OPTIMIZATION OF AROMA PRODUCTION IN GRAPE CELL SUSPENSIONS INDUCED BY CHEMICAL ELICITORS•

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1. INTRODUCTION

In many functional genomic studies, cell suspension cultures have been very useful experimental tools. Experiments with grape cell suspensions are quick and convenient compared to studies on whole vines and berries, they make it easy to control the environment conditions and to manipulate with accuracy the experiment conditions, and it is possible to carry out experiments all year.

The aim of this project is the discovery and functional characterizations of genes involved in flavour biosynthetic pathways by elicitation of grape cell suspension. Here we report the optimization of sesquiterpenes production in the methyl-jasmonate (MeJA) elicited grape cell suspensions that have been utilized for real-time RT-PCR and microarray analysis experiments reported in D'Onofrio *et al.*, 2009.

2. MATERIALS AND METHODS

Cell suspensions of 'Cabernet sauvignon' have been set up in Gamborgs B5 liquid medium with minimal organics supplemented with 30 gL^{-1} sucrose, 0.25 gL^{-1} casein hydrolysate, 0.93 μM kinetin and 0.54 μM naphthaleneacetic acid by callus cultures established from young berries on the same medium solidified with and 0.8 % (w/v) of agar. The cultures were incubated in darkness on an orbital shaker at 100 rpm. The cell suspensions were sub-cultured every 2 weeks using an initial packed cell volume (PCV) of 15%. In a set of preliminary experiments the effect of some elicitors on induction of secondary metabolites in cell suspensions was tested (tab. 1). Among those, methyl-jasmonate (MeJA) induced the production of at least 25 compounds with sesquiterpene-like mass spectra. Sesquiterpene production was assayed with different concentrations of MeJA (0, 50, 100, 250, 500, 750, 1000, 1500, 2000, 3000 μM) added to a cell suspension with a PCV of 35 % or 70 %. To study the interaction between MeJA concentration and PCV at the moment of elicitor addition, the production of volatile compounds was assayed in cell suspensions added with 1000 μM of MeJA at 0, 1, 2, 3, 4, 5 and 6 days after cell suspension subcultures with an initial PCV of 28 %. Finally, sesquiterpene production was assayed in the cell cultures with a initial PCV of 70 % at 0, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120 hours after the addition of 1000 μM MeJA.

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Tab. 1 - Elicitors tested for the induction of flavour compounds in 'Cabernet sauvignon' berry cell suspension.

Elicitor	Solvent	Mother solution concentration	Concentration in cell suspension
jasmonic acid	ethanol	1 M	1000 μM
Methyl jasmonate	-	4.76 M	1000 μM
salycilic acid	ethanol	1 M	1000 μM
Methyl salicylate	ethanol	1 M	1000 μM
Ethephon	water	0.1 M	100 μM
ABA	NaOH 1M	0.2 M	50 μM
Sucrose	water	100 g L^{-1}	100 g L^{-1}
Mannitol	water	0.5 M	0.5 M
Sorbitol	water	0.5 M	0.5 M
PEG	water	50 g L^{-1}	50 g L^{-1}

3. RESULTS AND DISCUSSION

Among the elicitors tested, the addition of methyl-jasmonate (MeJA) in 'Cabernet sauvignon' cell suspensions induced the production of at least 25 compounds with sesquiterpene-like mass spectra.

