Emerging and re-emerging *Ralstonia solanacearum* species complex strains causing bacterial wilt of potato in Sub-Saharan Africa

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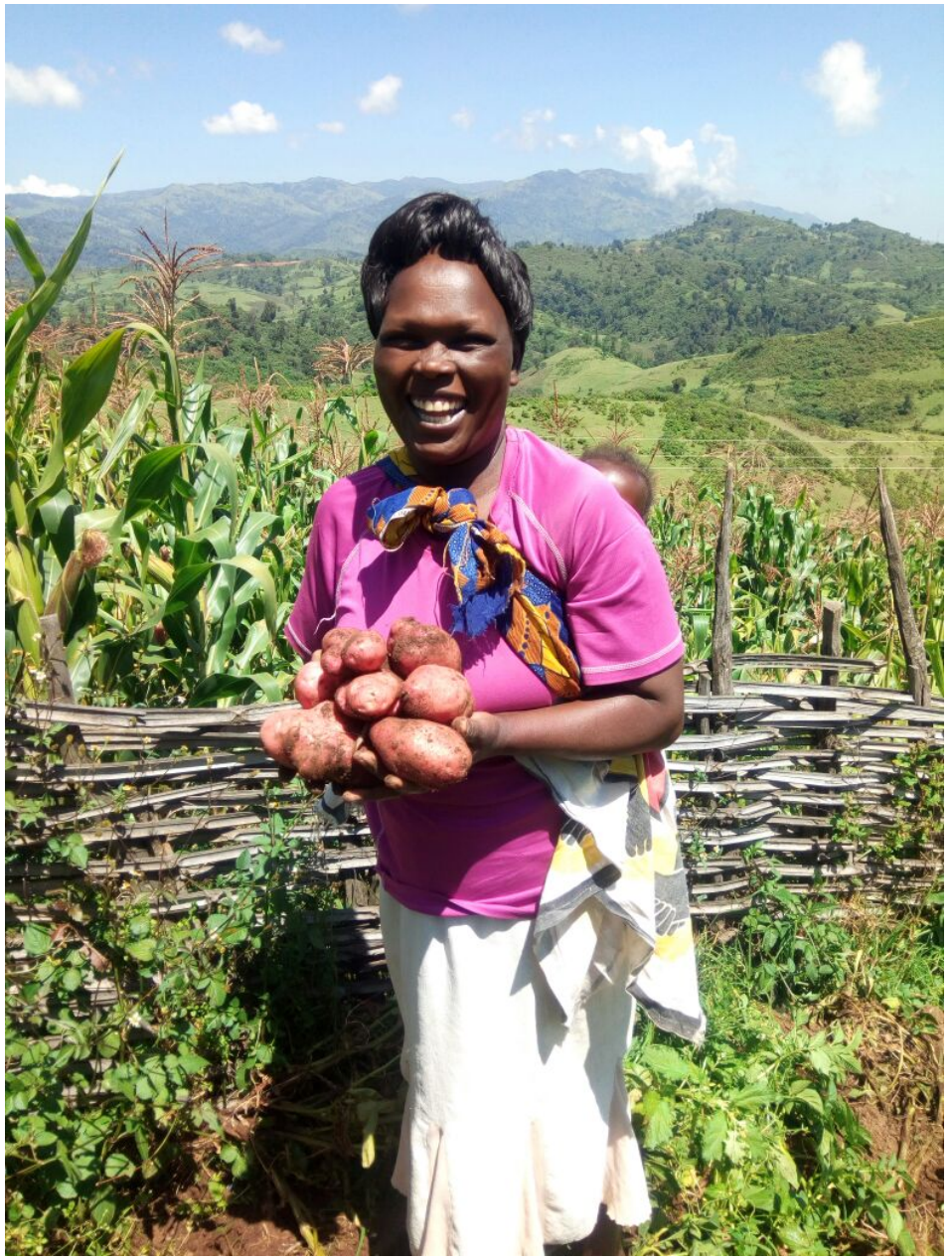
Potato in Sub-Saharan Africa (SSA)

- Food security crop for ~ 5 million potato farmers
- Area has increased 2 to 6x in past 25 years, ~1.6 million ha
- Short cropping cycle of 3-4 months
- An important crop for the “hunger months”
- Average yield 8-10 t/ha
- Potential yield goes up to 45-50 t/ha; opportunities to increase food availability without increasing resources, especially land



