Very ApPEELing DNA: DNA Barcodes of Different Types of Bananas

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Abstract:

We researched three types of Bananas from the genus Musa. The goal of our project was to find the differences and similarities in the chloroplast DNA of a cavendish banana, lady finger banana and plantain. Our hypothesis is that the different types of bananas will have many genetic similarities because they fall under the same genus, grow in the same way, and have similar peel structures.We predicted there will be differences among these species because they differ in size and taste. We amplified the DNA after grinding up the bananas by using rbcl primers. We made a gel electrophoresis of our samples and analysed the final results on the DNA subway website. The results were that the cavendish bananas and the plantains are more closely related than the lady finger bananas on the phylogenetic tree.

Introduction:

Scientists have started using DNA barcoding to find more genetic similarities within species. They take these new findings and create phylogenetic trees to diagram new ways the species are related. DNA barcoding is a way to identify, classify, and examine relationships between living things. This is done by ultimately comparing the sequences to other DNA sequences. Before DNA Barcoding, the way to classify species was by sight. This method was not always accurate, for example, dogs and wolves are the same species, genetically but look very different phenotypically.

There are many different types of banana-like fruits. These include cavendish bananas, lady finger bananas and plantains. All of these banana-like fruit are from the genus Musa. but from different species. Cavendish bananas are from the *Balbisiana* species, lady finger bananas are from the *Acuminata* species and plantains are from the *Paradisiaca* species.We want to find out if these organisms have any genetic similarities. We hypothesise that they will have genetic similarities because they are from the same genus, have similar growth patterns, and physically look similar. We will analyze our results by comparing and contrasting the DNA of the cavendish banana, lady finger banana and plantain and use this data to create a phylogenetic tree. The type of DNA obtained is chloroplast DNA. This is a form of cytoplasmic inheritance inherited only from the mother-maternal egg- and can't give any DNA evidence about paternal relationship, which can only be obtained from the diploid nuclear DNA of the plant.

Materials and Methods:

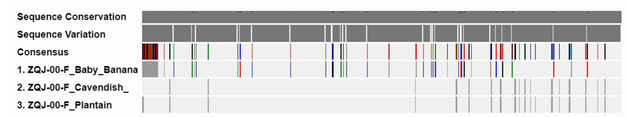
To perform our experiment we bought cavendish bananas, lady finger bananas and plantains in a store close to our school. The cavendish bananas will act as the control group in our experiment. The lady finger bananas and the plantains will be the experimental groups. We brought them to Yeshiva University High School for Girls and kept them there until we started our project. We started isolating the DNA from the plant by collecting seeds from the organism. We obtained three samples of each plant to make sure our results would be accurate. We mixed our samples with many solutions and centrifuged. In order to PCR, we added *rbc*L primers (rbcLaF / rbcLa rev) to each tube and allowed the beads to dissolve. We then placed our PCR tube in a thermal cycler that was programmed with the appropriate PCR protocol. After the thermal cycle, we stored the amplified DNA at -20 °C. The last step was to analyze the PCR products. We poured the 2% agarose solution into the tray to a depth that covers about one-third the height of the comb teeth and allowed the gel to solidify. Once it solidified, we placed the gel into the electrophoresis chamber and added enough 1x TBE buffer to cover the surface of the gel. We then carefully removed the comb and added additional 1x TBE buffer to fill in the wells and just cover the gel, creating a smooth buffer surface. We then loaded each sample from the previous step into our assigned wells with a ladder sample on the far left. We saw separation as the cresol red dye front moved at least 50 mm from the wells after we ran the gel for approximately 30 minutes at 130V. Lastly, we viewed the gel using UV transillumination and we photographed the gel using a phone camera. Once we determined this we sent our samples to Genewiz for sequencing and they uploaded our results onto the database. Then, using DNA subway we were able to analyze our results, first by cleaning the sequence data, using BLASTN to search a known database of sequences for matches. We then compared the samples and made a phylogenetic tree.

Results:

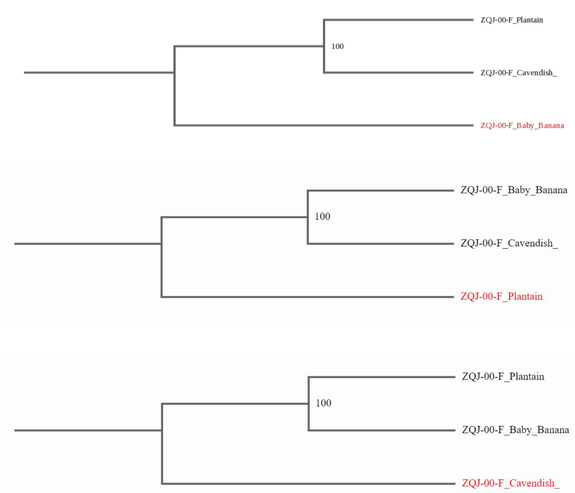
Once the DNA sequences were uploaded on the database we reviewed them.. We used BLASTN to search a known database of sequences for matches. The top three matches for the Cavendish Bananas were *Musa environmental sample*; *Musa, velutina* and *Musa, acuminata* with two mismatches. The plantains had very similar results to the cavendish bananas with the top three matches being *Musa environmental sample*, *Musa, velutina*, *Musa, acuminata* with two mismatches. The lady finger bananas' top three results were *withania, 'Ashwagandha'* with 18 mismatches, *Solanum, lycopersicum* and *Datura, stramonium* with 19 mismatches. Next, we aligned with MUSCLE to generate the multiple sequence alignments. Because we assume they all share a common ancestor, the alignments of DNA sequences show mutations between the different species.

Tables and Figures:

Location of sample collection.

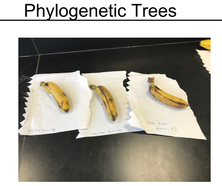


MUSCLE alignment

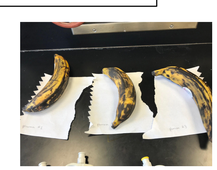


Phylogenetic Trees

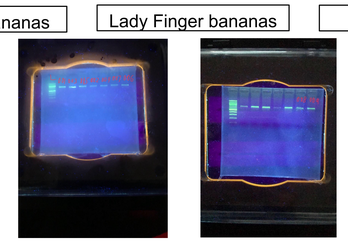
Cavendish bananas



Lady Finger bananas



Plantains



Gel Electrophoresis

Discussion:

From the results of BLASTN the banana sequences were analyzed. The Cavendish bananas and plantains had identical matches, meaning they are very closely related. We expected these two organisms to be closely related because the top matches had some sort of connection to bananas. Although, we were also expecting lady finger bananas to be as closely related as the others the closest matches were tomatoes and eggplants, which is very unexpected. Then we analyzed the phylogenetic tree made by Phylip NJ. This tree shows that they all have one common ancestor but the cavendish and plantains are more closely related than the lady finger bananas. A possible reason for this is, because lady finger bananas aren't as phenotypically similar to cavendish bananas as plantains, they aren't as similar genetically. Our hypothesis is proven because according to this, each of the bananas have very genetic similarities. Additional research by repeating the experiment with a bigger sample size can confirm these results.

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