Asian Journal of Agriculture and Biology
https://doi.org/10.35495/ajab.2024.142

Original Article

Characterization of wheat leaf rust resistance genes in promising genotypes from Kazakhstan: Molecular screening and field evaluation

Madina Kumarbayeva*, Alma Kokhmetova*, Zhenis Keishilov, Ardak Bolatbekova, Assiya Kokhmetova, Kanat Mukhametzhanov, Kanat Bakhytuly

Institute of Plant Biology and Biotechnology, Almaty 050040, Kazakhstan

**Corresponding author's emails:* madina_kumar90@mail.ru; akokhmetova@gmail.com

Received: 10 September 2024 / Accepted: 26 November 2024 / Published Online: 23 January 2025

Abstract

Leaf rust is one of the most prevalent and harmful wheat diseases in the world. Wheat leaf rust is best controlled over the sustainable development of genetic resistance, which requires thorough testing via field trials and marker-assisted selection. In this research, we studied sixty wheat genotypes to assess their resistance to foliage rust over two growing seasons: 2019/2020 and 2020/2021. The adult plant resistance (APR) severity and the area under the disease progress curve (AUDPC) were calculated for each wheat entry studied. The results of molecular screening showed that five *Lr* genes, namely, *Lr19*, *Lr24*, *Lr*34, *Lr50*, and *Lr68*, both independently and in combination, were identified in 25 wheat genotypes. The genes *Lr24* (8.3%), *Lr37* (6.7%) and *Lr50* (25%) were characterized by the highest frequency of occurrence. Five genotypes were identified as carriers of two *Lr* resistance genes: CP_13 (*Lr19* and *Lr68*), CP_21 and CP_22 (*Lr24* and *Lr50*), CP_21 and 388_SP2 (*Lr50* and *Lr68*). These genotypes may be used to introduce *Lr* genes into Kazakhstani wheat cultivars acclimating to leaf rust, since most showed high to moderate resistance to the disease in mature plants. Principal component analysis (PCA) biplots demonstrated the strong correlation between each spike productivity attribute. The study's sources of leaf rust resistance may be leveraged to improve resistance to leaf rust in Kazakhstani and other relevant international wheat breeding programs.

Keywords: Wheat, Leaf rust, *Puccinia triticina*, Molecular markers, *Lr* genes

How to cite this article:

Kumarbayeva M, Kokhmetova A, Keishilov Z, Bolatbekova A, Kokhmetova A, Mukhametzhanov K and Bakhytuly K. Characterization of wheat leaf rust resistance genes in promising genotypes from Kazakhstan: Molecular screening and field evaluation. Asian J. Agric. Biol. 2025: 2024142. DOI: https://doi.org/10.35495/ajab.2024.142

Introduction

In 2023, global it is anticipated that 785.1 million tons of wheat would be produced, marking a 2.2% decline (about 18 million tonnes) from the previous year. This decrease is attributed to adverse weather conditions that affected both winter and spring crops, leading to overall grain production falling underneath the five years average (Vanongeval and Gobin, 2023). Specifically, Kazakhstan's wheat output is projected at 12.1 million tonnes, which is significantly lower than the five years average and about 25% less than last year's figures. In Northern Kazakhstan, drought and excessive rainfall have reduced yields and compromised grain quality (FAO, 2023). A major factor contributing to the decline in wheat yields in Kazakhstan is the prevalence of airborne diseases, Specifically, rust and leaf spot diseases that impact wheat crop (Kokhmetova and Atishova, 2012; Morgounov et al., 2015; Kokhmetova et al., 2016; Kokhmetova et al., 2018a; Kokhmetova et al., 2020a; Kokhmetova et al., 2021a; Kokhmetova et al., 2018b; Kokhmetova et al., 2019; Kokhmetova et al., 2020b; Kokhmetova et al., 2021b; Kumarbayeva et al., 2022a; Olivera et al, 2022; Kumarbayeva et al., 2022b; Kokhmetova et al., 2023; Kokhmetova et al., 2024; Kenzhebayeva et al, 2024).

Research indicates that wheat resistance to diseases, such as leaf rust, is becoming increasingly relevant in the context of climate change and the rising aggressiveness of pathogens. Recent studies highlight significant advancements in understanding and utilizing *Lr* genes to enhance wheat resistance to leaf rust, as well as emphasize the importance of integrating genetic diversity into breeding programs for effective management of evolving pathogens (Kumar et al., 2022; Ren et al., 2023; Sharma et al., 2023). This translation maintains the scientific integrity and clarity of your original text.

Climate change leads to fluctuations in temperature and increased humidity, creating favorable conditions for the spread of pathogens such as *Puccinia triticina*. The optimal temperatures for leaf rust infection range from 11 to 23°C, and high humidity promotes spore germination and plant infection. This means that under rising temperature and humidity conditions, the risk of epidemics may increase. Weather conditions, such as the frequency and intensity of precipitation, affect the development of leaf rust. In regions with increased rainfall during the growing season, more intense disease development is observed. Climate change may also contribute to changes in pathogen virulence. Pathogens can adapt to new conditions, making existing wheat varieties more vulnerable. For example, rising temperatures may alter the pathogen's life cycle and enhance its ability to infect. Climate change can facilitate the migration of new pathogen races into regions where they were previously absent, leading to unexpected disease outbreaks and loss of resistance in traditional wheat varieties (Miedaner and Juroszek, 2021; Singh et al., 2023). Long-term selection for high yield can result in reduced genetic diversity among wheat varieties. This makes crops less resilient to changing climatic conditions and new pathogen races. Therefore, resistant varieties must be developed with genetic diversity in mind to ensure protection against future threats. The need for developing new varieties with high disease resistance becomes particularly urgent in the context of climate change. Breeding programs should focus on utilizing resistance genes (e.g., *Lr* genes) and their combinations to create varieties capable of adapting to new conditions. Given the impact of climatic factors on disease epidemiology, it is essential to continue exploring these relationships to develop effective management strategies and breed resistant wheat varieties (Miedaner and Juroszek, 2021; Singh et al., 2023).

According to Riaz and Wong (2017), leaf rust is a serious disease that affects wheat and may cause output declines of up to 50%. at contrast to other rust illnesses like stem and stripe rust, leaf rust grows best at temperatures between 10 and 25 degrees Celsius. However, the geographic distribution of leaf rust has significantly increased due to climate change, and its active infection period has extended (Ren et al., 2023). Finding the genetic mechanisms behind this disease and creating wheat cultivars resistant to leaf rust are two important areas of study interest (Kolmer and Liu, 2002; Oelke and Kolmer, 2005; Datta et al., 2008; Rosa et al., 2016; Aoun et al., 2017). It is necessary for Avr, the avirulence gene that mates with each distinct *Lr* gene to bestow confrontation to certain races of *P. triticina* (Pt), to exist. The "gene-for-gene" theory states that an Avr gene in the pathogen and a specific *Lr* genetic factor in the wheat host correlate in this regard (Bakkeren and Szabo, 2022). It's possible for new strains to spread from other locations and bring new Avr genes that the host doesn't have corresponding resistance genes for. Through Avr gene recombination or mutation, the virus continues to produce new, virulent races (Bolton et al., 2008).

https://doi.org/10.35495/ajab.2024.142

Consequently, host resistance often decreased over time, indicating that most race-specific *Lr* genes did not provide long-lasting resistance (Ellis et al., 2014). Based on their phenotypic effects, *LR* genes may be divided into two types: adult plant resistance (APR) and seedling resistance (SR), often referred to as all-stage resistance (ASR). Only in the APR, namely after the booting step, can the latter manifest itself. APR genes are linked to more resilient resistance, while ASR genes often provide resistance that may fade after a period of time. Breeders and pathologists are increasingly in agreement that, in order to guarantee long-term efficacy in agricultural methods, more attention should be paid in finding, defining, and exploiting APR genes rather than depending only on R genes, which often lack durability (Ellis et al., 2014). Recombination and mutation of the Avr gene can have significant implications for resistance to leaf rust in wheat. Here are several key aspects: 1) Changes in Pathogen Virulence: recombination can lead to the emergence of new pathogen strains with altered virulence. This may reduce the effectiveness of existing resistance genes in plants, as new Avr variants can evade recognition by the host's R genes. For example, if a pathogen loses its Avr function, it may be able to infect previously resistant plants. 2) Adaptation to New Conditions: Mutations in Avr genes may allow pathogens to adapt to environmental changes or selection pressures, which can also diminish resistance effectiveness. Changes in the structure or function of Avr may render pathogens less specific, enabling them to infect a broader range of wheat varieties. 3) Long-term Resistance: Resistance based on one or a few R genes may be temporary if pathogens rapidly adapt through recombination and mutations. This underscores the necessity of employing polygenic approaches in breeding programs to slow down the adaptation process of pathogens. Research indicates that some resistance genes lose their effectiveness within a few years after being introduced into breeding programs due to changes in pathogen virulence structure (e.g., studies on *Lr* genes and their interaction with Avr) (Porotnikov et al., 2020). This reinforces the need for continuous monitoring and updating of genetic resources to maintain resistance.

In wheat, approximately 80 *Lr* genes leaf rust resistance genes have been originated in recent investigations. While some of these genes are sourced from wild cousins like Aegilops, Agropyron, Secale, and Thinopyrum, others have been introgressed from durum or bread wheat cultivars (Safavi and Afshari, 2012; McIntosh et al., 2017, 2020). Since the 14 genes in question haven't been examined for alleles with known *Lr* genes to verify their originality, they haven't been given new numbers in the *Lr* series (Kumar et al., 2022; Talebi et al., 2023).

The introgression of new *Lr* genes may result in reduced expression due to interactions with the recipient genome. This can lead to situations were, even in the presence of target genes, the level of resistance may not be sufficient to protect against pathogens (Leonova, 2018). The introduction of foreign genes can cause cytological instability and sterility in first-generation hybrids, complicating further selection and the practical use of such varieties in agriculture. The integration of foreign genes may also affect other economically valuable traits, such as yield, grain quality, and resistance to other stresses. For instance, the presence of additional fragments from foreign genomes can negatively impact plant growth and development (Leonova, 2018). The use of a limited number of *Lr* genes may lead to a reduction in overall genetic diversity within the wheat population, making varieties more vulnerable to new pathogen races and other stress factors. Several strategies are known to overcome these issues: 1) Combined breeding approach: to enhance disease resistance, it is recommended to use not only a single *Lr* gene but also combinations of multiple genes. This can provide more reliable protection and reduce the risk of losing expression from individual genes; 2) Use of molecular markers: the application of molecular markers for monitoring and selecting resistant lines will allow for more precise tracking of the effectiveness of new *Lr* gene introgressions and their impact on other agronomic traits. 3) Genetic modification and CRISPR technologies: utilizing modern genome editing technologies, such as CRISPR, can facilitate the precise introgression of desired *Lr* genes without the negative effects associated with transferring large fragments of foreign DNA. 4) Long-term trials and monitoring: It is essential to conduct prolonged trials of new varieties with introrse *Lr* genes under various agronomic conditions to assess their resistance and adaptability. This will help identify potential negative effects before widespread adoption in agriculture.

There are several limitations to the use of PCR-based molecular markers for the identification of *Lr* genes: 1) Marker Specificity: insufficient specificity of primers can lead to false-positive results, complicating

the accurate identification of target genes; 2) Method Sensitivity: the sensitivity of PCR can vary depending on sample quality and the presence of inhibitors, which may result in false-negative outcomes; 3) Reproducibility of Results: it is important to consider that PCR conditions can impact the reproducibility of results. Variations in temperature protocols and reagent quality can lead to discrepancies in outcomes, highlighting the need for stringent quality control throughout the analysis process; 4) Alternative Methods: exploring alternative methods, such as next-generation sequencing (NGS), may help overcome the limitations of PCR by providing higher accuracy and sensitivity (Leonova, 2018).

The application of a comprehensive breeding approach, the use of molecular markers, and modern genome editing technologies can significantly enhance the efficiency of developing resistant wheat varieties while minimizing risks associated with the introgression of foreign genes (Li et al., 2023). New developments in molecular marker technology have made it possible to detect disease resistant genes with greater accuracy. When compared to conventional phenotypic selection techniques, the use of PCR with DNA-based molecular markers has many advantages (Aktar et al., 2017). By addressing issues with traditional phenotypic screening, marker-assisted selection (MAS) has been extensively used to target rust resistance genes, increasing the effectiveness of plant breeding (Schachermayr et al., 1995; Herrera-Foessel et al., 2012). Early plant growth may benefit from MAS since it allows for the simultaneous screening of many genes using different DNA markers (Aktar et al., 2017). Accurate data may be obtained using molecular markers to evaluate the variety of diseases and host plants. Agribusiness crop tolerance to biotic stressors, like as leaf rot, has been effectively increased by techniques including gene pyramiding and cloning (Ijaz et al., 2023). Finding more sources of resistance is crucial in creating new wheat varieties. In order to promote the development of wheat varieties with greater resistance to this important disease, our study focuses on discovering resistance *Lr* genes among a collection of promising wheat lines from Kazakhstan. To do this, we have utilized molecular screening and field assessments.

