The Presence of LL601 Rice Within the Connecticut Community

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I Abstract

The LL601 rice is a variety developed by Bayer Crop Science in the late 1990's . This variety was engineered by Bayer Crop Science to be tolerant to Liberty Link herbicide. The DNA of the LL601 variety contains a bacterial gene which creates phosphinothricin acetyl transferase, PAT, proteins. The PAT proteins provide the rice with resistance to glufosinate ammonium, the active ingredient in the Liberty Link herbicide. In 2006, this experimental variety of rice contaminated the supply of commercial rice within the United States. After the contamination scare, LL601 rice was never deregulated.

This study investigated whether or not the LL601 rice is still within the commercial supply of rice within the country. A negative control (water) and specimens of commercial rice were collected. After DNA extraction, each of the samples went through polymerase chain reaction, also known as PCR. To start the PCR, certain primers were added to each of the samples. The primers targeted the desired DNA sequence, the PAT gene, and amplified it (if the target DNA was present). Once PCR was complete, the results were further analyzed through electrophoresis.

Once the samples were placed in the lanes of the agarose gels and then put through electrophoresis, the gels were observed and analyzed. The agarose gels containing the samples combined with the LL601 primers contained no DNA bands. Thus, it was determined that the rice samples collected did not contain the LL601 gene.

II Introduction

Rice

Providing 20% of the world's dietary energy supply, rice is one of the world's staple crops and evidently, the most important grain produce known to mankind (FAO of the UN, 2009). Every year, rice provides, in some countries, up to 70% of the calories in the human population's diet (beta.irri.org, 2009). Annual worldly donations, over the past 30 years, amount anywhere from 500 tons to 2000 tons of rice (beta.irri.org, 2009). One might say rice is salvation for the hungry throughout the world, providing vital nutrition to one third of its population (Smith, 2009).

Rice is cultivated from the grass plant Oryza (Smith, 2009). Numerous varieties of this grain crop exist (for instance: brown rice, long- grain rice, Jasmine rice). Rice is mainly cultivated through 2 methods: lowland rice is grown in flooded plains and upland rice is grown in soil (in areas where water is not always so readily available). In technologically advanced countries, such as United States of America, rice is grown in fields which receive hydration through irrigation or rainwater. However, most rice is grown in China and India in flooded plains. Accordingly, Asia provides for 90% of the production of this staple crop (Hijmans, 2007).

A form of grain, rice contributes to the production of various grain products such as bread, pasta, and cereal. It also serves as the main component of many meals and recipes in every country and culture of the world. Such a large part of the human diet, rice contains many nutrients such as iron, folic acid, niacin, and Vitamin B (micronutrient.org, n.d.).

Because rice is the staple crop of many nations, a provider of various nutrients, and an ingredient in meals worldwide, it is extremely important that rice, destined for human consumption, be deemed safe. In order to protect the lives of the billions of people who consume this crop daily, the world must be fully aware of the contents, chemical make-up, and genetic make-up of the tons of rice imports and exports.

Genetically Modified Organisms

In today's technological society, the genetics of the plants can be explored and means of altering the genetic make-up of numerous plant species can be generated. With new technology, genes can be chosen to be added or removed or possibly changed in the genome of the selected species. This process is called genetic modification or engineering.

As a result of genetic engineering, crops can be made more productive through insertion of a gene which provides a plant with resistance to insects or pesticides. For example, *Bacillus thuringiensis* is added to the DNA of various crops, specifically corn, to give the crop insect resistance. Bt, a bacteria, will kill the insects that attempt to snack on the genetically modified crop (bionetonline.org, n.d.). Crops can also be genetically modified to yield more harvest and profit through the removal of a certain gene. For example, the genome of tomatoes contains a gene which sparks decomposition of the organism once it is removed from the vine. Such a gene serves as an agricultural obstacle - it begins the decomposition of tomatoes before they have reached consumers. Genetic engineering has allowed for a solution to this problem. Scientists can simply turn off the gene which causes the tomato to begin decomposition at such an early stage.

Genetic engineering also allows for the improvement of nutritional value of crops grown for human consumption. Nutrients can be added to a crop through the alteration of its DNA. For example, a variety of rice is modified to contain a large amount of beta-carotene, found in carrots, which the body converts to Vitamin A (bionetonline.org, n.d.). Thus through genetic engineering, this specific variety of rice will provide additional nutrients to its consumer.

