

Puccinia graminis: Molecular Analysis

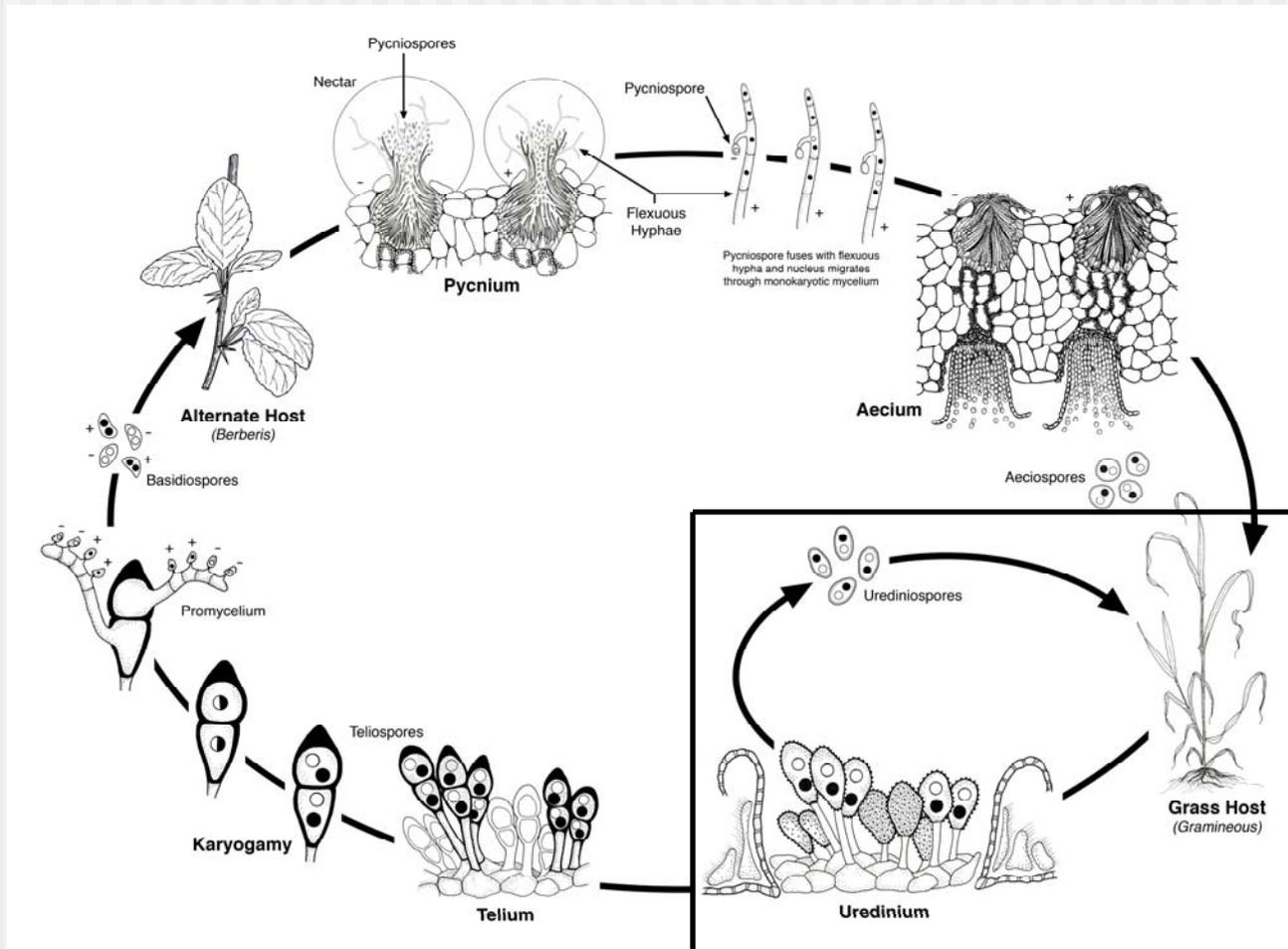
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May 20, 2009