Material and Methods

Plant material

This study utilized 60 promising winter wheat lines (*Triticum aestivum* L.) developed in the Genetics and Breeding Laboratory at IPBB (Table 4). The cultivar Morocco, which is very sensitive, served as the negative control. The resistant cultivar Parula, carrying *Lr68*, was used as the positive check during identifying the *Lr* gene. Additionally, near-isogenic lines (NILs) were used: Agrus/6Thatcher *(Lr19)*, Payne/tam W-101/Amigo *(Lr24)*, VPM 1/Moisson 421//2Tyee (*Lr37*) and TA 870 (*Lr50*).

Mature plant resistance investigation

At the Kazakh Research Institute of Agriculture and Plant Growing (KRIAPG) in Almalibak (43°14'330" N 076°44'797"E, 790 meters above sea level), assessments of resistance to *Puccinia triticina* were conducted during the growing seasons of 2021 and 2022. These evaluations focused on mature plants from the SP2 and CP breeding nurseriers (7-8 year adult plants) and were carried out under field conditions, providing critical insights into the resistance levels of various wheat varieties against this significant pathogen. Three replications and a fully randomized block design were used in the experiment. Buffer rows one meter wide, planted with the very sensitive cultivar Morocco, surrounded each area. Each plot measured 3 m², or in a layout of 3 meters by 7 rows separated by 15 cm. The techniques used for fertilizer application and cultivation complied with local recommendations (Dospekhov, 1985). Nitrogen oxide (60 kg/ha) and phosphorus oxide (30 kg/ha) were the fertilizers utilized. Every year, the trials were planted in early October and harvested at the end of July. Experimental plots received irrigation three times during the growth period, with 600 m³/ha applied each time, and were maintained weed-free. All recommended cultivation practices for commercial fields, including fertilization, irrigation, and other management techniques, were implemented.

The weather conditions during the research period were favourable for the development of wheat leaf rust. Precipitation exceeded normal levels, increasing environmental humidity and facilitating effective plant infection by *P. triticina* spores (http://weatherarchive.ru, accessed on 22 February 2024); Table 1).

Table-1. Mean daily temperature and relative humidity data for KRIAPG during the years 2021–2022.

Month	2020-2021		2021-2022	
	Temp (0C)	Amount of precipitation (mm)	Temp (0C)	Amount of precipitation (mm)
Oct	9.7° (-1.8°, 25.0°)*	9 mm	7.9° (-2.3°,21.1°)*	76 mm
Nov	0.2° (-11.7°, 21.5°)*	23 mm	1.1° (-14.6°,15.8°)*	39 mm
Dec	-6.3° (-12.6°, 6.9°)*	13 mm	1.3° (-8.8°,12.7°)*	13 mm
Jan	-5.7° (-17.2°,11.2)*	13 mm	-0.1° (-8.5°,13.0°)*	16 mm
Feb	1.7° (-14.4°,17.4°)*	51 mm	-1.1° (-11.0°,13.5°)*	33 mm
Mar	4.9° (-8.0°,22.0°)*	113 mm	5.6° (-2.0°,17.5°)*	116 mm
Apr	12.5° (-4.2°,32.5°)*	54 mm	16.7° (5.6°,30.5°)*	45 mm
May	19.5° (6.5°,32.0°)*	70 mm	19.0° (8.1°,32.5°)*	142 mm
Jun	23.0° (10.3°,34.5°)*	20 mm	24.3° (14.0°,36.5°)*	36 mm
Jul	27.2° (16.2°,39.7°)*	23 mm	26.4° (16.1°,39.3°)*	15 mm
Total		389 mm		531 mm
Notes: * - Mean (Min, Max).				

A variety of *P. triticina* (Pt) races acquired from 80–100 randomly selected infected leaf samples of the susceptible cv. Steklovidnaya, taken from Kazakhstan's primary spring wheat-growing areas were used to inoculate the field plots. The highly susceptible variety Morocco was used to multiply the inoculum in a greenhouse. The collected urediniospores were mixed with talc at a ratio of 1:100 (20 mg/m²) and sprayed during the spring tillering stage. Data on the kind and severity of leaf rust infections were documented on flag leaves in midle May and early June, which corresponded to the boot and milk stages of the plots. The second assessment was scheduled to begin when the susceptible control cultivar Morocco's rust severity increased to 60–80%.

The CIMMYT technique was used in evaluating the symptoms of leaf rust (Roelfs et al., 1992). There were five categories for infection types (IT): MR is moderately resistant (damage no more than 10–25% of the leaf surface), MS is moderately susceptible (damage up to 40–50% of the leaf surface), S is susceptible (damage 50–100% of the leaf surface), and 0 is immune (absence of damage of uredinia or other macroscopic signs of infection). In the booting and milking periods, partial resistance was assessed in the field using a modified Cobb scale (Malysheva et al., 2023). Table 2 summarizes the infection type scale used in the CIMMYT assessment of leaf rust symptoms.

Table-2. Infection Type (IT) Scale for Leaf Rust Evaluation

Infection Type (IT)	Description	Leaf Surface Damage (%)
0	Immune (absence of uredinia or other macroscopic signs of infection)	0%
MR	Moderately Resistant (damage no more than 10–25% of the leaf surface)	10–25%
MS	Moderately Susceptible (damage up to 40–50% of the leaf surface)	40–50%
S	Susceptible (damage 50–100% of the leaf surface)	50–100%

Data collection commenced upon the appearance of initial symptoms on the susceptible check plant (Morocco); severity data gathered by the ripening and milky-wax maturity stages at the end of May and early June, respectively. The extent of leaf rust infection was recorded across three replications, and mean values were calculated from the collected data.

Agronomic characteristics

Wheat yield depends on several factors, one of which is the yield components, which include: the number of grains in an ear, the number of ears per unit area and the weight of the grain, which are some of the main indicators affecting grain production. Agronomic traits of different cultivars were evaluated under field conditions. The average plant height in the plot, measured from the soil surface to the top of the spike, was recorded in centimeters to calculate plant height (PH). Days to heading (DH) was the time interval measured from planting to the point at which half of the spikes protruded from the flag leaf. Ten randomly chosen spikes were measured for length, with the awns excluded, from the base of the first spike to the tip. These ten spikes at maturity were used to calculate the average amount of grains per spike and spikes per plant for each genotype. In addition, the thousand grain weight (TKW) is a key indicator in agriculture, determined by weighing 1000 seeds from a given sample of the crop. It is measured in grams and serves several important purposes in crop management. Four measurements of the Normalized Difference Vegetation Index (NDVI) were conducted using the GreenSeeker (Trimble Nav. Ltd., a company based in Sunnyvale, California, USA) starting on May 24, June 2, 12, and June 23 in both 2021 and 2022 in order to assess genotypic diversity in thermotolerant and high-yielding genotypes. According to Zadoks et al. (1974), these measures roughly matched the development phases of Z40-49 (booting), Z60-69 (flowering/anthesis), Z70-79 (milk development), and Z83-85 (dough ripe). The Vegetation Index values were consistent across the same growth stages in both years. Upon maturity, grain yield was assessed from the 3 m² plots.

Molecular screening of *Lr* genes in a wheat collection using molecular markers

Using the CTAB procedure, genomic DNA was isolated from fresh leaves of individual plants at the two-leaf seedling stage for each genotype (Riede and Anderson, 1996). Using a NanoDrop One spectrophotometer, the extracted DNA's concentration and purity were assessed, and the DNA concentration for PCR was set at 20 ng/µL. *Lr* gene-related primers were used in accordance with certain, authorized methods. For every *Lr* gene, specific primers and annealing temperature settings were used to perform the polymerase chain reaction (PCR) (Table 3). A Bio-Rad T100TM Thermal Cycler (Thermo Fisher Scientific SimpliAmp, Thermo Cycler, Singapore) was used to conduct PCR tests. The PCR was performed in 25 µL of reaction mixture contained 2.5 µL of genomic DNA (30 ng), 1 pmol of both forward and reverse primers (Sigma-Aldrich, Germany), 2.5 µL of dNTP mixture (SibEnzyme, Russia), 25 mM $MgCl_2$, 0.2 U Taq polymerase (5 units / µL) (ZAO Sileks, Russia), 2.5 µL 10xPCR buffer and 12.8 µL ddH20. The amplification products were separated using TBE buffer (45 mM Tris-borate, 1 mM EDTA, pH 8), and ethidium bromide was added for visualization (Chen et al., 1998). A 100 base pair (bp) DNA ladder (Fermentas, Vilnius, Lithuania) was employed to gauge the size of the amplification fragment. The Gel Documentation System (BIO-RAD Laboratories Inc, Hercules, California, USA) was used to visualize the results. Each genotypes underwent three separate tests.

Table-3. Molecular markers linked to *Lr* genes of wheat resistance to leaf rust

Lr gene	Chr	Marker type	Molecular marker	Forward primer	Anneling t^0C	Fregment size, b.p.	Reference
Lr19/ Sr25	*7DL*	*SSR*	*Xwmc221*	ACGATAATGCAGCGGGGA AT GCTGGGATCAAG GGA TCAAT	61^0C	200	Gupta et al., 2006a
Lr37/ Yr17/ Sr38	*2AS*	*SCAR*	*Venttriup/LN2*	AGGGGCTACTGACCAAGGCT TGCAGCTACAGCAGTATGTACACAAAA	65^0C	262	Herrera-Foessel et al., 2012
Lr24	*3 DL*	*STS*	*J09*	TCTAGTCTGTACATGGGGGC TGGCACATGAACTCCATACG	58^0C	310	Schachermayr et al., 1995
Lr68	*7 BL*	*STS*	*csGS*	AAGATTGTTCACAGATCCATGTCA GAGTATTCCGGCTCAAAAAGG	60^0C	385	Herrera-Foessel et al., 2012
Lr50	*2BL*	*SSR*	*GWM382*	GTCAGATAACGCCGTCCAAT CTACGTGCACCACCATTTTG	60^0C	139	Brown-Guedira et al., 2003

https://doi.org/10.35495/ajab.2024.142

Statistical analysis

The resistance behavior to leaf rust of all tested samples was evaluated using the Average Coefficient of Infection (ACI) and the Area Under the Disease Progress Curve (AUDPC). The Average Coefficient of Infection (ACI) was calculated following the methodology outlined by Saari and Wilcoxson (1974). AUDPC was calculated using the formula proposed by Wilcoxson et al. (1974). This approach allows for a quantitative assessment of disease progression over time, providing valuable insights into the resistance levels of different wheat genotypes.

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{yi + yi + 1}{2} X(ti + 1 - \text{ti} \right)$$

where y_i is the average coefficient of infection for the i-th assessment yi_{+1} is the average coefficient of infection for the (i+1)-th assessment, t_{i+1} - t_i is the number of days between the i-th and (i+1)-th assessments, and n is the total number of observations. The susceptibility index (φ) is determined by dividing the Area Under the Disease Progress Curve (AUDPC) of a specific genotype by the AUDPC of a known susceptible control genotype. This ratio provides a quantitative assessment of how susceptible or resistant a particular genotype is in comparison to the susceptible control.

R-studio software was used to conduct a one-way analysis of variance (ANOVA) in order to evaluate the differences in productivity and resistance to leaf rust among genotypes and across years. Pearson correlation coefficients were calculated based on the mean values of the evaluated traits. PCA was conducted, and biplots were generated using R-studio software version R 3.5.3 (R Core Team, 2018).

The broad-sense heritability index (Hb^2), which reflects the proportion of phenotypic variation due to genetic factors, was calculated from the ANOVA results using the specified formula:

$$H_b^2 = SSg/SSt,$$

where: SSg – represents the sum of squares for genotype, SSt – denotes the total sum of squares. This approach allows for a quantitative assessment of disease progression over time, providing valuable insights into the resistance levels of different wheat genotypes.

Results

Field phenotyping

According to the ANOVA analysis ($p < 0.001$), the severity of wheat leaf rust significantly differed among genotypes in both growing seasons. A high broad-sense heritability ($H_b^2 = 0.87$) for disease resistance was observed among wheat genotypes in 2021 (Table 4).

Table-4. A one-way analysis of variance (ANOVA) for resistance of wheat collection to leaf rust

Experiment	Factor	Sum of squares	Degree of freedom	Mean squares	F-value	p-value	H_b^2, %
AUDPC, field - 2021	Genotype	63884.7	59	10827.9	4.249	5,261E-08	0.87
	Year	35363.3	1	35363.3	13.88	0.000438	
	Residuals	150337	59	2548.08			
AUDPC, field - 2022	Genotype	74636.7	59	12650.3	0.988	0,5185	0.68
	Year	6453.33	1	64533.3	5.04	0.02853	
	Residuals	75546.7	59	12804.5			
Notes: H_b^2 – broad-sense heritability index. *** Significant difference at $p < 0.001$.							

