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Research note

Plant regeneration through shoot formation from callus of *Areca catechu*

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Abstract

Plantlet formation through shoot formation from callus of the important palm species *Areca catechu* L. is described. Greenish soft callus was formed from shoot tip explants of *Areca catechu* L. within 4 weeks, when cultured on Gelrite-gelled MS (Murashige and Skoog, 1962) basal medium (BM) supplemented with BA (0.2 mg l^{-1}) plus TDZ (0, 0.02 and 0.2 mg l^{-1}). The highest percentage of callus formation (100%) was found on the medium supplemented with 0.2 mg l^{-1} BA and 0.2 mg l^{-1} TDZ. During subculture on the same medium for callus induction, most of calluses proliferated and 50–60% formed shoots. About 90% of shoots formed roots on BM containing 0.1 mg l^{-1} NAA after 4 weeks in culture. Regeneration of plantlets from shoot tips via primary callus production and a two-step process of organogenesis, required about 20 weeks.

Abbreviations: MS – Murashige and Skoog (1962) medium; BA – N^6-benzyladenine; NAA – naphthaleneacetic acid; TDZ – 1-phenyl-3-(1,2,3-thiadiazol-5-yl)-urea

Areca catechu L. (Arecaceae), locally named betel nut, is the most economically important palm in Taiwan. The fresh fruit of this species is the major part of a natural masticatory in south-eastern Asia, called 'betel quid' (Bhonsle et al., 1992). The *Areca* palms usually grow as a single-stem and the way to propagate this species is through the seed. The seeds are short-lived, and the progeny from seeds are not uniform. Conventional vegetative propagation methods such as cutting, grafting or layering are not possible for many palms including Areca species. Tissue culture of palms, in general, is difficult and cultures are often slow growing (Tisserat, 1987). With an increasing demand for fruit production of *Areca* palms in Taiwan, there is a need to develop approaches for efficient propagation.

In this paper, we describe a protocol for the vegetative propagation of *Areca catechu* L. through *in vitro* adventitious shoot formation from shoot tip-derived callus. Mature fruits of *Areca catechu* L. were collected from a local farm in Taipei, Taiwan. These fruits were germinated on humid sand for 1 month in a greenhouse (Figure 1a). Shoots (4–8 cm in height) were taken from the stock seedlings and were washed under running water for 20 min, and then immersed in 70% alcohol for 1 min followed by surface sterilization by agitation for 20 min in a solution of 2% sodium hypoclorite and 0.05% Tween (1:1 v/v). All shoots were finally washed three times (2 min each) in sterile distilled water. Shoot tips (1–3 mm in diameter) after removing the rest were used as explants (Figure 1b) and were inoculated into test tubes containing full-strength macro- and micro-elements of MS (Murashige and Skoog, 1962) salts supplemented with (mg l^{-1}): myo-inositol (100), niacin (0.5), pyridoxine HCl (0.5), thiamine HCl (0.1), glycine (2.0), peptone (1000), NaH_2PO_4 (170), sucrose (30000), and Gelrite™ (2200). Plant growth regulators (0.2 mg l^{-1} BA plus 0, 0.02 and 0.2 mg l^{-1} TDZ) were added prior to autoclaving as optional additives according to the experimental objectives. The pH of the media was adjusted to 5.7 with 1 N

