Leaf nitrogen determination using a portable near infrared spectrometer

A.B. Blakeney, G.D. Batten and L.A. Welsh
NSW Agriculture, Yanco Agircultural Institute, PMB, Yanco, NSW 2703 Australia.

Introduction

For the past nine years, research at Yanco Agricultural Institute has concentrated on using near infrared instruments to rapidly measure nitrogen and other constituents, such as non-structural carbohydrates, in crop plant tissues. The results from this research have been utilised to develop tissue testing services for farmers for prediction of the fertiliser requirements of crops.[1,2] Research to-date has concentrated on uniformly sampling crops, microwave drying the plant material and analysing the ground tissue. Although the tissue test is rapid and well accepted by Australian rice and wheat farmers, these tests usually take a minimum of two days as the sample must be transported to a central laboratory, ground and the results phoned or faxed back to the farmer (Figure 1). Recently, portable infrared instruments have become commercially available, raising the hope that some analysis could be directly done in the field.

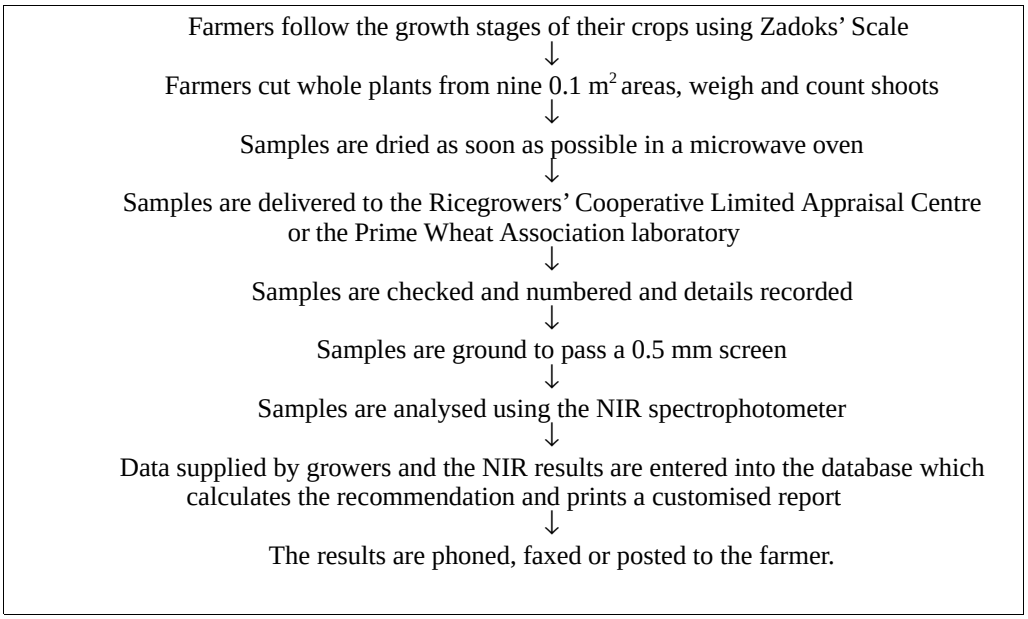

Figure 1. Flow diagram showing the operation of the NIR tissue test for cereal crops.

From Near Infrared Spectroscopy: The Future Waves

Materials

The instrument available for our experiments was a Zeltex 100F portable constituent analyser (Zeltex Inc., Maryland Parkway, Hagerstown, MD) fitted with an interactance probe. The probe is designed for direct contact with the sample surface. Light is conducted from the instrument down an fibre optic cable and emitted from a doughnut shaped area on the face of the probe. Some of the light that enters the sample is re-emitted and the centre of the probe face has another fibre optic cable that conducts this light back to the instrument. The instrument records readings at 14 wavelengths between 893 and 1045 nm, as well as measuring sample and analyser temperature. Data analysis is by Zeltex calibration and utility software. The latter facility includes an option that allows file conversion to ASCII and by renaming these files as .DAT instead of .ASI they can then be imported into Pirouette (Infometrix, Seattle, WA) for analysis.

Results and discussion

As we had no experience with interactance, or with the wavelengths used by this instrument, and also because it was not rice growing season, we decided to attempt calibration on dried ground samples of rice, wheat and grape petioles. Samples of ground tissues were placed in small plastic cups to a depth of 4 mm and the probe pressed directly into the sample. Care needed to be taken to maintain the probe directly perpendicular to the sample surface or anomalous readings occurred. The black rubber skirt provided by the manufacturer was used to minimise stray light.

Calibrations for nitrogen in rice, wheat and grape petiole tissue and starch in rice were developed using the partial least squares (PLS) options of Pirouette (Figure 2 and Figure 3). These are of lower accuracy than our current methods but would still be usable in a tissue testing service. However, fresh plant tissue initially proved more difficult. When samples are collected for our existing tissue tests, care is exercised to collect random samples. Rice farmers collect nine samples each 0.1 m^2 in area and these are subsampled to provide three samples for analysis. Each sample consists of 100 g fresh tissue that is then microwave dried and ground prior to analysis.[3,4] When fresh tissue is used only one or two leaves are sufficient to cover the end of the probe, so that averaging in this case is dependant on obtaining a number of readings from each crop sampled.

