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An analysis of the functionality of molecular markers related to the *Lr19* gene conditioning resistance to wheat leaf rust

Angelika KIEL, Dorota WEIGT, Martyna KARPIŃSKA, Danuta KURASIAK-POPOWSKA, Janetta NIEMANN, Agnieszka TOMKOWIAK, Sylwia MIKOŁAJCZYK, Jerzy NAWRACAŁA

Poznan University of Life Sciences, Department of Genetics and Plant Breeding
Dojazd 11, 60-637 Poznan, Poland
E-mail: angelina.kiel@up.poznan.pl

Abstract

Among all cereals, common wheat (*Triticum aestivum* L.) occupies the largest area of crops worldwide. Wheat leaf rust, caused by the pathogen *Puccinia recondita* f. sp. *tritici* considerably reduces the yield of wheat. Breeding for resistance combined with selection based on molecular markers may become an effective tool in the struggle against fungal diseases. *Lr19* is the gene that carries high resistance to wheat leaf rust. In recent years, we have seen the development of many molecular markers in close neighbourhood of the *Lr19* gene, e.g., SCS265, SCS253, GB, Xwmc221, XustSSR2001-7DL, Xgwm37 and Xgwm44. The aim of the study was to investigate the functionality of molecular markers related to the *Lr19* gene. The study was based on two reference genotypes with the *Lr19* gene ('Agatha' and Lr19) and three lines strongly infested by wheat leaf rust. Seven molecular markers were analysed, but only two of them (GB and Xwmc221) proved to be specific to the gene under study. These markers were used for analysis of 25 wheat genotypes, which were evaluated for leaf rust resistance in field conditions to confirm their usefulness for selection of breeding material.

These findings point to the need of continuous search for functional molecular markers giving repeatable and reliable results.

Key words: common wheat, *Lr19*, molecular markers, *Puccinia recondita* f. sp. *tritici*.

Introduction

Wheat leaf rust, which is caused by *Puccinia recondita* f. sp. *tritici*, is the most common fungal disease occurring in all regions where winter and spring wheat is cultivated (Vanzetti et al., 2011; Abdelbacki et al., 2013). Every year wheat leaf rust damages more than 10% of crops (Prabhu et al., 2004; Urbanovich et al., 2006), but severe infestation may affect as much as 40% of crops (Kassem et al., 2011). In Poland on average 9.1% of crops is lost to wheat leaf rust annually (Trawal, Walczak, 2012). These facts result in enormous economic significance of the disease. In recent years, there has been increasing focus on breeding for resistance. Improving resistance to fungal diseases, including wheat leaf rust, is one of the essential trends in cultivation (Matysik, Nita, 2008; Okoń et al., 2012).

In the last decade, the number of wheat leaf rust resistance genes has increased considerably. By 2013, 71 genes had been identified (Singh et al., 2013). The most effective genes are *Lr19*, *Lr24*, *Lr26*, *Lr28*, *Lr34*, *Lr42*, *Lr46* and *Lr67* (Gorash et al., 2014; Elangbam et al., 2018). The *Lr19* is considered the most effective resistance gene, carrying immunity against most races of the pathogen in many regions of the world (Sehgal et al., 2012). The gene was transferred into common wheat by translocation with *Thinopyrum ponticum* (syn. *Agropyron elongatum*) to the distal part of the long arm of chromosome 7D (Uhrin et al., 2008).

Marker-assisted selection (MAS) can be used at early stages of cultivation. It also enables genotype-based selection of plants, which is preferable to the laborious and time-consuming phenotype-based assessment (Święcicki et al., 2011). It considerably reduces the duration and costs of cultivation (Heffner et al., 2010). Due to the considerable number of molecular markers in close neighbourhood of the *Lr19* gene, it is necessary to verify their usefulness for early selection. A functional marker should give highly repeatable results of molecular analyses in different gene pools.

The aim of the study was to verify the functionality of molecular markers related to the *Lr19* gene conditioning resistance to wheat leaf rust.

Materials and methods

The *Lr19* gene-specific molecular markers were identified by means of reference genotypes with the *Lr19* gene: 'Agatha' (Agrus/6 × Thatcher) and Lr19 (Thatcher × 6/*Agropyron elongatum*) obtained from the National Small Grain Collection, United States Department of Agriculture, Agricultural Research

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Service (NSGC USDA-ARS) in Aberdeen, Idaho, USA. Three lines: STHT 001, STHA 003 and STHS 002, which were severely infested by wheat leaf rust, were used as negative reference samples. They came from the collection of the Department of Genetics and Plant Breeding, Poznań University of Life Sciences, Poland. Selected markers were used for the analysis of 25 wheat genotypes obtained from NSGC USDA-ARS (Table 1).

The leaf rust resistance of the genotypes above were examined in three (2015–2017) years in the field under natural infection conditions. The field experiment was established at the Dłoń Agricultural Research Station (lat. 51°41′ N, long. 17°3′ E), the Poznan University of Life Sciences, Poland in the years between 2015 and 2017. The 25 genotypes selected were sown in plots of 1 m^2 (1 × 1 m) laid out in a randomized block design in three

