

Heat Therapy of Cassava Infected with African Cassava Mosaic Disease

WALTER J. KAISER, Head, Plant Quarantine Station, and RAYMOND LOUIE, Research Plant Pathologist, Plant Pathology and Nematology Division, Kenya Agriculture Research Institute, Nairobi, Kenya

ABSTRACT

Kaiser, W. J., and Louie, R. 1982. Heat therapy of cassava infected with African cassava mosaic disease. *Plant Disease* 66:475-477.

Thermotherapy was used to free three cassava (*Manihot esculenta*) cultivars of African cassava mosaic, an important viruslike disease of cassava in East Africa. The pathogen was eradicated from 33-44% of tip cuttings (1.0-1.5 cm long) after hot-air treatment of mother plants at 37 C for 87-105 days. Survival of these tip cuttings after 35-105 days ranged from 22-73%. Exposure of entire cassava plants to hot-air treatments at 37 C for 42-96 days caused temporary remission of symptoms in most plants. Only one of 129 surviving plants was freed of disease. Two successive hot-water treatments of diseased stem cuttings at 50 or 55 C for varying intervals were not therapeutic.

Cassava (*Manihot esculenta* Crantz) is an important high carbohydrate, staple food crop in many African countries south of the Sahara. Cassava yields in Africa are among the lowest in the world (6,7). Diseases appear to be a major factor limiting production and contribut-

Contribution of the Kenya Agriculture Research Institute, Ministry of Agriculture, Muguga, Kenya; the U.S. Agency for International Development (Food Crops Research Project 618-11-110-657); the Animal and Plant Health Inspection Service, and Agricultural Research Service of the U.S. Department of Agriculture.

Present address of first author: Regional Plant Introduction Station, USDA-ARS, 59 Johnson Hall, Washington State University, Pullman, WA 99164. Present address of second author: USDA-ARS, OARDC, Wooster, OH 44691.

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Accepted for publication 7 July 1981.

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ing to low yields in Africa and elsewhere in the tropics (11,15). One important disease affecting cassava in most African countries is African cassava mosaic (ACMD). In East Africa (Kenya, Tanzania, and Uganda), it is considered the most important factor limiting cassava yields (2).

The etiology of ACMD is unknown, but recent evidence indicates a viral pathogen (1-3,16) that may be transmitted by rub-inoculation (2), grafting (11,12,15), and whiteflies (*Bemisia* spp.) (4,14).

Cassava is clonally propagated. Stem pieces (stakes) are used to establish new plantings. This method generally ensures perpetuation of systemic pathogens such as the ACMD agent(s) and appears to be a primary means by which ACMD is introduced into new areas (12,14).

Many legally imported plant materials, including cassava, are processed through the Plant Quarantine Station (PQS) at Muguga, Kenya, where they are assayed to ensure that only healthy material is distributed. Research with cassava at the Muguga PQS was designed to improve methods of detecting different pathogens and to develop techniques for producing disease-free plants. Earlier studies (9) had demonstrated the efficacy of using thermotherapy combined with tissue culture to produce ACMD-free cassava

plants. This paper describes the use of thermotherapy alone to free cassava of ACMD.

MATERIALS AND METHODS

Plant management. Stakes from cassava cultivars Kibandameno, 7301, and 7302 from the Coast Agricultural Research Station, Mtwapa, Mombasa, Kenya, and a cultivar from the Lake Nyasa region of Tanzania exhibiting symptoms of ACMD were propagated in steam-sterilized soil in greenhouses at the Muguga PQS and used in hot-air treatment studies. Only stakes of Kibandameno cassava collected from 12- to 16-mo-old ACMD-diseased plants were used in the hot-water treatments.

Before planting, stakes were cut into 15-cm lengths and treated for 10 min in solutions of benomyl (2.5 g a.i./L of H₂O) and malathion (1.2 ml a.i./L of H₂O). Apical ends were dried and then sealed with liquid wax to reduce dehydration. Stem pieces were planted vertically (7-8 cm deep) in a steam-sterilized mixture of soil and sand (2:1, v/v) in 1-L perforated black plastic bags.

After heat treatment, plants or cuttings were placed in insect-protected greenhouses at 23-30 C. Plants were sprayed at 1- to 2-wk intervals with pesticides.

Heat treatments. Sprouted cuttings, 20 to 30 days old, with ACMD were grown in a walk-in growth room (3 × 3 × 3 m) at 37 C ± 1 C, >75% RH, and an 18-hr day (5,000-6,000 lux). Tip cuttings (1.0-1.5 cm long) were excised from heat-treated cassava cultivars at biweekly to monthly intervals up to 105 days and planted in a mixture of moist sand and vermiculite (2:1, v/v) in 7.5-cm-diameter pots. Pots were covered with clear plastic bags to maintain high relative humidity and placed beneath fluorescent lights (8,000-9,000 lux) at 23-28 C. Rooted tip

cuttings were transplanted to steam-sterilized soil in 1-L plastic bags and transferred to a moist chamber until well established.

In another series of hot-air experiments, Kibandameno plants were removed from the heat chamber at 7- to 14-day intervals between 42 and 96 days and incubated in a greenhouse where plants were observed and indexed for inactivation of ACMD.

Stem cuttings of Kibandameno cultivar were also treated in a circulating hot-water bath at different temperatures and two time intervals over 2 days. After the second hot-water treatment, cuttings were treated with benomyl and malathion, waxed, planted in a steam-sterilized soil-sand mixture (2:1, v/v) in 1-L plastic bags, and placed in a greenhouse.

