Wheat Germplasm Screening for Stem Rust Resistance Using Conventional and Molecular Techniques

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Abstract: In Central Asia, stem rust (*Puccinia graminis* f.sp. *tritici*) causes considerable damage, especially during growing seasons with high rainfall. Ug99 is a race of stem rust that is virulent to the majority of wheat varieties. To develop disease-free germplasm, wheat material was screened using the predominant stem rust races of Kazakhstan and tested in two nurseries; CIMMYT-Turkey and the Plant Breeding Station at Njoro, Kenya. A total of 11 pathotypes of *P. graminis* f.sp. *tritici* were identified in Kazakhstan from the stem rust samples collected in 2008–2009. In particular, pathotypes TDT/H, TPS/H, TTH/K, TKH/R, TKT/C and TFK/R were highly virulent. Of the 170 advanced lines of wheat, 21 CIMMYT lines resistant to 5 aggressive Kazakhstani pathotypes of *P. graminis* were identified. A high level of resistance was observed in 11 wheat cultivars and advanced lines: Taza, E-19, E-99, E-102, E-572, E-796, E-809 (Kazakhstan), Ekinchi (Azerbaijan), Dostlik, Ulugbek 600 (Uzbekistan) and Umanka (Russia). Based on data obtained from Turkey-CIMMYT and the Plant Breeding Station Njoro, Kenya nurseries, out of 13 tested entries, 6 wheat breeding lines which were resistant to both stem and yellow rust and 10 wheat lines which showed high and moderate levels of resistance to Ug99 were selected. Using the sequence tagged site (STS) molecular marker Sr24#12, associated with *Sr24*/*Lr24*, seven wheat entries resistant to stem rust were identified. These results will assist breeders in choosing parents for crossing in programmes aimed at developing varieties with desirable levels of stem rust resistance in Kazakhstan and they will also facilitate stacking the resistance genes into advanced breeding lines.

Keywords: molecular markers; pathotypes; resistance; stem rust; wheat

The region of Central Asia is one of the most important wheat growing areas in the world. Wheat is grown on 15 mil ha, including 5 mil ha of winter or facultative wheat, and 10 mil ha of spring wheat. Kazakhstan is one of the largest wheat producers in Central Asia. Wheat rusts are an important problem in Kazakhstan and are one of the major factors reducing the productivity of wheat. Stem rust (*Puccinia graminis* f.sp. *tritici*) causes considerable damage, especially during growing seasons with higher rainfall. The stem rust fungus attacks the aboveground parts of the plant. In turn, infected plants produce fewer tillers and set fewer seed. In cases of extremely bad infection the plant may die. Currently, more than 60 numbered or temporarily designated stem rust (*Sr*) genes for resistance to stem rust have been listed in the Komugi Wheat Genetics Resource Database (http:// www.shigen.nig.ac.jp, accessed 26 June 2011). Utilization of many of these genes in breeding programmes has resulted in the effective control of stem rust in most countries (Visser *et al.* 2011). Ug99, which has the designation of TTKSK, is a race of stem rust that is virulent to the majority of wheat varieties. Unlike other rusts, which only partially affect crop yields, stem rust can cause 100% crop loss. Up to 80% of all Asian and African wheat varieties are susceptible to the fungus and according to the FAO, major wheat-producing nations east of Iran – such as Afghanistan, India, Pakistan, Turkmenistan, Uzbekistan and Kazakhstan – should be on high alert (http://www.agro-delo.ru/news/5572.html, accessed 5 March 2008). To combat the menace of rust, screening of various nurseries from national and international breeding programmes was initiated. The aim of this work was to find sources of stem rust resistance and to develop disease-resistant germplasm.

MATERIAL AND METHODS

Wheat genotypes used in this study included a set of 16 isogenic lines and differentials for *Sr* genes, 44 wheat commercial cultivars and breeding lines from national breeding programmes of Kazakhstan, Kyrgyzstan, Uzbekistan, Tadjikistan, Azerbijan and Russia, and 170 advanced lines from International Trials Nurseries, CIMMYT.

