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ORIGINAL PAPER

An introgression on wheat chromosome 4DL in RL6077 (Thatcher*6/PI 250413) confers adult plant resistance to stripe rust and leaf rust (*Lr67*)

Colin W. Hiebert · Julian B. Thomas · Brent D. McCallum · D. Gavin Humphreys · Ronald M. DePauw · Matthew J. Hayden · Rohit Mago · Wendelin Schnippenkoetter · Wolfgang Spielmeyer

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Abstract Adult plant resistance (APR) to leaf rust and stripe rust derived from the wheat (*Triticum aestivum* L.) line PI250413 was previously identified in RL6077 (=Thatcher*6/PI250413). The leaf rust resistance gene in RL6077 is phenotypically similar to *Lr34* which is located on chromosome 7D. It was previously hypothesized that the gene in RL6077 could be *Lr34* translocated to another chromosome. Hybrids between RL6077 and Thatcher and between RL6077 and 7DS and 7DL ditelocentric stocks were examined for first meiotic metaphase pairing. RL6077 formed chain quadrivalents and trivalents relative to Thatcher and Chinese Spring; however both 7D telocentrics paired only as heteromorphic bivalents and never with the multivalents. Thus, chromosome 7D is not involved in any translocation carried by RL6077. A genome-wide scan of SSR markers detected an introgression from chromosome 4D of PI250413 transferred to RL6077 through five cycles of backcrossing to Thatcher. Haplotype analysis of lines from crosses of Thatcher × RL6077 and RL6058 (Thatcher*6/PI58548) × RL6077 showed highly significant associations between introgressed markers (including SSR marker *cfd71*) and leaf rust resistance. In a separate RL6077-derived population, APR to stripe rust was also tightly linked with *cfd71* on chromosome 4DL. An allele survey of linked SSR markers *cfd71* and *cfd23* on a set of 247 wheat lines from diverse origins indicated that these markers can be used to select for the donor segment in most wheat backgrounds. Comparison of RL6077 with Thatcher in field trials showed no effect of the APR gene on important agronomic or quality traits. Since no other known Lr genes exist on chromosome 4DL, the APR gene in RL6077 has been assigned the name *Lr67*.

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C. W. Hiebert (✉) · J. B. Thomas · B. D. McCallum · D. G. Humphreys
Cereal Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Rd, Winnipeg, MB R3T 2M9, Canada
e-mail: colin.hiebert@agr.gc.ca

R. M. DePauw
Semiarid Prairie Agricultural Research Centre, Agriculture and Agri-Food Canada, P.O. Box 1030, Airport Rd, Swift Current, SK S9H 3X2, Canada

M. J. Hayden
Department of Primary Industries, Victorian AgriBioscience Centre, La Trobe Research and Development Park, Bundoora, VIC 3082, Australia

R. Mago · W. Schnippenkoetter · W. Spielmeyer
CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia

Introduction

Leaf and stripe rust, incited by *Puccinia triticina* Eriks. and *Puccinia striiformis* Westend. f. sp. *tritici,* respectively, are globally distributed diseases of wheat (*Triticum aestivum* L.). These diseases cause significant reductions in grain yield and quality but can be controlled by effective deployment of rust resistance genes (Samborski 1985). Most of the resistance genes that have been identified to date are expressed at the seedling stage and confer a race-specific resistance response that includes necrosis or chlorosis surrounding the site of infection (McIntosh et al. 1995). Two named leaf rust resistance (Lr) genes, *Lr34* and *Lr46*,

confer partial, race non-specific, adult-plant resistance (APR) (Dyck 1987; Singh et al. 1998). Both genes also confer partial resistance to stripe rust (*Yr18* and *Yr29* respectively) and to powdery mildew (*Pm38* and *Pm39*) (Spielmeyer et al. 2005; Lillemo et al. 2008). These genes are also associated with leaf tip necrosis of the flag leaf and are widely believed to confer a durable form of partial resistance (William et al. 2003; Rosewarne et al. 2006). *Lr34* was also associated with stem rust resistance in the Thatcher background (Dyck 1987).

