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Microsatellite mapping of adult-plant leaf rust resistance gene *Lr22a* in wheat

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Abstract This study was conducted to identify microsatellite markers (SSR) linked to the adult-plant leaf rust resistance gene *Lr22a* and examine their cross-applicability for marker-assisted selection in different genetic backgrounds. *Lr22a* was previously introgressed from *Aegilops tauschii* Coss. to wheat (*Triticum aestivum* L.) and located to chromosome 2DS. Comparing SSR alleles from the donor of *Lr22a* to two backcross lines and their recurrent parents showed that between two and five SSR markers were co-introgressed with *Lr22a* and the size range of the *Ae. tauschii* introgression was 9–20 cM. An F_2 population from the cross of 98B34-T4B × 98B26-N1C01 confirmed linkage between the introgressed markers and *Lr22a* on chromosome 2DS. The closest marker, GWM296, was 2.9 cM from *Lr22a*. One hundred and eighteen cultivars and breeding lines of different geographical origins were tested with GWM296. In total 14 alleles were amplified, however, only those lines predicted or known to carry *Lr22a* had the unique *Ae. tauschii* allele at GWM296 with fragments of 121 and 131 bp. Thus, GWM296 is useful for selecting *Lr22a* in diverse genetic backgrounds. Genotypes carrying *Lr22a* showed strong resistance to leaf rust in the field from 2002 to 2006. *Lr22a* is an ideal candidate to be included in a stack of leaf rust resistance genes because of its strong adult-plant resistance, low frequency of commercial deployment, and the availability of a unique marker.

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Introduction

Leaf rust (*Puccinia triticina* Eriks.) of wheat (*Triticum aestivum* L.) is a widespread foliar disease that causes significant reductions in grain yield and quality (Samborski 1985). Host genetic resistance is a desirable and proven method of leaf rust control. However, deployment of single race-specific genes allows the pathogen to evolve and accumulate new virulence (Dyck and Kerber 1985). This creates the need to identify new effective genes and can lead an ongoing cycle of breeding, deployment and subsequent erosion of resistance.

Over 50 leaf rust resistance (Lr) genes have been identified in wheat. Of these, approximately half were introgressed from related species (McIntosh et al. 1995). Five named Lr genes have been introgressed into common wheat from *Aegilops tauschii* Coss.; these are *Lr21* (Rowland and Kerber 1974), *Lr32* (Kerber 1987), *Lr41* (Cox et al. 1994; Singh et al. 2004), *Lr42* (Cox et al. 1994), and the adult-plant resistance (APR) gene *Lr22a* (Rowland and Kerber 1974). It should be noted that while *Lr22a* is expressed only at the adult-plant stage, the degree of resistance conferred is comparable to highly resistant seedling Lr genes in contrast to the slow-rusting type APR conferred by genes like *Lr34*.

In the search for improved genetic solutions for leaf rust resistance it is widely believed that combinations or stacks of multiple Lr genes would confer a more durable resistance than the same genes deployed individually. Such gene stacks might include race non-specific adult plant resistance genes (e.g. *Lr34*), "undefeated" genes for which no virulence has been detected such as *Lr22a*, and partially "defeated" genes, for which virulence already exists in the pathogen population (e.g. *Lr16*). One advantage of creating gene stacks is the synergistic interaction of leaf rust resistance

genes which often confers a higher level of resistance than would be expected from the level of resistance demonstrated by the genes in isolation (Samborski and Dyck 1982). The resistance genes *Lr34* (German and Kolmer 1992) and *Lr13* (Kolmer 1992) were both demonstrated to enhance the level of resistance synergistically when in combination with other Lr genes. Complex stacks of Lr genes can be difficult to construct using phenotypic selection. For example the Canadian cultivar Pasqua carries five Lr genes including *Lr11*, *Lr13*, *Lr14b*, *Lr30* and *Lr34*, but failed to retain *Lr16* and *Lr22a* from the parental cross (Dyck 1993a). With suitable markers a stack of seven Lr genes could have been stabilized in this cross.

Close molecular markers for all Lr genes can help in the assembly and dissection of complex gene stacks in any cross. *Lr22a* has been physically mapped to chromosome 2DS using telocentric mapping (Rowland and Kerber 1974). In this paper we report both the genetic location and the identification of a closely linked, unique molecular marker allele for *Lr22a* that was retained from *Ae. tauschii*. The application of this marker in different genetic backgrounds was examined on a broad range of wheat germplasm.

