



Leaf rust resistance genes in *Aegilops* genus: occurrence and efficiency

Lucia Koláriková · Leona Svobodová-Leišová · Alena Hanzalová · Vojtěch Holubec · Michaela Jungová · Minura Esimbekova

Accepted: 17 June 2023 / Published online: 28 June 2023
© The Author(s) 2023

Abstract Leaf rust is a common and costly wheat disease. The introduction of resistance genes derived from wheat wild relatives is one way to improve disease control. However, due to the rapid development of virulence in the casual pathogen *Puccinia triticina*, the long-term effectiveness of some leaf rust resistance (*Lr*) genes is questionable. To assess this, we examined 130 accessions of 13 wheat wild relative species from the *Aegilops* genus. The presence of 9 *Lr* genes was determined by PCR using primers complementary to *Lr* gene markers. The studied individuals were exposed to artificial inoculation with *P. triticina* under greenhouse and field

conditions, respectively. Based on infection rate, the degree of resistance for each individual was estimated. The relationship between the presence of *Lr* genes and the degree of manifested resistance was assessed using canonical correlation analysis (CCA) and analysis of variance (ANOVA). Our results indicate the effective participation of *Lr9* in seedling resistance while *Lr58* is involved in adult plant resistance.

Keywords *Lr* genes · *Aegilops* · Leaf rust · Resistance · Microsatellites

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1007/s10658-023-02712-0>.

L. Koláriková (✉) · L. Svobodová-Leišová ·
A. Hanzalová · V. Holubec · M. Jungová
Crop Research Institute, Drnovská 507/73,
Prague 6 – Ruzyně 161 06, Czech Republic
e-mail: lucia.kolarikova@vurv.cz; kolariku@vscht.cz

L. Koláriková
University of Chemistry and Technology, Technická 5,
Prague 6 – Dejvice 160 00, Czech Republic

M. Jungová
Department of Ecology, Faculty of Environmental
Sciences, Czech University of Life Sciences, Prague,
Kamýcká 129, Prague 6 – Suchbátka 165 00, Czech Republic

M. Esimbekova
Gene Bank, Kazakh Scientific Research Institute
of Agriculture and Plant Growing, Almalyk, Kazakhstan

Introduction

Wheat rust diseases pose a serious threat to wheat production worldwide. Although not as devastating as stem rust or stripe rust, the global and frequent occurrence of leaf rust makes it responsible for considerable annual wheat losses (Huerta-Espino et al., 2011). This fungal disease is caused by *Puccinia triticina* Eriks, an obligate biotrophic parasite with a lifecycle that consists of two phases: i) an asexual cycle (major phase) completed on wheat, and ii) a sexual cycle (minor phase) completed on an alternate host, *Thalictrum* sp., that gives the pathogen the advantage of acquiring genetic variability through recombination (Kolmer et al., 2013).

Various strategies have been developed to counter the adverse effects of leaf rust. However, agricultural methods, such as crop rotation, deep tillage and adjusting the sowing/harvesting time (Krupinsky

et al., 2002), as well as use of chemical applications are not successful in the long run. Although fungicide use has enabled farmers to intensify production, overall crop losses have not declined for decades (Oerke, 2006). Thus, the development of genetic resistance is now seen as the most efficient, economical and environment-friendly approach.

There are two types of host genetic resistance against *P. triticina*. The first is seedling resistance, which is expressed at all growth stages and is typically controlled by single genes that have a major effect. This type of resistance is generally qualitative and triggers a hypersensitive response that results in necrosis and prevents the pathogen from spreading. The second is adult plant resistance (APR), which occurs at a post-seedling stage and is commonly quantitative. The interplay of both types of resistance genes creates the overall host resistance to the pathogen.

To date, more than 80 leaf rust resistance (*Lr*) genes providing either seedling or adult plant resistance have been reported. However, the presence of a known *Lr* gene does not always guarantee resistance, with several studies documenting loss of efficiency over time (Kolmer, 2019; McCallum et al., 2021; Park et al., 2002). While single-gene resistance provides mostly short-term effectiveness, long term resistance is influenced by the specific combination of multiple resistance genes in the host (Nelson et al., 2018). For this reason, resistance breeding is an ongoing process that systematically tests wild relatives, landraces and other germplasm to identify new sources of genetic resistance to a successful pathogen. *Aegilops*, a genus of annual grass plants, is genetically related to bread wheat and carries *Lr* genes. It consists of 23 species with C, M, N, S, U and D genomes. The D genome, derived from *Ae. tauschii*, and the B genome, derived from a species closely related to *Ae. speltoides*, are part of the bread wheat (*Triticum aestivum*) hexaploid genome AABBDD. A notable number of *Lr* genes have been identified in various species of the *Aegilops* genus (Prasad et al., 2020). Since the first introgression of *Lr9* from *Ae. umbellulata* into the wheat genome (Sears, 1956), many more resistance genes have been introduced, including those from *Ae. tauschii* (*Lr21*, *Lr22a*, *Lr39*) and *Ae. speltoides* (*Lr28*, *Lr35*, *Lr36*, *Lr37*, *Lr51*). Despite the considerable number of *Lr* genes that have already been introduced

into wheat from *Aegilops* species, further screening studies are required to identify new gene(s) or combinations of genes in distinct genotypes.

To address this, 130 accessions of the *Aegilops* genus were screened for the presence of seedling and adult plant resistance *Lr* genes. After artificial exposure to *P. triticina* under greenhouse and field conditions, the seedlings and adult plants, respectively, were symptomatically evaluated. Comparing the outcomes enabled us to evaluate the efficiency of the tested genes and identify genotypes suitable for breeding. Moreover, we identified phenotypically resistant genotypes without the screened *Lr* genes whose genetically conditioned resistance is worth further investigation.

Material and methods

Plant material and phenotyping

The *ex-situ* gene bank *Triticeae* collection, maintained by the Czech Gene Bank, comprises 1763 accessions, of which the *Aegilops* genus accounts for 1230 accessions, encompassing 21 out of all 23 species. The material selected for this contribution was one set received from the Kazakh Gene Bank Almaty/Almaty on exchange basis. It comes partly from extensive collecting missions by Kazakh partners and partly from other sources. Selected 130 accessions, belonging to 13 species, (Table 1, S1) were regenerated and phenotyped, and data recorded to the national information system GRIN Czech (GRIN Czech Release 1.10.3 (vurv.cz)). A more detailed characterization of the origin and location of material collection is available in Table S2.

