THE USE OF TISSUE BLOTS TO SCREEN FOR VARIETAL RESISTANCE TO RATOON STUNTING DISEASE OF SUGARCANE

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No trials are currently conducted at SASEX to screen genotypes for resistance to ratoon stunting disease (RSD), a disease that is well established in most sugarcane growing regions of the world and considered to cause more yield losses worldwide than any other sugarcane disease. In South Africa, field trials have demonstrated that yield reductions under rainfed conditions can be as high as 40% in highly susceptible varieties (Bailey and Bechet, 1986). When cane is grown under irrigation, yield losses are less noticeable but still significant in many varieties (Bailey and Bechet, 1995). It is estimated that RSD currently results in a 1% reduction in industrial production in South Africa and between 10 and 20% in other African countries where South African varieties are grown (Bailey and McFarlane, 1999).

An integrated control programme could effectively reduce RSD incidence in crops to a low level. Despite this, the disease remains prevalent in a number of countries including South Africa where such a control programme has been in existence for many years. The use of resistant genotypes may offer an alternative strategy for minimising losses from RSD.

It was only recently that attempts were made to screen for varietal resistance to RSD on a large scale in Canal Point, Florida (Davis *et al,* 1994) although differences in varietal reactions to RSD have been recognised since 1950 (Steindl, 1950). This is mainly due to the fact that selection for disease resistance usually relies on the visual assessment of disease symptoms and RSD has no reliable external symptoms.

Yield response trials have shown that the released varieties grown in the South African sugar industry are tolerant, susceptible or highly susceptible to RSD; none have been shown to be resistant (Bailey and Bechet, 1986; 1995). Trials investigating varietal differences in the rate of spread of RSD on cane knives (Bailey and Tough, 1992) and levels of infection (McFarlane and Bailey, unpublished) have also been used to assign RSD resistance ratings. It is only possible to test a limited number of varieties each year using these methods since they require large areas of land and are time-consuming.

Harrison and Davis (1988) introduced the tissue blot-enzyme immunoassay (TB-EIA) to detect and enumerate vascular bundles colonized by *Leifsonia xyli* subsp *xyli,* the causal organism of RSD. They showed that the TB-EIA could be used to measure both the incidence and severity of RSD, two parameters commonly regarded as being necessary to breed for disease resistance in sugarcane. They found that the method correlated well with yield reductions caused by RSD. Davis *et al* (1994) made some modifications to the equipment used for the TB-EIA that enabled the screening of large numbers of genotypes for RSD resistance.

This poster presents data from preliminary experiments that show that the TB-EIA can be used to screen for RSD resistance in South African varieties.

Materials and Methods

The effect of variety and internode position on per cent colonized vascular bundles (CVB)

The lowest four internodes were cut from each of four stalks of varieties N12, N14, N17, N22, N23 and NCo376. The TB-EIA was conducted according to the procedures outlined by Davis *et al.* (1994). Briefly, longitudinal cores were removed from each internode. A 10 mm cross section was excised and placed on the surface of a nitrocellulose membrane within the wells of a filter-holding apparatus. Two sets of apparatus were centrifuged at 3 000 rpm for 10 minutes. The membranes were removed from the apparatus and dried at 80ºC for 1 hour.

A modified indirect ELISA procedure was used to label *L. xyli* subsp *xyli* cells deposited onto the membranes and the cells were stained with Fast Blue BB as described by Davis *et al* (1994). The membranes were air dried before examining the impressions of the tissue sections using a stereomicroscope at 8x magnification. All the vascular bundles in the tissue section made impressions on the membrane during centrifugation. Those colonized by *L. xyli* subsp *xyli* were visible as discrete areas of blue stain on the membranes and were counted and marked to determine the percentage CVB in each tissue section.

Within stool variability

All stalks were cut from stools of N14, N16, N17 and NCo376 to ascertain the variability within stools. The TB-EIA was performed on the lowest internode.

Results

The effect of variety and internode position on per cent CVB

The proportion of CVB of most varieties was significantly different (P=<0,05) with N17 having the highest per cent CVB, indicating that this variety was the most susceptible of those tested. There was no significant difference between N12 and N23, two varieties exhibiting a low per cent CVB and recognised to be more tolerant to RSD.

There was a difference in the per cent CVB from the first to the fourth internode, with the highest per cent CVB recorded in the third internode in most varieties. These differences were, however, not significant.

Within stool variability

The most mature stalks of each variety were consistently infected with RSD. The younger stalks of N16, the most resistant of the four varieties tested, were not infected with RSD and the range in per cent CVB amongst stalks was highest in this variety. The variation amongst the stalks taken from the more susceptible varieties was acceptable.

Discussion

The data indicate that the TB-EIA could be used to differentiate between the RSD tolerant and susceptible varieties tested, and therefore could be used to identify resistant genotypes. The ranking achieved by comparing the per cent CVB of the varieties corresponded, in most instances, to that obtained from yield trials conducted by Bailey and Bechet (1995), with N17 and N14 being the most susceptible and N12 and N23 being tolerant. N22 was ranked as the most resistant variety in the yield trial but was more susceptible than N23 according to the TB-EIA results. Such discrepancies will be investigated further in a combined trial using the TB-EIA and yield data to assess the reactions of varieties to RSD.

It is clear from these results that precise sampling procedures are critical to the success of this technique. In future trials, the two oldest stalks will be taken from each stool to be sampled and only the third internode will be tested. In this way, variability within a variety should be kept to a minimum.

If routine trials were to be introduced to screen genotypes for RSD resistance at SASEX, it would not necessarily result in genotypes being rejected solely on their RSD rating, but the ratings could be considered along with performance and susceptibility to other diseases. Resistant varieties could be retained despite other poor characteristics and used for breeding purposes. The data obtained from screening trials would provide valuable information for growers on the susceptibility of released varieties and thus enable them to take special precautions with those that are susceptible.

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