Materials and methods

Plant material and populations

Lr22a was previously transferred to a synthetic hexaploid RL5404 by crossing tetra-Canthatch ($2n = 2x = 28$, AABB) with *Aegilops tauschii* var. *strangulata* RL5271 ($2n = 14$, DD; Dyck and Kerber 1970; Rowland and Kerber 1974). This was followed by six backcrosses with Thatcher to produce the line RL6044 (Thatcher*7//tetra-Canthatch/RL5271) which was then backcrossed with AC Domain to produce the leaf rust resistant line 98B34-T4B (AC Domain*6/RL6044) with *Lr22a*. RL5404 was also crossed with Neepawa (Neepawa*6/RL5404) to create the *Lr22a*-carrying near-isogenic line (NIL) RL4495.

An F_2 population that segregated for *Lr22a* was created by crossing 98B34-T4B with the leaf rust susceptible line 98B26-N1C01 (AC Domain*2/Sumai3//Grandin*2/AC Domain).

Thatcher NILs carrying single Lr genes (*Lr11*, *Lr13*, *Lr16*, *Lr22a* and *Lr34*), cultivars carrying *Lr22a* (AC Minto) and cultivars believed to carry *Lr22a* (5500HR and 5600HR) were used in field tests to evaluate leaf rust resistance.

Disease rating

Segregation of *Lr22a* was followed in the 98B34-T4B × 98B26-N1C01 F_2 population by inoculating flag leaves with *Puccinia triticina* isolate TJBJ-77-2 (Long and Kolmer 1989; McCallum and Seto-Goh 2003) following

the procedures of McCallum and Seto-Goh (2003). Plants were grown with 16 h supplemental lighting in a greenhouse at approximately 20°C. Twelve days after inoculation plants were classified as resistant (infection types; to 1), moderately resistant (infection types 1+ to 2) and susceptible (infection types 3–4) as described by Stakman et al. (1962). Parents of the F_2 population were tested for leaf rust resistance at the seedling and adult stage.

Thatcher NILs and cultivars carrying *Lr22a* were grown in field plots at Glenlea, MB, Canada from 2003 to 2006 and 2002 to 2006, respectively, to observe Lr gene effectiveness. Plants were infected with natural leaf rust inoculum and rows of susceptible wheat throughout the nurseries were artificially inoculated with a mixture of virulence phenotypes found in western Canada during the previous year. Adult-plants were rated for leaf rust based on a modified Cobb scale for incidence (Peterson et al. 1948) and severity was scored as R = resistant (flecks and small uredinia with necrosis), MR = moderately resistant (large necrotic flecks and large uredinia), M = moderate (mixture of uredinia sizes), MS = moderately susceptible (moderate to large uredinia with chlorosis), and S = susceptible (large uredinia without chlorosis or necrosis).

Molecular mapping

DNA was extracted from lyophilized leaf tissue using a modified CTAB extraction (Kleinhoffs et al. 1993). PCR was performed with a cycle of 2 min 94°C, then 1 min 95°C, 1 min 50–60°C, and 50 s 73°C for 30 cycles, followed by 5 min 73°C, using the following conditions: PCR buffer 1×, dNTPs 0.2 mM each, MgCl$_2$ 1.5 mM, primers 10 pmol each, *Taq* DNA polymerase 1 U, and approximately 50 ng genomic DNA. PCR products were separated on 5% denaturing polyacrylamide gels in TBE buffer (89 mM tris, 89 mM boric acid, 20 mM EDTA) at 85 W for 2 h, and stained with silver (Promega, Madison, WI, USA). *Lr22a* has been previously located on chromosome 2DS (Rowland and Kerber 1974). Microsatellite marker (SSR) alleles located on chromosome 2DS were compared between RL5271, RL5404, RL6044, Thatcher, 98B34-T4B, and AC Domain. Polymorphic markers were tested for linkage to *Lr22a* using 68 of the susceptible individuals from the F_2 population of 98B34-T4B × 98B26-N1C01. Susceptible F_2 plants were used for mapping because their genotypes at the *Lr22a* locus were known. These susceptible individuals were selected for pustule uniformity to avoid assigning homozygous susceptible genotypes at the *Lr22* locus to heterozygous individuals that would falsely appear to be recombinants. The location of linked SSR alleles was confirmed using nulli/tetra and ditelocentric cytogenetic stocks (Sears 1966; Sears and Sears 1978).

