Conservation and Micropropagation of *Musa balbisiana* 'Kluai Hin'

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Lateral and apical buds of banana, *Musa balbisiana* 'Kluai Hin', were cultured on MS (Murashige and Skoog, 1962) medium supplemented with 22 µM BA and 15% (v/v) CW. The results showed that apical and lateral buds were suitable starting materials. MS medium supplemented with 44 µM BA and incubated at 25 $\pm$ 2°C with a 16-h photoperiod for 12 weeks was suitable for micropropagation of 'Kluai Hin' since 21.22 shoots per explant were obtained. Whereas, explants cultured on MS medium supplemented with TDZ differentiated to clusters. The storage of shoots over cotton saturated with 10 g l^{-1} sucrose and water at 25°C and a 16–h photoperiod could extend the survival time for 6 months. Surviving shoots were transferred to MS medium without plant growth regulators and then rooted normally. Rooted shoots, after acclimatization with vermiculite, had 100% survival when transplanted in the field.

Key words: *Musa balbisiana*, storage, tissue culture

Introduction

Dessert bananas and plantains (cooking bananas) are an important staple food crop in tropical and subtropical countries. Edible bananas evolved from two wild species, *Musa acuminata* Colla and *Musa balbisiana* Colla (Simmonds and Shepherd, 1956). Banana fruit production is limited by many diseases, pests, and traditional propagation methods. *Musa balbisiana* 'Kluai Hin'(BBB group), a leading commercial variety, is so far known only in the lower south of Thailand. It is found to have a sporadic distribution along the Pattani River and hence is facing the risk of losing accessions due to collapse of the river bank. It is propagated vegetatively by suckers and this is not sufficient to overcome the treat of extinction. Recent advances in biotechnology for crop improvement have had a great impact on plant cultivation. The development of tissue culture applications can be utilized for banana improvement and to supply the huge demand for planting materials. *In vitro* storage of plant germplasm is an alternative way to maintain a gene bank of plants that are threatened and for sustainable utilization. Different *in vitro* conservation methods such as cryopreservation, alginate encapsulation, and slow growth have been developed in recent times. Slow growth has been widely employed in bananas to reduce growth and prolong subculture intervals (Van den Houwe et al., 1995). In this communication we describe the usual *in vitro* strategies for propagation of *Musa balbisiana* 'Kluai Hin' by adventitious shoot formation from buds and the storage conditions for axillary bud conservation.

Methodology

1. Plant materials and micropropagation

The suckers of *Musa balbisiana* 'Kluai Hin' were used in this research. Suckers were cut into pieces (20-cm in diameter and 25-cm long) and washed in running water to remove dirt. Suckers were excised by removing the outer layers of tissue until the lateral buds were exposed. Tissue blocks (2 cm^2) containing lateral buds were cut off and suckers were further trimmed down to 5-6 cm long. Under aseptic conditions, tissue blocks and a portion containing the apical bud were immersed in 70% ethanol for 30 sec. Then, they were surface sterilized using a dilution of 10% (v/v) Clorox and 2 drops of Tween 20 per 100-ml solution for 15 min., followed by 5% (v/v) Clorox for 10 min. After sterilization, these explants were rinsed 3 times with sterilized distilled water to remove traces of disinfectant. Leaf sheaths were discarded until the diameters of the explants were about 1.5 cm, and then transferred to culture medium.

Apical and lateral buds were aseptically 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, 44 µM BA or 0.1, 0.5, 1, 5, 10 µM TDZ in combination with 15% (v/v) CW were used as the plant growth regulators either singly or in combination. All media contained 3% sucrose and was 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^{o}C for 20 min. All plant materials were cultured in 330 ml screw-topped jars each containing 20 ml of medium. One explant was implanted per culture and 10 cultures were raised for each treatment. Cultures were incubated at 25^{o}C with a 16-h photoperiod under an intensity of 20 µmolm^{-2}s^{-1} from white-fluorescent lamps (Philips 36W/54 Daylight). All experiments were conducted on three different days.

