Yr36 Confers Partial Resistance at Temperatures Below 18°C to U.K. Isolates of *Puccinia striiformis*

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ABSTRACT

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Wheat yellow (stripe) rust, caused by the obligate biotrophic fungus *Puccinia striiformis* f. sp. *tritici*, is a continual threat to wheat fields worldwide. New isolates with increased virulence have recently emerged driving breeding efforts to incorporate disease resistance genes which confer potentially more durable, albeit partial, resistance. *Yr36* is one such locus which was recently cloned (*WKS1*) and described as a high-temperature adult-plant gene being effective only at temperatures above

25°C. We examined the potential use of *Yr36* at temperatures below 25°C. Field experiments in the United Kingdom across 2 years show that lines carrying *Yr36* provide slow rusting resistance to the yellow rust pathogen. Juvenile and adult *Yr36* isogenic lines showed partial resistance at temperatures below 18°C under control environment conditions in tetraploid and hexaploid genetic backgrounds, but not at seedling stage, when inoculated with U.K. *P. striiformis* isolates. This partial resistance phenotype was similar to that observed previously at temperatures $\geq 25^\circ\text{C}$. Transgenic complementation tests and ethyl methanesulfonate mutants showed that the low-temperature partial resistance was due to the *WKS1* gene. This study indicates that *Yr36* has the potential to be an effective source of partial resistance in temperate wheat growing regions.

Wheat yellow rust caused by *Puccinia striiformis* f. sp. *tritici* is an important foliar disease in wheat capable of significant reductions in both grain quality and yield in susceptible cultivars (3,8,15). Historically, the use of major race-specific resistance (*R*) genes in varieties has been an effective method for disease control, although the appearance of *P. striiformis* races that overcome widely deployed *R* genes (such as *Yr2*, *Yr9*, *Yr17*, and *Yr27*) has led to large pandemics (39). The appearance of new and more aggressive *P. striiformis* races in recent years (15) and their rapid spread pose an increasing global threat to wheat production and food security (22).

Recent breeding efforts have focused on achieving durable resistance in the field by pyramiding non-race-specific resistance genes (4,21). These genes provide a moderate or partial control over a number of *P. striiformis* isolates and are often most effective at late plant development stages and are therefore referred to as adult plant resistance (APR) genes (28). The partial nature of the resistance response also implies that they delay disease progression (slow rusting), but can be overcome under heavy inoculum pressure (32). Several authors have further classified some APR genes as only effective under temperatures above 25°C, these being known as high-temperature adult-plant (HTAP) resistance genes (4,28).

The wheat yellow rust resistance gene *Yr36*, derived from wild emmer, was originally described as a partial resistance gene (37). Wheat lines carrying *Yr36* were susceptible to *P. striiformis* inoculations at seedling stage when cultivated at 20°C after inocu-

lation, but showed enhanced and partial resistance when inoculated at adult plant stage and maintained at 25 or 35°C after inoculation. This led to the classification of *Yr36* as an HTAP gene (37). Positional cloning of *Yr36* identified a gene encoding for a kinase-START domain protein as the underlying gene (named *WKS1* for *Wheat Kinase START 1* gene) (11). Ethyl methanesulfonate (EMS) mutants showed that both kinase and START domains were required for a resistance response (11), supporting a different molecular mechanism to traditional nucleotide-binding site leucine-rich repeat (NBS-LRR) domain resistance genes (6,13,16). Controlled environment and field studies in California (U.S.) corroborated the partial resistance provided by *Yr36* at temperatures $\geq 25^\circ\text{C}$ against a wide range of U.S. *P. striiformis* isolates. The gene has also been deployed successfully in other relatively high temperature growing areas of the United States, Canada, and India (12,19,29).