A gene for APR to *P. triticina* was identified in the common wheat accession PI250413 and transferred into Thatcher to produce the backcross line RL6077 (Thatcher*6/PI250413) (Dyck and Samborski 1979). Although RL6077 exhibited a lower level of resistance to leaf rust than that conferred by *Lr34*, the RL6077 gene was phenotypically similar to *Lr34* as it was also associated with resistance to both stem rust (Dyck et al. 1994) and stripe rust (Singh 1992a). While the gene in RL6077 remained unidentified, the presence of quadrivalents in the meiosis of segregating material and its phenotypic similarity to *Lr34* led Dyck et al. (1994) to suggest that a reciprocal translocation of chromosome 7DS carrying *Lr34* had persisted through the five cycles of backcrossing involved in the derivation of RL6077 and placed *Lr34* in a different chromosome location in RL6077 compared with the reference line of *Lr34* (RL6058 = Thatcher*6/PI58548). More recently, molecular markers derived from the *Lr34* gene suggest that *Lr34* is not present in RL6077 (Lagudah et al. 2009). The purpose of this study was to determine if RL6077 carries a translocation involving chromosome 7D and if no suitable translocation were found to determine the chromosomal location of the APR gene in RL6077. Furthermore, RL6077 was compared in field trials to its recurrent parent, Thatcher, for agronomic and end-use quality parameters to assess the suitability of using the resistance in RL6077 in breeding applications. Based on the evidence presented here, the coordinator of the wheat genetic map has given the APR gene in RL6077 the gene designation *Lr67*.

Materials and methods

Cytology

F_1 progeny from crosses of Thatcher × RL6077 (RL6077 = Thatcher*6/PI250413), RL6077 × Chinese Spring ditelo 7DS, RL6077 × Canthatch ditelo 7DL, and Chinese Spring ditelo 7DS × Canthatch ditelo 7DL were examined for metaphase I pairing configurations to determine if chromosome 7D (the location of *Lr34*) of RL6077 was involved in a reciprocal translocation. Pre-emergent heads were collected and fixed in Carnoy's solution II (6:3:1 95% ethanol:chloroform:acetic acid) at −20°C for 24 h and stored in ethanol at −20°C until examined. Anther smears were stained with aceto-carmine and pollen mother cells (PMC) in metaphase I were analyzed. Only cells that could be completely analyzed were scored.

Populations and leaf rust testing

A population of 135 F_2 plants from the cross of Thatcher × RL6077 was raised previously (Dyck et al. 1994). A preliminary field trial in 2005 identified 16 F_3 families that appeared to be homozygous for either resistance or susceptibility to leaf rust. Three plants from each of the selected F_3 families were grown in a growth cabinet (18°C/16°C 16/8 h light/dark). Fully emerged flag leaves of F_3 plants were inoculated with urediniospores of *Puccinia triticina* virulence phenotype 12-3 MBDS following the methods of McCallum and Seto-Goh (2003). Twelve days after inoculation, plants were rated for leaf rust infection types (McIntosh et al. 1995). The plant that was the most resistant or the most susceptible in each family was selected as the seed source for field testing. Two replicates of F_4 lines from selected F_3 plants were seeded in 0.6 m rows at Portage la Prairie, Manitoba, Canada in 2006 and 2007. Spreader rows susceptible to leaf rust were inoculated with a representative mixture of those virulence phenotypes found in Canada during the previous year. Spores were suspended in light mineral oil (Bayol 55, Imperial Oil Canada, Toronto, ON, Canada) and sprayed on the leaves at early tillering. Plots were rated for leaf rust severity and pustule type approximately at the onset of anthesis when the susceptible checks showed good infection and symptoms using the modified Cobb scale (Peterson et al. 1948). Rows were classified as homozygous resistant, homozygous susceptible or segregating. Seed of these 16 F_4 lines were sent to CSIRO, Australia for stripe rust testing (see below).

A second population of F_4 plants from the cross RL6077 × RL6058 (RL6058 = Thatcher with *Lr34* = Thatcher*6/PI58548) was raised previously by the late Dr. P.L. Dyck. F_3 families were grown in the field. Single individuals susceptible to leaf rust were selected from families segregating for resistance while those families fixed for resistance were harvested in bulk (Dr. P.L. Dyck). Fifty-eight F_4 families were retested in the field in 2007 in the same nursery as the Thatcher × RL6077 cross. Field inoculations and disease rating were performed as described earlier.