Life cycle



Sexual

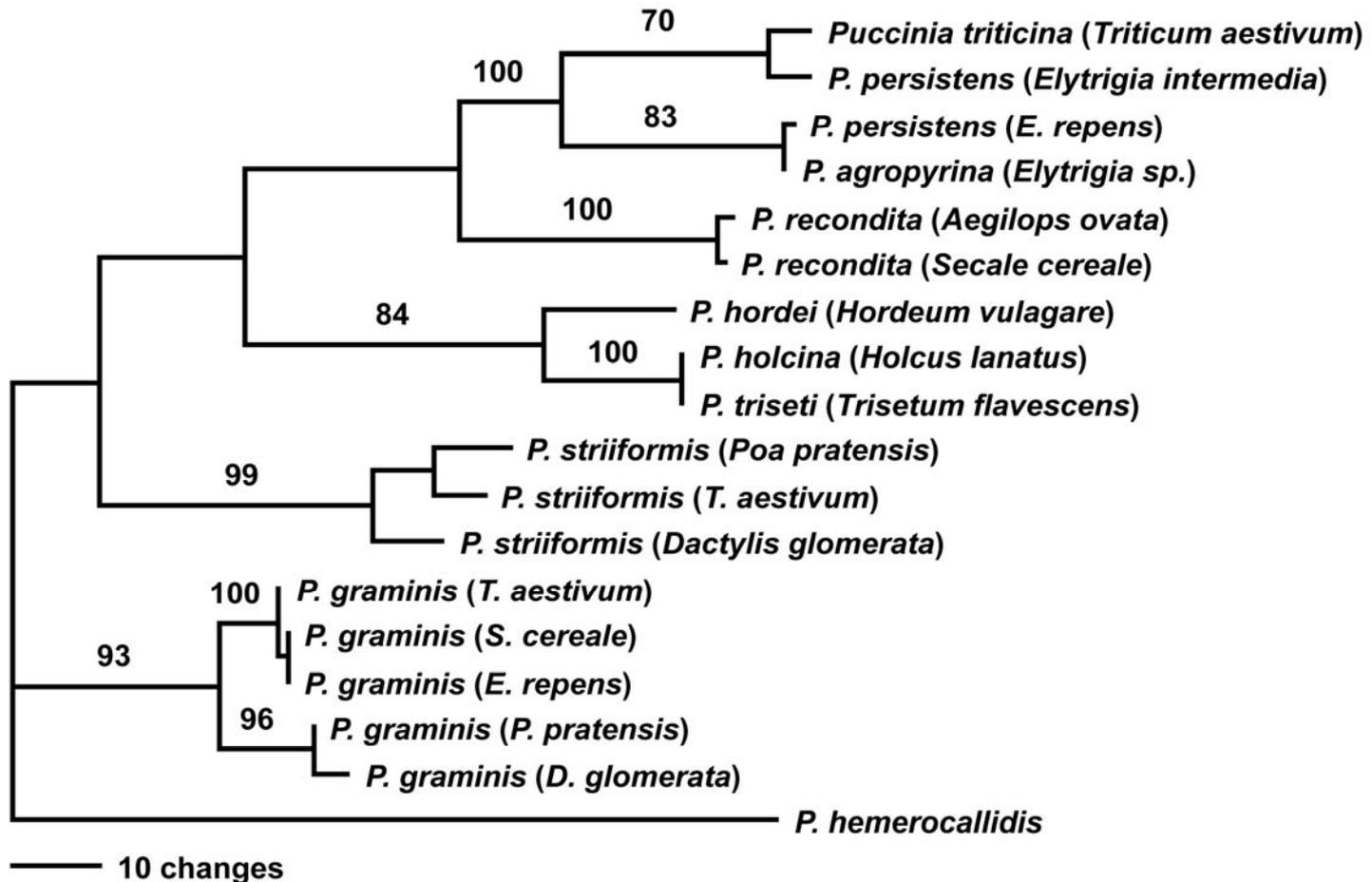
Asexual

Puccinia graminis

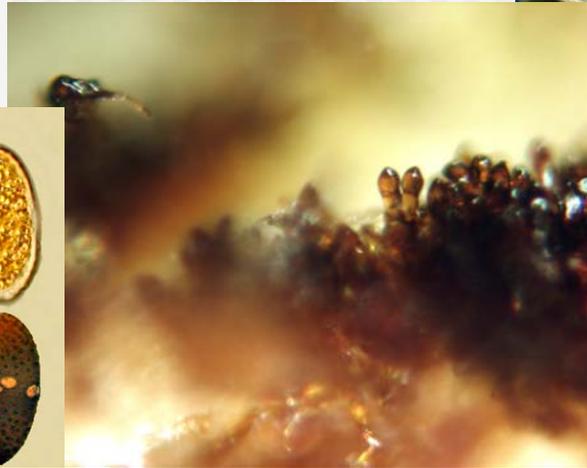
Puccinia graminis

- Broad Host range
 - 54 genera, 360 species
- Subdivided
 - Spore morphology (subspecies, varieties)
 - Host range (formae speciales)
 - *P. graminis* f.sp. *tritici*
 - Wheat, barley
