Genetic Improvement of Biofortified Common bean Varieties in Uganda for Multiple Disease Resistances using Marker assisted Backcrossing – Project Phase I and II

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Presented by Stanley T. Nkalubo

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1. Introduction

❑The first project phase :

- Started: **1st April 2019**
- Ended: **31st March 2022**

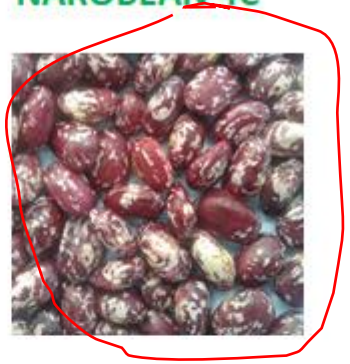
❑We are currently implementing the second project phase

- Started: **1st August, 2022**

❑This presentation highlights the achievements of the project during phase I and the current progress of phase II

Introduction …….

- At NaCRRI-NARO-Uganda we have released five biofortified (72-83ppm iron and 35-40ppm zinc) bean varieties in 2016

NAROBEAN 1	NAROBEAN 2	NAROBEAN 3	NAROBEAN 4C	NAROBEAN 5C
				
Bush bean Large seeded Iron: 65.8-72 ppm Zinc: 31.4-34.2ppm Yield potential: 1500 - 2000 kg/ha Maturity: 60-68 days Best suited for low-mid altitude area	Bush bean Medium seeded Iron: 66.1-72 ppm Zinc: 32.5-36.2ppm Yield potential: 1600 - 2200 kg/ha Maturity: 58-68 days Best suited for low-mid altitude area	Bush bean Medium seeded Iron: 65.4-69ppm Zinc: 35-38ppm Yield potential: 1500 - 2000 kg/ha Maturity: 58-68 days Best suited for low-mid altitude area	Climber bean Large seeded Iron: 77.4-83ppm Zinc: 32.1-36.2ppm Yield potential: 2500 - 3700 kg/ha Maturity: 82-88 days Best suited for Mid-high altitude areas	Climber bean Large seeded Iron: 72.2-80ppm Zinc: 34.7-38.5ppm Yield potential: 2500 - 3300 kg/ha Maturity:88-96 days Best suited for mid-high altitude areas

- Unfortunately, the released varieties succumb to a range of field pathogens in addition to postharvest pests (bruchids)

2. Overall Project objective

- To employ molecular markers and improve the productivity of two of these biofortified elite bean varieties (i.e. NAROBEAN 3 and NAROBEAN 4C) through introgression of multiple disease resistances to especially anthracnose and Pythium root rot

3. Specific objectives : project phase I

❑ **Project phase I was organized into six specific objectives:**

- Characterize and preserve *Colletotrichum lindemuthianum* isolates
- Identify new sources of anthracnose resistance genes
- Identify and validate disease resistance sources for anthracnose and Pythium root rot and the available resistance linked markers
- Develop populations combining the resistance traits into the background of biofortified market classes using marker assisted backcrossing procedure
- Conduct phenotypic and genotypic evaluation of the improved biofortified resistant lines for introgressed resistance genes
- Build human capacity through training of two MSc students

4. Methodology and Results

Specific objective 1. Characterize and preserve *Colletotrichum lindemuthianum* isolates

- Was handled by the MSc student, Mr. Allan Nkuboye
- Anthracnose infected plant samples were collected from 25 districts of Uganda
- The infected tissues were processed in the laboratory and cultured to isolate *C. lindemuthianum* and generated 130 isolates
- Morphological characterisation of *C. lindemuthianum* showed high variability basing on growth rate, colony colour, colony edges, shapes and sizes
- Pathogenicity and virulence characterisation showed 51 races of *C. lindemuthianum* from 25 districts of Uganda
- All the 130 isolates have been preserved at NaCRRI and CIAT-Kawanda for future use

