MIR162 Maize

Applications of Genetic Engineering and Biotechnology

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Table of Contents

Preface

Genetically modified organisms play an important part in everyday life, however their relevance is not necessarily as widely known as their presence. By taking a closer look at something as ordinary as maize we were interested in understanding the innovative developments to the yield of maize and the value that GMO's add to farming.

The development of our particular GMO is especially interesting in its path to discovery. Syngenta scientist Greg Warren returned from vacation to find spoiled milk, which had spilled in his refrigerator. He subsequently brought samples of this to the lab, ran tests, and found that proteins present on the milk were capable of controlling lepidopteran pests.

Some of the questions we posed were; what is the mode of action for MIR162? Is there any agricultural history leading up to the breakthrough of the product? If MIR162 is effective what would scientists need to research further? How can you prevent insect resistance? Does MIR162 course through the food chain?
Through research and communication with scientists involved with the agriculture industry, we hoped to answer our questions and learn about GMO's.

Introduction

Maize pests have been an incessant dilemma for farmers. The problem with controlling pests is that they attack in different places, at different times, and in different ways.
Companies were looking for a product to control pests that attacked maize. Prior to using Vip proteins the Cry protein from *Bacillus thuringiensis* had been used. The differences between Cry proteins and Vip proteins are the area of expression (Cry: during sporulation stage/ intracellular protein, Vip3: during vegetative stage/ extracellular secreted protein)
MIR162, also know as Agrisure™ Viptera, is used in corn and cotton fields in the following countries (see list)

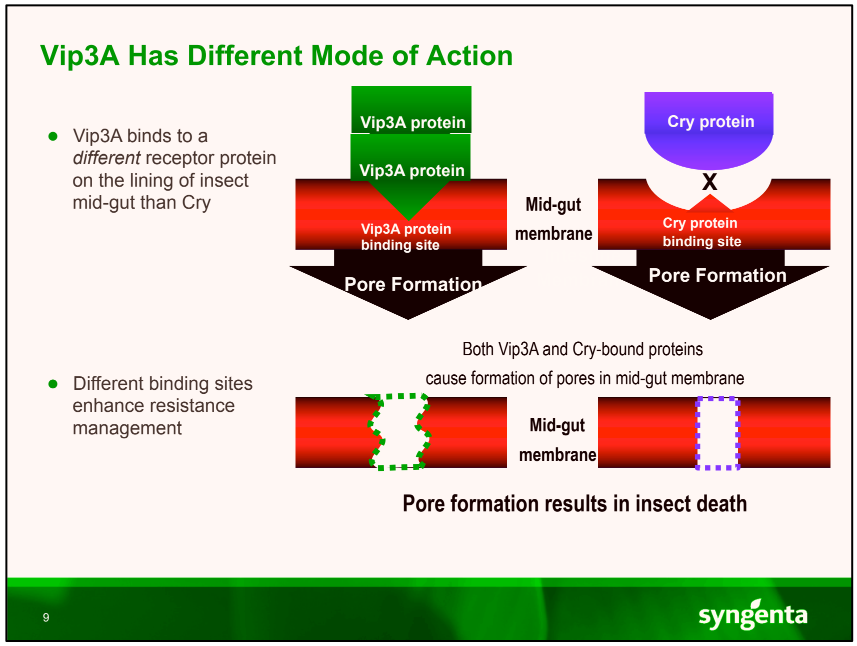
Argentina
United States
Brazil
Canada
Japan

This product (Vip3Aa20 Protein) is used in the Agriculture industry to control lepidopteran pests and a phosphomannose isomerase protein. MIR162 is used for conventional maize as well as for industrial purposes.

Alternative treatments to control pests of the same species have proven to be suitable however, less effective. Still other alternative is to take no action at all which would result in the damage of maize crops.

