Chemical Assay Potentially Suitable for Determination of Smut Resistance of Sugarcane Cultivars

H. L. LLOYD and G. NAIDOO, Department of Microbiology, University of Durban-Westville, Durban, 4000, South Africa

ABSTRACT

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Of 23 genetically diverse sugarcane cultivars tested, 21 had a chemically related group of substances in bud scales in concentrations highly correlated with resistance to smut caused by *Ustilago scitaminea*. The glycosidic substances were extracted quantitatively from fresh, homogenized bud scales with methanol followed by methanol/water (8:2) and partially purified by thoroughly drying extracts in vacuo and extracting with methanol, followed by precipitation from the methanolic solution at −5 C with diethyl ether. Quantification was done using HPLC with a μ-Bondpak C18 column and methanol/water (9:1) solvent. The prospects seem good for developing a chemical assay for rapid determination of smut resistance in sugarcane.

Traditional methods for screening sugarcane for resistance to smut (*Ustilago scitaminea* Syd.) commonly take 6–18 mo to complete, occupy large areas of cane land, and must be repeated over a number of seasons to be reliable. Evaluations for smut resistance are thus commonly delayed until fewer selections remain at the latter stages of the breeding cycle. The breeding cycle may be as long as 15 yr, and many cultivars are then found to be susceptible. A simple, rapid screening method would permit evaluation of larger numbers of progeny during preliminary selection cycles of a breeding program, which would expedite development of smut-resistant cultivars.

James (6) suggested that a more rapid evaluation system could be based on the inhibition of teliospore germination on internode surfaces of sugarcane stalks that he found to be highly correlated with field resistance in the 40 cultivars he tested. Despite this finding concerning germination inhibitors associated with the internode (6), the nodal bud is the primary site of infection of sugarcane by smut (1,2,7–9). Germination inhibitors would thus be expected to be present and most effective in resistance in the bud region. This expectation prompted an investigation (8) to determine the quantity of germination inhibitor in the bud scales as a possible indicator of resistance to smut. A chemically related group of substances in bud scales of the four cultivars tested were in concentrations closely related to resistance (8). Using a simpler and faster extraction procedure, we have now assayed 23 cultivars with a wide range of resistance to smut and with different geographic origins and pedigrees. The quantitative relationship between resistance and the concentration of the uncharacterized glycosidic substance in the bud scale persists over genetically diverse sugarcane cultivars. A report of these findings may promote a wider evaluation of the efficacy of the assay system, which seems to have every prospect of developing into a rapid, efficient procedure for screening sugarcane for smut resistance.

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MATERIALS AND METHODS

The 23 sugarcane cultivars assayed included traditional and modern cultivars with diverse pedigrees from Barbados (B47/419), Cuba (J59/3), Louisiana (CP61/37), Hawaii (H50/7209), India (Co1001, Co301), Natal, South Africa (N52/219, N7, N8, N11, N12, N13, N14, N15, N16, NCo339, NCo310, NCo376, NCo334, N55/805, NCo293), and Queensland, Australia (Q84, Q90). There was a wide range of resistance to smut among the cultivars. Resistance ratings are based on long-term, replicated field trials conducted by the South African Sugar Association (SASA) in smut-prone areas. All materials for analysis were harvested from the SASA Experiment Station Varietal Nursery situated on the Natal Coast (lat. 29°43′40″ S long. 31°3′32″ E).

Stalks were cut at their bases, leaving leaf sheaths and leaves intact, and immediately enclosed in polyethylene packets to minimize injury and drying in transit. The five most uniform stalks were selected from each cultivar and divided into equal upper, middle, and lower stalk sections. Bud regions of the mid-stalk nodes were cleansed with water and disks were cut from the center of the outer bud scale and held at 1–2 C. Approximately 30 bud scale disks were collected, and 0.2-g (fresh weight) samples were used for each replicate extraction and assay. Ten to 15 min elapsed between the removal of bud scale disks and the start of the solvent extraction procedure.

Separate collections of material were made over a period of 14 mo for the three or four replicate extractions and assays carried out on each cultivar. While all material within each collection (replication) was uniformly aged and included two standard cultivars, N52/219 and NCo293, the age of sugarcane from collection to collection varied from 8 to 15 mo and was taken from first and second ratoon crops.

