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Allelic variation for the rust resistance gene *Lr34/Yr18* in Canadian wheat cultivars

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Abstract The wheat (*Triticum aestivum* L.) gene *Lr34/Yr18* conditions resistance to leaf rust, stripe rust, and stem rust, along with other diseases such as powdery mildew. This makes it one of the most important genes in wheat. In Canada, *Lr34* has provided effective leaf rust resistance since it was first incorporated into the cultivar Glenlea, registered in 1972. Recently, molecular markers were discovered that are either closely linked to this locus, or contained within the gene. Canadian wheat cultivars released from 1900 to 2007, breeding lines and related parental lines, were tested for sequence based markers caSNP12, caIND11, caIND10, caSNP4, microsatellite markers wms1220, cam11, csLVMS1, swm10, csLV34, and insertion site based polymorphism marker caISBP1. Thirty different molecular marker haplotypes were found among the 375 lines tested; 5 haplotypes had the resistance allele for *Lr34*, and 25 haplotypes had a susceptibility allele at this locus. The numbers of lines in each haplotype group varied from 1 to 140. The largest group was represented by the leaf rust susceptible cultivar "Thatcher" and many lines derived from "Thatcher". The 5 haplotypes that had the resistance allele for *Lr34* were identical for the markers tested within the coding region of the gene but differed in the linked markers wms1220, caISBP1, cam11, and csLV34. The presence of the resistance or susceptibility allele at the *Lr34* locus was tracked through the ancestries of the Canadian wheat classes, revealing that the resistance allele was present in many cultivars released since the 1970s, but not generally in the older cultivars.

Keywords Brown rust · Slow rusting · Adult plant resistance

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Introduction

Rust resistance gene *Lr34* is one of the most important disease resistance genes in wheat. It was initially described by Dyck et al. (1966) as an adult plant leaf rust (caused by *Puccinia triticina* eriks.) resistance gene. This locus also confers resistance to stripe rust (*Yr18*, Singh 1992b), stem rust (Dyck et al. 1985; Hiebert et al. 2010), powdery mildew (*Pm38*,

Spielmeyer et al. 2005), and barley yellow dwarf virus (*Bdv1*, Singh 1993). *Lr34* conditions adult plant, slow rusting resistance and is completely linked with leaf tip necrosis (Singh 1992a). This resistance has not been overcome by *P. triticina* in a race-specific manner, over many years of widespread use throughout the world. This locus also enhances resistance conditioned by other leaf rust (German and Kolmer 1992) and stem rust (Hiebert et al. 2010) resistance genes. The *Lr34/Yr18* locus was cloned (Krattinger et al. 2009), and molecular markers were developed that are either closely linked to *Lr34*, or within the coding region of the gene (Dakouri et al. 2010; Lagudah et al. 2009).

The Western Canadian provinces of Alberta, Saskatchewan, and Manitoba produce approximately 96% of the wheat in Canada (Statistics Canada 2011). Canada Western Red Spring (CWRS) cultivars are grown on an average of 70% of the wheat area in Western Canada. Thatcher, the most popular CWRS cultivar from the 1940s to 1970s, is susceptible to leaf rust (McCallum and DePauw 2008). Breeding and research efforts resulted in the incorporation of resistance into subsequent cultivars, many of which were derived from Thatcher. Commonly used resistance genes in this class include *Lr10*, *Lr13*, *Lr14a*, *Lr16*, *Lr21*, *Lr22a,* and *Lr34* (McCallum et al. 2007a). Glenlea, registered in 1972, was the first major cultivar in Western Canada to carry *Lr34* (Dyck et al. 1985), although it did not have the end-use functionality to be a CWRS cultivar (McCallum and DePauw 2008). Since the 1970s, Glenlea and subsequent cultivars with *Lr34* have maintained a moderate level of resistance. Combinations of resistance genes involving *Lr34* have often been very effective, partially due to the ability of *Lr34* to enhance the expression of other resistance genes (German and Kolmer 1992). Laura, registered in 1986, was the first CWRS cultivar with *Lr34* (McCallum and DePauw 2008) and the gene was subsequently incorporated into more recently released CWRS cultivars. Determining the presence of *Lr34* in current cultivars should help to predict the field reaction and durability of cultivars and to aid wheat breeder's decisions on selecting parents for future crosses. The other main bread wheat classes in Western Canada include the Canada Prairie Spring (CPS) and Canada Western Extra Strong (CWES) which are distinguished from CWRS cultivars principally by their different end-use functionalities. Germplasm from these different classes tends to be relatively distinct (McCallum and DePauw 2008).

It is possible to postulate the presence or absence of *Lr34* in cultivars based on adult plant reaction. However, this is complicated by the presence of other resistance genes, the race non-specific nature of *Lr34*, and effects of environmental variability on expression. Molecular markers, tightly linked to *Lr34*, can help postulate the presence of this important gene. The csLV34 marker was reported to be tightly linked to *Lr34* (Lagudah et al. 2006) and diagnostic for the presence of the gene; two alleles were detected; *csLV34a* (229 bp) which is usually associated with the susceptibility allele and *csLV34b* (150 bp), normally associated with the *Lr34* resistance allele (Lagudah et al. 2006). This marker was used, in conjunction with phenotypic analysis, to postulate the presence of *Lr34* in 84 Australian wheat cultivars (Singh et al. 2007). The authors found good agreement between the presence of *csLV34b* and adult plant leaf rust resistance, although some susceptible lines also had a *csLV34b* allele in which recombination between *Lr34* and *csLV34* could have taken place, as was subsequently reported in other wheat lines (McCallum et al. 2008; Lagudah et al. 2009). The csLV34 marker was also used to assay the presence of *Lr34* in a larger, more diverse group of cultivars from many countries (Kolmer et al. 2008). Additional markers linked to *Lr34* that have been used for mapping include wms1220 (Lagudah et al. 2006), csLVMS1 (Spielmeyer et al. 2008), and swm10 (Bossolini et al. 2006).

Subsequently the coding region for *Lr34/Yr18* was identified, and reported to be an ABC transporter gene (Krattinger et al. 2009). Three mutations were originally described within the ABC transporter sequence, namely single nucleotide polymorphisms (SNPs) in intron 4 and exon 12, and a 3 bp indel in exon 11 (Krattinger et al. 2009). Gene specific markers within the ABC transporter gene were developed based on these sequence differences between the resistance and susceptibility alleles (Lagudah et al. 2009). Fine mapping of this region on chromosome 7D confirmed that the ABC transporter was the most likely valid candidate gene conditioning leaf rust resistance (Dakouri et al. 2010). Dakouri et al. (2010) described 10 new molecular markers spanning the *Lr34* locus, four of which were

within the ABC transporter gene. To determine haplotypes of wheat lines at the *Lr34* locus and vicinity the gene-based markers described by Lagudah et al. (2009), those described by Dakouri et al. (2010), and previously reported linked markers, are useful. The objective of our study was to determine the *Lr34* molecular marker profiles of a large number of Canadian wheat cultivars, related cultivars and lines, advanced breeding lines and other potential source materials to determine *Lr34* haplotype variation.

