

## Breeding for root rot resistance in East and Central Africa

### Acknowledgements

Robin Buruchara, Paul Kimani,  
Reuben Otsyula, Tusiime  
Geoffrey, Namayanja Annet,  
Pamela Paparu, Ongom Patrick,  
Obala Jimmy and Kamfwa  
Kelvin

[c.mukankusi@cgiar.org](mailto:c.mukankusi@cgiar.org)



# Outline

- Introduction
- Identification of sources of resistance
- Mechanisms of resistance
- Tagging molecular markers to resistance
- Development of root rot resistant varieties
- Dissemination and promotion of root rot resistant varieties
- Impact of root rot resistant varieties



# Introduction

- Major production constraint for beans in eastern Africa.
  - South-western highland regions of Uganda
  - Western Kenya
  - Some regions of the Republic of Rwanda
  - Regions of Democratic Republic of Congo
- Predicated to escalate in the east and central Africa due the increased precipitation in the coming years (Boko et al., 2007; Christensen et al., 2007; Burke et al., 2009; Seitz and Nyangena, 2009).
- Popular commercial varieties susceptible
- Known resistant varieties to specific pathogens but associated with undesirable characteristics



# Introduction

- Bean root rot caused by a complex of pathogens (*Pythium sp*, *Fusarium solani fsp. phaseoli*, *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Macrophomina phaseoli*)
- Research focus on *Pythium* and *Fusarium* root rot
- Increase in prevalence of *Sclerotium rolfsii* in major bean environments of Uganda (Paparou et al., 2015)
- Current projects targeting on all root pathogens except *Macrophomina sp.*

# Research plan to address root rots

- Identify sources of resistance
- Study mechanisms of resistance
- Develop breeding populations and select resistant progenies
- Tag molecular markers to resistance
- Disseminate and promotion resistant varieties
- Conduct impact studies on the usefulness of developed varieties



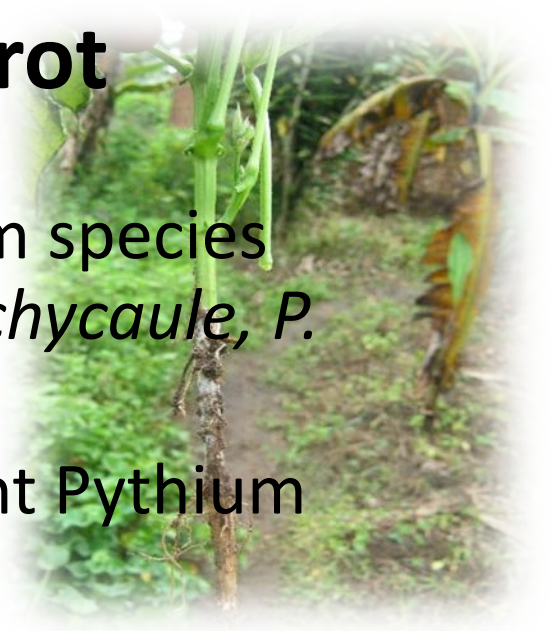
# Identification of sources of resistance to Pythium root rot

- Over 3000 screened on-station
- Nearly 500 lines evaluated in farmers' fields
- Over 300 lines under screen house conditions
  - Sixty eight tolerant and resistant lines (Buruchara and Kimani, 1999; Buruchara and Mayanja, 2000)
  - Regional root rot nursery
- Screening of regional nursery in BRR hot spots: Uganda, Rwanda, DRC, Burundi and Kenya
- Cvs. **MLB-49-89A, MLB-48-89A, RWR719, AND 620 and SCAM80-cm/15** selected as effective sources of resistance to Pythium root rot



# Race specificity of resistance to *Pythium* root rot

- Screening of regional nursery with ten isolates of seven *Pythium* species (*P. ultimum* var *ultimum*, *P. salpingophorum*, *P. spinosum*, *P. pachycaule*, *P. chamaehyphon*, *P. torulosum* and *P. ultimum*)
  - No significant differences among bean genotypes to different *Pythium* spp (Otsyula et al., 2010)
  - Good levels of resistance among: **MLB-49-89A, MLB-48-89A, AND1055, AND 620 and SCAM80-cm/15** previously selected as sources of resistance
- **Conclusion:** *Pythium* root rot resistance uniform and comparable for all races of *Pythium* and hence can be used to protect the commercial cultivars grown in different locations with different species.



# Host defense mechanisms of resistance to bean root rot

## Pythium root rot resistance

- Resistance to Pythium tagged to the small seeded meso American genepool.
  - Abawi and Pastor Corrales (1990), reported quantitatively controlled resistance in small seed beans of Meso-American origin and susceptibility of large seeded varieties
  - Similar finding reported by Kumar *et al.* (1991), Tu and Parker (1993), Parker and Tu (1994).
- Otsyula *et al.*, (1998), reported resistance, tolerance and susceptibility in small and medium seeded bean genotypes.
- However: Resistance in two large seeded Cvs. AND1055 and AND1062 (Buruchara and Kimani, 2001)



