Abstract

Auxin conjugation is one of the crucial metabolic processes regulating auxin activity in plant cells. Gretchen Hagen 3 (GH3) is a family of acyl amido synthetases that conjugates auxin with amino acids and belongs amongst important enzymes involved in auxin conjugation. Due to the existence of more sensitive methods to detect auxin metabolites and the current study of abiotic stress effects, research on GH3 enzymes is intensified these days. These enzymes are best known in thale cress (*Arabidopsis thaliana*), soya bean (*Glycine max*), rice (*Oryza sativa).* These models don't allow to study their activities in a biochemical way. Therefore, the aim of this work was to monitor the auxin metabolism in the established model tobacco BY-2 cell lines (*Nicotiana tabacum*). The *NtGH3.1* and *NtGH3.6* genes, which were shown to have a variability in their expression regulation by auxin, were targeted and mutated using tne CRISPR/Cas9 method. Mutations in the derived lines were detected by sequencing. In the derived lines, auxin metabolic profililing was analysed by LC/MS. Metabolic profiling showed a correlation between the *NtGH3.6d* form and the specific production of the metabolite oxIAA-Gln (N-(2-onindole-3-acetyl)-glutamine). The study of an eventual substitution of individual *GH3* gene forms in mutant lines using the RT-qPCR methode indicated that substitution is likely to be minor. Individual genes were labelled using GFP, expressed under inducible promoters, and their products were localised in the cytoplasm and nucleus of BY-2 tobacco cells by confocal microscopy. The results of this work represent the first complete analysis of the expression, function, and localisation of individual members of the auxin acyl amido synthetases in tobacco.