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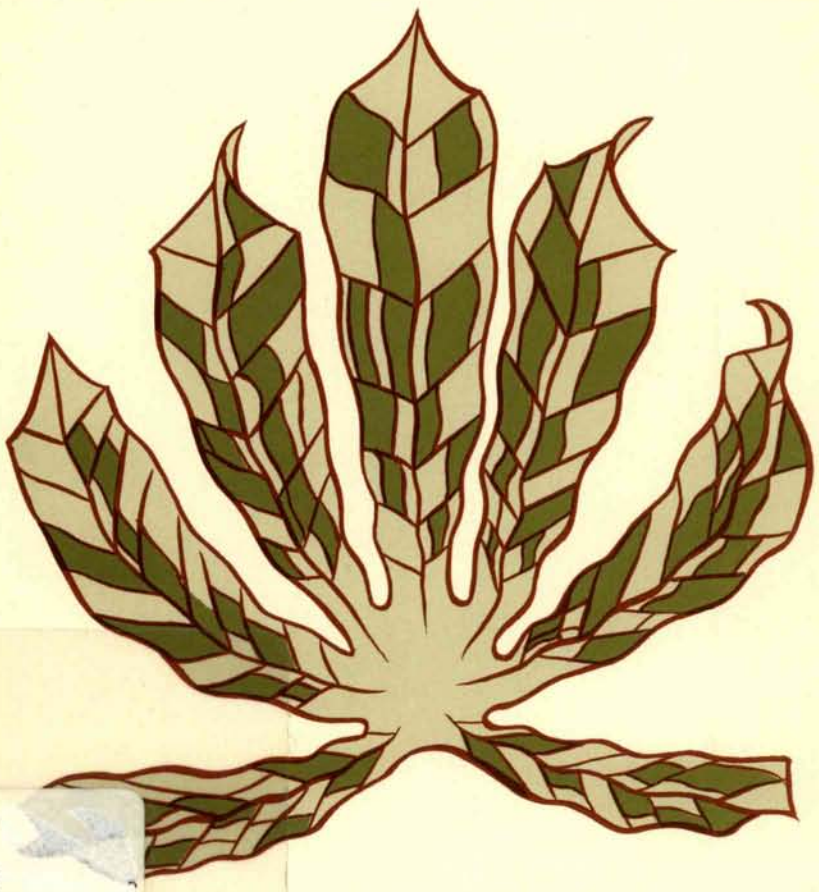
African Cassava MOSAIC



Report of an Interdisciplinary Workshop held at Muguga, Kenya, 19-22 February 1976

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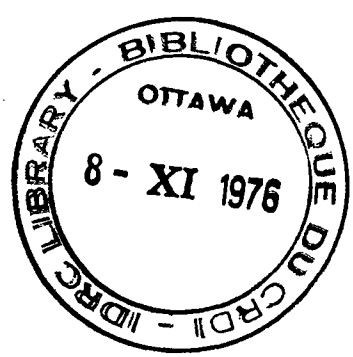

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Contents

In Vitro Techniques in the Control of Cassava Mosaic Disease

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Introduction

CASSAVA is a vital crop in many developing countries as a food source and is assuming increasing importance as an export product (Nestel 1973). Africa grows 35% of the world's production of cassava. The average yield of about 6 t/ha could be raised six to ten times this amount. One very significant factor contributing to low yield and which handicaps rapid expansion of cassava production is cassava mosaic disease (CMD) which may reduce yield by as much as 65–95% (Hahn 1972). The presence of this disease not only reduces yields, but also inhibits the movement of germ plasm for breeding purposes.

The effective solution to the mosaic problem requires the implementation of a wide range of both standard and new approaches to crop management and development. The present report focuses attention on the role that in vitro or plant cell culture methods might assume in cassava crop improvement with particular reference to cassava mosaic disease.

