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Poster Abstract - H.07

AN ALTERNATIVE TO ANTIBIOTICS FOR ALFALFA GENETIC ENGINEERING

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Concern over the presence of antibiotic resistance genes in genetically engineered plants has been expressed because of the risk that these genes are horizontally transmitted to pathogenic bacteria that become resistant to antibiotics. Although this risks has been judged to be very low, the scientific community has been stimulated to develop alternative selection systems, and several are available.
Gabaculine (3-amino-2,3-dihydrobenzoic acid) is toxic to a range of plants through a potent inhibition of chlorophyll synthesis via binding to the enzyme glutamate 1-semialdehyde aminotransferase (*GSA-AT*). In plants, this enzyme catalyses the conversion of glutamate-1-semialdehyde into aminolaevulinic acid, a step in the synthesis of tetrapyrrole compounds, including chlorophyll. Gabaculine has been used as a selecting substance for tobacco transformation (Gough et al. 2001). The gene conferring gabaculine resistance to tobacco encodes a mutant, gabaculine-insensitive *GSA-AT* form from *Synechococcus*. The coding sequence of the *hem*L gene with a chloroplast transit peptide (kindly provided by K. C. Gough) was introduced into the T-DNA of the pPZP201BK binary vector, along with the conventional selection marker gene for alfalfa, *Npt*II, conferring kanamycin resistance, in order to directly compare the efficiency of the two selection systems. Both genes were placed under the control of the dual CaMV*35S* promoter and the *Nos* terminator. This vector was introduced in the *Agrobacterium tumefaciens* strain LBA4404 for a transformation experiment using alfalfa (genotype Regen-SY1) leaf explants. Half of the *Agrobacterium*- treated leaf explants were placed on gabaculine selection (25 and 50 μM, previously shown to completely inhibit regeneration) and half on kanamycin (25 mg/l) selection. Sixty six % of the leaf explants produced at least one green somatic embryo on gabaculine selection *vs* 54% on kanamycin. In a functional test of marker gene expression, 78 % (95 in 121) of the embryos obtained with gabaculine selection regenerated again in a second regeneration cycle on gabaculine, whereas only 33% (32 in 94) of the embryos obtained with kanamycin regenerated again on kanamycin. PCR with primers specific for the bacterial *GSA-AT* demonstrated that the regenerated plants contained the gene. The higher efficiency of gabaculine vs kanamycin selection in this first experiment is probably due to the mechanism of action of the enzymes encoded for by the two genes: *Npt*II detoxifies the selective substance (kanamycin), whereas the mutant *GSA-AT* substitutes for the gabaculine-sensitive plant *GSA-AT*; therefore, gabaculine in the culture medium is not depleted and this would reduce the chance of non-transgenic plant cells to give rise to somatic embryos. Safety of *GSA-AT* for animal or human consumption has not yet been directly tested, but this enzyme is found in many organisms, including plants, and *Arabidopsis thaliana GSA-AT* is 69 % identical to the bacterial enzyme used in this experiment. Gabaculine-resistant *GSA-AT* could be an useful tool to develop more acceptable genetically engineered alfalfa varieties and may be applicable to a variety of crop species.

Reference:

Gough KC, et al. (2001) Cyanobacterial GR6 glutamate-1-semialdehyde aminotransferase: a novel enzyme-based selectable marker for plant transformation. Plant Cell Reports 20: 296-300