ACE-INHIBITORY ACTIVITY OF RECOMBINANT PROTEIN EXPRESED IN CELL SUSPENSION CULTURES OF *Nicotiana Tabacum* NT1

David U. Santos-Ballardo, Lourdes J. Germán-Báez, Abraham Cruz-Mendívil, Cindy I. Fuentes-Gutiérrez, Jorge Milán-Carrillo and Angel Valdez-Ortiz* Facultad de Ciencias Químico-Biológicas, Universidad Autónoma de Sinaloa, Av. de las Américas y Josefa Ortiz S/N, Culiacán, Sin., México 80030. *e-mail: angelvaldezortiz@yahoo.com.mx

Key words: plant cell suspensions, bioamarantin, antihypertensive

Introduction. Recently, the use of plant cell suspensions (PCS) has received considerable attention for the production of recombinant proteins, due to the fact that they have the same benefits of systems that use animal or microbial cell cultures, and whole plants (1). On the other hand, amarantin, is the most predominant seed storage protein of amaranth, the acidic-subunit of amarantin was modified by inserting four Val-Tyr (VY) antihypertensive biopeptides in tandem, after that it was named bioamarantin, resulting in an increase on the ACE-inhibitory activity of the recombinant protein expressed in *E. coli* (2). The ability to inhibit the angiotensin converting enzyme (ACE) activity has been usually considered for detection of therapeutic agents against chronic diseases such as hypertension (3). The aim of the present study was to express the bioamarantin, in a tobacco NT1 cell suspension and to evaluate its *in vitro* ACE-inhibitory activity.

Methods. The transformation of 11-days-old NT1 cell culture was performed by co-culture whit *Agrobacterium tumefaciens* EHA105::BioamarKDEL. For the PCR analysis pBioamarKDEL was used as positive control and DNA from *N tabacum* wt callus as negative control. The inmunodetection by Western blot was performed using polyclonal rabbit antibodies (0.5:30000 dilution) against amarantin and anti-rabbit IgG conjugated to horseradish peroxidase (HRP). The antihypertensive potential of transgenic and non-transformed hydrolyzates protein extracts was determined *in vitro* (IC_{50} value) (4).

Results. NT1 cells co-cultured with *Agrobacterium*, harboring pBioamarKDEL, developed into different colonies after 4 weeks of plating on selective medium. For pBioamarKDEL, the identity of transformants was confirmed by the amplification of the expected fragment (648-bp) in all the clones analyzed (Figure 1). Nevertheless, when the immunological detection was performed, only two of the clones tested were positive for bioamarantin (Figure 2, lanes 4 and 5), showing that this protein was stably expressed in some of the transgenic calli. The IC_{50} value obtained for the protein hydrolysates of transformed cells was 3.5 µg/mL, corresponding to nearly 10 times higher antihypertensive potential than the protein extracts of WT cells which IC_{50} obtained was 29.0 µg/mL, showing significant differences between them ($P \leq 0.05$).

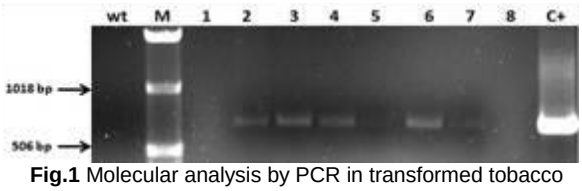

Fig.1 Molecular analysis by PCR in transformed tobacco cell cultures. (wt) wild type calli used as negative control, (M) molecular weight marker (1kb DNA ladder), (2-7) independent transformed tobacco cells, (1 and 8) empty, (C+) pBioamarKDEL DNA plasmidic used as positive control.

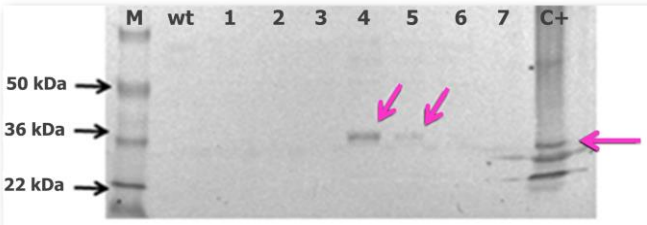

Fig.2 Western blot analysis of total protein extract. (M) molecular weight marker, (wt) protein extract from wild type calli used as negative control, (2-6) protein extracts of transformed cells, (1 and 7) empty, (C+) Bioamarantin expressed in *E. coli* used positive control.

Conclusions. The results shown in this work, can lead to further massive production of recombinant bioamarantin by using a bioreactor based on PCS.

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