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Use of X-ray microtomography for analysis of whole plant regeneration in *Passiflora organensis*

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Abstract

P. organensis is a native species in Brazil and the first species of the genus *Passiflora* to have its genome completely sequenced. The present work intends to elaborate an efficient protocol for *in vitro* regeneration of whole plants of *P. organensis* through tissue culture and the implementation of an innovative technology of 3D image acquisition - the computerized X-ray microtomography (micro-CT).

Key words:

Passiflora organensis, tissue culture, micro-CT.

Introduction

P. organensis is becoming more relevant since it is the first species of the genus *Passiflora* to have its genome completely sequenced (data not yet published from our group). The study of gene functions, resulting from the sequencing of the genome of a plant, implicates the application of transgenesis techniques. However, *in vitro* plant regeneration protocols needs to be optimized, especially for this species, since none was established yet¹. This present work aims to establish efficient protocols of regeneration of whole plants, from leaf and internode explants, besides testing the effect of different concentrations of the hormones Auxin (NAA) and Cytokinin (BAP), as well the effect of coconut water in the tissue culture media.

In addition, another aim of this work is the implementation of an innovative technology of 3D image acquisition - the computerized X-Ray microtomography (micro-CT) - which will allow a more refined description of the morphogenetic processes during *in vitro* tissue culture².

Results and Discussion

Preliminary results using leaf explant have shown better regeneration of *P. organensis* in media supplemented with 0,1 mg l⁻¹ NAA and 10,0 mg l⁻¹ BAP (Image 1). Concentrations above 0,1 mg l⁻¹ NAA were inefficient, inhibited the regeneration of whole plants since high levels of auxin shown to be harmful.

When the coconut water was added in culture media, the calli were bigger.

The micro-CT technique is being implemented and the morphogenesis processes are being analyzed.



Image 1. Regeneration of *P. organensis* through tissue culture using 0,1 mg l⁻¹ NAA and 10,0 mg l⁻¹ BAP after 93 days of culture.

Conclusions

Culture media supplemented with 0,1 mg l⁻¹ NAA and 10,0 mg l⁻¹ BAP and 10% coconut water, showed better results for leaf explants and may be useful for the establishment of an efficient protocol for the regeneration of whole plants of *P. organensis*.

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¹ Rosa, Y. B. C. J., Monte-Bello, C. C. & Dornelas, M. C. *Plant Cell Tissue Organ Cult.* **2015**, 120:69–77.

² Lee, K. J. I.; Calder, M. G., Hindle, C. R., Newman, J. L., Robinson, S. N., Avondo, J. J. H. Y. & Coen, E. S. *Journal of Experimental Botany*, **2017**, 68: 527–538.