Journal of Life Sciences 6 (2012) 353-362

The Screening of Wheat Germplasm for Resistance to Stripe and Leaf Rust in Kazakhstan Using Molecular Markers

Kokhmetova Alma[1], Yessenbekova Gulzat[1], Morgounov Alex[2] and Ogbonnaya Francis[3]

1. Institute of Plant Biology and Biotechnology, Timiryazev st. 45, Almaty 050040, Kazakhstan

2. CIMMYT-Turkey, P.K. 39 Emek, Ankara 06511, Turkey

3. International Centre for Agricultural Research in the Dry Areas, P.O. Box 5466, Aleppo, Syria

Received: July 26, 2011 / Accepted: November 16, 2011 / Published: April 30, 2012.

Abstract: Resistance to stripe and leaf rusts is the most important objectives in Kazakhstan, and they are the major factor that adversely affects wheat yield and quality and finally causes considerable economic damage. This study was aimed at characterizing elite wheat germplasm from Central Asia using molecular markers linked to the *Lr34/Yr18* dual rust resistance gene and to identify new wheat germplasm resistant to leaf and yellow rust. In experiment with germplasm developed from Kazakhstan and Central and West Asia yellow rust trap nursery (CIMMYT), the frequency of the csLV34b-allele linked to *Lr34/Yr18* (150 bp) was low and only seven of the 42 accessions had allele diagnostic of *Lr34/Yr18*. Two genotypes had high level of resistance, showing immune reaction to all three rusts. Disease severity from resistance to moderate susceptible was recorded in the lines having *Lr34/Yr18* genes, which is comparable to the disease severity observed on the cultivar, Cook (20MS-30MS), carrying *Lr34/Yr18* genes. The molecular screening of a set of additional 51 wheat genotypes, including commercial cultivars and breeding lines from different countries, showed that the *csLV34* marker was present in 20 genotypes. This allowed us to select lines that could be used for future breeding work. In all, a total of 269 lines possessed effective *Lr34/Yr18* gene complex: 28 lines of F4 Almaly/Opata-85, 34 lines of Almaly/Super Kauz, 26 lines of F4 Parula/(Almaly/Anza), 23 lines of F4 Babax 1/Opata 85, and 27 lines of Madsen/Cook populations. This further validates and confirms that the STS marker csLV34 and morphological marker leaf tip necrosis are reliable in the identification of carriers of effective slow rusting *Lr34/Yr18* gene. The germplasms identified are further being tested for end-used quality and could be released by NARS as varieties in the various countries of Central Asia.

Key words: Molecular markers, resistance, stripe rust, leaf rust, wheat.

1. Introduction

Kazakhstan is one of the great wheat producers in Central Asia. Today Kazakhstan steadily produces 16-18 million tons of wheat grain, half of which are used for domestic needs. The bread consumption in the region is very high (45%-60% of daily calories come from wheat), making wheat very important crop.

Resistance to stripe and leaf rusts is the most important objectives in our region and is the major factor that adversely affects wheat yield and quality and finally causes considerable economic damage. Yield losses reach 30%-50% in epidemic years. The FAO data indicate that annual yield losses from diseases may reach up to 10% of the world wheat production [1]. Worldwide, stripe, or yellow, rust of wheat, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most widespread and damaging diseases of wheat. Stripe rust infection can occur anytime from one-leaf stage to plant maturity provided plants are still green. Stripe rust reduces the photosynthetic capacity, increases transpiration, and reduces the

Corresponding author: Kokhmetova Alma, Ph.D., professor, research fields: genetics and plant breeding. E-mail: gen_kalma@yahoo.com.

accumulation of organic matter, resulting in shriveled grain with low quality. Stripe rust of wheat has been reported in more than 60 countries and on all continents except Antarctica. In most wheat producing areas, yield losses caused by stripe rust have ranged from 10% to 70% depending on susceptibility of the cultivar, earliness of the initial infection, rate of disease development, and duration of disease [2].

In south and southeast of Kazakhstan in the late 1990s and early 2000s, yield losses accounted for 20%-40% [3]. In 1999-2000 severe development of *Puccinia striiformis* was observed in highlands of Almaty region, South-Kazakhstan and Zhambyl regions. In 2002, in south and southeast regions of Kazakhstan epidemics of yellow rust were observed on susceptible commercial varieties of winter wheat. The pustules of yellow rust were also recorded on barley up to 60%-80% and 80%-100% in wild relatives of wheat (*Elimus*, *Aegilops cylindrica*) [4-6]. In 2009 and 2010 in the main wheat producing regions yield losses reached 30%-50%.

Puccinia triticina Eriks., which causes leaf rust, is one of the most important fungal diseases of wheat and causes substantial losses in grain yield worldwide. Yield losses due to leaf rust can be up to 30% or more [7]. More than 70 specific leaf rust resistance genes have been characterized so far in wheat [8]. But the ability of the pathogen to adapt to new resistances by single step mutation constitutes a never-ending challenge for breeders.

Leaf rust has historically been a major problem for north Kazakhstan. During the 2000-2001 epidemic, most widely grown spring wheat cultivars, suffered severely from the disease and grain yield losses, were up to 20%-75% [9]. Despite the unfavorable spring and summer, leaf rust has survived and spread in winter wheat growing areas of southern Kazakhstan, Uzbekistan and Kyrgyzstan in 2004-2010. During 2007-2010, the most widely grown high yielding, not only spring but also winter wheat cultivars, had severe leaf rust. In the period between 2001 and 2007 in north Kazakhstan, epidemic development of *Puccinia triticina* Ericks. occurred four times (2002, 2003, 2005, and 2007). In 2006, leaf rust appeared under severe development of *Septoria nodorum* [6]. In 2002, leaf rust had severe development in south and southeast of Kazakhstan. Infection on commercial varieties was up to 20%, collection accessions in demonstrative plots were damaged up to 20%-70%.