Materials and methods

Plant materials

Three hundred and seventy-five wheat lines were assembled for this study. They included nearly all Canadian cultivars from each of the major spring bread wheat (*Triticum aestivum* L.) classes, including CWRS, CPS, CWES, and, Canada Western Hard White spring (CWHWS) which represents most of the bread wheat production in Canada (McCallum and DePauw 2008). The collection also included breeding lines within each of these end-use functionality classes, parental lines for many of the cultivars, some unrelated cultivars, and lines with a diversity of origins throughout the world.

Genomic DNA extraction and molecular marker analysis

Plants from each line were grown to the 3–4 leaf stage and DNA was extracted from frozen, lyophilized leaf tissue, as described by Dakouri et al. (2010). The molecular markers linked to *Lr34* which were used in this study (in map order along chromosome 7D) were: wms1220 (Lagudah et al. 2006), caSNP12, caIND11, caIND10, caSNP4, caISBP1, cam11 (Dakouri et al. 2010), csLVMS1 (Spielmeyer et al. 2008), swm10 (Bossolini et al. 2006), csLV34 (Lagudah et al. 2006). Markers caSNP12, caIND11 and caSNP4 correspond to Krattinger et al. (2009) sequence variation within the ABC transporter gene, namely a C/T SNP in exon 12, a 3 bp indel in exon 11, and an A/T SNP in intron 4. The markers inside the ABC transporter coding sequence are caSNP12, caIND11, caIND10, and caSNP4, where caIN10 characterized a 1 bp indel in exon 10 (Dakouri et al. 2010). All other markers are located outside the coding region of *Lr34*.

Microsatellite markers wms1220, cam11, csLVMS1, swm10, as well as indel marker caIND11 and caIND10, were resolved on an ABI3100 using a modified amplification procedure with M13 tail primers (Schuelke 2000). Markers csISBP1, csLV34, caSNP12, caSNP4 were resolved on agarose gels as either co-dominant or dominant (SNP) markers. Allele sizes were determined by fragment analysis using a modified version of Genographer (Benham et al. 1999) and include an M13 tail of 19 bp.

Results

Haplotype groups and molecular marker profiles for 375 wheat cultivars and lines

The numbers of alleles differed among the markers for this diverse collection of germplasm (Table 1). There were only two alleles for all markers except wms1220, cam11, csLVMS1, and swm10 which had 9, 6, 4 and 5 alleles each, respectively. The markers within the coding sequence of the gene: caSNP12, caIND11, caIND10, caSNP4, divided the lines into four main groups. The assignment of these groups into those carrying the resistance or a susceptibility allele at the *Lr34* locus was based on the alleles of caSNP12 and caIND11 because these were shown to be diagnostic for resistance conditioned by *Lr34* in previous studies (Krattinger et al. 2009; Lagudah et al. 2009; Dakouri et al. 2010). Group 1 lines had the "C" allele at caSNP12, and the "null" allele at caIND11, whereas the other three groups had the "T" allele at caSNP12 and the "TTC" allele at caIND11. Group 1 consisted of all 108 lines that had the *Lr34* resistance allele, whereas the other three groups had a susceptibility allele (Table 2). The three groups with susceptibility alleles were differentiated from each other by alleles at caIND10 and caSNP4 with group 2 having the "A" and "T" alleles, group 3 had the "null" and "A" alleles, and group 4 had the "null" and "T" alleles, for caIND10 and caSNP4, respectively. These four main groups defined by the gene-based markers were then further divided into subgroups based on data from the linked markers (Table 2).

Table 1 Alleles for 10 molecular markers within or linked to the *Lr34/Yr18* coding region

Haplotype	wms1220	**caSNP12**	**caIND11**	**caIND10**	**caSNP4**	caISBP1	cam11	csLVMS1	swm10	csLV34
1	138[a]	T	TTC	N	T	509	N	224	194	175
2	146	C	N	A	A	391	298	226	208	255
3	148						299	228	210	
4	152						300	230	212	
5	157						301		214	
6	159						302			
7	161									
8	165									
9	169									

Markers caSNP12, caIND11, caIND10, caSNP4 in bold are inside the ABC transporter coding sequence, whereas the other markers are linked. Markers are listed in order along the chromosome

T thymine, *C* cytosine, *A* adenine, *N* null

[a] Allele sizes in base pairs include an M13 tail of 19 bp

Both caISBP1 and cam11 are linked tightly to the ABC transporter gene (Dakouri et al. 2010). There were only two alleles for caISBP1, a 509 bp fragment associated with the resistance allele for *Lr34* and a 391 bp fragment associated with the susceptibility allele at this locus. The only exceptions to this pattern among the 375 lines were RL6058 (Thatcher*6/PI 58548), a Thatcher near-isogenic line containing *Lr34*, and its PI 58548 donor parent, which both had the 391 bp fragment (sub-group 1E, Table 2, Supplementary Table 1). Similarly, these two lines also had a 300 bp allele for cam11 whereas all the other lines with the resistance allele were null. This appears to be a relatively rare haplotype which could be the result of a recombination event between the caSNP4 of the ABC transporter and cam11. There were four alleles for csLVMS1 (224, 226, 228, 230 bp) (Table 1). All the lines that carried *Lr34* had the 226 bp fragment, whereas most lines in the susceptible groups had the 228 bp fragment. Four sub-groups of lines without *Lr34* had the 226 bp fragment, but this represented only 13 of the 267 lines without *Lr34*. RL6058 and PI 58548 which differed at caISBP1 and cam11, had the same allele at csLVMS1 as the rest of the lines with *Lr34* suggesting a second recombination event between caISBP1 and csLVMS1. SSR marker swm10 had five alleles (Table 1) but only the 208 bp fragment was present in the lines that carried *Lr34*. The 208 bp allele was relatively rare among the lines without *Lr34*, found in only four lines from three groups. Lines without *Lr34* typically had the 210, 212 or 214 bp fragments although one line (Synthetic W7984, a parent of the ITMI population) had a rare 194 bp fragment.

