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Improvements in somatic embryogenesis protocol in Feijoa (*Acca sellowiana* (Berg) Burret): Induction, conversion and synthetic seeds

Gabriela Claudia Cangahuala-Inocente [a], Lírio Luiz Dal Vesco [a], Douglas Steinmacher [a], Antonio Carlos Torres [b], Miguel Pedro Guerra [a,*]

[a] *Laboratório de Fisiologia do Desenvolvimento e Genética Vegetal, Centro de Ciências Agrárias, Universidade Federal de Santa Catarina, 88.034-001 Florianópolis, SC, Brazil*
[b] *Embrapa Hortaliças, Caixa Postal 218, 70359-070 Brasília, DF, Brazil*

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Abstract

Pineapple guava (*Acca sellowiana*) syn. *Feijoa sellowiana*, a Brazilian indigenous Myrtaceae is under domestication in South Brazil. Previous works showed that this species is responsive to somatic embryogenesis and recalcitrant to conventional methods of clonal propagation. In the present work it was evaluated the role of components of culture medium in the induction and development of somatic embryos. The technology of synthetic seeds was also evaluated. Zygotic embryos were inoculated in LPm medium supplemented with 8 mM glutamic acid and 8 mM L-glutamine, 2,4-dichlophenoxiacetic acid (20 μM) and myo-inositol. For conversion of somatic embryos and synthetic seeds it was tested the effect of 6-benzylaminopurine and gibberellic acid combined or not with activated charcoal. The highest values for embryogenetic induction (100%) and number of somatic embryos/explant (113) were observed in the LPm medium supplemented with Glu (8 mM), and 2,4-D. The culture medium supplemented with BA (0.5 μM) and GA_3 (1 μM) and activated charcoal (1.5 g L^{-1}) enhanced the conversion of somatic embryos to plantlets. Pre-germinated somatic embryos encapsulated in sodium alginate with BA (0.5 μM) and GA_3 (1 μM) developed radicles. The use of synthetic seed was a requisite for the survival of plantlets.

Keywords: *Feijoa sellowiana*; Glutamic acid; Glutamin; Myo-inositol; Encapsulated; Activated charcoal

1. Introduction

Acca sellowiana (Berg) Burret *syn*. *Feijoa sellowiana* Berg is a native fruit species of South Brazil, now under domestication. This species is recalcitrant to propagation by conventional clonal methods. Thus, tissue culture based techniques had been developed to overcome such limitations.

The route of somatic embryogenesis of *A. sellowiana* was first described by Cruz et al. (1990). For the conversion of somatic embryos to plantlets it was used the modified MS media, supplemented with GA_3 and BA (Canhoto and Cruz, 1996).

A modified protocol of somatic embryogenesis for this species based on the use of LP media, 2,4-D, GA_3 and Kin was established (Guerra et al., 1997) using zygotic embryos as explants. In this species it was demonstrated that 2,4-D pulses enhanced the induction and development of somatic embryos from zygotic embryos (Guerra et al., 2001). Also, the addition of amino Asp, Gln or Arg (4 mM) in the LPm increased the number of somatic embryos (Dal Vesco and Guerra, 2001). The somatic embryogenesis of *F. sellowiana* from floral tissues was described by Stefanello et al. (2005).

An important application of the somatic embryos is its use in the production of synthetic seeds. Synthetic, artificial or somatic seeds are analogous seeds to the true or botanical seed, and consist of a somatic embryo surrounded by one or more artificial layers forming a capsule. This capsule serves of protection the somatic embryo against mechanical damages during the storage, and sowing (Onishi et al., 1994). In order to improve the conversion of the synthetic seeds the supplementation of nutrients has been evaluated, along with the use of plant growth regulators (PGR) and the addition of other substances to

* Corresponding author.
E-mail address: mpguerra@cca.ufsc.br (M.P. Guerra).

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the alginate matrix (Mamiya and Sakamoto, 2001; Dal Vesco et al., 2005).

Woody plants are considered recalcitrant a somatic embryogenesis. However, the works carried out in our laboratory show that *A. sellowiana* can be considered as a model system for the induction and control of this *in vitro* morphogenetic route (Guerra et al., 2001).

Several factors affect the efficiency of somatic embryogenesis induction, as amino acids (Dal Vesco and Guerra, 2001), myo-inositol (Vooková et al., 2001) as well as the development and conversion of somatic embryos (Dal Vesco et al., 2005). In the present work we employed a model system of *A. sellowiana* somatic embryogenesis in order to evaluate the effect of the glutamic acid and its neutral amide, glutamine, as well as the sugar-alcohols as myo-inositol in the induction and development of the somatic embryos. The effect of BAP, GA_3, and activated charcoal in the conversion of the somatic embryos in plantlets and in the reconstitution of artificial endosperm in synthetic seeds was also studied.

