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Poster Abstract – E.15

MODIFICATION AND IMPROVEMENT OF A PLASMID VECTOR FOR THE PRODUCTION OF ANTIGENIC MOLECULES IN GM TOBACCO, FOR VETERINARY USE

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The production of important molecules (as subunit vaccines) in plants is increasingly considered for relevant advantages: low costs of production, purification and delivery, no risks of contamination by pathogens and high scale production, but improvement and enhancement of transformation techniques are needed. MAR/SARs (Matrix/Scaffold Attachment Regions) are DNA sequences that have been reported to create a network with the proteinaceous fibrils of nuclear matrix, organizing chromatin into a series of topologically isolated loop domains of 5-200 kb. These sequences may influence the conformation of transgenes and their expression, possibly reducing or eliminating some forms of gene silencing.

Our research is addressed at the production of plant derived antigens, to be used in veterinary prophylaxis. In this field, the optimisation of transgene expression is crucial, also because of the necessity of plant containment during the whole cultivation period.

In particular, we sub-cloned *Rb7*, a MAR sequence from *Nicotiana tabacum*, in the binary vector pAMPAT, inside t-DNA close to LB and RB terminations, in its two possible orientations. The vector expression cassette carries a 511 bp portion of *Fib*, encoding Fibrinogen Binding Protein, from *Staphylococcus aureus*, under the control of 35SS constitutive promoter. The Fib protein fragment was proved to be effective against *S. aureus* mastitis in dairy cattle. *Nicotiana tabacum*, var. Samsun was transformed *via Agrobacterium tumefaciens* with the four constructs carrying *Rb7* elements in all their possible combinations. Statistical analysis was performed after four different experiments, showing an enhanced transformation efficiency for MAR-containing constructs (higher shoot number and shorter shooting time). Molecular and immunological analysis on transformed plants are now in progress, to define the transgene copy number and the resulting protein expression level.