Potato in SSA

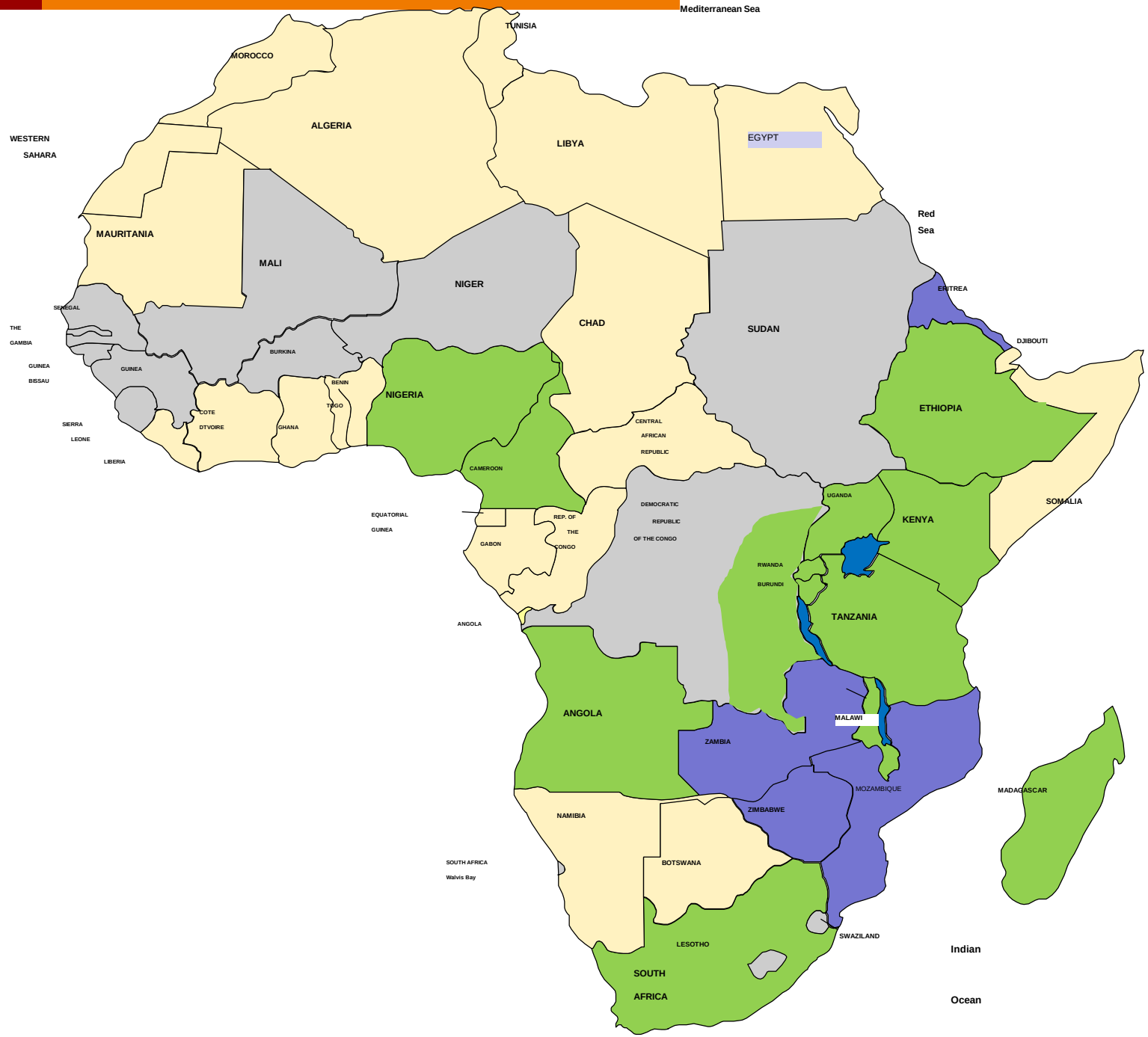
Potato among top consumed and strategic crop in many countries, importance is increasing!

- Highland crop, 1,500 – 3,500 masl
- East, Central and West Africa: rain-fed crop, 1-2 growing seasons/year
- Southern Africa: grown in cold- dry season with irrigation – 1 growing season/year

Potato in SSA

High potential for impact

Medium potential for impact



Profile of SSA Potato Farmer

- Moderate use of inputs
  - 25% spray 3x against late blight
- Limited access to seed, re-use seed over and over
- Limited knowledge on proper production practices
- Limited amount of quality assured and *Ralstonia* free seed

Yields 8-10 t/ha

Ralstonia solanacearum species complex (RSSC)

- Destructive pathogen, seed and soilborne
- Numerous economical crops important in tropics: potato, tomato, capsicum, tobacco, eggplant
- Widespread, essentially endemic in SSA
- Avoidance/quarantine impossible in many regions

Bacterial Wilt: re-emerging issue

- Increasing prevalence and incidence from 2017-2018 survey

Country	BW prevalence
Ethiopia	60% (158 out of 263 farms)
Kenya	72% (128 out of 176 farms)
Rwanda	80% (84 out of 104 farms)
Uganda	75% (170 out of 288 farms)

