Heat Treatment and Meristem Culture for the Production of Virus-Free Bananas

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ABSTRACT

Banana rhizomes were heat-treated at 35-43 C. Meristems from lateral buds which developed after 100 days of treatment were excised and grown on a modified Knudson's medium to which naphthaleneacetic acid was added to induce root formation. Virus could not be detected in plants derived from meristems by inoculations of indicator plants, *Chenopodium quinoa* and *C. amaranticolor*. Localized or systemic symptoms were often induced when indicator plants were inoculated with leaf material from control plants in commercial plantations.

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Gold (2) and Stover (10) reported that commercial cultivars of bananas ('Musa AAA') are infected with symptomless viruses. *Musa* seedlings have also been reported to carry virus (2). One of the viruses is cucumber mosaic virus (1, 11, 12), which produces local lesions on *Chenopodium quinoa* Willd. and *C. amaranticolor* Coste & Reyn. Another unidentified virus produces systemic yellowing and leaf distortion. Random testing indicated that, although banana plants do not display any virus symptoms, infection is general throughout all plantations. Research was initiated to produce a virus-free clone of bananas to determine what effect these symptomless viruses have on general vigor of the plant. Heat treatment and meristem culture, two techniques often used to free plants of viruses (6, 7), were employed. This is the first report on the establishment of banana plants from meristems. Resulting plants assayed virus-free when meristems were dissected from heat-treated rhizomes.

MATERIALS AND METHODS.—Rhizomes of the Cavendish group of banana cultivars measuring at least 20.3 cm (8 inches) in diam (after being peeled and having the roots removed) were obtained from banana plants which had not yet produced fruit. The central growing point was removed prior to heat treatment to stimulate development of lateral buds (3) during the treatment period. The rhizomes were placed in individual wooden boxes to which vermiculite was added to cover approximately two-thirds of the rhizome. Boxes containing the rhizomes were placed inside a chamber heated with hot water, giving an air temp of 35-43 C and a relative humidity of 100%. The lateral buds which developed after 100 days of heat treatment were removed and the meristems dissected.

The banana plant (8) is particularly suited for obtaining sterile meristems without surface disinfection. The rhizome or underground stem can be trimmed to about 2.5 cm square and 5.0-7.5 cm (1 inch square and 2-3 inches) long with the growing point on one end. The rhizome tissue then served as a handle while the meristem was dissected by systematically removing with a flamed scalpel one or more overlapping leaf bases, until the sterile meristem was exposed. For development of meristems, modifications of Neergard's medium (9) and Knudson's medium (4) presented in Table 1, along with additions to the latter of naphthaleneacetic acid (NAA), indolebutyric acid (IBA), indoleacetic acid (IAA), casamino acids, coconut milk, yeast extract, or nutrient broth were tested. These media were solidified with 0.6% agar. The pH of the media was adjusted to 5.8 prior to dispensing in 150 × 15-mm test tubes which were filled approximately one-third full, capped with aluminum foil, and autoclaved at 1.05 kg-force/cm^2 (15 psi) for 15 min. Cultures were maintained at ca. 27 C under continuous light of 40W Westinghouse incandescent bulbs.

Plants were assayed for virus by grinding the leaves in liquid nitrogen using a mortar and pestle. When leaf material was thoroughly ground, it was suspended in ca. 5 ml of buffer solution for each gram of fresh leaf material. The buffer solution used was 62.5% 0.1 M Na_2HPO_4 and 37.5% 0.1 M KH_2PO_4 plus 0.1% (w/v) Na_2SO_3. Leaves of one-month-old indicator plants (*C. quinoa* and *C. amaranticolor*) were dusted with 600-mesh Carborundum and inoculated by rubbing the leaves lightly with a cotton-tipped stick moistened with the inoculum. Following inoculation, leaves were washed with sterile distilled water and plants placed in a chamber maintained at 19-21 C, where they received diffuse sunlight.

RESULTS AND OBSERVATIONS.—Banana meristems, which are whitish when first excised, failed to "green-up" when placed on Neergard's medium,whereas on Knudson's medium chlorophyll production was apparent after 2 wk of incubation. When the former was prepared with sucrose, meristems still failed to turn green. Knudson's medium was used in subsequent studies.

