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In vitro micropropagation techniques for (*Musa* spp) Banana *grand-9 variety*.

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Abstract

It investigated the possibility of clonal banana propagation by tissue culture. Shoot tips isolated from the rhizomes were found to be sufficient for the *in vitro* development of plantlets. Excised shoot tips formed only one plantlet with the youngest leaves, but shoot tips regenerated several plantlets with several older sheathing leaf bases enclosing the axillary buds. A new crop of multiple shoots was produced by individual shoot lets, when separated and sub-cultured. The plantlets collected from both forms of ex-plants were successfully transplanted and matured into the soil. Shoot cultures are grown on MS (Murashige and Skoog) medium, supplemented with 30 g/l sucrose, 4.5 mg/l BA (6-benzyladenine) and 0.5 mg/l IAA (indole-3-acetic acid). In comparison with the culture medium on which shoot-tips are maintained, a tenfold decrease in cytokinin content (0.5 mg/l BA) induces regeneration of rooted plants. In contrast, adding 4.5 mg/l BA to the culture medium results in suppression of the apical dominance in shoot-tip cultures and a reduction of corm and leaf tissue between meristematic tissue.

Keywords: In vitro, Grand-9, micropropagation, Tissue culture, Murashige and Skoog.

Introduction

In recent years, clonal propagation of horticulturally important plants through shoot tip and apical meristem culture has significantly advanced (Murashige, 1974, 1978). However, (RODRIGUEZ W, et al., 1985) research on the clonal propagation by tissue culture of tropical fruit crops is very scarce and needs to be intensified. The

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banana is an example of a plant propagated vegetatively by suckers (ARIAS et al., 1987) The rate of propagation in nature is very sluggish, however. Defining the procedure necessary for a quick rate of in vitro propagation is highly desirable. This paper describes progress towards the efficient induction of plantlets from banana shoot tip explants. (ARIAS et al., 1987)

Material and methods

Banana (*Musa L.*) cultivar 'G-9', (SMITH, M.K et al., 1995) growing in the fields in Shimoga district, was utilized as the source material for obtaining shoot tips. sward suckers were carefully removed from field-grown banana plants and washed thoroughly with a solution of Tween 20. (ARIAS et al., 1987) All traces of Tween 20 were removed by repeated washing in running tap water. tissue was carefully removed with a stain less steel knife. Shoot tips, containing several sheathing leaf bases enclosing the axillary buds and subjacent corm tissue and measuring about 0.5—1.0 cm in length, were isolated. These shoot tips were washed with Tween 20 and surface-sterilized with hypochlorite solution for 15-20 min. (Ball et al., 1952) All traces of hypochlorite solution removed by washing several times with autoclaved distil- led water. From the sterilized shoot tips, 2 types of explants were prepared under aseptic conditions using stainless steel scalpels: (a) All the overlapping sheathing leaf bases and extra rhizome tissue were removed, leaving the youngest leaf bases covering the apical shoot bud; the explant thus prepared measured about 1-2 mm'. (b) Cut surface of the rhizomatous tissue and leaf bases were trimmed and the shoot tips finally contained at least 6-8 overlapping leaf bases enclosing the axillary buds. Morishige and Skoog's (1962) medium, containing sucrose (3%) and gelled with agar (0.8%), was used as the basal medium, which was further supplemented with benzyl adenine (BA), naphthalene acetic acid (NAA) and indole butyric acid (IBA), in various combinations. (BANERJEE et al., 1985) The pH of the medium was adjusted to 5.8. The medium was sterilized by autoclaving. Each treatment, containing 20 replicate cultures, was repeated twice. The cultures were incubated at 25 + 2° C in diffuse light (950-1000 lux) (BANERJEE et al., 1985).

Shoot-Tip Cultures

Stage 1: Initiation of shoot cultures

Banana shoot cultures typically start from any part of the plant containing a shoot meristem, (BHAGYALAKSHMI et al., 1995) i.e., the parental pseudo stem, small suckers, peepers and lateral buds. The inflorescence apex and axillary flower buds are also sufficient explants for the initiation of tissue culture. Overall, the collection of explanatory material from ideally mature individuals whose response to environmental factors is established and whose quality characteristics are determined by genotypical and environmental effects is essential (BHAGYALAKSHMI et al., 1995).