Furthermore, the world can benefit from the production of genetically modified organisms through their application to the pharmaceutical field. Certain drugs - hormones, proteins, antiviral and antibacterial treatments, can be mass produced through the insertion of their genetic material into the genome of a "pharmacrop" (ucsusa.org, n.d.) As the crop, containing the pharmaceutical DNA, goes through reproduction, so do the genetics of the selected drug. Then, the drug can be extracted from the cells of the "pharmacrop".

The actual process of genetic modification consists of various steps. First, the desired gene, which will give desired characteristics to the selected organisms, must be isolated and reproduced through PCR, polymerase chain reaction. Then the amounted DNA must be inserted into the desired organism. Each of these steps is complicated and requires much time, precision, and research to properly execute.

Risks of Genetic Modification

Although the process of genetic engineering provides the world with a more copious yield of crops as well as various medical benefits, it also has its disadvantages. It is unclear to today's scientists whether or not genetically modified organisms have a negative effect on those who consume them. The health risks of GMO consumption have

not yet been identified nor will they be fully understood anytime in the near future. The full knowledge of the purview of the effects of genetically engineered food will come with time only; GMOs have not been around long enough for their potential health risks to reveal themselves.

In addition, the production of genetically modified organisms yields various environmental risks. Insect-killing bacteria in crops could harm species which they are not intended to harm. Not all insects which are present in the environment of the crops pose threats the health of the crop. For example, farmers do not intend to harm butterflies with genetically modified crops which kill insects. However, because the butterflies live in the environments of the genetically modified crops they are also subject to this toxin (bionetonline.org, n.d.).

Another environmental disadvantage, cross cultivation can occur through the growth of genetically engineered crops. It is impossible to prevent the spread of genetically modified crops into the fields of non-genetically modified crops. The seeds and pollen of genetically modified crops can be carried by wind, birds, insects, and animals to the fields of non-genetically modified crops. Farmers may be unaware that their crops have been cross contaminated by a genetically modified species. Thus, crops that are grown free of genetic modification might be cultivated with a species that has been bioengineered.

Cross cultivation also poses problems with experimental crops. Before new varieties of genetically modified crops have been deregulated, they must go through various testing. The experimental fields, where genetically modified crops that have not been deregulated are grown, must be to a certain extent isolated from commercial fields.

However, there is still a risk that the seeds of the experimental crops might end up in the commercial fields. Thus, genetically modified organisms that have not been deregulated by the FDA can end up in food that humans will consume.

Rice as a GMO

Few genetically modified varieties of rice exist. The very idea of genetically modified versions of this staple crop has been rejected by nations and organizations worldwide. People insist that the crop has successfully been grown for thousands of years without the assistance of today's bioengineering technology (Oliver, 2007). It seems that the world is not ready for the unknown when it comes to a staple crop like rice.

However, GM supporters argue that the advantages of deregulating and commercializing genetically modified rice outweigh the risks (Oliver, 2007). Introducing genetically improved versions of the staple crop could benefit the world. By producing higher yields, genetically modified rice could lessen the percent of the world's population who struggle through starvation. In addition, genetically modified rice, engineered to contain extra nutrients, could be helpful instead of hurtful to human health.

Developed varieties of GM rice include LL601 and LL62 rice, which were engineered by Bayer Crop Science to be resistant to herbicides. Another variety is Roundup Ready rice, also engineered to be resistant to herbicides, specifically Roundup herbicide. Of the various genetically modified versions of rice produced, few have been deregulated by the FDA. Of those deregulated, which include the LL601, LL62, and Roundup Ready varieties, none have actually been commercialized due to lack of FDA

deregulation.   (Aruna). Thus, commercial rice, rice that we consume, should be genetic modification free. However, this is not always the case.

Even though the FDA has regulated a few of the GM rice varieties, there is a fair bit of uneasiness towards the commercialization of any form of genetically modified rice. Many countries refuse to even consider the genetic modification of this staple crop. Critics of genetically modified rice claim that this crop has been around for over 10,000 years and has been successfully produced without genetic modifications. Thus, they believe, there is no need to bioengineer rice and involve possible risks to human health.

LL601 Rice

LL601 rice, a Liberty Link variety, was designed by Aventis Crop Science, which is now owned by Bayer Crop Science, to be resistant to glufosinate ammonium, the active ingredient in a Liberty herbicide (Laws). The rice contains a bacterial gene which creates phosphinothricin acetyl transferase proteins, also known as PAT proteins (Lemaux). These proteins give the rice tolerance to the Liberty herbicide.

Other varieties of the Liberty Link rice, LL62 and LL06, are genetically modified to contain this same PAT gene, phosphinothricin acetyl transferase protein. In 1999, the LL62 and LL06 varieties were both declared safe and then deregulated by the FDA (Lemaux, 2007). However, neither was ever commercialized because they were not determined completely safe by the FDA. At this point in time, the LL601 variety was still being tested and grown only in experimental fields.

