Analysis of the *Lr34/Yr18* Rust Resistance Region in Wheat Germplasm

James A. Kolmer, Ravi P. Singh, David F. Garvin, Libby Viccars, Harinder M. William, Julio Huerta-Espino, Francis C. Ogbonnaya, Harsh Raman, Simon Orford, Harbans S. Bariana, and Evans S. Lagudah*

ABSTRACT

The *Lr34/Yr18* adult plant resistance gene contributes significantly to durable leaf rust (caused by *Puccinia triticina* Eriks.) resistance. Simple and robust molecular markers that enable early detection of *Lr34/Yr18* are a major advancement in wheat (*Triticum aestivum* L.) breeding. An insertion/deletion size variant located at the *csLV34* locus on chromosome 7D within an intron sequence of a sulfate transporter-like gene tightly linked to the *Lr34/Yr18* dual rust resistance gene was used to examine a global collection of wheat cultivars, landraces, and D genome–containing diploid and polyploid species of wheat relatives. Two predominant allelic size variants, *csLV34a* and *b*, found among the wheat cultivars showed disparate variation in different wheat growing zones. A strong association was observed between the presence of *Lr34/Yr18* and the *csLV34b* allele and wheat lines known to have *Lr34/Yr18* that had the *csLV34a* allele were rare. All landraces with the exception of those from China were predominantly of the *csLV34a* type. Only one size variant, *csLV34a*, was detected among the diploid and polyploid D genome–containing species, indicating that *csLV34b* arose subsequent to hexaploid bread wheat synthesis. The lineage of the *csLV34b* allele associated with *Lr34/Yr18* in modern wheat cultivars from North and South America, CIMMYT, Australia, and Russia was tracked back to the cultivars Mentana and Ardito developed in Italy by Nazareno Strampelli in the early 1900s. The robustness of the csLV34 marker in postulating the likely occurrence of *Lr34/Yr18* across a wide range of wheat germplasm and its utility in wheat breeding was confirmed.

J.A. Kolmer and D.F. Garvin, USDA-ARS Cereal Disease Laboratory and Dep. of Agronomy and Plant Genetics, Univ. of Minnesota, St. Paul, MN 55108; R.P. Singh and H.M. William, CIMMYT, Apdo. Postal 6-641, 06600, Mexico DF, Mexico; J. Huerta-Espino, Campo Experimental Valle de México INIFAP, Apdo. Postal 10, 56230, Chapingo, Edo de México, Mexico; L. Viccars and E.S. Lagudah, CSIRO Plant Industry, Canberra ACT 2601, Australia; F.C. Ogbonnaya, DNRE, Horsham, VIC, Australia; H. Raman, NSW Dep. of Primary Industries, Wagga Wagga, NSW 2650, Australia;. S. Orford, John Innes Centre, Norwich, UK; H.S. Bariana, Univ. of Sydney-Plant Breeding Institute, Camden, NSW, Australia. Received 23 Aug. 2007. *Corresponding author (evans.lagudah@csiro.au).

Abbreviations: APR, adult plant resistance; HRWW, hard red winter wheat; HRSW, hard red spring wheat; KUDS, kinase-U box domain; PCR, polymerase chain reaction; SRWW, soft red winter wheat.

Numerous genes conferring resistance to wheat rusts have been identified and used in wheat (*Triticum aestivum* L.) breeding. However, several of these genes have been rendered ineffective due to the emergence of new virulent races. Cultivars with the resistance gene *Lr34* such as Frontana have had effective durable resistance to the leaf rust fungus *Puccinia triticina* Eriks. (Dyck et al., 1966; Singh and Rajaram, 1992). Although *Lr34* has been used extensively in spring wheat grown in the United States, isolates of *P. triticina* with complete virulence to this gene have not been detected (Kolmer et al., 2003). Gene *Lr34*, first described by Dyck (1977, 1987), has been shown to enhance leaf rust resistance in combinations with other resistance genes (German and Kolmer, 1992). Another feature of *Lr34* resistance is that it has remained genetically inseparable from the adult plant resistance (APR) gene *Yr18* that

confers resistance to stripe rust (*P. striiformis* Westend. f. sp. *tritici*) (Singh 1992b, McIntosh 1992). Cosegregation of the dual APR gene *Lr34/Yr18* with other traits such as leaf tip necrosis (*Ltn1*), powdery mildew [*Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici* Em. Marchal] (recently designated *Pm38*), and tolerance to Barley yellow dwarf virus (*Bdv1*) have been documented (Singh 1992a, 1992b; McIntosh 1992; Spielmeyer et al., 2005; Liang et al., 2006). These multi–pathogen resistance traits have made the *Lr34/Yr18* locus one of the most valuable gene regions for disease resistance breeding in wheat.

Because *Lr34/Yr18* resistance is predominantly expressed at the adult plant stage and can also be masked by the effects of other major genes, there is considerable interest in developing effective methods for its easy detection. Phenotypes based on *Ltn1* (Singh 1992a) have been used; however, the background of the cultivar and the multigenic effects on overall leaf tip necrosis expression can lead to equivocal results, and *Ltn1* is not equally expressed in all environments. Development of molecular markers for *Lr34/Yr18* has long been a major objective for marker-assisted selection in wheat. Some of the earlier markers, such as *gwm295* and *gwm1220* identified on chromosome 7DS in quantitative trait loci regions associated with *Lr34/Yr18/Ltn1* (Suenaga et al., 2003; Schnurbusch et al., 2004), have been limited in breeding applications due to their low diagnostic capability across various wheat germplasm.

More recently two newly developed markers SWM10 (Bossolini et al., 2006) and csLV34 (Lagudah et al., 2006) closely linked to the *Lr34/Yr18/Ltn1/Pm38* locus have been shown to provide a much wider diagnostic ability for this multi–pathogen resistance trait in diverse wheat cultivar backgrounds. In this paper we explore the robustness of the csLV34 marker to assess a global wheat germplasm collection that includes wheat cultivars, landraces, bread wheat subspecies, and D genome–containing diploid and polyploid *Aegilops* species for variation at this locus. Knowledge of confirmed sources of *Lr34/Yr18*, donor parental lines and *csLV34* variants enabled inferences to be made on the likely presence or absence of this gene as well as to determine its origins in modern wheat cultivars.

MATERIALS AND METHODS
Plant Material
Hexaploid wheat cultivars from different market grades across the United States and Canada (Table 1) were analyzed for variation within an intron of the sulfate transporter-like gene based on the sequence tagged site *csLV34*. Bread wheat cultivars from other parts of the world included 114 tall and CIMMYT-derived semidwarf cultivars released in Mexico and various other countries, and 116 Australian cultivars, 80 winter wheat cultivars from Western Europe, which included the core lineages of the European Union wheat genetic diversity (GEDIFLUX set germplasm; http://www.jic.ac.uk/science/CropGen/GEDIFLUX/index.htm), and nine cultivars from Japan. Over 820 wheat landraces obtained from the Australian Winter Cereal collection (AWCC, Tamworth, NSW) with samples from Afghanistan, Armenia, Algeria, Azerbaijan, Bulgaria, China, Croatia, Cyprus, Egypt, Ethiopia, Georgia, Greece, India, Iran, Iraq, Italy, Jordan, Morocco, Nepal, Pakistan, Portugal, Spain, Syria, Tunisia, Turkey, Ukraine, former Yugoslavia, and parts of the former Soviet Union.