# Genetic mechanisms of resistance to *Pythium* root rot

- Genetics of resistance to *Pythium* root rot studied in MLB 49-89A, AND 1062 and RWR 719 (Otsyula et al., 2010)
- Single dominant gene governing resistance in MLB-49-89A, AND 1062 and RWR 719 suggested
- First evidence provided for inheritance of *Pythium* root rot in beans (Otsyula et al., 2003, Otsyula, 2010).
- Abawi and Pastor Corrales (1990) reported quantitatively controlled resistance for *Pythium* root rot

# Identifying and developing molecular markers linked to *Pythium* root rot resistance

- Four RAPD primers, (OPAA19, OPY20, OPG3 and OPBA08) and one RAMS marker (VHVGTT)<sub>5</sub>G segregating in coupling phase with the resistance gene in RWR719
- OPAA19, OPBA08 and (GT)<sub>n</sub> markers associated with resistance in MLB-49-89A, AND 1062, A 240, SCAM 80-CM/15
- Confirmed through allelism tests

**Implication:** resistant genotypes carry the same resistance gene locus, with the same or different alleles for conditioning *Pythium* root rot resistance.

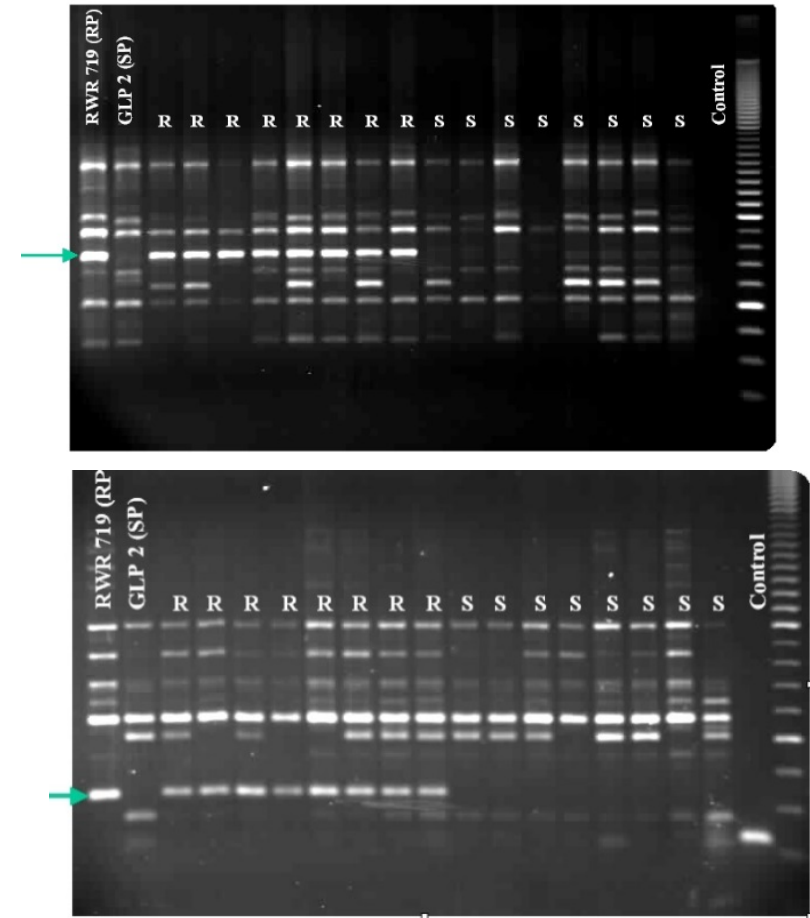


Figure 4: RAPD (A) OPAA19 and OPBA08, linked in coupling to the *Pythium* resistant gene in the common bean genotype, RWR 719.

# Identifying and developing molecular markers linked to Pythium root rot resistance

- SCAR markers developed for OPAA19, OPBA08 and (GT)<sub>n</sub> markers
- The SCAR marker derived from **OPAA19** was polymorphic and co-dominant
- The (GT)<sub>n</sub> and OPBA08 SCAR markers amplified similar sized fragment from susceptible and resistant plants.

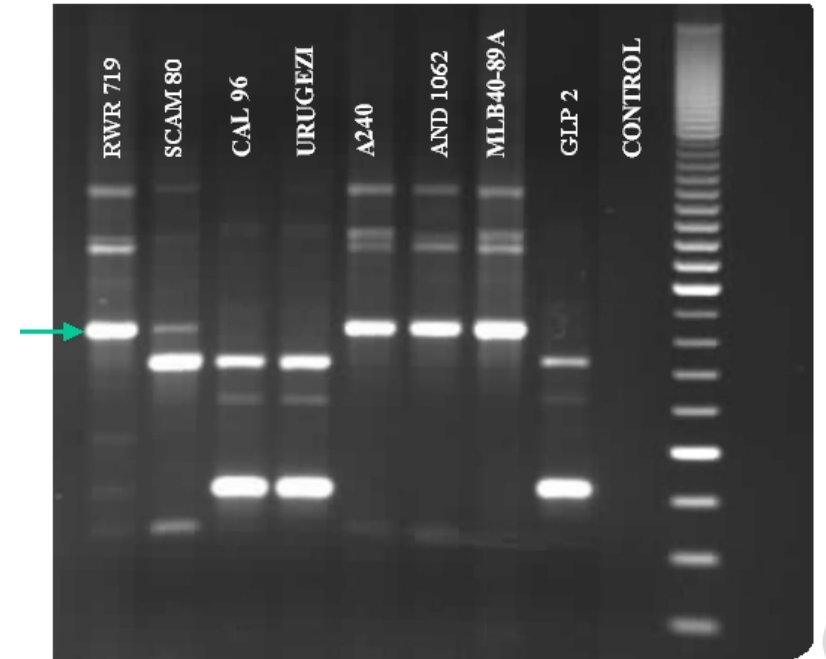


Figure 6: Amplification of resistant and susceptible bean genotypes using the SCAR primers derived from the OPAA19. The fragment associated with resistance was present in resistant and absent from susceptible genotypes.

