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Identification of new sources of wheat stem rust resistance genes

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ABSTRACT

Stem rust disease is widespread in the wheat-growing regions of Kazakhstan. Despite a large number of studies, the protection of wheat from the pathogen *Puccinia graminis* f. sp. *tritici* is considered one of the crucial problems. Chemical control has almost no impact on this disease and no exact result. The only effective way to control this disease is to cultivate resistant varieties and lines. Currently, 60 *Sr* resistance genes are known. Among them, *Sr2, Sr22, Sr25/Lr19, Sr28, Sr36*, and *Sr39* gene sources are efficient at stem rust disease in different conditions of Kazakhstan. The molecular markers Xgwm533, CFA2019, PSY-E1, wPt-7004, Xgwm319 and Sr39#50 linked to *Sr2, Sr22, Sr25/Lr19, Sr28, Sr36* and *Sr39* were used, respectively. As a result of PCR analysis, the *Sr2* gene was identified in six lines out of 16 lines, namely, GA951395-10-7/ WX98D011-U38, Select, GA961565-27-6/KS99U673, GA961662-1-7/TAM107, VA01W-283/ WX030513 and Sonalika. Five wheat lines were found as carriers of the *Sr22* gene: Line c-19SB, Lutescens 7-04-4, Lutescens 220-03-45, GA961662-1-7/TAM107 and Line D 25 77. There are three lines that are carriers of *Sr25/Lr19* gene sources, namely, Lutescens 220-03-45, Advance, and Line D 25 77. The *Sr28* gene was identified in four wheat genotypes (GA951395-10-7/WX98D011-U38, Select, Advance and VA01W-283/WX03ASHTS0513) and the positive control W2691Sr2Bkt. An expected fragment (170 bp) for Xgwm319 properly for *Sr36* gene was identified in four lines (GA951395-10-7/WX98D011-U38, Advance, VA01W-283/WX03ASHTS0513, GA961662-1-7/TAM107). The 10 wheat varieties were identified using primers Sr39 # 50R/F (Line C-19SB, Omskaya 37, Lutescens 7-04-4, Lutescens 220-03-45, Select, GA951395-10-7 / WX98D011-U38, Advance, GA961662-1-7 / TAM107', VA01W-283/WX03ASHTS0513 and Line D 25 77). The studied sources of resistance can be used in breeding programs to create varieties of common wheat with durable resistance to stem rust.

Key words: Genotypes, molecular markers, resistance genes, *Sr* genes, stem rust, wheat

INTRODUCTION

The growing area of spring wheat is more than 15 million hectares in western Siberia and northern Kazakhstan. Rust diseases are one of the serious issues like abiotic stresses, especially in heavy rainfall years which are suitable for the disease (Sapakhova *et al.*, 2022). Stem rust was not considered an economically devastating disease until the happening of the local epidemic that included more than 1 million hectares in the Omsk region of Russia and some regions of Kazakhstan were next to Omsk in 2015.

The spring wheat planting region of northern Kazakhstan and western Siberia covers about 15 million hectares, including the desert of central Kazakhstan and the boreal forests of Siberia extending 600-1000 km in width. The crop is grown under continental climate conditions that have long cold winters,

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short hot summers, and annual rainfall that varies from 280 to 300 mm in the south to 400-450 mm in the north. Spring wheat is commonly planted in mid-May and harvested in September in this area. There are different studies provided a detailed description of the production environment, biotic and abiotic stressors, varieties, and breeding challenges (Morgounov *et al.*, 2001; Kokhmetova *et al.*, 2014; Kokhmetova *et al.*, 2017; Kokhmetova *et al.*, 2018).

Wheat stem rust caused by *Puccinia graminis* f. sp. *tritici* (Pgt) is historically the most destructive wheat disease around the world (Roelfs *et al.*, 1992; Saunders *et al.,* 2019). Over the last 20 years, an increasing number of researchers have become concerned about the spread of the aggressive race Ug99, which was first identified in Uganda in 1999 (Pretorius *et al.*, 2000). Research work has been carried out in Kazakhstan relating to wheat diseases including leaf rust and common bunt which are caused by *Puccinia triticina* Eriks (Morgounov *et al.*, 2015; Kokhmetova *et al.*, 2016; Raghunandan *et al.,* 2022) and *Tiletia caries* (Madenova *et al.*, 2020; Madenova *et al.*, 2021), respectively.

Up until the present time, 13 races within the Ug99 race group have been identified in East Africa and the Middle East, and it is predicted to spread further, threatening critical wheat-growing regions over the world. (Patpour *et al.*, 2016; Saunders *et al.,* 2019). In the last few years, new races, non-members of the Ug99 race group, caused an epidemic in wheat crops in Europe (Bhattacharya, 2017; Lewis *et al.*, 2018), Africa (Olivera *et al.*, 2015), and the Caucasus region of Eurasia (Olivera *et al.*, 2019).

Recently, stem rust disease has caused a serious problem in the northern regions of Kazakhstan and in western Siberia which is the major wheat-growing area. There was a serious stem rust epidemic that affected more than 1 million ha of wheat crops, approximately, in the northern regions of Kazakhstan and close to the Omsk region of Russia in 2015 and 2016 (Shamanin *et al.*, 2016, 2020; Koishybayev, 2018; Rsaliyev and Rsaliyev, 2018).

Stem rust re-emerged led to crop failures in 2017 and 2018 in the northern and eastern regions of Kazakhstan, Omsk, Novosibirsk as well as the Altai Krai regions (Koishybayev, 2018; Gultyaeva *et al.*, 2020; Shamanin *et al.*, 2020; Skolotneva *et al.*, 2020). Disease severity and incidence wanted up enormously at 90 and 70%, respectively, in the main wheat-growing regions of Kazakhstan, and severity was higher than compared to previous years during 2015-2018 (Koishybayev, 2018; Rsaliyev *et al.*, 2019; Yskakova and Rsaliyev, 2019).

