Bacterial acid phosphatase activity and localisation in *Phaseolus vulgaris* nodules

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Localizing bacterial acid phosphatase activity is important to understand the role of bacteria for P availability in legume nodules. Using *in situ* RT-PCR and immuno-localization methodologies, we show increased transcript and protein levels of bacterial acid phosphatase within infected cells of common bean nodules.

Experimental procedure

P sufficient:
250 µmol.week^{-1}.plant^{-1}

X

P deficient:
75 µmol.week^{-1}.plant^{-1}

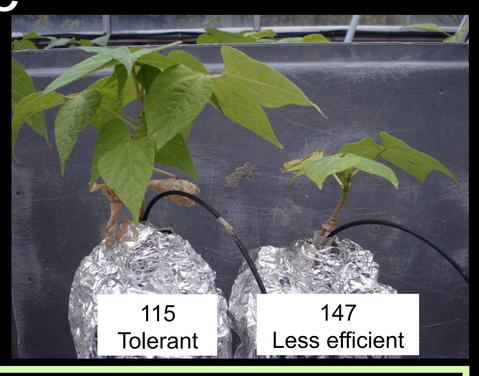

Figure 1: Production and *in situ* RT-PCR methodology for nodules.

Materials & methods

- Common bean recombinant inbred lines (RILs) were inoculated with *Rhizobium tropici* CIAT899, grown in hydro-aeroponic culture (Hernandez & Drevon 1991)
- Standard nodules (3-5 mm diameter) were harvested at flowering stage and immediately fixed
- *In situ* RT-PCR was performed on sections of the fixed nodules (Bargaz *et al*. 2012)
- Specific primers for bacterial acid phosphatase genes were designed online at Kazusa DNA Research Institute (http://genome.microbedb.jp/rhizobase/) with the known mRNA sequences of the homologous genes of *Rhizobium*, *Mesorhizobium* and *Sinorhizobium* spp.

Results

Bacterial acid phosphatase transcripts were primarily localized in infected cells, with some evident in vascular traces. The fluorescent signal intensity in infected cells was decreased under P deficiency (C-D) and was higher for the tolerant RIL115 (A-C) than for the less efficient RIL147 (B-D). Interestingly, the gene expression varied among infected cells. This suggest that P availability for bacteroids may depend upon the infected cell localization within the infected zone.

Acid phosphatase proteins were localized in inner cortex, distributing zone and infected cells (data not shown).

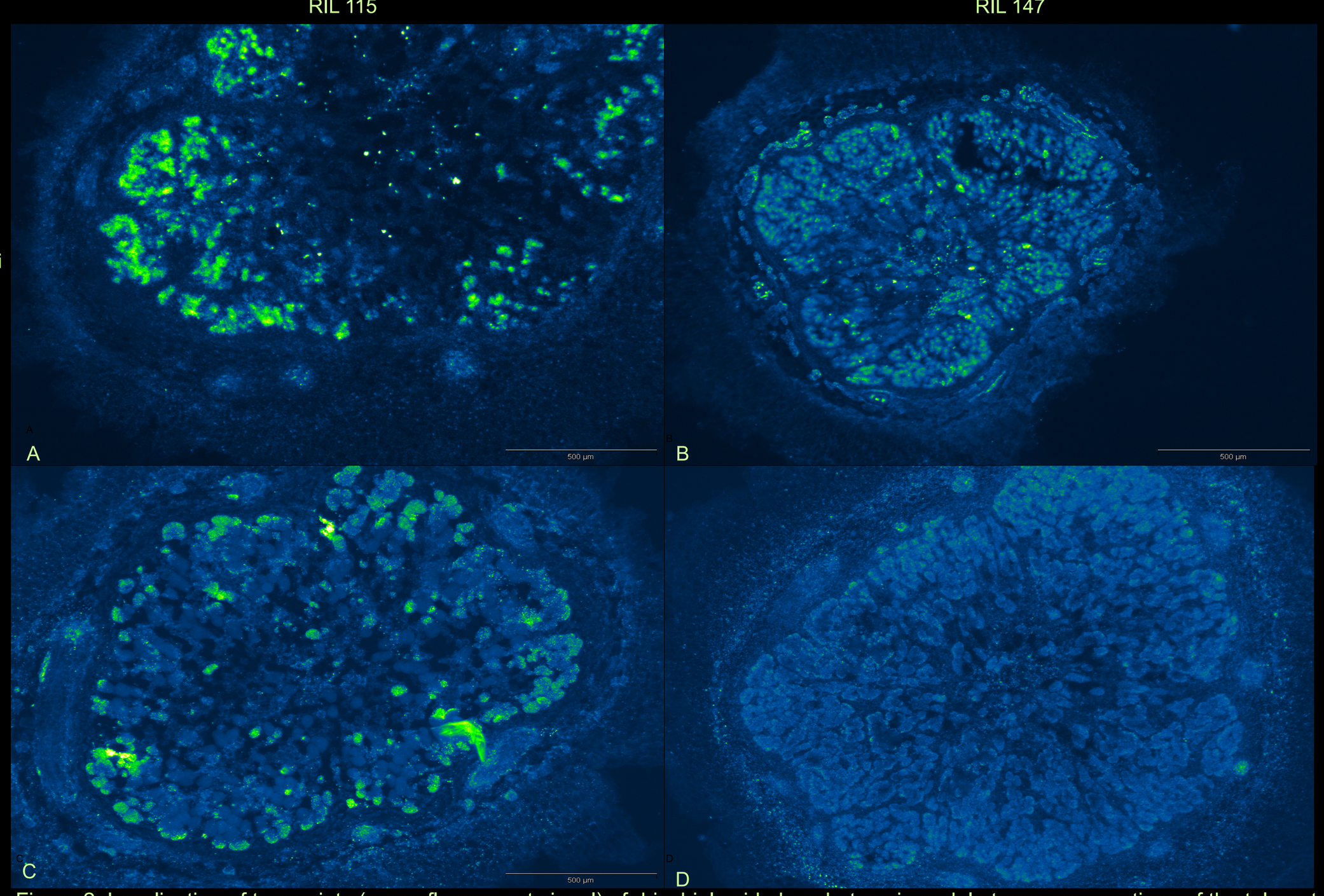

Figure 2: Localization of transcripts (green fluorescent signal) of rhizobial acid phosphatase in nodule transverse sections of the tolerant common bean RIL115 (A-C) and the less efficient common bean RIL147 (B-D) as a function of P treatments: 250 µmol Pi week^{-1} pl^{-1} as sufficient (A-B) versus 75 µmol Pi.week^{-1} pl^{-1} (C-D) as deficient.

Conclusion

To our knowledge, this *in situ* RT-PCR result is the first description of a rhizobial transcript within infected cells in legume nodules. From the variations of the signal intensity according to P supply, we can supposed that bacteroids are P-deprived under P deficiency, and that their P-induced acid phosphatase may contribute to the adaptation of the legume-rhizobia symbioses to low-P soils.

References: Bargaz, A, *et al*. (2012). J. Exp. Bot. 63: 4723-4730. ; van Aarle *et al.,* (2010). Mycorrhiza 17, 487–494. ; Hernandez, G. and Drevon, J.J. (1991). J. Plant Physiol. 138: 587-590.