Shoot Regeneration from Peduncles and Shoot-like Regeneration from Leaves of Babaco, *Carica pentagona*[1]

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ABSTRACT

Babaco, *Carica pentagona*, Heilborn (Badillo), a subtropical fruit native to Ecuador, has developed worldwide potential through micropropagation of shoot tips and lateral buds. Improvement of babaco is reliant on creating somaclonal variation. Direct regeneration has the potential for producing low-variation regenerants. Therefore, leaves and peduncles were examined for direct *in vitro* regeneration potential. MS media were used with combinations of the growth regulators BA (0.25 - 1.5 mg/l^{-1}), NAA (0.5 - 1.0 mg/l^{-1}), and/or IAA (0.5 - 3.0 mg/l^{-1}). Shoots were regenerated on peduncle sections when cultured on medium containing BA (0, 0.5 or 1.0 mg/l^{-1}) and IAA (0.5 or 1.0 mg/l^{-1}) under a 16 h or 18 h photoperiod and a light intensity of 16 µmols^{-1}•m^{-2}. Whole leaves from *in vitro*-stock material and leaf sections containing either a petiole or main vein from greenhouse plants regenerated roots and/or shoot-like structures on medium containing BA (1.0 or 1.5 mg/l^{-1}) and IAA (1.0 or 3.0 mg/l^{-1}). Histological sections of peduncles and leaves demonstrated that shoots appeared to regenerate directly from cells associated with the vascular systems.

Key words: Organogenesis, tissue culture, histology.

RESUMEN

Babaco, *Carica pentagona*, Heilborn (Badillo), es un fruto subtropical originario del Ecuador, que ha alcanzado distribución mundial con la micropropagación de yemas apicales y laterales. El mejoramiento genético del babaco depende de la inducción de mutaciones somaclonales. La regeneración directa tiene potencial suficiente para obtener plantas con ligeras variaciones. Por lo tanto, hojas y pedúnculos han sido probados para determinar su capacidad de regeneración *in vitro*. Se utilizó el medio de cultivo MS en combinación con los reguladores de crecimiento BA(0.25 - 1.5 mg/l^{-1}), ANA (0.5 - 1.0 mg/l^{-1}), y/o AIA (0.5 -3.0 mg/l^{-1}). Se produjeron brotes en secciones de pedúnculos cultivados en medio con BA (0, 0.5 - 1.0 mg/l^{-1}) y AIA (0.5 ó 1.0 mg/l^{-1}) bajo un fotoperíodo de 16 h a 18 h con una intensidad de luz de 16 µmols^{-1}•m^{-2}). Hojas enteras provenientes de material *in vitro* y secciones de hojas de plantas de invernadero, con pecíolo o vena principal, desarrollaron raíces y/o estructuras similares a brotes en medio con BA (1.0 ó 1.5 mg/l^{-1}) y AIA(1.0 ó 3.0 mg/l^{-1}). Cortes histológicos demostraron que los brotes parecen haberse regenerado directamente de células asociadas al sistema vascular.

INTRODUCTION

An increasing interest in *Carica pentagona* (Heilborn) Badillo (babaco) and the use of *in vitro* techniques for mass propagation (Cohen and Cooper 1982; Cossio 1988) have allowed for the expansion of babaco cultivation to other continents (Lost crops... 1989). However, there are still some commercial characteristics of babaco that need to be improved. Due to the absence of male plants and cross incompatibility with related species, babaco cannot be improved using conventional breeding techniques. Tissue culture regeneration techniques have been used to improve fruit crops (Mitra and Chatuverdi 1972; Srinivasan and Mullins 1980), and may allow for the improvement of babaco.

A number of *Carica* have been regenerated, including babaco (Cossio 1988; Jordan *et al.* 1983; Litz and Conover 1980; Tsay and Su 1985; Vega de Rojas 1989). Babaco's regenerative procedure as described for ovular callus is a lengthy, indirect

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process (Vega de Rojas and Kitto 1991). Indirect regeneration, especially as callus cultures age, has been associated with increased incidence of mutations and off-type regenerants (Larkin and Scowcroft 1981).

It would be desirable to recover plants directly from other explant sources such as petioles (Lal and Ahuja 1989), leaves (Seeni and Latha 1992), or peduncles (Julliard *et al.* 1992). The objective of this study was to examine peduncles and leaves for their ability to regenerate directly.