When Knudson's medium was supplemented with yeast extract, casamino acids, nutrient broth (1 g/liter), or coconut milk (100 ml/liter), banana meristems began to turn green after only 1 wk of incubation. However, after 3 mo, during which time the meristems were transferred once to fresh media, they had not begun to form roots, although the original size of the meristem had at least tripled. At this age, the meristems were transferred to Knudson's medium plus NAA (1 μg/ml). Root formation was observed on some of the meristems 2-5 wk after the transfer. Knudson's medium plus casamino acids and Knudson's medium plus coconut milk were the best starting media with ca. 60% of the meristems producing roots after being transferred to Knudson's medium, plus

TABLE 1. Composition of media tested for culturing meristems of bananas

	Neergard's medium (9)	Knudson's medium (4)
$Ca(NO_3)_2 \cdot 4H_2O$	1.0 g	1.0 g
$(NH_4)_2SO_4$	--	0.5 g
KNO_3	0.25 g	--
$MgSO_4 \cdot 7H_2O$	0.25 g	0.25 g
KH_2PO_4	0.25 g	0.25 g
Glucose	40.0 g	--
Sucrose	--	20.0 g
Thiamine	1.0 mg	1.0 mg
Berthelot's solution[a]	0.5 ml	0.5 ml
Iron solution[b]	5.0 ml	5.0 ml
Distilled water	1.0 liter	1.0 liter

[a] Berthelot's solution (9): $MnSO_4 \cdot 7H_2O$ 2.0 g, H_3BO_3 50 mg, KI 0.5 g, $NiCl_2 \cdot 6H_2O$ 50 mg, $CoCl \cdot 6H_2O$ 50 mg, $ZnSO_4 \cdot 7H_2O$ 100 mg, $CuSO_4 \cdot 5H_2O$ 50 mg, H_2SO_4 (conc.) 1.0 ml, plus distilled water 1.0 liter. The following compounds $Fe(SO_4)_3$, $TiSO_4 \cdot 5H_2O$, and $BeSO_4$ were omitted.

[b] Iron chelate solution (5): $FeSO_4 \cdot 7H_2O$ 5.57 g, Na_2-EDTA 7.45 g, plus distilled water 1.0 liter.

NAA. Roots of meristems initially on Knudson's medium plus casamino acids were thicker and more numerous than those produced on other media. When roots began to form, meristems were transferred to 125-ml flasks containing ca. 25 ml of solidified Knudson's medium. If NAA was added, roots continued to grow slowly, but leaf development did not occur except on rare occasions after long periods of incubation. However, if NAA was not added, the meristems began to produce leaves along with continued root growth. When these small plants had two to three leaves they could be successfully transferred to 10.2-cm (4-inch) diam plastic pots containing a 1:1 soil and sand mixture (1:1, v/v).

NAA, IAA, and IBA (1.0 μg/ml) were individually added to Knudson's medium (KM), KM + casamino acids, KM + coconut milk, and KM + casamino acids and coconut milk. Observations of meristem development on the 16 media, indicated that KM + casamino acids and coconut milk was the best basal medium. NAA appeared to be the most effective auxin in stimulating root formation; roots appeared ca. 2 mo after initially placing meristems on the medium. IAA was ineffective in stimulating root formation. Stages of development of the banana meristem are illustrated in Fig. 1, showing rooting (2-3 mo) and leaf formation (3-4 mo).

To date, 73 plants obtained by culturing meristems of heat-treated banana rhizomes have survived subsequent transplant to soil in pots. Seven banana plants which showed yellowing of leaves and 10 which produced symptoms in indicator plants were discarded. Twenty five plants have been repeatedly assayed for viruses on indicator plants with negative results. The 31 remaining plants have been assayed only once with negative results. Simultaneous inoculations of indicator plants with known infected plants resulted in typical symptoms.