Only two alleles (*csLV34a* and *csLV34b*) were reported to date for the csLV34 marker. The *csLV34a* allele (229 or 255 bp in our study) was associated with the susceptible allele of *Lr34*, whereas the *csLV34b* allele (150 or 175 bp in our study) was associated with the resistance allele of *Lr34* in lines and cultivars known to contain *Lr34* (Lagudah et al. 2006). The susceptible cultivar Thatcher had the expected 255 bp *csLV34a* allele, whereas the near-isogenic line Thatcher-*Lr34* (RL6058) had the 175 bp *csLV34b* allele. Recombination between csLV34 and *Lr34* was previously reported (McCallum et al. 2008; Lagudah et al. 2009). Lines without *Lr34* generally had the 255 bp allele except four sub-groups of lines, namely 2B (HY476), 2D (HY344), 4C (BW366 and BW807), and 4D (51 lines). Most of the 51 lines in sub-group 4D were related to the pre-harvest sprouting resistant line RL4137 (DePauw et al. 2009), also in this sub-group (Supplementary Table 1). AC Domain, previously postulated to have *Lr34* (Liu and Kolmer 1997), was also in this sub-group. All lines with *Lr34* had the 175 bp fragment except for group 1D (a breeding line designated 94B30-C6D) which had the 255 bp fragment. Recombination between csLV34 and *Lr34* was previously thought to be rare (Lagudah et al. 2006) although in the current study

Table 2 Profiles for 375 wheat cultivars and breeding lines based on 12 molecular markers within or linked to the *Lr34* coding region

Response allele	Haplotype	wms12220	**caSNP12**	**caInd11**	**caInd10**	**caSNP4**	caISBP1	cam11	csLVMS1	swm10	csLV34	No. lines	Representative lines
R	1A	148[a]	C	N	N	A	509	N	226	208	175	19	BW90, AC Cadillac, Lillian
	1B	159	C	N	N	A	509	N	226	208	175	12	Chinese Spring, Glenlea
	1C	161	C	N	N	A	509	N	226	208	175	74	Laura, CDC Teal, Frontana
	1D	161	C	N	N	A	509	N	226	208	255	1	94B30-C6D
	1E	165	C	N	N	A	391	300	226	208	175	2	RL6058-886C, PI 58548
												108	
S	2A	159	T	TTC	A	T	391	301	228	214	255	4	ND744, ND721, ND04/3-18, Glenn
	2B	161	T	TTC	A	T	391	299	228	210	175	1	HY476
	2C	161	T	TTC	A	T	391	300–301	228	212	255	2	Sonora 64, Kenya Farmer
	2D	161	T	TTC	A	T	391	300–301	228	212	175	1	HY344
	2E	161	T	TTC	A	T	391	298–301	228	214	255	21	Foremost, AC Taber, AC Karma
	2F	169	T	TTC	A	T	391	300–301	228	212	255	6	Garnet, Ruby
												35	
	3A	138	T	TTC	N	A	391	299	226	210	255	1	Buck Manantial
	3B	146	T	TTC	N	A	391	298	228	210	255	6	Invader, Prelude, Sinton, Lee
	3C	157	T	TTC	N	A	391	301	226	208	255	1	Ceres
	3D	157	T	TTC	N	A	391	300	228	212	255	3	Chinook, HW320, Fortuna
	3E	159	T	TTC	N	A	391	299	230	210	255	1	Salamouni
												12	
	4A	146	T	TTC	N	T	391	298	228	212	255	1	Code 15
	4B	148	T	TTC	N	T	391	300	228	194	255	1	Synthetic
	4C	148	T	TTC	N	T	391	298	228	208	175	2	BW366, BW807
	4D	148	T	TTC	N	T	391	298–302	228	210	175	51	RL4137, AC Domain, Kanata
	4E	148	T	TTC	N	T	391	298–301	228	210	255	140	Thatcher, AC Barrie, Superb
	4F	152	T	TTC	N	T	391	302	228	212	255	1	Kota
	4G	159	T	TTC	N	T	391	297–302	226	210	255	3	Lolo, Ladoga, Diamondbird
	4H	159	T	TTC	N	T	391	299–302	226	212	255	8	Lake, Stanley, Canus
	4I	159	T	TTC	N	T	391	298	228	210	255	1	ND694 = Parshall
	4J	159	T	TTC	N	T	391	297–301	228	214	255	2	McMurachy, McMurachy-Exchange

Table 2 continued

Response allele	Haplotype	wms12220	caSNP12	caInd11	caInd10	caSNP4	caISBP1	cam11	csLVMS1	swm10	csLV34	No. lines	Representative lines
	4K	159	T	TTC	N	T	391	301	230	210	255	1	93464
	4L	161	T	TTC	N	T	391	298–299	228	210	255	3	Milan 13, Atlas 66, Macon
	4M	161	T	TTC	N	T	391	300	224	208	255	1	Tybatt
	4N	165	T	TTC	N	T	391	298–302	226	212	255	5	Munk, Epos, Picoto, Taifun, Monsum
												220	
												375	Total

Markers caSNP12, caIND11, caIND10, caSNP4 in bold are inside the ABC transporter coding sequence, whereas the other markers are linked. Markers are listed in order along the chromosome

T thymine, *C* cytosine, *A* adenine, *N* null, *R* resistance allele, *S* susceptibility allele

[a] Allele sizes in base pairs include an M13 tail of 19 bp

there were five sub-groups (1D, 2B, 2D, 4C, 4D) in which there appeared to be a recombination between csLV34 and *Lr34*.

Wms1220 had nine alleles, the highest number of alleles among the markers in this study. This was the only marker tested on the proximal side of the ABC transporter gene (Dakouri et al. 2010). Haplotypes 1A, 1B, 1C/1D, and 1E with *Lr34* were differentiated by different alleles at wms1220. There was diversity among the haplotypes with the susceptibility allele, but a substantial proportion of the members from each of groups 2, 3 and 4 (as defined by the gene-based markers caSNP12, caIND11, caIND10, and caSNP4) belonged to a small number of sub-groups or haplotypes. Group 2 lines were CPS cultivars and breeding lines (2B, 2D, 2E), parental lines from CIMMYT (2C) and North Dakota (2A), and early Canadian cultivars such as Garnet and Ruby (2F). Group 3 lines were a mixture of Canadian cultivars and parental lines from South America (Buck Manantial) and the U.S.A. (Ceres). Group 4 was the largest group, containing 220 lines. There were two large sub-groups, viz. 4D, with 51 lines represented by RL4137 and the cultivar AC Domain, and group 4E with 140 lines represented by Thatcher and AC Barrie. Many lines had the 4E haplotype because Thatcher was used extensively as a recurrent parent in backcrossing to recover its excellent end-use functionality and stem rust resistance. The 4D haplotype was common because RL4137 and AC Domain with resistance to pre-harvest sprouting were used frequently as parents (Townley-Smith and Czarnecki 2008).

