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Peptone stimulates *in vitro* shoot and root regeneration of avocado (*Persea americana* Mill.)

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Abstract

The effect of peptone on *in vitro* regeneration of avocado's shoots and roots from juvenile or mature stem sections was studied. In this study, both mature and juvenile explants were used to study the effect of peptone on shoot regeneration. Explants were necrotized on Murashige and Skoog (MS) medium without or with plant growth regulators. On the MS medium supplemented with both peptone and benzyladenine or dichlorophenoxyacetic acid, explants survived but significantly delayed in shoot regeneration. Addition of peptone alone into the medium with an optimal amount of 2.0% (w/v) induced shoot formation. Shoot formation also occurred in nodal sections from mature plant but the regeneration rate remained low. All juvenile explant-derived shoots formed root on the medium supplemented with peptone and naphthaleneacetic acid.

Keywords: *In vitro*; Peptone; *Persea americana* Mill.; Rooting; Shoot regeneration

1. Introduction

Avocado (*Persea americana* Mill.) is the most economically important species of the Lauraceae family with the world total product of over 2.5 million tonnes in 2001 (FAOSTAT, 2001). Therefore, efficient propagation of this plant is highly required by the avocado industry; however, there are few *in vitro* culture studies which proved that tissue culture of avocado is very difficult because its tissue is easily browned and later necrotized (Castro et al., 1995).

Formerly, micropropagation using shoot tips from avocado seedlings was reported by Nel et al. (1982). Callus could be obtained from different avocado explants but the tissues were non-morphogenic (Schroeder, 1956, 1961, 1971, 1975, 1980; Blumenfeld and Gazit, 1971). Clonal propagation of avocado was mainly achieved by double grafting, an expensive and time consuming process (Brokaw, 1987); and juvenile tissues were used *in vitro* to obtain roots by microcuttings (Pliego-Alfaro et al., 1987). However, the use of juvenile materials has the disadvantage of genetic variability by the previously seed propagation of stock plants (Castro et al., 1995). Thus, effective regeneration from mature avocado explants would overcome this problem. In the present work, we studied shoot regeneration from *in vivo* juvenile and mature stem sections and found that peptone alone was able to enhance not only survival of explants but also shoot and root formation and development.

2. Materials and methods

2.1. Plant materials

The juvenile material was sprouts (25–30 cm) arising from avocado (*P. americana* Mill.) seeds germinated for 2 months in the greenhouse conditions. The mature material was active growing terminal shoots (30–35 cm) excised from a 10-year-old tree of an orchard in full production at Dalat Institute of Biology (Viet Nam). After removal of leaves, both *in vivo* juvenile and mature explants were surface-sterilized in diluted washing detergent powder (1% w/v, Viso, Viet Nam) for 20 min, washed with running tap water for 1 h and then disinfected in 70% ethanol for 30 s, and rinsed twice in sterile distilled water. Then, they were immersed in a 1‰ $HgCl_2$ solution containing two to three drops of Tween 80 for 7 min and rinsed three times in sterile distilled water. The sterilized explants were cut into 1–1.5 cm long nodal sections with a

Abbreviations: 2,4-D, dichlorophenoxyacetic acid; BA, benzyladenine; MS, Murashige and Skoog; NAA, naphthaleneacetic acid.

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lateral bud prior to inoculation onto solid medium. Juvenile and mature explants were used for shoot regeneration experiment, and just juvenile explant-derived shoots used for rooting experiment.

2.2. Medium and culture conditions

For shoot regeneration, explants were placed in 250 ml culture vessels containing 30 ml MS medium (Murashige and Skoog, 1962) without or with 2.2 μM plant growth regulators such as BA or 2,4-D. The medium also contained 3% (w/v) sucrose, 8% (w/v) agar and various concentrations of peptone (0.0, 1.5, 2.0 or 3.0%, w/v). For root induction, 3-month-old shoots obtained on shoot regeneration medium were sub-cultured in 500 ml vessels containing 80 ml MS medium containing 2.0% (w/v) peptone and 2.7 μM NAA. Explants were incubated at 25 ± 2 °C and 70–75% relative humidity under 10 h photoperiod with photosynthetic photon density of 2500 lx provided by cool-white fluorescent lamps. The pH of the medium was adjusted to pH 5.7 prior to the addition of agar and autoclaved at 121 °C for 30 min under 1 atm.