Phenotyping under infection

The National Program on Conservation and Utilization of Microbial Genetic Resources and Invertebrates of Agricultural Importance provided the pathotypes of the pathogen. The spores were collected from leaves from the previous seasons from different localities to simulate the range of races that appear naturally. A collection of 6 pathotypes with varying virulence levels were utilized for resistance tests, as shown in Table 2. Seedlings of the NILs (Nearly Isogenic Lines) were inoculated in the greenhouse by rubbing leaves with an urediniospore water suspension, then they were sprayed with water

Table 1 *Aegilops* species and number of accessions used in present study

Species	Genome	Number of accessions
<i>Ae. biuncialis</i> Vis	UM	2
<i>Ae. columnaris</i> Zhuk	UM	1
<i>Ae. comosa</i> Sibth. et Sm	M	3
<i>Ae. crassa</i> Boiss	DM	9
<i>Ae. cylindrica</i> Host	DC	67
<i>Ae. geniculata</i> Roth	UM	10
<i>Ae. juvenalis</i> (Thell.) Eig	DMU	1
<i>Ae. markgrafii</i> (Greuter) Hammer	C	1
<i>Ae. neglecta</i> Req. ex Bertol	UM	1
<i>Ae. peregrina</i> (Hackel) Maire et Weiller	US	2
<i>Ae. speltoides</i> Tausch	S	1
<i>Ae. tauschii</i> Coss	D	22
<i>Ae. triuncialis</i> L	UC	10

and placed in glass cylinders for 24–48 h. Thereafter plants were kept in the greenhouse at 18–22 °C. Infection types were scored after 10–14 days based on the scale by Stakman et al. (1962).

Due to pure germination, from overall 130 genotypes screened with molecular markers, 122 genotypes were evaluated in the field and only 77 were possible to evaluate in greenhouse conditions. To

provide clarity throughout the text, the SMM (Set tested with Molecular Markers) designation will now refer to 130 genotypes that were screened using molecular markers. The SF (Set tested on Field) designation will represent the 122 genotypes that were evaluated under field conditions, and the SG (Set tested in Greenhouse) designation will refer to the 77 genotypes that were evaluated in a greenhouse environment. It’s important to note that the smaller set always contains only representatives from the larger one. Rust spreaders (cv. Michigan Amber) regularly distributed in the trial were inoculated with a mixture of the same *P. triticina* urediniospores as used in greenhouse experiment to test SF cultivars for resistance (disease severity). Artificial infection was performed by injecting water suspension of urediniospores into the stems of the susceptible variety spreader at the beginning of stem elongation phase. Visual estimation of disease severity took place in several terms according to the development of the disease using a disease severity scale of 1–9, where 9 stands for no symptoms observed and 1 represents heaviest sporulation leading to defoliation.

DNA extraction

DNA of 130 accessions (Table S1) was purified from leaf tissue by following optimized CTAB

Table 2 Reaction of the 6 races on the NILs

Lr genes/races	9528 A	9668	9585	9528	9657	9712
<i>Lr1</i>	3	3	3	3	3	0
<i>Lr2a</i>	;	;	;0	;1	;1	;1
<i>Lr2b</i>	;	0	3	;	;1–2	;2
<i>Lr2c</i>	;1	;1	;1	;	;2	;2 N
<i>Lr3a</i>	;2	;2	;2	3	3-	3
<i>Lr9</i>	;N	;N	;	;	0;	0
<i>Lr11</i>	3	3	3	3	3	3
<i>Lr13</i>	3	3	3	3	3	3
<i>Lr15</i>	3	3	;2	3	2	3-
<i>Lr17</i>	3	3	3	3	3	3
<i>Lr19</i>	;0	;0	;0	;0	;0	;0
<i>Lr21</i>	3-	3	3	3	3	3
<i>Lr23</i>	;2	;2	;1	;2	;2	3
<i>Lr24</i>	;N	;1	;1	;1	;1 N	;1
<i>Lr26</i>	;	3	3	3	3	3
<i>Lr28</i>	0;	;	;	;	3	;

Infections types: ; = chloroses; N = necroses; 0, 1, 1–2, 2 = resistant; 3 = susceptible

procedure (Murray & Thompson, 1980). Homogenized plant tissue was incubated in 2% CTAB solution within 30 min at 65 °C and subsequently cooled for 5 min on ice. After incubation with chloroform-isoamylalcohol (24:1) solution and series of centrifugation, DNA was precipitated using 99% ethanol and afterwards purified by 75% ethanol. Dried nucleic acid was incubated with RNase A (20 mg/ml) for 10 min at 37 °C and diluted in TE solution. The integrity of isolated DNA was assessed on 0.8% gel supplemented with ethidium bromide and its concentration measured

spectrophotometrically. To set DNA work concentration on 100 ng/μl, DNA was further resuspended in nuclease-free water.

Lr genes detection

Polymerase chain reactions (PCR) were performed in 15 μl reaction volume with specific primers (Table 3) and DNA solution (100 ng/μl). The amplification profile and composition of reaction mixture are listed in Table S3. All amplification products were visualized using electrophoresis on 2% agarose gel stained with

Table 3 Molecular markers used in PCR reaction for resistance genes detection

Lr genes	Origin	Primer	Sequence 5'→3'	Amplicon length	References
<i>Lr1</i>	common wheat	WR003-F	GGG ACA GAG ACC TTG GTG GA	760 bp	Qiu et al., 2007
		WR003-R	GAC GAT GAT GAT TTG CTG CTG G		
<i>Lr9</i>	<i>Ae. umbellulata</i>	J13/1	CCA CAC TAC CCC AAA GAG ACG	1 100 bp	Schachermayr et al., 1994
		J13/2	TCC TTT TAT TCC GCA CGC CGG		
<i>Lr19</i>	<i>Thinopyrum ponticum</i>	GbF	CAT CCT TGG GGA CCT C	130 bp	Prins et al., 2001
		GbR	CCA GCT CGC ATA CAT CCA		
<i>Lr21</i>	<i>Ae. tauschii</i> var. <i>Meyeri</i>	D14-L	CGC TTT TAC CGA GAT TGG TC	885 bp	Huang & Gill, 2001
		D14-R	CCA AAG AGC ATC CAT GGT GT		
<i>Lr24</i>	<i>Thinopyrum ponticum</i>	SCS1326-F	GCA TCG TGC AGC TAG TTC TG	607 bp	Gupta et al., 2006
		SCS1326-R	AGG CAT CGT GAA AAG AGA ACA		
<i>Lr28</i>	<i>Ae. speltoides</i>	SCS421-1	ACA AGG TAA GTC TCC AAC CA	570 bp	Cherukuri et al., 2005
		SCS421-2	AGT CGA CCG AGA TTT TAA CC		
<i>Lr35</i>	<i>Ae. speltoides</i>	Sr39#22r-F	AGA GAA GAT AAG CAG TAA ACA TG	487 bp	Mago et al., 2009
		Sr39#22r-R	TGC TGT CAT GAG AGG AAC TCT G		
<i>Lr37</i>	<i>Ae. ventricosa</i>	Ventriup	AGG GGC TAC TGA CCA AGG CT	259 bp	Helguera et al., 2003
		LN2	TGC AGC TAC AGC AGT ATGTAC ACA AAA		
<i>Lr58</i>	<i>Ae. triuncialis</i>	Xncw-Lr58-1-F	TCA CTT TGG TCA GGG TAG GG	~404 bp	Kuraparthi et al., 2011
		Xncw-Lr58-1-R	CGA CGA GGT CCT GAT ATG GT		

ethidium bromide. GeneRuler 100 bp DNA ladder was used to estimate amplicon size.

SSR analysis

The microsatellite markers were selected primarily for the diversity evaluation of wheat by our and French lab (INRA) in collaboration as a selection from hundreds SSR markers. Before this study, we tested these markers on several species of *Aegilops* and their spontaneous hybrids with wheat (Holubec et al., 2019). It was clear, that only a part of them could be used and applied on genotypes in this study. The final set consisted of 38 markers. Microsatellite analyses were performed according to Röder et al. (1998). PCR products were separated using a capillary electrophoresis in ABI PRISM 3130 sequencer (Life Technology, Foster City, CA, USA). A multiplexed configuration of four reactions was used in one analysis. As to interne size standard LIZ500 (Applied Biosystems) was used. Electrophoretograms were processed by GeneMapper software.

