Rapid screening by near infrared spectroscopy for sugarcane smut resistance to improve breeding and selection outcomes

D.E. Purcell,[a,*] S. Kokot[b] and M.G. O'Shea[a]

[a]*BSES Limited, Indooroopilly, Queensland, 4068, Australia.*
E-mail: moshea@bses.org.au
[b]*School of Physical Science, Queensland University of Technology, Queensland, 4001, Australia*

Introduction

New and improved sugarcane varieties are the first line of defence against incursions of pests and diseases, and are largely responsible for maintaining high long-term yields of sugarcane per hectare. The overall process to produce a new sugarcane variety is time and labour intensive and requires around 10 years from the first production of new crosses as seedlings to the commercial release of a new variety. Because this process begins with very large numbers of plants, current disease resistance screening methods are not suitable to be applied until a late stage (year 7) of the breeding program. These traditional field trials for various diseases assess the performance of a new clone against that of a set of industry standard cultivars (ISC), which display reproducible tendencies with respect to a particular resistance parameter being screened.

In 2006, an incursion of sugarcane smut (*Ustilago scitaminea*), a fungal pathogen of sugarcane, was detected in Queensland for the first time. Plants infected with sugarcane smut can be severely stunted and stalks become extremely thin, thereby significantly decreasing yields. The severity of the symptoms depend upon the susceptibility of the variety to the disease and the environmental conditions present.[1,2]

Recently, we have described the use of near infrared (NIR) methods as a means of classifying the performance of sugarcane varieties,[3] and here we describe investigations of sugarcane smut resistance using NIR spectra collected from sugarcane bud scale tissue, and chemometrics data treatment methods. Specifically, this work sought a rapid method to rate sugarcane clones for resistance to smut without the need for infection or traditional field trials.

Figure 1. Obtaining an NIR spectrum of a sugarcane bud using a fibre optic probe assembly.

Materials and methods

A FOSS XDS NIR spectrometer fitted with a fibre optical probe accessory was used at the BSES Limited Southern Experiment Station (Bundaberg, Australia) for a blind validation trial in October 2008. Thirty-one blind samples were selected and NIR spectra of the bud scale tissue collected (Figure 1) from bud #15 (identified by counting down the stalk from the growing tip).

All spectra were subjected to N-point smoothing (NPS), converted to their 2nd derivative spectrum and applied as a standard normal variate (SNV) (Figure 2).

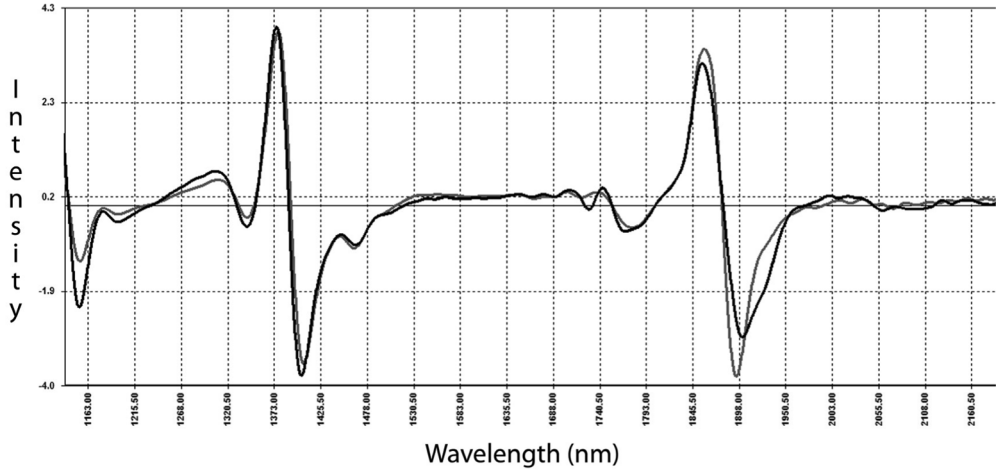

Figure 2. Overlay of NPS 2nd SNV spectra from Q117 (black trace) smut rating 9 (susceptible) and QN92-1234 (grey trace) smut rating 1 (resistant).

