RESISTANCE TO SUGARCANE YELLOW LEAF VIRUS IN COLOMBIA

By

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KEYWORDS: Disease Control, Breeding.

Abstract

SUGARCANE yellow leaf virus (SCYLV) causes disease in different commercial sugarcane varieties in Colombia. Therefore, the study of resistance as an effective control method is of utmost priority. Twenty-nine commercial varieties (Saccharum hybrids) and eight clones of Saccharum officinarum, S. spontaneum, S. sinense, S. barberi, and Erianthus sp. were evaluated through inoculation with SCYLV, using Melanaphis sacchari as the aphid vector. Levels of infection were determined monthly through the TBIA serological test. Later the progeny of crosses between susceptible and resistant varieties were examined using artificial inoculation and, finally, the behaviour of different naturally infected varieties was examined and compared with artificial inoculation. The results indicated that infection by SCYLV fluctuated from 0–100%. The varieties CC 86-29, CC 84-56, CC 82-15, CC 84-75, PR 61-632, CC 85-68, IN 84-103, CC 83-25, CC 87-473 and CCSP 89-43 had more than 50% infected plants. CC 91-1999, 18-95, CC 91-1880, CC 87-505, V 71-51, CC 86-33, CC 87-251, MZC 82-11, SP 71-6163 and CC 85-92 had levels of infection ranging from 10–50%. Varieties with less than 10% infection were MZC 74-275, CC 91-1555, CC 93-4223, CC 87-434, RD 75-11, CC 89-2000 and Desi Paunda. A group of ten varieties and cultivars showed no infection at all. These results were closely correlated with those of the naturally infected varieties ($R^2=0.7$). A cross between a susceptible female parent (CC 84-75) and resistant male parent (RD 75-11) produced mostly resistant progeny.

Introduction

Yellow leaf disease (previously called yellow leaf syndrome or YLS) has been reported to affect commercial sugarcane fields in different parts of the world (Ricaud, 1968; Lockhart et al., 1996). In Colombia, this disease was first found in 1998 on the variety SP 71-6163, introduced from Brazil in 1987. In Colombia, Victoria et al. (2000) found a high yield decrease on the susceptible variety CC 84-75, about 1.2 t cane/ha and 0.21 TSH for each percentage unit of infected stalks. Varieties CC 85-96, CC 87-434, V 71-51, MZC 74-275, CC 87-505 and MZC 82-11, in that order, had a smaller yield effect. The disease incidence in commercial lots of the Cauca Valley was 4.6% and 3.2% for 2002 and 2001, respectively (CENICAGA, 2002).

Additionally, a significant group of varieties (e.g. CC 85-63, CC 87-409, CC 87-474, CC 89-2000, Co 421, CC 85-92 and RD 75-11) was found free of the disease on a commercial scale (Cuervo et al., 2000). A characteristic symptom of the disease is an intense yellowing of the midrib of the leaf, which extends from the base to the distal part of the leaf (Lockhart et al., 1996). The disease is caused by Sugarcane yellow leaf virus (SCYLV) (Scagliusi and Lockhart, 2000). This virus is an unassigned member of the Luteoviridae family (Moonan and Mirkov, 2000; Smith et al., 2001).

Other gramineae, such as wheat (Triticum sativum), oat (Avena sativa) and barley (Hordeum vulgare) were found as host plants of SCYLV when inoculated with the virus by aphids (Schenck and Lehrer, 2000). SCYLV is transmitted by Melanaphis sacchari and Rhopalosiphum maidis, but its transmission has not yet been reported using mechanical means (Scagliusi and Lockhart, 2000; Avellaneda et al., 2001).

Different techniques can be used for diagnosis of the disease. The most widely known are ELISA (enzyme-linked immunosorbent assay), TBIA (tissue-blot immunoassay), DBIA (dot-blot immunoassay),
RT-PCR (transcription reverse polymerase chain reaction) and purification of the causal agent (Schenck et al., 1997; Scagliusi and Lockhart, 2000; Guzman and Victoria, 2001; Moonan and Mirkov, 2002).

Regarding plant resistance as a control for SCYLV, Comstock et al. (1999) in Canal Point (FL, USA) found resistance to SCYLV in seven varieties (CP 57-603, CP 89-1509, CP 92-1684, CP 57-614, CP 92-624, HoCP 93-741 and TCP 91-3543) using RT-PCR, ELISA and TBJA. They also found a high level of susceptibility in most of the commercial varieties used by the industry in Florida, Louisiana and Texas. Additionally, they determined that the incidence of SCYLV varied according to the parental clones. Most of the clones of *Saccharum spontaneum* and commercial varieties CL 61-620 and CP 85-1491 offered better resistance than most of the commercial varieties in Florida. Schenck and Lehrer (2000) found that the virus in Hawaii did not infect varieties H 78-3567, H 78-4153 and H 78-7750.