In this study, we examined the potential use of *Yr36* in more temperate growing regions, such as northern Europe, where temperatures remain below 25°C for much of the wheat growing season. In field experiments, we found significant reductions in the percentage of leaf area infected in lines carrying *Yr36* compared to their isogenic counterparts under both natural and artificial inoculations. These lines showed the characteristic partial resistance phenotype previously described at higher temperatures ($\geq 25^\circ\text{C}$). To confirm this effect, near-isogenic lines (NILs), transgenic lines, and previously characterized EMS mutants for the *WKS1* gene were evaluated in controlled environment conditions. Lines carrying a functional *WKS1* (*Yr36*) gene presented a partial resistance response at temperatures of 15 and 18°C when inoculated at juvenile and adult plants with U.K. *P. striiformis* isolates, whereas seedlings displayed a susceptible response. The low temperature resistance response was consistent in both tetraploid and hexaploid wheat varieties and transgenic plants. These results suggest that *Yr36* confers partial resistance to U.K. isolates of *P. striiformis* at temperatures below those previously reported and

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could therefore be an effective source of partial resistance in temperate wheat growing regions.

MATERIALS AND METHODS

Plant material and infection experiments. The materials used in this study have been previously described (5,11,37). Briefly, the tetraploid wheat (*Triticum turgidum* sp. *durum*) cultivar Langdon and the hexaploid wheat (*T. aestivum* L.) breeding line UC1041 were used as susceptible controls, whereas RSL65 (Langdon recurrent parent) and UC1041+Yr36 were used as Yr36-derived material. EMS mutants of the WKS1 gene (encoding for Yr36) in the UC1041+Yr36 genetic background have been previously described (11). Transgenic lines were generated in the hexaploid wheat spring cultivar Fielder as described below. Langdon, UC1041, and Fielder were susceptible to all *P. striiformis* isolates used in this study (Table 1).

The infection experiments were conducted in control environment rooms (CER) under long day conditions (8 h dark/16 h light) using a complete randomized design. Two temperature regimes were used in this study; 10°C during dark periods with a shift to 18°C (10/18°C) or 15°C (10/15°C) during light periods. Seeds were pregerminated in Petri dishes, sown in pots (7 × 7 cm), and placed immediately into the 10/18°C or 10/15°C temperature regime. Plants were infected with urediniospores of *P. striiformis* isolate 08/21, unless otherwise stated, at either two expanded leaves (seedling stage), fifth to sixth leaf stage (juvenile) or adult plants. For dosage experiments, 5, 20, and 80 mg of spores from isolate 08/21 were used for inoculation. After infection, plants were incubated in the dark at 10°C with high relative humidity for 24 h. Plants were then moved back to their original 10/18°C or 10/15°C growing conditions. After infection, 15 plants per line were evaluated and infection severity was recorded at either 14 days postinoculation (dpi) for plants maintained at 10/18°C, or 18 dpi for plants maintained at 10/15°C. The percentage of leaf area covered by pustules was scored using the modified Cobb scale (26) and two or three leaves per plant were averaged (depending on the wheat growth stage at infection) to provide a single plant score.

Field experiments. Field experiments were conducted at the National Institute of Agricultural Botany (NIAB) (Cambridge, UK) during the wheat growing seasons of 2009 to 2010 and 2010 to 2011. Plants were sown in autumn in both 2009 (28 October) and 2010 (21 October). Experimental units consisted of two adjacent 1 m rows which were organized in three (2010) or four (2011) randomized complete blocks. In both years, observations were recorded every 7 days starting at Zadok's growth stage (GS) 31 (first node detectable) until after anthesis (GS72) (42). The 2010 experiment was artificially inoculated with three *P. striiformis* isolates (08/501, 09/29, and 09/105) (Table 1). The 2011 experiment was performed under natural inoculation conditions with the predominant races having virulence profiles similar to 08/501,

08/21, and 03/7 (Table 1). Weather data were obtained from the NIAB weather station which was located less than 1 km from the experimental plots.

Transgenic plants. To generate transgenic wheat plants, the complete Yr36 gene was transferred from pWKS1 (11) as a Klenow polymerase-treated *PmeI*-*NcoI* fragment, ligated into the binary vector pRLF10 *PmeI* site, to create pEW210. This binary plasmid is a super clean vector (10), containing the *NPTII* (neomycin phosphotransferase II) gene under the control of the *Subterranean clover stunt virus* Sc4 promoter (33) and *Arabidopsis thaliana* *FAD2* intron (24) for selection on geneticin G418 in tissue culture. Plasmid pEW210 was electrotransformed into *Agrobacterium* strain LBA4404 pSB1 (17).

