

# The kinase-START domain gene *Yr36* confers partial resistance to stripe rust

J. Dubcovsky



Fu D., C. Uauy, A. Distelfeld, A. Blechl, L. Epstein, X. Chen, H. Sela, T. Fahima, and J. Dubcovsky.. **Positional cloning of the temperature-dependent stripe rust resistance gene *Yr36* . *Science* (2009)**

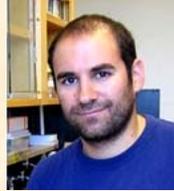


# Acknowledgements

- Daolin Fu



- Cristobal Uauy



- Dario Cantu



- Assaf Distelfeld



- Lynn Epstein and X. Chen



- Ann Blechl



- T. Fahima, H. Sela



*Funding provided by*

● BARD



United States  
Department of  
Agriculture

National Institute  
of Food and  
Agriculture



UNIVERSITY OF HAIFA, ISRAEL  
Institute of Evolution

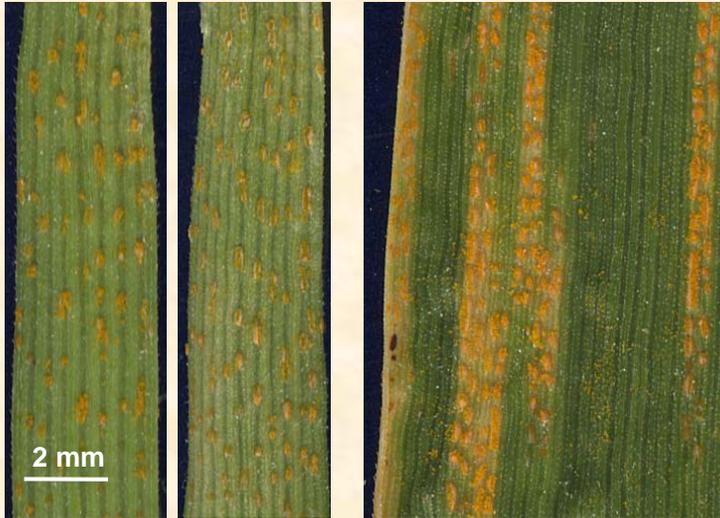
# *Yr36* is a high-temperature adult plant resistance gene

High temperature (25°C)

Seedling

Adult plant

UC 1041 (susceptible)

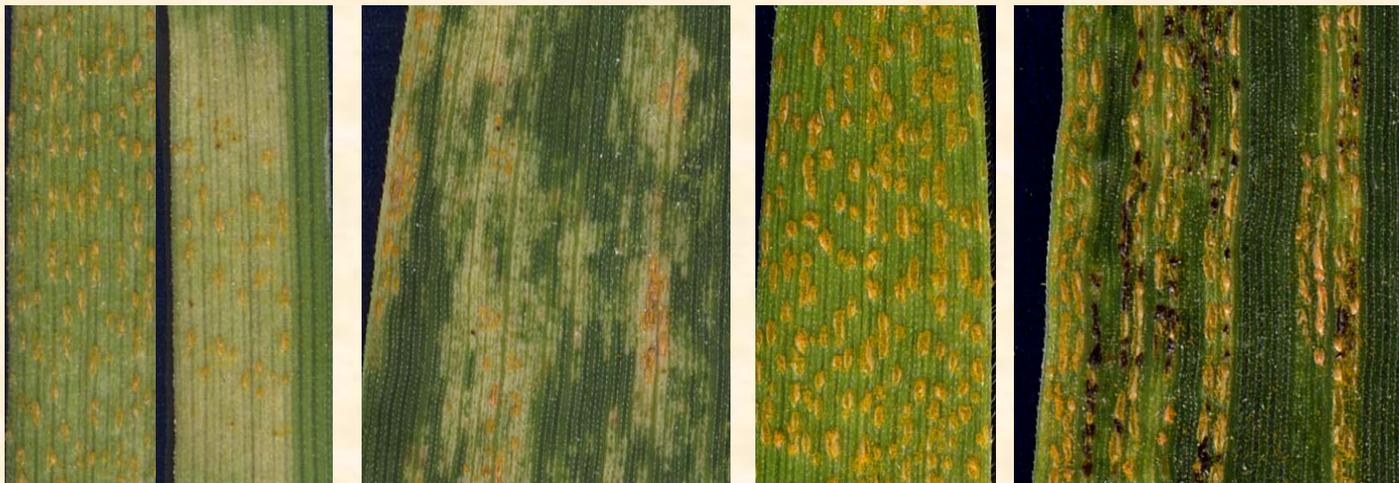


Low temperature (15°C)

Seedling

Adult plant

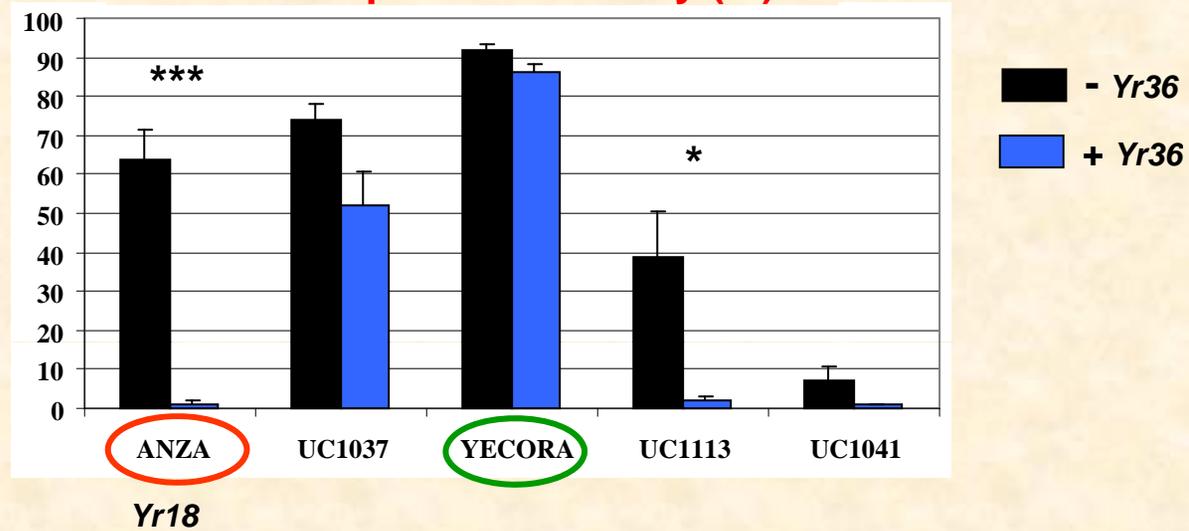
UC 1041 + *Yr36* (res.)



