Failure of Sugarcane Mosaic Virus to Survive in Cultured Sugarcane Tissue

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ABSTRACT

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Explants taken from immature spindle tissues above the apical meristerm of sugarcane plants systemically infected with sugarcane mosaic virus were callused on Murashige-Skoog culture medium. Of 57 plants regenerated from this callus, 55 were virus-free as indicated by lack of symptoms after 6 mo of growth in soil in the greenhouse and by bioassay on sorghum test plants. Bioassays of spindle tissues similar to those of the explants showed a steep infectivity gradient ranging from very low just above the growing point to very high about 4.5 cm above the growing point. The spindle tissues exhibited a reverse gradient with respect to callus formation, so that most of the callus came from tissues with the greatest infectivity. It was concluded that sugarcane mosaic virus was present in many, if not all, of the explants from which virus-free plants were regenerated.

Meristem culture (4,6) and a combination of heat therapy and meristem culture (10) have been used to free sugarcane (hybrids of *Saccharum* spp.) of sugarcane mosaic virus (SCMV). Leu (6) used tissue culture for the same purpose, but tissue culture is not a desirable method of obtaining disease-free plants in sugarcane because of the very high risk of genetic alteration in regenerated plants (3,5,7). Because genetic alteration has not been reported as a side effect in either meristem culture (4,6,10) or heat therapy (1), these methods appear superior to tissue culture for obtaining disease-free plants in sugarcane. There is another reason, however, to be interested in SCMV in cultured sugarcane tissue.

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Tissue culture could serve as a tool for the study of SCMV, provided that the virus can survive through the process. By analogy with other host-virus systems (9), and because of success in freeing sugarcane of SCMV through meristem culture, it may be supposed that SCMV is absent from the apical meristem of systemically infected sugarcane, but it is not known whether it is absent from immature leaf tissues above the apical meristem. These tissues are commonly used as explants in the tissue culture of sugarcane (8) and were used by Leu (6), who did not determine whether the virus was present in the explants. If the regenerated plants were derived from explants that were virus-free, there is hope that SCMV can be studied in tissue culture either by using known infected tissue as explants or by finding a method of inoculating callus in culture. If the virus was present, but failed to survive, there is much less hope.

The general purpose of this research was to explore the feasibility of establishing and maintaining SCMV in cultured sugarcane tissues, but the specific purpose of this paper is to report evidence that SCMV was present in explants from which virus-free plants were regenerated.

MATERIALS AND METHODS

The methods used to culture sugarcane tissue and to regenerate plants were described by Lyrene (8). Explants consisted of cross-sectional slices through the immature inner leaves of sugarcane spindles. Each slice was about 3 mm thick and 6 to 10 mm in diameter. The slices were taken aseptically from a 5-cm region just above the growing point. Four slices taken from each of eight spindles were bioassayed for SCMV to determine the distribution of virus in the spindles. The four slices were taken at 1.25-cm intervals above the growing point, as illustrated in Figure 1. Each assayed slice was crushed on a ground-glass surface with a ground-glass spatula in a few drops of water. The wet spatula was rubbed on Carborundum-dusted leaves of 1-wk-old sorghum (*Sorghum bicolor* (L.) Moench) cv. Mn 1056. Each slice was assayed on nine seedlings. Mn 1056 sorghum was chosen as an assay host because of its extreme susceptibility to SCMV and intense symptoms (2). Regenerated sugarcane plants were assayed for SCMV 8 wk after they were established in the greenhouse. A portion of the youngest visible leaf of

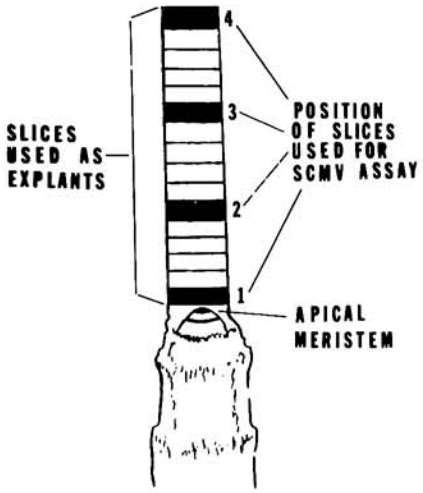

Fig. 1. Diagram of a sugarcane spindle showing the relative positions of the apical meristem, the slices used as explants, and the slices assayed for sugarcane mosaic virus.

each plant was triturated in a small quantity of distilled water, and the resulting inoculum was rubbed on the leaves of 1-wk-old Mn 1056 seedlings. Each slice was assayed on 125 plants.

