

Mesophyll Protoplasts of Some Wild and Cultivated *Cucumis* spp.

L. Rokytová, B. Navrátilová and A. Lebeda

Palacký University, Faculty of Science, Department of Botany, Šlechtitelů 11, 783 71 Olomouc, Czech Republic

e-mail: lebeda@prfholnt.upol.cz

Introduction: Isolation and somatic fusion of protoplasts may provide a means of genetic interchange with the aim to create interspecific or intergeneric hybrids between *Cucurbitaceae* (1,3). Recently there are available only a few results related to this topic (4,5,6,7,8). The aim of this work was to select suitable accessions of *Cucumis* spp. for protoplast isolation, culture and fusion.

Material and Methods: Seeds of *Cucumis anguria* ssp. *longipes* (09-H41-00569), *Cucumis melo* L. (line MR-1), *Cucumis metuliferus* E. Meyer ex Naudin (H41-0587), *C. sativus* (SM-6514) and *C. zeyheri* (09-H41-0196) originated from the Vegetable Germplasm Collection of the Research Institute of Crop Production (Prague), Gene Bank Division, Workplace Olomouc, Czech Republic.

Seeds of all accessions were deoiled, surface sterilized for 10 min in 2,5 % Chloramine B (sodiumbenzenesulfo-chloramidium), 3-5 times rinsed in sterile distilled water. Sterilized seeds were germinated onto OK medium: mineral salts and vitamins of basic M-S supplemented with 20 g sucrose, 0,01 mg.l⁻¹ IBA, 0,01 mg.l⁻¹ BAP and 20 mg.l⁻¹ ascorbic acid in glass tubes (in diameter 17 mm). After one or two weeks the seedlings were transferred onto fresh OK medium in Erlenmeyer flasks (100 cm³). Cultivation took place at light/dark cycles 16/8 hrs (irradiance of cca 35 μmol m⁻² s⁻¹) and temperature of 22 ± 2 °C. The plantlets were micropropagated to obtain clones (originated from one zygotic embryo).

The protoplasts were isolated from fully developed leaves (first and second) of plantlets cultivated *in vitro*, with good growing and rooting. Fresh leaves (about 100 mg) were cut into narrow strips, placed into 2 ml of enzyme solution consisting of 1% cellulase Onozuka R 10 (SERVA, Heidelberg) and 0,25% macerozyme R 10 (SERVA, Heidelberg) in wash medium PGly (2), then incubated 18 h in the dark at 25 °C (overnight). The crude protoplasts suspension was purified by filtration (polyamide net,

54 μm) and centrifugated at 100 G for 5 min, the pellet was resuspended in 4 ml of 20 % sucrose and overlaid by 2 ml of wash medium (PGly), centrifugated at 100 G for 5 min. Floated protoplasts (ring) were collected, resuspended in a wash medium (PGly), centrifugated and the final pellet was resuspended in a liquid cultivation medium LCM1 (2) at the density 1-2.10⁵ protoplasts.ml⁻¹ (by haemocytometr). The viability was determined by FDA staining after isolation.

The protoplasts were cultured for 2 weeks in the dark at 25 °C in Petri dishes (35 mm). After this period 1 ml of liquid media LCM2 (2) was added to each dish and cultivation continued in the light/dark 16/8 hrs cycles at 22 ± 2 °C.

Results and Discussion: Plant regeneration from protoplast culture has been reported for some important genotypes of *Cucumis* spp., mainly on *C. melo* (2) and *C. sativus* (8).

In our experiments there were used five *Cucumis* species for protoplast isolation. There were used mesophyll protoplasts for obtaining the homogenous material. The protoplasts were isolated from *in vitro* plants. Growing of *C. anguria*, *C. melo*, *C. metuliferus* and *C. zeyheri* plantlets was very good. The plants were green with efficient rooting and micropropagation. However, the growth of *C. sativus* genotype was questionable because the plantlets were small and soon flowering, the propagation *in vitro* was rather difficult.

The success of protoplast isolation was evaluated on the basis of the density and the viability immediately after isolation. *C. metuliferus* had the highest density in all experiments (7), *C. anguria* had the highest viability. The viability of other genotypes was high and suitable for protoplast cultivation, as well as the density. The Figs. 1 and 2 show the differences between studied genotypes.

The protoplasts of *C. sativus* and *C. zeyheri* were more damaged and plasmolysed after isolation in comparison with *C. anguria*, *C. metuliferus* and *C. melo*. The viability of protoplasts decreased on 50 % during 24 hrs of culture. Changes in the form and fragmentation of all studied genotypes were recorded in the culture period 24-48 hrs.

The first division in culture was observed from seven to ten days after isolation and viable protoplasts after two weeks. Then the division was stopped and there were no obtained microcalli, calli and plantlets. Protoplast culture of *C. anguria* was without the division, although the highest viability and good density of protoplast.

