Navigating the NaCRRI - ABC Project: Insights into Achievements and Research Implications

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Introduction

❑This presentation focusses on five specific highlights:

- Backcrossing program to transfer anthracnose (*Co-5*, *Co-4*^{2}) and Pythium root rot resistance genes into NAROBEAN 3 and NAROBEAN 4C
- Release of two MAS backcross-derived bean lines
- Progress made by the two MSc. Students and implications of their research findings for the breeding program
- Submitted materials to the PABRA (ECABYT) yield trials
  - Reconstitute into a PABRA –nursery that will be shared for evaluation by interested members within the region
- Publications

Project Achievements

1. Backcrossing program to transfer anthracnose (*Co-5, Co-4²*) and Pythium root rot resistance genes into NAROBEAN 3 and NAROBEAN 4C.

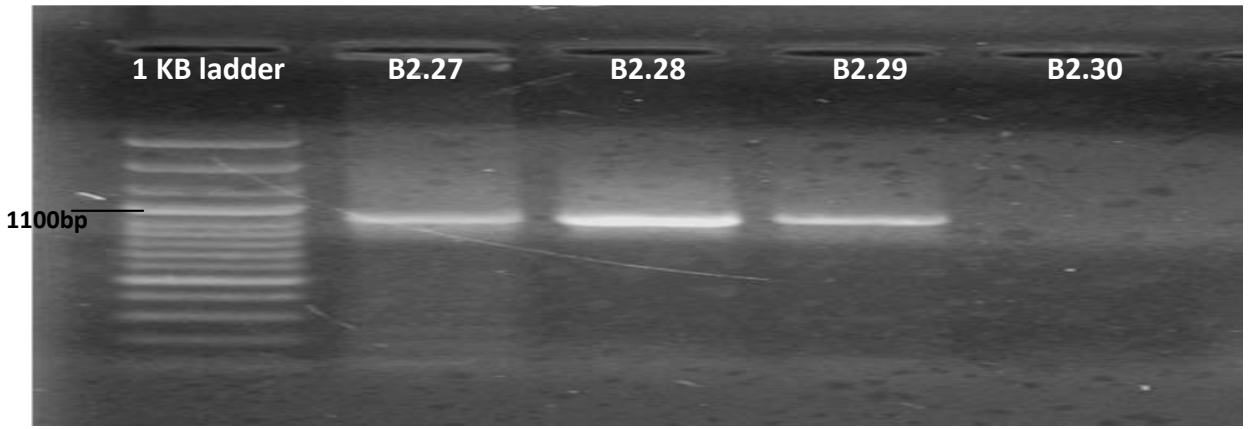
- A backcrossing program aimed at transferring of the *Co-4²* , *Co-5* and Pythium root rot resistance genes into NAROBEAN 3 (bush bean) and NAROBEAN 4C (climber) was initiated.

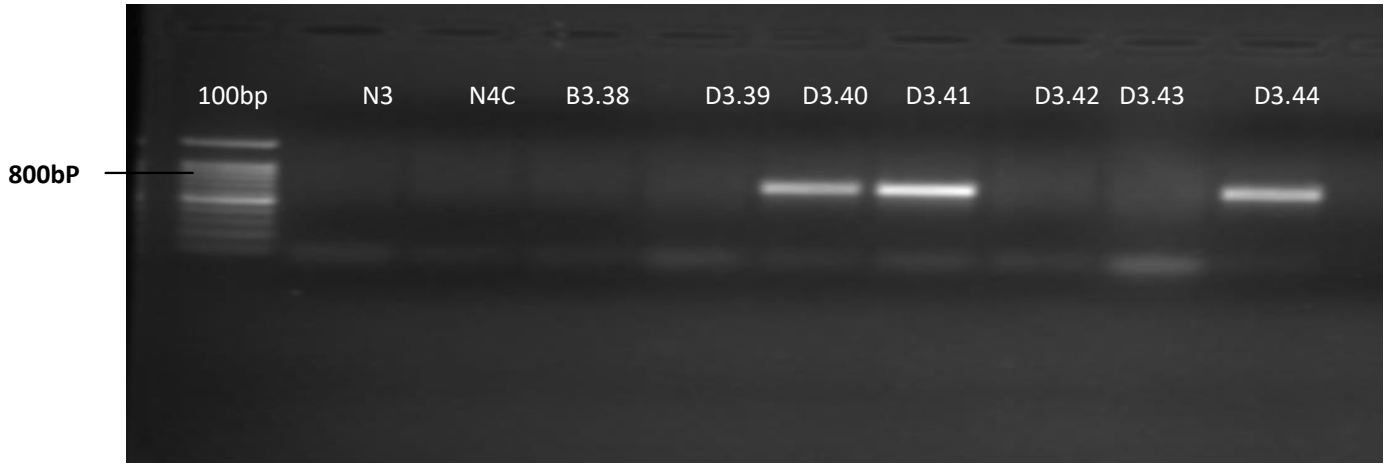
- The *Co-4²* gene was tagged by SH18 marker; the *Co-5* by SAB3 and the Pythium root rot by PYAA 19.