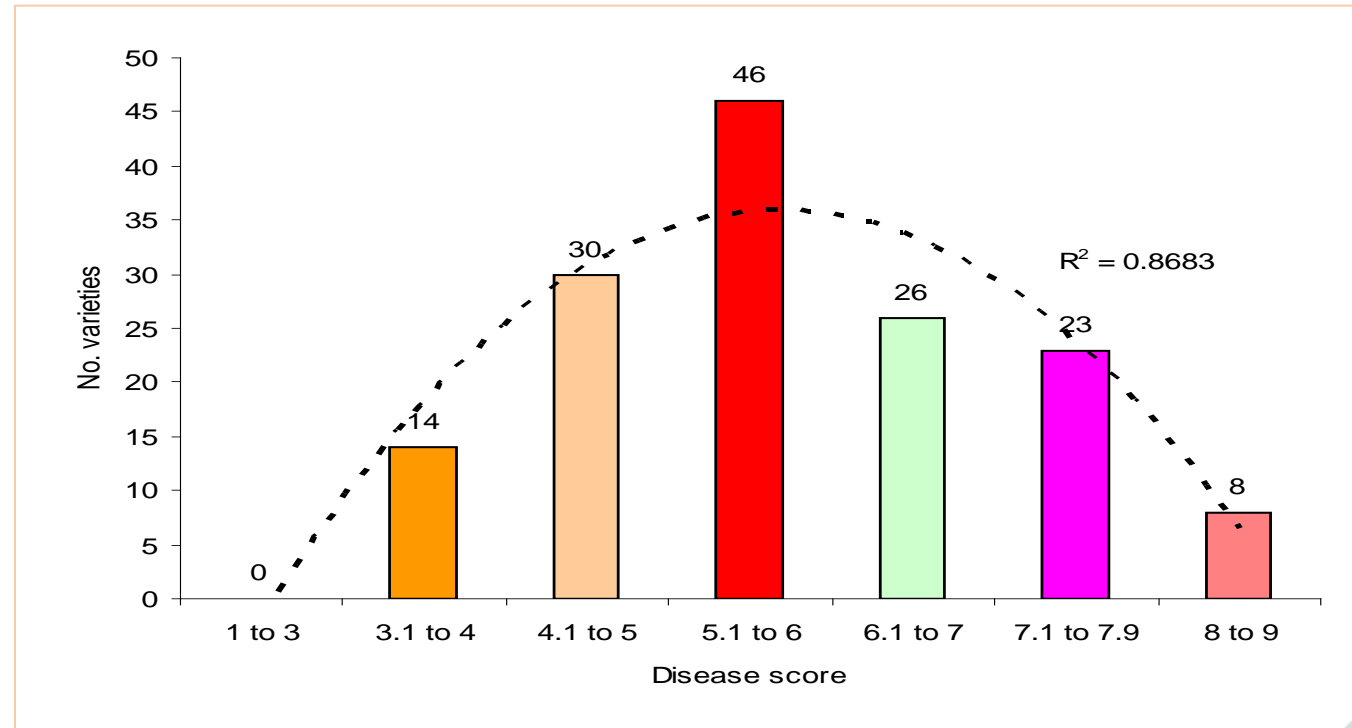


# Identification of sources of resistance to Fusarium root rot

- Screening of PRR regional nursery and documented sources of resistance and landraces
- All genotypes succumbed to disease infection though it varied among the genotypes (Tusiime, 2003; Mukankusi, 2008).
- Continuous distribution of FRR root rot severity scores characteristic of quantitative traits
- No genotype was scored as resistant (<3 on a score of 1-9),
- Lines ranked as **moderately resistant, tolerant and susceptible**

