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The Influence of Explant Types and Orientation on *in Vitro* Culture of *Musa balbisiana* 'Kluai Hin' (BBB group)

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Abstract

Inflorescence, apical and lateral buds of *Musa balbisiana* 'Kluai Hin' (BBB group) were used to culture on MS medium supplemented with 22 µM BA and 15% (v/v) coconut water. Comparison of bud orientation showed that the best response of *in vitro* shoot tip proliferation was obtained with abaxial surface of buds lying down i.e. one side touching the medium (tilt). Mass propagation of shoot tips was obtained when cultured buds on MS medium containing 44 µM BA. Rooting was achieved by transferring the isolated shoots to MS basal medium without growth regulators. Rooted plantlets were acclimatized and successfully established in soil.

Keywords: banana, bud culture, inflorescence culture, organogenesis, orientation

Introduction

Recent advances in plant biotechnology have had a great impact on crop improvement and cultivation. Dessert bananas and plantains (*Musa* spp.) are important export commodities in many tropical and subtropical countries. Edible bananas have evolved from two diploid *Musa* species, *Musa acuminata* Colla. and *Musa balbisiana* Colla. (Simmonds, 1966). Banana fruit production is threatened by many diseases caused by fungi, bacteria, or viruses and pests (Robinson, 1996). *Musa balbisiana* 'Kluai Hin' is a native banana known only in the lower south of Thailand and 'Kluai Hin' is seedless, tasty, and highly priced. It is found to have a sporadic distribution along the Pattani River and hence facing the risk of losing accessions due to the collapse of the bank. 'Kluai Hin' is propagated vegetative by suckers and not sufficient to overcome the treat of extinction. The development of tissue culture technology can be utilized for *M. balbisiana* 'Kluai Hin' improvement and the demand for huge planting materials.

In this communication, we reported the influence of explant types and orientation to optimize the *in vitro* shoot induction for efficient regenerating *M. balbisiana* 'Kluai Hin'.

Materials and methods

Plant materials

Suckers and inflorescence of *M. balbisiana* 'Kluai Hin' were collected from a commercial plantation located in Bannangsata District, Yala Province (Fig. 1a). Suckers were cut into pieces of 20 cm diameter and 25 cm long and washed in running water to remove dirt. They were trimmed down to 5-6 cm long until lateral buds were exposed (Fig. 1b). Tissue blocks (2 cm^3) containing either lateral buds or apical buds were immersed in 70% ethanol for 30 sec then surface sterilized using a dilution of commercial bleach 10% (v/v) Clorox™ containing 5.25% active chlorine and 2 drops of Tween 20 per 100 ml solution for 15 min, followed by 5% (v/v) Clorox™ for 10 min. After the surface decontamination was done, these explants were rinsed 3 times with sterilized distilled water to remove traces of disinfectant and transferred to culture medium. The inflorescence of *M. balbisiana* 'Kluai Hin' (Fig. 1c) was prepared by removing the enveloping bracts until the length of the inflorescence was 4-5 cm**.** The surface sterilization of inflorescence was the same as suckers. After the surface sterilization was done, explants were transferred to culture medium (Fig. 1d).

Media and culture conditions

Inflorescences, apical and lateral buds were first cultured on MS (Murashige and Skoog, 1962) medium supplemented with 22 µM BA and 15% (v/v) coconut water (CW). For rapid shoot multiplication 4.4, 22 or 44 µM BA and 15% (v/v) CW were used either singly or in combination. All media contained 3% sucrose and solidified with 0.9% commercial agar. The pH of all media was adjusted to 5.8 with 0.1 N NaOH or 0.1 N HCl prior to autoclaving at 1.05 kg/cm^2, 121°C for 20 min. Cultures were incubated at 25°C with a 16-h photoperiod under an illumination of 20 µmol m^{-2} s^{-1} from white-fluorescent lamps.