MATERIALS AND METHODS

Plant material

Peduncles were classified as young (< 2 months old) or old (> 2 months old) and ranged from 3 cm to 15 cm in length. The three most apical leaves collected from greenhouse-grown plants were cut into sections that contained either mainvein or petiole tissue. Leaves of uniform size with intact petioles also were collected from *in vitro* proliferating shoot cultures (Vega de Rojas 1989). The abaxial side of the leaf tissue was cultured in contact with the medium. Greenhouse-collected leaf pieces and peduncles (sectioned into 1 cm pieces) were disinfested for 20 min with 1.0% sodium hypochlorite (20% household bleach) plus 0.1% tween 20 (v/v) and rinsed three times with sterile distilled water previous to culture.

Medium

Peduncle media used were 1) White's (1963) supplemented with (in mg/l^{-1}) sucrose, 60 000; glutamine, 400; and filter-sterilized coconut water, 20% (v:v) or 2) MS salts and vitamins (Murashige and Skoog 1962) supplemented with sucrose, 30 000 mg/l^{-1}. Growth regulators included benzyladenine (BA) (0, 0.25, 0.5, 1.0 mg/l^{-1}), naphthaleneacetic acid (NAA) (0, 0.5, 1.0 mg/l^{-1}), or indoleacetic acid (IAA0 (0, 0.5, 1.0 mg/l^{-1}), alone or in combinations.

Leaf regeneration medium consisted of MS salts (half-strength) and vitamins and the following in $mg/liter^{-1}$, myoinositol, 1; glutamine, 400; and sucrose, 30 000. Growth regulators used were BA (0, 0.5, 1.0, 1.5 mg/l^{-1}) and IAA (0, 1.0, 2.0, 3.0 mg/l^{-1}) under different combinations. Media for leaves and peduncles were gelled with 8 g washed Difco Bacto agar per liter. Agar was washed with distilled water, oven-dried and reground. Media pH were adjusted to 5.6 - 5.7 prior to being autoclaved at 121°C, 124 kPa for 15 minutes.

Environmental conditions

Cultures were maintained under an 18 h photoperiod with light intensities of 12, 16 or 24 $\mu mol \cdot s^{-1} \cdot m^{-2}$ provided by cool-white fluorescent lamps at a temperature of 23±2°C. Two sizes of disposable Petri plates 100 mm x 15 mm and 60 mm x 15 mm were used depending upon the size of the explants. Jars (55 mm x 70 mm) or boxes (65 mm x 100 mm) were used for plantlet development.

Histological procedures

Tissues, both prior to and after culture for various lengths of time, were fixed in FAA (formalin, acetic acid, 30% ethanol; 1:1:10) for 48 h, dehydrated through an ethanol series and embedded in paraffin. Blocks were cut with a rotary microtome set at 12 to 15 microns, stained with safranin and fast green and mounted with Permount (Berlyn and Mikshe 1976).

Experimental design

Experiments were set up as completely randomized designs with 2 - 4 explants/container and 2 - 8 containers/treatment. All samples were selected for maximum uniformity. Factorial combinations of growth regulators were used as preliminary experiments. Only selected treatments were repeated due to limited availability of plant material.

RESULTS

Peduncles

Preliminary experiments examined White's versus MS media and peduncle age. Of 50 peduncle sections cultured for 8 weeks, 27 or 54% initiated callus and 1 (2%) peduncle less than two months old regenerated a root (data not presented). Peduncles were greener and more succulent when cultured on MS medium; therefore, subsequent experiments used MS-based medium.

Table 1. Effect of growth regulator concentration on shoot regeneration from peduncles of babaco.

Treatment			N*	No. shoots regenerated
NAA	BA (mg/l^{-1})	IAA		
0	0	0	2	0
0.5	0.25	0	2	0
0.5	0.5	0	2	0
1.0	0.25	0	2	0
1.0	0.5	0	2	0
0.5	0	0.5	2	0
0.5	0	1.0	2	0
1.0	0	0.5	2	0
1.0	0	1.0	2	0
0	0.25	0.5	2	0
0	0.5	0.5	2	1
0	0.5	1.0	2	0

* Number of Petri plates (four peduncle sections/plate)

Peduncle sections were cultured with combinations of NAA, BA, and IAA (Table 1). One shoot was regenerated on medium containing 0.5 mg each of BA and IAA $liter^{-1}$. In a subsequent experiment, peduncle sections were cultured on media containing BA (0, 0.5, 1.0 mg/l^{-1}) and IAA 0, 0.5, 1.0 mg/l^{-1}) (Table 2). IAA was required for shoot regeneration. The results suggest that as BA concentration increased shoot regeneration decreased (0 BA = 21%, 0.5 BA = 17%, 1.0 BA = 11%).