The objectives of this study were twofold. First, we aimed to determine the resistance of different sugarcane varieties to SCYLV in Colombia and, second, to study the inheritance of resistance of the progeny of a cross between a resistant and a susceptible variety.

**Materials and methods**

**Localisation and weather conditions**

This study was conducted at the Cenicafé Experiment Station in the Cauca Valley, Colombia. The station is located at 3°21' N, 76°18' W; altitude 1024 m and weather conditions are an average of 1160 mm of rainfall/year; 23.6°C temperature and 77% relative humidity.

**Artificial inoculation of SCYLV**

**Disease evaluation**

The assessment of resistance was performed with 37 genotypes. Of those, 27 were commercial varieties (*Saccharum* hybrids), eight cultivars of *S. officinarum*, *S. spontaneum*, *S. sinense*, *S. barberi*, and *Erianthus* sp. in addition to the susceptible SP 71-6163 and the resistant CC 85-92. For the study, around 20 healthy plants of each genotype were used. Single eyes were cut from stalks and certified SCYLV-free by TBJA (Guzmán and Victoria, 2001). The single-eye setts were grown in plastic pots containing a mixture of soil and sand. The plants were supplemented by fertilisation and irrigation according to their requirements.

**Source of inoculum and transmission of SCYLV**

Colonies of *M. sacchari* were fed on infected plants of SP 71-6163 for 48 to 96 hours to acquire the virus and then transferred to test plants for ten days as indicated by Scagliusi and Lockhart (2000) and Avellaneda et al. (2001). Groups of 20 aphids each were then transferred to each of the 20 healthy plants of every variety, using a camel-hair brush to locate them on leaves + 1 and + 2 (van Dillewijn, 1951). The aphids were then eradicated by applying a solution of 2mL/L of dimethoate insecticide. The inoculated plants were kept under glasshouse conditions for eight months (Scagliusi and Lockhart, 2000; Avellaneda et al., 2001).

**Verification of virus transmission**

Inoculated plants were assayed for presence of SCYLV every month until they reached seven months of age. Assessment of resistance/susceptibility was based on the percentage of infected plants: 0–10% infected plants = resistant genotype; 50–100% infected plants = susceptible genotype. The presence or absence of the virus was diagnosed by TBJA; and, in some cases, it was also verified by RT-PCR and EIM (Schenck et al., 1997; Guzmán and Victoria, 2001).

**Incidence of SCYLV in commercial varieties**

Since 1998, the disease diagnosis service at CENICAÑA assessed the natural infection of SCYLV in the main varieties planted in Colombia. Both commercial and experimental varieties used by the sugar industry were tested. The results were compared with those obtained under glasshouse conditions to determine the behaviour of the different varieties to SCYLV infection.

Additionally, incidence of SCYLV was determined in plant cane and first ratoon crops of 64 different varieties in nursery plots that were surrounded by infected plants. Presence of the SCYLV was determined by TBJA (Guzmán and Victoria, 2001).

**Segregation of resistance**

A cross between the susceptible variety CC 84-75 used as the female parent and the resistant variety RD 75-11 used as the male parent was made to examine the progeny for their resistance to SCYLV.

The resulting seeds were sown in plastic trays; seedlings were transplanted individually to small plastic pots and then grown in the field. Eight months later, stalks were taken from each of the 148 stools.
sampled, and their buds were excised and grown individually in small plastic pots. Five plants per clone were used for the evaluation of the progeny.

Table 1—Percentage of plants infected by SCYLV in 37 sugarcane cultivars and other Saccharum or related species, after inoculation with the virus via the aphid Melanaphis sacchari.

