Plant Regeneration from Cotyledons of *Cucumis melo* 'Topmark'

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Abstract. A procedure for the regeneration of muskmelon (*Cucumis melo* L.) cv. Topmark via shoot organogenesis from cotyledon explants is described. The best induction medium for a morphogenic response was MS salts and vitamins medium with BA at 1.0 mg·liter⁻¹. Further vegetative bud development was completed by transferring organogenic tissue to MS medium containing BA at 0.05 mg·liter⁻¹. The shoots were rooted in MS medium containing NAA at 0.01 mg·liter⁻¹. Morphologically normal plantlets were obtained. Chemical abbreviations used: 6-benzylaminopurine (BA); indoleacetic acid (IAA); naphthaleneacetic acid (NAA).

The literature descriptions of tissue culture methods applied to muskmelon are few. Blackmon and Reynolds (1982) first documented the development of adventitious shoots from cotyledon and primary leaf explants. Moreno et al. (1985), Kathal et al. (1988), Dirks and Buggenum (1989), and Niedz et al. (1989) also reported that muskmelon plants could be regenerated from cotyledon and leaf explants. Oridate and Oosawa (1986) observed plantlet regeneration from suspension culture-derived somatic embryos. These procedures are not necessarily applicable to other cultivars (D. Gonsalves, personal communication) because of the genotype specificity of the methods. This report describes a technique for the regeneration of whole muskmelon plants from cotyledon explants *C. melo* 'Topmark' through shoot organogenesis. The same procedure was also applicable to 'Perlita' muskmelon, another netted cultivar.

Seeds of 'Topmark' (Asgrow Seed Co., Kalamazoo, Mich.) were soaked in tap water

for ≈15 min. Seed coats were removed manually. De-coated seeds were surface sterilized for 10 min with 10% (v/v) solution of commercial bleach containing a trace amount of Tween-20 (Sigma, St. Louis) spreader and then rinsed three times with sterilized distilled water. Sterilized decoated seeds were germinated on MS medium (Murashige and Skoog, 1962) containing 0.1% activated charcoal (optional) and at 26C with a 16-h photoperiod under standard cool-white fluorescent lamp (4 klx). Unless otherwise stated, all media were supplemented with 3% sucrose and solidified with 0.6% Phytagar (Gibco). The pH of all media was adjusted to 5.8 before they were autoclave at 121C for 20 min.

Cotyledons from 9- to 10-day-old seedlings grown in vitro were cut longitudinally into halves and placed on induction medium with the abaxial side down. Induction medium was composed of MS medium (Murashige and Skoog, 1962) supplemented with either BA (0.0, 0.5, 0.75, 1.0 mg·liter⁻¹) or IAA at 1.5 mg·liter⁻¹ and kinetin at 6.0 mg·liter⁻¹ (I-K medium; Moreno et al., 1985). Explants were incubated at 26C under diffuse cool-white fluorescent lamps (4 klx). One hundred explants were evaluated per

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Table 1. Organogenic response (bud formation) of 'Topmark' muskmelon cotyledon explants on MS medium supplemented with growth regulators².

Growth regulator (mg·liter ⁻¹)	Explants producing organogenic tissues (%)
BA (1.0)	80
(0.75)	60
(0.5)	4
IAA (1.5) + KN (6.0)	20
Control (0)	0
LSD (5%)	10
CV	12

²Data represent the mean of two experiments (n = 200). The response is significantly different among treatments, χ^2 value is 450.5 ($P < 0.001$).

Table 2. Rooting response of 'Topmark' muskmelon shoots on MS medium supplemented with various levels of NAA².

NAA (mg·liter ⁻¹)	Rooting response of shoots (no./total)		
	Experiment		Mean (%)
	1	2	
0.000	0/15	3/19	8
0.001	1/15	2/17	9
0.010	6/15	16/36	42
0.100	3/15	4/16	22
LSD (5%)			18
CV (%)			31

²Data represent the mean of two experiments. The response is significantly different among treatments. χ^2 value is 16.7 ($P < 0.001$).

treatment. After 3 weeks, cultures were evaluated for the production of organogenic sectors. To resolve statistical differences between treatments, data from two independent experiments were entered into a contingency table and χ^2 values were computed. Agar-solidified MS medium supplemented with one of four levels of NAA (0.0, 0.001, 0.01, 0.1 mg·liter⁻¹) was evaluated for rooting of shoots. When plantlets developed an extensive root system, they were transplanted to pots containing a planting mix and covered with clear polyethylene storage bags for hardening-off. Subsequently, the regenerated plants were potted in soil and grown in a greenhouse.