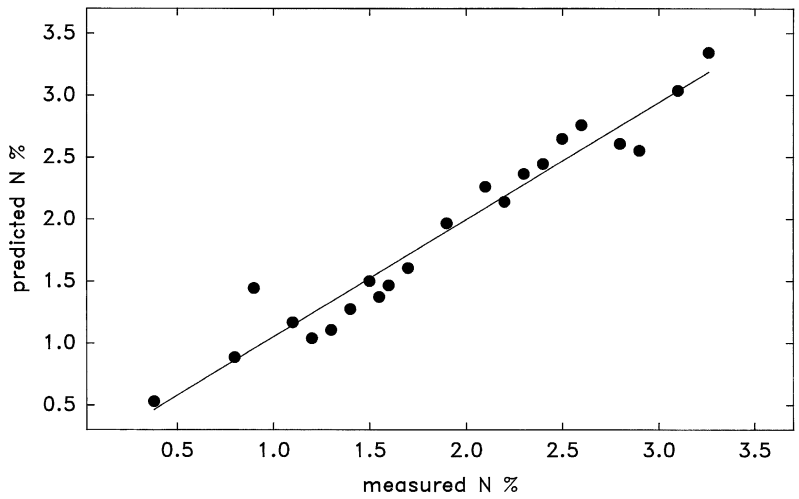

Figure 2. N in dried rice tissue (r = 0.97, *SEV* = 0.35, *SEC* = 0.22).

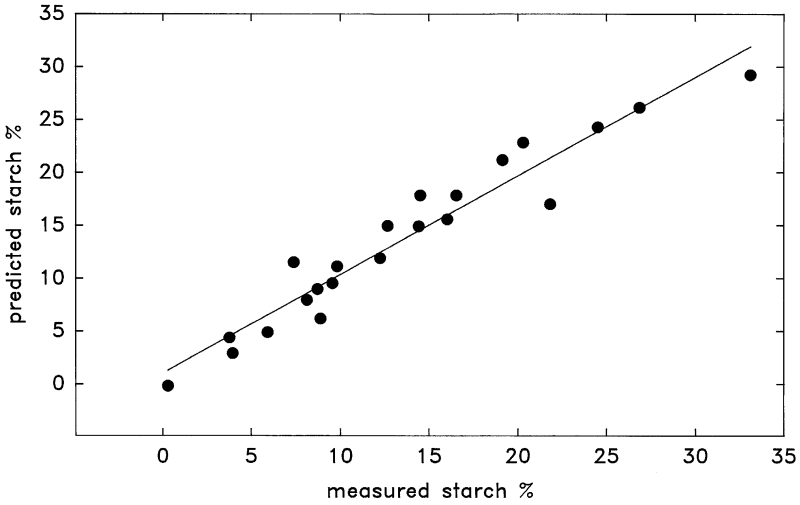

Figure 3. Starch in dried rice tissue (*r* = 0.97, *SEV* = 4.20, *SEC* = 2.54).

We have had similar difficulties in measuring crop colour and chloroplast content using a SPAD-502 chlorophyll meter.[5] In this case, leaf mid ribs and vertical differences contributed to difficulties in reproducible measurements. Initial tests using an analysis system similar to that used for dried ground samples showed that fresh tissue varied considerably in nitrogen content. We concluded that it would be necessary to select tissue at a particular morphological stage and to ensure there was a sufficient depth of tissue to achieve reproducibility.

Recent experiments on wheat have indicated that the sampling of the centre of mature single leaf blades from the central tiller is preferable to attempting to use whole shoots. Analysis is carried out in a small box made from black sealed foam rubber. Leaves are cut into 30 mm lengths and placed in the box, with leaf face up, to a depth of approximately 5 mm prior to analysis. We are hopeful these techniques will allow the collection of reliable data for calibration purposes.

Acknowledgments

We thank Ron Leaman of Graintec, Toowoomba, Queensland, for advice on the use and calibration of the instrument.

References

1. G.D. Batten and A.B. Blakeney, *NIR news*, **2(**4), 10 (1991).
2. G.D. Batten, A.B. Blakeney, V.B. McGrath and S. Ciavarella. “Non-structural Carbohydrate: Analysis by Near Infrared Reflectance Spectroscopy and its Importance as an Indicator of Plant Growth”, in *Plant Nutrition—from Genetic Engineering to Field Practice*, Ed by N.J. Barrow. Kluwer Academic Publishers, Dordrecht, Germany, pp. 269–72 (1993).
3. G.D. Batten, A.B. Blakeney and A.C. McCaffery, “Tissue Test of Rice Plant Nitrogen Using Near Infrared Reflectance”, in *Plant Nutrition—Physiology and Application*, Ed by M.L. van Beusichem . Kluwer Academic Publishers, Dordrecht, Germany, pp. 769–72 (1990).
4. A.B. Blakeney, G.D. Batten and S. Ciavarella, “An Interactive Database for Use with the Rice Tissue Test Service”, in *Temperate Rice—Achievements and Potential*, Volume 2, Ed by E.

Humphreys, E.A. Murray, W.S. Clampett and L.G. Lewin. Temperate Rice Conference Organising Committee, c/o NSW Agriculture, Griffith, pp. 477–484 (1994).
5. A.B. Blakeney, G.D. Batten and L.G. Lewin, "Comparison of Leaf Colour, Leaf Chlorophyll Content and Whole-plant Nitrogen Status in Temperate Rice Crops", in *Temperate Rice—Achievements and Potential*, Ed by E. Humphreys, E.A. Murray, W.S. Clampett and L.G. Lewin. NSW Agriculture, Griffith, pp. 469–472 (1994).