According to the latest reports (Hodson *et al.*, 2017), that was conducted by researchers who are from the International Maize and Wheat Improvement Center (CIMMYT) and the Global Rust Reference Center (GRRC) conducted for critical further research, to understand the quantitative approaches to understanding the pathogenesis and transmission of infectious diseases and to increase the proportion of resistant cultivars in regions. Because of the favourable weather conditions and high race diversity, the reported epidemics have serious consequences for the neighbouring regions (Hodson *et al.*, 2017). The Kazakhstan-Siberian Network for Wheat Improvement responded to the threat of stem rust by screening their bread and *durum* wheat varieties and locally developed germplasm in Kazakhstan, western Siberia and Kenya. The overwhelming majority of the locally cultivated and new varieties were highly susceptible to stem rust (Mathuria *et al.*, 2015; Shamanin *et al.*, 2016; Yskakova and Rsaliyev, 2019; Gultyaeva *et al.*, 2020). However, as a result of several years of screening established new spring bread and *durum* wheat varieties, breeding lines that are stem rust-resistant in Kazakhstan, Russia and Kenya (Kokhmetova and Atishova, 2012; Shamanin *et al.*, 2016; Gultyaeva *et al.*, 2020). Recently, the pathogenic variability of the Pgt population has been investigated across western Siberia. The virulence structure of the stem rust population was identified at the GRRC (Denmark) and in the laboratory of molecular phytopathology at the Institute of Cytology and Genetics (Russia) (Hovmøller *et al.,* 2018), which spread throughout the Omsk, Novosibirsk and Altai Krai regions 2017 (Shamanin *et al.*, 2020; Skolotneva *et al.*, 2020). Despite the increasing importance of research stem rust, the population structure of Pgt has not been characterized in Kazakhstan, thus, there have been no attempts to determine the effectiveness of the *Sr* genes for future

applications in local breeding programs. Hence, the present research work was carried out to describe the characteristics of the race structure of Pgt populations and the effectiveness of the *Sr* genes in the mainspring wheat planting regions of Kazakhstan from 2015 to 2018.

Severe epidemics of wheat stem rust caused by Pgt have been observed in Kazakhstan especially, in the major spring wheat-producing regions. However, there is no sufficient information about the virulence structure and race composition of Pgt. Stem rust isolates were collected from three regions of Kazakhstan between 2015-2018 years, in order to determine the virulence diversity and race distribution of the Pgt populations. A total of 203 single pustule isolates were derived and evaluated, meanwhile, 38 races were identified from the stem rust differential and supplemental lines. Among them, the races QHHSF and THMTF were found in all regions and during all years. The races RFRTF, RHMRF, TKRPF and MHCTC were the most common races in the Akmola and Kostanay regions, and the races LHCSF, QKCSF and LKCSF were only widely distributed in East Kazakhstan. The virulence complexity (i.e. number of *Sr* genes on which the races were virulent) ranged from 5 to 16, and about 40% of the races have 14 or more virulent genes. The stem rust resistance genes such as *Sr11, Sr13, Sr22, Sr26, Sr31, Sr33* and *Sr35* were found to confer resistance to all the races identified during the study period. Hence, these genes can be used as sources of resistance in wheat breeding programs in Kazakhstan (Rsaliyev *et al.*, 2020; Kokhmetova and Atishova, 2012).

According to the results of natural and artificial inoculation in different ecological regions of Kazakhstan during the period of 2016-2017 some stem rust-resistant wheat lines were found: namely, LINE-S-19SB, LUTESTSENS7-04-4, KARBINA, URALOSYBIRSKAYA, ERITROSPERMUM 85-08, LUTESTENS 6-04-4 (Amangeldikyzy *et al.,* 2018). According to Kokhmetova and Atishova (2012), pathotypes TDT/H, TPS/H, TTH/K, TKH/R, TKT/C and TFK/R were highly virulent in Kazakhstan. She reported that 21 CIMMYT wheat lines were resistant to five aggressive pathotypes of *P. graminis* in Kazakhstan (Kokhmetova and Atishova, 2012).

The objective of our study was to identify resistant wheat genotypes to stem rust among wheat lines, which were created by breeders from different countries such as Kazakhstan, Russia, the USA and Canada.

MATERIALS AND METHODS

This research was carried out in the fields of the Kazakh Research Institute of Agriculture and Crop Production (43°13′09″ N 76°41′17″E) during 2018-19, 2019-20, and 2020-21 seasons. Sixteen genotypes/varieties taken from the International CIMMYT Research Center were selected as the material of the study, and Sonalika was selected as the control variety. The local population of Pgt was used as an epidemic material for the Almaty region.

The Stakman scale was used to study the levels of seedling resistance to stem rust disease. Based on the Stakman scale, seedling infection types: 0 – immune, no visible uredia; - very resistant, hypersensitive spots; 1 – resistant, small uredia with necrosis; 2 – moderately resistant, small to medium-sized uredia with green islands and surrounded by necrosis or chlorosis; 3 – moderately susceptible, medium-sized uredia with or without chlorosis; 4 – susceptible, large uredia without chlorosis (Stakman, 1954).

McIntosh *et al.* (1995) suggested the scale of disease severity, according to this method, five types of reactions were considered: 0 – immune, no uredia or other macroscopic sign of infection; R – resistant, small uredia surrounded by necrosis; MR – moderately resistant, small to medium uredia surrounded by chlorosis or necrosis; MS – moderately susceptible, medium-sized uredia that may be associated with chlorosis; S – susceptible, large uredia without chlorosis or necrosis.

