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**The Proceedings of the International Plant Nutrition
Colloquium XVI**

Title

Effects of B deficiency on legume nodule organogenesis. Is boron involved in signalling during cell cycle regulation?

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Publication Date

2009-05-19

Peer reviewed

Introduction

Boron deficiency is an important constraint for nodule development (Bolaños et al. 1994; 1996; 2001; Bonilla et al. 1997) that leads to the formation of nodules with much smaller size than those developed with enough B, which also showed the appearance of different zones of tissue differentiation that were not distinguishable in – B nodules. Examination by optical microscopy showed that tissues of B-deficient nodules appeared abnormally developed, being most of cells of a small size, uninvaded by *Rhizobium* and apparently undifferentiated, in contrast to control nodules, which evidenced the presence of an apical meristem composed of small cells, an area of infection, and a zone of enlarged mature cells, full of bacteroids. The study by RT-PCR of the expression of key genes involved in cell cycle regulation during nodule organogenesis indicated that the gene encoding *cyclinD3*, involved in the reactivation of the cell cycle in response to Nod factors (Foucher and Kondorosi, 2000), was expressed in roots 10 h after inoculation with *Rhizobium* and during nodule development, both under control and B-deficient conditions. Meanwhile, the *ccs52a* gene, responsible of the transition from mitotic cell cycle to endoreduplication cycles prior to the generation of polyploid cells and triggering of the cell differentiation program (Cebolla et al. 1999; Vinardell et al. 2003) was not expressed in nodules developed in the absence of B. Confirming these results, quantification of nuclear DNA content by flow cytometry, indicated that the level of ploidy decreased in B-deficient nodules. Therefore, B does not seem necessary for the reactivation of the cell cycle after inoculation with *Rhizobium*, but it is required for the induction of mechanisms to gain ploidy prior to cell differentiation during organogenesis of symbiotic nodules in leguminous plants.

Acknowledgements

This work was supported by MICINN BIO2008-05736-CO2-01 and MICROAMBIENTE Comunidad de Madrid.

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