Three parallel backcrossing schemes, each targeting one resistance gene/ marker were conducted for each recurrent parent: from BC1F1 to BC4F1

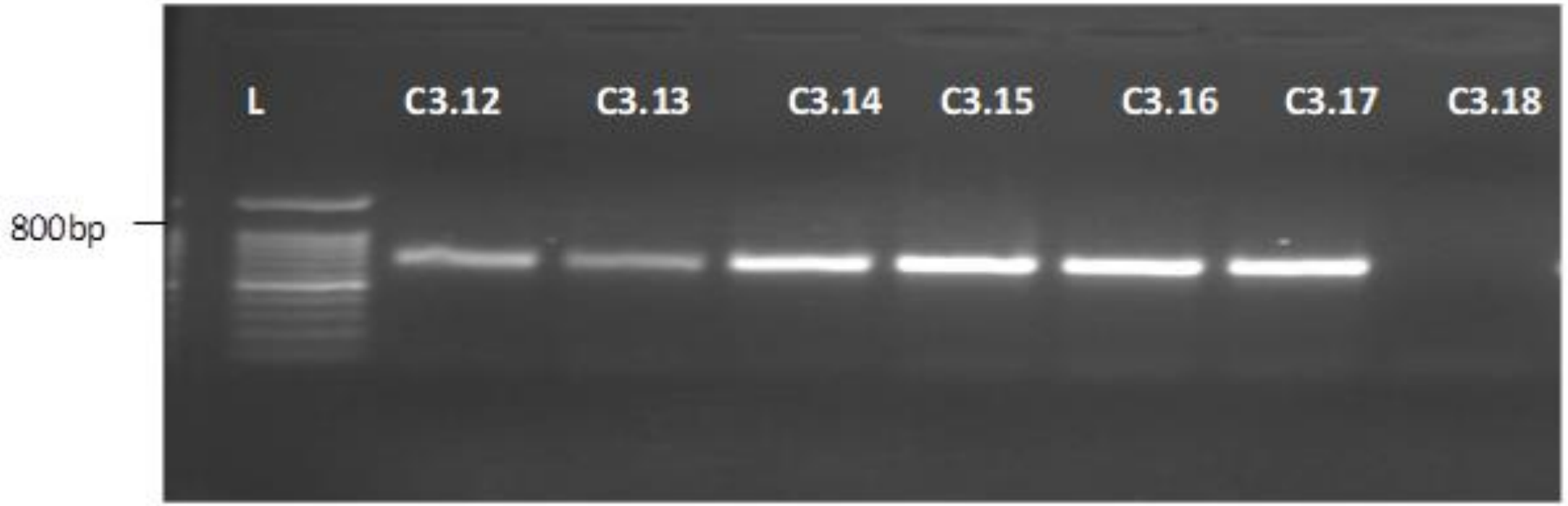
BC Scheme 1 (SH 18 marker/ Co-4²)	BC Scheme 2 (PYAA19 marker/Pythium)	BC Scheme 3 (SAB3 marker/ *Co-5*)
a) NARO BEAN 3 recurrent parent (NRB3)		
BC_1F_1 (NRB3/A4)- SH18 ↓ BC_4F_1 (NRB3/A4)- SH18	BC_1F_1(NRB3/C3)-PYAA 19 ↓ BC_4F_1(NRB3/C3)-PYAA 19	BC_1F_1 (NRB3/A4)- SAB 3 ↓ BC_4F_1 (NRB3/A4)- SAB 3
b) NARO BEAN 4C recurrent parent (NRB4C)		
BC_1F_1(NRB 4C/B2)- SH18 ↓ BC_4F_1(NRB 4C/B2)- SH18	BC_1F_1(NRB 4C/D3)–PYAA 19 ↓ BC_4F_1(NRB 4C/D3)–PYAA 19	BC_1F_1 (NRB 4C/B4)- SAB 3 ↓ BC_4F_1 (NRB 4C/B4)- SAB 3

