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

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Material & methods

Phaseolus vulgaris was inoculated with Rhizobium tropici CIAT899 and grown in hydroponic 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 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).

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.
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