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RESISTANCE OF COMMON WHEAT (*TRITICUM AESTIVUM* L.) MAPPING POPULATION PAMYATI AZIEVA × PARAGON TO LEAF AND STEM RUSTS IN CONDITIONS OF SOUTH-EAST KAZAKHSTAN

Abstract. Epiphitoty of wheat fungal diseases, particularly leaf (caused by Puccinia triticina Erikss.) and stem (caused by Puccinia graminis Pers.) rusts, leads for the serious grain yield losses up to 60-80% all over the Globe, including Kazakhstan. Such detrimental effect is explained by the parasitic nature of pathogens and the ability of their spores to be spread easily with the wind. The presence of intermediate pathogen's hosts near the fields also plays an important role in the rapid development of infection. Therefore, comprehensive studies of pathogens, including genetic and ecological aspects, are required. In the present study, the population including 98 recombinant inbred lines of crossing between Russian common wheat cultivar Pamyati Azieva and UK's cultivar Paragon was used for field trials of resistance to leaf and stem rusts. Experiments were conducted in 2018-2019 in the fields of South-East Kazakhstan. As a result of this study, the resistance status of each line and two parental cultivars was determined, the promising lines with relatively high resistance to two diseases and great yield potential were identified, a negative correlation between the severity of diseases and yield components was revealed. A better understanding of relationships among pathogen, its host and other organisms, ways of pathogen's distribution and its role in ecosystems will give reliable background for the developments of new common wheat cultivars.

Key words: Common wheat, ecological testing, genotype × environment interaction, leaf rust, stem rust.

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Қазақстанның оңтүстік-шығыс жағдайында жапырақ және сабақ татына жұмсақ бидайдың карталанатын Памяти Азиева × Парагон популяциясының төзімділігі

Аңдатпа. Бидайдың саңырауқұлақ ауруының эпифитотиии, әсіресе жапырақ (қоздырғыш Puccinia triticina Erikss.) және сабақ (қоздырғыш Puccinia graminis Pers.) татының таралуы, дүниежүзінде, оның ішінде Қазақстанда да өнімділікті 60-80% дейін төмендетеді. Мұндай зиянды әсер патогеннің паразиттік сипатымен және оның спораларының желмен оңай таралу қабілетімен түсіндіріледі. Егістік маңында патогендік аралық иелерінің болуы инфекцияның жедел дамуында маңызды рөл атқарады. Осыған байланысты, генетикалық және экологиялық аспектілерді қоса, қоздырғыштарды жан-жақты зерттеу қажет. Бұл зерттеуде Ресейдің Памяти Азиева мен британдық Парагон жұмсақ бидай сорттарын будандастыру арқылы алынған 98 рекомбинанттық инбредтік линиялардан тұратын популяциясы жапырақ пен сабақ татына төзімділігін егістік жағдайында сынау үшін қолданылды. Эксперимент 2018-2019 жылдар аралығында, Қазақстанның оңтүстік-шығыс егістік жағдайында жүргізілді. Нәтижесінде: әр линия мен ата-аналық сорттардың төзімділік статусы анықталды, екі ауруға салыстырмалы жоғары төзімділігі бар және жоғары өнімділігі бар перспективті линиялар анықталды, сонымен қатар, аурудың зақымдау ауырлығы мен өнімділік компоненттерінің арасында кері корреляция анықталды. Патоген, оның иесі және басқа ағзалар арасындағы қарым-қатынасты, патогеннің таралу жолдары және оның экожүйедегі рөлін жақсы түсіну жұмсақ бидайдың жаңа сорттарын дамытуға сенімді алғышарттар жасайды.

Түйін сөздер: жұмсақ бидай, Triticum aestivum, экологиялық сынақтар, генотип × қоршаған орта өзара әрекеттесуі, жапырақ таты, Puccinia triticina, сабақ таты, Puccinia graminis.

