In Vitro Propagation of ‘Guayabo del pais’
(Acca sellowiana (Berg.) Burret)

Silvia Ross* · Rafael Grasso

Departmento de Biología Vegetal, Facultad de Agronomía, Universidad de la República. Gral. E. Garzón 780. Montevideo, Uruguay

Corresponding author: *sross@fagro.edu.uy

ABSTRACT

* Acca sellowiana (Berg.) Burret, known as ‘Guayabo del pais’ or ‘Feijoa’, is a medium-sized tree of the Family Myrtaceae, native to southeastern Brazil and Uruguay. It can be found in regions with different climate conditions, it is somewhat resistant to frosts, it exhibits precocious productivity, high nutritional value, good postharvest life and is adequate for fresh consumption as well as for the elaboration of different manufactured products. (Cunda 2006).

INTRODUCTION

Acca sellowiana (Berg.) Burret, of the plant Family Myrtaceae, known as ‘Guayabo del pais’ or ‘Feijoa’, is one of the numerous native fruit tree species that are currently not part of any industrial process of breeding, production and commercialization in Uruguay. However, commercial cultures of this species are exploited in Europe, New Zealand and southeastern Brazil and Uruguay. It can be found in regions with different climate conditions, it is somewhat resistant to frosts, it exhibits

Keywords: bioreactors, liquid culture, micropropagation, phloroglucinol
Abbreviations: BAP, 6-benzylaminopurine; IBA, indole-3-butyric acid; 2iP, 2-isopentyl adenine; MS, Murashige and Skoog; NAA, 1-naphthaleneacetic acid; WPM, Woody Plant Medium

Received: 29 October, 2009. Accepted: 19 June, 2010.

Original Research Paper

© 2010 Global Science Books
1981) basic culture medium without plant growth regulators (PGRs) induced the best organogenic responses from nodal segments, both quantitative and qualitative; moreover, the organogenic responses observed by them largely depend on genotype. Cutting-grown plants take longer to establish in the orchard and grafted plants produce many suckers from their seedling rootstocks, both situations could complicate management techniques. Micropropagated plants have not yet been field-tested (Thorp and Bieleski 2002).

Research with *A. sellowiana* in Brazil includes breeding, studies on genetic diversity, germplasm conservation, propagation and technology transfer (Franzon 2004). At present there are two cultivars of this species developed in Brazil, ‘SCS 411-Alcantara’ and ‘SCS 412-Helena’, obtained in breeding programmes. These cultivars are self-fertile, complementary in terms of maturation, and present good resistance levels to the main diseases that affect the plant in Brazil (Epagri, s.f.).

As mentioned above, one of the main constraints for the commercial propagation of *A. sellowiana* in Uruguay is the yet unsuccessful vegetative propagation of selected materials (Vignale and Bisio 2005). In this work we consider the study of micropropagation as an alternative to conventional propagation techniques in this species, to produce high quality plant material at a big scale and pathogen-free. The main objective of this research was to standardize a micropropagation protocol for *A. sellowiana*, evaluating different in vitro techniques, including liquid medium in bioreactors during the multiplication stage.

MATERIALS AND METHODS

Plant material

Mother plants were selected from the Minor Fruit Trees Programme of the Faculty of Agronomy, Universidad de la República, Uruguay (http://www.fagro.edu.uy). They were cultivated in a greenhouse. Specimens represented eleven genotypes, corresponding to four localities of origin (Florida, Tacuarembó, Salto and San José). Materials are identified as Ca 70, Ca 74, Ca 75, Ca 127 (four clones from San José), Ch 128, Ch 162, Ch 304, Ch 24, Ch 168, Ch 246 (6 clones from Salto and Tacuarembó where weather conditions are similar) and C’Ch (one clone from Florida). Mother plants in the greenhouse were treated periodically with fungicide (Benlate®, 2 g l⁻¹) and spraying was induced by a cytokinin treatment of 4.4 mM 6-benzylaminopurine (BAP) (Sigma B-3408; Sigma-Aldrich). Basal sprouting was used as source of explants to introduce in vitro.

Disinfection of explants

Branches were washed with commercial detergent (Deterjane®) and tap water. Nodal segments were surface disinfected with 2% NaOCl for 20 min, washed three times with distilled water adding citric acid (0.1 g l⁻¹) in the third wash.

Culture media

Basal medium used in the different stages was WPM supplemented with MS vitamins (Murashige and Skoog 1962) and 3% sucrose. The gelling agent employed was 0.7% agar (Sigma A-4550; Sigma-Aldrich, St. Louis, MO, USA), and pH was adjusted to 5.8 prior to autoclaving in all cases. For the introduction stage, PGRs employed were 0.44 μM BAP (Sigma B-3408; Sigma-Aldrich) and 0.054 μM NAA (1-naphthalenacetic acid; Sigma N-0640; Sigma-Aldrich).

For the multiplication stage of the genotypes introduced, six treatments differing in type and concentration of PGRs were evaluated against a control free of PGRs. Treatments were as follows:

- **T1**: BAP 0.44 μM + NAA 0.054 μM
- **T2**: BAP 0.88 μM + NAA 0.054 μM
- **T3**: BAP 1.76 μM
- **T4**: 2iP (6-γ-γ-dimethylamino purine) 9.8 μM
- **T5**: 2iP 9.8 μM + NAA 0.108 μM
- **T6**: 2iP 19.6 μM

We also evaluated the performance of the liquid medium supplemented with 9.8 μM 2iP (Sigma D-7674; Sigma-Aldrich) alone, and with the addition of 40 mg l⁻¹ phloroglucinol (Sigma P-1178; Sigma-Aldrich) to prevent hyperhydricity by promoting lignification of shoots.

