Bacillus therungus Protein Mutations

In Agricultural

Maize

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Abstract:

Throughout the past 20 years, the field of biologically enhanced agriculture through genetic mutation has experienced a steady climb (Scanlon 2006). The most "tainted" crop affected by this steady climb is corn, also known as maize (Crispen 2003). A staple food, not only in the U.S, but the entire world, maize comprises almost 2/5 of the average adult's food intake (Associated Press 2004). In addition to individual consumption, Corn has also been called a staple in the worldwide economy, with corn derivatives being found in almost all processed and/ or prepackaged food. In fact, if you were to look in your pantry right now, chances are that most of the boxed goods have "Corn_______" written in the ingredients section of the nutrition label (Peterson 1997).

Combine the massive economical support provided by maize with the new-wave of organic living, and there is a definite potential for the birth of an extremely successful "super crop".

But what if the so called organic maize sold to unknowing consumers wasn't actually organic? My research project is focused on determining the true status of commercial maize, is it organic or not?

The main genetic mutation which is seen in falsely marked batches of agricultural maize is the use of Bt (*Bacillus therungus*) protein (Pertoldi 2006), a naturally birthed biological modifier created in maize crops through the synthetic insertion of the protein in the corn's genome. The protein, originally produced in pests such as locust which is deadly to them was never intended to be released into mainstream commerce, nor was it authorized to be sold commercially by the FDA (Peterson 1997)

Through the use of PCR testing, or Polymerase Chain Reaction testing (Rosenthal 2007), it will be attempted to isolate the Bt protein in three samples of maize collected from within 15 miles of the base of research, Oxford High School. All sites will be local farms selling corn which is claimed to be organic. In doing so, it will be attempted to prove the corn collected, and sold to consumers by the local farms, is actually genetically modified, therefore rendering it inorganic.

Introduction:

The field of genetic engineering, especially in consumer fields, has experienced an explosion in the past 10 years. Corporate companies, wishing to make their products more attractive to the public are incorporating genetic mutations into them to derive specific traits and/or qualities. One such example is the mutation of agricultural maize through the use of *Bacillus therungus*.

Scientists have isolated the gene which gives maize its specific qualities of color, nutrients, but most specifically – immunity. Recent advances in genetic sciences have allowed the transposon gene, *Bacillus therungus*, to be isolated and inserted into the corn's genome.

The altering of the gene by scientists allows for maize to remain in its natural environment, free of pests and disease, without the harmful effects of commonly used pesticides. What this means is that organic foods would be able to be produced by almost tenfold, without the loss of millions of crops and the use of harmful biological pesticides. This would benefit commerce in the area tremendously.

In the project, samples of agricultural maize were taken from surrounding farms in towns surrounding Oxford, CT. The samples were then brought to a lab where the maize was analyzed using PCR (Polymerase Chain Reaction) testing to identify the presence of the Bt – or *Bacillus therungus* gene in the corn samples.

Following the conduction of the PCR trials, it is expected that the Bt gene will be found in most, if not all, of the corn samples. (Crispen 2003). The successful isolation of the Bt gene in the corn samples would prove that although the samples obtained were said to be organic, or untainted by unnatural means, the organic classification would, in fact, be a false one – as the genetic makeup of the corn was altered to incorporate the Bt gene into the genome.

The discovery of the Bt gene in the sample maize would bear bad news for the farms who supplied the trial corn under an organic label. If any samples of the corn which have been marketed as organic are found to be inorganic by means of genetic mutation, the producers will be directly violating USDA and FDA standards. Such standards state that, "organic produce marketed as such, may not be, in any way, tampered with by human means."(Jeffery 2009)

The genetic transposon's creation is credited to a large Swiss pharmaceutical lab, Novartis, and was first acknowledged to be in testing in the year 1998.(Peterson 1997) Although the use of the Bt gene was acknowledged, its use in commercial sectors was never authorized, and still is not. The purpose of the presented research is to attempt to identify the presence of Bt in so-called "local organic maize" in order to disprove and prove two things; to disprove the corns' organic status, and to prove that the Bt gene has been leaked into the commercial sector illegally. It is very likely that the mutated corn may have been leaked to general commerce, and crossbred with organic strains of maize. (Scanlon 2006)