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Устойчивость картирующей популяции мягкой пшеницы Памяти Азиева × Парагон к листовой и стеблевой ржавчинам в условиях Юго-Востока Казахстана

Абстракт. Эпифитотии грибных болезней пшеницы, в особенности листовой (возбудитель Puccinia triticina Erikss.) и стеблевой (возбудитель Puccinia graminis Pers.) ржавчин, приводят к серьезным потерям урожая зерна до 60-80% по всему миру, включая Казахстан. Подобный пагубный эффект объясняется паразитической природой патогена и способностью его спор с легкостью распространяться ветром. Наличие промежуточных хозяев патогена вблизи полей также играет важную роль в быстром развитии инфекции. Исходя из этого, необходимо всестороннее изучение патогенов, в том числе генетические и экологические аспекты. В данном исследовании популяция 98 рекомбинантных инбредных линий, полученная путем скрещивания российского сорта мягкой пшеницы Памяти Азиева и британского сорта Парагон, была использована для полевых испытаний на устойчивость к листовой и стеблевой ржавчинам. Эксперимент проводился в течение 2018-2019 гг. на полях Юго-Востока Казахстана. В результате: был определен статус устойчивости каждой линии и родительских сортов, идентифицированы перспективные линии с относительно высокой устойчивостью к двум болезням и высоким потенциалом урожайности, а также была обнаружена негативная корреляция между тяжестью поражения болезнью и компонентами урожайности. Лучшее понимание взаимоотношений между патогеном, его хозяином и другими организмами, путей распространения патогена и его роли в экосистемах создадут надежные предпосылки для разработки новых сортов мягкой пшеницы.

Ключевые слова: мягкая пшеница, экологические испытания, взаимодействие генотип × среда, листовая ржавчина, стеблевая ржавчина.

Abbreviations

ANOVA – analysis of variance; I – immune; KRIAPI – Kazakh Research Institute of Agriculture and Plant Industry; LR – leaf rust; M – mixed reaction to disease; MAS – marker-assisted selection; MP – mapping population; MR – moderately resistant; MS – moderately susceptible; PCA – principal component analysis; R – resistant; RIL(s) – recombinant inbred line(s); S – susceptible; SR – stem rust.

Introduction

Common wheat (*Triticum aestivum* L.) is the most important cereal crop in the World. Annual production of wheat grain takes the first place not only globally, but also in Kazakhstan, where it gives 20-25 million tons of the grain per year with up to 5-7 million tons to be exported [1]. However, infection with leaf rust (LR) and stem rust (SR) pathogens causes serious yield reduction [2]. The majority of wheat cultivars used for commercial production on the territory of Kazakhstan are susceptible to both of these diseases [3].

Leaf or brown rust (caused by *Puccinia triticina* Erikss.) and stem or black rust (caused by *Puccinia graminis* Pers.) are parasitic rust fungi belonging to the order Uredinales [4]. Their lifecycle is complex and includes 5 stages and several intermediate hosts. For example, pathogen uses Berberis species and some wild grasses for the development of pycniospores and aeciospores; and cereal species, including wheat, are used for the development of urediniospores, teliospores, and basidiospores [5, 6]. The main dangers of this fungi for a wheat plant is the ability to use their nutrients and thereby reduce key yield parameters, such as size, weight of kernels and number of kernels per spike [7, 8]. Epiphytotic development of SR in 1964 on the territories of northern Kazakhstan caused yield losses up to 50% [9]. In 2000-2001 Akmola region was exposed to epiphytotic outbreak of LR which affected commercial wheat cultivars up to 50-100% [10]. Dry and hot climate in most of Kazakhstan territories contributes to the rapid spread of pathogens' spores along with the air masses to large areas. Despite this, pathogens' populations vary in different parts of Kazakhstan [2, 11]. Every year, new data on the pathogenic composition of LR and SR populations appear. In 1998 there was the first report on highly-virulent race Ug99 of SR on wheat detected in Uganda [12]. For the last 20 years this race had spread from Eastern Africa to Near East and keeps moving toward the center of Eurasia continent [13]. The Ug99 has not been detected in Kazakhstan yet [3], however, search and development of methods for combating both LR and SR are extremely important and relevant research areas for our country.

One of the most common ways to protect wheat crops from rust infections is the usage of fungicides [3, 14]. Chemical preventative methods are effective, but at the same time economically disadvantageous and dangerous to the environment and humans. The most optimal and safe option is to create and grow wheat cultivars that are resistant to disease and possesses high yield potential. The resistance of wheat to LR and SR pathogens is controlled by *Lr* and *Sr* genes respectively. Overall, approximately 80 *Lr* and 60 *Sr* genes have been identified and described in common wheat, durum wheat and diploid wheat species [15]. However, in addition to the genetic factor, the environment has a significant effect on the manifestation of resistance. Individual genes can be effective in all regions of the country or certain parts of it. For example, it was shown that gene *Lr9* was effective for South-East, East, North Kazakhstan, and Akmola region, but gene *Lr23* was highly specific and effective in Akmola region only [2]. Thus, the main task for wheat breeders is searching for sources of resistance genes for LR and SR, and development of new cultivars using both traditional and modern breeding methods, including marker-assisted selection (MAS) [16, 17].

