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EXPRESSION OF THE PLANT GSA GENE FROM THE PLASTID GENOME

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A quest for new selectable markers for plastid transformation is ongoing to apply plastome genetic engineering to a more significant number of plant species. We previously introduced a bacterial *hemL* mutant gene in the tobacco plastid genome, and showed that it confers resistance to the phytotoxin gabaculine, which inhibits the enzyme glutamate 1-semialdehyde aminotransferase (GSA). In this work, we have introduced the alfalfa GSA gene, devoid of its native chloroplast targeting peptide, into the tobacco plastome. We observed that gabaculine resistance was not sufficient to select transplastomic events during the first *in vitro* regeneration cycle, but conventional spectinomycin selection allowed us to regenerate 5 events. However, two further rounds of regeneration in the presence of gabaculine demonstrated that resistance was acquired and useful to attain the homoplastomic state. Molecular analyses confirmed correct homologous recombination, and sexual progenies demonstrated maternal inheritance of gabaculine and antibiotic resistance.
Like the bacterial *hem*L-encoded enzyme, the mutant plant GSA enzyme is either incompletely resistant to gabaculine or expressed at an insufficient level in the plastid for selection during the post-bombardment phase of the plastid transformation process in tobacco. However, nuclear expression of GSA is effective for the transformation of the nuclear genome of several species, and this selection system is based on a plant gene and does not employ antibiotics; for these reasons, an optimization effort for plastid transformation in other species may be worthwhile.