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ORIGINAL PAPER

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High-temperature adult-plant (HTAP) stripe rust resistance gene *Yr36* from *Triticum turgidum* ssp. *dicoccoides* is closely linked to the grain protein content locus *Gpc-B1*

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Abstract Several new races of the stripe rust pathogen have become frequent throughout the wheat growing regions of the United States since 2000. These new races are virulent to most of the wheat seedling resistance genes limiting the resistance sources that can be used to combat this pathogen. High-temperature adult-plant (HTAP) stripe rust resistance has proven to be more durable than seedling resistance due to its non-race-specific nature, but its use is limited by the lack of mapping information. We report here the identification of a new HTAP resistance gene from *Triticum turgidum* ssp. *dicoccoides* (DIC) designated as *Yr36*. Lines carrying this gene were susceptible to almost all the stripe rust pathogen races tested at the seedling stage but showed adult-plant resistance to the prevalent races in California when tested at high diurnal temperatures. Isogenic lines for this gene were developed by six backcross generations. Field tests in two locations showed increased levels of field resistance to stripe rust and increased yields in isogenic lines carrying the *Yr36* gene compared to those without the gene. Recombinant substitution lines of chromosome 6B from DIC in the isogenic background of durum cv. Langdon were used to map the *Yr36* gene on the short arm of chromosome 6B completely linked to *Xbarc101*, and within a 2-cM interval defined by PCR-based markers *Xucw71* and *Xbarc136*. Flanking locus *Xucw71* is also closely linked to the grain protein content locus *Gpc-B1* (0.3-cM). Marker-assisted selection strategies are presented to improve stripe rust resistance and simultaneously select for high or low *Gpc-B1* alleles.

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Introduction

Stripe rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici* (*Pst*), is a devastating disease in wheat. Since the late 1950s, stripe rust has been one of the most destructive diseases of wheat in the western United States, and in recent years, it has also become a major threat in the Great Plains and southeastern states (Line and Chen 1996; Chen and Moore 2002). The most economic and environmental friendly way to counter the continuous evolution of *Pst* populations is to develop wheat cultivars resistant to this pathogen.

Genetic resistance to *Pst* can be classified as race-specific or non-race-specific. Race-specific resistance can be detected at the seedling stage and remains effective at all stages of plant growth. It is conferred by single genes that follow the gene-for-gene interaction model (Flor 1971). Owing to their specific nature, seedling resistance genes have been frequently overcome by new races of the pathogen (Chen and Moore 2002).

In contrast, non-race-specific resistance genes are expressed at later stages of plant development, provide a broader range of resistance to pathogens, and tend to be more durable than seedling resistance genes. A particular class of adult-plant resistance genes are the high-temperature adult-plant (HTAP) resistance genes that are effective after stem elongation and when average night temperatures remain above 10°C and day temperatures are between 25°C and 30°C (Qayoum and Line 1985; Milus and Line 1986a, b; Line and Chen 1995). The level of resistance conferred by HTAP resistance

sources is usually rated as moderate and is affected by plant growth stage, temperature, and humidity.

More than 30 different genes for race-specific seedling resistance to wheat stripe rust have been identified and several of those genes have been mapped. In contrast, there are relatively few reports identifying markers for HTAP resistance to stripe rust in wheat (Börner et al. 2000; Bariana et al. 2001; Suenaga et al. 2003). Although this trait is polygenic, these studies have shown that single major genes can account for a significant portion of the variation in HTAP resistance.

The present paper reports the characterization of a new HTAP resistance gene derived from *Triticum turgidum* ssp. *dicoccoides* (Körn.) Thell. (hereafter referred to as DIC) accession FA15-3 from Israel (Avivi 1978). This new stripe rust resistance gene has been designated *Yr36* (McIntosh et al. 2005). The specific objectives of the present study were to (1) determine the seedling and adult-plant responses attributable to *Yr36* under controlled conditions, (2) determine the effect of *Yr36* on final disease severity and yield under field conditions in a set of tetraploid and hexaploid isogenic lines, and (3) develop a precise map of the *Yr36* gene region.

Materials and methods

Plant material

Joppa et al. (1997) developed a population of recombinant substitution lines (RSLs) from a cross between cultivar Langdon (LDN) and chromosome substitution line LDN(DIC6B) to map the high grain protein content (GPC) gene *Gpc-B1*. In field experiments performed at the University of California at Davis (UCD) to further characterize the *Gpc-B1* gene, we observed that RSLs 65 and 68, carrying a DIC chromosome segment between loci *Xgwm193* and *Xgwm508* (Khan et al. 2000) were more resistant to *Pst* than LDN and RSL14 (carrying a LDN segment in this region). These four lines were used to characterize the effect of stripe rust resistance gene *Yr36* on seedling and HTAP resistance in greenhouse experiments.