Immature donor wheat seeds (cultivar Fielder) were collected 14 to 16 days after anthesis and surface-sterilized (30). Before *Agrobacterium* infection, isolated embryos were pretreated with a centrifugation step essentially as detailed in Hiei et al. (14). Treated embryos were co-cultivated with the *Agrobacterium* strain described above at 23°C in the dark for 2 days. Following removal of the embryonic axis, subsequent tissue culture of the plant material was performed essentially as described in Hiei et al. (14). Positive lines were determined by quantitative polymerase chain reaction (PCR)-based amplification of the transgene transcript.

Quantitative reverse transcription (RT)-PCR. In transgenic wheat lines the fourth leaf was sampled before inoculation at the fifth to sixth leaf stage. For each sample, total RNA was extracted using TRIzol reagent (Invitrogen, UK) and treated with DNA-free DNase (Ambion, UK) and the Removal Reagents kit (Ambion, UK) following the manufacturer's instructions. The cDNA synthesis was done with 2.5 µg of total RNA as a template and synthesized with the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen, UK). Transcript levels of the WKS1 gene were determined using primers (WKS1_Fw: CACAAGTAC AATACCTTATGAAGATGG and WKS1_Rw: CATCTGACTC CTCTTCAAGCATTGT; 5'-3') with a LightCycler 480 instrument (Roche Applied Science, UK) using LightCycler 480 SYBR Green I Master Mix (Roche Applied Science, UK) and the following conditions: 5 min at 95°C; 40 cycles of 15 s at 95°C, 15 s at 60°C, 20 s at 72°C. The PCR amplification specificity was checked by dissociation curve analysis (from 60 to 95°C). Transcript levels were normalized with *actin* (11) and linearized values determined using the 2^{-ΔΔCT} method (20).

Microscopy. Leaf samples (5 cm long) were collected from infected plants at 10 days after inoculation (dpi) and stained as described in Ayliffe et al. (1). Briefly, samples were incubated in 1 M KOH and then washed with 50 mM Tris, pH 7.5, followed by a treatment with WGA-FITC (Sigma-Aldrich, UK) and then examined under the microscope with UV light. At least four independent biological replications were observed for each sample type.

Statistical analysis. Student *t* tests were used to determine the effect of Yr36 between lines used in the different experiments. For the EMS mutants, one way analysis of variance (ANOVA) was performed using arcsine-transformed values to meet the ANOVA assumption of normally distributed errors. Original values are reported in the text and all analyses were performed using GenStat 15th edition (<http://www.vsn.co.uk/>). CER experiments were repeated at least twice and the results are presented throughout the text as means ± standard error of the mean (SEM).

RESULTS

Yr36 confers partial resistance in adult plants under relatively cool field conditions. We examined the potential use of Yr36 as an APR gene under U.K. field conditions in 2010 and 2011. Naturally infected plots of hexaploid breeding line UC1041 and its BC₆ NIL containing the Yr36 locus (UC1041+Yr36)

TABLE 1. *Puccinia striiformis* isolates used in this study and their virulence profiles

Isolate	Year collected	Virulence profile on wheat ^a
87/7	1987	Yr1, Yr2, Yr3, Yr4, Yr6, Yr7, Yr9, Yr17, Yr27, Yr32
03/7	2003 ^b	Yr1, Yr2, Yr3, Yr4, Yr7
08/501	2002	Yr1, Yr2, Yr3, Yr4, Yr6, Yr9, Yr17
08/21	2008	Yr1, Yr2, Yr3, Yr4, Yr6, Yr9, Yr17, Yr32, YrRob, YrSol
09/29	2009	Yr1, Yr2, Yr3, Yr4, Yr6, Yr9, Yr17, Yr32, YrRob, YrSol
09/105	2009	Yr1, Yr2, Yr3, Yr4, Yr6, Yr9, Yr17, Yr32, YrRob, YrSol

^a YrRob and YrSol correspond to unmapped resistances in hexaploid cultivars Robigus and Solstice, respectively.

