*Proceedings of the 52nd Italian Society of Agricultural Genetics Annual Congress*
*Padova, Italy – 14/17 September, 2008*
ISBN **978-88-900622-8-5**

**Poster Abstract – E.14**

# ENZYME REPLACEMENT THERAPY: PRODUCTION OF α-MANNOSIDASE IN TRANSGENIC TOBACCO PLANTS

DE MARCHIS F.*, BALDUCCI C.**, ARCIONI S.*, BECCARI T.**, BELLUCCI M.*

*) Istituto di Genetica Vegetale – CNR, Via della Madonna Alta 130, 06128 Perugia (Italy)
**) Dipartimento di Medicina Interna, University of Perugia (Italy)

*α-mannosidosis, Nicotiana tabacum, α-mannosidase, enzyme replacement therapy*

α-Mannosidosis is a rare lysosomal storage disease with autosomal recessive inheritance that leads to mental and physical deterioration. This pathology is due to progressive accumulation of undegraded oligosaccharides inside lysosomes. The deficiency of the α-mannosidase (LAMAN) enzyme, which normally cleaves α-linked mannose residues from glycoproteins during their ordered degradation, is the cause of the disease. The enzyme contains 1011 amino acids (108 kDa), including the 49 N-terminal residues which constitute the signal peptide. It is synthesised as a single chain precursor and sorted to the lysosomes where is processed into three glycopeptides of 70, 42 and 15 kDa. In humans, the 70 kDa peptide is further partially proteolysed into three more peptides that are joined by disulfide bridges.

The objective of this study is to provide a plant-based method for the production of LAMAN to be used in "enzyme replacement therapy" (ERT). We report the expression of the human α-mannosidase gene in stable transformed tobacco plants. Two different constructs were produced: in the first one, pROK8-LAMAN, the full-lenght cDNA coding sequence of α-mannosidase was used. The gene was under the control of the rbcS (rubisco small subunit) promoter and NOS (nopaline synthase) terminator. In the second one, pGreen-LAMAN, the α-mannosidase cDNA expression was controlled by the CaMV 35S (cauliflower mosaic virus) promoter and terminator. Moreover, the original 49 N-terminal signal peptide was replaced by a specific plant signal peptide from PR1 protein and the FLAG epitope was added at the C-terminus of the protein.

Even if the α-mannosidase gene harboured by the transgenic pROK8-LAMAN tobacco plants showed a good transcription efficiency, no detectable levels of the corresponding enzyme were obtained both in Western blot and enzymatic assays.

On the contrary, pGreen-LAMAN tobacco transformed plants expressed the α-mannosidase enzyme. The main signal detected in Western blot experiments using the anti-FLAG antibody had a molecular mass of about 110 kDa corresponding to the entire protein precursor, indicating that the protein was correctly synthesised. Western blot experiments using antibodies specific for the α-mannosidase enzyme revealed several signals corresponding to the single chain precursor and glycopeptides derived from precursor proteolysis. Transformed plants expressing the protein exhibited an enzymatic activity significantly higher then the untransformed tobacco plants. The recombinant enzyme showed biochemical features comparable to those of the human enzyme. Our long-term goal is to offer a new therapeutic approach for α-mannosidosis.