SSR markers on chromosome 2DS were compared between RL5271, RL5404, RL6044, Thatcher, 98B34-T4B,

AC Domain, RL4495, Neepawa, BW63, AC Minto, 5500HR, and 5600HR to determine which markers were co-introgressed with *Lr22a* from *Ae. tauschii* into Canadian wheat.

New SSR markers were added to the Agriculture and Agri-Food Canada Cereal Research Centre (AAFC-CRC) International Triticeae Mapping Initiative (ITMI) genetic map (Opata85/Synthetic W-7984; Somers et al. unpublished, ITMI-CRC). A newly developed Opata85/Synthetic doubled haploid (DH) population was tested to confirm the marker order on 2DS found in the ITMI-CRC population. The ITMI-CRC map was used to align the *Lr22a* linkage group with the genetic map of 2DS. Genetic distances were calculated using the Kosambi mapping function (Kosambi 1944). All maps were constructed using MapMaker version 3.0 (Lander et al. 1987).

To asses the potential of GMW296, the closest marker to *Lr22a*, as a selection tool in varying genetic backgrounds 118 different cultivars and breeding lines were surveyed (Table 2). Alleles from these genotypes were compared to the allele amplified from RL5271, the *Ae. tauschii* donor of *Lr22a*.

Results

Fourteen polymorphic microsatellite markers on chromosome 2DS were evaluated in Thatcher, AC Domain, the *Aegilops tauschii* donor of *Lr22a* (RL5271), the original synthetic (RL5404 = Tetra-Canthatch/RL5271) and two backcross lines (RL6044 = Thatcher*7/RL5404 and 98B34-T4B = AC Domain*6/RL6044). Microsatellite alleles from GWM296 and GWM455 were introgressed from RL5271 via RL5404 to these two resistant backcross lines (Table 1; Fig. 1). Thus GWM296 and GWM455 alleles from RL5271 have remained associated with *Lr22a* through a minimum of 13 cycles of recombination in common wheat.

The same 14 SSR markers were used to assess the introgression of *Lr22a* into RL4495 (Neepawa*6/RL5404) and its derivatives which included BW63, AC Minto, 5500HR and 5600HR. RL4495 retained *Ae. tauschii* alleles from GWM455, GWM296, GWM261, WMC25, and WMC503, and transmitted them to its derivatives (Table 1). Two other introgression sizes were detected in RL4495, one with GWM455, GWM296, and GWM261, and one with GWM455 and GWM296. Since these introgressions are smaller than the one found in BW63 they cannot be in the line of descent to AC Minto, 5500HR and 5600HR as these cultivars have retained the largest introgression found in RL4495.

In the F_2 of 98B34-T4B × 98B26-N1C01, 88 plants were resistant, 216 plants had a moderately resistant infection type and 102 plants were susceptible. These numbers

Table 1 SSR alleles on chromosome 2DS introgressed along with *Lr22a* from *Ae. tauschii* into common wheat

Locus[a]	Alleles on 2DS[b]									
	RL5271[c]	RL5404[c]	RL4495[c]	BW63[c]	AC Minto	RL6044[c]	98B34-T4B[c]	Neepawa	Thatcher	AC Domain
BARC124	T	T	A	A	A	A	A	A	A	A
WMC111	T	T	A	A	A	A	A	A	A	A
GWM210	T	T	A	A	A	A	A	A	A	A
GWM455	T	T	T	T	T	T	T	A	A	A
GWM296	T							A	A	A
Lr22a	T	T	T	T	T	T	T	A	A	A
GWM261	T	T	T	T	T	A	A	A	A	A
WMC025	T	T	T	T	T	A	A	A	A	A
WMC503	T	T	T	T	T	A	A	A	A	A
WMC112	T	T	A	A	A	A	A	A	A	A
WMC470	T	T	A	A	A	A	A	A	A	A
GWM484	T	T	A	A	A	A	A	A	A	A
WMC453	T	T	A_1	A_1	A_2	A_1	A_2	A_1	A_1	A_2
GWM102	T	T	A_2	A_2	A_1	A_2	A_2	A_2	A_2	A_2
GWM515	T	T	A	A	A	A	A	A	A	A

[a] GWM markers are from Röder et al. (1998); WMC markers are from Somers et al. (2004); BARC markers are from Song et al. (2005)