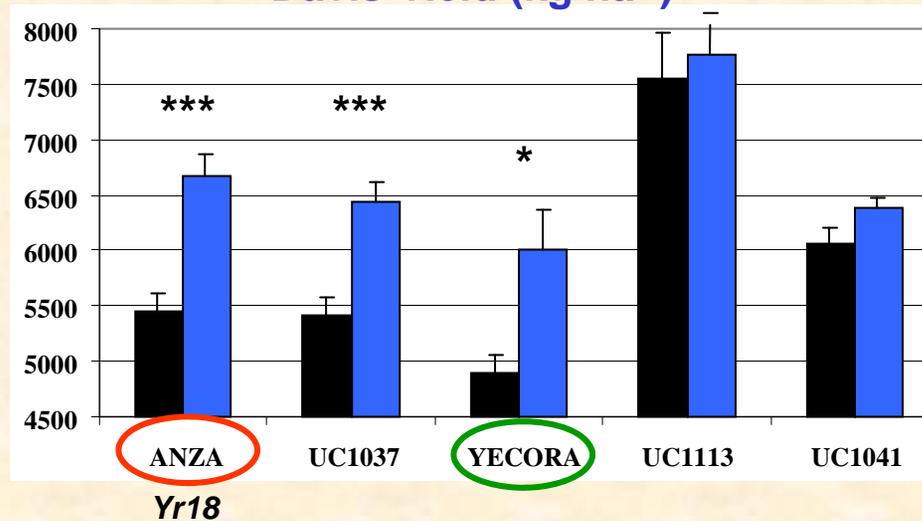
# Yr36 effect on field resistance and yield

Uauy *et al*,  
TAG 2005

### Davis-Stripe Rust Severity (%)

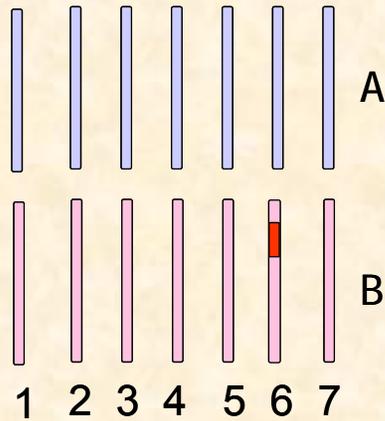


### Davis-Yield (kg ha<sup>-1</sup>)



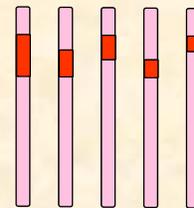
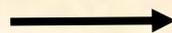
# Yr36 mapping using NILs

NIL for Yr36 region



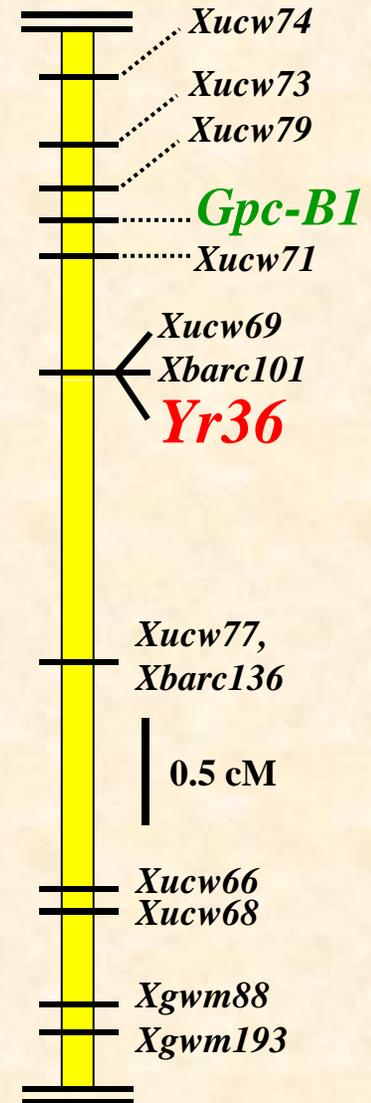
Precise genetic stocks:  
Reduced genetic variability

