EXPRESSION ANALYSIS OF DEFENCE-RELATED GENES IN *VITIS VINIFERA* CV. VINHÃO CELL CULTURES ELICITED WITH *PHAEOMONIELLA CHLAMYDOSPORA*

M.R.M. Lima and A.C.P. Dias*

Universidade do Minho - Departamento Biologia, Campus de Gualtar, 4710-057 Braga, Portugal

*acpdias@bio.uminho.pt

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.

To study the defence response, specifically gene activation, of grapevine to Pc we utilized *in vitro* cultures of *V. vinifera* cv. Vinhão (Vv) elicited with fungus extract. The expression of genes encoding pathogenesis-related proteins (class 6 and class 10 PR proteins, β-1,3-glucanase, and class III chitinases) and genes involved in the octadecanoid (lipoxigenase) and phenylpropanoid (phenylalanine ammonia lyase and stilbene synthase) pathways were monitored by semi-quantitative RT-PCR.

Experimental

In vitro cultures

- Vv suspension cell cultures 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

- Cultures were divided into 2 groups: a control group and a group elicited with Pc autoclaved biomass (0,5 mg/ml). Two independent experiments were made.
- Elicitation occurred on the 6th day of culture and samples were taken at 3, 12, 24 and 48 hours after Pc elicitation. Biomass was collected by centrifugation and immediately frozen at -80ºC.

Semi-quantitative RT-PCR

- RNA was extracted using the Rneasy Plant Mini Kit (Quiagen) and cDNA was synthesized using the First Stand cDNA Synthesis Kit (Fermentas). cDNA was stored at -20ºC.
- For each pair of primers, PCR conditions were determined to perform the amplification within the exponential phase
- PCR product was separated in 1% agarose gel stained with SYBR Safe (Invitrogen). Band density was analyzed using Quantity One software (Bio-Rad). Actin gene was used to normalize each sample.

Results

Figure 1 – Transcript accumulation of defense-related genes in control (C) and fungus elicited (F) samples at 3, 12, 24 and 48 hours after elicitation. Grapevine actin gene (ACT) was used as internal control to normalize each sample. Phenylalanine ammonia lyase (PAL); stilbene synthase (STSY); lipoxigenase (LOX); β-1,3-glucanase (GLUC); class III chitinase (CH3); class 6 pathogenesis-related protein (PR6) and class 10 pathogenesis-related protein (PR10) genes were studied.

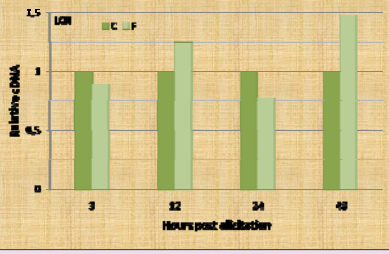

Figure 2 – Lipoxigenase (LOX) gene expression in control (C) and fungus elicited (F) samples at 3, 12, 24 and 48 hours after elicitation. Control samples of each time was defined as 1x expression level. Each sample was normalized by actin gene expression. Results represent two independent experiments.

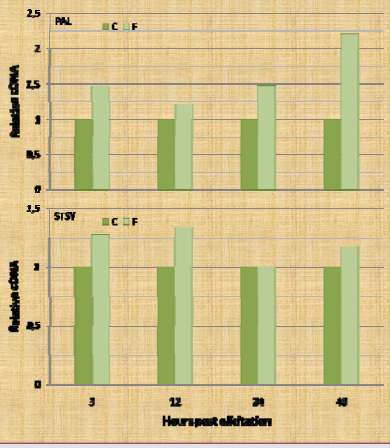

Figure 3 – Phenylalanine ammonia lyase (PAL) and Stilbene synthase (STSY) gene expression in control (C) and fungus elicited (F) samples at 3, 12, 24 and 48 hours after elicitation. Control samples of each time was defined as 1x expression level. Each sample was normalized by actin gene expression. Results represent two independent experiments.

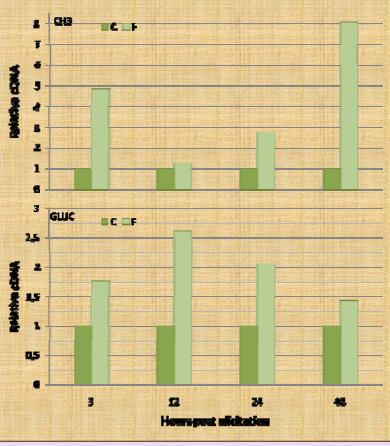

Figure 4 – Class III chitinase (CH3) and β-1,3-glucanase (GLUC) gene expression in control (C) and fungus elicited (F) samples at 3, 12, 24 and 48 hours after elicitation. Control samples of each time was defined as 1x expression level. Each sample was normalized by actin gene expression. Results represent two independent experiments.

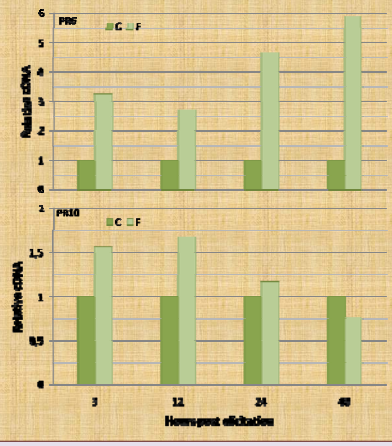

Figure 5 – Class 6 PR protein (PR6) and Class 10 PR protein (PR10) gene expression in control (C) and fungus elicited (F) samples at 3, 12, 24 and 48 hours after elicitation. Control samples of each time was defined as 1x expression level. Each sample was normalized by actin gene expression. Results represent two independent experiments.

Conclusions

Elicitation of *V. vinifera* cell cultures with Pc increases the transcription of all genes studied. Results suggest that transcription of these genes is regulated during the time course of infection.

PAL, CH3 and PR6 transcripts have a peak at 3 hours post elicitation followed by a second stronger increase from 24 hours on (fig. 3 top, fig 4 top and fig 5 top).

LOX and STSY transcripts reach a peak at 12 hours post elicitation and have a new increase at 48 hours (fig 2 and fig 3 bottom).

GLUC and PR10 transcripts seem to hit the highest level at 12 hours post elicitation (fig 4 bottom and fig 5 bottom).

Acknowledgements

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