Resumos do III Simpósio Brasileiro de Genética Molecular de Plantas
10 a 15 de abril de 2011 • Ilhéus • BA • Brasil

Identification of differentially expressed genes associated with pathogen-host interaction in 'Villard Blanc' and its expression analysis in grapevine cultivars resistant and susceptible to downy mildew.

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Keywords: grapevine, downy mildew, *Plasmopara viticola*, RDA, RT-qPCR

The downy mildew (*Plasmopara viticola*) is one of the most economically important fungal diseases for viticulture. In regions with favorable climatic conditions for its development, phytosanitary treatments represent about 30% of production cost. The identification of genes associated with resistance to downy mildew in grapevines may contribute to better understanding of resistance mechanisms and physiological responses to infection by pathogens, as well as for genetic improvement of this crop and handling the production. The aim of this study was to obtain a collection of differentially expressed genes during the pathogen-host interaction of the resistant cultivar 'Villard Blanc' inoculated with *P. viticola*, with subsequent sequencing of the library and selection of candidate genes for expression analysis by RT-qPCR. The *P. viticola* inoculum was obtained from infected leaves of Isabel cultivar and sporangia suspension ($3x10^5$ sporangia/mL^{-1}) was sprayed on the leaves of downy mildew resistant cultivar, 'Villard Blanc', and the downy mildew susceptible cultivar 'Cabernet Sauvignon'. The assay was conducted in a greenhouse with relative humidity of 100% at 25ºC. Inoculated and non-inoculated leaves with downy mildew were harvested at 0, 6, 12, 24, 48 and 72 hours after inoculation (hai). Total RNA was extracted and equimolar samples from each 'Villard Blanc' sample points were assembled and used for synthesis of a cDNA pool to obtain a collection of differentially expressed genes using the methodology of representational difference analysis (RDA). Two steps of subtractive hybridization were performed and the second subtraction amplicons were cloned into pGEM-T Easy (Promega, Madison). A total of 2,229 transformants were obtained, of which 384 were randomly chosen for sequencing. The resulting sequences were then analyzed using the SisGen Automatized System of Sequence Analysis (http://genoma.embrapa.br/genoma/), a unified tool that dynamically integrates data from various databases. Two candidate genes with possible orthologs in Arabidopsis were identified (GSVIVT00026172001 and GSVIVT00032424001). From the literature, other associated genes with downy mildew resistance were selected: stilbene synthase, β -1,3-glucanase and VvNHL1. Relative gene expression profiles using RT-qPCR, along with actin as reference-gene and the $2^{-\Delta\Delta Ct}$ method, were obtained for these genes from each sample point of the two cultivars challenged with downy mildew. The expression profile of each gene was similar between the resistant and the susceptible cultivars and the expression peak occurred at 6 hours after inoculation with downy mildew.

Financial support: Sistema Embrapa de Gestão