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 χ^2, 5% level.

Literature Cited

1. Abbott, A.J. and E. Whiteley. 1976. Culture of *Malus* tissues *in vitro*. I. Multiplication of apple plants from isolated shoot apices. Sci. Hort. 4:183-189.
2. Altman, A. and R. Goren. 1974. Growth and dormancy cycles in *Citrus* bud cultures and their hormonal control. Physiol. Plant. 30:240-245.
3. Boxus, P. 1975. La culture de meristemes de Prunus poor l'obtention de plants sains. Acta Hort. 44:43-46.
4. Dutcher, R.D. and L.E. Powell. 1972. Culture of apple shoots from buds *in vitro*. J. Amer. Soc. Hort. Sci. 97:511-514.
5. Feucht, W. and D. Dausend. 1976. Root induction *in vitro* of easy-to root *Prunus pseudocerasus* and difficult-to-root *Prunus avium*. Sci. Hort. 4:49-54.
6. Jones, O.P. and S.G.S. Hatfield. 1976. Root initiation in apple shoots cultured *in vitro* with auxins and phenolic compounds. J. Hort. Sci. 51:495-499.
7. Jones, O.P., M.E. Hopgood, and D. O'Ferrell. 1977. Propagation *in vitro* of M. 26 apple rootstocks. J. Hort. Sci. 52:235-238.
8. Jones, O.P. and M.E. Hopgood. 1979. The successful propagation in vitro of 2 rootstocks of Prunus the plum rootstock cultivar PIXY Prunus-insititia and the cherry rootstock cultivar F-12-1 *Prunus avium*. J. Hort. Sci. 54:63-66.
9. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.
10. Murashige, T. and D.P.H. Tucker. 1969. Growth factor requirements of citrus tissue culture. Proc. 1st Intern. Citrus Symp. 3:1155-1161.
11. Rosati, P., G. Marino, and C. Swierczewski. 1980. *In vitro* propagation of Japanese plum (*Prunus salicina* lindl. cv. Calita). J. Amer. Soc. Hort. Sci. 105:126-129.
12. Tabachnik, L. and D.E. Kester. 1977. Shoot culture for almond and almond-peach hybrid clones *in vitro*. HortScience 12:545-547.
13. White, P.R. 1954. The cultivation of animal and plant cells. Ronald Press, New York. p. 71-77.

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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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creasing firmness of the callus with increasing concentration of cytokinin also suggests that the water potential of the callus was changing. This paper reports the water potential, osmotic potential, and turgor potential of callus from *Echinopsis turbinata* grown with different concentrations of kinetin.

E. turbinata, a low, round cactus, has 13 to 14 ribs running longitudinally, with clusters of spines along the ribs (1). Callus tissue from the cactus was obtained according to Holder (5). After the spines were trimmed, the plant was soaked in a saturated aqueous solution of benomyl for 4 hr and rinsed successively in Tween 20 (0.10%), sodium hypochlorite (0.14%), and water for 15 min, followed by several rinsings with sterile water. The plant was sliced along the ridges of the cactus. Each part of the plant containing a cluster of clipped spines (an explant) was removed from the wedged slices and transferred to glass culture tubes (25 x 150 mm) containing 20 ml of medium. Each explant was 5 x 5 mm.

The basal medium was that of Murashige and Skoog (6), supplemented with kinetin (20, 30, 40, and 50 mg/liter) and p-CPA (5 and 10 mg/liter). The tubes were placed in a growth chamber with light intensity of 100 $\mu E\ m^{-2}sec^{-1}$ at the top of the tubes from 07:00 to 19:00 hr, provided by a combination of Cool-White fluorescent and incandescent lamps. The temperatures in the growth chamber were 24°C (day) and 10°C (night). The relative humidity varied between 36 and 42% (day) and 40 and 52% (night).

The callus tissue was grown for 11 weeks and then harvested for measurements of fresh and dry weights and potentials, after which a portion of the callus was put in a thermocouple psychrometer designed by Dalton and Rawlins (3). The thermocouple psychrometer consisted of a top, which held the thermocouple wires, and a bottom, which was a chamber. Water and osmotic potentials were measured using the technique of Ehlig (4). A piece of tissue about 2.5 x 2.5 x 2.5 cm was detached from the callus and placed in the chamber. The tip of the thermocouple junction was put in the chamber so that it was just above, but not touching, the tissue. After equilibration (about 2 hr), the water potential was measured using a microvoltmeter (Keithley 150B Microvolt Ammeter). The bottom of the thermocouple was removed and corked and the tissue was frozen. After thawing, the bottom was then secured to the top of the thermocouple and, after equilibration, the osmotic potential was measured using the microvoltmeter. The thermocouple junction was dipped in distilled water every time the tissue in the chamber was changed to insure lack of salts on the junction. The junction was air dried before it was placed in the thermocouple chamber. Turgor potential was calculated as the difference between water potential and osmotic potential.