Other hexaploid wheats tested included *T. aestivum* ssp. *spelta* (L.) Thell. (eight accessions), *T. aestivum* ssp. *macha* (Dekapr. and Menabde) MacKey (four accessions), *T. aestivum* ssp. *sphaerococcum* (Percival) MacKey (five accessions), *T. aestivum* ssp. *compactum* (Host) MacKey (five accessions), and *T. aestivum* ssp. *vavilovii* (Jakubz.) MacKey (five accessions). D genome–containing species including 50 accessions of *Aegilops tauschii* Coss. (D^t), seven accessions of *A. cylindrica* Host. (C^cD^c), 4 and 11 accessions of tetraploid and hexaploid *A. crassa* Boiss., respectively ($X^{cr}D^{cr1}$; $X^{cr}D^{cr1}D^{cr\ddagger}$), three accessions of *A. ventricosa* Tausch. (D^vN^v), two accessions of *A. vavilovii* (Zhuk.) Chennav ($X^{va}D^{va}S^{va}$), and one accession of *A. juvenale* (Thell). Eig ($X^jD^jU^j$).

DNA Extraction and Marker Analysis
Seeds of each cultivar from the North American set were sown in vermiculite, and leaf tips from three to four seedlings at the two-leaf stage were harvested and placed in 1.5-mL microfuge tubes for DNA extractions. DNA extractions for the majority of samples were completed by the method of Kasajima et al. (2004). Briefly, 200 µL of an extraction buffer (30 mM Tris-HCl [pH 8.0], 25 mM NaCl, 3.5 mM EDTA [pH 8.0], and 0.05% SDS) was added to each tube, and then tubes were boiled for 2 min, removed to ice, and stored at −20°C until use. For a subset of the cultivars, a small scale version of the DNA extraction protocol of Riede and Anderson (1996) was used. DNA from seeds of the set of wheat cultivars from Australia was extracted according to the method described in Kota et al. (2006) and those from CIMMYT following the method described in Hoisington et al. (1994).

Polymerase chain reaction (PCR) amplification using the primer pair csLV34F and R (described in Lagudah et al., 2006; Fig. 1) was undertaken in 20-µL volumes, in a PTC 100 thermocycler (MJ Research, Waltham, MA) for the North American wheats. The final PCR mixture consisted of 2 µL of DNA, 2 µL 10× buffer, 0.5 units of *Taq* DNA polymerase, dNTPs (final concentration 0.2 mM each), and primers (final concentration 0.1 mM each). Reagents were obtained from Applied Biosystems (Foster City, CA). Temperature profiles consisted of an initial denaturation at 94°C for 3 min, and then 45 cycles of the following program: 94°C for 15 s, 58°C for 15 s, and 72°C for 15 s. A final 5-min extension was also employed. Polymerase chain reaction amplification for the Australian and European cultivars, landraces, and other D genome–containing *Aegilops* and *Triticum* species were performed in a Hybaid PCR Express (Integrated Sciences, Sydney, NSW, Australia) using conditions specified by Lagudah et al. (2006); PCR amplification conditions for germplasm evaluated at CIMMYT are described in Hoisington et al. (1994).

The amplification products were separated on 1.5% agarose gels containing ethidium bromide and using 0.5× TBE buffer for the North American wheats and 1% agarose for the remainder of the genotypes tested; gels were visualized on a UV transilluminator for documentation of allele types in cultivars. Thatcher and RL6058 (Tc*6/PI58548) (Tc*Lr34*) served as standards.

RESULTS AND DISCUSSION
North American Wheats

In the 40 current and older soft red winter wheat (SRWW) cultivars and the 15 Pacific Northwest spring wheat cultivars evaluated, the only allele detected was the 229-bp *csLV34*a–allele associated with the absence of *Lr34* (Table 1). The absence of the *csLV34b* allele associated with *Lr34* was unexpected since Chinese Spring (which has *Lr34)* was used

Table 1. Distribution of *csLV34* alleles in North American wheat cultivars.

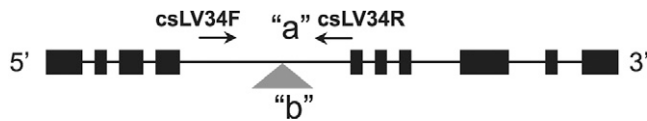| Cultivar class	csLV34[†]	Cultivar class	csLV34[†]
Soft red winter wheat current cultivars		2174	b
AGS 2000	a	Onaga	a
CK 9511	a	Dumas	a
CK 9436	a	Millennium	a
CK 9663	a	NuFrontier	a
CK 9835	a	Stanton	a
CK9803	a	2145	a
Crawford	a	Cutter	a
Featherstone 176	a	NuHills	b
Foster	a	TAM 111	a
Jackson	a	Santa Fe	b
Massey	a	Overley	a
McCormick	a	Deliver	b
NC Neuse	a	Endurance	a
Patton	a	Hallam	a
Pioneer 2580	a	**Hard red winter wheats older cultivars**	
Pioneer 26R12	a	Turkey	a
Pioneer 26R15	a	Triumph	a
Pioneer 26R61	a	Bison	a
Pochontas	a	Scout66	a
Renwood 3706	a	Eagle	a
Roane	a	Newton	b
S5 560	a	Arkan	a
Saluda	a	Siouxland	a
Sisson	a	Tomahawk	a
SS 520	a	Victory	a
SS 550	a	Karl92	a
Tribute	a	Ogallala	b
USG 3342	a	Sturdy	b
Truman	a	**Pacific Northwest wheats current cultivars**	
Soft red winter wheat older cultivars		Alpowa	a
Fultz	a	Alturas	a
Coastal	a	Blanca Grande	a
Taylor	a	Hank	a
Monon	a	Hyak	a
Knox62	a	ID0377S	a
Arthur	a	Jefferson	a
Caldwell	a	Louis	a
CK68-15	a	Madsen	a
Mediterranean	a	Nick	a
Vigo	a	Scarlet	a
Wabash	a	Stephens	a
Hard red winter wheat current cultivars		Wakanz	a
TAM 107	a	Westbred 936	a
Jagger	b	Zak	a
T81	a		
2137	a		

[†]a, *csLV34a*; b, *CSLV34b*.

Figure 1. Schematic representation of the sulfate transporter-like gene from the D genome of wheat showing region within an intron targeted by the *csLV34* F and R primers. The full length amplified product corresponds to the *csLV34a* allele and the triangle represents the deleted region resulting in the shorter *csLV34b* allele. The dark boxes correspond to exons.

as a parent in the development of the SRWW cultivar Knox from Purdue University. The frequency of the *csLV34b*-allele linked to *Lr34* (150 bp) was low in the hard red winter wheat (HRWW) cultivars evaluated, as only 8 of the 32 cultivars had the allele diagnostic of *Lr34* (Table 1). The presence of *Lr34* in the HRWW cultivars Jagger and Newton, is uncertain (R. Bowden, personal communication, 2007). The HRWW Sturdy was genetically determined to have *Lr34* (Dyck, 1991) and the accession used in this study had allele *csLV34b,* diagnostic of *Lr34.*

In contrast, the northern hard red spring wheat (HRSW) cultivars from the midwestern United States and Canada had the highest frequency of the *csLV34b*-allele associated with *Lr34* (Table 2). Of the 75 cultivars evaluated 32 had the *csLV34b* allele diagnostic of *Lr34.* Five HRSW cultivars displayed unique marker phenotypes. In four cultivars, PCR amplification resulted in a three-band pattern that included both *csLV34 a* and *b* alleles together with an additional band with a higher molecular weight (ca. 50 bp bigger than *csLV34a*). In the fifth cultivar (Pasqua), the *csLV34b* allele associated with *Lr34* was detected, in addition to a higher molecular weight band of approximately 320 bp.

