Abstract

Uncontrolled spreading of genetically modified (GM) plants is one of the main concerns about their cultivation. Inducible RNA interference against an essential gene could be a tool for control of GM plants. After spraying with a chemical inducer, the essential gene will be silenced so the treated GM plant will die. For testing this strategy we chose two key enzymes of nitrogen metabolism, glutamate synthase (GOGAT) and glutamine synthetase (GS). GS processes ammonium ions into glutamine, then GOGAT transfers the amide group from glutamine to 2-oxoglutarate to form two glutamates. GS/GOGAT cycle is the main pathway for assimilation of ammonium ions, which could be toxic to plants in a higher concentration. Disruption of ammonium assimilation during photorespiration causes a strong inhibition of photosynthesis.

The aim of this work was to describe the effects of silencing *GOGAT* and *GS* genes in *Arabidopsis thaliana*. To induce silencing, RNAi hairpin constructs under a control of constitutive or estradiol-inducible promoter were prepared.

In selected independent transformants with the inducible hairpin against GOGAT, chlorosis and reduced growth were observed after the estradiol treatment in *in vitro* conditions. However, the spraying with estradiol was tricky, at the whole plant level, the induction of silencing was not successful.

In the case of GS, we chose the gene for plastid isoform GS2, because knock-out mutant in *GS2* has not been hitherto described in *A. thaliana*. The function of GS2 in photorespiratory assimilation of ammonium is not clear. It is possible that mutation in *GS2* is lethal or the function of GS2 can be complemented by some of cytosolic GS1 isoforms. Using a promoter mutant in *GS2* and RNAi against *GS2*, we deduce that reduction in *GS2* expression causes decrease in biomass, yellowing and changes in some photosynthetic parameters. These phenotype effects were partially suppressed in an atmosphere with elevated CO_2, so GS2 appears to be involved in the fixation of ammonium ions during photorespiration.