A4/B2/B4= ANTH resistance donor : C3/D3 = Pythium root rot donor





Amplification of some BC_1F_1 backcross progenies using SH18 and PYAA 19 markers during MAS to generate the BC_2F_1



Amplification of some BC_2F_1 backcross progenies using PYAA 19 marker during MAS to generate the BC_3F_1

- This resulted into 3 BC4F1 populations, ie BC4F1 (SH18/*Co-4*^{2}), BC4F1 (SAB3/ *Co-5)* and BC4F1 (PYAA 19/PRR) for each recurrent parent

- Currently, the backcrossing program is towards the final stages of it's completion

  - Intercrossing has been done to combine three resistances genes/linked markers (SH18/SAB3/PYAA19) in a single background,

  - Selfing of the F1(SH18/SAB3/PYAA19) to F2 to F2:3 has been completed.

- **Next step**: Conducting artificial inoculation and genotypic evaluation of the F2:3 to identify lines homozygous for both anthracnose and Pythium root rot

2. Release of two MAS backcross-derived bean lines

❑ On the 29/02/2024, NaCRRI released two MAS backcross-derived bean lines

❑ **The first**, is a bush bean UGKT-B157-4

- Possessing the *Co-4*^{2} and *Co-5* anthracnose resistance genes from G2333
- Released as NABE 14R
- Targeting the mid to high altitude regions of Uganda

Field Agronomic Performance and Reaction to anthracnose disease of UGKT-B157-4 compared with other genotypes

Genotype	Seed yield (kgha)	Total Seeds	100 Seed weight (g)	No_of _pods	No_of_ seeds	DTF	DTM	Anth 1	Anth 2
UGKT- B73	1092bcd	267.3bc	37.70b	7.37bc	4.456b	43.00a	85.8a	1.1ab	1.5ab
UGKT- B93	1186bcd	339.0bc	39.49a	7.88bc	4.589b	44.45a	86.0a	1.5bc	1.8ab
UGKT- B133	1267bc	348.4bc	39.54ab	7.60bc	4.467b	44.30a	86.1a	1.4ab	2.0bc
UGKT- B119	943cd	222.3c	40.39a	5.90c	4.400b	44.30a	85.9a	1.9cd	2.8c
UGKT- B157-4	**1512b**	**453.1b**	**37.84b**	**8.81b**	**4.589b**	**44.25a**	**86.4a**	**1.3a**	**1.4a**
UGKT- B157-7	1288bc	409.9bc	37.50b	8.71b	4.644b	44.60a	86.8a	1.3ab	1.3a
UGKT- B160	1126bcd	366.7bc	38.48ab	7.61bc	4.733b	44.75a	86.8a	1.2ab	1.3a
UGKT- B264-3	800d	216.3c	39.47ab	5.95c	4.578b	44.00a	86.0a	2.2d	2.7c
NABE14 (recurrent)	**1136bcd**	**365.2bc**	**39.09ab**	**6.76bc**	**4.511b**	**44.90a**	**85.9a**	**2.0d**	**2.5c**
G2333 (Resistant)	1998a	1052.8a	23.03c	16.20a	7.189a	49.2b	90.1b	1.0a	1.0a
Mean	**1235**	**404**	**37.121**	**8.28**	**4.816**	**44.87**	**86.58**	**1.5**	**1.8**
s.e.d	**231.0**	**111.2**	**2.470**	**1.279**	**0.2063**	**0.917**	**2.732**	**0.46**	**0.34**
LSD (5%)	**455.1**	**219.2**	**4.897**	**2.524**	**0.4073**	**1.809**	**5.406**	**0.91**	**0.67**
F probability:									
Genotype	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Location	<.001	<.001	<.001	<.001	Ns	<.001	<.001	<.001	<.001
Season	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Gen. x loc.	Ns	<.001	Ns	Ns	Ns	Ns	<.001	Ns	Ns
Gen. x seas.	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Loc. x seas.	<.001	<.001	<.001	Ns	Ns	<.001	<.001	<.001	<.001
Gen. x loc. x seas.	<.001	<.001	<.001	<.001	Ns	Ns	Ns	<.001	<.001

Reaction to anthracnose disease under natural infestation

Resistant reaction to anthracnose on pods of UGKT-B157-4 compared with susceptible disease reaction observed on NABE 14 in Kabale- Kamuganguzi under natural infestation during 2021 second season.