Experimental materials were grown in 2008–2010 in two locations of Central Asia which differed in soil conditions, temperature and moisture. The experimental station in Almalybak, Almaty region, located at the foothill zone is a relatively well irrigated location. Wheat plants were irrigated three times during their development at a rate 600 m^3/ha. At this location altitude above sea level is 785 m and during the three years of this study annual rainfall ranged from 332 to 644 mm. Nitrogen fertilizer was applied at a rate of 60 kg/ha and phosphate fertilizer at a rate 30 kg/ha. The other location is an irrigated location in Gvardeysky, Research Institute for Biological Safety Problems (RIBSP), situated in the Zhambyl region, Kazakhstan in the desert-steppe zone, and therefore, the climate is extremely dry with great variation. The crop growth season is characterized by drought and dry winds, especially as the crop reaches maturity. The total rainfall is 200–220 mm. Plants were irrigated 3–4 times at a rate 650 m^3/ha. The soils in both testing locations are light, ranging from sandy losses 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 during autumn (20–25 September) of 2008–2010. The plots were inoculated in the spring at the tillering stage with a mixture of identified isolates representing the most prevailing races of the pathogen from Central Asia.

Seedlings of winter wheat cultivars and advanced breeding lines from Kazakhstan and CIMMYT were tested under the controlled greenhouse conditions at RIBSP. The structure of the stem rust population was determined according to the identification system of Roelfs and Martens (1988) based on inoculation of isogenic *Sr*-lines with *P. graminis* spores that had been modified. According to this system, the plant reaction is determined on 16 lines divided into 4 groups of 4 lines. The first group included near-isogenic lines for *Sr5*, *Sr21*, *Sr9e*, *Sr7b*; the second – *Sr11*, *Sr6*, *Sr8a*, *Sr9g*; the third group – *Sr36*, *Sr9b*, *Sr30*, *Sr17*. The fourth was the set of near-isogenic lines for *Sr24*, *Sr25*, *Sr27*, *Sr32* (Kazakhstani additional set). According to combination of responses of resistant (R) and susceptible (S) plants, each rust isolate was coded in letters. As a result, each pathotype has a code including 4 consonants of English alphabet from B through T. Virulence of the pathotypes was also studied using a set of *Sr* isogenic lines (Green *et al.* 1960). Stem rust pathotypes with virulence to 8–13 *Sr*-genes were used in the greenhouse for wheat seedling test (Table 1). For each plant, infection type (IT), based on a 0–4 scale, was recorded 20 days after inoculation. The IT data of seedling reactions were analyzed using the methods described by Stakman and Levine (1922). Disease severity was recorded following Peterson *et al.* (1948). Sampling of the spores, their storage, examination, reproduction and use were carried out according to methods described by Kiraly *et al.* (1970), Konovalova *et al.* (1977) and Roelfs *et al.* (1992).

The experimental material was screened with the predominant races in the region of Central Asia. Cultivars Bogarnaya 56 and Steklovidnaya 24 were used as susceptible checks, which were used for multiplication of the pathogen spores in the greenhouse and as spreaders in the field tests. Advanced lines of wheat were also tested in the nurseries of CIMMYT-Turkey and at the Plant Breeding Station, Njoro, Kenya.

Total genomic DNA was extracted from leaves following the protocol described by Riede and

Table 1. Characteristics of the Kazakhstani wheat stem rust pathotypes

Pathotype	Origin	Virulence to *Sr*-lines
TDT/H	North of Kazakhstan	*Sr5, 21, 9e, 7b, 8a, 9g, 36, 9b, 30, 17, 25, 32*
TTH/K	North of Kazakhstan	*Sr5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 17, 25, 27, 32*
TCK/H	North of Kazakhstan	*Sr5, 21, 9e, 7b, 9g, 9b, 30, 17, 25, 32*
TFK/R	North of Kazakhstan	*Sr5, 21, 9e, 7b, 8a, 9g, 9b, 30, 17, 24, 25, 32*
PCR/Q	South-east of Kazakhstan	*Sr5, 9e, 7b, 9g, 36, 17, 24, 25*
TKH/R	South-east of Kazakhstan	*Sr5, 21, 9e, 7b, 11, 8a, 9g, 9b, 17, 24, 25, 32*