Introduction and inheritance of *Lr34* in Canadian wheat cultivars

Over 70% of the bread wheat grown in Western Canada is from the CWRS class, which has good end-use functionality for producing high quality flour and bread (McCallum and DePauw 2008). The major cultivars which initiated this type of wheat in the early days of production were Red Fife (introduced in 1870) and Marquis (1909) (McCallum and DePauw 2008). Neither of these cultivars had *Lr34* (Fig. 1) and they are susceptible to leaf rust. Thatcher (released in 1935) replaced Marquis, due to stem rust resistance, but was very susceptible to leaf rust. Thatcher was partially replaced by Selkirk (1953),

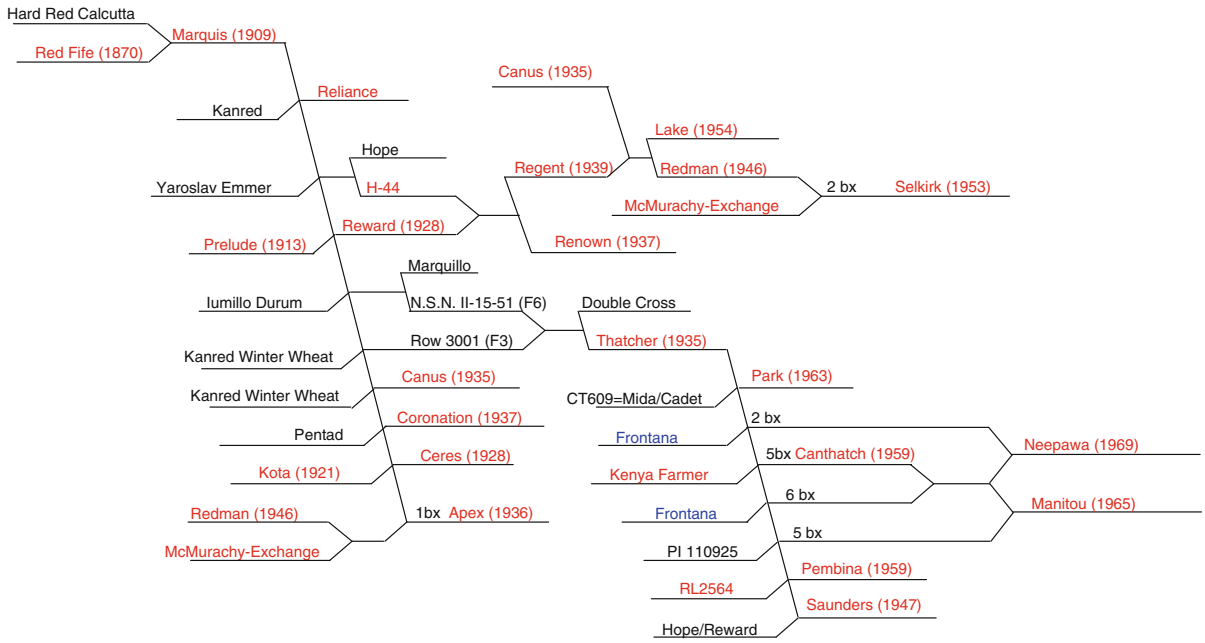

Fig. 1 Early CWRS wheat cultivars. Cultivars in *blue* font have the resistance allele for *Lr34*, those in *red* had the susceptibility allele, and those in *black* were not tested. The year of registration and/or release is designated in *brackets* for Canadian wheat cultivars

which had improved resistance to leaf rust due to *Lr10*, *Lr14a, Lr16* (Martens and Dyck 1989) but did not have *Lr34* (Fig. 1). Thatcher derivatives Manitou (1965) and then Neepawa (1969) replaced both Thatcher and Selkirk as dominant wheat cultivars in Western Canada and both were more resistant to leaf rust than Thatcher due to *Lr13*, donated by Frontana, but neither inherited *Lr34* from Frontana (Fig. 1). Neepawa was replaced as the most popular cultivar in 1986 by Katepwa which had a similar level of resistance to leaf rust but also lacked *Lr34* (Fig. 2). The first major CWRS cultivar to have *Lr34* was Laura (registered in 1986) (Fig. 3), which could have inherited *Lr34* from either Tobari 66 or Carazinho, as all three have the 1C haplotype (Table 2, Supplementary Table 1). CDC Teal (1991), another widely grown cultivar in Western Canada has *Lr34* from BW38 (Sonora 64/Tezanos Pintos Precoz//Neepawa). BW38 inherited *Lr34* from Tezanos Pintos Precoz since both Sonora 64 (2C) and Neepawa (4E) lack *Lr34*. CDC Teal, BW38 and Tezanos Pintos Precoz all belonged to haplotype 1C and carried the resistance allele of *Lr34* (Fig. 2, Supplementary Table 1). Roblin (registered in 1986) and Pacific (1983) were both important cultivars and parental lines with *Lr34* derived from the same parents (Fig. 3). Roblin inherited the 3C haplotype found in BW40, BW38 and BW15, which could have been inherited from either Tezanos Pintos Precoz (BW40 and BW38) or Tobari 66 (BW15) (Supplementary Table 1). However, Pacific inherited the 1C haplotype from RL4353 which likely was initially from Era, since it was reported to carry *Lr34* (Ezzahiri and Roelfs 1989) and the *csLV34b* allele (Kolmer et al. 2008). After 1998, AC Barrie became the leading CWRS cultivar, replacing CDC Teal, Roblin and other cultivars (McCallum and DePauw 2008). Since AC Barrie did not have *Lr34* (4E), the proportion the CWRS area seeded to cultivars with *Lr34* declined during the years 1998 to 2005 (Fig. 4). The proportion of the total CWRS area seeded to cultivars with *Lr34* increased slightly from 2007–2009 because many of the recently registered cultivars have *Lr34*, such as 5602HR, CDC Alsask, and AC Lillian, (Figs. 3, 6).

