Virus-Free Shoots from Cassava Stem Cuttings Infected with Cassava Latent Virus

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ABSTRACT

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Cassava stem cuttings infected with cassava latent virus (CLV) gave rise to shoots that showed CLV symptoms at emergence, shoots that showed CLV symptoms 3-5 wk later, and shoots that remained symptomless after 6 wk of observation. CLV symptoms appeared 5-11 days earlier when symptomless shoots were detopped than when they were left intact. Symptomless shoots severed 2 wk after emergence from CLV-infected stem cuttings and subsequently detopped did not develop CLV symptoms. These plants, however, readily developed symptoms of CLV when colonized with CLV-laden *Bemisia tabaci*. Therefore, symptomless shoots were considered free of CLV and could be used as healthy planting stock.

Additional key words: Manihot esculenta

Cassava (Manihot esculenta Crantz) is an important staple crop in most African countries. In Zaire, 90% of the population depends on this crop for subsistence. Recently, however, the cassava crop in Zaire has been devastated by a number of diseases, one of which is African cassava mosaic caused by cassava latent virus (CLV). The virus is transmitted by whiteflies (Bemisia tabaci) (11,14–16) and by sap from cassava to cassava and to other hosts, namely Nicotiana benthamiana and N. debneyi (7,10).

The "tree" cassava (hybrid between M. glaziovii and M. esculenta) and M. esculenta 'Kibandameno' were the only known symptomless carriers of CLV (9). Shoots from stem cuttings of other CLVinfected cassava cultivars showed immediate or delayed expression of CLV symptoms (10,15,16). This report describes procedures for obtaining CLVfree shoots from CLV-infected stem cuttings.

MATERIALS AND METHODS

Experiments were conducted during two cassava growing seasons (12 mo each) in 1977 and 1978 in Mankewa, M'vuazi, Republique du Zaire. Four field plots (5×11 m each) were planted with CLV-infected stem cuttings of a local CLV-susceptible cassava cultivar (02864) at a spacing of 1×1 m in November of each year. All border rows were planted

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with another local, susceptible cultivar (M'pelolongi).

At harvesttime, 16 CLV-infected stems 1.5 m long were selected at random and divided into four groups (treatments) of four stems each. Each stem in the first group was divided into 10 cuttings, each 15 cm long, that were planted in the field the same day. The other three groups were left in the field for 1, 2, or 3 wk after harvest, then divided, treated, and planted as described. Shoots that emerged from the planted cuttings were recorded as mosaic or mosaic-free. Symptomless shoots that remained attached to the stem cuttings were observed weekly for CLV symptoms for 5 wk.

In the first and second year of the experiment, 88 and 100 symptomless shoots, respectively, were severed at random from CLV-infected stem cuttings from all four treatment groups during the first and second week after shoots emerged from the ground. The severed shoots were allowed to develop roots in vials (22 mm in diameter \times 48 mm high) half filled with boiled tap water (1). Rooted plants were transferred into autoclaved earthen pots (16 cm in diameter \times 11 cm high) filled with soil. All transplants were then detopped (1-3 cm of the growing tips clipped) to hasten appearance of CLV symptoms, as has been demonstrated for virus-infected raspberry, tobacco, and sugar beet (2-6).

One-third of 60 and 66 detopped, symptomless plants in the first and second year of the experiment, respectively, were planted in the field and exposed to supposedly viruliferous *B. tabaci*, and one-third were colonized with 10–15 whiteflies reared on CLV-infected cassava plants under cheesecloth cages in the greenhouse. An equal number of caged, control plants was exposed to nonviruliferous whiteflies that had been reared on caged, symptomless plants. The whiteflies were killed with malathion sprays after 8 hr of feeding on the test plants.

Ten and 15 symptomless, unsevered shoots in the first and second year of the experiment, respectively, were also detopped the third week after shoots emerged from the ground. An equal number of unsevered symptomless shoots that were not detopped served as controls.

