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Investigation of plant cell culture as experimental model applied to the study of coumarin and chlorogenic acid biosynthesis pathway in Mikania glomerata and Mikania laevigata

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Abstract

Mikania glomerata and Mikania laevigata (guaco) are used as treatment for respiratory system diseases. Both are indistinctively used, however studies have shown differences between their chemical profiles. Therefore, there is a need to investigate the biosynthetic pathway of these metabolites in both species as way to guarantee the quality of these products. Plant cell culture can be an useful tool for studying changes in secondary metabolism as it provides a completely controlled environment and also allows manipulation of biosynthetic pathways for a more efficient production of metabolites. This study showed that plant cell cultures are viable experimental models and can be used to the study of biosynthetic pathways of coumarin and chlorogenic acid in both species.

Key words:

Phenylpropanoids, Plant cell cultures, Metabolic pathways.

Introduction

Mikania glomerata and *Mikania laevigata*, popularly known as guaco, are commonly used as treatment for respiratory system diseases due to their bronchodilator effect, bioactivity assigned to coumarin. Due to their morphological similarities both are indistinctively used by the general public, however studies have shown differences between their chemical profiles. It has been observed an accumulation of coumarin in *M. laevigata* and of chlorogenic acid in *M glomerata,* raising the question whether both could be used as medicine. Therefore, there is a need to investigate the biosynthetic pathway of these secondary metabolites in both species. Plant cell cultures are very promising for the production of secondary metabolites *in vitro*, since they allow a rapid cell proliferation. Moreover, they allow studies of regulation of a particular secondary metabolite under controlled conditions and are easy to manipulate. This research aim was to investigate the use of plant cell and tissue cultures as experimental models applied to the study of biosynthetic pathways of coumarin and chlorogenic acid in both species.

Results and Discussion

The explants obtained from leaves were collected from plants kept in the greenhouse of the Department of Plant Biology, Institute of Biology-Unicamp. Then they were washed and sterilized to prevent the growth of fungi and bacteria associated with the plant. Calli were maintained on MS medium[1] supplemented with 30 $g.L^{-1}$ sucrose (Image 1A). To determine the optimal concentrations of the hormones and ensure the best growing conditions, several tests were held with explants in media with different proportions of 2,4-dichlorophenoxyacetic acid and 6-benzilaminopurina. Media containing a 1:1 ratio of hormones were discarded because there was significant formation of roots (Image 1B). It was established that the best conditions for callus induction for these two species is 4.52 mM of 2,4-D and 26 uM BAP, since the development was fast and there was little root growth. Cell suspensions were established (Image 1C) by inoculating cells from friable calli into liquid MS medium with the same supplements, but it was observed that increasing the sucrose concentration of the medium to 50 $g.L^{-1}$ promotes the growth of the cells.

Analysis for identification and quantification of the phenylpropanoid biosynthetic pathway products were done by UPLC-MS, using methodology described by Melo[2]. Preliminary results from tests in which chlorogenic acid was inoculated in *M. glomerata* cell suspensions suggest that there is a positive metabolism modulation, which diverts the biosynthesis pathway to coumarin production. On the other hand, chlorogenic acid content was negatively correlated with an increasing chlorogenic acid concentration added to the suspensions.

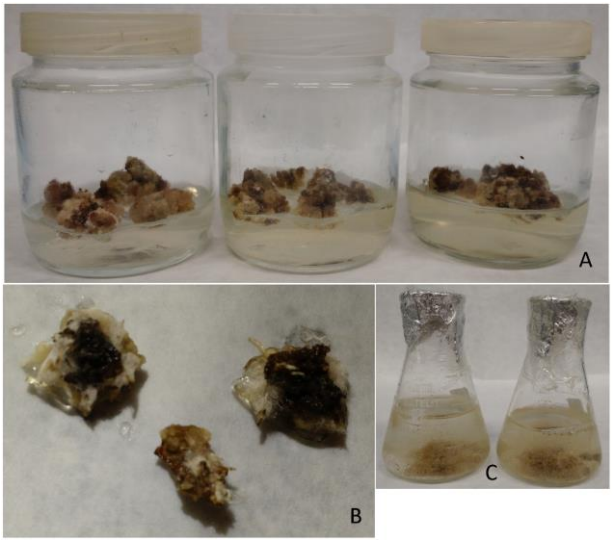

Image 1. *M. glomerata* e *M. laevigata* callus culture (A). Root growth on calli (B). *M. glomerata* cell suspensions (C).

Conclusions

M. Glomerata and *M. laevigata* cell cultures have shown viable as a biological model for application in studies of the biosynthetic pathway of coumarin and chlorogenic acid.

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