The *Lr34*–associated *csLV34b* allele was not present in the U.S. HRSW cultivars previous to 1965. In the mid-1960s with the release of Chris, which was the first spring wheat in the United States with leaf rust resistance derived from Frontana, cultivars with this allele were common until the early 1990s. Nearly all U.S. cultivars released between 1994 and 1998 had the *csLV34a* allele not associated with *Lr34.* This included the years after the major Fusarium head blight (caused by *Fusarium graminearum* Schwabe; FHB) epidemics of 1993 and following years in the upper Midwest. Wheat cultivars released in the mid-1990s were selected for some degree of tolerance to FHB, which may have eliminated germplasm lines with *Lr34* that were highly resistant to leaf rust. Cultivars with the *csLV34b* allele associated with *Lr34* began to reappear in 1999, and presently cultivars with this allele are common, though not at the levels observed before the mid-1990s. The frequency of *csLV34b* also varied among the spring wheat breeding programs in the United States. After 1965, 11 cultivars from the University of Minnesota had *csLV34b*, and four cultivars had *csLV34a*. From North

Dakota State University, nine cultivars had allele *csLV34a*, only one cultivar had *csLV34b*, and two cultivars had a three-band pattern. From South Dakota State University, four cultivars released after 1965 had *csLV34a* and four had *csLV34b.* From the cultivars developed by private breeding companies, six had *csLV34a*; nine had *csLV34b*, and one had a three-band pattern.

The presence of *csLV34a* and *csLV34b* in the U.S. and Canadian HRSW was highly correlated with the absence or presence of *Lr34* as determined in genetic analyses (Kolmer, 1996). The cultivars Era, Glenlea, Wheaton, Roblin, Laura, CDC Teal, Pasqua, Norm, AC Domain, Alsen, Knudson, and Briggs were determined to have *Lr34* and also had the corresponding *csLV34b* allele (Table 2). The cultivars Neepawa, Columbus, Katepwa, AC Taber, AC Cora, AC Karma, AC Barrie, Ingot, and Ivan were determined to lack *Lr34* and had the corresponding *csLV34a* allele. The cultivar Grandin was determined to have *Lr34*, yet had the *csLV34a* allele. Grandin was heterogeneous for *Lr16* (Liu and Kolmer 1997) and for bread-making quality traits. It is possible that Grandin was also heterogeneous for *Lr34*. AC Splendor was determined to have *Lr34* (Kolmer and Liu 2002) yet had the *csLV34a* allele. Recombination between the *csLV34* locus and *Lr34* may have occurred in the development of this cultivar.

CIMMYT-Derived Cultivars and Germplasm

Previous studies showed that a significant proportion of wheat cultivars derived from CIMMYT germplasm contained *Lr34/Yr18* (Singh and Rajaram, 1991). The presence of *Lr34/Yr18* was largely deduced by the phenotypic response of adult plants to leaf rust and stripe rust infection and under appropriate environmental conditions the expression of a leaf tip necrosis phenotype (Ltn). A total of 127 wheat cultivars were observed at CIMMYT (either Obregon or Toluca) for Ltn, using the phenotypes of Thatcher+*Lr34* (RL6058) and Thatcher as controls for the presence and absence of Ltn, respectively. With some of the cultivars previous tests investigating the presence or absence of *Lr34/Yr18* Ltn phenotypes had been established. Of the 127 wheat cultivars 52 were characterized as lacking Ltn (Table 3, Group 1) while 75 showed the phenotype (Table 3, Groups 2 and 3). Analysis with the csLV34 marker showed that all of the non-Ltn phenotypes carried the *csLV34a* allele (Fig. 2). The alternative allele, *csLV34b* (Fig. 2) associated with *Lr34/Yr18* was found in 62 out of the 75 cultivars that expressed the Ltn phenotype. All CIMMYT-derived cultivars and germplasm previously shown to have *Lr34/Yr18* based on a combination of APR for leaf rust, stripe rust, and temperature-sensitive seedling response to leaf rust as well as Ltn were among the 62 cultivars that had the *csLV34b* allele. These include Yaqui 50, Chapingo 53, Jaral 66, Bajio 67, Torim 73, Zacatecas 74, Tesopaco 76, Pima 77, Nainari 60, Penjamo 62, Nadadores

Table 2. Distribution of *csLV34* alleles in hard red spring wheat (HRSW) cultivars from North America.

HRSW	csLV34[†]	Released	Program[‡]	Lr34[§]	HRSW	csLV34	Released	Program	Lr34
Marquis	a	1909	CA		AC Splendor	a	1996	CA	+
Hope	a	1927	SDSU		BacUp	a	1996	UMN	
Thatcher	a	1935	UMN		AC Barrie	a	1996	CA	−
Renown	a	1937	CA		AC Cadillac	b	1996	CA	
Lee	a	1950	UMN		Forge	a	1997	SDSU	
Selkirk	a	1953	CA		Keene	a	1997	NDSU	
Crim	a	1963	UMN		Dandy	a	1998	IND	
Chris	b	1965	UMN		HJ98	a	1998	UMN	
Polk	b	1968	UMN		Ingot	a	1998	SDSU	−
Neepawa	a	1969	CA	−	McKenzie	m	1998	IND	
Waldron	a	1969	NDSU		Ember	a	1999	SDSU	
Era	b	1970	UMN	+	Ivan	a	1999	IND	−
Fletcher	b	1970	UMN		Parshall	a	1999	NDSU	
Glenlea	b	1972	CA	+	Reeder	a	1999	NDSU	
Columbus	a	1980	CA		AC Amazon	b	1999	CA	
Katepwa	a	1981	CA	−	McVey	b	1999	UMN	
Marshall	b	1982	UMN		Mercury	b	1999	IND	
Wheaton	b	1983	UMN	+	Norpro	b	1999	IND	
Stoa	a	1984	NDSU		Superb	a	2000	CA	
Butte 86	m	1986	NDSU		Alsen	b	2000	NDSU	+
Roblin	b	1986	CA	+	Keystone	b	2001	IND	
Laura	m	1986	CA	+	Knudson	b	2001	IND	+
Amidon	m	1988	NDSU		Walworth	b	2001	SDSU	
Grandin	a	1989	NDSU		Granite	a	2002	IND	
Vance	a	1990	UMN		Briggs	b	2002	SDSU	+
2375	b	1990	IND		Hanna	b	2002	IND	
CDC Teal	b	1990	CA	+	Dapps	a	2003	NDSU	
Minnpro	b	1990	UMN		Oklee	b	2003	UMN	
Pasqua	b*	1990	CA	+	Banton	a	2004	IND	
AC Taber	a	1991	CA	−	Steele-ND	a	2004	NDSU	
Norm	b	1992	UMN	+	Trooper	a	2004	IND	
AC Domain	b	1993	CA	+	Freyr	b	2004	IND	
AC Cora	a	1994	CA	−	Granger	b	2004	SDSU	
Gunner	a	1995	IND		Polaris	b	2004	IND	
Russ	a	1995	SDSU		Saturn	b	2004	IND	
Oxen	b	1995	SDSU		Glenn	a	2005	NDSU	
Verde	b	1995	UMN		Ulen	a	2005	UMN	−
AC Karma	a	1996	CA	−	AC Vista	b		CA	

[†]a, *csLV34a*; b, *csLV34b*; m, multiple bands; b*, b allele plus an additional fragment (320 bp).

[‡]CA, Canada; UMN, University of Minnesota; NDSU, North Dakota State University; SDSU, South Dakota State University; IND, independent company.

[§]+, *Lr34* genetically determined to be present; −, *Lr34* absent.

63, Lerma Rojo 64, Tobari 66, Cocraque 75, Nacozari 76, Tesia 79, Sonoita 81, Tonichi 81, Opata 85, Yaco, Esmeralda 86, Ocoroni 86, Curinda 87, Parula, Trap, and Mango (Singh and Rajaram, 1992; Singh, 1993).

Two exceptions (Singh, 1993) were the cultivars Salamanca 75 and Rayon 89 which were inferred to carry *Lr34* but possess the *csLV34a* allele. However, a recent allelism test using Rayon 89 and testers for *Lr34* and *Lr46* slow rusting genes conducted at CIMMYT has shown that resistance of Rayon 89 involved gene *Lr46* instead of *Lr34*. Because *Lr46* is also known to be associated with some degree of *Ltn* (Rosewarne et al., 2006), marker csLV34 was able to rectify the previous postulation. Either the resistance of Salamanca 75 also involved *Lr46* instead of *Lr34*. Alternatively, limited recombination that occurs between *csLV34* and *Lr34/Yr18*, estimated at 0.4 cM, may suggest Salamanca 75 as an example of cultivars derived from recombinants between *csLV34* and the dual APR genes. Because none of the newer CIMMYT wheat materials have shown this recombination status, we strongly

Table 3. Wheat cultivars and breeding lines evaluated at CIMMYT for leaf tip necrosis (Ltn).