Resistant reaction to anthracnose on pods of UGKT-B157-4 compared with susceptible disease reaction observed on NABE14 in Kabale- Kamuganguzi under natural infestation during 2021 second season

Reaction to anthracnose disease after artificial inoculation

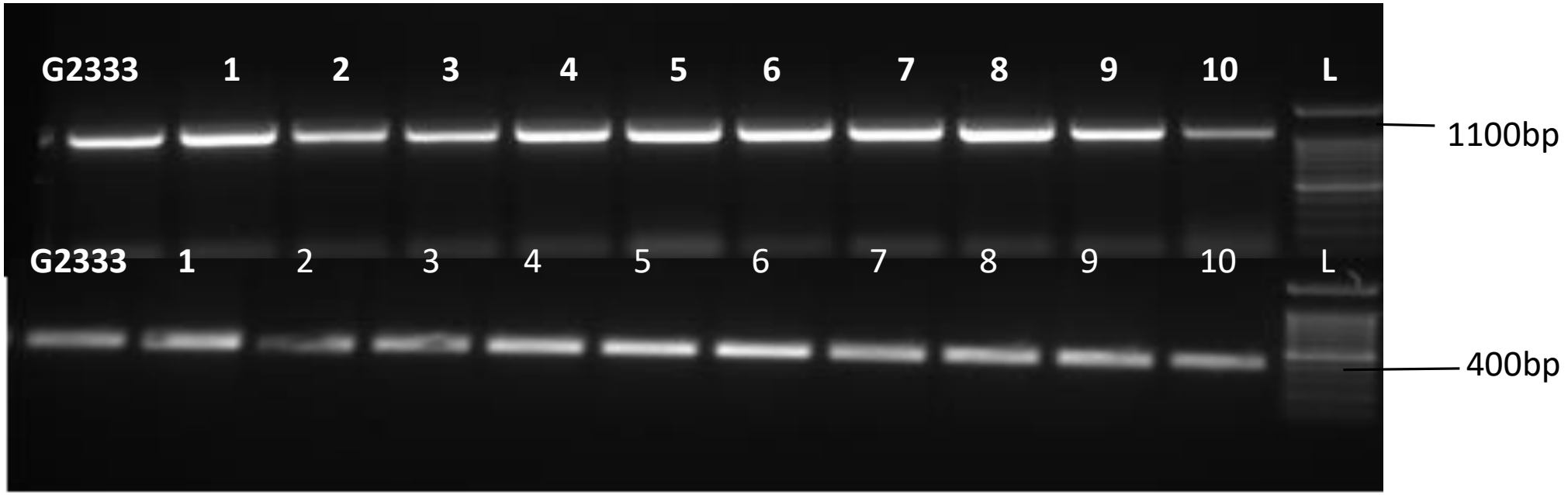
Line	Mean severity on the 1-9 scale when inoculated with six isolates of *Colletotrichum lindemuthianum*					
	204A (863)	**168A (10)**	**087A (15)**	**217A (64)**	**178-2A (254)**	**055A (111)**
UGKT-B157-4	2	1.5	1.3	2.3	1.5	1
UGKT-B160	1.5	1	3	1.8	1.3	1
UGKT-B133	1.5	1	1	2.3	2	2
UGKT-B157-7	1.3	1.3	1	2	1	2
NABE14	6	5	5	5.5	9	5
G2333	1	1	1	1	1	1

NABE14

UGKT-B157-4

Susceptible reaction on NABE14 compared with resistant reaction on UGKT- B157-4 seven days after inoculation with isolate 204A

Genotypic evaluation with the SCAR markers SH18 (*Co-4*^{2}) and SAB3 (*Co-5*) :



DNA amplification products obtained with SCAR markers SH18 (1100bp) and SAB3 (400 bp): lanes L: 100 bp DNA ladder, 1-10:. UGKT- B157-4

The SH18 and SAB3 amplified the expected band sizes of 1100 bp and 400 bp respectively in the resistant genotype (G2333) and in each of the ten individual plants of UGKT- B157-4.