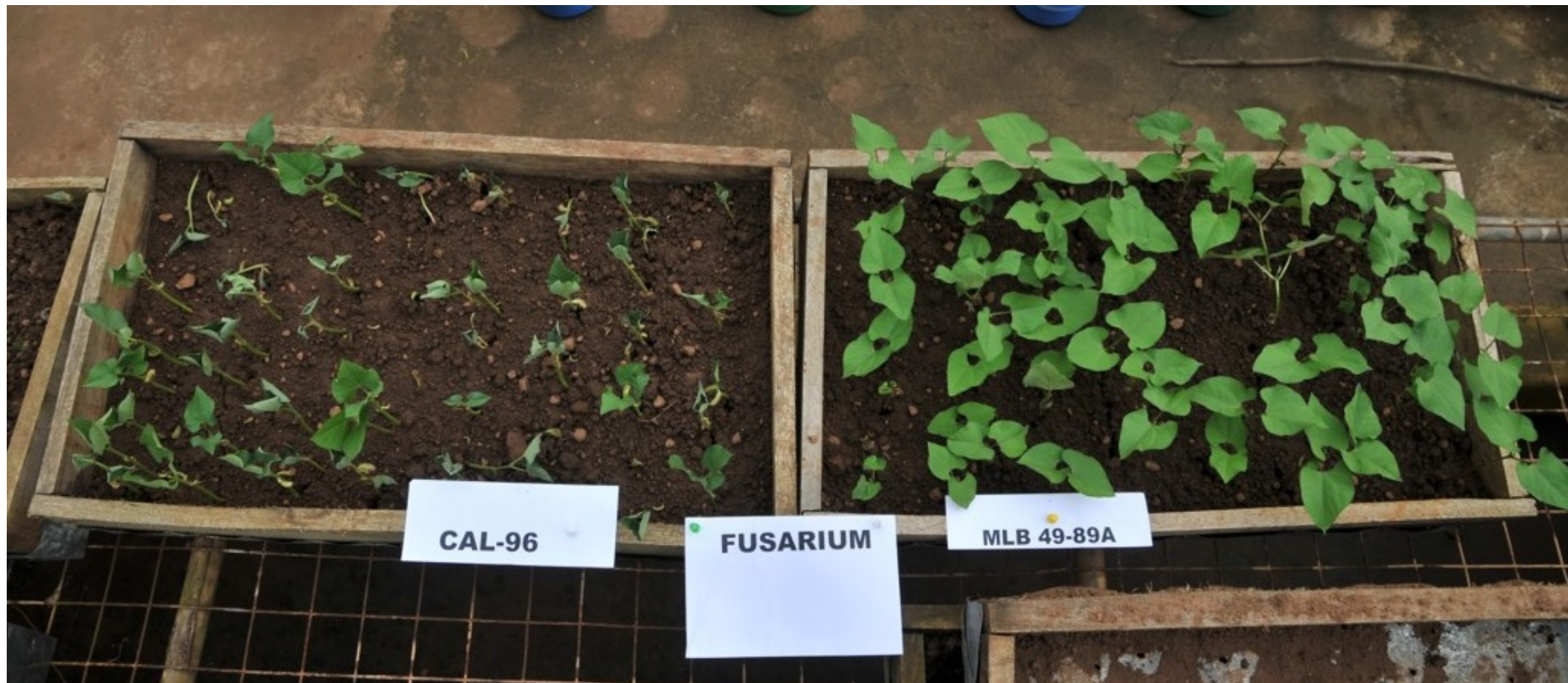
Implication:

- Quantitatively inherited mode of resistance implied



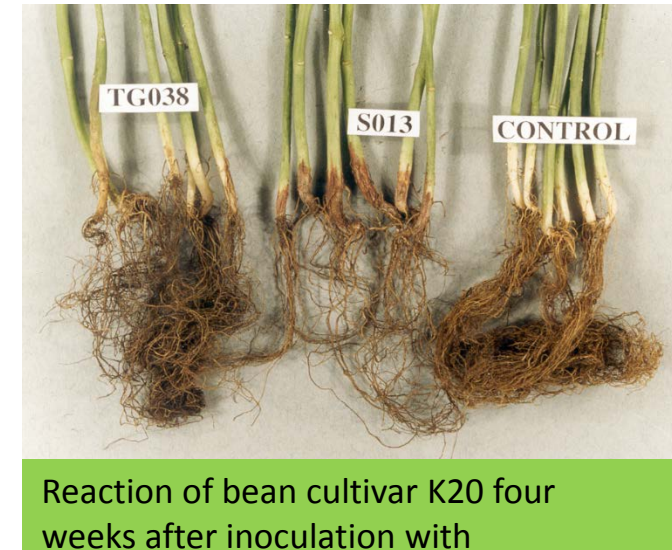
# Sources of resistance to Fusarium root rot

- Previously documented exotic resistant sources (CIAT, 1987) did not show high levels of resistance
- Landraces found very susceptible to the FSP-3 isolate.
- Cv. MLB-49-89A, RWR719, Vuninkingi, Hoima Kaki, G2333, Scam 80/15, Umgeni, MLB-48-89A, G1459 and G4795 selected as sources of resistance to FRR



# Race specificity of resistance to Fusarium root rot

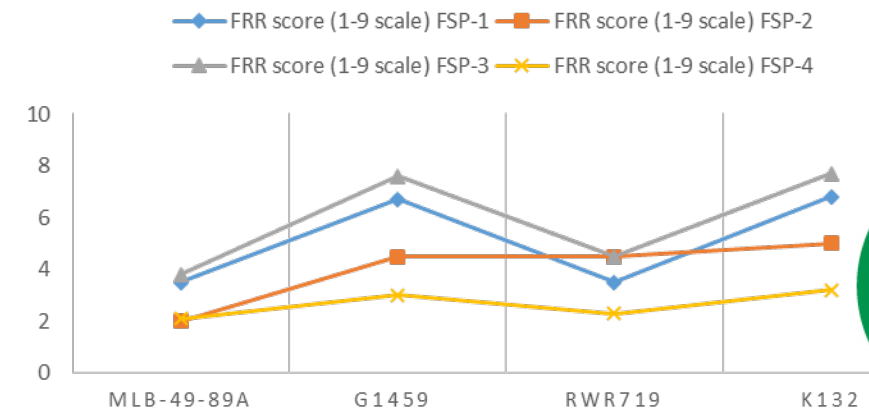
- Significant variety, isolate and variety x isolate effects on 14 Cvs. using four Fsp isolates with the most highly virulent isolates causing root rot on test genotypes (Tusiime, 2003)
- *F. solani* isolates TG038 and S013.
- Similar results reported by Mukankusi (2008)