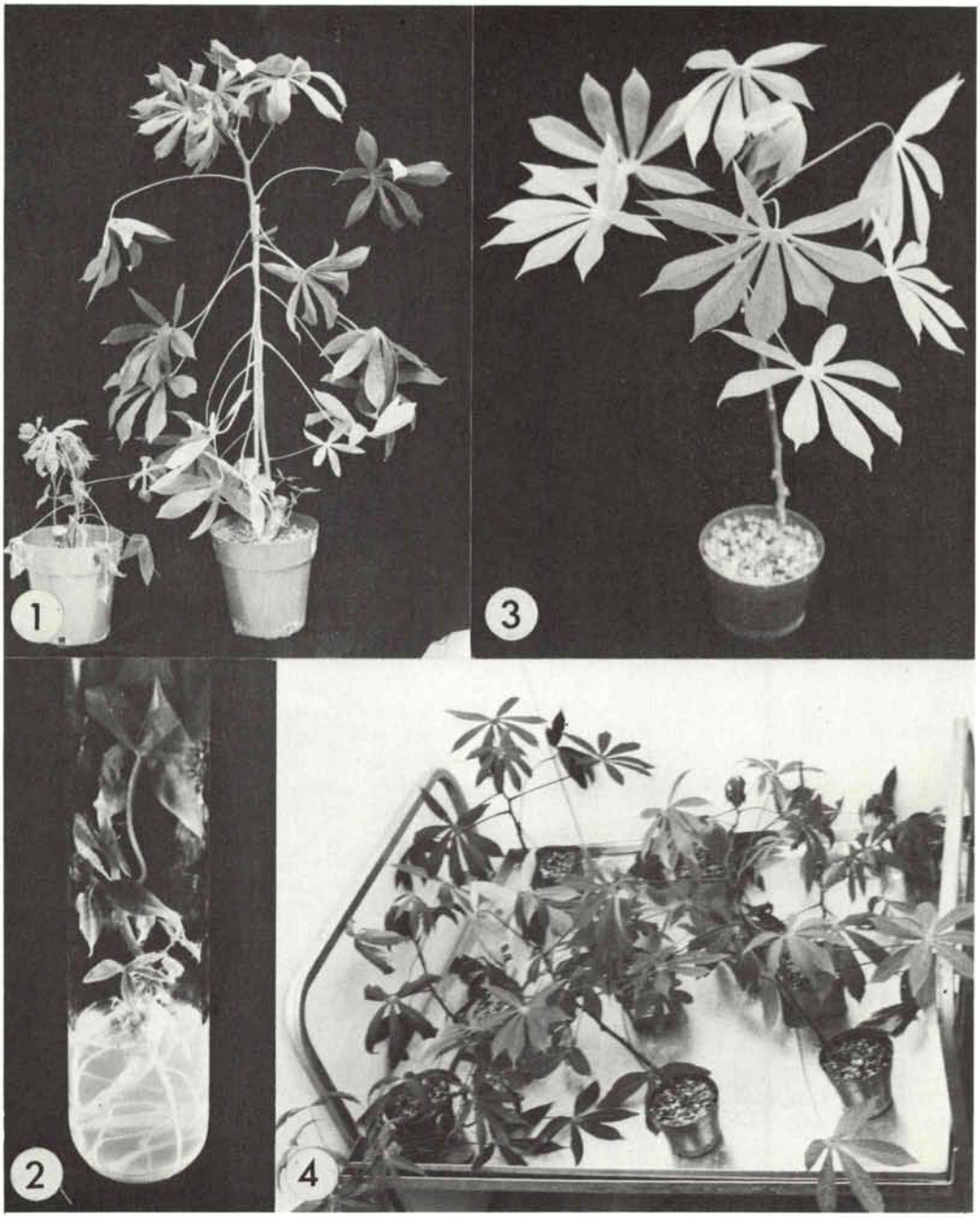
Recent advances in the development of in vitro techniques with higher plants permit their application to practical problems related to agricultural crops. Methods have become available for the growing of plant cells under controlled environmental conditions for indefinite periods. Using populations of diploid or haploid cells it is possible to carry out cloning and selection procedures analogous to those used with microorganisms. Since plants can be regenerated from cultured cells, the modifications induced in culture and expressed in the plants can be examined and the beneficial traits utilized.

Two of the immediate practical applications of tissue culture are the production of pathogen-free stock plant material by meristem culture, and the rapid propagation of new clones and cultivars by shoot tip and callus culture (Murashige 1974). Research has been undertaken in this laboratory to develop such methods for cassava. The meristem procedure that we have developed makes it possible to obtain large populations of disease-free plants (Kartha et al. 1974; Kartha and Gamborg 1975b). The results are presented and discussed below.

Elimination of the causal organism

The causal organism of CMD invades all tissues of cassava plants. The standard procedure of propagation from stem buds facilitates disease transfer from old to new plants. It has been known for some time that cells at the very tip of new shoots in plants are free of virus. These cell layers of the shoot tips can be removed, cultured in nutrient agar, and will then grow into complete plants. The method can be perfected to yield virus-free plants. This approach is used commercially for potato and several ornamental crops (Hollings 1965).

Although cells in the shoot tip region of higher plants have a high potential for division and formation of complete plants, the conditions must be ascertained for each species. Kartha et al. (1974) developed the method for regenerating complete plants from cassava which has been applied to Colombian cultivars and to infected plant material of Indian and African origin (Kartha and Gamborg 1975b).



