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Identification of carriers of *Puccinia striiformis* resistance genes in the population of recombinant inbred wheat lines

Abstract. Stripe rust (yellow rust) caused by *Puccinia striiformis* (*Pst*) f. sp. *tritici* is one of the most dangerous diseases of wheat. Marker assisted selection (MAS) accelerates the selection of resistance gene donors in wheat recombinant inbred lines Almaly/Avocet (S) and to evaluate their response to *Pst*. Evaluation of seedling resistance to *Pst* allowed us to select 2 lines simultaneously resistant to races 111E231 and 7E63. Race 7E63 was avirulent to most samples, and 111E231 was highly virulent. All the studied lines demonstrated a resistant and moderately resistant reaction to the causative agent of yellow rust at the adult plant stage (R-MR). Molecular screening revealed the presence of a marker allele associated with the *Yr18/Lr34* gene complex in 5 RIL. The frequency of resistant genotypes inheriting the *Yr18* gene was 22.7%. The results can be used in MAS wheat breeding programs to increase resistance to yellow rust of wheat.
Key words: wheat, yellow rust, *Puccinia striiformis,* resistance genes, molecular markers.

Introduction

Wheat (*Triticum aestivum L.*) is a significant food crop at the global level, and its production is the basis of food security throughout the world [1]. World wheat production currently reaches 777.8 million tons, and world consumption per capita is 67.6 kg/year [2]. On average Kazakhstan produces 18-20 million tons of wheat grain, but output is highly dependent on weather and in recent years has fluctuated between 10 and 17 million tons [3]. Wheat production in Kazakhstan is constrained due to rust diseases (stem, stripe and leaf rus) [4-10], as well as leaf spot diseases (tan spot and rust) [11-16].

Yellow rust caused by *Puccinia striiformis* Westend f. sp. *tritici* (*Pst*) is one of the most dangerous diseases of wheat worldwide. When epiphytotic occur, losses can vary from 20 to 40% or more [17-18]. A decrease in yield is observed as a result of a decrease in the number of grains in the ear and grain weight in highly sensitive wheat varieties [4, 19]. In the period from 2009 to 2016, stripe rust epidemics occurred annually in Kazakhstan, and yield losses of susceptible wheat varieties reached 20-50% [4]. The rust pathogen population overcomes the protection of resistant cultivars due to the emergence of virulent pathogen races [18]. The cultivation of resistant varieties is the most economical and environmentally friendly approach, which makes it possible to abandon the use of fungicides and reduce crop losses due to yellow rust [20]. However, traditional breeding methods are not always effective. Marker-assisted selection (MAS) using identified target genes makes the process of developing the wheat cultivars more accurate and reliable [21] MAS methods are effectively used to shorten the breeding cycle, increase resistance to biotic and abiotic stresses and maintain potential

wheat yields [22]. Currently, more than 80 genes for resistance to stripe rust have been identified [22]. Based on recent evaluations in Kazakhstan, genes *Yr5, Yr10, Yr15* and *Yr18* are still effective [9]. The locus *Lr34/Yr18/Pm38* confers partial and durable resistance against the devasting fungal pathogens leaf rust, stripe rust, and powdery mildew. *Yr18/Lr34* genes have been used in breeding programs for a century and so far, no pathogen adaptability has been found [23]. Wheat cultivars containing these genes occupy more than 26 million ha in various developing countries alone and contribute substantially to yield savings in epidemic years [24]. The gene complex *Yr18/Lr34* is of great interest as a donor of valuable traits. Therefore, identification of novel sources of resistance in a breeding material is of foremost importance for the effective disease control. In view of these facts, recombinant inbred lines of bread wheat developed in our laboratory were evaluated in this study for yellow rust resistance. The goal of this study was to determine the presence/absence of yellow rust resistance genes in wheat recombinant inbred lines Almaly/Avocet (S) and to evaluate their response to *Pst*.

