Regeneration in selected *Cucurbita* spp. germplasm

C. Gisbert, B. Picó and F. Nuez

Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV). Universitat Politècnica de València, Valencia, Spain 46022

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 (*C. 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 was 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 40g 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, 1mg 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-
significant 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 used for breeding with *C. pepo* and interesting as a genetic bridge, for transferring traits from wild *Cucurbita* species into cultivated *C. pepo* and *C. maxima*.

**References**


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

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Percentage of explants with shoots</th>
<th>Nº of shoots per explants</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. pepo</em></td>
<td>PI171628</td>
<td>100 b</td>
</tr>
<tr>
<td><em>C. pepo</em></td>
<td>V-CU-32</td>
<td>80 ab</td>
</tr>
<tr>
<td><em>C. pepo</em></td>
<td>TA</td>
<td>80 ab</td>
</tr>
<tr>
<td><em>C. moschata</em></td>
<td>AN-CU-45</td>
<td>100 b</td>
</tr>
<tr>
<td><em>C. moschata</em></td>
<td>AFR-CU-1</td>
<td>60 a</td>
</tr>
<tr>
<td><em>C. maxima</em></td>
<td>AN-CU-59</td>
<td>100 b</td>
</tr>
<tr>
<td><em>C. ficifolia</em></td>
<td>ECU-148 (BGV005955)</td>
<td>80 ab</td>
</tr>
<tr>
<td></td>
<td></td>
<td>67 a</td>
</tr>
</tbody>
</table>

*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
Table of Contents (article titles linked to pdf files)

1. Introduction
   - Comments from 34th CGC Business Meeting (2010)
   - Comments from the CGC Coordinating Committee
   - Cucurbit Genetics Cooperative Report Call for Papers
   - Comments from CGC Gene List Committee
   - Comments from CGC Gene Curators
   - 2010 Watermelon Research and Development Working Group - 30th Annual Meeting
   - 2011 Watermelon Research and Development Working Group - 31st Annual Meeting
   - Comment from the U.S. Cucurbit Crop Germplasm Committee OOB
   - Upcoming Meetings of Interest to Cucurbit Researchers
   - Cucurbit Genetics Cooperative Style Guide
   - In Memoriam

2. Cucumber (Cucumis sativus)
   1. The Cucurbit of Antiquity: A Case of Mistaken Identity
      Harry S. Paris and Jules Janik
      CGC 53 & 54: 2 (2010-2011)
   2. Origins and Characterization of the 'Lemon' Cucumber
      R.W. Robinson
      CGC 33 & 34: 3 (2010-2011)
   3. Yield of Sprinz-Planted Cucumber Using Row Covers, Polyethylene Mulch, and Chilling Resistant Cultivars
      Todd C. Webster, Gabrielle Gusman and Katherine B. Perry
      CGC 33 & 34: 5 (2010-2011)
   4. Genetic Control of Downy Mildew Resistance in Cucumber: A Review
      Adam D. Critwell, Adam D. Call and Todd C. Webster
      CGC 33 & 34: 13-16 (2010-2011)
   5. Antagonistic Acinonycte XN-1 from Phytophthora Microorganisms of Cucumber to Control Corynespora cassiicola
      Minggang Wang and Qing Ma
      CGC 33 & 34: 17-21 (2010-2011)

3. Melon (Cucumis melo)
   6. Powder Mildew Resistance of Cucumis at Different Locations
      R.W. Robinson
      CGC 33 & 34: 22-23 (2010-2011)
   7. Podosphaera xanthii but not Geotrichum cichoracearum Infects Cucumis in a Greenhouse at Salinas, California
      Cosme Bojorques Ramos, Karunatharan Maruthanam, James D. McCready and Raymundo S. Garcia Estrada
      CGC 33 & 34: 24-25 (2010-2011)
   8. Melon Trait and Germplasm Resources Survey 2011
      James D. McCready
      CGC 33 & 34: 59-71 (2010-2011)
   9. Variability and Correlation among Morphological, Vegetative, Fruit and Yield Parameters of Saale Melons (Cucumis melo var. saalei)
      CGC 33 & 34: 32-35 (2010-2011)

4. Watermelons (Citrullus lanatus)
   10. l-Citrulline Levels in Watermelon Cultivars from Three Locations
11. Breeding for Yield in Watermelons - A Review
Rakesh Kumar and Todd C. Wehner
CGC 33 & 34: 36-39 (2010-2011)

12. Natural Outcrossing in Watermelons - A Review
Rakesh Kumar and Todd C. Wehner
CGC 33 & 34: 40-41 (2010-2011)

13. Characterization of MI Generation of Polyploids in Watermelon Variety 'Sugar Baby'
T. Pradeep Kumar
CGC 33 & 34: 44-46 (2010-2011)

14. A "Hull-less" Seed Trait of Cucurbita maxima Duch. in Accession BGH 7653
Jose Raulindo Garlingo, Derly Jose Henrique da Silva, Vincente Wagner Dias Casal, Izails da Silva Lima Neto and Roseli Aparecida Ferrari
CGC 33 & 34: 47-50 (2010-2011)

15. Pollination of Squash Before and After the Day of Anthesis
R.K. Robinson
CGC 33 & 34: 51-52 (2010-2011)

16. Regeneration in Selected Cucurbita spp. Germplasm
C. Gilbert, B. Pico and F. Nuez
CGC 33 & 34: 53-54 (2010-2011)

17. "Exploding" Fruits not Unique to Watermelons; Fruit Cracking in Cucurbita moschata
Linda Weisell-Beaver
CGC 33 & 34: 55-56 (2010-2011)

A.D. Mwiriri, T.K. Behera, A.K. Sureja and Ravinder Kumar
CGC 33 & 34: 57-59 (2010-2011)

19. Use of Silver Thiosulfate and Gibberellic Acid for Induction of Hermaphrodite Flower in Gyneroeccous Lines of Bitter Gourd (Momordica charantia L.)
T.K. Behera, Samaranska Mishra and Anand Pal
CGC 33 & 34: 60-61 (2010-2011)

20. Phenotypic Diversity Analysis in Pointed Gourd (Trichosanthes dioica Roth)
L.K. Bharathi and Vimalnath
CGC 33 & 34: 62-64 (2010-2011)

21. Performance of Gyneroeccous x Monoeccous Hybrids of Bitter Gourd (Momordica charantia L.)
Swati Khan and T.K. Behera
CGC 33 & 34: 65-66 (2010-2011)

22. The Distribution and Application of Bitter Gourd in China
Chun-mei Duan, Zhen Liu and Hong-wen Cai
CGC 33 & 34: 67-68 (2010-2011)

23. Gene List 2010 for Cucumber
Adam D. Call and Todd Wehner
CGC 33 & 34: 69-101 (2010-2011)

24. Gene List 2011 for Melons
Catherine Dogimont
CGC 33 & 34: 104-133 (2010-2011)

Appendices:
- 2010-2011 CGC Membership Directory
  CGC 33 & 34: 134-138 (2010-2011)
- 2010-2011 CGC Membership by Country
  CGC 33 & 34: 139-140 (2010-2011)
- 2010-2011 United States CGC Membership by State
  CGC 33 & 34: 141 (2010-2011)
- By-Laws for the Cucurbit Genetics Cooperative