Data analysis

The gels were scored for the presence or absence of reproducible products with corresponding lengths. The molecular data from all genotypes screened with nine markers were transformed into a binary data matrix as discrete variables (1=presence, 0=absence). The results of symptomatic evaluation on field were transformed into a value between 0 and 1 depending on score in 1–9 scale. Data generated by symptomatic evaluation in greenhouse were also transformed into a value between 0 and 1 depending on number of resistant cases against 5 possible races. Canonical correlation analysis was used in R environment to identify and measure associations among genotypic and phenotypic data. One-way ANOVA was used to determine the significant difference in symptomatic response between genotypes with presence and absence of particular *Lr* gene.

A matrix of distances between all the samples was calculated from microsatellite data in the DARwin software (<http://darwin.cirad.fr/darwin>; Perrier & Jacquemoud-Collet, 2006). For clustering, a weighted Neighbour-joining method (W NJ) was used as its cophenetic coefficient r showed the highest value (0.947). The support for the phenogram branches was

obtained using 1,000 bootstrap resampling. Analysis of population structure was performed with STRU CTURE 2.3.4 (Pritchard et al., 2000). The number of populations (K) was selected by testing $K=1$ to $K=10$ using ten independent iterations of 50 000 MCMC Reps after 20,000 burnin period length. The optimum K -value was identified according to methodology of Evanno et al. (2005).

Results

Leaf rust resistance genes and their phenotypic manifestation

SMM set of genotypes was screened for the presence of 9 *Lr* genes (1, 9, 19, 21, 24, 28, 35, 37, 58) (Fig. 1a-c). Tested *Lr* genes were selected with respect to their role in APR/ASR resistance and based on their origin from wild relatives. All of genes were detected at least once with various frequencies (Table 4, Table S4), however in 32% of accessions none of tested gene was present. Marker for *Lr24* was present just in one accession representing the only representative of *Aegilops speltoides*. The frequency of the remaining eight markers ranged from 6.9% (*Lr35*) to 31.5% (*Lr37*). Three genotypes were tested positive for five resistance genes, although most genotypes were tested positive for just one resistance gene at once. Based on these results, SF genotypes were divided into three groups. First group was represented by genotypes with at least two detected resistance genes. Remaining two groups consisted of genotypes with one and no detected *Lr* gene, respectively. All of 122 SF accessions were evaluated in field tests with score 1–9 (Table S4) and their variances were analyzed by one-way ANOVA. Groups showed statistically significant differences ($F=11.973$; $p\leq 0.001$).

The relationship between genotypic and phenotypic data from SG genotypes was analyzed by CCA generating two canonical dimensions, both with statistical significance according to Wilks's lambdas. For the first dimension, the canonical correlation was equal to 0.557 ($p\leq 0.01$), for the second equal to 0.496 ($p\leq 0.01$). With respect to standardized canonical coefficients of genotypic variables, the first canonical dimension was most strongly influenced by *Lr24* (-0.47) and *Lr58* (-0.81), the second by *Lr1* (-0.45), *Lr9*

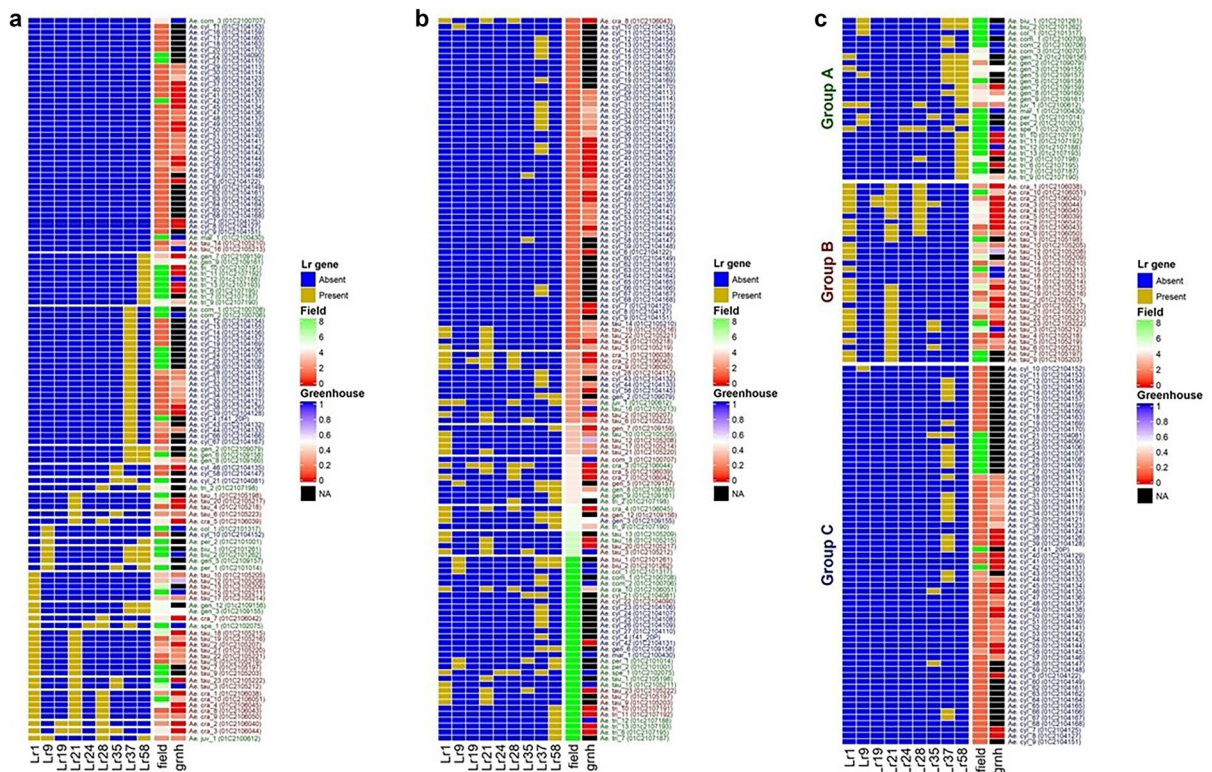


Fig. 1 Occurrence of *Lr* gene markers in each of 122 genotypes from SF set. Symptomatic evaluation in field tests was scored from 1 – susceptible to 9 – resistant, in greenhouse

(-0.58), *Lr21* (0.45), *Lr28* (0.55) and *Lr58* (0.53). Concerning phenotypic data, first dimension was influenced mainly by field evaluation (-0.97) and second by greenhouse (-1.12) variable.

For a more detailed interpretation of CCA results, one-way ANOVA was used. Statistically significant differences in symptomatic evaluation were observed in case of *Lr9* ($F=7.702$; $p \leq 0.01$) and *Lr24* ($F=5.865$; $p \leq 0.05$) in greenhouse conditions and *Lr58* ($F=17.9$; $p \leq 0.01$) in field conditions.