2. Slow growth treatment

The single isolated shoots obtained from the previous experiment were first transferred to MS medium supplemented with 4.4 µM BA for 16 days to heal the cut wound before being used in further experiments. These single shoots were then transferred to 330 ml screw-topped jars containing cotton submerged with 20 ml of distilled water, sucrose, glucose and sorbitol, with each sugar at the concentrations of 10, 30, or 50 g l^{-1}, respectively. A Parafilm M was wrapped around the jar lid to protect the isolated shoot from drying. The jars were stored under the following conditions: 4^{o}C (cold room) and in the dark; 16–h photoperiod at 4^{o}C and 20µmol m^{-2}s^{-1}; 25^{o}C and in the dark; 16–h photoperiod at 25^{o}C and 20µmolm^{-2}s^{-1}. After 6 months storage, stored shoots under the described conditions were transferred to MS medium supplemented with 22 µM BA and cultured for 8 weeks. Shoots from all treatments were taken from the culture vessels and planted in potted soil hereafter. The percentage of shoot survival was calculated for all treatments.

3. Root induction and acclimatization

Isolated shoots with 2-3 leaves from multiple shoots were transferred to MS basal medium for root induction. When adequate shoots and roots were obtained, the plantlets were transferred to 330 ml screw-topped jars containing sterile vermiculite for 2 weeks for hardening. Healthy plantlets were established in potting soils and transferred to the field.

4. Statistical analysis

The experiments were repeated three times according to a completely randomized design (CRD). One-way ANOVA was used to analyze the effect of treatment, and comparisons among specific pairs of treatment mean was made by Duncan's multiple range test (DMRT).

Results

1. Micropropagation

Clonal propagation by adventitious shoot induction was obtained on lateral and apical buds. All buds showed the same morphogenetic responses except for slight variation. After 3-4 weeks on MS medium supplemented with 22 µM BA and 15% CW, bulge buds were halved, transferred to fresh media, and serial subcultures were made every 3 weeks. By successive subculture, masses of proliferating shoot cultures were established. The cytokinin types and concentrations in the culture media dramatically influenced axillary shoot production after 12 weeks of culture. The data are presented in fig. 1. Each tested cytokinin promoted shoot regeneration with the concomitant concentrations used in the medium. Within the concentrations tested, the number of shoots was highest at 44 µM BA (21.22), then at 22 µM BA (15.3), and lowest at 4.4 µM BA (5.44). Although BA at 44 µM gave the highest number of shoot production, it was not significantly better than 22 µM BA. Experiments were conducted to determine in different combinations of BA and 15%CW. The results revealed that CW in combination with BA did not enhance axillary shoot production when compared to BA alone since an average of 12.33 shoots was recorded at 44 µM BA and15%CW. Furthermore, shoots obtained on MS medium supplemented with CW and BA were larger than BA alone. No root formation was observed in all treatments.

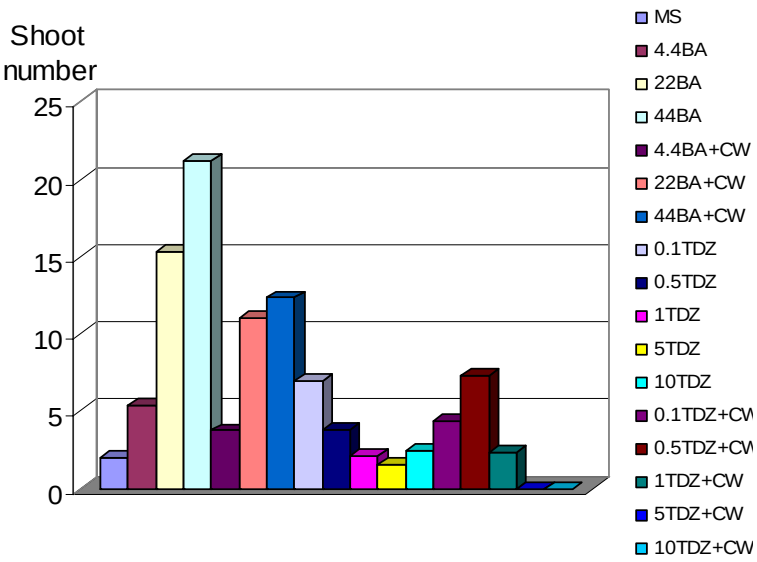

Figure 1. Effects of plant growth regulators on shoot multiplication of 'Kluai Hin' Bars represent means of shoot number ± standard error.