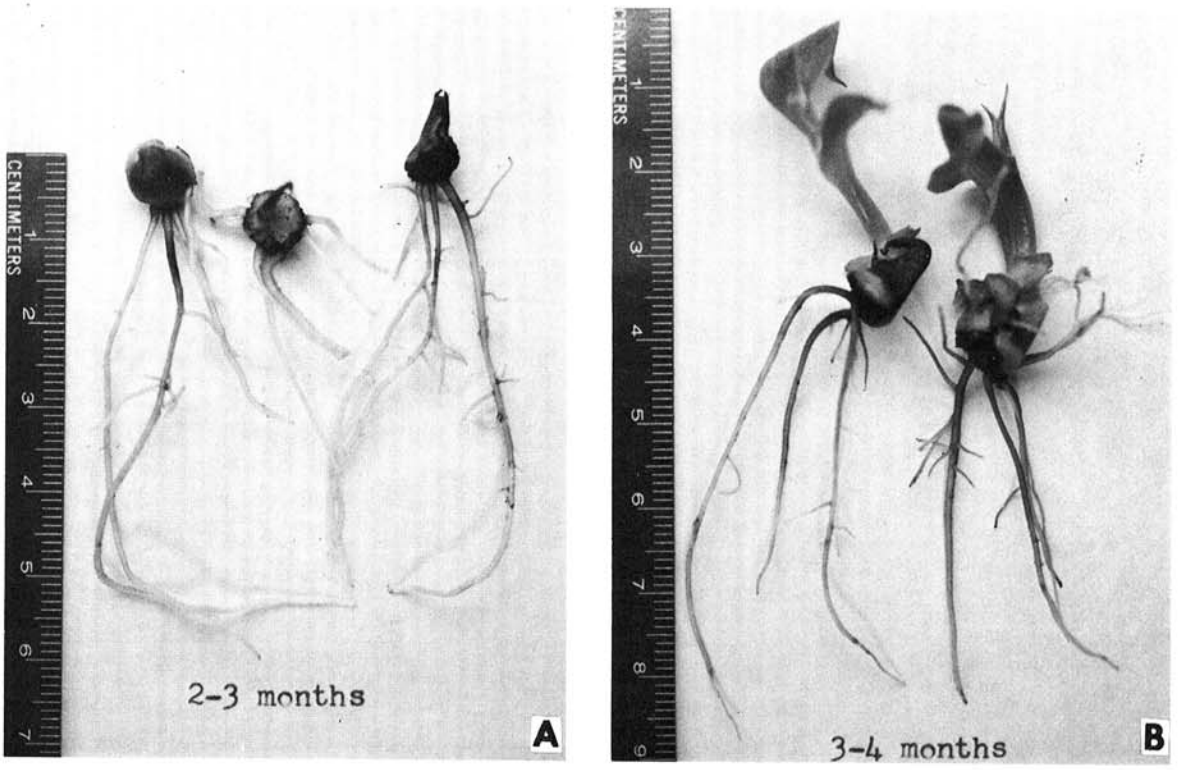

Fig. 1. Stages of development of banana meristem showing (A) rooting at 2-3 mo and (B) leaf formation at 3-4 mo.

Forty-five plants have been obtained from heat-treated rhizomes without meristem culture. Using indicator plants, 43 were infected with virus. The remaining two are still under observation and will be assayed again. In addition, 11 plants obtained by culturing meristems of rhizomes which were not heat treated produced symptoms when used to inoculate indicator plants.

DISCUSSION.—Meristems of bananas can be successfully grown on Knudson's medium, but fail to grow on Neergard's medium. The primary difference in the two media (Table 1) is the source of carbohydrate and nitrogen. When sucrose was used to prepare Neergard's medium meristems still failed to turn green, indicating that banana meristems probably require a more reduced form of nitrogen than the nitrate supplied in Neergard's medium. Although not essential, the addition of casamino acids or coconut milk to Knudson's medium resulted in more rapid growth of the meristems. NAA (1 μg/ml) was essential for root formation. Once roots were initiated, meristems had to be transferred to media without NAA to obtain leaf development.

Heat treatment of banana rhizomes or meristem culture alone is not enough to free bananas of viruses. When the two techniques were combined, approximately 75% of the plants were found to be free of the symptomless viruses which can be detected by indicator plants. Studies are being initiated to determine what effect these viruses have on plant vigor.

LITERATURE CITED

1. BIRD, J., and F. L. WELLMAN. 1962. A mosaic disease of musaceous crops in Puerto Rico. Phytopathology 52:286 (Abstr.).
2. GOLD, A. H. 1972. Seed transmission of banana viruses. Phytopathology 62:760 (Abstr.).
3. HAMILTON, K. S. 1965. Reproduction of banana from adventitious buds. Trop. Agric. 42:69-73.
4. KNUDSON, L. 1946. A new nutrient solution for the germination of orchid seed. Bull. Am. Orch. Soc. 15:214-217.
5. MURASHIGE, T., and F. SKOOG. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15:473-497.
6. NYLAND, G., and A. C. GOHEEN. 1969. Heat therapy of virus diseases of perennial plants. Annu. Rev. Phytopathol. 7:331-354.
7. PHILLIPS, D. J. 1968. Carnation shoot tip culture. Colo. State Univ. Exp. Stn. Tech. Bull. 102. 22 p.
8. SIMMONDS, N. W. 1966. Bananas. Longmans, Green and Co. Ltd., London. 512 p.
9. STONE, O. M. 1963. Factors affecting growth of carnation plants from shoot apices. Ann. Appl. Biol. 52:199-209.
10. STOVER, R. H. 1972. Banana, plantain and abaca diseases. Commonwealth Mycological Institute, Kew, Surrey, England. 316 p.
11. WAITE, B. H. 1960. Virus diseases of bananas in Central America. Proc. Caribb. Reg. Am. Soc. Hort. Sci. 4:26-30.
12. YOT-DAUTHY, D., and J. M. BOVÉ. 1966. Mosaique du bananier. Identification et purification de diverses souches du virus. Fruits d'Outre Mer. 21:449-466.