## Implication:

- Resistance not comparable for all races *and may not be* used to protect the commercial cultivars grown in different locations where different species exist
- Strategy adopted: Use the most virulent isolate for resistance breeding best or mixed isolates

## REACTION OF FOUR BEAN LINES TO FOUR FSP ISOLATES



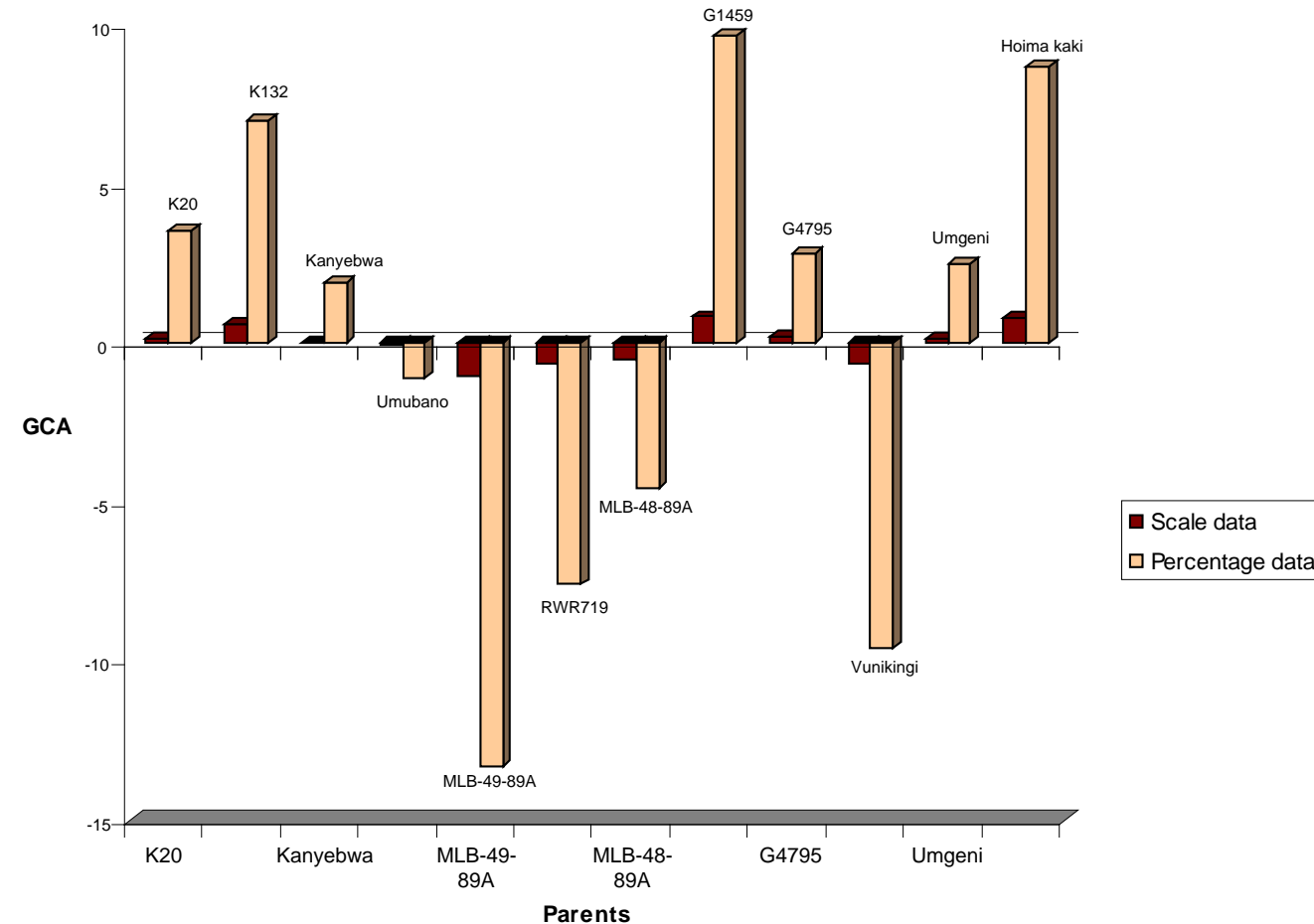


# Host defense mechanisms associated with Fusarium root rot resistance

- Mechanisms associated with host defense responses involved in resistance to FSP (Schneider et al., 2001; Román-Avilès and Kelly, 2005)
- Small-seeded meso American lines more resistant
- Colour of seed and hypocotyls related to the level of resistance (Staler, 1970; Beebe et al., 1981; Mukankusi, 2008)
  - Black seeded varieties and varieties with purple-coloured hypocotyls related to greater production of phenolic compounds inhibitory to fungal growth in the early stages of seedling growth.
  - Phytoalexins such as phaseolin produced in response to infection by FSP (Kendra and Hadwiger, 1989) greater and more rapid in resistant varieties.
  - Hypersensitive reaction to invasion by FSP (Pierre and Wilkinson (1970) limits growth of hyphae in resistant varieties.
  - Vigorous root system increases tolerance to root rot (Snapp et al., 2003; Román-Avilès et al., 2004.).
- **Selection, either direct or indirect, aimed at enhancing these traits should allow for rapid improvement of resistance to FRR in Andean bean genotypes**

