Abstract

RNA interference (RNAi) is a process mediated by small RNAs (sRNA), which is significantly involved in the regulation of gene expression in plants. Diverse RNAi pathways can be divided into two basic mechanisms, which are post-transcriptional and transcriptional gene silencing (PTGS and TGS). Production of sRNAs is dependent on the presence of a double-stranded RNA molecule (dsRNA), which is cleaved by one of DCL proteins to produce sRNAs usually of 21-24 nt in length. One strand of the sRNA is subsequently loaded onto AGO protein. During PTGS, the AGO-sRNA complex interacts with the target RNA based on its sequence complementarity to the sRNA and cleaves it or blocks its translation. In the case of TGS, AGO interacts with plant-specific RNA Pol V and its transcripts, which are again complementary to the sRNA. This interaction allows assembling of a protein complex facilitating DNA and histone methylation inhibiting RNA Pol II transcription.

There are numerous ways the dsRNA can arise. A significant part of dsRNA cell production is dependent on synthesising the complementary strand of the dsRNA by RDR6 (RNA-dependent RNA polymerase 6). RDR6 is also involved in the process of the secondary sRNA formation. The significance of RDR6 during PTGS was examined using a *GFP* reporter gene either during spontaneous silencing or during silencing induced by three different ways of the dsRNA production (inverted repeat, antisense RNA and aberrant unpolyadenylated RNA). The protein level of RDR6 was increased or decreased compared to the wild type. The work was also focused on studying the dynamics of PTGS, whose course has been compared with the process of TGS induced by an inverted repeat from a promoter sequence driving *GFP* expression.

As the plant model for this work, tobacco cell line BY-2 was used because of its high homogeneity and rapid division rate, which allows for an easy analysis of a high number of lines using macroscopic clusters of cells (so-called *calli*) and also for analysis at the single cell level using cell suspension cultures.