[Concepts] Prospects for Future Research on Bean Stem Maggot *Ophiomyia phaseoli* Resistance in Common Bean *Phaseolus vulgaris*: A Review of Research Outputs Supported by the KT-Funded Laboratory in Kakamega

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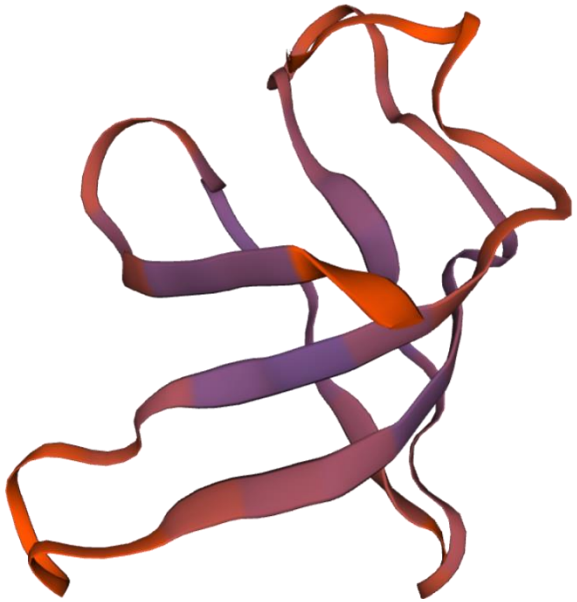
Background

- Under the guidance of Dr. Reuben Otsyula, a team of researchers was trained in the molecular laboratory, and we've made significant strides in advancing our understanding of common bean genetics and pathology.
- Some of the research work conducted in the molecular laboratory includes:

Background

- Research Area: Identification of novel candidate genes associated with scab disease resistance in common bean
- Key Outputs: Potential sources of scab resistance amongst a wide array of common bean accessions. LOC0003 (MCM 2001), ADP0030 (Rh.No 6), ADP551 (AFR 612), ADP526 (Cal 143), ADP0739 (UYOLE 03), and ADP0719 (NUA 59)
- The research successfully pinpointed the resistant gene locus associated with scab disease resistance to be in chromosome one of the *Phaseolus Vulgaris. EPL1* co-localized with *ABC* Transpoter (M to V)



Enhancer of polycomb-like protein

Gaps identified

- Bean stem maggot whose impact is felt when researcher or farmer fails to spray the field at specific timing. The populations within the plots is severely affected thus impeding on yield the validity of the data collected.
- This also reflects on the farmers' cost of productions through spraying against bean flies(BSM) using pesticide.
- However, one common bean (KK15) is thought to have this resistance, it is a black seeded common bean that has significantly shown resistance against the bean stem maggot.

Background

- The legume crop protects itself through defends itself against insect pest is through their structural conformation such as hair (trichome)
- Through, chemical defense mechanisms are more complex and more adapted ways the legume crop protects itself from insect pest BSM.
- To address this, we have to look for protease inhibitors in common bean which are not as abundantly synthesized as in other related species such as *Phaseolus coccineus* and *Phaseolus acutifolius.*

Problem statement and Justification

- Bean Stem Maggot insect pest in Kenya can cause complete crop loss under epidemic conditions affecting farmers as major stakeholders.
- Existing intervention such as cultural practices only contribute a little to the overall control of the pest
- To the best of our knowledge there are no known interventions to instill resistance in common beans as the most productive strategy.

Objectives

The general objective is to enhance the resistance of common beans to BSM through the identification of genes homologous to protease inhibitors, and the development of molecular markers and germplasm with increased resistance.

1. To conduct comparative genomics to identify *Phaseolus vulgaris* homologous genes to protease inhibitors among close relatives.
2. To develop molecular markers that target potential protease inhibitors homologs in common beans.
3. To screen common bean germplasm for Bean Stem Maggot resistant phenotype, using both molecular markers and phenotypic screening methods that target protease inhibition homologues.
4. To Introgress bean fly resistance genes into popular but susceptible germplasm.

