

Modulation of phenolic production in *Vitis vinifera* cell cultures with *Phaeomoniella chlamydospora* and other elicitors



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Introduction

Esca is a destructive disease that affects *Vitis vinifera* plants around the world leading to important losses in wine production. *Phaeomoniella chlamydospora* (Pc) is a fungus frequently associated with esca and grapevine decline. Information on the interaction of this fungus with *Vitis vinifera* is scarce.

To study the defense mechanisms of *Vitis* plants to Pc we utilized cell suspension cultures of *V. vinifera* cv. Vinhão (Vv) elicited with fungus Pc, methyl-jasmonate (MeJ) and salicylic acid (SA). We also used cultures primed with MeJ or SA 24h before elicitation with Pc (MeJ+Pc or SA+Pc, respectively). Several culture samples were taken during the experimental period and phenolic production was evaluated by HPLC-DAD-MS

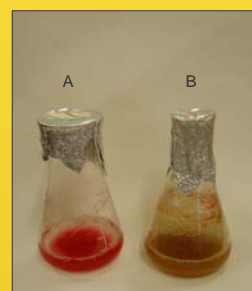


Fig 1 – Cell suspension cultures of *V. vinifera* cv. Vinhão. A – control; B – suspension elicited with fungus Pc.

Experimental

In vitro culture:

Vv cells suspensions were maintained in liquid medium (Gamborg B5 macronutrients, Murashige and Skoog micronutrients, 2% sucrose), at 25°C, under 16h/8h light/dark photoperiod and shaken at 100 rpm. Subculture occurred every 10 days.

Elicitation and sample collection:

Cultures were divided into 6 groups: a control group, a group elicited with MeJ (100µM), a group elicited with SA (100µM), a group elicited with autoclaved Pc biomass (0,5 mg/mL), a group primed with MeJ 24h before fungus elicitation and a group primed with SA 24h before fungus elicitation. Elicitation occurred on the 6th day of culture.

Samples of *V. vinifera* cells were taken 24h and 72h after fungus elicitation by centrifugation and lyophilized for 48h.

Phenolic Analysis:

Dried biomass was extracted in the dark with a 70% methanol solution acidified with 0,5% formic acid, during 1 day. The liquid phase was filtered and samples were stored at 4°C until HPLC analysis.

HPLC-DAD analysis of methanolic extracts: reverse phase column with gradient elution; quantification by the external standard method with pure reference compounds.

LC-MS analysis; Agilent 1100 LC/MSD Trap; ESI, negative ion mode; scan range 200-1500 amu.

Stilbene production of *Vitis vinifera* cell cultures

Anthocyanin production of *Vitis vinifera* cell cultures

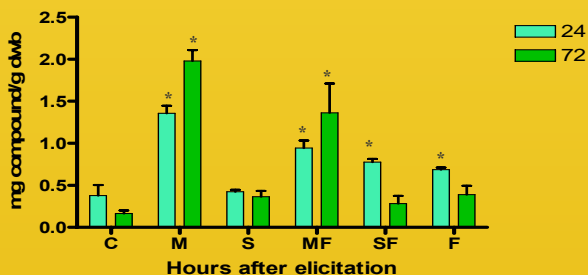


Fig 2 – Phenolic (stilbene) production of *Vitis vinifera* cell cultures. C – Control; M – MeJ (100µM); S – SA (100µM); MF – MeJ (100µM) + Fungus Pc (0,5mg/mL); SF – SA (100µM) + Fungus Pc (0,5mg/mL); F – Fungus Pc (0,5mg/mL). Bars signalised with and asterisk are significantly different from control ($P < 0.05$).

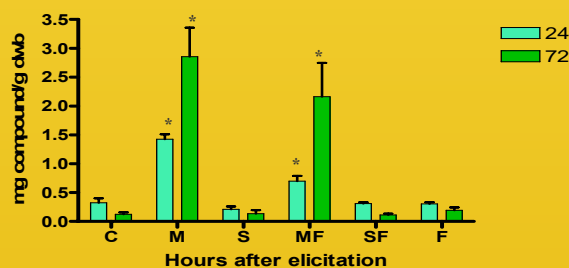


Fig 3 – Anthocyanin production of *Vitis vinifera* cell cultures. C – Control; M – MeJ (100µM); S – SA (100µM); MF – MeJ (100µM) + Fungus Pc (0,5mg/mL); SF – SA (100µM) + Fungus Pc (0,5mg/mL); F – Fungus Pc (0,5mg/mL). Bars signalised with and asterisk are significantly different from control ($P < 0.05$).

Conclusions

- There is a significant rise in stilbenic compounds in Vv cultures within a short period (24h) after elicitation with MeJ, MeJ+Pc and Pc. Priming with SA before elicitation with Pc also leads to a larger production of phenolic compounds.
- 72h after Pc elicitation phenolic production levels get lower except when MeJ is present.
- Anthocyanin levels significantly increase after elicitation with MeJ and MeJ+Pc. These high levels are maintained for a longer period, including 72h after elicitation.
- The rise in phenolic production, namely stilbens, after elicitation points out to a defense response.
- The results indicate that MeJ could possibly play an important part in the defense mechanism of Vv.