During the trials, a collection of wheat genotypes, including 60 genotypes, were evaluated for resistance to leaf rust under field conditions, and as a result, the genotypes were classified into resistance (0, R-MR) and susceptibility (MS-S) groups (Figure 1, Table 5). The disease development intensity in 2021 ranged from immune (0%) (379_SP2, 384_SP2, 386_SP2, 387_SP2, 389_SP2, 394_SP2, 423_SP2, 592_SP2, 517_SP2, 476_SP2, 475_SP2, 597_SP2, 599_SP2, 600_SP2, 603_SP2, 608_SP2, CP_15, CP_19, CP_22, CP_25, CP_26) to susceptible with 40% infection (520_SP2). In 2022, most of the varieties exhibited resistance to leaf rust. Eight entries exhibited an immune reaction (0% infection) (379_SP2, 384_SP2, 386_SP2, 387_SP2, 389_SP2, 394_SP2, 423_SP2, 592_SP2, 517_SP2, 476_SP2, 475_SP2, 597_SP2, 599_SP2, 600_SP2, 603_SP2, 608_SP2, CP_15, CP_19, CP_22, CP_25, CP_26) to leaf rust, while three promising lines displayed a moderately susceptible reaction (382_SP2, 384_SP2 and 520_SP2).

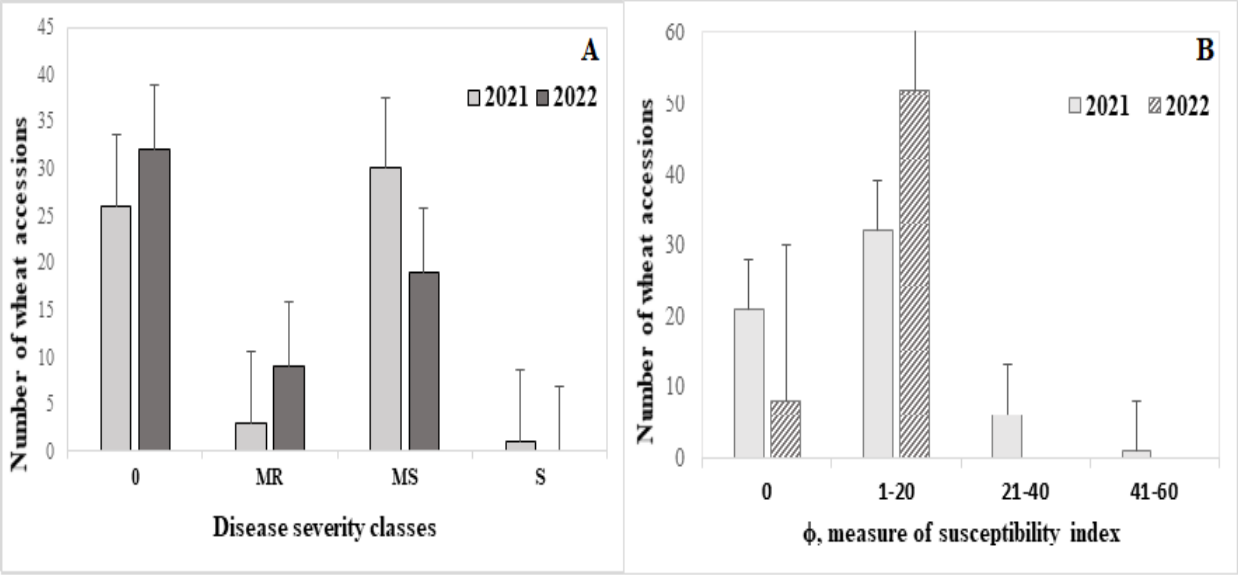

Figure-1. Frequency of occurrence of wheat accessions in different leaf rust resistance groups under field conditions (A) and distribution by susceptibility index values (B**).**

To determine the prevalence and progression of the disease, the area under the disease progress curve (AUDPC) was estimated. Among all 60 tested genotypes in 2021, twenty-one (35%) exhibited high resistance to leaf rust and AUDPC was of 0%. With an average resistance index of 3.0% and AUDPC of 42, 14 genotypes stood out, indicating moderate resistance to the disease. Twelve genotypes (20%) with a resistance index of 9.1% and AUDPC of 119 showed lower resistance to leaf rust. Eleven genotypes (18.3%) with an average φ value of 26.45% and AUDPC of 269.09 exhibited susceptibility to leaf rust.

In 2022, the AUDPC values and resistance index were significantly lower compared to 2021. Seven entries exhibited a maximum resistance index of 13% and an AUDPC of 91. Eighteen genotypes stood out for their resistance in field conditions, with a resistance index of 0.5% and AUDPC of 5.5. Thirty-five entries showed moderate susceptibility to stripe rust with a resistance index of 4.2% and AUDPC of 42.9. The susceptibility index (ϕ) allowed the grouping of wheat genotypes based on their susceptibility levels. Both years predominantly featured genotypes with a susceptibility index of 1–20% (Figure 1A).

In 2021, a wide range of leaf rust severity levels was observed, from 0 (complete resistance) to 40S (high susceptibility). In 2022, the severity levels also varied, but most genotypes demonstrated improvement or stability in their resistance ratings. Genotypes with *Lr*50 (386_SP2, 388_SP2, 392_SP2) showed a significant reduction in AUDPC in the second season. Three genotypes were identified with improved resistance: 378_SP2 (2021: Severity level of 10MS, AUDPC = 360; 2022: Improvement to 5MS, AUDPC = 100); 382_SP2 (2021: Severity level of 10MS, AUDPC = 360; 2022: Reduction to 10MR and improvement in AUDPC to 180); and 520_SP2 (2021: Severity level of 40S (high susceptibility), AUDPC = 460; 2022: Improvement to 30MS, AUDPC = 240). Two genotypes exhibited stable resistance: 386_SP2 (2021 and 2022: complete protection (0) in both seasons, confirming the effectiveness of *Lr*50) and 388_SP2 (no signs of infection in both seasons, also possessing *Lr*50 and *Lr*68). Some genotypes, such as 520_SP2 and 378_SP2, showed a significant decrease in resistance levels in the second season. This may be related to changes in pathogens or environmental conditions that affected the expression of resistance.

In most cases, a decrease in AUDPC values was observed in 2022 compared to 2021. This may indicate an increase in resistance or changes in environmental conditions. Genotypes with *Lr* genes exhibited higher AUDPC values, underscoring the importance of these genes for disease protection. Considering the impact of climatic factors on disease severity, regular monitoring of environmental conditions and adaptation of agronomic practices are necessary to enhance resistance.

https://doi.org/10.35495/ajab.2024.142

Table- 5. The assessment of leaf rust disease severity and the molecular analysis for the presence of *Lr* genes in a collection of winter wheat genotypes

Entries name	Leaf Rust Severity, 2021			AUDPC-2021	ϕ, %	Leaf Rust Severity, 2022			AUDPC-2022	ϕ, %	*LR* genes
	1st Score	2nd Score	3rd Score			1st Score	2nd Score	3rd Score			
378_SP2	10MS	30MS	20MS	360,0	35,0	5MS	5MS	10MS	100	10,00	-
379_SP2	0	0	0	0,0	0,0	0	5MR	0	20	2,00	-
382_SP2	10MS	30MS	20MS	360,0	36,0	10MR	5MS	30MS	180	17,00	-
383_SP2	5MS	30MS	30MS	380,0	38,0	5MR	5MS	10MS	90	9,00	-
384_SP2	30MS	10MS	30MS	210,0	20,0	5MS	10MS	30MS	60	6,00	-
386_SP2	0	0	0	0,0	0,0	0	5MR	0	20	2,00	*Lr50*
387_SP2	0	5MS	30MS	120,0	12,0	0	5MS	15MS	110	11,00	-
388_SP2	0	0	0	0,0	20,0	0	0	0	0	0,00	*Lr50, Lr68*
389_SP2	0	0	0	0,0	0,0	0	0	10MR	20	2,00	-
390_SP2	0	5MS	10MS	80,0	8,0	5MR	10MR	5MS	70	7,00	-
391_SP2	0	0	5MS	20,0	2,0	0	10MR	0	40	4,00	-
392_SP2	0	10MR	10MS	80,0	8,0	5MR	0	10MR	30	3,00	*Lr50*
393_SP2	0	5MS	20MS	120,0	11,0	0	5MS	10MS	80	8,00	-
394_SP2	0	0	0	0,0	0,0	5MR	0	0	10	1,00	-
395_SP2	0	0	10MS	40,0	4,0	0	5MS	0	40	4,00	-
423_SP2	0	0	0	0,0	0,0	0	0	10MR	20	2,00	-
592_SP2	0	0	0	0,0	0,0	0	0	0	0	0,00	*Lr19*
591_SP2	0	10MR	5MS	60,0	6,0	5MR	5MS	0	50	5,00	*Lr19*
590_SP2	10MS	0	0	40,0	4,0	10MR	0	0	20	2,00	-
589_SP2	0	0	10MS	40,0	4,0	5MS	0	0	20	2,00	-
588_SP2	0	5MS	10MS	80,0	8,0	0	10MR	5MS	60	6,00	-
587_SP2	10MS	0	0	40,0	4,0	0	5MS	10MS	80	8,00	-
520_SP2	5MS	30MS	40S	460,0	45,0	10MS	10MS	30MS	240	23,00	-
519_SP2	5MS	10MS	0	100,0	10,0	0	10MR	5MS	60	6,00	-
517_SP2	0	0	0	0,0	0,0	0	0	5MR	10	1,00	-
516_SP2	0	10MS	30MS	200,0	20,0	5MR	5MS	10MS	90	9,00	-
514_SP2	0	5MS	20MS	120,0	12,0	10MR	5MS	10MS	100	10,00	-
513_SP2	5MS	10MS	20MS	180,0	18,0	5MR	10MR	5MS	70	7,00	*Lr50*
476_SP2	0	0	0	0,0	0,0	0	0	0	0	0,00	*Lr37*
475_SP2	0	0	0	0,0	0,0	0	0	0	0	0,00	*Lr50*
424_SP2	5MS	5MS	10MS	100,0	10,0	5MR	5MR	5MS	50	5,00	-
593_SP2	5MS	10MS	15MS	160,0	16,0	0	10MR	5MS	60	6,00	-
595_SP2	0	10MS	10MS	120,0	12,0	10MR	5MS	0	60	6,00	-
597_SP2	10MR	10MS	30MS	220,0	22,0	5MS	10MS	0	100	10,00	-
599_SP2	0	0	0	0,0	0,0	0	0	0	0	0,00	*Lr50*
600_SP2	0	0	0	0,0	0,0	0	5MR	0	20	2,00	*Lr50*
601_SP2	0	10MR	5MS	60,0	6,0	5MR	10MR	0	50	5,00	-
603_SP2	0	0	0	0,0	0,0	0	0	0	0	0,00	*Lr24*
604_SP2	0	0	5MS	20,0	2,0	10MR	0	0	20	2,00	*Lr24*
605_SP2	0	10MR	5MS	60,0	6,0	0	0	5MR	10	1,00	-
608_SP2	0	0	0	0,0	0,0	5MR	0	0	10	1,00	-
469_SP2	5MR	10MS	30MS	210,0	21,0	5MS	0	10MS	60	6,00	-

473_SP2	5MR	10MR	5MS	70,0	7,0	0	5MR	0	20	2,00	-
CP_13	0	0	5MS	20,0	2,0	0	10MR	0	40	4,00	*Lr19, Lr68*
CP_14	0	10MR	0	40,0	4,0	0	0	5MR	10	1,00	-
CP_15	0	0	0	0,0	0,0	5MR	0	0	10	1,00	-
CP_16	0	10MR	0	40,0	4,0	0	5MR	0	20	2,00	*Lr50*
CP_17	0	0	5MS	20,0	2,0	10MR	0	0	20	2,00	*Lr50, Lr68*
CP_18	0	0	10MR	20,0	0,0	5MR	0	0	10	1,00	*Lr50*
CP_19	0	0	0	0,0	0,0	0	5MR	0	20	2,00	*Lr50*
CP_20	0	10MS	30MS	220,0	21,0	10MR	5MS	10MS	100	10,00	-
CP_21	0	0	0	0,0	0,0	0	0	0	0	0,00	*Lr24, Lr50*
CP_22	0	0	0	0,0	0,0	0	0	0	0	0,00	*Lr24, Lr50*
CP_23	0	0	10MR	20,0	2,0	0	5MR	0	20	2,00	-
CP_24	0	0	0	0,0	0,0	0	0	5MR	10	1,00	*Lr50*
CP_25	0	0	0	0,0	0,0	5MR	0	0	10	1,00	*Lr50*
CP_26	0	0	0	0,0	0,0	0	5MR	0	20	2,00	*Lr37*
CP_27	0	0	10MR	20,0	2,0	0	0	5MR	10	1,00	*Lr37*
CP_30	0	0	30MS	120,0	11,0	5MS	0	10MS	60	6,00	*Lr37*
77_SP2	0	0	10MS	40,0	4,0	10MR	0	0	20	2,00	*Lr24*
Controls for leaf rust											
Morocco	30MS	50S	80S	1020,0	100,0	30MS	60S	90	1170	100	none
Agrus/6*Thatcher	0	0	0	0,0	0,0	0	0	0	0	0	*Lr19*
Payne/tam W-101/Amigo	0	0	10MR	20,0	2,0	0	0	0	0	0	*Lr24*
VPM 1/Moisson 421//2*Tyee	0	20MS	30MS	280,0	27,0	0	0	10MR	20	2	*Lr37*
TM 870	0	0	0	0,0	0,0	0	0	0	0	0	*Lr50*
Parula	0	0	10MR	20,0	2,0	0	0	0		0	*Lr68*

To determine which lines were the most promising, a structural analysis of productivity was carried out. This involved measuring the plant height (PH, cm), calculating the days to heading (DH), and analyzing important variables like spike length (SL, cm), average number of spikes per spike (SS), number of grains per spike (GS), weight of grain per spike (WGS, g), and thousand kernel weight (TKW, g). In 2021, genotypes 388_SP2 (229 days), 394_SP2 (229 days), and 587_SP2 (214 days) showed the most variation in days to heading, which was 15 days. The genotypes 394_SP2 (235 days) and 384_SP2 (217 days) differed by 18 days in 2022. Plant height varied in 2022 from 63 cm (77_SP2) to 125 cm (513_SP2), while in 2021 it ranged from 60 cm (CP_25, 77_SP2) to 117 cm (592_SP2). From 16.41 g (601_SP2) to 36.76 g (394_SP2) in 2021 and from 27.9 g (CP_14) to 45.5 g (475_SP2) in 2022, the average TKW (thousand kernel weight) changed.