All species counting 10 and more genotypes were further evaluated by one-way ANOVA to compare the average infestation rate in the presence and absence of a particular *Lr* gene (Table 5). A statistically significant difference for both field and seedling resistance was observed only for *Lr37* in *Ae. cylindrica*. The rate of seedling infestation was significantly different for *Ae. tauschii* and *Lr21*, while *Lr28* gene significantly affected the infestation degree of *Ae. triuncialis* on the field.

from 0 – susceptible to 1 – resistant. Genotypes are sorted by pattern of marker presence (a), symptomatic evaluation in field conditions (b), clustering according to DARwin software (c)

Microsatellites

To determine whether genetic variability corresponds to the occurrence of *Lr* genes, and subsequently to the degree of pathogen infestation, SMM genotypes were evaluated using 38 microsatellite markers. A total of 339 alleles were detected from the 38 amplified loci. The number of alleles per locus ranged from 2 to 22, with an average of 8.9 alleles per locus in the range of analyzed genotypes.

Cluster analysis based on microsatellite data showed three main clusters (A, B, C) supported by bootstrap resampling. Individual clusters were further divided into subclusters, all (except A.1) representing individual species (Fig. 2).

Similarly, the results obtained within DARwin software generally aligned with population structure analyzed by STRUCTURE and thus support the idea of three main populations within investigated *Aegilops* representatives (Fig. 3).

Table 4 Frequency of *Lr* genes present in genotypes of 13 *Aegilops* species with level of field (1 to 9) and seedling (0 to 1) resistance. Frequencies of three different sets (SMM, SF, SG) are calculated given the number of individuals included

Species	Set	Frequency of <i>Lr</i> genes (%) present											Field resistance	Seedling resistance	
		<i>Lr1</i>	<i>Lr9</i>	<i>Lr19</i>	<i>Lr21</i>	<i>Lr24</i>	<i>Lr28</i>	<i>Lr35</i>	<i>Lr37</i>	<i>Lr58</i>					
<i>Ae. biu</i>	SMM	0	100	50	0	0	0	0	0	0	0	100	100	9.0	1.00
	SF	0	100	50	0	0	0	0	0	0	0	100	100	100	
	SG	0	100	0	0	0	0	0	0	0	0	100	100	100	
<i>Ae. col</i>	SMM	0	100	0	0	0	0	0	0	0	0	0	0	9.0	1.00
	SF	0	100	0	0	0	0	0	0	0	0	0	0	0	
	SG	0	100	0	0	0	0	0	0	0	0	0	0	0	
<i>Ae. com</i>	SMM	0	0	33	0	0	0	0	0	0	0	66	0	7.3	1.00
	SF	0	0	33	0	0	0	0	0	0	0	66	0	0	
	SG	0	0	0	0	0	0	0	0	0	0	50	0	0	
<i>Ae. cra</i>	SMM	89	0	33	89	0	100	29	0	0	29	0	0	3.7	0.06
	SF	89	0	33	89	0	100	29	0	0	29	0	0	0	
	SG	89	0	33	89	0	100	29	0	0	29	0	0	0	
<i>Ae. cyl</i>	SMM	0	1	15	0	0	0	4	38	0	4	38	0	2.2	0.11
	SF	0	0	15	0	0	0	5	36	0	5	36	0	0	
	SG	0	0	21	0	0	0	3	29	0	3	29	0	0	
<i>Ae. gen</i>	SMM	30	40	0	0	0	0	0	70	90	0	70	90	4.5	0.31
	SF	25	38	0	0	0	0	0	75	100	0	75	100	100	
	SG	20	20	0	0	0	0	0	50	100	0	50	100	100	
<i>Ae. jin</i>	SMM	100	100	100	0	0	100	0	0	0	0	0	0	2.0	0.20
	SF	100	100	100	0	0	100	0	0	0	0	0	0	100	
	SG	100	100	100	0	0	100	0	0	0	0	0	0	100	
<i>Ae. mar</i>	SMM	0	100	0	0	0	0	0	0	0	0	0	0	9.0	1.00
	SF	0	100	0	0	0	0	0	0	0	0	0	0	0	
	SG	0	100	0	0	0	0	0	0	0	0	0	0	0	
<i>Ae. neg</i>	SMM	100	100	0	0	0	0	0	100	0	0	100	0	-	-
	SF	100	100	0	0	0	0	0	100	0	0	100	0	0	
	SG	100	100	0	0	0	0	0	100	0	0	100	0	0	
<i>Ae. per</i>	SMM	0	100	50	0	0	0	50	0	0	50	0	100	9.0	-
	SF	0	0	0	0	0	0	0	0	0	0	0	0	0	
	SG	0	0	0	0	0	0	0	0	0	0	0	0	0	

Table 4 (continued)

Species	Set	Frequency of Lr genes (%) present										Field resistance		Seedling resistance
		Lr1	Lr9	Lr19	Lr21	Lr24	Lr28	Lr35	Lr37	Lr58				
<i>Ae. spe</i>	SMM	100	0	0	0	100	100	0	100	0	9.0			1.00
	SF	100	0	0	0	100	100	0	100	0				
	SG	100	0	0	0	100	100	0	100	0				
<i>Ae. tau</i>	SMM	73	0	0	68	0	0	14	0	0	4.4			0.31
	SF	71	0	0	67	0	0	14	0	0				
	SG	71	0	0	65	0	0	18	0	0				
<i>Ae. tri</i>	SMM	0	30	0	0	0	10	0	20	100	7.9			0.22
	SF	0	38	0	0	0	13	0	0	100				
	SG	0	50	0	0	0	0	0	0	100				

Discussion

The main aim of this study was to verify the extent to which the degree of resistance to the *Puccinia triticina* is influenced by the presence of *Lr* genes in *Aegilops* genus members. No *Lr* gene was present in one-third of the genotypes, yet some of these accessions showed resistance in both field and greenhouse conditions. These genotypes probably have other *Lr* genes contributing to their overall resistance and/or their resistance is based on various components of quantitative resistance (Azzimonti et al., 2013).

In our study, the marker for *Lr1* was detected in all genotypes of *Ae. juvenalis*, *Ae. neglecta* and *Ae. speltoides*, in 89% of *Ae. crassa* genotypes, 73% of *Ae. tauschii* genotypes and 30% of *Ae. geniculata* genotypes. *Lr1*, available in many wheat cultivars (McIntosh et al., 1995) and *Ae. tauschii* accessions, is located on the D genome. D genome is further part of *Ae. juvenalis* and *Ae. crassa*, however we also detected *Lr1* in accessions with absence of D genome, both *Ae. neglecta* and *Ae. geniculata* having the UM genome. This unlikely occurrence of *Lr1* could have resulted from spontaneous hybridization of these species and wheat (Holubec et al., 2019), followed by misidentification of the resulting hybrids as representatives of *Ae. geniculata* and *Ae. neglecta*. Previous studies conducted on *Ae. tauschii* have shown resistance in 3.5% (Ling et al., 2004) to 11% (Assefa & Fehrmann, 2000) of tested genotypes accessions, slightly lower than ours 18%. Ling et al., however, counted only the accessions that displayed infection type 0, while we considered as resistant also types 1 and 2.

