Morphological evaluation of *Nicotiana tabacum* plants transformed for the expression of verocytotoxic *Escherichia coli* antigens

Luciana Rossi[1], Elisabetta Onelli[1], Alessandra Moscatelli[1], Angela Lombardi[2], Antonella Baldi[2]

[1]*Department of Health, Animal Science and Food Safety, Università degli Studi di Milano, Via Celoria 10, 20133 Milan, Italy*
[2]*Department of Biosciences, Università degli Studi di Milano, Via Celoria 26, 20133 Milan, Italy*

Corresponding author: luciana.rossi@unimi.it

Two transgenic *Nicotiana tabacum* plants carrying respectively the F18 and the B subunit of verocytotoxin genes from O138 verocytotoxic *E.coli* serotype (VTEC), were developed by agroinfection as model of edible vaccine. VTEC causes severe enterotoxaemia in weaned piglets and is responsible of important economic losses. For the construction of transgenic plants, the bacterial genes were placed under control of GLOB for the seed specific expression. Previous studies demonstrated that the Vt2e-B and FedA fimbrial genes were stably incorporated into tobacco plant genome by being transcribed through the nuclear apparatus of the plant for specific expression in the seeds, and that these genes are inherited by the next generation. The dietary administration of transgenic tobacco seeds promoted a significant increase in the number of intestinal mucosal IgA-producing cells in mice and showed a protective effect against *VTEC* strain in piglets. *Agrobacterium tumefaciens* binary vector system is an efficient tool to transform plant cells; however, the exogenous gene integrates at semi-random into the nuclear chromosomes. In other words, the insertion of a transgene into the plant genome inevitably disrupts the sequence of the endogenous plant DNA and may be accompanied by other mutations. For these reasons, the aim of this study is the morphological evaluation of *Nicotiana tabacum* plants transformed for the expression of F18 and Vt2e-B proteins with respect to Nicotiana tabacum wild type (WT). Three lines of tobacco seeds (F18, VT2e-B and WT) were seeded in homogeneous conditions and were harvested simultaneously. Tobacco plants were analysed by optical microscope in different phases of growth. Germination of transgenic seeds was delayed of three and five days compared to WT in two replicated experiments, suggesting that genetic manipulation influenced mechanisms leading to germination. The analysis of F18 and VT2e-B seed polypeptides, following two different methods of protein extraction showed differences in the electrophoretic profiles with respect to WT. Furthermore, morphological observations using optical microscope, showed no difference in the embryos of tree samples. On the contrary, a large amount of storage material (oleosomes or aleuron grains) are observed in the endosperm of F18 seeds, with respect to WT endosperm, in which storage proteins and lipids were already mobilized. This data could explain the delayed germination of transgenic lines.

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