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Poster Abstract – F.03

EXPRESSION OF HUMAN LECITHIN-CHOLESTEROL ACYLTRANSFERASE (LCAT) GENE INTO TOBACCO PLANTS

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Lecithin: cholesterol acyl transferase (LCAT; EC 2.3.1.43) is a glycoprotein synthesised by liver and secreted into the plasma, where it circulates bound to HDL and LDL complexes. LCAT is responsible for the esterification of free cholesterol and allows formation of mature HDL complex. So, LCAT plays an essential role in reverse cholesterol transport. However, mutations in *lcat* gene cause two human rare forms of dyslipoproteinemia known as familial LCAT deficiency (FLD) and fish eye disease (FED).

In a frame of a Telethon project, in order to study *in vitro* wild and mutated LCAT enzymes to ascertain their physiological activities, it is necessary to have a large amount of those proteins, perhaps expressed in an heterologous system to avoid any interferences with other human or mammals proteins. In this presentation we report the LCAT wild type gene expression into *Nicotiana tabacum* plants. LCAT cDNA was transferred into a pGREEN binary vector, under the control of CAMV 35S promoter, giving rise to the plasmid called pG0029-LCAT. Nuclear genomic DNA amplification by PCR, using specific primer, showed the presence of *lcat* cDNA in 7 of 36 putative transgenic shoots rooted on MS medium added with kanamycin. To confirm the presence of *lcat* transcript, RT-PCR analysis was carried out. This analysis confirmed the presence of *lcat* transcript in 6 of 7 tested plants. Hence, Western analysis was performed to verify the expression of the LCAT enzyme in transformed tobacco plants. Rabbit polyclonal antibody against LCAT protein detected the presence of recombinant LCAT enzyme into transgenic plants. Identification of transgene copy number and quantification of LCAT recombinant enzyme are now in progress.