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Poster Abstract – E.01

IN SEARCH OF A SELECTABLE MARKER GENE FROM *MEDICAGO SATIVA*

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Selectable marker genes (SMG) for plant genetic engineering are mostly derived from bacteria, and very few derive from plants; however, plant SMG could be perceived as safer for human health and the environment.

In plants, the enzyme glutamate 1-semialdehyde aminotransferase (*GSA-AT*) catalyses the conversion of glutamate-1-semialdehyde into aminolaevulinic acid, a step in the synthesis of tetrapyrrole compounds including chlorophyll. This enzyme is irreversibly inhibited by gabaculine (3-amino-2,3-dihydrobenzoic acid). In a gabaculine resistant mutant strain of *Synechococcus elongatus*, a gene encoding a mutated GSA-AT was isolated, that has been successfully used as a selectable marker gene in genetic transformation of tobacco Gough et al. 2001) and *Medicago sativa* (Rosellini et al., unpublished). The mutant bacterial protein differs form the wild type four a 3-aminoacid deletion close to the ammino terminus and a substitution in the catalytic domain.

We found that plant GSA-AT have high similarity (more than 70% at the aminoacid level) with this bacterial enzyme. Therefore, we decided to test the possibility to obtain a gabaculine resistant plant GSA-AT that could be used as selectable marker gene.

To this purpose, we took advantage of a published *Medicago truncatula* tentative consensus sequence (TC93991) highly homologous to GSA-AT, and designed primers to amplify the coding sequence using *Medicago sativa* and *Medicago truncatula* cDNA as templates. Single PCR products of the expected size were obtained, cloned and sequenced. Sequence analysis revealed that the cDNAs from the two species indeed encode GSA-AT, and have about 98% identity at both the nucleotide and aminoacid level.

Multiple alignment was performed to investigate the conservation of the active domains of the protein and the phylogenetic realationships among GSA-AT from plants.

We hypothesize that a single aminoacid substitution in the highly conserved catalytic domain could be sufficient for gabaculine resistance.