Evaluating the Accuracy of Labels for Genetically Modified Rice Products Sold in Canada

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Abstract

Nearly half of the world's population depends on rice (*Oryza sativa*) as its major source of calories. Thus, scientists have developed genetically engineered rice (genetically modified, GM) to increase production yield and enhance plant resistance to human and environmental factors. The genetic modification of food crops has previously been met with general public concern due to the threat placed on native plant populations. Inevitable cross-pollination transfers GM genes from GM crops, resulting in devastating consequences for the health and fitness of wild plant populations. Currently, there is little to no global regulation that provides guidelines for the labelling of GM rice. Our study aimed to test the accuracy of labelling practices in Canada for GM rice products. Our research used twelve rice samples, half of which were unlabelled and the rest with one of the two following labels: Canada Organic or NON-GMO Project Verified. DNA products were generated using DNA isolation and PCR amplification of three primers. Subsequently, the DNA was examined through gel electrophoresis for the presence of these genes: sucrose phosphate synthase (SPS), Cauliflower Mosaic Virus 35S promoter sequence (P35S), and *Agrobacterium tumefaciens* nopaline synthase gene (T-nos). The results of gel electrophoresis showed no detection of the banding patterns for any rice sample used. This was likely due to high levels of starch contamination.

Introduction

828 million people face food insecurity worldwide (Wijerathna & Pathirana, 2022). Rice (Oryza sativa) contributes to addressing food insecurity, with 50% of the global population relying on it as a major calorie source (Valluru et al., 2015). Genetic engineering boosts crop production by improving yields, resistance to herbicides and insecticides, nutritional enhancements, and increasing plants' resilience to environmental factors. However, a challenge remains: establishing consistent standards for the commercialization of GM crops and clear labelling guidelines.

The current labelling standards for GM crops range between 0-5% across different countries and may not be mandatory (Fraiture et al., 2016). The presence of certain approved GM crops differs across countries and may result in the accidental planting and transfer of

genetic material to the wild type, which can have devastating ecological effects (Lu, 2009). Once wild-type rice has inherited the GM material, it is nearly impossible to remove from their native populations and may decrease biodiversity, change fitness, and introduce traits affecting the native populations' survival (Lu, 2009). Standards for the planting and commercialization of GM crops must be monitored. As a result, this experiment aims to test the different labelling systems used in Canada. Numerous studies have been done to detect GM in rice crops with similar methods and have found ranges of 2.4% positive in Tehran (Safaei et al., 2019) to 20.4% across various markets in Saudi Arabia (Elsanhoty et al., 2013).

In Canada, there are two main labelling systems: Canada Organic and NON-GMO Project Verified. To be labelled Canada Organic, organic content must exceed 95% of the product (Government of Canada, 2021). NON-GMO Project Verified is certified by a non-profit organisation known as the Non-GMO Project, but is not mandatory for labelling in Canada. Products that carry this label offer to have their products tested by this organization to certify that the product is not genetically modified (The Non-GMO Project, 2022).

This experiment aims to detect the presence of GM in rice across the two labels used in Canada. PCR is used to detect the presence of GMOs (Fraiture et al., 2016). The screening targets common transgenic elements, namely P35S (35S promoter sequence in the Cauliflower mosaic virus) and T-nos (nopaline synthase terminator in *Agrobacterium tumefaciens*). Twelve different rice products were obtained from various grocery stores with different labels and brands. The expected results are that the products labelled as NON-GMO Project Verified or Canada Organic will have no bands present for P35S and T-nos. Additionally, it is expected that the products with no labels will have a band present for either P35S or T-nos. All of the rice samples are expected to have one band for SPS to indicate proper preparation and DNA presence in the samples.

Methods

Sample Labelling

Table 1: This table displays each of the 12 rice samples used along with their labelling system for the experiment.

Sample number: Rice Product	**GMO-Related Labels**	**DNA Isolation Tube Label**	**PCR Labels**
Sample 1: Ben's Original Bistro Express: Jasmine Rice (Family Size)	No label present	B5-S1	SPS: 5.1.1
			P35S: 5.1.2
			T-nos: 5.1.3
Sample 2: Ben's Original Bistro Express: Long Grain & Wild Rice Mushroom Flavour	No label present	B5-S2	SPS: 5.2.1
			P35S: 5.2.2
			T-nos: 5.2.3
Sample 3: Lundberg Family Farms: Wild Blend	NON-GMO Project Verified	B5-S3	SPS: 5.3.1
			P35S: 5.3.2
			T-nos: 5.3.3
Sample 4: Lundberg Family Farms: California White Jasmine	NON-GMO Project Verified	B5-S4	SPS: 5.4.1
			P35S: 5.4.2
			T-nos: 5.4.3
Sample 5: Lundberg Family Farms: California White Basmati	NON-GMO Project Verified	B5-S5	SPS: 5.5.1
			P35S: 5.5.2
			T-nos: 5.5.3
Sample 6: Floating Leaf: Wild Rice	NON-GMO Project Verified	B5-S6	SPS: 5.6.1
			P35S: 5.6.2
			T-nos: 5.6.3