- Detected from altitude of 3,300 masl seed producing areas

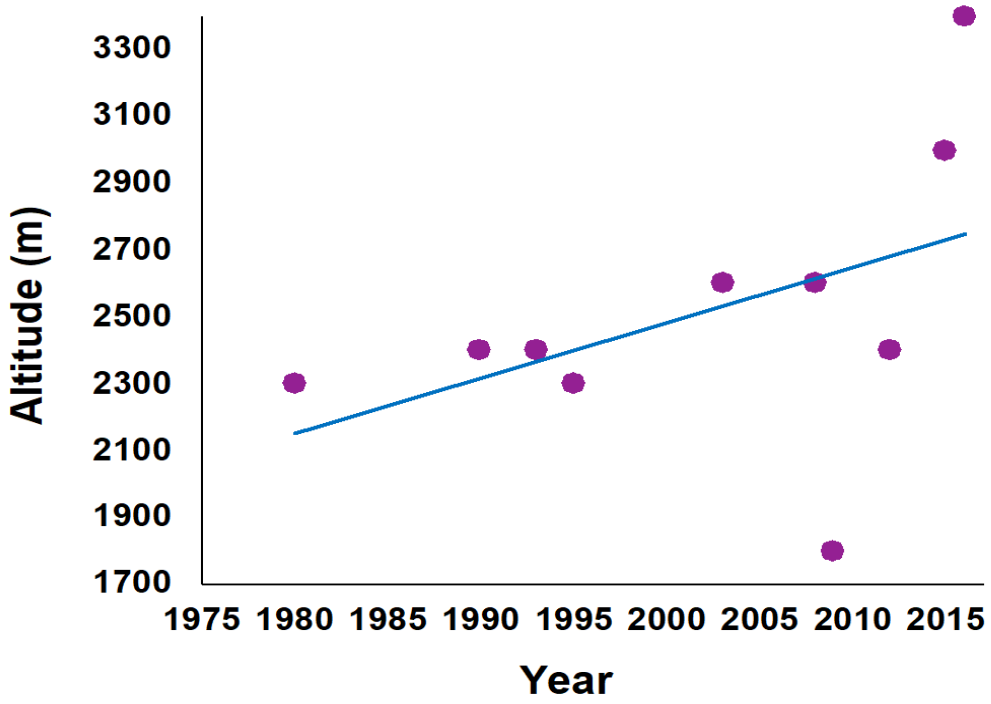
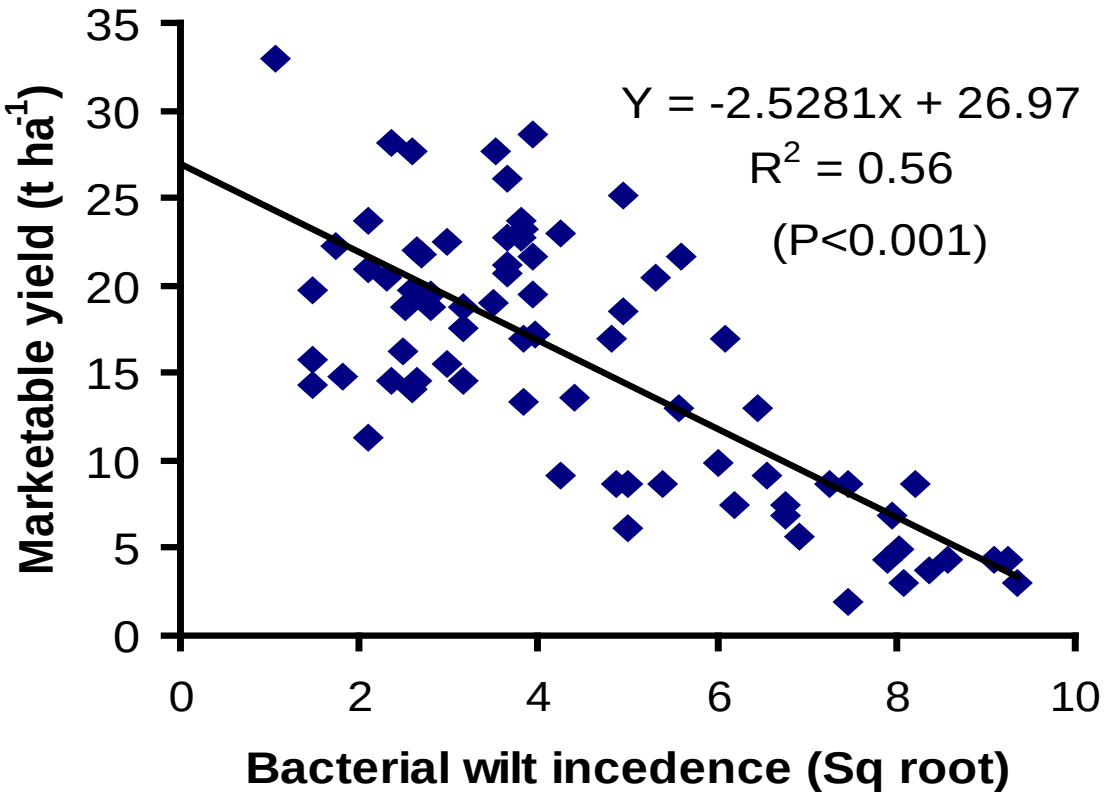