One of the methods which is widely used to find genetic factors associated with diseases resistance is linkage mapping. For these purposes biparental mapping populations (MP) are applied. There are several types of MP – F_2, Double haploid (DH), Back cross population, recombinant inbred lines (RIL), near-isogenic lines (NIL). This method has established itself as effective for the analysis of many quantitative traits of wheat, such as productivity [18, 19, 20], disease resistance [21, 22, 23], and tolerance to abiotic stress factors [24, 25]. The results of such work usually are the identification of new genomic regions responsible for the quantitative trait and the selection of promising lines for further breeding programs. In this study, the Pamyati Azieva × Paragon MP consisted from 98 RIL was used for studying the genetic basis of LR and SR resistance. Current field analysis was a part of QTL mapping study, and data obtained in this work allowed to reveal promising lines with the resistance to LR and SR, that could be used in the breeding of new promising cultivars.

Materials and methods

Mapping population. The MP comprising of 98 F8 recombinant inbred lines (RILs) was created via crossing between two spring wheat cultivars – "Pamyati Azieva" and "Paragon" – using single seed descent method [26, 27]. The first parental cultivar was Russian medium-early spring wheat cultivar "Pamyati Azieva" recommended for the Western Siberian region of Russian Federation [28], and approved for commercial cultivation in the North Kazakhstan [29] The second parental cultivar was the United Kingdom's elite spring wheat cultivar "Paragon". The MP was developed within the ADAPTAWHEAT project in greenhouse conditions by using facilities of the John Innes Centre (Norwich, UK) during 2011-2015 [30].

Field trials and ecological testing. Evaluations of the MP's resistance and major yield components were conducted in the field plots of Kazakh Research Institute of Agriculture and Plant Industry (KRIAPI, South-East Kazakhstan, Almaty region) in 2018-2019. Ninety-eight RILs, the parental cultivars ("Pamyati Azieva" and "Paragon"), and standard check cultivars ("Kazakhstanskaya 4" and "Kazakhstanskaya rannespelaya") were evaluated under a natural source of infection for the resistance to LR and SR. The MP was planted at each site in randomized doubled experiments. Plants were grown in 15 cm distance between rows and 5 cm distance between plants within a row. Each row contained 25 plants. LR and SR resistances were assessed visually during the phase of grain formation on 75 of Zadoks scale [31]. Averaged values for both diseases in two years were used for further analysis. Evaluation of resistance/susceptibility levels was performed using the scale of Mains and Jackson [32] for LR, the scale of Stakman [33] for SR. The severity of rust infection on leaf and stem surfaces was assessed using the modified Cobb scale [34, 35]. For more precise analysis of information, initial data was converted into 9-point scale (Table 1). The list of assessed yield components included several plant architecture traits – plant height (PH), peduncle length (PL), and number of productive spikes per plant (NPSP); spike architecture traits – spike length (SL), number of fertile spikelets in the main spike (NFS), and number of kernels in the main spike (NKS); productivity traits – weight of kernels in the main spike (WKS), weight of kernels per plant (WKP) and thousand kernels weight (TKW).

Table 1 – Correspondence between traditional scale and the 9-point scale for evaluation of resistance/susceptibility to leaf and stem rusts based on visual symptoms

Traditional scale	9-point scale	Plant's reaction	Symptoms
0	0	Immune	No visible signs of infection
R	1	Resistant	Visible chlorosis or necrosis, no uredia are present
10-40 MR	2	Moderately resistant	Small uredia are present and surrounded by either chlorotic or necrotic areas (10-40% of the leaf/stem surface)
50-100 MR	3		Small uredia are present and surrounded by either chlorotic or necrotic areas (50-100% of the leaf/stem surface)
10-40 M	4	Intermediate (mixed)	Variable sized uredia are present some with chlorosis, necrosis or both (10-40% of the leaf/stem surface)
50-100 M	5		Variable sized uredia are present some with chlorosis, necrosis or both (50-100% of the leaf/stem surface)
10-40 MS	6	Moderately susceptible	Medium sized uredia are present and possibly surrounded by some chlorotic areas (10-40% of the leaf/stem surface)
50-100 MS	7		Medium sized uredia are present and possibly surrounded by some chlorotic areas (50-100% of the leaf/stem surface)
10-40 S	8	Susceptible	Large uredia are present, generally with little or no chlorosis or necrosis (10-40% of the leaf/stem surface)
50-100 S	9		Large uredia are present, generally with little or no chlorosis or necrosis (50-100% of the leaf/stem surface)

Statistical analysis. MS Excel software was used to process and visualize numerical data. Analysis of variance (ANOVA), Pearson correlation tests and principle component analysis (PCA) were performed using the R statistical platform [36].