Lack of durable resistance in local wheat cultivars is the main reason for stripe and leaf rust epidemic which limits yields. Effective durable resistance is often based on additive interactions among slow rusting genes [10]. This resistance may be conditioned by groups of minor genes and may not be so easily overcome. A small group of leaf rust resistance genes is known as “slow rusting genes”, such as *Lr34* and *Lr46* [11]. The locus *Lr34/Yr18/Pm38* confers partial and durable resistance against the devasting fungal pathogens leaf rust, stripe rust, and powdery mildew. The gene *Lr34/Yr18*, leaf rust resistance gene is known as “slow rusting gene” which provides durable and non-specific APR and located on the short arm of chromosome 7D. In order to obtain cultivars with good levels of protection under high disease pressure, several of these “slow rusting” gene complexes need to be combined. *Lr34* is tightly linked to the leaf tip necrosis (LTN) locus, and it is also possible for the LTN phenotype to have a pleiotropic effect on *Lr34* itself [12]. *Lr34* provides an important source of partial resistance that is expressed in adult plants during the critical grain-filling stage and is most effective in the flag leaf. When deployed with other adult plant resistance genes, near-immunity can be achieved [13]. Wheat cultivars containing *Lr34* occupy more than 26 million ha in various developing countries alone and contribute substantially to yield savings in epidemic years [14].

Conventional breeding methods are not always effective, especially for such polygenic traits like non-race-specific disease resistance. Molecular markers should accelerate the development of wheat

cultivars with superior and durable resistance by rapid identification of related genes and their transfer into cultivars by conventional crossing and progeny analysis. Marker assisted selection (MAS) offers the possibility to trace resistance genes in cultivars in an easier and more efficient way. It also facilitates the pyramiding of genes.

The development of donors and potential breeding lines resistant to stripe rust is a very important task. This objective is especially important for Kazakhstan because of changing epidemic situations. In order to effectively combat stripe rust, it is necessary to characterize existing elite wheat germplasm using linked markers and to find potentially new donors of resistance. This study was aimed at characterizing elite wheat germplasm from Central Asia using molecular markers linked to the *Lr34/Yr18* dual rust resistance gene and to identify new wheat germplasm resistant to leaf and yellow rust.

2. Materials and Methods

2.1 Plant Materials and Evaluation for Stripe and Leaf Rust Resistance

The following wheat genotypes were used in this study: the differentials from CWARTN—Central and West Asia yellow rust trap nursery, including "World differentials", "European differentials", "Cobbity differentials" and "North American differentials"; advanced lines of winter wheat from different international trials nurseries including CIMMYT and ICARDA; entries from national breeding programs of Kazakhstan, Kyrgyzstan and Uzbekistan; and segregating populations and fixed lines, selected from progenies of crosses between adapted local cultivars and effective sources of resistance. Ninety three wheat lines and cultivars from different countries were used to identify sources of *Lr34/Yr18* dual rust resistance gene.

The experimental study was carried out during 2005-2010 crop seasons at the experimental station at Almalybak, Almaty region, located at the foothill zone. In this period, the experiments were relatively well irrigated three times during their development at a rate 600 m^3/ha. The altitude above the sea level is 785 m. The annual rainfall ranged from 332 to 644 mm in three years. Nitrogen fertilizer were applied at a rate of 60 kg/ha and phosphate fertilizer at a rate 30 kg/ha. The soils in testing location are light, ranging from sandy loess to brown semi-desert soils to light silt loams. Each experiment consisted of three randomized replications.

Field trials were conducted by sowing seed of the entries in each autumn (September 20-25) of 2005-2010. The plots were inoculated in the spring at the tillering stage, with a mixture of isolates representing the most prevailing races of the pathogens *Puccinia striiformis* f. sp. *tritici* and *Puccinia triticina* Eriks. from Central Asia.

Disease severity and adult plant response to stripe and leaf rust were recorded following [15]. The cultivar Morocco and local cultivar Steklovidnaya 24 were used as susceptible checks, for multiplication of the pathogen spores in the greenhouse and as spreaders in the field tests. Evaluation for agronomic traits was done each year in all selected materials by the most important traits of productivity.

2.2 DNA Extraction, PCR Amplification, Electrophoresis, and Gel Visualization

Leaves of two-week-old plants were frozen in liquid nitrogen and stored at -80 °C. DNA was extracted from leaf powder following the protocol described by Riede et al. [16], dissolved in 1 × TBE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), and stored at -20 °C. The primer pairs used in this study were csLV34F and csLV34R synthesized by Sigma (Sigma-Aldrich). Polymerase chain reaction (PCR) amplification using the primer pair csLV34F and R [17] undertook in 20 μL volumes, in a mastercycler personal (Eppendorf) for the identification of wheat lines carrying *Lr34/Yr18*. The final PCR mixture consisted of 2 μL of DNA, 2 μL 10 × TBE buffer, 0.5 units of *Taq* DNA polymerase, dNTPs (final concentration 0.2 mM each), and primers (0.1 mM

each). Reagents were obtained from Sileks (ZAO Sileks, Russia). 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. The amplification products were separated on 1.5% agarose gel containing ethidium bromide in 0.5 × TBE buffer; gels were visualized on UV transilluminator for documentation of allele sizes in the cultivars. Thatcher and RL6058 (Tc*6/PI58548) (TcLr34) served as standards check cultivars.