^b Isolate 03/7 was first detected in the field in 2003. A similar virulence profile was common in the races present in the 2011 field experiments.

were evaluated throughout the growing seasons. UC1041 is known to carry *Yr1*, which is not effective to any of the major U.K. *P. striiformis* isolates (Table 1), suggesting that any resistance observed in UC1041+*Yr36* could be attributed to the *Yr36* locus.

In 2010, incidence of the disease was detected in UC1041 the second week of May during early stem elongation stage (GS31) whereas pustules were not detected in UC1041+*Yr36* leaves until 2 weeks later in late stem elongation stage (GS35-39). At anthesis (GS60) significant differences in infection were observed in flag leaves between NILs (Fig. 1A and B), with UC1041+*Yr36* leaves

having a significantly lower percentage of leaf area infected compared with UC1041 leaves ($P < 0.05$; Fig. 1C). The *Yr36*-mediated resistance remained effective during the initial stages of grain filling (GS70-72), whereas susceptible UC1041 plants were fully covered with pustules at this stage. The observed *Yr36*-mediated partial resistance was similar to that previously documented in field conditions in California (U.S.) (37). Similar results were obtained in field experiments in 2011, where NILs harbouring *Yr36* were significantly more resistant to *P. striiformis* than the UC1041 control lines between GS32 and GS70 ($P < 0.05$; Fig. 1D). Despite the partial resistance and slower disease

Fig. 1. *Yr36* confers partial resistance to *Puccinia striiformis* in field trials across 2 years. Plots and individual leaves of **A**, UC1041+*Yr36* and **B**, UC1041 infected with *P. striiformis* at the National Institute of Agricultural Botany-Cambridge field station in 2010. UC1041+*Yr36* displays a partial resistant phenotype which limits pathogen spread compared with UC1041 which is covered by pustules. Average percentage of leaf area infected in **C**, 2010 and **D**, 2011 between Zadok's growth stage (GS) 31 (early stem elongation) and 72 (mid-grain fill) of UC1041 (filled circle) and UC1041+*Yr36* (empty circle). Significant *t* tests at each time point are reported (*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$). Error bars are standard error of the mean.

progression observed in *Yr36* NILs up to early grain filling (GS70), the final percentage of infected leaf area at mid grain fill (GS72) was 100% for both NILs, irrespective of their *Yr36* status. Overall, the 2 years of field data suggest that *Yr36* confers partial resistance under U.K. field conditions, similar to that observed in previous reports (37).

We examined the temperature records during the field experiments since *Yr36* was previously shown to confer partial resistance at temperatures $\geq 25^{\circ}\text{C}$, but not at temperatures $\leq 20^{\circ}\text{C}$ (37). The average maximum daily temperature between April and June was 16.9 and 18.8 $^{\circ}\text{C}$ in 2010 and 2011, respectively (Fig. 2). Maximum temperatures within the study period were higher than 25 $^{\circ}\text{C}$ on only 4 days in 2010 and only twice in 2011. This suggests that *Yr36*-mediated resistance is effective in relatively cooler conditions than previously reported. However, on at least two occasions maximum day temperatures were higher than 20 $^{\circ}\text{C}$ (but lower than 25 $^{\circ}\text{C}$) for 4 to 5 consecutive days in both years.

***Yr36* confers resistance at temperatures below 18 $^{\circ}\text{C}$ in controlled environment conditions.** Controlled environment experiments were performed to establish the effectiveness of *Yr36* at temperatures $\leq 18^{\circ}\text{C}$. Based on the field meteorological data, 15 and 18 $^{\circ}\text{C}$ were chosen as the maximum daytime temperatures to be tested. To account for potential background effects on the partial resistance observed in the field, we included (in addition to the UC1041 NILs) tetraploid wheat cultivar Langdon (LDN) and RSL65 which carries the *Yr36* locus in an otherwise LDN genetic background (37). Juvenile plants at the fifth to sixth leaf stage were inoculated with *P. striiformis* isolate 08/21 and scored at either 14 or 18 dpi depending on the temperature regime.

