Proceedings of the 52nd Italian Society of Agricultural Genetics Annual Congress
Padova, Italy – 14/17 September, 2008
ISBN **978-88-900622-8-5**

Poster Abstract – E.07

MUTAGENESIS OF *MEDICAGO SATIVA GSA-AT* FOR GABACULINE RESISTANCE

FERRADINI N., NICOLIA A., CAPOMACCIO S., VERONESI F., ROSELLINI D.

Department of Applied Biology, University of Perugia, Borgo XX Giugno 74, 06121 Perugia (Italy)

site-specific mutagenesis, GSA-AT, Alfalfa, gabaculine resistance

Selectable marker genes (SMG) of bacterial origin, especially antibiotic resistance genes, represent a valuable tool for plant engineering. However, the use of these SMG has raised concern about the safety of their use in agriculture. Several approaches are now available to eliminate or replace antibiotic resistance genes from transgenic plants. Plant-derived SMG could be a valid alternative to overcome these problems but there are few available. We have identified a possible SMG candidate in the *Medicago sativa* glutamate 1-semialdehyde aminotransferase (GSA-AT**)** gene**.** In plants, algae and cyanobacteria, the enzyme GSA-AT catalyses the conversion of glutamate-1-semialdehyde into aminolevulinic acid, a step in the synthesis of tetrapyrrole compounds including chlorophyll. This enzyme is irreversibly inhibited by gabaculine (3-amino-2,3-dihydrobenzoic acid). A mutant form of the *hemL* gene of *Synechococcus elongatus* encoding GSA-AT has been demonstrated to work well as SMG in tobacco (Gough et al. 2001) and particularly in alfalfa (*Medicago sativa* L.) (Rosellini et al. 2007).

We found that plant GSA-AT genes have high similarity (more than 70% at the aminoacid level) with *hemL*: the mutant bacterial protein differs form the wild type for a 3-aminoacid deletion close to the amino terminus and a point mutation resulting in a methionine to isoleucine substitution in the catalytic domain.

We are implementing two strategies of site–specific mutagenesis to induce gabaculine resistance in alfalfa GSA-AT. The first consists in the production of two variants of the alfalfa cDNA: 1) Substitution of the methionine 286 with a isoleucine in the catalytic domain; 2) The same substitution plus the replacement of the aminoterminal of the alfalfa protein with that of the *hemL* protein.

The second strategy aims to exploit the natural competence and the extremely efficient recombination system of *Synechococcus elongatus* PCC7942 in order to produce a recombinant strain in which the chromosomal gene *hem*L is replaced by the cDNA sequence of the alfalfa GSA-AT gene. This recombinant strain will be placed under selection with increasing concentration of gabaculine in the attempt to isolate a mutated, insensitive form of alfalfa GSA-AT (collaboration with the University of Leicester – UK, ADAS group – Garry Whitelam's lab).