RNA interference (RNAi) control of whitefly, Bemisia tabaci MEAM1

Cecilia Garcia1, Navneet Kaur2, William M. Wintermantel2
1. California State University, Monterey Bay, Seaside, CA
2. United States Department of Agriculture-Agriculture Research Service, Crop Improvement and Protection Research, Salinas, CA

Abstract
Whiteflies have been recognized as important crop pests for more than 120 years. Effects on diverse crops throughout the world result from damage caused by feeding on plants and transmigration of more than 200 plant viruses. These viruses impact agriculture by reducing crop yield and quality, in some cases destroying primary food sources in developing countries. Many whitefly populations are now developing resistance to pesticides making them more difficult to control. This study focuses on the development of a new treatment for management of whitefly through a sustainable method known as RNA interference (RNAi). RNAi selectively eliminates RNA coding for proteins that are important for whitefly survival. Six unique RNAi constructs were designed to target sequences of different genes or sets of genes in the whitefly, Bemisia tabaci MEAM1. These genes were delivered to plants by two methods: artificial feeding and spray application. Experimental results were evaluated to determine whether our constructs increased whitefly mortality, and if they produced higher mortality rates than negative controls. The combination construct “3-9” was the most effective with a mortality rate of 92.73%. Evaluation from our spray application test indicated, 4 out of 5 constructs had significantly higher mortality rates than controls, with construct “P” having the highest mortality rate. Results from both experiments suggest that not only is RNAi an effective strategy, but could also be an ecologically responsible alternative to pesticides, reducing both negative consequences on human health and further development of pesticide resistance.

Methods

RNA Interference (RNAi)

- A double stranded RNA(dsRNA) is injected into cell
- dsRNA is cleaved by an enzyme called dicer to form small RNAs (siRNA)
- siRNA is attached to RNA Inducing Silencing Complex(RISC) which degrades sense strand and uses the anti-sense as a template to match with mRNA within the cell
- Once RISC finds a match the mRNA is degraded preventing from translating into protein

I. Artificial Feeding

- 5 dsRNA constructs were tested combining 2 constructs for a total of 10 different treatments with 1 treatment consisting of all dsRNA constructs.
- Construct or combinations contained 40 ng/ul in 100ul of 20% sucrose solution.
- Controls were 1) 0.05%TNT2 solution itself, 2) watermelon gene target, 3) Negative controls were 1) 20% sucrose solution, 2) dsRNA construct

II. Spray Method

- 5 spray formulations were tested individually.
- Concentration of 500ng of construct/2.5ml of 0.05%TNT2 spray solution per leaf was used.
- Controls were 1) 0.05%TNT2 solution itself, 2) watermelon gene target.
- Constructs were sprayed onto tomato leaves and allowed to dry for 24 hours.
- Leaves were subsequently placed in vials with water and 20 WF each.
- Spray protocol conditions were same as artificial feeding method.
- WF mortality was observed every 24th for 11 days.
- Constructs and controls were replicated 3 times.

Results

I. Artificial Feeding

- All constructs showed significantly higher mortality in treated whiteflies compared to negative controls in artificial feed method (Table 1 & Fig 6).
- Combination constructs 3-9 proved to be the most effective with a mortality rate of 92.73% after the 5th day (Table 1 & Fig 6 blue line)

II. Spray Method

- 4/5 constructs showed significantly higher mortality in whitefly compared to sucrose and watermelon control using spray method (Fig 7).
- Construct “P” showed higher mortality (20%) compared to negative controls: sucrose (6.64%) and watermelon (4.98%) with P <0.05.

Conclusion

RNAi proved to be an effective strategy to manage whiteflies using 5 unique dsRNA constructs, with combination construct “3-9” & construct “P” showed the highest mortality compared to others using artificial feeding and spray method, respectively.

RNA interference showed potential as an alternative to pesticides. This new method can prevent whiteflies from becoming more resistant as well as decrease the negative effects pesticides have on the environment and humans.

References


Acknowledgements

Thanks to Western Human, USDA-ARS, Dr. Peter DeFalco for suggestions on gene targets for RNAi constructs and advice on using an array. Also, appreciation to Drs. Toneva and Zikic (USDA-ARS, Salinas, California for assistance with scholarly work, and USDA-ARS Salinas for giving me the opportunity to publish my experience on RNAi.

Funding from USDA-ARS for providing the opportunity to take part in summer research. Also grateful to all UROC staff for providing support and guidance during this project.

Funding for this research provided by USDA-ARS & USAID.