In Australia, a third population was developed from line H1777, which was one of the 16 F_4 lines from the Thatcher × RL6077 population described earlier. Line H1777 segregated for leaf rust when screened in Canada. A single plant was grown in Australia to produce sufficient

seed for stripe rust evaluation and population development. Once it was confirmed that progeny from the single H1777-derived plant were still segregating for stripe rust resistance, the remaining seed was used to raise 67 lines (equivalent to F_2) which were progeny tested in the field. Two replicate rows were sown together with parental lines at Cobbitty near Sydney, Australia, in 2009. *P. striiformis* f. sp. *tritici* pathotype 134 E16A+ was released as the predominant pathotype. Stripe rust infections on Thatcher (80S) were clearly higher than on RL6077 (5–30 MR). F_3-equivalent lines were rated according to the disease severity and response of the parental lines and were scored as homozygous resistant, homozygous susceptible or segregating.

From the remaining 15 F_4 lines derived from the Thatcher × RL6077 population (see above), 12 lines were advanced to F_5 and evaluated for stripe rust resistance in the field at Cobbitty in 2008 and 2009. Field inoculations and disease scoring were done as described earlier.

DNA markers

Leaf tissue was collected from seedlings of Thatcher, RL6077, PI250413, RL6058 and progeny lines from the Thatcher × RL6077, RL6058 × RL6077, and H1777-derived population. DNA was extracted using a modified ammonium acetate extraction (Chao and Somers, http://maswheat.ucdavis.edu/PDF/DNA0003.pdf, accessed July 2009) based on the procedures of Pallotta et al. (2003). Two independent microsatellite (simple sequence repeat—SSR) marker scans were performed in Canada and Australia. In Canada, a set of 336 SSR markers were screened (Röder et al. 1998; Somers et al. 2004; Song et al. 2005) representing 372 loci and previously selected based on genome coverage and simplicity of marker profile to facilitate association mapping experiments (D.J. Somers, unpublished data). This set of markers was used to compare Thatcher and RL6077. Markers polymorphic between Thatcher and RL6077 were tested on homozygous F_4 lines (13 resistant and 35 susceptible) from the Thatcher × RL6077 and RL6058 × RL6077 populations. The SSR marker csLVMS1 (Spielmeyer et al. 2008) was used to select resistant families in the RL6058 × RL6077 population that did not carry *Lr34*. PCR and fragment analysis (with an ABI 3100 genetic analyzer, Applied Biosystems, Streetsville, Ontario, Canada) were performed as described by Somers et al. (2004).

In Australia, a genome-wide scan based on 488 SSR markers was used to screen Thatcher, RL6077 and two homozygous resistant and one homozygous susceptible lines from the Thatcher × RL6077 population to identify markers associated with resistance. To confirm the location of the resistance gene, a chromosome-specific SSR scan was performed using bulked segregant analysis (Michelmore et al. 1991). The resistant and susceptible bulks contained equal amounts of pooled DNA from five homozygous resistant and five homozygous susceptible lines, respectively. SSR amplification was performed using multiplex-ready PCR (Hayden et al. 2008). SSR fragment analysis was performed on an ABI3730xl (Applied Biosystems), as described by Hayden et al. (2008).

Table 1 Plot dimensions and harvested area for yield tests at Glenlea, Morden, Brandon, Manitoba and Swift Current, Saskatchewan (2002–2004)

Location	No. of rows	Row length (m)	Row spacing (m)	Harvested area (m^2)
Glenlea	5	4.27	0.15	3.2
Morden (2002, 2003)	5	4	0.18	3.6
Morden (2004)	4	4	0.23	3.68
Brandon	4	4	0.23	3.68
Swift Current	4	3	0.23	2.76

Field testing for leaf rust resistance, agronomic and quality performance

RL6077 was compared with Thatcher and RL6106 (Thatcher*6/Terenzio = Thatcher + *Lr34*) to determine the effect of *Lr67* on leaf rust resistance, agronomics, and end use quality. Field tests were conducted in Canada at Glenlea, Morden and Brandon, Manitoba and Swift Current Saskatchewan in 2002, 2003, and 2004. Yield tests consisted of 36 entries including Thatcher and RL6077 using a 6 × 6 lattice design with two replicates. Yield plot dimensions and harvested areas are given in Table 1. Plots in Glenlea were inoculated with *P. triticina* as described earlier, while plots in Morden and Brandon were infected with natural inoculum. Plants were rated for leaf rust in Glenlea, Morden, and Brandon as described earlier. Agronomic traits were determined as outlined in McCartney et al. (2005) at all four locations. Wheat protein and particle size index were determined using a Dickey-John Instalab 600 instrument (Dickey-John Corporation, Cornwall, Ontario, Canada) that was calibrated using AACC method 46-30.01 and 55.30.01 (AACC 2000), respectively. Wheat sedimentation volume was determined as per AACC method 56.60.02.