For the mapping of *Yr36* we used two sets of recombinant lines. The first mapping set included 11 homozygous RSLs (8, 19, 36, 41, 47, 50, 54, 58, 59, 67, and 77) from the cross LDNxLDN(DIC6B) and the four control lines indicated above. These lines were selected from the mapping population of Joppa et al. (1997) for the presence of recombination events between *Xgwm193* and *Xgwm508*. Once the approximate location of *Yr36* was established, a more precise map was developed using a second set of 18 homozygous RSLs (108, 110, 115, 117, 118, 120, 121, 123, 128, 130, 135, 144, 149, 151, 158, 160, 209 and 291) from the cross RSL65xLDN (Olmos et al. 2003; Distelfeld et al. 2004). These lines have recombination events between RFLP markers *Xucw75* and *Xucw66*.

For the field experiments, isogenic lines for *Yr36* were developed by six backcross generations followed by self-pollination to produce BC_6F_2 homozygous lines. Homozygous plants carrying the *Yr36* gene were selected with molecular markers (Khan et al. 2000). RSL65 was the source of *Yr36* for the tetraploid breeding line UC1113, whereas common wheat cultivar 'Glupro' (Khan et al. 2000) was the source of *Yr36* backcrossed into the four hexaploid hard red spring cultivars and breeding lines 'Anza', 'Yecora Rojo', UC1037, and UC1041. BC_6F_3 seeds of homozygous lines were deposited at the National Small Grains Collection (NSGC) as PI 638740 (Yecora Rojo *Yr36*/*Gpc-B1*) and PI 638741 (UC1113 *Yr36*/*Gpc-B1*). These isogenic lines have been designated as the type germplasm for the *Yr36* gene in *T. aestivum* and *T. turgidum*, respectively (McIntosh et al. 2005).

Molecular markers

Plant nuclear DNA isolations, southern blots, and hybridization procedures have been described before (Dubcovsky et al. 1994; Dvorak et al. 1988). RFLP probes (*Xucw66, 68, 69, 73, 74, 75* and *77*) and PCR markers (*Xucw71* and *79*) were described by Distelfeld et al. (2004). These markers were previously used to genotype the RSLs used in this study. Microsatellite markers *Xbarc101* and *Xbarc136* were mapped on chromosome arm 6BS by D. Santra and K. Kidwell (personal communication) and *Xgwm88* was described by Röder et al. (1998).

Greenhouse experiments

In the initial test, the susceptible parent (LDN) and three recombinant substitution lines (RSL 14, 65 and 68) were evaluated for their reaction to *Pst* in both seedling and adult-plant stages. For the seedling test, five to seven seeds of each line were planted in a 7×7×7 cm pot filled with a potting mixture. The winter wheat 'Nugaines', susceptible to all races of *Pst* in the seedling stage, was used as a susceptible check to confirm successful infection. The seedlings were grown in a rust-free greenhouse at a diurnal temperature cycle of 10–25°C. Metal halide lights were used before and after inoculation to maintain an 8-h dark and 16-h light photoperiod. At the two-leaf stage, seedlings were inoculated with urediniospores of different races mixed with talc. A total of 15 isolates representing seven races (Table 1) were used in the seedling test. Inoculated plants were placed in a dew chamber without light at 10°C for 24 h. Seedlings were then moved to a growth chamber at temperatures programmed to change gradually between a minimum of 4°C at 2:00 am during the 8-h dark period and a maximum of 18–20°C at 2:00 pm during the 16-h light period (Chen and Line 1992).

For the adult-plant test, three to nine seeds for each line were planted in three 12×12×12 pots. The spring wheats 'Lemhi' and 'Alpowa' were used as susceptible and HTAP resistant checks, respectively. The conditions for growing plants before inoculation were the same as those for the seedling tests. One month after planting, plants of the susceptible parent and checks at the boot to heading stages and the RSLs in the stem elongation stage were inoculated with urediniospores mixed with talc. Cultures of three races (PST-100, PST-101, and PST-111, see Table 1) were used in the adult-plant tests. The inoculated plants were kept in a dew chamber at 10°C for 24 h and then grown in a greenhouse section under controlled diurnal temperature cycles gradually changing between a minimum of 10°C at 2:00 am during the 8-h dark period and a maximum of 35°C at 2:00 pm during the 16-h light period (Chen and Line 1995). The adult plants of four selected RSLs (RSL 14, 65, 68 and 110) were also tested at the low-temperature cycle described above for the seedling tests, to determine the effect of temperature on *Yr36* resistance at the adult-plant stage with the three races. Two plants of each line were used for all three race tests. Each flag leaf was inoculated with three races, and the inoculated sites were marked with colored tape to indicate the different races used. The inoculated plants were kept in a dew chamber at 10°C for 24 h and then grown under the same conditions as for the seedling tests.