Genomic DNA was isolated from five-day-old seedlings of wheat plants using a well-known method with cetyltrimethylammonium bromide (Murray and Thompson,1980). To identify resistance genes we used primers for 6 *Sr* genes (*Sr2, Sr22, Sr25, Sr28, Sr36* and *Sr39*). The PCR thermal cycling parameters are shown in Table 1. The most optimal conditions were selected for each primer pair. The amplification products were divided into 2% agarose gel and stained with ethidium bromide. GeneRulerTM 100 bp (Thermo Fisher)

Table 1. Description of the polymerase chain reaction (PCR) program and molecular markers used in gene identification

Primer name	Primary denaturation (°C, min)	No. of cycles	Denaturation (°C, sec)	Annealing temperature (°C, sec)	Extension (°C, sec)	Last extension (°C, min)
Xgwm533	95 (5)	35	95 (40)	60 (30)	72 (120)	72 (10)
CFA2019	95 (3)	35	94 (45)	60 (45)	72 (60)	72 (4)
PSY-E1	94 (4)	10	94 (20)	63 (20)	72 (90)	-
		35	94 (20)	58 (20)	72 (80)	72 (7)
wPt-7004	94 (7)	35	94 (60)	60 (60)	72 (60)	72 (5)
Xgwm319	94 (10)	35	94 (1)	51 (60)	72 (120)	72 (10)
Sr39#50	94(3)	7	92 (30)	65 (30)	72 (40)	-
		30	94 (30)	58 (30)	72 (40)	72 (10)

was used as a molecular weight marker in the agarose gel. Isogenic lines and varieties with known *Sr* genes served as a positive control. The PCR was performed in a 10 µl reaction mixture consisting of 50 ng genomic DNA, 0.2 mM dNTP, 1 × Thermo Fisher PCR buffer, 0.2 µl of each primer, and 0.25 U Taq polymerase (Thermo Fisher).

RESULTS AND DISCUSSION

Most of the wheat varieties in Kazakhstan are susceptible to the local stem rust population. Unfortunately, the effectiveness of *Sr* resistance genes is gradually being lost. In connection with these urgent problems, Pgt occurs in all periods of vegetation of the plant; therefore, it is important to identify resistance genes and varieties with these genes (or genotypes) that allow pathotypes to be effectively protected. For this purpose, 16 wheat lines were selected from the CIMMYT. The disease was studied under laboratory conditions and in an introduction nursery (Table 2).

As a result of the study on the resistance of wheat lines to stem rust during Pgt germination in a greenhouse, five wheat lines were identified as immunodeficient to the pathogen: GA951395-10-7/TX98D3447, GA951395-10-7/WX98D011-U38, GA961565-27-6/KS99U673, GA961662-1-7/tam107, and VA01W-283/WX03SHTS0513. The lines Line c-19sb, Omsk 37, Lutescens 7-04-4, Lutescens

Table 2. Identification of resistant genes to stem rust using molecular markers in artificial condition

Lines	Origin*	Resistance of the stem to rust in the artificial condition during germination		*Sr2 Xgwm 533*	*Sr22* CFA 2019	*Sr25/ Lr19 PSY-E1*	*Sr28 wPt-7004*	*Sr36 Xgwm 319*	*Sr39 Sr39# 50*
			at the adult stage	120 bp	238 bp	191 bp	194 bp	170 bp	250 bp
Line C-19SB	KZ	1	5 MR	-	+	-	-	-	+
Omskaya 37	RU	1	R	-	-	-	-	-	+
Lutescens 7-04-4	RU	1	R	-	+	-	-	-	+
Lutescens 220-03-45	RU	1	5 MR	-	+	+	-	-	+
Sy Ingmar	USA	1	R	-	-	-	-	-	-
GA951395-10-7/TX98D3447	USA	0	0	-	-	-	-	-	
GA951395-10-7/WX98D011-U38	USA	0	0	+	-	-	+	+	+
Select	USA	3	5 MR	+	-	-	+	-	+
Advance	USA	1	R	-	-	+	+	+	+
GA961565-27-6/KS99U673	USA	0	0	+	-	-	-	-	-
Brick	CA	3	20 MS	-	-	-	-	-	-
Carberry	CA	3	10 MS	-	-	-	-	-	-
Muchmore	RU	3	10 MS	-	-	-	-	-	-
Line D 25 77	RU	1	5 MR	-	+	+	-	-	+
GA961662-1-7/TAM107	USA	0	0	+	+	-	-	+	+
VA01W-283/WX03AIITC0513	USA	0	0	+	-	-	+	+	+
Sonalika (positive control)		2	5 MR	+	-	-	-	-	-

*R – resistant, MR – medium resistant, MS – medium susceptible and S – susceptible.
Note: KZ–Kazakhstan, RU–Russia, USA–United States of America and CA–Canada.

220-03-45, Sy Ingmar, Advance, and Line D 25 77, which showed "1" reaction to the disease, were found to be resistant. When wheat lines Select, Brick, Carberry, and Muchmore were infected by the pathogen, they had a reaction score of "3" and were found to be moderately susceptible.

An artificial epidemic environment in a field line, namely, GA951395-10-7/ TX98D3447, GA951395-10-7/WX98D011-U38, GA961565-27-6/KS99U673, GA961662-1-7/ The TAM107 and VA01W-283/WX03HTS0513 models were identified as immunity to the diseases, when Omsk 37, Lutescens 7-04-4, Sy Ingmar and Advance showed resistance to the pathogen with the reaction index "R". The average endurance of the lines Line c-19sb, Lutescens 220-03-45, Select and Line D 25 77 was 5 MR. The lines Brick, Carberry and Muchmore, infected with the disease from 10 to 20 MS, were moderately susceptible.

One of the ways to strengthen the results of breeding programs is the use of molecular markers, in addition to the use of classical methods. The use of molecular markers significantly expanded the ability to assess plant diseases and resistance genes.

Using molecular markers associated with genes that ensure plant resistance to the pathogen increased the speed of the selection process. With this method, research work can be carried out at any stage of plant development and independently of the state of the environment. One aim of this study was to identify wheat varieties resistant to stem rust. To achieve this goal, the genotypes of wheat varieties were screened using markers associated with rust resistance genes.

