Sac-like Structures in *Callicarpa saccata*

Tiffany Netz

Tsukaya Laboratory

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Introduction:

The genus *Callicarpa* is comprised of many different species. One of these species, *Callicarpa saccata,* stands out from the rest due to characteristic structures located at the base of the leaves. Structures similar to those found in this species have been found in a genetically modified blade-on-petiole (bop) mutant of the *Arabidopsis thaliana* species. Because of the similarities to this bop mutant we hypothesize that there may be a mutation within the BOP gene of the *Callicarpa saccata* species. One of the BOP homologs for the species has already been isolated and sequenced. This experiment is being conducted to isolate and sequence the second BOP homolog. The process to do this requires finding the correct PCR procedure and primer pairs to amplify the desired nucleotide segment. Once the segment is amplified the segment needed to be sequenced. The sequence then needed to be compared to previous experiments to confirm what was found was not the same as those found formerly.

Purpose:

The characteristic structures located at the base of the leaves of *Callicarpa saccata* are the focus of this experiment. These structures are sac-like and often inhabited by ant species that, it is believed, have a mutualistic relationship with the plant. In exchange for nutrients, the ants receive shelter. The purpose of this experiment is to genetically determine how these structures are developed. Although no other *Callicarpa* species show similar structures, there are morphological similarities found in the leaves of *Arabidopsis thaliana* BOP mutants. Because of this, the focus of our experiment was on the BOP homolog or *Callicarpa saccata.*

Background:

Arabidopsis thaliana is a model plant species. Previous experiments have produced mutant species that demonstrate how altering different sections of the genomic DNA can cause alterations within the plant. When alterations of a gene in *Arabidopsis thaliana* produce traits similar to those in other plant species the hypothesis can be made that the same gene was responsible for the trait's appearance.

The *Callicarpa saccata* species is a native plant of Borneo. This species is the only species of *Callicarpa* that has the structures at the base of the leaves. The similarities between the leaves of this species and those of the bop mutants in *Arabidopsis thaliana* caused us to believe the BOP homologs may be responsible. Previous experiments have identified one of the *Callicarpa saccata* BOP homologs, but our goal was to sequence the second one.

Methods:

From previous experiments we already had access to *Callicarpa saccata* genomic DNA. We used this DNA to run a nested touchdown PCR using degenerate primers. Through a series of heating and cooling cycles a PCR program uses primers to amplify a specific segment from template DNA. This makes multiple copies of the desired fragment found between the primers. In the nested PCR method, the products of one PCR are then used as template DNA for a second PCR. In our initial test, we used a drop cycle of 0.5 degrees Celsius per cycle. When this PCR did not succeed in producing bands we tried reducing the number of cycles from 25 to 20. When this also did not work, we tried using 1/100 PCR product as template DNA instead of 1/10 PCR product. When this also did not work, a new set of primers were used. When none of these primer pairs produced bands, we tried changing the temperatures within our PCR program and reducing the drop per cycle to 0.4 degrees Celsius. This also resulted in no bands being produced. The next PCR program tried increased the number of cycles back

to 25 and changed the primers pairings. This pairing did result in bands, but they were very light. Because of this, another nesting was done using the same PCR program and primers, but utilizing the previous PCR results as template DNA. The results were clear bands produced at 500 base pairs during electrophoresis.

Once bands were created, the next step was to extract them from the agarose gel and purify the samples. A FastGene gel/PCR extraction kit was used to do this. The bands were cut from the gel and solutions from the extraction kit were used to obtain the DNA. Once the DNA was extracted a sequencing PCR was used to amplify the DNA further. This solution was then run within a sequencing machine to display the nucleotide sequence for the DNA segment.

Results:

Once the amplified DNA is sequenced the genetic code can be seen:

TGGGGGGCCCGGACTTCGCAATCGGGCCTGGACCCGTTGAGGATGGGCCCAGGGAGTGGCTCTCCGAGAGGGCC
CATCGCACAGGTGATACCGGTGAATTCAGTGGGATATGAGGTTTTCTTGTTGATGCTGCAGTTCTTGTACTGTGGG
CAGGTCTCGATTGTACCCCAGAAGCATGAACCACGACCTGATTGTGGGGAGAGGGGTTGCTGGCACACACATTGC
TCCTCCGCCGTTGATCTTGCTGTCGAAACTCTTGCAGCCGCTAGATCTTTTGGTGTGGAACAGCTTGCTTTGCTCAC
TCAGGTTTTCGATGTTTTCATGTCTATATTTTTCATAAAAATTGCCACTGTTTGTGAATTTCCTCTGTCCTCTTTGTTTC
TCTCTAAAAGACATGTATGTTATGAGTGGTTGTCATGTGTTTGAACTGATTGGGCTTTGGAACTTGGATCAAGATTT
TCAGGTTAATAAAGATACAGTGAATTTCAGTTTTTTATACATATTTTTGTATAAAAGTTCTGTGGGATGGTTCGTCA
GAGACCAGGAAATTCTTGGTGGTTTCTTGACCACGTTTCTTGAAATGAAATGGTTGTGATCTCAAAGAAATATAGT
ATTTTTCTTCTTTTTGGGATGGATTCTTCTGTTTTAAAATTGCATACTTTCGGCCCCTTATGTACAACCTTGCCTTCTT
CTCCTCCTCCTCCTCCCCCCTTTTTTTTTGGGT

The resulting sequence was run through NCBI's nucleotide sequence blast. This process compares the sequence we extracted to previously sequenced segments of other plant species. This search resulted in a match to the *Nicotiana tabacum* BOP3 mRNA. This result means that the DNA segment that we isolated and sequenced is one of the BOP homologs.

To make sure this was the second homolog that we were looking for and not the homolog previously found, a similarity comparison was run between the nucleotide sequence we found with the sequence previously identified. The comparison showed many dissimilarities between the two, meaning that the two were not the same and that we had found the second BOP homolog.

Conclusion:

This experiment successfully found a portion of the second BOP genome for the Callicarpa saccata species, however the sequence needs to be extended to have the whole homolog. Additional PCR's need to be conducted to isolate and sequence the rest of the BOP homolog. These experiments should follow similar protocols, but utilize new primers to extend the range of the PCR. Further research also needs to be done to compare these genomes to those of closely related Callicarpa species to see if there is a mutation in the BOP genes that is causing the sac-like structures.