

Response of *Vitis vinifera* cell cultures to *Phaeomoniella chlamydospora*: changes in phenolic production, oxidative state and expression of defence-related genes

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Accepted: 18 August 2011
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Abstract Cell suspension cultures of *Vitis vinifera* cv. Vinhão (Vv) were used to study the putative response of *V. vinifera* to *Phaeomoniella chlamydospora* (Pc), a fungus frequently associated with esca and grapevine decline. Cells were elicited with a Pc autoclaved biomass extract and methyl jasmonate (MeJ). Phenolic production was evaluated by HPLC-DAD and HPLC-MS/MS. Phenolic production of Vv cells significantly changes after elicitation. Compared to control, Vv cells elicited by Pc extract increase their stilbene production 20-fold and those elicited by MeJ increase stilbenic production 9-fold. In both cases, there is *de novo* production of viniferin type compounds. We also analyzed the oxidative burst of Vv cells after elicitation with Pc extract and MeJ, using the probe 2',7'-dichlorodihydrofluorescein diacetate. Adding Pc extract induces an oxidative burst that shows a biphasic pattern in Vv cells. Moreover, the induction of 7 defence-related genes expression in Vv

cell cultures upon Pc extract elicitation was investigated employing semi-quantitative RT-PCR. Elicitation increases the expression of class 6 and class 10 pathogenesis-related proteins, β -1,3-glucanase, class III chitinase, lipoxygenase, phenylalanine ammonia lyase and stilbene synthase. Therefore, Vv in vitro cell cultures could be an important tool to study esca disease, since they offer a simple, rapid and selective way to evaluate plant/fungus interactions.

Keywords Cell suspensions · Defence-related genes · Esca disease · Oxidative burst · Phenolic production · *Vitis vinifera*

Introduction

Esca is a destructive disease that affects grapevines worldwide. Although esca-like symptoms are reported in ancient Greek and Latin works (Mugnai et al. 1999), only recently has the disease become a major concern due to its dramatic incidence increase. This recent increase has been related to a variety of factors such as changes in the management of vineyards, new cultural practices, introduction of new grapevine cultivars and rootstocks, reduced sanitary care of rootstocks and vineyard propagation material, poor protection of pruning wounds, increasing use of selective fungicides and banishment of arsenite treatment due to its toxicity (Graniti et al. 2000; Surico et al. 2006). Although arsenite treatment did not appear to affect esca-related

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