

# An unexpected actor in ammonium assimilation in conifer trees

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Conifers are tree species with enormous environmental and economic interests but with several characteristics that complicate their investigation (big size, secondary compounds, large long-life cycles, megagenomes...). However, they are well adapted to ammonium-rich soils being a good model to study ammonium assimilation in plants. Although they have a special feature, only two glutamine synthetase (GS, EC 6.3.1.2) genes, GS1a and GS1b, coding for cytosolic proteins, have been identified. In angiosperms and in the gymnosperm *Ginkgo biloba* there are two types of this enzyme responsible of the ammonium assimilation: GS1 expressed in the cytosol and GS2 in the plastids. Until the date, the searches of new GS1 and GS2 genes in conifers have been made with classical biochemical and molecular biology techniques without satisfactory results.

In the present context, the emergence of the next generation sequencing (NGS) techniques has open new opportunities in the resolution of old problems. They have allowed the whole sequencing of the massive conifer genomes and the analysis of their transcriptomes. Thus, in the framework of the European project ProCoGen, a gene expression atlas of the tissues of one-month seedlings was carried out using laser capture microdissection (LCM) and massive sequencing in maritime pine (*Pinus pinaster*), which is a conifer tree from the Southwestern Mediterranean region<sup>1</sup>. From the analysis of this work, a new gene coding for a new putative cytosolic GS has been identified, *PpGS1c*. This gene is expressed mainly in the apical and basal meristems at very low levels in comparison with the genes that code for GS1a and GS1b respectively. This has diffculted its previous identification by traditional Biochemical and Molecular Biology methods. This gene, GS1c, has been also found in the genomes of different conifers such as *Pinus taeda*, *Pinus lambertiana*, *Picea glauca* and *Picea abies*. At the same time, there is no gene coding for GS2 in the genomes of these species.

The role and regulation of *PpGS1c* in maritime pine are being studied through ammonium nutrition experiments at transcriptomic level using LCM. Finally, the GS1c enzymatic properties have been characterized in comparison to GS1b to better explain the function of GS1c in the nitrogen metabolism.

<sup>1</sup>Cañas, RA et al. (2017). *Plant J*, 91. 1064-1087

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