Elimination of Cassava Mosaic Disease by Meristem Culture

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ABSTRACT

The cassava mosaic agent was eliminated from two cultivars of Indian and Nigerian origin by culturing shoot apical meristems on a Murashige and Skoog medium supplemented with benzyladenine (5×10^{-7}M), naphthaleneacetic acid (10^{-6}M) and gibberellic acid (GA_3 10^{-7}M). Plant regeneration averaged 90 to 95% and 60% of the regenerated plants were mosaic symptom-free when explants of up to 0.4 mm in length were cultured. Growing diseased cuttings at 35 C for 30 days resulted in the production of plants without symptoms and from such plants meristem tips of up to 0.8 mm were cultured and mosaic disease-free plants regenerated. Results of graft transmission experiments demonstrated the absence of the causal agent in the plants regenerated by meristem culture technique.

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Mosaic disease of cassava (*Manihot esculenta* Crantz) poses serious problems to the crop and is prevalent in most of the cassava-growing regions of the world (10). The vegetative propagation of cassava promotes the devastating spread of the disease. Three types of cassava mosaic, namely, Indian, Nigerian, and Brazilian, have so far been reported and their mode of transmission studied (2, 4, 10, 13). The Indian and Nigerian cassava mosaic diseases are identical with regard to the type of symptoms produced on susceptible cultivars, host range, and transmission either by grafting or through the insect vector. Published reports so far indicate that no viral particles have been isolated from the diseased plants or demonstrated by electron microscopy. On the other hand, the Brazilian mosaic has been shown to be of viral origin and mechanically transmissible to susceptible cultivars of cassava and other herbaceous hosts (2, 9).

The technique of meristem culture is of economic importance in eliminating seed-borne viral infections (6). Meristem tip culture alone or in combination with heat treatment has been successfully employed to eliminate viral pathogens from a wide range of plant species (5, 12). Heat therapy and shoot tip culture have been employed to produce cassava plants free of leaf distortion symptoms (1). We have reported a procedure to regenerate plants from the shoot apical meristems of healthy cassava (8). The present investigation concerns the application of the procedure for the elimination of cassava mosaic diseases of Indian and Nigerian origin. A preliminary report on this investigation has already appeared (7).

MATERIALS AND METHODS.—Dormant diseased stakes of Indian and Nigerian cassava, cultivars 'Kalikalan' and 'Ogunjobi', respectively, were cut in sections with two nodes each. The upper cut ends were sealed with paraffin and the sections were planted in pots in a greenhouse where the cuttings sprouted within 5 to 7 days. Apical meristematic domes from the sprouts were aseptically dissected and cultured in 10×2.5 cm pyrex tubes on a Murashige and Skoog medium (11) containing vitamins as in B5 medium (3), and supplemented with benzyladenine (BA, 5×10^{-7}M), naphthaleneacetic acid (NAA, 10^{-6}M), and gibberellic acid (GA_3 10^{-7}M), as previously reported (8). The tubes containing the meristem tips were incubated in a growth cabinet programmed to provide a light and dark cycle of 16/8 hours (3,000 lux provided by cool-white fluorescent lamps), 26 C and 70% relative humidity (RH).

In other experiments, cuttings from diseased stakes were planted in vermiculite in pots and grown in a growth cabinet at 35 C (constant), 16-hour photoperiod (4,000 lux from banks of cool-white fluorescent lamps) and 70% relative humidity. After 30 days of growth, meristem tips were isolated and cultured as mentioned above. The control experiments consisted of cuttings originating from the same diseased stakes but grown under greenhouse conditions (21 C, 14 hours photoperiod, and 40 to 45% RH).

RESULTS AND DISCUSSION.—The diseased cuttings sprouted within 5 to 7 days and the foliage showed typical mosaic symptoms. As the plants grew older, symptoms became very severe and were manifested in the form of crinkling, distortion, and reduction in size of leaf laminae (Fig. 1).