Table 1. Wheat cultivars tested for leaf rust resistance in field experiments

Genotype	Plant ID	Origin	Pedigree	*Lr* gene
Agatha	CItr 14048	Canada	Agrus/6*Thatcher	*Lr19**
Aquila	PI 447040	England	Tadorna/Caribou	*Lr13*, *Lr23****
Agrus	CItr 13228	USA	Trumbull/Agropyron elongatum/4/Fultz sel./3/Trumbull//Hope/Hussar	*Lr19***
Century	PI 502912	USA	Payne//TAM W-101/Amigo	*Lr10*, *Lr24****
Express	PI 573003	USA	Veery/BH1146	
Fielder	Cltr 17268	USA	Yaktana-54-A*4//Norin-10/Brevor/3/2*YAQUI-50/4/Norin-10/ Brevor//Baart/Onas	
Freedom	PI 562382	USA	GR876/OH217 = GR876/4/Logan*3/3/VA63-52-12/Logan/Blueboy	*Lr26****
Grandin	PI 531005	USA	Len//Butte*2/ND507/3/ND593	*Lr2a*, *Lr3a*, *Lr10*, *Lr13*, *Lr16*, *Lr34****
Greer	CItr 17725	USA	WA 4765//Burt/PI 178383	
Hyak	PI 511674	USA	VPM 1/Moisson 421//2*Tyee	*Lr37****
Clasic	PI 486139	USA	Klein-Rendidor/2*Sonora-64//INIA-66/3/Ciano-67/4/Yecora-70	*Lr1*, *Lr10*
KS89WGRC7	PI 535770	USA	Wichita//TA 1649 (Aegilops squarrosa)/2*Wichita	*Lr40*, *Lr21****
Lee	CItr 12488	USA	Hope//2*Bobin/Gaza	*Lr10*, *Lr23****
Lemhi	CItr 11415	USA	Federation/Dicklow	
Lr19	GSTR 420	Canada	Thatcher*6/Agropyron elongatum	*Lr19**
Lr64	GSTR 445	Canada	Thatcher*6/Triticum dicoccoides (8404)	*Lr64**
NC8860-5	PI 664252	USA	NC96BGTA4 (PI 599034) / NC97BGTD7 (PI 604033) // NC96B-GTD3 (PI 597350) / NC96BGTA5 (PI 599035)	
Ok75Abd-380	CItr 17470	USA	Little Club/Agrus	*Lr19**
R04-156	GSTR 13847	USA	Coda/Brundage	
R04-268	GSTR 13962	USA	Coda/Brundage	
Thatcher	CItr 10003	USA	Marquis/Iumillo//Marquis/Kanred	*Lr22b***
Wawawai	PI 574538	USA	ID0046/7/ID0045/6/2*A6596S-A-21-1/5/2*A6535S-443-107/4/ A6316 7S-A-1-59-2-2/3/Thew/Federation//A63166S-A-2-8/8/Potam 70/Fielder/5/Tifton3725/Walladay/3/Fielder//Brons/Koelz7941S70-5/4/Lemhi66/3/Yaktana54A*4//Norin10/Brevor/4/IDO065/Potam 70	
XW591	PI 550697	USA	(Vahart/Frondoso/5/Vahart/4/(KY4097-37, CItr12658, Frondoso/3/ Trumbull//Hope/Hussar)/6/Asosan/7/Norin 10/Brevor/8/(VA55-16-23, CItr13351, Supreza/Fultz/5/Kawvale/4/Fultz/Hungarian//Illinois No. 1/Wabash/3/TrumBull*3//Hope/Hussar), VA66-54-10)/9/Arthur, IL71-5662)/10/(W9018A, Pioneer Line W521/Pioneer S76)/11/ (W689D-2, Coker 68-15/5/(MO7510, Etoile de Choisy//Thorne/ Clarkan/4/Pawnee/3/(Pd3848A5-5-1-26, CItr12454, Trumbull/W38// Fultz/Hungarian))	
XW571	PI 532913	USA	Hadden*2/3/GA1123//Norin 10/Brevor/Tenmarq/4/MOW6582/Red-coat/5/Coker 68-15/4/Etoile de Choisy//Thorne/Clarkan/3/ Pawnee/ Pur3848A5 Sel	
2737W	PI 561197	USA	Frankenmuth/2550//Pioneer line W9018A/Houser	

* – according to Plant Genetic Resources Documentation in the Czech Republic (http://genbank.vurv.cz/genetic/resources), ** – according to U.S. National Plant Germplasm System (https://npgsweb.ars-grin.gov/gringlobal/search.aspx), *** – according to Genetic Resources Information System for Wheat and Triticale (http://www.wheatpedigree.net)

replications. The infection rates of genotypes by *Puccinia recondita* f. sp. *tritici* were assessed on a scale from 1 to 9. The scoring standards were as follows: 1 – immune (no visible uredia), 2 – very resistant (hypersensitive flecks), 3 – resistant (small uredia with necrosis), 4 – resistant to moderately resistant (small to medium sized uredia surrounded by necrosis or chlorosis), 5 – moderately resistant (medium sized uredia with chlorosis), 6 – moderately resistant to moderately susceptible (medium sized uredia with or without chlorosis), 7 – moderately susceptible (medium to large sized uredia without clear chlorosis), 8 – susceptible (large sized uredia without chlorosis), 9 – very susceptible (large and very abundant uredia without chlorosis) (McIntosh et al., 1995). Assessments were conducted at the growth stages from the onset of flowering to full anthesis (BBCH 61–65) of common wheat (*Triticum aestivum* L.).

Rainfall and temperature in the seasons of 2015–2017 in Dłoń Agricultural Research Station, Poland are presented in Table 2.

Table 2. Means for rainfall and temperature in the seasons of 2015–2017 in Dłoń Agricultural Research Station, Poland and their percentage relationships to the means for the last four decades

Month	2015	2016	2017
	Rainfall		
April	26 (81%)	54.5 (170%)	56 (175%)
May	30 (54%)	57.5 (103%)	43 (78%)
June	33 (50%)	72.5 (109%)	58.5 (88%)
July	53 (64.5%)	128.1 (156%)	108.5 (132%)
Total	142	312.7	266
	Temperature		
April	8.3 (101%)	8.8 (107%)	8.1 (98%)
May	14.8 (109.3%)	16.3 (120%)	14.9 (110%)
June	17.7 (105%)	21 (125%)	19 (113%)
July	21.7 (118%)	19.5 (106%)	19.5 (106%)
Mean	15.6	16.4	15.4

The column method was used to isolate the DNA from young seedlings by means of a kit *Genomic Mini AX PLANT* (A&A Biotechnology) according to the manufacturer's methodology. Protocol of DNA purification is available on the website: http://www.aabiot.home.pl/aabiop/PDF/Genomic%20Mini%20AX%20Plant_EN.pdf. Samples were diluted with distilled water to a concentration of 25 ng μl^{-1}. Data provided in the literature were used to select molecular markers for the experiment (Prins et al., 2001; Groenewald et al., 2003; Gupta et al., 2006; Li et al., 2006). Two sequences characterized amplified region (SCAR) markers, one sequence tagged site (STS) marker and four microsatellite (SSR) markers were selected for analyses. Table 3 shows the primer sequences for individual molecular markers. A polymerase chain reaction (PCR) was carried out in a mixture with a total volume of 12.5 µl. The mixture used in the PCR was composed of water – 5 µl, DreamTaq™Green PCR Master Mix – 6.25 µl, primers – 2 × 0.25 µl, DNA matrix – 1 µl. The PCR was repeated at least ten times for each marker to check the repeatability of results. If the amplification did not give a desirable product, there were attempts to optimise the conditions of the reaction by using a temperature gradient and changing the duration of individual phases of the reaction.