For the majority of the agronomic parameters evaluated over both growing seasons, statistical analysis revealed substantial genotype changes (Table 6). Days to heading (0.82) and plant height (0.89) showed evidence of heritability.

Table-6. A one-way analysis of variance (ANOVA) was conducted to evaluate the differences in productivity traits among the tested winter wheat genotypes

Experiment	Factor	Sum of squares	Degree of freedom	Mean squares	F-value	p-value	H_b^2, %
NDVI	Genotype	0,10572	59	0,001791	0,4916	0,9964	0.37
	Year	0,280333	1	0,280333	76,9	2,771E-12	
	Residuals	0,215067	59	0,003645			
Days to heading	Genotype	2011,49	59	34,0931	44,28	2,278E-33	0.82
	Year	378,075	1	378,075	491,1	2,732E-30	
	Residuals	45,425	59	0,769915		5,897E-75	
Plant height	Genotype	41294,1	59	699,9	1186	1,351E-30	0.89
	Year	297,675	1	297,675	504,3		
	Residuals	34,825	59	0,590254			
Spike lengths	Genotype	69,538	59	1,17861	5,882	7,805E-11	0.51
	Year	54,0558	1	54,0558	269,8	1,116E-23	
	Residuals	11,8219	59	0,200372			
The mean number of spikelets/spike	Genotype	253,958	59	4,30438	5,212	9,696E-10	0.63
	Year	98,3554	1	98,3554	119,1	8,811E-16	
	Residuals	48,7284	59	0,825905			
Grains/spike	Genotype	1362,68	59	23,0963	4,806	4,924E-09	0.60
	Year	622,304	1	622,304	129,5	1,628E-16	
	Residuals	283,526	59	4,80553			
The weight of grain/spike	Genotype	4,08504	59	0,069238	2,058	0,00315	0.55
	Year	1,27102	1	1,27102	37,79	7,365E-08	
	Residuals	1,98463	59	0,033637			
Thousand kernel weights	Genotype	843,202	59	14,2916	0,6907	0,9209	0.53
	Year	4062,01	1	4062,01	196,3	2,003E-20	
	Residuals	1220,87	59	20,6928			

Plant height (PH) and the Normalized Difference Vegetation Index (NDVI) showed a strong negative connection in 2021 (r = -0.31**). There were significant positive correlations (r = 0.26*) between AUDPC and TKW (r = 0.37**), SS and SL (r = 0.53***), and GS and SL (r = 0.33**) across all of the analysis's relationships. The attributes TKW and WGS (r = 0.74***), GS and SS (r = 0.54***), WGS and GS (r = 0.50***), and TKW and WGS (r = 0.74***) exhibited the greatest degree of association, according to Table 7.

Table-7. Pearson correlation analysis between major agronomic traits and area under the disease progress curve (AUDPC) in 2021

Variables	AUDPC	NDVI	DH	PH	SL	SS	GS	WGS	TKW
AUDPC	1	0,080	0,139	0,168	-0,028	-0,026	-0,071	0,262*	0,376**
NDVI	0,080	1	-0,033	-0,312**	0,368**	0,176	0,197	-0,022	-0,234
DH	0,139	-0,033	1	0,216	-0,008	0,082	-0,026	-0,026	0,026
PH	0,168	-0,312**	0,216	1	-0,070	0,192	0,153	0,192	0,207
SL	-0,028	0,368**	-0,008	-0,070	1	0,534***	0,337**	0,137	-0,180
SS	-0,026	0,176	0,082	0,192	0,534***	1	0,545***	0,240	-0,129
GS	-0,071	0,197	-0,026	0,153	0,337**	0,545***	1	0,502***	-0,127
WGS	0,262*	-0,022	-0,026	0,192	0,137	0,240	0,502***	1	0,746
TKW	0,376**	-0,234	0,026	0,207	-0,180	-0,129	-0,127	0,746***	1
Note: Values in bold are significantly different from 0 at the specified significance level, *** – p<0.001; ** – p<0.01; * – p<0.05									

According to the research data in 2022, positive correlations were observed between PH and WGS (r = 0.25*), SL and SS (r = 0.41**), GS (r = 0.26*), WGS (r = 0.28*) and TKW (r = 0.29*), and a significant positive correlation was found between WGS and GS (r = 0.88***). Whereas, TKW was negatively correlated with NDVI (r = -0.35**) and PH (r = -0.42***) (Table 8).

Table-8. Pearson correlation analysis between major agronomic traits and area under the disease progress curve (AUDPC) in 2022

Variables	AUDPC	NDVI	DH	PH	SL	SS	GS	WGS	TKW
AUDPC	-	0,068	0,139	0,169	-0,093	-0,222	0,079	0,104	-0,070
NDVI	0,068	-	-0,134	0,682***	0,179	0,091	0,022	0,054	-0,358**
DH	0,139	-0,134	-	0,121	-0,014	0,024	0,102	0,074	0,065
PH	0,169	0,682***	0,121	-	0,114	0,118	0,171	0,258*	-0,426***
SL	-0,093	0,179	-0,014	0,114	-	0,411***	0,265*	0,286*	0,298*
SS	-0,222	0,091	0,024	0,118	0,411***	-	0,361**	0,341**	0,216
GS	0,079	0,022	0,102	0,171	0,265*	0,361**	-	0,880***	0,479***
WGS	0,104	0,054	0,074	0,258*	0,286*	0,341**	0,880***	-	0,338**
TKW	-0,070	-0,358**	0,065	-0,426***	0,298*	0,216	0,479***	0,338**	-
Note: Values in bold are significantly different from 0 at the specified significance level, *** – p<0.001; ** – p<0.01; * – p<0.05									

To visualize the relationship between agronomic characteristics, a principal component analysis (PCA) was performed, the results of which are presented as biplots for 2021 and 2022 (Figure 2). The productivity components and AUDPC parameters served as the foundation for PCA. 49.38% of the variance was explained by the first two main components, according to this study. The influences of NDVI, wheat grain yield (WGY), thousand kernel weight (TKW), grains per spike (GS), spikes per square meter (SS), and spike length (SL) were combined in 2021 by PC1 (25.7%). AUDPC, GS, and PH made the largest contributions to PC2, accounting for 23.68% of the variance. There was strong correlation between all productivity attributes (Figure 2A).

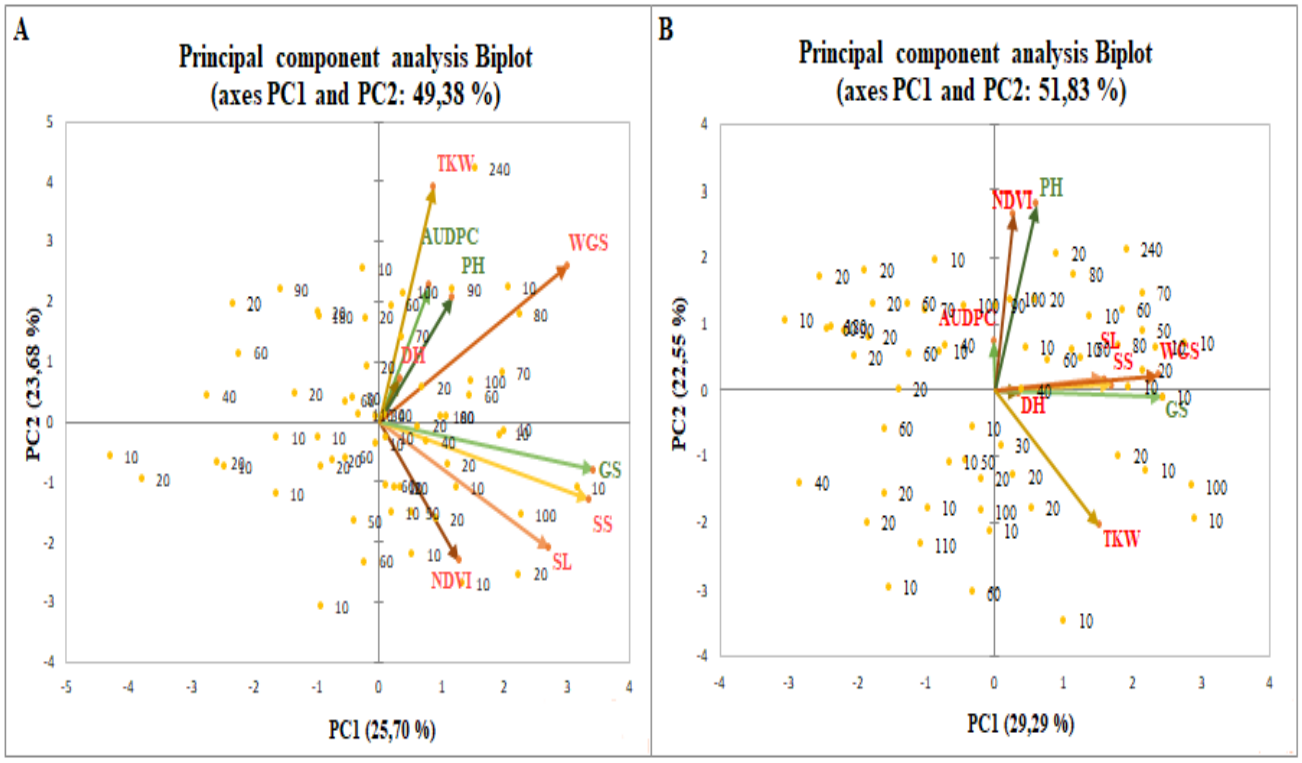

Figure-2. PCA Biplots of leaf rust severity and productivity traits in 2021 (a) and 2022 (b): Visualization of Observations and Variable Contributions.

In 2022, the first two principal components accounted for 51.8% of the total variation. Principal Component 1 (PC1) explained 29.3% of the variation, with the most significant contributions from the NDVI, TKW, WGS, SS, and SL parameters. All spike productivity traits exhibited strong correlations. Principal component 2 (PC2) explained 22.5% of the variation and integrated the GS, AUDPC, DH and PH traits. Notably, AUDPC significantly negatively affected TKW and WGS across the two growing seasons (Figure 2B). A boxplot analysis of quantitative parameters was conducted to validate the PCA results. Figure 3 presents the boxplot analysis of quantitative data (descriptive statistics) for AUDPC, phenotypic data, and yield components of winter wheat. The results are illustrated through graphical data analysis, where the box encompasses 50% of the data. The upper edge of the box (hinge) represents the 75th percentile of the dataset, while the lower hinge indicates the 25th percentile. A horizontal line within the box illustrates the median yield. The upper and lower whiskers of the plot extend to the yields corresponding to the 10th and 90th percentiles, respectively. Points beyond the whiskers correspond to outliers (Figure 3).