Table 2. Regeneration of shoots from peduncles of babaco cultured with BA and IAA.

Treatment		N*	Peduncle response No. shoots regenerated
BA	IAA (mg/l^{-1})		
0.0	0.5	4	4
0.0	1.0	4	1
0.5	0.0	4	0
0.5	0.5	4	2
0.5	1.0	4	4
1.0	0.0	4	0
1.0	0.5	4	1
1.0	1.0	4	4

* Number of Petri plates (three peduncle sections/plate)

Peduncle sections were cultured under one of three light intensities; 10, 16, or 24 $\mu mol \cdot s^{-1} \cdot m^{-2}$ with a 16 h or 18 h photoperiod (Table 3). The greatest number of shoots (9/32 or 28%) developed from sections cultured under 10 $\mu mol \cdot s^{-1} \cdot m^{-2}$ (Table 3), while peduncles under 24 $\mu mol \cdot s^{-1} \cdot m^{-2}$ usually became chlorotic within 4 to 5 weeks and produced callus that was watery and clear. There appears to be no difference in shoot production between peduncles cultured for 16 h or 18 h under 16 $\mu mol \cdot s^{-1} \cdot m^{-2}$ (Table 3).

Callus initiated from the subepidermal layers of the peduncle (observation not presented). Internodal sections of peduncle only produced callus compared to nodal sections that regenerated shoots (Fig. 1) or roots. Histological sections of peduncles demonstrated that floral buds were necrotic and that shoots regenerated from cells associated with the vascular bundles (Fig. 1).

Leaves

Leaf sections from *in vitro*-maintained stock cultures were cultured for six weeks on media containing a 3 x 3 factorial combination of BA (0.5, 1.0, 1.5 mg/l^{-1}) and IAA (1.0, 2.0, 3.0 mg/l^{-1}) plus a control without growth regulators. One shoot regenerated in each of 3 media that contained in mg l^{-1}; 1.0 IAA +

