INVESTIGATION OF SUGARCANE EPICUTICULAR WAX USING FT-RAMAN SPECTROSCOPY

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Abstract: This paper describes the use of a phenotypic property of sugarcane, its epicuticular wax, as a potential genetic maker and predictor of a desirable plant trait. Principal Component Analysis (PCA) was used to investigate FT-Raman spectra obtained from refined epicuticular stalk wax samples collected from six varieties of sugarcane. Clustering of varieties according to an arbitrary rating range of poorly performing, mid performing and well performing varieties was observed with respect to a sugar quality parameter.

In most agricultural industries, the development of new varieties is a prime means of delivering both productivity and quality advances. The continual development of new plant varieties takes considerable time and physical resources, particularly with respect to the selection process where large numbers of prospective varieties are rated against one another for traits which are considered important. In this regard, sugarcane is no different from any other crop. Thus, new varieties of sugarcane are constantly produced and their quality must be assessed against a series of appropriate parameters. Previous BSES research has established a number of varieties with reproducible tendencies across these parameters and these are referred to in this paper as industry standards varieties (ISV). These ISVs are used extensively in traditional field trials and for many traits, the sampled sugarcane is classified on an arbitrary rating scale from 1-9, with 1 showing the highest quality or resistance and 9 showing the least quality or susceptibility. These parameters include measures of sucrose content, fibre, yield and susceptibility to diseases and pests of economic importance.

Most of this conventional testing requires a reasonable supply of mature sugarcane, while some testing is not suited to large sample numbers and thus can be performed only in the later years of the breeding and selection cycle which typically takes around 10 years. As a direct consequence of the length of the plant breeding cycle, the cost of rejecting cultivars that may become new varieties is very high if it takes place late in the cycle. The development of tools to enable plant breeders to rate plants at earlier stages in the selection cycle will significantly improve the cost and overall efficiency of this program.

Sugarcane samples were sourced from the BSES Central Experiment Station (Mackay) from statistically designed experimental trials[1], with replicate samples taken from different row-plot areas. Wax sampling involved exposing the bands of epicuticular wax by carefully removing the leaves from cane stalks. The wax bands and wax covering the internodal areas from the first six consecutive evenly spaced nodes below the apical meristem were removed by scraping with a scalpel blade and were combined to produce one single sample. A wax refining procedure was developed and all refined wax samples were stored at -20ºC.

FT-Raman spectra were acquired by placing the refined wax directly in the sample cup for analysis. Spectral data as displayed in Figure 1 were collected over the wavelength range of 3800 – 200 Raman shift/cm^{-1}, using a neodymium-doped yttrium aluminium garnet (Nd:YAG) solid-state continuous wave laser operating at 1.064 μm. Data for chemometric analysis were in the form of

Raman Peak intensities obtained from the different varieties, covering the well performing, intermediate and poorly performing varieties. The spectra provided 250 variables and the raw data matrix was block normalised and y-mean centered prior to PCA. The PC1 versus PC2 score plot (Figure 2) accounts for 99% of data variance with sample replicates clustering well except for variety Q117. Varietal separation has occurred and provides some differentiation with respect to the quality rating of interest. Poorly performing varieties have clustered with slightly negative scores on PC1, while mid performing and well performing varieties have positive and negative scores respectively on PC1. These initial results provided the corroboration needed and justified for further development of spectroscopic methodologies to provide rapid tools of analysis, which may permit faster selection of prospective new sugarcane varieties.

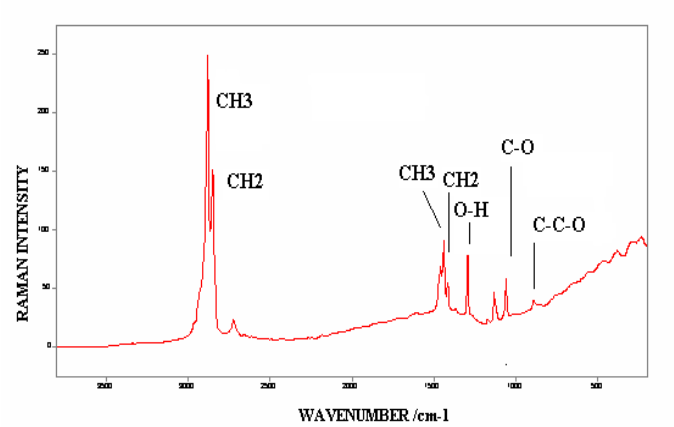

Figure 1. FT-Raman spectrum of refined stalk wax from variety Q117.

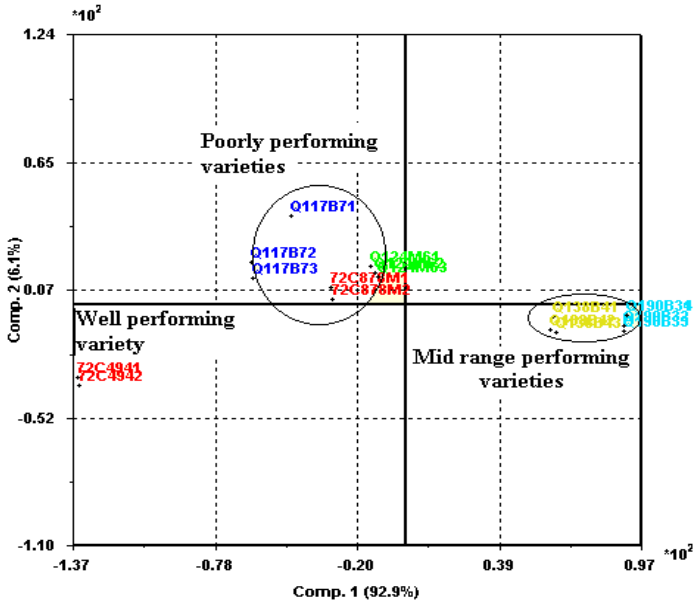

Figure 2. PC1 versus PC2 score plot of 17 FT-Raman spectroscopic analyses of refined stalk wax from 6 varieties.

References:

(1) D. M. Hogarth, in BSES Plant Breeding Manual, BSES Publications, 2002, p 32.