RSL#65 x LDN



Adequate replication:  
Reduced env. variability

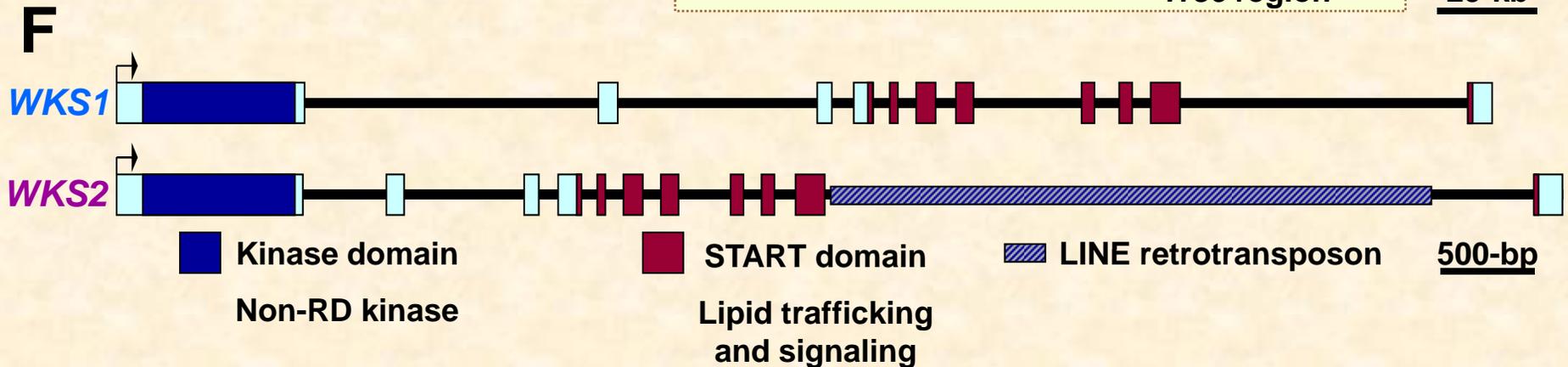
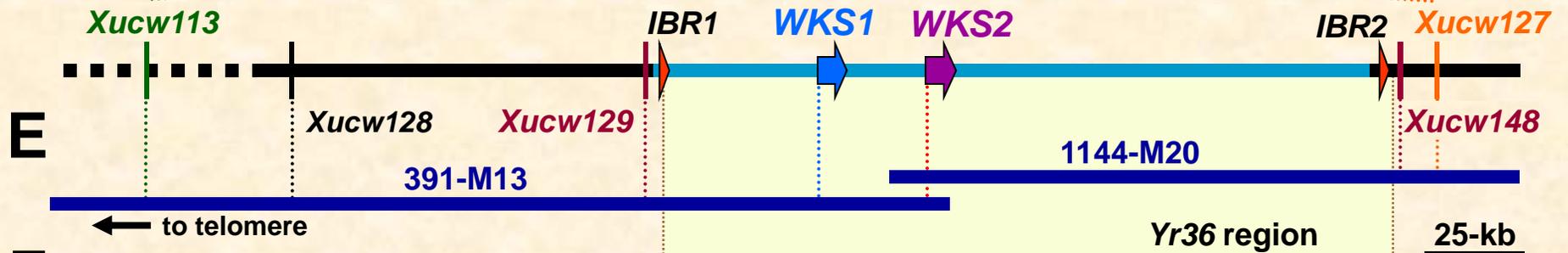
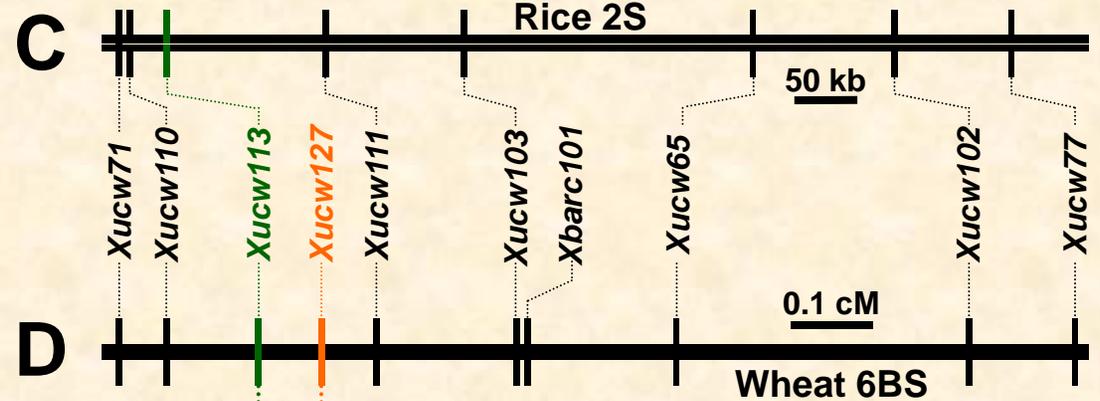
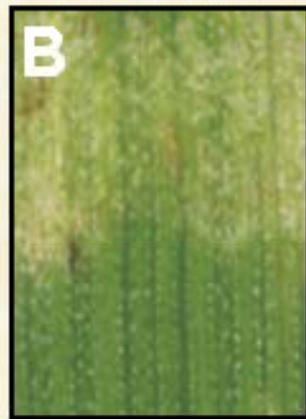
Yr36 region



