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AVOCADO CALLUS AND BUD CULTURE

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ABSTRACT

Parameters influencing viability, callusing and shoot elongation of avocado (*Persea Americana* Mill.) leaf sections, axillary buds and stem sections were evaluated. Seedlings of 'Lula' and 'Waldin' were used. Explants taken from older leaves, oriented with the abaxial surface in direct contact with the medium, with 2,4-(dichlorophenoxy)acetic acid (2,4-D) (1 mg/liter) and maintained at 27°C exhibited optimum callusing. Elongation of axillary buds was enhanced by inclusion of gibberellic acid (1 mg/liter) in the medium and culture at 30°C. Callusing of stem sections was better in the light and when the medium was supplemented with benzyladenine (1 mg/liter).

Micropropagation using shoot tips has been experimentally achieved with numerous woody plant species. Recently, success using shoot tips from avocado seedlings was reported (5). An alternative to using shoot tips in micropropagation involves formation of callus from various parts of the plant followed by differentiation into shoots or embryoids. Proliferation and differentiation of the callus formed may be influenced by several factors. The cotyledons (2, 3, 4, 6), fruit mesocarp (2, 3), peduncle (9), buds (8, 11) and stem tissue (10, 11) of avocado have all been found capable of forming callus. Cultures maintained in the dark at 25-27°C compared to other temperatures formed callus more readily (3, 4, 7). Cytokinins were necessary for the growth of callus from fruit medocarp tissue but not for that derived from cotyledons (2). Culture of avocado leaves and callus formation from them has not been reported.

The present study examines the influence of: 1) explant source, orientation in the media and culture temperature on viability and callusing of leaf explants, 2) gibberellic acid (GA) and temperature on elongation of axillary buds, and 3) light intensity, 2,4-D and benzyladenine (BA) on callusing of stem sections of avocado.

Materials and Methods

Plant material was obtained from 'Lula' and 'Waldin' avocado seedlings maintained in a lighted, air conditioned growth room. Culture media consisted of Anderson's (1) mineral salts and vitamins, caScin hydrolysate (1 g/liter) and sucrose (30 g/liter). The medium for leaf cultures was modified by adding BA (1 mg/liter); for axillary buds by adding BA

and IBA, both at 0.2 mg/liter. Media for leaf and stem cultures was gelled with 4 g/liter and for axillary buds with 6 g/liter Bactoagar. The pH of all media was adjusted to 5.7 ± 0.1 prior to dispensing 10 ml into 150 ml baby food jars. Jars were capped with polypropylene closures (Magenta) and autoclaved at 1.05 kg/ cm^2 and 121°C for 15 min. Unless otherwise indicated, light intensity was 1800 lux for 16 hr a day. Illumination was provided by Sylvania Lifeline fluorescent lights.

Leaf section explants were 25 mm^2 (5 mm x 5 mm), axillary buds 1-2 mm and stem sections 5 mm in length. Except for the orientation study, leaf sections were placed with their abaxial (upper) surface in direct contact with the medium. Leaf and axillary bud cultures were replicated 20 times with 2 explants per jar and evaluated after 8 wk. Stem section cultures were replicated 8 times with 5 explants per jar and evaluated after 6 wk.

Disinfestation was achieved by first soaking the plant material in a solution containing benomyl (1.46 g/liter) and captan (2.3 g/liter) with constant agitation for 15-30 min, draining, then storing overnight at 5°C. Final disinfestation was facilitated by soaking in filtered CaOCl (9%) plus 0.1% Tween 20 for 30-60 min followed by 3 rinses with sterile water.

Viability, callusing and shoot elongation were evaluated where appropriate. Viability of leaf sections refers to the overall condition of the explant at the time of evaluation. Only cultures in which at least 75% of the leaf section was "normal" in regards to retention of green coloration and absence of injury were counted.

Results and Discussion

Leaf sections. Viability of cultures maintained at 21°C was better than that at 27°C (Table 1). However, those at 27 °C were more likely to form callus. In addition, leaves at 27 °C enlarged more than those kept at the lower temperature.

