Resistance to the H Strain of Sugarcane Mosaic Virus Among Wild Forms of Sugarcane and Relatives

M. P. GRISHAM, Research Plant Pathologist, D. M. BURNER, Research Geneticist, and B. L. LEGENDRE, Research Agronomist, U.S. Department of Agriculture, Agricultural Research Service, Sugarcane Research Unit, Houma, LA 70361-0470

ABSTRACT

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One hundred and three clones of sugarcane relatives, including *Saccharum* interspecific hybrids, were inoculated with the H strain of sugarcane mosaic virus (SCMV) in two greenhouse experiments. Wild relatives included *Erianthus* spp., *S. spontaneum, S. barberi/S. sinense, S. officinarum*, and *S. robustum*. Among the six taxa represented in the first experiment, *Erianthus, S. spontaneum*, and *S. barberi/S. sinense* clones were the most resistant and *S. robustum* clones were the most susceptible, with the interspecific hybrid and *S. officinarum* clones intermediate. Clones in the second experiment, which were predominantly *S. spontaneum*, were assigned to one of three groups that reflected their geographic origin. The mean percent infection differed ($P < 0.05$) among the clones from India (6%), the Philippines (21%), and Indonesia (42%).

Mosaic of sugarcane caused by sugarcane mosaic virus (SCMV) contributed to the decline of Louisiana's sugarcane industry during the early part of this century (12). The highly susceptible noble cultivars (*Saccharum officinarum* L.) were replaced initially by interspecific hybrid derivatives that were tolerant to the disease, then later by hybrids that were resistant to the various strains of SCMV (4,5,10). New cultivars have been released as new strains of SCMV have developed. Currently, strain H is the predominant strain recovered from Louisiana sugarcane, and strains I and M are recovered occasionally (*unpublished*). Shukla et al (22) have proposed that strains H, I, and M be designated as strains of sorghum mosaic virus on the basis of serological and chemical properties.

Sugarcane cultivars currently planted throughout the world are interspecific hybrids derived primarily from a few clones of four *Saccharum* spp.: *S. officinarum* L., *S. spontaneum* L., *S. robustum* Brandes & Jesw. ex Grassl, and *S. sinense* Roxb. (3). To increase the diversity of germ plasm available for development of commercial cultivars, several collections of wild sugarcane relatives were made and deposited in repositories in India and the United States (5). A breeding program was established in 1964 by the U.S. Department of Agriculture, Agricultural Research Service, Houma, Louisiana, with the objective being to incorporate mosaic resistance of this diverse wild germ plasm into new cultivars (10). The objective of this study was to identify mosaic resistance among a more recently introduced collection of wild clones of sugarcane and its relatives for possible future use in the breeding program.

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MATERIALS AND METHODS

Clones of sugarcane and near relatives were tested for susceptibility to mosaic in two greenhouse experiments. In one experiment, 51 clones representing interspecific hybrids of sugarcane and five taxa of near relatives were challenged with the H strain of SCMV. Clones of *S. barberi* Jeswiet and *S. sinense* are frequently grouped together because of their close phylogenetic relationship (8,24). Among the clones of the genus *Erianthus* Michx. sect. *Ripidium* Henrard were the following species: clone US63-006, *E. brevibarbis* Michx.; clone SES288, *E. arundinaceus* (Retz.) Jeswiet; clone US57-11-2, *E. longisetosus* Anderss.; clone Mardon, species unknown; clone Kalimpong, *E. procerus* (Roxb.) Raiz.; clone US57-60-2, *E. rufipilus* (Stevd.) Griseb.; clone SES372, *E. ravennae* (L.) P. Beauv.; and cane 2886, *E. bengalense* (Retz.) Bharadw.

The second experiment included 52 clones from three geographic regions. All but three of the clones—IND81-53 (*Vetiveria*) sp., IND81-47 (*E. bengalense*), and IS76-182 (*Miscanthus* sp.)—were of *S. spontaneum*. Seven clones were from India (IND81-47, IND81-53, IND81-80, IND81-142, IND81-144, IND81-161, and IND81-166); 27 clones were from Indonesia (IN84-9, IN84-10, IN84-11, IN84-12, IN84-12-F1, IN84-12-F2, IN84-12-F3, IN84-12-F8, IN84-12-F10, IN84-12-F13, IN84-12-F14, IN84-12-F16, IN84-13, IN84-16, IN84-18, IN84-21, IN84-22, IN84-27, IN84-39, IN84-42, IN84-66, IN84-69, IN84-70, IN84-88, IN84-89, IN84-91, and IS76-182); and 18 clones were from the Philippines (PAL-84-1, PAL-84-4, PCA-SUR-84-5, PCA-NOR-84-7, PCAV-84-5, PCAV-84-6, PCAV-84-7, PCAV-84-11, PCAV-84-12, PCAV-84-20, PLAG-84-8, PPGN-84-2, PPGN-84-5, PPGN-84-8, PQ-84-3, PQ-84-4, PTAR-84-1, and PTAR-84-2).