The marker associated with *Lr9* was detected with various frequencies in *Ae. biuncialis*, *Ae. columnaris*, *Ae. geniculata*, *Ae. juvenalis*, *Ae. neglecta*, *Ae. peregrina* and *Ae. triuncialis*. All these species contain the U genome of diploid progenitor *Aegilops umbellulate* in which *Lr9* was identified for the first time (Sears, 1961). Interestingly, *Lr9* was also detected in *Ae. markgrafii* containing C genome only. Similar results to ours were reported for *Ae. columnaris* in a study of Aliakbari Sadeghabad et al. (2020) where the presence of *Lr9* corresponded with observed seedling resistance.

Although the band corresponding to *Lr19* marker was previously found in accessions of *Ae. tauschii* (Aliakbari Sadeghabad et al., 2020), this study

Table 5 Mean values of field and seedling resistance of 5 *Aegilops* species (*Ae. cylindrica*, *Ae. geniculata*, *Ae. tauschii*, *Ae. triuncialis*) depending on presence (+) or absence (-) of each of eight *Lr* genes. Means were analyzed by one-way ANOVA. (* $p \leq 0.05$)

<i>Lr</i> gene presence (+) / absence (-)	Mean field resistance				Mean seedling resistance				
	<i>Ae. cyl</i>	<i>Ae. gen</i>	<i>Ae. tau</i>	<i>Ae. tri</i>	<i>Ae. cyl</i>	<i>Ae. gen</i>	<i>Ae. tau</i>	<i>Ae. tri</i>	
<i>Lr1</i>	+	x	5.00	4.73	x	x	0.50	0.33	x
	-	x	4.33	3.50	x	x	0.25	0.28	x
<i>Lr9</i>	+	1.00	4.67	x	9.00	x	0.50	x	0.33
	-	2.25	4.40	x	7.20	0.12	0.25	x	0.11
<i>Lr19</i>	+	3.50	x	x	x	0.14	x	x	x
	-	2.00	x	x	x	0.13	x	x	x
<i>Lr21</i>	+	x	x	4.64	x	x	x	0.19*	x
	-	x	x	3.86	x	x	x	0.55*	x
<i>Lr28</i>	+	x	x	x	4.00*	x	x	x	x
	-	x	x	x	8.43*	x	x	x	0.22
<i>Lr35</i>	+	3.67	x	5.67	x	0.00	x	0.39	x
	-	2.16	x	4.17	x	0.13	x	0.30	x
<i>Lr37</i>	+	3.29*	4.83	x	x	0.20*	0.38	x	x
	-	1.61*	3.50	x	7.88	0.09*	0.25	x	0.22
<i>Lr58</i>	+	x	4.22	x	7.88	x	0.31	x	0.22
	-	x	x	x	x	x	x	x	x

observed it in genotypes of *Ae. biuncialis*, *Ae. comosa*, *Ae. crassa*, *Ae. cylindrica*, *Ae. juvenalis* and *Ae. peregrina* without conferring any observable resistance.

Lr21, first time identified in an Iranian *Ae. tauschii* accession (Huang et al., 2009), is claimed to provide complete resistance at seedling stage and partial resistance at adult stage under field conditions (Naz et al., 2021). However, there are studies identifying *Lr21* as gene carrying APR (Draz et al., 2015; Kumar et al., 2022). Here, *Lr21* was present not only in *Ae. tauschii* genotypes, but also in *Ae. crassa*. These two species had an equal representation of *Lr1*, *Lr21* and *Lr35* genes, but *Ae. crassa* possess two more *Lr* genes, namely *Lr19* and *Lr28*. Still, *Ae. crassa* performed worse in both field and greenhouse tests compared to *Ae. tauschii*. The comparison of *Ae. tauschii* accessions revealed significantly lower resistance at seedling stage in presence of *Lr21*. Although this is in contrast with work of Naz et al., 2021, one of the causes might be high virulence of Czech isolates against *Lr21* with average of 94% between 2002–2019 (Hanzalová et al., 2021).

The marker for *Lr24*, originating from *Thinopyrum ponticum*, was present in only one representative of the species *Ae. speltoides*. Although its presence in this species is surprising it has been previously

recorded (Aliakbari Sadeghabad et al., 2020). The observed high resistance of this genotype in greenhouse and field conditions could also be mediated by the presence of *Lr1*, *Lr28* and *Lr37*, whose individual presence did not show increased resistance.

All accessions of *Ae. crassa*, *Ae. juvenalis*, *Ae. speltoides* and one of *Ae. triuncialis* have been shown to be the residence of the *Lr28* gene. The presence of this gene was also observed outside the genus *Aegilops*, in *Triticum timopheevii* – a tetraploid member of the *Triticeae* tribe (Gulyaeva et al., 2014). Out of 12 genotypes possessing this gene, only two showed the maximal resistance in the field (9) and just one was resistant against all examined isolates, despite the satisfactory results of *Lr28* in Czech wheat cultivars (Hanzalová & Zelba, 2022). Moreover, the presence of *Lr28* in *Ae. triuncialis* accessions was associated with significantly higher infestation level on the field. Two reasons may explain this result. The first, there was a single genotype of *Ae. triuncialis* carrying the *Lr28* rated 4 on the symptomatic scale. The field resistances of the remaining genotypes were mostly the highest possible shifting the results against the presence of *Lr28*. Secondly, *Lr28* is considered a juvenile resistance gene and therefore may not have such an effect on adult plant resistance.

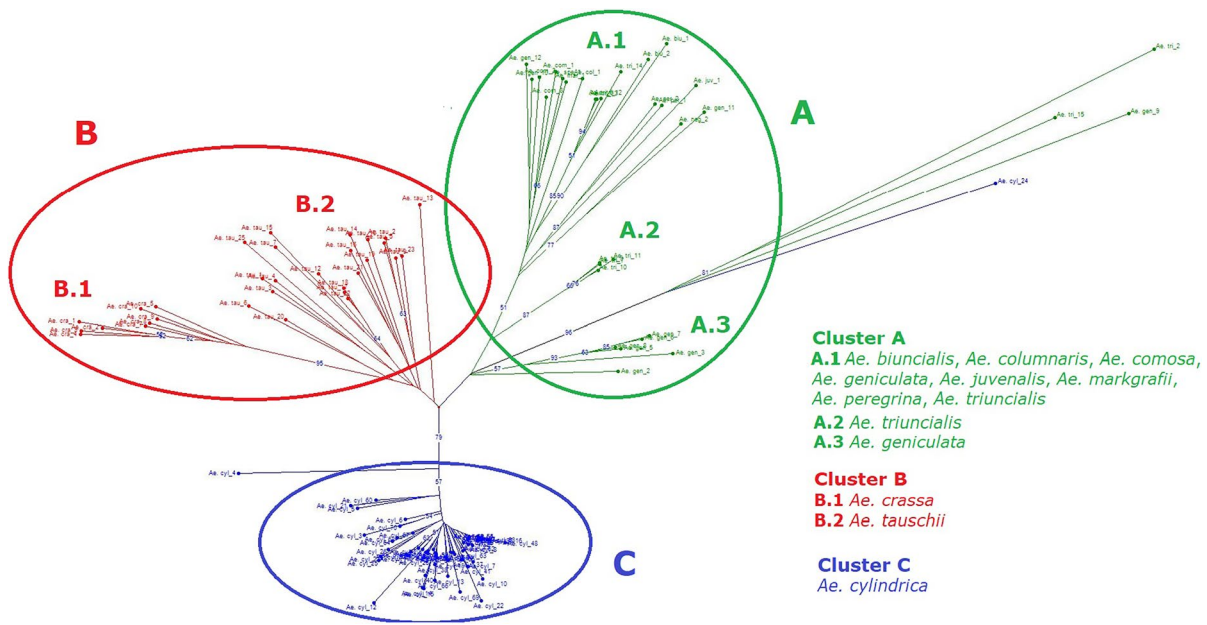


Fig. 2 Phylogenetic tree formed by unweighted neighbor-joining method based on microsatellite analysis