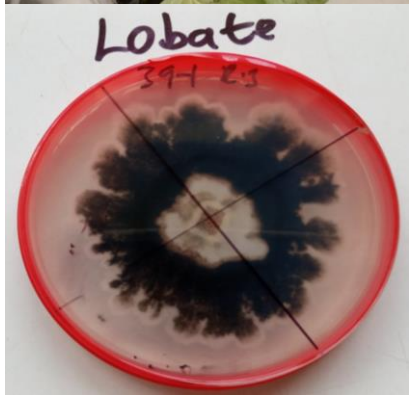
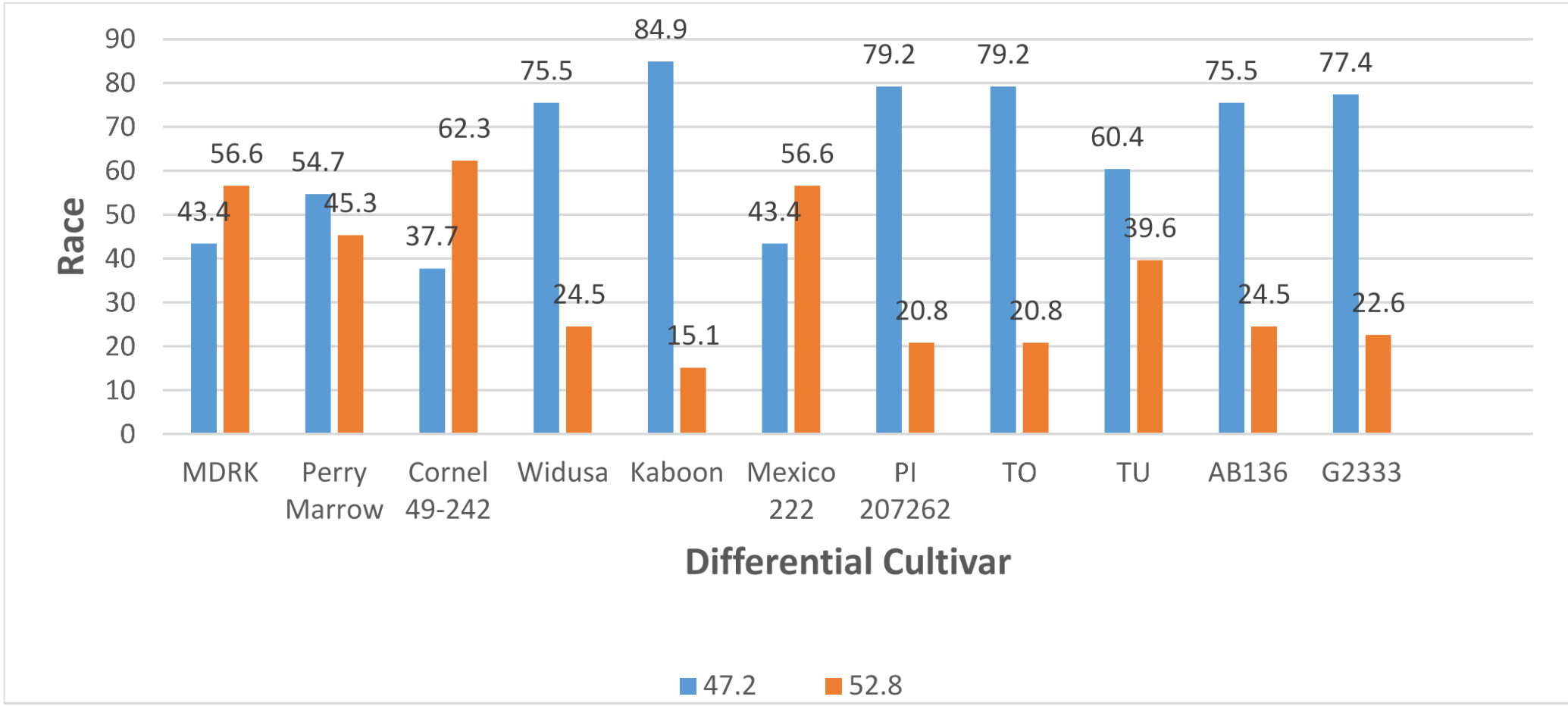


