

Reactivation of *VaSTS1* expression in transgenic *Arabidopsis thaliana* plants by retransformation with *2b* from *Cucumber mosaic virus*, isolate NK

Nitiagovsky N.N.^{1,2*}, Tyunin A.P.¹, Kiselev K.V.¹

¹ *Laboratory of Biotechnology, Federal Scientific Center of East Asia Terrestrial Biodiversity, FEB RAS, Vladivostok, Russia*

² *Department of Biochemistry and Biotechnology, Far Eastern Federal University, Vladivostok, Russia*

* e-mail: niknit1996@gmail.com

Since transgene silencing in genetically transformed plants is a serious limitation for a wide application of genetic engineering techniques, studying mechanisms ensuring stability of transgene expression is vital. Molecular mechanism underlying transgene silencing is RNA interference (RNAi). RNAi protects plant cells from the expression of viral and foreign DNA. Small interfering RNAs (siRNAs) are key components in RNAi. The siRNA-protein complexes inhibit transgene expression at the post-transcriptional and transcriptional levels by degrading target mRNA transcripts and establishing DNA methylation within transgene nucleotide sequences, respectively. Multiple investigations concerning viral suppressors of gene silencing revealed that *2b* protein from *Cucumovirus* (CMV) effectively represses assembly and targeting of the RNA-induced silencing complex. Current study presents unique data on using the *2b* gene from CMV-NK isolate for transgene silencing reduction in *A. thaliana* plants earlier transformed with *VaSTS1*. In our study, two *VaSTS1*-transgenic lines were retransformed with *2b* and derived plants were analyzed. Our data demonstrated that *A. thaliana* plants with decreased expression of *VaSTS1* transgene increased transgene expression in up to 3.0-fold upon retransformation with *2b* from CMV NK. Interestingly, the more pronounced effect of *2b* retransformation regarding increase in transgene expression was shown for *nptII* used as selective marker for transformants selection upon transformation with *VaSTS1* of *A. thaliana* plants. The *nptII* gene expression increased in more than 10.0-fold in lines retransformed with *2b* being compared to initial plants transformed with *VaSTS1*. Moreover, the decrease in the level of *VaSTS1* expression in transgenic *A. thaliana* plants was associated with enhancement of the cytosine DNA methylation level within *VaSTS1* sequence. The mentioned fact implies that *VaSTS1* expression was repressed at transcriptional level and our data demonstrates that *2b* from CMV NK can reactivate a silenced transgene.