Cultivar Grandin was used as a parent to develop several CWRS cultivars. It was heterogeneous for the presence of *Lr34* (results not shown) and did not donate *Lr34* to the CWRS cultivars Superb, CDC Abound or CDC Go (Fig. 5). A group of CWRS cultivars have resistance to stem sawfly by having

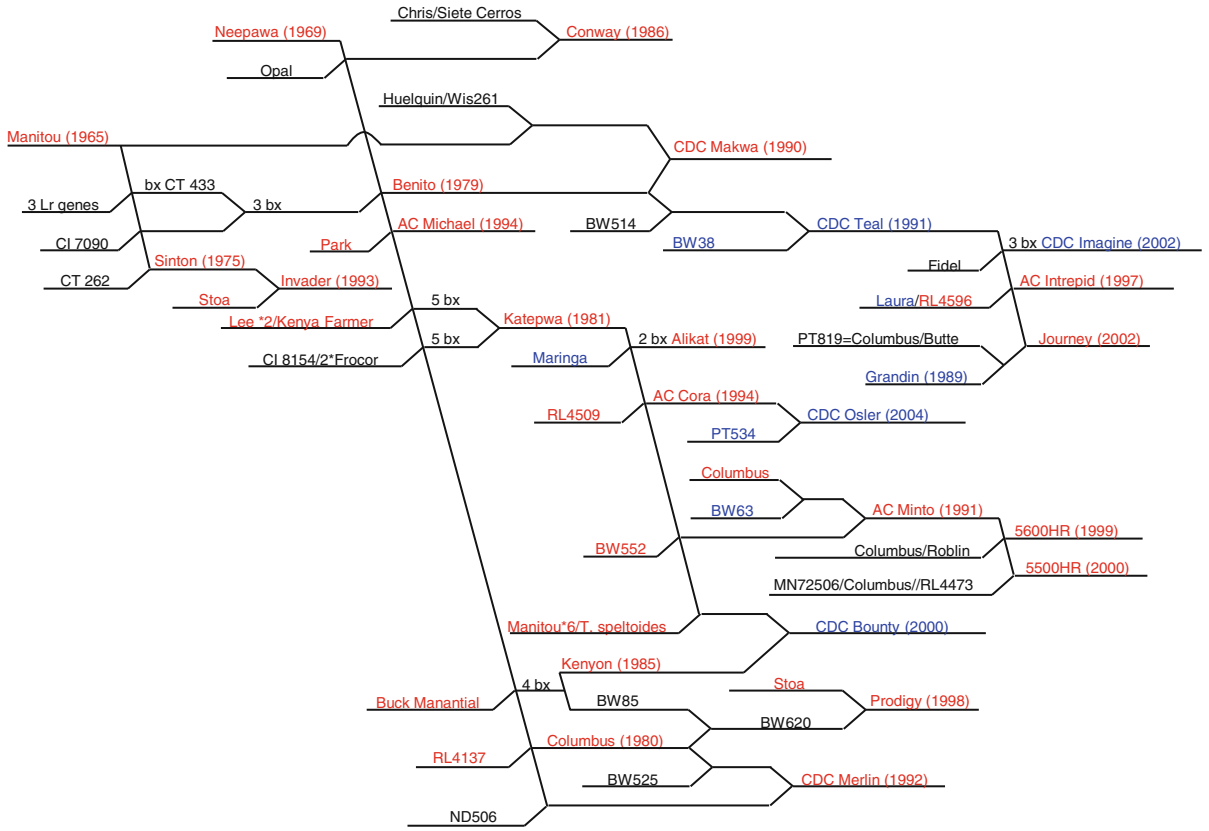

Fig. 2 Neepawa derived CWRS wheat cultivars. Cultivars in *blue* font have the resistance allele for *Lr34*, those in *red* had the susceptibility allele, and those in *black* were not tested. The year of registration and/or release is designated in *brackets* for Canadian wheat cultivars

solid stem, derived from the line S-615 (Fig. 6). Early solid stem cultivars such as Rescue (1946), Chinook (1952), Cypress (1962) and Chester (1976) did not have *Lr34*, but it was introduced into the later solid stem cultivars Leader (1981), Lancer (1985) and AC Eatonia (1993) through the U.S. cultivar Chris. AC Lillian (2003), the most popular CWRS cultivar in 2007–2009, inherited *Lr34* through BW621 since AC Lillian and BW621 have the 1A haplotype. AC Lillian's other potential *Lr34* donor parent Pasqua had the 1C haplotype (Fig. 6, Supplementary Table 1).

Canada Prairie Springs cultivars were originally produced from CIMMYT semi-dwarf cultivars (McCallum and DePauw 2008), which commonly carry *Lr34*. Despite the relationship to CIMMYT germplasm, nearly all the CPS cultivars registered to date lack *Lr34* (Fig. 7). The exceptions are Genesis (1990) which inherited *Lr34* from Pitic 62, and 5701HR which inherited *Lr34* from either N89-3004 or N87-0446. Most CPS lines are derived from HY320, which has the susceptibility haplotype (2E), inherited by many of its progeny including AC Taber, AC Crystal and AC Foremost (Supplementary Table 1). This haplotype was also in Romany, a parent of HY320, but was not found among the tested CWRS cultivars or breeding lines.

CWES cultivars are primarily derived from Glenlea (registered in 1972). Glenlea was the first major cultivar in Canada to have *Lr34*; it continues to be moderately resistant to leaf rust. Glenlea donated *Lr34* to CWES cultivars Bluesky (1987), Amazon (1998), AC Corinne (1999), Glenavon (1999), Burnside (2004), CDC Rama (2002), and CDC Walrus (2004) since all these cultivars belong to the group 1B haplotype, whereas other potential donors Kitt, McNeal and Pasqua had the 1C haplotype (Fig. 8, Supplementary Table 1).

The Canada Western Hard White Spring wheat class was initiated with the registration of Snowbird