Figure 1: Resistance (R) and Susceptibility (S) of the bean anthracnose differential cultivars to races of *C. lindemuthianum*



The study revealed high resistance indices for Kaboon (84.9%), PI207262 (79.2%), TO (79.2%), G2333 (77.4%), AB136 (75.5%) and Widusa (75.5%)

❑Genetic diversity

- Two mitochondrial genome primers (SEQ1, SEQ2) were used for genetic diversity study of *C. Lindemuthianum*
- DNA for 70 isolates was used
- The PCR products for 70 *C. lindemuthianum* samples were sequenced at Macrogen Europe, Amsterdam, Netherlands
- 58 sequences were generated after editing using Mega 7 software. These are being used to study Genetic diversity, population genetic structure, haplotype distribution, Population structuring, Phylogenetic inference and Isolation by distance (IBD).
- **Challenges**

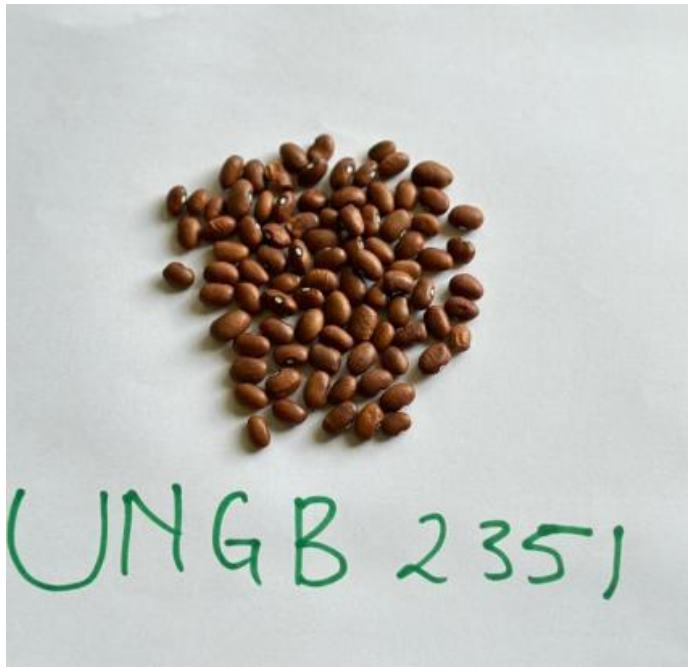
There was a delay to complete genetic diversity studies because of lack of enough expertise for molecular data analysis for especially data concerning genomic sequencing.

- Consequently delaying submission of thesis for examination

Specific objective 2. Identify new sources of anthracnose resistance genes

- This objective was addressed by the second Msc student, M/s Prossy Namuli
- Seed increase of 90 local landraces and 201 introduced germplasm was conducted at NaCRRI over 3 seasons
  - Local landraces were previously collected from different districts in Uganda and were being conserved in the national gene bank
  - Introductions were from CIAT-Kawanda
- The student conducted: Field evaluation of the germplasm for reaction to *Colletotrichum lindemuthianum* under natural infestation during 2021 first season and selected 40 resistant genotypes
- The selected 40 resistant genotypes: were further subjected to artificial inoculation for reaction to anthracnose using the most virulent and prevalent pathotypes identified in objective 1

- Results showed two resistant landraces UNGB 2351 and UNGB 746 that were resistance most virulent and prevalent pathotypes



- Good level of resistance was also observed in cultivar Uyole98 as it showed resistance to most of the pathotypes

Specific objective 3. Identify and validate disease resistance sources for anthracnose and Pythium root rot and the available resistance linked markers

❑Under this objective we :

- Evaluated selected disease resistance sources for anthracnose (*Co-5*,*Co-4*^{2}) and Pythium root rot and,
- Validated selected molecular markers linked to the above resistance genes