The genetic transposon which is located in the corns chromosomal DNA acts to increase the hardiness of the crop. In other words, Bt acts to coat the cell walls of the corn which are responsible as the first line of defense from outer elements such as harmful pests like locust which kill thousands of crops every year. (Rosenthal 2007). Many alternatives to genetically enhancing the corn have been made, however, said options are not attractive to the public who is purchasing the product. Some proposals include: importing alien corn from surrounding countries where it can be grown year-round without the presence of such pests as locusts which consistently damage crops, or growing the corn in specialized hydro-ponic chambers in which they can be grown without the harmful pests. Although both options seem to be logical alternate solutions to the irritating pest problems, the options are both extremely expensive and illogical. For example, the size of the hydro-ponic chamber which would be necessary to the cultivation would have to be 100's of acres long and wide. Options such as this one are simply, not possible.

Through the Bt gene's use, the problems associated with harmful pests will be remedied. With the area available for cultivation increased by the extraction of the pest factor crop production will be increased tremendously, and therefore the need for imported maize will decline accordingly. Not only will this boast a massive boost to commerce in the area, but it will also help to comfort the 30 million citizens in the U.S. who worry every day about the safety of themselves and their families.

The benefits which can be reaped from the integration of the Bacillus Therungus gene are immense. Not only can the gene increase the specific hardiness of maize crops, but as a result the commerce involving maize with Bt gene insertion will skyrocket. (Peterson 1997)

Many corporate financiers are saying that with the introduction of such change in the corn industry which is already and explosive market, the benefits to be reaped are astronomical. As of now, 3/5 of organic crops which are grown in the U.S. make it through the farming process to be sold in stores (Pertoldi 2006). This is because 2/5 of the time, the crops do not survive pest interference, the same problems which have plagued farmers for centuries.

The USDA recently issued an article in “The Atlantic”, in which it was said that, “through the use of the *Bacillus therungus* gene, production of organic maize can be increased two fold” (Newhouse 2004). What this means for business is less loss in revenue, and a spike in profit. In addition the crops will also help to create thousands of jobs for eager citizens in the U.S.

A 2009 U.S. census ranked the third most unemployed job type in the U.S. as “Agricultural Engineers” or people employed in the farming industry; below factory workers (1) and government enlisted employees (2). (Jeffery 2009) The approximation of jobs currently filled by workers for the “Agricultural Engineer” category is 15 million. This current census shows a decreased by almost 5 million people in the past five years. Out of the 15 million people currently employed in the farming industry, 7 million operate farms on which organic maize is grown; approximately half of the farming population Imagine the tremendous improvement in job security if the existing 3/5 survival rate for organic maize was increased to a miraculous 100 percent. The increased survival rate would translate almost directly into that of America’s farming engineers.

The most amazing aspect of the introduction of Bt into the maize genome are the possibilities which emerge from the creation of this hybrid corn. Now that the gene responsible for the changes in organic maize has been identified, the possibility that it can be applied to any other cash crop is very real. Imagine if the same benefits from the implementation of Bt in corn could be multiplied for every crop to which it was applied. The results would surely be tremendous.

Second only to the corn industry is the “greens” industry – as financial analysts like to call it. The “greens” industry includes, by definition, any crop which, when harvested contains the chemical chlorophyll (Scanlon 2006). What this means for commerce is that all bagged salads, avocados, cucumbers etc. could be grown as almost entirely an organic endeavor. The concept would be like the entire produce section in groceries throughout the U.S. being completely organic; without all of the price inflation and political correctness currently smothering the organic movement.

Despite the cost effectiveness of supplying only organically grown crops to consumers, consumers would also stand to gain great health benefits from an “all organic” lifestyle. As organic foods do not contain any artificial hormones or pesticides, the crops which consumers will be feeding to themselves and to their families will be far healthier than if the crops had been contaminated with harsh pesticides. One such pesticide which has had extremely detrimental effects on biological life is the formerly widely used D.D.T. D.D.T. was responsible for the almost complete extermination of the Bald Eagle until bans were created in the late 1990’s prohibiting its use (Rosenthal 2007). The *Bacillus therungus* would render the use of such harmful pesticides obsolete.

In addition to the other benefits of using the Bt gene in agriculture, the use of it as an organic gene can also act to lower federal taxes placed on farms for the use of pesticides. With no pesticides being used, the taxes levied upon already financially stressed farm owners would decrease exponentially. The results of which may be the creation of even more jobs with a higher pay grade.