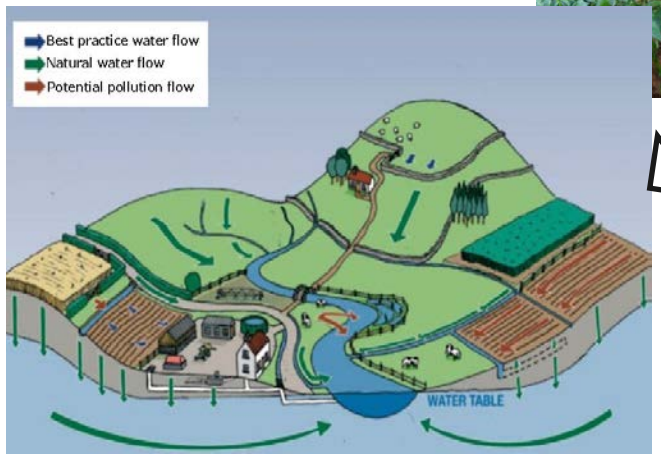
Figure 1. Bacterial wilt incidence at different altitude. Positive correlation between observed incidence of bacterial wilt at higher altitudes in Ethiopia over years.

Bacterial Wilt – Yield Losses



COUNTRY	% yield loss
Burundi, Rwanda	30–70
Ethiopia	45–60
Kenya	50–70
Uganda	26–100

How did *RSSC* strains become so widespread in SSA?



- **Lack of crop rotation**
- **Shared farm tools**
- **Sloppy land**

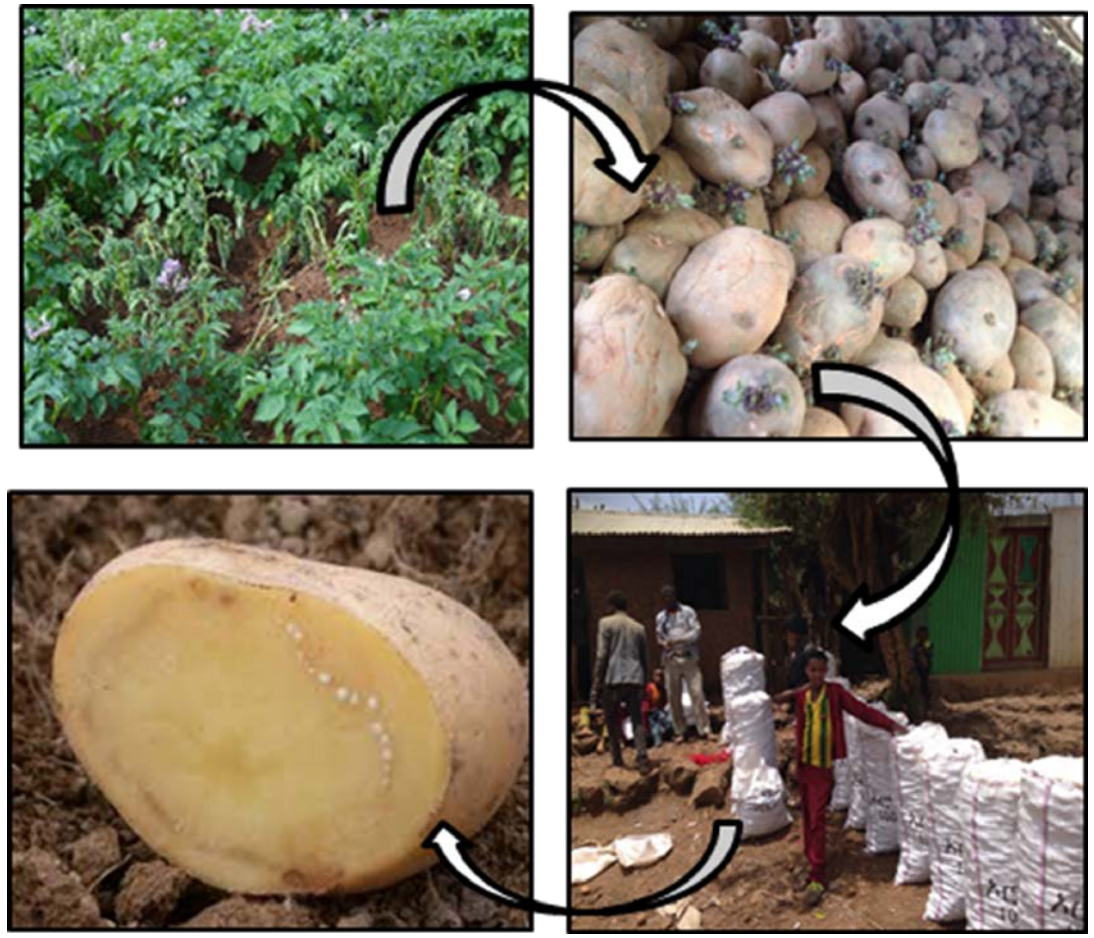
Seed systems in SSA

Certified seed (less than 2%) →

Informal seed

- **Decentralised seed production (mostly informal)**
- **Farmer-based seed market**
- **Farm-saved seed**

Bacterial wilt present in all stages of potato value chain



Ralstonia is a major cause of poor seed quality

Farmers predominately use poor quality seed, & consequently, low yields

Ongoing Research Efforts

- **Risk assessment, climate change impact**
- **RSSC diversity**
- **Molecular epidemiology of RSSC strains causing bacterial wilt of potato**
- **Diagnostics**
- **Breeding for tolerant varieties**

