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USE OF TISSUE CULTURE AND THERMOTHERAPY TO FREE EAST AFRICAN CASSAVA CULTIVARS OF AFRICAN CASSAVA MOSAIC AND CASSAVA BROWN STREAK DISEASES

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ABSTRACT

Meristem-tip and thermotherapy techniques were used to free several cassava (Manihot esculenta) cultivars from two important viruslike diseases that affect the crop in East Africa, namely African cassava mosaic (ACMD) and cassava brown streak (CBSD). Meristematic tips (0.2-0.4 mm long) cultured on a modified Murashige and Skoog medium supplemented with gibberellic acid, naphthaleneacetic acid, and benzyladenine regenerated into complete plants within 60 days. Mechanical inoculation (CBSD) and grafting tests (CBSD and ACMD) were used to index regenerated plants for disease. Some plants were heat-treated (37°C for 30-36 days) before excision of meristems. From a total of 93 meristems excised after heat treatment, 19 regenerated into complete plants, compared with 20 from 30 that were not heat-treated. Heat treatment increased the percentage of disease-free plants that developed from five cassava lines affected by ACMD and CBSD, or both. Rapid multiplication techniques were used to propagate the disease-free cassava plants.

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Additional key words: meristem-tip culture, graft transmission.

Cassava (Manihot esculenta) is an important food crop in East Africa (Kenya, Tanzania, and Uganda), particularly at elevations below 1200-1500 m. The most important disease of cassava in East Africa is African cassava mosaic (ACMD). The etiology of ACMD is unknown, but evidence suggests that the pathogen is a virus (2, 3, 14). It is transmissible by grafting, whiteflies (Bemisia tabaci) (4, 14), and mechanical (sap) inoculation (3). Another important viruslike disease present in East Africa, particularly in the coastal regions of Kenya and Tanzania, is cassava brown streak (CBSD) (6, 13). It is transmissible by grafting and mechanical inoculation (10). Transmission by vectors has not been demonstrated. Rod-shaped (9) and isometric (2) particles have been associated with CBSD-infected cassava. Although CBSD appears to be caused by a virus, the etiology will remain in doubt until the full range of typical disease symptoms can be produced in healthy cassava inoculated with purified preparations of the pathogen. Foliar symptoms of CBSD (Fig. 1) and ACMD (Fig. 2) differ markedly from one another. Cassava brown streak disease affects mature or nearly mature leaves and produces little or no distortion of the leaf laminae (13), whereas ACMD induces leaf distortion symptoms on both old and young leaves (14).

Distribution of cassava vegetative material into and within East Africa for research and crop improvement purposes is hampered because of the difficulty of ensuring the health status of the material. The present study was undertaken to alleviate this problem and to improve methods of producing cassava free from ACMD and CBSD, through meristem-tip culture and thermotherapy. This approach has been successful in freeing cassava of a mosaic disease (8).

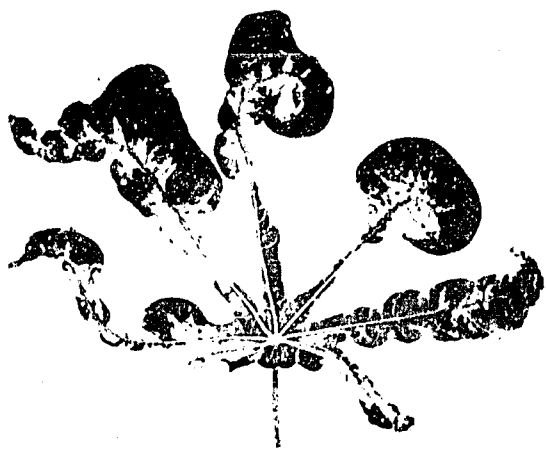

FIGURE 1. (Above) Leaf of a healthy cassava plant (right) and a plant infected with cassava brown streak disease (left). Chlorotic veinbanding patterns occur on mature or nearly mature leaves of infected plants, the laminae of which generally are not distorted.

FIGURE 2. (Left) Leaf of cassava plant infected with African cassava mosaic disease. Mosaic and distortion and reduction in size of laminae are typical symptoms of the disease.

MATERIALS AND METHODS

Stakes (stem pieces) of cassava varieties F 279, Kibandameno, Aipen Valenca, 46106/27, and a local cultivar exhibiting symptoms of ACMD, CBSD, or both, were collected from the Coast Agricultural Research Station, Mtwapa, Mombasa, Kenya. The stakes with dormant buds were cut into sections with at least four nodes. The upper exposed end of each stake was sealed with wax and the stakes were planted in 2-liter plastic pots containing a steam-sterilized mixture of sand and soil (3 : 1, v/v). Pots were placed in a walk-in growth room (3 m x 3 m x 3 m) at a temperature of 30°C, and relative humidity (RH) of 70-90% with fluorescent lights (tropical daylight with 3,500-4,000 lux) programmed for 18 hr of light and 6 hr of darkness per day. Sprouts began appearing in 5-6 days. Some meristems were excised from axillary shoots 9 days after planting (nonheat-treatment series). The temperature then was increased by 1°C increments over a 14-day period until it reached 37°C. Meristems were excised from plants that remained at 37°C for 30-36 days (heat-treatment series).

