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Assessment of Medicago based systems for the production of human proteins: microscopy analysis of the subcellular deposition patterns of the recombinant product

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The use of transgenic plants for the large scale production of recombinant proteins with commercial and therapeutic value has emerged as an alternative to conventional platforms. Plant based systems, including whole plants and plant cell cultures offer many advantages particularly regarding safety and cost effectiveness. In our laboratory we have been using the model plant *Medicago truncatula* as a system to express recombinant proteins with a variety of applications [1].

In this study, we have generated transgenic Medicago plants stably expressing a recombinant enzyme – lipocalin-type prostaglandin D synthase (L-PGDS) or beta-trace – the major component of the human cerebrospinal fluid which is involved in various physiological and pathological functions including Alzheimer's Disease. Given the high number of potential applications for this enzyme, the production of recombinant human L-PGDS can be a promising field of research.

L-PGDS is highly glycolysated and therefore its transit through the secretory pathway is necessary for correct post-translational modifications to occur and the resulting proper biological activity. Understanding the different pathways that a protein can take within a plant cell, and how these pathways can vary among different tissues of the plant, is essential for assessing and optimizing plant systems for the production of biotherapeutics. Microscopy preceded by imunolabeling of the recombinant protein is one of the most important techniques to gain this knowledge, since it reveals the localization of the protein within the plant tissue. Whether the protein is present intra or intercellularly, or where exactly it is located inside the cell, is most relevant to assess possible different protein glycoforms that may be produced. This information can be crucial to evaluate if the product is suitable for its intended application and also to devise the best procedures for its purification.

In our study, we analyzed the expression of human L-PGDS (secreted version, containing a signal peptide) in different organs of Medicago plants (leaves, roots, and seeds) and in cultured cells in suspension. The results obtained by SDS-PAGE followed by Western blotting, where single or multiple band patterns were identified which can suggest different glycosylation levels, were compared against our images of localization with confocal microscopy of tissue sections labeled with an anti-L-PGDS antibody coupled with fluorescence.

Our results indicate, for example, that there are obvious differences between leaves and roots. Figure 1 shows a western blot of leaf and root tissue where there is a striking variation between the two. Whereas the leaf tissue (lane 4) shows heterogeneity of bands which may correspond to different glycoforms of L-PGDS, the root tissue (lane 5) presents a single band (arrow head).

We then studied the localization of the protein in leaves and roots by immunolabeling of tissue sections followed by observation under the confocal microscope (Figure 2). In the leaf, we observed a homogeneous distribution of the protein in the extracellular space (a), while in the root the protein seems to have a punctuated pattern in the apoplast (b). These results, although expected since the protein has a signal peptide for secretion to the apoplast, do not account for the obvious differences detected by the western blot analysis. We are now further analyzing the deposition pattern of the recombinant protein by electron microscopy in order to elucidate the precise location of the protein in these two tissues and correlate these results with different glycoforms.

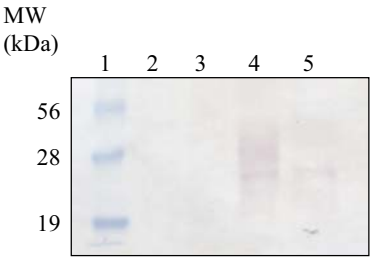

Figure 1. Western Blot with anti L-PGDS antibody. Lane 1: Molecular marker Low Range (BioRad); Lane 2: wild-type leaf; Lane 3: wild-type root; Lane 4: leaf sample of transgenic *M. truncatula;* Lane 5: root sample of transgenic *M. truncatula*.

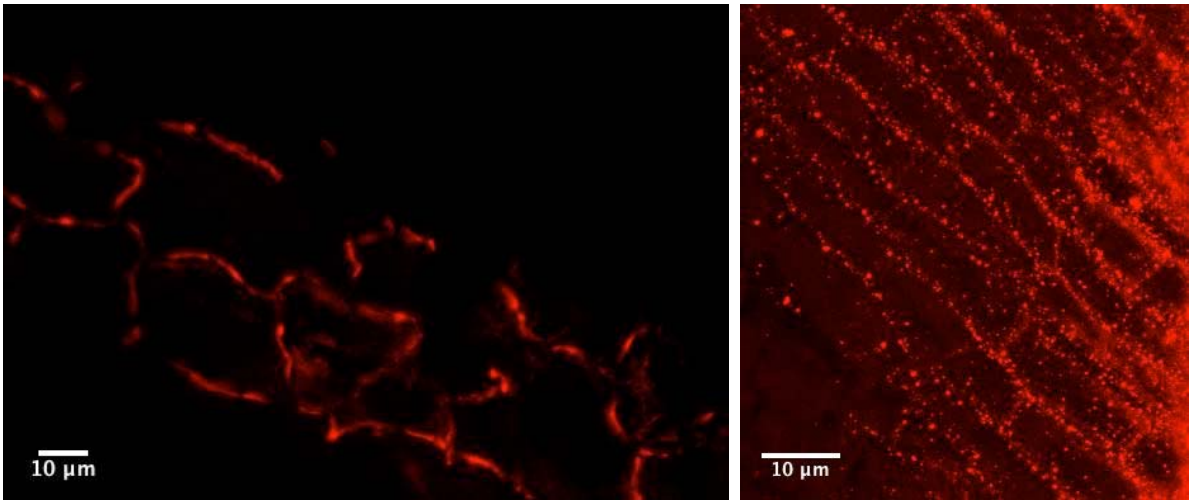

Figure 2. Immunolocalization of L-PGDS by confocal microscopy in the leaf (a) and root (b) tissue of transgenic *Medicago truncatula* (projection of optical sections). Incubation with primary antibody anti-L-PGDS (ab61866, Abcam) and secondary antibody anti-rabbit conjugated with a red fluorochrome (Alexa Fluor 594, Invitrogen).

In conclusion, the secreted version of L-PGDS presents a distinct deposition pattern in leaves and roots of *Medicago truncatula* transgenic plants, which is relevant not only for the characterization of this plant production platform but also to help unraveling the secretory pathway in plant cells. We are now analyzing seed tissue and cultured cells, in order to have a better understanding of the tissue specific factors that affect the final destination of the protein.

References

1. Abranches R., Marcel S., Arcalis E., Altmann F., Fevereiro P. and Stoger E., Plants as bioreactors: a comparative study suggests that *Medicago truncatula* is a promising production system. Journal of Biotechnology 120: 121-134, 2005

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