Sample 7: Unico: Italian Style Rice	No label present	B5-S7	SPS: 5.7.1
			P35S: 5.7.2
			T-nos: 5.7.3
Sample 8: President's Choice Organics: Brown & Wild Rice	Canada Organic	B5-S8	SPS: 5.8.1
			P35S: 5.8.2
			T-nos: 5.8.3
Sample 9: Kokuho: Rose Brown Rice	No label present	B5-S9	SPS: 5.9.1
			P35S: 5.9.2
			T-nos: 5.9.3
Sample 10: President's Choice: Jasmine	No label present	B5-S10	SPS: 5.10.1
			P35S: 5.10.2
			T-nos: 5.10.3
Sample 11: President's Choice Organics: White Rice	Canada Organic	B5-S11	SPS: 5.11.1
			P35S: 5.11.2
			T-nos: 5.11.3
Sample 12: Minute Rice: Basmati	No label present	B5-S12	SPS: 5.12.1
			P35S: 5.12.2
			T-nos: 5.12.3

Preparing Samples

Twelve rice samples were collected from various grocery stores; Real Canadian Superstore, Whole Foods, and Save-On Foods with varying labels or no labels related to GMO content.

Each sample underwent a dual washing procedure in order to eliminate any starch content. Next, the rice samples were finely ground to a homogenous, mushy texture using a mortar and then transferred to individual Eppendorf tubes to about the 1.0 line. Then, 150 microlitres of lysis buffer was introduced to each individual tube.

DNA Isolation

The samples were ground with a toothpick until a homogenous mixture was obtained then 150 microlitres of cell lysis solution with proteinase K was added. Next the tubes were placed in an incubator set at 65 degrees Celsius for 15 minutes, with intermittent vortexing every 5 minutes, followed by being placed on ice for five minutes. Then 150 microlitres of Protein reciprocate Reagent was added to the tube, vortexed for ten seconds and centrifuged at maximum speed for 10 minutes. The supernatant was transferred to another labelled tube. Then, 500 microlitres of ice-cold isopropanol was added, inverted 30-40 times and centrifuged at maximum speed for 10 minutes. Then the isopropanol was carefully removed, followed by two ethanol washes where 500 microlitres of ethanol was added to each DNA pellet. Each ethanol rinse was followed by removing the excess ethanol with a pipette ensuring to not disrupt the pellet. All the samples were left with the cap open overnight.

Spectrophotometry

995 microlitres of sterile distilled water was placed into a cuvette along with 5 microlitres of DNA from the Eppendorf tube. After running a blank with 1000 microlitres of sterile water, the sample with the DNA was placed and measured.

PCR

Each sample received 30 microlitres of TE buffer and was resuspended in order to make a homogenous mixture. Then master mixes were then made for each respective primer. For the SPS primer the master mix included: 11µL Sterile distilled water, 5µL 50% glycerol,

1µL 10milliM forward primer, 1µL 10milliM reverse primer, 2.5µL 10X PCR buffer, 0.5µL 10mM dNTP, 1.5µL 25mM MgCL2 and 0.5µL Taq polymerase. For the P35S-cf3/cf4 and t-nos-118f/118r primers the master mix included: 10µL Sterile distilled water, 5µL 50% glycerol, 1µL 10milliM forward primer, 1µL 10milliM reverse primer, 2.5µL 10X PCR buffer, 0.5µL 10mM dNTP, 1.5µL 25mM MgCL2 and 0.5µL Taq polymerase. In preparation of the master mix, the chemicals were added from the highest amount to lowest amount. Then, 23 microlitres of master mix were transferred to a new PCR tube followed by 2 microlitres of DNA and placed on ice. Each specific primer had a different incubation time:

a. SPS
    i. 95 degrees Celsius for 5 minutes
    ii. 95 degrees Celsius for 30 seconds
    iii. 58 degrees Celsius for 45 seconds
    iv. 72 degrees Celsius for 75 seconds
    v. Repeat steps ii to iv 35 times
    vi. 72 degrees Celsius for 8 minutes
b. P35S-cf3/P35S-cf4 and T-nos-118f/T-nos-118r
    i. 95 degrees Celsius for 5 minutes
    ii. 95 degrees Celsius for 30 seconds
    iii. 60 degrees Celsius for 30 seconds
    iv. 72 degrees Celsius for 30 seconds
    v. Repeat steps ii to iv 35 times
    vi. 72 degrees Celsius for 5 minutes

Samples were then left at 4 degrees Celsius overnight.