Description of engineering technique

MIR162 maize is genetically modified maize, which expresses a protein (Vip3Aa20), which in turn controls pests and allows plant cells to use mannose as their primary carbon source. The area of insertion for Viptera contains a gene (*Vip3Aa19*) from *Bacillus thuringiensis.* This gene is under the predomination of a ZmUbiInt promoter and a nopaline synthase (NOS) terminator.
A *pmi* gene encodes the phosphomannose isomerase, which is under the control of a polyubiquitin promoter and NOS terminator. Consequent to the development of the a19 gene the a20 gene was designed and the encoded protein Vip3Aa20 was produced.



The way that Vip3 proteins function as an insecticide is similar to the *B. thuringiensis* proteins. After ingestion, complete Vip3 proteins are processed into active fragments, which bind to the receptors in the stomach of the targeted insects. Subsequent to receptor binding is the formation of damaging pores in the epithelial membranes, which kills the insect.
PMI is used to mark the transformation of selected plant species. These plant cells, which have been transformed with a *manA* gene, are able to survive using mannose as their sole energy source.
In areas where this is the case, nontransformed plant cells aren't active and are overruled by transformed plant tissue.
No changes in reproduction, dissemination, survivability in MIR162 maize were reported when compared to non-GM maize. Analysis did result in the discovery that traits have been stably integrated into the genetically modified maize.

Graph 1:

Vector component	**Description**
ZmUbiInt	Promoter derived from the maize (Zea *mays)* polyubiquitin gene
Pmi	*E. coli pmi* gene encoding the enzyme phosphomannose isomerase (PMI). This gene is also known as *manA.*
NOS	Polyadenylation region from the nopaline synthase gene from *Agrobacterium tumefaciens.*

Documentation

We interviewed Scientist Greg Warren (25/3/14) who discovered and produced the Vip proteins used in MIR162. The following interview is adapted from recording and paraphrased into writing.

I've read all the documents and applications of Viptera, it's quite impressive, one thing that wasn't explicitly documented was the mode and site of action?

So if you look at Vip3, we're positive it works in the insect's gut. It eats it, it swallows it and because of the particular shape of the protein it will only bind to certain receptors, just like a lock and key. What happens is when Vip is activated it opens up a hole in the insect's stomach and the insect dies. Not directly from the protein itself but because of the damage to it's gut lining.

Tell me a bit about the discovery process of Vip3?

When I started this project, Cry (crystal) was the only known protein to work in the growing plant as a pesticide. Now while Cry proteins have a very narrow spectrum, I thought we should look for something broader and possibly insecticidal proteins in the vegetative state. I started collecting samples from everywhere: soil from my garden, dust from my garage…so when I returned home from vacation to find spoiled milk, I thought why not? And it turned out to be exactly what I needed.

How did you determine that MIR162 wasn't toxic to other organisms, the soil or groundwater?

What happens is we test the functionality of the pesticide and look into how many organisms it affects, both pests and beneficial insects. We test for general toxicity first, then transform it into plants and once again the plants are rigorously tested (fish, mammals, insects)

The endangered Karner Butterfly is at potential risk for being affected, have you had any reports of toxicity?

No. We've tested many beneficial butterfly species and Vips don't seem to affect them. The spectrum is still quite narrow and we do monitor those things, we also follow up on any reports that we get.

Lepidopterans could eventually become resistant. How would you go about solving that problem?

They could. Resistance usually occurs because you rely solely on one protein. If that's the case, insects are under heavy selection pressure to become resistant. But what one of the best ways to overcome that is not to use one protein in a plant, but many proteins with different modes of action. That's what we've done with Vip and Cry proteins. If an insect were to become resistant to one, it would still be affected by the other and couldn't pass on it's resistance genes, because it would die before it could reproduce. We call this, dual mode of action.

At what point did you decide that the cost of the product was worth the effect that it had?

Good question, one that is asked at the beginning of all research. We do research to provide customers with a product. So we have analysis done to find potential business by seeing who would pay for a certain product. Researchers are then asked what it would take to find a result. The worth (in the market price) and the cost for research are then compared and agreed on or not.

Were there any surprises when developing MIR162?

