Proceedings of the 51st Italian Society of Agricultural Genetics Annual Congress
Riva del Garda, Italy – 23/26 September, 2007
ISBN **978-88-900622-7-8**

Poster Abstract – F.11

GAD65 EXPRESSION BY COWPEA MOSAIC VIRUS-BASED SYSTEM

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GAD65, autoimmune diabetes, CPMV, transgenic plants

Type 1 insulin-dependent diabetes mellitus (T1DM) which afflicts 0.2-0.3% of population is caused by autoimmune destruction of insulin-secreting beta cells. The young age of affected patients, the need for life-long insulin therapy and the high prevalence of late-onset complications make T1DM a major health problem. The smaller isoform of glutamic acid decarboxylase of 65 kDa (GAD65) is a major autoantigen in human T1DM. Induction of oral tolerance has been reported to modify the natural history of several autoimmune diseases both in experimental models and in pilot human trials. Studies in animal models of spontaneous autoimmune diabetes have shown that parenteral administration of GAD65 can prevent (or delay) the onset of disease.

Poor GAD protein solubility in bacteria and inadequate production from eukaryotic cells have so far precluded the use of this approach for the large scale production of GAD65 for oral tolerance studies . Transgenic plants that express high level of recombinant human GAD65 could be the source of food for oral administration of the autoantigen. We previously reported the production and characterization of transgenic plants expressing membrane-anchored hGAD65 (Porceddu *et al.*, 1999) and the production and characterization of plants expressing a cytosolic form of the recombinant protein (GAD67/65) (Avesani *et al.*, 2003). By using a radio-immuno assay with human serum from a GAD65 autoantibody positive T1DM patient, the highest expression level of the recombinant GAD67/65 protein was estimated to be 0.19% of total soluble protein, compared to only 0.04% of hGAD65.

In a recent study we tested the hypothesis that the expression level of recombinant hGAD65 could be improved by using a cowpea mosaic virus-based system. This system is based on the bipartite RNA plant virus, cowpea mosaic virus (CPMV) and involves the amplification of integrated copy of a deleted version of RNA-2 carrying the human GAD65 cDNA.

The CPMV-based systems have advantages over existing plant expression systems in terms of the expression levels obtainable and the simplicity and flexibility of use, and should be of great practical benefit in the development of plants as bioreactors.