

Abstract

RNA interference (RNAi) is one of the key mechanisms that are involved in many biological processes such as control of plant gene expression, influence on chromatin arrangement or providing protection against invasive DNA or RNA transposons, viruses and transgenes. In plants, RNAi is triggered by double stranded RNA (dsRNA) that is cleaved by DICER LIKE (DCL) proteins to small RNAs (sRNAs). The size of these sRNAs is in range of 21 - 24 nucleotides (nt). Small RNA acts in the place of origin and they are also a mobile signal which in plants can move to a short distance through plasmodesmata and to a long distance through phloem. sRNA and Argonaute (AGO) protein form RNA-induced silencing complex (RISC). Together, they recognize the target RNA molecule and contribute to an efficient RNAi phase which may be exhibited by gene silencing at posttranscriptional level (PTGS) or transcriptional level (TGS).

The purpose of this study was to compare the effects of silencing constructs, which in a controlled way differently trigger RNAi directed against the expression of the *GFP* reporter gene in the model organism *Arabidopsis thaliana*. Silencing constructs were placed under an inducible promoter activated by the presence of 17- β -estradiol (XVE system). They differed in the way of the dsRNA formation and in the level of silencing (PTGS or TGS). These were: *GFP* in antisense orientation (AS), *GFP* without terminator (BT), *GFP* as inverted repeat (IR) and an inverted repeat against the 35S promoter (35SIR) under which the reporter gene was placed.

During this study, four constructs carrying a reporter gene together with one of the silencing constructs were created. These constructs were transformed into plants of *A. thaliana*. Experiments made on these plants showed a high frequency of spontaneous silencing independently of the carried construct variant, which may be due to an unexpected spontaneous activation of XVE system. Clones generated from the same plant of origin exhibited mostly uniform reaction, but different clones showed a high degree of variability within the same variant. The observed differences between the variants, especially in the localization and the spreading of silencing, will be the subject of further research.

Keywords: RNA interference (RNAi), PTGS (posttranscriptional gene silencing), TGS (transcriptional gene silencing), RNA-dependent DNA methylation (RdDM), *Arabidopsis thaliana*, GFP, controlled gene silencing, XVE system