IMPROVE SMALL RNA-MEDIATED GENE SILENCING IN SOYBEAN BY USING *GMFAD3* AS A TEST MODEL

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ABSTRACT

The primary goal of this work is to improve RNAi technology as a tool to analyze gene function and manipulate commercial traits in soybean. We have developed two RNAi approaches towards the silencing of soybean genes: one involved the creation of silenced lines that result from hpRNA-producing transgene, and the second emphasized on the use of an atasiRNA expression cassette. In the first approach, all three family members of *GmFAD3* were successfully silenced and the silencing phenotype was stably inherited. Silencing levels of *FAD3A, FAD3B* and *FAD3C* correlate to degrees of sequence homology between the inverted repeats (IR) of hpRNA and *GmFAD3* transcripts in the RNAi lines. siRNAs generated from the 318-bp IR were characterized and associated with the inferred cleavage sites on target transcripts. Small RNAs corresponding to the loop portion of the hairpin transcript were detected, implicating possible transitive self-silencing of the hairpin transgene. In contrast, much less RNAs were found outside of the target region, suggesting that transitivity along endogenous transcripts is prohibited by some inherent protective feature. Strikingly, transgenes in two of the three RNAi lines were heavily methylated, leading to a dramatic reduction of hpRNA-derived siRNAs. Small RNAs encoding part of the transgene promoter as well as the *bar* gene coding sequences were also detected by deep sequencing, but whether they induced the methylation of transgenes still need further exploration. In the second approach, we developed two *Arabidopsis TAS1a*-based atasiRNA constructs targeting the *GmFAD3* gene family using online siRNA design tool OligoWalk. However,

computational predicted siRNAs does not represent their *in vivo* efficacy. Further investigation is needed to detrmine whether siRNA candidates could conduct efficient silencing of target genes in plants. Furthermore, to simplify the deployment of atasiRNA platform and investigate the utility of miR390 and *TAS3* as a gene silencing tool in soybean, spacial and temporal analysis of miR390 was performed. Our results implicated that miR390 is consistently expressed in all sampled tissues with the highest abundance in flowers and early stage of pod development, which makes miR390 a good candidate to trigger the formation of atasiRNA in soybean.