Results and discussion

The resistance of RILs, parental cultivars and check cultivars to leaf and stem rusts. Studied RILs population had demonstrated different levels of resistance to LR and SR observed in two years. Severity distribution for both diseases showed transgressive segregation. In 2018 the average score of LR resistance was 6.5±2.5 (ranged from 5 to 7) and remained stable in 2019 when it was 6.6±2.7 (ranged from 2 to 9) on 9-point scale, both of which corresponded to moderately susceptible level (MS). The major part of lines – 92% – in 2018 belonged to MS group and 8% was highly susceptible (S), but in 2019 the situation had changed (Figure 1A). In the second year of observations, amount of MS lines had reduced to 64% and number of S lines had increased up to 29%. At the same time, 9 lines had demonstrated moderate resistance (MR) which was not observed in 2018. Parental cultivars Pamyati Azieva and Paragon had demonstrated high susceptibility to LR in 2018 (20S and 40S respectively or 8 on 9-point scale), which were higher than the average value among the RILs; and moderate susceptibility in 2019 (30MS and 40MS respectively or 6 on 9-point scale), which were lower than the average score of RILs population.

As for SR, the severity scores had changed dramatically between two years. The average score in 2018 was on the level of 7.4±2.3

points (ranged from 3 to 8) with the following distribution: 77% of lines were defined as S, 14% were MS, 6% demonstrated mixed reaction, and three lines were MR (Figure 1B). SR severity level of Pamyati Azieva and Paragon corresponded to 10MS and 40MS respectively or 6 points for both in 2018 and were lower than the average score in the population. In 2019 scores moved towards resistance, and average score was 1.8±2.0 (ranged from 0 to 8) with 47% of lines being immune (I), 9% were resistant (R), 27% were MR, 16% demonstrated MS level and only one line was highly susceptible. Parental cultivars were generally resistant to SR in 2019 – 5R for Pamyati Azieva and 20R for Paragon.

Check cultivars Kazakhstanskaya rannespelaya and Kazakhstanskaya 4 had demonstrated moderate susceptibility to LR – 40MS or 6 on 9-point scale for two diseases and both resistant to SR – 5R or 1 on 9-point scale.

Transgressive segregation for resistance in cereals has been previously reported in wheat for resistance to leaf rust [37, 38] and stem rust [39, 40]. The current study, therefore, confirms the quantitative effect of loci explaining resistance to the two rust diseases. However, both parental cultivars are described as susceptible to two diseases [3, 41], and variations like that can be possibly explained by differences in the composition of pathogen's races in population of particular region and/or weather conditions. Nevertheless, their offspring lines had different levels of resistance to LR and SR in two years what may be explained by the genetic background of parental cultivars.

