Go Bananas for Kiwi and Papaya

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Funded by the Thompson Family Foundation

Abstract:

This experiment studied the possible evolutionary relationship between bananas, green kiwi, and papaya in both an American wholesale market and an Asian market. Due to similar characteristics in these fruits such as: containing digestive enzymes, having black seeds at their core, and being common allergies for people and giving similar allergic reactions, we hypothesize that these fruits have an evolutionary relationship to each other. In order to test our hypothesis we took a sample of each fruit and isolated and amplified the rbcl sequence in chloroplast DNA by polymerase chain reaction (PCR). We confirmed that we isolated DNA from each sample by running the samples through gel electrophoresis, and then sending the samples to the DNA Learning Center for DNA sequencing. Our results found a high percentage of similarities between the rbcl sequence of the chloroplast DNA of each fruit showing a relationship between each sample of fruit, proving that these fruits may have an evolutionary relationship.

Introduction:

The importation of fruits from areas outside of the United States has introduced new allergens to the populations of people living in America. Being so culturally diverse, New York City has a variety of tropical fruits in its many markets, including bananas, green kiwi, golden kiwi, and papaya. A common factor of these specific fruits that we would like to analyze is the fact that when a person is allergic to one of these fruits they are likely to have an allergy to another one. Symptoms of an allergic reaction to a banana can include an itchy mouth and throat, rash (urticaria), skin or mucosal swellings (angioedema), and in rare cases narrowing of the throat, wheezing, and even collapsing. Mild allergic reaction symptoms to a kiwifruit can include an itchy mouth and rashes in the areas where the fruit made contact with skin. However, in severe allergic reactions to kiwifruit, some people can experience anaphylaxis, where tingling in the mouth and throat lead to swelling in the tongue, lips, or throat, and difficulty breathing. Allergic reaction symptoms to a papaya fruit can lead to atopic sensitization in the gut mucosa with symptoms of oral itching, hives, shortness of breath, and even severe gastrointestinal shock. The fact that there is a common allergy to all of these fruits indicates that there is a similar molecular quality that triggers a response. Because of the common threads in these fruits, are they related through a shared ancestor?

Materials & Methods:

Samples of each of the following were purchased from an American wholesale market and an Asian market in New York City: green kiwi, golden kiwi, banana, and papaya. Upon attaining our samples we documented each sample accurately, which included taking pictures of the specimen and entering all of the data collected in the barcode project sample database. We brought all collected samples to our school lab and prepared the samples for analysis.

We cut small samples from each of the specimens that were about 10 mg and preserved the rest of the samples by keeping them frozen at -20 C in separate, labeled bags. We took precautions not to cross-contaminate our samples by using different tools for each sample. We then placed the samples in separate, labeled centrifuge tubes. We added 300 µL of lysis solution to each tube which dissolves membrane-bound organelles so that DNA can be accessible and forcefully twisted with a clean plastic pestle on the inside of the tube for at least 2 minutes to grind the tissue until the sample is ground into fine particles. We then incubated the tubes in a water bath at 65° C for 10 minutes. Then, we placed the tubes in a balanced centrifuge, with its cap hinges pointed upward for one minute at maximum speed. After that, we transferred 150 µL of each of the supernatants into new, labeled tubes. We added 3 µL of silica resin to each of the new tubes and ensured that it was mixed well with the supernatant by pipetting up and down. The silica resin binded to the DNA and separated it from the rest of the solution. The closed tubes were then incubated for 5 minutes in a water bath at 57° C. To isolate the resin pellet, we placed the tubes in a centrifuge for 30 seconds at maximum speed. Using a micropipette with a fresh tip, we removed all the supernatant, without disrupting the resin pellet at the bottom of each tube.