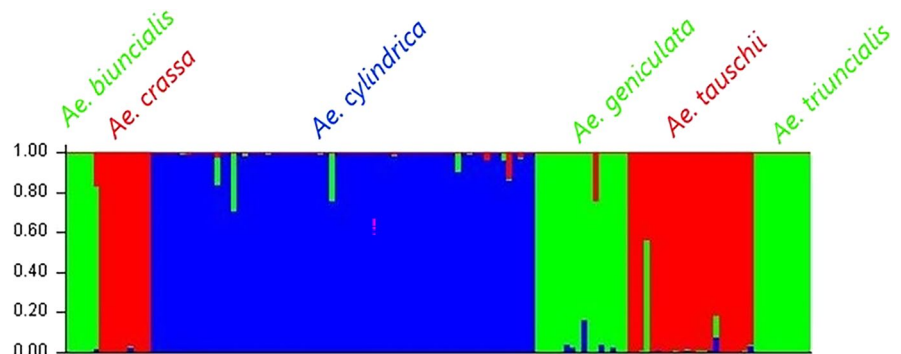
Interestingly, the presence of the gene *Lr35* was not observed in *Ae. speltoides*, the species from which it was derived. Similarly, overall distribution of this gene was low with its presence in 29% of *Ae. crassa* accessions, 14% of *Ae. tauschii* accessions and 4% of *Ae. cylindrica* accessions, all D-genome species.

Lr37, the most abundant *Lr* gene in this study, is also the most represented gene conferring adult plant resistance in Czech wheat cultivars (Hanzalová & Zelba, 2022; Holubec et al., 2014). Although *Lr37* did not show a population-wide effect, within *Ae. cylindrica* was indicated to be a possible factor positively affecting resistance not only of adult plants, but also of seedlings.

Lr58 originates in *Ae. triuncialis* with accessions carrying effective resistance against leaf rust for many years (Arora et al., 2021). Although it is not yet clear whether *Lr58* is more of juvenile resistance or adult plant resistance gene, its performance appeared to be more pronounced in the field. The benefiting genotypes were of *Ae. geniculata*, *Ae. peregrina* and *Ae. triuncialis*. However, the presence of this gene did not translate into increased resistance of species *Ae. juvenalis*.

Based on the results of ANOVA, one *Lr* gene was sufficient to observe a significant difference in the rate of infestation in the field (Fig. 1b). However, each additional *Lr* gene did not result in a statistically

Fig. 3 Bar plot of individual *Aegilops* genotypes, using $K=3$, the dataset of 130 individuals and 38 SSR loci



significant increase in resistance. This trend is mostly prevalent in *Ae. triuncialis* accession, where *Lr58* alone is sufficient to maintain high levels of resistance (Fig. 1a).

The results verified by canonical correlation analysis and subsequent ANOVA showed that the genotypes carrying *Lr9* performed significantly better under greenhouse conditions. This is in agreement with reports declaring efficiency of *Lr9* in Europe (Aktar-Uz-Zaman et al., 2017; Volkova et al., 2022) and against Czech isolates used also in this study (Hanzalová et al., 2021). Similarly, if the genotype carried *Lr58*, it was more likely to be resistant in the field. This was true in all genotypes of *Ae. biuncialis* and *Ae. peregrina* and 75% of *Ae. triuncialis* genotypes with scoring of 9. In *Ae. geniculata* the presence of *Lr58* provided only mean resistance of 4.5.

Genotypes belonging to 13 different species of the genus *Aegilops* were analyzed to assess genetic variability. Cluster analysis revealed the existence of three groups in the examined population. The largest observed variability was in the group A (mainly from the sect. *Aegilops*), which clustered into three smaller groups. Within the group B there are two closely related D-genome species, splitting in two subgroups, each representing a different species. The last group C showed the least variability and contained exclusively genotypes of the species *Ae. cylindrica* (Fig. 2). The results from STRUCTURE confirmed the division of genotypes into three main groups, but the division into subgroups was beyond the distinctiveness of the program (Fig. 3). Abbasov et al. (2019) used microsatellite markers to evaluate the genetic diversity of *Aegilops* genotypes from Azerbaijan and Georgia evaluating *Ae. crassa* and *Ae. vavilovi* as the most diverse species. On the other hand, *Ae. cylindrica* possessed the lowest diversity, which is congruent not only with our results, but also with findings of previous studies (Goryunova et al., 2004; Pester et al., 2003). Contrary to this, Moradkhani et al. (2015) based on microsatellites and Pour-Aboughadareh et al. (2017) based on SCoT markers, determined a relatively higher diversity in *Ae. cylindrica* compared with other species. These discrepancies might be caused by the different sample size and *Ae. cylindrica* germplasm from different countries. However, considering the fact *Ae. cylindrica* is evolutionary younger species (Gandhi et al., 2005) then their

diploid progenitors *Ae. markgrafii* and *Ae. tauschii*, our results could be of more accuracy.

In addition, the application of DARwin-based clustering on *Lr* gene occurrence and symptomatic evaluation revealed that group A consists mainly of resistant genotypes compared to representatives in group B. This is even more pronounced when comparing the group A with group C accessions (Fig. 1c).

In conclusion, we have shown that leaf rust resistance in *Aegilops* is not strictly based on separate resistance genes, but rather the combination of specific genes is needed to ensure higher resistance levels. Our results will not only contribute to knowledge of the genetically based resistance of the *Aegilops* genus, but also indicate how the genetic composition translates into a phenotype measured by symptomatic evaluation, given that our results suggest an effective contribution of *Lr9* on seedling resistance and *Lr58* on resistance of adult plants. Moreover, identified resistant genotypes may serve as suitable source of resistance in further breeding. The fact that some of accessions showed increased resistance without any *Lr* gene present made these accessions worth for further investigation with potential of finding new, yet undiscovered resistance contributors. Although we screened with only nine molecular markers, these were the once with the highest probability of detection due to their origin in wild relatives. However, there are more markers that can reflect on overall resistance and thus a broader set of molecular markers could be used in future studies.

Authors' contributions VH and ME provided the materials. AH performed the greenhouse and VH the field experiments. LK and LS performed the molecular methods. LK and MJ analyzed the data. LK wrote and LS, AH and VH reviewed and edited the manuscript. All authors read and approved the manuscript.

Funding Open access publishing supported by the National Technical Library in Prague. This work received funding from the Ministry of Agriculture of the Czech Republic under the project number MZE-RO0423.

Data Availability All relevant data are available either in manuscript or in the supplementary file1.