3. Results and Discussion

3.1 Identification Wheat Germplasm Resistant to Leaf and Yellow Rust Developed from Kazakhstan and CIMMYT

The leaf rust resistance gene, *Lr34/Yr18*, is known as "slow rusting gene" which provides durable and non-specific APR and locates on the short arm of chromosome 7D. To identify the genotypes carrying this gene in our germplasm, F4 derived lines including parents known to carry the resistance gene Anza, Opata 85, Super Kauz, Parula and Cook were used. The family of 130-150 F4 lines was phenotyped for adult plant leaf and stripe rust resistance and leaf tip necrosis, *Ltn*. Molecular screening of these lines was done with a specific co-dominant STS marker csLV34, which is a bi-allelic locus. Genetic linkage between *csLV34* and *Lr34/Yr18* was estimated at 0.4 cM [17]. The robustness of the csLV34 marker in postulating the likely occurrence of *Lr34/Yr18* across a wide range of germplasm was earlier confirmed [18].

Further, a collection of 42 wheat advanced lines derived from CIMMYT and Kazakhstan germplasm was phenotyped for adult plant leaf and stripe rust resistance (Table 1). Most of lines expressed resistance

Table 1 Distribution of csLV34 alleles in Winter Wheat Lines and Cultivars, Field Response to three Rusts of the Wheat Germplasm Derived from Kazakhstan and CIMMYT.

No. entry	No. accession	Parentage/cross	Source/origin	Field response diseases			csLV34 allele*	Lr/Yr gene (s)**
				Stem rust	Stripe rust	Leaf rust		
1	23/23S-286-1454-5	Zhenis/*T. aestivum*/Agropyron repens	KZ	30 MS	0	20 MS	*a*	–
2	23/23S-286-1455-6	Zhenis/*T. aestivum*/Agropyron repens	KZ	40 S	10 MS	20 MS	*a*	–
3	28/28S-289-6/478-2	Zhenis/*T. dicoccum*	KZ	5 MR	20 MR	30 MS	*a*	–
4	28/480-4	Zhenis/*T. dicoccum*	KZ	10 MR	30 MS	30 MS	–	–
5	28/481-5	Zhenis/*T. dicoccum*	KZ	5 R	20 MR	20 MR	*a*	–
6	28/483-7	Zhenis/*T. dicoccum*	KZ	10 MR	10 MR	30 MS	*a*	–
7	32/32S-266-4/85-21	Saratovskaya29/*T. macha*	KZ	70 S	0	40 MS	*a*	–
8	32/86-22	Saratovskaya29/*T. macha*	KZ	70 S	0	10 MS	*a*	–
9	32/89-25	Saratovskaya29/*T. macha*	KZ	80 S	0	10 S	*a*	–
10	32/97-33	Saratovskaya29/*T. macha*	KZ	70 S	0	0	*a*	–
11	36/36S-274-6/138-2	Zhenis/*T. compactum*	KZ	70 S	10 MR	30 MS	*a*	–
12	36/152-18	Zhenis/*T. compactum*	KZ	80 S	0	5 MS	*a*	–
13	36/154-20	Zhenis/*T. compactum*	KZ	70 S	0	20 MS	*a*	–
14	36/155-21	Zhenis/*T. compactum*	KZ	90 S	0	0	*a*	–
15	1W	Ramınal	KZ	20 MS	40 S	15 MS	*a*	–
16	4W-108	Zhetısu/Argus (Lr19Sr25)	KZ	5 MR	10 MS	30 MS	*a*	–
17	5W-104	Komsomolskaya 1/95Sr25	KZ	10 MR	0	15 MS	*a*	–
18	19W-207	Progress/94Sr36	KZ	0	0	0	*a*	–
18	23W-241	241 *T. monococcum*/Progress	KZ	15 MR	10 MR	20 MS	–	–
20	24W-242	242/*T. tımopheevii*	KZ	0	0	0	–	–

(Table 1 continued)