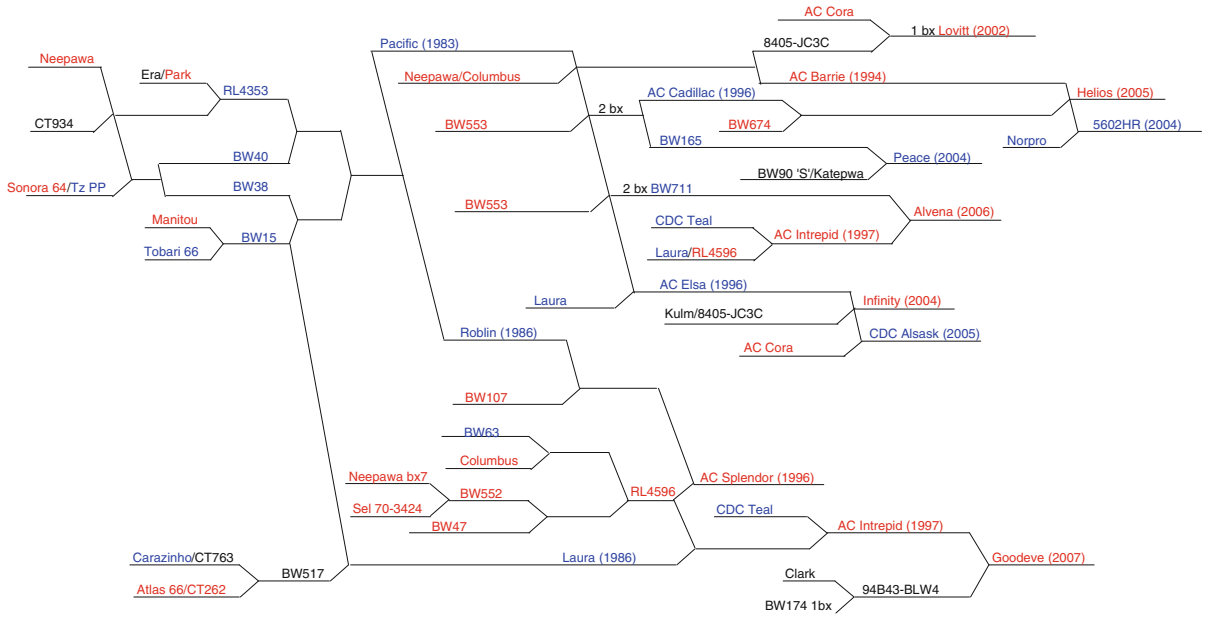

Fig. 3 Pacific, Roblin and Laura derived CWRS wheat cultivars. Cultivars in *blue* font have the resistance allele for *Lr34*, those in *red* had the susceptibility allele, and those in *black* were not tested. The year of registration and/or release is designated in *brackets* for Canadian wheat cultivars

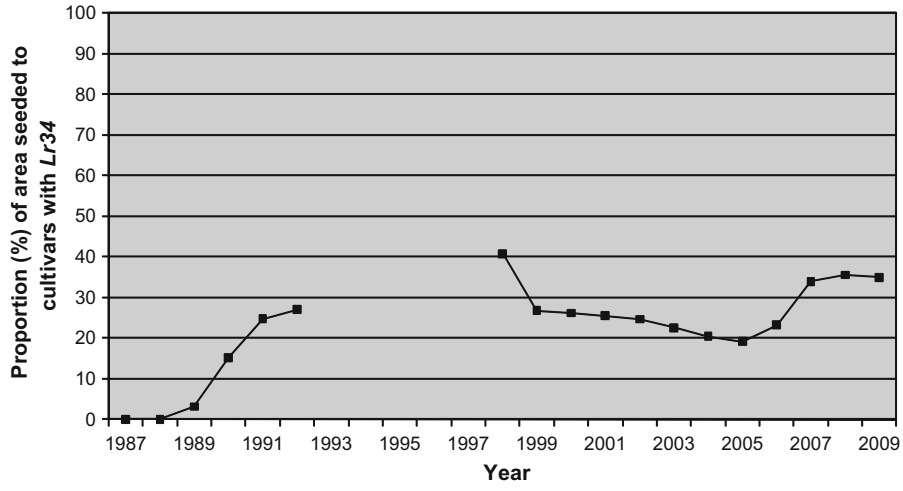

Fig. 4 Proportion of the total CWRS seeded area which was seeded to *Lr34* carrying cultivars. Data from Canadian Wheat Board varietal surveys (McCallum and DePauw 2008). No prairie wide variety surveys were conducted 1993–1997

and Kanata in 2001. Both have a susceptibility haplotype at the *Lr34* locus (group 4D). Two more recent hard white cultivars in this class namely Snowhite 475 and 476 also have susceptibility haplotypes belonging to groups 2E and 2B, respectively (Supplementary Table 1). The different haplotypes for these cultivars reflect their parentage since Snowbird and Kanata were derived partially from AC Domain (group 4D), whereas Snowhite 475 and 476 were derived from CPS parental cultivars in groups 2E and 2B.

Discussion

The only markers truly diagnostic for the presence of the resistance allele for *Lr34* were those within the coding sequence of the ABC transporter gene,

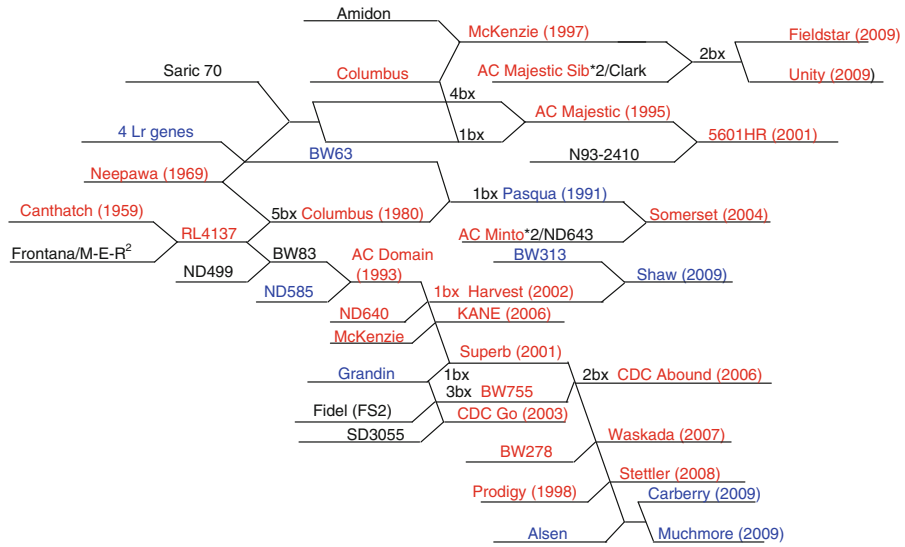

Fig. 5 Modern CWRS wheat cultivars. Cultivars in *blue* font have the resistance allele for *Lr34*, those in *red* had the susceptibility allele, and those in *black* were not tested. The year of registration and/or release is designated in *brackets* for Canadian wheat cultivars