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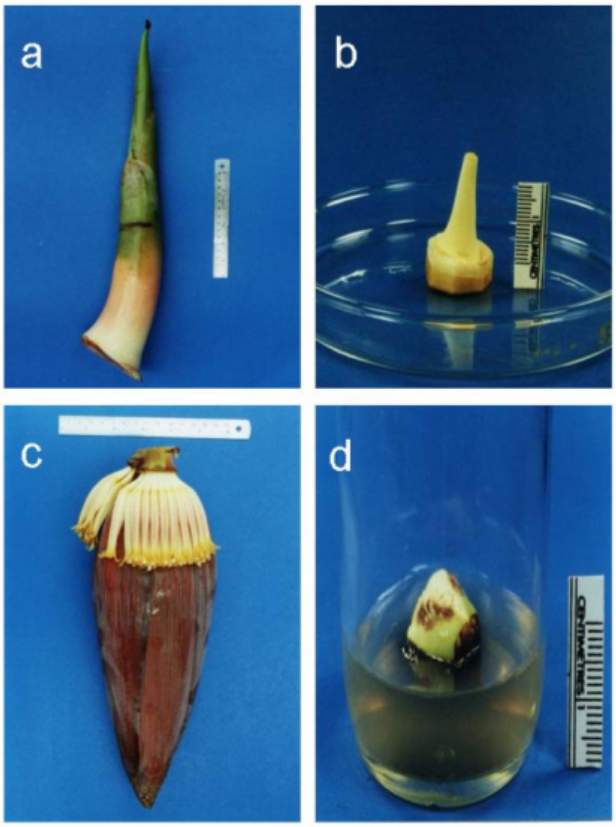

Fig. 1. Plant materials of *M. balbisiana* 'Kluai Hin'. a) Sucker excised from mother plant; b) Sucker was trimmed down to 5-6 cm long containing lateral buds; c) Inflorescence excised from mother plant; d) Inflorescence explants produced phenolic compound in the culture medium

Effect of orientation of explants on shoot multiplication

Buds were cultured on MS medium supplemented with 22 μM BA and 15% (v/v) CW at different orientation with the abaxial surface down i.e. one side touching the medium (tilt) or both sides in contact with the medium (down). Some explants revealed an orientation with the abaxial surface up i.e. both sides not touching the medium (up). While some explants had one side pushed into the medium (vertical) (Fig. 2).

Root induction and acclimatization

Isolated shoots with 2-3 leaves from multiple shoots were transferred to MS basal medium for root induction. Rooted plants were acclimatized in sterilized vermiculite. Healthy plantlets were established in potting soils and transferred to field.

Statistical analysis

One explant was implanted per culture and all experiments were conducted on three different days with 20 replicates per treatment. Data were submitted to ANOVA and the difference between the means was compared using Duncan's multiple range test (DMRT).

Results and discussion

Effect of explant types

Inflorescences, apical and lateral buds were cultured for shoot induction on MS medium supplemented with 22 μM BA and 15% CW. It is most likely that all explants showed the same morphogenetic responses except for slight variation. During the first two weeks, all buds swelled considerably due to the development of leaf primordia and changed from white cream to green color and shoots developed from these buds within forty-nine days (Fig. 3 a, b). The development of shoots from inflorescence took eighty-four days (Fig. 3 c, d). This is in agreement with our previous finding that apical and lateral buds of *M. acuminata* 'Kluai Hom Thong' (AAA group, 'Gros Michal') when cultured on MS medium containing 22 μM BA and 15% CW developed to shoot within forty-two days whereas inflorescence developed to shoot within seven months (Kanchanapoom and Chanadang, 2000). Therefore lateral and apical buds were selected for further experiments. Silayoi (2001) also reported that the growth of inflorescences of *M. acuminata* 'Kluai Khai' took longer time than buds from sucker when cultured in both liquid and semi solid medium. At the initial stage of culture, all buds produced phenolic substances into the media and inflorescences produced phenolic exudates in higher amount than buds. The problem of phenolic compounds exudated to the medium could be overcome by the frequent subcultures at 3-week-interval to new fresh medium.