Group 1 = *csLV34a* without Ltn		Group 2 = *csLV34b* with Ltn		Group 3 = *csLV34a* with Ltn	
Cultivar	Country	Cultivar	Country	Cultivar	Country
Ahome 70	Mexico	Anza	USA	Chilero[†]	Mexico
Anahuac 75	Mexico	Bacanora 88	Mexico	Eagle	Australia
Apache 81	Mexico	Bajio 67	Mexico	Gamenya[‡]	Australia
Azteca 67	Mexico	BH 1146[‡]	Brazil	Kenya Kongoni	Kenya
Bobwhite[†]	Mexico	Buck Atlantico	Argentina	Kundan	India
Cajeme 71	Mexico	C271[‡]	Pakistan	Marcos Juarez	Argentina
Ciano 79	Mexico	Chapingo 53[‡]	Mexico	NP846[‡]	India
Cleopatra 74	Mexico	Cocoraque 75	Mexico	NP876[‡]	India
Diamante INTA	Argentina	Condor	Australia	Ponta Grossa 1[‡]	Brazil
Galvez 87	Mexico	Cook	Australia	Rayon 89	Mexico
Genaro 81	Mexico	CSP44	Australia	Salamanca 75	Mexico
Glennson 81	Mexico	Cumpas 88	Mexico	Sujata[‡]	India
Guasave 81	Mexico	Curinda 87	Mexico	Tarachi 2000	Mexico
HD2281	India	Era	USA		
HD2329	India	Esmerealda 86	Mexico		
Hermosillo 77	Mexico	Fret 2[†]	Mexico		
Hira	India	Frontana[‡]	Brazil		
Inia 66	Mexico	Girija	India		
Jupateco 73S	Mexico	Huanca[‡]	Peru		
K7410	India	HUW234+ Lr34	India		
Kehan 1	China	Jaral 66	Mexico		
Kenya Nyati	Kenya	Jauhar 78	Pakistan		
Mayo 64	Mexico	Kenya Nigri[‡]	Kenya		
Mexico 82	Mexico	Kenya Fahari[‡]	Kenya		
Millewa	Australia	Kenya Zabadi[‡]	Kenya		
Mochis 73	Mexico	Klein Cartucho	Argentina		
Narro 74	Mexico	Kung Chiao #284	China		
Noroeste 66	Mexico	Lerma Rojo 64	Mexico		
Papago 86	Mexico	Mango[†]	Mexico		
Pavon 76	Mexico	Maringa[‡]	Brazil		
PBW343	India	Nacozari 76	Mexico		
Potam 70	Mexico	Nadadores 63	Mexico		
RL6077	Canada	Nainari 60	Mexico		
Saric 70	Mexico	Neelkant	Algeria		
Seri 82	Mexico	Norteño 67	Mexico		
Sham 2	Syria	Ocoroni 86	Mexico		
Siete cerros 66	Mexico	Opata 85	Mexico		
Sonalika	India	Parula[†]	Mexico		
Sonora 64	Mexico	Penjamo 62	Mexico		
Spica	Australia	Pima 77	Mexico		
Tacupeto 2001	Mexico	Pionero INTA	Argentina		
Tanori 71	Mexico	RL6058	Canada		
Thatcher	Canada	Shailja	India		
UP201	India	Sinchi[‡]	Peru		
Valluno 78	Bolivia	Sonoita 81	Mexico		
Vicam 71	Mexico	Tanager[†]	Mexico		
Victoria INTA	Argentina	Tesia 79	Mexico		
Weebill 1[†]	Mexico	Tesopaco 76	Mexico		
WH147	India	Tobari 66	Mexico		
WL711	India	Tonichi 81	Mexico		
Yecora 70-Lr34	Mexico	Torim 73	Mexico		
Zaragoza 75	Mexico	Toropi[‡]	Brazil		
		Trap[†]	Mexico		
		T. Pintos Precoz	Argentina		
		Veranopolis[‡]	Brazil		
		VL 404	India		
		Wheaton	USA		
		Yaco[†]	Mexico		
		Yaqui 50[‡]	Mexico		
		Yecora 70+Lr34	Mexico		
		Yehe T50 #2	China		
		Zacatecas 74	Mexico		

[†]CIMMYT line
[‡]Tall cultivar.

believe that postulation of *Lr34* in Salamanca 75, released in Mexico in 1975, is another case of wrong postulation based on Ltn and slow rusting characteristics. A near-isogenic line of the cultivar Thatcher, RL6077, described by Dyck et al. (1994) as carrying *Lr34*-like gene on a reciprocally translocated chromosome, was observed to have a smaller Ltn phenotype that was equivalent to Thatcher and had more leaf and stripe rust severity compared to RL6058. RL6077 carried the *csLV34a* allele in contrast to the *csLV34b* allele present in RL6058. It remains to be determined if the leaf and stripe rust resistance genes as well as the Ltn phenotype found in RL6077 are identical to *Lr34/Yr18* and originated from chromosome 7D or represent a different gene.

Australian Cultivars

A set of Australian cultivars released from 1990 to 2005 and some of the older cultivars were tested for variation at the *csLV34* locus. The *csLV34b* allele occurred in more than half of the cultivars released in the southeastern states of Victoria, New South Wales, and Queensland (Table 4). In contrast almost all the cultivars from Western and South Australia had the *csLV34a* allele associated with the absence of *Lr34/Yr18*. Most of the cultivars with the *csLV34b* allele have pedigrees that trace back to the cultivars Condor, Cook, and Oxley. These three cultivars have been shown to carry *Lr34/Yr18*, which tracks back to a common CIMMYT-derived line WW15 and has identical pedigree as the cultivar Anza (Fig. 2).

Near-isogenic lines and genetic stocks (Avocet+*Lr34/Yr18*, Lalbahadur+*Lr34/Yr18*, Thatcher+*Lr34/Yr18*, and Jupateco 73R) all of which contain the *csLV34b* allele provide moderate levels of resistance against a recently detected and highly aggressive pathotype, Pt134E16A+ of *Puccinia striiformis* f. sp. *tritici*, in Australia. The corresponding recurrent parents

of these isolines, which have the *csLV34a* allele are susceptible. The Australian cultivar H45, from New South Wales, which possesses *csLV34b*, is susceptible to this stripe rust race. However the rate of stripe rust development on H45 was observed to be slower than on the highly susceptible cultivar Morocco (H.S. Bariana, unpublished data, 2007). If H45 carries *Yr18* it suggests that the presence of *Yr18* alone does not provide adequate resistance under high disease pressure. H45 had leaf rust resistance similar to cultivars with *Lr34* in field plots at Obregon in 2006 (H.S. Bariana, unpublished data, 2007). Alternatively, H45 may be considered an example of a cultivar with recombination between *Yr18* and *csLV34b*. Further allelism tests will be necessary to confirm this observation.

The higher frequency of the *Lr34/Yr18* associated *csLV34b* allele in cultivars from the southeastern wheat regions of Australia could be attributed to the utilization of CIMMYT derived germplasm. The paucity of *Lr34/Yr18* containing wheats in West and South Australia as inferred from the presence of the *csLV34a* allele provides an opportunity to specifically incorporate these genes by marker–assisted selection into genotypes adapted to these areas of Australia. A significant benefit of *Lr34/Yr18* in Australian cultivars is their inability to sustain high levels of leaf rust infections during late winter and early spring. This is in contrast with cultivars commonly grown in Western Australia which have high levels of leaf rust severity throughout the entire growing season (McIntosh et al., 1995; Singh et al., 2007).

