

Tracking Ug99 and Other Races of the Wheat Stem Rust Pathogen Using SNP Technologies



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Wheat Stem Rust

Pathogen: *Puccinia graminis* f. sp. *tritici* (Pgt)

Disease: Wheat stem rust or Black rust

Host: Wheat, barley and few grasses

Life cycle stages:

- Urediniospores (n + n)
- Teliospores (n + n => 2n)
- Basidiospores (n)
- Pycniospores (n)
- Aeciospores (n + n)



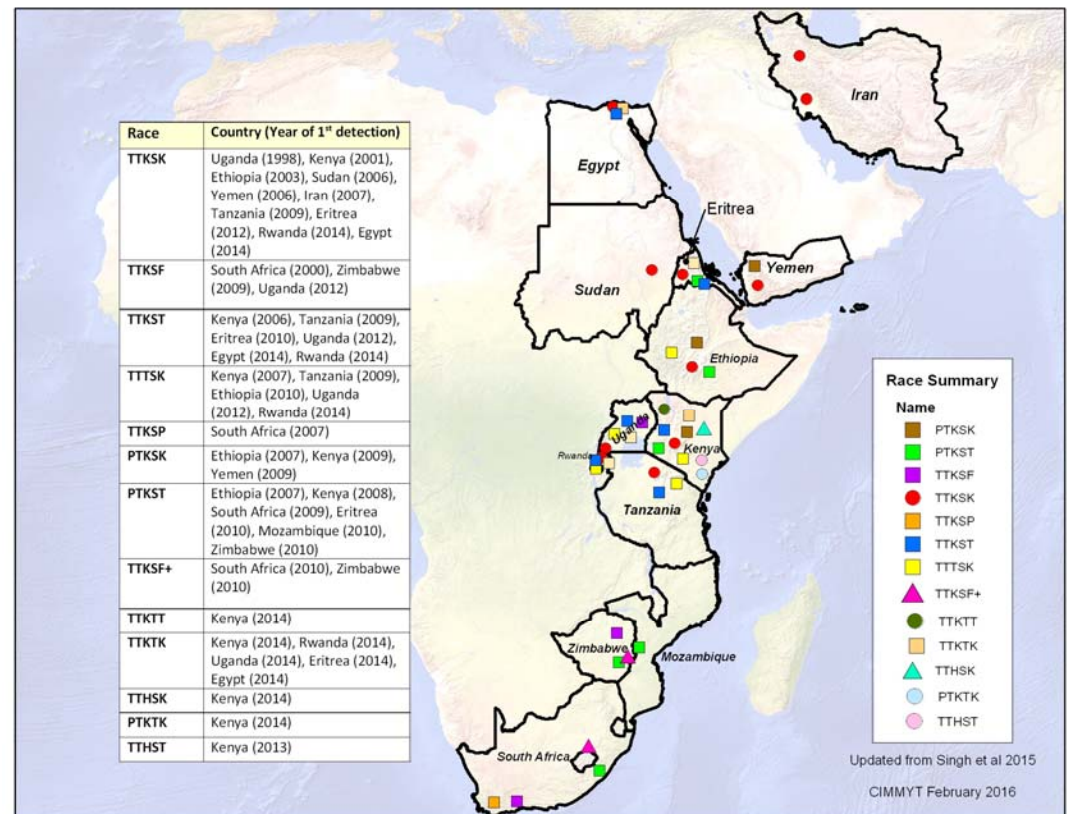
Wheat Stem Rust: Ug99

- First found in Uganda in 1998 and characterized in 1999.
- Ug99 = *Pgt* race TTKSK
- In 2004 Ug99 caused a major epidemic in Kenya.
- Ug99 and its variants pose a critical threat to wheat production worldwide.



Ug99 Race Group

➔ Ug99 race group is a complex of related races that belong to the same genetic lineage.



Molecular diagnostics – Ug99 RG

- Two stage Assay
 - Stage-1
 - Is it Ug99 RG?
 - Stage -2
 - What member of the Ug99 RG?