Clear and significant differences were observed between lines carrying *Yr36* and the isogenic control lines LDN and UC1041 at 15 $^{\circ}\text{C}$ (Fig. 3A to D). Pustules were present in all the germplasm tested, but lines carrying *Yr36* displayed characteristic chlorotic patches that restricted pathogen growth (Fig. 3A and C). The LDN and UC1041 control lines did not exhibit chlorotic patches and pustules were larger in size compared with those formed in the *Yr36* lines (Fig. 3B and D). Disease incidence as determined by the percentage of leaf area covered by pustules was significantly less ($P < 0.001$) in UC1041+*Yr36* ($16.5 \pm 3.1\%$) and RSL65 ($29.7 \pm 6.8\%$) compared with their isogenic counterparts without *Yr36* ($90.8 \pm 2.8\%$ and $91.1 \pm 2.4\%$, respectively). Similar results were obtained in juvenile UC1041 NILs evaluated at 10/18 $^{\circ}\text{C}$. UC1041+*Yr36* displayed chlorotic patches and had an average percentage of leaf area covered by pustules of $19.7 \pm 3.1\%$, whereas UC1041 had an average of $82.7 \pm 6.7\%$ ($P < 0.001$;

Fig. 3E and F) when infected with isolate 08/21. These data provide strong evidence that *Yr36* confers resistance to U.K. *P. striiformis* isolates at temperatures $\leq 18^{\circ}\text{C}$ in controlled environment conditions.

Resistance at temperatures below 18 $^{\circ}\text{C}$ is conferred by *WKS1*. The gene encoding for the *Yr36*-mediated resistance was previously identified as a wheat kinase-START domain gene (*WKS1*) (11). Transgenic lines expressing a full genomic contig of *WKS1* were developed in the hexaploid wheat cultivar Fielder which is susceptible to U.K. *P. striiformis* isolate 08/21. Two independent transgenic events (lines 1 and 3) were selected at the T₁ generation based on the presence of the genomic construct as a single insertion event and the expression of *WKS1* transcript in leaves. T₂ plants from both events were segregating for the presence of the *WKS1* construct as determined by PCR. Expression levels of *WKS1* were determined by qPCR and 10 positive and 10 negative plants were selected for *P. striiformis* virulence assays at the 10/15 $^{\circ}\text{C}$ temperature regime (Fig. 4). Transgenic lines expressing *WKS1* had an average percentage of leaf area

Fig. 3. *Yr36*-mediated resistance at A to D, 10/15 $^{\circ}\text{C}$ and E to F, 10/18 $^{\circ}\text{C}$ temperature regimes. Pictures of representative leaves of tetraploid (4x) durum wheat cultivar Langdon and hexaploid (6x) UC1041 near-isogenic lines with the presence (A, C, and E) and absence (B, D, and F) of *Yr36*. Plants were inoculated with *Puccinia striiformis* isolate 08/21 at the fifth to sixth leaf stage and pictures were taken at 18 (A to D) and 14 (E to F) days postinoculation. Scale bar; 5 mm.

Fig. 2. Maximum daily temperatures at the National Institute of Agricultural Botany-Cambridge field station in 2010 and 2011. Values range from April to June 2010 (black diamond) and 2011 (gray squares) encompassing the scoring dates (gray triangles) reported in Figure 1. The threshold temperatures of 20 and 25 $^{\circ}\text{C}$ are indicated across the graph. Zadok's growth stage (GS) as in Figure 1.

