

Plant Regeneration from Explant-Derived Calli of Cucumis anguria L. var longipes.

Garcia-Sogo, M., I. Granelli, B. Garcia-Sogo, L. A. Roig and V. Moreno
Departments of Genetics and Microbiology, E.T.S. Ingenieros Agronomos,
Universidad Politecnica de Valencia, 46022 Valencia, Spain.

Resistances to Tetranychus urticae KOCH, nematodes and cucumber green mottle mosaic virus have been described in Cucumis anguria L. var. longipes. Because of the difficulty in obtaining sexual crosses between this species and C. melo L., we are trying protoplast fusion to achieve hybridization. Basic studies aiming at acquiring knowledge of how in vitro cultured wild species behave have been carried out in our laboratory. In this paper we show the results of some of those experiments devoted to achieving morphogenesis in explant derived calli of C. anguria.

Seeds of C. anguria L. var longipes (kindly supplied by Dr. Jacobs, University of Stellenboch, South Africa) were surface sterilized and germinated on MG medium as previously described (4, 5). When the first true leaf appeared, the shoot apex (1-3 mm length) was excised and aseptically planted in 200 ml bottles containing 40 ml of MB3 solid medium (7). Leaf segments of about 1 cm² surface as well as internode stem segments of 1 cm length from 15-20 day old axenic plants were used as explants. The explants were cultured in a way similar to that described previously (5) for cotyledon and hypocotyl explants, respectively except that the stem fragments were longitudinally divided in two halves before they were put horizontally onto the solid medium. The culture media consisted of MB3 + 1 mg/l 6-benzylaminopurine + 100 ml/l coconut milk and indole-3-acetic acid (IAA) at the following concentrations: 0.00, 0.01, 0.05, 0.10, 0.50, 1.50, and 3.00 mg/l. All the cultures were incubated for 30 days in a 16 h photoperiod (1,500-1,800 lux) at 27+°C continuous temperature and 60-70% relative humidity.

Most of the growth of the explants on the assayed media was organized tissue. Unorganized proliferation of callus with organized zones was observed only on media with high levels of IAA (1.5 and 3.0 mg/l). Leaf-derived calli were morphologically different from stem derived ones. The former presented numerous, uniformly distributed trichomes, while the latter had fewer trichomes that were in discrete zones.

Table 1 shows the morphogenic responses of both kinds of explants. Calli arising from stem segments produced roots at frequencies higher than leaf-derived calli. The frequencies of shoot-buds and shoots producing calli were greater in leaf calli than in stem ones. Moreover, the number of shoots per callus was also greater in the leaf calli, where they were practically innumerable.

The influence of the type of explant on the neoformation of shoots observed was similar to the one previously reported in explants of C. melo (5). Nevertheless, regarding the kind of apices developed, there exists a notable difference between both plant species: C. anguria produces well developed shoots which can be easily excised and rooted in MB3 medium to give whole plants; whereas C. melo usually gave few shoots, and, in order to increase the number of individual ones, organogenic pieces of calli must be subcultured in a shoot-developing medium.

Relative to shoot formation, the morphogenic response of primary explants was higher in C. anguria than in C. melo. The nutritional requirements, however, seem to be more restrictive than those of the latter. In preliminary studies (data not shown) media with different IAA and kinetin concentrations but without coconut milk, C. anguria explants did not produce shoots whereas C. melo explants (especially those from cotyledons) did generate shoots.

Literature cited

1. Kho, Y. O., A.P.M. Den Nijs and J. Franken. 1980. Interspecific hybridization in Cucumis L. species. 2. An investigation of "in vivo" pollen tube growth and seed set. *Euphytica* 29:661-672.
2. Knipping, P.A., L.G. Patterson, D.E. Navel and S.G. Rodriguez. 1975. Resistance of cucurbits to twospotted spider mite. *Env. Ent.* 4:507-508.
3. Kroon, G.H., J.B.M. Custers, Y.O. Kho, A.P.M. Den Nijs and H.Q. Varekamp. 1979. Interspecific hybridization in Cucumis L. 1. Need for genetic variation, biosystematic relations and possibilities to overcome crossability barriers. *Euphytica* 28:723-728.
4. Moreno, V., L. Zubeldia and L.A. Roig. 1984. A method for obtaining callus cultures from mesophyll protoplasts of melon (Cucumis melo L.). *Plant Sci. Let.* 34:195-201.
5. Moreno, V., M. Garcia-Sogo, I. Granell, B. Garcia-Sogo and L.A. Roig. 1985. Plant regeneration from calli of melon (Cucumis melo L.) cv. Amarillo Oro. *Plant Cell Tissue and Organ Culture.* (in press).
6. Ponti de, O.M.B. 1978. Resistance in Cucumis sativus L. to Tetranychus urticae KOCH. I. Search for sources of resistance. *Euphytica* 27:167-176.
7. Roche, M.V., L.A. Roig and V. Moreno. 1986. Callus formation, plant regeneration and clonal propagation in vitro of Gynura aurantiaca (Blume) DC. *Plant Cell Physiol.* 27(1):79-84.

This research has been supported by Grant No. 3021/83 from the C.A.I.C.Y.T. (Spanish Government) Fund. B. Garcia-Sogo is grateful for a Grant from the Caja de Ahorros de Valencia Foundation. M. Garcia-Sogo present address: Instituto de Investigaciones Citologicas de la Caja de Ahorros de Valencia, Spain.