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Title: Developing RNAi-mediated resistance against whitefly (*Bemisia tabaci*) infestation in tobacco and cotton

Keywords: *Bemisia tabaci*, RNAi, miRNA, ATP synthase, Begomovirus, *N. tabacum*, *G. hirsutum*

ABSTRACT

Whiteflies (*Bemisia tabaci*) are hemipterous insects of the family *Aleyrodidae*. These insects are capable of feeding on over 600 plant species, thereby damaging the crops by phloem feeding, honey dew excretion and transmission of more then 100 species of plant viruses. RNA interference (RNAi) has been proven as important approach for regulation of gene expression. This could be accomplished by the use of dsRNA, siRNA or miRNA. Insect gene function studies have shown that gene silencing can dramatically affect insect growth and development. In the present study, *G. hirsutum* miRNAs showing potential of targeting Expressed Sequence Tags (ESTs) of *B. tabaci*, based on sequence complimentarity and binding enthalpy of the duplex (miRNA-target mRNA), were *in silico* identified. Based on identity with model insect *Drosophila melanogaster*, conserved domains were Blast searched in the ESTs of *Bemisia tabaci*. A total of 55 *G. hirsutum* miRNAs were seen targeting protein coding ESTs of *B. tabaci*. Out of all the miRNAs ghr-miR166b was choosen owing to its capability of exclusively targeting ATP synthase of *B. tabaci*. The role of ghr-miR166b was validated by cloning of precursor sequence of ghr-miR166b (ghr-MIR166b) into binary vector pBI121, following its stable expression in model plant tobacco (*Nicotiana tabacum* cv. Xanthi) as well as *G. hirsutum*. The presence of transgene in the regenerated shoots of *N. tabacum* and *G. hirsutum* were detected via PCR-based amplification of the selectable marker gene *npt*II. A total of 5 *N. tabacum* and 7 *G. hirsutum* transformed (T_0) lines overexpressing ghr-MIR166b were obtained following molecular analysis. Southern hybridization demonstrated integration of one and two copies of transgene in *N. tabacum* lines while *G. hirsutum* cv. HS6 lines showed a one, two and three copies of the transgene. The transgenic *N. tabacum* cv. Xanthi lines exhibited 3 to 8 fold enhanced expression of ghr-MIR166b as compared to non-transformed plants, however transformed *G. hirsutum* (T_0) lines showed 2.0 to 17-fold enhancement. The presence of the ghr-miR166b was confirmed in the sRNAs isolated from the transformed plants via Northern blot analysis. In biological

assay it was demonstrated that whitefly population was reduced upto 80% and 91%, after feeding on the leaves of transgenic *N. tabacum* and *G. hirsutun* lines (NT-5 and HS6-miR166-30, respectively) exhibiting the highest level of ghr-miR166b, compared to non-transformed tobacco and cotton leaves. Further, the whole plant assay revealed maximum of 70% and minimum of 30% decline in *B. tabaci* population on *N. tabacum* transgenic (T_1) line NT-5 and line NT-1, respectively. Similarly, transgenic *G. hirsutum* line HS6-miR166-30 revealed a maximum of 78% whitefly mortality, while HS6-miR166-11 showed 23% mortality. Moreover non-transformed *N. tabacum* and *G. hirsutum plants* exhibited an increase in whitefly population. Our results revealed the potency of ghr-miR166b against whitefly population and indicates towards its use as a biopesticide for controlling the population of whitefly and the spread of whitefly-transmitted viruses.