The size of the explant influenced the number of plants regenerated by culturing meristems which were symptom-free as well as the number of plants differentiating from the total meristems cultured (i.e., regeneration potential expressed as per cent). Only explants exceeding 0.2 mm in length formed complete plants. Those less than 0.2 mm in length produced either callus or callus with roots. The regeneration potential of meristem tips exceeding 0.2 mm (0.3-0.5) ranged from 90 to 95% in both the Indian and Nigerian cultivars. The presence of leaf primordia on the meristem explants was not essential for plant regeneration. The meristem tips regenerated into complete plants within 26 days had attained a size of 4-5 cm. At this stage they were transferred to pots of vermiculite and the plants were grown either in a greenhouse or in a growth room. The plants were frequently observed for symptom expression for a period of 6 months.

In a population of 135 plants regenerated in several experiments from 150 meristems (size 0.4 mm) of mosaic-diseased cultivar Kalikalan, 60% showed no symptoms of mosaic even after 6 months. The mortality of plants subsequent to potting averaged 4-5%. In subsequent experiments, when the meristem tip size exceeded 0.4 mm (0.5-0.8 mm), all the resulting plants exhibited mosaic symptoms. In a test on Nigerian cultivar 'Ogunjobi' out of 42 plants regenerated from 45 meristem tips, 40 were symptom-free. The healthy regenerated plants had deep green foliage, and grew vigorously (Fig. 4, 5).

Transmission experiments were conducted by grafting the scions from 50 regenerated plants onto healthy, but

