Elicitor-mediated enhanced accumulation of secondary metabolites in apple cell cultures

Carmen Laezza (carmen.laezza@unina.it)

Dept. Agricultural Sciences, University of Naples Federico II, Italy

Tutor: Prof. Maria Manuela Rigano

Our work first aimed to the development of pulp-derived callus cultures from an apple landrace native to southern Italy. Thereafter, the content of secondary metabolites (SMs) within pulp-derived calli was analysed, showing the presence of a good amount of bioactive compounds. In light of this, the use of elicitors was tested in order to boost the production of these molecules.

Incrementato accumulo di metaboliti secondari in colture cellulari di mela mediato da elicitori

Il nostro lavoro ha avuto come primo obiettivo lo sviluppo di colture di callo vegetale derivate dalla polpa di una cultivar di mela originaria dell'Italia meridionale. Successivamente, è stato analizzato il contenuto di metaboliti secondari all'interno dei calli derivati dalla polpa, evidenziando la presenza di una buona quantità di composti bioattivi. Alla luce di ciò, è stato testato l'uso di elicitori per incrementare la produzione di queste molecole.

**Key words**: apple, callus cultures, secondary metabolism, elicitor, polyphenols, triterpenic acids.

# **1. Introduction**

In accordance with the PhD thesis project previously described (Laezza, 2022), this poster reports the main results of the first two activities concerning:

(A1) the production of apple pulp calli and the analysis of the content of bioactive compounds within these cell cultures.

(A2) the use of salicylic acid as elicitor to determine an increase in the production of secondary metabolites. The analysis of the content of bioactive compounds after elicitation.

# **2. Materials and Methods**

Apple pulp was sterilized, cut into small pieces, and placed on Gamborg B5 medium (Dixon, 1985) with auxin and cytokinin in a ratio 2:1. Cell cultures were incubated in the dark at 25 ± 2 °C. Pulp-derived calli from exponential developmental stage were inoculated on control medium and on medium with the addition of 50 and 100 mg/L salicylic acid (SA). Thereafter, extracts from freeze-dried apple calli were obtained with 1 mL of 1% formic acid methanol-water (80:20; v/v) mixture and with the use of vortex, ultrasonic bath and centrifuge at 9000 rpm for 10 minutes. The HPLC-DAD/ESI-MSn analysis was performed as described by Tenore et al. (2013).

# **3. Results and Discussion**

## **3.1 Development of apple pulp-derived callus cultures and analysis of secondary metabolites.**

Several studies have suggested that plant cell cultures (PCCs) enhance the production of secondary metabolites. Consequently, PCCs have been long studied especially in terms of their nutritional properties as food products. Here, we reported our results demonstrating the development of apple pulp callus culture, starting from raw material obtained from *Malus pumila* Miller cv Annurca, which is a native apple landrace to southern Italy (Figure 1.).

***Figure 1*** *(a) Apple pulp pieces placed in agar medium; (b) Pulp-derived calli.* 

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| ***Table 1*** *The content of metabolites presents within pulp-derived calli compared to lyophilized apple.* |
| **Secondary metabolites(mg) / callus(g)** |
| **Compounds** | **Pulp** | **Callus** |
| Procyanidin B1 | 0.421±0.060 | 0.318±0.044 |
| Procyanidin B2 | 0.519±0.013 | 0.028±0.0006\*\*\* |
| Epicatechin | 0.408±0.019 | 0.001±0.0003\*\*\* |
| Gallic acid | 0.120±0.017 | 0.102±0.025 |
| Ursolic acid | 4.020±0.010 | 2.190±0.292\*\* |
| Values are means ± SE (n = 3) and asterisks denote statistically significant differences of each treatment compared to the apple lyophilized (∗∗p ≤ 0.001; ∗∗∗p ≤ 0.0001) according to Student’s *t*-test. |

Furthermore, the SMs content was assessed. As shown in Table 1, the bioactive compounds present in the lyophilized apple are also present in the pulp-derived calli, proving that there is accumulation of these molecules also in cell cultures. In some cases, the amount of the SMs contained in the cell culture is similar to that contained in the starting material.

## **3.2 Use of salicylic acid as elicitor within callus culture.**

One of the several methods adopted to prompt the production of natural compounds within plants cell cultures is the use of elicitors, biotic or abiotic molecules that triggers a signal and thus activates specific pathways within cells. Given this, SA as elicitor was tested, as it is known for its several functions, such as inducing seed germination and flowering, controlling photosynthesis and enzyme activities, therefore stimulating the SM production (Ali et al. 2021).

The addition of SA 50 and 100 mg/L to the medium for the cultivation of pulp-derived calli determined the increase of specific SMs whose amount was comparable or even greater than the one detected in the control and the lyophilized apple (Table 2). Specifically, the addition of SA 100 mg/L induced a major increase in procyanidin B1, gallic acid and ursolic acid. The first two belong to the polyphenol category. These phytochemicals are usually known for their important antioxidant activity and found in much higher concentrations in apple peel (Boyer & Liu, 2004). The third one belongs to the category of triterpenic acid. In particular, ursolic acid has long been investigated for its activity against several types of cancers and its low toxicity after administration (Zafar et. al 2022). These results indicate that pulp-derived cell cultures can be used as bio-factories for the production of valuable quantity of secondary metabolites to be employed for food purposes. Indeed, nutritionally valuable compounds were produced in quantitative amounts that are comparable or better than reference material by optimizing the cell culture with the elicitation.

Since there is a need to increase the intake of plant nutrients in the diet, PCCs can facilitate the shift from an animal-based to a plant-based food, with minimal waste of our planet's primary resources.

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| ***Table 2*** *The content of metabolites present within pulp-derived calli treated with SA and the control.* |
| **Secondary metabolites(mg) / callus(g)** |
| **Compounds** | **Control** | **Treated with 50 mg/L SA** | **Treated with 100 mg/L SA** |
| Procyanidin B1 | 0.310±0.044 | 0.689±0.006\*\*\* | 0.744±0.028\*\*\* |
| Procyanidin B2 | 0.02 ±0.0006 | 0.041±0.002\*\*\* | 0.040±0.001\*\*\* |
| Epicatechin | 0.001±0.0003 | 0.017±0.002\*\*\* | 0.020±0.0002\*\*\* |
| Gallic acid | 0.102±0.025 | 0.127±0.004 | 0.207±0.002\*\* |
| Ursolic acid | 2.910±0.292 | 2.890±0.288 | 4.220±0.123\*\* |
| Values are means ± SE (n = 3) and asterisks denote statistically significant differences of each treatment compared to the control (∗∗p ≤ 0.001; ∗∗∗p ≤ 0.0001) according to Student’s *t*-test. |

# **4. References**

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