The *Bacillus therungus* gene functions by inserting itself into the DNA of the corn between genes B4 and B5 (Crispen 2003). To insert, the transposon first activates a enzyme called transposase splits the two genes which border its future spot in the chromosomal DNA. The gene, then, after inserting successfully into the maize's genome used a reverse enzyme called, reverse transposase to "seal" itself in place between B4 and B5. In order to insert itself into the DNA, the gene must first bond covalently to the hydrogen bonds of the genes bordering it. Once this step has been completed the Bt gene has successfully been inserted into the maize's genome and is ready to deliver its radical benefits.

Although the cause of the activation of the Bt gene is still unknown, what is known is that the reason for activation is dependent on certain external catalysts. Some of which are temperature, human interaction, or genetic pre-disposition. In the cases which involve gene activation on the basis of pre-disposition, the result is a corn in which certain kernels are multicolored; this corn is sometimes referred to as "Indian Corn". Temperature catalyzed reactions usually result in deformations of the ear of corn (Associated Press 2004). For example the lack of kernels at either tip of the ear, or a half sized ear growing in a full sized pocket (the leafy covering around an ear in which it grows). Fortunately, in all instances, the benefits of the Bt gene against harmful pests remain just as effective.

Instances such as these, in which deformations and such appear in the maize only occur when two or more Bt genes are inserted into one genome. If the gene was used to increase agricultural benefits (once being certified by the USDA), this deformity would be avoided, as the transposon would be inserted into one genome at a time in a stable lab environment, and then replicated in a controlled manner. The lab produced corn will then be cloned as is necessary to produce enough exact replicates to populate the produce producing farms located throughout the U.S. The cloned maize which is essentially immune to harm by pests will then be harvested and sold to consumers everywhere, legally and safely. Bt mutated agricultural maize has far more benefits than simply being immune to harm by natural or manmade nuisances the hybrid maize can also be used increase the nutritional value of feed used for animals. What this means is that not only will commerce in the corn industry increase, but in addition, meat products that are distributed to the public will not be contaminated with all the harmful pesticides used in the feed which they consume. The hybrid maize can also be used increase the nutritional value of feed used for animals.

Materials:

Chemicals/ Consumables	***Supplies***	***Equipment***

• **Bt Primers**
  • **Primer 1:** **CTG GTG GAC ATC ATC TGG GGC ATC TTC G**
  • **Primer 2:** **TTG GTA CAG GTT GCT CAG GCC CTC C**
• **GMO Primers**
• **3 samples of local organic maize**
• **Schreiber's Farm**
• **Gazy Farm**
• **Belinsky Farm**
• **1 sample of GMO-negative control**
• **PSII positive Bio-Rad maize sample**
• **Bio-Rad genetic testing kit**
	• **1, 100 mL beaker**
	• **1, 600 mL beaker**
	• **Ring stand/gauze pad**
	• **Bunsen burner**
	• **Foam floats/ thermometer**
	• **PCR tubes**
	• **Microtubes (w/wo caps**
	• **Mortar and pestle**
	• **400mL of distilled water**
		• **PCR thermal cycling machine**
		• **Centrifuge**
		• **Agarose gel and**
		• **Electrophoresis equipment**

Procedure:

-Part 1:

Find screwcap tubes and label one“non-GMO” and one “test”. Weigh out 0.5–2 g of certified non-GMO food and put it into the mortar. Add 5 ml of distilled water for every gram of food. To calculate the volumes of water you need, multiply the mass in grams of the food weighed out by 5 and add that many milliliters. Grind with pestle for at least 2 min to form a slurry. Add 5 volumes of water again and mix or grind further with pestle until smooth enough to pipet. Pipet 50 µl of ground slurry to the screwcap tube containing 500 µl of InstaGene labeled“non-GMO” using the 50 µl mark on a graduated pipet. Recap tube. Repeat steps 2–5 to prepare the test food sample. Pipet 50 µl of ground test food slurry to the screwcap tube labeled “test”. Recap tube. Shake or flick the non-GMO food and test food InstaGene tubes and place tubes in 95°C water bath for 5 min. Place tubes in a centrifuge in a balanced
conformation and centrifuge for 5 min at max speed. Store tubes in a refrigerator.