Table 3. Sequences of primers used for the identification of molecular markers specific to the *Lr19* gene

Marker	Marker type	Sequence of primer 5→3'	Reference
SCS253	SCAR	GCTGGTTCCACAAAGCAAA GGCTGGTTCCTTAGATAGGTG	Gupta et al., 2006
SCS265	SCAR	GGCGGATAAGCAGAGCAGAG GGCGGATAAGTGGGTTATGG	Gupta et al., 2006
GB	STS	CATCCTTGGGGACCTC CCAGCTCGCATACATCCA	Prins et al., 2001
Xwmc221	SSR	ACGATAATGCAGCGGGGAAT GCTGGGATCAAGGGATCAAT	Gupta et al., 2006
XustSSR2001-7DL	SSR	CATCGTGTGGCCAACTTGTT TTCCTCGTGTCTAGTGTCTC	Groenewald et al., 2003
Xgwm37	SSR	ACTTCATTGTTGATCTTGCATG CGACGAATTCCCAGCTAAAC	Li et al., 2006
Xgwm44	SSR	ACTGGCATCCACTGAGCTG GTT- GAGCTTTTCAGTTCGGC	Li et al., 2006

SCAR – sequences characterized amplified region, STS – sequence tagged site, SSR – simple sequence repeats

Table 4 shows the PCR conditions selected for each marker according to reports in the literature and our own modifications. The PCR products were separated in 2.5% agarose gel with ethidium bromide added at 1 µl per 100 ml of gel.

Table 4. The polymerase chain reaction (PCR) conditions used for the identification of molecular markers specific to the *Lr19* gene

Marker	Cycle conditions
SCS253	94°C – 5 min, 35 cycles (94°C – 1 min, 63°C – 1 min, 72°C – 1 min); 72°C – 7 min
SCS265	94°C – 5 min, 35 cycles (94°C – 1 min, 65°C – 1 min, 72°C – 1 min); 72°C – 7 min
GB	94°C – 4 min, 35 cycles (94°C – 30 s, 60°C – 30 s, 72°C – 30 s); 72°C – 5 min
Xwmc221	94°C – 3 min, 35 cycles (94°C – 30 s, 55°C – 30 s, 72°C – 30 s); 72°C – 5 min
XustSSR2001-7DL	94°C – 3 min, 30 cycles (94°C – 30 s, 55°C – 30 s, 72°C – 30 s); 72°C – 5 min
Xgwm37	94°C – 4 min 30 s, 48 cycles (94°C – 1 min, 50°C – 1 min, 72°C – 2 min); 72°C – 7 min
Xgwm44	94°C – 4 min, 35 cycles (94°C – 30 s, 55°C – 30 s, 72°C – 30 s); 72°C – 5 min

Results and discussion

The identification of molecular markers coupled with resistance genes facilitates breeding. As a result, it is possible to breed new cultivars and pyramid genes in individual genotypes (Pietrusińska, 2010). Due to the considerable number of molecular markers located close to the *Lr19* gene, it is important to verify their functionality for this gene. The functionality of 7 molecular markers for the *Lr19* gene was investigated in this study (Fig. 1).

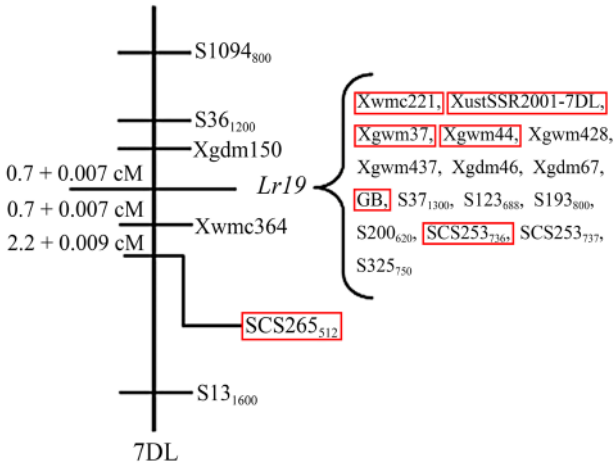


Figure 1. A genetic map of chromosome 7DL of wheat

Gupta et al. (2006) converted molecular markers SCS265 and SCS253 from random amplified polymorphic DNA (RAPD) markers. The authors successfully identified both markers on a mapping population, used them to identify 44 near isogenic lines and found that the markers were specific to the *Lr19* gene. Uhrin et al. (2008) confirmed validation of the SCS265 marker, which they used to investigate 21 common wheat cultivars. They used near isogenic line TcLr19 as a positive reference sample. Four cultivars were used as negative reference samples (three cultivars were resistant to wheat leaf rust, but did not have the *Lr19* gene and one cultivar was severely infested by the pathogen). The SCS253 marker was successfully used by Okoń et al. (2012), who identified the *Lr19* gene in Polish breeding lines. They observed an amplicon of 736 bp, which proved the lack of resistance in 213 samples. The lack of an amplification product, which proved the presence of the *Lr19* gene, was noted in 33 lines. Seghal et al. (2012) analysed 20 Pakistani common wheat cultivars and found that the presence of a product with 736 bp proved the occurrence of the *Lr19* gene.

In this study, the attempt to identify the *Lr19* gene with two SCAR (SCS265 and SCS253) markers did not confirm their specificity to this gene. As far as the SCS265 marker is concerned, an amplicon of 512 bp was observed in reference genotypes with the *Lr19* gene ('Agatha' and Lr19), as can be seen in Figure 2A. However, the analyses were not repeatable. Apart from that, the product also appeared in the line (STHT 001) susceptible to wheat leaf rust. The analysis with a pair of SCS253 primers revealed an amplification product with 736 bp only in one of three lines without the *Lr19* gene (Fig. 2B).