❑ **The second**, is a climbing bean UGKT-KS1 1073-1

- Possessing the Pythium root rot resistance genes from RWR 719
- Released as NABE 12CR
- Targeting the Mid to high altitude regions of Uganda

Field Agronomic Performance and Reaction to anthracnose and root rot diseases of UGKT-KS1 1073-1 compared with other genotypes

Genotype	Seed yield (kg/ha)	100 Seed weight (g)	No_of_ pods	No_of_ seeds	DTF	DTM	Anth 1	Anth 2	Root rot
UGKT-KS1 299-2	1941	43.79b	16b	6.6b	58	100	1.6bc	1.97ab	1
UGKT-KS1 299-8	2284	45.50ab	17b	6-2c	59	100	1.7bc	2.2bc	1
UGKT-KS1 649-3	2190	47.09a	15b	6.4bc	58	101	1.5b	1.8ab	1
UGKT-KS1 41-5	2097	45.17ab	17b	6.7 b	58	100	1.7bc	2.13b	1
UGKT-KS1 1073-1	2195	44.20b	16b	6.6 b	58	100	1.8cd	2.2 bc	1
NABE 12C (susceptible check)	2022	45.94ab	16b	6.5 bc	58	100	2.0d	2.7c	1
G2333 (Resistant check)	2213	23.90 c	20a	7.9a	55	96	1.2a	1.6a	1
Mean	**2135**	**42.24**	**17**	**6.7**	**55.**	**100**	**1.6**	**2.1**	**1**
s.e.d.	**319**	**1.368**	**1.8**	**0.2**	**1.4**	**1.8**	**0.1**	**0.3**	**0.02**
LSD(5%)	**629**	**2.697**	**3.6**	**0.4**	**2.7**	**3.5**	**0.3**	**0.5**	**0.06**

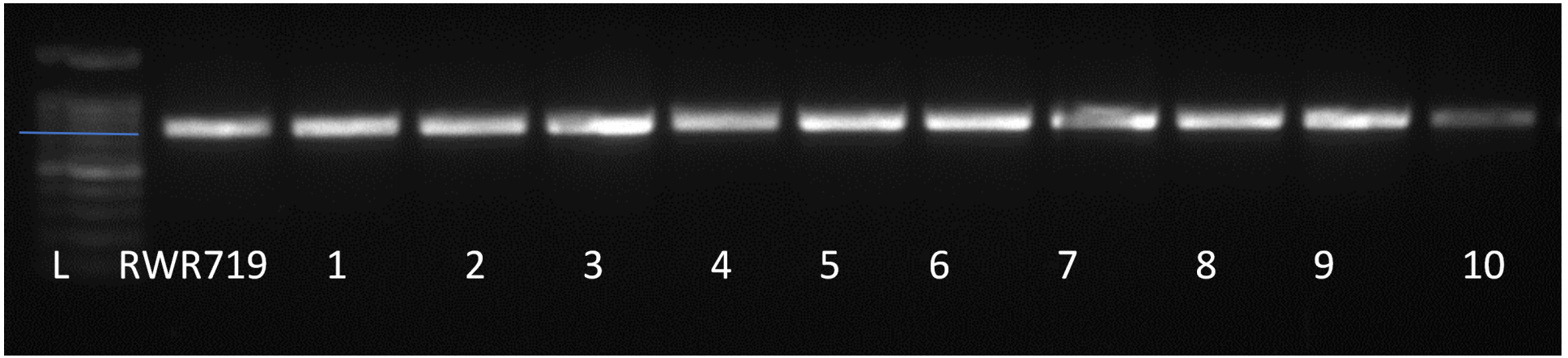
Reaction of UGKT-KS1 1073-1 compared with other genotypes after inoculation with *Pythium ultimum var ultimum* (Ms 61)

Line/Genotype	Description	Mean disease severity on the CIAT scale of 1-9
UGKT-KSI-299-2	NABE12C backcross derived line	2.7
UGKT-KSI-1073-1	"	2.5
UGKT-KSI-41-5	"	3.0
UGKT-KS1-649		2.8
UGKT-KSI-299-8	"	3.3
RWR 719	Resistant control	2.0
NABE12C	Susceptible control	7.6
CAL 96	Susceptible control	9.0

Susceptible disease reaction of NABE12C and CAL 96 compared with resistant reaction of UGKT-KSI-1073-1 and resistant check RWR 719, 21 days after inoculation with *Pythium ultimum var ultimum* (Ms 61)