❑In order to identify those suitable for use in a MAS backcrossing procedure to introgress the *Co-5* and *Co-4*^{2} anthracnose and Pythium root rot resistance genes into the two bio fortified bean varieties , NAROBEAN 3 and NAROBEAN 4C

- The study germplasm consisted of the following:
  - Selected NABE14/G2333 backcross derived lines :
    - ✓ previously reported to show the markers linked to the *Co-5* and the *Co-4*^{2} anthracnose resistance genes
  - Selected NABE12C backcross derived lines:
    - ✓ previously reported to show the marker linked to the *Pythium root rot* resistance gene
  - 2 Recurrent parents (NAROBEAN 3, NAROBEAN 4C)
  - Controls (RWR 719, G2333, NABE14, NABE12C)

- The germplasm was artificially inoculated with race 7 of *Colletotrichum lindemuthianum* and isolate of *Pythium ultimum var ultimum* (Ms 61) obtained from CIAT-Kawanda where they were previously characterized and preserved

❑The germplasm was also validated for the presence /absence of the resistance genes using the linked molecular markers :

- **<u>3 Dominant markers</u> :**

✓ **SAB3 linked to the *Co-5* anthracnose resistance gene**

✓ **SH18 linked to the *Co-4*^{2} anthracnose resistance gene**

✓ **PYAA19** linked to the ***Pythium root rot*** resistance gene

- **<u>1 Co-dominant marker</u>:**

✓ **BMB 588 linked to the *Co-4*^{2} anthracnose resistance gene**

- **DNA extraction/ analysis followed previously reported procedures**

❑Results showed that :

- ✓the studied NABE14/G2333 and NABE12C backcross derived lines can be used as potential sources of anthracnose (Co-5, Co-4^2) and Pythium root rot resistance genes respectively in the backcrossing program to improve NAROBEAN 3 and NAROBEAN 4C for multiple disease resistance

Line / Genotype	SH 18 marker	SAB-3 marker	Average disease severity
24-1	+	+	1.0
B93	+	+	2.3
B 133	+	+	1.0
B 157	+	+	1.0
B 160	+	+	2.0
B 189	+	+	2.0
35-5	+	+	1.0
G2333	+	+	1.0
NABE14	-	-	4.6
NAROBEAN 3	-	-	1.9
NAROBEAN 4C	-	-	2.2

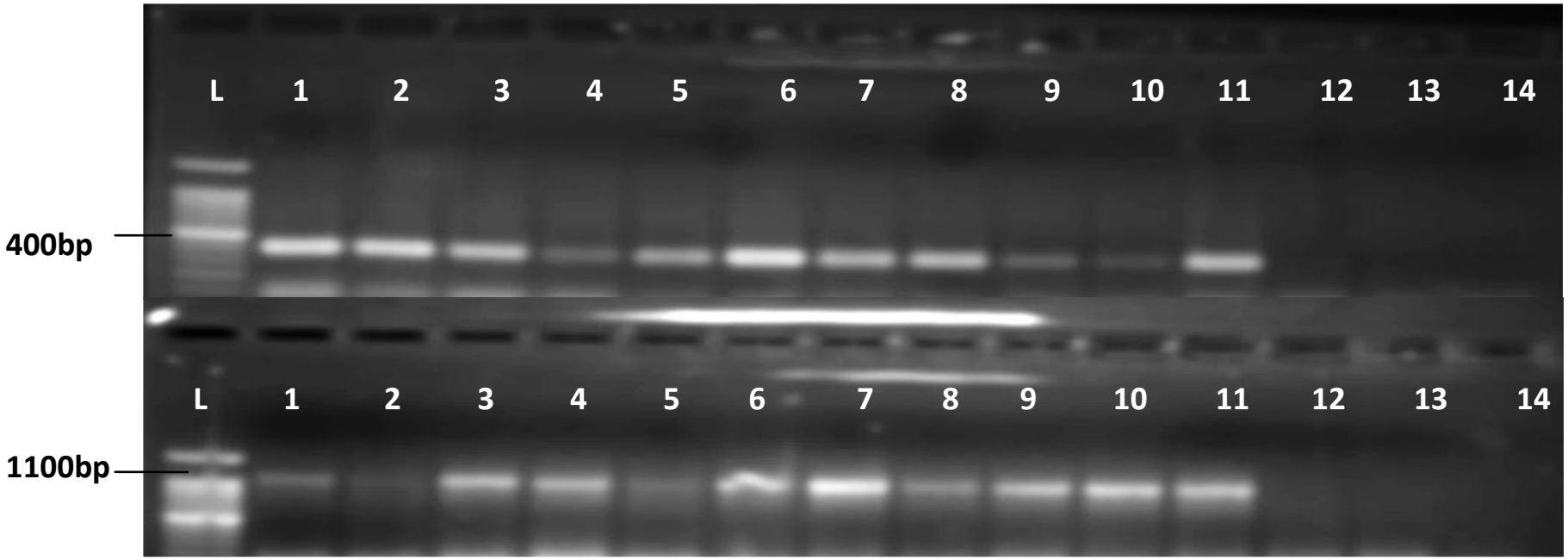
❑Results further showed that :

