Gene expression profiles of rice plants challenged with the root-knot nematode *Meloidogyne graminicola* after pre-inoculation with the endophytic fungi *Fusarium oxysporum* strain S2A1 and *Talaromyces purpurogenus*.

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Background and purpose

The endophytic strain, S2A1, of the fungal species *F. oxysporum* was recently isolated from upland rice in Kenya. Preliminary infection tests have shown that this strain can promote rice growth. As a potential bio-fertilizer, its biocontrol capacity against rice nematodes has never been analysed in detail. In this short mobility program, we have studied the tripartite interaction of rice, the root-knot nematode *Meloidogyne graminicola* and the fungi S2A1 or *T. purpurogenus* (TP), by mainly focussing on the gene expression profiles of inoculated and non-inoculated rice plants. *T. purpurogenus*, also a rice endophytic fungus, was selected over *F. oxysporum* strain (Fo162) because of its strong pathogenic effects on rice. In this way we can compare the expression of marker genes between a pathogenic and non-pathogenic (endophytic) interaction with a fungus in the absence and presence of nematodes. Several genes involved in hormone signalling pathways, general defence responses and transcription

factors were selected. The plant-specific NAC (**N**AM/**A**TAF/**C**UC) transcription factors were particularly interesting based on emergence information that they play fundamental roles in regulating plant growth, development and stress responses.

Description of the work carried out during the STSM

The rice cv. Nipponbare (GSOR-100; Genetic Stocks Oryza Collection, Washington DC, USA) was used in this study. De-husked seeds were surface-sterilised by soaking in 70% ethanol for 2 min and 5% sodium hypochlorite (NaOCl) for 15 minutes. Pre-germinated seedlings were transplanted singly in PVC tubes filled with autoclaved synthetic absorbent polymer (SAP) substrate, and inoculated with $1.5x10^6$ fungal spores on the second and third week after transplanting. Samples for RNA extraction were collected at 0, 3, 7, 14 and 21 dpi (fungal inoculated). For plants inoculated with fungi and nematodes, samples were collected at 24 and 28 days after fungal infection. Total RNA was extracted using NucleoSpin® RNA plant kit (Macherey-Nagel, Düren, Germany). First-strand cDNA was synthesised from 1µg of the total RNA using the high capacity reverse transcriptase (Promega, USA). The 20µl reaction mixture contained 10xRT buffer, 25mM dNTP mix, 10mM random primers, 1U ul^{-1} RNase-inhibitor (RNasin®), 5U ul^{-1} Reverse transcriptase, Nuclease-free water and the RNA template. The cDNA-reaction steps were performed as follows: 10 min at 25°C, 2 hours at 37°C and 5 min at 85°C. The quality of the cDNA was tested by performing a normal PCR with OsEXP reference genes, and the amplicons visualised in 1% agarose gel. The q-PCRs were performed using the SensiMix™ SYBR No-ROX Kit (GC biotech, Netherlands), in a 20 µl reaction mixture containing 10µl SensiMix™, 7µl RNAse-free water, 1µl cDNA (1:10 dilution) and 1µl 10mM of each primer. All reactions were performed in three technical replicates and two independent biological replicates using the StepOnePlus software (Applied Biosystems, USA). The PCR conditions were as follows: 95°C for 10 min followed by 45 cycles of 95°C for 15 sec, 60°C for 15 sec and 72°C for 15 sec. Later, a melting curve was

generated by a gradual increase of the temperature to 95°C to test the amplicons specificity. Three reference genes: OsUBQ5, OsEXPnarsai and OsEXP were used to normalize the expression levels of the target genes. Data was analyzed using the REST 2009 software (Qiagen).

Results

Six genes: the ONA131 (transcription factor), OsPR10/PBZ1 (general defence), OsERF1 (Ethylene response), OsEIN2 (ethylene signalling), OsNPR1 (Salicylic acid response) and PAL (SA biosynthesis), have been analysed. For TP-inoculated plants, data is available for most of the time points, however, for S2A1-inoculated plants, some time-points are yet to be analysed. For these samples, analysis of these genes will be continued at Ghent University, Belgium, in a close collaboration with the host institution. It may be too early to draw any conclusions. However what seems to be clear is that these genes are differentially expressed in plants when these endophytes and/ or nematodes are inoculated. To determine whether these biochemical changes have any biological importance, additional experiments focussing on nematodes infection, development and reproduction may be required. We anticipate to gather a significant and relevant data set when the analyses of these materials and experiments are completed.

Future collaboration with the host institution

Bonn University has signed a memorandum of understanding (MoU) with Ghent University.