Table 1. Culture temperature, viability and callusing of 'Waldin' and 'Lula' avocado leaf cultures.

Temp (°C)	(%) Viable	Callusing
21	40	16
27	15	23

Placing the lower (adaxial) or upper (abaxial) surface of the explant in direct contact with the medium resulted in greatest viability and callusing (Table 2). For the other orientations, the portion of the explant inserted into the medium generally became necrotic with callus forming only at or near the surface.

Viability of the explants removed from the basal portion of and from older leaves was considerably better (Table 3). Although callusing potential was not consistently influenced by the explant position, older leaves were more responsive in this regard.

Leaf viability was greatest in cultures supplemented with naphthalene acetic acid (NAA)

or 2,4-D (Table 4). Callusing was promoted by all auxins but was greatest using 2,4-D.

Table 2. Explant orientation in media, viability and callusing of 'Waldin' and 'Lula' avocado leaf cultures.

Orientation	(%) Viable	Callusing
adaxial	49	34
adaxial	39	41
proximal	22	17
distal	23	25
inside	13	21
outside	7	19

Table 3. Leaf section and position effects on stem, viability and callusing of 'Lula' avocado leaf cultures.

Leaf Section	(%) Viable	Callusing	Position on Stem	(%) Viable	Callusing
			1	25	5
Tip	28	15	3	11	4
Middle	25	6	5	14	2
Base	43	16	7	36	13

Table 4. Auxins, viability and callusing of 'Lula' avocado leaf cultures.

Auxin (1 mg/liter)	(%) Viable	Callusing
Control	89	0
IBA	88	36
NAA	97	42
2,4-D	98	52

In general, callus formation on leaf sections occurred at isolated points, especially near the cut ends of major veins.

Axillary buds. Maximum elongation of axillary buds occurred when the culture medium was supplemented with 1 mg/liter GA (Table 5). Shoot elongation was much better in cultures maintained at 30 compared to 20 °C (Table 6).

Table 5. Gibberellic acid and elongation of 'Lula' avocado axillary bud cultures.

Gibberellic acid concn (mg/liter)	Shoot length (mm)		
	<4	5-9	>10
		(%)	
0.0	63	26	11
0.2	25	31	44
1.0	17	28	55
5.0	67	22	11

Table 6. Culture temperature and elongation of 'Lula' avocado axillary bud cultures.

Temp (°C)	Shoot length (mm)		
	<4	5-9	>10
		(%)	
20	56	32	12
30	32	22	46

Stem sections. Callusing of stem sections was promoted by illuminating the cultures (Table 7). Both 2,4-D (0.2 mg/ liter) and BA (1 mg/liter) were effective in promoting callusing of stem sections (Table 8). Inclusion of either hormone at 5 mg/liter inhibited callus formation.

Table 7. Light and callusing of 'Lula' avocado stem section cultures.

Light regime	Callusing cultures (%)
Dark	47
Light (1800 Lux, 16 hr)	63

Table 8. Effect of 2,4-D and BA on callusing of 'Lula' avocado stem section cultures.

Growth regulator	Concn (mg/liter)	Callusing cultures (%)
Control	—	48
2,4-D	0.2	67
2,4-D	1.0	55
2,4-D	5.0	31
BA	0.2	60
BA	1.0	82
BA	5.0	40

In both studies, callus formation occurred primarily at the cut ends of the explant.

However, at isolated points it formed along the elongated and enlarged stem sections. In general, callus was whitish and very friable. A second type of callus occasionally formed which did not correspond to any treatment. It was very firm, dense and greenish-white.

Viability and callusing ability of avocado leaf explants can be maximized by proper selection of the explant source and orientation in the medium. Culture temperature and inclusion of an auxin in the culture medium promote callusing. Elongation of axillary buds is promoted by GA and elevated culture temperature. Callusing of stem sections is optimized by illuminating cultures as well as including 1.0 mg/liter BA in the medium.

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