Poster

ANTHOCYANINS BIOSYNTHESIS OPTIMIZATION IN *IN VITRO* VITIS VINIFERA LONG-TERM CALLUS BY USING NANOCARBONS MATERIALS

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Abstract

The aim of the present paper is to present an original protocol for improving the *in vitro* anthocyanin pigments biosynthesis using as ellicitors nanocarbon materials in callus cultures of Vitis vinifera. Anthocyanins represent a class of secondary metabolites that constitute the largest category of plant pigments. They have an important antioxidant role against free radicals, improving and maintaining human health. Secondary metabolytes represent usually roughly 1-3% of the dry weight of plant tissues. Their complex chemical structure and the presence within the plant of unwanted compounds, some toxic, makes their extraction and purification difficult. Due to these difficulties coupled with the limited capacity of harvesting large quantities of medicinal plants from the wild - new alternative methods for the production of secondary metabolites are required [1-5]. *In vitro* biotechnologies offer an alternative that is not hindered by these limitations. The proposed method can prove useful for the large scale production of those ones.

The starting work hypothesis is that the used nanocarbon materials could induce effects on the plant culture resembling to those induced by the activated charcoal. This study has investigated the influence of two types of nanocarbons based on graphite intercalated compounds (GICs) on cell proliferation and pigment biosynthesis in grape callus culture.

Graphite has an unique capability to form so-called graphite intercalated compounds (GICs), because of its layered structure. Molecules of intercalated species fill interlayer spaces and form monomolecular layers during intercalation process. In this work we synthesized two types of expanded graphite, based on thermal exfoliation of GICs, along with nanosized metal particles placed on the surface of expanded graphite. These compounds were notified as: type **A** without Fe nanoparticles and type **B** with Fe nanoparticles. Type A nanocarbon exhibit a surface area of 150 m^2/g with a porous system composed of micro and mezopores (2-50 nm) and type B nanocarbon shows a surface area of 10 m^2/g and is primary microporous (pore smaller than 2 nm). Figure 1 show a SEM image of type A nanocarbon material.

We used as inocula source a long-term callus culture of Vitis vinifera L. cv. Isabell. The nanocarbons (type A and B) have been separately included in the growth medium after medium sterilization, in concentrations ranging between 10 and 100 mg/12.5 ml nutritive medium/Petri dish.

Biological parameters measured

It has been estimated the following parameters: (i) callus growth rate, from the increase in wet weight at the end of the experiment, (ii) callus dry weight and (iii) concentration of anthocyanins measured spectrophotometrically at 525 nm. The measurements have been performed in a first attempt after 30 days of cultivation. Both tested compounds (A and B) had a significant effect on the experimental system.

Cell proliferation was slightly inhibited, while anthocyanins biosynthesis was stimulated up to 2.5 times compared to the control, in the presence of 30 mg **B**/Petri dish. The dry weight was significantly higher in both variants as compared to the control. The different cell lines showed

a high variability in their responses. This is a consequence of the heterogeneous cell composition of the calli.
From squash microscopically observations, the callus cells were extremely different in size, shape and pigment content. This suggests a need for selecting lines with increased yields in both growth rate and anthocyanins biosynthesis.

Conclusions

Is for the first time when significant effects of stimulation of some biological processes – biosynthesis of secondary metabolites such as anthocyanines- in *in vitro* vegetal systems are evidenced by using nanocarbons based on GICs. According to the above described method it has been registrated a strong secondary metabolites biosynthesis of 225% related to average.

References:

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Figures:

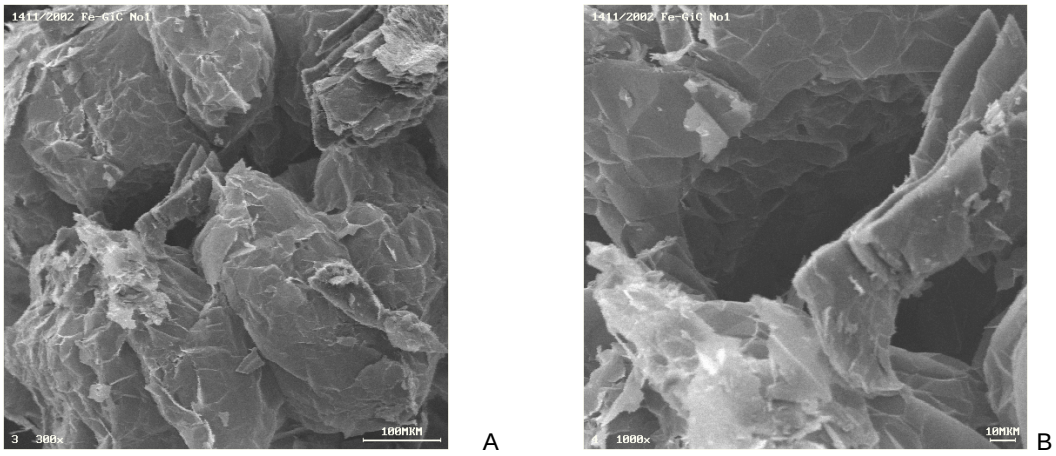

Figure 1. SEM images of type A nanocarbon used for antocyanins biosynthesis optimisation:
B enlarged image of A