Results

Cytology

The F_1 from ditelo 7DS × ditelo 7DL had two telocentric univalents in all PMC analyzed (Table 2). This confirms that both telosomes in use were correctly identified since this pairing configuration ($20^{II} + t + t$) is diagnostic for

Table 2 Mean frequencies of chromosome pairing configurations in pollen mother cells (PMC) of F_1 plants from crosses involving RL6077 and chromosome 7D ditelosomics

Cross	No. of PMC	PMC with two multivalets	Mean frequency of pairing configurations						
			IV	III	Ring II	Rod II	It	I	t
RL6077/Thatcher	14	2	0.57	0.07	17.00	2.50	–	0.50	–
Ditelo 7DS/Ditelo 7DL	10	0	–	–	16.90	2.90	–	0.40	2.00
RL6077/Ditelo 7DS	61	3	0.72	0.03	15.56	2.87	0.98	0.18	0.02
RL6077/Ditelo 7DL	51	3	0.67	0.06	15.61	2.78	1.00	0.37	–

IV quadrivalent, *III* trivalent, *Ring II* a ring (closed) bivalent, *Rod II* rod (open) bivalent, *It* a heteromorphic bivalent involving a standard chromosome paired with a telosome, *I* univalent, *t* univalent that is a telosome

telosomes carrying opposite arms of the same chromosome. As reported by Dyck et al. (1994), quadrivalents or trivalents were frequently (≈70%) recorded in PMC of all hybrids involving RL6077 (Table 2). In hybrids between RL6077 and the two 7D ditelocentric lines, both telocentric arms were observed to pair at very high frequency (98–100%) exclusively as heteromorphic bivalents. Some of the multivalents were closed (present as rings); none of the multivalents showed tri-radial pairing or were ever observed to pair with telo 7DS ($n = 44$ among 61 PMC) or telo 7DL ($n = 34$ among 51 PMC). As reported by Dyck et al. (1994) a second multivalent was noted in about 3% of cells (Table 2). Not one quadrivalent was noted in the small ($n = 10$) sample of PMC recorded for the hybrid between Chinese Spring ditelo 7DS × Canthatch ditelo 7DL. This result was unexpected given prior reports of a translocation in Thatcher relative to Chinese Spring (Sears 1953) and Canthatch relative to Chinese Spring (Kerber and Aung 1999). However, these latter studies provided no details of pairing rates or sample size.

Populations and rust testing

Thirteen of the 16 F_4 lines from selected F_3 plants in the Thatcher × RL6077 population were homozygous (8 resistant, 5 susceptible). These 13 lines showed quantitatively different levels of leaf rust resistance in 2006 compared with 2007, although trends were consistent across years. Mean severity among resistant lines in 2006 was 48.1% and among susceptible lines was 80%. In 2007, the mean severity of resistant lines was 5% while the mean severity of susceptible lines was 74%. The improved expression of resistance conferred by *Lr67* in 2007 allowed a clear identification of segregating lines compared with homozygous resistant and homozygous susceptible lines in replicated field trials. The F_4 lines from the RL6058 × RL6077 population selected for DNA analysis were also tested in Portage la Prairie, Manitoba, Canada, in 2007. The difference between lines carrying *Lr67* compared with susceptible lines was clear. Thirty-five of the 58 F_4 lines were homozygous (5 resistant, 30 susceptible). The mean severity of resistant lines carrying only *Lr67* was 3% and the susceptible lines had a mean severity of 72%.

