# OXIDATIVE RESPONSE OF *VITIS VINIFERA* CELLS ELICITED WITH *PHAEOMONIELLA CHLAMYDOSPORA*

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## Introduction

Esca is a destructive disease that affects vineyards leading to important losses in wine production, which incidence has dramatically increased during the last few years. *Phaeomoniella chlamydospora* (Pc) is a fungus frequently associated with esca and grapevine decline. Information on the interaction of this fungus with *Vitis* plants is scarce.

To study early defense mechanisms, namely the oxidative response, of *Vitis* plants to Pc we utilized *in vitro* cell suspension cultures of *V. vinifera* cv. Vinhão (Vv) and a Pc elicitor (hydrolyzed extract).

## Experimental

**In vitro cultures**

- Vv cells suspensions were maintained in liquid medium (Gamborg B5 macronutrients, Murashige and Skoog micronutrients, 2% sucrose), at 25ºC, under 16h/8h light/dark photoperiod and shaken at 100 rpm. Subculture occurred every 14 days.

**Elicitation and sample collection**

- A first experiment was made to observe de oxidative response of Vv cell cultures to Pc extract elicitation; cultures were divided into 2 groups: a control group and a group elicited with Pc extract (0,5 mg/ml).
- In a second experiment we studied the relationship between $Ca^{2+}$ signalling and oxidative burst; cultures were elicited with Pc extract (0,5 mg/ml), EGTA (5mM), $LaCl_3$ (100 µM), nifedipine (100 µM) and ruthenium red (100 µM), as well as primed with EGTA 20 minutes prior to Pc elicitation and with nifedipine, ruthenium red and $LaCl_3$ 30 seconds prior to Pc elicitation (using the above concentrations).
- In a third experiment we assayed the involvement of NADPHoxidase in the oxidative burst: cultures were elicited with Pc extract (0,5 mg/ml) and DPI (10 µM) as well as primed with DPI 10 minutes before Pc elicitation (using the above concentrations).
- In every experiment elicitation occurred on the 6th day of culture and samples were taken during the first 7 hours after Pc elicitation.

**ROS levels analysis**

- ROS levels were detected(excitation: 488 nm; emission: 525 nm) in a fluorimeter (Perkin Elmer LS50 Luminescence Spectrometer, Buckinghamshire, England) as proportional to fluorescence emitted by DCFH-DA (Molecular Probes, Eugene, OR USA) probe.

## Results

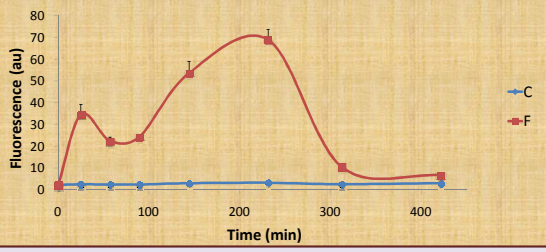


Figure 1 – Intracellular ROS levels in *Vitis vinifera* cv. Vinhão cells. C – control; F – Pc extract (0,5 mg/ml).

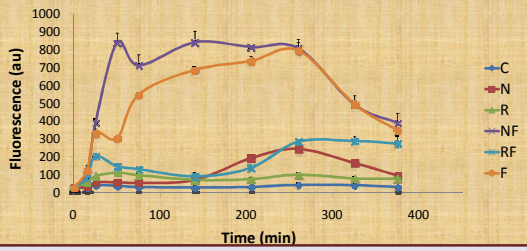


Figure 2 – Intracellular ROS levels in *Vitis vinifera* cv. Vinhão cells. C – control; N – nifedipine (100 µM); R – ruthenium red (100 µM); NF – nifedipine (100 µM) + Pc extract (0,5 mg/ml); RF – ruthenium red (100 µM) + Pc extract (0,5 mg/ml); F – Pc extract (0,5 mg/ml).

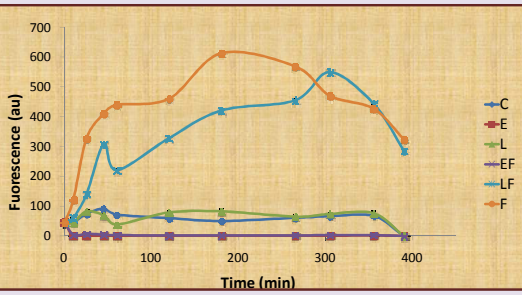


Figure 3 – Intracellular ROS levels in *Vitis vinifera* cv. Vinhão cells. C – control; E – EGTA (5 mM); L – $LaCl_3$ (100 µM); EF – EGTA (5 mM) + Pc extract (0,5 mg/ml); LF – $LaCl_3$ (100 µM) + Pc extract (0,5 mg/ml); F – Pc extract (0,5 mg/ml).

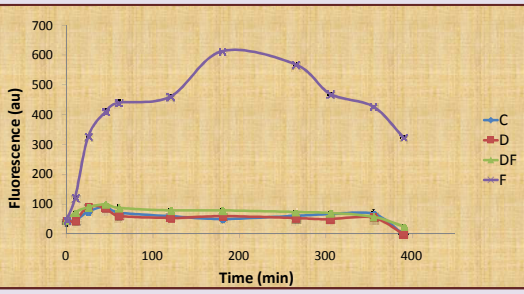


Figure 4 – Intracellular ROS levels in *Vitis vinifera* cv. Vinhão cells. C – control; D – DPI (10 µM); DF – DPI (10 µM) + Pc extract (0,5 mg/ml); F – Pc extract (0,5 mg/ml).

## Conclusions

**Oxidative burst**

- Elicitation of *V. vinifera* cell cultures with Pc induces a typical biphasic oxidative burst profile after infection, with a smaller and quicker first burst followed by a more intense and longer second burst (Figure 1).

**Oxidative burst and $Ca^{2+}$ signaling**

- Both EGTA and ruthenium red strongly inhibit oxidative burst, indicating that oxidative response depends on $Ca^{2+}$ availability and that $Ca^{2+}$ channels specifically inhibited by ruthenium red are critical in this event (Figures 2 and 3).

**Oxidative burst and NADPHoxidase**

- DPI strongly inhibits oxidative burst, suggesting that NADPHoxidase plays a major role in ROS production during oxidative burst (Figure 4).

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