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## Data in Brief

# Microarray analyses during early and later stages of the *Arabidopsis*/*Piriformospora indica* interaction



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## ABSTRACT

Colonization of the roots of different plant species by *Piriformospora indica* results in better plant performance and biotic and abiotic stress tolerance. An increase of the biomass and seed yield is other beneficial effect of *P. indica* for the host plants. The interaction of *P. indica* with *Arabidopsis thaliana* roots is a unique model system to study symbiotic relationships. We describe a co-cultivation system which allows us to investigate the effects of fungal exudates on the root transcriptome before and after the establishment of a physical contact, and during early phases of root colonization. We present a detailed protocol which facilitates easy reproduction of the results (NCBI GEO accession number GSE58771) published by Vahabi et al. (2015) in BMC Plant Biology [1].

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## Specifications

Organism/cell line/tissue	<i>Arabidopsis thaliana</i> /wild type, Col-0/root tissue
Sex	N/A
Sequencer or array type	Arabidopsis Genome Array ATH1 (Affymetrix, USA)
Data format	Raw and analyzed
Experimental factors	Colonized plant roots with <i>Piriformospora indica</i> vs. non colonized roots before and after establishment of a physical contact of the two symbionts
Experimental features	<i>Arabidopsis</i> roots co-cultivated with or without <i>P. indica</i> for 2 and 6 days
Consent	N/A
Sample source location	N/A

## 1. Direct link to deposited data

(<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE58771>, submission number GSE58771).

## 2. Experimental design, materials and methods

### 2.1. *A. thaliana* growth conditions

Surface-sterilized seeds (20 seeds/plate) of *A. thaliana* WT (Columbia-0) were placed on Petri dishes with MS medium using a 1 ml micro-pipette [2], and kept at 4 °C for 48 h. Plates were kept at 22 °C under continuous illumination ( $65 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) from the side (distance from light source = 30 cm) for 10 days.

### 2.2. *P. indica* growth conditions

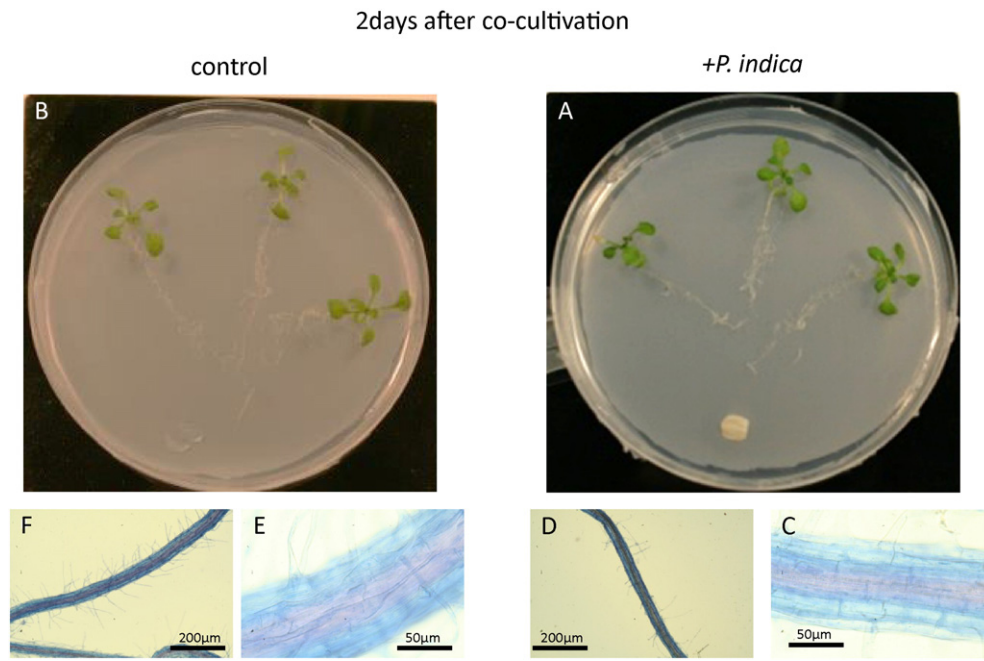
*Aspergillus*-minimal medium was used to propagate *P. indica* for 3 weeks [3,4] (Section A) at 22 °C in the dark. An *Aspergillus*-minimal medium plaque of 5 mm diameter with fungal hyphae (or without fungal hyphae; control) was used as inoculum.