[b] T = *Ae. tauschii* allele; A = *T. aestivum* allele; subscript numbers differentiate between alleles within the same species

[c] RL5271 = *Ae. tauschii* donor of *Lr22a*; RL5404 = synthetic hexaploid from tetra-Canthatch/RL5271; RL4495 = Neepawa*6/RL5404; BW63 is derived from RL4495 and is the donor of *Lr22a* in all currently registered Canadian wheat cultivars; RL6044 = Thatcher*6/RL5404; 98B34-T4B = AC Domain*6/RL6044. Two other introgressions were detected in RL4495 but are not shown because they lack the full introgression transferred to AC Minto via BW63
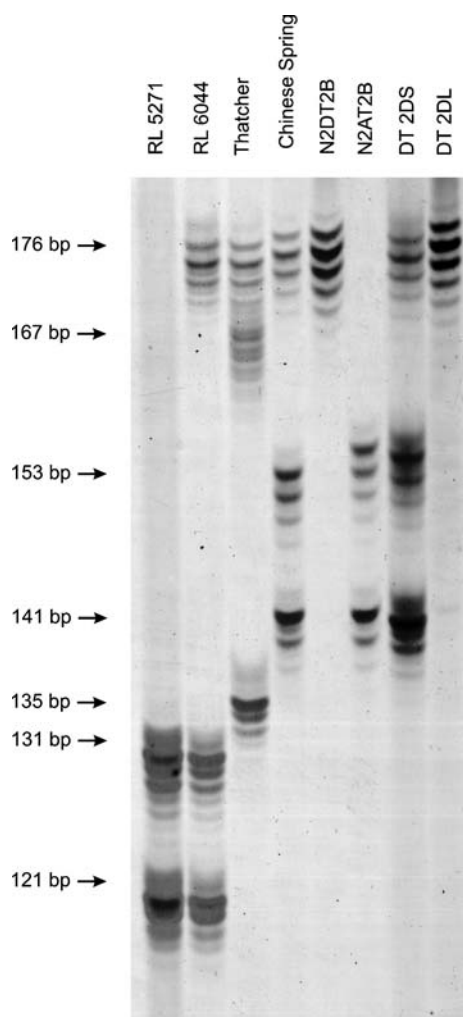
Fig. 1 Alleles of GWM296 found in RL5271 (*Lr22a* donor), RL6044 (Thatcher*6/RL5271; carries *Lr22a*), Thatcher (recurrent wheat parent), Chinese Spring, nulli-2D/tetra-2B, nulli-2A/tetra-2B, ditelo 2DS, and ditelo 2DL on a silver-stained polyacrylamide gel. The allele associated with *Lr22a* has DNA fragments that are 131 and 121 bp in size. A common chromosome 2DS allele in the North American wheat lines tested has the 167 and 135 bp fragments. The 176 bp fragment is from the chromosome 2A locus

fit both a 3:1 ratio (resistant plus moderate resistant: susceptible; $\chi^2_{3:1} = 0.02$, $P = 0.87$), and a 1:2:1 ratio (resistant: moderate resistant: susceptible; $\chi^2_{1:2:1} = 2.64$, $P = 0.27$). As adult plants 98B34-T4B was resistant to *P. triticina* and 98B26-N1C01 was susceptible, while as seedlings both lines were susceptible. This confirms that the F_2 population segregated for the APR conferred by *Lr22a*. The *Ae. tauschii* alleles of GWM296 and GWM455 (both present in 98B34-T4B) were both polymorphic with respect to 98B26-N1C01. Based on 68 susceptible F_2 plants representing 136 gametes, GWM296 was 2.9 cM and GWM455 was 4.4 cM from the *Lr22a* locus (Fig. 2). Testing with nulli-2D/tetra-2B, nulli-2A/tetra-2B, ditelo 2DS, and ditelo 2DL confirmed that the locus of GWM296 shown to be

linked to the APR of *Lr22a* was on chromosome 2DS (Fig. 1).

A minor conflict exists between our proposed order for markers close to *Lr22a* and those published elsewhere (Raupp et al. 2001). The marker order was clarified by re-genotyping lines recombinant in the region and adding additional markers to the ITMI-CRC map. An alternate DH population from the ITMI cross was also used to make a comparative map. The DH map agreed with the ITMI-CRC map (data not shown). Based on these results, we concluded that the marker order on 2DS is as shown (Table 1; Fig. 2) with the GWM296 locus 2.9 cM distal to *Lr22a*.