For the mapping of *Yr36*, the susceptible parent (LDN), 23 RSLs, and the susceptible check Lemhi were evaluated with three races (PST-100, PST-101, and PST-111). The conditions and methods for the seedling test and the adult-plant test at the high-temperature cycles were the same as in the initial test, except that four plants were used for each race test for the high-temperature adult-plant tests. Infection type (IT) data were recorded based on a 0–9 scale at 20 days after inoculation (Line and Qayoum 1992). Infection types between 0 and 3 were considered resistant reactions, while infection types higher than 5 were considered susceptible.

Field experiments

The two field experiments grown at Davis, CA and Madera, CA in 2003 were organized in a split-plot design in five randomized complete blocks. The pairs of isogenic lines were sown such that cultivar or breeding lines were assigned to the main plots, whereas presence or absence of *Yr36* was assigned to the sub-plots. This design was adopted to maximize the sensitivity of the comparison within the isogenic pairs. Experimental units consisted of plots of 6.3 m^2 at Madera and 5.0 m^2 at Davis and a total of five pairs of isogenic BC_6F_2 lines were used. Stripe rust was evaluated at Feekes stage 10.3 (Large 1954). Severity was assessed on a 0 to 100% leaf area damage scale. Plots were machine harvested at maturity and yield per plot was adjusted using individual plot lengths to correct for possible small differences during planting.

Statistical analysis

Analyses of variance were performed using the SAS Version 8.2 program (SAS Institute 2003). For field trials, the general linear model (PROC GLM) was used to assess the effect of the DIC segment in the isogenic lines. In order to meet the assumptions of the model, data were transformed when necessary using logarithmic and power transformations. Each location was analyzed separately. Values after the "±" sign are standard errors of the mean throughout the text.

Table 1 Races and isolates of *Puccinia striiformis* f. sp. *tritici* (PST) used in seedling and adult-plant stripe rust resistance tests

PST race[a]	Isolate	Susceptible differential lines[b]	State origin	Year collected
PST-58	98−24	1,11,12,16	CA	1998
PST-59	2K-129	1,3,11,12,16	CA	2000
PST-78	02−114	1,3,11,12,16,17,18,19,20	CA	2002
PST-98	02-305-10	1,3,8,10,11,12,16,17,18,19,20	WA	2002
PST-98	02-305-18	1,3,8,10,11,12,16,17,18,19,20	WA	2002
PST-98	03−5	1,3,8,10,11,12,16,17,18,19,20	CA	2003
PST-100	03−4	1,3,8,9,10,11,12,16,17,18,19,20	CA	2003
PST-100[c]	03-202-10-sp-1	1,3,8,9,10,11,12,16,17,18,19,20	OR	2003
PST-100	04−110	1,3,8,9,10,11,12,16,17,18,19,20,21	CA	2004
PST-100	04−116	1,3,6,8,9,10,11,12,16,17,18,19,20,21	CA	2004
PST-100	04−128	1,3,8,9,10,11,12,16,17,18,19,20,21	CA	2004
PST-101	03−190-2	1,2,3,8,9,10,11,12,16,17,18,19,20	CA	2003
PST-101	04−111	1,2,3,8,9,10,11,12,16,17,18,19,20,21	CA	2004
PST-101[c]	04−134	1,2,3,8,9,10,11,12,16,17,18,19,20,21	CA	2004
PST-111[c]	04−301	1,3,5,8,9,10,11,12,16,17,18,19,20	CA	2004

[a]Chen et al. (2002, 2004), and Line and Qayoum (1992). Race PST-111 is unpublished
[b]Numbers indicate the susceptible differential lines used to identify the race: 1, Lemhi; 2, Chinese 166; 3, Heines VII; 4, Moro; 5, Paha; 6, Druchamp; 7, Ribesel 47/51*; 8, Produra; 9, Yamhill; 10, Stephens; 11, Lee; 12, Fielder; 13, Tyee; 14, Tres; 15, Hyak; 16, Express; 17, Avocet S + *Yr8;* 18, Avocet S + *Yr9;* 19, Clement; 20, Compair. (*Since 2004, *7*, Avocet S + *Yr5*); and 21, Summit
[c]Cultures used for adult-plant tests

Results

Yr36 resistance in seedling and adult plants under greenhouse conditions

Seedlings from RSL65 and RSL68 (carrying the DIC *Xgwm193–Xgwm508* segment) and LDN and RSL14 (carrying the LDN alleles in this region) had high infection types (IT values 8 or 9) similar to the susceptible control Nugaines for all of the races listed in Table 1 (data not shown). On the basis of the contrasting results observed between the *Pst* reactions in the greenhouse experiments at the seedling stage and the original observation of resistance under field conditions, we decided to test the same lines for adult-plant resistance.