Field Sampling Procedure

Select a well separated pustule from *Pgt* infected stem



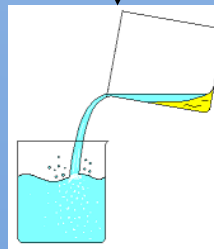
Excision of single pustule



Place specimen in microfuge tube



Ethanol Treatment (75%)
for 5-7 days



Decant ethanol and let air dry



Send sample

Stage 1: qPCR assay

Race/isolate	Ug99RG-P01	Ug99RG-P02	Ug99RG-P03	Ug99RG-P04
TTKSK/04KEN56-4	24.6 (23.6)	27.1 (28.9)	25.8 (26.7)	30.4 (26.2)
TTKSK/09ETH04-4	22.0 (21.5)	24.5 (25.7)	23.0 (24.9)	28.6 (24.9)
TTTSK/07KEN24-4	24.6 (23.6)	27.1 (28.9)	26.0 (26.7)	30.1 (26.9)
TTKST/06KEN19-v-3	21.1 (20.1)	23.9 (23.2)	23.0 (23.3)	26.3 (22.6)
TTKSF/09ZIM01-1	25.3 (25.0)	26.1 (24.7)	25.6 (26.1)	30.8 (26.6)
Non-Ug99 Race group isolates				
TRTTF/06YEM34-1	Neg. (20.8)	Neg. (26.0)	Neg. (28.8)	26.9 (23.2)
JRCQC/09ETH04-1	Neg. (22.1)	24.9 (26.2)	23.1 (25.3)	Neg. (25.2)
BKCS/75MEX1830	Neg. (27.6)	27.8 (29.6)	Neg. (27.7)	Neg. (26.9)
HFCQ/84CSK759c	25.1 (24.7)	Neg. (29.2)	Neg. (28.4)	31.1 (27.8)

Stage 2: SNP marker set

Race	Isolate	Genotype	A003	A005	A007	A010	A011	A012	A013	A014	A017	A020	A021	A022	A026	A030
TTKSK	98UgA_1	AF-001aa	CC	GG	GG	TT	CC	TT	CC	AA	CC	CC	TT	CT	GG	AA
TTKSK	04Ken156-4	AF-001ac	CC	GG	GG	TT	CC	TT	CC	AA	CC	CT	TT	CT	GG	AA
TTKSK	07Ken34-1	AF-001ad	CC	GG	GG	TT	CC	TT	CC	AA	CC	CT	TT	CT	GG	AC
TTKSK	09Tan06-2	AF-001af	CC	GG	GG	TT	CC	TT	CC	AA	TT	CT	TT	CT	GG	AC
TTKST	06Ken19v3	AF-001ba	CT	GG	GA	TT	CC	TT	TT	AA	CC	CT	TT	CT	GG	AC
TTKST	07Ken18	AF-001bb	CC	GG	GG	TT	CC	TT	TT	AA	CC	CT	TT	CT	GG	AC
TTKST	09Tan10-9	AF-001bc	CC	GG	GG	TT	CC	TT	CT	AA	CC	CT	TT	CT	GG	AC
TTTSK	07Ken24-4	AF-001ca	CC	GA	GG	TT	CC	TT	CC	AA	TT	CT	TT	CT	GG	AC
TTTSK	09Tan08-1	AF-001cc	CC	GG	GG	GG	CC	TT	CC	AA	CC	CT	TT	CT	GG	AC
TTTSK	09Tan08-2	AF-001cd	CC	GG	GG	GG	CC	TT	CC	AA	CC	CT	AT	CT	GG	AC
TTKSF	UvPgt55.1	AF-001da	CC	GG	GG	TT	CC	TT	CC	AG	CC	CC	AA	TT	GA	AA
TTKSF	09Zim01-1	AF-001db	CC	GG	GG	TT	CC	TT	CC	AA	CC	CC	AA	CT	GG	AA
TTKSF	UvPgt61.2	AF-001dd	CC	GG	GG	TT	CC	TT	CC	AA	CC	CC	AA	CT	GA	AA
TTKSP	UvPgt59	AF-001ea	CC	GG	GG	TT	CT	TG	CC	AA	CC	CC	AA	TT	GA	AA
PTKST	UvPgt60	AF-001fa	CT	GG	GG	TT	CC	TT	TT	AA	CC	CT	TT	CT	GG	AC