Declarations

Research involving human participants and/or animals The authors confirm there was no research on any humans or animals.

Consent to participate All authors have reviewed the manuscript and consented the submission to the European Journal of Plant Pathology.

Competing interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Abbasov, M., Brueggeman, R., Raupp, W., et al. (2019). Genetic diversity of *Aegilops* L. species from Azerbaijan and Georgia using SSR markers. *Genetic Resources and Crop Evolution*, 66, 1–11. <https://doi.org/10.1007/s10722-018-0725-3>
- Aktar-Uz-Zaman, M., Tuhina-Khatun, M., Hanafi, M. M., & Sahebi, M. (2017). Genetic analysis of rust resistance genes in global wheat cultivars: An overview. *Biotechnology and Biotechnological Equipment*, 31(3), 431–445. <https://doi.org/10.1080/13102818.2017.1304180>
- Aliakbari Sadeghabad, A., Dadkhodaie, A., & Heidari, B. (2020). Phenotypic and genetic diversity of leaf rust resistance in wheat wild relatives. *Journal of Phytopathology*, 168, 428–438. <https://doi.org/10.1111/jph.12907>
- Arora, S., Kaur, S., Dhillon, G. S., Singh, R., Kaur, J., Sharma, A., & Chhujena, P. (2021). Introgression and genetic mapping of leaf rust and stripe rust resistance in *Aegilops triuncialis*. *Journal of Genetics*, 100, 6. <https://doi.org/10.1007/s12041-020-01253-3>
- Assefa, S., & Fehrman, H. (2000). Resistance to wheat leaf rust in *Aegilops tauschii* Coss. and inheritance of resistance in hexaploid wheat. *Genetic Resources and Crop Evolution*, 47(2), 135–140.
- Azzimonti, G., Lannou, C., Sache, I., & Goyeau, H. (2013). Components of quantitative resistance to leaf rust in wheat cultivars: Diversity, variability and specificity. *Plant Pathology*, 62, 970–981. <https://doi.org/10.1111/ppa.12029>
- Cherukuri, D. P., Gupta, S. K., Charpe, A., Koul, S., Prabhu, K. V., Singh, R. B., & Haq, Q. (2005). Molecular mapping of *Aegilops speltoides* derived leaf rust resistance gene *Lr28* in wheat. *Euphytica*, 143, 19–26. <https://doi.org/10.1007/s10681-005-1680-6>
- Draz, I. S., Abou-Elseoud, M. S., Kamara, A. E., Alaa-Eldein, O. A., & El-Bebany, A. F. (2015). Screening of wheat genotypes for leaf rust resistance along with grain yield. *Annals of Agricultural Science*, 60(1), 29–39. <https://doi.org/10.1016/j.aos.2015.01.001>
- Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure: A simulation study. *Molecular Ecology*, 14, 2611–2620. <https://doi.org/10.1111/j.1365-294X.2005.02553.x>
- Gandhi, H. T., Vales, M. I., Watson, C. J., Mallory-Smith, C. A., Mori, N., Rehman, M., Zemetra, R. S., & Riera-Lizarazu, O. (2005). Chloroplast and nuclear microsatellite analysis of *Aegilops cylindrica*. *TAG. Theoretical and Applied Genetics*, 111(3), 561–572.
- Goryunova, S. V., Kochieva, E. Z., Chikida, N. N., & Pukhalskiy, V. A. (2004). Phylogenetic relationships and intraspecific variation of D-genome *Aegilops* L. as revealed by RAPD analysis. *Russian Journal of Genetics (Translation of Genetika (Moscow, Russian Federation))*, 40, 515–523.
- Gulyaeva, E. I., Orina, A. S., Gannibal, P. B., Mitrofanova, O. P., Odintsova, I. G., & Laikova, L. I. (2014). The effectiveness of molecular markers for the identification of *Lr28*, *Lr35*, and *Lr47* genes in common wheat. *Russian Journal of Genetics (Translation of Genetika (Moscow, Russian Federation))*, 50, 131–139. <https://doi.org/10.1134/S1022795414020069>
- Gupta, S., Charpe, A., Koul, S., Haq, Q., & Prabhu, K. (2006). Development and validation of SCAR markers co-segregating with an *Agropyron elongatum* derived leaf rust resistance gene *Lr24* in wheat. *Euphytica*, 150, 233–240. <https://doi.org/10.1007/s10681-006-9113-8>
- Hanzalová, A., Dumaslová, V., & Zelba, O. (2021). Virulence in the *Puccinia triticina* population in the Czech Republic and resistance genes in registered cultivars 1966–2019. *Euphytica*, 217, 4. <https://doi.org/10.1007/s10681-020-02733-4>
- Hanzalová, A., & Zelba, O. (2022). Leaf rust (*Puccinia triticina* Eriks) resistance genes in wheat cultivars registered in the Czech Republic. *J Plant Dis Protect*. <https://doi.org/10.1007/s41348-022-00625-4>
- Helguera, M., Khan, I. A., Kolmer, J. A., Lijavetzky, D., Zhong-qi, L., & Dubcovsky, J. (2003). PCR assays for the *Lr37-Yr17-Sr38* cluster of rust resistance genes and their use to develop isogenic hard red spring wheat lines. *Crop Science*, 43, 1839–1847.
- Holubec, V., Hanzalová, A., Dumaslová, V., & Bartoš, P. (2014). *Aegilops* conservation and collection evaluation in the Czech Republic. *Journal of Systematics and Evolution*, 52, 783–789. <https://doi.org/10.1111/jse.12117>
- Holubec, V., Leišová-Svobodová, L., & Matějovič, M. (2019). Spontaneous hybridisation within *Aegilops* collection and biobanking of crop wild relatives (CWR). *Genetic Resources and Crop Evolution*, 66, 311–319. <https://doi.org/10.1007/s10722-018-0723-5>
- Huang, L., Brooks, S., Li, W., Fellers, J., Nelson, J. C., & Gill, B. (2009). Evolution of new disease specificity at a simple resistance locus in a crop–weed complex: Reconstitution of the *Lr21* gene in wheat. *Genetics*, 182(2), 595–602. <https://doi.org/10.1534/genetics.108.099614>