Variation in Western European Wheats

A representative collection of Western European winter wheat cultivars was evaluated for variation at the *csLV34* locus (Table 5). Wheat cultivars from this collection form part of the European Union project on Genetic Diversity in Agriculture: Temporal Flux (GEDIFLUX) genotypes chosen to represent the most diversely related winter wheats in the United Kingdom, Austria, Belgium, Denmark, France, Germany, Netherlands, and Sweden. A total of 80 cultivars were analyzed and with the exception of Kavkaz all had the *csLV34a* allele associated with the lack of *Lr34/Yr18* (Table 5).

The prevalence of the *csLV34a* allele suggests sources of the dual APR genes tightly linked to *csLV34b* have rarely been used in Western European winter wheat breeding. Very few European winter wheats, for example, the Swiss cultivar Forno and the Russian cultivar Bezostaya–1, have been documented to carry *Lr34* (Schnurbusch et al., 2004). Both cultivars contain the *csLV34b* allele (Lagudah et al., 2006) and the presence of this allele in Kavkaz may have been derived from one of its parents, Bezostaya–1.

The predominance of the *csLV34a* allele in Western European winter wheat is similar to the fixation of this allele among the North American SRWW. Given that
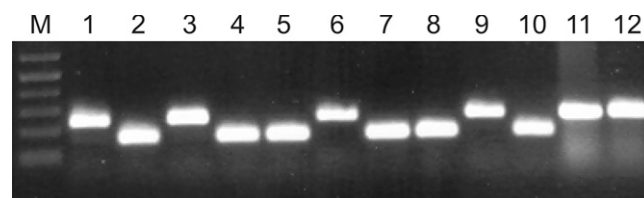

Figure 2. Polymerase chain reaction amplification products from wheat cultivars using *csLV34* F and R primers. M, 100-bp ladder size marker; 1, Pavon; 2, Frontana; 3, Seri 82; 4, WW15; 5, Anza; 6, Renan; 7, Mentana; 8, Condor; 9, Sonora 64; 10, RL6058 = Thatcher +*Lr34*; 11, Thatcher; 12, Hope. The larger amplification product (229 bp) corresponds to *csLV34a* and the smaller product (150 bp) is the *csLV34b* allele.

successful cultivars have been bred from winter wheats such as Bezostaya–1 and Forno, which carry the *csLV34b* allele and *Lr34* there is no reason to expect that this region of wheat chromosome 7D would be unfavorable to winter wheat selection and adaptation.

Variation in Japanese Wheats

Japanese cultivars were tested following reports that Fukuho, a Japanese cultivar, carried *Lr34/Yr18* and Ltn (Suenaga et al., 2003) and possessed the *csLV34b* allele (Lagudah et al., 2006). Of nine cultivars analyzed, Norin10, Norin26, Norin130, Chihoku, and Kitakami-komugi carried the *csLV34b* allele while Norin44, Norin67, Kitami33, and Akakomugi had the *csLV34a* allele. From previous observations in Japan, the cultivars Haru-yutaka (Norin130), Chihoku, and Kitakami-komugi had Ltn and adult plant leaf rust resistance (K. Suenaga, personal communication, 2007). Akakomugi and a line derived from Norin10/Brevor14 are among the founder parents used in the introduction of dwarfing genes *Rht-B1b* and *Rht-D1b* into CIMMYT wheat. If the Norin10/Brevor14 derivative was fixed for the *csLV34b* allele derived from Norin10, it suggests that in addition to Frontana, the primary source of *Lr34/Yr18* and *csLV34b* in CIMMYT wheat, Norin 10 may have also contributed this allele to the CIMMYT gene pool. The Argentinean cultivar Tezanos Pintos Precoz (TZPP) which contains the *csLV34b* allele is also considered among the contributors of *Lr34* into CIMMYT germplasm.

Landraces vs. Improved Cultivars

We examined 870 landraces from 29 countries distributed across North Africa, the Middle East, south Western Europe, South and Central Asia, and China (Fig. 3). Similar to the cultivated wheats two alleles corresponding to *csLV34a* and *csLV34b* were detected within the targeted region of *csLV34*. However the *csLV34b* allele was absent in most of the landraces and was at low frequency (Fig. 3) when compared with the overall frequency in improved cultivated wheats. This disparate distribution between cultivars and landraces at the *csLV34* locus may be directly

Table 4. Distribution of *csLV34* alleles in wheat cultivars from different states in Australia.

Cultivar	csLV34[†]	State[‡]	Cultivar	csLV34[†]	State[‡]
Amhem	a	QLD	Kewell	a	VIC
Hartog	a	QLD	Matong	a	VIC
Kennedy	a	QLD	Monad	a	VIC
Leichardt	a	QLD	More	a	VIC
Mawson	a	QLD	Muchmore	a	VIC
Banks	b	QLD	Olympic	a	VIC
Bass	b	QLD	Wyuna	a	VIC
Batavia	b	QLD	Annuello	b	VIC
Baxter	b	QLD	Beulah	b	VIC
Cook	b	QLD	Chara	b	VIC
Cunningham	b	QLD	Goroke	b	VIC
Diaz	b	QLD	Kiata	b	VIC
Flinders	b	QLD	Meering	b	VIC
Giles	b	QLD	Minto	b	VIC
Hume	b	QLD	Mira	b	VIC
Janz	b	QLD	Mitre	b	VIC
Lang	b	QLD	Cocamba	b	VIC
Petrie	b	QLD	Aroona	a	SA
Strzelecki	b	QLD	Bindawarra	a	SA
Tasman	b	QLD	Bowie	a	SA
Vasco	b	QLD	Dagger	a	SA
Bowerbird	a	NSW	Excalibur	a	SA
Chough	a	NSW	Frame	a	SA
Diamondbird	a	NSW	Halberd	a	SA
Gamenya	a	NSW	Krichauff	a	SA
Lark	a	NSW	Kukri	a	SA
Mendos	a	NSW	Machete	a	SA
Qual2000	a	NSW	Molineux	a	SA
Sunbrook	a	NSW	Schomburgk	a	SA
Suneca	a	NSW	Spear	a	SA
Sunkota	a	NSW	Stileto	a	SA
Sunlin	a	NSW	Tatiara	a	SA
Triller	a	NSW	Trident	a	SA
Babbler	b	NSW	Warigal	a	SA
Currawong	b	NSW	Yitpi	a	SA
Egret	b	NSW	Ajana	a	WA
Festival	b	NSW	Arrino	a	WA
H45	b	NSW	Bodallin	a	WA
Lorikeet	b	NSW	Cadoux	a	WA
Mercury H	b	NSW	Calingri	a	WA
Mulgara	b	NSW	Camm	a	WA
Petrel	b	NSW	Carnamah	a	WA
Quarrion	b	NSW	Cascades	a	WA
Rosella	b	NSW	Cirrigin	a	WA
Snipe	b	NSW	Cranbrook	a	WA
Sunbri	b	NSW	Cunderlin	a	WA
Sunco	b	NSW	Gutha	a	WA
Sunpict	b	NSW	Kalgarin	a	WA
Sunsoft 98	b	NSW	Kulin	a	WA
Sunstar	b	NSW	Madden	a	WA
Sunvale	b	NSW	Perenjori	a	WA
Thornbill	b	NSW	Ruby	a	WA
Vulcan	b	NSW	Tasman	a	WA
Whistler	b	NSW	Westonia	a	WA
Wylah	b	NSW	Wyalkatchem	a	WA
Warbler	b	NSW	Sapphire	b	WA
Declic	a	VIC			
Goldmark	a	VIC			
Kelalc	a	VIC			

[†]a, *csLV34a*; b, *csLV34b*.