# Mechanisms of resistance to Fusarium root rot

- Highly significant ( $P \leq 0.01$ ) GCA effects (Mukankusi, 2011, Ongom et al., 2013)
  - Additive gene action more important than the non-additive gene action
  - Predictability based on GCA high: 85-98% (Mukankusi et al.; 2009; Ongom et al., 2013)
  - **Implication:** Most resistant progeny may be produced by crossing the two parents with the highest GCA effects



**Fig. 1.** General combining ability effects of 12 parents for resistance to isolate FSP-3 of *Fusarium solani* f. sp. *phaseoli* in the F<sub>2</sub> generation

# Mechanisms of resistance to Fusarium root rot

- Significant maternal and non-maternal
  - Reciprocal effects were significant ( $P \leq 0.05$ ) and ranged from 25% to 49% of the genotypic variation in F1 to F3 generations, respectively
  - **Implication:** Cytoplasmic genes and cytoplasmic x nuclear gene interaction effects important

Table1. Maternal and non-maternal effects of 12 bean parents for resistance to isolate FSP-3 of *Fusarium solani* f. sp. *phaseoli* at F<sub>1</sub> generation

	K2	K3	KN	UB	M49	RW	M48	G1	G4	VN	UM	HK
K2	<u>0.06</u>	-0.40	-0.15	0.21	-0.29	-0.30	0.65	-0.82	-0.12	0.28	0.10	0.83
K3		<u>-0.01</u>	0.69	-0.17	0.02	0.57	0.17	0.05	-0.48	0.43	<u>-1.08*</u>	-0.60
KN			<u>0.44**</u>	0.13	-0.43	-0.61	-0.66	-0.29	<u>1.81***</u>	0.45	0.81	-0.34
UB				<u>-0.31*</u>	0.19	0.26	-0.94	0.27	0.16	-0.39	-0.01	0.61
M49					<u>-0.28</u>	0.05	0.51	<u>-0.99*</u>	-0.56	0.29	0.43	-0.24
RW						<u>-0.12</u>	-0.06	0.46	-0.44	0.18	0.22	-0.40
M48							<u>0.28</u>	0.53	0.10	-0.38	-0.51	-0.41
G1								<u>0.33*</u>	-0.23	-0.32	-0.18	-0.03
G4									<u>0.09</u>	0.20	-0.33	0.38
VN										<u>-0.37*</u>	0.34	0.40
UM											<u>-0.28</u>	-0.21
HK												<u>0.41**</u>
S.e.d <sub>ME</sub> (P=0.05)	0.148											
S.e.d <sub>NM</sub> (P=0.05)	0.489											

[1] Above Diagonal are the non-maternal effects and In Diagonal are the maternal effects.

K2=K20, KN=Kanyebwa, K3=K132, UB=Umubano, M49=MLB-49-89A, RW=RWR719, M48=MLB-48-89A, G1=G1459, G4= G4795, VN=Vuninikingi, UM=Umgeni, HK=Hoima kaki



# Mechanisms of resistance to Fusarium root rot

Heritability estimates:

Medium to high heritability reported and greatly affected by the environment

- 38%-45% (Mukankusi et al., 2011)
- 25.9% - 44.3% (Hassan et al. (1971)
- 35%-71% (Schneider et al. 2001; Román-Avilés and Kelly 2005).
- 76-86% (Ongom et al., 2013)

# Mechanisms of resistance to Fusarium root rot

- 2-9 genetic factors (Mukankusi et al., 2011)
  - Recessive resistance genes
  - Epistasis
  - Susceptibility genes
- 1-3 partially dominant loci, modified by epistasis (Ongom et al., (2013)
- 2-4 independent genes (Obala et al., 2012)
- Two duplicate recessive genes (McRostie, 1921)
- Three recessive genes (Azzam 1958).
- One recessive and one dominant gene (Smith and Houston (1960)
- >3 dominant genes in N203 and *P. coccineus* (Bravo et al., 1969; Hassan et al. ,1971)

Table 2. Chi square testing for goodness of fit of phenotypic classes in  $F_2$

Cross	Hypothesis	$\chi^2$ Value	Df	P value	Implication
MLB-49-89A x Vuninkingi	49:15	0.93	1	0.336	one dominant and two recessive genes
MLB-49-89A x G4759	9:7	0.49	1	0.482	two complementary dominant genes
MLB-49-89A x Umubano	57:7	0.01	1	0.931	one dominant and two complementary genes
MLB-49-89A x MLB-48-89A	57:7	0.60	1	0.438	one dominant and two complementary genes
MLB-48-89A x G4759	27:37	0.16	1	0.693	three complementary dominant genes
RWR719 x Vuninkingi	27:37	0.29	1	0.591	three complementary dominant genes

# Breeding strategies for Fusarium root rot

- Gene accumulation of the resistance genes on different loci from the different resistant parents (Young and Kelly 1996; Pastor-Corrales et al. 1998)
- Simple backcross to improving resistance in large-seeded Andean beans (Mukankusi et al., 2011; Ongom et al, 2013)
- Selection with multiple backcrosses alternating between the recurrent parent and donor parent: resistance modified by cytoplasmic gene effects and their interaction with nuclear genes (Mukankusi, 2008)
- Gene pyramiding: lower FRR symptom severity in crosses involving four FRR resistance parents [(G2333 x G685)x (MLB-48-89A x MLB-48-89A)] compared to two way crosses G2333 x G685 and MLB-48-89A x MLB-48-89A (Obala, 2012)
- Recurrent selection?