No entry	No. accession	Parentage/cross	Source/origin	Field response diseases			csLV34 allele*	Lr/Yr gene (s)**
				Stem rust	Stripe rust	Leaf rust		
21	29W-306	94Sr36/Progress	KZ	10 MR	20 MS	30 MS	–	–
22	5-TR	MV10-2000/4/AGRI/NAC//KAUZ/3/1D13.1/MLT	CIMMYT	0	60 S	40 S	*b*	*Lr34/Yr18*
23	20-TR	338-K1-1//ANB/BUC/3/GS50A/4/059E//JAGGER/PECOS/5/ZARGANA-4	CIMMYT	0	40 S	40 S	*b*	*Lr34/Yr18*
24	22- TR	KALYOZ-18//8229/OK81306/4/AGRI/NAC//KAUZ/3/1D13.1/MLT	CIMMYT	10 MS	30 MS	0	*a*	–
25	38-TR	451-KAZ/BLOYKA/4/AGRI/NAC//KAUZ/3/1D13.1/MLT	CIMMYT	20 MS	30 MS	20 MS	*a*	–
26	46-TR	338-K1-1//ANB/BUC/3/GS50A/4/TX71A1039. V1*3/AMI//BUC/CHRC	CIMMYT	30 MS	5 R	5 R	*a*	–
27	73-TR	TX69A509-2//BBY2/FOX/3/PKL70/LIRA/4/YMH/TOB//MCD/3/LIRA/5/F10S-1//ATAY/GALVEZ87	CIMMYT	10 S	10 MS	20 MS	*b*	*Lr34/Yr18*
28	76-TR	KAPKA-I.P./3/F10S-1//STOZHER/KARL	CIMMYT	0	60 S	0	–	–
29	79-TR	ARDEAL/BOEMA//F135U2-1/5/TX69A509-2//BBY2/FOX/3/PKL70/LIRA/4/YMH/TOB//MCD/3/LIRA	CIMMYT	0	20 MS	0	*h*	*Lr34lr34/Yr18yr18*
30	92-TR	AUS 4930.7/2*PASTOR//NALIM-3/5/TX69A509-2//BBY2/FOX/3/PKL70/LIRA/4/YMH/TOB//MCD/3/LIRA	CIMMYT	10 MR	10 R	0	*a*	–
31	93-TR	AUS 4930.7/2*PASTOR/4/338-K1-1//ANB/BUC/3/GS50A/5/TAM200/KAUZ	CIMMYT	5 MS	10 MR	0	–	–
32	95-TR	SILVERSTAR/4/338-K1-1//ANB/BUC/3/GS50A/5/TAM200/KAUZ	CIMMYT	0	20 MS	0	*a*	–
33	103-TR	KATEA-1/3/059E//JAGGER/PECOS/4/AU/CO652337//2*CA8-155/3/F474S1-1.1	CIMMYT	0	0	0	*a*	–
34	111-TR	338-K1-1//ANB/BUC/3/GS50A/4/TREGO/JGR 8W/5/TX69A509-2//BBY2/FOX/3/KL70/LIRA/4/YMH/TOB//MCD/3/LIRA	CIMMYT	0	40 MS	0	*a*	–
35	113-TR	338-K1-1//ANB/BUC/3/GS50A/4/4_22/5/BAYRAKTAR	CIMMYT	0	30 MS	0	–	–
36	128-TR	BONITO-37/STEKLOVIDNAYA24//TAM200/KAUZ	CIMMYT	5 MR	20 MS	0	*h*	*Lr34lr34/Yr18yr18*
37	140-TR	TAM200/KAUZ//KRASNODAR/FRTL/3/WELS-2	CIMMYT	0	20 MS	20 S	*b*	*Lr34/Yr18*
38	141-TR	NGDA146/4/YMH/TOB//MCD/3/LIRA/5/F130L1.12/6/GALLYA-ARAL1/7/TAM200/KAUZ	CIMMYT	0	60 S	5 MR	*b*	*Lr34/Yr18*
39	142-TR	KAPKA-I.P./BILINMIYEN 96.55//BEZOSTAYA1	CIMMYT	20 S	20 MS	0	*b*	*Lr34/Yr18*
40	160-TR	TREGO/BTY SIB//ZARGANA-3/3/TAM200/KAUZ	CIMMYT	0	20 MS	10 MS	*h*	*Lr34lr34/Yr18yr18*
41	167-TR	BZA/CAL//BB/3/P221-35/7907//AU/4/AGRI/BJY//VEE/5/CEP17/ND9257//SD94160/6/BEZOSTAYA1	CIMMYT	20 S	10 MS	0	*b*	*Lr34/Yr18*
42	CWARTN	Cook	Australia	20 MS	20 MS	30 MS	*b*	*Lr34/Yr18*
43	Susceptible check	Morocco	Morocco	30 S	100 S	60 S	*a*	–

* The codominant amplification products obtained as either "*a*" or "*b*" or "*h*" allele of csLV34 correspond with homozygous susceptible, resistant and heterozygote allele of *Lr34/Yr18* gene, respectively;

– lack of *Lr34/Yr18* gene.

which ranged from moderate-resistant to moderate-susceptible (20MR-40MS). The csLV34 DNA marker was present in seven lines derived from CIMMYT (Fig. 1). Amplification products obtained as "b" allele of csLV34 corresponds with homozygous resistant allele of *Lr34/Yr18* gene were detected in wheat lines: MV10-2000/4/AGRI/NAC//KAUZ/3/1D13.1/MLT, 338-K1-1//ANB/BUC/3/GS50A/4/059E//JAGGER/PECOS/5/ZARGANA-4, TX69A509-2//BBY2/FOX/3/PKL70/LIRA/4/YMH/TOB//MCD/3/LIRA/5/F10S-1//ATAY/GALVEZ87, TAM200/KAUZ//KRASNODAR/FRTL/3/WELS-2, NGDA146/4/YMH/TOB//MCD/3/LIRA/5/F130L1.12/6/GALLYA-ARAL1/7/TAM200/KAUZ, KAPKA-I.P./BILINMIYEN96.55//BEZOSTAYA1 and BZA/CAL//BB/3/P221-35/7907//AU/4/AGRI/BJY//VEE/5/CEP17/ND9257//SD94160/6/BEZOSTAYA1. Further, three genotypes of the 42 entries showed presence of codominant amplification products obtained as both "a" and "b" alleles of csLV34 which corresponds with heterozygote state of allele of Lr34/Yr18 gene, respectively. Among them, the lines ARDEAL/BOEMA//F135U2-1/5/TX69A509-2//BBY2/FOX/3/PKL70/LIRA/4/YMH/TOB//MCD/3/LIRA, BONITO-37/STEKLOVIDNAYA24//TAM200/KAUZ and TREGO/BTYSIB//ZARGANA-3/3/TAM200/KAUZ. PCR of other wheat genotypes showed the presence of a DNA fragment corresponding with homozygous susceptible allele of *Lr34/Yr18* gene. No germplasms containing *Lr34/Yr18* gene were found in advanced lines derived from Kazakhstan.