### 2.3. *Arabidopsis*/*P. indica* co-cultivation

Three seedlings (equal in size and number of leaves) from twelve days-old plants (described above) were picked from the MS plates and their roots were laid onto the surface of a nylon membrane in a distance of 3 cm from the plaques. All plates were transferred to 22 °C under continuous illumination ( $80 \pm 5 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) from the top (distance from light source = 30 cm) for 2 or 6 days. While no physical contact has been established after 2 days of co-cultivation, microscopic staining and PCR analyses confirmed the presence of fungal mycelium in and around the roots after 6 days of co-cultivation (Figs. 1 and 2).

### 2.4. RT-PCR

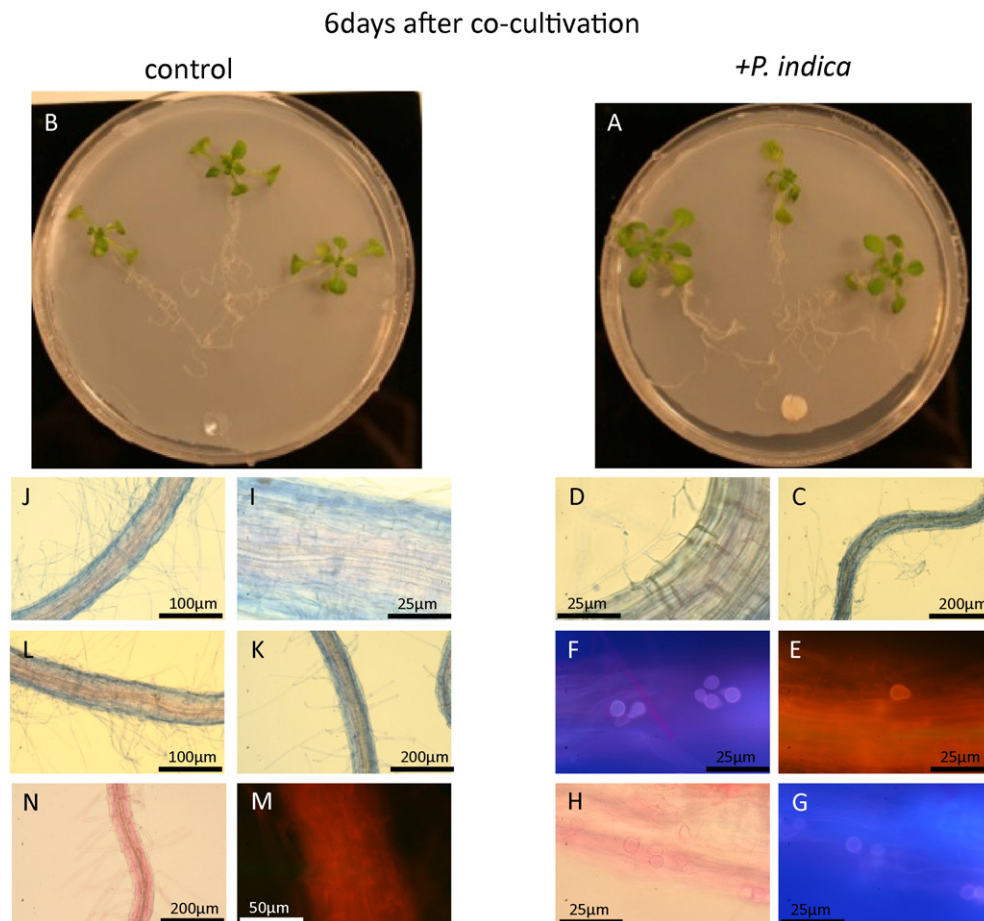
Total RNA was isolated from the roots co-cultivated with the fungus (or mock-treated) after 2 and 6 days of co-cultivation using the RNeasy Plant Mini Kit (Qiagen). After reverse transcription, 1  $\mu\text{g}$  of total RNA was used for cDNA synthesis by the Omniscript RT Kit (Qiagen) and oligo (dT)<sub>20</sub> in 20  $\mu\text{l}$  reaction volume. To confirm the absence of any mycelium in the 2 day sample, or the presence of fungal mycelium in the 6 day sample, RT-PCR was conducted with the primer pairs for



**Fig. 1.** *A. thaliana* co-cultivation with *P. indica* (A, C, D) or mock-treated (B, E, F) after 2 days. Visible light microscopic view of *A. thaliana* root stained with trypan blue (C, D, E, F) after 2 days of co-cultivation with *P. indica* (C, D) or mock-treated (E, F). The roots were stained with trypan blue.