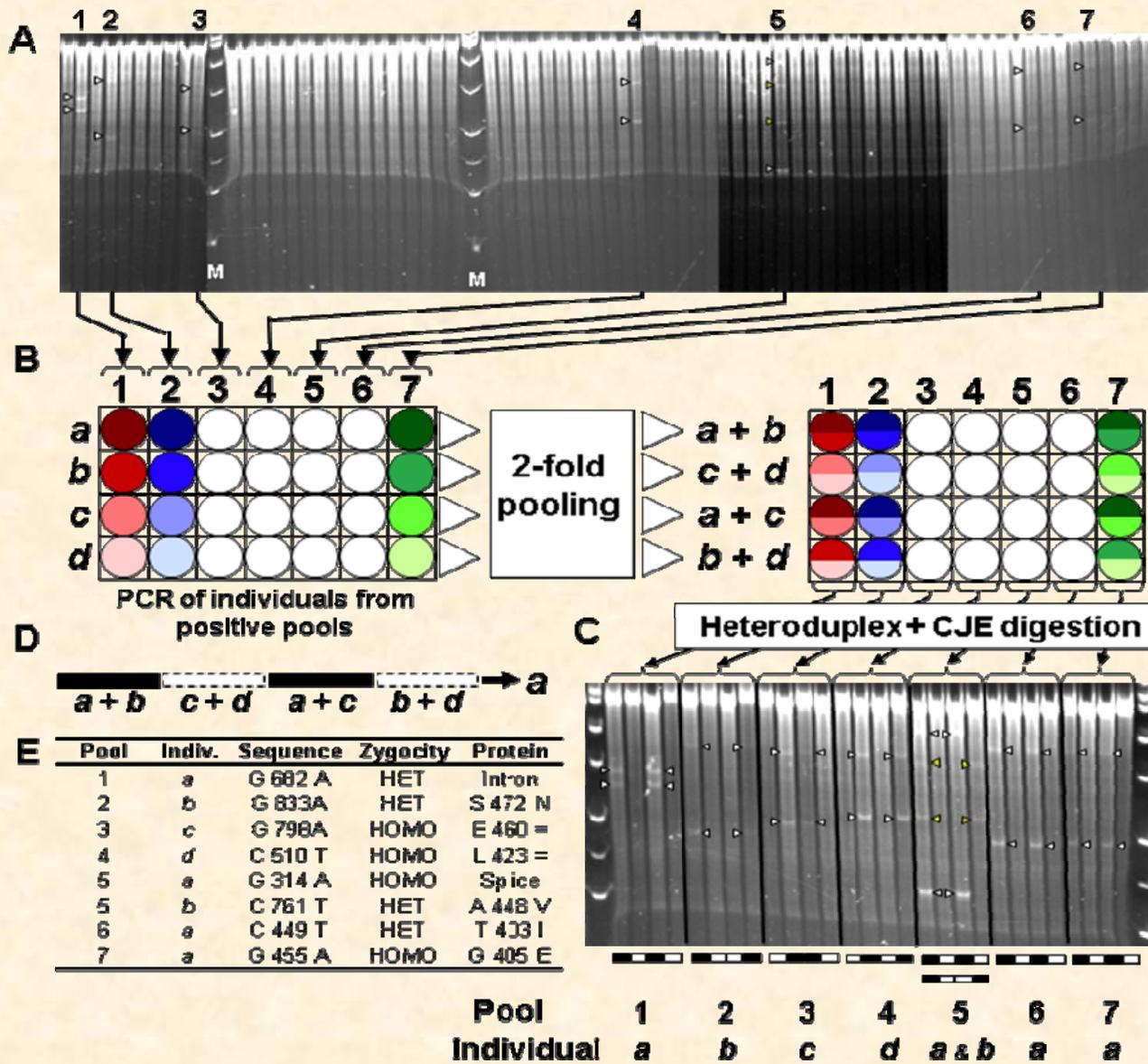
**Yr36** was  
converted into a  
Mendelian gene  
and mapped 0.3  
cM distal to GPC  
and linked to  
*Xucw69*

# Yr36 map-based cloning

High-density map 4500 BCF2 plants (9000 gametes)



# TILLING: screening with non-fluorescent primers



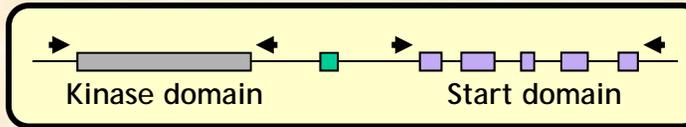
Uauy et al. 2009 BMC  
Plant Biology. 9:115-128

- 1,368 tetraploid lines  
25 mutations/kb

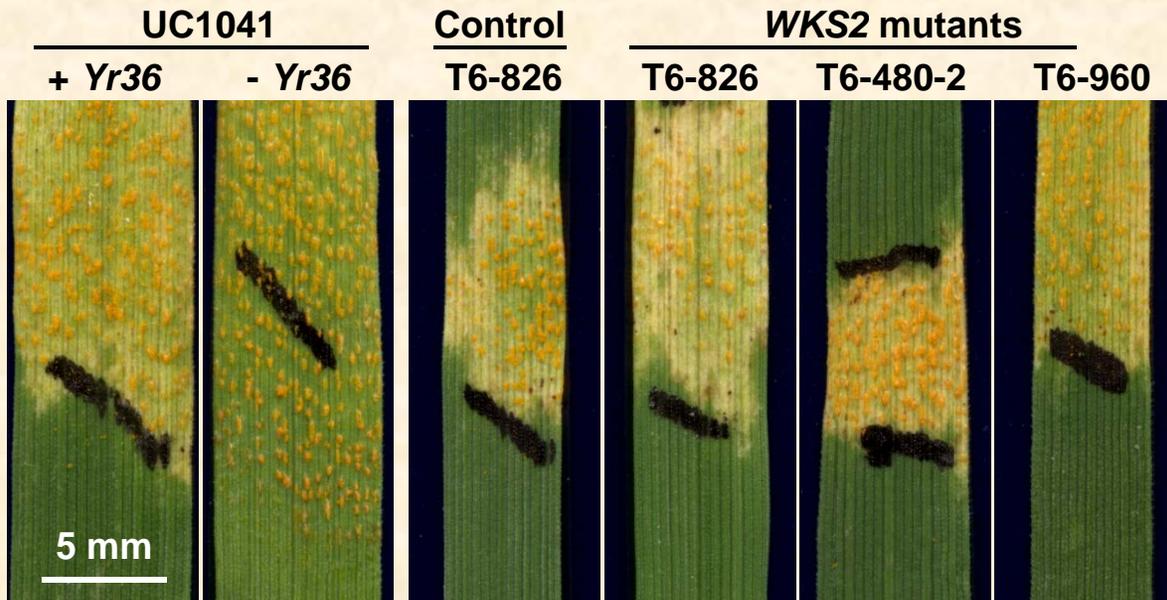
- 1,536 hexaploid lines  
38 mutations/kb