In order to remove contaminants from the samples, we added 500 µL of ice-cold wash buffer to each pellet and resuspended the silica resin by pipetting up and down. The tubes were then placed in a centrifuge for 30 seconds at maximum speed. We then used a micropipette with a fresh tip to remove the supernatant. 500 µL of ice-cold wash buffer was added to the pellets and resuspended the pellet by pipetting up and down. We placed the tubes in a centrifuge for another 30 seconds. Then, we removed the supernatant using a micropipette with a fresh tip. To separate the rbcl sequence of the chloroplast DNA from the silica resin, we added 100 µL of distilled water to the resin pellet and mixed it well by pipetting up and down. The tubes were incubated at 57° C for 5 minutes and placed in a centrifuge for 30 seconds. We then transferred 50 µL of the supernatant to a new, labeled tube. We stored the samples at -20° C until we were ready to begin the PCR reaction.

In order to make gel electrophoresis we sealed the ends of a gel-casting tray and inserted a well-forming comb, poured 2% pre-stained agarose solution into the tray to a depth that covers about ⅓ of the height of the comb teeth. We allowed the gel to solidify for about a half-hour. After the gel solidified, we placed the gel into the electrophoresis chamber, removed the combs, and added enough 1xTBE buffer to cover the surface of the gel and fill the wells. Using a micropipette with a fresh tip, we loaded 5µL of each PCR product to the prepared wells. We then stored the remaining 20µL of the PCR at -20 degrees Celsius, until we were ready to submit our samples for sequencing. The gel ran for about 30 minutes at 130V. We then viewed the gel using UV light and took a picture of the results.

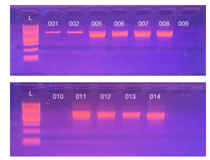
Next, we sent our prepared samples to be sequenced and uploaded onto the DNA subway website. When our sequences were available, we analyzed them on the website and determined the extent of evolution between the fruits and the genetic differences and similarities between the samples.

Results:

According to the gel electrophoresis results, all of the samples showed the presence of the rbcl sequence of chloroplast DNA except for sample 010 (Asian banana) so it wasn't able to be sequenced. Sample 009 (Asian banana) had low sequencing quality which means that there might be some errors in the rbcl sequence results and analysis. The sequenced results show a strong relationship between all of the fruits. The lowest sequence similarity percentage is 77.71. There is a 100% sequence similarity between all of the papaya samples and between all of the green kiwi samples. The banana samples have the lowest sequence similarity percentage compared to the kiwis and papayas. The phylogenetic tree (Figure 4.) shows that bananas evolved from their common ancestor before kiwis and papayas did.

American Market:

Asian Market:



Figures 1&2 Gel results

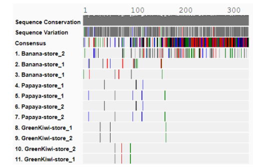


Figure 3. DNA Subway alignment viewer

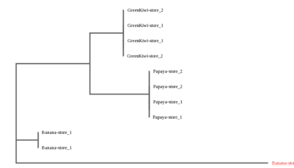


Figure 4. Phylogenetic Tree (ML)

Sample #	Fruit Type	Gel Results	Sequencing Quality
KXQ-001	Banana (1)	DNA present	Good quality
KXQ-002	Banana (1)	DNA present	Good quality
KXQ-003	Golden Kiwi (1)	n/a	n/a
KXQ-004	Golden Kiwi (1)	n/a	n/a
KXQ-005	Green Kiwi (1)	DNA present	Good quality
KXQ-006	Green Kiwi (1)	DNA present	Good quality
KXQ-007	Papaya (1)	DNA present	Good quality
KXQ-008	Papaya (1)	DNA present	Good quality
KXQ-009	Banana (2)	DNA present	Low Quality
KXQ-010	Banana (2)	No DNA	n/a
KXQ-011	Papaya (2)	DNA present	Good quality
KXQ-012	Papaya (2)	DNA present	Good quality
KXQ-013	Green Kiwi (2)	DNA present	Good quality
KXQ-014	Green Kiwi (2)	DNA present	Good quality
KXQ-015	Golden Kiwi (2)	n/a	n/a
KXQ-016	Golden Kiwi (2)	n/a	n/a