Materials and methods

Twenty-two RILs used in this study were established at F_6 generation by the single-seed descent method (SSD) from an F_2 family between a high yielding Kazakh winter wheat variety 'Almaly' and an Australian spring wheat variety 'Avocet (S)'. Almaly is a Kazakh variety of common winter wheat, developed in 2002, pedigree [(R6862/50431) xBezostaya1], is widely used as a parent in breeding programs in Kazakhstan and Central Asian countries. Almaly has a high yield potential and moderately high resistance to 3 species of rust, is a carrier of genes *Lr1, Lr28* and *Lr34* [6]. The pedigree of the cultivar contains Bezostaya1, a carrier of the leaf rust resistance genes *Lr34, Lr3a* and *Lr13* (https://maswheat.ucdavis.edu). Second parent is 'Avocet (S)', spring wheat cultivar, is considered to be universally susceptible to the three types of rust and is widely used in rust resistance tests. The parents were selected on the basis of their contrasting phenotypic expression of resistance to stripe rust. The highly susceptible Morocco, as well as the near-isogenic lines (NIL *Lr34*/TC*6/PI58548 for *Yr18*) of Thatcher, are used in field and laboratory tests as controls.

It is known that in order to develop a mapping population, it is necessary to cross parents that are contrasting in terms of the target trait. Therefore, the Almaly variety, moderately resistant to the disease, served as the maternal parent, and the Avoset (S) variety, susceptible to stripe rust, was used as the father. This made it possible to obtain a mapping population consisting of 186 RILs with a wide range of genetic diversity for stripe rust, susceptibility to which varied from 0 to 100%. These RILs will be the objects of further studies: on the basis of QTL mapping, genetic loci of quantitative traits associated with resistance to YR of wheat will be identified and mapped. However, in this study, we will study only 22 RILs that demonstrated field resistance to stripe rust. These lines were selected based on their field and laboratory phytopathological screening for resistance to *Pst*, where cv. Almaty was resistant and Avocet (S) was susceptible.

The phenotyping of the material was carried out in the conditions of the Kazakh Research Institute of Agriculture and Crop Production (KazNIIZiR), Almalybak (43°13'09"N, 76°36'17"E), Almaty region in 2019-2020 cropping season. Each entry was planted in 1 m^2 plot in the middle of September. Experiment was conducted using randomized complete block design with two replications and recommended cultural practices were used for trial management. For the replicated data means were calculated. The stripe rust susceptible cultivar Morocco was planted in every 10th row and as a spreader border around the nursery to ensure uniform infection. In mid-April, stripe rust induced susceptible cultivar Morocco, was inoculated with mixed races of Pst at seedling stage in the field in Kazakhstan to serve as spreader of stripe rust pathogen to the experimental plots. Weather conditions in Almaty in 2019 and in 2020 were favorable for the development of stripe rust, and the infection on susceptible checks reached 100S. The resistant assessment was carried out according to the method developed by CIMMYT [25]. Five infection types described as the following: 0 – immune; R – resistant; MR – moderately resistant; MS – moderately susceptible; and S – susceptible. Severity of disease was recorded in terms of per cent leaf area infection and pustule type was recorded as response.

Spore collection, storage, and reproduction were then conducted in accordance with the methods of Roelfs et al. [25]. Spores of *P. striiformis* were used to determine the pathotypes of stripe rust isolated from wheat leaves in the different regions of Kazakhstan. The determination of race–specific seedling resistance

was performed according to the method of Roelfs et al. (1992) [25] using highly virulent *Pst* races – 111E231 (virulence 60%) and 7E63 (virulence 73%). The 111E231 pathotype was characterized by avirulence to varieties-differentiators with *Yr* genes *SU, SD, Yr10, Yr3v, Yr2,* and *Sp* and virulence to varieties with *Yr6, Yr7, Yr1, Yr12, Yr8, 3N, Yr6+, Yr7+,* and *Yr4+* genes. The 7E63 pathotype showed avirulence to the differentials with *SU, SD, Yr10,* and *Sp* genes and virulence to varieties with *Yr3v, Yr6, Yr7, Yr1, Yr2, Yr12, Yr8, 3N, Yr6+, Yr7+,* and *Yr4+* genes. The results were evaluated on 15-20 days according to the Gassner and Straib accounting scale (IT from 0 to 4) [26].