A few years later, in 2006, the LL601 – still an experimental crop, showed up in commercial samples of rice across the globe. The LL601 gene was discovered in bins of

rice in Missouri and Arkansas. Soon after this alarming news of American rice contamination, 9 European countries found traces of the LL601 gene in US rice imports (Lemaux, 2007). A frenzied European Union enacted a "zero tolerance" policy for the LL601 rice and required that all US rice imports go through extensive testing before entering the European market (swissinfo.ch).

Soon after the contamination scare, the FDA deregulated the LL601 rice and declared it ready for commercialization (deltafarmpress.com). However, the rice market – still recovering from LL601 contamination, was not a welcoming environment to any genetically modified rice. Thus, the LL601 variety was never commercialized.

Despite deregulation of LL601, uneasiness towards this genetically modified variety of rice remains. Apprehension towards the LL601 variety is natural for society today. There is no complete assurance that any genetically modified organism is not hazardous toward human health. Additionally, rice is a crop, in some places, which the human population survives on. Thus, introducing a genetically modified version of rice to the world is risky. Therefore, it is important to continue to test commercial rice and check for the presence of the LL601 gene.

Testing for LL601

Testing for the LL601 genetics in commercial rice requires access to a sample of commercial rice, a sample of LL601 rice, and control sample (water). Each of the samples must go through PCR, polymerase chain reaction. Then, the resulting DNA of PCR, or lack of DNA in some cases, can be prepared for electrophoresis. After the samples are put through electrophoresis, observations and comparisons can be made on

the results of the PCR. From these results, it can be determined whether or not the sample of commercial rice contains the gene of the LL601 rice.

PCR

Testing methods which identify the presence of a genetic modification include PCR, polymer chain reaction. With the appropriate primers, PCR will commence by first targeting the desired segment of the DNA and then making copies of this segment. In a very short period of time, the segment of DNA can be multiplied so that millions of copies are created. Then, the amounted DNA can be further analyzed.

When using PCR to test for the presence of LL601 genetics, the target segment of DNA is the gene which encodes the PAT protein. Primers used to target this segment and begin PCR process include MDB498 and DPA143. The PCR must be set up in a thermo cycler. In this thermo cycler, the samples and primers go through various cycles. In the first step of the cycle, initial denaturation, the DNA is prepared for amplication (the DNA is separated into strands). In the next step, annealing, the desired DNA sequence is targeted by the primers. Then in amplication, the target DNA is copied and duplicated. This should result in the formation of millions of copies of the targeted PAT bar, if it is present in the DNA. The procedure for the PCR of the LL601 rice was developed by Bayer Crop Science – also the company which developed the Liberty Link variations of rice.

If PCR is successful, the negative sample will not have any amplified DNA, the positive sample will have amplified DNA, and the unknown will have amplified DNA only if it contains the LL601 gene. To further analyze whether the PCR results, all of the samples can be put through electrophoresis.

Electrophoresis

In electrophoresis, DNA samples are set up in the lanes of agarose gel, in an electrophoresis apparatus. In electrophoresis, distinctive patterns are created by certain DNA sequences in the agarose gel as a result of their distinctive combinations of base pairs. The distinctive patterns can be compared and contrasted.

For the electrophoresis with the LL601 rice, the PAT positive sample should display a distinctive pattern in the agarose gel. The PAT negative sample should display no pattern of bands whatsoever because no DNA should have been extracted in the PCR process. The unknown sample of DNA, the sample of commercial rice, will either show no pattern of DNA, like the control sample, or will display a similar pattern to that of the PAT positive sample.

If the unknown sample of DNA displays a pattern identical to that of the PAT positive sample, it can be determined that the sample of commercial rice does, indeed, contain the LL601 gene. This means that, in PCR, the targeted gene was found in the DNA of the unknown sample. However, if the DNA does not display a pattern in the agarose gel, the targeted DNA sequence was not extracted from the unknown sample. This means, in PCR, the targeted DNA was not found in the DNA of the commercial rice. Thus, the commercial rice would not contain the LL601 genetics.