Anderson (1996). PCR was performed as described by Chen *et al.* (1998). DNA samples from the parents and breeding lines were screened using STS marker Sr24#12, which co-segregates with the *Sr24/Lr24* locus (Mago *et al.* 2005) and is used for identification of wheat lines in breeding populations with resistance to stem rust. PCR products were resolved on 8% polyacrilamide gels stained with ethidium bromide.

RESULS AND DISCUSSION

Seedling tests of wheat genotypes for resistance to races of *Puccinia graminis* f.sp. *tritici* detected in Kazakhstan

A total of 11 pathotypes of *P. graminis* f.sp. *tritici* were identified from the stem rust samples collected in 2008–2009 in Kazakhstan. The study of virulent pathotypes using differentials for *Sr*-genes indicated that the pathotypes PCP/C and PCR/Q were virulent only to 8 out of 16 *Sr*-genes studied (Table 2). Isolates of the pathogen studied in 2008–2009 were highly virulent. Isogenic lines of *Sr5*, *Sr11*, and *Sr25* in 2009 became susceptible to pathotypes TPS/H, TTH/K, TMR/H and TKH/R. Results presented in Table 2 indicate that 6 pathotypes (TDT/H, TPS/H, TTH/K, TKH/R, TKT/C and TFK/R) were highly virulent (part of virulent *Sr*-lines was 75% and more). They were selected from durum wheat cultivars (Bezenchukskaya 139, Damsinskaya 90 and Navryz 2).

The presence of highly virulent pathotypes in the population of stem rust represents a great threat to commercial varieties of wheat in Kazakhstan. The results of tests of wheat cultivars to four pathotypes show that the tested cultivars differ in their resist-

Table 2. Virulence of pathotypes *Puccinia graminis* f.sp. *tritici* detected in Kazakhstan, 2008–2009

Source of infection	Pathotype	Virulence formulae (virulent/avirulent)	Part of virulent *Sr*-lines (%)
Bezenchukskaya 139	TDT/H	*Sr5, 21, 9e, 7b, 8a, 9g, 36, 9b, 30, 17, 25, 32/Sr11, 6, 24, 27*	75.0
Bogarnaya 56	TPS/H	*Sr5, 21, 9e, 7b, 11, 8a, 9g, 36, 9b, 30, 25, 32/Sr6, 17, 24, 27*	75.0
Damsinskaya 90	TTH/K	*Sr5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 17, 25, 27, 32/Sr36, 30, 24*	81.2
K-84107, F_4-00-42	TCP/H	*Sr5, 21, 9e, 7b, 9g, 36, 30, 17, 25, 32/Sr11, 6, 8a, 9b, 24, 27*	62.5
K-84112, 242/93-10	PCP/C	*Sr5, 9e, 7b, 9g, 36, 30, 17, 32/Sr21, 11, 6, 8a, 9b, 24, 25, 27*	50.0
Seymur	PCR/Q	*Sr5, 9e, 7b, 9g, 36, 17, 24, 25/Sr21, 11, 6, 8a, 9b, 30, 27, 32*	50.0
Navryz 2	TCK/H	*Sr5, 21, 9e, 7b, 9g, 9b, 30, 17, 25, 32/Sr11, 6, 8a, 36, 24, 27*	62.5
K-84482, 18297	TMR/H	*Sr5, 21, 9e, 7b, 11, 9g, 36, 9b, 17, 25, 32/Sr6, 8a, 30, 24, 27*	68.7
Omskaya 19	TKH/R	*Sr5, 21, 9e, 7b, 11, 8a, 9g, 9b, 17, 24, 25, 32/Sr6, 36, 30, 27*	75.0
Saratovskaya 29	TKT/C	*Sr5, 21, 9e, 7b, 6, 8a, 9g, 36, 9b, 30, 17, 32/Sr11, 24, 25, 27*	75.0
Shortandinskaya 95	TFK/R	*Sr5, 21, 9e, 7b, 8a, 9g, 9b, 30, 17, 24, 25, 32/Sr11, 6, 36, 27*	75.0