Three of the most virulent races and isolates from the seedling tests (PST-100, PST-101 and PST-111) were used to test adult plants of the lines carrying a complete DIC or LDN chromosome segment in the region encompassed by *Xgwm193* and *Xgwm508*. Under a low-temperature cycle, all four RSLs tested (RSL 14 and 110 carrying the LDN segment and RSL 65 and 68 carrying the DIC segment) had similar susceptible infection types (IT values of 8 or 9) (data not shown). In contrast, adult plants inoculated under a high-temperature regime yielded significant differences in IT for all three PST races. Plants with the LDN segment (LDN and RSL14) were susceptible to stripe rust (average IT value of 7.1 ± 0.06), whereas lines with the complete DIC segment (RSL65 and 68) were resistant (average IT value of 2.2 ± 0.04) (Fig. 1). Susceptible control Lemhi had an average IT value of 9, whereas the resistant control Alpowa had an average IT value of 4.1. This bimodal difference in IT phenotype was also seen in the RSLs used for mapping (Fig. 1). These results confirm the presence of a previously uncharacterized HTAP resistance gene in the DIC interval defined by microsatellite loci *Xgwm193* and *Xgwm508*.

Effect of *Yr36* on stripe rust response and yield under field conditions

To test the effect of *Yr36* in the field and in different genetic backgrounds, five isogenic lines with and without *Yr36* were grown at Davis and Madera. Four of the five cultivars and advanced breeding lines tested were classified as susceptible to the stripe rust pathogen (Anza, Yecora Rojo, UC1037 and UC1113), whereas UC1041 was resistant to the races present at both locations in 2003. Although the first four cultivars were classified as susceptible, quantitative differences were observed in their responses (Fig. 2). At both locations, Yecora Rojo was the most susceptible cultivar, Anza and UC1037 showed intermediate final infection scores, and UC1113 was the least susceptible of the four.

At Madera, the presence of *Yr36* significantly reduced stripe rust infection on the flag leaf of the four susceptible cultivars tested, while not affecting the level of infection on the resistant line (Fig. 2a). This increased resistance led to significant yield gains in the UC1037 and Yecora Rojo isogenic lines carrying *Yr36*, and to non-significant yield increases in Anza and UC1113 lines carrying *Yr36* (Fig. 2b). In the susceptible cultivars, the average yield of the four isogenic lines carrying *Yr36* was 15.4% higher than the isogenic lines without *Yr36*. The presence of *Yr36* in resistant line UC1041 had a non-significant effect on yield.

At Davis, severity of stripe rust infection was also significantly lower in Anza and UC1113 carrying *Yr36* compared with the isogenic lines without *Yr36*. It was also lower, but not significantly, in the UC1037 and Yecora Rojo isogenic lines carrying *Yr36* (Fig. 2c) compared to their respective recurrent parents without

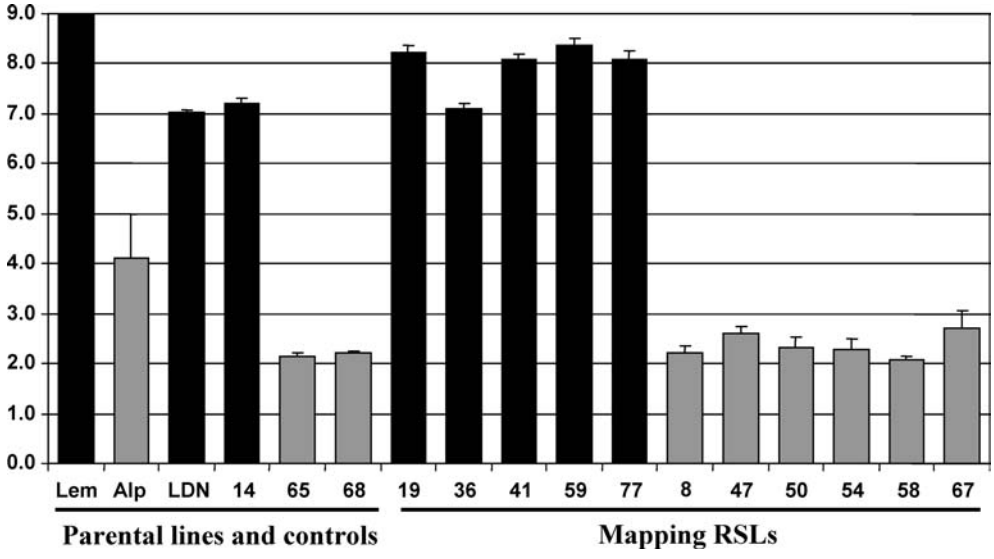

Fig. 1 Average adult-plant infection types with stripe rust races PST-100, PST-101, and PST-111, under the high-temperature cycle. Infection type data were recorded based on a 0–9 scale (Line and Qayoum 1992). Infection types of susceptible control Lemhi (Lem) and resistant control Alpowa (Alp), two lines carrying a complete LDN segment between loci *Xgwm508* and *Xgwm193* (LDN and 14) and two RSLs carrying the complete DIC segment (65 and 68). RSLs used for mapping of *Yr36* were classified into susceptible (*black bars*) and resistant (*grey bars*) lines. *Error bars* are standard errors of the means