One hundred and five North American wheat cultivars and breeding lines, four Asian lines, eight European cultivars and one South American wheat cultivar were surveyed for alleles of GWM296 (Table 2). In total 14 alleles were detected, with the most common being that found in Thatcher (Fig. 1). Most Canadian hard-red spring wheat cultivars are derived from Thatcher (B. McCallum and R. DePauw unpublished). In this collection of wheat, the allele from *Ae. tauschii* RL5271 was present in those lines and cultivars that were expected to carry *Lr22a* and was absent from all others (Table 2). BW63 was heterogeneous for GWM296 with half the individuals tested carrying the RL5271 allele. Two products were amplified from RL5271 that were 131 and 121 bp in size (Fig. 1). The size range of all other chromosome 2DS alleles found were between 167 and 135 bp.

Cultivars expected to carry *Lr22a*, including AC Minto, 5500HR, and 5600HR, showed strong resistance to *P. triticina* in the field from 2002 to 2006 (Table 3). Furthermore, no isolates of *P. triticina* that are virulent to *Lr22a* have been found in Canadian virulence surveys between 2002 and 2005 (McCallum and Seto-Goh 2005; McCallum and Seto-Goh unpublished data; Table 4). The Thatcher NILs tested in the field showed that *Lr22a* provided good resistance singly, while *Lr34* showed moderate resistance, *Lr16* provided moderate to poor resistance, and *Lr11* and *Lr13* showed no improved resistance compared to Thatcher (Table 3).

Discussion

Wheat SSR maps reveal that coverage by polymorphic markers is non-uniform (Somers et al. 2004). Of the three genomes of common wheat, the D genome was added most recently and has the shortest evolutionary life span at the hexaploid level. Therefore, the degree of polymorphism is lowest for the D genome chromosomes. By contrast, *Ae. tauschii* is an older, polymorphic, broadly distributed diploid that has contributed the D genome to many polyploids in the Triticeae. These differences in polymorphism were

Fig. 2 Alignment of the *Lr22a* and ITMI-CRC maps with two previously published maps of chromosome 2DS. The map of 98B34-T4B/98B26-N1C01 was based on 68 F₂ plants while the ITMI-CRC map was derived from 68 recombinant inbred lines from the cross of Opata85/W-7984 (W-7984 = *Ae. tauscii*/Altar84 durum; this cross is commonly called Opata/Synthetic). The map of Raupp et al. (2001) reports the mapping of *Lr39*, however, Singh et al. (2004) found that *Lr39* = *Lr41*
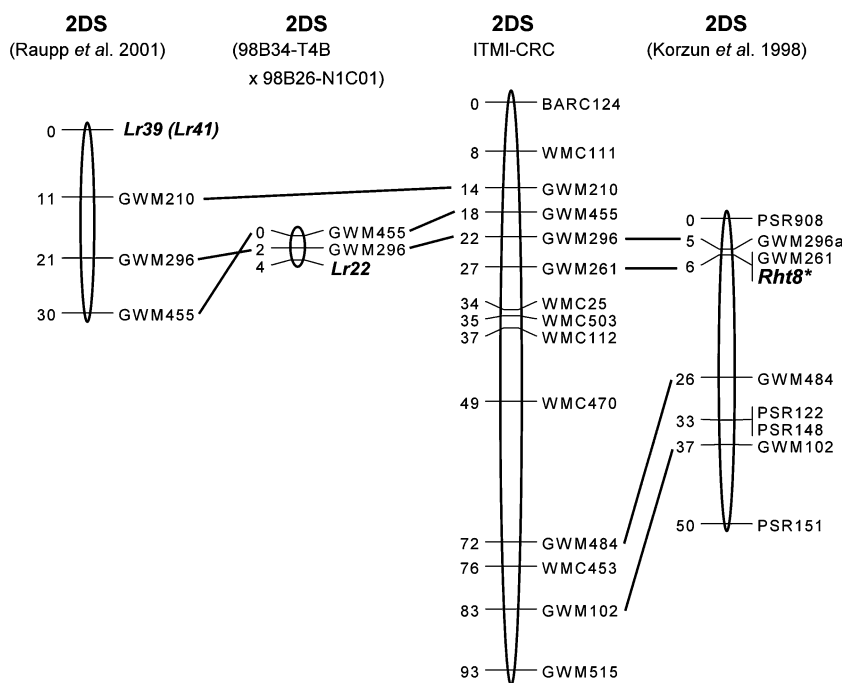
evident in this study as all 14 SSR markers tested on chromosome 2DS were polymorphic between the *Ae. tauschii* accession RL5271 and the two recurrent parents, Thatcher and AC Domain. Thatcher and AC Domain were monomorphic for 13 of the 14 SSR markers (Table 1).

