ABSTRACT

In order to cope with pathogenic attacks of different nature, plants display constitutive natural barriers and accumulation of certain defensive compounds. In addition, plants have developed a series of inducible mechanisms such as the immune response and RNA silencing, which are activated after the pathogen entry.

Secondary metabolites of phenolic nature play a very important role in the defensive response. These metabolites can be classified into three major groups: *signal molecules*, which are able to activate the response, *phytoanticipins*, which are constitutively present or preformed in the plant, and *phytoalexins*, which are either synthesized *de novo* or their synthesis increase in response to microbial attack. This work focuses on studying the role played by salicylic acid (SA), gentisic acid (GA) and hydroxycinnamic acid amides (HCAA) in the plant defensive response. Among these phenolics, SA and GA could be classified as *signal molecules*, whereas HCAA, considered *phytoalexins*, acting directly on the pathogenic organism due to their antibacterial, anti-fungal and/or antioxidant properties.

To study the role of SA and GA in plant defence, exogenous treatments have been performed using these compounds on Gynura and tomato plants infected with the Citrus Exocortis Viroid (CEVd) and the tomato Mosaic Virus (ToMV), respectively. Treatments produced a delay in the onset of symptoms, thus emphasizing the ability of these metabolites to effectively activate the defensive response. Also, we have observed that these treatments produced the activation of RNA silencing mechanisms. Our results establish a correlation between SA or GA-mediated resistance and RNA gene silencing, and confirm the role of these phenolic compounds in the defensive response.

Transgenic plants showing altered levels of the studied metabolites have been obtained. Overexpression of GAGT, a GA-glycosyltransferase identified in tomato, in Arabidopsis and tomato plants has resulted in a metabolic phenotype for both species, which display a larger percentage of GA conjugation and, consequently, a lower accumulation of free GA, which is the active form of the compound. In Arabidopsis, the metabolic phenotype of the transgenic plants was accompanied by a phenotype of susceptibility to infection with *Pseudomonas syringae* pv. *tomato RpmI* as compared to control plants. We focused our efforts to characterize the function of Twi1, a putative glycosyltransferase with a possible role in the defensive response. Overexpression of the protein has allowed the analysis of its enzymatic activity *in vitro*, which displays glucosyltransferase activity against the phenolics 2,4-dihydroxybenzoic acid (2,4-DHBA) and 2,4,6-trihidroxibenzoic acid (2,4,6-THBA). Exogenous applications of these polyphenols to *Twi1*-silencing transgenic plants corroborated its activity *in vivo*, since

these plants showed a reduced conjugation percentage of 2,4-DHBA and 2,4,6-THBA and, consequently, a larger accumulation of both metabolites in their free form. Finally, we have generated transgenic tomato plants overexpressing *THT* gene, which encodes a key enzyme in the HCAA biosynthesis. These transgenic plants presented an enhanced constitutive accumulation of amides in leaf, flower and fruit tissues as compared to control plants. In addition, the amount of HCAA found in leaves was also higher in these plants in response to wounding. Transgenic THT plants infected with the bacterial pathogen *Pseudomonas syringae* pv. *tomato* showed a high accumulation of HCAA and SA, compared with control plants. As a result, these plants resulted to be more resistant to the bacterial infection, confirming the role of the HCAA in defence.

Both exogenous treatment with phenolic compounds and the generation of transgenic plants with altered levels of them have resulted to be very useful strategies in carrying out this work, allowing us to further study in greater depth on the function of these metabolites in the plant defensive response against pathogen attack.