 - *P. graminis* f.sp. *secalis*
 - Rye, quack grass
 - *P. graminis* f.sp. *avenae*
 - oat

Phylogenetic analysis

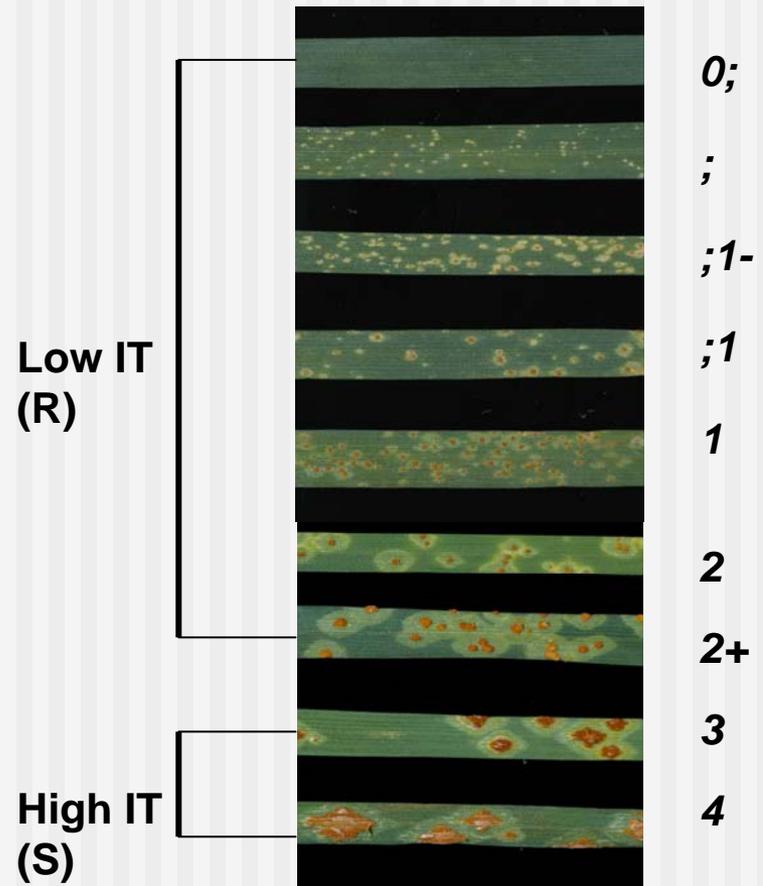


Race Nomenclature



Wheat Stem Rust Differentials

- Set includes 20 lines
- Most are single R gene lines with a common background
- Infection types (IT) are characteristic of particular R gene