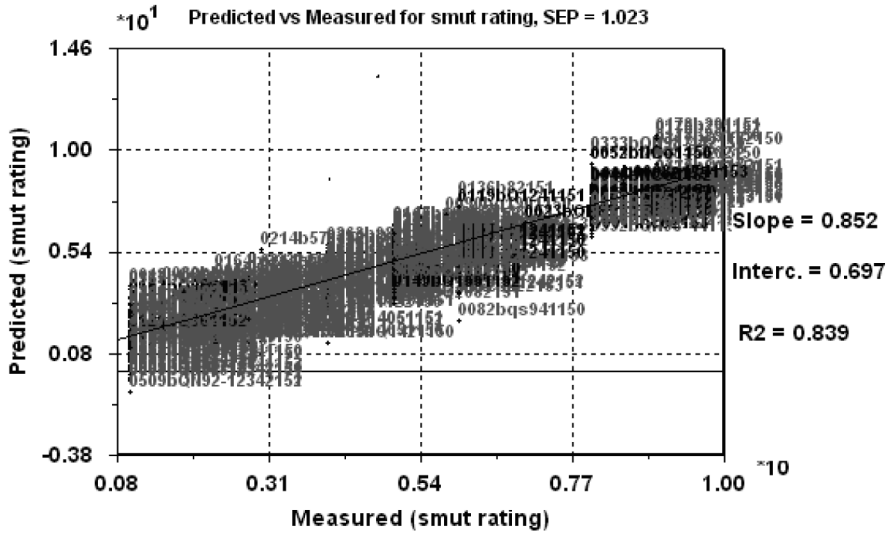

Figure 3. PLS regression of predicted versus measured smut ratings.

These data were imported into Sirius 6.5, (Pattern Recognition System (PRS), Bergen, Norway) and predicted upon using a previously developed partial least squares (PLS) regression model. The original calibration set was constructed using a set of 20 ISC to which additional clones were progressively added. These additional clones were initially treated as blinds and after the model successfully predicted their smut rating, they were added into the calibration set. The final regression model (Figure 3), used in this experiment to predict the thirty one blind samples, contained spectra collected from five different geographic regions and multiple harvesting seasons.

The data matrix consisted of 1424 objects (spectra) each containing 2002 wavelength variables (collected at 0.5 nm intervals between 1200-2200 nm) as well as the traditionally derived smut rating as the response variable. This model was constructed using 14 principal components (PC), with every 5th spectrum used for model validation. Other model properties are given in Figure 3.

Results and discussion

The outer bud scale tissue is well known as the point of entry of the fungal pathogen[2] and as such, bud scale morphology is likely to play a role in determining the resistance of a sugarcane variety to the disease. This work sought to characterise such differences between varieties via their NIR spectra. Previous reports have shown the effective infrared (IR) radiation penetration depth based on a sample of microcrystalline cellulose to be 2.5 mm according to the variable layer thickness method.[4] Assuming a similar penetration depth for the sugarcane sample, the IR beam would penetrate into the cell layer structure of the bud scale where ground tissue and vascular bundles

Table 1. Classification outcomes for the Bundaberg blind validation experiment. Correct classifications are shown in bold font.

	Resistant (1–3)	Intermediate (4–6)	Susceptible (> 7)	Total
Resistant	**4**	6	1	11
Intermediate	0	**4**	3	7
Susceptible	1	3	**9**	13
				31

are located. This infers that the NIR spectra obtained should be a composite of various structural features of the sugarcane bud scale tissue, and potentially be capable of varietal discrimination.

Previous blind experiments have showed that earlier prediction models (results not shown) were predicting considerably better at the susceptible end of the rating scale (smut rating >7.0). However, the model shown in Figure 3 was developed to better discriminate between resistant clones. Due to the complementary nature of these models, they have been used in tandem— if the final model generated a predicted rating above 5.5, the earlier model was used to determine whether that variety was likely to be susceptible or not.

The predicted outcomes of the blind validation trial are summarised in Table 1, where the results are shown on a classification basis. For example, for the 11 resistant clones examined, four were classified correctly as resistant, a further six were classified as having intermediate resistance, while only one was classified as susceptible. These results indicate that only one of the eleven resistant clones would have been rejected on the basis of NIR screening (i.e. possess a predicted smut rating above 7). Assuming these predictions hold across a larger sample set, this would indicate that only 9% of resistant clones would be rejected on the basis of NIR screening. Similarly for the seven intermediate clones, four were correctly classified, with the remaining three predicted to be susceptible. For the 13 susceptible clones, nine were correctly classified, three were predicted with intermediate resistance, while just one was predicted to be resistant. This is a very encouraging result with around 70% of susceptible varieties correctly identified.

Conclusion

Overall, these results provide considerable encouragement for the development of an early screening tool for sugarcane smut. The earlier that susceptible clones can be discarded from the selection program, the greater the efficiency of the overall program. Potentially, the breeding program can make similar gains in productivity with fewer resources if susceptible clones that would eventually be discarded anyway, can be eliminated earlier. Alternatively, the same number of clones can be tested with an even greater potential for productivity gains.

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References

1. B.J. Croft and K.S. Braithwaite, *Australasian Plant Pathology* **35,** 113 (2006).
2. P. Rott, R.A. Bailey, J.C. Comstock, B.J. Croft and A.S. Saumtally, *A Guide to sugarcane diseases*. CIRAD Publications Service, Brisbane, Australia (2000).
3. D.E. Purcell, M.G. O'Shea and S. Kokot, *Chemometr. Intell. Lab. Syst*. **87,** 139 (2007).
4. J.S. Shenk, J.J. Workman and M.O. Westerhaus, in *Handbook of Near-Infrared Spectroscopy*, Ed by D.A. Burns and E.W. Ciurczak. Marcel Dekker, New York, USA, p. 419 (2001).