How to visualisation and localisation of acid phosphatase transcripts in common bean nodules (Phaseolus vulgaris) by in situ RT-PCR

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In situ RT-PCR to visualise the distribution of transcripts in *symbiotic-root tissues*

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Objectif : In order to detect gene expression in different tissues by *in situ* Reverse Transcriptase Polymerase Chain Reaction (RT-PCR), enzyme-labelled fluorescent substrate was used to *in situ* with epi-fluorescence microscopy and to visualise it at the level of transcripts. Tissues used were root-nodules of *Phaseolus vulgaris* and hypae of *Hebeloma cylindrosporum*.

Material & methods

Phaseolus vulgaris was inoculated with *Rhizobium tropici* CIAT899 and grown in hydroaeroponic cultures for 5 weeks under P deficiency.

Nodules of 3 mm diameter were fixed and stored in 4% (w/v) paraformaldehyde, 45% (v/v) ethanol and 5% (v/v) acetic acid, before embedding in low melting point agarose 9% (w/v).

The ectomycorrhizal fungus *Hebeloma cylindrosporum* was grown for 5 days in liquid medium with or without P. Sections of mycelium of 2 mm length were immediately fixed in 2% (w/v) paraformaldehyde, 63% (v/v) ethanol and 5% (v/v) acetic acid.

The RT-PCR was performed with specific primers either on 50 µm vibratome nodule-sections in order to amplify a 250 bp sequence in the 3' acid phosphatase cDNA, or on 2 mm mycelium-sections in order to amplify a 400 bp sequence in the 3' fungal phosphate transporter gene *HcPT1* cDNA (Fig. 1).

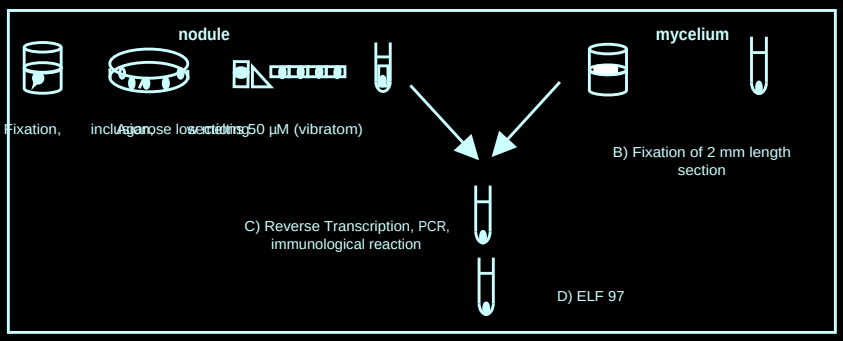

Figure 1: In situ RT-PCR methodology :

A) nodules sections were fixed and embedded in low melting agarose 9%.; B) mycelium was fixed.; C) M-MLV reverse transcriptase (Promega) mix was added to the tissues and PCR carried out with Taq Polymerase (Invitrogen) and Digoxygenin-11-dUTP (Roche). This protocol was modified from Koltai and Bird (2000). After PCR, the sections were transferred into blocking buffer (BSA 0,1%; PBS 1X). The blocking solution was replaced with blocking buffer containing anti-digoxigenin (Roche) 1/1000 and the sections were incubated for 1h at 37°C; D) The ELF-97 kit (Molecular Probes) was used for the detection of alkaline phosphatase-conjugated anti-digoxigenin.

The amplified gene products were visualized using the ELF-97 kit (Molecular Probes) and a BX61 epi-fluorescence microscope (Olympus) for the detection of alkaline phosphatase-conjugated anti-digoxigenin.