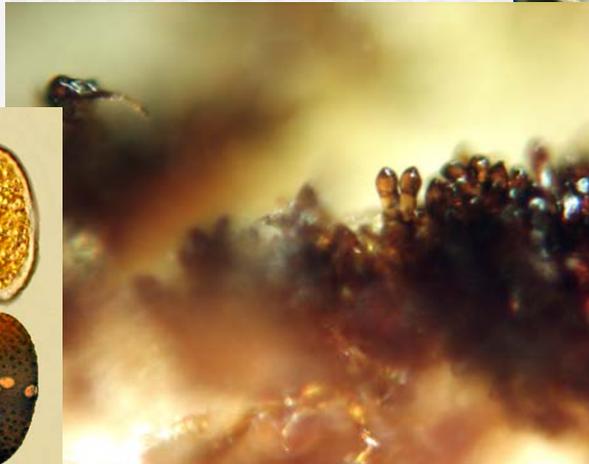
Race Nomenclature

Set	Line	Sr Gene	IT	Race
1	ISr5Ra	5	4	T
	T.m.der.	21	3+	
	Verstein	9e	4	
	ISr7bRa	7b	3	
2	ISr11Ra	11	4	T
	ISr6Ra	6	4	
	ISr8Ra	8a	4	
	CnsSr9g	9g	4	
3	W2691SrTt-1	36	0	K
	W2691Sr9b	9b	4	
	BtSr30Wst	30	4	
	Comb. VII	17+13	2++	
4	ISr9aRa	9a	4	S
	ISr9dRa	9d	4	
	W2691Sr10	10	4	
	CnsSrTmp	Tmp	2+	
5	LcSr24Ag	24	2	K
	Sr31/LMPG	31	4	
	VPM1 38	38	4	
	McNair701	Mc	4	

B	R	R	R	R
C	R	R	R	S
D	R	R	S	R
F	R	R	S	S
G	R	S	R	R
H	R	S	R	S
J	R	S	S	R
K	R	S	S	S
L	S	R	R	R
M	S	R	R	S
N	S	R	S	R
P	S	R	S	S
Q	S	S	R	R
R	S	S	R	S
S	S	S	S	R
T	S	S	S	S



Ug99



Ug99 - History

First reported in Uganda in 1999

--Pretorius et al. 2000 *Plant Dis* 84:203

- Virulent on *Sr31*
- *Sr31* is located on 1BL.1RS translocation.
 - 1BL.1RS translocation is widely spread in wheat worldwide due to increased adaptation and higher yield.
 - 1BL.1RS carries *Lr26*, *Yr9*.
 - Virulence to *Yr9*, originated in the eastern Africa in mid 80s, caused worldwide epidemics.

Ug99 - History

- In 2002 and 2004, CIMMYT nursery planted in Njoro, Kenya were severely infected by stem rust.
- Kenyan isolates from 2004 collections were characterized as race TTKS with *Sr31* virulence.
 - Wanyera et al., 2006 Plant Dis.
- Ug99 (98UGA1) = race TTKS with *Sr31* virulence
- Expanded set of wheat differentials developed to include *Sr31* (race TTKSK).
 - Jin et al., 2008 Plant Dis.

Sr24 Virulence

In 2006, scattered MS to S pustules with low frequency were seen in many *Sr24* lines in the Kenyan stem rust nursery. *Sr24* virulence was suspected.

- Seedling tests confirmed the variant was virulent on *Sr 24* as well as *Sr 31*
- Race TTKST



