

**Genetic analysis of halo blight
(*Pseudomonas syringae* pv. *Phaseolicola*
(*Psph*)) resistance and yield components
in common bean**

PhD Proposal

Muhammed Sitote (PhD Student)

Kirkhouse Trust Combined Meetings

Arusha (Tanzania) June (2024)



Introduction

- The common bean (*Phaseolus vulgaris* L.) is one of **major pulse** crop grown in the Ethiopian farming systems
- Mainly produced for its nutritional, food securities, and economic values
- The annual average area coverage and production were 0.55 million tons from 0.31 million hectares with a productivity of 1.8 tons per hectare (CSA, 2021)
- It has two production seasons in Ethiopia farming system;
 - First; from **February- May** and second season sets; from **July - October**



Statement of Problem

- Diseases specifically ALS, CBB, ANT, rust, and **HB** are becoming among the most biotic limiting factors in southern Ethiopia (affecting 40-100% yield reduction)
- **Halo blight disease** (*Pseudomonas syringae pv.Phaseolicola* (**Psph**)) is one of the major diseases affecting the productivity of common bean (as much as **up to 45%**)
- **“Hawassa Dume”** is one of the **most preferred** but **susceptible to halo blight diseases** in Ethiopia, and **“PI150414”** is one of the most known deferential having the HB resistance gene
- The development and use of resistance cultivars are the most effective, economic and environmentally sound strategy of disease control



Justification

- In the previous study, a KT-supported project in Ethiopia deployed resistance genes for ALS, ANT, CBB, and Rust through MAS on the common bean varieties that farmers preferred and were adapted
- But it hasn't been done in Ethiopia yet for **the bean Halo blight** (HB) disease
- Recent survey results (unpublished) with KT-supported project in 2023 showed that **HB is one of the major common bean diseases** in southern Ethiopia
- However, it lacks information on race variability and its geographic spread in southern Ethiopia, which is very important for cultivar development.



Cont.

- Knowing HB race variability is insufficient; additional research is required
 - **Identifying novel sources of resistance and**
 - **Map the resistance genes** for HB, which is crucial for resistance breeding and cultivar development.
- Regardless of how early data on halo blight resistance is characterized,
 - **it is likely that additional resistance will be discovered in the populations under study.**
- **Genetic resistance** provides the only viable control of this disease and
- Is essential to the reliable production of disease-free seed, especially in East and Central Africa where smallholder farmers rely on seed saved from a previous harvest (Miklas et al. 2014)



Cont.

- Even though, “**HB4.2 R**” gene which is race non-specific and having broad spectrum protection against HB was used for durable resistance in common bean (Tock eta 2017)
- However, its gene action didn't studied in yet in Hawassa Dume (farmers **preferred** but **susceptible** cultivar) since, it differs among the variety
- Ethiopian research **generally lacks the aforementioned crucial information**, which makes it difficult for the common bean improvement program to deploy the HB resistance gene into farmers' preferred cultivars
- Generally, the current study **will fill the above-mentioned gaps.**



Significance of the study

- The research will integrate and generate information on;
 - the **variability pattern and its distribution** of HB throughout the region,
 - investigate **novel sources of resistance, map resistance genes** using GWAS, and
 - ascertain how **the HB4.2 R- gene affects the yield** of the Hawassa Dume cultivar

- Thus, this study will be the continuation of the Mega project activity (KT-Project), which will include additional samples (at least 100-150) from major bean growing areas and build on the existing results

- The study will contribute to an increase of the production of this important crop under farmer condition in Ethiopia

- It will have a significant benefit and implication for regional national common bean improvement programs and ABC project



Objectives

General Objective

- ➡ To identify pathological races, sources of resistance, and resistance genes against halo blight diseases of common bean for resistance breeding and cultivar development.

Specific objective

- ⊕ To determine the **prevalence and severity** of bean halo blight disease from the major bean-growing areas of southern Ethiopia
- ⊕ To assess **new sources of resistance** for bean HB disease under field condition
- ⊕ To **map HB disease resistance genes** through GWAS
- ⊕ To **determine the effect of HB4.2 introgression** on the yield of the released cultivar 'Hawasssa Dume'



Material & Methods

Study area

- Assessment study;
 - seven major bean growing areas of southern Ethiopia;
 - **Sidama, Wolaita, Siltie, Gamo-Gofa, Guraghe, Halaba and Hadiya zones of southern Ethiopia**

- Study on;
 - race variability, exploring new source of resistance, mapping resistance gene and determining the effect of HB4.2 R gene;
 - **Hawassa Molecular Laboratory and screen house**

Objective 1.

determine the prevalence and severity of bean halo blight disease

Activity 1.