Bud scale disks (0.2 g) were homogenized for 2 min in 6 ml of methanol at 10,000 r/min^{-1} with an Ultra Turrax homogenizer (Junke & Kunkel KG, Breisgau). The sides of the vial were washed down with 2 ml of methanol and the homogenate stirred in the vial on a magnetic stirrer at 60 r/min^{-1} for 5 min. The stirred homogenate was filtered (90-mm Whatman GF/C) and the residue was washed sequentially with 8 ml of methanol and 8 ml of methanol/water (8:2). The filtrate and washings were rotary evaporated in vacuo to dryness at 40 C. The dried film was reextracted with 8 ml of methanol and centrifuged at 12,000 *g* for 5 min, and the pellet was washed with 2 ml of methanol.

To the supernatant (about 10 ml) was added and mixed 18 ml of diethyl ether, and the mixture, in a tightly capped tube, was held for 16 hr at − 5 C. The resulting precipitate was pelleted at 2,000 *g* for 10 min, then dried and stored for not longer than 2 days in a vacuum desiccator over silica gel at 21–23 C. For chromatographic analysis, the dried extracts were redissolved in 2 ml of distilled water, the only solvent in which the dried extract was readily soluble.

The concentration of the substance to be assayed was determined by quantitative high-performance liquid chromatographic (HPLC) analysis using a Waters HPLC fitted with a μ-Bondpak C18 column, a 10 × 2 mm (i.d.) phenyl/corasil guard column, and an ultraviolet detector set at 280 nm. A methanol/water (9:1) solvent, delivered at 1.5 ml/min^{-1}, resolved the structurally related substances as a single sharp peak and separated them from phenolic and flavonoid glycosides, which had longer retention times under the chromato-

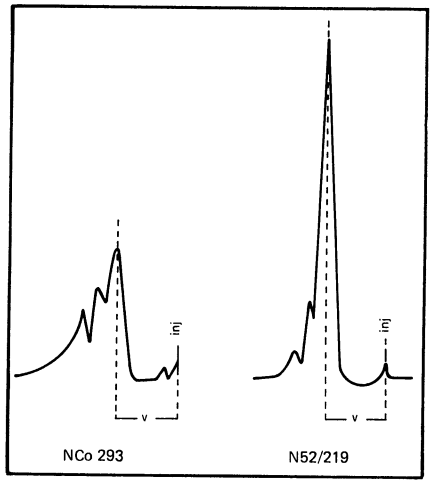

Fig. 1. Recordings of HPLC analyses of extracts from two standard cultivars, N52/219 and NCo293, which are, respectively, highly resistant (rating 1) and susceptible (rating 9). Peak area (by triangulation) was used as a measure of the concentration of substances eluted as a single peak with retention, v = 15 mm ± 1 mm, under the following chromatographic conditions: Waters μ-Bondpak C18 column, methanol/water (9:1) solvent, flow rate 1.5 ml/min^{-1}, recorder speed 600 mm/hr^{-1}, recorder sensitivity 20 mV fs and 280 nm UV detection.

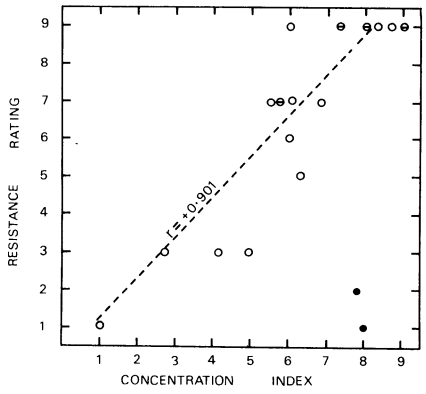

Fig. 2. Relationship between resistance ratings and concentration indices of the assayed substances of 23 sugarcane cultivars. The cultivars seem to fall into two groups. The major group of 21 cultivars (o), to which the regression line is fitted, includes those falling together on the same points on the diagram (ɵ). The two cultivars (•), Co1001 and J59/3, in the second group have an inverse relationship between resistance and the concentration index. The linear regression line of y (resistance rating) on x (concentration index) was estimated using mean values of x.

graphic conditions used (Fig. 1).

