EXPRESSION PATTERNS OF WRKY GENES DURING INTERACTION BETWEEN ROOTS OF *ARABIDOPSIS* AND THE *TRICHODERMA ATROVIRIDE*

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Introduction. The plants are capable of reprogramming their transcriptome in a highly dynamic and temporal manner when are exposed to microbes (1, 2). In *Arabidopsis*, the plant WRKY transcription factors (TFs) superfamily consists of an estimated 72 members subdivided into three groups. These TFs appears to play a major role in the regulation of plant-microbe response (3, 4). *Trichoderma* spp. are free-living fungi that have shown to have direct effects on plants (5, 6). The establishment of Arabidopsis-Trichoderma association results from a complex, dynamic and still largely unknown signaling mechanism involving major changes in fungal and plant gene expression (7).
In this study, we analyzed the gene expression patterns of the *Arabidopsis thaliana* WRKY TFs under interaction with the fungus *T. atroviride*. The genes selected for this study code for WRKY8, WRKY33, and WRKY57 (Group I), WRKY42 and WRKY60 (Group II) and, WRKY38, WRKY54, and WRKY70 (Group III). Thus, data obtained here will increases the knowledge of how is activated the defense response of *Arabidopsis thaliana* in the interaction with the beneficial fungus *T. atroviride.*

Methods.

RNA Extraction and Real-Time qRT-PCR
Total RNA was extracted from inoculated and non-inoculated plants using the Concert™ Plant RNA reagent (Invitrogen, Carlsbad, CA, USA). Real-time PCR was performed using a StepOne Real-Time PCR Detection System and StepOne Software v2.1 (Applied Biosystems). These PCR reactions were repeated by triplicate for each condition. Quantification of WRKY TFs group I, group II, and group III gene expression was based on a cycle threshold value and normalized to the *actin 8* gene values.

Results. WRKY TFs were selected from a cDNA microarray experiment obtained after 48 h of interaction of 25-day-old A. thaliana roots with T. atroviride. We focused on 8 WRKY TFs showing a significant induction during this interaction. To expand the expression analysis, we analyzed by qRT-PCR six times of interaction (24, 48, 72, 96, 120 and 144 hpi), where T. atroviride was inoculated at 3 cm distance from the root tips (Fig. 1).

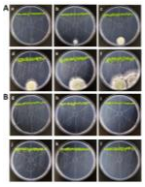

Fig 1. Time course development of interaction between *A. thaliana* plantlets and *T. atroviride* in MS plates. (**A**) Development of interaction of *Arabidopsis thaliana* plantlets with *T. atroviride*. (**B**) Photographs of 17 day-old Arabidopsis (Col-0) control non-inoculated plantlets.

The figure 2 showed the diverses transcriptional responses of *WRKY Arabidopsis* TFs to *Trichoderma* interaction.

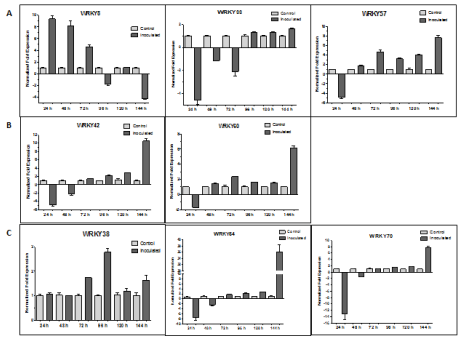

Fig 2. Expression analysis of WRKY TFs from *A. thaliana* in interaction with *T. atroviride.* Quantification of (A) group I WRKY TFs, (B) group II WRKY TFs, and (C) group III WRKY TFs by qRT-PCR, expressed as relative mRNA level compared with a control conditions, was calculated after normalization to the Arabidopsis *actin 8* gene using the comparative threshold method. Control conditions or non-inoculated plantlets (Light grey bars) and tester conditions or inoculated plantlets (Dark bars) at 24, 48, 72, 96, 120 and 144 hpi, respectively.

Conclusions. The analysis of WRKY TFs expression in various stages of Arabidopsis-Trichoderma interaction provided evidence of diverse and complex signaling and transcriptional networks of plant response to beneficial interaction establishment.

References.

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