Regeneration of mosaic disease-free cassava plant by thermotherapy coupled with meristem tip culture. 1—Cutting from diseased plant, cv. Kalikalan, grown under greenhouse conditions (*left*) and growth cabinet conditions (*right*) at 35°C for 30 days. 2—Cutting from mosaic-diseased plant, cv. Ogunjobi, grown at 35°C for 30 days. Note the disappearance of mosaic symptoms and the increased vegetative growth in 1 (*right*) and 2.3—In vitro regeneration of cassava plantlet from the meristem tip of a diseased plant grown at 35°C. 4—Mosaic disease-free cassava plants grown from in vitro culture of meristem tips.

The procedure consists of several steps (Kartha and Gamborg 1975a, b; Fig. 1–4) (*1*) Sections with two nodes cut from diseased stakes were planted in pots containing vermiculite and watered by nutrient solution: (*2*) The stakes were incubated at 26°C for 18 h at 70% RH to permit sprouting: (*3*) Sections of 0.2–0.5 mm were dissected aseptically from the shoot apex of the new sprouts under a microscope in a laminar flow cabinet: (*4*) The sections were placed on agar nutrient medium supplemented with hormones (benzyladenine, naphthalenacetic acid, gibberellic acid) in tubes and incubated at 26°C for 16 h at 70% RH: (*5*) Complete plantlets regenerated within 30 days and were transferred to pots and grown to mature plants: (*6*) Visual observations and diagnostic procedures were performed over a 6-month period. The diagnostic methods included transmission experiments by grafting scions from regenerated plants into stocks of healthy, susceptible cultivars and using appropriate controls.

Results

Data with cv Kalikalan (Indian cultivar provided by Dr M. R. Menon): From 150 cultured meristems, 135 plants were obtained. Of these 70 were symptom-free.

Data with cv Ogunjobi (Nigerian cultivar from Dr S. K. Hahn): From 45 meristems, a total of 42 plants were obtained and 40 of these were symptom-free.

Data employing high temperature: Further experiments were performed with meristems taken from sprouts of stakes incubated at 35°C for 30 days. Tip sections of 0.5–0.8 mm were used. Kalikalan — 50 meristems, 45 plants (all symptom-free). Ogunjobi — 50 meristems, 46 plants (all symptom-free).

Plant regeneration is highly reproducible and occurs at 90% efficiency. The percentage of symptom-free plants is 90–100.

These procedures were effective in yielding symptom-free plants. The plants also are free of any virulent or transmissible causal agent.

One or more types of virus have been implicated, but information on the causal agent(s) is sparse (Beck 1971). Until the causal organism can be fully identified, the elimination of the disease agent from a stock cannot be satisfactorily verified (Appendix A).

Liu (1975) at the Taiwan Sugar Research Institute reported recently on the success of using nearly identical procedures for regenerating plants from cassava cultivars grown in Taiwan. Reports have also appeared recently on similar although less successful methods to eliminate viruses in sweet potato, taro, cocoyam, and bananas (Alconero et al. 1975; Hartman 1974; Berg and Bustamante 1974).

Morphogenesis and propagation of healthy stock

The very high efficiency of plant regeneration from cassava meristems makes it feasible to employ the methods for the production of healthy stock (Appendix A). The further vegetative propagation of such stock can proceed by using tip cuttings and rooting hormones in humidity chambers. These chambers can be equipped with a mist system and fine gravel used as growth support (Wholey and Cock 1973).

The meristem culture operation requires relatively simple facilities and a modest working area (Appendix A). The propagation of healthy stock plants requires a larger area. Such an operation may be most economically established in a region in which vegetative growth can occur throughout the year. Other requirements are a location isolated from commercial cassava production and the absence of vectors transmitting the causal agent of mosaic. A comparable operation has been in existence for some time to produce healthy potato stock in Canada (Agriculture Canada Research Station, Vancouver, B.C.). At this station the meristem technique is used to obtain virus-free plantlets. The plantlets are then transferred to an isolated region in the interior of British Columbia where elite growers produce seed potatoes for commercial growers and for export to the United States and Holland. In tests with the healthy potato stocks, the reported yields reached 38% above that of infected plants. A similar arrangement can be envisaged for producing mosaic-free cassava stock.

Propagation by tissue culture In recent years methods have been developed for propagating plants through explants other than meristems (Murashige 1974). Procedures are employed for several horticultural crops by commercial growers. The explant material is often a shoot apex tissue. The plants obtained by this means are genetically the same. Two examples may serve to illustrate the prolific nature inherent in such procedures.

Earle and Langhans (1974) designed methods for obtaining multiple plantlets from shoot tips of chrysanthemums. These plants are normally propagated by cuttings using succulent shoots. The shoot tip technique enables one tip to produce 125 plantlets in 3 months, 15.000 in 6 months, and more than 200 million in 1 year. Dr Murashige,

who is one of the pioneers in devising these methods for commercial growers, also estimated that it should be possible to produce 300,000 plantlets of asparagus from a single shoot tip in 1 year (Hasegawa et al. 1973).