ance to disease (Table 3). For example, cultivars Almaly and Tungush were susceptible to all four pathotypes (IT 3, 4, 4+). The cultivars Zhalyn, Zhetisu, Steklovidnaya 24, Aichureck, Alex and Ozoda showed a high level of resistance to one or more rust pathotypes (IT 0, 1, 2). Studying the dynamics of disease within 7 days confirmed the infection type of studied cultivars. Resistances to races TTH/K, TCK/H and TFK/R were observed in cultivars Zhalyn (Kazakhstan) and Aichureck (Kyrgyzstan). The high level of resistance to all 4 pathotypes was observed in Taza, E-19, E-99, E-102, E-572, E-796, E-809 (Kazakhstan), Ekinchi (Azerbaijan), Dostlik, Ulugbek 600 (Uzbekistan) and Umanka (Russia). The use of these cultivars and advanced lines in the breeding process will allow the development of new wheat cultivars resistant to stem rust.

Race-specific resistance to stem rust

Within greenhouse conditions, 170 F_3 lines carrying *Sr*-genes were tested for resistance to five races of *P. graminis* (TDT/H, PCR/Q, TKH/R, TTH/K and TFK/R) that are currently prevalent in Kazakhstan. As a result, 21 CIMMYT lines resistant or moderately resistant to all 5 aggressive pathotypes were identified (Table 4). When testing the resistance of wheat lines to the field collection of *P. graminis*, lines that were immune or had moderately resistant reactions were identified. Nine wheat lines, which showed '0' infection type (Table 4) in the field, were found to be most resistant to this pathogen. All of the selected lines were highly or moderately resistant to all five pathotypes. A consistent resistant reaction both in the field and in the greenhouse to five specific races was observed in 11 of the F_3 lines.

Table 3. Infection types of wheat genotypes grown in Central Asia produced by four Kazakhstan races of *Puccinia graminis* f.sp. *tritici* in greenhouse tests on seedlings

Wheat genotype	Origin	Infection type to races			
		TDT/H	TTH/K	TCK/H	TFK/R
Almaly	Kazakhstan	4, 4+	4	3+, 4	3
Zhalyn	Kazakhstan	4, 4+	0;	0;	2
Zhetisu	Kazakhstan	4, 4+	4, 4+	3+	0;
Egemen	Kazakhstan	4, 4+	4, 4+	2, 2+	2+
Steklovidnaya 24	Kazakhstan	4	4, 4+	0	4
Tungush	Kazakhstan	4, 4+	4, 4+	4, 4+	4, 4+
Taza	Kazakhstan	1	1+, 2	0;	0;
E-19	Kazakhstan	1	1+, 2	0	0
E-99	Kazakhstan	0; 1	0;	0	1
E-102	Kazakhstan	0	0	0	0
E-572	Kazakhstan	1	0	0;	1+
E-796	Kazakhstan	0	0	0;	0;
E-809	Kazakhstan	2	0;	0	0
Ekinchi	Azerbaijan	0	2	0;	1
Aichureck	Kyrgyzstan	3+, 4	0	0	2+
Alex	Tajikistan	4, 4+	3+, 4	3, 3+	0
Ozoda	Tajikistan	3+,4	0;	4, 4+	1+
Dostlik	Uzbekistan	2+	1	0	0
Ulugbek 600	Uzbekistan	2+	0;	0;	0
Umanka	Russia	1	1	1–, 1	1

Table 4. Infection types of CIMMYT lines produced by five Kazakhstani races and field responses to the field collection of *Puccinia graminis* f.sp. *tritici*, 2010