The overall variable indicated that the distribution of genotypes between groups in 2021 and 2022 falls within the following range: Min – 10.0; 1st Quartile – 10.0; Median – 20.0; 3rd Quartile – 60.0; Max – 65.0; variance (n-1) – 1924.8; and standard deviation – 43.87.

https://doi.org/10.35495/ajab.2024.142

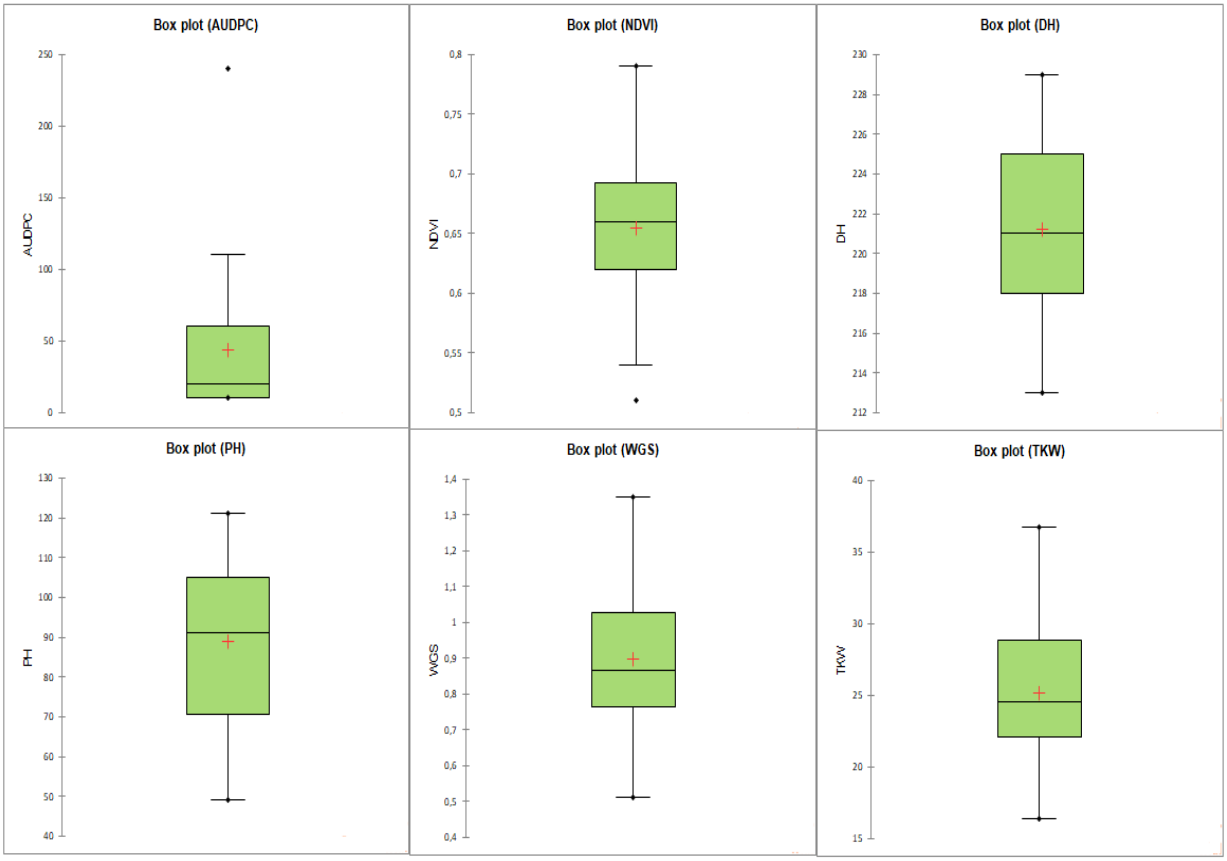

Figure-3. Boxplots of AUDPC, agronomic traits and yield components of wheat genotypes

Identification of *Lr* genes using molecular markers

Molecular analysis of 60 promising winter wheat genotypes using markers markers linked to specific *Lr* genes revealed the presence of six *Lr* genes (*Lr19*, *Lr24*, *Lr37*, *Lr50* and *Lr68*) in 26 tested lines. Table 4 and Figures 4–8 provide specifics of the findings of the molecular screening for the respective *Lr* genes. Globally, immunization against a broad spectrum of pathogen races is provided by the *Lr19* gene, which is acknowledged as one of the most efficient resistance genes (Gultyaeva and Shaydayuk, 2021; Sehgal et al., 2012). Thinopyrum ponticum (previously Agropyron elongatum) was translocated to the distal region of chromosome 7D's long arm, introducing this gene into wheat (Uhrin et al., 2008). According to Singh et al. (2011), tolerance to leaf rust, the Ug99 race, and its variations is bestowed by the 7D.7Ag translocation, which carries both *Lr19* and Sr25. Furthermore, *Lr19* increases grain production when circumstances are ideal (Herrera-Foessel et al., 2012). To select for genotypes that contain the *Lr19*/Sr25 gene complex, many studies have used molecular markers such Xwmc221 (Prins et al., 2001; Somers et al., 2004), PSY1-E1 (Zhang and Dubcovsky, 2008), and Gb (Liu et al., 2010). To identify *Lr19*/Sr25 carriers, the line Argus/6 *Thatcher was used as a positive check for gene carrier identification, and the Xwmc221 primer was utilized. Two products are amplified by the *Xwmc221* primer: one at 200 bp for *Lr19* gene carriers and another at -220 bp for wheat entry sensitive to leaf rust (Gupta et al., 2006a; Kiel et al., 2020). To determine whether wheat genotypes in the collection under study had the *Lr19* gene, an analysis was performed. Three genotypes—591_SP2, 592_SP2, and CP_13—among the sixty wheat entries that were evaluated showed the predicted marker fragment linked to *Lr19* (Figure 4).

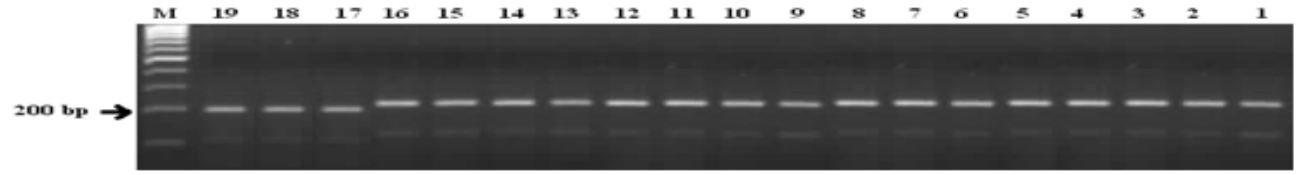

Figure-4. Amplification products using specific marker for *Lr19* (*Xwmc221*) in the 19 wheat genotypes. 1-379_SP2, 2-382_SP2, 3- 386_SP2, 4- 388_SP2, 5- 390_SP2, 6- 394_SP2, 7- 423_SP2, 8- 517_SP2, 9- 588_SP2, 10- 605_SP2, 11-424_SP2, 12- CP_14, 13- CP_15, 14- CP_20, 15 CP_23, 16- 77_SP2, 17-592_SP2, 18- CP_13, 19 – Agrus/6*Thatcher (positive control), M- 100 bp DNA Ladder.

The *Lr24* gene, derived from *Agropyron elongatum*, has been mapped to the distal region of chromosome 3DL (Schachermayr et al., 1995). Subsequent investigations have successfully developed SCAR (Sequence Characterized Amplified Region) markers that co-segregate with and are tightly linked to the leaf rust resistance gene *Lr24* (Gupta et al., 2006b), and this gene has been extensively employed in breeding programs (Ren et al., 2023). The *Lr24* gene was detected using primers targeting the J9J9 locus, yielding a 310 bp amplification product. The isogenic line Payne/TAM W-101/Amigo served as a positive control. As a result of PCR amplification, the expected fragment associated with the *Lr24* gene was identified in five genotypes, confirming the presence of the *Lr24* resistance gene in these lines (603_SP, 604_SP2, CP_21, CP_22, 77_SP2) (Figure 5).

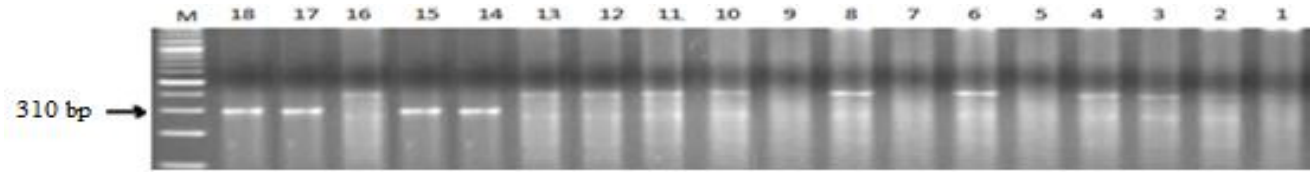

Figure-5. Amplification products using specific marker for *Lr24* (*J9J9 F/R*) in the 17 wheat genotypes. 1- ddH2O (negative control), 2-379_SP2, 3- 384_SP2, 4- 386_SP2, 5- 390_SP2, 6- 394_SP2, 7- 395_SP2, 8- 590_SP2, 9- CP_14, 10- CP_15, 11- CP_20, 12- CP_23, 13- CP_26, 14- 603_SP2, 15- CP_21, 16- 150-597_SP2, 17- 77_SP2, 18- Payne/tam W-1010/Amigo (positive control), M- 100 bp DNA Ladder.

Cultivated wheat has been injected with the *Aegilops ventricosa* Tausch-derived leaf rust resistance gene *Lr37*. Despite the identification of new virulent leaf rust races in many countries, the *Lr37* gene still provides long-lasting resistance to a broad spectrum of races. The *Lr37* gene is particularly useful in combination with other effective resistance genes. This gene was first was initially integrated into the winter wheat cultivar VPM1 and is located within the 2NS-2AS translocation (Xue et al., 2018). Since then, breeders from various locations have made considerable use of it (Bulos et al., 2006). Identification of *Lr37* was performed using *LN/VENTRIUP* primers, confirming the presence of a 262 bp amplification product. Our study identified the resistance gene complex *Lr37*/Yr17/Sr38 against rusts in four wheat lines (476_SP2, CP_26, CP_27, CP_30) from the examined entries (Figure 6).

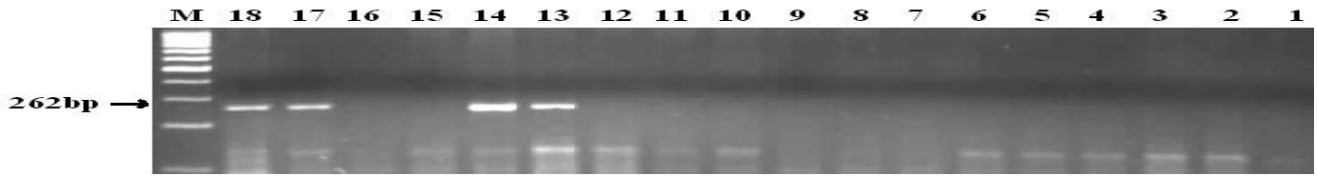

Figure-6. Amplification products using specific marker for *Lr37* (*Ln/Ventriup-F/R*) in the 17 wheat genotypes. 1-77_SP2, 2-CP_16, 3- CP_23, 4- 379_SP2, 5- 382_SP2, 6- 383_SP2, 7- 387_SP2, 8- CP_14, 9- CP_15, 10- CP_20, 11- 424_SP2, 12-593_SP2, 13- CP_27, 14- 476_SP2, 15- 595_SP2, 16- ddH2O (negative control), 17- VPM 1/Moisson 421//2*Tyee (positive control), 18- CP_30, M- 100 bp DNA Ladder.

https://doi.org/10.35495/ajab.2024.142

The *Lr50* gene was inserted into three background cultivars of durum wheat, Karl 92 (red), TAM 107 (red), and Arlin (white), from four entries of *Triticum timopheevii* subsp. armeniacum: TA870, TA874, TA895, and TA145. For *Lr50* backcrossing, the embryo plasma is line U2657 (Karl92*3/TA874). On the long arm of wheat chromosome 2B, *Lr50* is flanked by the microsatellite markers Xgwm382 (6.7 cM) and Xgdm87 (9.4 cM) (Brown-Guedira et al., 2003). As a result of PCP amplification, a fragment of 139 bp in size was detected in 15 promising wheat lines (CP_24, CP_25, 386_SP2, 388_SP2, CP_22, 392_SP2, 513_SP2, CP_18, 475_SP2, 599_SP2, 600_SP2, CP_16, CP_17, CP_19, CP_21), indicating the presence of a resistant allele of the *Lr50* gene (Figure 7).

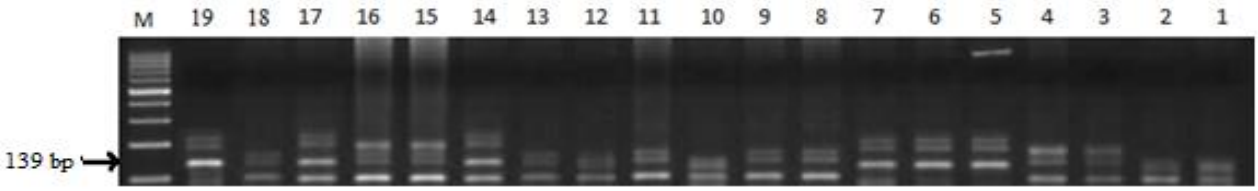

Figure-7. Amplification products using specific marker for *Lr50* (*Xgwm382 F/R*) in the 17 wheat genotypes. 1-378_SP2, 2-379_SP2, 3- 382_SP2, 4- 384_SP2, 5-386_SP2, 6- 388_SP2, 7- 513_SP2, 8- 592_SP2, 9-590_SP2, 10- 520_SP2, 11-519_SP2, 12- 517_SP2, 13- 514_SP2, 14- CP_17, 15- CP_13, 16- CP_14, 17- CP_25, 18- ddH2O (negative control), 19- TA 870 (positive control), M- 100 bp DNA Ladder.

The codominant marker cs7BLN*LR*R, which is 0.8 cM from the gene, and the dominant marker csGs, which is 1.2 cM from the gene, have been shown to be placed in a particular gene-rich area on chromosome 7BL between the Psy1-1 locus and the *Lr68* gene (Herrera-Foessel et al., 2012; El-Orabey et al., 2019). The csGs-specific marker for *Lr68* identifies a single distinct PCR product of 385 bp, according to the findings of molecular screening. Out of the sixty wheat cultivars that were evaluated, three wheat lines (CP_13, CP_17, and 388_SP) showed a 385 bp fragment, suggesting that these genotypes had the *Lr68* gene (Figure 8, Table 4).

