Development of Genetically Engineered Banker Plants for Biological Control of Whiteflies in Greenhouses

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ABSTRACT

Greenhouse and nursery production is becoming a stronger segment of U.S. agricultural markets. However, controlling whitefly remains a great challenge for greenhouse and nursery production. Whitefly (*Bemisia tabaci*) can infest more than 600 plant species by sucking phloem-sap and spread over 300 plant pathogenic viruses, which causes substantial plant losses and poses a serious threat to greenhouse and nursery production. Whiteflies are notorious for their chemical control due to their high fecundity, broad host range, and quick ability to develop pesticide resistance. Natural enemies of *B.tabaci* have been employed as a means of biological control, however this approach is expensive and reliable natural enemies are not commercially available. Moreover, the presence of predators of whiteflies in plant products will affect consumer-shopping preferences. Genetically engineered plants expressing δ-endotoxin(s) from *Bacillus thuringiensis* (Bt) have been demonstrated to have high tolerance to chewing insects, however this toxic Bt protein was much less effective on controlling sap-sucking insects including whiteflies, aphids and thrips. In this study, we have expressed an insecticidal protein, *Tma12*, from an edible fern in both lettuce and tobacco, which are the two most favored plant species by whiteflies. We have generated 11 and 13 independent transgenic lettuce and tobacco lines. Segregated lettuce seedlings contain T-DNA of *Tma12* and a control lettuce line have been used for examining the effect on controlling whitefly development. Our results showed that *Tma12*-expressing lettuce caused significant developmental defects of whiteflies with much less numbers of eggs and nymph developments. Further investigation will be performed to examine whether these plants have negative effects on predators of whitefly. The results here will provide fundamental evidences for developing an integrated pest management practice using a trapper plants expressing insecticidal protein in combination with other banker plants.

OBJECTIVES

The goal of this project is to develop genetically engineered plants with expressing insecticidal protein for biological control of whiteflies for greenhouse and nursery production.

METHODS

Generation of Tma12-expression construct

A 651bp cDNA sequence (accession: JQ438776.1) encoding a 216-aa chitin binding protein (*Tma12*) in *Tectaria macrodonta* was synthesized by Gene-Universal Company (Newwark, DE, USA). The synthesized DNA was tagged by BP sequences for Gateway cloning. The *Tma12* was subsequently cloned into a binary expression vector, pGWB402Ω through LR reaction to create a *Tma12*-expressing construct. The *Tma12* gene is driven by a 2 x CaMV35s promoter. The plasmid DNA was transformed into the agrobacterium strain EHA105. Colonial PCR was applied to confirm the success of plasmid DNA transformation into EHA105.

Plant Transformation

Seeds of lettuce and tobacco were surfaced sterilized in 70% alcohol for 2mins, followed by a 12-min sterilization in a solution of 20% of Clorox® bleach. The seeds were then washed for 5 times with sterile water before being transferred to MS medium for germination. Cotyledons from 10-d old germinated seeds were cut off for agrobacterium infection. Agrobacterium EHA105 containing *Tma12* plasmid DNA were cultured overnight and centrifuged for harvesting pellets. The EHA105 pellet was diluted with liquid MS medium to an OD600 of 0.8, which was used for cotyledon incubation for 20 mins. The excessive agrobacterium was removed with blotting in sterile filter papers before the infected cotyledons were transferred callus and shoot induction medium (MS+1mg/L NAA and 0.1mg/L BAP+50μg/L Kanamycin+30g sucrose/L+8g Agar/L). The regenerated shoots were transferred to root induction medium (MS+50μg/L Kanamycin+30g sucrose/L+8g Agar/L).

In-planta bioassay of transgenic plants

The F2 transgenic lettuce were genotyped with NPT II primers and used for examining their effects on controlling whitefly. F2 seeds of transgenic lettuces were germinated and transplanted to 1-Gallon pots. Seedlings were grown under 74°F, 60% relative humidity and a 16 h light/8 h dark photoperiod. The 4-week old seedlings were placed into net chambers (Fig. 1), and 30 whiteflies per plant were released into each chamber. The number of live adult, eggs, and instar nymph (2nd to 4th) were investigated after 4 weeks.

Fig. 1. A representative picture showing in planta bioassay using a net chamber.

RESULTS

Using a set of primers binding NPT II that confers kanamycin resistance, we have confirmed that there are T-DNA insertion in multiple transgenic lettuce and tobacco plants (Fig. 2).

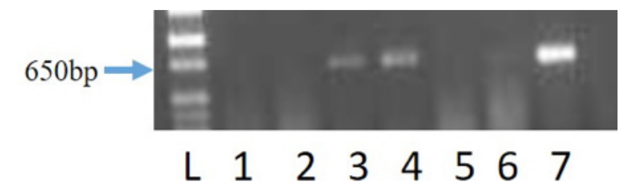

Fig. 2. Genotyping of regenerated lettuces with a NPTII primer set. L, a DNA ladder showing a fragment of 650bp(arrow). 1-7, independently regenerated lettuce lines. A fragment of 651bp indicates that one or more *Tam12* T-DNA inserts exist in the tested plant genome.