III Material and Procedure

Materials

Chemicals/Consumables	*Supplies*	*Equipment*
MDB498 – Forward Primers TATCCTTCGCAAGACCCTTCC DPA143 Reverse Primer ATGTCGGCCGGGCGTCGTTCTG Universal PCR Master Mix Ethium Bromide Agarose Gel Water Molecular Weight Ruler Orange Marker	Rice Microfuge Tubes PCR Adapters PCR Test Tubes	Micropipet Electrophoresis Apparatus Thermo Cycler Microcentrifuge

Procedure

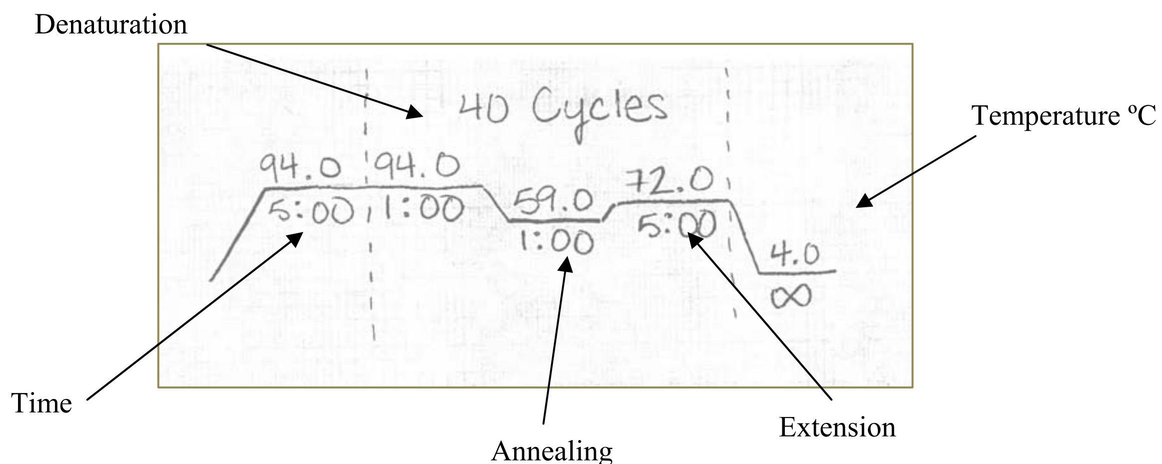
First, .5 grams of each sample, Uncle Ben's Rice and Stop and Shop Instant Rice, were combined with 25mL of water. The water and sample were then ground in a mortar and pestle for 2 minutes until a slurry was formed. 50µl of Insta Gene matrix were placed within a microfuge tube. Another 50µl of the Insta Gene matrix were placed in an additional microfuge tube. Each of the samples was placed within one of the tubes containing the Insta Gene matrix, the substance which makes the DNA extraction possible. This whole process was repeated so that there were 2 microfuge tubes containing samples of Uncle Ben's Rice and 2 microfuge tubes containing samples of Stop and Shop Instant Rice. These 4 microfuge test tubes were then placed in a water bath set at 95°C for 5 minutes. Then, they were spun in a microcentrifuge for 5 minutes.

After the DNA was extracted, the PCR was set up. First, 150µl of the Universal PCR Master Mix were combined with 4µl of the plant primers (colored green). Another 150µl were combined with 4µl of the genetic modification primers (colored red). Another 150µl were

combined with 4µl of the LL601 primers, 2µl of the MDB498 and 2µl of the DPA143. (Before, each of the LL601 primers was hydrated with 500µl of sterile water.) Then, 20µl of each of the of the DNA samples, extracted with a micropipet from the microfuge tube, were placed into a PCR tube which was seated in a PCR adapter. It was made sure that the DNA material was not contaminated with the beads of the Insta Gene matrix located at the bottoms of the tubes. This was repeated 2 times so that there were 12 different PCR test tubes set up, 3 of each sample. Then, 20µl of sterile water were placed in a PCR test tube. This was repeated 2 times so that there were three PCR test tubes containing sterile water, the control samples. Then 20µl of the master mix/plant primer solution were added to 5 of the PCR test tubes, 2 containing the Uncle's Ben's rice samples, 2 containing the Stop and Shop Instant Rice samples, and 1 containing the water. This was repeated for each of the other master mix/primer solutions: the genetic modification and the LL601 primers. Thus, at this point, there were 15 PCR test tubes set up, 5 combined with the plant primers, 5 combined with the genetic modification primers, and 5 combined with the LL601 primers.

These 15 PCR test tubes, once removed from the PCR adapters, were each placed within the thermo cycler. The thermo cycler was set to run these specific cycles.

Temperature °C	Time (Minutes)	
94.0	5:00	
94.0	1:00	
59.0	1:00	Repeated 40 times
72.0	5:00	
4.0	∞	



The first part of the cycle, denaturation, separated the DNA strands. Next, during annealing, the DNA sequence was targeted by the primers. Then, in extension, the targeted DNA sequence was amplified, with the aid of the PCR Master Mix.