Sr36 Virulence

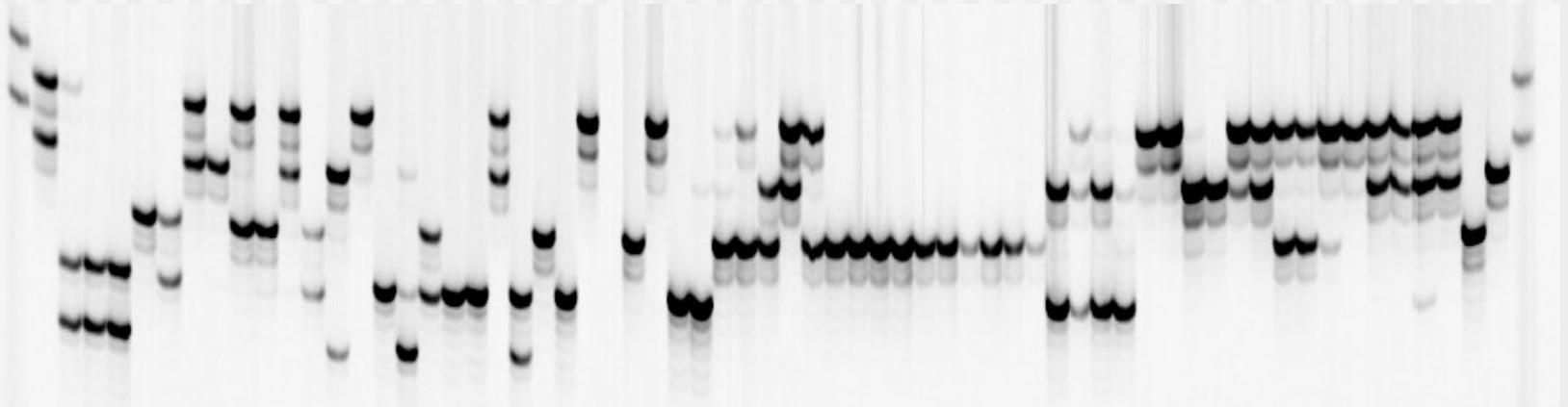
- In 2007, scattered MS pustules were observed on *Sr36* lines in Kenya plots.
- Seedling tests confirmed the variant was virulent on *Sr 36* as well as *Sr 31*
- Race TTTKS



Race Nomenclature: Ug99

Set	Line	Sr Gene	04KEN156 TTKSK	06KEN19v TTKST	07KEN24-1 TTTSK
1	ISr5Ra	5	4	4	4
	T.m.der.	21	3+	3+	3
	Verstein	9e	4	4	4
	ISr7bRa	7b	3	3+	4
2	ISr11Ra	11	4	4	4
	ISr6Ra	6	4	4	4
	ISr8Ra	8a	4	4	4
	CnsSr9g	9g	4	4	4
3	W2691SrTt-1	36	0	0	4
	W2691Sr9b	9b	4	4	4
	BtSr30Wst	30	4	3+	4
	Comb. VII	17+13	2++	2++	2++
4	ISr9aRa	9a	4	4	4
	ISr9dRa	9d	4	4	4
	W2691Sr10	10	4	4	4
	CnsSrTmp	Tmp	2+	2+	2+
5	LcSr24Ag	24	2	3	2
	Sr31/LMPG	31	4	4	4
	VPM1 38	38	4	4	4
	McNair701	McN	4	4	4