RESULTS AND DISCUSSION

Incidence of symptomless shoots (averaged over 2 yr) that emerged from CLV-infected stem cuttings during the first week ranged from 16.1 to 62.7% among the four treatment groups (Table 1). Numbers of symptomless shoots decreased as the shoots became older or until the fifth week, when the incidence of symptomless shoots ranged from 0 to 23.1%. Significantly more symptomless shoots occurred in treatment 3, in which stems lay in the field for 2 wk before division into cuttings.

One hundred eighty-eight symptomless shoots severed from CLV-infected stem cuttings and subsequently detopped did not show symptoms of CLV. Furthermore, these detopped symptomless plants readily developed CLV symptoms within 20-26 days when colonized with viruliferous whiteflies in the greenhouse (36 infected of 42 plants inoculated) or from 17 to 23 days when these plants were exposed to virus-laden whiteflies in the field (42 infected of 42 plants planted). These results indicated that the severed and detopped symptomless plants were virus-free.

The occurrence of asymptomatic shoots from CLV-infected cassava plants was first reported in 1938 (15), and Kartha and Gamborg (12) reported that shoots from cultured apical meristems of CLV-infected plants gave 90–95% CLVfree plants. Storey and Nichols (16) found that plantings made at the beginning of June remained symptomless for months, whereas those made from December to April were largely diseased after 3 mo of growth. In this report, shoots attached to CLV-infected stem cuttings remained symptomless as long as 6 wk.

Bock et al (8,9) reported a strain of CLV (CLV-C or CLV-T) that was mostly confined to East Africa, west of the Rift Valley, and latent in tree cassava, *M. esculenta* 'Kibandameno,' and coral plants (*Hewittia sublobata* and *Jatropha*

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 Table 1. Incidence of symptomless shoots emerging from stem cuttings of 02864 cassava infected with cassava latent virus (CLV) during two growing seasons

| Treatment ^x | Average incidence (%) at weekly intervals after emergence ^y | | | | |
|------------------------|--|--------------|--------------|--------------|--------------|
| | 1st wk | 2nd wk | 3rd wk | 4th wk | 5th wk |
| 1 | $28.0 c^{2}$ (159) | 16.7 b (145) | 5.8 b (135) | 3.7 b (135) | 0.0 b (135) |
| 2 | 41.3 b (187) | 13.3 b (142) | 7.2 b (137) | 5.2 b (137) | 3.6 b (137) |
| 3 | 62.7 a (178) | 48.0 a (139) | 34.6 a (122) | 28.2 a (122) | 23.1 a (122) |
| 4 | 16.1 d (149) | 11.4 b (151) | 7.4 b (151) | 4.6 b (151) | 2.6 b (151) |

^xRandomly selected CLV-infected stems in the field were divided into cuttings and planted the same day (treatment 1) or left lying in the field for 1 wk (treatment 2), 2 wk (treatment 3), or 3 wk (treatment 4) before planting.

^y Data are averages for two growing seasons; numbers in parentheses are total shoots observed each week.

^z Means in columns followed by the same letter were not significantly different (P = 0.05) according to Duncan's multiple range test.

multifida). This CLV strain was different and was never recovered from CLVinfected cassava plants from Nigeria or other cassava-growing countries of western Africa (8). The data obtained from my study agrees with the report of Bock et al (8).

Detopped, symptomless shoots attached to CLV-infected stem cuttings developed mosaic symptoms within 5–11 days of detopping (25 infected of 25 plants detopped), whereas symptomless shoots that were not detopped showed mosaic symptoms within 21–35 days (17 infected of 25 plants observed). The long incubation period in unsevered, symptomless shoots suggests slow movement of CLV particles within tissues of the stem cuttings and of the young cassava shoots. A similar phenomenon was reported in cassava (15) and in suckers arising from abaca corms infected with abaca mosaic virus (13).

From this study, it appears that symptomless shoots severed from CLVinfected stem cuttings within 2 wk of emergence could be used as virus-free sources for propagation of planting materials.

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