Gupta et al. (2006) related the XustSSR2001-7DL marker to the *Lr19* gene. They analysed about 30 microsatellite markers and found that 9 of them were related to the *Lr19* gene, whereas the XustSSR2001-7DL marker (along with the Xwmc221, Xgwm37, Xgwm428, Xgwm437, Xgdm46 and Xgdm67 markers) co-segregated with the locus of the *Lr19* gene. As far as the XustSSR2001-7DL marker is concerned, the presence of the *Lr19* gene was proved by the occurrence of an amplification product with 300 bp. When the gene was absent, there was an amplification product with 310 bp. In this study, the analysis with a pair of primers of the XustSSR2001-7DL marker did not confirm its specificity to the *Lr19* gene. There was no amplification product with 300 bp in any of the reference genotypes, which would indicate resistance to wheat leaf rust (Fig. 2C). On the other hand, in the susceptible lines there was no product with 310 bp.

Pillard et al. (2003) proved that the Xgwm37 marker was coupled with the *Lr19* gene in a genetic map of wheat. Prabhu et al. (2009) identified this marker at a distance of 0.7 cM from the *Lr19* gene. Gupta et al. (2006) identified the *Lr19* gene in common wheat forms made by crossbreeding the near isogenic line 'Thatcher' including the *Lr19* gene with the susceptible cultivar 'Agra Local'. The analysis with the Xgwm37 marker proved the presence of a product with 130 bp, which indicated the absence of the *Lr19* gene from the susceptible genotypes. Haque et al. (2014) analysed 20 common wheat genotypes with a pair of Xgwm37 primers and they related the presence of the *Lr19* gene with the amplification of a product with 189 bp. This study did not reveal the presence of a product with 130 bp, which indicated the absence of the *Lr19* gene both among the genotypes with and without the *Lr19* gene.

Gupta et al. (2002) genetically mapped 66 microsatellite loci in wheat and reported that the Xgwm44 marker was located near the *Lr19* gene. Pillard et al. (2003) provided similar information when they made an integrated genetic map of wheat. Li et al. (2006) found that the Xgwm44 microsatellite marker was specific to the *Lr19* gene. They made a molecular analysis of near isogenic lines (TcLr13, TcLr19, TcLr21, TcLr37, TcLr38 and TcLr44) with the Xgwm44 marker and observed an amplification product with 139 bp only in the line with the *Lr19* gene. They confirmed the same result in a study of 120 plants from generation F_2, which were obtained by crossbreeding the wheat leaf rust-resistant near isogenic line TcLr19 with the susceptible cultivar 'Thatcher'. The marker was present in all 84 resistant plants and in one of 36 susceptible plants. Our experiment did not prove the specificity of the Xgwm44 marker. An amplicon with 139 bp was observed both in the genotypes with the *Lr19* gene and in some genotypes susceptible to wheat leaf rust, but the results were not repeatable (Fig. 2D).

Prins et al. (2001) converted the GB (STS) marker from the amplified fragment length polymorphism (AFLP) marker. They found that it was related to the *Lr19* gene – its presence was proved by an amplification product with 130 bp. Numerous data in literature prove that it is possible to identify the *Lr19* gene using the GB marker. Stępień et al. (2003) analysed 37 European common wheat cultivars and 15 breeding lines. They proved the presence of the *Lr19* gene in three genotypes. Abou-Elseoud et al. (2014) reported the presence of the gene in two out of seven Egyptian common wheat cultivars under analysis. This study confirmed the functionality of the GB marker for the *Lr19* gene. Amplification products with 130 bp were identified in both reference genotypes, which proved the presence of the *Lr19* gene (Fig. 2E).

Gupta et al. (2006) found that the Xwmc221 microsatellite marker was useful for selection of wheat genotypes resistant to wheat leaf rust. They analysed 20 plants (10 resistant and 10 susceptible plants) from generation F_2, which resulted from the crossbreeding of the isogenic line 'Thatcher' (Tc + Lr19) with genotype 'Agra Local'. They obtained an amplification product with 200 bp, which proved the presence of the *Lr19* gene in all the

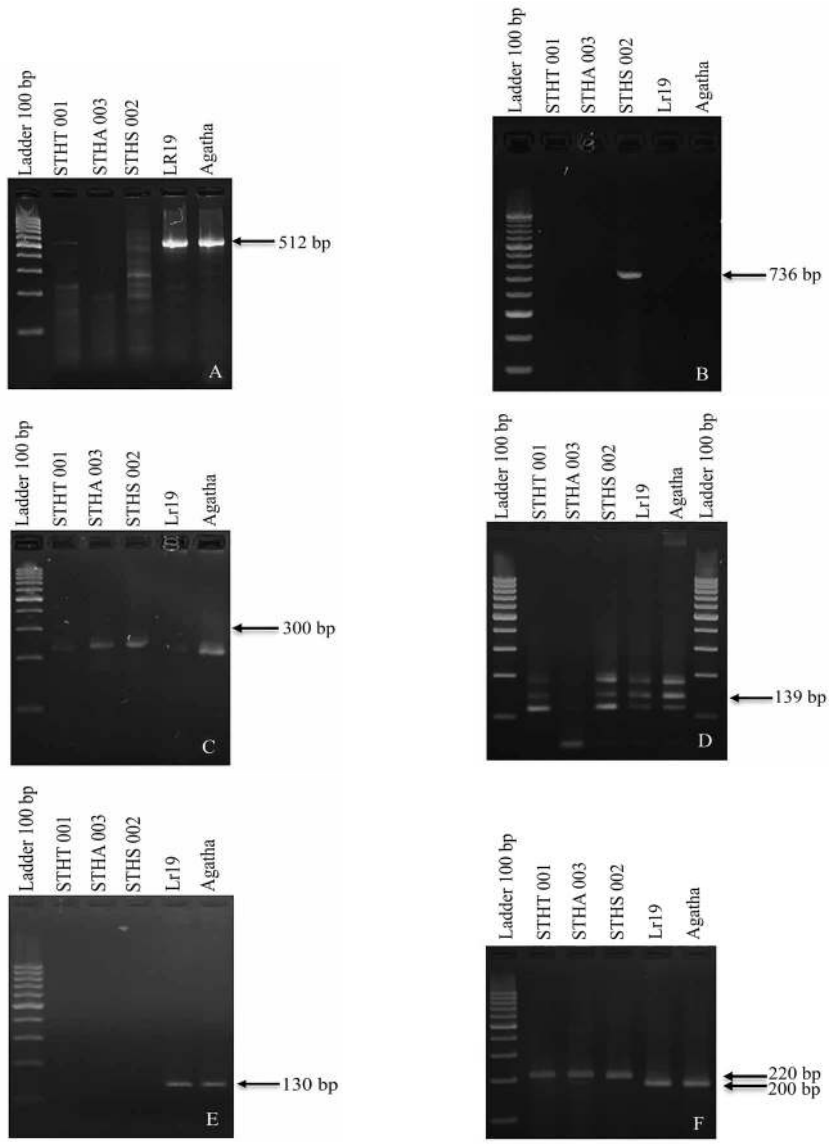


Figure 2. Electrophoretic separation of polymerase chain reaction (PCR) amplification products using the SCS265 (A), SCS253 (B), XustSSR2001-7DL (C), Xgwm44 (D), GB (E) and Xwmc221 (F) molecular markers

