Identification of a Histone Lysine Methyltransferase (ATX3) in Common Bean (*Phaseolus vulgaris*)

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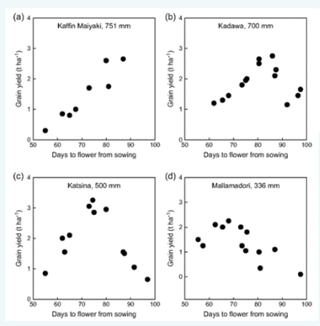
1. Background Research

- Common bean (*Phaseolus vulgaris*) is one of the most important legumes consumed worldwide
  - Annual production of dry beans: 15 million tons [3]
  - Primary source of dietary protein for over half a billion people, mainly in Latin America [3]
- Climate change has been shown to reduce the yield of annual crops due to earlier flowering [2] (Figure 1)

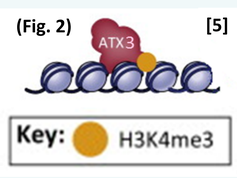


(Figure 1) **[2]**

This graph displays how plants that flower earlier produce a smaller yield.

- Epigenetics is the study of gene expression without changes in the DNA sequences [9]
- The study of epigenetic modifications in common bean will allow for the understanding of how important traits in common bean are regulated, such as flowering time
- Histone methyltransferases are one of the many epigenetic factors involved in gene expression
- ATX3 adds a tri-methyl group to the fourth lysine of the third histone (H3K4me3) [5]
  - This alters gene transcription for the following genes: LTP, AG, AP1, AP3, PI, FLC, AB1, FUS3, LTP3, and AT2S3 [8]
  - These genes have been associated with flowering time in *Arabidopsis thaliana* [8]

(Fig. 2) [5]



(Fig. 3) [5]

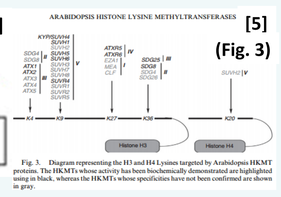
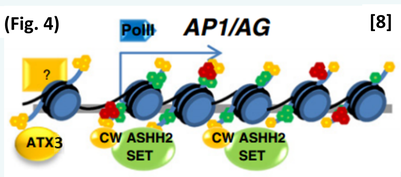


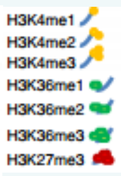
Fig. 3. Diagram representing the H3 and H4 Lysines targeted by Arabidopsis HKMT proteins. The HKMTs whose activity has been biochemically demonstrated are highlighted using in black, whereas the HKMTs whose specificities have not been confirmed are shown in gray.

The two pictures to the right display the function of the gene ATX3, and where it alters gene expression.

(Fig. 4)



[8]



Maintenance of correct tissue-specific expression of floral organ identity genes like AP1 and AG is needed for flower development.

2. Outline

- The sequences of ATX3 were found by performing a BLAST search in the model species, *Arabidopsis thaliana,* using the NCBI and Phytozome databases.
- The molecular biological techniques utilized in this research include DNA isolation, RNA isolation, cDNA synthesis, polymerase chain reaction, and agarose gel electrophoresis.
- The expected results consist of the amplification and cloning of the ATX3 gene from Common Bean.
- Future research may include sequencing and differential expression.

3. Objective

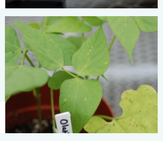
The goal of this research is to amplify the ATX3 gene from Common Bean cDNA.

4. Material and Methods

- **Genotypes: Sierra and Olathe (Figures 5-6)**
  - Sierra is the disease-resistant, cultivated genotype [4]
  - Olathe is the non-resistant, wild genotype [4]
- **DNA isolation:**
  - DNA was extracted from Sierra leaf tissue
  - DNA was used as a template for PCR
  - DNA amplifies the genomic sequence

(Fig. 5)

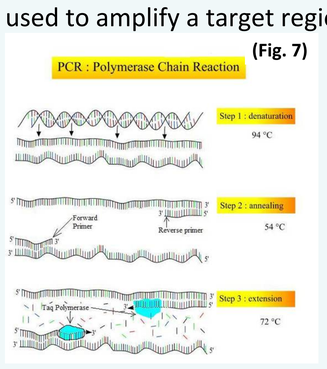
- **RNA isolation:**
  - RNA was extracted from Sierra leaf tissue
  - RNA was used to synthesize cDNA



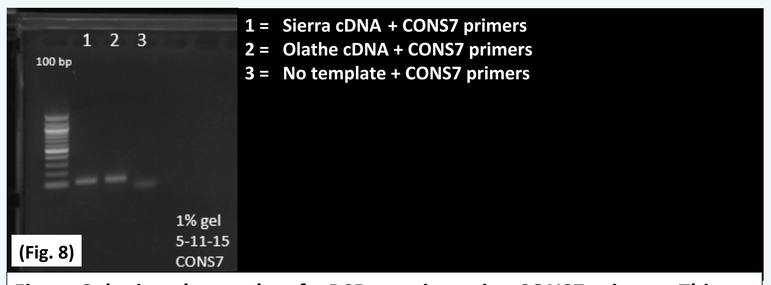
(Fig. 6)

