Conservation of Black Sigatoka tolerant Musa Spp. in Plant Tissue Culture

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Background

Banana is one of the most important agricultural commodities in Saint Lucia. It is grown extensively for local, regional and international markets. For the past half century banana exports brought in revenue that circulated throughout the length and breadth of Saint Lucia. At one point it was called “green gold” because of its profitability and far reaching benefits to the Island’s economy.

Over the past few years the industry has suffered tremendously for reasons ranging from change in global trading regimes to pest and disease challenges in particular Banana black Sigatoka. Given the importance of the sector to the economy it is felt that steps should be taken in preserving improve Musa Spp. that are believed and identified as being tolerant to Black Sigatoka. One approach is the conservation of selected Musa Spp. through the use of Plant Tissue Culture.

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History of Banana Tissue Culture

The first report ever on banana micropropagation was published by Ma and Shii, 1972, 1974 from Republic of China, Taiwan. The researchers used male flower explants and Kinetin plant growth regulator to induce shoots *in vitro* for subsequent banana plantlets. Banana tissue culture plantlets have several advantages. They have a higher survival rate than traditionally grown suckers, reduce the cost of disease and pest control, show uniform and vigorous growth, and have a shorter harvesting period.

Meristem culture can eliminate a number of pests and diseases by the explant cultures' rapid growth of pathogen-free tissues, which would surpass the growth and development of infected tissues in *in vitro* conditions. However, it is possible for viruses to be transmitted through micro propagated materials.

Micropropagation Protocol

The micropropagation of banana plantlets consists of five stages: the preparative stage, the culture initiation (establishment of the culture), the multiplication of shoots, the regeneration of plantlets and acclimatization of the plantlets in the nursery.

The Preparative Stage

Young suckers 2-3 feet tall are selected and uprooted from healthy and true-to-type mother plants. Subsequently the suckers are virus indexed (checked) for disease symptoms. (Sword suckers that are 2-3 feet tall are uprooted). This plant material is washed thoroughly in running water and detergent to dislodge all loose soil particles out-doors in order to prevent the spread of soil microorganisms inside the sterile environment of the laboratory. The suckers are then cut by trimming down (removing) the outer leaf sheathes (leaves) and corm with a (using) a sterile knife and then using alcohol to disinfect the blade after each cut to a desirable portion and a length (size) of approximately 30 cm (in height). After every cut, the trimmed psuedostem is also wiped thoroughly with tissue paper or cotton soaked in 75% alcohol, in order to maintain the corm and leaf sheathes initial disinfection. The plant material was packed in an upside-down position e.g., the corm tissue facing downwards on the psuedostem.

Culture Initiation

The outer layer of leaf sheathes (leaves) and the corm tissues of the sucker are removed. What remains is a piece 10 cm long and 6-8 cm in diameter. The plant material (piece) is wiped clean with 75% alcohol. Under aseptic conditions (in the transfer room), the sheaths and bases of the leaves are further trimmed to expose the meristematic region.

The shoot tip is decapitated, and a block of tissue measuring 1.5 x 1.5 x 1.0 cm is excised and inoculated onto multiplication medium.

Banana Initiation/Multiplication medium

Component	Quantity/Volume
Ma	100 ml
Mi	5 ml
MS-5	5 ml
MS-vit	5 ml
Inositol	0.1g
IAA	2 mg
BA	5 mg
NAA	-
pH	5.7
Agar/phytagel	6.5g/2g
Sucrose	30g

The explant is incubated at 26-28 ^{O} C with a 16-hour light and 8-hours dark cycle. After four or five weeks, 1shoot / bud can be induced from each explant.

Multiplication of Adventitious buds

The adventitious buds induced from the meristematic tissue can be divided into smaller pieces, such as large, medium and small, and sub-cultured onto fresh multiplication medium. The multiplication rate of buds is genotype dependent (depends on the cultivar); the multiplication rate also depends on the type and concentration of cytokinin, as well as (and) the number of subcultures. The number of subcultures should be limited to six or seven, in order to reduce the incidence of off-types arising through somaclonal variation.

Regeneration of plantlets

Elongation and rooting of adventitious buds is accomplished by adding semi-solid or liquid regeneration medium to the established shoot/bud culture. The regeneration medium:

Component	Quantity/Volume
HYPONEX 2 (20.20.20)	6g
pH	5.7
Sucrose	30g
Agar	6.5g
Charcoal	1.5g

Varieties identified

SABA

NAMWA

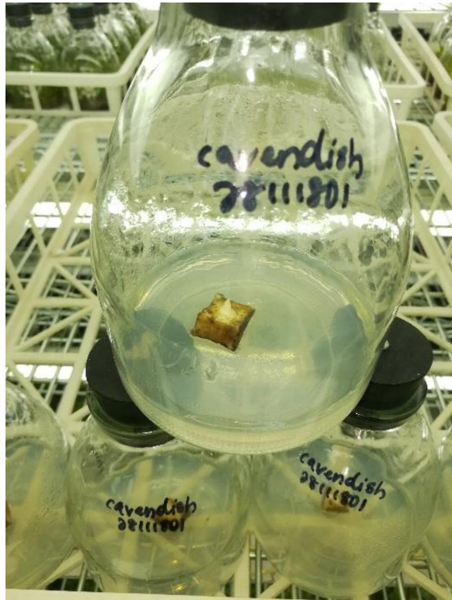
Other Varieties

Tissue Culture of Selected tolerant Musa Spp.

Laboratory procedures

SABA

NAMWA



Cavendish

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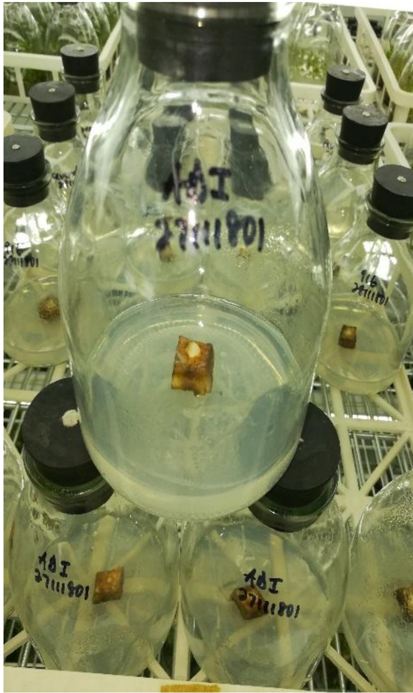

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