- ✓ the two anthracnose resistance linked markers (SAB3 , SH18) can be used in the MAS backcrossing procedure to introgress the *Co-5* and *Co-4²* anthracnose resistance genes into NAROBEAN 3 and NAROBEAN 4C backgrounds

- ✓ The PYAA19 marker can also be used for MAS for the Pythium root rot resistance gene in the same background

- ✓ **Only the BMB 588 marker did not show the expected polymorphism , hence not useful for MAS for the *Co-4²* anthracnose resistance gene in the studied backgrounds**

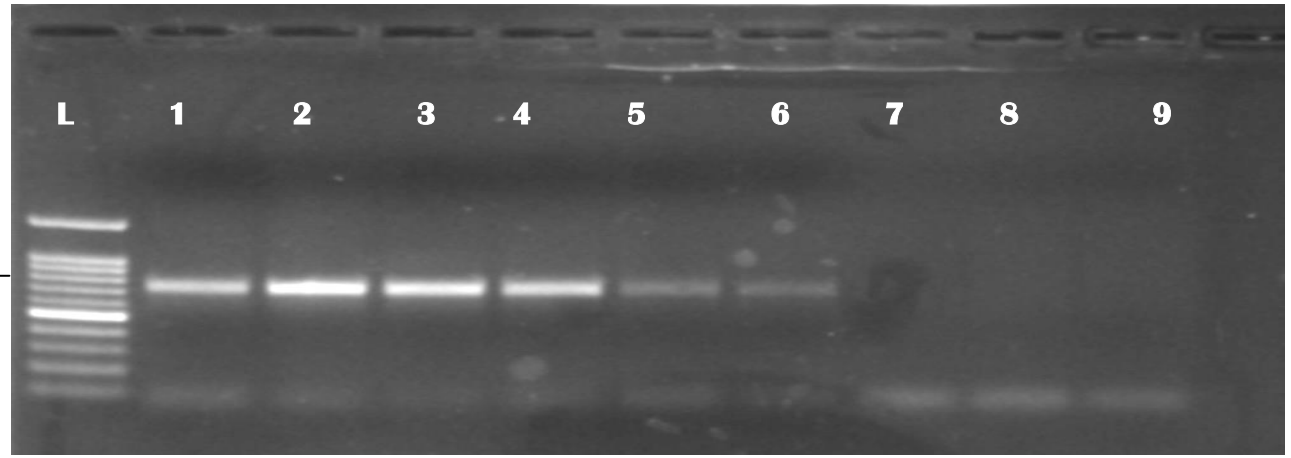
DNA amplification products obtained with SAB3 and SH18 markers