Cultivars, lines	Field response	Infection types to *P. graminis* races				
		TDT/H	PCR/Q	TKH/R	TTH/K	TFK/R
T-2003//TREGO/JGR 8W/4/AGRI/NAC//KAUZ/3/1D13.1/MLT	0	;	1+	0	0	2
TAM 105/3/NE70654/BBY//BOW"S"/4/CENTURY × 3/TA2450/5/ZARGANA-3/6/BONITO-36	0	2+	1	0	2	1
TX87V1613/KS91WGRC11//BETTY/3/AGRI/NAC//ATTILA	5 MS	1+ 2	2	1	1	2
338-K1-1//ANB/BUC/3/GS50A/4/TX71A1039.V1 × 3/AMI//BUC/CHRC	10 S	1+	1	1	2+	1
ERIT58-87//KS82W409/SPN/3/KRC66/SERI/5/TX69A509-2//BBY2/FOX/3/PKL70/LIRA/4/YMH/TOB//MCD/3/LIRA	0	;	2	2	2	2
FRTL/NEMURA//KROSHKA	30 S	1	2	1	1	1
PASTOR/MILAN/3/F10S-1//STOZHER/KARL	0	2	1	1	0	0
EMB16/CBRD//CBRD/5/TX69A509-2//BBY2/FOX/3/PKL70/LIRA/4/YMH/TOB//MCD/3/LIRA	0	1	2+	1+	0	0
AUS 4930.6/2 × PASTOR/4/338-K1-1//ANB/BUC/3/GS50A/4/ZARGANA-4	0	2	2+	1	1	1
AUS 4930.7/2 × PASTOR//JI5418/MARAS/3/TAM200/KAUZ	0	1+	1	1	2+	2
AUS 4930.7/2 × PASTOR/4/338-K1-1//ANB/BUC/3/GS50A/5/TAM200/KAUZ	5 MS	2-	2	1+	1	0
338-K1-1//ANB/BUC/3/GS50A/4/TREGO/JGR 8W/5/ZARGANA-3	10 MR	1	2	2	2+	1+
338-K1-1//ANB/BUC/3/GS50A/4/4_22/7/ZCL /3/ PGFN//CNO67/SN64/4/SERI/5/UA.2837/6/ATTILA/3 × BCN	0	2	2	2+	2	0
GRISET-9/4/AGRI/NAC//KAUZ/3/1D13.1/MLT/5/SHI#4414/CROWS"//GK SAGVARI/CA8055	5 S	2-	2	2	0	1
DALNITSKAYA/4/AGRI/NAC//KAUZ/3/1D13.1/MLT/5/F10S-1//ATAY/GALVEZ87	40 S	2	2	2	0	0
KS00F5-14-7/KARLYGASH/5/TX69A509-//BBY2 /FOX/3/PKL70/LIRA/4/YMH /TOB// MCD/3/LIRA	20 MR	1	2	1	0	2
TREGO/BTY SIB//BAYRAKTAR/3/ZARGANA-4	60 S	2	2	1	2	0
TREGO/BTY SIB//ZARGANA-3/3/TAM200/ KAUZ	0	1	0	0	0	0
WARIGAL//PYN/2 × BAU/3/BEZOSTAYA1	30 S	2	1	1	0	0
DIAMONDBIRD/GALLYA-ARAL1//TAM200/KAUZ	5 S	0	2	0	2	2
PYN/2 × BAU/5/FRET2 × 2/4/SNI/TRAP#1/3/KAUZ × 2/TRAP//KAUZ/6/TAM200/KAUZ	40 S	2	2	2	0	1+
BEZOSTAYA 1 (Check)	60 S	4	2+, 3	2+	4	2

MR – moderately resistant; MS – moderately susceptible; S – susceptible

Some of the wheat entries were resistant in the field (IT 0), but were susceptible to individual pathotypes in the greenhouse. However, we also identified a number of lines combining resistance in the field and race-specific resistance to stem rust (T-2003//TREGO/JGR8W/4/AGRI/NAC//KAUZ/3/1D13.1/MLT, PASTOR/MILAN/3/F10S-1//STOZHER/KARL, EMB16/CBRD//CBRD/5/TX69A509-2//BBY2/FOX/3/PKL70/LIRA/4/YMH/TOB//MCD/3/LIRA and TREGO/BTY SIB//ZARGANA-3/3/TAM200/KAUZ).