Survey samples: Africa 2013-15

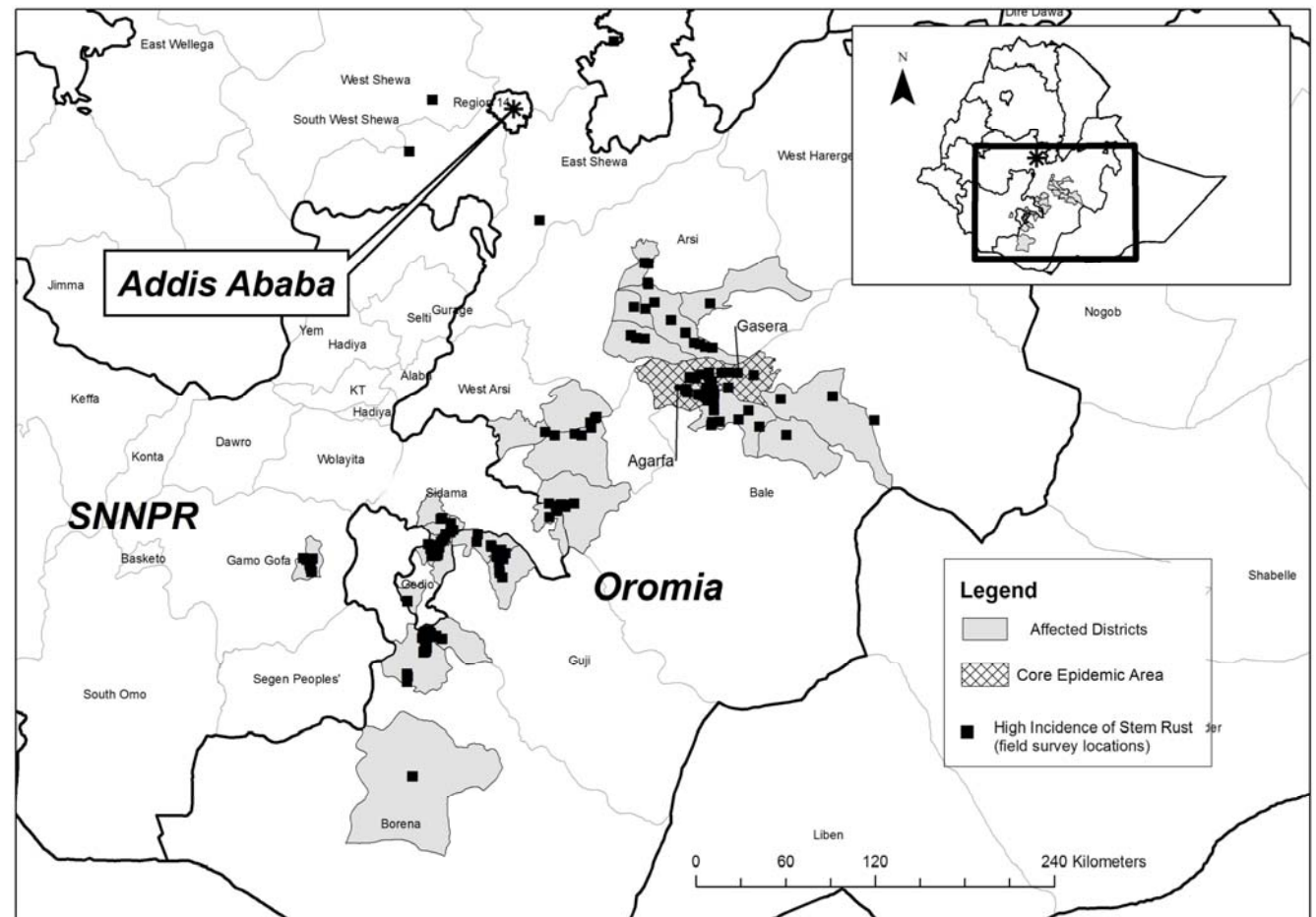


Total = 815 samples

TKTTF

➔ 2013 wheat stem epidemic in Southern Ethiopia.