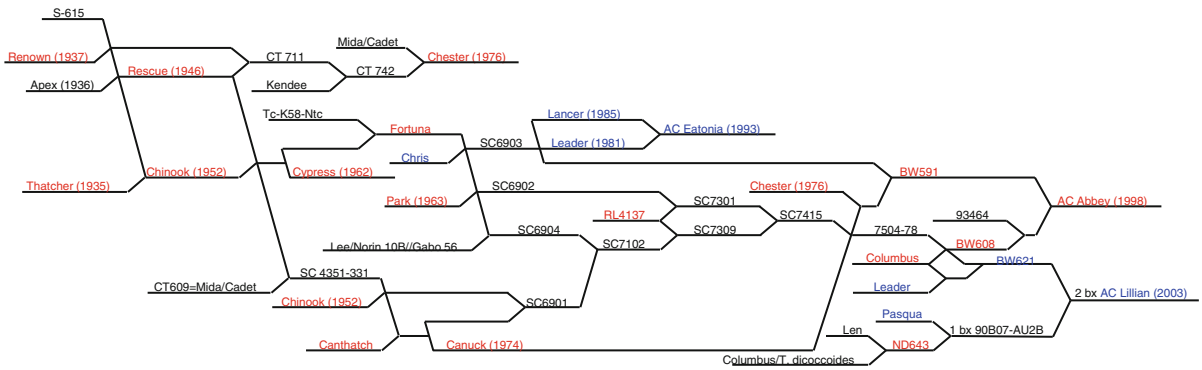

Fig. 6 Solid stem CWRS wheat cultivars. Cultivars in *blue* font have the resistance allele for *Lr34*, those in *red* had the susceptibility allele, and those in *black* were not tested. The year of registration and/or release is designated in *brackets* for Canadian wheat cultivars

namely caSNP12 and caIND11. However, in another analysis of a broader world wheat collection, recombination between SNP4 and caIND11 was observed in two lines, but such a rare event was not observed in the 375 lines studied herein (Dakouri et al. 2010). Markers caISBP1 and cam11, located just upstream of the ABC transporter gene, were also diagnostic for *Lr34*, with the exception of RL6058 and its donor parent PI 58548. All lines without *Lr34* had the 391 bp fragment for caISBP1 and the 297–302 bp fragment for cam11. The csLV34 marker was previously thought to be fairly diagnostic for the presence of *Lr34* (Singh et al. 2007; Kolmer et al. 2008). While only two alleles were found for csLV34 in our study this marker was not diagnostic for *Lr34* because five groups appeared to have recombination events between csLV34 and *Lr34* (1D, 2B, 2D, 4C, and 4D). Recombinants included one resistant line (94B30-C6D) with the 255 bp fragment and 55 susceptible lines with the 175 bp fragment usually associated with resistance. In this latter case, the large number of lines within this recombinant group,

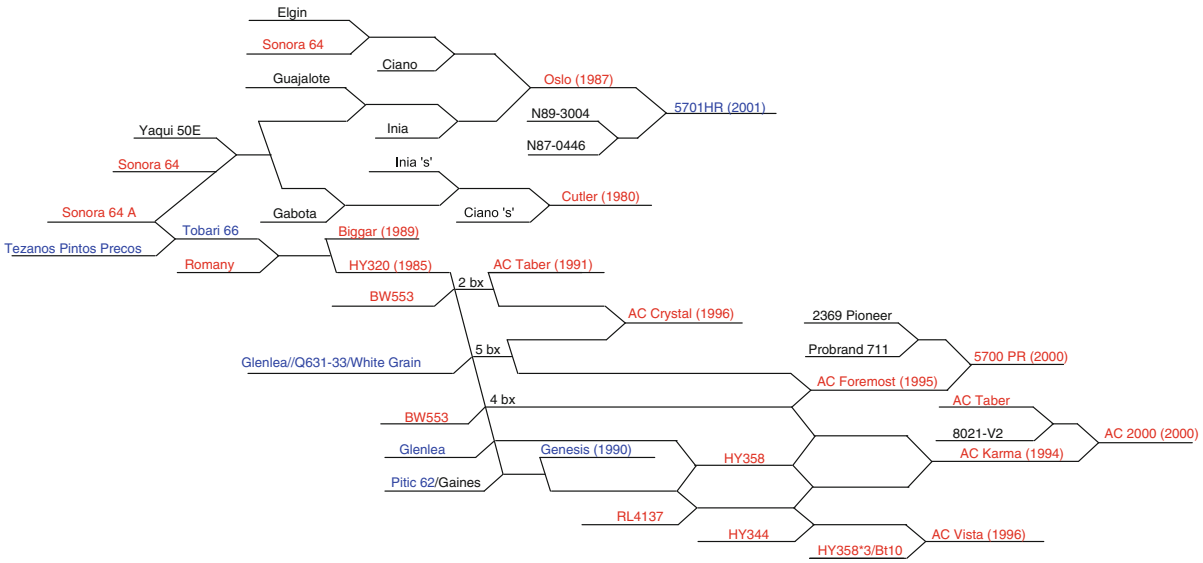

Fig. 7 CPS wheat cultivars. Cultivars in *blue* font have the resistance allele for *Lr34*, those in *red* had the susceptibility allele, and those in *black* were not tested. The year of registration and/or release is designated in *brackets* for Canadian wheat cultivars

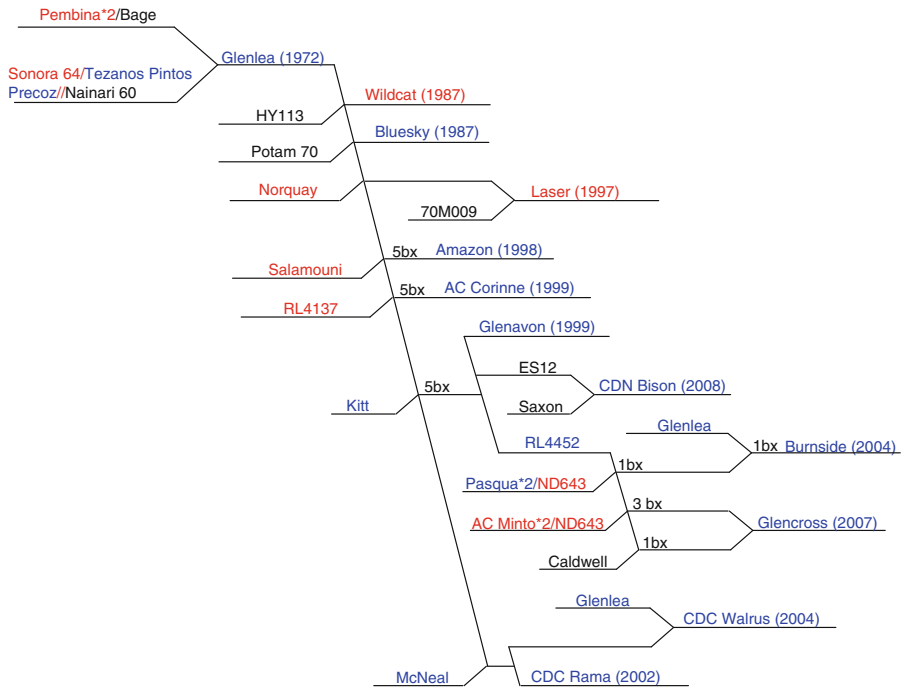

Fig. 8 CWES wheat cultivars. Cultivars in *blue* font have the resistance allele for *Lr34*, those in *red* had the susceptibility allele, and those in *black* were not tested. The year of registration and/or release is designated in *brackets* for Canadian wheat cultivars

probably reflects the relatedness of the Canadian cultivars in Group 4D. McCallum et al. (2008) and Lagudah et al. (2009) also reported recombination between csLV34 and *Lr34* in a small number of cultivars. These results highlight the problems of using linked markers to postulate the presence or absence of a resistance gene. Since gene-based diagnostic markers are now available for *Lr34*, they

should be used preferentially to identify the presence of this resistance allele.