International cooperation

Because Ug99 is virulent to the majority of wheat varieties, we tested promising material at the Plant Breeding Station, Njoro, Kenya, 2009. Experimental material tested in Kenya was developed using local spring commercial varieties and wild relatives of wheat. Table 5 presents the resistance of 13 winter wheats to stem and yellow rust. Screening of winter wheat germplasm allowed selection of 6 from these 13 breeding lines which were resistant to both stem and yellow rust: KSI3/97Sr25, 95SR/Progress, 186Al/159 Arthur, Progress/94SR36, 241F4/*Tr. monococum* CP-1223 and 242T/*Tr. timopheevii*. Ten of the thirteen lines demonstrated high or moderate levels of resistance to stem rust, among them entries #2070, 2071, 2075, 2080, 2081, 2084, 2085, 2089, 2090 and 2095. At the same time nine of the entries (#2080, 2081, 2084, 2085, 2089, 2090, 2093, 2096 and 2097) showed an immune reaction to yellow rust. Thus, all 13 wheat lines tested at Plant Breeding Station, Njoro are valuable for breeding for both stem and yellow rust resistance.

Identification of stem rust resistance genes in wheat germplasm from Kazakhstan using molecular markers

The aim of this part of the study was to screen elite advanced wheat lines from Kazakhstan and CIMMYT with molecular markers linked to stem rust gene *Sr24/Lr24*. The genotypes analyzed in this study are presented in Table 6. It is known that gene *Sr24* is located on the *Agropyron elongatum* tranlocation on chromosome 3DL where leaf rust resistance gene *Lr24* is present (McIntosh *et al.* 1977). Sequence tagged site (STS) marker Sr24#12 is closely linked to *Sr24*. This dominant

Table 5. Resistance of winter wheat to stem and yellow rust, Turkey-CIMMYT nursery, planted at Plant Breeding Station Njoro, Kenya, 2009

No.	Entry	Accession No.	Disease note – Kenya	
			Yellow rust	Stem rust
2070	Zhetisu/96 Argus	4W-108	10 S	1 R
2071	Komsomolskaya 1/95SR	5W-104	10 S	TR
2075	KSI21/159 Artur	9W-130	10 S	5 MR
2080	KSI3/97Sr25	14W-143	0	5 MR
2081	95SR/Progress	15W-147	0	1 R
2084	186AI/159 Arthur	18-179	0	10 MR
2085	Progress/94SR36	19W-207	0	TR
2089	241 F4 *Tr. monoccum*/CP-1223	23W-241	0	0
2090	242T/*Tr. timopheevii*	24W-242	0	0
2093	KSI3/96 LR19SR25	27W-291	0	5 MS
2095	94SR36/Progress	29W-306	20 S	5R
2096	Agatha/Komsomolskaya1	30W-313	TR	20 MS
2097	Progress/*Ae .taushii*	31W-318	0	15 MS