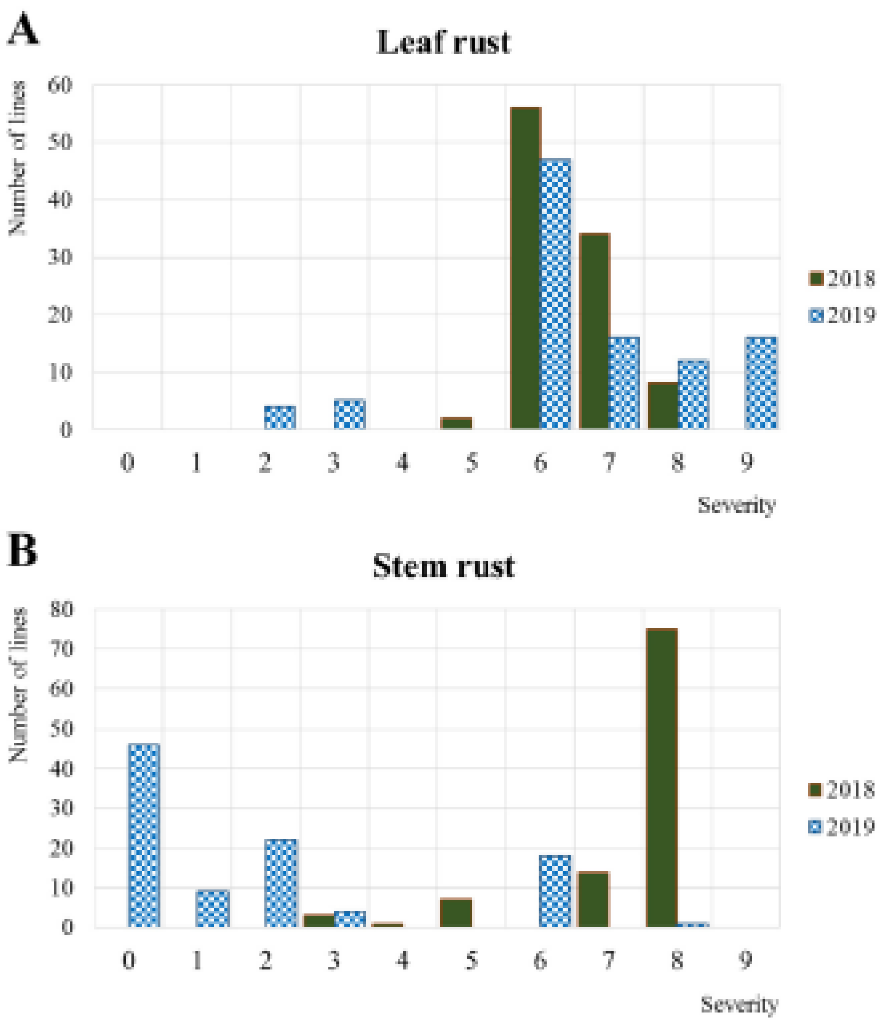

Figure 1 – Phenotypic distribution of Pamyati Azieva x Paragon recombinant inbred lines for leaf (A) and stem (B) rusts severity observed in two years

Analysis of variance indicated a moderately significant influence of genotype ($p<0.001$) and weaker influence of genotype × environment combination ($p<0.05$) on the severity of LR (Table 2). As for SR, there were highly significant differences ($p<2e^{-16}$) among the 98 RILs explained by the test environments or two years and weaker influence of genotype on the trait ($p<0.05$) (Table 2).

Table 2 – ANOVA of genotype and environment effects and proportion of phenotypic variation for leaf and stem rusts severity obtained across two testing environments (two years) in Pamyati Azieva x Paragon population

Leaf rust					
Factor	**Df**	**Sum sq**	**Mean sq**	**F-value**	**p-value**
Genotype	1	13.89	13.891	8.858	0.003**
Environment (year)	1	0.4	0.405	0.258	0.612
Genotype×Environment	1	8.21	8.207	5.233	0.023*
Residuals	196	307.37	1.568		
Stem rust					
Factor	**Df**	**Sum sq**	**Mean sq**	**F-value**	**p-value**
Genotype	1	16.2	16.2	5.011	0.026*
Environment (year)	1	1596.1	1596.1	494.673	<2e-16***
Genotype×Environment	1	7.6	7.6	2.368	0.125
Residuals	196	632.4	3.2		
Notes: Df – degree of freedom; F-value – effect of the factor; p-value – probability; * – p<0.05, ** – p<0.01, *** – p<0.001					

Correlation between severities of LR and SR and its influence on yield components. In the course of present work, a moderate positive correlation was observed for LR severity between 2018 and 2019, as well as positive correlation between LS and SR in 2019 (Table 3).

Table 3 – Pearson correlation between severities of leaf and stem rusts in two environments (years) of the experiment

Disease/Environment (year)		Leaf rust		Stem rust	
		2018	2019	2018	2019
Leaf rust	2018	-			
	2019	0.306**	-		
Stem rust	2018	-0.014	0.044	-	
	2019	0.027	0.244*	0.051	-
Notes: * – p<0.05, ** – p<0.01, *** – p<0.001					

In the case of LR severity in two years, such phenomena can indicate consistency and repeatability of field data collected from the testing environments in two years. As for the correlation between LR and SR in 2019, high correlation coefficient may suggest that genes conferring leaf rust resistance were either closely linked or pleiotropic to genes that condition stem rust resistance. Therefore, the interactions between loci conditioning resistance to both LR and SR diseases in the current MP should be studied more deeply.

