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In Vitro Propagation of Carrizo Citrange[1]

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Abstract. In vitro proliferation of shoot tips was attempted with nucellar seedlings of sour orange (*Citrus aurantium* L.), Carrizo citrange ('Washington' navel x *Poncirus trifoliata* (L.) Raf.), and Cleopatra mandarin (*C. reshni* Hort. ex Tanaka). Shoot multiplication was achieved with Carrizo citrange shoot tips cultured on Knop's medium (with organics) supplemented with Murashige and Skoog microelements, 5 mg/liter 6-benzylamino purine (BA), 3 — 4% sucrose and at a light intensity of 2.2 klx. No effect on shoot proliferation was obtained with 6-furfurylamino purine (kinetin), 6 γ-γ-dimethallyl amino purine (2iP), agar concentration, addition of orange juice, or nitrogen source. Proliferated shoot tips of Carrizo citrange rooted when cultured on Murashige and Tucker's medium containing 1 mg/liter napthaleneacetic acid and gelled with 0.5% agar. Plants were successfully established in soil.

Several fruit tree species, including apple (1, 6, 7), plum (3, 8, 11) and cherry (5, 8) have been clonally propagated by *in vitro* techniques. Although clonal propagation by nucellar seedlings is possible with some citrus rootstocks, micropropagation from non-nucellar vegetative tissue would be desirable as several years are required before sufficient seed can be produced from a newly introduced rootstock. Some rootstock clones, although desirable in other respects, may not produce nucellar seedlings, may be seedless, or produce few seed; and scion cultivars may fruit more precociously on rootstocks derived from vegetatively propagated shoots than vegetative seeds. The following research was undertaken to determine the feasibility of tissue culture-propagation of citrus by shoot tip culture.

Media. On the basis of a preliminary study, the basal medium for shoot proliferation consisted of Knop's macroelements (13) plus the microelements of the Murashige and Skoog (MS) high salt medium (9). The following organics were added to the basal medium, in mg/liter: pyridoxine·HCl, 0.5; thiamine·HCl, 0.4; nicotinic acid, 0.5; myo-inositol, 100; adenine sulfate, 80; sucrose, 3000; and agar, 1000. Rooting of proliferated shoots was evaluated using Murashige and Tucker medium (10) with the following addenda, in mg/liter: pyridoxine·HCl, 10; thiamine·HCl, 10; nicotinic acid, 5; myo-inositol, 100; adenine sulfate, 80; sucrose, 5000; and agar, 1000. The pH of the media was adjusted to 5.7 ± 0.1; 25 ml of medium were placed in 25 x 150 mm culture tubes capped with polypropylene closures and autoclaved for 15 minutes at 121°C.

Rootstocks. Seeds of sour orange, Carrizo citrange, and Cleopatra mandarin, currently the most commonly propagated rootstocks in Florida, were sown on January 23, 1979 in flats containing a mixture of 1 perlite:1 vermiculite:1 peat (v/v/v) and grown in a growth chamber illuminated with Westinghouse Cool White bulbs (0.9 klx at plant height) 16 hr a day. The temperature averaged 26° (day) and 21°C (night).

The explant. Shoot tips 2-cm long were excised when seedlings were 12 weeks old and every 6 weeks thereafter. Larger leaves were removed and shoots were trimmed to 1 cm. Groups of 10 prepared shoot tips were placed in small cheesecloth bags and disinfested as follows: 70% ethanol for 30 sec, 1 rinse with sterile water, 0.5% sodium hypochlorite (10% commercial laundry bleach) plus 0.1% Tween 20 for 5 min followed by 3 rinses with sterile water. The tips were then trimmed to 5 mm, treated again with 0.5% sodium hypochlorite for 1 min, and rinsed once with sterile water before explanting. To obtain shoots for rooting experiments, 10 tips obtained from *in vitro*-proliferated shoots were further multiplied in 125 ml Erlenmeyer flasks each containing 30 ml of medium. Illumination was provided by Sylvania Lifeline bulbs for 16 hr per day with an average light intensity of 2.2 klx. Culture room temperature averaged 29°C. Experiments were terminated after 6 weeks.

Proliferation. The effect of 3 cytokinins (BA, kinetin, and 2iP) at 0, 0.2, 1.0, and 5.0 mg/liter on shoot tip proliferation of each of the 3 rootstocks was tested (Table 1). BA induced meaningful prolif-

Table 1. Influence of cytokinins on shoot proliferation of 3 citrus rootstocks (10 shoots per treatment).

		No. shoots per explant		
Cytokinin	Concn (mg/liter)	Carrizo citrange	Sour orange	Cleopatra mandarin
Control	0.0	1.0a[z]	1.0a	1.0a
BA	0.2	1.2a	1.0a	1.0a
	1.0	1.8b	1.1a	1.2a
	5.0	3.1c	0.9a	1.3a
Kinetin	0.2	1.0a	1.0a	1.0a
	1.0	1.0a	1.0a	1.0a
	5.0	1.0a	1.0a	1.0a
2iP	0.2	1.0a	1.1a	1.0a
	1.0	1.0a	1.1a	1.0a
	5.0	1.5a	1.2a	1.0a

[z]Mean separation in columns by Duncan's multiple range test, 5% level.