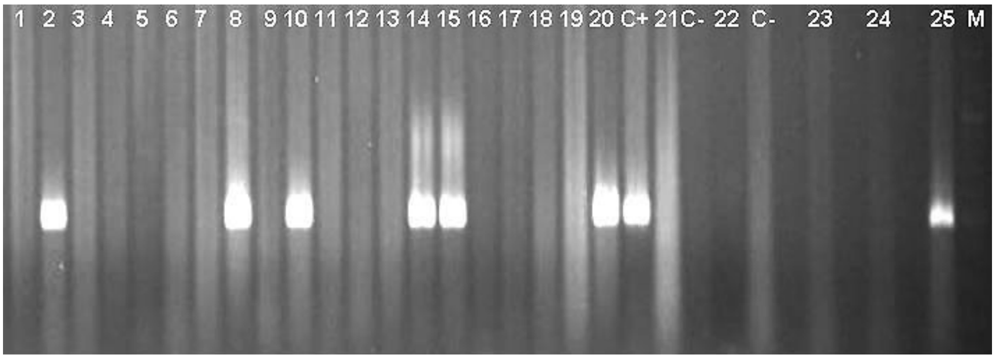
R – resistant; MR – moderately resistant; MS – moderately susceptible; S – susceptible; TR – trace severity with a resistant field response

Table 6. Wheat cultivars and breeding lines evaluated for stem rust resistance, Almaty region, 2010

#	Accession No.	Parentages/Cross	Field responses to stem rust
1	38-TK	451-KAZ/BLOYKA/4/AGRI/NAC//KAUZ/3/1D13.1/MLT	50 S
2	19W-207	Progress/94-W2691SrTt-1(*Sr36*)	0
3	46-TK	338-K1-1//ANB/BUC/3/GS50A/4/TX71A1039.V1 × 3/AMI//BUC/CHRC	20 MS
4	23W-241	241 F4 *T. monococcum*/Progress	15 MS
5	73-TK	TX69A509-2//BBY2/FOX/3/PKL70/LIRA/4/YMH/TOB//MCD/3/LIRA/5/ F10S-1//ATAY/GALVEZ87	10 S
6	24W-242	242/*T. timopheevii*	0
7	76-TK	KAPKA-I.P./3/F10S-1//STOZHER/KARL	0
8	29W-306	94-W2691SrTt-1(*Sr36*)/Progress	10 MR
9	79-TK	ARDEAL/BOEMA//F135U2-1/5/TX69A509-2//BBY2/FOX/3/PKL70/LIRA/4/ YMH/TOB//MCD/3/LIRA	0
10	5-TK	MV10-2000/4/AGRI/NAC//KAUZ/3/1D13.1/MLT	0
11	92-TK	AUS 4930.7/2 × PASTOR//NALIM-3/5/TX69A509-2//BBY2/FOX/3/PKL70/ LIRA/4/YMH/TOB//MCD/3/LIRA	10 MR
12	20-TK	338-K1-1//ANB/BUC/3/GS50A/4/059E//JAGGER/PECOS/5/ZARGANA-4	0
13	93-TK	AUS 4930.7/2 × PASTOR/4/338-K1-1//NB/BUC/3/GS50A/5/TAM200/KAUZ	5 MS
14	22-TK	KALYOZ-18//8229/OK81306/4/AGRI/NAC//KAUZ/3/1D13.1/MLT	10 MS
15	95-TK	SILVERSTAR/4/338-K1-1//ANB/BUC/3/GS50A/5/TAM200/KAUZ	0
16	103-TK	KATEA-1/3/059E//JAGGER/PECOS/4/AU/CO652337//2 × CA8-155/3/F474S1-1.1	0
17	167-TK	BZA/CAL//BB/3/P221-35/7907//AU/4/AGRI/BJY//VEE/5/CEP17/ND9257// SD94160/6/BEZOSTAYA1	20 S
18	111-TK	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	0
19	32/32S-266-4	Saratovskaya29/*T. Maha*	70 S
20	113-TK	338-K1-1//ANB/BUC/3/GS50A/4/4_22/5/BAYRAKTAR	0
21	128-TK	BONITO-37/STEKLOVIDNAYA24//TAM200/KAUZ	5 MR
22	140-TK	TAM200/KAUZ//KRASNODAR/FRTL/3/WELS-2	0
23	141-TK	NGDA146/4/YMH/TOB//MCD/3/LIRA/5/F130L1.12/6/GALLYA-ARAL1/7/ TAM200/KAUZ	0
24	142-TK	KAPKA-I.P./BILINMIYEN96.55//BEZOSTAYA1	20 S
25	160-TK	TREGO/BTY SIB//ZARGANA-3/3/TAM200/KAUZ	0