In this study SSR markers previously mapped to 2DS were compared between backcross lines and the recurrent parents in order to identify candidate markers that might be linked to *Lr22a*. Out of the 14 loci investigated, two polymorphic markers were transferred through thirteen rounds of backcrossing and both were then shown to be closely linked to the target gene. This screening strategy was successful because of the high marker density and high degree of polymorphism between chromosome 2DS of *Ae. tauschii* and the corresponding chromosome of common wheat.

Based on recombination in the F₂ population, the minimum size of the segment introgressed into RL6044 and 98B34-T4B was 4.4 cM and the probable size was 9 cM (assuming the crossovers occurred at the midpoint of the flanking intervals; Fig. 2). In contrast, the *Lr22a* introgression from RL5271, via RL5405, into RL4495 included up to five SSR markers. The largest introgression represents a minimum of 17 cM and a probable size of 20 cM as estimated from the ITMI-CRC map (Table 1; Fig. 2). This larger introgression has persisted from RL4495 into three Canadian cultivars, AC Minto, 5500HR, and 5600HR.

Three-point linkage values observed in the F₂ population (98B34-T4B/98B26-N1C01) confirmed that *Lr22a*, GWM296 and GWM455 are closely linked (Fig. 2). In a study of durable leaf rust resistance in Swiss winter wheat, QTL analysis revealed a significant narrow QTL close to the GWM296 locus (Schnurbusch et al. 2004). Schnurbusch et al. (2004) speculated that this QTL on 2DS could represent *Lr22* and our data supports this hypothesis. GWM455 was previously reported to be polymorphic between Thatcher and RL6044 in an effort to exclude *Lr22a* as an allele of other *Lr* genes introgressed from *Ae. tauschii*, but linkage experiments between *Lr22a* and GWM455 were not performed (Raupp et al. 2001).

Other genes of interest are found near the *Lr22* locus on chromosome 2DS. *Lr41* (*Lr41* = *Lr39*; Singh et al. 2004) is distal to GWM210 (Raupp et al. 2001) whereas *Lr22a* is proximal. The order of loci near *Lr22* is inconsistent between our map (ITMI-CRC) and the map of Raupp et al. (2001). After re-genotyping lines recombinant on 2DS, the marker order as presented in the ITMI-CRC map is the most likely (Fig. 2). However, the marker orders in the ITMI-CRC map and the map presented by Raupp et al. (2001) both place *Lr22a* and *Lr41* on opposite sides of GWM210 (Fig. 2). Both introgressions of *Lr22a* into wheat, RL6044 and RL4495, retained SSR markers proximal to GWM210. Furthermore, *Lr41* is a seedling resistance gene and *Lr22a* is an adult plant resistance gene. McIntosh et al. (1995) recorded no instances of an adult-plant resistance gene with an allele that confers seedling resistance for any of the three rusts of wheat. In the particular case of *Lr22* there are two alleles that have been reported and both provide adult-plant resistance (Rowland and Kerber 1974; Dyck 1979). This data agrees with the conclusion of Raupp et al. (2001) that *Lr41* is not allelic to *Lr22a*. The dwarfing gene *Rht8* is closely linked with GWM261 (Korzun et al. 1998) and is proximal to the *Lr22* locus (Fig. 2).

Table 2 Cultivars from different geographical origins screened with GWM296, the closest marker to *Lr22a*, to survey allele diversity and cross-applicability

Ae. tauschii allele	Other alleles[a]		
North America	*North America*	CDC Teal	Superb
5500HR	5601HR	Columbus	Thatcher
5600HR	AC Barrie	Grandin	*South America*
AC Minto	AC Bellatrix	Invader	Frontana
BW63[b]	AC Cadillac	Kanata	*Asia*
	AC Cora	Katepwa	Chinese Spring
	AC Domain	Lancer	Nyu Bai
	AC Elsa	Laura	Wuhan-1
	AC Intrepid	McClintock	Sumai-3
	AC Majestic	McKenzie	*Europe*
	AC Readymade	Neepawa	Alcedo
	AC Splendor	Norstar	Alidos
	AC Tempest	Park	Altos
	Alikat	Pasqua	Bezostaya
	Alsen	Prodigy	Kontrast
	CDC Falcon	Roblin	Milan 13
	CDC Kestrel	Snowbird	Welford
	CDC Osprey	Somerset	Zentos

