

Abstract

Accidental discovery of RNA interference in plants a few decades ago and its description by Fire et al. (1998) brought a real revolution in molecular biology and in many other branches of biology. Researchers have uncovered a large and unexpected mechanism of regulation of gene expression at many levels in almost all studied eukaryotes. The first studies have revealed silencing of both exogenous and endogenous genes in plants. So, it soon became clear that this process serves to organisms not only in the defense against viruses, but also against mobile genetic elements as well as in modulation of expression own genes. The key role in all RNAi mechanisms is played by dsRNA that is processed into small RNAs. In association with a number of other proteins, small RNAs can direct either cleavage of complementary or nearly complementary mRNAs or block their translation in the process of posttranscriptional gene silencing (PTGS). Additionally, sRNAs can affect gene expression at transcriptional level *via* modifying DNA in a process called RNA dependent DNA methylation (RdDM).

In plant transformation, RNAi cause variability amongst transgene lines and instability in the expression of introduced genes, which complicates the use of transgenic lines, because many biotechnological approaches require a defined stable transgene expression.

The main aim of the thesis was to evaluate the impact of selected factors on the degree of silencing of transgene expression and to find such conditions or treatments, which would cause rapid silencing of the transgene expression in lines that are prone to silence. This would select immediately after transformation the lines with stable transgene expression, which is very desirable for both basic and applied research. The materials for this research were transgenic potato lines with silenced, restored *GFP* and long-term stable *GFP* expression and tobacco cell line BY-2, which was transformed with newly prepared constructs.

In the frame of the master thesis, the impact of the transgene coding sequence on silencing was proven by comparing the *GFP* fluorescence in BY-2 lines transformed with prepared constructs. The rate of transgene silencing was also affected by exposure to stress conditions during potato *de novo* regeneration. The transition of potato plants through the dormant tuber stage did not reveal any changes in the expression of the transgene. Reactivation of expression from silenced transgenic single-copy line of potato was successful only for the *NPTII* gene. Only temporary restoration of expression was seen in the case of *GFP*.

Keywords: RNA interference, PTGS (posttranscriptional gene silencing), TGS (transcriptional gene silencing), tobacco BY-2 cell line, *Solanum tuberosum*, RdDM (RNA-directed DNA methylation), *GFP*, transgene silencing