covered by pustules of $6.7 \pm 1.9\%$, whereas the negative sister lines showed significantly higher infection of $46.8 \pm 7.7\%$ ($P < 0.001$). The lines with highest *WKS1* expression (Fig. 4: 3b among others) had well defined chlorotic patches and only a few narrow pustules were observed. Lines with relatively lower *WKS1* expression (Fig. 4: 1b and c and 3c) displayed a wider chlorotic area and increased number of pustules, although they were smaller and less developed compared with those found in plants not expressing *WKS1* (Fig. 4: 1a and 3a). Similar results were observed in transgenic plants grown under the 10/18°C temperature regime. Leaves of negative transgenic lines as determined by PCR were susceptible ($80.0 \pm 3.8\%$) at 14 dpi, whereas in positive transgenic lines, the percentage of leaf area covered by pustules was significantly reduced ($22.2 \pm 5.9\%$; $P < 0.001$) (Supplemental Figure 1).

We performed functional assays on *WKS1* EMS mutants to determine if both domains of the *WKS1* gene were necessary for *Yr36*-mediated resistance at 15°C with U.K. *P. striiformis* isolate 08/21 (Fig. 5). Three mutants were selected with changes in conserved amino acids in the kinase (T6-138 and T6-569) or

START (T6-567) domains. In addition to the UC1041 NILs, a sister line of T6-567 which was homozygous for the absence of the START domain mutation (i.e., functional *WKS1* gene) was included as a control. The three *WKS1* mutants showed a susceptible reaction similar to the UC1041 susceptible control (Fig. 5), whereas UC1041+*Yr36* and the T6-567 control line showed a resistant phenotype. *WKS1* mutants had an average percentage of leaf area covered by pustules of $79.6 \pm 3.5\%$, whereas the controls had an average of $31.3 \pm 6.0\%$ ($P < 0.001$). These mutants and controls had been previously characterized at 25°C (11) and shown similar results. These results suggest that both the kinase and START domains are necessary for the low temperature resistance response.

***Yr36* partial resistance is affected by plant developmental stage and inoculum pressure.** *Yr36* NILs and Fielder transgenic plants maintained at the 10/15°C regime were inoculated with *P. striiformis* isolate 08/21 at seedling stage with two expanded leaves. UC1041+*Yr36* and UC1041 NILs were both susceptible regardless of their *Yr36* status (Fig. 6A), with lines showing no significant differences in disease incidence determined by the percentage of leaf area covered by pustules ($90.8 \pm 3.6\%$ and $93.3 \pm 3.6\%$, respectively). Microscopic observations showed profuse hyphae formation in both NILs independent of the presence of *Yr36* (Fig. 6B). Likewise, all positive and negative T₂ plants from two independent transgenic events (lines 1 and 3) were susceptible when infected at seedling stage with *P. striiformis* isolate 08/21. However, positive transgenic plants displayed the *Yr36* resistance phenotype when infected at the adult plant stage (Supplemental Figure 2).

The UC1041 NILs were also inoculated at adult plant stage under the 10/15°C temperature regime when flag leaves were fully expanded. Infected flag leaves from UC1041+*Yr36* showed chlorotic stripes clearly defined along the leaf surface and scarce pustule formation ($7.7 \pm 1.0\%$), contrasting with UC1041 where flag leaves were covered by pustules ($70.8 \pm 7.3\%$; Fig. 6A). Similar results were obtained when inoculating adult plants of UC1041 NILs with *P. striiformis* isolate 87/7 at the 10/18°C temperature regime (Supplemental Figure 3). Microscopic observations revealed that in *Yr36* adult plants, the pathogen had restricted hyphal development and failed to develop the extensive mycelial network seen in UC1041 leaves which were fully colonized by 10 dpi (Fig. 6B).

We also assessed the effect of increased inoculum pressure on the *Yr36*-mediated resistance response at juvenile stage (10/15°C regime). Inoculation of UC1041 and UC1041+*Yr36* NILs with the standard inoculum (5 mg) resulted in similar results to those

Fig. 4. Characterization of T₂ plants from independent transgenic events 1 and 3 in hexaploid wheat cultivar Fielder at the 10/15°C temperature regime. Pictures of representative leaves from transgenic events 1 and 3 at 18 days postinoculation with *Puccinia striiformis* isolate 08/21. *WKS1* transcript levels preinoculation were determined by quantitative polymerase chain reaction relative to *actin*. Values are averages of two technical replications $\pm$ standard error of the mean. Scale bar; 5mm.