Gel Electrophoresis

To the PCR tubes 5 microlitres of 6X loading dye were added, mixing well by suspending the solution. Then 15 microlitres of each sample were loaded into 2% agarose gel, following a diagram previously made.

Ladder	5.1.1	5.1.2	5.1.3	5.2.1	5.2.2	5.2.3	5.3.1	5.3.2	5.3.3	Ladder	5.4.1	5.4.2	5.4.3	5.5.1	5.5.2	5.5.3	5.6.1	5.6.2	5.6.3
	Ladder	5.7.1	5.7.2	5.7.3	5.9.1	5.9.2	5.9.3		Ladder	5.10.1	5.10.2	5.10.3	5.11.1	5.11.2	5.11.3	5..12.1	5.12.2	5.12.3	

Table 2: Diagram for gel loading. Two rows were used in the agarose gel to load our samples. The diagram above depicts what is present in each well after loading.

The samples were first put on 50V for 10 minutes until they started to migrate out of the well then, the voltage increased to 120V for 1-2 hours.

Results

After gel electrophoresis, the appearance of any bands that indicated the presence of the three genes was noted. The first gene, sucrose phosphate synthase (SPS), encodes a protein that is responsible for photosynthetic sucrose synthesis (UniProt, n.d.). The SPS band is expected at 277 base pairs and should be present in all samples. SPS serves as a control to indicate whether or not PCR and gel electrophoresis was successful.

The second gene, P35S, or the Cauliflower Mosaic Virus 35S promoter sequence, is a common target to detect genetically modified rice and is found in most GMOs. This gene was derived from the plant pathogen Cauliflower Mosaic Virus and is used as a vector for foreign gene insertion into plants (Amack & Antunes, 2020). The P35S gene is expected at 123 base pairs for most GM rice samples that were tested and should not be present for non-GMO rice samples.

The final gene, T-nos, is derived from the *Agrobacterium tumefaciens* nopaline synthase gene. It serves as a polyadenylation site for vector constructs for plant transformation (Holden et al., 2010). This gene may be expected in conjunction with the P35S gene in GM rice samples and is around 151 base pairs. T-nos is not expected to be seen in rice samples that are non-GMO.

Table 3: Primer name, sequence, target and expected base pair (bp) length

Primer name	Sequence (5′ – 3′)	Target	Length (bp)
SPS-F	TTG CGC CTG AAC GGA TAT	SPS	277
SPS-R	GGA GAA GCA CTG GAC GAG G	SPS	277
P35S-cf3	CCA CGT CTT CAA AGC AAG TGG	P35S	123
P35S-cr4	TCC TCT CCA AAT GAA ATG AAC TTC C	P35S	123
T-nos 2-5’	GTC TTG CGA TGA TTA TCA TAT AAT TTC TG	T-nos	151
T-nos 2-3’	CGC TAT ATT TTG TTT TCT ATC GCG T	T-nos	151

Results of gel electrophoresis are noted in Table 2. For our experiment, all results were inconclusive, meaning there were no distinct bands and only smearing for each sample in the gel.

Table 4: Results for detection of SPS, P35S, and T-nos in Rice Samples

Sample number	SPS	P35S	T-nos
1	Not detected	Not detected	Not detected
2	Not detected	Not detected	Not detected
3	Not detected	Not detected	Not detected

4	Not detected	Not detected	Not detected
5	Not detected	Not detected	Not detected
6	Not detected	Not detected	Not detected
7	Not detected	Not detected	Not detected
8	Not detected	Not detected	Not detected
9	Not detected	Not detected	Not detected
10	Not detected	Not detected	Not detected
11	Not detected	Not detected	Not detected
12	Not detected	Not detected	Not detected

Discussion

The experimental goal was to determine if each rice sample was genetically modified or not, and if that matches with the labels on the product. We aimed to accomplish this goal through DNA extraction, PCR, and gel electrophoresis. We expected to see the products labelled with NON-GMO Project Verified or Canada Organic would have no bands for P35S or T-nos, and one band for SPS. We expected the products with no labels to have one band for SPS and at least one band for either P35S or T-nos. Following the completion of gel electrophoresis, we determined the results were inconclusive for all samples. There were no distinct bands for any samples at any expected base pair lengths (SPS: 277, P35S: 123, T-nos: 151). The probable cause is starch contamination. Our team took precautions to remove starch such as thorough rinsing of the rice, however it was likely not completely removed. Discussed below are potential errors to explain the inconclusive results.