*Arabidopsis GAPDH* (forward: GAGCTGACTACGTTGTTGAG and reverse: GGAGACAATGTCAAGGTCGG) and *P. indica ITS* (forward: CAACACATGT GCACGTCGAT and reverse: CCAATGTGCATTGACAACGA) as housekeeping

genes for the two organisms. CFX connect Real-time system and the CFX manager software version 3.1 (Bio-Rad) were used for quantitative PCR. For the amplification of the PCR products, iQ SYBR Supermix (Bio-Rad)



**Fig. 2.** *A. thaliana* co-cultivation with *P. indica* (A, C–H) or mock-treated (B, I–N) after 6 days. Visible light and fluorescent microscopic view of *A. thaliana* root stained with trypan blue (C–E, I–K) and fuchsin acid (H, G, N, M) after 6 days of co-cultivation with *P. indica* (C–H) or mock-treated (I–M) as described in Vahabi et al. [6].

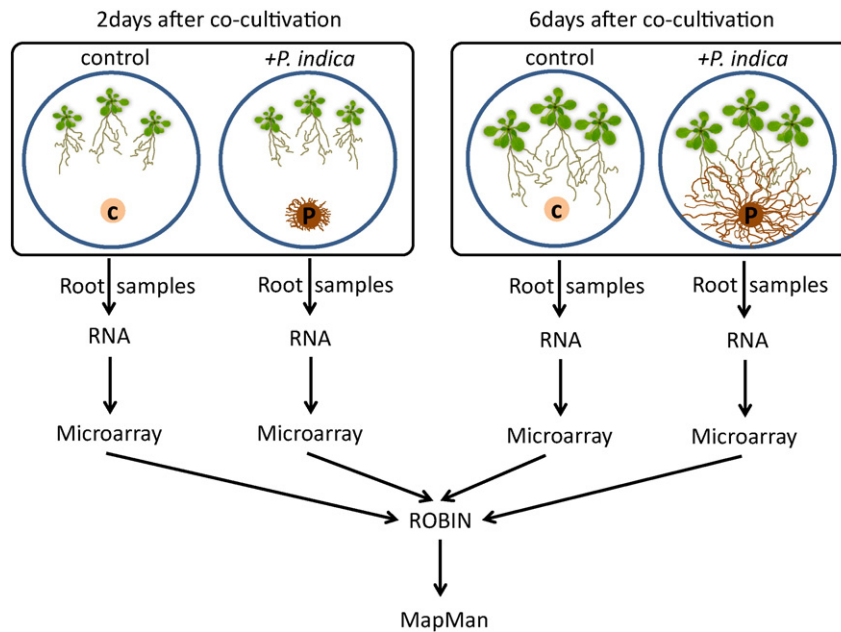


Fig. 3. Experiment design and steps in schematic view.

was used according to the manufacturer's instructions in a final volume of 20  $\mu$ l. The iCycler was programmed to 95  $^{\circ}$ C 2 min, 35  $\times$  (95  $^{\circ}$ C 30 s, 55  $^{\circ}$ C 40 s, 72  $^{\circ}$ C 45 s), 72  $^{\circ}$ C 10 min followed by a melting curve (55–95  $^{\circ}$ C in increasing steps of 0.5  $^{\circ}$ C). All reactions were repeated twice.

### 2.5. Microarray analysis, data processing

RNA was extracted from root samples of 3 biological independent experiments, and hybridized to the Arabidopsis Genome Array ATH1 (Affymetrix, USA) at the Kompetenzzentrum für Fluoreszente Bioanalytik, Regensburg, Germany. Hybridization was performed by mixing equal amounts of RNA from the 3 biological samples. ROBIN (<http://mapman.gabipd.org/web/guest/robin-download>) and MapMan [5] programs were used for analysis of hybridization signal data followed by statistical analysis with the *t*-test (Fig. 3). The raw and normalized data have been submitted to GEO (<http://www.ncbi.nlm.nih.gov/geo>, submission number GSE58771) [1].

### 2.6. Roots microscopy

To investigate whether a physical contact exists between the roots and the *P. indica* mycelium, the roots were stained with trypan blue/

fuchsin acid and the colonization was analyzed by light and fluorescent microscopy as described in Vahabi et al. [6].

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