As the samples went through the PCR cycles, electrophoresis was set up. The agarose gel was made by combining 3 grams of agarose with 100mL of 1x TAE electrophoresis buffer in a flask. The flask was shaken thoroughly, in order to suspend the agarose, and then was heated in the microwave for 1 minute. Then, the agarose gel solution poured into the 4 electrophoresis apparatuses which contained the casting combs. Once the gel was set, the combs were removed and the electrophoresis apparatuses were filled with 1x TAE electrophoresis buffer. Then the PCR molecular mass ruler was combined with 10µl of the Orange G loading gel. 20µl of the ruler was then placed in the first lane of each of the agarose gels.

Once the PCR was complete, the contents of each PCR test tube was combined with 10µl of the Orange G loading gel. Then, 20µl of each of the test tubes was placed into a lane in the electrophoresis gels. All of the samples combined with the plant primers were placed in one gel. All of the samples combined with the genetic modification primers were placed in another. All of samples combined with the LL601 primers were placed in another. The last gel contained 20µl of each of the LL601 primers, which were also combined with 10µl of the Orange G loading gel. The gel set up now complete, the electrophoresis apparatuses were run for a half hour.

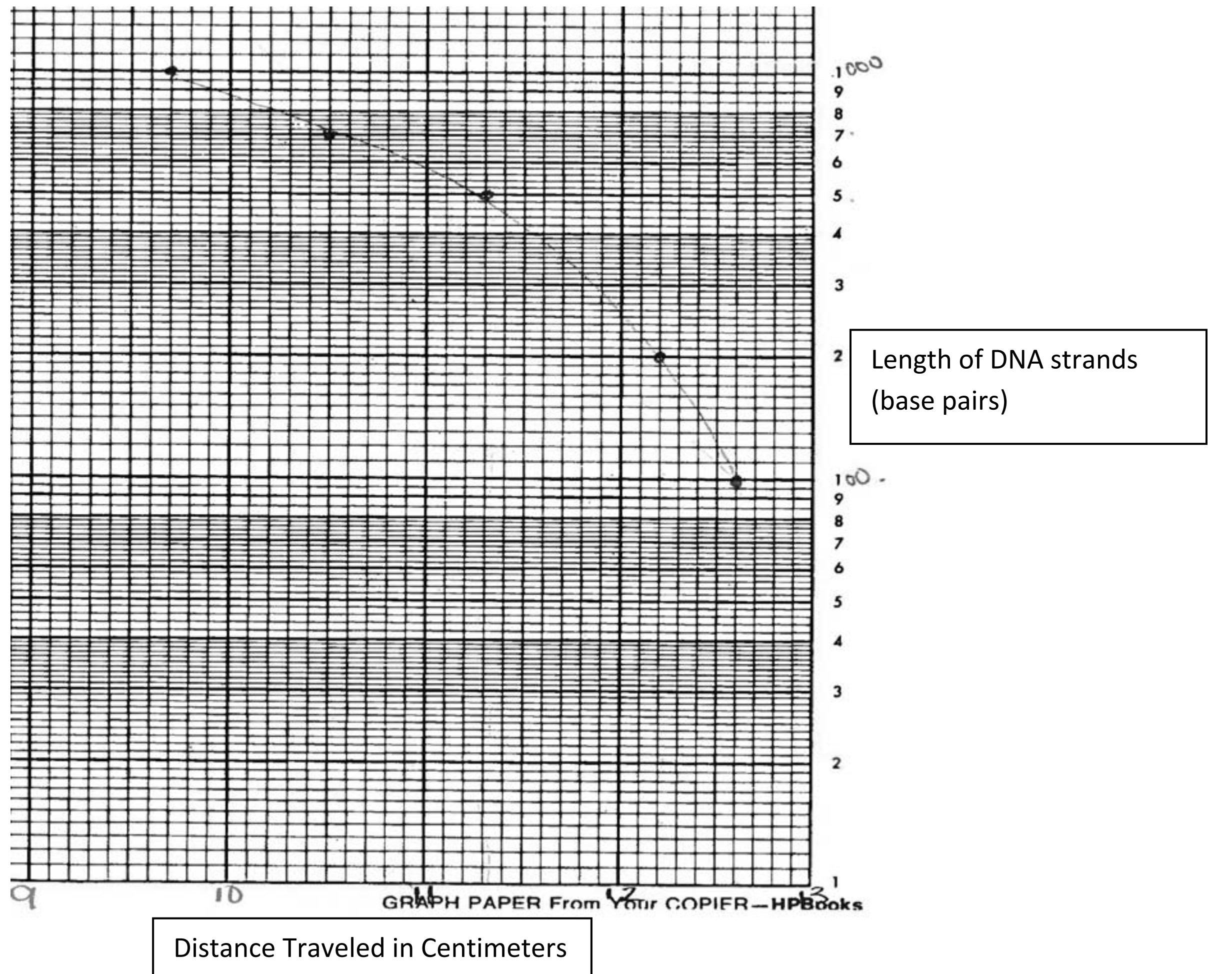
The gels were removed from the apparatuses and stained with ethium bromide. They were rinsed in water afterwards and then observed under ultraviolet light. There, the DNA bands within the gels were observed, recorded, and analyzed.

