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Molecular biology**MOLECULAR ASSESSMENT OF THE ALLELIC STATE OF *Lr34* LEAF RUST RESISTANCE GENE IN BREAD WHEAT GERMPLASM IN AZERBAIJAN**

**Academician of ANAS I.M. Huseynova, S.M. Rustamova, F.B. Guliyeva,
Academician of ANAS J.A. Aliyev**

*The aim of the present study was to determine the allelic state of the *Lr34* locus associated with leaf rust resistance in bread wheat (*Triticum aestivum* L.) collection of 61 cultivars and advanced lines from the Research Institute of Crop Husbandry (Azerbaijan). For the molecular screening codominant STS marker *csLV34* has been used. The presence of 150-bp PCR fragment linked to the *csLV34b* allele, which is associated with *Lr34*, was detected in 15% of the tested samples. A 229-bp band belonging to *csLV34a* allele has been amplified in 34% of genotypes, indicating the absence of such resistance. However, both alleles for the *Lr34/Yr18* gene complex are present in cultivars *Zirva-80* and *Gyrmyzy gul-1*. According to the general analysis with both markers, 54% of investigated wheat genotypes did not produce any bands.*

Keywords: *Triticum aestivum* L., leaf rust, *Lr34* gene, allelic state

Introduction

Leaf rust, caused by the fungal pathogen *Puccinia triticina* Eriks., is the most prevalent disease of wheat (Sehgal et al., 2012; Kolmer, 2013; Casassola et al., 2015). Therefore, the creation of new cultivars, having an effective and long-term resistance to brown rust, is one of the key tasks of the modern wheat breeding. To date, more than 70 leaf rust resistance genes and QTLs in wheat have been described (<http://www.shigen.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp>). Most of the genes controlling race specific resistance to rust and other harmful fungi (R-genes) are effective for only a few years. In contrast, the gene *Lr34*, providing stability at the level of the adult plant (adult plant resistance, APR), is effective for decades. Race nonspecific manifestation of the effectiveness of the gene *Lr34* in a "stable" allelic state was also established (Kolmer et al., 2008; Galaev and Siveolap, 2015).

Lr34 was first described in cultivar Frontana in 1966 (Dyck et al., 1966). It is located on the short arm of chromosome 7D, close to locus *Xgwm295*. *Lr34* is tightly linked to the leaf tip necrosis (LTN) locus, it is also possible that the LTN phenotype could be a pleiotropic effect of *Lr34* itself. Krattinger et al. (2009) made

a detailed physical and genetic map of the locus *Lr34* and suggested its functional role in wheat cells. According to them, this gene encodes ABC transporter (pleiotropic drug resistance (PDR)-like ATP-binding cassette (ABC) transporter), which determines the availability of the plant resistance type. The nucleotide sequence of *Lr34* has a length of 11805 bp and contains 24 exons (Krattinger et al., 2011).

Development and application of molecular markers for *Lr34/Yr18* have been a major objective for marker-assisted selection in wheat breeding program for durable leaf rust resistance. Several SSR, STS and CAPS-markers were proposed to identify this gene. The *csLV34* marker was reported to be tightly linked to *Lr34* (Lagudah et al. 2006) and diagnostic for the presence of the gene. This marker was recently used in conjunction with phenotypic analysis to postulate the presence of *Lr34* in Australian, Canadian, Indian, Russian, etc. wheat cultivars (Karelov et al., 2011; Galaev and Siveolap, 2015).

The present study was conducted to assess allelic variation at *Lr34* locus controlling leaf rust resistance in bread wheat germoplasm collection in Azerbaijan.

Table 1

Specific primers used for the DNA amplification for rust leaf resistance genes in wheat

Gene	Marker	Sequence of primer (5'-3')	The size of amplified marker fragments
<i>Lr34</i>	csLV34a csLV34b	GTTGGTTAAGACTGGTGATGG TGCTTGCTATTGCTGAATAGT	229 bp 150 bp

Material and methods

The objectives of this study were 61 bread wheat (*Triticum aestivum* L.) cultivars and lines collected in the gene pool of the Research Institute of Crop Husbandry. Plants were cultivated in field conditions. PCR was performed in the "Applied Biosystems 2720 Thermal Cycler" (Singapore) thermocycler under the following conditions: 1 cycle - 3 minutes at 94°C; 38 cycles - 1 min at 94°C, 1 min at 60 and 63°C (for SCS123 and SCS253 respectively), 2 minutes at 72°C; the final elongation cycle was performed at 72°C for 10 min, then kept at 4°C.