➔ New *Pgt* race in Africa (TKTTF).



SNP analysis of *Pgt* isolates from Ethiopia

- *Pgt* isolates
 - 41 isolates derived from collections made in 2013 main wheat growing season.
 - Genotyped samples with PgtSNP 1.5k chip

Phylogenetic analysis



Data: 918 SNP loci

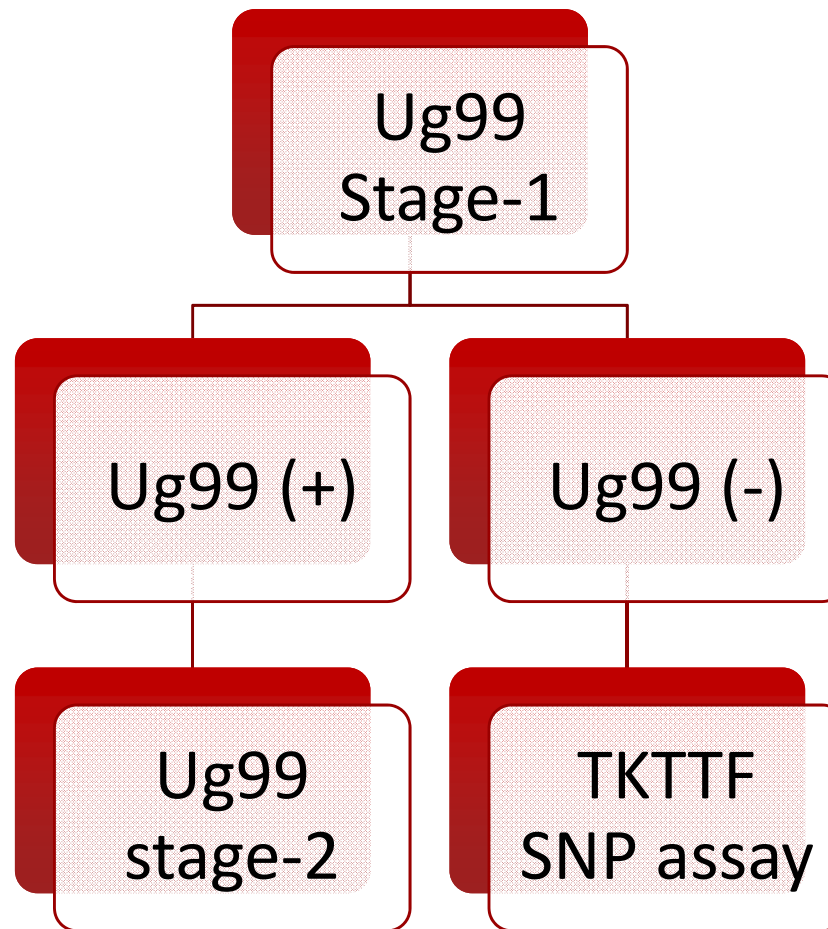
Analysis: Neighbor-Joining, 5,000 bootstrap replicates