- Huang, L., & Gill, B. S. (2001). An RGA-like marker detects all known *Lr21* leaf rust resistance gene family members in *Aegilops tauschii* and wheat. *TAG. Theoretical and Applied Genetics*, 103(6–7), 1007–1013.
- Huerta-Espino, J., Singh, R., Germán, S., McAllum, B. D., Park, R. F., Chen, W. Q., Bhardwaj, S. C., & Goyeau, H. (2011). Global status of wheat leaf rust caused by *Puccinia triticina*. *Euphytica*, 179, 143–160. <https://doi.org/10.1007/s10681-011-0361-x>
- Kolmer, J. A. (2019). Virulence of *Puccinia triticina*, the wheat leaf rust fungus, in the United States in 2017. *Plant Disease*, 103(8), 2113–2120. <https://doi.org/10.1094/PDIS-09-18-1638-SR>
- Kolmer, J. A., Hanzalová, A., Goyeau, H., Bayles, R., & Morgounov, A. (2013). Genetic differentiation of the wheat leaf rust fungus *Puccinia triticina* in Europe. *Plant Pathology*, 62(1), 21–31. <https://doi.org/10.1111/j.1365-3059.2012.02626.x>
- Krupinsky, J. M., Bailey, K. L., McMullen, M. P., Gossen, B. D., & Turkington, T. K. (2002). Managing plant disease risk in diversified cropping systems. *Journal of Agronomy*, 94, 198–209. <https://doi.org/10.2134/agronj2002.1980>
- Kumar, K., Jan, I., Saripalli, G., Sharma, P., Mir, R., Balyan, H., & Gupta, P. (2022). An update on resistance genes and their use in the development of leaf rust resistant cultivars in wheat. *Frontiers in Genetics*, 13, 816057. <https://doi.org/10.3389/fgene.2022.816057>
- Kuraparthi, V., Sood, S., Guedira, G., & Gill, B. (2011). Development of a PCR assay and marker-assisted transfer of leaf rust resistance gene *Lr58* into adapted winter wheats. *Euphytica*, 180, 227–234. <https://doi.org/10.1007/s10681-011-0383-4>
- Ling, H. Q., Qiu, J. W., Singh, R. P., & Keller, B. (2004). Identification and genetic characterization of an *Aegilops tauschii* ortholog of the wheat leaf rust disease resistance gene *Lr1*. *Theoretical and Applied Genetics*, 109, 1133–1138. <https://doi.org/10.1007/s00122-004-1734-5>
- Mago, R., Zhang, P., Bariana, H., Verlin, D., Bansal, U., Ellis, J., & Dundas, I. (2009). Development of wheat lines carrying stem rust resistance gene *Sr39* with reduced *Aegilops speltoides* chromatin and simple PCR markers for marker-assisted selection. *Theoretical and Applied Genetics*, 119, 1441–1450. <https://doi.org/10.1007/s00122-009-1146-7>
- McCallum, B. D., Reimer, E., McNabb, W., Foster, A., Rosa, S., & Xue, A. (2021). Physiologic specialization of *Puccinia triticina*, the causal agent of wheat leaf rust, in Canada in 2015–2019. *Canadian Journal of Plant Pathology*, 43, 333–346. <https://doi.org/10.1080/07060661.2021.1888156>
- McIntosh, R. A., Wellings, C. R., & Park, R. F. (1995). *Wheat rusts: An atlas of resistance genes*. CSIRO Publications.
- Moradkhani, H., Mehrabi, A. A., Etminan, A., & Pour-Aboughadareh, A. (2015). Molecular diversity and phylogeny of *Triticum-Aegilops* species possessing D genome revealed by SSR and ISSR markers. *Plant Breeding and Seed Science*, 71(1), 81–95.
- Murray, M. G., & Thompson, W. F. (1980). Rapid isolation of high molecular weight plant. *Nucleic Acids Research*, 8, 4321–4325.
- Naz, A. A., Bungartz, A., Serfling, A., Kamruzzaman, M., Schneider, M., Wulff, B. B. H., Pillen, K., Ballvora, A., Oerke, E. C., Ordon, F., & Léon, J. (2021). *Lr21* diversity unveils footprints of wheat evolution and its new role in broad-spectrum leaf rust resistance. *Plant, Cell and Environment*, 44(10), 3445–3458. <https://doi.org/10.1111/pce.14144>
- Nelson, R., Wiesner-Hanks, T., Wisser, R., & Balint-Kurti, P. (2018). Navigating complexity to breed disease-resistant crops. *Nature Reviews Genetics*, 19(1), 21–33. <https://doi.org/10.1038/nrg.2017.82>
- Oerke, E. (2006). Crop losses to pests. *Journal of Agricultural Science*, 144(1), 31–43. <https://doi.org/10.1017/S0021859605005708>
- Park, R. F., Bariana, H. S., Wellings, C. R., & Wallwork, H. (2002). Detection and occurrence of a new pathotype of *Puccinia triticina* with virulence for *Lr24* in Australia. *Australian Journal of Agricultural Research*, 53, 1069–1076.
- Perrier, X., & Jacquemoud-Collet, J. P. (2006). DARwin Software. <http://darwin.cirad.fr/darwin>.
- Pester, T. A., Ward, S. M., Fenwick, A. L., Westra, P., & Nissen, S. J. (2003). Genetic diversity of jointed goatgrass (*Aegilops cylindrica*) determined with RAPD and AFLP markers. *Weed Science*, 51, 287–293.
- Pour-Aboughadareh, A., Ahmadi, J., Mehrabi, A. A., Etminan, A., & Moghaddam, M. (2017). Insight into the genetic variability analysis and relationships among some *Aegilops* and *Triticum* species, as genome progenitors of bread wheat, using SCoT markers. *Plant Biosyst*, 152(4), 694–703.
- Prasad, P., Savadi, S., Bhardwaj, S. C., & Gupta, P. K. (2020). The progress of leaf rust research in wheat. *Fungal Biology*, 124(6), 537–550. <https://doi.org/10.1016/j.funbio.2020.02.013>
- Prins, R., Groenewald, J., Marais, G., Sanpe, J., & Koebner, R. (2001). AFLP and STS tagging of *Lr19*, a gene conferring resistance to leaf rust in wheat. *Theoretical and Applied Genetics*, 103, 618–624. <https://doi.org/10.1017/S002185960500570810.1007/PL00002918>
- Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155(2), 945–959. <https://doi.org/10.1093/genetics/155.2.945>
- Qiu, J. W., Schürch, A. C., Yahiaoui, N., Dong, L. L., Fan, H. J., Zhang, Z. J., Keller, B., & Ling, H. Q. (2007). Physical mapping and identification of a candidate for the leaf rust resistance gene *Lr1* of wheat. *TAG. Theoretical and Applied Genetics*, 115(2), 159–168. <https://doi.org/10.1007/s00122-007-0551-z>
- Röder, M. S., Korzun, V., Wendehake, K., Plaschke, J., Tixier, M. H., Leroy, P., & Ganal, M. W. (1998). A microsatellite map of wheat. *Genetics*, 149(4), 2007–2023. <https://doi.org/10.1093/genetics/149.4.2007>
- Schachermayr, G., Siedler, H., Gale, M. D., Winzeler, H., Winzeler, M., & Keller, B. (1994). Identification and localization of molecular markers linked to the *Lr9* leaf rust resistance gene of wheat. *TAG. Theoretical and Applied Genetics*, 88(1), 110–115. <https://doi.org/10.1007/BF00222402>

- Sears, E. R. (1956). The transfer of leaf rust resistance from *Aegilops umbellulata* into wheat. Brookhaven Symposia in Biology: 1–22.
- Sears, E. R. (1961). Identification of the wheat chromosome carrying leaf rust resistance from *Aegilops umbellulata*. *Wheat Information Service*, 12, 12–13.
- Stakman, E. C., Stewart, D. M., & Loegering, W. O. (1962). Identification of physiologic races of *Puccinia graminis* var. *tritici*. US department of agriculture Agricultural Research Service E-617, Washington DC.
- Volkova, G., Kudinova, O., Vaganova, O., & Agapova, V. (2022). Effectiveness of leaf rust resistance genes in the adult and juvenile stages in southern russia in 2011–2020. *Plants*, 11, 793. <https://doi.org/10.3390/plants11060793>