

Regeneration in selected *Cucurbita* spp. germplasm

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Introduction

A system for plant regeneration from individual cells or explants is essential for the application of genetic engineering. In Cucurbitaceae, *in vitro* regeneration has been reported across a wide spectrum of crops, including summer squash (*Cucurbita pepo* L.) (7), winter squash (*Cucurbita maxima* Duch.) (9), bottle gourd (*Lagenaria siceraria* Standl.) (6) and, recently, figleaf gourd (*Cucurbita ficifolia* Bouché.) (8). Genetic transformation was applied in this family for different purposes, for example to improve several cucurbits with potential to function as rootstocks (4, 6, 11). Regeneration ability is highly influenced by genotype, and then it is necessary to develop and adjust the appropriate regeneration protocols for each cultivar. In this work we assay different *in vitro* conditions useful for regeneration in eight *Cucurbita* cultivars which presented interesting characteristics for breeding. They include: three *C. pepo*, two *C. ficifolia* one *C. maxima* and two *C. moschata* species. To the best of our knowledge this is the first study reporting *in vitro* regeneration for the latter species.

Materials and Methods

C. pepo cultivars PI 171628 (a pumpkin from Turkey), V-CU-32 (a Spanish Zucchini commercial cultivar) and the commercial hybrid Temprano de Argelia (Vilmorin; TA), *C. moschata* cultivars AN-CU-45 (a Spanish Butternut cultivar) and AFR-CU-1 (a globular landrace from Morocco); *C. maxima* cultivar AN-CU-59 (a Spanish globular landrace), and the ECU-148 and Pa 06 cultivars of figleaf gourd (from Ecuador and Spain respectively) were used. The PI accession was kindly provided by NPSG-USDA Genebank and the remainder accessions, with the exception of the commercial hybrid, were maintained at the COMAV Genebank and fixed by selfing, and were morphologically and molecularly characterized by the Cucurbits Breeding group of the COMAV (1-3). Manually de-coated seeds were sequentially surface-sterilized by immersion in ethyl alcohol (96%) for 1 min, followed by 2 min in a solution of 50% commercial bleach (containing 40 g L⁻¹ active chloride), and 10 min in NaOCl (20%). Between each step, the seeds were rinsed once with sterile distilled water and three times

at the end of the process. Most degenerated perisperms were removed from the embryos during the sterilization procedure. The embryos were blot dried on sterile filter paper for about 3 min, and then seeds were sown in Petri dishes (5 seeds per plate) containing 25 ml of hormone-free MS medium (10) solidified with 7 g L⁻¹ plant agar (Duchefa Biochemie, Haarlem, The Netherlands) and 15 g L⁻¹ sucrose (Duchefa Biochemie). The pH of the medium was adjusted to 5.8 prior to autoclaving at 121°C for 20 min. The seeds were incubated at 25°C under a 16-h photoperiod with cool white light provided by fluorescent lamps (light intensity 90 μmol m⁻² s⁻¹). Organogenic medium consists of MS salts, 30 g L⁻¹ sucrose, 1 mg L⁻¹ 6-bencilaminopurine (BA) added after sterilization, and 7 g L⁻¹ plant agar. For the accession AN-CU-45, three types of explants were used, two from cotyledons (proximal and distal from the plumule that was excised previously) and one from hypocotyls. Proximal cotyledon explants were employed in the rest of genotypes. Five explants per plate and 3 plates per genotype and treatment were used. Adventitious shoots induced from explants were isolated and cultured on MS medium or MS with 1 mg L⁻¹ indole butyric acid (IBA). Results were analyzed by one-way analysis of variance and means were separated using Duncan's multiple range test.

Results and Conclusions

Explants from proximal cotyledons of *C. moschata* were found to display a markedly enhanced production of adventitious shoots compared to distal cotyledons and hypocotyls (P value <0.0000). An average of 1.33 shoots/explant was obtained from proximal explants, whereas only 0.13 were obtained from the distal and hypocotyls. These results confirm our previous results with *C. pepo* where the best performance was observed in proximal cotyledons (5) and are also similar to that recently reported in *C. ficifolia* (8). These data suggest that cells at this zone display an enhanced response to morphogenesis induction and/or that, in *Cucurbita* species, the endogenous growth regulators that promote morphogenesis accumulated at this area. Regeneration from proximal cotyledon explants was also observed in all tested genotypes on medium containing 6BA, a common growth regulator for organogenic induction (7), (Table 1). Sig-

nificant differences among genotypes and cultivars belonging to similar species have been observed. *C. moschata* AFR-CU-1 had the lower regeneration response, whereas *C. pepo* cultivars PI171628 and TA showed the highest level of regeneration. The number of shoots per explant after 20 days of culture ranged from 1 to 4. Individual mean values are shown in Table 1. About 50% of isolated shoots (on average) were able to root on MB without growth regulators but, this percentage increased since 100% when IBA was added. The plantlets that were obtained were satisfactorily acclimatized. The information presented here is useful in order to select the most appropriate combination of explants/medium for regeneration in different species of *Cucurbita*. The highest regeneration ability has been found in *C. pepo*, the most economically important *Cucurbita* species. We have also reported here for the first time the regeneration ability of proximal explants in *C. moschata*, a species frequently use for breeding *C. pepo* and interesting as genetic bridge, for transferring traits from wild *Cucurbita* species into cultivated *C. pepo* and *C. maxima*.

