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

Laurie Amenc, Ingrid van Aarle, Gaëlle Viennois, Saber Kouas, Doan Luu, Claude Plassard, Jean-Jacques Drevon

To cite this version:
Laurie Amenc, Ingrid van Aarle, Gaëlle Viennois, Saber Kouas, Doan Luu, et al.. How to visualisation and localisation of acid phosphatase transcripts in common bean nodules (Phaseolus vulgaris) by in situ RT-PCR. Rhizosphere 2, Aug 2007, Montpellier, France. 2007. hal-02756069

HAL Id: hal-02756069
https://hal.inrae.fr/hal-02756069
Submitted on 3 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
In situ RT-PCR to visualise the distribution of transcripts in symbiotic-root tissues

L.K. Amenc1, I.M. van Aarle1, G. Viennois2, L. Bouhmama1, D. Luu2, C. Plassard1, J.J. Dreyvon1
1 SUPAGRO M-INRA UMR 1222, Place Viala, 34060 Montpellier Cedex 1, France.  2 SUPAGRO M-INRA UMR BPMP, Place Viala, 34060 Montpellier Cedex 1, France.

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 epifluorescence microscopy and to visualise it at the level of transcripts. Tissues used were root-nodules of *Phaseolus vulgaris* and hyphae 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).

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

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 digoxigenin-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 visualised 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).

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.