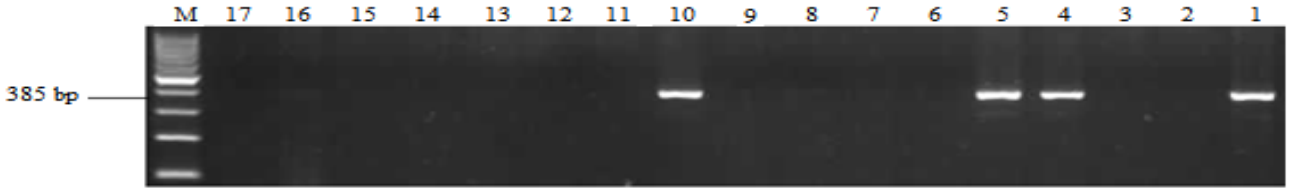

Figure-8. Amplification products using specific marker for *Lr68* (*csGS F/R*) in the 16 wheat genotypes. 1- Parula (positive control), 2- ddH2O (negative control), 3- 393_SP2, 4- 388_SP2, 5- CP_13, 6- 394_SP2, 7- 475_SP2, 8- 476_SP2, 9-605_SP2, 10- 608_SP2, 11- 469_SP2, 12- 473_SP2, 13- CP_14, 14- CP_20, 15- CP26, 16- CP_30, 17- Parula, (positive control), M-100 bp DNA Ladder.

Based on the results of molecular screening, markers identifying *Lr* genes individually and in various combinations were detected in 41.7% of the wheat entries studied (Table 4). Effective combinations of *Lr* genes were identified in 8.3% of the wheat entries. The results of the comparative analysis showed that the identified carriers of *Lr* genes also showed resistance to leaf rust in the field. During the two-year study period, the winter wheat genotypes 388_SP2 (harboring *Lr50* and *Lr68*), 592_SP2 (*Lr19*), 475_SP2 (*Lr50*), 476_SP2 (*Lr37*), 599_SP2 (*Lr50*), 603_SP2 (*Lr24*), CP_21 (*Lr24* and *Lr50*), and CP_22 (*Lr24* and *Lr50*) exhibited a high degree of resistance to leaf rust at the adult plant growth stage under field conditions. Based on the results of molecular screening, the following *Lr* genes were identified among 60 wheat genotypes: *Lr*50: detected in 5 genotypes (386_SP2, 384_SP2, 392_SP2, 475_SP2, 599_SP2, 600_SP2); *Lr*68: present in 2 genotypes (388_SP2, CP_17); *Lr*19: identified in 3 genotypes (592_SP2, 591_SP2, CP_13); *Lr*24: found in 4 genotypes (603_SP2, 604_SP2, CP_21, CP_22); *Lr*37: present in 4 genotypes (476_SP2, CP_26, CP_27, CP_30). The data presented indicate a significant genetic diversity among the studied wheat genotypes. The detection of

multiple *Lr* genes in various combinations across different varieties suggests a high level of polymorphism. For instance, some varieties possess two or three resistance genes simultaneously (e.g., *Lr*50 and *Lr*68 in 388_SP2), which may provide more reliable protection against pathogens. It is important to note the patterns in the combinations of *Lr* genes. The presence of both *Lr*9 and *Lr*26 in a single variety may indicate a synergistic effect between these genes that enhances resistance to leaf rust. This opens up opportunities for selecting varieties with combined resistance. Thus, a more complete analysis of genetic diversity among the studied wheat genotypes and the distribution patterns of *Lr* genes has significantly enriched the understanding of disease resistance mechanisms. This will improve breeding strategies and lead to the development of more resistant wheat varieties for different agronomic conditions.

The results of our study on the identification of *Lr* genes in wheat genotypes from Kazakhstan open new opportunities for enhancing resistance to leaf rust. To maximize these benefits, we recommend the following strategies: 1) Use of Molecular Markers: we recommend employing molecular markers for the rapid and accurate selection of plants containing *Lr* genes. This will expedite the breeding process and increase the likelihood of developing resistant varieties; 2) Polygenic Approaches: It is important to combine multiple *Lr* genes to create more resilient varieties, which will help slow down pathogen adaptation and enhance the long-term effectiveness of resistance.

Potential Benefits of Incorporating *Lr* Genes: 1) Increased Yield: Varieties containing *Lr* genes may demonstrate significant yield increases due to enhanced disease resistance, thereby reducing losses from leaf rust. 2) Improved Grain Quality: The inclusion of these genes may also contribute to better baking quality and nutritional properties of the grain. 2) Reduced Pesticide Use: Resistant varieties require fewer chemical treatments, promoting more sustainable agriculture and minimizing negative environmental impacts.

It is recommended to regularly monitor the performance of the incorporated Lr genes under real agricultural conditions and adapt breeding strategies depending on changes in the pathogen population. We emphasize the importance of collaboration between research institutes and breeders to share knowledge and resources, which will allow for faster introduction of new varieties with improved resistance. These recommendations will help not only to improve wheat resistance to leaf rust, but also to increase overall agricultural productivity and sustainability in the regions.

Discussion

Undoubtedly, rust diseases continue to threaten present and future maximization of wheat yields.

To counter these serious concerns, developing wheat cultivars resistant to rust diseases, especially leaf rust, and genetic host resistance remain viable approaches (Gorash et al., 2014; Ren et al., 2023). The use of molecular-assisted breeding has become more commonplace thanks to recent developments in molecular marker methods and marker-assisted selection (MAS). With the availability of PCR-based markers for around 80 distinct resistance genes and alleles, this is particularly relevant for breeding wheat for resistance to leaf rust. Using MAS, individual progeny populations may be monitored for all effective resistance genes discovered to date (Singh et al., 2013; Elangbam and Deepshikha, 2018; Uhrin et al., 2008).

Molecular markers may not always accurately reflect the presence or activity of resistance genes. For example, some markers may be associated with other genes unrelated to resistance, leading to false conclusions about the presence of resistance in certain genotypes. The pathogen *Puccinia triticina* can mutate and develop new races that can circumvent existing resistance mechanisms. This creates a need for continuous monitoring and updating of breeding programs to account for changes in pathogens. Disease resistance is often controlled by multiple genes, and interactions among these genes can be complex. The use of molecular markers to identify individual *Lr* genes may not capture these interactions, which reduces the effectiveness of selection. Plant resistance to diseases can depend on numerous environmental factors, including climatic conditions and agronomic practices. Molecular markers do not always take into account the influence of these factors on the expression of resistance. For a more accurate assessment of resistance, it is recommended to combine molecular methods with traditional selection techniques and phytopathological testing. This approach will provide a more comprehensive understanding of the genetic basis of resistance and its expression under various conditions.

In Kazakhstan's winter wheat breeding material,

https://doi.org/10.35495/ajab.2024.142

different sources of genes conferring resistance to *Puccinia* fungal diseases have been identified over time (Kokhmetova and Atishova 2012; Yessenbekova et al., 2014; Kokhmetova et al., 2016; Kokhmetova et al., 2020a). These genes include *Lr*1, *Lr19*, *Lr*26, *Lr37*, *Lr*34, *Lr*72, *Lr*10, and *Lr68*. Local cultivars were subjected to molecular screening in 2021, which discovered *Lr*1, *Lr*9, *Lr*10, *Lr*28, and *Lr68*, among other significant variations in their frequencies (Kokhmetova et al., 2021c). Nine *Lr* genes (*Lr*9, *Lr*10, *Lr19*, *Lr*26, *Lr*28, *Lr*34, *Lr37*, *Lr*46, and *Lr68*) were found in 47 different wheat genotypes from Kazakhstan, CIMMYT, and ICARDA, either alone or in combinations (Malysheva et al., 2023). The best breeding method to prevent leaf rust in wheat is still to exploit genetic loci that influence resistance sanely (Ren et al., 2023). In this work, carriers of certain *Lr* resistance genes and gene complexes were identified by molecular screening of sixty wheat genotypes utilizing five markers. There were found to be twenty-five (41.7%) resistance gene bearers. Under field circumstances, adult plant resistance to *P. triticina* was strong for the genotypes 388_SP2 (IT-0), 592_SP2 (IT-0), 475_SP2 (IT-0), 476_SP2 (IT-0), 599_SP2 (IT-0), 603_SP2 (IT-0), CP_21 (IT-0), and CP_22 (IT-0), suggesting that these genotypes had extra *Lr* genes conferring resistance. Twenty-one (35%) of the sixty genotypes that were evaluated in 2021 showed strong resistance to wheat leaf rust, with a AUDPC of 0% and a resistance index (φ). But in 2022, the resistance index and the AUDPC levels were both much lower than in 2021. Genes *Lr*9, *Lr19*, *Lr24*, *Lr*28, *Lr*29, *Lr*41(=39), *Lr*42, *Lr*45, *Lr*47, *Lr50*, *Lr*51, *Lr*53, and *Lr*57 were shown to be extremely efficient in North Caucasus populations of *P. triticina* in research conducted by Gultyaeva and Shaydayuk (2021). These genes, with the exception of *Lr*9 and *Lr19*, also shown efficacy in other Russian grain-producing areas. Stable genetic protection for wheat is provided by genes such as *Lr*26, *Lr*34, and *Lr37*, which are extensively distributed across North Caucasus cultivars (Gultyaeva et al., 2020; Gultyaeva et al., 2021). Genes *Lr*1, *Lr19*, *Lr*26, and *Lr*34 were found in local wheat cultivars by Chinese researchers (Ren et al., 2018); eight *Lr* genes (*Lr*1, *Lr*10, *Lr*17, *Lr*20, *Lr*26, *Lr*34, *Lr37*, and *Lr*46) were found, either alone or in combination, in 32 cultivars from foreign sources (Liu et al., 2021). Using linked molecular markers (csLV34, Xgwm259, CFD71, and csGSR), four resistance genes (*Lr*34, *Lr*46, *Lr*67, and *Lr68*) were found in wheat cultivars in Egypt (El-Orabey et al., 2019).

The leaf rust resistance gene *Lr19* was present in three (5%) of the genotypes, while *Lr68* was present in another three (5%) of them, according to the findings of the molecular screening. In addition, the *Lr24* gene was present in five genotypes (8.3%). Additionally, genotypes harboring *Lr19*, *Lr24*, and *Lr37* were shown to have strong resistance in mature plants, indicating that these plants may be included in future breeding initiatives to increase effective resistance to leaf rust. These results align with earlier research conducted by Abdul (2011), Tariq et al. (2003), Liu and Kolmer (1997) and Kolmer (1996).

The identified *Lr* genes, such as *Lr*50, *Lr*19, and *Lr*24, can significantly enhance the resistance of wheat varieties to leaf rust. This is particularly important for Kazakhstan, where climatic conditions favor the spread of diseases. The incorporation of these genes into breeding programs can help develop varieties capable of adapting to local conditions and ensuring stable yields. It is crucial to consider that genetic diversity is a key factor for resilience against changing climatic conditions and pathogens. The reduction in genetic diversity mentioned in European studies may also be relevant for Kazakhstan. Breeding programs should aim to create varieties with diverse combinations of *Lr* genes, which will help improve their resistance and adaptability. Kazakhstan ranks third in the CIS market for grain production and is one of the major exporters of flour. Resistant wheat varieties can contribute to increased export volumes and strengthen the country's economy. The development of high-yielding varieties with improved quality characteristics can also enhance the competitiveness of Kazakh wheat in international markets. Climate change poses a serious threat to agriculture worldwide. Breeding efforts should focus not only on increasing yield but also on creating varieties capable of withstanding extreme weather conditions, such as drought or increased humidity. This is especially pertinent for Kazakhstan, where such conditions can significantly impact yields. Successful breeding requires active collaboration among research institutions, farmers, and government agencies. The exchange of data regarding new varieties, their resistance, and yield will help improve breeding programs both in Kazakhstan and in other countries in the region. Thus, the findings of this study on *Lr* genes have important implications for wheat breeding in Kazakhstan and beyond. Resistant varieties utilizing the identified genes could serve as a

foundation for enhancing productivity and resilience in agriculture under changing climate conditions and increasing pathogen threats. Continued research into the genetic basis of resistance and the implementation of new varieties into production are essential for ensuring the country's food security.

The genetic basis of resistance to leaf rust, represented by *Lr* genes, is a key factor in wheat breeding. These genes provide protection to plants against pathogens, which is particularly relevant in the context of climate change and the emergence of new fungal races. The introduction of varieties with resistant *Lr* genes can significantly increase wheat yield by reducing losses due to diseases. This is crucial for ensuring food security and stability of supply. This study emphasizes the importance of considering genetic diversity in breeding programs to enhance wheat resistance to diseases.

The adult plant resistance gene *Lr37* ensures effectiveness in field conditions during later stages of wheat ontogenesis, post seedling emergence (Gultyaeva et al., 2021). Molecular analysis of Romanian wheat breeding lines revealed the presence of the *Lr37* resistance allele in 20 cultivars (40%) out of the total genotypes analyzed (Cristina et al., 2015). In our study, four entries (6.7%) exhibited a linked gene complex *Lr37/Yr17/Sr38*.