- **cDNA synthesis:**
  - RNA was used to synthesize complementary DNA
  - cDNA was used as a template in PCR
  - cDNA amplifies the coding sequence
- **Designing Primers:**
  - The sequences obtained from the NCBI and Phytozome databases are used to design primers that amplify ATX3
- **Polymerase Chain Reaction (Figure 7):**
  - Primers are used to amplify a target region

(Fig. 7)

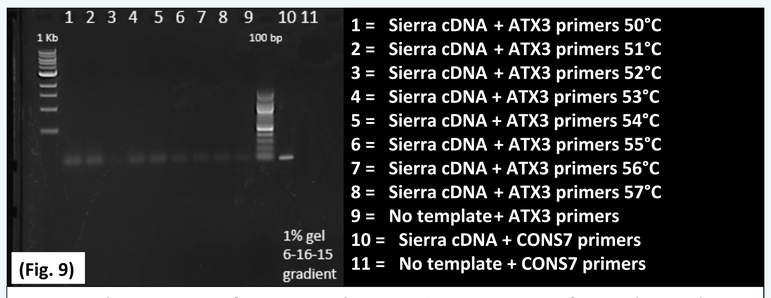


5. Results



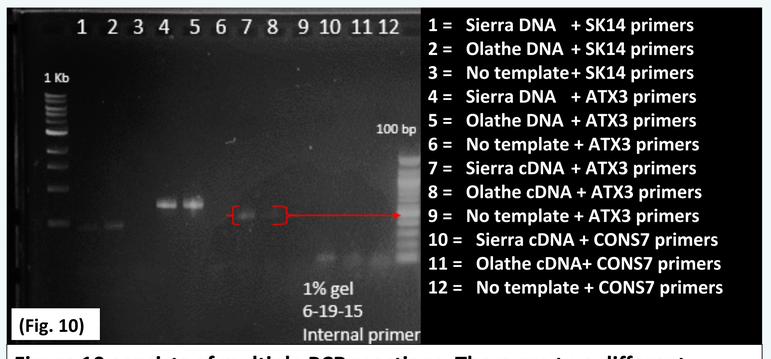
(Fig. 8)

Figure 8 depicts the results of a PCR reaction using CONS7 primers. This gene has successfully been amplified. This gene is known as a "housekeeping" gene. This means that It is found in all cells and is needed for basic cell functions. The purpose of this gel was to validate the reagents used in other reactions; it is the positive control.



(Fig. 9)

Figure 9 depicts one of many gradient PCR reactions performed in order to discover the best annealing temperature of the ATX3 primers used. There has been no amplification using the full-length ATX3 primers, but the positive control has amplified. This suggests that the issue is not in the reagents and template, but in the primers.



(Fig. 10)

Figure 10 consists of multiple PCR reactions. There are two different positive controls. The SK14 primers validate the genomic DNA, while the CONS7 primers validate the cDNA. The amplicons in the red brackets are from internal primers that are meant to amplify a 509 base pair region of ATX3. According to the gel, the amplicon is of the correct size. This means that the gene ATX3 is indeed in Common Bean. The amplicons from wells 4 and 5 are using the same primers, but with genomic DNA instead of cDNA. It is larger in size because the genomic DNA contains both introns and exons, while the cDNA has only exons.

6. Discussion

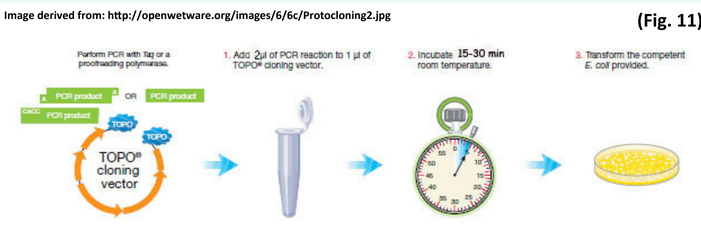
- There has not been any successful amplifications of the gene ATX3.
- However, this research proved that the gene is present in common bean because the internal primers amplified the appropriate segment of the gene.

7. Future Research

Future research may include redesigning primers to amplify the full length CDS of ATX3. The gene could then be cloned (figure 11). Another project may include the study of differential expression through quantitative PCR, so that its relevance to flowering time can be affirmed by measuring its expression in the different tissues of the plant.

Image derived from: http://openwetware.org/images/6/6c/Protocloning2.jpg

(Fig. 11)



The picture above depicts the protocol that would have been used for cloning ATX3. The process consists of the following steps:

1. Amplification of the gene ATX3 through PCR.
2. Gel extraction and purification of the amplified gene.
3. Insertion of the purified gene into the cloning vector (depicted above).
4. Insertion of the vector into the competent cells (depicted above).
5. Growth of cell cultures.

This process clones, or makes multiple copies of, the gene of interest. As the cells multiply, so does the vector along with the gene of interest.

8. References

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9. Acknowledgements

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