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

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Institute of Evolution
**Yr36 is a high-temperature adult plant resistance gene**

<table>
<thead>
<tr>
<th>High temperature (25°C)</th>
<th>Low temperature (15°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seedling</td>
<td>Adult plant</td>
</tr>
<tr>
<td>UC 1041 (susceptible)</td>
<td>UC 1041+Yr36 (res.)</td>
</tr>
</tbody>
</table>

2 mm
**Yr36 effect on field resistance and yield**

**Davis-Stripe Rust Severity (%)**

- **ANZA**
- **UC1037**
- **YECORA**
- **UC1113**
- **UC1041**

**Davis-Yield (kg ha⁻¹)**

- **ANZA**
- **UC1037**
- **YECORA**
- **UC1113**
- **UC1041**
**Yr36 mapping using NILs**

NIL for **Yr36** region

Precise genetic stocks:
- Reduced genetic variability

Adequate replication:
- Reduced env. variability

**Yr36 region**

- **Xucw74**
- **Xucw73**
- **Xucw79**
- **Gpc-B1**
- **Xucw71**
- **Xucw69**
- **Xbarc101**

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

- **Xucw77**, **Xbarc136**
- 0.5 cM

- **Xucw66**
- **Xucw68**

- **Xgwm88**
- **Xgwm193**
**Yr36 map-based cloning**

High-density map 4500 BCF2 plants (9000 gametes)

A

B

C

Rice 2S

Xucw71

Xucw110

Xucw113

Xucw127

Xucw111

Xucw103

Xbarc101

Xucw127

Xucw111

Xucw103

Xucw111

Xucw127

Xucw111

Xucw127

Wheat 6BS

0.1 cM

50 kb

D

**Yr36 region**

25-kb

1144-M20

391-M13

Xucw113

Xucw128

Xucw129

IBR1

WKS1

WKS2

IBR2

Xucw127

Xucw148

E

F

**WKS1**

**WKS2**

Kinase domain

Non-RD kinase

START domain

Lipid trafficking and signaling

LINE retrotransposon

500-bp

25-kb

to telomere
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

<table>
<thead>
<tr>
<th>UC1041</th>
<th>Control</th>
<th>WKS2 mutants</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ Yr36</td>
<td>T6-826</td>
<td>T6-826</td>
</tr>
<tr>
<td>- Yr36</td>
<td>T6-826</td>
<td>T6-480-2</td>
</tr>
<tr>
<td></td>
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<td>T6-960</td>
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</tbody>
</table>

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

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**WKS2 is not essential to confer resistance**

**Mutations in WKS1 kinase or START domains result in susceptible plants**
Yr36 TILLING

<table>
<thead>
<tr>
<th>UC1041</th>
<th>T6-138</th>
<th>T6-312</th>
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</thead>
<tbody>
<tr>
<td>ΔYr36</td>
<td>+Yr36</td>
<td>Mutant</td>
</tr>
<tr>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>8.1 ± 0.9%</td>
<td>0.2 ± 0.1%</td>
<td>8.5 ± 1.2%</td>
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ΔYr36

+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

<table>
<thead>
<tr>
<th>Southern pop.</th>
<th>Northern pop.</th>
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<tr>
<td></td>
<td>- +</td>
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<tr>
<td>TCD76</td>
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<td>TCD100</td>
<td>LDN</td>
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<td>LDN65</td>
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*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**

![Diagram showing the WKS1 alternative splicing site with different splice variants and corresponding kinase and START domains.](image)

- **TV1**: Kinase domain
- **TV2**: Kinase domain
- **TV3**: Kinase domain
- **TV4**: Kinase domain
- **TV5**: Kinase domain
- **TV6**: Kinase domain

*Stop codon*

500-bp

**Model of START domain transport of sterols in humans**
The transcription profiles of TV1 shows significant interactions between Temperature, inoculation, and time after inoculation.

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.
**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 from 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

* *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.