resistant genotypes. They also obtained a product with 220 bp in the susceptible genotypes. Tomkowiak et al. (2016) also used the Xwmc221 marker to analyse foreign winter wheat cultivars. They identified the *Lr19* gene in 2 out of 47 cultivars under analysis. This experiment confirmed the functionality of the Xwmc221 marker for the *Lr19* gene. The analysis revealed that the marker amplified two products: one with 200 bp for reference genotypes (with the *Lr19* gene) and one with 220 bp for the wheat leaf rust negative reference sample (Fig. 2F).

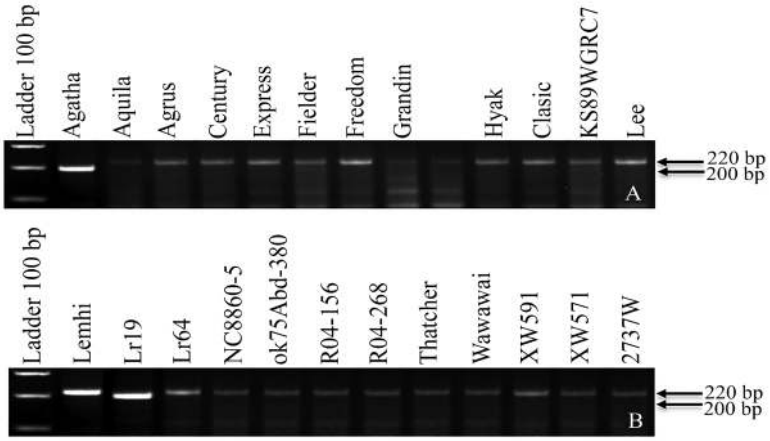
Among seven molecular markers under analysis only two gave repeatable results: the GB marker, which was dominant, and the Xwmc221 microsatellite marker, which was a codominant marker. The SCS265, SCS253, Xgwm37, Xgwm44 and XustSSR2001-7DL markers did not clearly identify PCR products characteristic of the *Lr19* gene.

Xwmc221 and GB markers were used in selection of 25 wheat genotypes evaluated for leaf rust resistance in the field in natural infection conditions. A product of 200 bp indicating the presence of the *Lr19* gene was identified only in two ('Agatha' and *Lr19*) of the 25 genotypes examined using Xwmc221 marker (Fig. 3A-B).

In the remaining genotypes 220 bp band was found suggesting the lack of *Lr19* gene. These findings were confirmed in the analysis with the GB marker. A 130 bp product was observed only in genotypes 'Agatha' and *Lr19* (Fig. 4A-B). The genotypes 'Agrus' and Ok75Abd-380, which should have *Lr19* gene as described in U.S. National Plant Germplasm System and Czech Information System on Plant Genetic Resources, did not reveal the product of amplification characteristic of this gene in any of the tested markers (Figs 3 and 4).

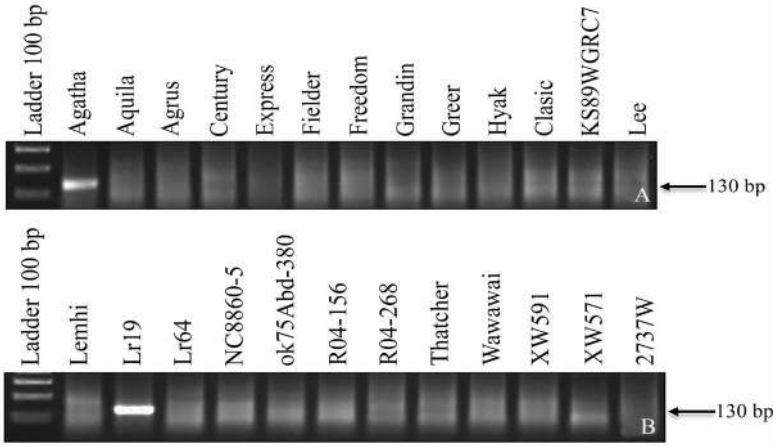
The field experiment scores from a 3-year evaluation of cultivars, in which the presence of the gene was confirmed, were on average 3.7 ('Agatha') and 5.0 (GSTR 420). These two cultivars indicated "resistant to moderate" and "resistant" infection rates on 9-step scale (Table 5).

Such results were probably caused by unfavourable weather conditions in the second year of assessment when scores of these genotypes were 6.0 and 7.7, respectively. In 2016, higher rainfall and higher temperature than the means for the last four decades occurred (Table 2). Such conditions promoted the intensive development of fungal diseases. These findings suggest that *Lr19* resistance gene is not effective during every year in Central Europe and its effectiveness is strongly affected by the weather conditions.



Note. The *Lr19* gene marker was found in genotypes 'Agatha' and Lr19.

Figure 3. An electrophoretic image with separation of polymerase chain reaction (PCR) products in the analysis of 25 wheat genotypes using the Xwmc221 marker



Note. The *Lr19* gene marker was found in genotypes 'Agatha' and Lr19.