2.3. Data collection and statistical analyses

Shoot regeneration (SR), shoot height (SH) and number of shoots were recorded after 50 days or 5 months culture. Root length and number of roots of 5-month-old plantlets were determined. Each experiment was conducted with 50 explants and done in triplicate, data were analyzed for significance by Duncan's multiple range test (Duncan, 1995).

3. Results and discussion

3.1. Effect of growth factors on shoot regeneration and development

MS medium containing growth factors were tested for regeneration of shoot from avocado juvenile and mature samples prepared as described in Section 2. T1 (MS medium), T2 (MS medium containing BA), T3 (MS medium containing 2,4-D) and T5 (MS medium containing peptone and BA) did not allow shoot regeneration. The explants on T1 and T2 medium turned browning and necrotic, while the ones on the T5 medium survived but lost the regeneration capacity. However, on T6 (MS medium containing peptone and 2,4-D) exhibited shoot regeneration at a low rate and the growth was retarded. Addition of only peptone into MS medium (T4) induced regeneration and good development of shoots with the height of 65 mm after 5 months incubations (Table 1, Fig. 1a).

Gorzalez-Rosas and Salazar (1984), Pliego-Alfaro et al. (1987) and Barceló-Muñoz et al. (1990) suggested that MS medium might induce toxicity during *in vitro* cultivation of avocado that may explain the necrosis of explants. Addition of peptone and 2,4-D and/or BA in T5 and T6 medium was not suitable for tissue growth and shoot formation of avocado. However, the combination of peptone and 2,4-D (T6) slightly and stimulated shoot formation. Auxin (in the combination with cytokinin) might promote shoot growth and formation of new shoot apical meristems from parenchyma (Gutierrez et al., 2005), so formation of retarded shoots on T6 medium may be induced by combined effect of the supplemented 2,4-D and endogenous cytokinin.

Table 1
Comparative effects of peptone and BA or 2,4-D on shoot regeneration from juvenile explants after 5 months of culture

Medium	SR (%)	SH (mm)[a]	Number of shoots/explant
T1 (base)	0	0	0.0
T2 (+BA)	0	0	0.0
T3 (+2,4-D)	0	0	0.0
T4 (+peptone)	100	50 a	1.0 a
T5 (+peptone + BA)	0	0	0.0
T6 (+peptone + 2,4-D)	10	3 b	0.1 b

Juvenile explants were placed on MS medium (base) or MS medium supplemented with peptone (2%) and/or the indicated plant growth regulators. The shoot height (SH) and shoot regeneration (SR) after 5 months of culture are reported.

[a] Different letters within a column indicate significant differences at $P = 0.05$ by Duncan's multiple range test.

BA was used in medium for avocado shoot regeneration (Barceló-Muñoz et al., 1990; Capote de Sol et al., 2000) and shoot elongation (Cooper, 1987; Witjaksono et al., 1999). However, when used at high concentrations in multiplication medium and/or in maintaining medium, BA suppressed the growth or reduced shoot length, or both (Barceló-Muñoz and Pliego-Alfaro, 2003).

The inclusion of plant growth regulators into MS medium or MS medium containing peptone did not give the desired shoot formation. Therefore, we tested medium containing only peptone.

3.2. Effect of peptone alone on shoot regeneration

On MS medium (without peptone supplementation), explants were browned and necrotized (Fig. 1e). However, addition of peptone (2.0%) induced shoot formation after 50 days culture (Table 2, Fig. 1b–d). On this medium, shoots developed more uniformly to highest average lengths. Previously, peptone was used added as the carbon and

Table 2
Effects of various concentration of peptone on shoot regeneration from juvenile explants after 50 days of culture

Peptone (%)	SR (%)	SH (mm)[a]	Number of shoots/explant
0.0	0	0.0	0.0
1.5	97	5.6 c	0.9 a
2.0	100	8.3 a	1.0 a
3.0	90	7.8 b	0.8 a

Juvenile explants were placed on MS medium containing indicated amounts of peptone. The shoot height and shoot regeneration (SR) in 50 days old cultures on the MS medium containing peptone (2%) are reported.