IV Data

Observations of Bands within the Electrophoresis Gels of the 3 Primers and 3 Samples

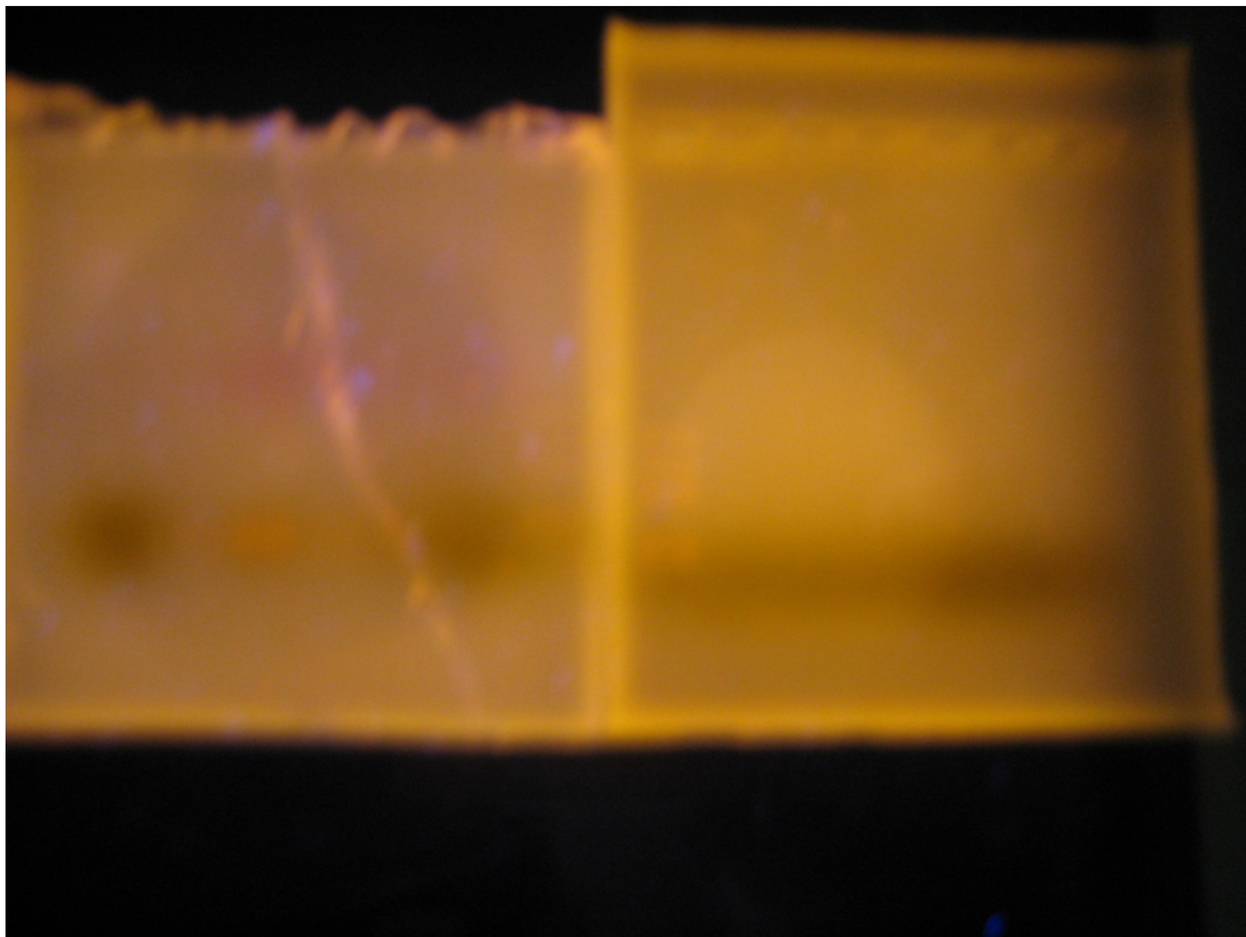
	GMO Primers	Plant Primers	LL601 Primers
Rice 1 – Uncle Ben's	No bands	Bands – 410 base pairs	No bands
Rice 2 – Instant Rice	No bands	No bands	No bands
Control – Water	No bands	No bands	No bands