Shoots (2-4 cm) were surface sterilized in 70% ethyl alcohol for 1 min and rinsed 3-5 times in sterile distilled water. A portion of the apex (0.2-0.4 mm) was excised with a disposable scalpel beneath a dissecting microscope. Excised tips were placed on the surface of the agar culture medium (see below) in test tubes that were plugged and sealed with Parafilm. Cultures were incubated in a growth room with a temperature of 26-28°C, RH of 65-75%, and a 16-hr photoperiod (3,500-4,000 lux from banks of tropical daylight fluorescent lamps). Regenerated plantlets 4-5 cm tall were transferred from culture tubes to plastic pots containing sterilized coarse sand and incubated in the growth room for about 14 days before being moved to insect-protected greenhouses.

The culture medium used was that of Murashige and Skoog (12) as modified by Kartha and Gamborg (8). It contained the following (mg/liter): NH_4NO_3, 1,650; KNO_3, 1,900; $MgSO_4 \cdot 7H_2O$, 370; KH_2PO_4, 170; $CaCl_2 \cdot 2H_2O$, 435; H_3BO_3, 6.2; $MnSO_4 \cdot 4H_2O$, 22.3; $ZnSO_4 \cdot 7H_2O$, 8.6; $Na_2MoO_4 \cdot 2H_2O$, 0.25; KI, 0.75; $CuSO_4 \cdot 5H_2O$, 0.025; $CoCl_2 \cdot 6H_2O$, 0.025; ferric sodium ethylenediaminetetraacetate, 40; thiamine hydrochloride, 1.0; nicotinic acid, 0.1; m-inositol, 100; pyridoxine hydrochloride, 0.1; plus sucrose, 20 g and Difco Bacto agar, 6 g. Growth hormones (naphthaleneacetic acid, 10^{-6} M; benzyladenine, 5×10^{-7} M; gibberellic acid, 10^{-7} M) were added to the medium after the agar was melted. The ph of the culture medium was adjusted to 5.7 with 0.2 N KOH before sterilization. Medium in 2.5 ml aliquots was dispensed into pyrex test tubes (100 x 25 mm) that were plugged with nonabsorbent cotton and autoclaved at 1.46 kg/cm^2 (20 psi) for 20 minutes.

Regenerated plants were indexed for CBSD and ACMD at various intervals during the course of these studies. Plants were indexed for CBSD by triturating leaves (young and old from each plant) in 1% K_2HPO_4 and applying the sap to leaves of young Nicotiana clevelandii test plants dusted with 600-mesh (0.22 μm) carborundum. Test plants were rinsed with tap water immediately after inoculation. Scions from regenerated plants also were grafted onto healthy cassava test plants (cultivars M Col 22, M Col 113, and N Mex 55) from CIAT (Centro Internacional de Agricultura Tropical), Cali, Colombia. Plants were indexed for ACMD by grafting scions from the heat-treated and nonheat-treated series onto healthy M Col 22, M Col 113, and N Mex 55 cassava plants (and vice versa). Infected controls were included in the indexing tests. All regenerated plants were cut back, and the stem pieces were rooted. Developing axillary shoots on plants that were cut back and rooted stem cuttings were observed periodically for disease symptoms.

RESULTS

Callus tissue began forming from the basal portions of excised meristems within 4-5 days, and shoot differentiation started in 10-14 days. Root formation on the developing callus tissues was poor or absent on the complete culture medium. Roots developed profusely when callus tissues with shoot growth were transferred after 20-26 days to the complete culture medium minus gibberellic acid. Regenerated plants (4-6 cm high) were ready for transfer from culture tubes to sand in less than 60 days.

The number of regenerated plants developing from meristems in the heat-treated and nonheat-treated series varied (Table 1). Regeneration of meristems into complete plants in two nonheat-treated cultivars was 20 of 30, whereas that in five cultivars in the heat-treated series was 19 of 93.

Heat treatment of cassava plants before excision of meristems significantly increased the proportion of regenerated plants that were apparently free of ACMD and CBSD (Table 1). All regenerated plants that received a heat treatment, except two from a doubly infected cultivar, were free of disease symptoms. For example, the numbers of ACMD-free regenerated Kibandameno plants were 4 of 4 and [illegible] of 10 in the heat and nonheat-treated series, respectively.

Table 1. Effect of meristem-tip culture and heat treatment on production of cassava plants free of symptoms of African cassava mosaic (ACMD) and cassava brown streak (CBSD) diseases.

Cassava variety	Disease	Heat treatment[a]	No. Meristem-tips cultured	No. Regenerated plants	No. Regenerated plants free of disease symptoms
F 279	CBSD	-	10	10	0
F 279	CBSD	+	24	2	2
Local	CBSD	+	19	5	5
Kibandameno	ACMD	-	20	10	1
Kibandameno	ACMD	+	30	4	4
Aipin Valenca	ACMD	+	10	5	5
46106/27	ACMD and CBSD	+	10	3	1[b]

[a]- = no heat treatment; + = treatment at 37°C for 30-36 days.

