Validation of Histone Methyltransferase SUVH4 (KRYPTONITE) in Common Bean (*Phaseolus vulgaris* L.)

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1. Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most important food legumes in the world. Common bean provides proteins, complex carbohydrates, and micronutrients to more than 500 million people in developing countries (1). Epigenetic regulation is very important in control of gene expression. One very important epigenetic modification is histone methylation which involves histone methyltransferases (HMTs) that carry out gene expression and repression. During histone methylation, one to three methyl groups are transported to a lysine or arginine residue on a histone protein allowing for the regulation of developmental processes (2). Specifically we chose to understand proteins involved in the methylation of histone H3 and lysine 9 (H3K9me) which controls chromatin modification. SET-domain proteins have been studied in the model plant *Arabidopsis thaliana* and found to be involved in chromatin modification through their HMTs, SUVH1-10 and SUVR 1-5 (4). We chose to validate HMT SUVH4 also known as KRYPTONITE (KYP) in the Sierra genotype of common bean. This particular HMT is involved in seed dormancy of the plant *A. thaliana*, as well as the maintenance of DNA methylation (3). We identified SUVH4 in common bean by designing primers specific to this HMT and amplified SUVH4 using PCR verification. SUVH4 was also cloned and then ten positive bacterial colonies were analyzed. Out of those ten positive colonies, three were selected and confirmed by performing double digest with restriction enzymes and then submitted for sequencing. The results from sequencing confirmed that SUVH4 is found in the genotype Sierra of common bean. This is the first report of cloning and sequencing of HMT SUVH4 in common bean.

2. Objectives

Identify HMTs in common bean using model species *Arabidopsis thaliana*

- Select HMTs using NCBI and Phytozome BLAST
- Design primers to express the full length HMT in common bean
- Clone and sequence genes using coding sequences
- Analyze sequences to confirm SUVH4 in the genotype Sierra

3. Materials and Methods

- **Plant material**: Sierra genotypes were grown and stored at DSU
- **RNA isolation**: Using TRIzol from Life Technologies (Grand Island, NY) protocol, total RNA was isolated from plant material
- **cDNA Synthesis**: Using New England BioLabs (Ipswich, MA) protocol; 1-5 µL of previously isolated RNA was converted to cDNA using reverse transcription
- **Selection of HMT**: NCBI and Phytozome databases were utilized to select SUVH4 in *A. thaliana* and *P. vulgaris*
- **Primer design**: Full length primers containing zero cutter restriction enzymes were designed with the help of SEQbuilder and NEB Tm calculator
- **PCR**: Standard PCR using genomic DNA was performed to verify primers, high fidelity PCR was performed to isolate and purify cDNA and create blunt ends for cloning, and colony PCR was performed to verify inserts of SUVH4
- **Cloning**: Using Life Technologies (Grand Island, NY) protocol, SUVH4 was cloned using a bacterial vector
- **Double digestion**: Using purified plasmid DNA, double digestion with restriction enzymes was performed using standard lab protocol to verify positive bacterial colonies
- **Sequencing**: Three positive colonies were submitted for Sanger sequencing using the ABI3130 machine

4. Results

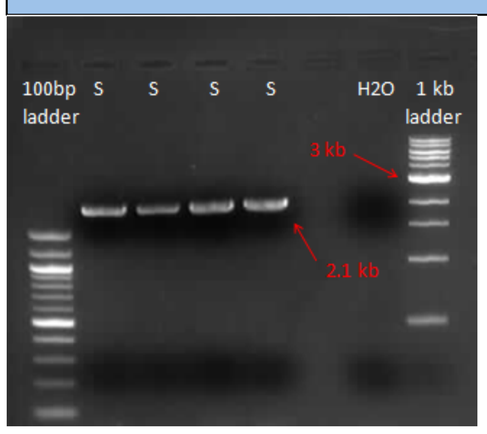

Fig. 1: Verification of SUVH4 using Sierra cDNA and full length primers

- Primers were designed to verify the HMT SUVH4 in Sierra genotype of common bean in Fig. 1
- SUVH4 is approximately 2.1 kb in size and as seen in Fig. 1, the PCR band is amplified just above the 2 kb marker
- H2O was used as a control in Fig. 1 to test for any contaminants in the PCR

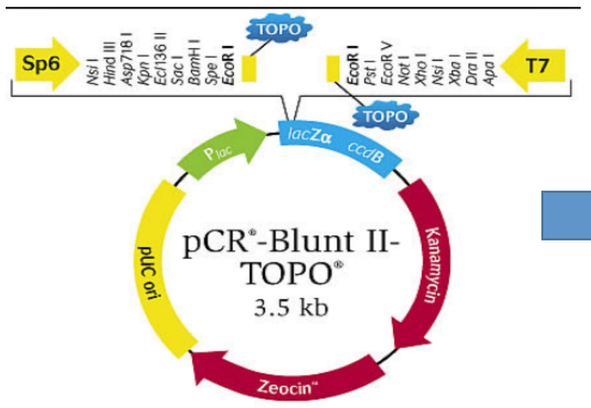

Fig. 2: pCR-Blunt TOPO cloning vector with restriction enzymes obtained from www.lifetechnologies.com