- Screening method with  
non-fluorescent primers

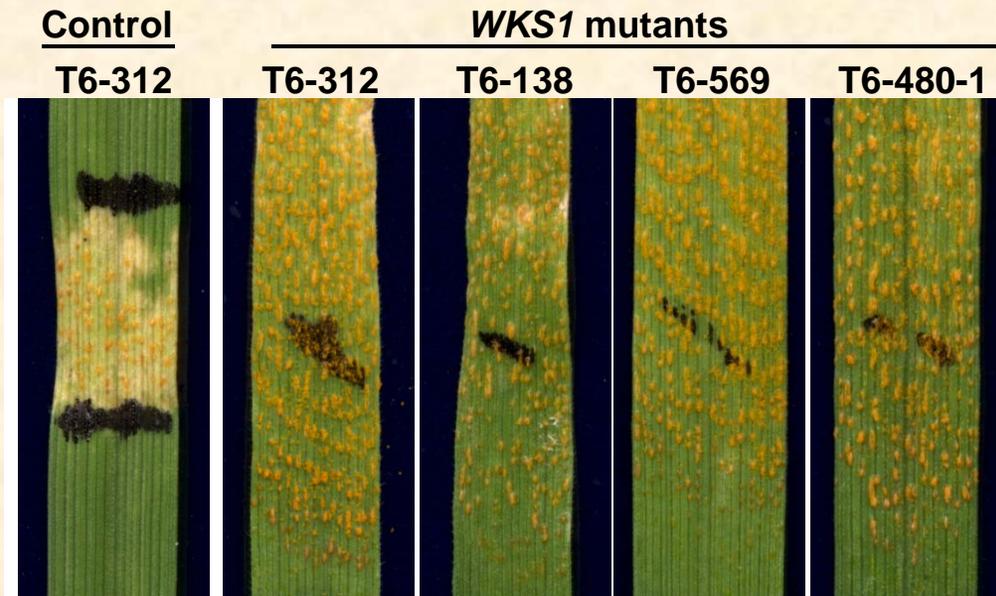
# WKS TILLING



- 2 genes and 2 domains
- 117 mutants detected,
- 9 mutants selected

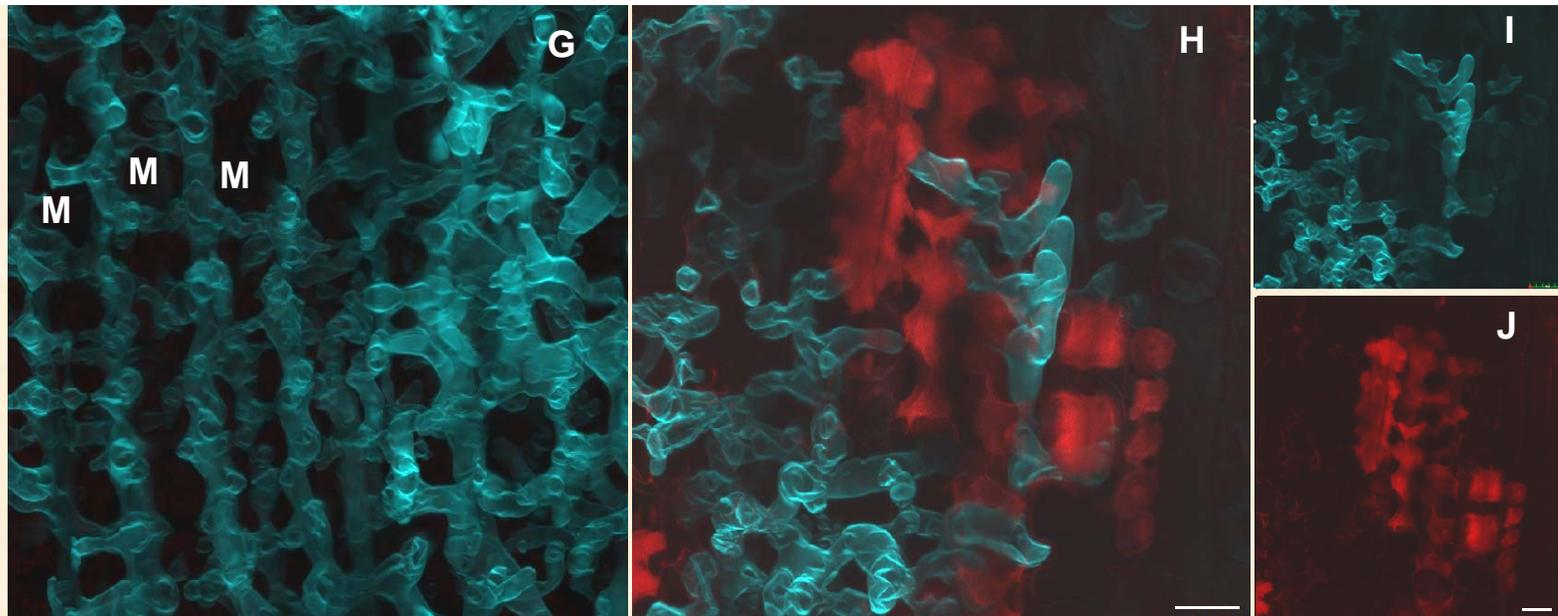
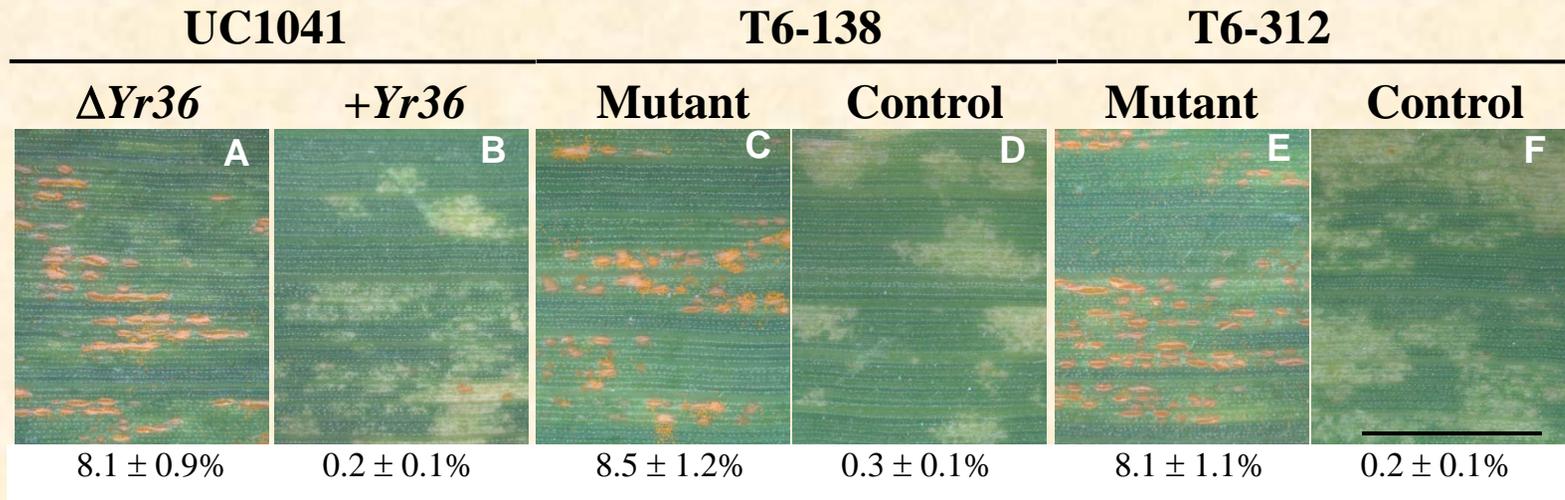