- Part 2:

Number PCR tubes 1–6 and initial them. Place each tube in a capless microtube adaptor and place in the foam float on ice. Referring to the table and using a fresh tip for each addition, add 20 µl of the indicated master mix to each PCR tube, cap tubes. Referring to the table and using a fresh tip for each tube, add 20 µl of the indicated DNA to each PCR tube, being sure to avoid the InstaGene pellet at the bottom of the tubes. Mix by pipetting gently up and down; recap tubes. When instructed, place PCR tubes in thermal cycler.

Tube number	Master Mix	DNA
1 20 µl	Plant MM (green)	20 µl Non-GMO food control DNA
2 20 µl	GMO MM (red)	20 µl Non-GMO food control DNA
3 20 µl	Plant MM (green)	20 µl Test food DNA
4 20 µl	GMO MM (red)	20 µl Test food DNA
5 20 µl	Plant MM (green)	20 µl GMO positive control DNA
6 20 µl	GMO MM (red)	20 µl GMO positive control DNA

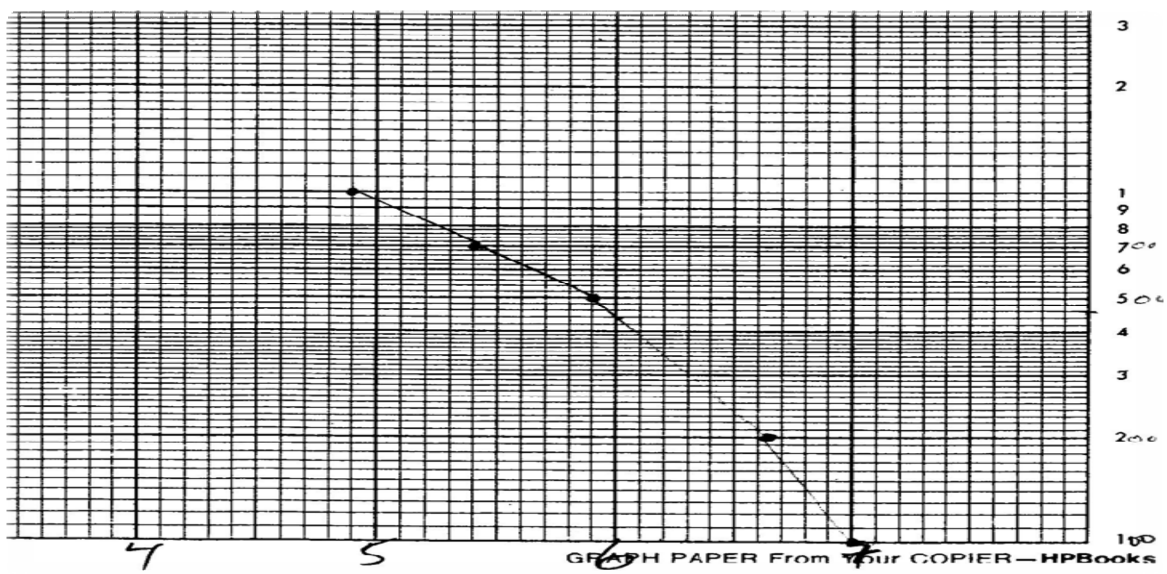
-Part 3:

Set up your gel electrophoresis apparatus as instructed. Obtain your PCR tube from the thermal cycler and place in the capless microtube adaptor. Pulse-spin the tube for ~3 seconds. Using a fresh tip each time, add 10 µl of Orange G loading dye (LD) to each sample and mix well. Load 20 µl of the molecular weight ruler and 20 µl each sample into your gel in the order indicated. Run an Agarose gel for 30 min at 100 V and run a polyacrylamide gel at 200 V for 20 min. Stain in Fast Blast DNA stain.

Data/Results:

The results of the research project yielded an unexpected composite of results. The trials which tested for GMO in the maize samples, tested positive. Every sample tested showed a definitive band during electrophoresis showing the presence of GMO.

