

Plant Regeneration from Callus of Cucumis melo L.

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Tissue culture is a promising tool for the recovery of important traits such as disease resistance. Selection in vitro would allow for rapid screening of large populations of cells if suitable selection agents are available and if cell culture techniques exist for the regeneration of plants from single cells or clumps of cells. Recently, techniques for the regeneration of Cucumis melo L. have been reported (1,2,3,4,5). We report here on the regeneration of plants from calli of three muskmelon cultivars ('Hales Best', 'Perlita', and 'Iroquois') using modifications of the technique originally reported by Moreno et al. (2).

Surface disinfection was accomplished by removing the seedcoat and immersing the embryo in a continuously stirred 10% Clorox solution with 0.1% Tween 20 for 20 minutes. After three rinses in sterile distilled water, the cotyledons were removed from the embryonic axes and plated directly on callus induction medium, which consisted of a basal medium of MS salts, 3% sucrose, $100 \text{ mg} \cdot \text{l}^{-1}$ myo-inositol, $1 \text{ mg} \cdot \text{l}^{-1}$ thiamine-HCL, and 0.8% agar supplemented with $6.0 \text{ mg} \cdot \text{l}^{-1}$ kinetin and $1.5 \text{ mg} \cdot \text{l}^{-1}$ indoleacetic acid (IAA). The pH was adjusted to 5.7 with NaOH and HCL prior to autoclaving for 20 minutes at 121°C , 124 kPa for 15 minutes. Cultures were grown either in the dark or under 16 hr of fluorescent lights ($\sim 50 \mu\text{Em}^{-2}\text{s}^{-1}$) at 25°C .

Cotyledon cultures placed in the light expanded and turned green in approximately five days and formed green nodular callus within two weeks. Visible shoots emerged in three weeks. At the end of four weeks, shoots were excised and placed on rooting medium. Rooting medium consisted of basal medium supplemented with $0.001 \text{ mg} \cdot \text{l}^{-1}$ 1-naphthaleneacetic acid (NAA). The green nodular callus was maintained by subculturing every four weeks on basal medium supplemented with $0.1 \text{ mg} \cdot \text{l}^{-1}$ benzyladenine, with emerging shoots removed at the end of each subculture period. Table 1 lists the number of plants established at the end of three subcultures for each genotype. Of the 32 plants recovered only one exhibited a mutant phenotype, with a blind apical meristem and no apparent lateral meristems. The other plants flowered normally.

Cotyledon cultures placed in the dark formed a creamy white, friable callus within two weeks. Only one shoot primordia formed among the 120 cotyledons in the dark. This shoot did not develop when subsequently placed in the light. The dark-grown callus was subcultured and transferred to the light at the end of four weeks. The calli developed small green zones throughout the callus clumps. Subculture of the green zones of calli resulted in uniformly green callus, but no shoot formation occurred during five months of subculturing. Transfer of this callus to basal medium supplemented with IAA (0, 0.15, 0.75, or $1.5 \text{ mg} \cdot \text{l}^{-1}$) and kinetin (6.0, 9.0, or $12.0 \text{ mg} \cdot \text{l}^{-1}$) in a factorial combination resulted in altered callus growth rates, with the greatest on the original medium of $6.0 \text{ mg} \cdot \text{l}^{-1}$ kinetin and $1.5 \text{ mg} \cdot \text{l}^{-1}$ IAA and the least on the

media lacking IAA. However, no change in callus morphology was evident on any of the media tested.

In an effort to increase the number of shoots formed, cotyledon and hypocotyl segments from axenically grown seedlings were placed on basal medium supplemented with kinetin (0.1, 0.5, 1.0, or 2.5 mg·l⁻¹) and NAA (0.1, 0.5, or 1.0 mg·l⁻¹) in a factorial combination. Of the 180 cotyledon segments, 150 formed roots with only one forming a single shoot (0.5 mg·l⁻¹ NAA and 0.5 mg·l⁻¹ kinetin). In contrast, hypocotyl segments formed roots in only 31 of the 180 cultures with no shoots formed in any culture.

Thus far these experiments demonstrate that 'Hales Best' has a greater morphogenetic potential than 'Iroquois' or 'Perlita', and that the number of subcultures may affect morphogenetic potential. Current experiments are focused on alternative growth regulator combinations and concentrations, both to improve the number of shoots formed in culture and to recover shoots from longterm subcultured callus.

Table 1. Number of regenerated plants by cultivar and subculture number.

Cultivar	Subculture no.			Total
	1	2	3	
Hales Best	8	4	11	23
Iroquois	5	1	1	7
Perlita	2	0	0	2

Literature Cited

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