Pressure of climate change

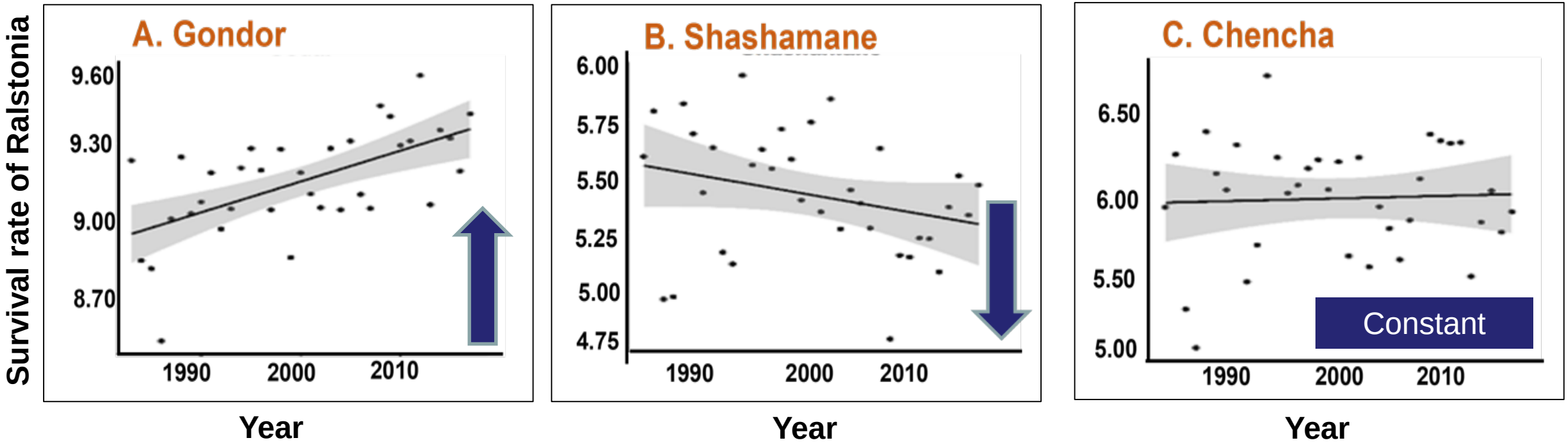


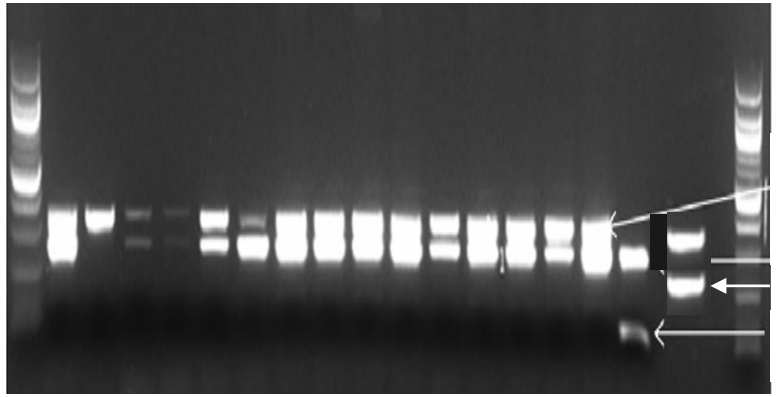
Figure. Estimating the survival rate of bacterial wilt pathogen by years (1983-2017) in three different regions of Ethiopia. Each dot represents the survival of *RSSC* strains and shaded region being 95% confidence interval.

- Simulations were done to study the survival rate of Ralstonia over years of potato growing season.
- Model assumes survival to be minimum of 0.01 at 4℃ to start the epidemic. A polynomial regression was used to estimate the parameter values. The model uses hourly temperature. Daily survival was calculated by averaging every 3-hour survival.

Shakya et al. under preparation……..

RSSC diversity

- Nation wide Ralstonia survey in Ethiopia, Kenya, Rwanda and Uganda
- Screened using RSSC specific primers pair 759/760
- Phylotyped by multiplex PCR, Sequenced



RSSC diversity from Eastern Africa**			
East Africa	P I	P II	P III
Ethiopia	x	x	x
Kenya	x	x	x
Rwanda	x	x	x
Uganda	x	x	x

**Based on new classification- Phylotype I: Asia; Phylotype II: America; Phylotype III: Africa; Phylotype IV: Indonesia

Molecular epidemiology of RSSC strains causing bacterial wilt of potato

- Isolates of the same sequevar were then haplotyped using multi-locus Tandem Repeat Sequence Typing (TRST) schemes to map and trace the movement of epidemiological RSSC strains
- Evidence based findings on regional and cross border seed movement

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Molecular characterization of *Ralstonia solanacearum* strains from Ethiopia and tracing potential source of bacterial wilt disease outbreak in seed potatoes

