Comparative proteomic analysis of Arabidopsis wild-type, mutants, and *Fa WRKY1* transgenic plants to characterize the function of the strawberry (*Fragaria* x *ananassa*) Fa WRKY1 protein and its Arabidopsis homolog, At WRKY75, two positive regulators of resistance

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WRKY factors are central players orchestrating the efficient activation of plant defense responses. We have generated Arabidopsis plants overexpressing the strawberry Fa WRKY1 gene that exhibit enhanced resistance to pathogen infection, and used a proteomic approach to identify differentially accumulated proteins when compared to the corresponding WT and mutant At *wrky*75 parental lines. The identification of the proteins showing significant differences will give us some clues on the signaling networks in which these two factors might be involved.

Plant resistance involves the reprogramming of host cells which relies on major changes in gene expression. The identification of the molecular mechanisms that translate recognition of pathogens into appropriate transcriptional outputs resulting in resistance is a valuable tool in directing programs aimed at generating resistant varieties [1]. Nevertheless, relevant crops of agronomical interest usually contain complex genomes making difficult molecular studies. This is the case of the interaction between strawberry (*Fragaria* x *ananassa*), an important agronomical fruit crop, and *Colletotrichum acutatum*, a pathogen which infection causes severe yield losses.

In a previous work we used the molecular resources of the model plant Arabidopsis for studying this plant-pathogen interaction, and characterize Fa *WRKY1*, the first member of the WRKY family of transcriptional regulators identified in strawberry [2]. WRKY factors are central players of plant physiology and orchestrate the defense transcriptome for efficient activation of the plant defense response against a range of biotic and abiotic stresses [3]. In strawberry, *Fa WRKY1* gene is modulated during fruit ripening and strongly up-regulated following *Colletotrichum* infection, and treatments with defense-related hormones. We demonstrated that Fa WRKY1 and its Arabidopsis homolog, At WRKY75, act as positive regulators during the activation of basal (the first line of active defense in plants) and R-mediated resistance (a strong form of race-specific resistance) in Arabidopsis. At *wrky75* T-DNA insertion mutants were more susceptible to virulent and avirulent isolates of the pathogenic bacteria *Pseudomonas syringae*, while overexpression of *Fa WRKY1* in At *wrky75* mutant and wild-type reverts the enhanced susceptible phenotype of the mutant, and even increases resistance. The resistance phenotype of these transgenic plants was uncoupled to the expression of the tipical PATHOGENESIS RELATED (PR) signalling pathway associated with resistance, suggesting differences in the way of action between both factors in order to activate the defense response.

In order to investigate the mode of action of At WRKY75 and Fa WRKY1 and to identify the targets of these factors we are using a differential expression proteomics strategy, consisting in 2- DE combined with MS [4].

Here we present the proteomic analysis carried out to compare the leaf protein profile of transgenic Arabidopsis plants overexpressing *Fa WRKY1* and the corresponding WT and mutant At *wrky75* parental lines to identify proteome alterations due to the expression of this strawberry gene under normal physiological conditions. This constitutes a previous and neccesary step for further comparisons of these lines, that exhibit enhanced resistance and

susceptibility, in response to pathogen infection or hormone treatment.

The identification of the proteins showing significant differences between the *FAWRKY1* overexpressing lines and their controls (mutant and wild type plants) will give us some clues on the signalling networks in which these two factors might be involved, in addition to defense-related signaling.

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