After measurements of potentials, the 2 parts of a callus (one part used for potentials) were dried at 70°C to constant weight and weighed for dry weight. Results presented for the callus are the average of the 4 replications. The potential of the growth media was measured using the thermocouple psychrometers. No difference in potential of the media was noted before and after freezing. Also, no difference in potential was detectable among the different media. The average potential of the media was –0.3 MPa.

Water and turgor potentials increased as the kinetin concentration increased (Table 1). For 5 and 10 mg/liter p-CPA, water potential increased 0.34 and 0.88 MPa, and turgor potential increased 0.49 and 0.95 MPa, respectively, for increase in kinetin concentration from 20 to 50 mg/liter. At 50 mg/liter kinetin, callus grown with 5 mg/liter p-CPA had a lower water potential than callus grown with 10 mg/liter p-CPA. At lower concentrations of kinetin, callus grown with 5 mg/liter p-CPA had water potentials similar to callus grown with 10 mg/liter p-CPA. At each concentration of kinetin, turgor potentials of callus grown with 5 mg/liter p-CPA were not significantly different from those of callus grown with 10 mg/liter p-CPA. The osmotic potential showed no consistent trend (either increase or decrease) with increase in concentration of kinetin or p-CPA.

Table 1. Water, osmotic and turgor potentials, plus standard deviations, of calli from the cactus *Echinopsis turbinata*, grown with p-chlorophenoxyacetic acid and kinetin.

Kinetin (mg/liter)	p-Chlorophenoxyacetic acid (mg/liter)	MPa		
		Water potential	Osmotic potential	Turgor potential
20	5	–1.09 ± 0.16	–1.70 ± 0.34	0.61 ± 0.25
	10	–1.21 ± 0.18	–1.63 ± 0.44	0.42 ± 0.02
30	5	–0.98 ± 0.15	–1.79 ± 0.14	0.81 ± 0.22
	10	–0.81 ± 0.12	–1.56 ± 0.38	0.75 ± 0.46
40	5	–0.87 ± 0.28	–1.52 ± 0.32	0.65 ± 0.20
	10	–0.88 ± 0.13	–1.62 ± 0.42	0.74 ± 0.08
50	5	–0.75 ± 0.04	–1.85 ± 0.37	1.10 ± 0.23
	10	–0.33 ± 0.07	–1.70 ± 0.43	1.37 ± 0.42

Table 2. Fresh weight and standard deviations of callus from the cactus *Echinopsis turbinata*, grown with p-chlorophenoxyacetic acid and kinetin.

Kinetin (mg/liter)	Callus fresh wt (g) ± SD	
	p-Chlorophenoxyacetic acid	
	5 mg/liter	10 mg/liter
20	4.99 ± 0.40	5.68 ± 1.04
30	4.30 ± 0.75	3.79 ± 0.55
40	2.94 ± 0.74	2.63 ± 0.72
50	2.87 ± 0.88	3.21 ± 0.19

The water potential of the medium (–0.3 MPa) was always higher (less negative) than the water potential of the callus. Water flowed along the water-potential gradient, from a higher to a lower water potential (from the medium to the callus). Dead callus had a water potential similar to that of the medium. Therefore, the medium and the callus were in water-potential equilibrium only when the callus was dead.

The fresh weight of callus grown with p-CPA at 5 mg/liter was similar to that of callus grown at 10 mg/liter (Table 2). Dry weights (not shown) paralleled fresh weights. Fresh weight of callus decreased as kinetin concentration increased. For both 5 and 10 mg/liter p-CPA, fresh weight of callus grown with 20 mg/liter kinetin was 1.7 times more than that of callus grown with 50 mg/liter kinetin.

Literature Cited

1. Bailey, L.H. and E.Z. Bailey. 1941. Hortus second. Macmillan, New York.
2. Brown, D.C.W. and T.A. Thorpe. 1980. Changes in water potential and its components during shoot formation in tobacco callus. Physiol. Plant. 49:83-87.
3. Dalton, F.N. and S.L. Rawlins. 1968. Design criteria for Peltier-effect thermocouple psychrometers. Soil Sci. 105:12-17.
4. Ehlig, C.F. 1962. Measurement of energy status of water in plants with a thermocouple psychrometer. Plant Physiol. 37:288-290.
5. Holder, P.L. 1980. Tissue culture and drought resistance: growth of cactus (*Echinopsis turbinata* L.) and wheat (*Triticum aestivum* L. em. Thell. 'Ponca' and 'KanKing'). MS Thesis, Okla. State Univ., Stillwater.
6. Murashige, T. and F. Skoog. 1952. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.
7. Skoog, F. and R.Y. Schmitz. 1972. Cytokinins. p. 181-213. In: F.C. Steward (ed.) Plant physiology. A treatise. Vol. VIB: Physiology of development: the hormones. Academic Press, New York.