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Rapid Multiplication of Bananas and Plantains by *In Vitro* Shoot Tip Culture

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Abstract. **Rapidly multiplying cultures of dessert banana clones ('Philippine Lacatan' and 'Grande Naine') and plantain clones ('Saba' and 'Pelipita') were established from isolated shoot tips on a modified Murashige and Skoog medium supplemented with 5.0 mg/liter 6-benzylamino purine (BA). The growth rates of these cultures, expressed as increase in fresh weight over a 4-week period, were assessed. Rooted plantlets were produced using the auxins naphthaleneacetic acid (NAA), indole-3-butyric acid (IBA), or indole-3-acetic acid (IAA) at 1 mg/liter with low levels of activated charcoal (0.025% w/v).**

Bananas and plantains normally are propagated vegetatively using suckers of various sizes or pieces of the corm since the fruits of the edible clones are seedless. Since forcing buds under field conditions is a tedious and time-consuming procedure, the amassing of a sufficient amount of suitable planting material is slow at best (1, 8). This is a problem when a new clone is being generated to replace older plantings or when a large amount of planting material of a specific pathogen-tolerant clone is needed for field testing and assessment. The prevalence of disease problems and the need for generating clean planting stock in large quantities has stimulated recently a surge of interest in the production of clonal material of both cooking and dessert bananas by the use of aseptic micropropagation techniques (4). Published procedures emphasize the use of large shoot tip explants which are relatively slow to multiply (2, 5, 12). Moreover, the majority of effort has been placed on clones of dessert bananas destined for export (4).

We describe here a shoot multiplication procedure utilizing small primary explants which can produce thousands of clonal plantlets in a short period of time.

Apices from 2 important dessert banana clones ['Grande Naine' and 'Philippine Lacatan', AAA designation of Simmonds and Shepherd (9)] and 2 ABB plantains ['Saba' and 'Pelipita', ABB designation of Simmonds and Shepherd (9)] were isolated by removing the sheathing leaf bases which make up the pseudostem until the leaf sheaths became too small to remove by hand. The remaining leaves were removed with the aid of a dissecting microscope, forceps, and a scalpel. Shoot tips (the vegetative meristem plus 1 or 2 leaf primordia) were removed and surface-sterilized for 5 min in a 0.0525% solution of NaOCl (1% commercial Clorox) with a few drops of Tween 20. They were rinsed then 4 times with sterile distilled water and transferred to culture medium comprised of the mineral salts of Murashige and Skoog (6), here abbreviated as B_{MS}, supplemented with (per liter) 100 mg inositol, 40 g sucrose, 1 mg thiamine · HCl, and 5 mg BA; pH was adjusted to 5.8 and the medium solidified with 0.7% Difco Bacto agar. Fifteen ml of medium were poured into 30-ml, French square, screw-cap glass jars which were autoclaved for 20 min at 127°C. Cultures were maintained at 30° ± 2° at 50% relative humidity in a 16-hr daily illumination cycle at 10.2 Klx (Sylvania Grolux wide spectrum). Once the cultures had become established, they were moved into 100-ml screw-cap jars with 50 ml of medium and were subcultured routinely every 3 to 4 weeks by subdividing the shoot clusters with a few scalpel incisions and transferring the divided clusters to fresh medium.

The growth rate of established shoot cultures was monitored by weighing under aseptic conditions small shoot clusters representative of the type of tissue used during normal subculturing and placing them on maintenance medium. The tissue pieces were removed from the jars, reweighed, and returned to the same jar at weekly intervals. The increase in fresh weight and the increase as a percentage of original weight were calculated. Each clone had 16 replicates.

Isolated apices from each of the 4 clones almost always grew into individual small shoots (Fig. 1a). This was true even if they were cultured on media that contained high levels of cytokinins such as BA and/or kinetin. Multiple shoot cultures could be induced by longitudinally splitting a young cultured shoot through the apex and placing each half upright on semi-solid medium. I have shown that the addition of 5 mg/liter BA promotes rapid shoot multiplication (Fig. 1b) with each half shoot yielding, in the case of 'Pelipita', an average of 9.1 new shoots in 3 weeks. The other clones showed comparable rates of shoot multiplication.

Established cultures continue to multiply at a rapid rate. This is reflected by the increases in fresh weight during a 4-week culture period (Fig. 2). The 2 dessert banana clones ('Philippine Lacatan' and 'Grande Naine') and the plantain 'Saba' all had fresh weight increases in excess of 1000%, while the plantain 'Pelipita' showed a fresh weight increase of 500%. Although shoot cultures of 'Pelipita' did not multiply as rapidly as the other clones, it still yielded a very satisfactory number of new shoots.