Resistant disease reaction of UGKT-KSI-1073-1, RWR 719 compared with susceptible reaction of CAL 96, on uprooting 21 days after inoculation with *Pythium ultimum var ultimum* (Ms 61)

Genotypic evaluation with the SCAR marker PYAA 19 :



DNA amplification products obtained with SCAR marker PYAA19 : lanes L: 100 bp DNA ladder, 1-10:. UGKT-KSI-1073-1

The PYAA 19 amplified the expected band size of 800 bp in the resistant genotype (RWR719) and in each of the ten individual plants of UGKT-KSI-1073-1

❑Implication of the two released varieties to the breeding program

- In addition to being released for farmers' use
  - They can also be used as sources of resistance genes (Anth & RR), especially considering that our Pythium root rot resistance gene source, RWR 719 is becoming increasing susceptible to BCMNV to the extent that total crop loss has been observed in some seasons and locations.

❑ Seed increase and dissemination.

3. Progress made by the two MSc. Students and implications of their research findings for the breeding program

- The project had two MSc students registered at Makerere University, Uganda
  - The **first student**, Mr, Allan Nkuboye, graduated on 29th January 2024
- His research thesis was titled, "Diversity of the Bean Anthracnose Pathogen, *Colletotrichum lindemuthianum* (Sacc. and Magn.) from Major Bean Agroecological Zones of Uganda."

- The most important out put of his research to the breeding program was two fold namely:

✓A collection of characterized *Colletotrichum lindemuthianum* isolates that have been preserved for future screening purposes

- Before this study, lack of preserved characterized isolates of *Colletotrichum lindemuthianum* was a major gap.
- There had not been adequate deliberate preservation of the pathogen for quick reference

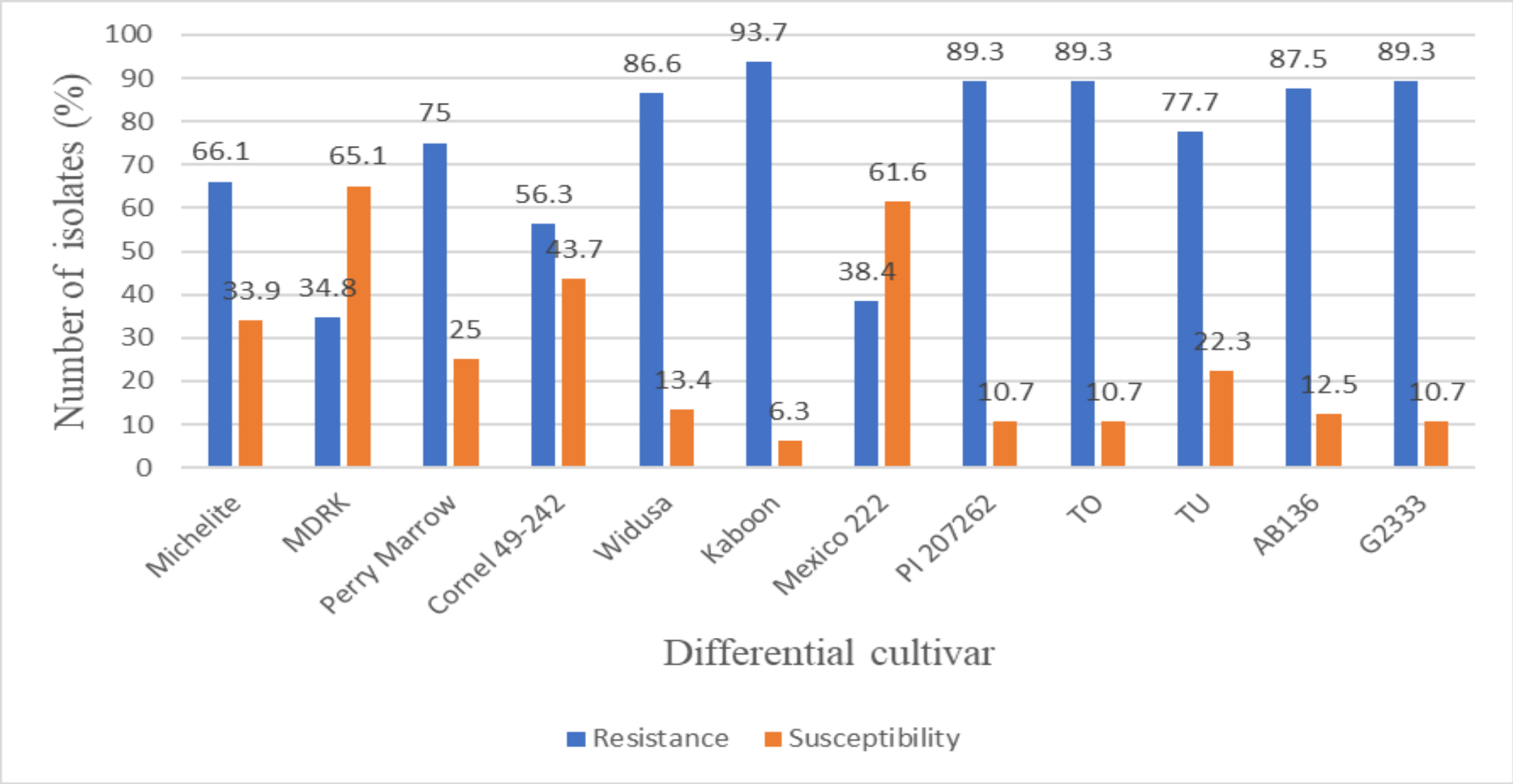
✓However there will be need for rejuvenation, inoculation, re-isolataion and storage at specified intervals so as to maintain them