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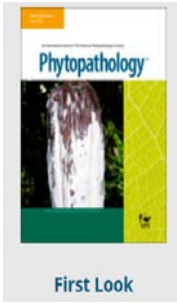

Molecular epidemiology of *Ralstonia solanacearum* species complex strains causing bacterial wilt of potato in Uganda

Abdulwahab Abdurahman, Monica L Parker, Jan Kreuze, John Elphinstone, Paul Christiaan Struik, Andrew Kiggundu, Esther Arengo, and kalpana Sharma

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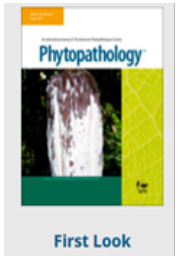
****Surveyed districts, host plants, and distribution of the pathogen by Phylotype, Sequevar, TRST haplotype- Uganda**

Region	District	Host of RSSC[2]	RSSC strains	Sequevar	TRST haplotypes[4]
Central	Mubende[1]				
	Mityana	Tomato, Eggplant, Chili	Phylotype I (Rps)	31	Rps-TRST 2
	Butambala[1]				
	Kayunga	Tomato, Eggplant, Garden egg, Green pepper, Sodom apple	Phylotype I (Rps)	31	
	Mukono	Tomato, Eggplant, Green pepper	Phylotype I (Rps)	31	Rps-TRST 1
	Wakiso	Tomato, Eggplant	Phylotype I (Rps)	31	Rps-TRST 1, 4, 5
		Hot pepper	Phylotype II (Rs)	1	
	Nakaseke	Nakati, Tomato, Eggplant, Garden egg, Green pepper, Sodom apple	Phylotype I (Rps)	31	Rps-TRST 1, 2, 3
	Luwero[1]				
Eastern	Kween	Potato	Phylotype II (Rs)	1	Rs-TRST 1, 4, 6, 16, 27
	Kapchorwa	Potato	Phylotype II (Rs)	1	Rs-TRST 14
	Bulambuli	Potato	Phylotype II (Rs)	1	Rs-TRST 1, 2, 4, 5, 10, 15, 18
	Mbale	Potato	Phylotype II (Rs)	1	Rs-TRST 1, 2, 4, 9, 10, 20
	Ngora[1]				
Northern	Zombo	Potato	Phylotype II (Rs)	1	Rs-TRST 2, 13, 26
		Potato	Phylotype III (Rps)	-3	
Western	Kabale	Potato	Phylotype II (Rs)	1	Rs-TRST 1, 2, 3, 5, 7, 8, 12, 17, 19, 21, 22, 23
	Kisoro	Potato	Phylotype II (Rs)	1	Rs-TRST 2, 11, 24
	Kasese	Potato	Phylotype II (Rs)	1	Rs-TRST 25
	Kabarole	Potato	Phylotype II (Rs)	1	
	Kyenjejo	Potato	Phylotype II (Rs)	1	

Based on new classification- **Phylotype I and III: *R. pseudosolanacearum*; ***Phylotype II: R. solanacearum***; ***Phylotype IV: R. syzygi***



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- Blue arrows indicate the main seed potato distribution pattern
- Red arrow indicates secondary seed distribution pattern