Twelve out of 13 homozygous lines from the Thatcher × RL6077 population were evaluated for stripe rust resistance in Australia. Leaf rust resistant lines were resistant to stripe rust while leaf rust susceptible lines were also susceptible to stripe rust. Disease severity ranged from 5 to 30% in resistant lines and 80% in susceptible lines. Another segregating population was developed from line H1777, progeny derived from Thatcher × RL6077 which segregated for leaf rust resistance in Canada and for stripe rust resistance under Australian field conditions. Selfed seed from one heterozygous H1777-derived plant was used to generate 67 lines that were progeny tested for stripe rust resistance in the field. The frequency distribution of homozygous resistant (5–30MR), homozygous susceptible (80 MS), and segregating lines was consistent with the expected ratio for single gene segregation (observed 20 h:27Het:20HS; expected 1:2:1 $\chi^2 = 2.5$, $df = 2$, $p > 0.05$).

Chromosomal location and linked SSR markers

Among 372 genome-wide loci tested by 336 SSR primer pairs, polymorphisms between RL6077 and Thatcher were detected on chromosomes 4D (cfd71), 2A (wmc522), 5D (cfd10) and 6B (gwm193). Markers on chromosomes 2A, 5D, and 6B were not associated with *Lr67* ($p > 0.15$); however, the 4D-specific fragment of cfd71 was significantly associated with *Lr67* ($p < 0.001$). Further testing of adjacent markers reported on 4D showed the presence of an introgression containing five polymorphic SSR loci (*cfd71*, *barc98*, *cfd23*, *wmc457,* and *wmc48*). These markers were examined on eight homozygous resistant and five homozygous susceptible F_4 lines from the Thatcher × RL6077 population (Table 3). Four out of the five markers showed a perfect association with *Lr67,* while one marker (*wmc48*) recombined (Table 3). The association between non-recombinant parental markers on chromosome 4D and the

Table 3 Haplotypes of SSR markers on chromosome 4D for parental lines and progeny lines from segregating populations

Line/Cross	No. of lines	*Lr67*	*Yr4D*	4DL deletion bins				C[b]	4DS deletion bin		
				0.41–0.38[a]	0.38–0.31	0.31–0.09	0.09-C		C-0.53		
				cfd71	*barc98*	*cfd23*	*wmc457*		*wmc48*	*barc288*	*wmc574*
RL6077	–	+	+	A	A	A	A		A	A	A
RL6058	–	–	–	B	B	B	B		B	B	B
Thatcher	–	–	–	B	B	B	B		B	B	B
PI250413	–	+	ns	A	A	A	A		A	A	A
H1777 derived F3s	18	n/s	+	A	B	B	B		B	B	B
	20	n/s	–	B	B	B	B		B	B	B
	27	n/s	+/–	H	B	B	B		B	B	B
	2	n/s	+	H	B	B	B		B	B	B
Thatcher × RL6077	7 (6)[c]	+	+	A	A	A	A		A	–	–
	1	+	+	A	A	A	A		B	–	–
	5	–	–	B	B	B	B		B	–	–
RL6058 × RL6077[d]	4	+	n/s	A	A	A	A		A	–	–
	1	+	n/s	B	B	B	B		B	–	–
	26	–	n/s	B	B	B	B		B	–	–
	3	–	n/s	H	H	H	H		H	–	–
	1	–	n/s	A	A	A	A		A	–	–

Markers are reported in order from left to right according to physical map and published consensus maps. A = homozygous for the RL6077 allele, B = homozygous for the Thatcher allele and H = heterozygous. barc markers are from Song et al. (2005); wmc and cfd markers are from Somers et al. (2004)

ns not scored

[a] Values represent fraction length measurements (FLM) of chromosome arms

[b] C = centromere

[c] Seven lines were screened for leaf rust; only six lines were evaluated for stripe rust resistance

[d] Resistant families reported from this cross were screened with marker csLVMS1 to select resistant families that did not carry *Lr34*

resistance phenotype (*Lr67*) was significant ($\chi^2 = 13.0$, $df = 1$, $n = 13$, $p < 0.001$). Further, among the 35 homozygous lines from the cross of RL6058 × RL6077, five resistant lines (all lacked *Lr34*) and 30 susceptible lines showed a significant association between the *Lr67* phenotype and the same introgressed block of five SSR markers on 4D ($\chi^2 = 17.86$, $df = 1$, $n = 35$, $p < 0.001$). Five individuals were recombinant between the *Lr67* and haplotypes of 4D markers near the centromere placing the gene distal to this linkage block (Table 3).