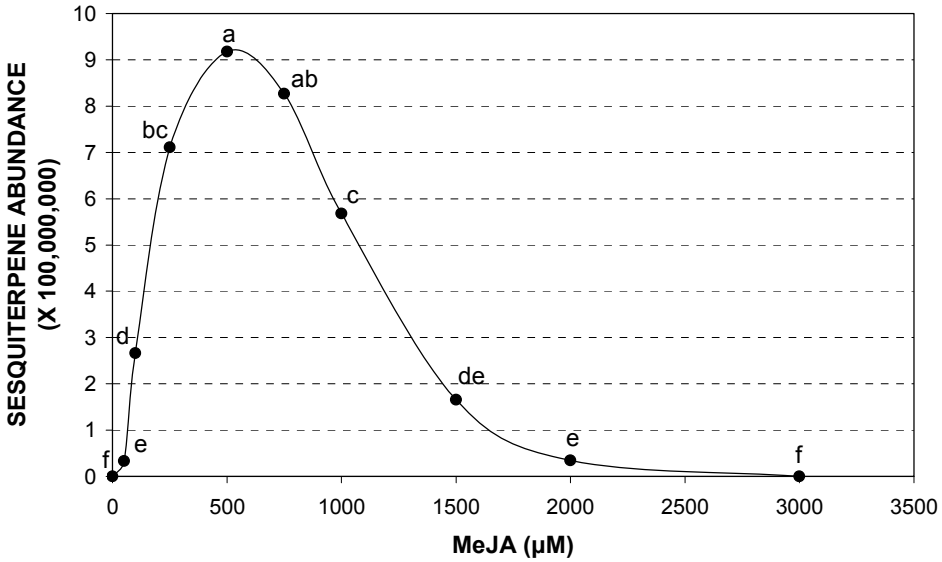

Fig. 1 - Sequiterpene production induced by different MeJA concentrations (0, 50, 100, 250, 500, 750, 1000, 1500, 2000, 3000 μM) in 'Cabernet sauvignon' berry cell suspensions with a initial PCV of 35 %. In this and in the following figures different letters indicate statistically different values ($P<0.05$) according to Tukey's test.

The most effective concentration of MeJA in stimulating the production of sesquiterpenes was found to be 500 μM if added when the cell suspensions had a PCV of 35 %, and 1000 if added when the cell suspensions had a PCV of 70 % (fig. 1).

So the optimal concentration of MeJA for induction of sesquiterpenes appeared to be 1.43 µmol per ml of PCV. These results suggest that in the interaction elicitor/density of cell in suspension the real important parameter is the concentration of elicitor per PCV unit and not the concentration of elicitor per total cell suspension volume.

The production of sesquiterpenes quickly decreased with the increase of cell suspension PCV at the moment of MeJA addition, suggesting a strong interaction between PCV and MeJA concentration (fig. 2).

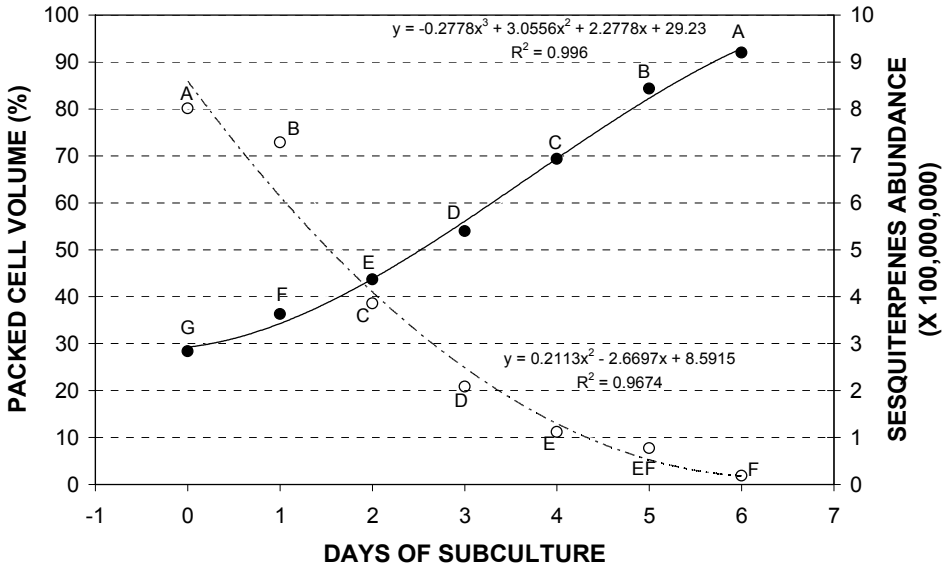

Fig. 2 - Sequiterpene production induced in 'Cabernet sauvignon' berry cell suspensions by 1000 µM of MeJA added at different days after subculturing with an initial PCV of 28 %.