The active protein was incredibly hard to purify. Also it was really hard to identify what the gene sequence was, but once they found it we could use it, Vip3 stands for **V**egetative **I**nsecticidal **P**rotein and the 3 for my 3rd protein used as insecticides.

What is the scientific history regarding Lepidopterans and the search for an insecticide?
If you look at Biotech prior to Vip3, we could control certain corn pests. You see this was a corn project to begin with. If you look at the spectrum of maize pests, you see that they attack different parts of the plant at different times during a season. The question was how to find something to control more pests with one product. Actually when the project started out we were just interested in (Black cut worms) because it attacks in the small plant, which ruins the whole cycle. We got lucky because Vip3 controls all lepidopteran maize pests, so there was a lot of incentive to develop this. Another benefit is that the harvested corn (which is susceptible to mycotoxins and diseases) isn't at risk because the insects don't make holes in the leaf through which bacteria could enter. Disease through these secondary invaders is avoided and the whole grain quality is improved.

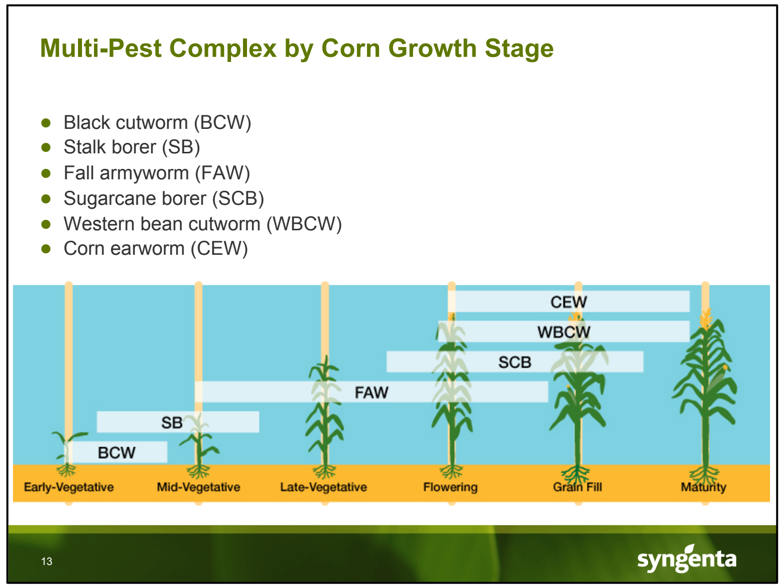
So does the corn itself act as the insecticide? Can you explain the process?
Yes. What happens is you need a promoter and terminator. The whole thing is put into what is called a vector and cloned into a bacterium, we transfer the whole vector into plant cells and a plant is grown. The DNA directs Vip proteins to be produced. So wherever you want the gene to be expressed you affix the promoter to the designated region (for example only expressed in roots). MIR162 wants it everywhere that pests feed. Lepidopterans feed above ground so we don't need it to be expressed in the roots, we have it expressed everywhere else. For reasons of control we like promoters that don't express Vip in pollen.

So pollenating GM corn won't contaminate organic cornfields?
Corn pollen is very heavy, so it pretty much only pollenates in the immediate area around itself. Of course it depends on the proximity of both cornfields. This is a known concern of organic growers, but there have been very few incidents of crop contamination and even fewer regarding corn, especially because of the way it's grown; in big, open fields surrounded by farmers' other products.

Are there any disadvantages to MIR162?
So far we haven't found any, specific to MIR162 or Vip3. Naturally you have to balance the interaction of proteins so as not to have negative effects. We haven't identified any side effects of Viptera, but we do continually look for those kinds of things.

Is MIR162 just a name?
The product name is actually Viptera. MIR stands for Maize Insect Resistant. The transformation of plant cells each produce many plants. We then look for the optimal level and effect of Vip3. That's called an event. Each event has different characteristics. We had the best results with the 162nd event, and so we had MIR162.