[a] Different letters within a column indicate significant differences at $P = 0.05$ by Duncan's multiple range test.

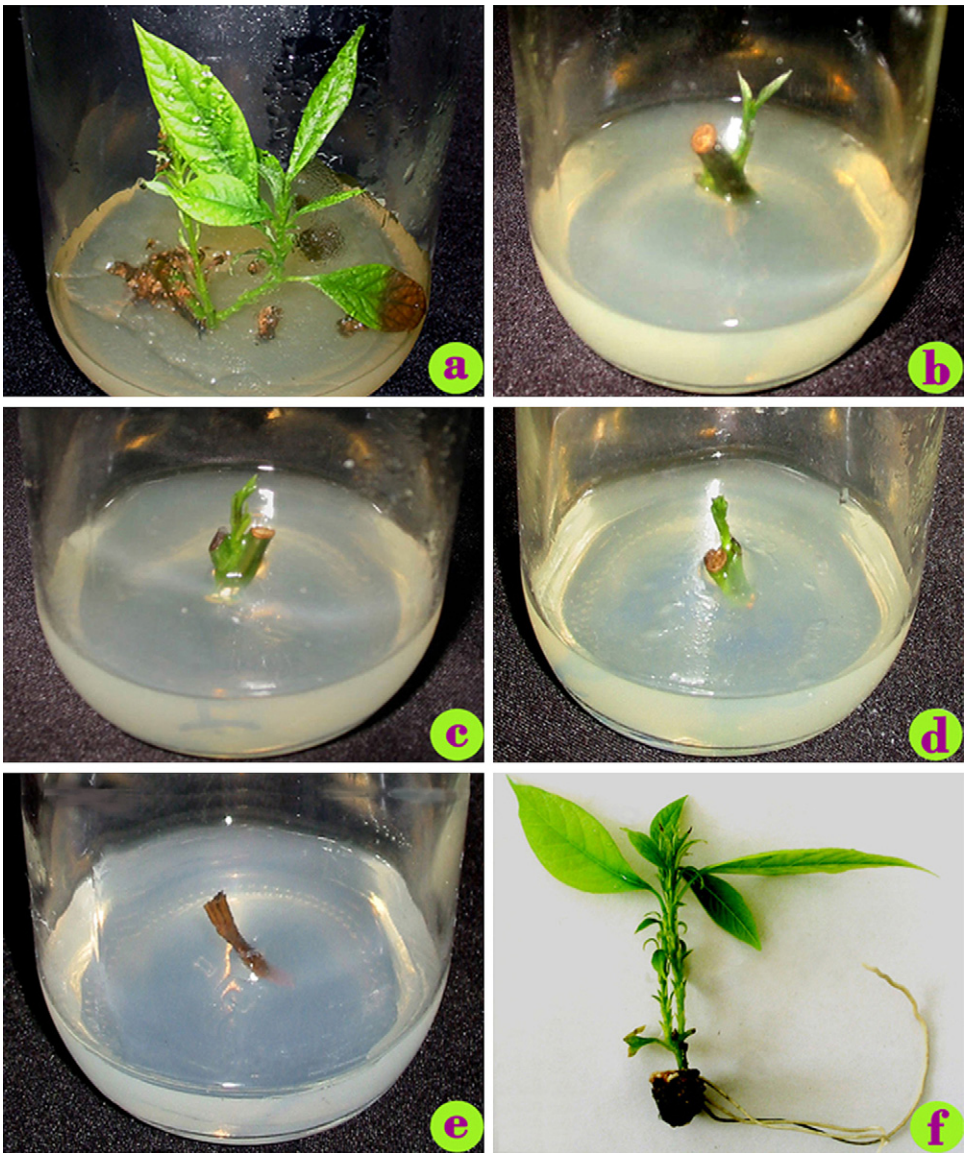

Fig. 1. Avocado shoot and root formation *in vitro*. (a) Avocado shoot after 5 months culture. (b)–(e) Shoots formed from juvenile explains on MS media containing 1.5, 2.0, 3.0 and 0.0% of peptone, respectively. (f) Shoot formed root on MS solid medium supplemented with 2.0% peptone plus 2.7 nM NAA.