L-DNA ladder, 1-G2333; 2-B93; 3-B133; 4-B157; 5-B160; 6-B189; 7-B264; 8-24-1; 9-6-2;10-35-3; 11-35-5; 12-NAROBEAN 3; 13-NAROBEAN 4C; 14-NABE14

The SAB3 marker amplified a band of 400bp and the SH18 amplified a band of 1100bp in only the NABE14/G2333 backcross derived lines and the genotype G2333 as expected

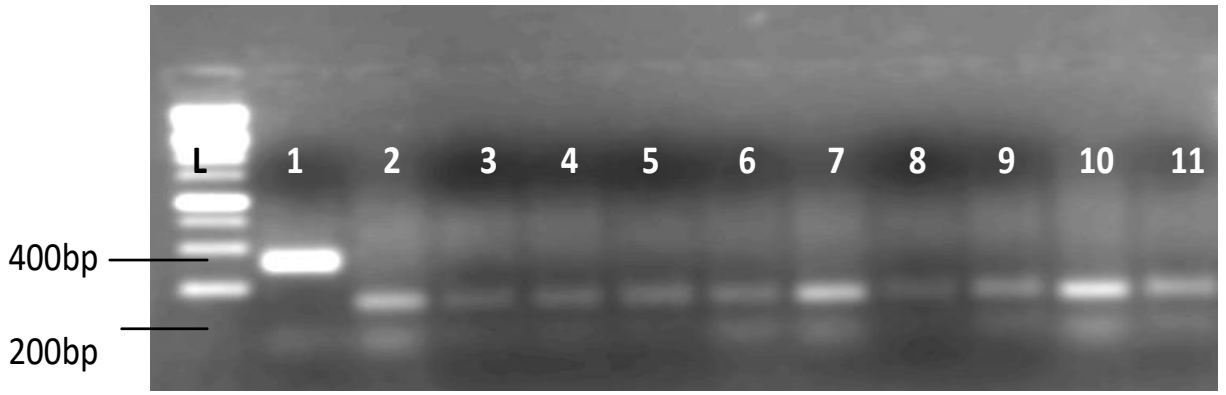
DNA amplification products obtained with the PYAA 19 marker



L- DNA ladder, 1-RWR719, 2-KS1-650, 3- KS1-299, 4-KS1-649, 5- KS1-1073, 6- KS1-146, 7-NAROBEAN 3, 8-NAROBEAN4C, 9-NABE 12C

The PYAA 19 marker amplified the expected band size of 800 bp in only the NABE12C backcross derived lines and the genotype RWR 719

DNA amplification products obtained with the BMB 588 marker



1-G2333, 2-NABE14,3-B93, 4 –B160, 5-B73, 6-B119, 7-B133, 8-35-5-9, 8-35-3-1,

9-NAROBEAN 4C,10-NAROBEAN 3, 11- NABE12C, L-1KB ladder

The BMB 588 marker did not show expected amplification in the NABE14/G2333 backcross derived lines: hence cannot be used to tag the *Co-4*^{2} gene

Specific Objective 4. Develop populations combining the resistance traits into the background of biofortified market classes using marker assisted backcrossing procedure