2. Material and methods

2.1. Plant material

Mature fruits the *A. sellowiana* maintained at the germplasm collection of the EPAGRI's Experimental Station of São Joaquim, Santa Catarina State, Brazil, were collected and transported to the Laboratory of Developmental Physiology and Plant Genetics, of the Federal University of Santa Catarina, Florianópolis, Brazil. The seeds were rinsed with water and commercial detergent, placed on a solution of 1.0% NaOCl overnight, and then rinsed three times in sterile water. The zygotic embryos were excised in an aseptic chamber and inoculated in test tubes (25 mm × 150 mm) containing 15 mL of basal medium LPm (von Arnold and Eriksson, 1981) supplemented with Morel's vitamins (Morel and Wetmore, 1951) and sucrose (3%). The pH was adjusted to 5.8 prior to autoclaving. This culture medium is designated as basal medium.

All steps of somatic embryogenesis induction were undertaken in chamber room, in the dark at 25 °C. For somatic embryo and synthetic seed conversion, the cultures were maintained in room culture at 25 °C with 16 h light (60 μmol m^{-2} s^{-1}) provided by fluorescent cool white lamps.

2.2. Influence of the glutamic acid, the glutamine and myo-inositol in the induction of somatic embryogenesis

Zygotic embryos of the genotype 291 × 458 were excised and then inoculated in the basal LPm medium gelled with Phytagel® (0.2%), and supplement with 2,4-D (20 μM), myo-inositol (0, 0.1, 0.25 and 0.5 g L^{-1}) in combination with L-glutamine – Gln (8 mM) or glutamic acid – Glu (8 mM) and amino acid free control. This culture medium was dispensed in test tubes (150 mm × 25 mm) with approximately 15 mL each and autoclaved at 121 °C during 15 min. Each experimental sample consisted of 10 test tubes arranged in a completely randomized design (CRD), replicated four times. The percentage of induction and the number of somatic embryos per explant were recorded by 15 days (0–120 days) after induction.

2.3. Conversion of the somatic embryos

Torpedo-staged somatic embryos of the genotype 101 were used for the experiment of pre-germination. The somatic embryos were cultured in basal medium describe above in the presence or absence of activated charcoal (1.5 g L^{-1}), combined with BAP (0.5 μM) and GA_3 (1 μM). Each experimental sample consisted of 40 somatic embryos inoculated in Petri dishes containing 25 mL of the medium, arranged in CRD, and replicated three times. The percentages of complete plantlets, green somatic embryos, somatic embryos with green cotyledon and somatic embryos without responses were recorded after 15, 30 and 45 days in culture.

2.4. Reconstitution of artificial endosperm and synthetic seeds

Torpedo-stages somatic embryos of the genotype 101 were cultured before encapsulation during 15 days in the basal medium describe above in the presence or absence of activated charcoal (1.5 g L^{-1}), and in basal medium containing BAP (0.5 μM) and GA_3 (1 μM) in the presence or absence of activated charcoal (1.5 g L^{-1}). For the reconstitution of endosperm the same treatments evaluated for embryo pre-germinated were employed in the sodium alginate matrix. Each experimental unit was consisted of eighteen somatic embryos distributed in CRD, with three repetitions.

The synthetic seeds were established by capturing somatic embryos in a capsule of sodium alginate (1%) diluted in distilled and sterile water and then releasing the embryo containing capsule in a solution of $CaCl_2$ (50 mM) during 15 min. The resulting synthetic seeds were rinsed in distilled water.

2.5. Statistical analysis

Data of percentage, number and conversion of somatic embryos were submitted to test of F-maximum. When necessary, the data were transformed to $\log(x+2)$ or $(x+0.5)^{0.5}$. The data were then submitted to analysis of variance, to the Student–Newman–Kuels (SNK) test, and to the regression analysis according to Steel and Torrie (1988).

3. Results

3.1. Influence of the glutamic acid, the glutamine and myo-inositol in the induction of somatic embryogenesis

The rate of somatic embryogenesis induction was enhanced by Gln, Glu (Fig. 1). There were not significant differences in response to the concentrations of myo-inositol tested. The interactions among Glu (8 mM) and myo-inositol (0.1 g L^{-1}) resulted in 100% of embryogenetic induction 120 days after