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Introduction

In 1994 the Flavr Savr tomato was the first transgenic plant to be grown commercially in the United States. These tomatoes were developed by Calgene to have improved taste and longer shelf life compared to the conventional tomatoes available in the supermarket at that time. The ripening process was altered by silencing a gene using "antisense RNA". The scientists who developed the Flavr Savr tomato, and many other biotechnologists who were attempting to modify plants at that time, did not understand the details of how antisense RNA functioned. It would take a few more years before the mechanism was elucidated and for this Andrew Fire and Craig Mello were awarded the Nobel Prize in 2006. The antisense RNA produced in the Flavr Savr tomato inadvertently tapped into a mechanism called RNA interference that is used by plants and animals to control the expression of genes.

The trigger for RNA interference is double stranded RNA (dsRNA). When dsRNA is detected inside a cell it is cut into short pieces that are incorporated into a protein complex. This RNA-protein complex functions like a molecular guided missile. The short RNA provides the guidance mechanism by identifying any RNA molecules that contain a complementary sequence of bases. The base-pairing rules for DNA (A pairs with T, C pairs with G) operate in a similar way for RNA except that A pairs with U. When the RNA-protein complex finds a complementary RNA, the RNA is destroyed by the protein complex. If the target RNA is a messenger RNA encoding a protein, the RNA interference mechanism prevents that protein from being produced and the gene is effectively silenced. This URL takes you to a video from the PBS program Nova that provides a straightforward explanation of how RNA interference works:
http://www.pbs.org/wgbh/nova/body/rnai.html

RNA interference provides a relatively straightforward method to silence specific target genes in transgenic plants. Here is an outline of the steps required to silence a gene:

1. Determine the DNA sequence of the target gene you want to silence. This information is used to design a transgene that will produce dsRNA containing this sequence.
2. Develop a transgenic plant that produces dsRNA with the same sequence as the target gene. This initiates the RNA interference mechanism.
3. The mRNA produced by the target gene is destroyed by the RNA interference mechanism. The protein encoded by that gene is not produced.
4. The target gene is silenced.

RNA interference to modify plant traits

Several transgenic crop varieties that use RNA interference to modify specific plant traits have been developed and approved for commercial production. Examples of these include:

- Papaya with resistance to papaya ringspot virus (Cornell, University of Hawaii and USDA)
- Yellow squash with resistance to several viruses (Monsanto)
- Alfalfa with reduced lignin (Monsanto)
- Soybeans with modified oil profile (DuPont Pioneer and Monsanto)
- Apples with reduced browning (Okanagan Specialty Fruits)
- Potatoes with reduced bruising and lower acrylamide potential (JR Simplot)
- Tobacco with reduced nicotine content (Vector Tobacco)

RNA interference to control pests

All of these transgenic plants were designed to alter a specific property or characteristic of the plant itself. Research on the mechanism of RNA interference demonstrated that some organisms are able to take up dsRNA from the environment (in their food, for example). The ingested dsRNA is, somewhat surprisingly, able to silence genes in the organism that consumed the RNA. This led to the development of transgenic host plants producing dsRNA that silenced genes in pests such as insects and nematodes that feed on those plants. This is commonly referred to as host plant-induced gene silencing (HIGS).

The steps required to produce a transgenic plant that makes dsRNA for HIGS are the same as those outlined above except that in the first step the target gene must be identified in the genome of the pest rather than the host plant. Expression of dsRNA in the transgenic plant can be targeted to a specific organ or tissue by using gene promoters that are active only in those parts of the plant. For example, dsRNA targeting corn rootworm could be expressed only in roots using an appropriate root-specific promoter.

This approach has been used to develop transgenic plants that are less susceptible to damage caused by insects, nematodes, fungi and parasitic plants. Research on this topic is summarized in a review by Koch and Kogel (2014). As of late 2016, USDA has approved only one transgenic event that uses dsRNA to control a pest. MON87411 developed by Monsanto expresses both a Bt toxin (Cry3Bb1) and dsRNA targeting an essential gene (Snf7) in the Western corn rootworm (WCR) to reduce injury caused by WCR. Stacking multiple transgenes is highly recommended as a strategy to delay the evolution of insects with resistance to Bt toxins. This gene stack of a Bt toxin combined with a dsRNA has the advantage that it uses two different modes of action that are unlikely to be overcome by a single mutation in WCR.

Recent examples of the successful application of this dsRNA HIGS approach include potato plants with resistance to the Colorado potato beetle (Zhang et al., 2015), fruits and vegetables with resistance to Botrytis (Wang et al. 2016), and additional target genes in WCR that have been used in transgenic maize (Hu et al., 2016). It is likely that HIGS will be a very active area for research and development in crop improvement in the future.

dsRNA as a pest control spray

dsRNA can silence genes in insects and nematodes when ingested by these organisms. Can dsRNA also provide protection if it is sprayed onto plants in the same way as many other pesticides? This would seem unlikely to succeed because RNA is generally thought of as a very unstable molecule that is rapidly degraded in the environment. However, research results from both universities and companies have demonstrated some success with this approach.