Key:
Store **1** = American Market
Store **2** = Asian Market

	C	1	2	3	4	5	6	7	8	9	10	11
C	-	78.36	94.42	94.31	96.68	96.69	96.67	96.69	97.85	98.03	98.07	98.01
1	78.36	-	80.35	80.35	77.71	77.71	77.71	77.71	77.13	77.13	77.13	77.13
2	94.42	80.35	-	100.00	91.20	91.24	91.18	91.24	92.57	92.76	92.90	92.72
3	94.31	80.35	100.00	-	91.20	91.24	91.18	91.24	92.57	92.76	92.78	92.72
4	96.68	77.71	91.20	91.20	-	100.00	100.00	100.00	94.68	94.68	94.68	94.68
5	96.69	77.71	91.24	91.24	100.00	-	100.00	100.00	94.71	94.68	94.71	94.70
6	96.67	77.71	91.18	91.18	100.00	100.00	-	100.00	94.68	94.68	94.68	94.68
7	96.69	77.71	91.24	91.24	100.00	100.00	100.00	-	94.71	94.68	94.71	94.70
8	97.85	77.13	92.57	92.57	94.68	94.71	94.68	94.71	-	100.00	99.83	100.00
9	98.03	77.13	92.76	92.76	94.68	94.68	94.68	94.68	100.00	-	100.00	100.00
10	98.07	77.13	92.90	92.78	94.68	94.71	94.68	94.71	99.83	100.00	-	100.00
11	98.01	77.13	92.72	92.72	94.68	94.70	94.68	94.70	100.00	100.00	100.00	-

Figure 5. The Percentage of Similarities between DNA Sequences of Various Samples

Discussion:

The fruits that were analyzed in this experiment were papaya (*Carica papaya),* banana (*Musa acuminata)*, and green kiwi (*Actinidia deliciosa).* All of the fruits showed sequence similarities that were greater than 77%. These results show a high percentage of genetic resemblance and a strong relationship between all of the fruits. Therefore, we can conclude that there is an evolutionary relationship between these species. This experiment was conducted twice as we encountered an unexpected result. In the first attempt of this experiment, the gel electrophoresis results showed that there was DNA present in all samples, except for 002 (American banana), 007 (American papaya), and 009 (Asian banana). When sent to the lab for sequencing, however, the results came back inconclusive. A possible error was that the primer that was used was defective. At the time that we attempted our experiment for the second time, golden kiwis were not in season. Therefore, we decided to eliminate golden kiwi from the experiment. The American kiwi and the Asian kiwi showed a 100% similarity in their DNA. The American papaya and the Asian papaya showed a 100% similarity in their DNA as well. However, the American banana and the Asian banana only showed an 80.35% similarity in their DNA. After further research, we discovered that bananas are exceptionally diverse, as there are over 1,000 different types of bananas in the world ("8 things you didn't know about bananas"). This is known as intraspecies variation. Comparing the DNA of different banana varieties is further research for another project. Because of the differences between the rbcl sequence of the bananas from each market, we compared each of the fruits within each separate market. The American kiwi and banana showed a 92.57% similarity in their DNA and the American papaya and banana showed 91.20% similarity. The papaya and kiwi overlapped between the two markets and showed 94.68% similarity in their DNA. The Asian Market papaya and banana displayed 77.71% similarity in their DNA and the Asian kiwi and banana displayed 77.13% similarity. The phylogenetic tree (Figure 4.) exhibits the relationships between all of the samples. Comparatively, there is a relatively high percentage of sequence similarities between all of the species that were sampled. This might explain the common characteristics such as: having black seeds, containing digestive enzymes, and common allergies that people tend to have to all of these fruits. If a person is aware that they are allergic to one of these fruits, they may go for an allergy test to see if they have these common allergies and prevent themselves from potentially experiencing bothersome symptoms of an allergic reaction. A suggestion for future studies would be to prepare duplicate samples and use a different batch of primer on each sample duplicate, in order to have backup samples. The duplicate samples will also help in avoiding having to redo the entire experiment if conditions such as food supply have changed.

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Acknowledgements:

With deep gratitude to Mrs. Shulamith Biderman, Mrs. Ruth Fried, Mr. Jason Williams, The Cold Spring Harbor Laboratory, and Yeshiva University High School for Girls for helping us with our project.