Genomic DNA was extracted at the stage of 3–5-day-old wheat seedlings using the CTAB method [27]. The presence of the *Lr34/Yr18* gene complex was identified using a specific codominant STS marker csLV34 (*Yr18*) [28], PCR was performed in a Bio-Rad T100TM amplifier (Bio-RAD, Hercules, California, USA). The PCR mixture contained 2.5 µl of genomic DNA (30 ng), 1 µl of each primer (1 pM/µl) (SigmaAldrich, St. Louis, Michigan, USA), 2.5 µl of dNTP mixture (2.5 mM, dCTP, dGTP, dTTP and dATP (aqueous solution) (Silex CJSC, Russia), 2.5 µl MgCl2 (25 mM), 0.2 µl Taq polymerase (5 units. mcl) (CJSC "Silex", Russia), 2.5 mcl 10X buffer for PCR and 12.8 mcl ddH20. PCR was performed at initial denaturation of 94 °C for 5 minutes, 40 cycles: 94°C – 40 s., 55°C – 30 s., 72°C – 1 min., final elongation at 72 °C – 7 min. The separation of PCR products was carried out in a 2% agarose gel using a TBE buffer (45 mM Tris-borate, 1 mM EDTA, pH 8) with the addition of ethidium bromide. The lengths of the amplicon fragments were determined using a 100-bp DNA marker. Visualization of the results was performed using the gel documentation system (Gel Doc XR+, BIO-RAD, Hercules, California, USA)

Results and discussion

The parent variety Almaly is characterized by high yields and a moderate degree of resistance to stripe rust. There is a variety of Bezostaya 1 in Almaly's pedigree, which is a carrier of the *Yr18* gene [29]. The Australian variety Avocet (S), on the contrary, has a high degree of susceptibility to stripe rust. The results of phytopathological evaluation of the breeding material under artificial infectious background allowed us to select 22 RIL Almaly/Avocet(S) lines that showed a high degree of resistance to the stripe rust pathogen *Pst* at the adult plant stage (0-20MR). From the two parents, 'Almaly' showed a moderate degree of resistance (20MR), while 'Avocet(S)' and the susceptible control Morocco showed a high degree of susceptibility (100S, both) (Table 1). The other control line NIL *Lr34*/TC*6/PI58548 for *Yr18*, the carrier of gene *Yr18* showed a moderate susceptibility with low severity (10MS).

In greenhouse experiment for seedling resistance lines investigated showed a different infectious type (ITs) for the two races of *Pst*. Avirulence of race 7E63 was shown to the majority of the studied RIL entries (91%) (IT-0), while 2 lines (9%) had only moderate resistance to the pathogen (IT-2). Evaluations for race 111E231 showed the following results: 14 RILs had a high degree of susceptibility (IT-4), 6 lines were moderately susceptible (IT-3), one line was moderately resistant and one immune (IT-2 and IT-0, respectively). The susceptible parent 'Avocet (S)' and the control 'Morocco' both showed a high susceptibility (IT-4) for both races, while the parent 'Almaty' and the isogenic line *Lr34*/TC*6/PI58548 (carrier of gene *Yr18*) were moderately resistant (IT-2) to race 111E231 and was immune (IT-0) to race 7E63.

Table 1 – Yellow rust severity and the presence of the *Yr18* gene in the genotypes of the population of recombinant inbred lines Almaly/Avocet

#	Genotype	Origin[a]	APR[b]	111E231[c]	7E63[c]	CSLV34[d]	Yr gene detected based on linked marker
♀	Almaly	KZ	20MR	2	0	+	Yr18
♂	Avocet (S)	AUS	100S	4	4	-	-
1	RIL Al/Av(S)-951-664	KZ	0	3	0	-	-
2	RIL Al/Av(S)-1094-817	KZ	0	3	0	+	Yr18
3	RIL Al/Av(S)-1085-808	KZ	20MR	3	0	-	-
4	RIL Al/Av(S)-1086-809	KZ	10MR	3	0	-	-