Similar to the transgenic HIGS dsRNA strategy, the first step is to identify a specific gene in the target organism that, when silenced by RNA interference, will impair growth of that organism. dsRNA is produced that contains the same sequence as the target gene and applied to the plant. When the dsRNA is taken up by the insect or pathogen it silences the target gene and compromises growth of the pest. Recently published research has shown that this approach provides protection against the Colorado potato beetle (San Miguel and Scott, 2015) and the fungal pathogens Fusarium (Koch et al., 2016) and Botrytis (Wang et al., 2016).

Although there are few peer reviewed research papers on this topic from the private sector, it is evident that several companies are active in this area. In 2015 Monsanto was awarded a patent on the use of RNA interference to help manage herbicide resistant weeds (Sammons et al., 2015). The concept is to use dsRNA to silence the gene that encodes the protein site of action of a herbicide. For example, glyphosate-resistant weeds are sprayed with dsRNA to silence the gene encoding EPSP synthase, the enzyme that is inhibited by glyphosate. Reducing the expression of EPSP synthase makes these weeds amenable to control with glyphosate once again. Monsanto has a number of R&D collaborations with other companies working on biomedical applications of RNA interference and is developing a product platform called BioDirect that is based on RNA interference (http://www.monsanto.com/products/pages/biodirect.aspx). Syngenta is also active in RNA interference R&D and in 2013 acquired Devgen, a biotechnology company developing methods to manage nematodes.

There are significant challenges that must be resolved before dsRNA can be developed into a viable pest control product. Many of these are technical issues similar to what is encountered with other pesticides, including the scale and cost of production for dsRNA, appropriate formulation of dsRNA for efficient uptake, stability of dsRNA in the field, and dosage and efficacy with different target organisms. If these can be addressed, the next hurdle will be regulatory approval. Releasing genetic material into the environment raises several important issues that will have to be addressed by regulatory agencies. Some of the more obvious concerns are: the safety of dsRNA for humans (will human genes be silenced by exposure to dsRNA?); the potential impact of dsRNA on non-target organisms (how can dsRNAs be designed to limit off-target effects?); and the development of resistance to dsRNA technology (there are several paths that could lead to resistance to dsRNA). The regulatory approval process will also influence consumer acceptance of dsRNA technology. While “spray-on dsRNA” does not involve release of transgenic plants into the environment, this does not guarantee willing adoption and ready acceptance of this technology by the public, especially when there are on-going concerns from consumers about how their food is produced.

Much of the interest in RNA interference and dsRNA technology has centered around pest management. However, there are many other areas where dsRNA could be utilized in crop production. For example, at the onset of a prolonged period of dry weather, dsRNA could be applied to regulate the expression of genes that control transpiration in order to conserve limited water. To facilitate the production of hybrid seed, male sterility could be induced by silencing genes required for pollen development. Disease resistance responses could be activated to protect crops from an emerging pathogen threat. It is possible that dsRNA could rival or even displace synthetic pesticides as a critical tool for sustaining crop production in the future.

References

Hu, X., Richtman, N.M., Zhao, J.Z., Duncan, K.E., Niu, X., Procyk, L.A., Oneal, M.A., Kernodle, B.M., Steimel, J.P., Crane, V.C., Sandahl, G., Ritland, J.L., Howard, R.J., Presnail, J.K., Lu, A.L., and Wu, G. 2016. Discovery of midgut genes for the RNA interference control of corn rootworm. Scientific Reports 6: 30542.

Koch, A., Biedenkopf, D., Furch, A., Weber, L., Rossbach, O., Abdellatef, E., Linicus, L., Johansmeier, J., Jelonek, L., Goesmann, A., Cardoza, V., McMillan, J., Mentzel, T., and Kogel, H. 2016. An RNAi-based control of Fusarium graminearum infections through spraying of long dsRNAs involves a plant passage and is controlled by the fungal silencing machinery. PLoS Pathogens 12: e1005901.

Koch, A. and Kogel, K.H. 2014. New wind in the sails: improving the agronomic value of crop plants through RNAi-mediated gene silencing. Plant Biotechnology Journal 12: 821-831.

Sammons, R.D., Ivashutu, S., Liu, H., Wang, D., Feng, P.C.C., Kouranov, A.Y., and Andersen, S.E. 2015. Method for controlling herbicide-resistant plants. U.S. Patent 9,121,022.

San Miguel, K. and Scott, J.G. 2015. The next generation of insecticides: dsRNA is stable as a foliar-applied insecticide. Pest Management Science 72: 801-809.

Wang, M., Weiberg, A., Lin, F.M., Thomma, B.P.H.J., Huang, H.D., and Jin, H. 2016. Bidirectional cross-kingdom RNAi and fungal uptake of external RNAs confer plant protection. Nature Plants 2: 16151.

Zhang, J., Khan, S.A., Hasse, C., Ruf, S., Heckel, D.G., and Bock, R. 2015. Full crop protection from an insect pest by expression of long double-stranded RNAs in plastids. Science 347: 991-994.