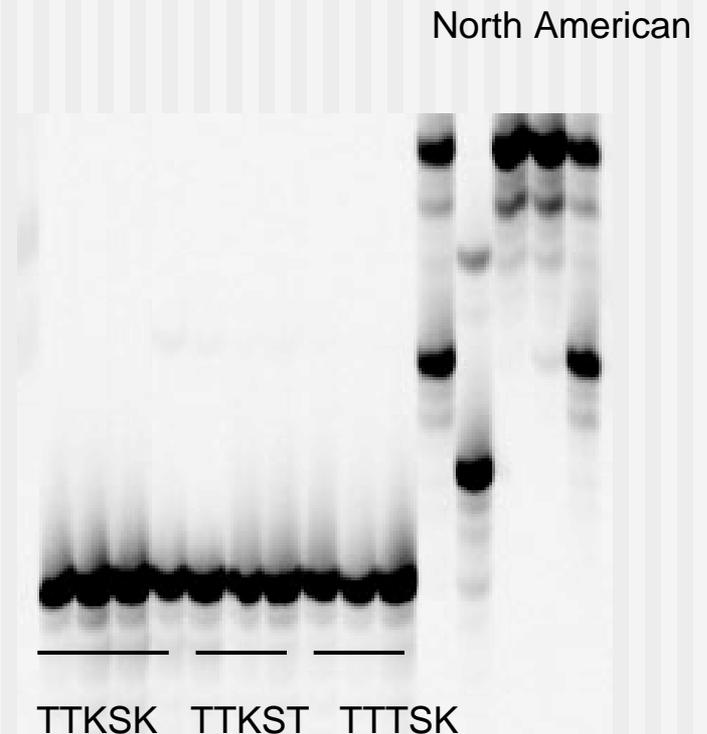
SSR Marker Analysis



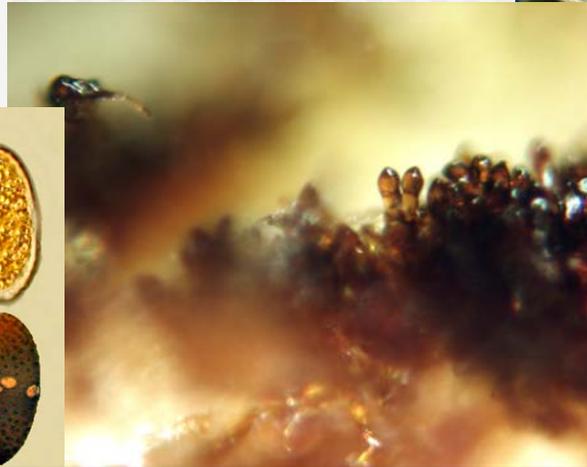
PgtSSR47

SSR Marker Analysis

- Compared 18 isolates from Kenya representing races:
 - TTKSK (Ug99)
 - TTKST (*Sr24 vir*)
 - TTTSK (*Sr36 vir*)
- 13 SSR markers tested.
- All isolates of these 3 races had identical SSR genotypes.
- SSR genotypes for Ug99 was distinct from North American isolates.



Pgt Genomic Resources



Pgt Genome Sequence

- U.S. Isolate
 - CRL 75-36-700-3
- Standard ABI/Sanger sequence
 - 12X
 - Assembly (7X)
 - 4,557 contigs (81.5 Mb)
 - 392 supercontigs
 - http://www.broad.mit.edu/annotation/genome/puccinia_graminis.3

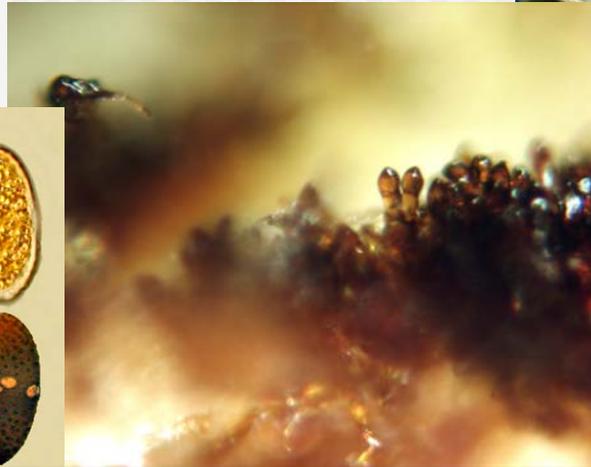
“Ug99” sequencing

- Illumina
 - 75 base read length
- Isolate: 04KEN156/04
 - Race: TTKSK (“Ug99”)
 - Approximately 176 million reads (16 lanes)
- Reference isolate: CDL 75-36-700-3 (Ref)
 - Race: SCCL
 - Approximately 21 million reads (2 lanes)

PCR assay development

- Comparison of DNA sequence data from reference isolate and Ug99 was used to develop a SNP database.
- DNA regions containing 2 SNPs within 10 base were chosen as targets for assay development.
- Primers and TaqMan probes are being tested for a selected set of target regions.

Summary



Summary

- Ug99
 - Lineage contains at least three members:
 - TTKSK Ug99 (vir *Sr31*)
 - TTKST (vir *Sr31* & *Sr24*)
 - TTTSK (vir *Sr31* & *Sr36*)
 - SSR genotypes of isolates from these three races are identical indicating that the variants (TTKST and TTTSK) are derived by mutation rather than sexual recombination.

Summary

- Genomic resources
 - Standard ABI/Sanger sequence
 - U.S. isolate (CRL 75-36-700-3)
 - Assembled and annotated
 - Illumina
 - Ug99 (04KEN156)
 - Reference U.S. isolate
 - Using SNP data to develop PCR assay specific for Ug99.