The sugarcane clone Branchue (*Saccharum officinarum* L.) was chosen for this study because it is very susceptible to infection by SCMV, which has a short incubation period (5–8 days) in it; it shows intense symptoms; and it never recovers spontaneously from SCMV infection. The virus was SCMV-E (American Type Culture Collection PV-115).

Explants from both healthy and SCMV-infected Branchue plants were started on 7 June 1977 on Murashige-Skoog (MS) medium containing 2,4-D at 3 mg/L. On 29 June, callus was transferred to MS containing 2,4-D at 1 mg/L. On 3 August, differentiating callus was transferred to MS lacking 2,4-D. On 12 September, rooted plantlets were transplanted into soil. The plants were grown in the greenhouse for 6 mo and monitored almost daily for mosaic symptoms.

RESULTS AND DISCUSSION

A total of 57 plants was derived from SCMV-infected plants and a similar number from healthy plants. Only 12 of those derived from healthy plants were maintained as controls; the others were discarded. Only two regenerated plants, both derived from infected plants, ever showed symptoms. One of these showed symptoms before it was transplanted into soil; the other showed symptoms 4 days after it was transplanted.

Table 1. Assay of tissue slices from sugarcane spindles for sugarcane mosaic virus

Spindle no.	No. of assay plants infected[a]			
	Slice 1[b]	Slice 2	Slice 3	Slice 4
1	0	0	2	7
2	1	5	8	9
3	0	0	0	2
4	0	1	0	4
5	0	1	4	7
6	0	0	7	8
7	0	0	1	1
8	0	7	9	2
Total	1	14	31	40

[a] Each slice was assayed on nine sorghum seedlings.

[b] Slice 1 was taken just above the growing point, and slices 2, 3, and 4 were taken at successive 1.25-cm increments above slice 1.

All regenerated plants, including the 12 controls and the two symptom-bearing plants, were assayed for SCMV 8 wk after they were established in soil. The two symptom-bearing plants assayed positive; all others assayed negative. None of the regenerated plants showed obvious genetic deviation from normal Branchue.

Eight spindles from the originally infected Branchue plants were assayed for SCMV. The results (Table 1) suggest a viral concentration gradient (or at least an infectivity gradient) in the spindles starting at near zero in the slice nearest the growing point and reaching a high level in the slice farthest from the growing point (about 4.5 cm). The existence of this gradient was shown clearly by linear regression of the mean number of infected assay plants on the distance of the assayed slice from the growing point ($r^2 = 0.993$).

The assay of tissue slices was very insensitive because the glass spatula inoculation method is very inefficient on sorghum seedlings and because each tissue slice provided only enough inoculum to inoculate nine assay plants. A viral concentration high enough to infect 10% of the assay plants on the average would be expected to assay negative more often than not by this assay. It is likely that all slices contained virus. In spite of the insensitive assay, virus was detected in all of the slices taken from the upper end of the spindle. This information and the observation in this and previous experiments that callus forms more abundantly on the more mature slices from this region of the spindle indicate that most, if not all, of the plants derived from originally infected plants were regenerated from explants containing virus.

The assay of regenerated plants was more sensitive than the assay of tissue slices because of the large amount of inoculum available and the greater number of assay plants. However, it should be noted that an assay was not really necessary to establish lack of virus in the symptomless Branchue plants. Branchue is a very susceptible clone with very intense symptom expression. It was grown under conditions favorable for symptom expression, as indicated by the two exceptional symptom-bearing plants. The possibility that SCMV was present in Branchue for 6 mo without reaching a concentration high enough to produce symptoms seemingly would require a genetic change in all 55 of the symptomless plants or in the virus in all 55 plants.

The evidence indicates that SCMV was present in the tissues going into tissue culture and absent in the plants coming out. Somehow the virus did not survive the process.

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