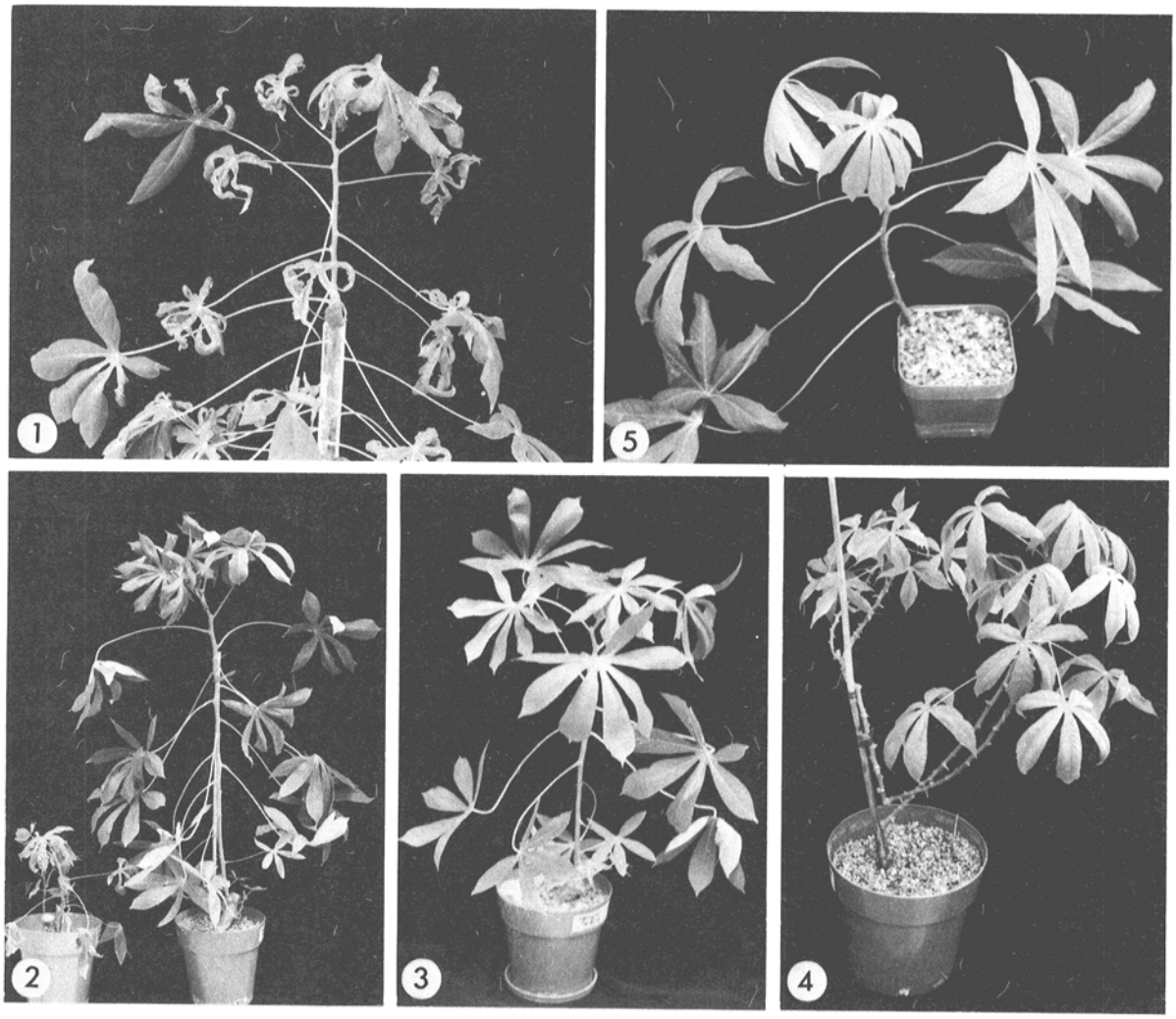

Fig. 1-5. Regeneration of mosaic disease-free cassava plants by thermotherapy coupled with meristem tip culture. **1)** An adult plant showing typical symptoms of cassava mosaic disease. **2)** Cuttings from diseased plant, cultivar Kalikalan, grown under greenhouse (left) and growth cabinet conditions at 35 C (right) for 30 days. **3)** Cuttings from mosaic-diseased plant, cultivar Ogunjobi, grown at 35 C for 30 days. Note the disappearance of mosaic symptoms and increased vegetative growth in 2 (right and 3. **4 and 5)** Three-month-old symptom-free plants regenerated by culturing shoot apical meristems from mosaic diseased cassava cultivars Kalikalan (4) and Ogunjobi (5).

susceptible South American (Colombia) cassava cultivars Llanera and Colombia #800, at monthly intervals for a period of 6 months. Control experiments consisted of grafting scions from diseased plants. No visible symptoms were observed on the stock plants grafted with healthy plants regenerated from meristems. The control plants using diseased scions, however, exhibited typical mosaic symptoms on the newly formed axillary shoots of the stock plants within 21 to 28 days.

Cuttings from diseased stakes of cultivars Kalikalan and Ogunjobi when grown at 35 C (constant), 16-hour photoperiod (4,000 lux) and 70% RH exhibited vigorous growth as compared to similar plants grown under greenhouse conditions. The new leaves produced from day 15 showed slight mosaic symptoms and those appearing by day 30 displayed none of the mosaic symptoms (Fig. 2, 3). The plants of the same age at 21 C developed severe mosaic symptoms on all leaves (Fig. 2). In the plants grown at 35 C for 30 days with symptom-free leaves, typical mosaic symptoms appeared within 7 to 10 days when they were transferred to the greenhouse environment (21 C).

Meristems were cultured from the plants grown at 35 C after day 30 which were still maintained under growth cabinet conditions and plants regenerated. The regeneration potential averaged 90 to 95% when the explant size exceeded 0.2 mm, thus confirming results of other experiments. Mosaic symptoms could not be detected in any plant regenerated from meristem tips of up to 0.8 mm in length. Plantlets developed from larger meristem tips (0.9 to 1 mm) developed mosaic symptoms. Fifty plants of each cultivar have been produced and

grown to adult plants by the combined 35 C growth and meristem culture technique. Transmission tests on healthy cultivars (susceptible) carried out at monthly intervals for a period of 6 months gave no indication that the disease agent was present.

Under normal growing conditions the causative agent of cassava mosaic disease appeared to be absent from the top 0.4 mm of the shoot apical region of a diseased plant. The size of the meristematic dome explant, therefore, was critical for regenerating mosaic symptom-free plants. Growing the infected cuttings at 35 C resulted in rapid vegatative growth and explants up to 0.8 mm could be obtained free of pathogen. The higher temperature favored plant growth and appeared to have retarded invasion and multiplication of the causative agent. The results reported in this communication suggest that the causative agent of cassava mosaic disease in the Indian and Nigerian cassava can be eliminated by meristem culture. Alternatively this procedure can be combined with thermotherapy with the advantage of using larger meristem tips and producing higher percentage of symptom-free plants.

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