MR – moderately resistant; MS – moderately susceptible; S – susceptible

marker typically amplifies only one band, this was used to screen for *Sr24/Lr24* genes. The expected size of this marker is 500bp. Resistant germplasm source of *Sr24*, LcSr24Ag, was positive for the marker (Figure 1). Seven out of twenty five genotypes (#2 Progress/94-W2691SrTt-1-*Sr36*, #8 94-W2691SrTt-1(*Sr36*)/Progress, #10 MV10-2000/4/AGRI/NAC//KAUZ/3/1D13.1/MLT, #14 KALYOZ-18//8229/OK81306/4/AGRI/ NAC//KAUZ/3/1D13.1/MLT, #15 SILVERSTAR/4/338-K1-1//ANB/BUC/3/GS50A /5/TAM200/KAUZ, #20 338-K1-1//ANB/BUC/3/GS50A/4/4.22/5/



← 500 bp

Figure 1. STS-marker-assisted screening of stem and leaf rust resistance gene *Sr24/Lr24* in wheat breeding lines; fragment 500 bp amplified by Sr24#12 for detection of *Sr24/Lr24*; M, Marker (Gene-Ruler, 100bp DNA Ladder); C+, LcSr24Ag; C- Morocco; 1–25, set of breeding lines presented in Table 1

BAYRAKTAR and #25 TREGO/BTYSIB//ZARGANA-3/3/TAM200/KAUZ) were positive for the Sr24#12 marker and displayed the DNA-fragment associated with *Sr24/Lr24* resistance genes. These seven breeding lines are likely to possess *Sr24/Lr24* resistance. They also demonstrated immunity or moderate resistance level in the field, except the line #14 with the rating 10MS (Table 5).

CONCLUSION

The screening of winter wheat germplasm from different nurseries in the field and greenhouse allowed the evaluation of resistance to stem rust. From the results obtained in our studies, it can be concluded that the populations of stem rust in Kazakhstan include highly virulent pathotypes. A total of 11 pathotypes of *P. graminis* f.sp. *tritici* were identified from the stem rust samples collected in 2008–2009. The pathotypes TDT/H, TPS/H, TTH/K, TKH/R, TKT/C and TFK/R were highly virulent (75% and more). The use of these pathotypes for evaluating wheat germplasm for resistance could help to improve breeding for stem rust resistance.

A high level of resistance to all four aggressive pathotypes was observed in wheat cultivars and advanced lines Taza, E-19, E-99, E-102, E-572, E-796, E-809 (Kazakhstan), Ekinchi (Azerbaijan), Dostlik and Ulugbek 600 (Uzbekistan) and Umanka (Russia).

Of the 170 advanced lines, 21 CIMMYT lines resistant to 5 aggressive Kazakhstani pathotypes of *P. graminis* were identified. A consistent resistant reaction to the field collection of rust and to five specific races was identified in eleven advanced lines of wheat. These entries can be used as donors in the breeding programmes aimed at stem rust improvement.

Based on the data obtained from Turkey-CIMMYT and the Plant Breeding Station Njoro, Kenya, out of the 13 tested entries, 6 valuable winter wheat breeding lines resistant to both stem and yellow rust and 10 wheat lines, showing high or moderate levels of resistance to Ug99 were selected.

Using the STS molecular marker Sr24#12, associated with *Sr24/Lr24,* seven carriers of wheat stem rust resistance genes were identified. These results will assist breeders in choosing parents for crossing in developing varieties with desirable levels of stem rust resistance in Kazakhstan and will facilitate the stacking of resistance genes into advanced breeding lines.

Thus, this genetic study of stem rust resistance allowed the identification of disease-resistant germplasm of wheat. A number of advanced lines showed a high level of yield potential combined with resistance to the Ug99 stem rust race and to other races which are predominant in the region of Central Asia.

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