A separate genome-wide SSR marker screen was carried out in Australia on DNA bulks of five homozygous resistant and five homozygous susceptible lines together with near-isogenic parents RL6077 and Thatcher. In addition to the 4D markers identified earlier, *barc288* and *wmc574* were polymorphic between near-isogenic lines and were associated with rust resistance as the RL6077 alleles were only detected in the resistant bulk while the Thatcher alleles were only present in the susceptible bulk. To map chromosome 4D markers in the H1777 population, markers were first assayed on five homozygous resistant and five homozygous susceptible lines. With the exception of marker *cfd71*, other 4D markers were fixed for Thatcher alleles and failed to segregate in the population suggesting that previous recombination event(s) have reduced the donor segment in line H1777 carrying leaf and stripe rust resistance. Marker *cfd71* recombined in two out of 67 lines and was located ~1.5 cM from the stripe rust resistance gene on chromosome 4D (Table 3).

Physical mapping of 4D markers linked to rust resistance

Previously published data suggested that 4D markers identified in this study were located on both sides of the centromere. We used aneuploid lines developed in the Chinese Spring background to physically map markers to chromosome arms and deletion bins. On the basis of PCR assays of the 4DS and 4DL ditelosomic lines, markers *wmc48*, *wmc574,* and *barc288* were placed on the short arm and markers *wmc457*, *cfd71*, *cfd23,* and *barc98* on the long arm of chromosome 4D (Table 3). Lines containing deletions of different fraction length measurements (FLM) from the

telomeric end of chromosome 4DS and 4DL were used to further refine physical map locations. The 4DS markers were located in the most proximal deletion bin on the short arm delineated by the breakpoint FLM C-0.53. Markers *wmc457* and *cfd23* were placed in the proximal deletion bin of the long arm delineated by the breakpoints FLM 0.09–0.31. Marker *barc98* was mapped to deletion bin FLM 0.31–0.38 and *cfd71* in the very small distal deletion bin FLM 0.38-0.41 (Table 3). From these results, we conclude that the size of the 4D introgression from PI250413 after five backcrosses into Thatcher includes part of the short arm and at least 40% of the long arm of chromosome 4D. Because there was no evidence for recombination between markers on the long arm in the Thatcher × RL6077 and RL6058 × RL6077 populations, *Lr67* is closest to *cfd71* and probably on the distal side on chromosome 4DL. This map location is consistent with results obtained for the stripe rust resistance gene which was tightly linked to marker *cfd71* in the H1777 population. Given that line H1777 also segregated for leaf rust resistance and carried a reduced donor segment, it is possible that both leaf and stripe rust resistance map to the same locus and are conferred by the same gene as it has been postulated for multiple resistance locus *Lr46/Yr29* (E.S. Lagudah, pers. comm.) and demonstrated for *Lr34/Yr18* (Krattinger et al. 2009).

Allele survey of linked markers in diverse wheat germplasm

To evaluate linked markers for use in marker-assisted breeding we compared the size of the RL6077 alleles amplified by markers *cfd71* and *cfd23* to allele sizes found within a diverse set of 247 international wheat germplasm including cultivars and breeding lines from Australia, North America, CIMMYT, Asia and Europe (Table S1). Marker *cfd71* amplified a 214-bp allele from chromosome 4DL in RL6077, the same size product was present in 22 out 247 lines (~9%) tested (Table S1). The marker *cfd23* amplified a 211-bp fragment from RL6077 and this allele was also present in 13 of lines tested (~5%) (Table S1). These 13 lines which contained the RL6077-like *cfd23* allele also carried the RL6077-like allele for marker *cfd71*, suggesting that this marker haplotype was retained in breeding programs across the world. While pedigree information will need to be analyzed to determine if these lines share a common ancestor, it appears possible that the RL6077 haplotype was introduced into Australian and Canadian germplasm through Sonora64. However, no conclusive genetic data are available to confirm if this represents the transmission of *Lr67*. While genetic linkage between *cfd23* and rust resistance was not measured in this study, it is likely that linkage of this marker is sufficient in at least some breeding applications given the reduced rate of recombination commonly associated with the proximal region of chromosomes.