● *Pgt* reference isolates

Clade IV (TKTTF) assay

Race/Clade	Isolate	Clade IV										Clade IV sub-groups										
		C286	E077	E246	F066	F824	C073	D588	D606	D779	E951	C064	C728	E324	F782	C203	C293	D938	E720	E824	F008	
TKTTF, IV-A	13ETH18_1	AA	GG	CC	CC	AG	CC	GG	AA	AA	GG	GG	AC	AG	AG	AG	AC	AG	AG	TT	GG	AG
TKTTF, IV-A	13ETH22_1	AA	GG	CC	CC	AG	CC	GG	AA	AA	GG	GG	AC	AG	AG	AG	AC	AG	AG	TT	GG	AG
TKTTF, IV-A	13TUR13_1	AA	GG	CC	CC	AG	CC	GG	AA	AA	GG	GG	AC	AG	AG	AG	AC	AG	AG	TT	GG	AG
TKTTF, IV-A	13TUR19_2	AA	GG	CC	CC	AG	CC	GG	AA	AA	GG	GG	AC	AG	AG	AG	AC	AG	AG	TT	GG	AG
TKTTF, IV-B3	13ETH20_1	AA	GG	CC	CC	AG	AA	AG	AG	AG	CC	AA	CC	GG	AA	AG	AA	AG	CT	GG	AG	
TKTTF, IV-B3	13ETH21_3	AA	GG	CC	CC	AG	AA	AG	AG	AG	CC	AA	CC	GG	AA	AG	AA	AG	CT	GG	AG	
TKTTF, IV-B3	13ETH23_1	AA	GG	CC	CC	AG	AA	AG	AG	AG	CC	AA	CC	GG	AA	AG	AA	AG	CT	GG	AG	
TKTT	13TUR4_1	AA	GG	CC	CC	AG	AC	AG	AG	AG	CG	GG	AC	AG	AG	GG	AC	GG	CT	CC	AA	
TKTT	13TUR9_1	AA	GG	CC	CC	AG	AC	AG	AG	AG	CG	GG	AC	AG	AG	GG	AC	GG	CT	CC	AA	
TTKSK, I	04KEN156-4	AG	AG	AC	AC	AA	AC	AG	AG	AG	CG	AG	AC	AG	GG	AG	AC	AG	TT	CG	GG	
TTKST, I	06KEN19-V-3	AG	AG	AC	AC	AA	AC	AG	AG	AG	CG	AG	AC	AG	GG	AG	AC	AG	TT	CG	GG	
TTTSK, I	07KEN24-4	AG	AG	AC	AC	AA	AC	AG	AG	AG	CG	AG	AC	AG	GG	AG	AC	AG	TT	CG	GG	
JRCQC, II	13ETH25_2	AG	GG	AC	AC	AA	AC	GG	GG	GG	CG	GG	AC	AG	GG	AG	AC	AA	TT	CG	GG	
JRCQC, II	13ETH28_2	AG	GG	AC	AC	AA	AC	GG	GG	GG	CG	GG	AC	AG	GG	AG	AC	AA	TT	CG	GG	
TRTTF, III	06YEM34-1	AG	AG	AA	AC	AA	AC	AA	AG	AG	CG	GG	AC	AG	AG	AA	AC	AG	CT	GG	AG	

Flow chart



Analysis of *Pgt* field samples

➤ 131 *Pgt* field samples.