[‡]QLD, Queensland; NSW, New South Wales; VIC, Victoria; SA, South Australia; WA, Western Australia.

or indirectly due to selection and breeding efforts aimed at incorporating *Lr34/Yr18* into modern cultivars.

Among the landraces those from China had the highest frequency (30%) of the *csLV34b* allele. Wheat genotypes obtained from China, including Chinese Spring, were among the earliest documented sources of *Lr34* (Dyck 1977). In addition, the source of *Lr34/Yr18* in the reference genetic stock, RL6058 (= Thatcher +*Lr34/Yr18/Ltn1*) was from a Chinese wheat introduction (Dyck and Samborski, 1982).

D Genome Containing Species and Synthetic Hexaploids

Specificity of *csLV34* primers for an intron region of the sulfate transporter-like gene located on chromosome 7D from both *T. aestivum* and the diploid D genome progenitor (Lagudah et al., 2006) served as the basis for investigating variation in a representative collection of *A. tauschii* distributed across their primary centers of origin. These included 50 *A. tauschii* accessions from Turkey, Armenia, Azerbaijan, Georgia, Iran, Iraq, Afghanistan, Pakistan, and other regions from the former Soviet Union. All accessions carried the *csLV34a* allele; the accession AL8/78 from Armenia reported by Dvořák et al. (1998) to be the closest to the D genome of *T. aestivum* also contained the *csLV34a* allele. Further analysis of other D genome–containing *Aegilops* polyploids, *A. cylindrica*, *A. crassa*, *A. ventricosa*, *A. vavilovii*, and *A. juvenalis*, also had the *csLV34a* allele. Similarly all the other *T. aestivum* subspecies (*spelta*, *macha*, *compactum*, *sphaerococcum*, and *vailovii*) analyzed in this study were invariant and carried the *csLV34a* allele. The absence of the *csLV34b* allele in the diploid D genome progenitor from the current *A. tauschii* gene pool, suggests that this allele may have arisen subsequent to hexaploid wheat synthesis. While it is common to find wider allelic variation within the *A. tauschii* gene pool relative to the D genome of *T. aestivum* (Ogbonnaya et al., 2006) there are some rare examples of genetic variants found in bread wheat that are yet to be discovered in the diploid D genome progenitor. A case in point is the *GluD1d* allele of significance to bread-making that encode a variant gene of the high molecular weight subunit of glutenin with an extra cysteine residue present in *T. aestivum*, which to date is nonexistent at the corresponding *GluD^{t}1* locus from *A. tauschii* (Pflueger et al., 2001).

We also investigated 250 synthetic hexaploids reconstituted from crosses between different tetraploid wheat and *A. tauschii* accessions. Of these, 230 synthetic hexaploids carried the *csLV34a* allele whereas 15 (6%) had the *csLV34b* allele (Fig. 4). As expected the parental tetraploid lines gave no amplification product (Fig. 4) confirming the specificity of the *csLV34* primers for the D genome. However, the presence of the *csLV34b* alleles in 6% of the synthetics was unexpected as none of the *A. tauschii* accessions

Table 5. Distribution of *csLV34* alleles in Western European wheat.

GEDIFLUX wheats[†]		Historic UK wheats	
Cultivar	*csLV34*[‡]	Cultivar	*csLV34*
Albatross	a	Admiral	a
Avalon	a	Apollo	a
Beaver	a	Aquila	a
Cadenza	a	Armada	a
Cezanne	a	Bersee	a
Erla	a	Bouquet	a
Kolben	a	Brigadier	a
Etoile de Choisy	a	Brigand	a
Extrem	a	Cappelle Desprez	a
Flair	a	Elite Le Peuple	a
Flame	a	Fenman	a
Florida	a	Flamingo	a
Hereward	a	Galahad	a
Mega	a	Genesis	a
Multiweiss	a	Haven	a
Nautica	a	Holdfast	a
Palur	a	Hunter	a
Perlo	a	Hustler	a
Recital	a	Hybrid 46	a
Regina	a	Juliana	a
Renan	a	Lingbow	a
Riband	a	Marchal	a
Rimpaus Braun	a	Maris Huntsman	a
Schweigers Taca	a	Maris Nimrod	a
Soissons	a	Maris Ranger	a
Sperber	a	Maris Widgeon	a
Stamm101	a	Mercia	a
Svale	a	Minister	a
Tadorna	a	Mission	a
Virgo	a	Norman	a
Kavkaz	b	Pilot	a
		Rapier	a
		Redman	a
		Spark	a
		Staring	a
		Steadfast	a
		Stetson	a
		Thor	a
		Torfrida	a
		Virtue	a
		Warden	a

[†]GEDIFLUX, European Union Genetic Diversity in Agriculture: Temporal Flux wheat genotypes.

[‡]a, *CSLV34a*; b, *CSLV34b*.

carried this allele. Some individual plants within these synthetics were found to carry both alleles indicative of heterozygosity at the locus (Fig. 4) which may have arisen from limited outcrossing with *T. aestivum* genotypes carrying *csLV34b* allele. Further tests using DNA sequences from a gene encoding a kinase-U box domain (KUDS)
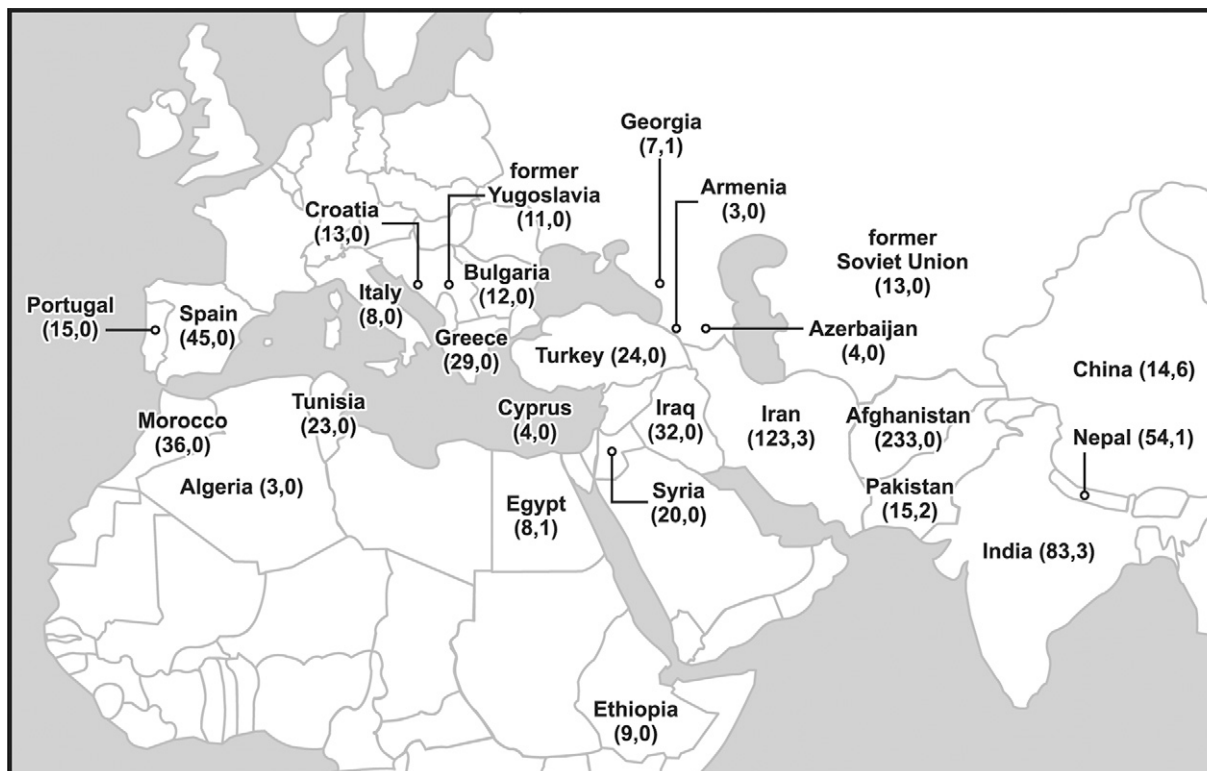
Figure 3. Distribution of *csLV34* alleles in wheat landraces. Numbers in parentheses refer to the a and b alleles, respectively.

that differentiate the D genomes of *T. aestivum* (DD) from *A. tauschii* (D^tD^t) (Lagudah et al., 2006) were conducted on the synthetic hexaploids. The KUDS locus flanks the *Lr34/Yr18* locus and was estimated at 2.5 cM from *csLV34*. All 15 synthetic hexaploids that possessed the *csLV34b* allele were also shown to contain a *T. aestivum* D genome for the KUDS locus while the remaining synthetics were confirmed as carrying the expected D^t genome (data not shown). These observations lend further support that the unusual occurrence of *csLV34b* allele in a small proportion of synthetic hexaploid genotypes may have been derived from *T. aestivum* through outcrossing.

