

The RNA interference is a mechanism, which allows cells to regulate their genes functions, to establish and maintain heterochromatin and to defend them against invasive nucleic acids. In plants, RNA interference is initiated by double-stranded RNA, which is processed by Dicer into small RNAs, usually 20-24nt long. These small RNAs form a complex with Argonaut protein that participates in different processes based on sequence complementarity. This complex can guide mRNA cleavage, translation blocking and chromatin modifications, resulting either into posttranscriptional silencing (by preventing translation of already existing mRNA, PTGS) or transcriptional silencing (by preventing transcription of mRNA, TGS). The first step of this thesis was to establish different ways of triggering PTGS and to evaluate their functionality and efficiency. The next step was a preparation of a system which would allow to study the transition from posttranscriptional to transcriptional silencing. These so called "indicator lines" should allow to observe the timing and dynamics of this process by utilizing fluorescent proteins. This system is also going to enable to evaluate, how different factors are involved in this process – one of the factors is RNA-dependent RNA polymerase 6 (RDR6) which plays an essential role in spontaneous triggering of PTGS. In this work, the tobacco BY-2 cell line was used as a model organism. Large number of independent clones can be established using this cell line and it also enables to study cell populations or single cells in precisely controlled conditions. Three different constructs for induction of silencing were prepared and their silencing potential was assessed. All three constructs were able to trigger gene silencing. The silencing based on hairpin RNA was the most efficient. Two different constructs for preparation of indicator lines were created and the feasibility of preparing such lines was evaluated. The gene for RDR6 was successfully isolated.