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eration only in Carrizo citrange. The degree of proliferation in Carrizo was proportional to BA concentration, a response previously reported in various woody fruit crops (2, 3, 4, 12).

In another experiment a comparison was made with shoot tip and nodal sections of Carrizo citrange using BA at 5 mg/liter. Nodal sections were the first node below the 5 mm shoot tip explant containing an axillary bud. Shoot tip explants proliferated significantly (5% level) more than nodal sections (3.0 vs 1.7 shoots per explant), perhaps because they contained more unexpanded buds.

Levels of various other factors were tested with Carrizo using the optimum BA concentration (5 mg/liter). These include concentration of agar, reconstituted orange juice, sucrose, light intensity and N source (Table 2). Shoot proliferation showed no response to agar, reconstituted orange juice, and N source. However proliferation was higher at 3 or 4% sucrose as compared to 5 or 6% and 2.2 klx as compared to higher or lower intensities. Thus, shoot proliferation was influenced by genotype, explant source, cytokinin, sucrose level, and light intensity.

Rooting. Rooting studies were of necessity confined to Carrizo citrange. NAA enhanced rooting (80% rooting; 10.2 roots per shoot) as compared to IBA or IAA (Table 3). Root generation usually occurred near the cut surface of the basal ends of the shoot tip explants or from leaves touching the medium. The NAA response was only found at 1 mg/liter and as a result all further variables were tested using NAA at 1 mg/liter. Among other factors tested (agar, sucrose, orange juice, light intensity, and nitrogen source) only agar affected rooting with increasing response as agar concentration decreased from 2.0 to 0.5% (Table 3).

Establishment. Establishment of rooted and unrooted proliferated shoots in soil was achieved after 7 weeks under mist (10 sec every 10 min for 10 hr a day). Nineteen of 43 unrooted Carrizo citrange shoots rooted when placed under mist and 4 of 5 shoots that already rooted in culture survived.

Table 2. Influence of agar, sucrose, orange juice, light intensity and nitrogen source on proliferation of Carrizo citrange shoot tips grown with 5.0 mg/liter BA (10 shoots per treatment).

Variable	Concn or level	No. shoots per explant
	%	
Agar	0.5	2.8a[z]
	1.0	3.0a
	2.0	2.7a
Sucrose	3	3.0b
	4	3.4b
	5	2.3a
	6	2.2a
Orange juice	0	3.0a
	3	2.3a
	10	3.2a
	30	2.8a
	(klx)	
Light intensity	0.0	1.3a
	2.2	3.0c
	5.7	2.1b
Nitrogen source	high NH_4[y]	2.8a
	high NO_3[x]	3.0a

[z]Mean separation within variable by Duncan's multiple range test, 5% level.
[y]High level of ammonium and a low level of nitrate nitrogen (Ca) $(NO_3)\cdot 4H_2O$ omitted from standard Knop's medium and $(NH_4)_2SO_4$ (650 mg/liter) and $CaCl_2\cdot 2H_2O$ (717.8 mg/liter) added.
[x]Nitrate nitrogen only (standard Knop's medium).

Table 3. Influence of auxins and agar on rooting of Carrizo citrange shoots (10 shoots per treatment).

Variable	Concn	Rooting (%)	No. roots/ shoot tip[z]
	(mg/liter)		
Auxin Control	0.0	0a[y]	0.0a
IAA	0.2	0a	0.0a
	1.0	0a	0.0a
	5.0	0a	0.0a
IBA	0.2	0a	0.0a
	1.0	0a	0.0a
	5.0	10a	0.1a
NAA	0.2	0a	0.0a
	1.0	80b	10.2b
	5.0	0a	0.0a
	(%)		
Agar	0.5	70a	2.3a
+ NAA (1 mg/liter)	1.0	40ab	0.8b
	2.0	10b	0.1b

[z]Mean separation within variable by Duncan's multiple range test, 5% level.
[y]Mean separation within variable by x^2, 5% level.

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Water, Osmotic, and Turgor Potentials of Kinetin-treated Callus[1]

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Abstract. Water and turgor potentials of callus tissue from the cactus *Echinopsis turbinata* L. increased with increasing concentration of kinetin. Osmotic potential showed no consistent trend with an increase in concentration of kinetin or p-chlorophenoxyacetic acid (p-CPA).

Although water, osmotic, and turgor potentials of shoot-forming and non-shoot-forming callus tissue have been published (2) little is known about the water relations of callus tissue grown with varying concentrations of cytokinin. Skoog and Schmitz (7) reported that watery tissue was formed in tobacco-callus cultures in the presence of 10^{-4} μM 6-Δ^2-isopentenylaminopurine (2iP). Higher concentrations of 2iP, to 5 μM, produced progressively more compact tissues. The increasing compactness of the callus suggests that the higher cytokinin concentrations were inducing higher rates of cell division relative to cell expansion. The in-

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