Semi-log analysis of DNA migration to determine size of unknowns



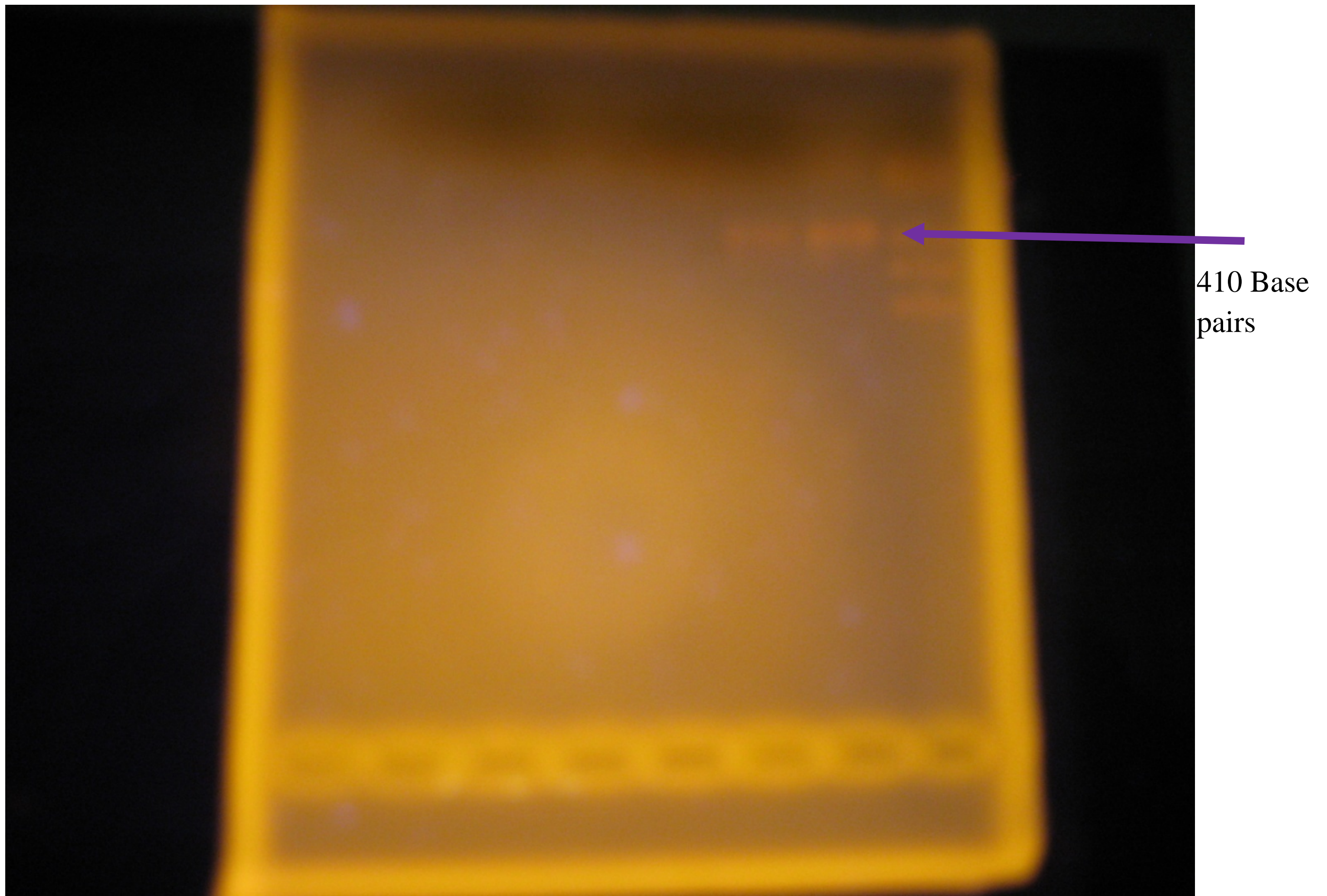
The analysis was done using a picture of the electrophoresis gels scaled to an 8.5 x 11 inch piece of paper.

Electrophoresis Gels of Samples Combined with LL601 Primers (Left) and GMO Primers (Right)



As it can be observed, no bands appeared in either of these gels.

Electrophoresis Gel of Samples Combined with Plant Primers



Here, bands of 410 base pairs appeared in the second and third lanes of the gel, those containing the 2 samples of Uncle Ben's Rice.