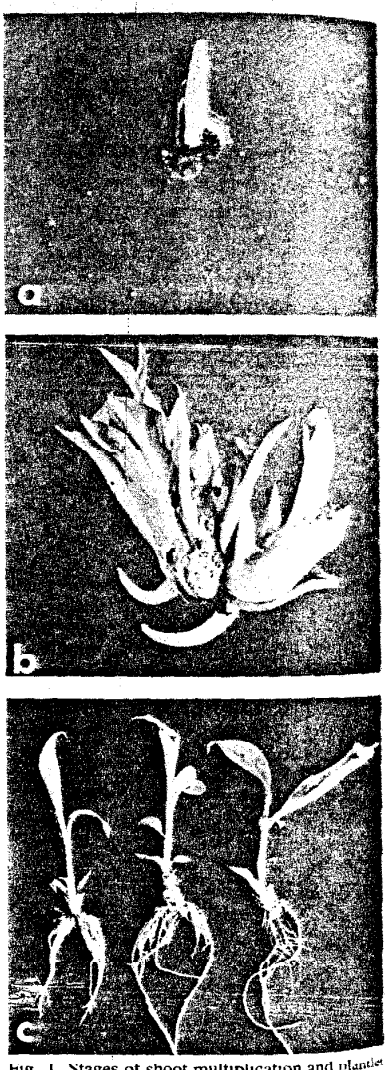

Fig. 1. Stages of shoot multiplication and plantlet production. **a.** 'Philippine Lacatan' banana shoot tip cultured for 34 days on semi-solid medium composed of B_{MS} + 5 mg/liter BA (×2). **b.** Representative piece of an established multiple shoot culture of 'Grande Naine' (×1); and **c.** Rooted 'Saba' plantlets 4 weeks after transfer to root-inducing medium (× 0.3).

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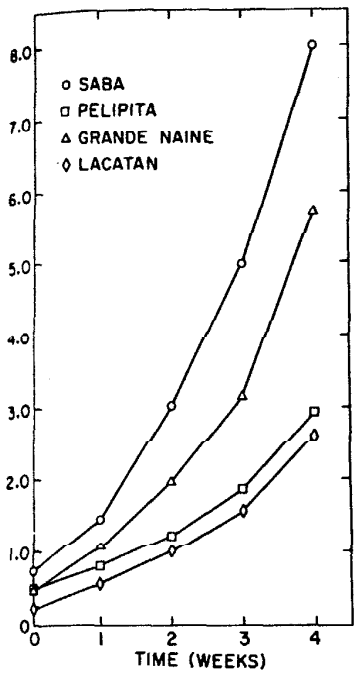

g. 2. Growth of shoot cultures of 4 *Musa* clones as reflected by the increase in fresh weight over a 4-week period. Each data point represents 16 replicates.

Plantlets were produced from all 4 clones by separating the clusters into individual shoots and transferring them to a root-inducing medium which contained, in addition to the basal medium, either NAA, IAA, or IBA at 1 mg/liter with a low level of activated charcoal (0.025%). Roots generally appeared within 5 to 7 days. A sufficient number of roots had formed on 100% of the shoots after an additional 1 to 2 weeks so as to enable transferral to the greenhouse. All of the plantlets survived transferral to the greenhouse when rooting was allowed to proceed for a total of 4 weeks (Fig 1c). These plantlets were potted in 1 Pro Mix BX (Premier Brands, Inc., New Rochelle, N.Y.) : 1 vermiculite in "3 square" plastic pots and placed in a misting bed on a 15 min cycle for 7 to 10 days. Afterwards they were moved to 23° day: 18°C night greenhouse conditions.

The major banana and plantain growing regions of this hemisphere currently are threatened by the spread of the disease 'Black Sigatoka' caused by the fungus *Mycosphaerella fijiensis* var. *difformis* (11). The clones 'Pelipita' and 'Saba' possess a high degree of resistance to this disease (7, 11). There has been a widespread shortage of suitable planting material because of the increased demand for these clones. Strict quarantine regulations in producer-exporter countries further exacerbates the problems of widespread germplasm transfer (10). Implementation of a multiplication system such as the one described here could help alleviate these shortages. It also will be useful in the rapid distribution of any new disease-resistant clones which may be produced via conventional breeding or aseptic culture-based efforts (3).

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