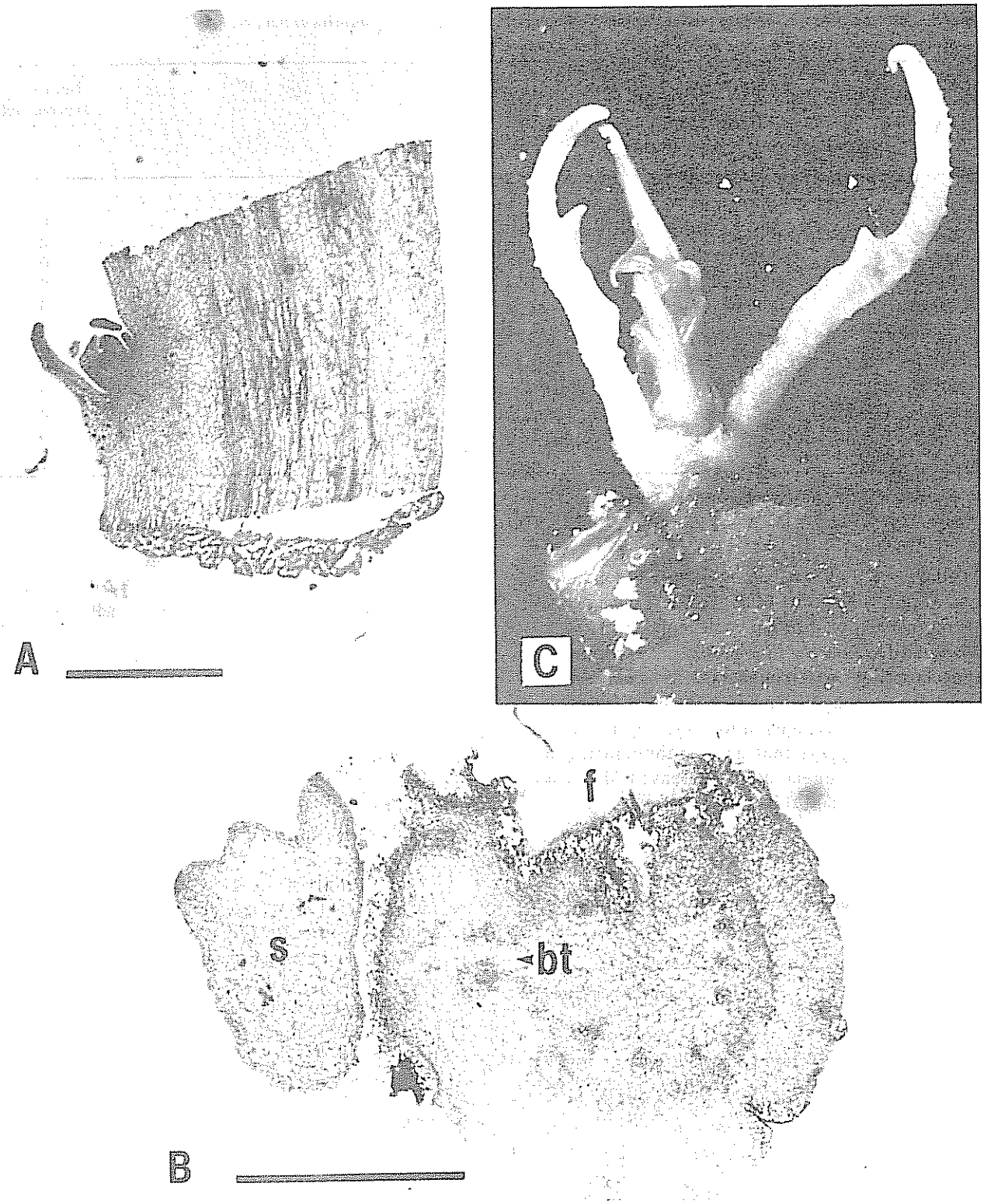

Fig. 1. Regeneration of shoots from peduncle sections. (A) Longitudinal section of peduncle with regenerated shoot. Bar = 1 mm; (B) Cross-section of regenerated shoot (s) with branch trace (bt) and necrotic floral shoot (f). Bar = 1 mm; (C) Peduncle with regenerated shoot. Bar = 1 mm.