Therefore, the frequency of the csLV34b-allele linked to *Lr34/Yr*18 (150 bp) was low in the germplasm derived from Kazakhstan and CIMMYT, as only seven of the 42 accessions had allele diagnostic of *Lr34/Yr*18. In three lines, PCR amplification resulted in a two band pattern that included both csLV34 "a" and "b" alleles. The newly identified lines possessing the *Lr34/Yr18* resistance gene are being used for further breeding and improvement in end-use quality.

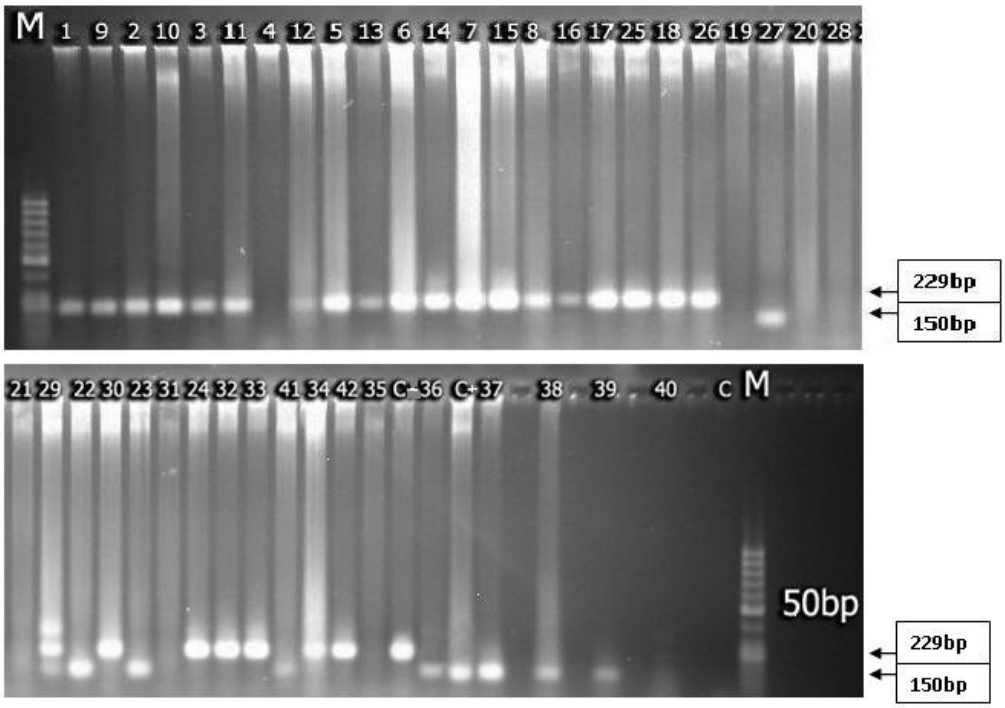

Fig. 1 Polymerase chain reaction amplification products from wheat germplasm derived from Kazakhstan and SIMMYT using csLV34F and R primers.

M, 50 bp ladder size marker; 1-42 wheat lines in order as in Table 1; C- and C+ negative (-*Lr34* Thatcher) and positive (+*Lr34* Thatcher RL6058) controls, respectively; the larger amplification product (229 bp) corresponds to *csLV34a* and the smaller product (150 bp) is the *csLV34b* allele.