From TDZ experiments, the number of axillary shoots per explant was greater for TDZ at low concentration (0.1 µM) compared to TDZ at a higher concentration than 0.1 µM. An increase in TDZ level progressively decreased shoot number and retarded shoot elongation. When CW was incorporated into the TDZ containing medium, explants showed poor shoot multiplication except in the treatment with 0.5 µM TDZ whereas no shoots were produced at 5 and 10 µM TDZ. Similarly all TDZ and CW concentrations hindered root growth.

2. *Slow growth treatment*

Sucrose, glucose and sorbitol solutions at 10, 30, or 50 g l^{-1} were used to test for their ability to cause slow-growth of tissue culture-derived shoots. The results showed that storage of shoots over cotton saturated with sugars and water could extend survival time for 6 months and sucrose was the best followed by glucose and sorbitol. Our results also showed that 25°C and a 16–h photoperiod were better than 4°C and in the dark (Table 1). The shoots that survived slow-growth storage had greater shoot height than those not exposed to slow-growth. The leaves curled, became yellow and dried during the storage period. After 6 months, these shoots were transferred to fresh MS medium supplemented with 22 µM BA and cultured for 8 weeks. The survival of shoots incubated on water and 10 g l^{-1} sucrose was 25% and these shoot developed into plantlets whereas shoots incubated on glucose and sorbitol at all concentrations eventually died (Table 1). The new shoots were transferred to MS medium without plant growth regulators and then rooted normally.

Table 1. Survival rate of 'Kluai Hin' shoots preserved with 0, 1, 3, and 5% sucrose solution under various conditions for 6 months.

Preserved conditions (temperature; °C /photoperiod; h)	**Survival (%) at different concentrations of sucrose (%)**			
	0	**1**	**3**	**5**
4/0	0	0	0	0
4/16	0	0	0	0
25/0	0	0	0	0
25/16	25	25	0	0

3. *Root induction and plant acclimatization*

The multiple shoots thus obtained from multiple shoot experiments or slow growth treatment developed roots *per se*. Roots originating from shoots were thick, long and fibrous and easy to handle. When rooted plantlets reached 4 to 5 cm in height, they were transferred to sterile vermiculite for 2 weeks (Fig. 2). These plantlets were then moved to a mixture of manure and soil (1:1). All plants derived from tissue culture survived and grew extremely vigorously and reached a 3 to 4 m height after 4 months cultivation in the glasshouse. Total time from sucker excision to field establishment was about 5 months.

igure 2. A 'Kluai Hin' plantlet after storage with 10 g l-1 sucrose at 25°C and a 16–h photoperiod for 6 month.

Discussion

TDZ, a synthetic phenylurea derivative, increases shoot formation of several woody plant species more efficiently than adenine derivatives. TDZ explants showed poor shoot multiplication except in treatment with 0.5 μM TDZ whereas no shoots were produced at 5 and 10 Our study indicated that BA, an adenine derivative, induces more shoot multiplication than TDZ suggesting that BA is more suitable for shoot proliferation of *Musa balbisiana* 'Kluai Hin' than TDZ. This result agrees with our previous findings with *Musa balbisiana* 'Kluai Hom Thong' (Kanchanapoom and Chanadang, 2001). TDZ may inhibit shoot elongation due to an apical dominance release that accelerates axillary bud formation and stunts the length of explants (Huetteman and Preece, 1993) and these events were also observed in this study.

The protocol described in this study was effective for medium-term storage. The subculture interval was prolonged up to 6 months. After 6 months, growth was recovered from *in vitro* shoot tip storage in the treatment of water and 10 g l^{-1} sucrose. This indicates, sucrose allowed the slow growth of stored materials and maintained their viability during storage and subsequent micropropagation. Light regimes did not improve the capacity to the regrowth of *in vitro* shoot tips. Similarly, shoot tip cultures did not tolerate to cold (4^{o}C) treatment during the storage period. These results are in accordance with Banerjee and de Langhe (1985) who were able to keep 25% of meristem tips of 'Cavendish' at reduced temperature and low light intensity. Surviving cultures at 25^{o}C exhibited no carry-over effect of slow growth storage similar to the control in subsequent subcultures. The consistently reduced survival at cold storage suggests that *Musa balbisiana* 'Kluai Hin', of tropical origin, is chilling sensitive and will suffer physical dysfunction when stored below 16^{o}C (Blakesley et al., 1996).

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