Isolation of Cells and Protoplasts from Muskmelon Leaves

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Techniques exist for the isolation of cells and protoplasts from a wide range of plant species (3), but few reports describe methods for isolating mesophyll cells and protoplasts from cucurbits (2,4). Moreno et al. (4) produced cells and protoplasts of muskmelon by inducing callus, producing cell suspensions from the callus, and isolating protoplasts from the suspension cell cultures (4). In this study, we report conditions for obtaining protoplasts and cells directly from muskmelon leaves.

Mesophyll Cell Isolation: The muskmelon breeding lines MD 8518 and MD 2042 were grown under greenhouse conditions. Young leaves (approx 4 cm diam and 0.5 g FW) were detached and surface sterilized with 95% ethanol for 15 sec, washed three times in sterile distilled water and aseptically cut into 1.0 mm strips. The strips were transferred into petri dishes (35 x 10 mm) containing 5 ml of 2%, 5% or 10% (w/v) macerase (Calbiochem) and 0.7 M-mannitol in modified Murashige and Skoog (MS) mineral solution (5) at pH 5.7. The dishes were incubated overnight at 25-30°C with constant orbital agitation. The number of isolated cells per dish were counted with an inverted microscope and expressed as a percentage of total observable cells. There were four replicates of each genotype-enzyme combination.

Protoplast Isolation and Culture: Leaf strips were obtained as described above. In one experiment, strips were directly suspended in solutions containing 2% or 5% macerase, 5% cellulysin (Calbiochem) and 0.65 M-mannitol in MS mineral solution at pH 5.7. In another experiment, strips were suspended in solutions containing 2% or 5% macerase, 5% cellulysin, 0.5% (w/v) pectolyase (Sigma) and 0.65 M-mannitol in MS solution at pH 5.7. All treatments were incubated at 25-30°C with constant agitation for 24 hr. Isolated protoplasts was quantified using an inverted microscope, then filtered through sterilized stainless steel mesh (61 µ) and centrifuged three times before resuspension in 0.65 M-mannitol in MS solution. Four protoplast counts per replicate were made using a hemacytometer and protoplast viability was assessed using the procedures of Bornman and Bornman (1). Each genotype-enzyme treatment was replicated three times.

Results and Discussion: The 5% macerase gave a significantly higher percentage of isolated cells than either 2% or 10% macerase (Table 1) but the yield of isolated mesophyll cells was still low (about 8%). As few plant species give cell yields greater than 50% when leaves are treated directly with enzymes (3), high cell yields are probably better obtained through production of cell suspensions from callus cultures. For protoplast isolations, the solution containing 2% macerase, 5% cellulysin and 0.5% pectolyase gave the highest yield (Table 2). Protoplast viability was also highest with this enzyme combination, and viability declined with increasing macerase levels. Although protoplast yields obtained in this study are generally lower than those obtained from other plant species, they are comparable to or greater than those obtained by others working with cucurbits (2,4). These methods should be useful to those interested in isolating cells and protoplasts from muskmelon leaf tissue.

Table 1. Effect of macerase concentrations on production of intact mesophyll cells from two muskmelon genotypes.

Genotype	Enzyme treatment	% Cells isolated
MD 2024	2% macerase	2.3
	5% macerase	4.6
	10% macerase	2.7
MD 8518	2% macerase	3.4
	5% macerase	7.8
	10% macerase	3.5

Table 2. Effect of enzyme combinations on protoplast yield and viability from leaves of two muskmelon genotypes.

Genotype	Enzyme combination			Protoplast yield (10^6/ml)	Protoplast viability (%)
	macerase	cellulysin	pectolyase		
MD 2024	2%	5%		0.26	53
	5%	5%		0.13	40
	2%	5%	0.5%	1.60	72
	5%	5%	0.5%	0.84	56
MD 8518	2%	5%		3.41	64
	5%	5%		1.09	47
	2%	5%	0.5%	14.62	92
	5%	5%	0.5%	7.42	81

Literature Cited

1. Bornman, C.H., and J.F. Bornman. 1985. Plant protoplast viability. In: P.E. Pilet (ed.). The physiological properties of plant protoplasts. p.29-35. Springer Verlag, Berlin.

2. Coults, R.H.A. 1977. Improved isolation and culture methods for cucumber mesophyll protoplasts. Plant Science Letters 9:45-51.

3. Kohlenbach, Hans W. 1984. Culture of isolated mesophyll cells. In: I.K. Vasil (ed.). Cell culture and somatic cell genetics. p.204-210. Academic Press, N.Y.

4. Moreno, V., L. Zubeldia, and L.A. Roig. 1984. A method for obtaining callus cultures from mesophyll protoplasts of muskmelon (Cucumis melo L). Plant Science Letters 34:195-201.

5. Murashige, T., and F. Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol. Plant. 15:473-497.