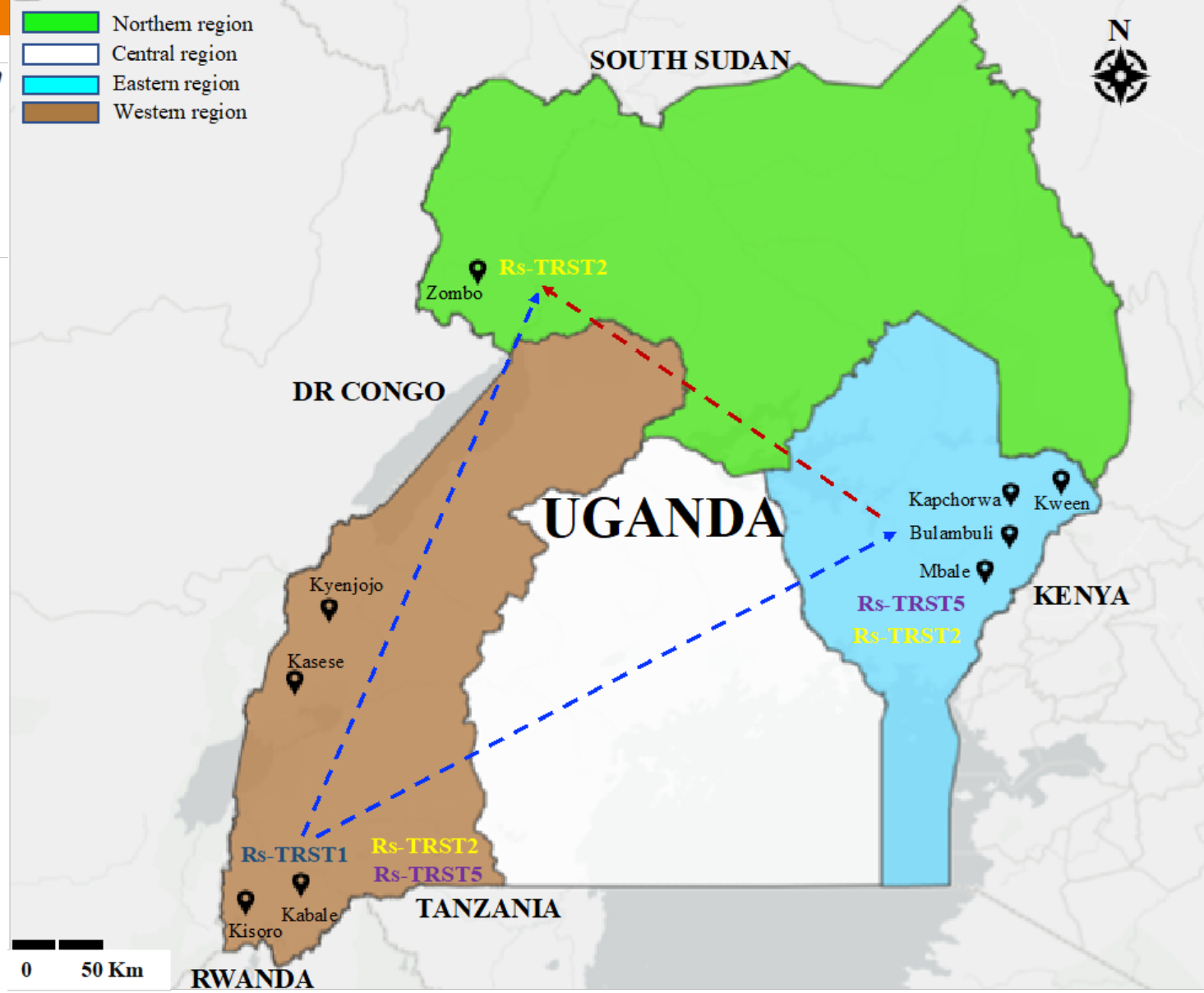
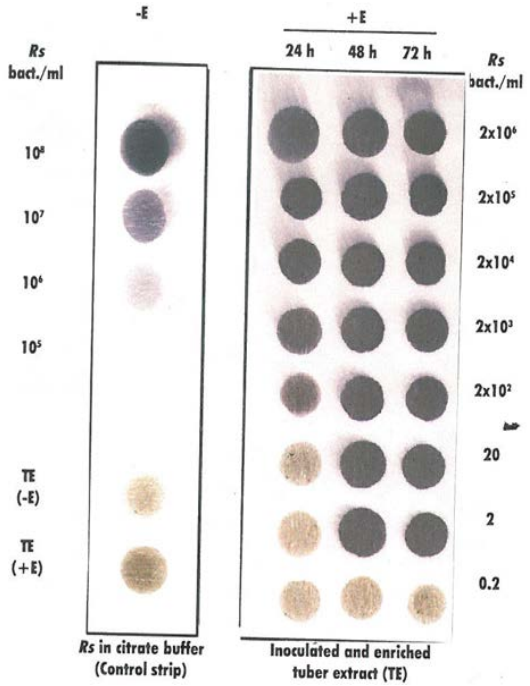


Figure. Tandem repeat sequence haplotype localization of epidemiologically important Phylotype II Sequevar 1 strains of RSCC in Uganda.

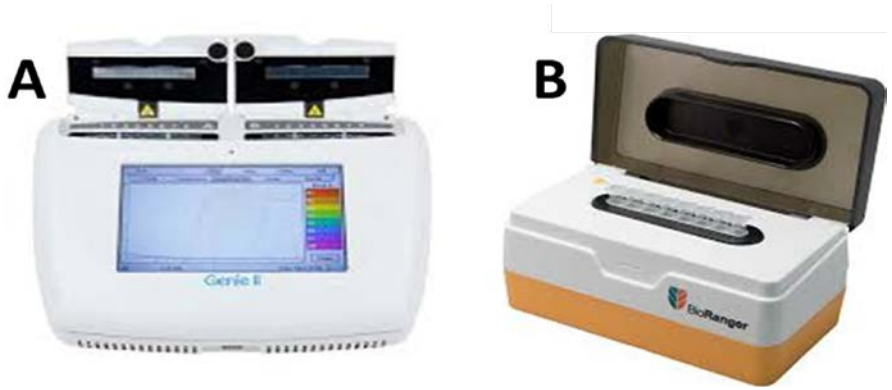
Enhancing efficiency and efficacy of diagnostics

ELISA test is current method to detect viruses and *Ralstonia solanacearum* for seed quality



- False positives for Rs
- Sample management- transport, processing!
- 14 days waiting
- Delays – seed overgrows

- Improve efficiency and efficacy
- Increase flexibility



LAMP (loop mediated isothermal amplification) assay to detect *Ralstonia* in the field and at the port of entry/exit

Figure 1. Field deployable LAMP instruments, A) Genie II (Optigene, UK) and B) BioRanger (Diagenetix, USA)