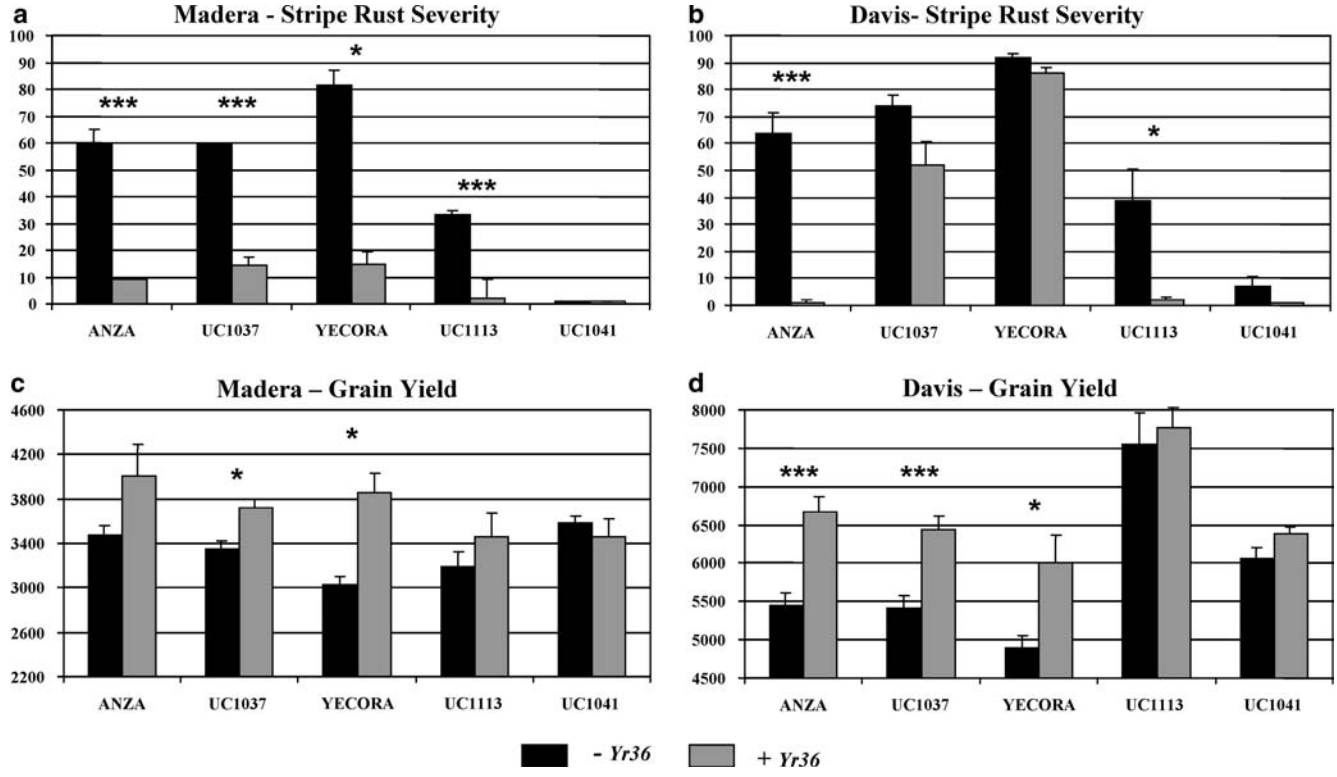

Fig. 2 *Top panels,* Effect of *Yr36* on final severity of stripe rust (%) in isogenic lines at Madera (**a**) and Davis (**c**). *Lower panels*, Effect of *Yr36* on yield (kg ha^{-1}) of isogenic lines at Madera (**b**) and Davis (**d**). *Black bars* represent lines without *Yr36* (-*Yr36*) and *grey bars* represent lines with *Yr36* (+ *Yr36*). * significance level at 5%, *** significance level at 1%. *Error bars* are standard errors of the means

Yr36. No significant differences in stripe rust severity were observed between the isogenic lines of resistant cultivar UC1041. Yields were significantly higher in the *Yr36* carrying isogenic lines of Anza, UC1037, and Yecora Rojo compared to the recurrent parents (Fig. 2d). Durum line UC1113 carrying *Yr36* also showed a higher yield than the line without *Yr36* but the differences were not significant (Fig. 2d). In the four susceptible cultivars the average yield of the isogenic lines carrying *Yr36* was 15.3% higher than the isogenic lines without *Yr36*, a similar value to that observed in Madera. The near-isogenic lines of UC1041 showed non-significant differences in yield.