V Conclusion

Through the PCR procedure and electrophoresis, the inquiries of this project were answered. The results were displayed directly through the electrophoresis gels. The gel containing the genetic modification primers and samples of rice displayed no bands. The gel containing LL601 primers and samples of rice also displayed no bands. The gel containing the plant primers and samples of rice displayed two bands of DNA. The bands appeared in the second and third lanes of the gel. Both lanes contained the primers combined with rice sample # 2, the Uncle Ben's rice. Through the semi-log analysis of the DNA bands of the ruler, it was determined that each of these DNA segments were 410 base pairs.

From these results, various conclusions can be made. First, through evidence of the control, it can be concluded that the PCR procedure was successful. Lanes of the electrophoresis gel displayed DNA bands. This demonstrates that the actual procedure worked which verifies the actual results of the gels containing the genetic modification primers and LL601 primers. There were no DNA bands present within the LL601 and GMO gels. This is an indication that DNA was not amplified in any of these polymer chain reactions. If no DNA was amplified, the targeted sequence (in this case the LL601 or genetic modification sequence) was not present in the first place. Thus, because the LL601 and genetic modification electrophoresis gels did not display DNA bands, the rice samples which were tested did not contain any genetic modifications, including the LL601 modification.

These results have extremely significant implications. First, it is evident that the methods which have been taken to test for and eliminate the presence of LL601 rice have been sufficient. Because neither sample tested contained LL601 DNA, it can be assumed that rice in the local

Connecticut area is free of this genetic modification. It might also be inferred that the LL601 gene is out of the commercial rice system. Thus, these results are beneficial to the community in that they verify the testing methods the federal government has enforced. In addition, they eliminate some of the reason for consumers to question the safety of the products they purchase. So, it can be stated with certainty that these samples did not contain LL601 DNA modification. Accordingly, it can be assumed that the LL601 rice is no longer present within the local rice market.

However, if the results were positive for the LL601 DNA, the implications would have been significantly different. First, the presence of LL601 DNA in commercial rice would imply that testing is not being done sufficiently on commercial rice to check for the presence of genetic modifications. This would mean that the general public may be consuming genetically modified rice and have no knowledge that they are doing so.

The presence of LL601 gene in the DNA of the commercial rice could also raise the concern of cross cultivation. Even though the LL601 rice is not being grown commercially, it would still be contaminating non genetically modified rice. This would demonstrate the system's lack of ability to keep genetically modified organisms separate from non genetically modified organisms. Thus, the results, if the LL601 DNA was proved present in the experiment, would implicate that the procedures to keep non- commercial genetically modified organism genes out of the genes of commercial crops must be considered void. If the experiment did reveal the commercial rice to contain the PAT bar, such procedures would need to be reformed.

In addition, an LL601 gene positive sample could lead to the questioning of the safety of the rice the world consumes. The United States does export rice to countries across the globe.

Thus, if US rice still contains the LL601 gene, then so could the rice which is brought to different places all around the world. This would mean that people from United States all the way to Japan could be consuming a genetically modified organism.

So, although the sample did not contain the LL601 gene, it is entirely possible that all of these implications for a positive LL601 DNA result remain applicable. Only a few samples of rice were tested. Thus, the conclusions do not fully answer the question: is LL601 rice still present within the commercial market? It is true that the samples tested did not contain the LL601 modification. However, the millions of other grains of rice circulating within the system could still contain LL601 DNA. Thus, the extremely miniscule purview of this experiment is a significant limitation.

Furthermore, this experiment lacks a positive control. Many attempts were made to attain this positive control, a sample of actual LL601 rice. However, due to legal conflicts, it was impossible to get a hold of a positive control. So, the experiment had to be completed with merely a negative control, water. This is another significant limitation of the project.

Various other validity issues existed. Primarily, only one trial was completed. In addition, there were some flaws in the execution of this single trial. At one point in the procedure, the samples were dropped on the floor and mixed up. Nonetheless, the results contained some significance and supplied answers to the question of the project. However, the final results lack validity and confidence.

Accordingly, there are various improvements that can be made in the project's procedure. First, another trial can be completed. A wider variety of samples could be tested. More caution could be taken during the procedure. The PCR procedure is a lengthy and meticulous process

and it does require a careful hand and plenty of time. It is fairly impossible to obtain a positive control for the experiment. Thus, this is the one part that cannot be improved on in the future.

In conclusion, this project (although of course providing an answer to the question at hang) was beneficial in that it explored foreign procedures within the biological field. Thus, it provided, along the way, extremely significant learning experiences.

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