According to CIMMYT, the *Ug99* race *Sr28, Sr29, SrTmp, Sr2, Sr13, Sr14, Sr22, Sr35, Sr36, Sr37, Sr32, Sr39, Sr47, Sr33, Sr45, Sr40, Sr24, Sr25, Sr26, Sr43, Sr44, Sr27,* 1A, and 1R gene sources have high efficiency (Shamanin *et al.,* 2016). Therefore, a molecular analysis of 16 wheat lines was performed to identify effective genes *Sr2, Sr22, Sr25, Sr28, Sr36* and *Sr39*.

The *Sr2* gene is located on the short arm of wheat chromosome 3B. The *Sr2* gene (*Triticum turgidum*) was widely used in the works of other authors and was presented in marker-assisted selection (MAS) using the SSR marker Xgwm533, which is closely related to this gene (Saccomanno *et al.,* 2018). The recessive *Sr2* gene determined the stability of the plant during the growth period, making it difficult to identify its carriers. The expected size of diagnostic fragments for Xgwm533 was 120 bp. In PCR, Xgwm533 (5´AAGGCGAATCAAA CGGAATA-3´) and (5´GTTGCTTTAGGGGGAA AAGCC3´) primers were used (Spielmeyer *et al.*, 2003), and the Sonalika variety was used as a positive control.

The PCR analysis of 16 wheat lines obtained from the CIMMYT Research Center was performed to identify the sources of the thymus *Sr2* gene. As a result of the study, it was found that six lines were carriers of the *Sr2* gene, namely, GA951395-10-7/WX98D011-U38, Select, GA961565-27-6/KS99U673, GA961662-1-7/TAM107, VA01W-283/WX0 30513 and Sonalika (Fig. 1).

The *Sr22* gene of wheat provides pathogen resistance. The *Sr22* gene of *T. monococcum* L. ssp. *aegilopoides* (synonym of *T. boeoticum* Boiss.) was obtained by introgression.

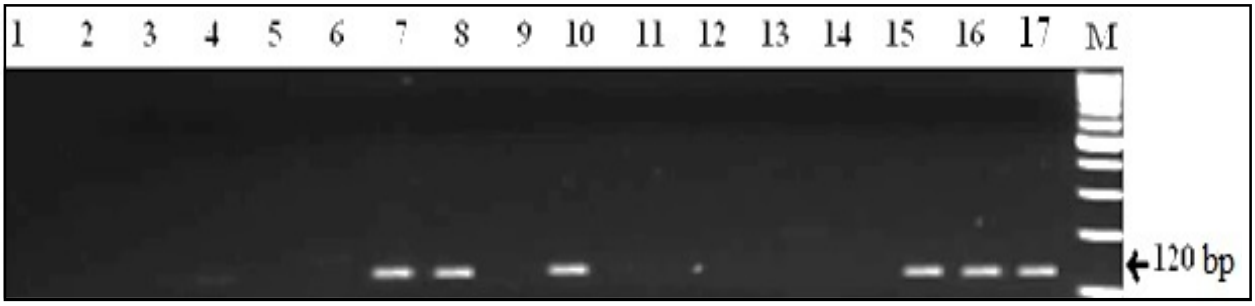

Fig. 1. Amplified polymerase chain reaction product of a hybrid wheat population with Xgwm533 locus primers associated with the *Sr2* resistance gene. 1–Line C-19SB, 2–Omskaya 37, 3–Lutescens 7-04-4, 4–Lutescens 220-03-45, 5–Sy Ingmar, 6–GA951395-10-7/TX98D3447, 7–GA951395-10-7/ WX98D011-U38, 8–Select, 9–Advance, 10–GA961565-27-6/KS99U673, 11–Brick, 12–Carberry, 13–Muchmore, 14–Line D 25 77, 15–GA961662-1-7/TAM107, 16–VA01W-283/WX03AIIITC0513, 17–Sonalika (positive control), M–molecular weight marker (GeneRuler 100 bp DNA Ladder).

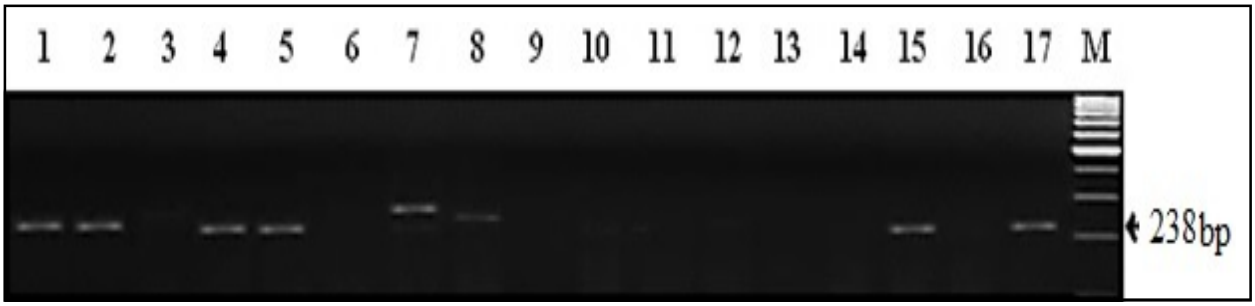

Fig. 2. Amplified polymerase chain reaction product of a hybrid wheat population with CFA2019-F locus primers associated with the *Sr22* resistance gene. 1–Line C-19SB, 2–Lutescens 7-04-4, 3–Omskaya 37, 4–Lutescens 220-03-45, 5–GA961662-1-7/TAM107, 6–Select, 7–GA951395-10-7/WX98D011-U38, 8–GA951395-10-7/TX98D3447, 9–Advance, 10–GA961565-27-6/KS99U673, 11–Brick, 12–Carberry, 13–Muchmore, 14–Sy Ingmar, 15–Line D 25 77, 16–VA01W-283/WX03AIIITC0513, 17–*Sr22TB* (positive control), M–molecular weight marker (GeneRuler 100 bp DNA Ladder).