Precise mapping of *Yr36*

The adult-plant screening of RSL65 and RSL68 established that the *Yr36* gene was located within the overlapping 30-cM DIC segment present in these two lines. To define more precisely the location of *Yr36* within this segment, we tested eleven RSLs developed by Joppa et al. (1997) which had recombination events within this 30-cM 6BS region. RSLs 8, 47, 50, 54, 58, and 67 showed significantly lower infection types (IT values ranged from 2.1 to 2.7) than RSLs 19, 36, 41, 59, 77 (IT values ranged from 7.1 to 8.4) when tested with races PST-100, PST-101, and PST-111 at the adult-plant stage under the high-temperature cycle (Fig. 1). Values from the susceptible and resistant RSLs were in the same range as the susceptible and resistant controls, indicating segregation for a single gene (Fig. 1). Since all lines were classified as susceptible or resistant, with no overlap among the values in the two groups, *Yr36* was mapped as a single Mendelian gene within a 2.8-cM interval between markers *Xucw74* and *Xucw77* (Fig. 3). All six resistant lines carry DIC alleles for these two markers, whereas all the susceptible lines carry the LDN alleles for the same markers.

A second set of RSLs was screened under the high-temperature cycle to further delimit the *Yr36* region. Graphical genotypes (Young and Tanksley 1989) and IT values for these RSLs are presented in Table 2. On the basis of the phenotype of these critical lines, the *Yr36* locus was mapped completely linked to *Xucw69* and *Xbarc101* inside a 2-cM interval defined by loci *Xucw71* and *Xucw77* (Fig. 3). All other lines with recombination events outside of the *Xucw71-Xucw77* interval showed consistent results with this map location (Table 2). Lines carrying a DIC chromosome segment between the two flanking markers showed significantly lower infection types (IT values ranged from 1.8 to 3.0) than lines carrying the LDN chromosome segment (IT values ranged from 5.8 to 7.8).

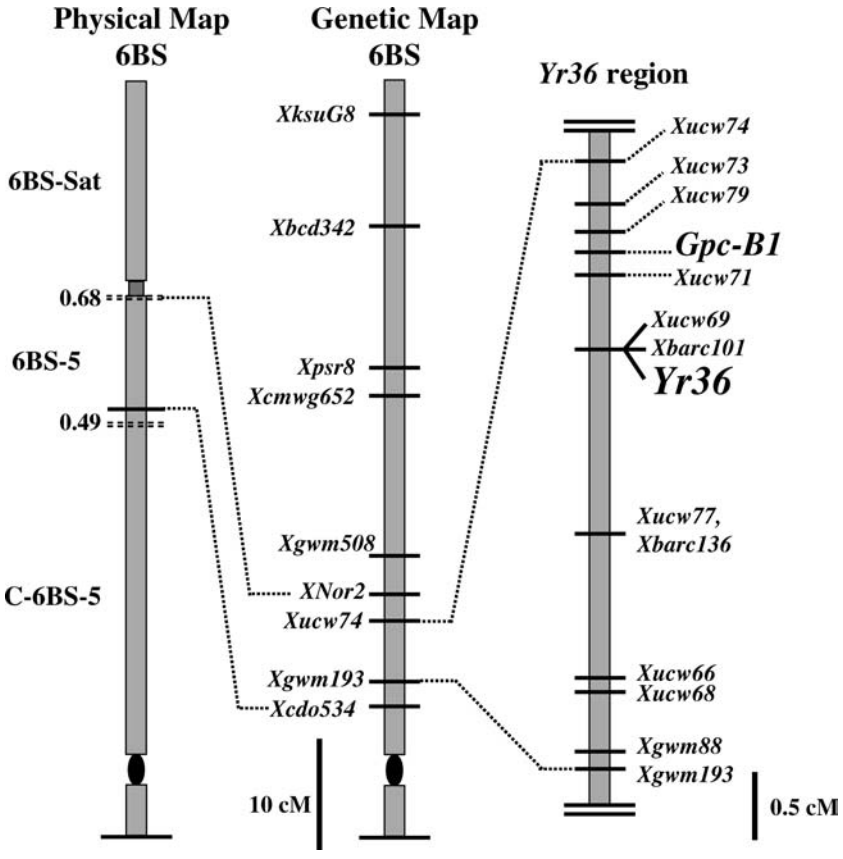

Fig. 3 Current map of the *Yr36* gene region in relation to the physical (Gill et al. 1993) and genetic maps of chromosome arm 6BS. *Yr36* was mapped completely linked to *Xucw69* and *Xbarc101*, between markers *Xucw71* and *Xucw77* based on results from the *T. turgidum* ssp. *dicoccoides* RSLs greenhouse experiments at adult-plant stage under high-temperature cycle. Centromeres are represented by *black ovals* and chromosomal breakpoints in the physical map with double *dashed lines*

Discussion

Mapping of *Yr36*: a novel HTAP resistance gene

Adult-plant resistance (APR) genes are useful for the implementation of long-term disease resistance strategies due to their non-specific nature and, thus, have greater potential for durable resistance. We report here the characterization of *Yr36*, a previously unidentified stripe rust resistance gene from *Triticum turgidum* ssp. *dicoccoides* located on chromosome arm 6BS. This gene confers adult-plant resistance under a high-temperature greenhouse testing regime and under field conditions.

