A New Strategy of Insect Pest Control: Down-regulating Cotton Bollworm Gene Expression by Engineering Plant Double Stranded RNA

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Cotton bollworm (*Helicoverpa armigera*) is an important agricultural pest that causes severe yield loss to crops, particularly to cotton. Transgenic *Bt* crops have been successful in protecting plants, however, *Bt* proteins are toxic to all lepidopteran insects but have little effects to sucking pests, such as aphids. Furthermore, the continuous use of *Bt* crops increases insect resistance. These call for new transgenic approaches of pest control. The bollworms have adapted to cotton plants, despite that most cotton cultivars accumulate a high level of gossypol and related sesquiterpene phytoalexins. In insects, cytochrome P450 monooxygenases are commonly involved in xenobiotic metabolism. We are interested in gossypol biosynthesis and its regulation. Recently, we isolated a gossypol-inducible P450 monooxygenase gene, *CYP6AE14*, from *H. armigera*. This gene is highly expressed in midgut. When gossypol was supplemented in diet, larval growth was correlated with *CYP6AE14* expression level. In order to impair the bollworm tolerance to gossypol, we tried to knockdown *CYP6AE14* expression by RNA interference (RNAi). We generated transgenic *Arabidopsis*, tobacco, and cotton plants that expressed double stranded RNA of *CYP6AE14* (ds*CYP6AE14*). Several lines produced the effective form of dsRNA. When the bollworm larvae were fed with the transgenic *Arabidopsis* or tobacco leaves, small RNAs of *CYP6AE14* could be detected from the midgut after two days, and the *CYP6AE14* transcript abundance was then decreased. The larvae fed on transgenic *Arabidopsis* plants showed a slightly retarded growth in comparison with the control, and the inhibition became much more dramatic when gossypol was administrated. The transgenic ds*CYP6AE14* cotton plants also showed a strong inhibitive effect on larval growth. To examine if other genes of the bollworm were suppressible by plant-mediated insect RNAi, we tested *H. armigera GST1* gene (EF033109), coding for glutathione-S-transferase. We found that when cotton bollworm larvae were transferred to leaves of *Atds GST1* plants, *GST1* transcript level in midgut reduced. Our data demonstrate a novel phenomenon of plant-mediated insect RNAi, which has potential applications in entomological research and in field control of herbivorous insect pests.