While a number of leaf rust and stripe rust resistance gene loci have been described in the D^t genome of *A. tauschii* and synthetic wheats, there are no reports of

gene(s) *Lr34/Yr18*. Masking effects of major genes for rust resistance can confound the presence of *Lr34/Yr18* genes if they exist in the diploid D genome. Exploiting natural variation in *Lr34/Yr18* for novel variants is an attractive objective if these genes occur in the D^t gene pool. In the absence of a cloned gene(s) for *Lr34/Yr18*, preliminary insights into this region of the genome can be deduced from the strong linkage disequilibrium observed with the bi-allelic *csLV34* variants in wheat cultivars. Barring any fixed recombination events, the occurrence of the monomorphic *csLV34a* allele in the D^t genome suggests a low probability of finding *Lr34/Yr18* within this gene pool. Definitive conclusions on the presence or absence of *Lr34/Yr18* in the D^t gene pool will become evident from the eventual cloning of the gene(s).

Origin(s) of *Lr34* in Wheat Germplasm

A combination of the simple molecular genotyping tool provided by the *csLV34* marker together with the knowledge of some of the well-established cultivars with and without *Lr34/Yr18* enabled the most likely origin(s) of this important pair of APR genes to be tracked. The Brazilian cultivar Frontana has been traced as the source of *Lr34/Yr18* in a significant proportion of CIMMYT cultivars as well as HRSWs from North America. In this study we tracked the inheritance of the *csLV34b* allele within the lineage of Frontana back to the crosses made in Italy by Nazareno Strampelli in the early 1900s (Borghi, 2001). The cultivar Mentana, was introduced from Italy to South America and
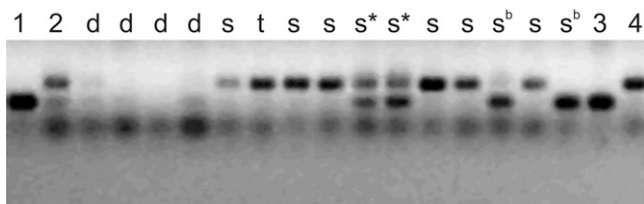

Figure 4. Polymerase chain reaction amplification products using *csLV34* F and R in synthetic hexaploids (s), durum cultivars (d), and *Aegilops tauschii* (t). Lanes designated with s* are synthetic hexaploids where both alleles were amplified and s^b are those rare synthetic hexaploids (6%) that were found to possess the *csLV34b* allele. Lanes 1 to 4 represent bread wheat controls with either the *csLV34a* or *b* alleles; Frontana (b), Hope (a), Chara (b), and Yitpi (a), respectively.

crossed with Frontiera to produce Frontana; Mentana carried the *csLV34b* allele while Frontiera had the *csLV34a* allele (Fig. 5). Mentana was also introduced to other parts of the world such as Turkey and China (reselected as Nanda2419) and grown in large areas over long periods due in part to its reported rust resistance (Borghi, 2001; Braun et al., 2001). A sib cultivar to Mentana, developed from the same program in Italy by Strampelli and released as Ardito was also found to carry the *csLV34b* allele; Ardito serves as the most likely donor of the *Lr34/Yr18* associated *csLV34b* allele as traced through the lineage of European winter wheat Bezostaya-1 and Kavkaz (Fig. 5). The origin of the *csLV34b* allele from the likely parental donor, 'Selezione 21' from the heterogeneous landrace 'Rieti' used by Strampelli (Borghi, 2001), has yet to be confirmed. Currently available accessions of 'Rieti' contain the *csLV34a* allele (A. Gennaro and C. Ceoloni, unpublished data, 2008).

With Mentana traced as the donor of the *csLV34b* allele found in Frontana, subsequent derivatives of crosses with Frontana in the CIMMYT program led to cultivars such as Penjamo 62, Lerma Rojo, and Nainari 60. These, cultivars in turn became the founders for germplasm with *Lr34/Yr18* as found in WW15, and Anza that served as key donors for this pair of APR genes in the Australian wheat gene pool. Frontana was the source of *Lr34* for the U.S. wheats Chris and Era, which were used extensively as parents, and was also the source of *Lr34* in the high quality Canadian spring bread wheats. Other donors outside of the Mentana/Ardito lineage, in particular those of Chinese origin, contributed to the *Lr34/Yr18/csLV34b* haplotype in Japanese cultivars.

CONCLUSIONS

The use of the tightly linked intron size variants (*csLV34*) of a sulfate transporter-like gene tightly linked to the adult plant rust resistance genes *Lr34/Yr18* provided insights into variation in this region of a diverse wheat gene pool that included, landraces, synthetic hexaploids, modern and historic wheat cultivars, and D genome–containing polyploid species. Two prevalent alleles at the csLV34 locus occur in modern cultivars of which the allele *csLV34b* shows strong association with *Lr34/Yr18*. In contrast, only one size variant, *csLV34a*, was detected in a collection of putative D-genome progenitors, which indicated that the genes *Lr34/Yr18* arose in hexaploid wheats after the hybridization of the AABB tetraploid wheats with the diploid D-genome progenitors. Genotypic postulations of *Lr34/Yr18* based on the csLV34 marker in
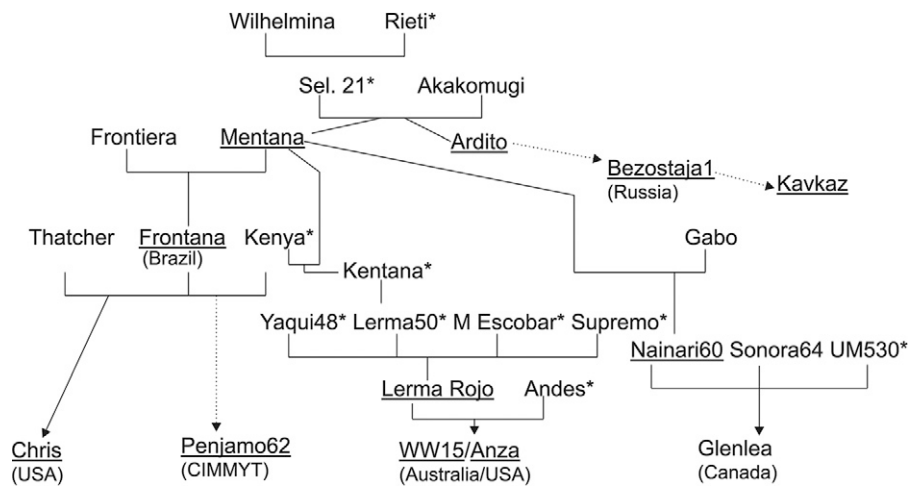

Figure 5. Pedigree and *csLV34* classification of *Lr34/Yr18* cultivar entries into different wheat breeding programs. Cultivars underlined carry *csLV34b* and the others carry *csLV34a* allele except those with an asterisk which were not tested. The dotted lines refer to the lineage.

wheat germplasm will enhance the prospects of determining other uncharacterized dual APR genes that may exist elsewhere in the wheat genome.

