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Poster Abstract – F.02

PRODUCTION OF AN ANTIMICROBIAL KILLER PEPTIDE IN TRANSGENIC TOBACCO

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The decapeptide Killer Peptide (KP) derived from the sequence of a single-chain anti-idiotypic antibody, was shown to exert a strong microbicidal activity against both human and plant pathogen. (Polonelli *et al.* 2003, Infect. Immun. 71: 6205-6212; Donini *et al.* 2005, Appl. Env. Microbiol., 71: 6360-6367). It was also shown that the KP expressed in plants by using a Potato Virus X (PVX)-derived vector as a fusion to the viral coat protein (CP), yields chimeric virus particles (CVPs) displaying the heterologous peptide endowed with a potent microbicidal activity. Furthermore in planta challenge assays demonstrated that the KP-CVPs protect Nicotiana benthamiana plants from the bacterial infection of Pseudomonas syringae pv. Tabaci (Donini *et al.* 2005, Appl. Env. Microbiol., 71: 6360-6367).

In this work we focused on the expression of KP in Nicotiana tabacum by using *Agrobacterium tumefaciens* mediated transformation in order to evaluate the protection conferred to a broad spectrum of both bacterial and fungal pathogens. Moreover transgenic plants are evaluated for their potential as biofactories for large-scale production of the microbicide. To this aim different transformation vector cloning strategies of the KP were pursued. We constructed five different KP expression vectors all harbouring the nptII Kan resistance gene, the CaMV 35S promoter and the tobacco mosaic virus (TMV) Ω translational enhancer sequence.

The KP sequence was cloned as a single ORF, however due to the stability issues of this peptide, we also designed a new molecule introducing two identical KP sequences fused by a linker peptide able to form intrachain disulfide bonds. The rationale behind this construct was to stabilize the molecule maintaining its antimicrobial activity. The other constructs were obtained through the N-terminal fusion of the KP peptide to a non functional form of the PVX CP that was used as a carrier to stabilize and enhance the production yields of the peptide. The expression of these constructs was directed either in the cytoplasm or to the secretory pathway by adding a polygalacturonase inhibiting protein (PGIP) signal peptide; moreover a specific restriction site for proteolysis was inserted between the KP and the CP sequence to facilitate the purification process of the peptide. To rapidly evaluate the in planta behaviour of the different gene constructs, we performed transient expression analysis through agroinfiltration of N. tabacum leaves. We evaluated the expression of the different PK constructs through Western Blot analysis and ELISA. N. tabacum plants were stably transformed with the different gene constructs, obtaining 50 independent transgenic lines for each construct. Transgenic plants will be evaluated for both pathogen resistance and expression yield of the heterologous protein.