WKS2 is not essential to confer resistance



Mutations in WKS1 kinase or START domains result in susceptible plants

# *Yr36* TILLING

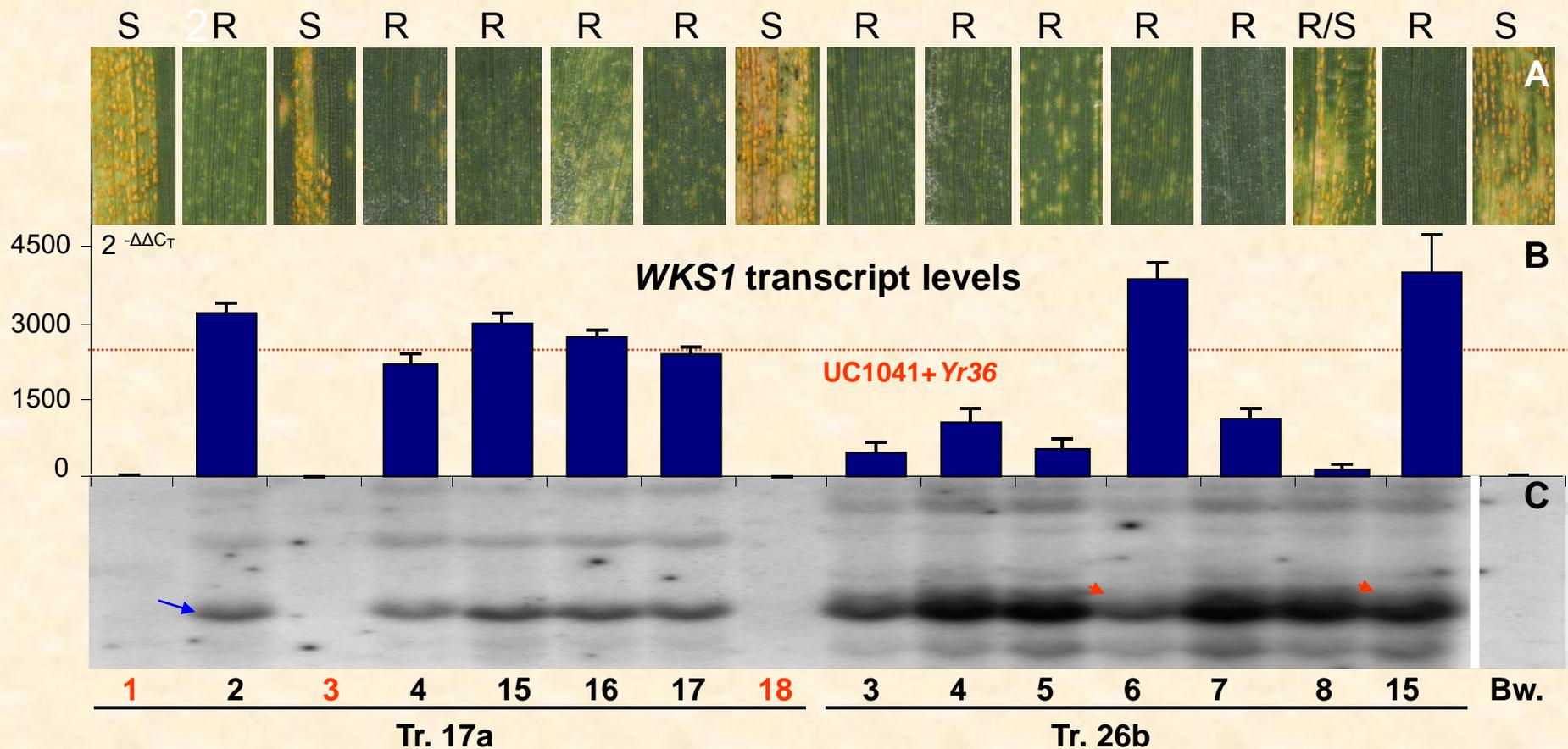


$\Delta Yr36$

+*Yr36*

# *WKS1* complementation

Bobwhite. 12.2 kb genomic *WKS1* endogenous promoter



Based on 5 independent EMS mutants and 2 independent transgenic events we concluded that *WKS1* is *Yr36*