Table 4 Leaf rust severity ratings for Thatcher, RL6077 and RL6106 from 2002 to 2004

Year	Location	Leaf rust severity (%)[b]		
		Thatcher	RL6077 (*Lr67*)	RL6106 (*Lr34*)
2002	Glenlea[a]	80	55	25
2002	Brandon	60	30	15
2002	Morden	90	40	5
2003	Glenlea[a]	77	32	10
2003	Morden[a]	80	30	20
2004	Glenlea	50	10	5
2004	Brandon	60	20	10
2004	Morden	50	15	3
Mean		67.4	27.4	12.0

[a] These sites included multiple replicates that were rated for leaf rust severity. The mean leaf rust severities are shown

[b] Modified Cobb scale (Peterson et al. 1948)

Field testing for leaf rust resistance, agronomic and quality performance

Over 4 years of field testing, both *Lr34* and *Lr67* conditioned improved leaf rust resistance compared with Thatcher (Table 4). *Lr34* (RL6106) conferred a higher level of resistance compared with *Lr67* (Table 4). The amount of leaf rust infection varied by both year and location as evident by differences in severity ratings on all three lines. RL6106 showed lower severity of leaf rust compared with RL6077 regardless of the relative level of infection (Table 4).

The average yield (measured across 3 years and four diversely located test sites) and other agronomic (height, maturity, lodging, bulk density and kernel weight) did not significantly differ between Thatcher and RL6077. Similarly, end-use quality traits also did not differ significantly for RL6077 and Thatcher (Table 5). This shows that the introgression carrying *Lr67* conferred no adverse effect on the economic performance in this case and may be predictive of its performance in future plant breeding.

Discussion

Frequent multivalent associations observed here and previously in hybrids of RL6077 confirm that this line contains a translocation relative to its recurrent parent, Thatcher, and other wheats (Dyck et al. 1994). Contrary to the suggestion of Dyck et al. (1994), the translocation breakpoint cannot be associated with chromosome 7D or *Lr34* because neither

Table 5 Comparison of agronomic and quality traits for Thatcher and a single-gene backcross line (RL6077) for the leaf rust resistance gene *Lr67*

Entry	Agronomic traits									Quality traits		
										Whole wheat	Particle size	SDS sedimentation
	Grain yield (g/m^2)				Height	Maturity	Lodging[a]	Bulk density	Kernel wt.	Protein	Index	Volume
	2002	2003	2004	Overall	(cm)	(days)	(1–9)	(g/l)	(mg)	(%)	(%)	(ml)
Thatcher	224	229	333	262	91	94.6	3.6	748.5	27.7	12.7	57.2	47.0
RL6077	255	210	319	261	91	94.2	3.3	750.2	28.6	13.4	58.0	45.6
LSD(0.05)	38	35	33	21	3	1.7	0.8	8.7	1.0	0.8	1.4	3.8
N	4	4	4	12	11	8	9	7	7	4	4	4

[a] Lodging scale: 1 = plants are upright to 9 = plants are completely lodged

of the two 7D telocentrics paired with any of the observed multivalents (Table 2). However, failure to re-establish the Thatcher karyotype in RL6077 through five backcrosses suggests that, if the translocation breakpoints derive from PI250413, they are plausibly linked to *Lr67*.

Markers on chromosome 4D were significantly associated with *Lr67* and stripe rust resistance (Table 3). Based on the physical marker order and the observed haplotypes, it was possible to assign resistance to both pathogens to the proximal region on the long arm in a relatively small physical interval and on the distal side of the closest marker *cfd71*. Since no other named Lr genes have been mapped to chromosome 4D, the APR gene in RL6077 was assigned the name *Lr67*. This confirms the assertion of Lagudah et al. (2009) that the resistance in RL6077 is not *Lr34*.

There are intriguing similarities between phenotypes conferred by *Lr34* and those postulated for *Lr46* and *Lr67* (Table 6). With *Lr34*, it was shown that a single gene encoding an ABC transporter-like protein was responsible for resistance to leaf rust, stripe rust, and powdery mildew (Krattinger et al. 2009). The same gene was associated with enhanced APR for stem rust by probably interacting with unlinked gene(s) in the Thatcher background (Vanegas et al. 2008; Spielmeyer et al. 2008). Thatcher lines carrying *Lr67* also had improved stem rust resistance (Dyck et al. 1994). *Lr46* is tightly linked to stripe rust resistance gene *Yr29* and powdery mildew resistance, but its effect on stem rust resistance in the Thatcher background remains undetermined. Leaf tip necrosis, which is associated with *Lr34* and *Lr46*, was also recorded in segregants carrying *Lr67* (Dyck and Samborski 1979) and in single gene lines carrying *Lr67* from the present study (P.L. Dyck unpublished; see Kolmer et al. 2008). Thus it appears there is a distinct class of APR genes in wheat characterized by leaf tip necrosis and conferring partial resistance to several of the major biotrophic pathogens (Lillemo et al. 2007). Widespread international interest in *Lr34* and similar genes is based on the inference that these genes confer durable, broad spectrum resistance

