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Poster Abstract – E.04

MARKER GENE REMOVAL AND ALTERNATIVE MARKER GENES FOR AN IMPROVED GENE TRANSFER IN GRAPE

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Improvement of grape molecular breeding was attempted by exploiting two strategies based respectively on the marker gene excision and the use of marker genes alternative to antibiotic resistance.

To exploit the self-excision, embryogenic calli of *V. vinifera* (cvs. Chardonnay, Brachetto), and the rootstock 110 Richter were co-cultured with *Agrobacterium tumefaciens* carrying the chemically-inducible site-specific *cre/loxP* pX6 vector with the genes for the Green Fluorescent Protein (GFP) and for the neomicin phosphotransferase (NPTII). In this construct, kindly provided by prof. Nam-Hai Chua (The Rockefeller University, New York), the expression of the *cre* recombinase is regulated by the 17-β-estradiol (Zuo *et al.*, 2001, *Nature Biotechnol.* 19 :157-161) and should result in the NPTII gene excision and the consequent GFP gene expression. Besides, the construct was also tested on *Nicotiana benthamiana* as model system. Putatively transgenic cultures were selected on kanamycin, and individual somatic embryos were isolated and converted into plantlets. Preliminary molecular assays showed the transfer of the GFP gene into the plant genome, and observations at the fluorescence stereomicroscope proved its expression, as the result of the successful induction of the marker gene self-excision mechanism.

To test the efficiency of the constructs based on marker genes alternative to antibiotic resistance genes, we verified the possibility to make cells able to metabolize specific carbon sources, such as mannose as substitute to sucrose (Posytech, Sygenta licence). This strategy has proved successful with various plants, however gave variable results in grapes (Reed *et al.*, 2001, In Vitro Cellular & Devel. Biolol. Plant, 37:127-132; Kieffer *et al.*, 2004, Vitis 43:35-39). The effect of the selective medium on the callus growth ability and morphogenic potential was verified. Embryogenic calli of *V. vinifera* cv. Brachetto were grown and monthly subcultured on different formulations of the same medium containing mannose, sucrose or free of the carbohydrate source, respectively. Calli cultured on the substrate containing mannose or sucrose showed a similar growth rate. As expected, no growth was observed in calli placed on the medium without carbohydrate source, which progressively turned brown and died.

At this point of the work, the removal of the marker gene seems to be the most promising between this two strategies compared for grape gene transfer. First of all, tissue culture conditions related to the PMI gene still require a labor-intensive optimization in the view of accurately selecting transgenic- from non-transgenic cells. On the contrary, the presence of the NPTII gene in the pX6 vector allows an effective selection among cell lines. Moreover, the elimination of the

marker gene after induction with 17-β-estradiol allows to take advantage of an effective selection method, and also to perform the actual elimination from the grape cells of the non-desired genetic material that were inserted during the gene transfer process.

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