To determine if this gene is located on the long arm of wheat chromosome 7A, three closely related molecular markers were usually used: *CFA2019*, *CFA2123* and *BARC121* (Saccomanno *et al.,* 2018).

The primers used in PCR analysis to determine carriers of the *Sr22* gene were CFA2019-F 5 '- GAC GAG CTA ACT GCA GAC CC -3' and CFA2019-R 5'- CTC AAT CCT GAT GCG GAG AT -3'. The *Sr22*TB line was used as the positive control. Among the studied wheat genotypes, five lines had a PCR product fragment of 238 bp and were identified as carriers of the *Sr22* gene: Line c-19SB, Lutescens 7-04-4, Lutescens 220-03-45, GA961662-1-7/TAM107 and Line D 25 77. No amplification characteristic of the *Sr22* gene was observed in the remaining 11 wheat lines; thus, these genotypes were not carriers of the *Sr22* gene (Fig. 2).

GB STS is a marker for identifying the *Sr25/Lr19* gene (Prins *et al.*, 2001). Additionally, SCAR markers SCS265 and SCS253 (Bhardwaj *et al.,* 2021), the codominant marker BF145935, and the codominant markers PSY-E1 can be used. The source of the *Sr25* gene was *Agropyron elongatum (Thinopyrom elongatum)*. The gene was translocated on chromosome 7DL.

As a result of PCR analysis performed using a primer pair PSY-E1 (5'- CTA CGT TGC GGG CAC CGT T -3', 5'- AGA GAA AAC CAT TGC ATC TGT A -3') to determine the sources of the *Sr25/Lr19* gene from wheat, the product fragment was identified as a carrier of the *Sr25* gene in the following three lines with 191 nucleotide pairs: Lutescens 220-03-45, Advance and Line D 25 77 lines. A sample of *LcSr2691Sr25Ars* was used as a positive control (Fig. 3).

The *Sr28* gene: A PCR marker derived from the DaRT locus and associated with *Sr28* was named wPt-7004-PCR (Rouse *et al.*, 2012). In this study, the wPt-7004 marker (F 5'-CTC CCA CCA AAA CAG CCT AC -3 ', R 5'-AGA TGC GAA TGG GCA GTT AG -3') was found in

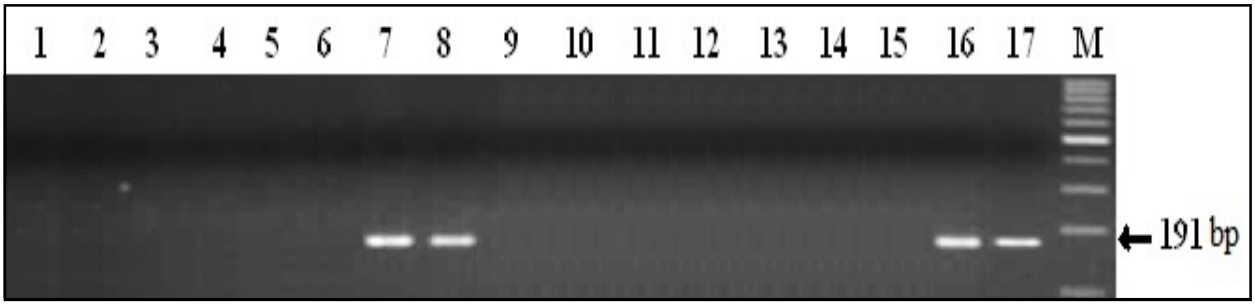

Fig. 3. Amplified polymerase chain reaction product of a hybrid wheat population with PSY-E1 locus primers associated with the *Sr25/Lr19* resistance gene. 1–Line C-19SB, 2–Omskaya 37, 3–Lutescens 7-04-4, 4–GA951395-10-7/WX98D011-U38, 5–Sy Ingmar, 6–Select, 7–Lutescens 220-03-45, 8–Advance, 9–GA951395-10-7/TX98D3447, 10–GA961565-27-6/KS99U673, 11–Brick, 12–Carberry, 13–Muchmore, 14–GA961662-1-7/TAM107, 15–VA01W-283/WX03AIIITC0513, 16–Line D 25 77, 17–*LcSr2691Sr25Ars* (positive control), M–molecular weight marker (GeneRuler 100 bp DNA Ladder).

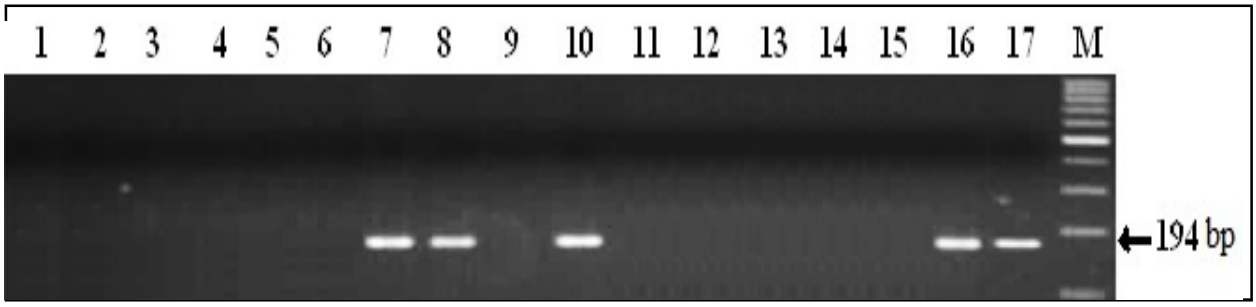

Fig. 4. Amplified polymerase chain reaction product of a wheat lines with wPt-7004 primers associated with the *Sr28* gene. 1–Line C-19SB, 2–Omskaya 37, 3–Lutescens 7-04-4, 4–Lutescens 220-03-45, 5–Sy Ingmar, 6–GA951395-10-7/TX98D3447, 7–GA951395-10-7/WX98D011-U38, 8–Select, 9–GA961565-27-6/KS99U673, 10–Advance, 11–Brick, 12–Carberry, 13–Muchmore, 14–GA961662-1-7/TAM107, 15–Line D 25 77, 16–VA01W-283/WX03AIIITC0513, 17–W2691Sr2Bkt (positive control), M–molecular weight marker (Gene-Ruler 100bp DNA Ladder).

cultivars with *Sr28* resistance genes due to the presence of an amplified fragment 194 bp (Fig. 4).