Effect of orientation of explants on shoot multiplication

After 5-7 weeks on BA-CW containing media, bulge buds were halved, transferred to fresh media, serial subcultures were made every 3 weeks. In *M. balbisiana* 'Kluai Hin', shoot tip proliferation was observed in all cultured buds in regard to their orientation after inoculation. Tilt placement (5.8±0.8 shoots/explants) of buds was found to be superior to other placement for shoot tip proliferation ($p \leq 0.05$). Up orientation and control (intact bud) were significantly less effective ($p \leq 0.05$) in shoot multiplication when compared to other orientations (Tab. 1). Results revealed that in *M. balbisiana* 'Kluai Hin', bud positioning is an important critical factor in determining the efficiency

Tab. 1 Effect of explant orientation on shoot multiplication of *M. balbisiana* 'Kluai Hin'

Orientation of explants	Shoot number (Mean ± SE)
Tilt (one side touching the medium)	5.8 ± 0.8^{a}
Down (both sides contact with the medium)	5.3 ± 0.5^{ab}
Vertical (one side pushed into the medium)	4.8 ± 0.7^{ab}
Up (both sides not touching the medium)	3.0 ± 0.6^{bc}
Control (intact buds)	1.0 ± 0.0^{c}

The different letters within column show significant differences by ANOVA and Duncan's multiple range tests at $p \leq 0.05$

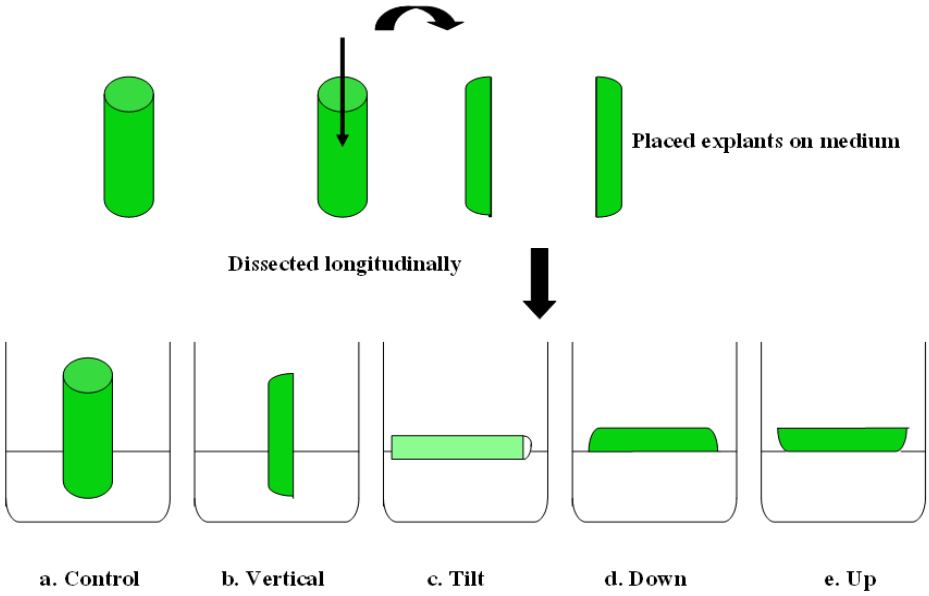

Fig. 2. Effect of explant orientation on shoot multiplication of *M. balbisiana* 'Kluai Hin' buds on response in culture

