



It's a Grape Big World: Determining the Differences Between Red, Green, and Black Grapes

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Abstract

Grapes are an extremely important part of the cultures and religions of the people who live in New York City, so we wanted to learn more about how different colored grapes differ genetically from each other. We hypothesized that there would be a genetic difference between different cultivars of grapes. Samples of red, green, and black grapes were obtained and various solutions were added to the samples to isolate the DNA of the grapes in preparation for PCR. The samples were sequenced and the data was analyzed using DNA Subway. A phylogenetic tree was generated, but the results were not as expected. The tree did not show that the samples from the grapes of the same color were the most closely related, even though those samples were obtained from the same grape. The most likely reason for this occurrence is because of the low quality and slight errors in the DNA sequences found on DNA Subway. The experiment should be repeated in order to obtain more accurate results. We were not able to use our data to support or reject our hypothesis that differences exist between different cultivars of grapes, but further experimentation may reveal whether this hypothesis can be supported.

Introduction

New York City is a cultural melting pot, with as many as eight hundred languages spoken throughout the five boroughs and approximately 36% of its population foreign-born (Dan 2014). In addition, more than half of the people living in New York City are affiliated with a religion, including Christianity, Islam, Judaism, Hinduism, and Buddhism (Jones 2015). Although these cultures and religions have very different customs and values, wine is an element common to many cultures and religions. Since grapes and wine play a significant role in the religion and culture of New York City residents, we wanted to learn more about different types of grapes. Often, all types of grapes are lumped together into one category; however, there are actually thousands of different kinds of grapes. In doing this Urban Barcoding project, we aimed to investigate and display the genetic variation between three cultivars of grapes: red, green, and black, and by doing so we learned more about the grapes that are so crucial to our religion and culture. We hypothesized that there would be differences between the DNA sequences of red, green, and black grapes, and we examined a region of the chloroplast gene *rbcL*, RuBisCo large subunit, when we barcoded our grapes.

Materials & Methods

Red, green, and black grapes were purchased from a supermarket in Flushing, NY, on February 20, 2017 since it was not possible to obtain grapes from a winery as it was winter at the time that our samples were obtained. Three samples each of red, green, and black grapes were originally taken by cutting a piece of the flesh of the grape. DNA was not found in the gel electrophoresis, and it was recommended to use the skin of the grape instead. Three samples of skin from each grape were then collected. Lysis solution was added to each tube and a plastic pestle was used to crush the samples and help break open the cells. To separate nucleic acids, silica resin was added to each tube. After pouring out the supernatant, wash buffer was added to the pellet to remove residue of protein and RNA and the solution was mixed to resuspend the silica resin. The supernatant was again removed and wash buffer was added to the pellet. Then distilled water was added to the pellets and the tubes were centrifuged to pellet the resin. The DNA was transferred by removing the supernatant and putting it into PCR tubes. Samples were amplified in the thermal cycler that was programmed with a PCR protocol. Then the samples were mixed with loading dye and run through gel electrophoresis to confirm that DNA was present in the solution. Eight out of nine of the samples were sequenced (one of the samples of the red grape was not sequenced). Once sequencing was complete, the data was analyzed using the Blue Line of DNA Subway, where it was viewed using Sequence Viewer and trimmed using Sequence Trimmer. Next, pairs were matched to each other using Pair Builder and the DNA was edited using Consensus Editor. BLASTN was used to find sequences in the database that have similar sequences to the sequences of our samples, and Reference Data was used to add common plants as a reference. Finally, data was selected and analyzed with MUSCLE before being made into a phylogenetic tree using PHYLIP NJ and PHYLIP ML.

Tables & Figures

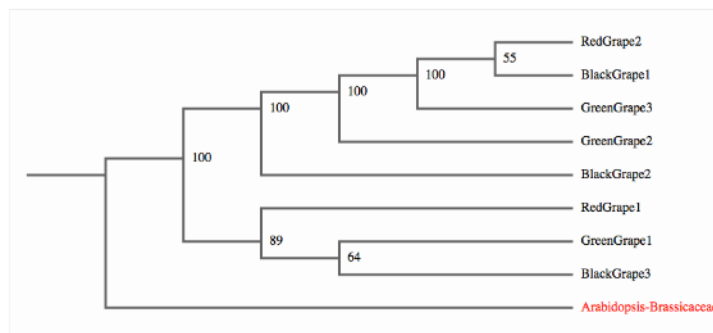


Figure 1: Phylogenetic tree showing the relationships between different cultivars of grapes

Results

The grape samples of the same color did not seem to be closely linked based on the phylogenetic tree generated by PHYLIP NJ.

Discussion

At first glance, our results deviated from what we expected. We hypothesized that the grapes would be grouped by color in the phylogenetic tree since all of the same colored grapes were obtained from the same grape. The samples of red grape were obtained from one red grape, the samples of green grape were obtained from one green grape, and the samples of black grape were obtained from one black grape. Since the samples taken from identical grapes should have the same DNA, the expectation was that the grape samples would be organized by color in the phylogenetic tree. However, when we interpreted the phylogenetic tree, the samples from identical cultivars were not on the same node. The most likely reason for this occurrence is because of the low quality and slight errors in the DNA sequences found on DNA Subway. Specific evidence of this was found with the green grape samples. One of the samples has a high Phred score, while the other samples have low quality. In the case of the red grape, one sample has a good quality Phred score, and one does not. The Phred scores of the black grape samples also varied. Even a slight variation in the genetic sequence could create a vast change in the identity of the sample. These small errors account for the deviations in the phylogenetic tree.

References

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