The production of sequiterpenes began at 12 hours after the addition of MeJA, and reached a maximum 48 hours after MeJA addition (fig. 3).

The viability of cells drastically decreased after the addition of MeJA and this decrease depended on the concentration of MeJA added.

MeJA also induced a significantly greater amount of proanthocyanidins and stilbenes in the grape cell cultures. The analysis of gene expressions by real-time RT-PCR and microarray analysis revealed that the mechanism by which jasmonates induced the production of secondary metabolites in cultured grape cells varied depending on the pathway (D'Onofrio *et al*., 2009).

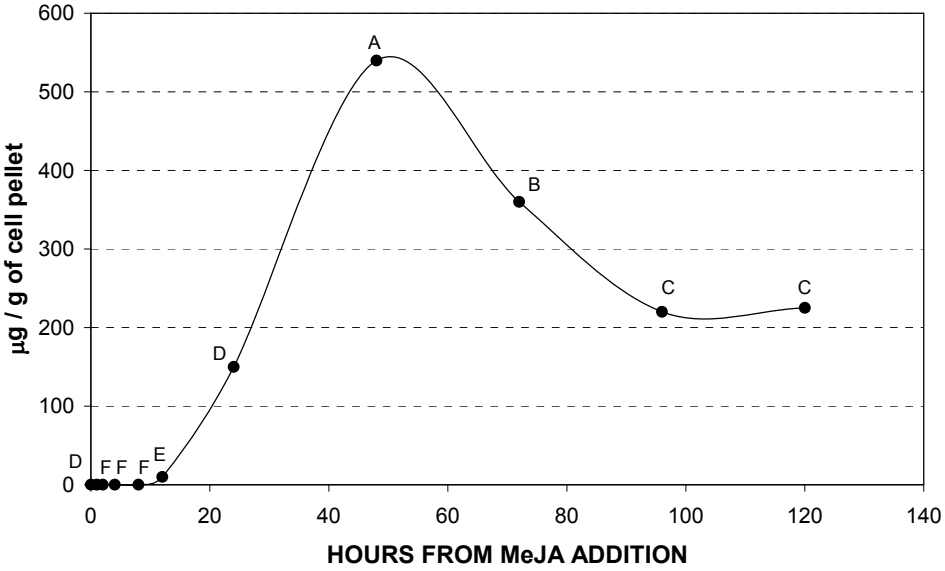

Fig. 3 - Sesquiterpene production detected at different times (0, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120 hours) after the addition of 1000 µM MeJA to 'Cabernet sauvignon' berry cell suspensions with an initial PCV of 70 %.

Abstract

Methyl-jasmonate (MeJA) induces the production of at least 25 compounds with sesquiterpene-like mass spectra in 'Cabernet sauvignon'. The most effective concentration of MeJA in stimulating the production of sesquiterpenes was found to be 500 µM if added when the cell suspensions had a PCV of 35 %, and 1000 if added when the cell suspensions had a PCV of 70 %. The production of sesquiterpenes quickly decreased with the increase of cell suspension PCV at the moment of MeJA addition. The production of sequiterpenes began 12 hours after the addition of MeJA, and reached a maximum 72 hours after MeJA addition. MeJA also induced a significantly greater amount of proanthocyanidins and stilbenes in the grape cell cultures.

Literature cited

D'Onofrio C., Cox A., Davies C., Boss PK. – 2009 - Induction of secondary metabolism in grape cell cultures by jasmonates. *Functional Plant Biology,* 36, 323–338.