A phenotypic association study among the 98 RILs was conducted using principal component analysis (PCA) (Figure 2) in order to detect similarities in the lines' responses to LR and SR in two studied environments, as well as to determine the extent and direction of correlations between the two rusts and determine promising resistant lines. The PCA separated the RILs into two distinct principal components: PC1 and PC2, which explained 35.1% and 25.9% of the total variation, respectively. The relatively resistant RIL_01 was plotted on a significant distance from the main mass of RILs, as well as lines RIL_10, RIL_12, RIL_33, RIL_43, and RIL_44, which were also highlighted as promising for the breeding (Figure 2).

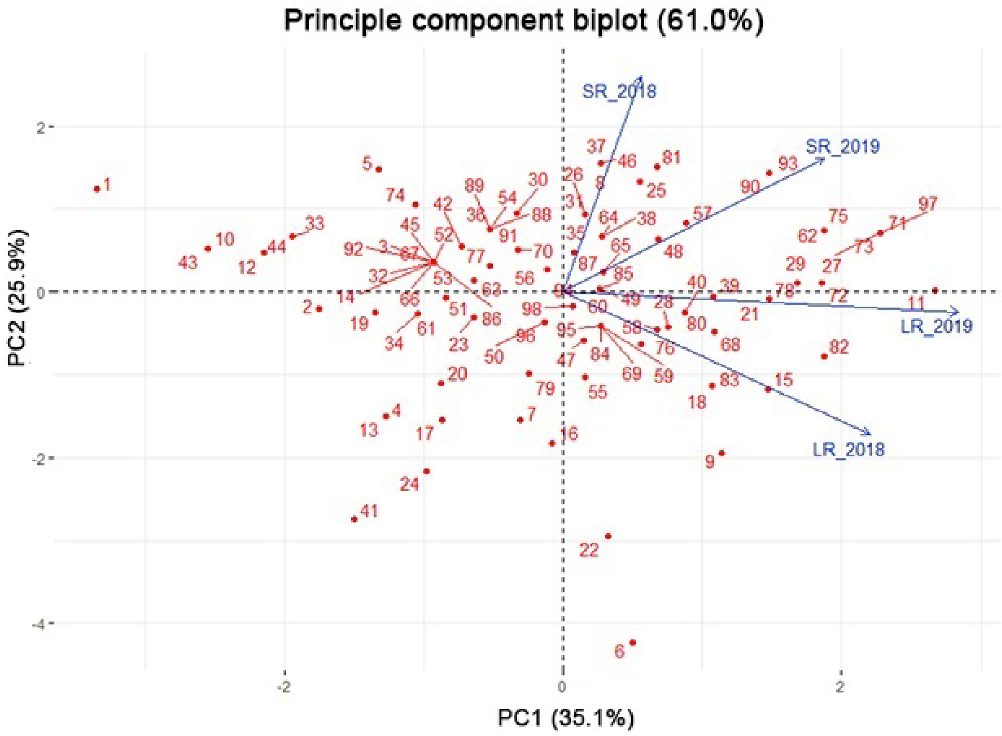

Figure 2 – Association between 98 Pamyati Azieva × Paragon RILs on the basis of the first two principal components (PC1 and PC2) obtained from a principal component analysis based on leaf and stem rust severity scores in two environments. LR – leaf rust, SR – stem rust. Numbers indicate the genotypes

In previous study of yield and its components of this MP in 2015-2018 in Almaty region several RILs were observed to be better than check and parental cultivars [20]. Analysis of weight of kernels per plant (WKP) has indicated that 40 RILs, including five best RILs: RIL_48, RIL_36, RIL_83, RIL_01, and RIL_46, outperformed the local parent cultivar Pamyati Azieva. They showed the best averaged yield values over the indicated period, which is highly correlated with PH, NFS, NKS, and TKW, as indicated by the Pearson correlation index. Individuals with favorable values for all yield-related traits were identified for their incorporation into the breeding studies [20].

In Table 4, there is summarized information about six RILs that had shown relatively high level of resistance to both rust diseases compared with the averaged level, as well as values of important yield components. RIL_01 confirmed its high-yielding significance in the last years [20].