We have closely tracked the adults and found that live adult (Fig. 3 A) can possibly be killed by toxin of transgenic lettuce (Fig. 3B). We also check whether there is any effect on nymphal development. Development of some eggs was greatly arrested although some of them can reach advanced nymphal stages (Fig. 3 C and D). We tested 11 different *Tma12*-expressing lettuce on their effect of laying egg, nymphal development and adult lethality.

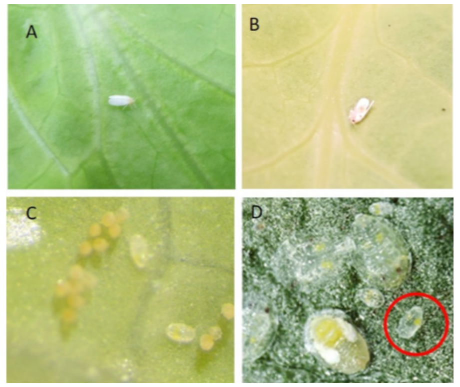

Fig. 3. Effect of *Tma12*-expressing lettuce on whiteflies. A) live adult whitefly, B) Dead adult whitefly, C) eggs and hatching eggs, D) advanced nymphal stages. The circles indicated poor adult emergence.

Results from Figure 4 showed that higher number of advanced nymphs were observed in the control lettuce and two *Tma12*-expressing lettuce lines (*Tma12-9* and *Tma12-5*) (Fig. 4). However, the inhibitory effect on laying eggs, nyphmal development and adult whitefly were significant in rest of tested plants. The lowest number of eggs were observed in *Tma12-3, Tma12-10, Tma12-11 and Tma12-8* (1 egg each); by contrast, control and *Tma12-9* have 4 and 6 eggs, respectively (Fig. 4). Regarding of live adults, the least number of live adults (1 adult) were also observed in *Tma12-8* and *Tma12-7* (Fig. 4), and seven, six and four live adults were observed in *Tma12-5, Tma12-9* and the control line, respectively (Fig. 4). Significant variations were observed for nymphs with advanced stages among tested lettuce lines. The higher numbers of nymphs were found in *Tma12-9* (26 nymphs), *Tma12-5* (20 nymphs) *Tma12-11* (15 nymphs), *Tma12-4* (13 nymphs) and the control lettuce line (23 nymphs). By contrast, nymphal development were substantially arrested in *Tma12-8* (3 nymphs), *Tma12-10* (3 nymphs), *Tma12-3* (4 nymphs) and *Tma12-6* (4 nymphs). Collectively, the best *Tma12*-expressing lettuce lines are *Tma12-8, Tma12-10* and *Tma12-3.*

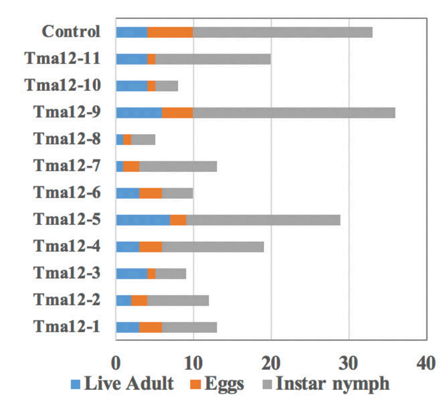

Fig. 4. Effect of *Tma12*-expressing lettuce on numbers of adult, eggs and instar nymphs of whitefly. Eleven different *Tma12*-expressing lettuce and one control lettuce line were tested for their effect on laying egg, nymph development and adult lethality.

CONCLUSIONS

The number of live adult, eggs and nymphs in control lettuce line is 6 times higher than the most effective transgenic lettuce line *Tma12-3*, suggesting that expression of *Tma12* in lettuce could effectively inhibit whitefly fecundity. In this study, we have used a 2 x CaMV35s promoter for expressing *Tma12*. The phloem-specific promoter has been reported to be more effective in controlling phloem-sap sucking insects. Transgenic lines of *Tma12* under a phloem-specific promoter like *Sus2* will be developed to compare their efficacy with current *Tma12-3* transgenic lines. Tetraploid transgenic tobacco lines for expressing *Tma12* have been developed in this study, Comparison between the efficacy of transgenic tobacco and lettuce in controlling whitefly fecundity will be performed in the future. Development of these pest-resistant trapper plants will not effective if an integrated biological control approach is not be considered. Therefore, whether *Tma12* negatively affect predators of whitefly that are used for biological control should be addressed before we put these genetically engineered plants in practical uses.