Efficient use of artificial micro-RNA to downregulate the expression of genes at the post-transcriptional level in *Arabidopsis thaliana*

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Genet. Mol. Res. 15 (2): gmr.15027439
Received August 13, 2015
Accepted December 7, 2015
Published April 7, 2016
DOI http://dx.doi.org/10.4238/gmr.15027439

ABSTRACT. Micro-RNAs are cellular components regulating gene expression at the post-transcription level. In the present study, artificial micro-RNAs were used to decrease the transcript level of two genes, *AtExpA8* (encoding an expansin) and *AHL25* (encoding an AT-hook motif nuclear localized protein) in *Arabidopsis thaliana*. The backbone of the *Arabidopsis* endogenous *MIR319a* micro-RNA was used in a site-directed mutagenesis approach for the generation of artificial micro-RNAs targeting two genes. The recombinant cassettes were expressed under the control of the CaMV 35S promoter in individual *A. thaliana* plants. Transgenic lines of the third generation were tested by isolating total RNA and by subsequent cDNA synthesis using oligo-dT18 primers and mRNAs as templates. The

expression of the two target genes was checked through quantitative real-time polymerase chain reaction to confirm reduced transcript levels for *AtExpA8* and *AHL25*. Downregulation of *AtExpA8* resulted in the formation of short hypocotyls compared with those of the wild-type control in response to low pH and high salt concentration. This technology could be used to prevent the expression of exogenous and invading genes posing a threat to the normal cellular physiology of the host plant.

Key words: Artificial micro-RNA; *Arabidopsis thaliana*; qRT-PCR; AtExpA8; AHL25