nitrogen sources for plant tissue culture. It was suggested that at an efficient concentration, organic and inorganic nitrogen sources can promote the growth of explants (Chen and Chang, 2002). Recently, special role of peptone as factor to enhance the growth and development of plant tissues was observed. When used in the initial medium, peptone functioned as an elicitor of ginseng hairy root formation (Sivakumar et al., 2005) and embryo production of *Oncidium* (Chen and Chang, 2002). Seyring (2005) suggested that 0.25% (w/v) peptone is appropriate for differentiation and germination of *Cyclamen*. On other hand, flower buds of *Dianthus* that have vitrification required a higher peptone concentration (3.0%) (Seiki et al., 1993). Our results indicated that 2.0% peptone in the MS medium was most sufficient for shoot regeneration of avocado. This is a simple and low-cost medium for avocado tissue culture without the need of plant growth regulators.

3.3. Effect of plant material age on shoot regeneration

Explant age affects on number as well as height of shoots. Juvenile and mature explants of avocado were able to regenerate shoots after 50 days culture (Table 3, Fig. 1f and g). However,

Table 3
Comparative of shoot regeneration capacity of plant materials on MS medium containing 2% peptone

Explant source	SR (%)	SH (mm)[a]	Number of shoots/explant
Juvenile explant	100	8.3 a	1.0 a
Mature explant	44	4.5 b	0.4 b

The shoot height (SH) and shoot regeneration (SR) in 50 days old cultures on the MS medium containing peptone (2%) are reported.
[a] Different letters within a column indicate significant differences at $P = 0.05$ by Duncan's multiple range test.

Table 4
Effects of different concentrations of peptone on root formation from juvenil explant-derived shoots after 5 months of culture on MS medium contaning 2.7 μM NAA

Peptone concentration (%)	Root length (cm)[a]	Number of roots/explant
0.0	0.0	0.0
2.0	14.0 a	2.3 a

Shoots generated from juvenile explants were placed on MS medium supplemented with NAA in the absence or presence of peptone. The results of 5 months old cultures were recorded.

[a] Different letters within a column indicate significant differences at $P = 0.05$ by Duncan's multiple range test.

regeneration rate of the juvenile explants was much higher than mature explants. Although mature explants formed shoots but they developed slowly. It has been widely reported for many plants that juvenile materials usually give higher *in vitro* responses while mature explants, in some cases such as sugarcane, passion fruit and neem, did not form even callus (Virupakshi et al., 2002; Becerra et al., 2004; Quraishi et al., 2004). In addition, shoot regenerated from mature plants was not of satisfactory quality and after some proliferations, shoots exhibit the symptoms of necrosis, leading to death afterward (Harty, 1985; Cooper, 1987; Capote de Sol et al., 2000). Necrosis is a major problem in multiplication of mature explants. Pliego-Alfaro et al. (1987) prevented the necrosis in IV-8 rootstock by using double-phase medium (solid and liquid medium) and observed that mature explants retained some regeneration capacity and development of shoot. In addition, the explants produced callus at the base with vitrification symptoms (Pliego-Alfaro et al., 1987). Zirari and Lionakis (1994) used half-strength MS medium supplemented with BA (2.9 and 0.4 μM in solid and liquid phases, respectively) to multiply some mature plants (Fuerte, Hass, Topa-Topa and Duke cultivars). However, they did not report properties of subcultured tissues. In our experiment, mature explants of avocado were able to regenerate on MS medium containing peptone, albeit it at a lower efficiency than the juvenile ones. We further characterized the ability to form roots of these shoots since this may be indicative for the future success of avocado tissue propagation.

3.4. Effect of peptone on root formation

NAA was used as elicitor for rooting of Hass and Hopkins resulted in 100% rooting by microcuttings of seedlings, and the rooted plants showed adequate survival and subsequent growth (Cooper, 1987). Therefore, we used the MS medium containing NAA for rooting of avocado shoots. However, no root was formed in this medium. When peptone was added into the medium, roots were formed and developed well from avocado shoots (Table 4, Fig. 1h). It is possible that peptone in root induction medium might have an important role in maintaining shoot growth while NAA promoted rooting. As the result, shoots may die before rooting in the medium without peptone.

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