<table>
<thead>
<tr>
<th>Cultivar or genotype</th>
<th>Origin</th>
<th>Number of inoculated plants</th>
<th>Percentage of infectiona at 4 mths</th>
<th>Percentage of infectiona at 5 mths</th>
<th>Percentage of infectiona at 6 mths</th>
<th>Percentage of infectiona at 7 mths</th>
<th>Infection levelb</th>
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<td>CC 84-56</td>
<td>Hybrid</td>
<td>19</td>
<td>63</td>
<td>84</td>
<td>95</td>
<td>84</td>
<td>HI</td>
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<tr>
<td>IN 84-103</td>
<td>S. officinarum</td>
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<td>56</td>
<td>61</td>
<td>72</td>
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<td>80</td>
<td>70</td>
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<td>6</td>
<td>24</td>
<td>94</td>
<td>65</td>
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<td>59</td>
<td>65</td>
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<td>54</td>
<td>15</td>
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<td>63</td>
<td>37</td>
<td>53</td>
<td>HI</td>
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<td>V 71-51</td>
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<td>15</td>
<td>23</td>
<td>15</td>
<td>15</td>
<td>II</td>
</tr>
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<td>CC 91-1999</td>
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<td>22</td>
<td>44</td>
<td>33</td>
<td>II</td>
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<td>36</td>
<td>14</td>
<td>36</td>
<td>29</td>
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<td>Erianthus sp.</td>
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<td>0</td>
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<td>0</td>
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<td>II</td>
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<td>CC 85-92</td>
<td>Hybrid</td>
<td>36</td>
<td>3</td>
<td>4</td>
<td>6</td>
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<tr>
<td>CC 93-4223</td>
<td>Hybrid</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
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<td>0</td>
<td>6</td>
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<td>LI</td>
</tr>
<tr>
<td>Desi Paunda</td>
<td>S. sinense</td>
<td>19</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>0</td>
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<td>MZC 74-275</td>
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<td>8</td>
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<tr>
<td>RD 75-11</td>
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<td>18</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>6</td>
<td>LI</td>
</tr>
<tr>
<td>CC 87-434</td>
<td>Hybrid</td>
<td>17</td>
<td>0</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>LI</td>
</tr>
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<td>CC 92-2358</td>
<td>Hybrid</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>CC 85-63</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NI</td>
</tr>
<tr>
<td>CC 87-474</td>
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<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NI</td>
</tr>
<tr>
<td>CC 90-1160</td>
<td>Hybrid</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>NI</td>
</tr>
<tr>
<td>CP 57-603</td>
<td>Hybrid</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NI</td>
</tr>
<tr>
<td>19-95</td>
<td>S. spontaneum</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>Keong-Java</td>
<td>S. officinarum</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NI</td>
</tr>
<tr>
<td>Mid Land</td>
<td>S. sinense</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>NI</td>
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<tr>
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<td>S. barberi</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>NI</td>
</tr>
<tr>
<td>Paunra</td>
<td>S. barberi</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NI</td>
</tr>
</tbody>
</table>

a Presence of SCYLV was determined by tissue-blot immunoassay (TBIA).

b HI = High infection, II = Intermediate infection, LI = Low infection, and NI = No infection.
Transmission of SCYLV to the progeny

Virus transmission to the progeny was performed in the greenhouse as described above, except that the aphid colony was established on susceptible parent CC 84-75 infected with SCYLV. Each clone of the 148 clones was exposed to infection by the virus-bearing aphids for ten days. Aphids were then removed manually and the plants sprayed with dimethoate insecticide. Evaluation of SCYLV in the progeny. Infection by SCYLV was determined by TBIA every month as previously described. The clones were classified into three groups depending on the level of disease incidence: susceptible (S), when three or more plants out of five were infected by SCYLV; intermediate (I), when one or two plants out of five were infected by SCYLV; and resistant (R), when none of the plants was infected by SCYLV.

Results and discussion

Resistance evaluated by artificial inoculation of plants with SCYLV

We aimed for 20 plants per genotype for disease resistance assessment. However, due to some germination and growth problems, not all of the varieties had a full set (Table 1). Varieties were grouped according to the level of infection: null (no infection detected), low (less than 10% of plants infected), intermediate (between 10% and 50% of plants infected) and high (more than 50% of plants infected). According to the glasshouse assessment, commercial varieties MZC 74-275, RD 75-11, CC 89-2000, CC 91-1555, CC 93-4223, CC 87-434, CC 92-2358, CC 85-63, CC 87-474, CC 90-1160, and CP 57-603 had the greatest resistance to SCYLV. Genotypes 19-95 (S. spontaneum), Desi Paunda (S. sinense), Keong Java (S. officinarum), Mid Land (S. sinense), Patarki Mango (S. barberi) and Paunra (S. barberi) also showed high levels of resistance. Genotypes IN 84-103 (S. officinarum) and 18-95 (Erianthus sp.) were susceptible. Finally, CC 85-92 showed 11.1% infection, and this result is in agreement with those obtained for field tests where this cultivar was resistant.

