Nature Reviews Genetics | AOP, published online 10 March 2015; doi:10.1038/nrg3926

BIOTECHNOLOGY

Making a meal of RNA

Insect pathogens are a major burden on global agriculture, and some, such as the Colorado potato beetle (CPB), have developed resistance to all the major insecticide classes. A new study shows how engineering plant chloroplasts to express insect-targeted double-stranded RNAs (dsRNAs) can effectively control CPB pests.

When insects ingest dsRNAs, they can be taken up by midgut cells and enter the insect's endogenous RNA interference (RNAi) pathway to mediate gene repression. This has motivated attempts to engineer plant-expressed dsRNAs that target essential insect genes as a strategy to defend against insect pathogens. However, such approaches have so far provided limited pest protection, partly because the plant's own RNAi machinery processes long dsRNAs into small interfering RNAs (siRNAs), which curtails uptake and repressive activity in insects.

To address this limitation, Zhang *et al.* engineered constructs expressing long dsRNAs into the chloroplast genome, reasoning that the origination of this organelle from cyanobacteria and lack of an endogenous RNAi pathway could protect the dsRNAs from nucleolytic processing in the plant. They generated a variety of linear and hairpin-based expression constructs for producing long dsRNAs that either singly target or co-target the essential CPB genes beta-actin (*ACT*) or Shrub (*SHR*).

Initial *in vivo* testing of the engineered chloroplasts in tobacco plants revealed that a construct in which linear sense and antisense RNA strands are transcribed separately from convergent promoters was the best type for maximal expression of full-length dsRNAs. Encouragingly, no adverse plant phenotypes were observed from dsRNA expression.

The investigators then focused on this particular construct type and progressed to test engineered chloroplasts *in vivo* in potato plants, which are the main hosts for CPB. Constructs targeting *ACT*, *SHR* and *ACT* + *SHR* all resulted in the accumulation of long dsRNAs in leaves and inhibited the growth of CPB larvae on the plants. Particularly striking was the construct that singly targets *ACT*: in these transgenic plants, a CPB mortality rate of 100% was achieved within 5 days, while minimal biomass of leaves (the food source for CPB) was consumed by the insects. Consistent with an on-target effect, observations in the insect gut prior to death included an accumulation of *ACT*-targeted siRNAs, *ACT* downregulation and disruption of actin filaments. Justifying the chloroplast strategy, equivalent constructs expressed from the plant nuclear genome resulted in extensive processing of long dsRNAs into siRNAs in the leaves and considerably less control of CPB infestations.

Crucially, despite the high accumulation of chloroplast-expressed dsRNAs in leaves, the dsRNAs were barely detectable in the potato tubers; hence, concerns about human consumption might be limited.

It will be important to further test this chloroplast expression approach for efficacy in additional plant–pest contexts, to thoroughly verify food safety and to monitor whether insects can also develop resistance to this new method of combating them.

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ORIGINAL RESEARCH PAPER Zhang, J. *et al.* Full crop protection from an insect pest by expression of long double-stranded RNAs in plastids. *Science* **347**, 991–994 (2015)