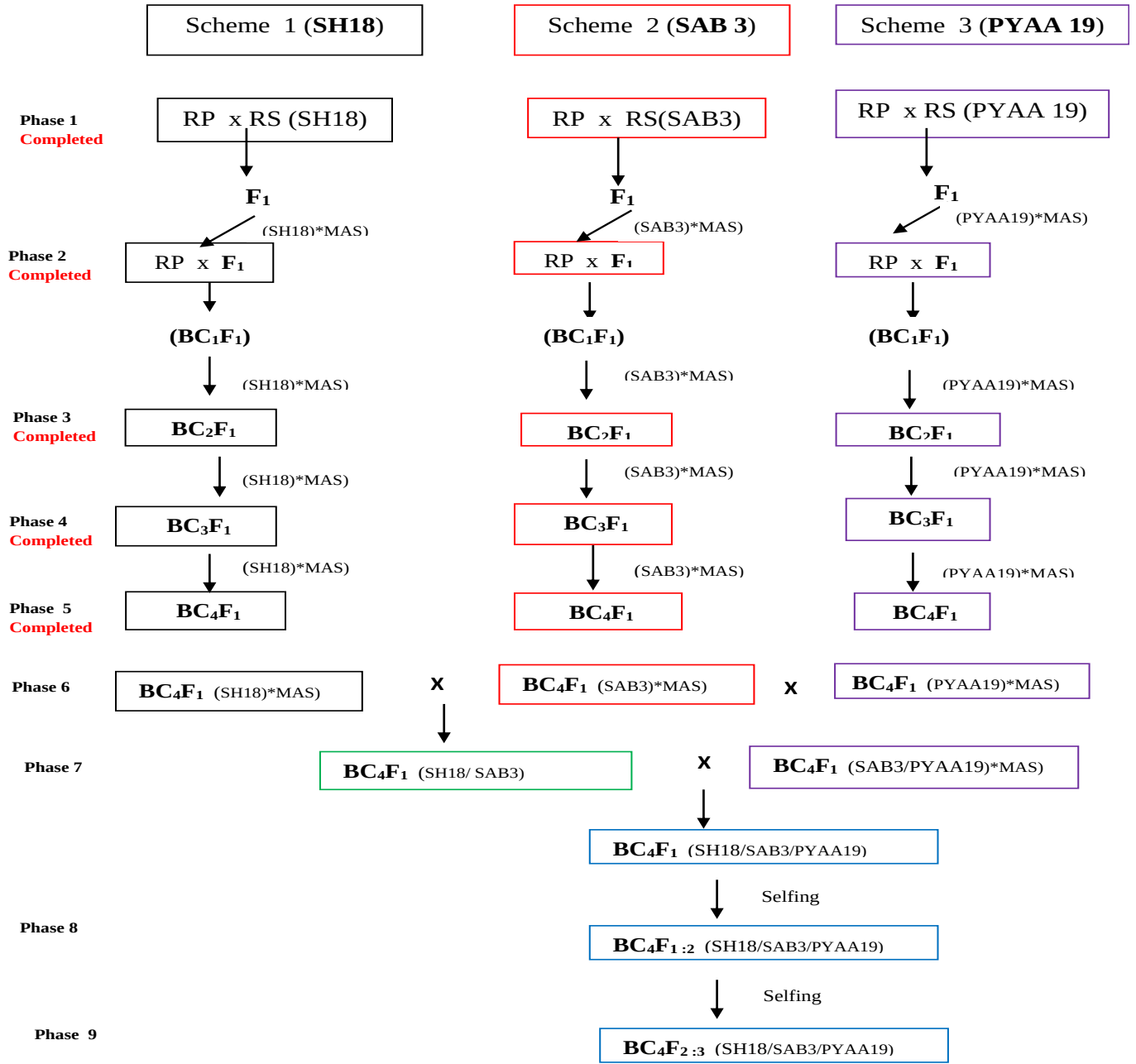
- We initiated a parallel backcrossing program to transfer anthracnose (Co-5, Co-4^2) and Pythium root rot resistance genes into NAROBEAN 3 and NAROBEAN 4C

- Two backcross derived lines evaluated in objective 3 were selected among the sources of the resistance genes

Specific Objective 4 cont'd……

- The SH18, SAB 3 and PYAA 19 markers were used to check for the presence of the resistance genes in the BC_1F_1
- Similarly, the BC_2F_1s and BC_3F_1s were also checked with the markers
- Only backcross F_1 plants whose DNA showed the presence of positive bands associated with each target marker were selected for crossing to the recurrent parents
- By the end of the project period, backcrossing had progressed through four cycles (BC_1F_1 to BC_4F_1).