The concentration of the substance assayed was transformed, by negative direct proportion, on a numerical scale of 0–9, using two cultivars as standards. Concentrations in the two standards, NCo293 and N52/219, with field resistance ratings of 9 (susceptible) and 1 (highly resistant), respectively, were set at the lower and upper limits of the scale. The concentrations of assayed chemicals in other cultivars were transformed by interpolation relative to the two standard

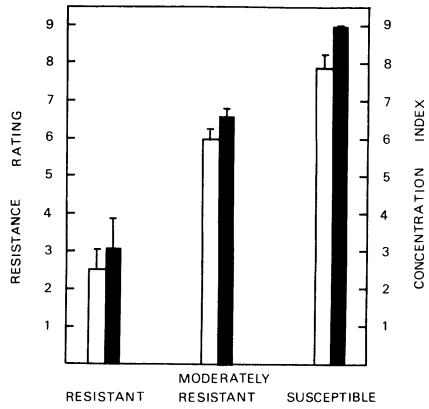

Fig. 3. The mean concentration index (black bars) and mean resistance ratings (white bars) of cultivars grouped as resistant (ratings 1, 2, and 3), moderately resistant (ratings 4, 5, and 6), and susceptible (ratings 7, 8, and 9). Vertical bar represents the standard error.

cultivars. The 0–9 scale is used internationally for rating resistance to smut and other sugarcane diseases (5).

RESULTS AND DISCUSSION

The points on the scatter diagram (Fig. 2) are dispersed into two groups. Sugarcane cultivars falling into group one, to which the regression line is fitted, contain the assayed substances in bud scales at concentrations highly correlated ($r = +0.901$) with resistance to smut. The second group contains two cultivars highly resistant to smut but without a commensurately high concentration of the quantified substance in the bud scale. One, Co1001, is the same cultivar in which inhibition of teliospore germination on internodes was not correlated with resistance (6).

The nature of resistance of sugarcane to smut is unknown (4) and the role in resistance to smut of the assayed chemicals extracted from bud scale has not yet been ascertained. To gain access to the inner tissues of the bud (1,8,9), however, *U. scitaminea* germinates on, then penetrates (8) or grows underneath (1), the bud scale. At some time during the initial infection process, teliospore promycelia are in the vicinity of the bud scale and thus are likely to be influenced by bud scale diffusates. The partly purified substances assayed in our studies inhibit teliospore germination and are present in bud scale diffusates (8). Diffusates in crude form from the bud scales of resistant cultivars are known to inhibit teliospore germination (3). The extracted chemicals used in this assay may thus play a direct role in resistance by reducing teliospore germination, thereby diminishing the probability of infection of nodal buds. As a corollary, the two populations represented in Figure 2 would have different forms of resistance. Group two cultivars would have a form of resistance to smut in which the preformed (assayed) substances in the bud scale play little or no part.

The relationship between the concentration of the quantified substances in the bud scale and resistance of cultivars in group one is shown in Figure 3, in which histograms of the mean chemical concentration indices and resistance indices for resistant, moderately resistant, and susceptible cultivar groups are plotted. It is common practice in early screening of breeding material to broadly categorize progeny into susceptible (resistance ratings 7, 8, and 9), moderately resistant (ratings 4, 5, and 6), and resistant (ratings 1, 2, and 3). The chemical assay being developed seems particularly appropriate for screening and broadly categorizing breeding material for resistance to smut during early selection cycles.

Two features of the assay detract from its usefulness. First, the quantitative extraction procedure is somewhat cumbersome and involves several purification steps, which can lead to errors in quantification. While at present it is practical to minimize the effect of analytical errors by replication, the most attractive alternative is to simplify the extraction and quantification procedure. Determining the chemical nature of the assayable substance(s) and empirical methods are being pursued with a view to simplifying the assay procedure. Second, any forms of resistance with which the concentration of substance in the bud scale is not correlated will be overlooked and discarded if preliminary screening is based on this chemical assay procedure alone. These forms of resistance seem to be relatively rare in modern sugarcane cultivars and breeding lines, however. In any event, breeders will have to weigh the advantages of rapid genetic gains that result from large-scale, early screening with the disadvantage of narrowing the gene base by discarding material with useful alternative forms of resistance to smut.

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