Table 6 Comparison of phenotypic properties of three genes for partial adult-plant resistance (APR) to leaf rust

Characteristic	Gene		
	Lr34[a]	*Lr46*[b]	*Lr67*[c]
Chromosome location	7DS	1BL	4DS
Leaf tip necrosis	+	+	+
Leaf rust resistance	+	+	+
Stripe rust resistance	+	+	+
Powdery mildew resistance	+	+	?
Association with stem rust resistance	+	?	+

The resistance in RL6077 may be conditioned by a single, pleiotropic gene (*Lr67*) or by several linked genes

[a] See Dyck (1987, 1993), Dyck et al. (1994), Singh (1992a, b), and Lillemo et al. (2008)

[b] See William et al. (2003), Lillemo et al. (2008), and Rosewarne et al. (2006)

[c] See present study; Dyck and Samborski (1979), Dyck et al. (1994), Singh (1992b), and Kolmer et al. (2008)

to a range of biotrophic pathogens, particularly when these genes are stacked. However, little is presently known about the pros and cons of these proposed gene stacks. With tightly linked or flanking markers it becomes possible to select a range of lines that carry known combinations of these partial APR genes and assess them as suitable sources for durable, broad-spectrum resistance in yield-based breeding.

For example, five lines from the cross of RL6058 × RL6077 were selected as having *Lr34* and *Lr67* based on field resistance (Dyck et al. 1994). We assessed four of these lines using markers and found that the classification based on phenotype was unreliable. Two lines were fixed for both genes, one was fixed for *Lr34* and segregated for *Lr67*, and one was fixed for *Lr67* and segregated for *Lr34* (data not shown). This latter line (90RN2447 from Dyck et al. 1994) showed the weakest field resistance to leaf rust of the four putative digenic lines characterized by

markers. One of the two confirmed two-gene lines (90RN2491 = Thatcher + *Lr34* + *Lr67*) plus an *Lr34* line (RL6058 = Thatcher + *Lr34*) were used by Agarwal and Saini (2009) to study the co-inheritance of leaf rust and stripe rust resistance in crosses with a susceptible parent (WL711). As expected, these crosses suggested that *Lr34* was present in both RL6058 and 90RN2491 and conferred resistance to both rusts. However, the additional resistance that was detected did not fit the profile expected for *Lr67* (i.e. additional resistance to both rusts was present in both crosses but showed no co-inheritance). Therefore, the cross with RL6058 unexpectedly showed segregation for two additional genes; this suggests that Thatcher may have contributed some unknown resistance not present in WL711 and not evident in North American conditions or with North American rust isolates and that *Lr67* was not detected. With the genotyping that is provided by markers it will be easier in future to design experiments that permit less contingent conclusions.

Combinations of *Lr34*, *Lr46,* and *Lr67* represent an attractive option to breeders for durable multi-pathogen resistance to leaf rust, stripe rust, stem rust, and powdery mildew. To generate these gene stacks, breeders require robust markers which are already available for *Lr34* and *Lr46* (Lagudah et al. 2009). Results from the allele survey of markers linked to *Lr67* suggest that SSR markers *cfd71* and *cfd23* will also be useful in most genetic backgrounds, although a more breeder friendly marker needs to be developed.

In conclusion, we report new, effective APR for leaf rust (*Lr67*) and stripe rust located in the centromeric region of chromosome 4DL. While *Lr67* phenotypically resembled *Lr34*, the degree of resistance conferred by *Lr67* was less than that conferred by *Lr34*. Furthermore, the translocation present in RL6077 did not involve chromosome 7D or *Lr34*. *Lr67* and any associated genetic material present in the introgression from PI250413 had no apparent deleterious effect on agronomic performance and quality traits; thus, *Lr67* is suitable for use in wheat breeding programs and is a valuable genetic resource.

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