Figure 1: GMO Semi Log Graph



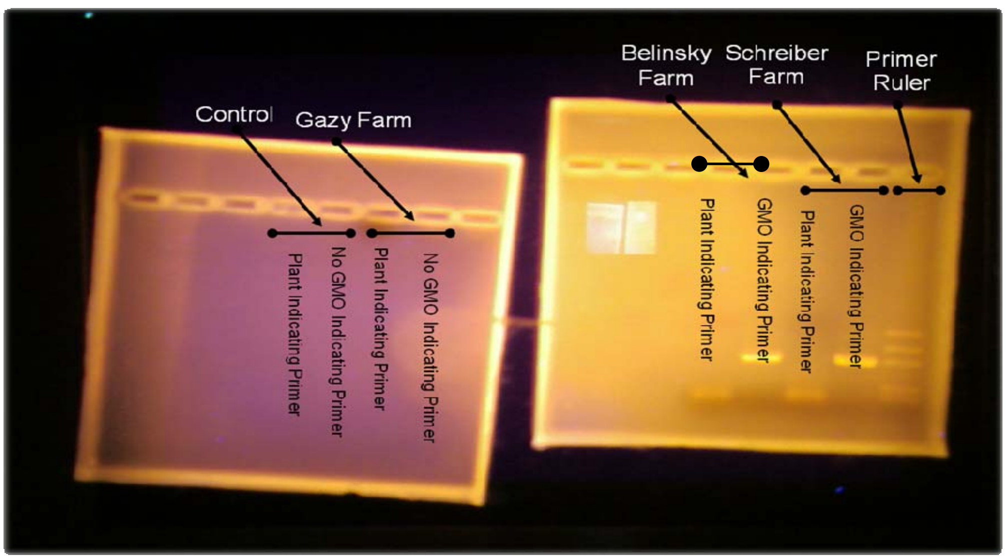
The graph shown above, known as a "semi log graph" shows the quantification of the GMO bands (base pairs) which were created during electrophoresis of the samples.

Table 1: Band existence & Base Pairing

	GMO Primers	Plant Matter Primers	Bt Primers
Gazy Farms	**No Bands**	**Bands - 100 BP**	**No Bands**
Schreiber's Farm	**Bands - 460 BP**	**Bands - 100 BP**	**No Bands**
Belinsky Farm	**Bands - 390 BP**	**Bands - 100 BP**	**No Bands**
Non GMO Control	**No Bands**	**Bands - 100 BP**	**No Bands**

- The semi log graph shows the base pairs of each band on the graph.
- Gazy Farms maize samples showed no GMO bands, Plant Matter bands at 100 BP, and no Bt bands.
- Schreiber's Farm maize samples showed GMO at 460 BP, Plant Matter bands at 100 BP, and no Bt bands.
- Belinsky Farm maize samples showed GMO at 390 BP, Plant Matter bands at 100 BP, and no Bt bands.

Figure 2: Electrophoresis Results



In addition to the semi log graph shown above, a picture of the Agarose gels produced during electrophoresis also shows visible bands which indicate both the presence of GMO and plant matter in all of the samples, except for the Gazy Farm samples which show only the presence of plant matter, as it should.

Conclusion:

The trials conducted during experimentation in this research yielded opposite results to the ones predicted in the hypothesis. What did correspond with the hypothesis, however, was the presence of GMO in each of the corn samples EXCEPT the maize sample taken from Gazy Farm. The sample maize taken from Schreiber's Farm yielded the expected outcome of testing positive for GMO, however the sample did not show presence of Bt. Similarly, the maize sample taken from Belinsky Farms tested positive only for GMO – not Bt. As expected the control maize tested did not test positive for GMO nor Bt as it was classified as non GMO. The Gazy farms sample was dissimilar from the other three, however, in that this sample did not test positive for GMO. Therefore, from said results I can conclude that 2 out of 3 of the samples of local maize tested positive for GMO, the sample which did not test positive being the Gazy Farms sample.

Although the two samples did test positive for GMO, suggesting that in the trial for Bt they would test positive, all maize samples yielded no evidence of Bt contamination. As expected, the control remained without presence of Bt. What can be concluded from this trial is that all of the maize samples tested, although positive for GMO, do not necessarily contain the protein, *Bacillus therungus*. Two explanations exist for this unexpected result; the maize samples were, conversely to what was expected, "clean", or the Bt trial was conducted in an incorrect manner. Both possibilities are equally likely, however, the second is most likely the culprit for the surprising outcome. During PCR any slight mistake in measurement or calculation could have put the entire experiment in jeopardy.

The results of this project definitively shows that two out of the three samples of local “organic” maize were shown to be genetically modified (Schreiber’s Farm, and Belinsky Farm) In addition, the results showed that the sample maize did not contain Bt, thus partially disproving the original hypothesis in that no samples tested positive for Bt, but did show GMO.

In conclusion, the results show that although the samples tested were said to be non GMO, 2 out of 3 were, with the exception of Gazy Farms samples.

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