Only named cultivars are listed. An additional 65 North American breeding lines were tested. Six of these breeding lines carried the *Ae. tauschii* allele. Five of these had AC Minto as a parent while the sixth line was 98B34-T4B (see "Materials and methods")

[a] Thirteen alleles of GWM296 were found in addition to the allele introgressed from *Ae. tauschii*

[b] BW63 was not a released cultivar, however, it was an important Lr gene source in Canadian wheat breeding programs. The allele scores for BW63 indicate it was heterogeneous for the GWM296 marker and presumably for *Lr22a*

Even though the D genome of wheat has reduced polymorphism, 14 alleles for GWM296 were identified on 2DS. Although both of the 2DS alleles illustrated in Fig. 1 have two fragments, other genotypes tested exhibited a single fragment. For example, the 2DS allele of GWM296 found in AC Splendor has a single fragment of 167 bp while Superb has fragments of 149 and 137 bp (not shown). Thus heterozygotes may have two, three or four fragments from chromosome 2DS while homozygotes may have one or two. Therefore caution is required when classifying an individual's genotype without prior knowledge of parental haplotypes and the degree of homozygosity. It should be noted that GWM296 also amplified a locus on chromosome 2A; however, this larger fragment (176 ± 4 bp) did not overlap with the 2DS fragments in any of the genotypes tested. Despite the allele diversity of GWM296 on chromosome 2D, the allele that was co-introgressed with *Lr22a* from *Ae. tauschii* was not found in any common wheat lines tested except for those believed to carry *Lr22a*

(Table 2). The uniqueness of the GWM296 allele, ease of scoring, and its close association with *Lr22a* provides a means to include *Lr22a* in complex gene stacks and to classify existing lines for the distribution of *Lr22a* provisionally.

In the survey of wheat breeding lines and cultivars GWM296 was useful in tracking the transmission of *Lr22a* through the genealogy of Canadian wheat. BW63 is an important source of leaf rust resistance in the pedigree of several Canadian bread wheat varieties containing *Lr11*, *Lr14b*, *Lr22a*, *Lr30* and *Lr34* (Dyck 1993a). The data indicated that BW63 was heterogeneous for the presence of *Lr22a* and this could explain why AC Minto (Columbus/ BW63//Katepwa/BW552; Townley-Smith et al. 1993) inherited *Lr22a* from BW63 while Pasqua (BW63*2/ Columbus) did not (Dyck 1993a). Since BW63 would have demonstrated a high degree of adult-plant resistance it would have been difficult to track the incorporation *Lr22a* during the development of Pasqua. All of the Canadian cultivars (Table 2) and breeding lines tested that inherited the RL5271 allele of GWM296, and presumably *Lr22a*, were derived from AC Minto except for 98B34-T4B (AC Domain*6/3/Thatcher*7//tetra-Canthatch/*Ae. tauschii* RL5271).

In Canada *Lr22a* is deployed in AC Minto (registered in 1991) and putatively in 5500HR (2000) and 5600HR (1999). There are no reports of US cultivars that carry *Lr22a* (JA Kolmer, personal communication). The Canadian cultivars that carry *Lr22a* have only occupied small percentages (0.13–0.95%) of the wheat production area in Canada from 1998 to 2006 (Canadian Wheat Board, http:// www.cwb.ca). Thus *Lr22a* has had relatively low exposure to *P. triticina* in Canada and the US. The detection of an ineffective race-specific "*b*" allele of *Lr22* (Dyck 1979) implies that *Lr22a* will also prove to be race specific. The absence of virulence on *Lr22a* could be explained in part by its lack of exposure to *P. triticina*.