➤ Collect 2014

➤ Ethiopia

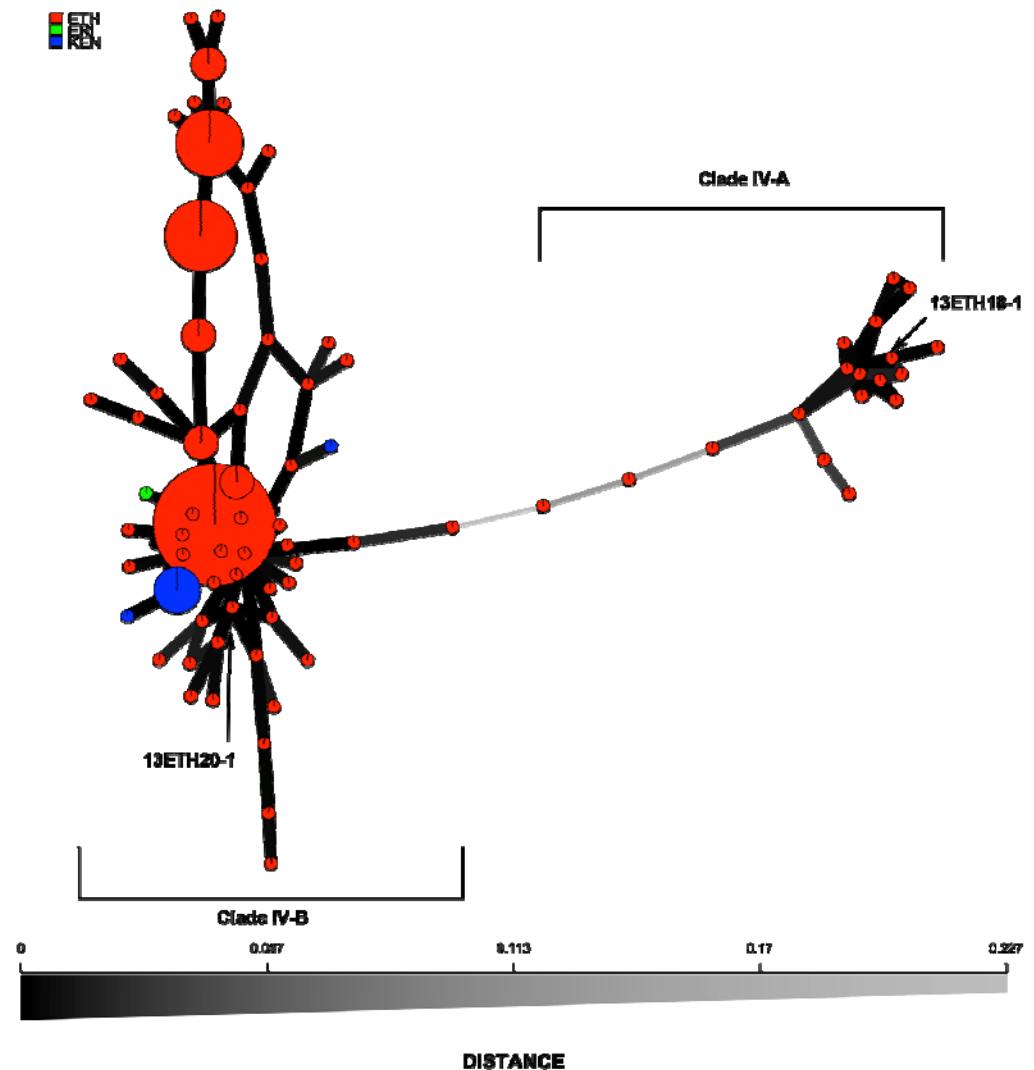
➤ Eritrea

➤ Kenya

➤ SNP analysis:

➤ TKTTF assay

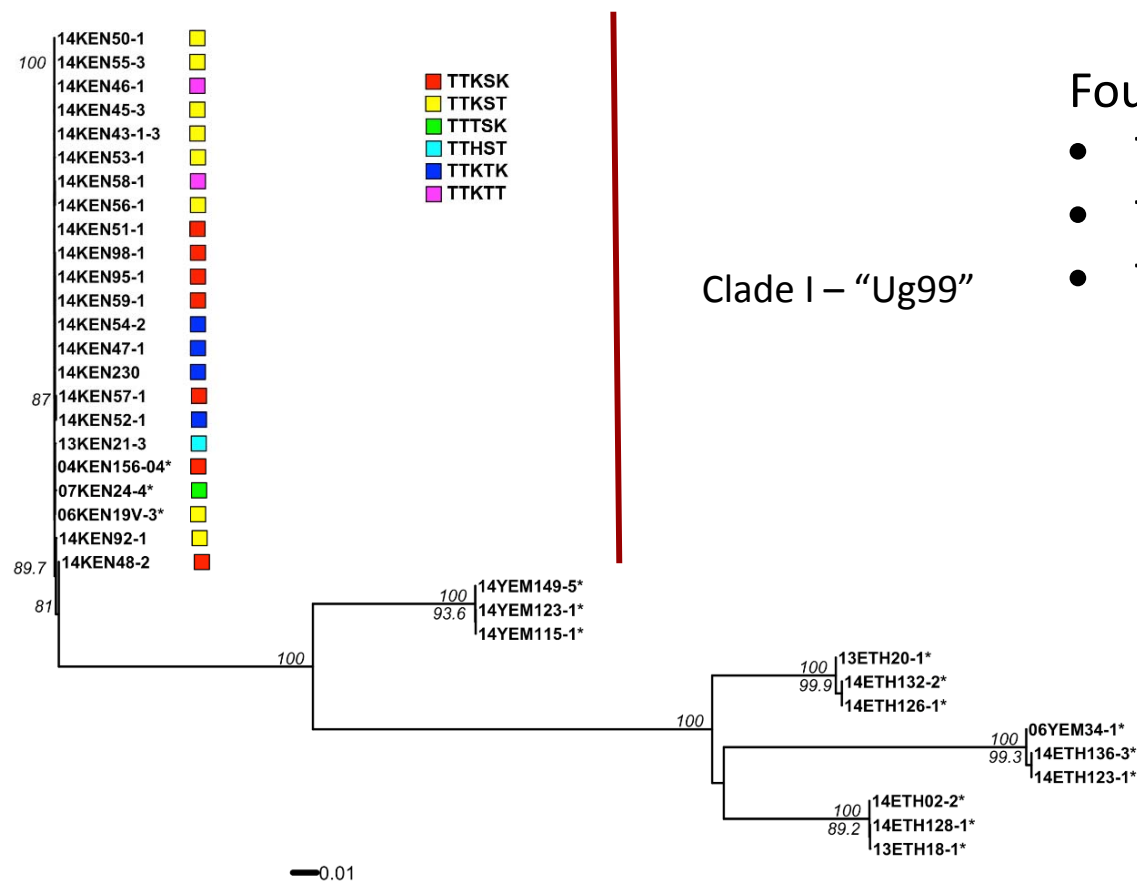
➤ PgtSNP 3.0k chip



Pgt Ug99 race group:

- Three new races
 - Kenya 2013 and 2014
 - TTKTK
 - TTKTT
 - TTHST

Kenya 2013/14: Race survey



1,634 SNP loci
 NJ tree
 10,000 bootstrap replicates

Newcomb et al Phytopathology (in review)

