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Poster Abstract – E.01

'CLEAN' STRATEGIES FOR EXOGENOUS GENE TRANSFER IN GRAPEVINE

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In grapevine, the gene transfer technique is based mainly on *in vitro* co-cultures of plant tissues with *Agrobacterium tumefaciens*. The selection of transgenic cells is one of the crucial steps of the overall strategy. Established selection techniques are usually based on resistance genes, that confer to the cells the ability to survive on media supplemented with antibiotic. Thus, their function is limited to the selection phase and their further presence in the plants may be undesirable. Moreover, their use is becoming controversial (regulation 2001/18/CE).

We are exploiting some strategies for transferring the exogenous genes into grapevine plants, and we are focusing on (i) the elimination of the antibiotic resistance genes after the selection step and (ii) the use of alternative marker genes.

The elimination of the marker gene is based on the pX6 vector (provided by The Rockefeller University - N.Y., Prof. Nam-Hai Chua) that contains the *gfp* gene, the *cre/loxP* recombination system induced with β-estradiol for the site-specific excision of the *nptII* gene (Zuo J. *et al*. 2001, Nat. Biotech., 19: 157-161). In a second construct, the *gfp* gene was replaced with a sequence of Grapevine Virus A (GVA) coat protein in sense and antisense orientation, resulting in a hairpin RNA (pX6-pKcpGVA, Turturo *et al.*, 2003, Proc. 14th ICVG Conference, Locorotondo, Italy, 12-17 Sept.). Successful marker gene removal leads to the expression of the reporter gene (*gfp*) or the GVA sequence respectively. Brachetto and Chardonnay embryogenic calli were co-cultured with *Agrobacterium* alternatively carrying one of these two constructs, putatively transgenic cultures were selected on kanamycin and individual somatic embryos were converted into plantlets. Several tests were performed to optimise the induction with β-estradiol. Best results were obtained with the application for one month of 10 mM β-estradiol on buds before stimulating plant elongation. Southern Blot analyses proved transgene integration and the *nptII* gene removal was quantified with Real-time PCR. Efficiency of *nptII* gene excision was observed to vary along the plant, decreasing from the roots to the apical tissues.

Plant cells expressing the *manA* gene from *Agrobacterium* can be made capable of metabolising mannose, otherwise toxic, as an alternative to sucrose. This strategy produced controversial results in grapes (Kieffer et al., 2004 Vitis 43:35-39). For this reason, extended preliminary assays were necessary to fully understand the effect of mannose on the cultures, before starting the gene transfer experiments. Wild-type embryogenic calli of 'Brachetto', 'Chardonnay' and '110 Richter' were grown and subcultured monthly on media added with mannose or sucrose or free of the carbohydrate source. No growth and a gradual death was observed in the carbohydrate-free media, while calli cultured on mannose showed no clear evidence of a fatal damage even after a long time (14 months). Similar results were obtained on mature somatic embryos induced to plantlet

conversion. Brachetto, Chardonnay and 110 Richter embryogenic calli were co-cultured with *Agrobacterium* carrying the pNOV2819 construct (courtesy of Syngenta, licensed) containing the *manA* gene. The maintenance of calli and the induction of plant recovery from somatic embryos were performed on mannose-containing media, and molecular analysis are in progress for assessing and quantifying the presence of the exogene insertion. (Thanks to the Autonomous Province of Trento, Project *EcoGenEtic.Com*.)