With the advent of QTL studies, several APR genes for the stripe rust pathogen have been identified in wheat. Specifically on chromosome arm 6BS, Suenaga et al. (2003) detected a minor QTL (average explained variance 2%) in a region similar to the present location of *Yr36* in a population derived from the cross of 'Fukuho-Komugi' and 'Oligoculm'. This QTL was 1 of 11 found in the study for stripe rust severity and was not assigned a formal name. Line et al. (1996) identified a QTL for HTAP resistance in cultivar 'Stephens' on the same chromosome arm. This QTL region has been recently narrowed to a 2.2-cM interval flanked by microsatellite markers *Xbarc101* and *Xgwm88* (D. Santra and K. Kidwell, personal communication). This segment partially overlaps with the current 2-cM region including *Yr36*, suggesting the possibility that the Stephens HTAP QTL might be allelic to *Yr36*. Alternatively, *Yr36* and the Stephens QTL might represent two closely linked loci since disease resistance genes tend to occur in clusters (Michelmore and Meyers 1998). These results taken together suggest that one or more APR genes are located on chromosome arm 6BS.

In order to precisely map this quantitative trait, we used isogenic RSLs to decrease genetic variation and controlled greenhouse conditions to reduce environmental variance. This resulted in clear and non-overlapping phenotypes between susceptible and resistant lines which allowed us to map *Yr36* as a single Mendelian locus. The precise mapping of *Yr36* presented here represents a first step in our positional cloning effort for this gene. This objective will be facilitated by the existence of a BAC library from RSL65 (Cenci et al. 2003) that includes the *Yr36* allele from DIC.

Effect of *Yr36* is temperature dependent

The ability of *Yr36* to confer resistance to stripe rust is temperature dependent, bestowing resistance only under a high-temperature regime while not affecting the plant's susceptibility at cooler temperatures. Several other APR genes for stripe rust have also been shown to function either more effectively at higher temperatures (Qayoum and Line 1985) or exclusively under high temperatures

Table 2 Genotypes of recombinant substitution lines (RSL) used for mapping

RSL	*Xucw75*	*Xucw73*	*Xucw79*	*Xucw71*	*Yr36*	*Xucw69 Xbarc101*	*Xucw77 Xbarc136*	*Xucw66*	*Xucw68*	*Xgwm88*	*Xwms193*	IT values	
14	L	L	L	L	L	L	L	L	L	L	L	6.67	S
LDN	L	L	L	L	L	L	L	L	L	L	L	6.80	S
135	D	L	L	L	L	L	L	L	L	L	L	6.00	S
158	L	D	D	D	D	D	D	D	D	D	D	2.18	R
121	L	L	L	D	D	D	D	D	D	D	D	1.83	R
110	D	D	D	D	L	L	L	L	L	L	L	6.75	S
117	D	D	D	D	L	L	L	L	L	L	L	6.42	S
291	L	-	L	L	D	D	D	-	-	D	D	2.00	R
209	-	L	L	L	D	D	D	-	-	D	D	2.17	R
50	D	D	D	D	D	D	L	L	L	L	L	2.33	R
54	D	D	D	D	D	D	L	L	L	L	L	2.30	R
115	D	D	D	D	D	D	L	L	L	L	L	2.25	R
118	D	D	D	D	D	D	L	L	L	L	L	2.17	R
128	D	D	D	D	D	D	L	L	L	L	L	2.22	R
130	D	D	D	D	D	D	L	L	L	L	L	2.92	R
151	D	D	D	D	D	D	L	L	L	L	L	2.75	R
144	L	L	L	L	L	L	D	D	D	D	D	6.75	S
149	L	L	L	L	L	L	D	D	D	D	D	7.30	S
160	L	L	L	L	L	L	D	D	D	D	D	6.00	S
123	D	D	D	D	D	D	D	L	L	L	L	3.00	R
108	L	L	L	L	L	L	L	D	D	D	D	7.75	S
120	L	L	L	L	L	L	-	L	D	D	D	5.75	S
65	D	D	D	D	D	D	D	D	D	D	D	2.00	R
68	D	D	D	D	D	D	D	D	D	D	D	2.00	R

White Cells with an 'L' indicate LDN allele, whereas gray cells with a 'D' indicate DIC allele. A change between gray and white cells indicates a recombination event. Last column indicates infection type (IT), and classification as resistant (R) or susceptible (S)

(Singh et al. 2000). This suggests that temperature plays a key role in determining the effect of these APR genes.