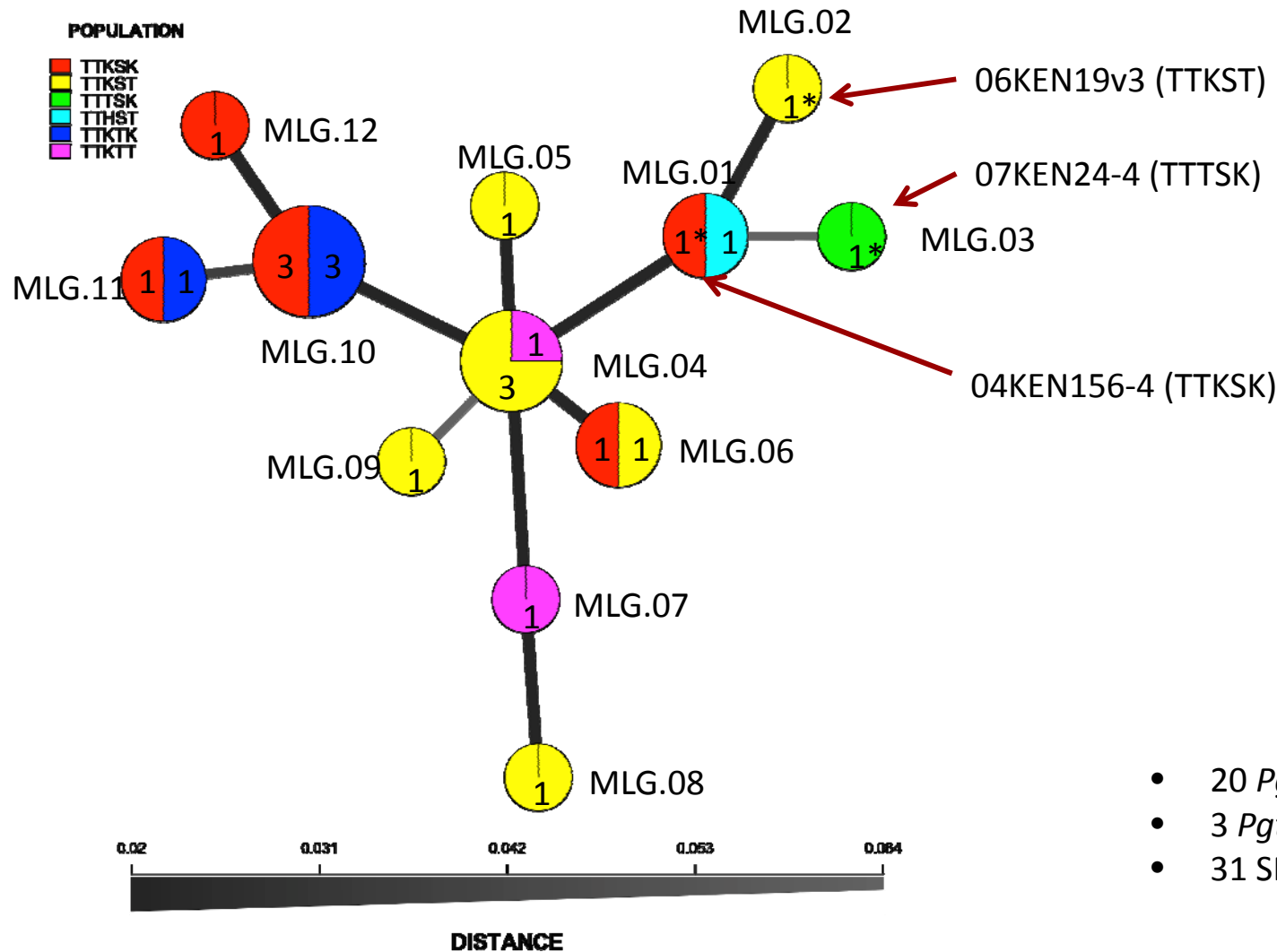
Ug99 race group assay

Isolate	Race	Stage-1	Stage-2
UG99-A-1	TTKSK	Positive	
07KEN34-1	TTKST	Positive	
06KEN19-v-3	TTKST	Positive	
14KEN48-1	TTKTK	Positive	
14KEN46-1	TTKTT	Positive	
14KEN21-3	TTHST	Positive	

Ug99 race group assay

Isolate	Race	Stage-1	Stage-2
UG99-A-1	TTKSK	Positive	AF-001aa
07KEN34-1	TTKST	Positive	AF-001ad
06KEN19-v-3	TTKST	Positive	AF-001ba
14KEN48-1	TTKTK	Positive	AF-001aa
14KEN46-1	TTKTT	Positive	AF-001ad
14KEN21-3	TTHST	Positive	AF-001ad

Minimal Spanning Network



- 20 *Pgt* isolates (2013/14)
- 3 *Pgt* standards (*)
- 31 SNP loci

Summary

- Ug99 RG assay is being successfully used to monitor *Pgt* Ug99 race group in Africa and spread into parts of Central Asia.
- Mining SNP chip data based allowed for the quick development to the new assay for *Pgt* clade IV (TKTTF).
- The molecular assays complements the ongoing *Pgt* race surveys.

Summary (cont.)

- *Pgt* is a dynamic pathogen that is continuing to change and therefore requires constant monitoring and updating of molecular assays.
- “*Rust is a shifty enemy*” EC Stakman
- “*Rust never sleeps*” N Borlaug

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