

Candidate plant gene homologues in grapevine involved in *Agrobacterium* transformation

Research Article

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Abstract: The grapevine (*Vitis vinifera*) genome was analyzed *in silico* for homologues of plant genes involved in *Agrobacterium* transformation in *Arabidopsis thaliana* and *Nicotiana* spp. Grapevine homologues of the glucomannan 4-beta-mannosyltransferase 9 gene *CsIA-09* involved in bacterial attachment to the cell wall, homologues of reticulon-like proteins BT11, 2, 3 and RAB8 GTPases, both involved in T-DNA transfer to the host cell, homologues of VirE2 interacting protein VIP1 that contributes to the targeting of T-DNA into the nucleus and to its integration, and homologues of the histone protein H2A, which promotes the expression of T-DNA encoded genes, were selected. Sequences homologous to the arabinogalactan-protein AtAGP17 were not found in the grape genome. Seventeen selected candidates were tested by semiquantitative RT-PCR analysis for changes in their expression levels upon inoculation with *Agrobacterium tumefaciens* C58. Of the tested homologues, the expression of *VvRab8a*, *VvVip1a* and two histone genes (*VvHta2* and *VvHta10*) increased significantly, therefore we hypothesize that these might be involved in *Agrobacterium* transformation of *V. vinifera*.

Keywords: *Vitis vinifera* • Crown gall disease • Pathogen-induced gene expression • Semiquantitative PCR

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1. Introduction

Crown gall caused by the pathogenic *Agrobacterium* species is a serious disease affecting several crop plants including fruit trees, berries, ornamental plants and grapevines. However, genetic transformation of crop plants by non-tumorigenic (“disarmed”) *Agrobacterium tumefaciens* strains has become a widely used method to introduce foreign genes into plants to improve agronomical traits [1,2].

Tumorigenic agrobacteria harbour a large plasmid called tumor inducing plasmid (pTi) that carries the genes which are essential for crown gall tumor induction. During the transformation process a region of this

plasmid, the transferred DNA (T-DNA) is transported into the host cell and it becomes stably integrated into the chromosomal DNA. The T-DNA transport through a type IV secretion system [3] from the prokaryotic bacterium into the eukaryotic plant cell is determined by the virulence (*vir*) genes located also on the pTi, but outside the T-DNA. The T-DNA is transported in a single-stranded form (T-strand), and this process is directed by the VirD2 protein that covalently binds to the 5' end of the T-strand [2]. Additionally, for T-DNA import the VirE2 protein forms an anion selective channel on the plasma membrane of the plant cells [4] and binds to the VirD2/T-DNA during the transport process. The VirD2/VirE2/T-strand called T-complex is targeted to the nucleus of

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