Bioreactors

Evaluation of liquid cultures was done in permanent immersion bioreactors (Fig. 1). Bioreactors were built with a 2-L Erlenmeyer, rubber lids with two ports for inlet and outlet filtered air (0.2 μm Millipore filters), and aeration was supplied by a Resun Air-Pump (AC-9803).

Growth conditions

Cultures were incubated at 20 ± 2°C, with a photon flux of 30 μmol.m⁻².s⁻¹, and a 16-h photoperiod.

Rooting

Two rooting conditions were evaluated for the microshoots obtained: *in vitro* and *ex vitro*.

*In vitro*, MS media supplemented with 2 mg l⁻¹ IBA (indol-3-butric acid) (Sigma I-5386; Sigma-Aldrich) for 1 week and then transfer to MS media devoid of auxin, was compared against a control without IBA. Rooted explants were transferred to pots for acclimation prior to transfer to the greenhouse.

*Ex vitro*, the base of the microshoots were immersed in IBA solution (500 ppm) for 1 min, and then placed in plastic trays with sterile substrate.

Statistical analysis

The experiments were repeated at least 5 times, 30 explants per treatment when using semi-solid medium and 15 explants per bioreactor in experiments with liquid culture medium. Comparisons were done using one-way analysis of variance and least significant difference (LSD) means separation with a confidence level of $P = 0.05$. STATISTICA® software was used.
Micropropagation of Acca sellowiana. Ross and Grasso

RESULTS AND DISCUSSION

Establishment

The main limitation found at this stage was fungal contamination, with strong differences according to genotype (Fig. 2).

Browning due to oxidation of phenolic compounds was not significant, as it would be expected for a woody species.

In some genotypes, proliferation of callus around the axillary buds was observed, which prevented buds from developing.

Multiplication on semi-solid media

Fig. 3 shows the multiplication rate of three genotypes evaluated. Multiplication rate of Ca 75 material did not show significant differences for any of the PGR treatments. Cº Ch and Ca 74 showed higher proliferation rates in all cases, highest when 2iP was used.

The average multiplication rate for the different materials is shown in Fig. 4, highest when 2iP was the cytokinin used. Similar results were reported by Oltramari et al. (2000) in Acca sellowiana, probably due to the low stability of this hormone (George 1993).

Progress in multiplication with bioreactors

Preliminary experiments employing bioreactors in stage 2 showed a significant increment in the multiplication rate of the genotypes evaluated.

A strong effect of genotype was observed, confirming the tendency observed on semi-solid media; Ca 74 was the plant material with the highest multiplication rate in permanent immersion in liquid media (Fig. 5).

The higher proliferation rate was associated to the immersion of the explant in the medium, probably because of a better contact of the plant tissue with the medium and thus optimal nutrient and supply of PGRs. In addition, aeration of the medium resulted in permanent movement of the explants which produced a gradual loss of apical dominance and promoted the growth of several axillary buds (Fig. 6).

The addition of phloroglucinol to the liquid culture medium to prevent hyperhydricity via promotion of lignification of the explants avoided the occurrence of plant malformations. Phloroglucinol can be used as an alternative precursor in the lignin biosynthesis pathway, and has been successfully employed to prevent hyperhydricity in other woody species in our laboratory (Ross 2006).
Rooting and acclimation

All the rooting conditions evaluated promoted root differentiation. Our results did not show significant differences for ex vitro and in vitro rooting with AIB, neither in number of microshoots which differentiated roots nor in the number of roots per explant or root length, as is shown in Table 1. Explants that rooted in vitro survived the acclimation stage without significant losses.

Fig. 7 shows rooting conditions evaluated, and acclimated growing plants obtained in the greenhouse.

Acknowledgements

This research was carried out as part of the interdisciplinary project “First systematic study of Acca sellowiana (Berg.) Burret populations as genetic resource” (PDT 32/35).

References

Lloyd G, McCown B (1981) Commercially-urable micropropagation of Feijoa sellowiana after 30 days in the bioreactor, showing axillary bud development.

Table 1 Average rooting percentages and number of roots per explant for all the genotypes evaluated. (T1: MS without growth regulators, T2: MS plus IBA (9.8 μM) for 7 days).

<table>
<thead>
<tr>
<th>Rooting (%)</th>
<th>Number of roots/explant</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro T1</td>
<td>22.7</td>
</tr>
<tr>
<td>In vitro T2</td>
<td>44.4</td>
</tr>
<tr>
<td>Ex vitro</td>
<td>48.0</td>
</tr>
</tbody>
</table>

Fig. 6 Explants of Acca sellowiana after 30 days in the bioreactor, showing axillary bud development.

Fig. 7 Rooting of Acca sellowiana. (A) Plant rooted in vitro (T2). (B) Acclimation of in vitro rooted plants. (C) and (D) Plants obtained, rooted in vitro. (E) Ex vitro rooting conditions.
Micropropagation of *Acca sellowiana*. Ross and Grasso