Therefore, the *Sr28* gene was identified in four wheat genotypes (GA951395-10-7/WX98D011-U38, Select, Advance, VA01W-283/WX03ASHTS0513) and in the control line W2691Sr2Bkt.

The *Sr36* gene: The *Sr36* resistance gene to stem rust transferred to common wheat from *Triticum timopheevi* and effective to *Ug99* race of *Puccinia graminis* f. sp. *tritici*. However, other races are virulent to this gene, so it was recommended that this gene be used in complex with other resistance genes as a pyramid genes system. It is known that hard spring varieties W1656 (CItr 12632) and W1657 (CItr 12633) were the main primary sources of the gene and *Sr36* spread from those varieties to many other varieties in the world. Among the genes that provide resistance to Ug99, *Sr36* is most commonly found in breeding lines in the United States. The *Sr36* was located on the short arm of chromosome 2BS. This gene was widely used in the breeding process and was widespread in western commercial wheat varieties. The Sr36 gene was effective against the Ug99 race, but ineffective against its species (TTKST and TTTSK) (Lin *et al.*, 2021).

The *Sr36* gene was effective against most other races of stem rust and it was used to create pyramids with other *Sr* genes when selecting resistant wheat varieties. PCR amplification was performed using the Xgwm319 primers (F 5'- GGT TGC TGT ACA AGT GTT CAC G -3 ‘, R 5’- CGG GTG CTG TGT GTA ATG AC - 3') to identify *Sr36* gene carriers (Fig. 5).

An expected fragment identified for Xgwm319 (170 bp) was identified in four lines (GA951395-10-7/WX98D011-U38, Advance, VA01W-283/WX03ASHTS0513, GA961662-1-7/TAM107) W2691SrTt-1 and in the positive control (W2691SrTt-1).

The *Sr39* stem rust resistance gene

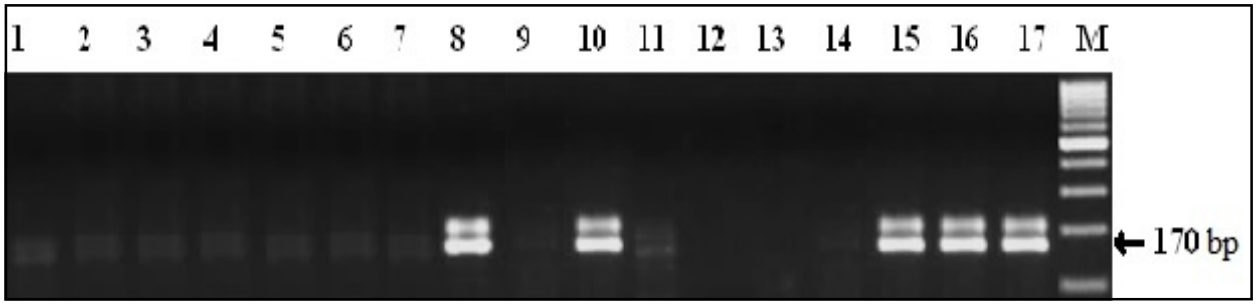

Fig. 5. Amplified polymerase chain reaction product of a wheat lines with Xgwm319 primers associated with the *Sr36* gene. 1–Line C-19SB, 2–Omskaya 37, 3–Lutescens 7-04-4, 4–Lutescens 220-03-45, 5–Sy Ingmar, 6–Select, 7–GA951395-10-7/TX98D3447, 8–GA951395-10-7/WX98D011-U38, 9–GA961565-27-6/KS99U673, 10–Advance, 11–Brick, 12–Carberry, 13–Muchmore, 14–Line D 25 77, 15–VA01W-283/WX03AIIITC0513, 16–GA961662-1-7/TAM107, 17–W2691SrTt-1 (positive control), M–molecular weight marker (Gene-Ruler 100bp DNA Ladder).

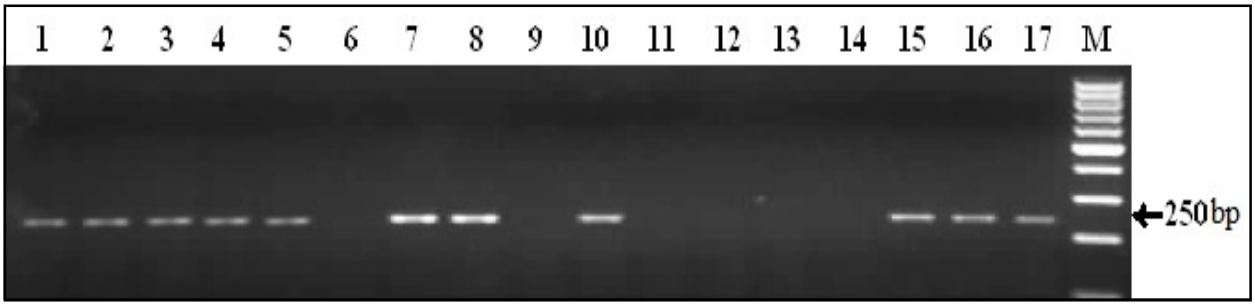

Fig. 6. Amplified polymerase chain reaction product of a wheat lines with Sr39#50 primers associated with the *Sr39* gene. 1–Line C-19SB, 2–Omskaya 37, 3–Lutescens 7-04-4, 4–Lutescens 220-03-45, 5–Select, 6–Sy Ingmar, 7–GA951395-10-7/WX98D011-U38, 8–Advance, 9–GA951395-10-7/TX98D3447, 10–GA961662-1-7/TAM107, 11– Brick, 12–Carberry, 13–Muchmore, 14–GA961565-27-6/KS99U673, 15–VA01W-283/WX03ASHTS0513, 16–Line D 25 77, 17–Isogenic lines Sr39 (RL6082) (control), M–molecular weight marker (Gene-Ruler 100bp DNA Ladder).