Shoot tips from young suckers of 40-100 cm height are most frequently used as explants for rapid in vitro banana multiplication. A cube of tissue of about 1-2 cm3 containing the apical meristem is excised from the

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selected sucker. This tissue block is dipped in 70% ethanol for 10 s, sterilized on the surface in a 2% sodium hypochlorite solution, and rinsed in sterile water three times for 10 min after 20 minutes. Variants of this protocol for decontamination exist (MORA et al., 1986). They vary in the form and size of the explant, the method of disinfection (single or double sterilization), the type of disinfectant (calcium hypochlorite instead of sodium hypochlorite) and its concentration and length of treatment. A shoot tip of approximately 1-2 mm, consisting of an apical dome covered with many leaf primordia and a thin layer of corm tissue, is then dissected aseptically. The merit of larger explants is that they consist of a shoot apex with more lateral buds that rapidly grow into shoots (MORA et al., 1986).

The optimum explant size depends on the intent. A comparatively larger explant (3-10 mm) is desirable for rapid multiplication, considering its higher sensitivity to blackening and contamination. Meristem-tip culture is the preferred choice when virus or bacteria removal is needed. The size (0.5-1 mm length) of the explant is then further decreased, leaving a meristematic dome with one or two leaf initials. The downside of Meristem cultures is that they might have a higher mortality rate and an initial slower growth rate.

The explant is placed directly on a multiplication-inducing culture medium. For banana micropropagation, (Murashige, et al 1974). M S-based media are widely adopted. Generally, they are supplemented with sucrose as a carbon source at a concentration of 30 g/l. Banana tissue cultures often suffer from excessive blackening caused by oxidation of polyphenolic compounds released from wounded tissues (Murashige, et al 1974). These undesirable exudates form a barrier round the tissue, preventing nutrient uptake and hindering growth. Therefore, during the first 4-6 weeks, fresh shoot-tips are transferred to new medium every 1–2 weeks. Alternatively, freshly initiated cultures can be kept in complete darkness for three weeks. Antioxidants, such as ascorbic acid or citric acid in concentrations ranging from 10–150 mg/l, are added to the growth medium to reduce blackening, or the explants are dipped in antioxidant solution prior to their transfer to culture medium.

Usually two types of growth regulators, a cytokinin and an auxin, are added to the banana growth medium. Their concentration and ratio determine the growth and morphogenesis of the banana tissue. We routinely add 4.5 mg/l 6-benzyladenine (BA) and 0.5 mg/l indole-3-acetic acid (IAA) to the initiation medium.

Stage 2. Multiplication of shoot-tip cultures

The formation of multiple shoots and buds is promoted by supplementing the medium with relatively high concentrations of cytokinin. (Raman et al., 1985). In banana, BA is the preferred cytokinin and is usually added in a concentration of 0.1–20 mg/l. For the multiplication of propagules, we use the same medium as for the initiation of shoot (Raman et al., 1985). If the production of highly proliferating meristem cultures is required. Higher concentrations of the cytokinin BA tend to have an adverse effect on the multiplication rate and morphology

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of the culture and should therefore be avoided.

The rate of multiplication depends both on the cytokinin concentration and the genotype. In general, shoot tips of cultivars having only A genomes produce 2-4 new shoots, whereas cultivars having one or two genomes produce a cluster of many shoots and buds at each subculture cycle. Approximately 6-12 weeks after culture initiation, depending on the initial explant size, new axillary and adventitious shoots may arise directly from the shoot-tip explant. Clusters can be separated, trimmed and repeatedly sub cultured at 4-6-week intervals.

Stage 3: Regeneration of plants

Individual shoot or shoot clumps are transferred to a nutrient medium which does not promote further shoot proliferation but stimulates root formation. The cytokinin in the regeneration medium is greatly reduced or even completely omitted. Within 2 weeks, shoot tips develop into unrooted shoots. To initiate rhizogenesis IAA, NAA (2-naphthalene acetic acid) or IBA (indole-3-butyric acid) are commonly included in the medium at between 0.5 and 2.0 mg/l. We use the same auxin concentration as in the proliferation medium (0.5 mg/l IAA), but a tenfold lower BA concentration (0.225 mg/l). For some genotypes (*Musa* spp.) that produce compact proliferating masses of buds, activated charcoal (0.1–0.25%) is added to the regeneration/rooting medium to enhance shoot elongation and rooting. After rooting, plants are hardened *in vitro* for 2-4 extra weeks on the regeneration/rooting medium prior to transplantation to soil.