Results

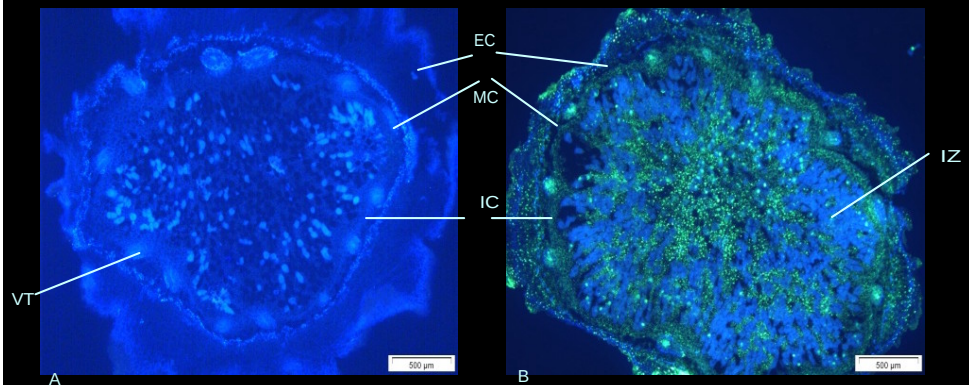

Figure 2 : plant acid phosphatase transcripts in Phaseolus vulgaris/Rhizobium tropici nodules : Nodule sections of A) control without RT; B) acid phosphatase specific RT-PCR. The green spots represent the transcripts under epi-fluorescence microscopy with filter settings at 360-370 nm for excitation and 420 nm for emission. IC, inner cortex; MC, middle cortex; EC, External Cortex; VT, Vascular Trace; IZ, Infected Zone.

The signal, corresponding to acid phosphatase gene expression in nodules sections, was not observed in control samples (without RT: fig 2A) by contrast with), green spots corresponding to the digoxygenin-labelled cDNA in samples with RT (Figs. 2B, 3B-3C).

Thus, the procedure was specific for cDNA and did not amplify genomic DNA. The signal was found in all nodule tissues except in the mid-cortex (MC) and the infected zone (IZ); i.e. acid phosphatase mRNA transcripts were visualized in: i) non infected cells of the infected zone; ii) vascular trace (VT) localised in the cortex surrounding the infected zone; iii) inner cortex (IC, i.e. cell-layers localised between the vascular trace and the infected zone); iv) external cortex (EC, i.e. cell-layers beyond the endodermis)

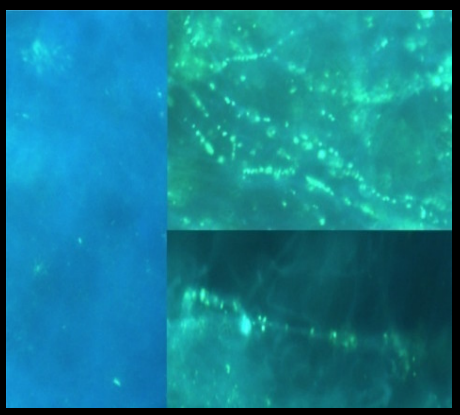

Figure 3 : plant acid phosphatase transcripts in Hebeloma cylindrosporum :
H. cylindrosporum was grown in plus P(A,B) or no P (C) containing medium. A) control without RT ; B) C) phosphate transporter HcPT1 specific RT-PCR.

The signal, corresponding to expression of phosphate transporter gene *HcPT1* in hyphae was observed in RT-samples (fig 3B-3C), but not in control samples (i.e. without RT: fig 3A). Thus, the following PCR reaction did not amplify genomic DNA.

Conclusion

The use of the ELF substrate to reveal activity of the alkaline phosphatase-conjugated antibody used in the technique enabled visualisation of gene expression in different organisms that could be related to specific structures.

References: Cellier F, Conéjéro G, Ricaud L, Luu DT, Lepetit M, Gosti F, Casse F (2004) Plant J 39: 834–846. Jebara M., Aouani E.A., Payre H., Drevon J.J. (2004) **J Plant Physiol** 162: 309-315. Koltai H, Bird DM (2000) Plant Physiol 123: 1203–1212. I.M. van Aarle, G.Viennois, L.K. Amenc, M.V. Tatry, Doan T. Luu, C. Plassard.. (2007). **Mycorrhiza,** Published online. Tatry M.V. (2003) PhD