Fungal infection of wheat usually leads to the reduced number of kernels per spike and lower kernel weights due to the parasitic consumption of host nutrients, which leads to apparent yield losses and poor quality of the grains [7]. Correlation analysis of the most important yield components of the studied MP allowed to reveal strong ($p<0.001$) in 2018 and weak ($p<0.05$) in 2019 negative influence of LR severity on the thousand kernel weight (Table 5).

Table 4 – Best-performing RILs displaying leaf rust/stem rust resistance and their values of important yield components compared with averaged levels of 2018-2019

Trait/RIL	Average*	RIL_01	RIL_10	RIL_12	RIL_33	RIL_43	RIL_44
LR	6.6±1.0	3.5±2.0	4.0±2.5	4.5±2.1	4.5±2.1	4.0±2.0	4.5±2.1
SR	4.6±.1.3	4.0±2.5	4.0±3.0	4.0±3.0	4.5±3.0	4.0±3.0	4.0±3.0
PH	97.3±8.7	106.2±5.9	93.1±1.2	104.7±1.5	92.0±6.1	97.3±4.7	93.0±0.5
PL	38.1±5.0	40.9±4.4	34.0±4.8	38.2±0.8	34.7±2.4	37.2±1.2	40.5±8.2
NPSP	3.3±0.7	5.3±1.4	3.4±1.1	3.6±1.1	2.7±0.0	3.8±0.7	2.8±0.7
SL	10.7±1.2	10.9±0.6	10.8±0.4	10.0±0.4	10.3±1.4	9.3±0.8	10.8±0.9
NFS	48.6±6.5	45.5±3.3	48.6±2.6	45.2±0.9	46.8±11.5	49.8±2.8	51.8±3.1
NKS	19.7±1.4	20.0±2.1	19.2±1.3	18.7±0.7	19.0±0.0	17.7±12.0	19.8±10.1
WKS	1.7±0.3	1.5±0.4	1.8±0.1	1.8±0.0	1.7±0.5	1.7±0.4	1.6±0.1
WKP	4.4±1.1	5.0±0.1	4.9±1.1	5.0±0.8	3.5±0.8	4.3±0.6	3.4±0.5
TKW	32.6±3.1	30.5±4.1	32.6±3.1	36.1±0.5	32.3±2.1	31.4±0.3	32.7±1.2
Notes: * – Average value of the trait per RIL measured in two years;							

Table 5 – Pearson correlation among severities of leaf rust and stem rust and key yield components observed in two years

Dis.	Env.	PH	PL	NPSP	SL	NFS	NKS	WKS	WKP	TKW
LR	2018	-0.006	-0.060	-0.214*	0.122	0.179	0.090	-0.015	-0.042	-0.340***
	2019	-0.185	-0.090	-0.260**	0.036	0.252	0.140	-0.069	-0.144	-0.067*
SR	2018	0.238	0.241	-0.032	0.221	-0.077	0.202	-0.083	-0.168	-0.106
	2019	-0.140	-0.108	-0.172	-0.085	0.019	-0.070	-0.064	-0.154*	0.136
Notes: Dis. – disease; Env. – environment (year); * – p<0.05, ** – p<0.01, *** – p<0.001										

Also moderate ($p<0.01$) and weak ($p<0.05$) negative correlations were observed in 2019 and 2018, respectively, for the number of productive spikes per plant. In the case of SR, negative correlation was detected in 2019 between the severity of infection and the weight of kernels per plant (Table 5).

Thus, the resistance status of each line and two parental cultivars was determined, the promising lines with relatively high resistance to two diseases and great yield potential were identified, a negative correlation between the severity of diseases and yield components was revealed.

Conclusion

The present study reported the diverse resistance of RILS of Pamyati Azieva × Paragon mapping population to leaf rust and stem rust resistance. The analysis was conducted based on field evaluation of 98 newly developed RILs, two parental cultivars and two checks in 2018-2019 on the experimental fields of KRIAPI (South-East Kazakhstan). As the results of this work, 6 best-performing RILs for leaf rust and stem rust resistance were selected for the further wheat breeding and genetic studies. Therefore, the newly developed RILs in the genetic background of Pamyati Azieva and Paragon cultivars can be used for breeding resistant accessions to these two fungal diseases. Further studies using the selected lines are required to understand the genetic basis explaining reaction of lines to leaf rust and stem rust resistance in more diverse environments. The results of this study are invaluable for resistance breeding and contribute to the surge to fight two of the most dangerous wheat diseases.

Conflict of interest

All authors are familiar with the text of the article and declare that they have no conflict of interest.

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