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Poster Abstract – E.03

CO-TRANSFORMATION FOR MARKER-FREE GENETICALLY ENGINEERED ALFALFA

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Among the arguments against Genetically Modified Plants (GMPs) is the fact that they contain, linked to a useful gene, an antibiotic or herbicide resistance gene serving as selectable marker (SMG) to facilitate the process of *in vitro* selection of transgenic cells. The development of techniques to avoid the use of these SMGs could improve public perception of plant genetic engineering.

In co-transformation, the useful gene and the SMG are introduced together, but using separate vectors, so that they are integrated in different genomic sites. Progeny plants carrying only the useful gene can then be obtained thanks to genetic segregation.

A mutant *Synechococcus* gene encoding glutamate 1-semialdehyde aminotransferase was used as the SMG for alfalfa genetic transformation in this experiment. This gene has been shown to confer resistance to gabaculine (3-amino-2,3-dihydrobenzoic acid) in tobacco (Gough et al. 2001) and performs very well in alfalfa (unpubished data from our lab). The conventional SMG *Npt*II played the part of the useful gene in this experiment. Both genes were placed under the control of the Dual CaMV35S promoter and the *Nos* polyadenylation sequence.

Alfalfa leaf explants were co-cultivated with a mixture of two *Agrobacterium* (strain LBA4404) cultures, each harbouring a pPZP201BK binary vector containing either the *hem*L or the *Npt*II cassette. Regeneration was performed on gabaculine-containing media, and each mature somatic embryo was divided into two parts and transferred onto regeneration medium containing either gabaculine or kanamycin in order to check for the expression of *hem*L and *Npt*II.

In a first experiment, 10 kanamycin resistant events were obtained in 107 gabaculine resistant events. So far, 5 of these 10 events have been tested by PCR for the presence of the *hem*L and *Npt*II genes, and three were positive for both.

In a second experiment we have 5 kanamycin resistance events in 83 gabaculine resistant events.

The relatively low efficiency of co-transformation that we obtained may be due to the presence of homologous control regions in the two constructs, involving silencing of the non selected gene (*Npt*II) during the first regeneration cycle on gabaculine. To check this hypothesis the experiment will be repeated using different control sequences for the two genes. In any case, our results indicate that an acceptable number of co-transformed plants can be obtained without much effort. The number of copies and independence of the two genes is being assessed by Southern blot experiments. Three plants containing both genes were crossed to a non transgenic, unrelated pollen parent and the segregation of the two genes will be assessed in the progeny.