AC Minto carries *Lr11*, *Lr13* and *Lr22a* (Kolmer 1997). Presence of *Lr22a* in AC Minto is confirmed by presence of the five *Ae. tauschii* alleles found in BW63 (Table 1). Each year AC Minto showed field resistance that was better than the *Lr22a* NIL, indicating that *Lr13* and/or *Lr11* had the ability to interact synergistically with *Lr22a* even though they were ineffective independently (Table 3). *Lr13* is known to interact synergistically with some other Lr genes (Kolmer 1992), however, no data has been reported on the potential interaction between *Lr13* and *Lr22a*. Cultivars 5600HR (AC Minto//Columbus/Roblin) and 5500HR (AC Minto/3/MN72506/Columbus//RL4473) both received the *Ae. tauschii* alleles found in BW63, and presumably *Lr22a*, via AC Minto (Table 1). In addition to *Lr22a*, 5600HR and 5500HR may also carry *Lr11*, *Lr13*, and *Lr16*, and *Lr11* and *Lr16*, respectively, based on pedigree and their

Table 3 Virulence of *P. triticina* on *Lr22a* and to selected cultivars and NILs in Canada

Year	No. of isolates[a]	Virulence on *Lr22a*	Field reaction to *P. triticina*[e]								
			AC Minto[b]	5600 HR[c]	5500 HR[c]	Tc[d]	Tc-*Lr11*[d]	Tc-*Lr13*[d]	Tc-*Lr16*[d]	Tc-*Lr22a*[d]	Tc-*Lr34*[d]
2002	71	0	TR R	TR R	10 RMR	80 S	–	–	–	–	–
2003	29	0	TR R	0 R	0 R	80 S	70 S	80 S	65 M	7 RMR	25 M
2004	58	0	3 MR	0 R	15 MR	80 S	80 S	80 S	60 M	19 MR	20 M
2005	81	0	15 RMR	5 RMR	5 RMR	80 S	80 S	80 S	70 MSS	20 M	40 M
2006	–	–	0 R	0 R	0 R	80 S	80 S	80 S	50 M	10 RMR	40 M

[a] Survey of Canadian *P. triticina* isolates for 2002 is from McCallum and Seto-Goh (2005) and (2003) to (2005) is from McCallum and Seto-Goh (unpublished data)

[b] AC Minto is reported to have *Lr11*, *Lr13* and *Lr22a* (Kolmer 1992)

[c] 5600 HR and 5500 HR are derivatives of AC Minto that carry the SSR allele linked to *Lr22a*. Our evidence suggests that these cultivars carry *Lr11*, *Lr16* and *Lr22a*. It is also possible that these two carry *Lr34*

[d] Tc = Thatcher, Tc-*Lr11* = RL6053, Tc-*Lr13* = RL4031, Tc-*Lr16* = RL6005, Tc-*Lr22a* = RL6044, Tc-*Lr34* = RL6058

[e] Field ratings are based on a modified Cobb scale for incidence (Peterson et al. 1948) and severity was scored as *R* resistant (flecks and small uredinia with necrosis), *MR* moderately resistant (large necrotic flecks and large uredinia), *M* moderate (mixture of uredinia sizes), *MS* moderately susceptible (moderate to large uredinia with chlorosis), and *S* susceptible (large uredinia without chlorosis or necrosis)

reactions to different *P. triticina* virulence phenotypes (Samborski and Dyck 1982; Dyck 1993b; B. McCallum unpublished). In the field all three Canadian cultivars that carry *Lr22a* showed leaf rust resistance that is better than the individual genetic components of their resistance (Table 3). Although *Lr22a* appeared to be the key component of the leaf rust resistance in these cultivars, the undesirable scenario where *Lr22a* is deployed as a single resistance gene has not occurred.

It would be desirable to stack *Lr22a* with other broadly effective resistance genes such as *Lr21* (Huang and Gill 2001; Huang et al. 2003) or *Lr34* (Lagudah et al. 2006) that have been characterized with reliable DNA markers. While the markers and mapping we have described can facilitate the stacking of *Lr22a* with *Lr21*, in fact markers are not needed to stack these two genes together. *Lr21*, or other seedling Lr genes, could be detected in the presence of *Lr22a* with seedling tests while *Lr22a* could be fixed by top crossing or back crossing with one or more "single gene" lines. Markers may prove more beneficial in complex crosses with several Lr genes segregating.

The low level of deployment in commercial cultivars and the lack of reported virulence to *Lr22a* (Table 3; Park and McIntosh 1994; McCallum and Seto-Goh 2005; Kolmer et al. 2005) make it a good candidate for use in Lr gene stacks. While various strategies can be used to include *Lr22a* in complex gene combinations, the marker reported here will facilitate gene stacking without the need to backcross. With careful management *Lr22a* could be widely deployed while maintaining its status as a broad-spectrum and effective Lr gene.

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