Hypothesis

1. By comparing the genome of P. vulgaris and its close relatives, homologous genes to protease inhibitors can be identified in common beans.
2. Molecular markers targeting potential protease inhibitors homologs in common beans can be developed using genomic analysis.
3. Common bean germplasm can be screened for Bean Stem Maggot resistant phenotypically and through molecular markers targeting protease inhibition homologues, which can help in identifying potential sources of resistance.
4. By introgressing bean fly resistance genes into popular but susceptible germplasm, the resulting hybrid can exhibit resistance to Bean Stem Maggot infestation.

Materials and Methods

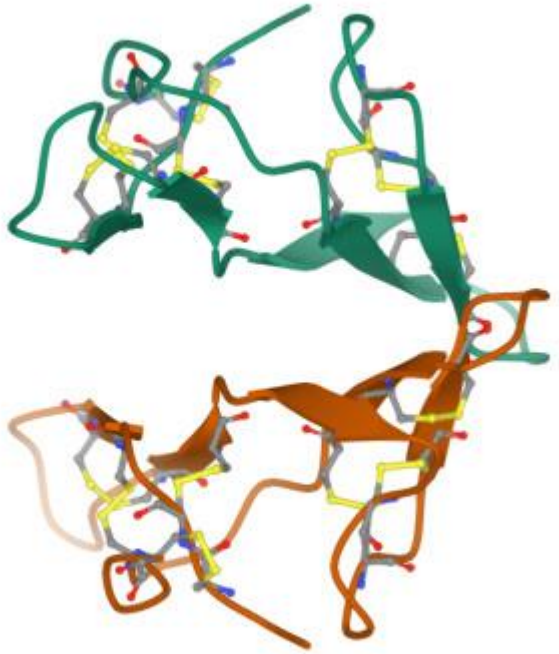
- Through molecular means, investigating if there are homologous genes to *protease inhibitors* identified in resistant *Phaseolus coccineus* and *Phaseolus acutifolius*.
- Use PDB database to look for protein sequences for protease inhibitors in legume crops
- Do a BLAST search for sequences among P. vulgaris distant relatives
- Design primers targeting these homologous sequences in common beans using Primer BLAST software and using the guiding principles for primer design to ensure successful amplification. (Avoid hairpin loops) target GC rich regions.

Materials and Methods

- Use common beans germplasm sets
  - Landraces in Kenya 99
  - Andean Diversity Panel 174
  - KT – Lines 49
  - Calima Beans – 34
  - Red Beans – 25
  - Other – 200
- Experimental design of Partial Replication in alpha lattice experimental design (R DiGGer or Bean Management System)
- Experiment planted under environmental conditions for an endemic infestation of BSM
- Data collected using a scale of 1 to 3 (Mbugua, 2016)

Materials and Methods

- Sample leaf will be collected for DNA extraction at KT-KALRO Laboratory using available protocols.(CTAB, TES, FTA)
- Amplification of target regions using the designed primers and visualization using Gel electrophoresis.
- Sequencing of amplified regions and functional genomics by homologous modeling of the crystal structures to define mode of action and active binding sites for the candidate proteins. (PyMol, PDB)



Crystal structure of Trypsin Inhibitor *Phaseolus lunatus* (Debreczeni, et al 2003)

Expected Outputs

- Expect to identify homologous genes to protease inhibitors in common bean and identify potential resistant common beans in existing germplasm.
- Define the function and mode of resistance in identified common beans with resistance.
- Subsequent continuation and adoption of potential lines to breed and introgress resistance to common beans

Way Forward

- Overall, the KT funded laboratory in Kakamega has made significant progress in advancing our understanding of common bean genetics and pathology. With continued support and investment, this facility has the potential to drive further innovation and progress in the field.

Thank You

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