An error in the DNA isolation process is inadequate removal of starch. During rice soaking on Day 1 of our experiment, two distinct challenges emerged. Firstly, we noticed a notable concentration of starch on the surface of rice grains, identified by the milky water present during washing. To address this, we washed the rice samples repeatedly. The second

challenge involved the complexity of extracting DNA from plant cells due to the physical properties of their cell walls. Our strategy included grinding the rice with a mortar to rupture the cell walls, followed by soaking. This procedure was designed to soften the cells so genetic material could be extracted more successfully. Despite these precautionary measures, the starch was likely not completely removed, resulting in contamination and inconclusive results.

To determine if there's DNA in our samples, we used spectrophotometry, looking at the absorbance at 260 nm and 280 nm. A 260nm/280nm ratio of 1.8 signifies DNA is pure, indicating the absence of any contamination. Values less than 1.8 indicate protein contamination, while values above 1.8 suggest RNA contamination. We performed spectrophotometry on three of our samples. We determined DNA was present with RNA contamination in sample 1, indicated by a ratio of 2.3. Sample 12 had a ratio of 1.78, indicating the presence of DNA with slight protein contamination. Sample 8 results revealed no DNA was present, leading us to exclude this sample from further analysis. Due to the limited scope of our spectrophotometry and one sample lacking DNA, there's a possibility that other samples may lack DNA due to an error in the DNA isolation process. In future experiments, it could be beneficial to perform spectrophotometry on all samples to determine DNA presence.

Our investigation revealed that agarose gel electrophoresis is a valuable tool for identifying common issues associated with PCR (Millipore Sigma. (n.d.)). After completing gel electrophoresis, the results revealed the absence of distinct bands for any of the rice samples. Given this, several potential reasons are considered, including a high primer annealing temperature, low or unbalanced primer concentration, poor DNA template quality, and contamination of reaction tubes (Millipore Sigma. (n.d.); New England Biolabs. (n.d.)). In the event of repeating this experiment, there are solutions available to prevent the errors

that may lead to the absence of distinct bands (Millipore Sigma. (n.d.); New England Biolabs. (n.d.)) For instance, to address a high primer annealing temperature, consider reducing it in increments of 2°C (Millipore Sigma. (n.d.); New England Biolabs. (n.d.)). If the primer concentration is low or unbalanced, ensure it falls within the recommended range and that both PCR primers have equal concentration (Millipore Sigma. (n.d.); New England Biolabs. (n.d.)). Poor DNA template quality may result from impure DNA, leading to inadequate amplification; consider using freshly prepared DNA or isolating the template using an alternative method (Millipore Sigma. (n.d.); New England Biolabs. (n.d.)). In case of contamination in reaction tubes, choose new reagents and tubes (Millipore Sigma. (n.d.); New England Biolabs. (n.d.)).

Some suggestions for improvement to this experiment include soaking the rice longer and re-evaluation of PCR technique. Soaking the rice longer will reduce starch contamination and allow for easier grinding. Additionally, consider excluding wild rice which has a hard shell. Another way to improve breakdown of rice is using stronger alternatives for the lysis solution. Furthermore, re-evaluating the PCR technique may reduce any potential errors during this step. Our team prepared the master mixes for the three primers individually which could have led to errors, for future experiments it would be beneficial if multiple team members work together to prepare each master mix.

Conclusion

Our team hoped to determine the validity of the labels on our rice samples and in general observe whether rice sold in Canadian supermarkets is genetically modified or not. The results of this experiment were inconclusive, likely because of starch contamination and other procedural errors. However, this experiment still provided valuable information to our team. We were able to practice many laboratory skills such as pipetting, data analysis, use of various machinery for PCR and gel electrophoresis, and more. Also, through background

research, our team determined the labelling requirements in Canada for GMO and non-GMO products need improvement. Currently there are no requirements for products to be labelled, it is voluntary. Additionally, the use of the word ‘organic’ in Canada is not heavily restricted which can be misleading to consumers as products with low organic content can still incorporate the word into their packaging or advertising. These types of experiments that look to validate the accuracy of labels and uncover the truth of unlabelled products are essential to bring forward information so consumers can make informed decisions about the products they purchase.

Acknowledgements

We would like to first acknowledge that we are learning on the traditional, ancestral and unceded territory of the xwməθkwəy̓əm (Musqueam) peoples. We would also like to thank UBC for providing the opportunity to take this course and conduct this research. Our sincerest gratitude also extends to the entire teaching team for this course. We are grateful for the guidance of Dr. Celeste Leander who assisted with making the procedure as well as guiding us in the lab. Additionally, thank you to Mindy Chow for assisting us with our materials list, ordering our equipment, proofreading our procedure, and conducting additional tests for our experiment. We are also very appreciative of our Teachers Aids, Tessa Blaunchard, for providing information and instructions on formatting and writing a scientific paper, and Magnus Macaulay, for aiding us throughout the learning processes of key elements of our research such as DNA isolation, PCR, and gel electrophoresis..

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