Resistance gene *Lr34* was not found in Canadian cultivars registered prior to Glenlea in 1972. Although Frontana, which carries *Lr34* (Fig. 1), was used to add leaf rust resistance gene *Lr13* to Manitou (1965) and Neepawa (1969) (McCallum and DePauw 2008), it did not transfer *Lr34* to these cultivars. Once *Lr34* was transferred into CWRS cultivars such as Laura (1986), Roblin (1986) and CDC Teal (1991), the wheat growing area seeded to cultivars with *Lr34* rapidly increased and has remained relatively high ever since (Fig. 4). Because many recently released CWRS cultivars have *Lr34*, such as AC Lillian, CDC Alsask and 5602HR (Figs. 3, 6), it is possible to fix *Lr34* in crosses by choosing adapted parental lines that harbour it. Retaining it in segregating crosses should be much easier using the diagnostic markers reported (Lagudah et al. 2009, Dakouri et al. 2010). Cultivars with *Lr34*, like Glenlea and Laura, have maintained at least a moderate level of resistance over many years of production in Canada. However, cultivars without *Lr34* such as AC Barrie and Superb were resistant when released, due to race-specific resistance genes, but became susceptible over time due to changes in the pathogen population (McCallum et al. 2007a). This type of "resistance breakdown" has not happened to Canadian cultivars with *Lr34*.

Most CPS cultivars do not have *Lr34* and the level of leaf rust resistance has generally not been as good within this class as within the CWRS class. Recent cultivar 5701HR has *Lr34* and was the third most popular cultivar with approximately 15% of the CPS area seeded to this cultivar in 2007 (McCallum and DePauw 2008). This cultivar should facilitate and accelerate the incorporation of *Lr34* in this class as it becomes used as a parental line for the development of new CPS cultivars. By contrast, the CWES class, which was founded by cultivar Glenlea, is essentially fixed for *Lr34*, with the exceptions of Wildcat (1987) and Laser (1997). Laser was a relatively popular cultivar from 2000 to 2007, ranking third during many years, although much of its production was outside of the leaf rust prone area.

Resistance gene *Lr34* works in combination with other genes such as *Lr10*, *Lr13*, *Lr14a*, *Lr16*, *Lr21* and *Lr22a* to condition resistance in Canadian wheat cultivars (McCallum et al. 2007a). There is a synergistic effect of *Lr34* on other leaf rust resistance genes when it is used in combinations of two or more genes (German and Kolmer 1992). Cultivar Pasqua, with resistance genes *Lr11*, *Lr13*, *Lr14b*, *Lr30* and *Lr34* (Dyck 1993), has remained immune since it was registered in 1991 (unpublished data). Of those genes *Lr11*, *Lr13* and *Lr14b* do not condition resistance when used individually, and *Lr30* only gives an intermediate level of protection. However, in backcross Thatcher lines derived from Pasqua, combinations of one or more of these genes with *Lr34* resulted in good resistance, and combinations of three or four genes involving *Lr34* resulted in near immunity (McCallum and Thomas 2011).

A few cultivars with susceptibility haplotypes at the *Lr34* locus are still very resistant to leaf rust due to other resistance genes. McKenzie, for example, is currently resistant due to the combination of *Lr10*, *Lr13*, *Lr16* and *Lr21* (McCallum and Seto-Goh 2010). Since *Lr10* and *Lr13* are currently not effective when used alone and *Lr16* conditions only partial resistance, *Lr21* essentially provides all the resistance in McKenzie. If virulence to *Lr21* were to develop in the pathogen population, McKenzie and other Canadian cultivars that depend on *Lr21* could become susceptible. If *Lr34* could be incorporated into cultivars with other effective genes such as *Lr21*, a more durable and effective level of resistance might be achieved. Virulence to *Lr21* has recently been reported in the U.S.A. (Kolmer 2011) which could jeopardize the resistance of cultivars that currently rely on *Lr21* for leaf rust resistance.

Most Canadian wheat cultivars are susceptible to stripe rust, except those with the moderate resistance conditioned by the *Lr34/Yr18* locus (McCallum et al. 2007b). Stripe rust has become an increasing problem in the Central Great Plains of the U.S. since 2000 and could threaten wheat production in Canada (Chen et al. 2010). Therefore, increased incorporation of *Lr34/Yr18* into future cultivars could be useful in protecting the Canadian wheat crop against stripe rust. Stem rust resistance is also conditioned by the *Lr34/Yr18* locus (Dyck et al. 1985, Hiebert et al. 2010, McCallum et al. 2011). The central role of *Lr34/Yr18* in multiple disease resistance makes knowledge of this gene an important consideration in any set of wheat germplasm. Given the availability of functional gene-based markers, a good initial step in developing rust resistance for wheat breeding

programs is to determine the presence or absence of *Lr34/Yr18* in cultivars, lines in development and potential parental lines. This was previously done for wheat cultivars from Australia (Singh et al. 2007) and an international collection (Kolmer et al. 2008) using linked markers. Gene pyramids which involve *Lr34*, such as Pasqua (Dyck 1993; McCallum and Thomas 2011), which have been demonstrated to be effective and durable, can be developed once *Lr34* is fixed within parental lines or selected in progeny populations.

This study demonstrates the utility of gene-based molecular markers within the coding sequence of *Lr34* and linked markers. The determination of the presence or absence of this important resistance allele in all the major bread wheat classes in Canada should be instructive in its incorporation and retention in these breeding programs. The introduction of *Lr34* occurred repeatedly within these various classes, as CIMMYT derived cultivars, such as Tezanos Printos Precoz, were common donors in Canadian breeding programs. Recently released Canadian wheat cultivars increasingly rely, at least in part, on *Lr34/Yr18* for rust resistance. To minimize losses due to cereal rusts, future breeding efforts should focus on its incorporation into wheat cultivars in Canada and internationally.

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