{cal} of 0.6-0.7; R^2_{val} of 0.5-0.6).

These values obtained for nutrient content of flag leaf rice in Madagascar are comparable to those reported in other studies on the determination of N, P and K with NIRS (Laio et al., 2012; Petisco et al, 2008; Zhai et al., 2012, Martin et al. 2008, Pimstein et al., 2011), for Mg (Petisco et al., 2008). The result obtained for the Carbon can be compared to the study conducted by Grossmann et al. 1996, which showed a calibration coefficient ranging from 0.60 to 0.90 varying according to the type of treatment (log 1/R, 1st derivate, 2nd derivate).

Table 2: **Statistics of NIRS calibration equation for best fit and cross validation, including SEC, coefficient of determination (R^2) and standard error of cross-validation (SECV)**

variables	units	N	R^2_{Cal}	SEC	R^2_{val}	SECV
$N_{Kjeldahll}$	g.kg^{-1}	110	**0,85**	0,92	**0,75**	1,17
N_{CHN}	g.kg^{-1}	130	**0,86**	1,00	**0,82**	1,11
P total	g.kg^{-1}	130	0.72	0.07	0.63	0.08
C	g.kg^{-1}	130	0,83	0,27	0,78	0,31
Ca	g.kg^{-1}	130	0,71	0,03	0,64	0,16
Mg	g.kg^{-1}	130	**0,68**	0,03	**0,60**	0,03
K	g.kg^{-1}	130	**0,57**	1,87	**0,48**	2,11

N: number of samples, $R^2_{Cal:}$ coefficient of calibrtaion, R^2_{val} : coefficient of cross-validation, SEC: Standard Error of Calibration, SECV: Standard Error of Validation.

These studies give an initial overview of the possibility of using NIRS to estimate the element contents in flag leaf rice. Indeed, according to the study performed by Williams (2003), a coefficient calibration value for R^2 between 0, 66 and 0, 81 indicated approximate quantitative predictions. Whereas value for R^2 between 0.82 and 0.90 reveald good predictions. Calibration models having a value for R^2 above 0.91 were considered to be excellent. Then, the NIR prediction of element contents was good for C and N, approximate for P, Ca and Mg, and poor for K.

Conclusion

The results obtained in the current work showed that C and N can be well predicted by NIRS, despite the strong heterogeneity of the samples considered. This technique also afforded acceptable accuracy in the prediction of P content. The prediction potential for cations Ca, Mg, K elements was moderate. This method could be considered as an alternative for the analysis of the mineral contents of large amount of plant samples.

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