Contamination:

Contamination in tissue cultures may be caused by endogenous bacteria that escape initial disinfection or by micro-organisms introduced during tissue-culture manipulations. Both types of contaminants may survive in the plant material for several subculture cycles and over extended periods of time without expressing symptoms in the tissue or visible signs in the medium (VAN DEN HOUWE et al., 1998).

'Internal' bacteria are a considerable source of concern in all aspects of plant cell, tissue and organ culture because they hinder the international exchange of germplasm or become a nuisance when contaminated tissues are used as explant material for cryopreservation or for the initiation of embryogenic cell suspensions (VAN DEN HOUWE et al., 1998). It is therefore important that control measures are taken at every tissue-culture step. At the, plant material is tested for endophytic bacteria on a broad-spectrum bacteriological medium at tissue culture initiation and during annual subculturing. Prior to placing each shoot-tip on culture medium, the base of the explant is streaked onto nutrient agar (VAN DEN HOUWE et al., 2000). Testing reveals the presence of cryptic contaminants in 5% of the stored germplasm. In mass propagation systems, positive stock materials should immediately be destroyed. However, germplasm in collections or cultures that are not readily replaceable with fresh material (e.g., from field or from greenhouse stock plants) are 'cleaned up' (VAN DEN HOUWE et al., 2000).

Some antibiotics are successful in controlling bacterial contaminants in banana

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tissue cultures. Streptomycin (100 mg/l) added to liquid cultures for 10–30 days was found most suitable for controlling frequently occurring Gram-positive bacteria in banana shoot tips without affecting plant growth (VUYLSTEKE et al., 1989). However, culturing small meristem tips (1 mm) isolated from contaminated *in vitro* plants or from greenhouse plants obtained from contaminated *in vitro* plants was found to eliminate any bacterial contaminant (VUYLSTEKE et al., 1993).

Results

Murashige and skoog media SB supplemented with BAP (5 mg /l) + IBA (0.5 mg/l) remained quiescent for nearly 2 weeks. After about 3-4 weeks, ex-plants showed signs of growth and the first pair of leaves emerged (Fig. 1 A, B). At the end of 6 weeks, leaves were fully formed (Fig. 1 C) and eoneomitantly, root development took place (Fig. 1 D). A single fully-developed plantlet resulted after about 7-8 weeks, which was the same as for apical shoot buds cultured on MS supplemented with IBA (0.5 mg/1). and after root formation the plants will be taken into primary hardening process. (Fig. 1 E) The time course of plantlet development was also similar. A variety of combinations and concentrations of cytokinin and auxins failed to induce multiple plantlet formation from apical buds. Some of the *in vitro* produced plantlets have been successfully transplanted to soil and raised to maturity. (Fig. 1 F)

Socialization and training activities are able to increase the knowledge, insight and skills of farmers and students about the propagation of bananas in vitro, propagation by suckers, mycorrhiza applications and enlargement of banana seeds.

Discussion

The present investigation as have demonstrated. The terminal buds produce only one plantlet, whereas a larger explant with axillary buds can produce multiple plants. decapitation was necessary for releasing axillary buds from apical dominance. However, in the present investigation decapitation was not necessary. Multiple plantlets have also been produced from isolated axillary buds in grand 9 banana variety, *musa spp.* The ability to regenerate plants from the excised shoot tips is well documented. Shoot tips will regenerate when cut into 4 or more equal segments. Similar responses obtained with banana suggest that this technique could be used for rapid multiplication of clones with desirable qualities. All techniques of banana propagation have limitations ranging from low multiplication rates, genetic fidelity, disease elimination and affordability of the generated suckers. If the central objective of the seed system is to generate high quality planting materials, tissue culture technique offers the highest seed quantity and quality and therefore, is highly recommended. The technique can be integrated in the downstream propagation and dissemination of cleaned suckers to address the problem of seed quantity and quality.

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Fig. l: A

Fig. l: B

Fig. l: C, D

Fig. l: E

Fig. l: F