

Differential phenolic production of *Vitis vinifera* cell cultures induced by *Phaeomoniella chlamydospora* elicitation



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Introduction

Esca is a destructive disease, of complex aetiology, that affects grapevines in several countries around the world, specially in the Mediterranean, South Africa and California (where is known as “black measles”). In the last few years the disease became a major concern given to its dramatic incidence increase. Till now, does not exist a method to treat plants attacked by Esca.

To study the putative response of *V. vinifera* plants to *Paeomoniella chlamydospora* (Pc), a fungus directly related with Esca, we utilized *in cells* cultures of *Vitis vinifera* cv. Vinhão (Vv) elicited with fungi Pc autoclaved extracts and methyl jasmonate (MeJ).



Vitis vinifera leave showing esca symptoms.

Culture changes after Pc elicitation



A – Control. B – 3 days after Pc biomass addition.

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 3 groups: a control group, a group elicited with MeJ (100µM), and a group elicited with autoclaved Pc biomass (30 mg/culture flask). Elicitation occurred on the 5th day of culture. Biomass samples were taken during culture time and lyophilised.

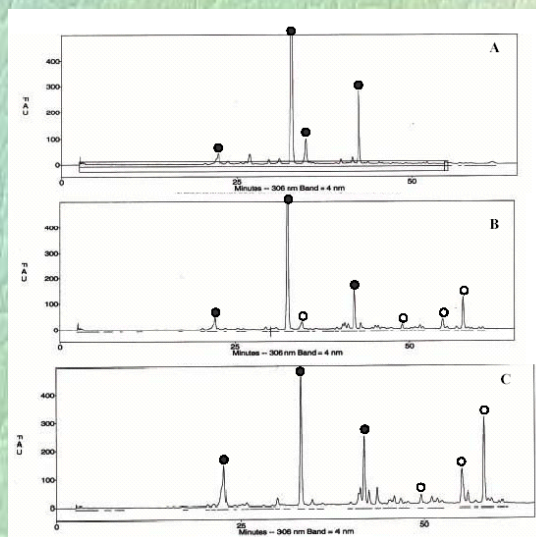
Phenolic Analysis:

Dried biomass was extracted with methanol 70%.

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

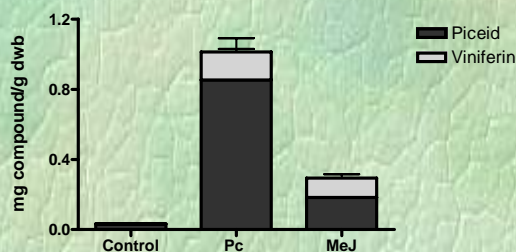
LC-MS analysis; Agilent 1100 LC/MSD Trap; ESI, negative ion mode; scan range 200-1500 amu.

HPLC profiles of *Vitis vinifera* phenolic extracts



A – Control. B – Cultures elicited with Pc biomass (30 mg/culture flask). C – Cultures elicited with MeJ (100µM).

Overall phenolic production of *Vitis vinifera* cell cultures



Control; Cultures elicited with Pc biomass (30mg); Cultures elicited with MeJ (100µM).

Conclusions

Elicitation of Vv cell cultures with Pc induces changes in phenolic production, including the production of new viniferin type compounds. These substances might be involved in the defense mechanism against Pc, since they have been described as antifungal compounds.

Elicitation with MeJ induces similar changes in Vv phenolic production, as those induced by Pc. So, it is possible that this molecule is involved in the defense mechanisms against Pc, namely as a signal molecule.

In vitro cultures of Vv could be an important tool, since they offer a simple, rapid and selective way to study plant/esca interactions.