# *WKS1* and *WKS2* were both lost during wheat domestication



## *T. dicoccoides*

brittle rachis  
hulled grain

122 accessions



## *T. dicoccon*

non-brittle rachis  
hulled grain

23 accessions

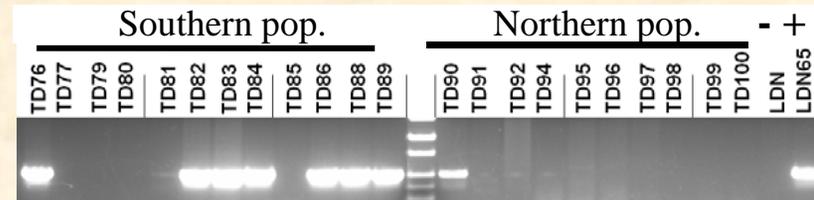


## *T. durum*

non-brittle rachis  
naked seeds

40 accessions

*Yr36* was detected in 38 accessions  
All in the Southern populations

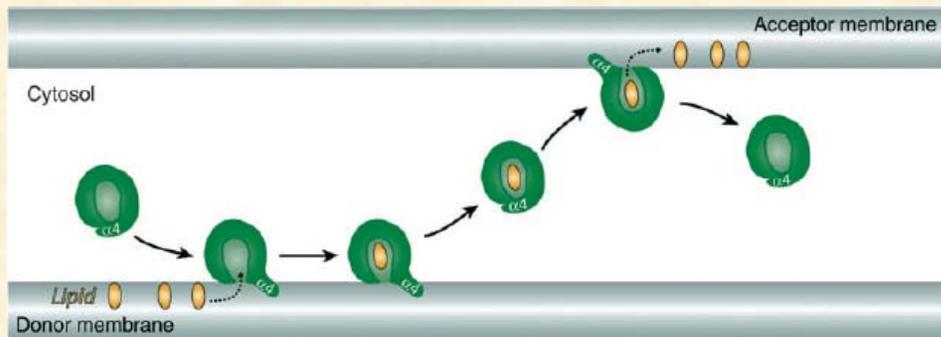
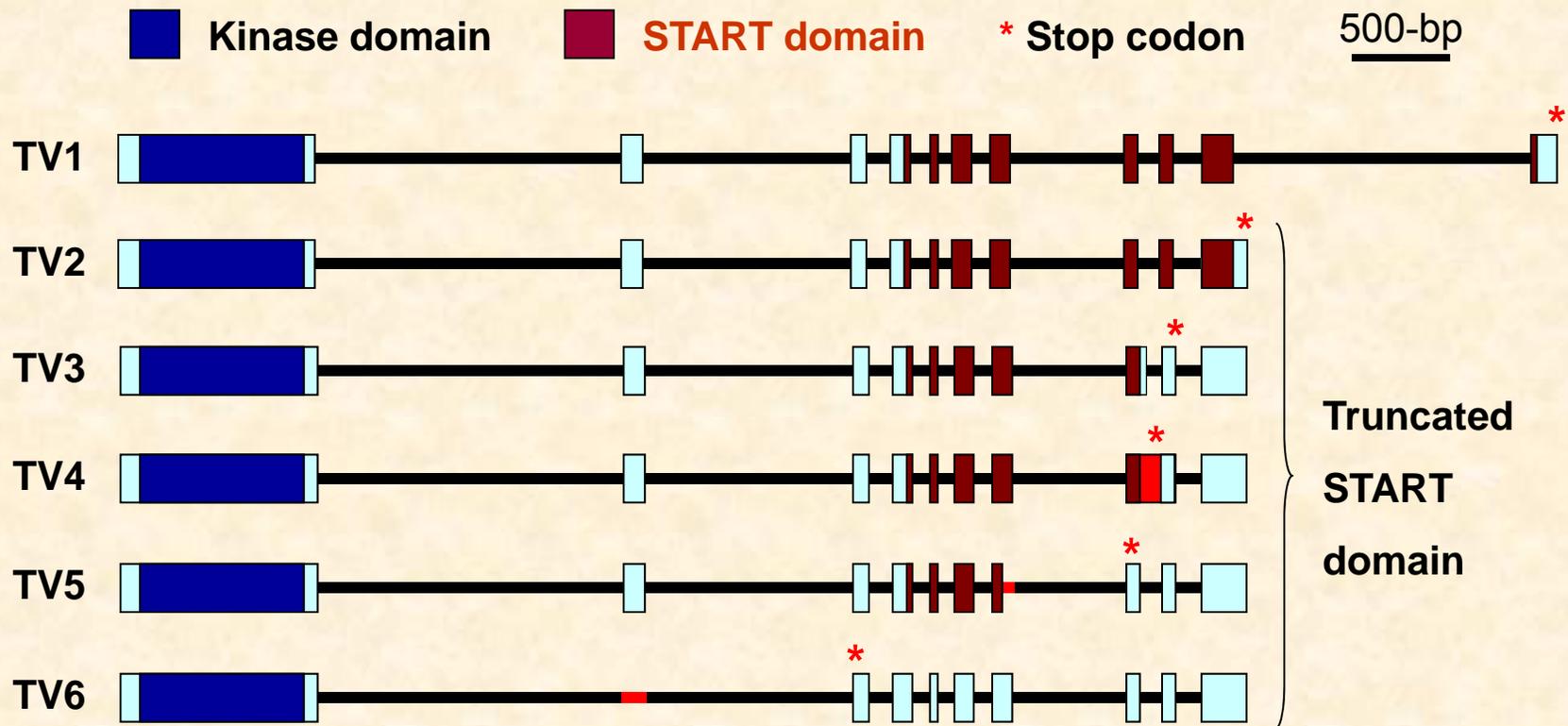