In addition to meristems and shoot tip morphogenesis, plant regeneration has also been achieved from leaf sections, e.g. tomato (Kartha et al. 1976) and rapeseed (Kartha, unpublished data). This approach has recently been applied to tobacco where plant regeneration occurred from sections of the dark green "islands" of tobacco leaves infected with TMV. Approximately half the plants were virus-free (Murakishi and Carlson 1976). Plant regeneration from callus sugar cane on a larger scale has been employed successfully to obtain new selections which are Fiji disease-resistant (Krishnamurthi and Tlaskal 1974).

Breeding resistant cultivars

The claim has been made that the most efficient, practical and economical attack on the mosaic disease problem is breeding for resistance (Beck 1971; Jennings 1972), and some success has been achieved in introducing resistance into cultivated varieties (Beck 1971; Bock and Guthrie 1976).

The germ plasm of most varieties is derived from *Manihot esculenta*, which apparently possesses no resistance. One of the wild species, *M. glaziovii*, is resistant to cassava mosaic. Crosses have been made between *esculenta* and *glaziovii* but natural barriers lower the efficiency of the process (Beck 1971) and resistance in the progeny may not persist (Hahn 1972).

The problems inherent in producing crosses between genera and species impose restrictions to crop improvement and are not limited to cassava. The transfer of desirable characteristics in plants is achieved by making sexual crosses. Incompatibility barriers of various types prevent wide crosses, which would be necessary for effectively introducing beneficial characteristics (Bates and Deyoe 1973). The most recent innovation of in vitro methods is the production of somatic hybrids between different plant genera and families (Gamborg et al. 1974). The process of hybridization involves a series of techniques in which protoplasts (cells with walls removed) are fused and on division form hybrid cells. The hybrids are isolated and recognized through the use of specific selection procedures. The final step is the growing of plants from hybrid cells.

Sufficient progress has been made to establish that fusion products from species of different plant families can form dividing cells. By fusion of protoplasts from two separate chlorophyll mutants of tobacco, both hybrid cells and subsequent plants were obtained. The hybrids were recognized as green plants produced as a result of genetic complementation (recombination).

Advances in the technology have reached the stage where predictions can be made about its potential use in plant-breeding programs. Somatic hybridization permits expanding the genetic base far beyond what is now possible by conventional methods. It allows for new and desirable crosses and thus increases the scope for crop development. This technique holds great potential, not only for introducing genetic variability but also for making crosses which at present create problems because of lack of floral development (e.g. yams).

Another procedure with a potential practical application is pollen culture. Both anther and pollen can be cultured to yield haploid cells. In some species plants can be regenerated directly from pollen. By applying chromosome doubling procedures, the technique becomes an expedient, facile, time-saving device in plant-breeding procedures (Kasha 1975).

To date there have been no reports on research on somatic hybridization and anther culture of cassava. Most of the research in these two fields has involved cereals, legumes, and plants such as tobacco. The technique may be particularly useful in the improvement of cassava and other root and tuber crops because of the difficulties in making sexual crosses and because a somatic hybrid can be readily multiplied by vegetative propagation.

Conclusions

Cassava mosaic disease causes substantial losses in yields of cassava, particularly in Africa. In vitro techniques can be employed to supplement conventional procedures for combatting disease problems. A new and efficient meristem culture method has been developed for obtaining plants free of cassava mosaic disease symptoms. The method can be utilized in the production of disease-free planting stock. Other tissue culture methods, such as morphogenesis and somatic cell genetic techniques hold potential in providing new and effective procedures to amplify conventional techniques in cassava crop improvement.

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Appendix A
Facilities and Equipment Required for a Unit Station to Produce Cassava Stock by Meristem Culture

A. Facilities

(1) An area for medium preparation, cleaning of glassware and storage facilities
(2) In the same or preferably in an adjacent area, space for a transfer cabinet and stereomicroscope
(3) Area for environmental growth cabinets and rooms
(4) An area for potting and growing plants

B. Equipment

(1) Laminar flow sterile transfer cabinet (available in several sizes and produced by a number of companies)
(2) Stereomicroscope
(3) Equipment for preparing sterile media
    a) autoclave

b) filter sterilizing units
c) sterilizing oven

(4) Growth cabinets. The number and size depends on the operation
Walk-in growth rooms, e.g. 10 × 14 ft (3 × 4.2 m) may be the most useful for meristem culture and plantlet formation.

(5) Small equipment – test tubes, flasks, knives and other dissecting instruments

(6) Refrigerator and freezer

(7) Pots and growing materials (vermiculite)

(8) Chemicals required for media presentation.