Natural infection of commercial varieties by SCYLV

A close correlation was observed when comparing natural plant infections by SCYLV with results obtained in the glasshouse. Varieties with the highest infection in the field (e.g., CC 84-75, CC 85-68, CC 86-29, and CC 87-505) were also susceptible in the glasshouse. Varieties CC 85-92, MZC 74-275, RD 75-11, CC 93-4223 and CC 87-434 showed low levels of infection both in the glasshouse and the field (Table 2).

**Table 2**—Correlation between SCYLV infections in inoculated and in field infected varieties.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Percentage of infection* in the Glasshouse</th>
<th>Field</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC 86-29</td>
<td>100</td>
<td>100</td>
</tr>
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<td>CC 84-66</td>
<td>95</td>
<td>90</td>
</tr>
<tr>
<td>CC 84-75</td>
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<td>PR 61-632</td>
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<td>50</td>
<td>10</td>
</tr>
<tr>
<td>CC 91-1999</td>
<td>44</td>
<td>15</td>
</tr>
<tr>
<td>CC 91-1880</td>
<td>36</td>
<td>25</td>
</tr>
<tr>
<td>CC 87-505</td>
<td>32</td>
<td>100</td>
</tr>
<tr>
<td>V 71-51</td>
<td>23</td>
<td>11</td>
</tr>
<tr>
<td>MZC 82-11</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>CC 85-92</td>
<td>11</td>
<td>1</td>
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<td>MZC 74-275</td>
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<td>2</td>
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<tr>
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<td>0</td>
</tr>
<tr>
<td>CP 57-603</td>
<td>0</td>
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</tr>
</tbody>
</table>

*Highest infection level obtained/observed.
Correlation between these two sources of data was 0.83 ($R^2=0.7$). These results showed that the methodology used to determine resistance to SCYLV in the glasshouse is an efficient tool for estimating resistance to the disease.

Additionally, incidence of SCYLV was determined in plant cane and first ratoon crops of 64 different varieties in nursery plots that were surrounded by infected plants. The virus was not found in 28 varieties whatever the crop cycle. Varieties CC 93-7510, CC 93-7513, CC 93-3801, CC 93-3803, CC 93-3826; CC 93-3895, CC 92-2154, and CC 92-2198, which are outstanding for their health, also showed good commercial results. A group of 17 varieties was not infected by SCYLV in plant cane but was infected in first ratoon crop.

Two varieties were infected only in plant cane while the virus was found in 17 varieties in both plant and first ratoon crops. Incidence was higher in some varieties in first ratoon crop than in plant cane. When analysing the progenitors of varieties, we noticed that, if MZC 74-275 or CP 57-603 (considered resistant to SCYLV) was the male parent of the varieties, the progeny had no virus or only a low level of infection. In contrast, incidence of SCYLV was high when the female parent was Mex 64-1487 (considered highly susceptible to SCYLV) and the male parent different from MZC 74-275.

### Table 3—Natural infection by SCYLV of nurseries surrounded by infected varieties.

<table>
<thead>
<tr>
<th>Number of varieties</th>
<th>Female parent</th>
<th>Male parent</th>
<th>% SCYLV-infected varieties in Plant cane</th>
<th>Ratoon crop</th>
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<tbody>
<tr>
<td>10</td>
<td>Mex 64-1487</td>
<td>Any</td>
<td>35</td>
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<tr>
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<td>Any</td>
<td>Mex 64-1487</td>
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<td>20</td>
</tr>
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<td>5</td>
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<td>50</td>
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<td>Any</td>
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<td>16</td>
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</tbody>
</table>

*Different from MZC 74-275 and CP 57-603.

### Segregation of resistance to SCYLV

The number of clones infected by SCYLV in the progeny only slightly increased between 3 and 8 months of growth in the field (Table 4). At the third evaluation (8 months of growth), 3–5 plants were infected for 13 clones, 1–2 plants were infected for 32 clones while 103 clones were apparently free of SCYLV (Table 4).

### Table 4—Number of clones infected by SCYLV in the progeny of a cross between a susceptible and a resistant sugarcane variety (virus infection was determined by tissue blot immunoassay-TBIA).

<table>
<thead>
<tr>
<th>Months after inoculation</th>
<th>Number of clones showing*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High infection$^b$</td>
</tr>
<tr>
<td></td>
<td>(3 to 5 plants)</td>
</tr>
<tr>
<td>3</td>
<td>8 (5.3%)</td>
</tr>
<tr>
<td>5</td>
<td>8 (5.4%)</td>
</tr>
<tr>
<td>8</td>
<td>13 (8.7%)</td>
</tr>
</tbody>
</table>

*High infection = 3-5 plants infected by SCYLV, medium infection = 1–2 plants infected by SCYLV, no infection = SCYLV not detected in any of the 5 plants.