Assessment of halo blight prevalence and severity

Activity 2.

Identification of HB race variability

I. Isolation of bacterial isolate
Using Schaad et al 1998 method

II. Identification of bacterial isolate

III. Race identification

IV. Pathogenicity test

Objective 2.

Assess new sources of resistance for HB

Activity 1. Evaluating MAGIC Pop for HB resistance under field conditions

At least 250 MAGIC lines will be used

Augmented design will be used

Two rows with 0.4 m and 0.8 b/n row
and plot respectively

1-9 score for HB disease severity

Activity 2. Evaluating the selected MAGIC Pop under screen house conditions

Selected lines from field trial will be
used

Bacterial suspensions will be
prepared and inoculated

HB disease severity score and
candidate line will be identified for
GWAS analysis

Objective 3 .

Map halo blight disease resistance genes through GWAS

MAGIC Populations



Phenotyping MAGIC Pop



Genotyping MAGIC Pop
Will be send for NDSU



GWAS study



Candidate gene identification

Steps for Mapping of Resistance gene

DNA extraction using CTAB



Genotype quality control
Variant calling and exclusions



Imputation of genotype



Adjustment for ancestry and population
stratification



GWAS Analysis , Reporting and annotation
and Post GWAS analysis

Steps for GWAS strategy

Objective 4.

To determine the effect of HB4.2 introgression on the yield of the released cultivar 'Hawassa Dume'

Activity 1.

HB4.2 R gene introgression

Crossing

Hawassa Dume*PI150414

Developing F1 and F2 Pop

Targeting generate up to 300 F2 Pop

DNA extraction from F1 and F2
Pop for HB4.2 R gene tracing

Identifying F2 Pop with HB4.2 R
gene

Evaluating for HB resistance using
developed screening protocol
(Rezene et al. 2018)

Activity 2.

Evaluating the effect of HB4.2 on
Hawassa Dume

Planting introgressed F2 Pop and parental
lines in screen house using pot

CRD with four replication will be adopted

Tagging each genotype and
Yield, yield related and disease data will be
collected

Analyzing the collected data using R-
software

Data to be collected and analysis

- Quantitative and qualitative data will be collected
 - Quantitative data
 - Yield and yield related data;
 - Days to flowering, days to maturity, plant height, pod per plant, seed per pod, seed yield per plant and hundred seeds weight
 - Qualitative data ;
 - Seed size, seed color and growth habit
- Model used for analysis;
 - MLM equation in GWAS analysis :

$$Y = X\alpha + P\beta + K\mu + e$$

- Where Y is phenotype, X is SNP, p is the PCA matrix and both X and p represents fixed effects, K is the relative kinship matrix value, and e is for residual effects
- For evaluation trial :

$$X_{ij} = \mu + a_i + \varepsilon_{ij}$$

- where μ = grand mean a_i = treatment effect and ε_{ij} = random error
- For disease severity:

$$X_{ijk} = \mu + a_i + \tau_j + a^* \tau_{ij} + \varepsilon_{ijk}$$

- where μ = grand mean a_i = treatment effect, τ_j =race effect, $a^* \tau_{ij}$ = treatment*race effect and ε_{ijk} = random error

- All collected data will be subjected to R software



Expected Out put

- PhD Thesis
 - 4 publications
- Information on
 - halo blight virulence in Ethiopia will be generated
- At list one/ two new source of resistance will be explored
- The effect of HB4.2 gene on the yield of Hawassa Dume cultivar will be examined



The Timeline of the activities

Activities	2024-25											
	Sep	Oct	Nov	Dec	Jan	Feb	Mar	April	May	June	July	August
Registration	X											
Class attendance		X	X	X	X	X	X	X	X	X	X	X
2025												
Proposal defense					X							
Inputs fulfilment for field and green house study												X
Conducting survey and Colleting samples	X	X	X									
Characterizing collected sample		X	X									
Planting study materials	X	X										
Management and crossing	X	X	X	X	X	X	X	X	X	X	X	X
2026												
Characterizing the lines	X	X	X	X								
Data management and analysis	X	X	X	X	X	X	X	X				
2027												
Literature Review	X	X	X	X	X	X	X	X	X	X	X	X
Thesis write up	X	X	X	X	X	X	X	X	X	X	X	X
Thesis Submission									X			
Thesis defense										X		



**Many Thanks
for your nice attention's**

Acknowledgement

SIRARI