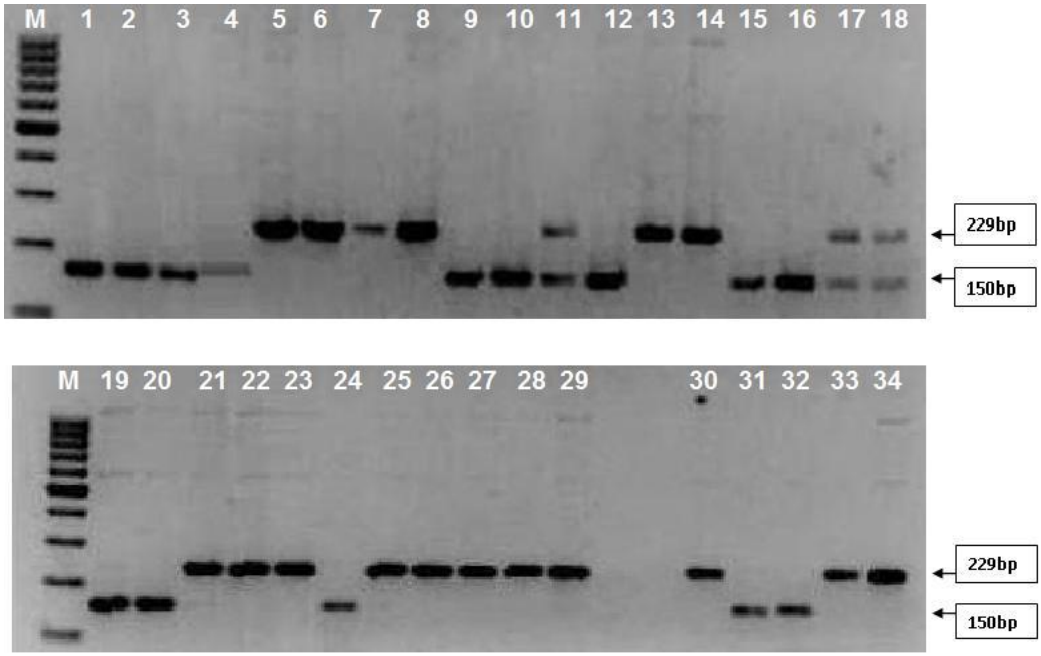

Fig. 2 Polymerase chain reaction amplification products from wheat cultivars and hybrid lines using csLV34F and R primers. M, 100 bp ladder size marker; 1, Positive control, Thatcher +*Lr34*; 2, Opata 85; 3, Super Kauz; 4, Anza; 5, (Almaly/Knyazhna)/Super Kauz; 6, Almaly-1; 7, BWKLDN33; 8, Zhetisu; 9, Madsen/Cook; 10, Parula/(Almaly/Anza); 11, Almaly/Super Kauz-1; 12, Almaly/Super Kauz-2; 13, Bermet; 14, Knyazhna; 15, Almaly-2; 16, Babax2/(Bermet/MK3797); 17, Babax 1/Opata 85-1; 18, (Almaly/Umanka)/Bermet; 19, control, positive Thatcher +*Lr34*; 20, Almaly/Opata 85; 21, MK 3797; 22, Krasnovodopadskaya 210; 23, Clement; 24, Parula; 25, Naz/GF-55; 26, (Naz/GF-55)/Clement; 27, Almaly/Umanka; 28, (Almaly/Umanka) Bermet; 29, (Almaly/Umanka) Zhetisu; 30, Bermet/Knyazhna; 31, Cook; 32, Progress/Anza; 33, Almaly/Knyazhna; 34, Babax1/Almaly. The larger amplification product (229 bp) corresponds to *csLV34a* and the smaller product (150 bp) is the *csLV34b* allele.

The resistance evaluation results are listed in Table 1. In field tests 23 of the 42 lines were resistant to stem rust, 20 accessions were resistant to stem rust and 18 lines shown resistance to leaf rust. Of 42 lines, two genotypes (19W-207 and 103-TR) had high level of resistance, showing immune reaction to all three rusts. Disease severity of 5-20 MR to three rusts (stem, stripe and leaf) was recorded in lines 28/481-5 and 92-TR. Based on the molecular analysis, none of these four genotypes had polymorphic bands linked with the *Lr34/Yr18*. Disease severity from resistance to moderate susceptible (0-40 S) was recorded in the lines having *Lr34/Yr18* genes, which is comparable to the disease severity observed on the cultivar Cook (20-30 MS), carrying *Lr34/Yr18* genes. The disease severity in the susceptible cultivar Morocco was estimated to lie between 30 S and 100 S.

3.2 Identification of Lr34/Yr18 Gene in a Set of Commercial Cultivars and Breeding Lines

The presence of *Lr34/Yr18* gene complex was studied in another set of 51 wheats, including commercial cultivars and breeding lines (Table 2). The csLV34 DNA marker was present in 13 cultivars and breeding lines. The csLV34 marker was present in 28 and 34 lines of F4 Almaly/Opata 85 and Almaly/Super Kauz populations respectively (Fig. 2). Similarly, 26 and 23 lines contained the csLV34 fragment associated with resistance in F4 of Parula/(Almaly/Anza), and Babax 1/Opata 85 populations while 27 lines in Madsen/Cook population exhibited csLV fragment linked to the *Lr34/Yr18* resistance. A few in the three populations appeared to be heterozygotes exhibiting the csLV34 “a” and “b” fragments. The results obtained with this marker were

Table 2 Association of Lr34/Yr18 with the Molecular marker csLV34 and MAS in Wheat Breeding lines and Cultivars.

Cultivar, breeding line	Lr/Yr genes	Field response to LR/YR	csLV34 allele	Source/ origin	Presence of *Ltn* locus	No. selected lines
Thatcher (RL6058)	*Lr34/Yr18*	20 MS/30 S	b	Canada	+	–
Yr18/3*Avocet S	*Yr18*	10 MR/90 S	b	Australia	+	–
Avocet S	–	60 S/90 S	–	Australia	–	–
Jupateco R	*Lr34/Yr18*	5 R/5 MR	b	CIMMYT	+	–
Jupateco 73S	–	70 S/60 S	a	CIMMYT	–	–
Frontana	*Lr34/Yr18*		b	Brazil	+	
Cook	*Lr34/Yr18*	30 MS/20 MS	b	Australia	+	–
Opata 85	*Lr34/Yr18, Lr10, Lr27+Lr31*	5 R/30 MS	b	CIMMYT	+	–
Super Kauz	*Lr26, Lr34/Yr18 +Slow Rusting Gene*	50 S/20 MR	b	CIMMYT	+	–
Parula	*Lr13, Lr34/Yr18+Lr46/Yr29+ Yr30, Sr2*	5 R/30 MS	b	CIMMYT	+	–
Compair	*Yr8, Yr19*	70 S/50 MS	b		–	–
Tonichi 81	*Lr1, Lr13, Lr27+Lr31, Lr34, + Slow Rusting Gene*	20 MR/10 MR	b	CIMMYT	+	–
Cranbrook (S)	*Yr7, Lr27*	20 MR/15 MR	–	Australia	–	–
Clement	*Yr9, Yr25, Cle*	40 MS/30 MS	a		–	–
CAR422/ANA/YACO/3/KAUZ*2/TRAP//KAUZ (CG84-099Y-099M-1Y-2M-2Y-OB)	*Slow Rusting Gene*	5 R/15 MS	a + b	CIMMYT	–	–
TRAP#1/YACO/3/KAUZ*2/TRAP//KAUZ (CG96-099Y-099M-17Y-5M-5Y-08)	*Slow Rusting Gene*	5 R/10 R	–	CIMMYT	–	–
SNI/PBW65/3/KAUZ*2/TRAP//KAUZ 099Y-099M-27Y-5M-4Y-OB	*Slow Rusting Gene*	5 R/5 R	b	CIMMYT	–	–
Babax 2	*Lr27+Lr31+Major Adult Plant Resistance gene*	40 S/10 MR	a	CIMMYT	–	–
Babax 1	*Lr26+Lr27+Lr31+Major Adult Plant Resistance gene*	5 R/30 MS	a	CIMMYT	–	–
Sharora	*Lr26/Yr9*	5 R/40 S	a	Tajikistan	–	–
Arap	*Lr34/Yr18*	30 MS/50 S	b	KZ	+	
Almaly-1	–	5 MS/40 S	a	KZ	+	–
Almaly-2	*Lr34/Yr18*	20 MR/30 MS	b	KZ	+	–
Karlygash	*Lr34/Yr18*		b	KZ	+	–
BWKLDN#33 NS732/HER/4/2CH-542C/SKOROSPELKA//NEUZUCHT/3/NAC76/JCW92-0840-OAP-1AP-04-OBR-2AP-2AP-OAP	*Lr34/Yr18*	0/40 S	b	ICARDA	+	–
FAWWON MK3797	–	20 MS/50 S	a	CIMMYT	–	–
Zhetisu	–	40 S/50 S	a	KZ	+	–
Steklovidnaya 24	–	30 S/80 S	a	KZ	–	–
Yuzhnaya 12	–	0/100 S	a	KZ	–	–
Krasnovodopadskaya 210	–	40 S/80 S	a	KZ	–	–
Bermet	–	60 S/20 MS	a	KG	+	–
Knyazhna	–	0/40 S	a	Russia	–	–
Kupava	–	40 MS/40 S	a	Russia	–	–
Umanka	–	20 MS/50 S	–	Russia	–	–
Ulugbek 600	*Yr9*	10 MS/70 S	a	Uzbekistan	–	–
Anza	*YrA, Lr34/Yr18*	40 MS/30 MS	b	USA	+	–