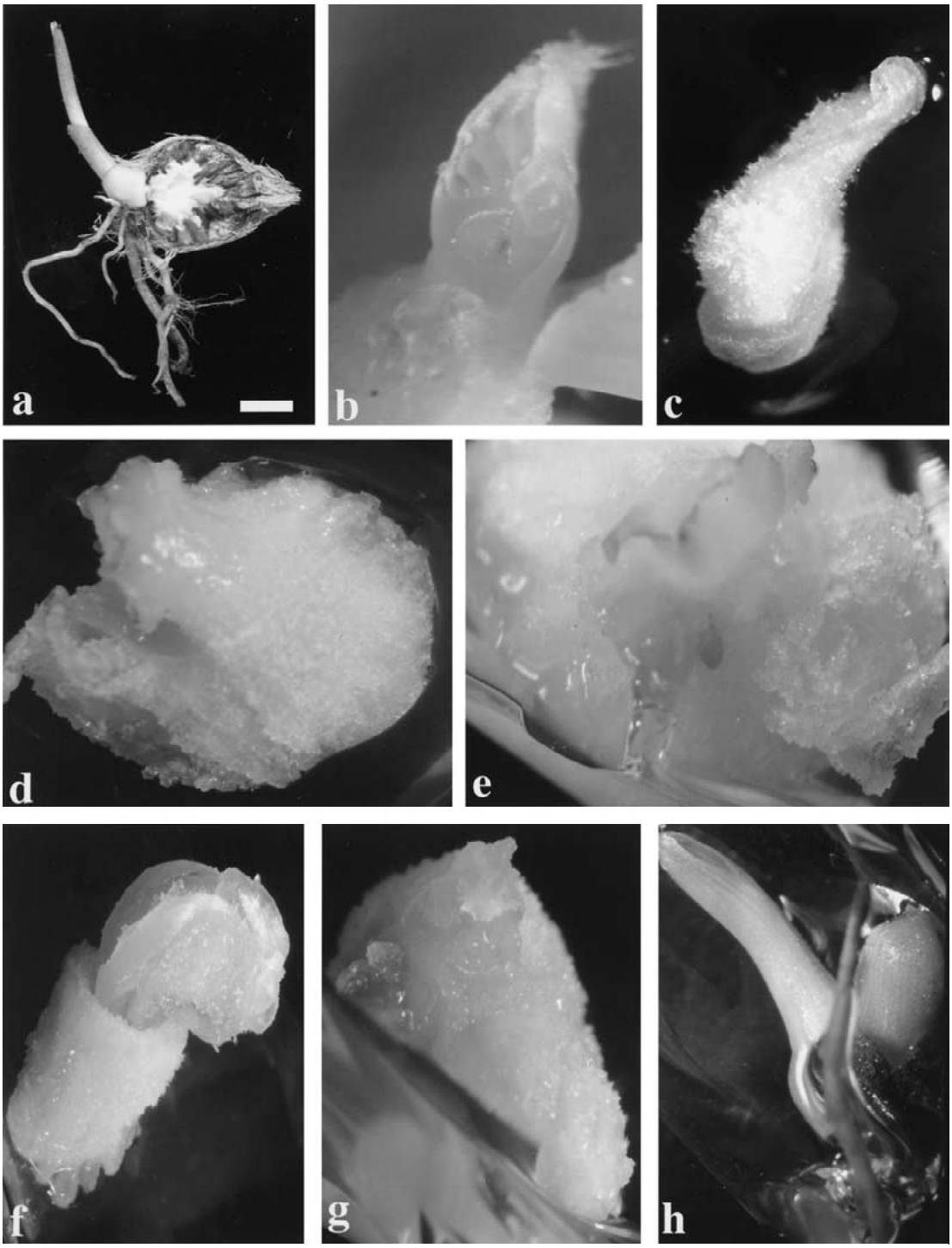

Figure 1. Plant regeneration through shoot formation from shoot tip-derived callus of *Areca catechu* L. (a) A germinating fruit (bar: 0.9 cm). (b) A shoot tip explant (bar: 0.4 mm). (c) The shoot tip explant turned green and enlarged (bar: 0.5 mm). (d) Greenish callus formed from the shoot tip explant after 4 weeks of culture at 0.2 mg l^{-1} BA (bar: 0.5 mm). (e) Primary callus formed shoots (bar: 0.5 mm). (f) Callus formed from the shoot tip explant after 4 weeks of culture at 0.2 mg l^{-1} TDZ (bar: 0.5 mm). (g) Callus formed from the shoot tip explant after 4 weeks of culture in the presence of 0.2 mg l^{-1} BA plus 0.2 mg l^{-1} TDZ (bar: 0.5 mm). (h) A rooted plantlets developed from regenerated shoots on basal medium supplemented with 0.1 mg l^{-1} NAA (bar: 0.3 cm). (The bar is fitting for all figures.).

KOH or HCl prior to autoclaving for 15 min at 121 °C. The cultures were incubated in 20×156 mm test tubes under a 16:8 h photoperiod at 28–36 μmol m^{-2} s^{-1} (daylight fluorescent tubes) and 26±1 °C. Fifteen explants were taken for each treatment. The percentages of callus formation were determined for each trial. Cultures were examined and photographed with a stereozoom microscope (SZH, Olympus). Differences between means were scored with Duncan's multiple range test (Duncan, 1955). Regenerated shoots were cultured on basal medium containing 0.1 mg l^{-1} NAA for root induction.

In palm tissue culture, high levels of 2,4-D or synthetic auxins cocktails were usually used to induce callus formation and proliferation (Gabr and Tisserat, 1985; Bhaskaran and Smith, 1992; Dias et al., 1994; Guerra and Handro, 1998; Fernando and Gamage, 2000). However, in our preliminary experiment, explants of *Areca catechu* L. were found to be necrosied even in the presence of low concentrations of 2,4-D (data not shown). Initially, the shoot tip explant turned green and enlarged for 1–2 weeks (Figure 1c). After 3–4 weeks of culture, we obtained greenish callus from shoot tip explants, when cultured on MS basal medium (BM) supplemented with the combinations of 0.2 mg l^{-1} BA, 0.02 and 0.2 mg l^{-1} TDZ (Figure 1d,f,g). Gabr and Tisserat (1985) reported that in *Phoenix dactylifera* (Arecaceae), explant size affected the contamination rate and the ability to form callus and 3 mm in diameter was most suitable explant size. In this paper, shoot tip explants were excised to be 1–3 mm in diameter, and we obtained an acceptable contamination rate.