provided resistance to all currently known pathogens of *Puccinia graminis f. sp. tritici*, including race *Ug99* (TTKSK) and its variants TTKST and TTTSK.

The *Sr39* was transferred from *Aegilops speltoides* to the hexaploid wheat variety Marquis. Marker *Sr39 # 50* was obtained to identify the *Sr39* gene. The *Sr39* resistance gene was located on chromosome 2B (Bernardo *et al.*, 2013). The *Sr39* gene was identified in the RL6082 line and 113/00i-4 wheat-egilops line, which carried the genetic material of *A. speltoides* (Saccomanno *et al.,* 2018).

The PCR amplification was performed using *Sr39#50* primers (F 5'-CCA ATG AGA AGA TCA AAA CAA CC-3 ‘, R5’-CTA GCA AGG ACC AAG CAA TCT TG-3') to identify *Sr39* gene carriers with an expected fragment size of 250 bp. The *Sr39* (RL6082) was used as a positive control (Fig. 6).

The 10 varieties of wheat were identified using primers *Sr39* # 50R/F (Line C-19SB, Omskaya 37, Lutescens 7-04-4, Lutescens 220-03-45, Select, GA951395-10-7/WX98D011-U38, Advance, GA961662-1-7/TAM107’, VA01W-283/WX03ASHTS0513, Line D 25 77).

In the conditions of Kazakhstan, there were no varieties resistant to the local population of stem rust, according to the studies of stem rust researchers (Koyshibayev, 2018; Amangeldikyzy *et al.*, 2018). Although the vast majority of wheat varieties were resistant during the seedling, they were very susceptible to stem rust in the adult stage, and most of them were resistant to one or more pathotypes of stem rust, while can be affected by two or more pathotypes. Due to these topical issues, it was important to find resistance gene sources to provide effective protection against *Puccinia graminis* f.sp. *tritici*. For this purpose, wheat genotypes obtained from Dr. Morgunov (Morgounov *et al.*, 2001) were studied in the field and the laboratory in the experimental fields of South-East (Almalybak), north and west Kazakhstan under artificial infection of stem rust pathogen (Table 3).

The study showed that the wheat varieties were resistant to TTKSK race on the seedling stage under the greenhouse test conditions, as well as in the adult stage in the field to local isolates of *Puccinia graminis* f.sp. *tritici*.

The source of the *Sr25* gene Lutescens 220-03-45 was recognized by the reaction 5 MR – 10 MR, while Omskaya 37 (*Sr31, Sr25*), and

Table 3. Seedling infection types and adult plant infection responses of wheat lines to stem rust in the artificial condition

Name of lines	Origin*	Seedling	Adult			Sources of *Sr* genes
		TTKSK	Kenya	Nur-Sultan	Almaty	
Lutescens 220-03-45	RU	2+	10MR	10MR	5MR	*Sr25/Lr19, Sr22,Sr39*
Omskaya 37	RU	2	20MR	10MR	R	*Sr31, Sr25/Lr19, Sr39*
Lutescens 7-04-4	RU	1+	5MR	5MR	R	*Sr31, Sr25, Sr22,Sr39*
GA951395-10-7/WX98D011-U38	USA	0;	0;	0	0	*Sr36, Sr2 Lr24/Sr24*
GA961565-27-6/KS99U673	USA	0	0	0	0	*Sr36, Sr2*

Lutescens 7-04-4 (*Sr31, Sr25, Sr22, Sr39*) showed R-MR reaction type. According to greenhouse and field tests, two lines, namely, Omskaya 37 and Lutescens 7-04-4 were avirulent to the local population of stem rust pathogen and characterized as highly resistant. These results showed that *Sr31* and *Sr25* genes had high efficiency in application to protect wheat regions of Kazakhstan.

Wheat stem rust (*Puccinia graminis* f. sp. *tritici*) has historically been the most destructive wheat disease. After infected with this disease, wheat spikes intensively turned black and were filled with rotting grains during harvest time, which looked healthy a few weeks before harvest. In some cases, this disease could cause 70% or more yield loss. The creation and introduction of disease-resistant varieties into production was the fastest, cheapest, and most centralized way to combat winter wheat diseases. In addition, the creation and cultivation of resistant varieties eliminated the widespread use of pesticides and protected the environment from pollution and destruction (Zhuchenko, 2001). A continuous program was carried out in order to breed disease resistance because pathogens evolved with the host. The variety of varieties with the different genetic basis of resistance, and frequent variety change allowed to outstrip the evolution of the pathogen in time and to limit and divide the ranges of the host and the parasite in space (Kumar *et al.,* 2022). Shamanin *et al.* (2016) suggested that Kazakhstan should consider stem rust-resistant genes (including APR) field screening, as well as identification of genes and types of resistance in order to create rust-resistant varieties. Rsaliyev *et al.* (2020) identified a number of advanced lines, which combined rust resistance to stem and leaf rust and revealed that wheat varieties carrying *Sr11, Sr13, Sr22, Sr26, Sr31, Sr33* and *Sr35* were resistant to all the races of *Puccinia graminis.* According to several reports (Bhavani *et al.,* 2019; Afzal *et al.* 2021), *Sr2, Sr22, Sr25, Sr28* and *Sr39* genes are effective or partially effective in all races of the Ug99.