Fig. 5. Effect of *WKS1* mutations on resistance to *Puccinia striiformis* isolate 08/21 at 10/15°C. Near-isogenic lines A, UC1041 and B, UC1041+*Yr36* were inoculated at the fifth to sixth leaf stage together with C to E, three *WKS1* mutants. Lines T6-569 and T6-138 carry nonsynonymous mutations in the kinase domain, whereas line T6-567 carries a nonsynonymous mutation in the START domain. F, A sister line of mutant T6-567 homozygous for the absence of the *WKS1* mutation was also included as a control. Scale bar; 5 mm.

reported above. As the amount of inoculum increased, the *Yr36*-mediated resistance eventually broke down, with both NILs being classified as fully susceptible when inoculated with 80 mg of spores (Supplemental Figure 4).

DISCUSSION

Yr36 was originally classified as an HTAP resistance gene which was able to confer partial resistance to *P. striiformis* in adult plants at temperatures $\geq 25^{\circ}\text{C}$ (37). Subsequent studies showed that *Yr36* was effective in conferring partial resistance in juvenile plants (fourth to fifth leaf stage) at high temperatures ($\geq 25^{\circ}\text{C}$) (11). In this study we show that *Yr36* confers partial resistance, similar to that seen at 25 and 35°C , when evaluated at temperatures $\leq 18^{\circ}\text{C}$ with U.K. *P. striiformis* isolates.

Our field experiments across two seasons show that *Yr36* is able to confer partial resistance to multiple U.K. *P. striiformis* isolates under more temperate growing conditions than previously reported (11,12,29,37). The adult plant resistance observed in the field resembled a typical slow rusting response (41), with less pustule formation and delayed pathogen proliferation in the UC1041 line containing *Yr36*. This response was similar to previously reported *Yr36*-mediated field resistance (11,12,37). Despite the effective partial control in the field during the majority of the growing season, lines carrying *Yr36* can sometimes show high levels of infection and sporulation towards the end of the season ([37] this study). We attribute this to high inoculum pressure in certain years. This was supported by the CER dosage experiment in which the *Yr36*-mediated resistance observed at $10/18^{\circ}\text{C}$ was overcome at high levels of inoculum.

Across both years maximum day temperatures rarely surpassed 25°C , the threshold for effective *Yr36*-mediated resistance based on previous studies. This implied that temperatures between 20 and 25°C could be sufficient to trigger the *Yr36*-mediated resistance response in UC1041, or that *Yr36* functions at temperatures well below those previously reported. To clarify these possibilities, we performed control environment experiments with plants grown at diurnal constant temperature of either 15 or 18°C , before and after inoculation. *Yr36*-mediated partial resistance was observed when inoculated at fifth to sixth leaf stage in both temperature regimes to a similar degree to that observed at 25°C (11). This was confirmed in both tetraploid (Langdon) and hexaploid (UC1041) wheat for the $10/15^{\circ}\text{C}$ regime, suggesting that the effect was stable across genetic backgrounds. Adult plant *Yr36*-mediated resistance under the $10/15^{\circ}\text{C}$ and $10/18^{\circ}\text{C}$ temperature regime was also shown using the same UC1041 NILs and *P. striiformis* isolates (08/21 and 87/7).

The gene underlying *Yr36* was previously identified as a wheat kinase-START domain gene (*WKS1*) (11). To confirm that the low-temperature resistance observed was due to *WKS1* and not to other genes within the larger *Yr36* introgression, we generated transgenic hexaploid wheat lines expressing *WKS1* and evaluated *WKS1* EMS mutants. Both sets of experiments confirmed that *WKS1* was the underlying gene conferring partial resistance at temperatures below 18°C and that both kinase and START domains were necessary for resistance.