[b]Two of the regenerated plants were free of ACMD symptoms; one of these was also free of CBSD symptoms.

From cassava line 46106/27, which was affected with both ACMD and CBSD, 1 of 3 regenerated plants indexed negative for both diseases (Table 1).

Symptoms of CBSD were not observed on the young foliage of regenerated plants that were still infected, but developed later on mature leaves of 4- to 7-month-old plants Fig. 1). Symptoms were seldom observed on the stem. The causal agent of CBSD was mechanically transmitted to N. clevelandii test plants from regenerated cassava plants with foliar symptoms of CBSD. The disease was also transmitted by grafting scions from infected plants onto healthy cassava test plants. The causal agent(s) of CBSD was not recovered from symptom-free regenerated mother plants in either grafting or mechanical-inoculation tests conducted at intervals over a 10- to 12-month period. Six- to 8-month-old cuttings from symptomless mother plants also remained free of CBSD symptoms, and a pathogen could not be detected in transmission studies.

Foliar symptoms of ACMD, characterized by deformed, misshapen laminae (Fig. 2), usually were noticeable on the first leaves that formed on diseased regenerated plants while they were still in culture tubes. Grafting was the only transmission test reliable for detection of ACMD in regenerated plants in M Col 22, M Col 113, and N Mex 55 indicators. Symptoms of ACMD developed most quickly and were most pronounced on variety M Col 22, which was included in all grafting tests. At times, symptoms were latent in ACMD-affected plants; however, unmistakable symptoms of ACMD invariably developed rapidly, even from symptomless infected plants, on the young foliage arising from axillary buds of plants that had been cut back 1-3 weeks earlier. After 2-6 months, there was considerable difference in size between healthy and ACMD-affected plants.

After 10-12 months, all regenerated plants that remained symptom-free and repeatedly indexed negative for ACMD, CBSD, or both, were multiplied with the rapid propagation techniques developed at CIAT (5, 11).

DISCUSSION

This study demonstrates the value of meristem culture in conjunction with thermotherapy to free cassava cultivars of two important viruslike diseases, ACMD and CBSD, that affect the crop in East Africa. This is the first report of the use of these techniques to produce CBSD-free cassava. Apparently, it is also the first published report of the successful use of tissue culture on the African continent to eliminate the ACMD pathogen(s) from infected cassava. Similar results were obtained in Canada by Kartha and Gamborg (8), who used meristem-tip culture to free cassava cultivars of Nigerian and Indian origin of a mosaic disease of cassava. In our trials, heat treatment of infected mother plants for 30-36 days before excision of meristems greatly increased the percentage of regenerated plants that were free of ACMD and CBSD. The beneficial effect of heat also was observed by Kartha and Gamborg (8).

Cassava is generally propagated and distributed as vegetative material. Several important vascular pathogens, including bacteria (Xanthomonas manihotis), viruses, viruslike organisms, and mycoplasmas (11), are systemic in diseased plants and are difficult to detect unless special indexing tests are used. Because of the high risks of introducing new pathogens and pests of cassava in the transfer of planting material, the importation of cassava germplasm into and within the African continent is restricted, or prohibited, or both, by various African governments and the Interafrican Phytosanitary Council (1). It may be possible, however, to promote the international exchange of cassava germplasm by shipping pathogen-free plants in the form of meristem-tip cultures, which would considerably reduce the hazards of germplasm exchange. Kahn (7) demonstrated the feasibility of using tissue culture to improve the phytosanitary aspects of asparagus plant introductions.

Presently, the most reliable and sensitive test for indexing cassava plants for ACMD is grafting. The use of cassava test plants that might be carriers of the pathogens of ACMD and CBSD was avoided by use of scionwood from cassava cultivars imported from CIAT, Cali, Colombia. ACMD and CBSD are not known to be present on the American continent; however, healthy CIAT indicators developed typical symptoms of ACMD or CBSD when grafted with affected cassava.

In East Africa, Bock and Guthrie (2, 3) are trying to purify and characterize the causal agents of ACMD and CBSD and other viruses and viruslike pathogens of cassava. They succeeded recently in mechanically transmitting the pathogen(s) of ACMD in sap from infected cassava to healthy cassava (3). These recent advances in the transmission, characterization, and purification of viruses and viruslike agents of cassava may lead to the development of new testing methods, such as serology, that will complement existing indexing procedures.

Researchers at CIAT have developed rapid propagation techniques with cassava (5, 11) whereby one mature plant can be multiplied to produce about 36,000 cuttings in one year (5). We have used these techniques to propagate disease-free plants of each cassava cultivar to establish a disease-free repository in the highlands at Muguga, Kenya, where cassava is not cultivated, and for distribution to researchers and crop-improvement schemes in East Africa.

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