✓In addition, important information on the best differential cultivars to provide anthracnose resistance genes was generated.

- Where as G2333 still remains an important source of anthracnose resistance genes, the findings from this research further emphasize the need for the Uganda bean breeding program to also focus on exploiting the *Co - 1*^{2} in Kaboon for more durable resistance.

  - Studies by Nkalubo, 2006; Mwesigwa, 2008; Kiryowa et al., 2017 and Nkuboye, 2024, have shown that some pathotypes that pathogen to G2333 are not pathogenic to Kaboon.

Resistance (R) and Susceptibility (S) of the bean anthracnose differential cultivars to 112 races of *C. lindemuthianum*



❑The second student, Ms., Prossy Namuli, successfully passed the VIVA VOCE examination on 25th April 2024.

✓Her research thesis was titled, "Identification of new sources of resistance to common bean anthracnose (*Colletotrichum lindemuthianum*) among the common bean (*Phaseolus vulgaris*. L) germplasm in Uganda"

- Identified two new resistant local genotypes UNGB 2351 and UNGB 746 that could be exploited by the breeding program.

4. Submitted material to the PABRA (ECABYT) yield trials

- Materials submitted include
  - 5 Bush lines
    - UGKT-B119, UGKT-B133, UGKT-B157-7, UGKT-B160 and UGKT-B157
  - 5 Climber lines
    - UGKT-KS1 649-3, UGKT-KS1 41-5, UGKT-KS1 1073-1, UGKT-KS1 299-8, UGKT-KS1 649
- Will be incorporated and reconstituted into a PABRA –nursery that will be shared for evaluation by interested members within the region
  - Data on performance across the region
  - Informed decision making for any further selection and possible release within the country and across the region.

5. Research Publications

- Nkalubo et al., 2024. Agronomic Performance, Stability Analysis and Evaluation of Anthracnose Disease Resistance of Common Bean Lines Derived by Marker-Assisted Backcrossing in Uganda. Agricultural Sciences, (Vol.15 No.3 2024),
- Nkalubo et al., 2022. Validation and deployment of resistance-linked scar molecular markers for marker-assisted improvement of multiple disease resistance in biofortified common bean. *Bean Improvement Cooperative (BIC) vol.65:85-86.* *http://www.bic.uprm.edu/*
- Nkuboye et al., 2023. Pathogenic Variability of Colletotrichum lindemuthianum and Implications for Anthracnose Resistant Bean Variety Development in Uganda. *Paper Presented at the 3rd NARO-MAK Conference, 14-16th March 2023, Speke Resort Munyonyo, Entebbe, Uganda.*

Conclusion:

- During this period, we have experienced a few challenges in implementing some activities due to various reasons: Resulting into delays
  - ✓ For example the backcrossing program still experienced failure of the expected amplification of the SH18 marker.
  - ✓ We also usually experience outbreaks of white flies in the screen house which often necessitates a closed season.
- Despite the challenges and delays, the project is making satisfactory progress towards achieving the set objectives/activities.

Acknowledgement

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