No callus was observed on plant growth regulator-free BM after 60 days of culture, as the explants elongated directly into shoots. Except for 0.02 mg l^{-1} BA, other treatments all enhanced the percentage of callus formation. BA (0.2 mg l^{-1}) plus TDZ (0.2 mg l^{-1}) induced the highest percentage (100%) of callus formation. Gabr and Tisserat (1985) reported that embryogenic callus was obtained from shoot tips of *Phoenix dactylifera* on MS medium supplemented with 100 mg l^{-1} 2,4-D, but plantlets conversion from embryos failed. Bhaskaran and Smith (1992) also established callus culture from shoot tips of in the presence *Phoenix dactylifera* in the presence of 100 mg l^{-1} 2,4-D, and they successfully regenerated plantlets through somatic embryogenesis. In *Calamus flagellum*, which also belongs to Arecaceae, a relatively low dosage of 2,4-D (9.025 μM) was used to induce callus formation from the shoot tip culture, and they obtained plantlets via organogenesis (Kundu and Sett, 1999). In this paper, we used BA and TDZ (0.02 and 0.2 mg l^{-1}) to induced callus from shoot tips (Table 1). The regenerated plantlets were successfully established through shoot formation.

During subculture, most of the calluses proliferated and about 50–60% of cultures formed shoots (Figure 1e). Each clump of callus formed 2–5 shoots, and these shoots elongated to a length of 2–3 cm in 30 days after they appeared. The numbers of shoot buds could be further increased many fold by subdivision of calluses prior to the onset of organogenesis on BM supplemented with 0.2 mg l^{-1} TDZ. To induce rooting and thus to complete the formation of plantlets, the shoots were excised and cultured on BM supplemented with 0.1 mg l^{-1} NAA (Figure 1h). Within 10 days after the transfer to rooting medium, several root initials appeared and by 30 days many were 3–4 cm long. About 90% of regenerated shoots successfully rooted in this medium. From the inoculation of a shoot tip explant to regeneration of plantlets through primary callus formation and organogenesis required 20 weeks. *In vitro* regenerated plantlets were established in soil with 10% survival when acclimated in greenhouses.

Table 1. Effects of BA and TDZ on callus formation from shoot tip explants of *Areca catechu* L.

BA (mg l^{-1})	TDZ (mg l^{-1})	% callusing
0	0	0 b
0	0.02	20.0 b
0	0.2	93.3 a
0.2	0	86.6 a
0.2	0.02	80.0 a
0.2	0.2	100.0 a

Percentages of callus formation were scored on each explants after 60 days of culture. Means of 15 replicates (explants) with the same letters are not significantly different at $p<0.05$ (Duncan, 1955).

In conclusion, a protocol for *Areca catechu* L. propagation was established. Regenerated plantlets via adventitious shoot formation from callus cultures could be obtained by using shoot tips as explants on MS medium supplemented with BA (0.2 mg l^{-1}) plus TDZ (0, 0.02 and 0.2 mg l^{-1}).

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References

Bhaskaran S & Smith RH (1992) Somatic embryogenesis from shoot tip and immature infloresence of *Phoenix dactylifera* cv. Barhee. Plant Cell Rep. 12: 22–25

Bhonsle RB, Murti PR & Gupta PC (1992) Tobacco habits in India. In: Prakash C, Hamner JE & Murti PR (eds) The Control of Tobacco-related Cancers and Other Disease (pp. 25–46). Oxford University Press, Bombay

Dias AC, Guerra MP, Cordoba AS & Kemper EL (1994) Somatic embryogenesis and plant regeneration in the tissue culture of *Genoma gamiova* (Arecaceae). Acta Hortic. 360: 167–171

Duncan DB (1955) Multiple range and multiple *F* test. Biometrics 11: 1–42

Fernando SC & Gamage CKA (2000) Abscisic acid induced somatic embryogenesis in immature embryo explants of coconut (*Cocos nucifera* L.). Plant Sci. 151: 193–198

Gabr MF & Tisserat B (1985) Propagating palms *in vitro* with special emphasis on the date palm (*Phoenix dactylifera*). Sci. Hortic. 25: 255–262

Guerra MP & Handro W (1998) Somatic embryogenesis and plant regeneration in different organs of *Euterpe edulis* Mart. (Palmae): Control and structural features. J. Plant Res. 111: 65–71

Kundu M & Sett R (1999) Regeneration through organogenesis in rattan. Plant Cell Tiss. Org. Cult. 59: 219–222

Murashige T & Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant 15: 495–497

Tisserat B (1987) Palms. In: Bonga JM & Durzan DJ (eds) Cell and Tissue Culture in Forestry Vol. 3 (pp. 338–356). Nijhoff, Dordrecht, Boston, Lancaster