When possible, lateral buds were used to propagate clones. Clones that did not form nodes with viable buds were propagated from tillers or rhizomes. Germinating buds or cuttings were randomly transplanted to cells (5.2 × 5.2 × 7.6 cm cavities) of speedling trays containing steam-sterilized vermiculite. Each of the five replicates included one to six plants (not all transplants survived) of each clone.

Inoculum was prepared from leaves of young plants of *Sorghum bicolor* (L.) Moench 'Rio' infected with SCMV strain H. The virus was recovered from sugarcane with mosaic symptoms in Louisiana. The symptoms on differential hosts described by Abbott and Tippett (1) were used to identify the strain. Juice was extracted from the leaves with a grinding mill, filtered through cheesecloth, and mixed with 0.005 M sodium sulfite buffer (1:1, v/v) and silicon carbide particles. Plants were inoculated at the one- to two-leaf stage with the extract by the airbrush technique (20). Plants not showing mosaic symptoms after 2 wk were inoculated a second time.

Plants were examined for mosaic symptoms at 3-wk intervals for 9 wk after the second inoculation. Diagnosis was confirmed for plants showing very weak or no symptoms by extracting juice from individual plants as described above; the extract was used to inoculate young Rio sorghum plants at the three-leaf stage. Inoculated sorghum plants were examined for symptoms of mosaic at weekly intervals for 4 wk.

Plants were maintained throughout the experiment in a screened (30-mesh) greenhouse at temperatures between 24

and 35 C. Plants were treated weekly with diazinon for insect control and fertilized weekly with a commercial 15-30-15 fertilizer (Miracle-Gro).

The two experiments were arranged in a randomized complete block design with five replications. Most plots had six plants per clone but some had as few as one plant. Data were presented as simple percentage infection and as $\sin^{-1}y^{1/2}$ transformed expressed in degrees (25), where y = infection percentage in decimal form. Zero percentages were equal to $\sin^{-1}(1/4n)^{1/2}$, where n = six plants for most plots. Because of missing data, percentage and transformed data did not always correspond exactly. Transformed data were analyzed using the GLM procedure (21). Means were reported as least squares means. Negative least squares means for percentage data were assumed to be equal to zero. Conclusions were based on analysis of transformed data.

RESULTS

Taxa differed in mean percent infection (Table 1). Taxon means ranged from 2.8% of the *Erianthus* spp. plants infected to 66.2% of the *S. robustum* plants infected. Clone differences were noted within each taxon. Among the *Erianthus* spp. clones, the highest level of infection (11%) was in Mardon. Of the *S. spontaneum* clones, three (Mandalay, Tainan 2*n*=96, and US56-15-8) had no infected plants, two (SES184B and SES189) had 17% infected plants, and the other three (SH249, Tainan 2*n*=112, and an unknown local volunteer clone) had 37–47% infected plants. *S. barberi* clones Matki Mango, Kalari, and Semari had infection levels of 7, 18, and 33%, respectively, and *S. sinense* clones Zwinga, Katha, and China had infection levels of 7, 30, and 31%, respectively.

The two interspecific hybrid cultivars with the highest levels of infection were CP 73-351 (76%) and NCo 310 (92%). Only 3% of CP 76-356 plants became infected. Infection levels in the other five cultivars ranged from 26 to 61%. *S. officinarum* clones NG77-117 and NG28-211 had infection levels of 0 and 17%, whereas clones Badila, Creoula, IN84-071, NG28-002, NG51-146, NG57-067, NG77-062, and Sylva had infection levels of 30% or higher. Except for NG77-021, with an infection level of 18%, *S. robustum* clones also had infection levels higher than 30%; these clones were IJ76-293, IJ76-314, IJ76-522, Molokai 5099, NG57-134, NG77-038, NG77-077, NG77-147, NG77-160, and NG77-199.

In the second experiment, in which clones were collected from three geographic regions, origin means differed significantly ($P < 0.05$), with clones from India being the most resistant and those from Indonesia, the least resistant (Table 2). Among the seven clones from India, IND81-53 and IND81-144 had infection levels of 7 and 30%, respectively; the other five showed no symptoms of mosaic infection. Among the 18 clones from the Philippines, PAL-84-1, PAL-84-4, PCAV-84-5, PCAV-84-20, PQ-84-4, and PTAR-84-1 had no infected plants, and the other 12 clones had from 10 to 60% infected plants. Among the 27 clones from Indonesia, IN84-11, IN84-12, IN84-12-F16, and IN84-18 had infection levels of 10% or lower, and the other 23 clones had infection levels ranging from 15 to 90%.