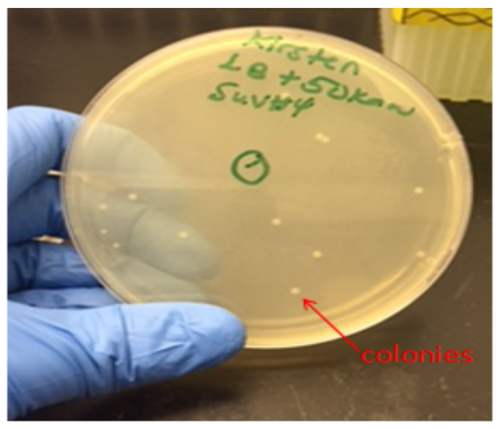

Fig. 3: Bacterial colonies shown on a plate of media from cloning of SUVH4

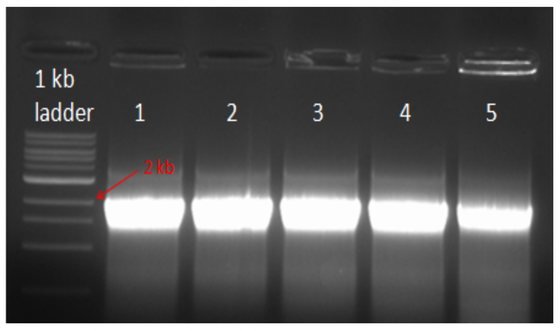

Fig. 4: Colony PCR of five bacterial colonies

- Wells 2 – 6 of Fig. 4 represent five bacterial colonies that were selected for verification
- Amplified bands at the 2 kb marker in Fig. 4 show that selected bacterial colonies were positive for the SUVH4 gene

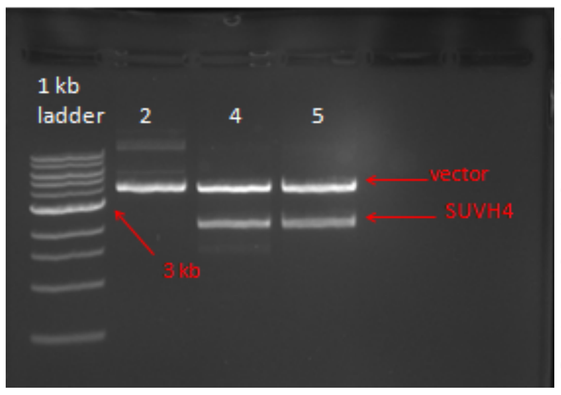

Fig. 5: PCR verification of double digestion from positive bacterial colonies # 2, 4, and 5

- Double digestion of colonies # 2, 4, and 5 was performed to verify positive colonies as shown in Fig. 5
- Products were amplified in Fig. 5 at approximately 2 Kb to show the insertion of SUVH4 into the cloning vector for colonies # 4 and #5
- There was a possible error in the protocol for colony # 2 due to it being positive in Fig. 4, but no insert amplifying in Fig. 5
- Alignment results from sequencing three positive SUVH4 colonies
- Expected value of zero and 100% identities confirms that extraction, cloning, and sequencing were successful

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ALIGNMENTS
>ref|XM_007157675.1| Phaseolus vulgaris hypothetical protein (PHAVU_002G094200g) mRNA, complete cds
          Length=2557

 Score = 3864.1 bits (4284),  Expect = 0E00
 Identities = 2142/2142 (100), Gaps = 0/2142 (0)
 Strand = Plus/Plus

Query  1     ATGGTTGTGGAGGCGCTCCAAGTTGTTCATCAGCATTTAATGGTTTCCACCAGGGCGGAT  60   <- SUVH4 clone
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  68    ATGGTTGTGGAGGCGCTCCAAGTTGTTCATCAGCATTTAATGGTTTCCACCAGGGCGGAT  127  <- P.vulgaris G19
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5. Discussion

Validating genes in model legumes such as common bean is important for future epigenetic research. Histone methylation is a major epigenetic modification that involves the methylation of a lysine or arginine residue. Histone methyltransferase SUVH4 has been studied in the model plant *A. thaliana* and is known to play a role in chromatin modification. If we can validate this HMT in common bean, we can begin research on protein and differential expression. SUVH4 has been amplified in the common bean genotype Sierra and has been successfully cloned in a TOPO cloning vector for the first time. Three samples have been sent for sequencing to verify. Once the sequences were analyzed, BLAST confirmed that SUVH4 is found in the genotype Sierra of common bean.

6. Future Work

- Perform special and temporal expression analysis of SUVH4 by qRT-PCR
- Perform differential expression by analyzing abiotic and biotic stress such as drought or fungal infection
- Observe the protein interaction through chromatin immunoprecipitation sequencing (ChIP-Seq)

7. Acknowledgements

This study was supported by the NSF – funded Delaware ESPCoR program at DSU and by the Center for Integrated Biological and Environmental Research. Special thanks to colleagues in the MGEL lab.

8. References

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