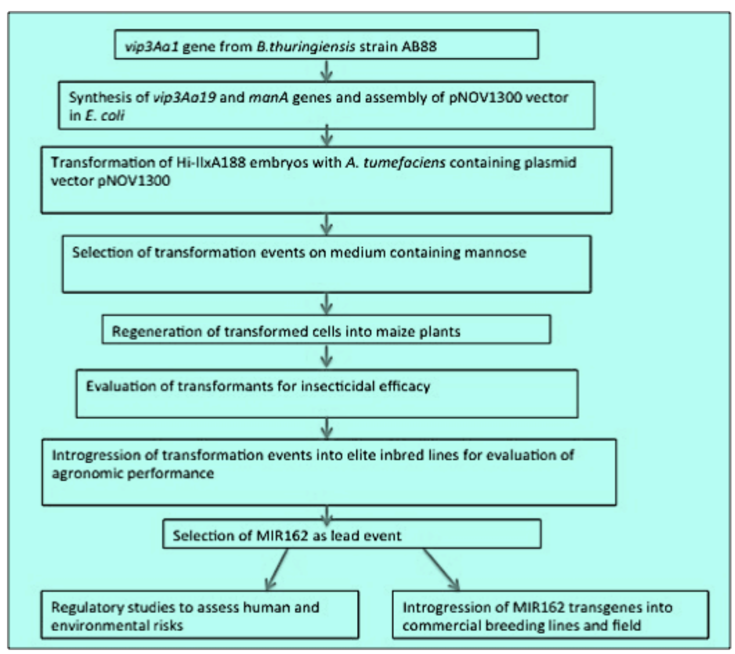
Discussion



MIR162 has been successfully introduced to the market as an insecticide. The farmers who have applied it have reported visibly improved maize crops.
To precede the resistance of the pest, research has been implemented to develop a different mechanism of action to the present: mediated mechanism of action. Tests on toxicity as well as resistance are conducted on a regular basis so as to fulfill requirements regarding follow-up procedure. Many oppose GMO technology, most commonly on either health and environmental grounds, or citing religious and moral grounds. For the former, the weight of reasoning that there haven't been sufficient tests to prove that GMO's aren't deleterious to off-target organisms, or may cause allergenicity. Religious opponents suggest that GMO's go against nature and God himself. Scientists answer that tests conducted for more than 25 years show no human health concerns. With respect to morale grounds, that GMO is counter to nature, the underlying biology is a natural phenomenon that had been observed and since replicated, merely using different plants and strains of bacteria.
Pros: Agrisure™ Viptera is a new addition to the Agrisure maize traits; it has a new and improved mode of action. MIR162 has an excellent resistance profile and controls a broad spectrum of maize pests (illustration above). The use of MIR162 has significantly improved the grain quality of corn in regard to the reduction of disease.
Contras: Although MIR162 has a dual mode of action to diminish the potential for selective resistance, the overuse of any pesticide can invariably result in the promotion of resistant pests.

Summary

MIR162 is a protein, which is inserted into maize plantlets so as to modify the plant into a self-projecting insecticide. This protein was developed to combat a huge spectrum of maize pests; ranging from early feeders, Black Cutworms, all the way to mature maize feeders, Corn Earworms. The insecticide is ingested and binds to the pest's mid-gut membrane. There the proteins create holes in the insect's stomach lining which causes death. Mr. Greg Warren of Syngenta was the scientist who discovered the potential of Vip proteins as a pesticide and who lead the development of Agrisure™ Viptera. MIR162 is not only shielding against pests but also mycotoxins, which are extremely harmful to humans. The product Agrisure™ Viptera is sold in numerous countries worldwide and has reduced Lepidopteran pests on an equally large scale. This Graph 2: shows the process of creating MIR162



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Pictures/Graphs: (24/4/14)

Graph 1 adapted from: http://www.gmo-compass.org/pdf/regulation/maize/MIR162_maize_application_food_feed
Graph 2 from: http://www.aphis.usda.gov/brs/aphisdocs/07_25301p.pdf

Slides from Syngenta Presentation by Greg Warren
Picture of Mr. Warren taken by Annora