(Table 2 continued)

Cultivar, breeding line	Lr/Yr genes	Field response to LR/YR	csLV34 allele	Source/ origin	Presence of *Ltn* locus	No. selected lines
Almaly//Opata 85	*Lr34/Yr18*	20 MR/30 MS	b	KZ	+	28
Almaly/Super Kauz-1	*Lr34lr34/Yr18yr18*	20 MR/20 S	a + b	KZ	+	16
Almaly//Super Kauz-2	*Lr34/Yr18*	30 MR/20 MS	b	KZ	+	18
(Almaly/Knyazhna)/Super Kauz	–	10 MS/30 S	a	KZ	–	17
(Almaly/Knyazhna)/Super Kauz	*Lr34lr34/Yr18yr18*	10 MS/30 S	a + b	KZ	+	19
Madsen//Cook	*Lr34/Yr18*	R/20 MS	b	KZ	+	27
Babax 1/Opata 85	*Lr34lr34/Yr18yr18*	R/30 MR	a + b	KZ	+	23
Babax2//(F5Bermet/MK3797)	*Lr34/Yr18*	20 MR/30 MS	b	KZ	+	18
Parula/(Almaly/Anza)	–	30 MS/40 S	a	KZ	+	26
Naz/GF-55	–	30 MS/30 S	a	KZ	–	12
(Naz/GF-55)/Pastor	–	20 MR/30 MS	a	KZ	–	17
Almaly/Umanka	–	30 MS/40 MS	a	KZ	–	11
(Almaly/Umanka)/Bermet	–	30 MS/40 MS	a	KZ	–	12
(Almaly/Umanka)/Zhetisu	–	30 S/30 MS	a	KZ	–	11
Bermet/Knyazhna	–	30 S/30 MS	a	KZ	–	14

* The codominant amplification products obtained as either "a" or "b" alleles of csLV34 correspond with homozygous susceptible and resistant allele of *Lr34/Yr18* gene, respectively.

compared with determinations of *Lr34/Yr18* by adult plant rust resistance and presence or absence of morphological marker, *Ltn* screening of segregating populations using *Ltn* marker confirmed the data obtained from molecular analysis using csLV34 and allowed the selection of a number of lines as potential carriers of *Lr34/Yr18 g*ene. Thus, using both morphological *Ltn* and molecular marker, associated with *Lr34/Yr18* gene, 269 carriers of leaf and stripe rust resistance genes were identified.

4. Conclusions

This study aimed to identify new wheat germplasm resistant to leaf and yellow rust using molecular markers linked to the *Lr34/Yr18* dual rust resistance gene. In experiment with germplasm developed from Kazakhstan and CIMMYT, the frequency of the csLV34 b-allele linked to *Lr34/Yr*18 (150 bp) was low and only seven of the 42 accessions had allele diagnostic of *Lr34/Yr*18. Three lines were heterozygotes. Of 42 lines, two genotypes (19W-207 and 103-TR) had high level of resistance, showing immune reaction to all three rusts. Disease severity of 5-20 MR to three rusts (stem, stripe and leaf) was recorded in lines 28/481-5 and 92-TR. Based on the molecular analysis, none of these four genotypes had polymorphic bands linked to *Lr34/Yr18* gene. Disease severity from resistance to moderate susceptible (0-40 S) was recorded in the lines having *Lr34/Yr18* genes, which is comparable to the disease severity observed on the cultivar, Cook (20-30 MS), carrying *Lr34/Yr18* genes.