Continuation of the table

#	Genotype	Origin[a]	APR[b]	111E231[c]	7E63[c]	CSLV34[d]	Yr gene detected based on linked marker
5	RIL Al/Av(S)-852-560	KZ	20R	4	0	+	Yr18
6	RIL Al/Av(S)-882-589	KZ	0	4	0	-	-
7	RIL Al/Av(S)-883-590	KZ	0	4	0	-	-
8	RIL Al/Av(S)-968-682	KZ	20MR	4	0	-	-
9	RIL Al/Av(S)-975-691	KZ	0	4	0	-	-
10	RIL Al/Av(S)-976-692	KZ	0	4	0	-	-
11	RIL Al/Av(S)-982-698	KZ	20MR	4	0	-	-
12	RIL Al/Av(S)-986-702	KZ	0	4	0	-	-
13	RIL Al/Av(S)-993-710	KZ	20MR	4	0	-	-
14	RIL Al/Av(S)-994-711	KZ	20MR	4	0	-	-
15	RIL Al/Av(S)-1058-781	KZ	5R	4	0	-	-
16	RIL Al/Av(S)-1097-821	KZ	0	4	0	-	-
17	RIL Al/Av(S)-1099-823	KZ	0	4	0	-	-
18	RIL Al/Av(S)-1053-776	KZ	10MR	3	2	+	Yr18
19	RIL Al/Av(S)-1054-777	KZ	10MR	3	2	+	Yr18
20	RIL Al/Av(S)-1052-775	KZ	0	4	2	+	Yr18
21	RIL Al/Av(S)-1067-790	KZ	0	0	0	-	-
22	RIL Al/Av(S)-1009-725	KZ	20MR	2	0	-	-
	Controls						
23	NIL Lr34/TC*6/PI58548	USA	10MS	2	0	+	Yr18
24	Morocco	MA	100S	4	4	-	-

a – Origin include countries and organizations: KZ – Kazakhstan, AUS – Australia, USA – United States of America, MA – Marocco, Almaty – Institute of Plant Biology and Biotechnology;

b – Values indicate severity;

c – IT– infection typeaction type;

d – "+", "-" – indicate the presence and absence allele of corresponding gene, respectively.

The STS marker csLV34 is a diagnostic marker for the *Yr18/Lr34* gene complex, which allows determining the allelic state of a gene. Two alleles demonstrate amplicons that are clearly distinguishable in the agarose gel (150 bp for the dominant state and 229 bp for the recessive state). Molecular screening revealed the presence of a marker allele associated with *Yr18/Lr34* in 5 RIL Almaly/Avocet(S) (1094-817, 852-560, 1053-776, 1054-777, 1052-775) (Table 1, Figures 1 and 2). Thus, the frequency of resistant genotypes inheriting the *Yr18* gene was 22.7%. The heterozygous state of alleles of the *Yr18*/*Lr34* gene was not detected. (Figures 1 and 2.)

The complex of resistance genes *Yr18/Lr34/ Sr57/ Pm38* is important in the breeding of resistant varieties to yellow, leaf, and also partially to stem rust and powdery mildew [30]. This gene retains its effectiveness for about 100 years, which is due to the molecular characteristics of the defense mechanism. The activity of *Yr18/Lr34* induces necrosis of the tip of the flag leaf.

However, the observed reaction cannot be used as a phenotypic marker for *Yr18/Lr34*, which also manifests itself with other resistance genes (such as *Lr46/Yr29/Pm39*) [31], which indicates the need for molecular screening. *Yr18/Lr34* is a gene for adult plant resistance (APR), nevertheless able to impart resistance at the seedling stage to some rust races [32]. It is possible that the processes induced by *Yr18/ Lr34* make the tissue less favorable for biotrophic pathogens [33].