Figure 4. An electrophoretic image with separation of polymerase chain reaction (PCR) products in the analysis of 25 wheat genotypes using the GB marker

Table 5. Results of leaf rust resistance, field and markers evaluation of 25 wheat genotypes

Genotype	2015		2016		2017		Mean		Gene *Lr19*	
	average	min–max	average	min–max	average	min–max	average	min–max	Xwmc221	GB
Agatha	**2.7**	**(2–3)**	**6.0**	**(6)**	**2.3**	**(2–3)**	**3.7**	**(2–6)**	+	+
Aguila	6.3	(6–7)	7.7	(7–8)	8.0	(8)	7.3	(6–8)	–	–
Argus	2.7	(2–3)	3.0	(2–4)	1.3	(1–2)	**2.3**	(1–4)	–	–
Century	7.3	(7–8)	8.0	(8)	8.7	(8–9)	8.0	(7–9)	–	–
Express	5.3	(5–6)	7.0	(6–8)	7.7	(7–8)	6.7	(5–8)	–	–
Fielder	3.7	(3–4)	1.3	(1–2)	2.0	(2)	2.3	(1–4)	–	–
Freedom	1.3	(1–2)	3.0	(3)	2.7	(2–3)	2.3	(1–3)	–	–
Grandin	7.0	(7)	7.7	(7–8)	4.3	(4–5)	6.3	(4–8)	–	–
Greer	5.3	(5–6)	5.0	(4–6)	6.7	(6–7)	5.7	(4–7)	–	–
Hyak	1.3	(1–2)	4.0	(3–5)	2.7	(2–3)	2.7	(1–5)	–	–
Clasic	8.3	(8–9)	7.7	(7–8)	8.0	(7–9)	8.0	(7–9)	–	–
KS89WGRC7	6.7	(6–7)	5.3	(5–6)	7.0	(7)	6.3	(5–7)	–	–
Lee	6.0	(5–7)	7.7	(7–8)	5.3	(5–6)	6.3	(5–8)	–	–
Lemhi	8.7	(8–9)	7.3	(7–8)	8.0	(7–9)	8.0	(7–9)	–	–
Lr19	**6.0**	**(5–7)**	**7.7**	**(7–8)**	**1.3**	**(1–2)**	**5.0**	**(1–8)**	+	+
Lr64	5.3	(5–6)	4.7	(4–5)	4.0	(4)	4.7	(4–6)	–	–
NC8860-5	7.3	(7–8)	7.0	(7)	7.7	(7–8)	7.3	(7–8)	–	–
Ok75Abd-380	5.0	(4–6)	2.0	(2)	1.0	(1)	2.7	(1–6)	–	–
R04-156	8.0	(8)	5.3	(5–6)	8.7	(8–9)	7.3	(5–9)	–	–
R04-268	7.3	(7–8)	7.7	(7–8)	9.0	(9)	8.0	(1–3)	–	–
Thatcher	8.7	(8–9)	9.0	(9)	8.3	(8–9)	8.7	(8–9)	–	–
Wawawai	6.3	(6–7)	7.0	(6–8)	6.7	(6–7)	6.7	(6–8)	–	–
XW591	1.3	(1–2)	2.3	(2–3)	2.3	(2–3)	2.0	(1–3)	–	–
XW571	6.7	(5–8)	4.3	(4–5)	4.0	(3–5)	5.0	(3–8)	–	–
2737W	1.7	(1–2)	6.0	(6)	1.3	(1–2)	3.7	(1–6)	–	–

According to scale 1–9: 1 – immune, 9 – very susceptible

The best scores of a three-year field resistance evaluation ("very resistant") were received for three genotypes: 'Argus', 'Fielder' and 'Freedom'. Two genotypes: KS89WGRC7 and Ok75Abd-380, were "resistant", reaching average score of 2.7 on the 9-step scale. In none of these genotypes the marker of *Lr19* gene was identified. Moreover, only genotypes 'Freedom' and KS89WGRC7 of the most resistant genotypes have identified other *Lr* gene (*Lr37* and *Lr40*, *Lr21*, respectively). The result could be caused by large diversity of pathotypes in the European population of *Puccina triticina* characterized by different combinations of virulence / avirulence against the *Lr* genes (Mesterhazy et al., 2000). The Polish population is also composed of many different pathotypes. In the years 2013–2015, 21 pathotypes of *P. triticina* were identified (Czajowski et al., 2016). Pathotypes marked with code 12722 and 12723 clearly dominated, but constituting only 17.2–27.6% of the fungal isolates tested. In the previous period of research carried out by Czajowski et al. (2011) and by Hanzalová et al. (2013; 2016), the pathotypes 12720, 12724 and 12762 appeared more frequently. A common feature of these pathotypes is virulence against the following genes: *Lr1*, *Lr3*, *Lr11*, *Lr15*, *Lr17* and *Lr21* (Czajowski et al., 2016). Large diversity of leaf rust pathotypes and changing weather conditions cause the differences in resistance of individual cultivars, depending on *Lr* genes that occur in their genotypes. Due to the pathogen's ability to evolve and overcome host resistance, single major genes are often broken in a short time from their application by new pathotypes. Therefore, combinations of two or more resistance genes in one genotype are very desirable. In order to obtain high resistance to leaf rust in new wheat cultivars, it is recommended to pyramidize the *Lr19* resistance gene with other *Lr* genes, which are effective in Central Europe. This approach could provide good resistance at variable weather conditions. According to Kolmer (2009), a combination of genes *Lr34+Lr2a+Lr9+Lr26* in winter wheat gave high resistance, while plants with gene combination of *Lr34+Lr10+Lr11+Lr18* were evaluated as moderately or low resistant. Leonardo et al. (2011) have reported that combinations of seedling resistance genes (e.g., *Lr16*, *Lr19*, *Lr21*, *Lr25*, *Lr29*, *Lr41*, 42 and *Lr47*) with adult plant resistance genes (*Lr34*, *Lr42* and *Lr46*) could be good approach to provide durable leaf rust resistance. Prabhu et al. (2009) showed that the following gene pyramids can be successfully used in breeding programs: *Lr9+Lr24*, *Lr19+Lr24*, *Lr19+Lr28* and *Lr9+Lr24+Lr28*.

Conclusions

1. Only two of the seven markers under analysis were functional for wheat leaf rust resistance gene *Lr19*: dominant marker GB and the codominant Xwmc221 microsatellite marker. In the case of these two markers, in all replications of analyses there were obtained repeatable results. The appropriate PCR products (139 and 200 bp, respectively) were observed only in genotypes with *Lr19* gene.

2. Molecular markers GB and Xwmc221 are recommended for marker assisted selection of breeding material for leaf resistance gene *Lr19*.