The adult plant-resistant gene Sr2, which provided a durable broad-spectrum to Pgt was difficult to screen under field conditions (Hayden *et al.,* 2004). A closely linked and codominant SSR marker, Xgwm533 (120bp), was used to track *Sr2* in wheat genotypes (Kolmer *et al.*, 2008). The recessive gene *Sr2* determined adult resistance, thus, it complicated the selection. At present, Sr2 was widely used in combination with other genes in breeding programs for resistance to all virulent races of stem rust. Currently, *Sr2* was widely used in combination with other genes in breeding programs for resistance to all virulent races of stem rust (Bhavani *et al.,* 2019). This gene was common in commercial wheat varieties in the USA (Hope, Arthur 71, Ottawa, Scout), Canada (Lancer, Selsirk, Pembina), Australia (Baxter, Diamondbird, Hartog, Sunbrook), India (Sonalika), as well as CIMMYT (Nuri 70, Pavon76, Siete-Cerros). In this study, this gene was found in advanced wheat lines GA951395-10-7/WX98D011-U38, Select, GA961565-27-6/KS99U673, GA961662-1-7/TAM107, and VA01W-283/WX030513.

The *Sr22* gene was located on chromosome 7A (Aoun *et al.*, 2019). The *Sr22* gene confered resistance to Ug99 race and was effective to use in the breeding program (Steuernagel *et al.*, 2016). This study confirmed that the lines, namely, Line c-19SB, Lutescens 7-04-4, Lutescens 220-03-45, GA961662-1-7/TAM107 and Line D 25 77 (*Sr22* gene sources) were identified to confer resistance to Ug99 races. When analyzing PCR products for the CFA 2019 marker, our results were similar to those of Sharma *et al.* (2022).

The *Sr25* gene was transferred from wheatgrass *Agropyron elongatum* (*Thinopyrom elongatum*) to common wheat. Very rarely appearing virulent pathotypes did not have to possess aggressiveness to this gene (Procunier *et al.*, 1995; Prins *et al.*, 2001; Wu *et al.*, 2020). This gene was identified in lines Lutescens 220-03-45, Advance and Line D 25 77.

The stem rust resistance gene *Sr28* was effective in the race Ug99, which was reported by Rouse *et al.* (2012). Rehman *et al.* (2020) reported that the marker wPt-7004 was identified as linked to *Sr28* gene (Elshafei *et al.*, 2022) The *Sr28* gene was found in three Russian wheat varieties, namely, Murat, Pamyat and Mafe. Our study confirmed that GA951395-10-7/WX98D011-U38, Select, Advance, VA01W-283/WX03ASHTS0513 advanced lines confered resistance to stem rust.

The *Sr36* gene was located on the short arm of chromosome 2B. These genes were

widely used in breeding and common in commercial varieties. This gene was effective for the Ug99 race (Jin *et al*, 2022). This gene was effective against most other stem rust races; they were used to create pyramids in combination with other *Sr* genes in the breeding program of resistant wheat varieties. In this study, this gene was identified in GA951395-10-7/WX98D011-U38, Advance, VA01W-283/WX03ASHTS0513 and GA961662-1-7/TAM107.

The stem rust resistance gene *Sr39* provided resistance to all currently known pathotypes of Ug99 (TTKSK) and its variants TTKST and TTTSK. The *Sr39* gene transferred from *T. speltoides* to common wheat variety Marquis and located on chromosome 2B (Kerber and Dyke, 1990). According to molecular analysis the *Sr39* gene was detected in lines Line C-19SB, Omskaya 37, Lutescens 7-04-4, Lutescens 220-03-45, Select, GA951395-10-7/WX98D011-U38, Advance, GA961662-1-7/TAM107', VA01W-283/WX03ASHTS0513 and Line D 25 77, which had the gene for resistance to leaf rust *Lr35*. Both of these genes were linked to each other.

The studied sources of resistance can be used in breeding programs to create varieties of common wheat with durable resistance to stem rust, as well as to create pyramids of the *Sr2, Sr22, Sr25, Sr28, Sr36* and *Sr39* genes using molecular markers.

CONCLUSION

In conclusion, phytopathological and molecular genetic studies of 16 wheat lines taken from the CIMMYT International Research Center were carried out. In the Almaty region, using the populations of Pgt during the wheat germination period, five wheat lines were identified as immune; these were GA951395-10-7/TX98D3447, GA951395-10-7/WX98D011-U38, GA961565-27-6/KS99U673, GA961662-1-7/TAM107 and VA01W-283/WX03AIIITC0513. Using an artificial epidemic environment under field conditions, the above five lines were identified as immune to disease. The PCR analysis was performed to identify rust-resistant sources of the *Sr2, Sr22, Sr25, Sr28, Sr36* and *Sr39*. The advanced line Omskaya 37 had 1 *Sr*gene (*Sr39*). Two lines Line C-19SB (*Sr22, Sr39*) and Lutescens 7-04-4 (*Sr22, Sr39*) had two genes. Three studied lines Lutescens 220-03-45 (Sr22, Sr25, Sr39) Select (*Sr2, Sr28, Sr39*), and Line D 25 77 (*Sr22, Sr25, Sr39*) were sources of three resistance genes to stem rust. Four lines were sources of four resistance genes: GA951395-10-7/WX98D011-U38 (*Sr2, Sr28, Sr36, Sr39*), Advance (*Sr25, Sr28, Sr36, Sr39*), GA961662-1-7/TAM107 (*Sr2, Sr22, Sr36, Sr39*), as well as VA01W-283/WX03AIIITC0513 (*Sr2, Sr28, Sr36, Sr39*). These 10 advanced lines were found as gene sources to stem rust. These lines will be useful for further breeding program to stem rust resistance.

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