All healthy-appearing cassava plants were assayed for ACMD at 2-4 wk intervals over 12-18 mo by grafting treated scions onto healthy test plants of cassava cultivar M Col 22 (from Centro

Internacional de Agricultura Tropical, Cali, Colombia), or vice versa. At 1-mo intervals, heat-treated plants also were cut back and the stem pieces rooted. Axillary shoots developed on these plants and rooted cuttings from them were observed for disease. Plants with ACMD that were not treated with heat were included as controls in all assays.

RESULTS

The ACMD pathogen(s) was eradicated from 11 of 28 tip cuttings of three cassava cultivars by hot-air treatment of mother plants at 37 C for 87-105 days (Table 1). Tip cuttings from the four cassava cultivars survived at rates of 22-73% when heat-treated for 35-105 days. Foliar symptoms of ACMD, characterized by chlorosis and distortion of laminae (Fig. 1), were observed in more than 95% of tip cuttings 3-5 wk after rooting. Plants freed of ACMD by heat treatment were darker green and grew faster than

diseased plants. At least 9 mo elapsed between heat treatment and final indexing of all plants that appeared to be freed of ACMD by heat treatment at 37 C for up to 105 days.

Hot-air treatment of Kibandameno cassava plants at 37 C for 42-98 days was an inefficient method of freeing plants of ACMD. Only one of 129 heat-treated plants was freed of ACMD (data not shown). Survival of heat-treated plants averaged 89% at 42 days and 58% at 98 days. Heat treatment resulted in temporary remission of symptoms in most plants, but characteristic ACMD symptoms developed on new foliage 1-2 wk after heat treatment in more than 95% of surviving plants. Grafting of scions from heat-treated plants onto cassava cultivar M Col 22 (and vice versa) was a reliable and sensitive method (9) of assaying heat-treated and control plants for ACMD. Repeated indexing at monthly intervals over 18 mo failed to detect ACMD in one plant that was heat-treated for 84 days.

Hot-water treatment at 50 or 55 C for 10 min on day 1 followed in 24 hr by treatment at 50 C for 90-180 min or 55 C for 15-120 min did not eradicate ACMD from dormant Kibandameno stem cuttings (data not shown). Pretreatment of stem cuttings at 50 or 55 C for 10 min on the first day did not appear to adversely affect survival of cuttings treated the following day at 50 C for 90-180 min. However, hot-water treatment at 55 C after 24 hr resulted in a decrease in survival of cuttings from 75% at 15 min to 0% at 120 min.

DISCUSSION

In 1959, Chant (5) used thermotherapy to eradicate ACMD from tip cuttings and entire plants of a Nigerian hybrid cassava line. However, it appears that thermotherapy was rarely, if ever, used in Africa to develop ACMD-free cassava plants. We confirmed Chant's observation that hot-water therapy at 50-55 C failed to free cassava stem cuttings of ACMD.

Chant (5) reported eradicating ACMD from entire cassava plants and green shoot cuttings (>5 cm long) by hot-air treatment at 37 C for 28-42 days. In our tests, the time required to free cassava plants and tip cuttings (<1.5 cm long) of ACMD at 37 C was >84 days. Several factors could have contributed to this discrepancy. Storey and Nichols (14) were first to recognize mild and severe strains of the ACMD pathogen(s). East African strains may be more heat resistant than those in West Africa. Cassava germ plasm differs in susceptibility to ACMD (7) and could also differ in heat tolerance. Pathogen detection techniques could also contribute to the discrepancy. Cassava can be assayed for ACMD by mechanical (sap) inoculation (3), grafting (10,11,14,15), and vector transmission (4,14). Chant (5) assayed

Table 1. Incidence of African cassava mosaic disease in tip cuttings from diseased plants of four cassava cultivars maintained at 37 C^a

Cultivar	Treatment period (days)	Total plants treated	Surviving plants	
			Infected	Healthy
Kibandameno	35	38	19	0
	67	32	8	0
	90	33	9	4
	105	63	10	4
7301	35	14	6	0
	99	17	3	1
7302	42	14	6	0
	87	11	6	2
Lake Nyasa collection	35	10	7	0
	90	6	4	0

^a After heat treatment, tip cuttings (1.0-1.5 cm long) were planted in a sterilized sand-vermiculite mixture at 23-28 C. Cuttings were maintained at >75% RH until rooted and then transplanted to steam-sterilized soil in the greenhouse before indexing.



Fig. 1. Kibandameno cassava plant (right) with foliar symptoms of African cassava mosaic disease (ACMD). The healthy plant (left) was derived from a tip cutting (1.0-1.5 cm long) taken from a diseased Kibandameno plant after hot-air treatment at 37 C for 95 days. Photograph was taken 85 days after tip cuttings were made.

heat-treated cassava plants and rooted shoot cuttings by whitefly (*Bemisia* spp.) transmission, whereas we used grafting. Indexing by grafting is considered more reliable than assay by sap transmission or insect vectors (13).

Methods that have been used to control ACMD on the African continent include propagation of mosaic-free cuttings (1,2,11,12,15), roguing of diseased plants (1), use of resistant cultivars (7,11,15), thermotherapy (5), and meristem-tip culture (9,10). Heat therapy and tissue culture are frequently used at plant quarantine stations to free imported, vegetatively propagated plant materials of systemic pathogens such as the ACMD agent (9) and potato viruses (8). At Muguga PQS, tissue culture combined with thermotherapy was used to free five East African cassava lines of ACMD and another viruslike disease, cassava brown streak (9). The present study also demonstrated the feasibility of using thermotherapy alone to produce ACMD-free cassava.

ACKNOWLEDGMENTS

We are grateful to Richard Ransom, former U.S. Peace Corps volunteer, and to Loise M. Muriithi, laboratory technician, Plant Quarantine Station, Kenya Agriculture Research Institute, Muguga, for assistance. We thank E. J. Guthrie of the Institute for the photograph in this article.

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