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References

1. Abdelbacki A., Soliman N., Najeeb M., Omara R. 2013. Postulation and identification of resistance genes against *Puccina triticina* in new wheat cultivars in Egypt using molecular markers. International Journal of Chemical Environmental and Biological Sciences, 1 (1): 2320–4087.
2. Abou-Elseoud M. S., Kamara A. M., Alaa-Eldein O. A., El-Bebany A. F., Ashmawy N. A., Draz I. S. 2014. Identification of leaf rust resistance genes in Egyptian wheat cultivars by multipathotypes and molecular markers. Journal of Plant Sciences, 2 (5): 145–151. https://doi.org/10.11648/j.jps.20140205.11
3. Czajowski G., Strzembicka A., Karska K. 2011. Wirulencja populacji *Puccinia triticina* sprawcy rdzy brunatnej pszenicy i pszenżyta w Polsce w latach 2008–2010. Biuletyn Instytutu Hodowli i Aklimatyzacji Roślin, 260–261: 145–153 (in Polish).
4. Czajowski G., Czembor P., Radecka-Janusik M. 2016. Virulence of *Puccinia triticina* the causal agents of wheat leaf rust in Poland in the years 2013–2015. Biuletyn Instytutu Hodowli i Aklimatyzacji Roślin, 280: 13–19 (in Polish).
5. Elangbam P. D., Deepshikha, Jaiswal J. P. 2018. Revealing of brown rust resistance genes by molecular markers in wheat: a review. International Journal of Pure and Applied Bioscience, 6 (1): 407–417. https://doi.org/10.18782/2320-7051.4095
6. Gorash A., Galaev A., Babayants O., Babayants L. 2014. Leaf rust resistance of bread wheat (*Triticum aestivum* L.) lines derived from interspecific crosses. Zemdirbyste-Agriculture, 101 (3): 295–302. https://doi.org/10.13080/z-a.2014.101.038
7. Groenewald J. Z., Marais A. S., Marais G. F. 2003. Amplified fragment length polymorphism-derived microsatellite sequence linked to the *Pch1* and *Ep-D1* loci in common wheat. Plant Breeding, 122: 83–85. https://doi.org/10.1046/j.1439-0523.2003.00801.x
8. Gupta P. K., Balyan H. S., Edwards K. J., Isaac P., Korzun V., Roder M., Gautier M., Jourdrier P., Schlatter A. R., Dubcovsky J., De La Pena R. C., Khairallah M., Penner G., Hayden M. J., Sharp P., Keller B., Wang R. C. C., Hardouin J. P., Jack P., Leroy P. 2002. Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. Theoretical and Applied Genetics, 105: 413–422. https://doi.org/10.1007/s00122-002-0865-9
9. Gupta S. K., Charpe A., Prabhu K. V., Haque Q. M. R. 2006. Identification and validation of molecular markers linked to the leaf rust resistance gene *Lr19* in wheat. Theoretical and Applied Genetics, 113 (6): 1027–1036. https://doi.org/10.1007/s00122-006-0362-7
10. Hanzalová A., Bartoš P., Sumíková T. 2013. Physiological specialization of wheat leaf rust (*Puccinia triticina* Eriks.) in the Czech Republic in 2009–2011. Czech Journal of Genetics and Plant Breeding 49: 103–108. https://doi.org/10.17221/221/2012-CJGPB
11. Hanzalová A., Bartoš P., Sumíková T. 2016. Virulence of wheat leaf rust (*Puccinia triticina* Eriks.) in the years 2013–2015 and resistance of wheat cultivars in Slovakia. Cereal Research Communnication, 44 (4): 585–593. https://doi.org/10.1556/0806.44.2016.030
12. Haque A., Shaheen T., Gulzar T., Rahman M., Jalal F., Sattar S., Ehsan B., Zafar I., Younas M. 2014. Study of rust resistance genes in wheat germplasm with DNA markers. Bioinformation, 10 (6): 371–377. https://doi.org/10.6026/97320630010371
13. Heffner E. L., Lorenz A. J., Jannink J., Sorrells M. E. 2010. Plant breeding with genomic selection: gain per unit time and cost. Crop Science, 50: 1681–1690. https://doi.org/10.2135/cropsci2009.11.0662
14. Kassem M., El-Ahmed A., Hakim M. S., Al-Saleh A., El-Khalifeh M., Nachit M. 2011. Identifying leaf rust resistance gene *Lr19* in durum wheat using simple sequence repeat (SSR) marker. African Journal of Biotechnology, 10 (44): 8716–8719. https://doi.org/10.5897/AJB10.1526
15. Kolmer J. A. 2009. Postulation of leaf rust resistant genes in selected soft red winter wheat. Crop Science, 43: 1266–1274. https://doi.org/10.2135/cropsci2003.1266
16. Leonardo S. V., Campos P., Demichelis M., Lombardo L. A., Aurelia R. P., Vaschetto L. M., Bainotti T., Helguera M. 2011. Identification of leaf rust resistance genes in selected Argentinean bread wheat cultivars by gene postulation and molecular markers. Electronic Journal of Biotechnology, 14 (3): 1–17. https://doi.org/10.2225/vol14-issue3-fulltext-14