References

Borghi, B. 2001. Italian wheat pool. p. 289–309. *In* A.P. Bonjean and W.J. Angus (ed.) The world wheat book: A history of wheat breeding. Intercept, London.

Bossolini, E., S.G. Krattinger, and B. Keller. 2006. Development of SSR markers specific for the Lr34 resistance region of wheat using sequence information from rice and *Aegilops tauschii*. Theor. Appl. Genet. 113:1049–1062.

Braun, H.J., N. Zencirci, F. Altay, A. Atli, A. Muzaffer, V. Esser, M. Kambertay, and T.S. Payne. 2001. Turkish wheat pool. p. 851–879. *In* A.P. Bonjean and W.J. Angus (ed.) The world wheat book: A history of wheat breeding. Intercept, London.

Dvořák, J., M.-C. Luo, Z.-L. Yang, and H.-B. Zhang. 1998. The structure of the *Aegilops tauschii* genepool and the evolution of hexaploid wheat. Theor. Appl. Genet. 97:657–670.

Dyck, P.L. 1977. Genetics of leaf rust reaction in three introductions of common wheat. Can. J. Genet. Cytol. 19:711–716.

Dyck, P.L. 1987. The association of a gene for leaf rust resistance with the chromosome 7D suppressor of stem rust resistance in common wheat. Genome 29:467–469.

Dyck, P.L. 1991. Genetics of adult-plant leaf rust resistance in Chinese Spring and Sturdy wheats. Crop Sci. 31:309–311.

Dyck, P.L., and D.J. Samborski. 1982. The inheritance of resistance to *Puccinia recondita* in a group of common wheat cultivars. Can. J. Genet. Cytol. 24:273–283.

Dyck, P.L., E.R. Kerber, and T. Aung. 1994. An interchromosomal reciprocal translocation in wheat involving leaf rust resistance gene *Lr34*. Genome 37:556–559.

Dyck, P.L., D.J. Samborski, and R.G. Anderson. 1966. Inheritance of adult plant leaf rust resistance derived from the common wheat varieties Exchange and Frontana. Can. J. Genet. Cytol. 8:665–671.

German, S.E., and J.A. Kolmer. 1992. Effect of gene *Lr34* on the enhancement of resistance to leaf rust of wheat. Theor. Appl. Genet. 84:97–105.

Hoisington, D.N., M. Khairallah, and D. González-de-León. 1994. Laboratory protocols: CIMMYT Applied Molecular Genetics Laboratory. 2nd ed. CIMMYT, Mexico, D.F.

Kasajima, I., Y. Ide, N. Ohkama-Ohtsu, H. Hayashi, T. Yoneyama, and T. Fujiwara. 2004. A protocol for rapid DNA extraction from *Arabidopsis thaliana* for PCR analysis. Plant Mol. Biol. Rep. 22:49–52.

Kolmer, J.A. 1996. Genetics of resistance to wheat leaf rust. Annu. Rev. Phytopathol. 34:435–455.

Kolmer, J.A., and J.Q. Liu. 2002. Inheritance of leaf rust resistance in the wheat cultivars AC Majestic, AC Splendor, and AC Karma. Can. J. Plant Pathol. 24:327–331.

Kolmer, J.A., D.L. Long, E. Kosman, and M.E. Hughes. 2003. Physiologic specialization of *Puccinia triticina* on wheat in the United States in 2001. Plant Dis. 87:859–866.

Kota, R., W. Spielmeyer, R.A. McIntosh, and E.S. Lagudah. 2006. Fine genetic mapping fails to dissociate durable stem rust resistance gene Sr2 from pseudo-black chaff in common wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 112:492–499.

Lagudah, E.S., H. McFadden, R.P. Singh, J. Huerta-Espino, H.S. Bariana, and W. Spielmeyer. 2006. Molecular genetic characterization of the Lr34/Yr18 slow rusting resistance gene region in wheat. Theor. Appl. Genet. 114:21–30.

Liang, S.S., K. Suenaga, Z.H. He, Z.L. Wang, H.Y. Liu, D.S. Wang, R.P. Singh, P. Sourdille, and X.C. Xia. 2006. Quantitative trait loci mapping for adult-plant resistance to powdery mildew in bread wheat. Phytopathology 96:784–789.

Liu, J.Q., and J.A. Kolmer. 1997. Inheritance of leaf rust resistance in wheat cultivars Grandin and CDC Teal. Plant Dis. 81:505–508.

McIntosh, R.A. 1992. Close genetic linkage of genes conferring adult-plant resistance to leaf rust and stripe rust in wheat. Plant Pathol. 41:523–527.

McIntosh, R.A., C.R. Wellings, and R.F. Park. 1995. Wheat rusts: An atlas of resistance genes. CSIRO Publications, Melbourne, Australia.

Ogbonnaya, F.C., G.M. Halloran, and E.S. Lagudah. 2006. D genome of wheat: 60 years on from Kihara, Sears and McFadden. p. 205–220. *In* K. Tsunewaki (ed.) Frontiers of wheat bioscience. Kihara Memorial Foundation for the Advancement of Life Sciences, Yokohama, Japan.

Pflueger, L.A., R. D'Ovidio, B. Margiotta, R. Pena, A. Mujeeb Kazi, and D. Lafiandra. 2001. Characterisation of high- and low-molecular weight glutenin subunits associated to the D genome of *Aegilops tauschii* in a collection of synthetic hexaploid wheats. Theor. Appl. Genet. 103:1293–1301.

Riede, C.R., and J.A. Anderson. 1996. Linkage of RFLP markers to an aluminum tolerance gene in wheat. Crop Sci. 36:905–909.

Rosewarne, G.M., R.S. Singh, J. Huerta-Espino, H.M. William, S. Bouchet, S. Cloutier, H. McFadden, and E.S. Lagudah. 2006. Leaf tip necrosis, molecular markers and $\beta1$-proteasome subunits associated with the slow rusting resistance genes *Lr46/Yr29*. Theor. Appl. Genet. 112:500–508.

Schnurbusch, T., E. Bossolini, M. Messmer, and B. Keller. 2004. Tagging and validation of a major quantitative trait locus for leaf rust resistance and leaf tip necrosis in winter wheat cultivar Forno. Phytopathology 94:1036–1041.

Singh, D., R.F. Park, and R.A. McIntosh. 2007. Characterisation of wheat leaf rust resistance gene *Lr34* in Australian wheats using components of partial resistance and molecular markers. Aust. J. Agric. Res. 58:1106–1114.

Singh, R.P. 1992b. Genetic association of leaf rust resistance gene *Lr34* with adult plant resistance to stripe rust in bread wheat. Phytopathology 82:835–838.

Singh, R.P. 1992a. Association between gene *Lr34* for leaf rust resistance and leaf tip necrosis in wheat. Crop Sci. 32:874–878.

Singh, R.P. 1993. Resistance to leaf rust in 26 Mexican wheat cultivars. Crop Sci. 33:633–637.

Singh, R.P., and S. Rajaram. 1991. Resistance to *Puccinia recondita* f. sp. *tritici* in 50 Mexican bread wheat cultivars. Crop Sci. 31:1472–1479.

Singh, R.P., and S. Rajaram. 1992. Genetics of adult-plant resistance to leaf rust in 'Frontana' and three CIMMYT wheats. Genome 35:24–31.

Suenaga, K., R.P. Singh, J. Huerta-Espino, and H.M. William. 2003. Microsatellite markers for genes *Lr34/Yr18* and other quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. Phytopathology 93:881–890.

Spielmeyer, W., R.A. McIntosh, J. Kolmer, and E.S. Lagudah. 2005. Powdery mildew resistance and *Lr34/Yr18* genes for durable resistance to leaf and stripe rust cosegregate at a locus on the short arm of chromosome 7D of wheat. Theor. Appl. Genet. 111:731–735.