# Tagging molecular markers to Fusarium root rot

- Two SSR markers (PVBR87 and PVBR109) polymorphic for resistance to Fusarium root rot (Kamfwa, 2003)
- $R^2$  of 34% of identified markers for the major QTL in MLB-49-89A in a mapping population of MLB-49-89A x CAL96) of 90 individuals (Kamfwa et al., (2013)
- The same markers significantly associated with resistance in the MLB-49-89A x K20 population ( $R^2 = 14\%$ ,  $P < 0.001$ )
- Lack of association between PYAA19800 (*Pythium ultimum* resistance) and *Fusarium solani* resistance (Ongom et al., 2014)



# Wild Phaseolus sp as sources of resistance to root rot

- *P. acutifolius* and *P. lunatus* and interspecific lines of *P. coccineus* and *P. acutifolius*
- *P. acutifolius* and *P. lunatus* lines found resistant to Pythium root rot but responded differently to Fusarium root rot (PABRA report, 2012)
- 50% of *P. lunatus* lines and 44% of the *P. acutifolius* were resistant to Fusarium root rot
- 36% of *P. acutifolius* lines and 50% of the *P. lunatus* lines displayed combined resistance to Pythium and Fusarium root rots

Wild Phaseolus lines with combined resistance to Pythium and Fusarium root rot under screen house condition

Phaseolus acutifolius							Phaseolus lunatus						
Entry	Pythium root rot			Fusarium root rot			Entry	Pythium root rot			Fusarium root rot		
	Md	Mn	Me	Md	Mn	Me		Md	Mn	Me	Md	Mn	Me
G40001	2	2	2	2	3	2	G25758	2	2	2	2	4	2
G40005	2	2	2	2	3	2	G25008	2	2	2	2	3	2
G40019	2	2	2	2	2	2	G25047	2	2	2	2	4	2
G40023	2	2	2	2	4	2	G25059	2	2	2	2	3	2
G40062	2	2	2	2	4	3	G25224A	2	2	2	2	4	2
G40066A	2	2	2	2	3	2	G25366	2	2	2	2	4	2
G40068	2	2	2	2	4	2	G25695	2	2	2	2	3	2
G40125	2	1	2	2	3	3	G25701	2	2	2	2	3	2
G40137	2	2	2	2	4	2	G25718	2	2	2	2	4	2
G40139	2	1	2	2	4	2	G25728	2	2	2	2	2	2
G40144A	2	2	2	2	2	2	G25732	2	2	2	2	3	2
G40145	2	2	2	2	3	2	G25734	2	2	2	2	3	2
G40147	2	2	2	2	3	2	G25743	2	2	2	2	2	2
G40150	2	2	2	2	4	2	G25813	2	2	2	2	3	2
G40157	2	2	2	2	2	2	G25975	2	2	2	2	4	2
G40201	2	2	2	2	3	2	G26021	2	2	2	2	3	2
G40237	2	2	2	2	3	2	G26035	2	2	2	2	3	2
G40158	2	2	2	2	3	2	G26065	2	2	2	2	4	3
							G26068	2	2	2	2	4	2
							G26298	2	2	2	2	4	2
							G26363	2	2	2	2	3	2
							G26440	2	2	2	2	4	2
							G26489	2	2	2	2	3	2
							G26524	2	2	2	2	2	2
							G25028	2	2	2	2	3	2
CAL 96	9	9	9	9	9	9	CAL 96	9	9	9	9	9	9
RWR 719	2	2	2	-	-	-	RWR 719	2	2	2	2	2	2
MLB-49	-	-	-	2	2	2	MLB-49	-	-	-	-	-	-
CV (%)	9.4	11.8	11.7	59.9	28.6	56.5	-	0	0.04	0	55.9	21.6	48.9
Se	0.20	0.23	0.24	2.65	1.32	2.33	-	0	2.00	0	2.30	0.99	1.85
Sed	0.28	0.33	0.34	2.16	1.08	1.91	-	0	0.04	0	1.88	0.81	1.51
Lsd (5%)	0.4	0.5	0.5	4.3	2.1	3.8	-	ns	0.08	ns	3.7	1.6	3.0

# Wild Phaseolus sp: as sources of resistance to root rot

- 34% of 186 interspecific lines (*P. coccineus* G35346 x *P. vulgaris*; SER 16) resistant to PRR and 74% to FRR
- 31% resistant to both root rot pathogens
- Sources of resistance identified
- **Observation:** Resistance to Fusarium root rot infrequent among the wild *Phaseolus* sp. than among the interspecific and vice versa for resistance to Pythium root rot



# Development of root rot resistant varieties

1. Identification of root rot resistant materials with regional and international nurseries
2. Protection of I gene in root rot resistant varieties from Uganda and Rwanda (RWR1956, RWR2075, CAB2) through introgression of bc3 gene
3. Introgression of root rot resistance into commercial backgrounds of commercial bean varieties in Kenya, Uganda and Rwanda (Urguezi, GLP2, GLP585, and CAL96)
4. MAS using SCAR PAA19 marker