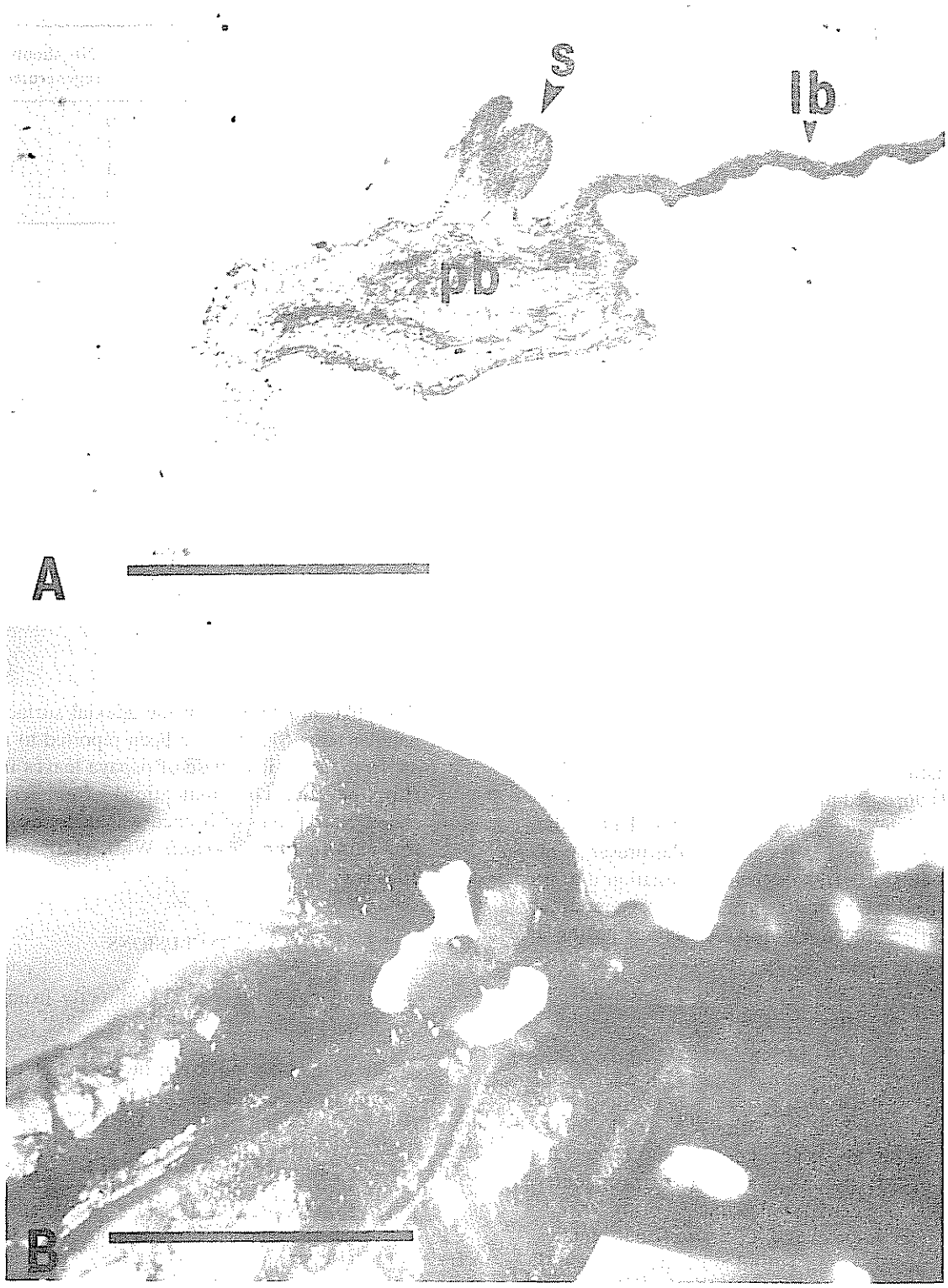

Fig. 2. **Initiation of shoot-like structures from leaves of babaco. (A) Longitudinal section of leaf with regenerated shoot-like structure (s). lb = leaf blade, pb = petiolar base. Bar = 1 mm; (B) Leaf with regenerated shoot-like structures. Bar = 1 mm.**

Table 3. Influence of light intensity and photoperiod on shoot regeneration from peduncles of babaco.

Light intensity ($\mu mol \cdot s^{-1} \cdot m^{-2}$)	Photoperiod (h)	N*	No. shoots regenerated	
10	16	8	9	(28%)
16	16	8	4	(17%)
16	18	8	3	(13%)
24	16	8	0	(0%)

* Number of Petri plates (2 - 4 sections/plate). Percent of sections initiating shoots.

Table 4. Regeneration of shoot-like structure from leaves of babaco.

Treatment		N*	Leaf response			
IAA	BA (mg/l^{-1})		Green	Yellow	Callus	No. shoots regenerated
1.0	1.5	8	8	6	7	1
3.0	1.0	8	8	2	11	1
3.0	1.5	8	6	12	6	1

* Number of jars; 2 - 3 leaves/jar

1.5 BA, 3.0 IAA + 1.0 BA, or 3.0 IAA + 1.5 BA (data not presented). Similar results were obtained in a subsequent experiment with whole leaves (Table 4). Shoots appeared to initiate from the joint between leaf and petiole (Fig. 2). Approximately half of the leaves cultured produced callus in the presence of growth regulators. Histological examination during an early development stage of shoot regeneration showed differentiated shoot-like structures having a vascular connection (Fig. 2).

DISCUSSION

The development stage of the peduncle may be a critical factor for either shoot or root development, as in none of the sections did both organs regenerate concurrently. Histological analysis of peduncular shoots of babaco demonstrated that shoots regenerated from cells associated with the vascular bundles (Fig. 1) as has been found with peduncles of *Brassica napus* L. (Julliard *et al.* 1992).

Leaves obtained from either *in vitro* shoot cultures or greenhouse stock plants of babaco regenerated shoot-like structures from the adaxial surface at the petiolar base. Shoots have been reported to regenerate from the petiolar base of papaya leaves in nature (Litz, personal communication). Regeneration of shoots from leaves of babaco was direct as has been reported in *Rheum emodi* Wall. (Lal and Ahuja 1989).

CONCLUSIONS

— Shoots can be regenerated directly from peduncles of babaco.

— Shoot-like structures can be regenerated directly from leaves of babaco.

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