Field deployable LAMP assay

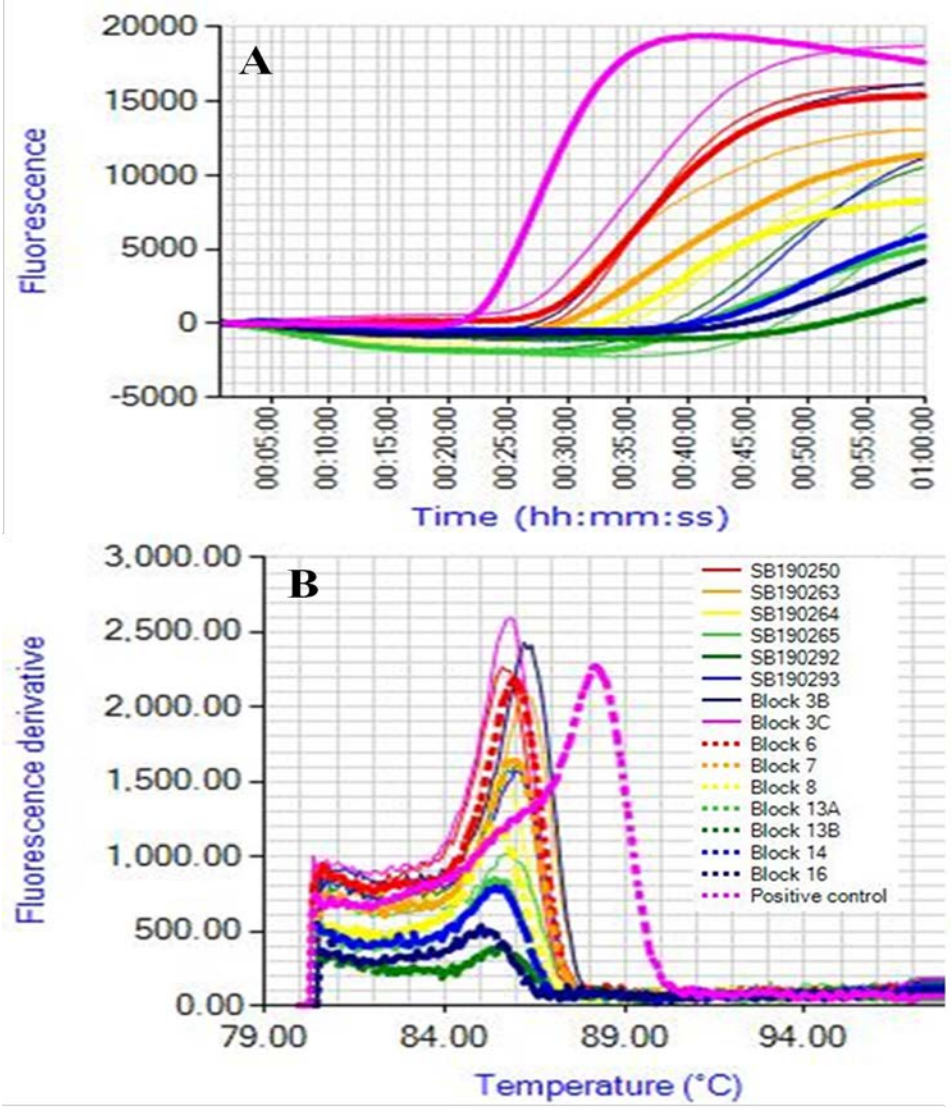


Figure 2. A) Amplification curve, soil samples lysed in Alkaline PEG200 solution; B) Anneal (melting) temperature, soil samples lysed in Alkaline PEG200

Table 1. Comparison of LAMP assay over alternative tests for BW disease diagnostic tools, ELISA, PCR, and qPCR

Diagnostic Tools	On-site Testing[1]	Time to Get Results (hr)[2]	Cost/100 Samples ($)[3]	Strain Specificity[4]
LAMP	Yes	0.5–0.8	300	Very high
ELISA	No	96-120	350	Low
PCR	No	5	700	High
qPCR	No	2	1,000	Very high

[1]Testing on on-farm potato fields or at point of entry (quarantine control).

[2]From receiving samples to getting test results for decision-making. Current turnaround time for ELISA is at least 1 week.

[3]Excluding labour costs, which are significantly higher than LAMP.

[4]Detection of the entire RSSC strains or specific to potato brown rot strains.

Bacterial wilt tolerant potato varieties?

- Evaluation trials in Western Kenya = humid tropical zone
- Trials in farmers' fields, green house
- Bacterial wilt was a greater challenge than anticipated
- Surviving clones, 9/20 clones had latent Infection

Hybrid breeding/ True potato seed (TSP)?

Concluding Remarks

- Ralstonia has reached all corners of the potato value chain
- Tackling seed systems is a key to disease management
- Climatic shifts are also contributing to the expansion of bacterial wilt, more research is needed
- Integrated seed health strategy
- Systems approach is required in bacterial wilt management, combining crop-rotation, host plant resistance, quality assured and Ralstonia free seed .....

  -trainings, capacity development

  -growers behavior and attitude change

Thank you

Ethiopian Institute of Agricultural Research (EIAR)

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