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HOUSEKEEPING GENE SELECTION USING AN EXTERNAL CONTROL FOR RT-PCR ANALYSIS OF DIFFERENTIALLY EXPRESSED GENES IN EGGPLANT ROOTS DURING FUNGAL INOCULATIONS

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Fungal inoculation, quantitative RT-PCR, housekeeping gene, external reference

Fungal wilts caused by *Verticillium dahliae* and *Fusarium oxysporum f. sp. melongenae* are among the most serious diseases harming the eggplant production both in greenhouses and open field cultivation. In a previous work three cDNA libraries of differentially expressed genes involved in the plant-pathogen interaction have been isolated from roots of a *Fusarium* resistant introgression line after inoculation with *Fusarium, Verticillium* and both fungi together. Molecular studies enabled to identify a number of genes putatively involved in the plant pathogen interaction which need to be validated and subjected to characterization. We want to compare the expression profiles of these genes following the three different inoculations (*Fusarium, Verticillium* and *Fusarium+Verticillium*) and considering 0, 4, 8 and 24 hours after roots dipping in the fungal suspension.

Real-time PCR has greatly improved the ease and sensitivity of quantitative gene expression studies. Accurate measurement of gene expression with this method relies on the choice of a valid reference gene for data normalization which should be unaffected by experimental conditions. Usually, an internal control gene is used for this purpose. The most common housekeeping genes known in literature for plant-pathogen interaction studies are β tubulin, elongation factor 1-α, ubiquitin, 18s rRNA and glyceraldeyde-3-phosphate dehydrogenase (GAPDH). However, many recent studies showed that also internal standard genes could vary depending on different experimental conditions, and not often a reliable control has been reported. Therefore, in order to find the most suitable housekeeping gene for our study, a preliminary characterization was performed to confirm that the internal control gene chosen is expressed at a constant level in the three different fungal inoculations.

We first compared each other the expression of the above mentioned housekeeping genes and, in addition, we decided to use an exogenous reference gene to confirm the expression profiles of the internal control genes. A heterologous kanamycin transcript was co-transcribed with total RNA from eggplant roots to have an external reference in each sample. The expression of the external reference gene was invariable among all our experimental conditions. Therefore, we can use heterologous kanamycin transcript both as a reference gene and to check the expression profiles of putative housekeeping genes. Applying this approach to all our experimental treatments (i.e. type of fungal inoculation and timings), GAPDH showed an suitable stability in gene expression and it may be employed as internal reference for the evaluation of the expression of our collection of genes involved in eggplant/fungi interaction.