The high-temperature cycle under controlled environment mimics the natural field conditions of high diurnal temperatures followed by cool nights. Therefore, we expected to obtain similar results in the field to those observed in controlled conditions. We tested three susceptible RSLs (110, 117 and 135) and two resistant RSLs (121, 158) under field conditions and obtained similar results for all five lines to those observed under controlled high-temperature conditions (data not shown). This result confirms that our controlled greenhouse environment mimicked well the field conditions. The good correlation observed here between greenhouse and field phenotypes is important since there are contrasting reports about the reproducibility of field evaluation results under greenhouse conditions (Ramburan et al. 2004).

Effect of *Yr36* on resistance and yield under field conditions

We were able to validate the effect of *Yr36* on final infection severity and yield in five sets of isogenic lines (both tetraploid and hexaploid) under field conditions at two sites with high diurnal temperatures during the growing season. At Madera, *Yr36* consistently decreased stripe rust severity when introgressed into the susceptible lines Anza, UC1037, Yecora Rojo, and UC1113. Low levels of disease were also maintained when the gene was introgressed into the stripe rust resistant line UC1041. At Davis, the effect of *Yr36* on final infection levels was more difficult to observe due to the intense pathogen pressure present at this location in 2003. Yecora Rojo and UC1037 carrying *Yr36* had severity values similar to those of the original susceptible parents. These results suggest that the presence of *Yr36* alone would not be sufficient to completely control stripe rust under high pathogen pressure. In other genetic backgrounds, such as Anza and UC1113, the decrease in infection conferred by *Yr36* was significant under these same conditions. The lower levels of disease severity in Anza, UC1037, and UC1113 relative to Yecora Rojo could be attributed to the presence of other APR genes for stripe rust. Anza carries the *Lr34*/*Yr18* gene complex for leaf rust and stripe rust APR. Therefore, the interaction of two different APR genes (*Yr36* and *Yr18*) might have contributed to the larger reduction in stripe rust severity observed in this cultivar at Davis. This result supports the idea that the pyramiding of APR genes may be a good strategy to maintain low levels of infection under high disease pressure.

No yield penalty was detected with the incorporation of *Yr36* in hexaploid and tetraploid wheat in both locations. As expected, significantly higher resistance of lines carrying *Yr36* led to increases in yield at both locations. Even in the isogenic lines of Yecora Rojo and UC1037 that presented non-significant differences in stripe rust severity at Davis, significantly higher yields were observed in the isogenic lines carrying *Yr36*. This may be explained by the fact that only a single measurement of final disease severity was taken during the growing season. Thus, *Yr36* could have exerted a

resistance effect earlier in the season delaying the progression of the infection and allowing for an increase in yield, but later presented similar scores during the final severity measurements.

Use of *Yr36* in marker assisted selection (MAS)

Traditional breeding efforts to improve adult-plant disease resistance tend to be complicated due to the small effect of individual APR genes and the difficulty to detect them when major seedling resistant genes are present. With the precise mapping of *Yr36*, a MAS strategy can be used to accelerate the incorporation of this HTAP resistance gene into adapted germplasm. Selection for *Yr36* with flanking markers *Xucw71* and *Xbarc136* will generally result in the incorporation of the high grain protein allele of *Gpc-B1* because of the close linkage between *Xucw71* and *Gpc-B1* (0.3-cM). *Gpc-B1* is located within the region flanked by *Xucw79* and *Xucw71* (Distelfeld et al. 2004), whereas *Yr36* lies within the region encompassed by loci *Xucw71* and *Xbarc136*. By using PCR-based markers *Xucw79* and *Xbarc136*, the complete DIC region can be introgressed with both *Yr36* and *Gpc-B1*.

A drawback to this strategy is that both *Xucw79* and *Xucw71* are CAPS markers that require digestion after PCR amplification, increasing screening costs. Therefore, an alternative strategy would be to use microsatellite markers *Xgwm508* and *Xbarc136* for the selection of *Yr36* and *Gpc-B1*, thus reducing costs, but increasing the amount of introgressed foreign DNA.

If the objective is to reduce linkage drag, markers *Xucw79* and *Xbarc136* can be used to select for the DIC alleles, whereas external microsatellite markers *Xgwm508* and *Xgwm193* (Khan et al. 2000) can be used to select against the DIC allele.

In specific cases, such as soft wheats for cookies and pastries, high grain protein content negatively affects the quality characteristics of the products and therefore the introgression of *Yr36* needs to be complemented with selection against the *Gpc-B1* DIC allele. Recombinant substitution lines 209 and 291(Table 2) have the *Yr36* gene combined with the low grain protein content allele of *Gpc-B1*. Although these lines are available as donor parents of this particular allele combination, their tetraploid nature may complicate its introgression in hexaploid soft wheats. We are currently developing hexaploid recombinant lines that will have the same allelic combination.

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