Two types of tissue were produced by the cotyledon explants, a compact green organogenic tissue and a green friable callus. The best induction medium was MS medium supplemented with 1.0 mg BA/liter; 80% of explants cultured on this medium produced organogenic tissue (Table 1). The difference in response between all media formulations was highly significant. The difference between 1 and 0.75 mg·liter⁻¹ was also highly significant ($\chi^2 = 19.1^{**}$). The least organogenic tissue was generated from explants cultured on a medium with 0.5 mg BA/liter other than from the control. Only one-fourth as many explants with I-K medium produced organogenic tissue as those with 1 mg BA/liter.

Organogenic tissues could be serially propagated by subculture on MS with BA at 0.75 or 1.0 mg·liter⁻¹ (Fig. 1). Generally,

Fig. 1. Organogenic tissue derived from cotyledon explant.

Fig. 2. Organogenic tissue with developing vegetative buds.

organogenic tissues lost morphogenic potential and produced friable callus when serially subculture on I-K medium. Shoot development was subsequently promoted by transfer of the organogenic tissues to MS with 0.05 mg BA/liter. Forty percent of the organogenic tissue then produced many shoot buds (Fig. 2). Shoots developed within 20 days of transfer to this latter medium (Fig. 3). Further shoot development was encouraged by transfer of organogenic tissue to Magenta boxes (Magenta Corp., Chicago) containing four sheets of sterile paper towel saturated with ≈ 20 ml of MS liquid medium containing BA at 0.05 mg·liter⁻¹. Fresh medium was added to boxes every 2 weeks.

Developing shoots, ≈ 1 cm in length, were dissected from the mass of organogenic tissues and transferred to rooting medium. The remainder of the organogenic tissue was returned to liquid medium in Magenta boxes for further shoot development. The differences in the frequency of root development due to NAA concentration were significant. The highest rooting percentage was obtained in a medium containing 0.01 mg NAA/liter (Table 2). Although medium containing no NAA was ineffective for root formation, a difference between NAA at 0, 0.001, and 0.1 mg·liter⁻¹ could not be resolved ($\chi^2 = 2.63^{NS}$). The difference between 0.01 mg·liter⁻¹ and all other treatments pooled was highly significant ($\chi^2 = 14.96^{**}$). The medium containing NAA at 0.1 mg·liter⁻¹ stimulated callus growth at the shoot bases. Rooted plants flowered and produced fruits with seed.

Organogenesis from cotyledon of *Cucumis melo* has been reported earlier (Dirks and van Buggenum, 1989; Niedz et al., 1989).

Fig. 3. Shoot formation from organogenic tissues.

Fig. 4. Regeneration stages of muskmelon from cotyledon explant. (Upper left) Initiation of organogenic tissue. (Upper right) Differentiation of organogenic tissue. (Lower left) Plantlet development. (Lower right) Regenerated muskmelon plant.

The differences between the present results and the earlier ones are the cultivar used, concentration of growth regulators, and the ability to propagate organogenic tissues. We used cotyledons of 'Topmark' and 'Perlita' as explant sources; while the others used cotyledons of 'Hale's Best Jumbo' (Niedz et al., 1989), 'Accent', 'Galila', 4215, 'Presto', and 'Viva' (Dirks and van Buggenum, 1989). We were able to induce organogenic callus tissues by culture of the explants on MS medium with 1.0 mg BA/liter and shoot development could be promoted by transfer of the organogenic tissues to MS medium with 0.05 mg BA/liter. In contrast, Niedz et al. (1989) reported that 75% of the explants were induced to form organogenic callus with the use of IAA at 0.875 mg·liter⁻¹ and abscisic acid at 0.26 mg·liter⁻¹ in combination with BA at 1.0 mg as inductive growth regulators. Shoot formation (30%) was obtained by transfer of shoot buds to MS medium with BA at 0.675 mg·liter⁻¹. In addition, Dirks and van Buggenum (1989) obtained shoot formation directly from every cotyledon explant cultured on MS medium that contained

BA at 1.0 mg-liter⁻¹. In the work reported here, the competence of organogenic tissue was maintained by serial propagation on MS medium with 1.0 mg BA/liter.

Moreno et al. (1985) maintained organogenic tissues of 'Amarillo Oro' muskmelon for 1 year using I-K medium. However, when organogenic calli of 'Topmark' were serially subculture onto I-K medium, morphogenic competency was lost and only friable tissues were produced. Such difference in morphogenic responses is likely due to genetic differences between the cultivars. The procedure reported here (Fig. 4) may be applicable to other muskmelon cultivars, as 'Perlita' and 'Topmark' responded similarly. Cotyledons have proven to be an excellent source of transformation experiments in *Cucumis sativus* (Chee, 1990a, 1990b). The simple regeneration method described may be useful

in gene transfer using either *Agrobacteria* or microprojectile bombardment.

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