References

1. Ferriol, M., B. Picó, and F. Nuez. 2003a. Genetic diversity of some accessions of *Cucurbita maxima* from Spain using RAPD and SBAP markers. *Genetic Research and Crop Evolution*. 50:227-238.
2. Ferriol, M., B. Picó, and F. Nuez. 2003b. Genetic diversity of a germ-plasm collection of *Cucurbita pepo* using SRAP and AFLP markers. *Theoretical and Applied Genetics*, 107:271-282.
3. Ferriol, M., B. Picó, P. Fernández de Córdoba, and F. Nuez. 2004. Molecular Diversity of a Germplasm Collection of Squash (*Cucurbita moschata*) Determined by SRAP and AFLP Markers. *Crop Science* 44:653-664.
4. Gal-On, A., D. Wolf, Y. Antignus, L. Patlis, K.H. Ryu, B.E. Min, M. Pearlsman, O. Lachman, V. Gaba, Y. Wang, Y.M. Shibolet, J. Yang and A. Zelcer. 2005. Transgenic cucumbers harboring the 54-kDa putative gene of Cucumber fruit mottle mosaic tobacco virus are highly resistant to viral infection and protect nontransgenic scions from soil infection. *Transgenic Research* 14:81-93.
5. Gisbert, C., B. Picó, and F. Nuez. 2009. Regeneración de plantas a partir de explantes de cotiledón de calabacines de interés horticola. Resúmenes VIII Reunión de la Sociedad Española de cultivo in vitro, p.51.
6. Han, J.S., C.K. Kim, S.H. Park, K.D. Hirschi, I.G. Mok. 2005. Agrobacterium-mediated transformation of bottle gourd (*Lagenaria siceraria* Standl.). *Plant Cell Reports* 23:692-698.
7. Kathiravan, K., G. Vengedesan, S. Singer, B. Steinitz, H. S. Paris and V. Gaba. 2006. Adventitious regeneration *in vitro* occurs across a wide spectrum of squash (*Cucurbita pepo*) genotypes. *Plant Cell, Tissue and Organ Culture* 85: 285-295.
8. Kyung-Min, K., K. Chang Kil, and H. Jeung-Sul. 2010. *In vitro* regeneration from cotyledon explants in figleaf gourd (*Cucurbita ficifolia* Bouche'), a rootstock for Cucurbitaceae. *Plant Biotechnology Reports* 4:101-107.
9. Lee, Y.K., W.I. Chung and H. Ezura. 2003. Efficient plant regeneration via organogenesis in winter squash (*Cucurbita maxima* Duch.). *Plant Science* 164:413-418.
10. Murashige, T., and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* 15:473-497.
11. Park, S.M., J.S. Lee, S. Jegal, B.Y. Jeon, M. Jung, Y.S. Park, S.L. Han, Y.S. Shin, N.H. Her, J.H. Lee, M.Y. Lee, K.H. Ryu, S.G. Yang, and C.H. Harn. 2005. Transgenic watermelon rootstock resistant to CGMMV(Cucumber Green Mottle mosaic Virus) infection. *Plant Cell Reports* 24:350-356.

Table 1. Regeneration of cotyledon explants* of *Cucurbita* spp. on medium with 6BA mgL⁻¹ after 20 days of culture

	<i>Genotype</i>	<i>Percentage of explants with shoots</i>	<i>Nº of shoots per explants</i>
<i>C. pepo</i>	PI171628	100 b	2.33 d
<i>C. pepo</i>	V-CU-32	80 ab	1.00 ab
<i>C. pepo</i>	TA	80 ab	2.40 d
<i>C. moschata</i>	AN-CU-45	100 b	1.33 c
<i>C. moschata</i>	AFR-CU-1	60 a	0.80 a
<i>C. maxima</i>	AN-CU-59	100 b	1.13 ab
<i>C. ficifolia</i>	ECU-148	80 ab	0.80 a
<i>C. ficifolia</i>	Pascual 06	67 a	0.80 a

*Cotyledons containing the region proximal to the plumule which has been previously excised. Means followed by different letter are significantly different by Duncan's multiple comparison test.