The gene *Lr50* has demonstrated high effectiveness in Russia and is a foundation for developing rust-resistant cultivars (Gultyaeva and Shaydayuk, 2021a). In this study, *Lr50* was identified at a high frequency (25%) among fifteen genotypes, highlighting its importance. These rust resistance genes provide robust protection in the studied genotypes. Identifying microsatellite markers associated with *Lr50* offers tools for integrating this gene into pyramids with other effective resistance genes (Brown-Guedira et al., 2003).

The inclusion of *Lr19*, *Lr50*, and *Lr68*—whose carriers showed a low illness susceptibility index (φ − 0)—was the most beneficial combination in our investigation. Genes *Lr24* and *Lr50* were found in genotypes CP_21 and CP_22; *Lr50* and *Lr68* were found in genotypes CP_21 and 388_SP2. The combination of two genes, *Lr19* and *Lr68*, was found in genotype CP_13. During the whole growth season (φ – 0), these cultivars exhibited strong resistance levels at the adult plant stage and good production. The disparate reactions seen in cultivars with identical *Lr* resistance genes might potentially be ascribed to undiscovered genes, fluctuating gene expression levels, and other biotic and abiotic elements (Tomkowiak et al., 2023). Increased cooperation between wheat researchers and breeders is necessary for the potential use of wheat rust resistance genes in the future. Pyramids of slow rusting resistance peculiar to a certain race are expected to prove to be useful tools in the development of long-lasting resistance (Ren et al., 2023). When it comes to improving agricultural crops' tolerance to both biotic and abiotic challenges, parents from different backgrounds produce more desired results (Ijaz et al., 2023). Thus, novel genomic areas causing resistance to leaf rust may be quickly identified using contemporary genotyping systems. Breeders can remain ahead of the quickly changing pathogen with the use of these techniques (Ijaz et al., 2023). The identification of resistance genes has aided in the research of host-pathogen interactions and enhanced our knowledge of the dynamics of virulence in *P. triticina* populations. The data obtained regarding the presence and distribution of *Lr* genes can be utilized to develop more effective breeding programs. Varieties with a high level of resistance to leaf rust may be recommended for widespread cultivation in regions with high disease incidence. The use of molecular markers for monitoring and selecting resistant lines within breeding programs will expedite the process of creating new varieties with desired traits. These findings underscore the importance of employing modern molecular genetics techniques to enhance breeding programs in Kazakhstan and other regions.

Conclusion

Sixty winter wheat genotypes showed phenotypic variability in their resistance to leaf rot in this investigation. The findings showed that in 2021, there was a substantial positive association between mature plant resistance and thousand-grain weight. Eight wheat entrants were chosen for direct inclusion in breeding programs to increase wheat resistance to leaf rust because they had a combination of two *Lr* genes and demonstrated an immune response at the adult plant stage. Twenty carriers of a single efficient *Lr* resistance gene and five carriers of two *Lr* genes were found by molecular screening. A constant supply of novel, persistent genetic resistance is required for the development of rust-resistant genotypes since increasing crop yields depends on the genetic diversity of the host. In order to stay up with the quickly

https://doi.org/10.35495/ajab.2024.142

changing pathogens, breeding efforts for rust resistance must be maintained. This calls for continuous integration of novel resistance genes and rigorous phenotypic screening. To sum up, this study offers academics and wheat breeders useful tools to help them better understand the genetics of wheat resistance to leaf rust. The results may help breeders incorporate carriers of advantageous *Lr* genes found in this research into breeding programs, which would help Kazakhstan produce new cultivars resistant to leaf rust.

Acknowledgment

Authors acknowledge financial support by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: This research was financially supported by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan (Grant No. BR18574149 "Development of highly productive cultivars and lines of crops using innovative technologies", the task 01 Breeding of promising wheat lines resistant to wheat rust based on introduction of Marker Assisted Gene Pyramiding technology into the breeding process).

Contribution of Authors

Kokhmetova AM, Kumarbayeva MT & Nurzhuma M: Performed experiments and wrote the first draft of manuscript.

Kokhmetova AM & Nurzhuma M: Developed study designs and reviewed literature.

Keishilov ZhS, Bolatbekova A & Bakhytuly K: Edited the draft and revised the final article.

Kokhmetova AM: Developed study designs, edited and revised the final draft.

References

Abdul SD, 2011. Expression of the wheat leaf rust resistance gene *Lr*34 alone and in combination with other leaf rust genes against UK isolates of Puccinia triticina. Emirates J. Food Agric. 23 (5): 413–423.

Aktar UZM, Tuhina-Khatun M, Hanafi MM and Sahebi M, 2017. Genetic analysis of rust resistance genes in global wheat cultivars: an overview. Biotechnol. Biotechnol. Equip. 31(3):431-445. doi 10.1080/13102818.2017.1304180

Aoun M, Kolmer JA, Rouse MN, Chao S, Bulbula WD and Elias EM, 2017. Inheritance and Bulked Segregant Analysis of Leaf Rust and Stem Rust Resistance in Durum Wheat Genotypes. Phytopathol. 107:1496–1506. doi:10.1094/phyto-12- 16-0444-r

Bakkeren G and Szabo LJ, 2022. Progress on Molecular Genetics and Manipulation of Rust Fungi. Phytopathol. 110:532-543. doi: 10.1094/PHYTO-07-19-0228-IA

Bolton MD, Kolmer JA and Garvin DF, 2008. Wheat Leaf Rust Caused by Pucciniatriticina. Mol. Plant Pathol. 9:563–575. doi:10.1111/j.1364-3703.2008. 00487.x

Brown-Guedira GL, Singh S and Fritz AK, 2003. Performance and Mapping of Leaf Rust Resistance Transferred to Wheat from *Triticum timopheevii* subsp. Armeniacum. Phytopathol. 93(7):784-789. doi: 10.1094/PHYTO.2003.93.7.784.

Bulos M, Echarte M and Sala C, 2006. Occurrence of the rust resistance gene *Lr37* from *Aegilops ventricosa* in Argentine cultivars of wheat. Electron. J. Biotechnol. (9)5. doi: 10.4067/S0717-34582006000500014

Chen X, Line R and Leung H, 1998. Genome scanning for resistance-gene analogs in rice, barley, and wheat by 432 high-resolution electrophoresis. Theor. Appl. Genet. 97:345–355. doi: org/10.1007/s001220050905

Cristina D, Turcu A-G and Ciuca M, 2015. Molecular detection of resistance genes to leaf rust Lr34 and Lr37 in wheat germplasm. Agric. Agric. Sci. Procedia. 6: 533–537.

Datta D, Nayar SK, Bhardwaj SC, Prashar M and Kumar S, 2008. Detection and Inheritance of Leaf Rust Resistance in Common Wheat Lines Agra Local and IWP94. Euphytica. 159:343–351. doi:10.1007/s10681-007-9522-3

Dospekhov BA, 1985. Methods of Field Experience (With the Basics of Statistical Processing of Research Results), 5th ed. Kolos, Kovalivka, Ukraine.

Elangbam PD and Deepshikha JP, 2018. Revealing of brown rust resistance genes by molecular markers in wheat: a review. Int. J. Pure Appl. Biosci. 6(1): 407–417. doi: http://dx.doi.org/10.18782/2320-

7051.4095

Ellis JG, Lagudah ES, Spielmeyer W and Dodds PN, 2014. The Past, Present and Future of Breeding Rust Resistant Wheat. Front. Plant Sci. 5:641. doi:10.3389/fpls.2014.00641

El-Orabey WM, Hamwieh A and Ahmed SM, 2019. Molecular markers and phenotypic characterization of adult plant resistance genes *Lr* 34, *Lr* 46, *Lr* 67 and *Lr* 68 and their association with partial resistance to leaf rust in wheat. J. Genet. 98:82. doi: 10.1007/s12041-019-1122-1

FAO, 2023. Crop Prospects and Food Situation – Triannual Global Report No. 3, November 2023. Rome. doi: 10.4060/cc8566en

Gorash A, Galaev A, Babayants O and Babayants L, 2014. Leaf rust resistance of bread wheat (*Triticum aestivum L.*) lines derived from interspecific crosses. Zemdirbyste-Agriculture. 101(3): 295–302. doi: 10.13080/z-a.2014.101.038

Gultyaeva EI, Kokhmetova AM, Shreyder ER, Shaydayuk EL, Atishova MN, Madenova A, Malysheva A and Galymbek K, 2020. Genetic variability of perspective breeding material of spring bread wheat for resistance to leaf rust in Russia and Kazakhstan. Bull. Nat. Acad. Sci. Repub. Kazakh. 3:385, 60-68. doi: doi:10.32014/2020.2518-1467.70

Gultyaeva EI and Shaydayuk EL, 2021. Identification of leaf rust resistance genes in the new Russian cultivars of common wheat. Biotekhnologiya i Selektsiya Rasteniy. Plant Biotechnol. Breed. 4(2):15-27. doi: 10.30901/2658-6266-2021-2-o2 (in Russian)

Gultyaeva E, Shaydayuk E and Gannibal P, 2021. Leaf Rust Resistance Genes in Wheat Cultivars Registered in Russia and Their Influence on Adaptation Processes in Pathogen Populations. Agriculture. 11: 319. doi:10.3390/ agriculture11040319

Gupta SK, Charpe A, Prabhu KV and Haque QMZ, 2006a. Identification and validation of molecular markers linked to the leaf rust resistance gene *Lr19* in wheat. Theor. Appl. Genet. 113:1027-1036.

Gupta S, Charpe A, Koul S, Haque Q and Prabhu K, 2006b. Development and validation of SCAR markers co-segregating with an Agropyron elongatum derived leaf rust resistance gene *Lr24* in wheat. Euphytica. 150, 233-240.

Herrera-Foessel SA, Singh RP, Huerta-Espino J, Rosewarne GM, Periyannan SK, Viccars L, Calvo-Salazar V, Lan C and Lagudah ES, 2012. *Lr68*: a new gene conferring slow rusting resistance to leaf rust in wheat. Theor. Appl. Genet. 124(8):1475-86. doi: 10.1007/s00122-012-1802-1.

Ijaz M, Afzal A, Shabbir G, Iqbal J and Rafique M, 2023. Breeding wheat for leaf rust resistance: past, present and future. Asian J. Agric. Biol. 2023(1): 2021426 doi: https://doi.org/10.35495/ajab.2021.426

Kenzhebayeva S, Mazkirat S, Shoinbekova S, Atabayeva S, Abekova A, Omirbekova N, Doktyrbay G, Asrandina S, Zharassova D and Amirova A, 2024. Phenotyping and Exploitation of Kompetitive Allele-Specific PCR Assays for Genes Underpinning Leaf Rust Resistance in New Spring Wheat Mutant Lines. Curr. Issues Mol. Biol. 4: 689-709. doi: 10.3390/cimb46010045

Kiel A, Weigt D, Karpińska M, Kurasiak-Popowska D, Niemann J, Tomkowiak A, Mikołajczyk S and Nawracała J, 2020. An analysis of the functionality of molecular markers related to the *Lr19* gene conditioning resistance to wheat leaf rust. Zemdirbyste-Agriculture. 107(1): 63–70. doi: 10.13080/z-a.2020.107.009

Kokhmetova AM and Atishova MN, 2012. Identification of sources of resistance to wheat stem rust using molecular markers. Russ. J. Genet. Appl. Res. 2:486–493.

Kokhmetova A, Madenova M, Purnhauser L, Kampitova G, Urazaliev R and Yessimbekova M, 2016. Identification of leaf rust resistance genes in wheat cultivars produced in Kazakhstan. Cereal Res. Commun. 44(2):240-250-301. doi: org/10.1556/0806.43.2015.056

Kokhmetova A, Sharma R, Rsaliyev S, Galymbek K, Baymagambetova K, Ziyaev Z and Morgounov A, 2018a. Evaluation of Central Asian wheat germplasm for stripe rust resistance. Plant Genet. Resour. 16: 178–184.

Kokhmetova A, Ali S, Sapakhova Z and Atishova M, 2018b. Identification of genotypes-carriers of resistance to tan spot Ptr ToxA and Ptr ToxB of *Pyrenophora tritici-repentis* in common wheat collection. Vavilov. J. Genet. Breed. 22: 978–986.

Kokhmetova A, Atishova M, Madenova A and Kumarbayeva M, 2019. Genotyping of wheat germplasm for resistance to toxins of tan spot *Pyrenophora tritici-repentis*. J. Biotechnol. 305: 53.

Kokhmetova AM, Atishova MN and Galymbek K, 2020a. Identification of wheat germplasm

resistant to leaf, stripe and stem rust using molecular markers. Bull. NAS RK. 2(384): 45-52. doi: org/10.32014/2020.2518-1467.40

Kokhmetova A, Kovalenko N and Kumarbaeva M, 2020b. Pyrenophora tritici-repentis population structure in the Republic of Kazakhstan and identification of wheat germplasm resistant to tan spot. Vavilov J. Genet. Breed. 24: 722–729.