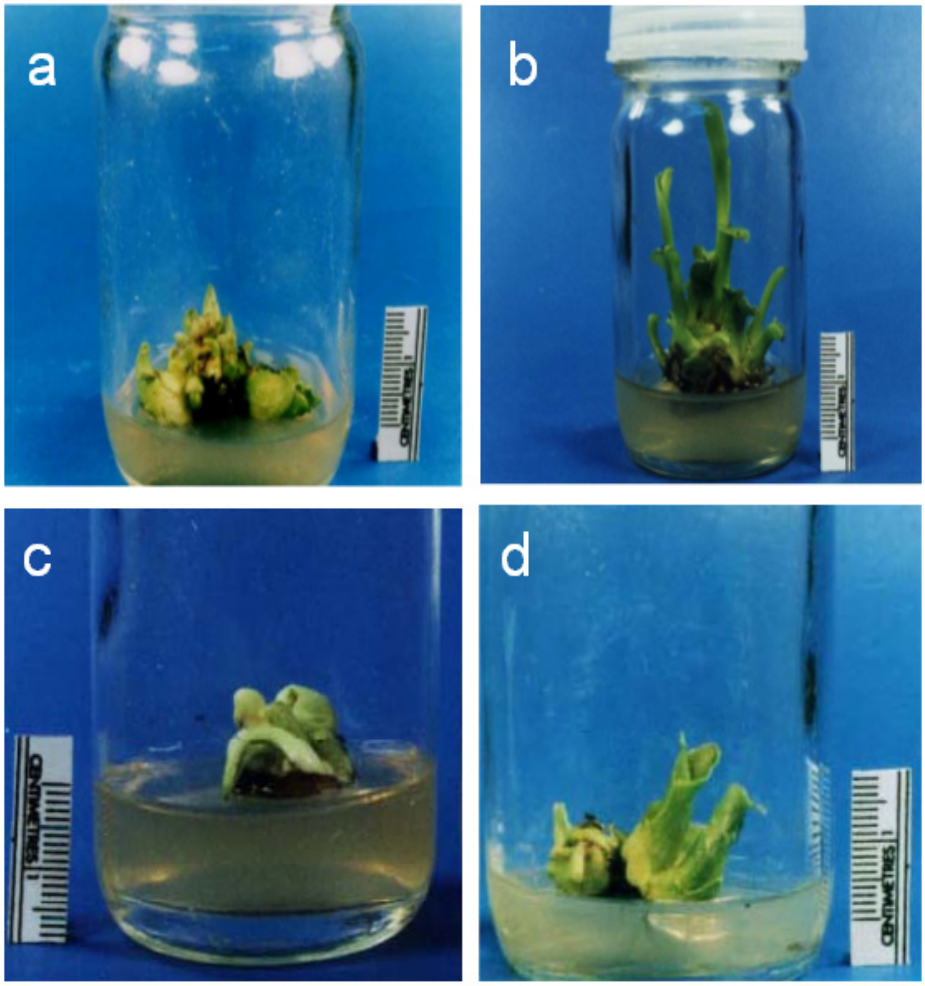

Fig. 3. Development of multiple shoots in *M. balbisiana* 'Kluai Hin'. a) Small shoots developed from apical buds after cultured for 15 days; b) Same as a) after cultured for 49 days; c) Inflorescence enlarged after cultured for 15 days; d) Same as c) after cultured for 84 days

of shoot tip regeneration from cultured buds. Effects of orientation in response to *in vitro* culture have been reported in many plants such as *Oryza* (Mercy and Zapata, 1987) and *Brassica* (Arnison *et al*., 1990). However, there has been one report on the orientation of banana embryos (Asif *et al*., 2001). According to this report, embryo orientation did not have significant effect on number of days to shoot emergence, number of roots and root length.

Tab. 2. Effect of different combinations of BA and CW on shoot multiplication in *M. balbisiana* 'Kluai Hin' bud explants cultured on MS medium

BA (μM)	CW (%)	Number of shoots (Mean±SE)
0	0	2.0 ± 0.2^{e}
4.4	0	5.4 ± 0.9^{d}
22	0	15.3 ± 2.5^{b}
44	0	21.2 ± 4.1^{a}
4.4	15	3.8 ± 0.6^{d}
22	15	11.0 ± 2.9^{c}
44	15	12.3 ± 3.6^{c}

The different letters within column show significant differences by ANOVA and Duncan's multiple range tests at $p \leq 0.05$

Shoot multiplication and root induction

BA and CW concentrations in the culture media influenced axillary shoot production. BA at 44 μM gave the highest number of shoot production (21.2). CW did not enhance axillary shoot production (Tab. 2). No root formation was observed in all treatments. By successive subculture, masses of proliferating shoot cultures have been established. The single isolated shoots thus obtained from inflorescence and bud experiments on BA-CW supplemented media developed thick, long and fibrous roots *per se*. Rooted shoots were acclimatized in sterile vermiculite for 3 weeks before being transferred to potting soil. All plants derived from tissue culture survived and were morphologically the same as mother plants.

In conclusion, a micropropagation system for *M. balbisiana* 'Kluai Hin' has been worked out utilizing bud and inflorescence explants. Shoot tip proliferation was observed in all cultured buds in regard to their orientation. Micropropagated plants were rooted and established in soil successfully.

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