**Table Pythium root rot severity, presence of root rot marker and yield of advanced lines**

Cross	No. lines	Pythium severity (1-9 scale)				Presence of marker		Yield (kg/ha)		
		1-3	4-6	7-9		+	-	<500	500-1000	>1000
F6 BC- S4 (GLP 585 x RWR 719)	9	5	4	0		3	6	0	3	6
F6 BC- S4 (Uruezi x RWR 719)	20	10	9	1		3	17	2	6	6
F7 BC- S5 (GLP 2 x RWR 719)	9	8	1	0		5	4	2	8	5
F8 (GLP 2 x RWR 719)	24	10	12	2		16	8	1	8	9
F8 ( CAL 96 x RWR 719)	8	4	3	1		6	2	0	6	2
F8 (Uruezi x RWR 719)	1			1		1	0	0	1	0
F8 (GLP 585 x RWR 719)	3	-	3	-		2	1	0	1	2
F5 BC- S5 (GLP2 x RWR719)	27	15	11	1		11	16	1	7	5
Total	101	52	43	6		47	54	6	40	35

# Dissemination and promotion of root rot resistant varieties

## Participatory variety selection

- Root rot nursery of entries evaluated by farmer groups in SW Uganda and W. Kenya.
- A number of entries selected for further examination by farmer groups.
- Selection criteria included adaptation to their conditions, resistance to root rots, vigor, yield and seed characteristics
- ***Application of farmer participatory approaches during evaluation and dissemination of the improved bean varieties enhanced adoption***
- ***However, there is was a call for more involvement of farmers in variety development***



# Dissemination and promotion of root rot resistant varieties

## Modified farmer field schools (MFFS)

- PVS trials utilized as modified farmer field schools as learning tools to address identified farmer knowledge gaps
- Appropriate capacity building tool that enabled detailed-learning among beneficiaries resulting into empowerment in terms of knowledge and skills.

# Impact of root rot resistant varieties (Kenya)

- Adoption of five root rot resistant bush (KK8, KK14, KK15, KK20 and KK22) and five climbing bean varieties (Umubano, Gisenyi, Flora, Puebla and Ngwinurare) introduced in Kakamega and Vihiga Districts of Western Kenya assessed (Odendo (2010)
- This was in response to the significant decline in bean production due to the bean root rot disease crisis reported in the late 1980s and early 1990's.
- 35-80% of the farmers mainly adopted three bush bean varieties KK22, KK15 and KK8.
- 8-18 % adopted the climbing beans, especially Umubano and Gisenyi
- Low adoption of climbers due to high labour demand, especially for staggered harvesting and staking.
  - Shortage of stakes,
  - Bird damage
  - Inability to be intercropped in maize.

# Impact of root rot management technologies in Uganda)

- Study conducted in 2010 to assess technologies introduced in Kabale district, SW Uganda in 2001 (Lubega, 2010)
- The study examined knowledge of BRR, its management practices and how the IPDM technologies were diffusing into the communities
- Findings showed an increased and more detailed understanding of BRR disease, its stages of development and the effects it causes
  - Farmers' description of damage caused on the different plant parts was a combination of the different stages of disease development as well as the severity

# Technologies adopted to control bean root rot in Uganda and their impact

Technology	How the technology has helped	Sustainability plan
Application of compost manure (46%)	Increased production (26%), soil fertility improvement (6%), reduced disease (5%), cheap (4%) tasty bean leaves, controlled BSM	Dig compost pits (18%) Rare livestock (11%) Plant fodder (6%)
<b>Changed varieties (8%)</b>	<b>Increased yield (4%)</b>	<b>Save own seed (5%)</b>
Use of FYM (64%)	Increase yields (37%), increases soil fertility (8%), cheap (6%), controls pests/diseases (4%)	Rare livestock (19%) Dig compost pits (15%) Plant fodder for animals (6%)
Planting in lines (15%)	Use less seed (3%), get high yield (5%) and reduced time in weeding & harvesting	Continue practice (8%) Planning fields (2%)
Digging trenches & stabilizing bunds with agro-forestry trees and grasses (22%)	Stops erosion (3%), controls floods (9%) provide stakes, firewood and animal fodder & improves soil fertility	Construction of drainage channels (16%)



# Impact of improved varieties

- New bean root rot varieties released in 2003 in Uganda and 2014 in W. Kenya
- More recent impact studies indicate that the bean root rot resistant varieties are of greater value to farmers when used in combination with soil management practices such as use of soil amendments (both organic and inorganic), terracing, contour planting on sloppy land, crop rotation and use of clean seed (Kankwatsa unpublished)
- In general, farmers do not perceive any negative effects of the technologies on the environment but they observed reductions in pests and diseases and improvements in soil fertility.

# Conclusions !!



“Some investigators believe that factors, such as the ability of the seed to germinate in the cold, the ability to develop a large, vigorous root system, and the presence of inhibitory substances in the seed coat and hypocotyls may increase the level of genetic tolerance.” (Zaumeyer & Meiners, 1975).

- Future Publication:

**“100-years of Breeding for Root Rot Resistance”**

What will it say ????