Cascade diagram of the Parallel Marker assisted backcrossing program (represents either NAROBEAN 3 or NAROBAEN 4C): RP = Recurrent parent; RS= Resistant source



Specific Objective 5. Conduct phenotypic and genotypic evaluation of the improved biofortified resistant lines for introgressed resistance genes

- ❑This objective was to be implemented
  - ✓At the end of the parallel marker assisted backcrossing procedure and selfing of the BC_4F_1
- ❑ Was not implemented during the project phase 1
- ❑ Because we experienced some delays

Specific Objective 6. Build human capacity through training of two MSc students

- The project had two MSc students registered at Makerere University, Uganda

- ✓ The first student, Mr, Allan Nkuboye implemented specific objective 1

- ✓ The second student, M/s Prossy Namuli implemented specific objective 2

- ✓ Both students are expected to submit their thesises for examination in April 2023. Both have already signed and submitted the intend to submit forms.

5.Conclusion :

- During the project phase 1, we have experienced challenges in implementing some activities due to various reasons : :

✓ Resulting into delays

✓ such as the Covid-19 Pandemic. This almost paralysed the students' program as the Universities were not accessible for a full year and half.

✓ The parallel backcrossing program also experienced some delays mainly due to failure of the expected amplification of the SH18 marker. Time was lost trying to address this issue. This involved getting new sets of primers

- Despite the challenges and delays, the project made satisfactory progress towards achieving the set objectives/activities.

6. PROJECT Phase II:

- This second project phase is aimed at consolidating on the achievements of the first phase of the project

Specific objectives

- To combine the *Co-5* and *Co-4*^{2} anthracnose resistance genes and the Pythium root rot resistance gene into the same background of biofortified bean varieties using marker assisted selection procedure

- To validate and maintain selected *C. lindemuthianum* isolates/races

Specific objective 1. To combine the *Co-5* and *Co-4²* anthracnose resistance genes and the Pythium root rot resistance gene into the same background of biofortified bean varieties using marker assisted selection procedure

Major activities

- ✓ **Activity 1:1** Intercrossing BC_4F_1 (SAB3/*Co-5*) with BC_4F_1 (SH18/*Co-4²*) and BC_4F_1 (SAB3/*Co-5*) with BC_4F_1 (PYAA19/PRR) to get two resistances genes/linked markers in a single background
- ✓ **Activity 1:2** Intercrossing F_1 (SH18/SAB3) with F_1 (SAB3/ PYAA19) to get three resistances genes/linked markers in a single background
- ✓ **Activity 1:3** Selecting for progeny carrying all three markers, F_1(SH18/SAB3/PYAA19)
- ✓ **Activity 1:4** Selfing of the BC_4F_1 to $BC_4F_{2:3}$
- ✓ **Activity 1:5** Conducting artificial inoculation and genotypic evaluation of the $BC_4F_{2:3}$ to identify lines homozygous for both anthracnose and Pythium root rot
- ✓ **Activity 1:6** Selfing of the $BC_4F_{2:3}$ non-segregating/homozygous lines for both anthracnose (*Co-5* and *Co-4²*. and Pythium root rot up to F_4
- ✓ **Activity 1:7** Conducting genotypic evaluation at the end of selfing

Specific objective 2. To validate and maintain selected *C. lindemuthianum* isolates/races

Major activities

- ✓ **Activity 2:1** Validating of *Colletotrichum lindemuthianum* races for discriminating between NAROBEAN 3 and NAROBEAN 4C and the resistant parents, UGKT-B133 and G2333

- ✓ **Activity 2:2** Maintenance of selected *C. lindemuthianum* isolates/races

This will involve rejuvenation, inoculation, re-isolataion and storage of preserved *C. lindemuthianum* isolates of selected races of the pathogen at determined intervals in the year to keep them virulent

Acknowledgement

- **We are grateful to the Kirkhouse Trust for the continued financial support**

- **CIAT Kawanda –** provided the previously characterised Pythium root rot isolate and race 7 of *Colletotrichum lindemuthianum*
- **MUARIC –**Training of MSc students