Effect of transient expression of two grapevine chitinases upon infection by Plasmopara viticola and Erysiphe necator

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Effect of transient expression of two grapevine chitinases upon infection by *Plasmopara viticola* and *Erysiphe necator*

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Powdery mildew caused by the Ascomycete *Erysiphe necator* and downy mildew caused by the Oomycete *Plasmopara viticola* are two of the most important grapevine diseases worldwide. These obligate biotrophs can drastically reduce yield and quality of grapes leading to important economic losses. Control of these pathogens is mainly based on the use of fungicides. These chemical treatments can cause damage to the environment and increase production costs considerably. Thus, there is a need for alternative strategies to reduce these treatments. An alternative, cost-effective and environmentally friendly strategy is the use of varieties showing resistance to the pathogens.

Accordingly, at INRA Colmar we are developing a breeding program for resistance to grapevine downy and powdery mildew. The program exploits mainly the resistance to *P. viticola* and *E. necator* found in *Muscadinia rotundifolia*, whereas other sources of resistance are being characterised. The analysis of the genetic basis of the resistance from *M. rotundifolia* resulted in the identification of two resistance genes against downy mildew, named *Rpv1* and *Rpv2*, and one resistance gene against powdery mildew, named *Run1*. Based on the position of these genes on the grapevine genetic map, they are members of the NBS-LRR class of canonical plant disease resistance genes.

Genes for resistance to fungal diseases are of special interest for improving grapevine cultivars, so it is important to have efficient tools to study their function. Stable genetic transformation of grapevines is today performed with both *Agrobacterium*-mediated and biolistic systems, but it is time consuming and shows low efficiency. In order to evaluate gene function in grapevine leaves, a method of transient expression of genes using *Agrobacterium* was developed at our laboratory (Santos-Rosa *et al.*, 2008). While this method allowed the transient expression of marker genes, it remains to be confirmed as suitable to analyse the function of plant disease resistance genes.

Since no NBS-LRR genes from grapevine have been cloned to date, we used other genes to test the suitability of our method for the analysis of disease resistance. Genes encoding hydrolytic enzymes such as chitinases, which can degrade fungal cell wall components, are attractive candidates for improving disease resistance. As a matter of fact, it has been shown that the introduction of rice chitinase RCC2 into grapevine enhances resistance against powdery mildew (Yamamoto *et al.*, 1999). Thus, we searched the grapevine genome sequence for chitinases highly similar to RCC2 and found two chitinases showing 59% and 58% identity at the amino acid level, which we called respectively Chi22 and Chi88. Both chitinases were cloned from cDNA and genomic DNA from the *Vitis vinifera* line 40024 into binary vector pBIN 61 to be used in transient expression experiments. The identity of the cloned genes was confirmed by sequencing.

Evaluation of the effect of Chi22 and Chi88 expression on pathogen infection will be done by infiltrating *Agrobacterium* containing those genes into grapevine leaves and challenging the infiltrated leaves with the pathogens. *Agrobacterium* suspensions will be performed as described (Santos-Rosa *et al.*, 2008). The first and second fully expanded leaves from *in vitro* grown plants of *Vitis vinifera* cultivar Syrah and *Vitis vinifera* line 40024 will be detached and submerged abaxial face down in an *Agrobacterium* suspension containing the chitinase constructs to be vacuum-infiltrated. The leaves will be rinsed in sterile water and placed on sterile water-soaked filter paper in a sealed 90 mm Petri dish. Gene expression is expected within 3 days. Thus, 3 days after infiltration, leaves will be inoculated with *P. viticola* or *E. necator*. The Petri dishes will be incubated in a growth chamber at 21°C. Non-infiltrated leaves, or leaves infiltrated with an empty vector, will be used as controls. Susceptibility to powdery or downy mildew infection will be evaluated after inoculation, based on a disease index. The number of spores produced will be assessed using a Beckmann-Coulter cell counter.

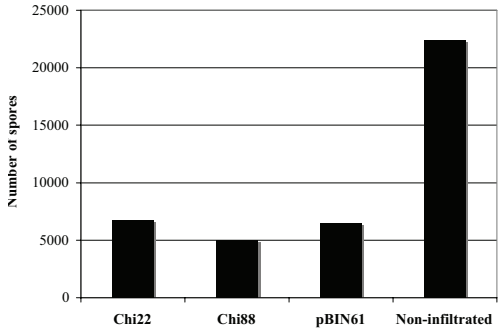

Figure 1: Quantification of powdery mildew infection in leaves, expressed as number of spores produced 11 days after inoculation with *E. necator*. Data shown represent the total spore number of seven leaves.

Preliminary results for inoculation with *E. necator* showed that the number of spores produced on non-infiltrated leaves was more than 3 times higher than on infiltrated leaves. However, we did not find differences between the number of spores produced on leaves infiltrated with chitinases or with pBIN61 (Figure 1). Experiments aiming to confirm these results as well as to study the effect of the expression of chitinases on downy mildew infection are on course.

Literature Cited

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