Kokhmetova A, Kumarbayeva M, Atishova M, Nehe A, Riley I and Morgounov A, 2021a. Identification of high-yielding wheat genotypes resistant to *Pyrenophora tritici-repentis* (tan spot). Euphytica. 217: 97. doi: 10.1007/s10681-021-02822-y

Kokhmetova A, Rsaliyev A, Malysheva A, Atishova M, Kumarbayeva M and Keishilov Z, 2021b. Identification of stripe rust resistance genes in common wheat cultivars and breeding lines from Kazakhstan. Plants. 10: 2303. doi: 10.3390/plants10112303

Kokhmetova A, Rsaliyev S, Atishova M, Kumarbayeva M, Malysheva A, Keishilov Z, Zhanuzak D and Bolatbekova A, 2021c. Evaluation of wheat germplasm for resistance to leaf rust (*Puccinia triticina)* and Identification of the Sources of *Lr* Resistance Genes Using Molecular Markers. Plants. 10: 1484. doi: 10.3390/plants10071484

Kokhmetova A, Rathan ND, Sehgal D, Malysheva A, Kumarbayeva M, Nurzhuma M, Bolatbekova A, Krishnappa G, Gultyaeva E, Kokhmetova A, Keishilov Z and Bakhytuly K, 2023. QTL mapping for seedling and adult plant resistance to stripe and leaf rust in two winter wheat populations. Front. Genet. 14:1265859. doi: 10.3389/fgene.2023.1265859

Kokhmetova A, Bolatbekova A, Zeleneva Y, Malysheva A, Bastaubayeva S, Bakhytuly K, Dutbayev Y and Tsygankov V, 2024. Identification of Wheat *Septoria tritici* Resistance Genes in Wheat Germplasm Using Molecular Markers. Plants. 13: 1113. doi: 10.3390/ plants13081113

Kolmer JA, 1996. Genetics of resistance to wheat leaf rust. Annu. Rev. Phytopathol. 34:435–455.

Kolmer JA and Liu JQ, 2002. Inheritance of Leaf Rust Resistance in the Wheat Cultivars AC Majestic, AC Splendor, and AC Karma. Canad J. Plant Pathol. 24:327–331. doi:10.1080/07060660209507017

Kumar A, Choudhary A, Kaur H and Mehta S, 2022. A Walk towards Wild Grasses to Unlock the Clandestine of Gene Pools for Wheat Improvement: A Review. Plant Stress 3. doi: 10.1016/j.stress.2021.100048

Kumar K, Jan I, Saripalli G, Sharma PK, Mir RR, Balyan HS and Gupta PK, 2022. An Update on Resistance Genes and Their Use in the Development of Leaf Rust Resistant Cultivars in Wheat. Front. Genet. 13: 816057. doi: 10.3389/fgene.2022.816057

Kumarbayeva M, Kokhmetova A, Kovalenko N, Atishova M, Keishilov Z and Aitymbetova K, 2022a. Characterization of *Pyrenophora tritici-repentis* (tan spot of wheat) races in Kazakhstan. Phytopathol. Mediterr. 61: 243–257. doi: 10.36253/phyto-13178

Kumarbayeva MT, Kovalenko NM, Kremneva OYu, Atishova MN. Keishilov ZhS, Malysheva AA, Zhanuzak DK, Bolatbekova AA and Kokhmetova AM, 2022b. Identification of Wheat Samples for Resistance to Toxins of Pyrenophora Tritici-Repentis (Ptr). Int. J. Biol. Chem. 15 (1):64-72. doi: 10.26577/ijbch.2022.v15.i1.07

Leonova IN, 2018. Influence of alien genetic material on the manifestation of agronomically important traits of common wheat (*T. aestivum* L.). Vavilov J. Genet. Breed. 22(3): 321-328. doi: 10.18699/VJ18.367 (in Russian)

Li H, Hua L, Zhao S, Hao M, Song R, Pang S, Liu Y, Chen H, Zhang W, Shen T, Gou J, Mao H, Wang G, Hao X, Li J, Song B, Lan C, Li Z, Wang Deng X, Dubcovsky J, Wang X and Chen S, 2023. Cloning of the wheat leaf rust resistance gene *Lr47* introgressed from *Aegilops speltoides*. Nat. Commun. 14:6072. doi: org/10.1038/s41467-023-41833-2

Liu JQ and Kolmer JA, 1997. Inheritance of leaf rust resistance in wheat cultivars Grandin and CDC Teal. Plant Dis. 81: 505–508.

Liu S, Yu LX, Singh RP, Jin Y, Sorrells ME and Anderson JA, 2010. Diagnostic and co-dominant PCR markers for wheat stem rust resistance genes Sr25 and Sr2. Theor. Appl. Genet. 120:691-697.

Liu Y, Gebrewahid TW, Zhang P, Li Z and Liu D, 2021. Identification of leaf rust resistance genes in common wheat cultivars from China and foreign countries. J. Integr. Agric. 20:5. doi: 10.1016/S2095-3119(20)63371-8

Malysheva A, Kokhmetova A, Urazaliev R, Kumarbayeva M, Keishilov Zh, Nurzhuma M, Bolatbekova A and Kokhmetova As, 2023.

Phenotyping and Identification of Molecular Markers Associated with Leaf Rust Resistance in the Wheat Germplasm from Kazakhstan, CIMMYT and ICARDA. Plants 12(15): 2786. doi: 10.3390/plants12152786

McIntosh RA, Dubcovsky J, Rogers WJ, Morris C and Xia XC, 2017. Catalog of Gene Symbols for Wheat. Aberdeen, USA: supplement.

McIntosh RA, Dubcovsky J, Rogers WJ, Xia XC and Raupp WJ, 2020. Catalogue of Gene Symbols for Wheat. Supplement. Ann. Wheat Newslett. 66.

Miedaner T and Juroszek P, 2021. Climate change will influence disease resistance breeding in wheat in Northwestern Europe. Theor. Appl. Genet. 134(6): 1771-1785. doi: 10.1007/s00122-021-03807-0.

Morgounov A, Akin B, Demir L, Keser M, Kokhmetova A, Martynov S and Yessimbekova M, 2015. Yield gain due to fungicide application in cultivars of winter wheat (*Triticum aestivum*) resistant and susceptible to leaf rust. Crop Pasture Sci. 66: 649.

Oelke LM and Kolmer JA, 2005. Genetics of Leaf Rust Resistance in spring Wheat Cultivars Alsen and Norm. Phytopathol. 95:773–778. doi:10.1094/phyto95-0773

Olivera PD, Szabo LJ, Kokhmetova A, Morgounov A, Luster DG and Jin Y, 2022. *Puccinia graminis f. sp. tritici* Population Causing Recent Wheat Stem Rust Epidemics in Kazakhstan Is Highly Diverse and Includes Novel Virulence Pathotypes. Phytopathol. 112(11): 2403-2415. doi: 10.1094/PHYTO-08-21-0320-R

Porotnikov IV, Antonova OY and Mitrofanova OP, 2020. Molecular markers in the genetic analysis of crossability of bread wheat with rye. Vavilov. J. Genet. Breed. 24(6): 557-567. (Russian). doi: 10.18699/VJ20.649.

Prins R, Groenewaid JZ, Marais GF, Snape JW and Koebner RMD, 2001. AFLP and STS tagging of *Lr19*, a gene conferring resistance to leaf rust in wheat. Theor. Appl. Genet. 103: 618-624.

R Core Team, 2018. R: A Language and Environment for Statistical Computing. The R Foundation for Statistical Computing: Vienna. Austria. Available online: http://www.R-project.org/ (accessed on 21 April 2023).

Ren X, Wang C, Ren Z, Wang J, Zhang P, Zhao S, Li M, Yuan M, Yu X and Li Z, 2023. Genetics of Resistance to Leaf Rust in Wheat: An Overview in a Genome-Wide Level. Sustainability. 15:3247. doi: org/10.3390/su15043247

Riaz M and Wong Y, 2017. Estimation of Yield Losses Due to Leaf Rust and Late Seeding on Wheat (*Triticum aestivum L*) Cultivar Seher-06 in District Faisalabad, Punjab, Pakistan. Adv. Biotech. Micro 4. doi: 10.19080/AIBM.2017. 04.555657

Riede CR and Anderson JA, 1996. Linkage of RFLP markers to an aluminum tolerance gene in wheat. Crop Sci. 36(4):407:905-909.

Roelfs AP, Singh RP and Saari EE, 1992. Rust Diseases of Wheat: Concept and methods of disease management. Mexico, 395 D.F.: CIMMYT.

Rosa SB, McCallum B, Brûlé-Babel A, Hiebert C, Shorter S and Randhawa HS, 2016. Inheritance of Leaf Rust and Stripe Rust Resistance in the Brazilian Wheat Cultivar Toropi. Plant Dis. 100:1132–1137. doi:10.1094/pdis-10-15- 1128-re

Saari E and Wilcoxson R, 1974. Plant disease situation of high-yielding durum wheat in Asia and Africa. Annu. Rev. Phytopathol. 2: 49–68.

Safavi SA and Afshari F, 2012. Quantitative resistance of some Elite wheat lines to *Puccinia striiformis f. sp. tritici*. Arch. Phytopathol. Plant Prot. 45: 740–749.

Schachermayr GM, Messmer MM, Feuillet C, Winzeler H, Winzeler M and Keller B, 1995. Identification of molecular markers linked to the Agropyron elongatum-derived leaf rust resistance gene *Lr24* in wheat. Theor. Appl. Genet. 90: 982-990.

Sehgal SA, Tahir RA, Anwar Z, Abbas G, Kausar Nawaz SM and Zaman Khan KJ, 2012. Molecular genetic characterization of rust in wheat genotypes. Asian J. Agric. Sci. 4:337-340.

Sharma JS, McCartney CA, McCallum BD and Hiebert CW, 2023. Fine mapping and marker development for the wheat leaf rust resistance gene Lr32. G3 (Bethesda). 13(2): jkac274. doi: 10.1093/g3journal/jkac274.

Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Bhavani S, Njau P, Herrera-Foessel S, Singh PK, Singh S and Govindan V, 2011. The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. Ann. Rev. Phytopathol. 49: 465-481.

Singh D, Mohler V and Park RF, 2013. Discovery, characterisation and mapping of wheat leaf rust resistance gene *Lr*71. Euphytica. 190:131–136.

Singh BK, Delgado-Baquerizo M, Egidi E, Guirado E,

Jan EL, Liu H and Trivedi P, 2023. Climate change impacts on plant pathogens, food security and paths forward. Nat. Rev. Microbiol. 21: 640–656. doi: org/10.1038/s41579-023-00900-7

Somers DJ, Isaac P and Edwards K, 2004. A highdensity microsatellite consensus map for bread wheat (*Triticum aestivum L.)*. Theor. Appl. Genet. 109: 1105-1114.

Talebi R, Mahboubi M, Naji AM and Mehrabi R, 2023. Physiological specialization of Puccinia triticina and genome-wide association mapping provide insights into the genetics of wheat leaf rust resistance in Iran. Sci. Reports. 13:4398. doi: 10.1038/s41598-023-31559-y

Tariq AH, Ahmad S, Hussain MA, Akhtar LH, Arshad M and Siddiqi SZ, 2003. The virulence spectrum of wheat leaf rust in Southern Punjab. Pakistan. Plant Pathol. 2:80–84.

Tomkowiak A, Bobrowska R, Kwiatek M, Spychala J, Kuczynski J, Tyczewska A, Kowalczewski P, Weigt D and Kosiada T, 2023. Analysis of miRNA expression associated with gene *Lr*34 responsible for resistance mechanisms to wheat leaf rust. Pak. J. Bot. 55:379–385.

Uhrin A, Láng L and Bedő Z, 2008. Comparison of PCR-based DNA Markers for Using Different *Lr19* and *Lr24* Leaf Rust Resistance Wheat Sources. Cereal Res. Commun. 36(4):533-541. doi: 10.1556/CRC.36.2008.4.2

Vanongeval F and Gobin A, 2023. Adverse Weather Impacts on Winter Wheat, Maize and Potato Yield Gaps in northern Belgium. Agron. 13:1104. https://doi.org/10.3390/agronomy13041104

Wilcoxson R, Skovmand B and Atif A, 1974. Evaluation of wheat cultivars ability to retard development of stem rust. Ann. Appl. Biol. 80:275–281.

Xue S, Kolmer JA, Wang S and Yan L, 2018. Mapping of Leaf Rust Resistance Genes and Molecular Characterization of the 2NS/2AS Translocation in the Wheat Cultivar Jagger. G3. 8(6):2059–2065. doi: 10.1534/g3.118.200058

Yessenbekova G, Kokhmetova A, Madenova A, Amanov O, Dutbayev Y and Kampitova G, 2014. Identification of 387 *Lr*34/Yr18 gene in wheat germplasm in Kazakhstan. APS-CPS Joint meeting, Aug. 9-13, 388:252, Minneapolis, Minnesota, USA.

Zadoks JC, Chang TT and Konzak MM, 1974. A decimal code for the growth stages of wheat. Weed Res 14:415–421. https://doi.org/10.1111/j.1365-3180.1974.tb01084.x

Zhang W and Dubcovsky J, 2008. Association between allelic variation at the phytotene synthase1 gene and yellow pigment content in the wheat grain. Theor. Appl. Genet. 116:635-645.