17. Li X., Yang W., Li Y., Liu D., Yan H., Meng Q., Zhang T. 2006. A SSR marker for leaf rust resistance gene *Lr19* in wheat. Agricultural Sciences in China, 5 (2): 111–115. https://doi.org/10.1016/S1671-2927(06)60027-8
18. Matysik P., Nita Z. 2008. Progress in winter wheat resistance breeding on the example of advanced breeding lines. Progress in Plant Protection, 48 (1): 41–46.
19. McIntosh R. A., Wellings C. R., Park R. F. (eds). 1995. Wheat rusts: an atlas of resistance genes, 200 p. https://doi.org/10.1071/9780643101463
20. Mesterhazy A., Bartos P., Goyeau H., Niks R. E., Csosz M., Andersen O., Casulli F., Ittu M., Jones E., Manisterski J., Manninger K., Pasquini M., Rubiales D., Schachermayr G., Strzembicka A., Szunics L., Todorova M., Unger O., Vanco B., Vida G., Walther U. 2000. European virulence survey for leaf rust in wheat. Agronomie, 20: 793–804. https://doi.org/10.1051/agro:2000104
21. Okoń S., Matysik P., Nita Z., Bichoński A., Rubrycki K., Woźna-Pawlak U., Kowalczyk K. 2012. Identification of *Lr19* gene in Polish common wheat (*Triticum aestivum* L.) breeding lines. Annals Universitatis Mariae Curie Sklodowska, Sectio E, 67 (3): 39–43.
22. Pietrusińska A. 2010. The use of molecular markers for introduction of leaf rust (*Puccinia recondita* f. sp. *tritici*) and powdery mildew (*Blumeria graminis* f. sp. *tritici*) resistance genes in winter wheat (*Triticum aestivum*). Biuletyn Instytutu Hodowli i Aklimatyzacji Roślin, 256: 31–44 (in Polish).
23. Pillard S., Schnurbush T., Winzeler M., Messmer M., Sourdile P., Abderhalden O., Keller B., Schachermayr G. 2003. An integrative genetic linkage map of winter wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics, 107: 1235–1242. https://doi.org/10.1007/s00122-003-1361-6
24. Prabhu K. V., Gupta S. K., Charpe A., Koul S. 2004. SCAR marker tagged to the alien leaf rust resistance gene *Lr19* uniquely marking the *Agropyron elongatum*-derived gene *Lr24* in wheat: a revision. Plant Breeding, 123: 417–420. https://doi.org/10.1111/j.1439-0523.2004.00971.x
25. Prabhu K. V., Singh A. K., Basavaraj S. H., Cherukuri D. P., Charpe A., Krishan A. G., Gupta S. K., Joseph M., Koul S., Mohaparta T., Pallavi J. K., Samsampour D., Singh A., Singh V. K., Singh A., Singh V. P. 2009. Marker assisted selection for biotic stress resistance in wheat and rice. Indian Journal of Genetics, 69 (4): 305–314.
26. Prins R., Groenewald J. Z., Marais G. F., Snape J. W., Koebner R. M. D. 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/10.1007/PL00002918
27. Sehgal S. A., Tahir R. A., Nawaz M. 2012. Molecular characterization of wheat genotypes using SSR markers. International Journal of Bioautomation, 16 (2): 119–128.
28. Singh D., Mohler V., Park R. F. 2013. Discovery, characterization and mapping of wheat leaf rust resistance gene *Lr19*. Euphytica, 190: 131–136. https://doi.org/10.1007/s10681-012-0786-x
29. Stępień Ł., Golka L., Chełkowski J. 2003. Leaf rust resistance genes of wheat: identification in cultivars and resistance sources. Journal of Applied Genetics, 44 (2): 139–149.
30. Święcicki W. K., Surma M., Koziara W., Skrzypczak G., Szukała J., Bartkowiak-Broda I., Zimny J., Banaszak Z., Marciniak K. 2011. Modern technologies in crop production – friendly for man and environment. Polish Journal of Agronomy, 7: 102–112 (in Polish).
31. Tomkowiak A., Kurasiak-Popowska D., Mikołajczyk S., Weigt D., Niemann J., Kiel A., Lisewska A., Nawracała J., Matysik P., Rokicki M., Bocianowski J. 2016. Identification of brown rust resistance gene *Lr19* caused by *Puccinia recondita* f. sp. *tritici* in foreign cultivars of winter wheat *Triticum aestivum* L. Progress in Plant Protection, 56 (3): 318–323.
32. Trawal A., Walczak F. 2012. Occurrence of more important diseases on winter wheat in Poland 2006–2010. Annals Universitatis Mariae Curie Sklodowska, Sectio E, 57 (2): 28–41.
33. Uhrin A., Láng L., Bedö Z. 2008. Comparison of PCR-based DNA markers for using different *Lr19* and *Lr24* leaf rust resistance wheat sources. Cereal Research Communications, 36 (4): 533–541. https://doi.org/10.1556/CRC.36.2008.4.2
34. Urbanovich O. Y., Malyshev S. V., Dolmatovich T. V., Kartel N. A. 2006. Identification of leaf rust resistance genes in wheat (*Triticum aestivum* L.) cultivars using molecular markers. Russian Journal of Genetics, 42 (5): 546–554. https://doi.org/10.1134/S1022795406050127
35. Vanzetti L. S., Campos P., Demichelis M., Lombardo L. A., Aurelia P. R., Vaschetto L. M., Bainotti C. T., Helguera M. 2011. Identification of leaf rust resistance genes in selected Argentinean bread wheat cultivars by gene postulation and molecular markers. Electronic Journal of Biotechnology, 14 (3): 1–17. https://doi.org/10.2225/vol14-issue3-fulltext-14

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Molekulinių žymeklių, susijusių su *Lr19* genu, lemiančiu kviečių atsparumą rudosioms rūdims, funkcionalumo analizė

A. Kiel, D. Weigt, M. Karpińska, D. Kurasiak-Popowska, J. Niemann, A. Tomkowiak, S. Mikołajczyk, J. Nawracała

Poznanės gyvybės mokslų universiteto Augalų genetikos ir selekcijos skyrius, Lenkija

Santrauka

Iš visų javų visame pasaulyje didžiausią plotą užima paprastasis kvietys (*Triticum aestivum* L.). Grūdų derlių reikšmingai sumažina kviečių rudosios rūdys – liga, kurią sukelia patogenas *Puccinia recondita* f. sp. *tritici*. Efektyvia kovos su grybinėmis ligomis priemone gali tapti selekcija, nukreipta į atsparių veislių sukūrimą kartu su atranka, paremta molekuliniais žymekliais. *Lr19* yra genas, lemiantis didelį atsparumą kviečių rudosioms rūdims. Pastaraisiais metais buvo atrasta daug molekulinių žymeklių, esančių šalia *Lr19* geno, pvz., SCS265, SCS253, GB, Xwmc221, XustSSR2001-7DL, Xgwm37 ir Xgwm44. Tyrimo tikslas – nustatyti molekulinių žymeklių, susijusių su *Lr19* genu, funkcionalumą. Tyrimo metu buvo naudoti du standartiniai kviečių genotipai su *Lr19* genu (‘Agatha’ ir Lr19) ir trys linijos, smarkiai pažeistos rudųjų rūdžių. Išanalizuoti septyni molekuliniai žymekliai, tačiau tik du iš jų (GB ir Xwmc221) buvo specifiniai tirtam genui. Šie žymekliai naudoti analizuojant 25 kviečių genotipus, kurie buvo vertinti dėl atsparumo rudosioms rūdims lauko sąlygomis, siekiant patvirtinti jų naudingumą atrenkant selekcinę medžiagą.
Tyrimo duomenys rodo, kad reikia nuolat ieškoti funkcionalių molekulinių žymeklių, kurie duotų pakartojamus ir patikimus rezultatus.

Reikšminiai žodžiai: molekuliniai žymekliai, paprastasis kvietys, *Lr19*, *Puccinia recondita* f. sp. *tritici*.