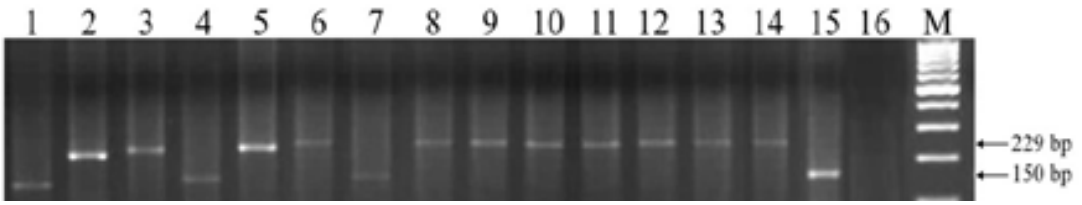

Figure 1 – Wheat DNA amplification products using primers to the STS csLV34 locus linked to the *Yr18/Lr34* resistance gene. Note: 1 – Almaly, 2 – Avocet(S), 3 –14 RIL Almaly/Avocet: 3 – RIL 951-664, 4 – RIL 1094-817, 5 – RIL 1085-808, 6 – RIL 1086-809, 7 – RIL 852-560, 8 – RIL 882-589, 9 – RIL 883-590, 10 – RIL 968-682, 11 – RIL 975-691, 12 – RIL 976-692, 13 – RIL 982-698, 14 – RIL 986-702; 15 – Yr18/NIL-Lr34/TC-6/PI58548, 16 – dd H_2O, M – Gene –Ruler 100 bp DNA Ladder

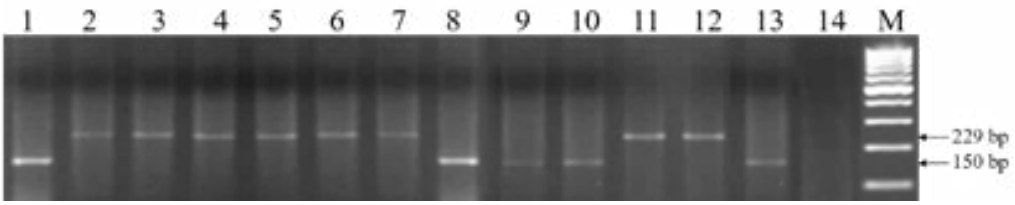

Figure 2 – Wheat DNA amplification products using primers to the STS csLV34 locus linked to the Yr18/Lr34 resistance gene (continued). Note: 1 – Almaly, 2 – Avocet(S), 3 –14 RIL Almaly/Avocet: 3 – RIL 993-710, 4 – RIL 994-711, 5 – RIL 1058-781, 6 – RIL 1097-821, 7 – RIL 1099-823, 8 – RIL 1053-776, 9 – RIL 1054-777, 10 – RIL 1052-777, 11 – RIL 1067-790, 12 – RIL 1009-725; 13 – Yr18/NIL-Lr34/TC-6/PI58548, 14 – dd H_2O, M – Gene –Ruler 100 bp DNA Ladder

It was previously shown that *Yr18/Lr34* provides a sufficiently high level of resistance in the conditions of an artificial epidemic. The effect of this gene is enhanced in combination with other resistance genes, such as *Yr5* and *Yr10* [5]. Molecular screening revealed 5 (22.7%) carriers of the *Yr18* gene (RIL Almaly/Avocet(S) 1094-817, 852-560, 1053-756, 1054-777 and 1052-775) from 22 studied samples, which indicates a high degree of its inheritance among resistant lines. No resistance genes were found in 71% of the studied samples, however, a high level of immunity to the pathogen *Pst* was noted in the field (0-20MR).

Conclusion

The results of field phytopathological screening of collection of RILs Almaly/Anza in field conditions to yellow rust caused by *Pst* allowed to select 22 resistance wheat lines, presumably carriers of resistance genes. The results of the evaluation of the selected lines of seedling resistance showed a contrasting reaction to two races of yellow rust: race 7E63 was avirulent to most samples, and 111E231 was highly virulent. Evaluation of the resistance of seedlings to *Pst* allowed us to identify 2 lines that were simultaneously resistant to both races. Molecular screening revealed the presence of a marker allele associated with the *Yr18/Lr34* gene complex in 5 samples. The frequency of resistant genotypes inheriting the *Yr18* gene was 22.7%.

The data obtained indicate the possibility of increasing the resistance of the material due to hybridization with productive wheat varieties and lines. The selected lines can be used in breeding programs to breed varieties resistant to yellow rust. Marker selection methods significantly simplify the process of selecting donors of resistance genes, which has a positive effect on the prospects for the development of the agricultural sector in Kazakhstan.

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