*Yr36* was not detected

*Yr36* was not detected

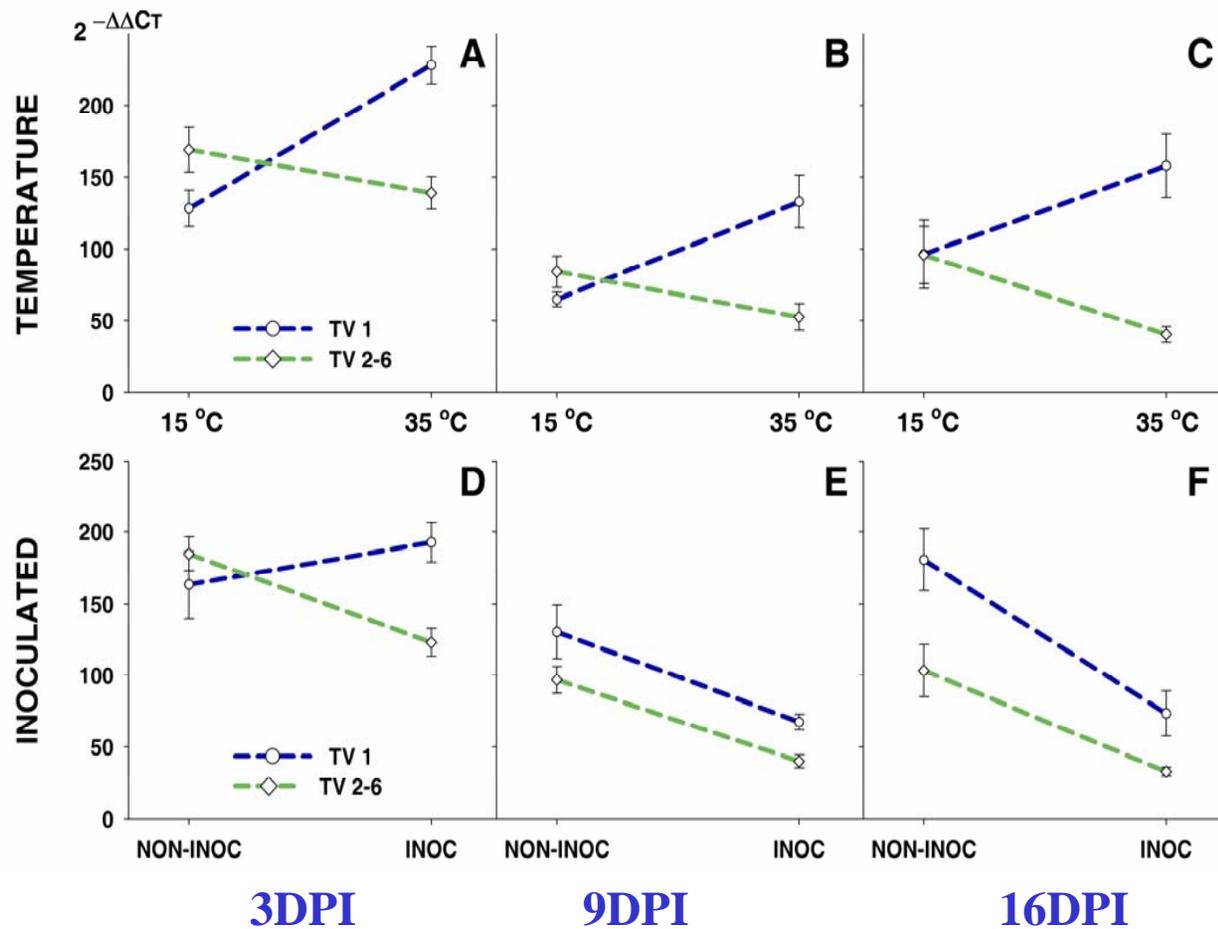
- *Yr36* was functional in all tetraploid and hexaploid lines in which it was introgressed
- *Yr36* can increase stripe rust resistance in almost all commercial pasta and bread wheat

# WKS1 alternative splicing site



Model of **START** domain transport of sterols in humans

# WKS1 transcription profile



TV1 transcripts increase at high T but TV2-6 decrease at high T

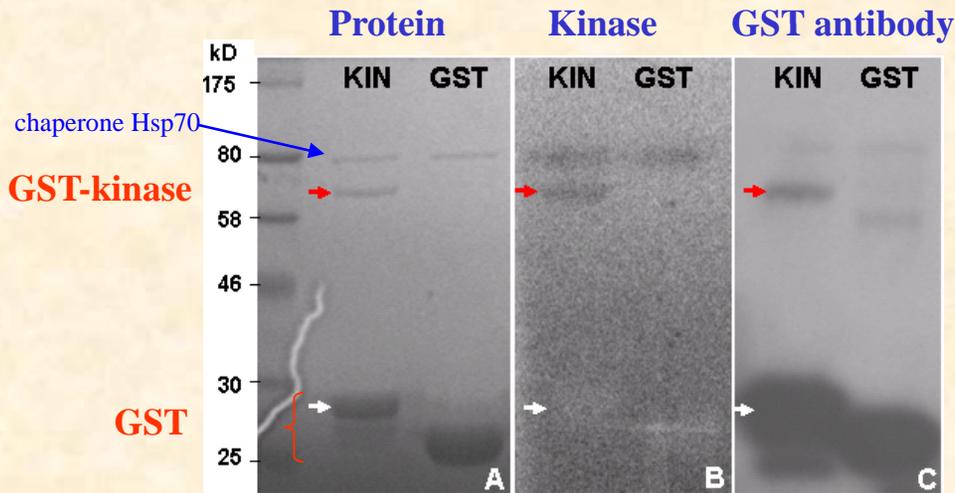
It parallels increased resistance at high T!

At 3 DPI TV1 increases and TV2-6 decreases with inoculation.

Later both decrease.

The transcription profiles of TV1 shows significant interactions between Temperature, inoculation, and time after inoculation

# WKS1 potential mechanism



- In-gel kinase assay using caseine as phosphorylation substrate.
- The GST:Kinase fusion protein (red arrow) showed kinase activity



A yeast-two hybrid assay shows that the kinase can form a dimer but does not interact with the complete WKS1

**Putative mechanism:** when the START domain binds a target lipid it alters the kinase activity and sends a signal to start the slow hypersensitive reaction characteristic of *Yr36*

# Summary

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- \* *Yr36* is a novel protein with a kinase and a START domain.
- \* *Yr36* was not incorporated or was lost during domestication.
- \* *Yr36* is effective when incorporated in 4x and 6x varieties.
- \* *Yr36* transcripts levels vary with temp. and pathogen attack
- \* *Yr36* is tentatively located in the chloroplast based on GFP fusion transgenic plants and Y2H interactions with chloroplast proteins
- \* *Yr36* kinase also interacts with itself but not with the complete protein.

The cloning of the first two wheat genes conferring partial resistance to rusts, *Yr36* and *Lr34/Yr18* (an ABC transporter) revealed resistance mechanisms different from the NB-LRR genes.