

Analysis of plant constituents using near infrared spectroscopy: intercorrelation effects

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Introduction

A significant feature of analysis using NIR is the ability to simultaneously determine the content of many constituents in a sample. This is particularly valuable when the traditional analyses involve diverse methodologies, equipment and technical skills. Rapid, multi-constituent analysis can reduce the cost of analysis per sample and reduce the turn around time at the laboratory. These features give commercial NIR laboratories advantages over laboratories which rely on more traditional analytical techniques.

The Australian rice industry has used NIR to analyse rice shoot samples since 1987.^{1,2} The original rice tissue test was based on the analysis of nitrogen, one of the earliest constituents determined using NIR.³ Analysis of nitrogenous compounds by NIR has been extended to many plant species and plant tissues.⁴ Analysis, using NIR for non-structural carbohydrates, which include starch and fructans, now assists in the interpretation of wheat and rice crop analysis.^{5,6} Since 1981 there have been several reports of analysis of mineral elements in plants using NIR^{4,7-9} and, more recently, calibrations have been developed to determine some plant-essential mineral elements in rice shoot samples.^{10,11}

In a sample set some of the constituents may be highly correlated leading to NIR calibrations which are unstable and produce unreliable estimates of constituent values.¹² This paper reports the intercorrelations between constituents in rice shoot samples for which NIR calibrations have been developed by the authors in association with the Australian Rice Industry and discusses the possible consequences of these on the reliability of NIR analyses used as a base for crop fertilizer management.

Materials and methods

Whole shoot samples of rice were collected by farmers from commercial crops in southern NSW Australia in the 1998 / 1999 season and dried using microwave energy.² The samples were ground to pass a 0.5 mm screen using a cyclone mill (Cereal Mill 6200, Newport Scientific, Warriewood NSW, Australia).¹³ The reflectance of energy from the samples was recorded using a visible-NIR spectrometer (NIRsystems 6500, Silver Spring MD, USA). Sub-samples of 1 g were digested in 10 mL nitric acid,¹⁴ and analysed for phosphorus, sulphur, potassium and magnesium by ICPS (ARL 3580B, simultaneous fitted with a Meinhard A type nebuliser). Total nitrogen and total non-structural carbohydrate (starch) in the samples were determined using NIR calibrations developed previously (Ciavarella *et al.* unpublished). The nitrogen calibration was developed with samples analysed for total nitrogen by the Dumas combustion technique (Leco). The starch calibration was developed

using samples analysed for total no-structural carbohydrate by an enzyme degradation method and colorimetric determination of glucose.¹⁵

Intercorrelations between constituents were determined as simple linear or exponential regressions with the assistance of Excel® (Microsoft International) while all NIR correlations used in this study were developed using WINISI software (Foss-NIRsystems Inc, Silver Spring MD, USA) and validated against a separate sample set.

Results and discussion

The constituent concentrations of the 619 samples used in this study are summarised in Table 1. The simple linear correlations between the concentrations of elements, as determined by ICPS, are presented in Table 2. The strongest correlation observed was that between nitrogen and sulphur ($r = 0.98$). This is a considerably stronger relationship than reported previously for rice shoots¹⁶ or for rice grains.¹⁷ The strength of the relationship reflects the interdependence of these elements in plant uptake.

Examination of the relationships revealed small curvilinear relations between N and starch ($R^2 = -0.79$), and sulphur and starch ($R^2 = -0.86$) for the equations $y = a + bx + cx^2$ and $y = a + bx + cx^2 + dx^3$, respectively. The negative relationships previously reported between N and non-structural carbohydrates in rice⁵ and wheat⁶ starch were confirmed by this study, which also indicates that there is also a strong negative correlation between sulphur and starch. The calibration for K is similar to that reported for rice shoots by Ciavarella,¹¹ while the calibrations for Mg and K are stronger than reported for rice leaves by Yoshikawa.¹⁸

Table 1. Summary of constituents in the 619 samples used in this study (element % dry matter).

Concentration	Mean	Minimum	Maximum	Standard deviation
Nitrogen	1.81	0.67	3.68	0.71
Starch	11.90	1.23	32.80	6.36
Phosphorus	0.30	0.11	0.48	0.05
Sulphur	0.16	0.10	0.24	0.03
Potassium	2.46	1.27	3.70	0.41
Magnesium	0.18	0.12	0.29	0.30

Table 2. Simple linear correlations between nutrients in rice shoots (r values).

	Nitrogen	Starch	Phosphorus	Sulphur	Potassium	Magnesium
Nitrogen	1.00					
Starch	-0.78	1.00				
Phosphorus	0.15	-0.16	1.00			
Sulphur	0.98	-0.85	0.15	1.00		
Potassium	0.39	-0.37	0.39	0.42	1.00	
Magnesium	0.40	-0.48	0.00	0.47	-0.32	1.00

The verifications of the calibrations developed by Ciavarella *et al.* (unpublished) revealed that the r^2 and SEP statistics for the nitrogen and starch calibrations were 0.99 and 0.66% for N and 0.98 and 0.73% for starch the equivalent statistics were only 0.78 and 0.23% for sulphur suggesting that NIR is not detecting sulphur directly in rice shoot samples. At present few Australian rice crops

appear to suffer from sulphur deficiency so growers seldom used sulphur data to determine crop fertilizer requirements.

The correlations between other elements (Table 2) were low and not expected to interfere with the reliability of NIR calibrations or the decisions made by rice growers who utilise the rice tissue testing service.

Conclusions

Adoption of NIR as the preferred analytical tool almost invariably leads to the desire to perform an increasingly wider range of analyses on each sample. This challenge is usually a desire to save time and costs per determination but may be made at the expense of analytical accuracy. This study suggest that the analysis of sulphur by NIR is inappropriate in rice shoot samples.

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