Contrasting results between the previous experiments (11,37) and our findings might be explained by several factors, although the use of the same germplasm across the different experiments rules out genetic background effects. First, it is well established that temperature affects plant pathogen interactions (4,9,25,27,28,34,38). Of relevance to this study is the early work of Qayoum and Line (28) who first defined HTAP in the wheat-*P. striiformis* pathosystem. They showed that in varieties with APR the infection type could change from low (resistant) to high (susceptible) when plants are first grown at high temperatures (maximum 34°C) and then transferred to low temperatures (18°C) after inoculation. Similar observations on the effects of the higher post-inoculation temperatures were described for Australian cultivars (25). In the previous *Yr36* study (37), an analogous experimental design was used possibly masking the low-temperature *Yr36* resistance response. Plants were grown preinoculation under a temperature regime with a maximum temperature of 25°C and then transferred to the low temperature regime (maximum 18 to 20°C) postinoculation. In this study, we maintained plants at the same temperature pre- and postinoculation to avoid this confounding effect. Recent work by Bryant et al. (2) supports this explanation as they found significantly increased susceptibility of UC1041+*Yr36* adult plants when shifted from 25°C (preinoculation) to 18°C (postinoculation) compared with plants grown constantly at 18°C pre- and postinoculation. In addition to the differences in the experimental design, different parameters between growth chambers (light intensity, relative humidity, gaseous exchange, speed of temperature changes, etc.) could also account for the contrasting results. Previous work used temperature profiles with a gradual temperature increase between the dark and light periods, whereas the chambers used in this study shifted rapidly and stayed at constant maximum temperatures during the entire light period. Lastly, the *P. striiformis* isolates used in this study are U.K. isolates best adapted to cooler temperatures compared to the U.S. isolates (37) which are best adapted to relatively higher temperatures (22,23). It will be important to assess in more detail the effect of *P. striiformis* isolates from different locations on *Yr36*-mediated low temperature resistance by controlling the temperature-related effects outlined above.

The interplay of disease resistance and plant development has been described before (7,40), with wheat APR genes providing a clear example of the developmental dependence of some resis-

Fig. 6. *Yr36* partial resistance is affected by plant developmental stage. Near isogenic plants maintained at $10/15^{\circ}\text{C}$ and infected with *Puccinia striiformis* isolate 08/21 at either seedling (two expanded leaves), juvenile (fifth to sixth leaf), or adult (expanded flag leaf) stage. **A**, Macroscopic observation. Scale bar; 5 mm. **B**, Microscopic observation. Scale bar; 20 μm .

tance responses (27,28,36). In this study we show that *Yr36* is only effective in juvenile and adult plants, but not in seedlings under low temperature. Microscopic observations show a restricted pathogen growth in juvenile and adult plants, whereas in seedlings the pathogen spread was equivalent between NILs. The cloned *Lr34/Yr18* APR gene (18) offers an interesting example of the interaction between developmental stage, temperature, and resistance response since *Lr34/Yr18* seedlings are only resistant when they are grown at low temperatures (<7°C). This has important agronomic and epidemiological consequences in many wheat growing regions since it reduces inoculum buildup during the initial part of the growing season (35). However, a recent study showed that transgenic expression of *Lr34/Yr18* could result in enhanced seedling resistance in the absence of the cold treatment in some genetic backgrounds (31). In our experiments we did not observe this phenomenon as *Yr36* transgenic seedlings were susceptible to *P. striiformis* in a similar manner to the nontransgenic *Yr36* lines.

The identified low-temperature *Yr36*-mediated resistance to U.K. *P. striiformis* isolates should be of interest to breeders who seek to deploy partial resistance in their programs. The different degrees of field resistance in 2010 and 2011 provide an illustrative example of the range of protection that can be expected from *Yr36* under different inoculum pressures. These results complement those reported in North America (12,29,37) which highlight that a range of resistances can be obtained when *Yr36* is combined with other resistance sources. Ultimately, the evaluation of the available *Yr36* genetic stocks (5,12) in specific breeding environments will provide further information of the potential of this gene to combat yellow rust in different wheat growing regions around the world.

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