In general, the best genotype (from the studied set) for protoplast isolation was *C. metuliferus* because of its easy mipropropagation, high density and viability of protoplasts after isolation. *C. melo*, *C. zeyheri* and *C. anguria* can be used too, but the optimisation of culture conditions is necessary. Finally, for successful regeneration of plants from protoplasts there is the most important to find the best genotype.

Literature Cited

1. Dabauza, M., L. González-Candelas, M. Bordas, L.A. Roig, D. Ramón and V. Moreno. 1998. Regeneration and characterisation of *Cucumis melo* L. (+) *Cucumis anguria* L. var. *longipes* (Hook. fil.) Meeuse somatic hybrids. *Plant Cell, Tissue and Organ Culture* **52**, 123-131.
2. Debeaujon, I., Branchard, M. 1992. Induction of somatic embryogenesis and caulogenesis from cotyledons and leaf protoplast-derived colonies of melon (*Cucumis melo* L.). *Plant Cell Reports* **12**, 37-40.
3. Fellner, M., Lebeda, A. 1998. Callus induction and protoplast isolation from tissues of *Cucumis sativus* L. and *C. melo* L. seedlings. *Biologia Plantarum* **41**, 11-24.
4. Lebeda, A., Křístková, E., Kubaláková, M. 1996. Interspecific hybridization of *Cucumis sativus* x *Cucumis melo* as a potential way to transfer resistance to *Pseudoperonospora cubensis*. In: Gómez-Guillamón, M.L., Soria, C., Cuartero, J., Torés, J.A., Fernández-Munoz, R. (eds): Cucurbits Towards 2000. Proceedings of the VIth Eucarpia Meeting on Cucurbit Genetics and Breeding, May 28-30, 1996, Málaga (Spain), 31-37.
5. Moreno, V., Zubeldia, L., Roig, L.A. 1984. A method for obtaining callus cultures from mesophyll protoplasts of melon (*Cucumis melo* L.). *Plant Science Letters* **34**, 195-201.
6. Navrátilová, B., Nisini, P.T., Crino, P., Lucretti, S. 1998. Somatic hybridization between Cucurbitaceae species: *Cucumis melo*, *Cucumis metuliferus* and *Benincasa hispida*. 8th Days of Plant Physiology, 7-10.7.1998, Olomouc (Czech Republic), Book of Abstracts, 161-162.
7. Navrátilová, B., Rokyťová, L., Lebeda, A. 2000. Isolation of mesophyll protoplasts of *Cucumis* spp. and *Cucurbita* spp. *Acta Horticulturae* **510**, 425-431.
8. Punja, Z.K., Tang, F.A., Sarmiento, G.C. 1990. Isolation, culture and plantlet regeneration from cotyledon and mesophyll protoplasts of two pickling cucumber (*Cucumis sativus* L.) genotypes. *Plant Cell Reports* **9**, 61-64.

Figure 1. The viability of *Cucumis* spp.

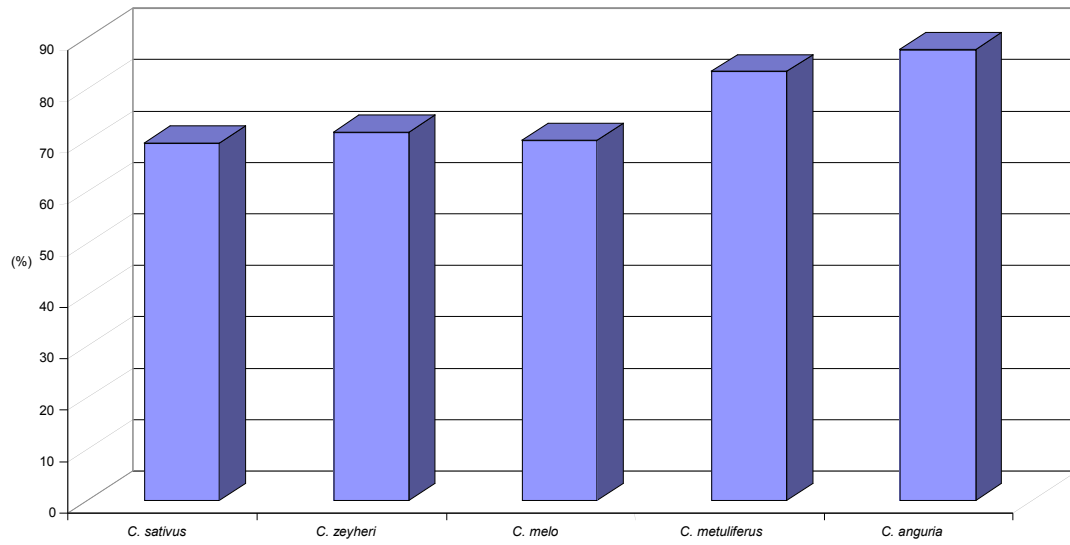


Figure 2. The density of *Cucumis* spp.