A more detailed analysis of data showed that clones with 1–2 infected plants at the first evaluation were highly infected at the last evaluation. Clones with medium and high infection levels were therefore considered susceptible.

The cross between CC 84-75 and RD 75-11 produced a high frequency of putative virus-free plants, suggesting high heritability of resistance to yellow leaf caused by SCYLV. Additional evaluations with a higher number of cross combinations are underway in order to test this hypothesis.
REFERENCES


RÉSISTANCE AU SUGARCANE YELLOW LEAF VIRUS EN COLOMBIE

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MOTS CLÉS: Lutte Contre les Maladies, Amélioration Variétale.

Résumé
PLUSIEURS variétés de canne à sucre sont infectées par Sugarcane yellow leaf virus (SCYLV) en Colombie. Par conséquent, l'étude sur la résistance des variétés comme méthode de lutte est une priorité. Vingt-neuf variétés commerciales (hybrides de Saccharum officinarum, S. spontaneum, S. sinense, S. barberi et Erianthus sp) furent évalués après inoculation par le vecteur Melanaphis sacchari. Le niveau d'infection a été déterminé mensuellement en utilisant la technique d'immuno-empreinte (IE). Les progénitures issues de croisements entre les variétés sensibles et les variétés résistantes ont été examinées après une inoculation artificielle et leurs réactions comparées à celles des variétés infectées naturellement. L'infection par le SCYLV variait de 0–100%. Plus de 50% de plantes des variétés suivantes étaient infectées: CC 86-29, CC 84-56, CC 82-15, CC 84-75, PR 61-632, CC 85-68, IN 84-103, CC 83-25, CC 87-473 et CCSP 89-43. Les variétés CC 91-1999, 18-95, CC 91-1880, CC 87-505, V 71-51, CC 86-33, CC 87-251, MZC 82-11, SP 71-6163 et CC 85-92 ont montré un taux d'infection de 10-50%; alors que moins de 10% d'infection était relevée chez les suivantes- MZC 74-275, CC 91-1555, CC 93-4223, CC 87-434, RD 75-11, CC 89-2000 et Desi Paunda. Par contre, aucune infection n'a été observée dans dix variétés. Ces résultats étaient fortement corrélés à ceux obtenus avec les variétés infectées naturellement (R²=0.7). Un croisement entre un parent femelle sensible (CC 84-75) et un parent mâle résistant (RD 75-11) a produit une majorité de variétés résistantes.

RESISTENCIA AL VIRUS DE LA HOJA AMARILLA DE LA CAÑA DE AZÚCAR EN COLOMBIA

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PALABRAS CLAVES: Control de Enfermedades, Mejoramiento.

Rusumen
El virus de la hoja amarilla (SCYLV) afecta diferentes variedades comerciales en Colombia, por tanto se estudió la resistencia como método efectivo de control de la enfermedad. Veintinueve variedades comerciales (híbridos de Saccharum spp.) y ocho clones de Saccharum officinarum, S. spontaneum, S. sinense, S. barberi, y Erianthus sp. fueron evaluados mediante inoculaciones del SCYLV empleando Melanaphis sacchari como vector. La infección se determinó mensualmente usando la técnica de TBIA. Los resultados fueron correlacionados con el comportamiento de variedades infectadas naturalmente. Posteriormente la progenie de cruzamientos entre variedades susceptibles y resistentes fue evaluada mediante inoculaciones artificiales. Los resultados mostraron que la infección de SCYLV fluctuó entre 0–100%. Las variedades CC 86-29, CC 84-56, CC 82-15, CC 84-75, PR 61-632, CC 85-68, IN 84-103, CC 83-25, CC 87-473 y CCSP 89-43 tuvieron más del 50% de plantas afectadas. CC 91-1999, 18-95, CC 91-1880, CC 87-505, V 71-51, CC 86-33, CC 87-251, MZC 82-11, SP 71-6163 y CC 85-92 mostraron niveles de infección de 10–50%. Las variedades con menos del 10% de infección fueron MZC 74-275, CC 91-1555, CC 93-4223, CC 87-434, RD 75-11, CC 89-2000 y Desi Paunda. Un grupo de diez variedades y cultivares no mostró infección alguna. Estos resultados correlacionaron con algunas variedades infectadas naturalmente (R²=0.7). Un cruzamiento entre una madre susceptible (CC 84-75) y un padre resistente (RD 75-11) produjo la mayoría de la progenie resistente.