The molecular screening a set of additional 51 wheat genotypes, including commercial cultivars and breeding lines from different countries showed that the csLV34 marker was present in 20 genotypes. This allowed us to select lines that could be used for future breeding work. In all, a total of 269 lines possessed effective *Lr34/Yr*18 gene complex: 28 lines of F4 Almaly/Opata-85, 34 lines of Almaly/Super Kauz, 26 lines of F4 Parula/(Almaly/Anza), 23 lines of F4 Babax 1/Opata 85, and 27 lines of Madsen/Cook populations. The results obtained with this marker were compared with phenotyping results by adult plant rust resistance and presence or absence of morphological marker, *Ltn* screening of segregating populations using *Ltn* marker confirmed the data obtained from molecular analysis using csLV34 and

allowed the selection of a number of lines as potential carriers of *Lr34/Yr18 g*ene. Thus, this further validates and confirms that the STS marker csLV34 and morphological marker leaf tip necrosis are reliable in the identification of carriers of effective slow rusting *Lr34/Yr18* gene. The germplasms identified are further being tested for end-sue quality and could be released by NARS as varieties in the various countries of Central Asia.

Acknowledgments

This study was supported by the Ministry of Education and Science Republic of Kazakhstan (Grant NO. 4.1.1/8-987FI). The authors would like to thank M. Atishova, K. Galymbek, Z. Sapakhova and D. Mukhametzhanova for technical assistance throughout this work.

References

[1] L.A. Zjivotkov, S.V. Birukov, A.Y. Stepanenko, Wheat, Urozhay, Kiev, 1989, p. 319. (in Russian)

[2] X.M. Chen, Epidemiology and control of stripe rust (*Puccinia striiformis* f. sp. *tritici*) on wheat, Can. J. Plant Pathol. 27 (2005) 314-337.

[3] A. Morgounov, M. Yessimbekova, S. Rsaliev, S. Boboev, H. Mumindjanov, M. Djunusova, High-yielding winter wheat varieties resistant to yellow and leaf rust in Central Asia, in: Proc. of the 11th Int. Conf. "Cereal Rust and Powdery Mildews", John Innes Centre, Norwich, UK, Aug. 22-27, 2004, Abstr. A2.52.

[4] M.K. Koishibayev, A.B. Zhanarbekova, N.D. Slamova, Immunological evaluation of spring wheat by fungal diseases, in: Book of Proc. Int. Conf. "Development of Key Directions of Agricultural Science in Kazkahstan: Plant Breeding, Biotechnology, Genetic Resources", Astana, Kazakhstan, Sep. 15-17, 2004, pp. 148-154. (in Russian)

[5] A. Yahaoui, Management of yellow rust in Central, Western Asia and Caucasus countries, Newsletter of CIMMYT 2 (5) (2003) 113-116.

[6] M.K. Koyshibayev, L.A. Ponomaryeva, Pathogenity of the air-borne diseases of spring wheat, Herald of Agricultural Science 8 (2008) 15-19. (in Russian)

[7] A.P. Roelfs., R.P. Singh, E.E. Saari, Rust Diseases of Wheat: Concept and methods of Disease management, Mexico, D.F., CIMMYT, 1992, p. 81.

[8] SHIGEN Website, Shared Information of Genetic Resources, http://www.shigen.nig.ac.jp (accessed June 26, 2011).

[9] M.K. Koishibayev, Crop Diseases, Bastau, Almaty, Kazakhstan, 2002, p. 367. (in Russian)

[10] R.P. Singh, J. Huerta-Espino, M. William, Genetics and Breeding for durable resistance to leaf and stripe rust of wheat, in: Book of Proc. First Central Asia Wheat Conf., Almaty, Kazakhstan, June 10-13, 2003, pp. 127-132.

[11] F. Martínez, R.E. Niks, R.P. Singh, D. Rubiales, Characterization of *Lr46*, gene conferring partial resistances to wheat leaf rust, Hereditas135 (2001) 111-114.

[12] T. Schnurbusch, S. Paillard, A. Schori, M. Messmer, G. Schachermayr, M. Winzeler, et al., Dissection of quantitative and durable leaf rust resistance in Swiss winter wheat reveals a major resistance QTL in the *Lr34* chromosomal region, Theoretical and Applied Genetics, 108 (2004) 477-484.

[13] R.P. Singh, R. Trethowan, Breeding spring bread wheat for irrigated and rainfed production systems of the developing world, in: M. Kang, Priyadarshan (Eds.), Breeding Major Food Staples, Blackwell, Iowa, 2007, pp 109-140.

[14] C.N. Marasa., M. Smale, R.P. Singh, The economic impact of productivity maintenance research: Breeding for leaf rust resistance in modern wheat, Agric. Econ. 29 (2003) 253-263.

[15] R.A. McIntosh, C.R. Wellings, R.F. Park, Wheat Rusts: An Atlas of Resistance Genes, CSIRO, Australia, 1995, p. 80.

[16] C.R. Riede, J.A. Anderson, Linkage of RFLP markers to an aluminum tolerance gene in wheat, Crop Science 36 (1996) 905-909.

[17] E.S. Lagudah, H. McFadden, R.P. Singh, J. Huerta-Espino, H.S. Bariana, W. Spielmeyer, Molecular genetic characterization of the *Lr34/Yr18* slow rusting resistance gene region in wheat, Theoretical and Applied Genetics 114 (2006) 21-30.

[18] J.A. Kolmer, R.P. Singh, D.F. Garvin, L.Viccars, H.M. William, J. Huerta-Espino, et al., Analysis of the *Lr34/Yr18* rust resistance region in wheat germplasm, Crop Science 48 (2008) 1841-1852.