DISCUSSION

Many sugarcane breeders and cytogeneticists believe that new developments in sugarcane cultivar improvement can be expected from the use of unexplored germ plasm. Early reports of resistance of *S. spontaneum* to SCMV indicated that, with few exceptions, clones were immune to SCMV (6,7,16,26). Abbott and Todd (2) and Koike (17) later found several clones of *S. spontaneum* that were susceptible to SCMV, possibly because they used different strains of the virus that had appeared since earlier studies. A considerable range of responses to SCMV strain H among the clones of *S. spontaneum* tested was also found in the present study. As has been shown from evaluation of clones in the greenhouse (11), only clones having the most resistance to SCMV are kept for breeding, and those may be tested for resistance repeatedly before being used in the breeding program. Dunckelman and Breaux (9) reported on the susceptibility of six of the eight *S. spontaneum* clones used in our first experiment. Their inoculum included a mixture of strains A, B, D, and H and, in later tests, I. Not tested were SES184B and the local volunteer clone. Except for the low level of infection in SES189 (16.7%) in that study, results of both studies were in general agreement.

With the exception of the cultivars and the *S. spontaneum* clones discussed above, the response to SCMV of the clones used in our two experiments was unknown or unreported before this study, and this is the first documented report of the disposition of these clones to their reaction to sugarcane mosaic. In their review of sugarcane mosaic, Koike and Gillaspie (18) concluded that among species of the genus *Saccharum, S. officinarum* is the most susceptible, *S. barberi* and *S. robustum* are moderately susceptible, and *S. sinense* and *S. spontaneum* are resistant, depending on the SCMV strain(s) involved. Among 13 clones of *Saccharum*-related genera, Koike (17) found that nine clones of the genera *Erianthus, Miscanthus, Sclerostachya*, and *Imperata* were resistant. Among the six taxa represented in our first experiment (Table 1), clones of *Erianthus* spp., *S. spontaneum*, and *S. barberi*/*S. sinense* were more resistant than clones of interspecific hybrids, *S. officinarum*, and *S. robustum*, which is in keeping with these earlier findings.

SCMV is present in the three geographic regions from which the clones in our second experiment originated (18). Strain H, which was used as the inoculum

Table 1. Differential reactions to sugarcane mosaic virus (SCMV) among sugarcane (*Saccharum*) taxa

Taxon	Number of clones tested	SCMV infection[y] Mean percent	Range	$\sin^{-1}y^{1/2}$ transformed
Erianthus	8	0	0–11	0.37 a[z]
S. spontaneum	8	18	0–47	0.43 a
S. barberi/*S. sinense*	6	21	7–33	0.46 a
Cultivars	8	48	3–92	0.76 b
S. officinarum	10	58	0–100	0.87 c
S. robustum	11	66	18–100	0.96 d
Grand mean		40	0–100	0.68
CV (%)		...		32.1

[y] Least square means.
[z] Means followed by the same letter do not differ significantly ($P < 0.05$) by paired t test.

Table 2. Differential reactions to sugarcane mosaic virus (SCMV) of *Saccharum spontaneum* clones from three geographic regions

Origin	Number of clones tested	SCMV infection[y] Mean percent	Range	$\sin^{-1}y^{1/2}$ transformed
India	7	6	0–30	0.34 a[z]
Philippines	18	21	0–60	0.52 b
Indonesia	27	42	0–90	0.71 c
Grand mean		30		0.59
CV (%)		...		38.0

[y] Least squares means.
[z] Means followed by the same letter do not differ significantly ($P < 0.05$) by paired t test.

in both experiments, has been reported from India and the Philippines but not from Indonesia (18). However, confirmation of the reports of strain H from India and the Philippines is needed. The behavior of these isolates was different from that of the Louisiana isolates originally used to describe strain H (15). The results of the present study indicate that the probability of finding resistance to SCMV strain H among populations of *S. spontaneum* is greater in India and the Philippines than in Indonesia; however, highly resistant clones were found among clones from each geographic region.

The need for improved mosaic resistance is evident among the cultivars tested. Six of the cultivars (CP 65-357, CP 70-321, CP 72-356, CP 72-370, CP 76-331, and CP 79-318) occupy 96% of the area planted to sugarcane in Louisiana (14). The levels of infection observed after artificial inoculation are similar to those observed in the field, which depend on the level of infection of the cane used for planting and the amount of disease pressure in the area (*unpublished*). The two most recently released recommended cultivars, CP 79-318 and LCP 82-89, are moderately resistant (13) and moderately susceptible (*unpublished*), respectively, to mosaic. The two cultivars that had the highest levels of mosaic infection, CP 73-351 and NCo 310, are no longer grown in Louisiana.

Interspecific crosses were made as early as 1893 in Java, with successful cultivars from this program first appearing in the 1920s (23). Most of these canes were derived from *S. spontaneum* clones backcrossed to noble types. The genetic base, however, has remained narrow (3). As few as 20 noble canes and 10 *S. spontaneum* or *S. spontaneum* derivatives form the base of the pedigrees of cultivars grown worldwide (23). As of 1987, 276 cultivars from 12 new basic lines, primarily *S. spontaneum*, have been assigned permanent CP (Canal Point) numbers at Houma (19). Further, the transfer of mosaic resistance from recently acquired wild relatives of sugarcane has been the major success of the basic breeding program (19), and clones identified as resistant to SCMV in this study will also be included in future crosses to further broaden the genetic base of sugarcane (10). Similar efforts have been initiated by other international sugarcane breeding programs (5).

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