The reaction products were separated by electrophoresis in a 1.2% agarose gel in the HR-2025-High Resolution («IBI SCIENTIFIC» U.S.) horizontal electrophoresis machine with addition of ethidium bromide. Digital molecular imager "Gel Documentation System UVITEK" (UK) was used for visualization and documentation of banding patterns.

DNA extraction. Genomic DNA was extracted from fresh leaves by CTAB method with some modifications (Murray and Thompson, 1980). DNA quantity and quality was estimated using a ULTROSPEC 3300 PRO spectrophotometer ("AMERSHAM", USA) by

measuring absorbance values at A₂₆₀ and A₂₈₀.

DNA amplification. Polymerase chain reaction was performed by Williams (1990) using codominant STS marker csLV34 (Eurofins mwg operon) (table 1). DNA amplification was performed in 25 µl volume of the reaction mixture, containing 10 × buffer, 20 ng of the genomic DNA, 0.2 µM primer, 200 µM of each of the following: dATP, dCTP, dGTP and dTTP, 2.5 mM MgCl₂, and 0.2 units of Taq-polymerase in the incubation buffer.

Results and discussion

Codominant STS-marker csLV34 is closely associated with the biallelic locus *Lr34* (0.4 cM), which is a locus was especially used in MAS programs (Lagudah et al. 2006). Therefore, we used markers *Lr34*/csLV34a and *Lr34*/csLV34b to identify a and b alleles of the gene *Lr34*. The specific marker for the allele *Lr34*/csLV34a have to lead to the amplification of 229 bp fragment (fig.). PCR analysis using this primer revealed corresponding locus only in 21 genotypes (table 2). This represents approximately 34% of all tested genotypes. This allele was not identified in 66% of genotypes.

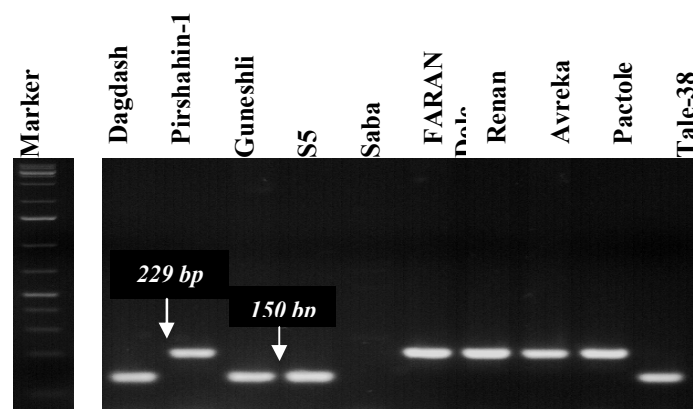


Figure. PCR - profiles of *Triticum aestivum* L. plants for *Lr 34*. Molecular weight marker - 100 bp.

Table 2

Results of the PCR analysis for the gene *r34*

Genotypes	csLV34a 229bp	csLV34b 150bp	Genotypes	csLV34a 229bp	csLV34b 150bp
Pirshahin-1	+	-	S1	-	-
Guneshli	-	+	Nurlu-99	-	-
Dagdash	-	+	Gyrmyzy gul-1	+	+
FARAN Dolc	+	-	Azamatli-95	+	-
Renan	+	-	Tale-38	-	+
Avreka	+	-	Ruzi-84	-	-
Pactole	+	-	12nd FAWWON №97(130/21)	+	-
S5	-	+	4th FEFWSN №50 (130/32)	+	-
TH1	+	-	FO2 N7-A (karlik)	+	-
D8 sechme №5	+	-	Tigre	-	+
S4	+	-	Bezostaya-1	-	+
S3	+	-	Kanada 2	-	-
Sechme SS	-	-	Sechme sunbul giz	-	-
Mirbashir-128	-	-	S9	-	-
Yegane	-	-	Sechme sunbul ag	-	-
Zirve-80	+	+	Bogdanka	-	-
Fatima	-	-	Kripsinka	-	-
Azan	+	-	16th FAWWON-IR (61)	-	-
Azeri	-	-	16th FAWWON-IR (46)	-	-
Murov	-	-	16th FAWWON-IR (52)	-	-
Murov-2	+	-	16th FAWWON-IR (90)	-	-
Saba	+	-	16th FAWWON-IR (47)	-	-
Taragi	+	-	29 ES WVT (7)	-	+
Beyaz	-	-	29 ES WVT (26)	-	-
Shafaq	+	-	29 ES WVT (38)	-	-
rsi-13(shafaq-2)	-	-	29 ES WVT (30)	-	-
Pirshahin	-	-	16 SAWWVT (29)	-	-
Ugur	-	-	16 SAWWVT (34)	-	-
Perzivan-1	-	-	39 IBWSN (97 №)	-	-
Perzivan-2	-	-	11st IWWT-R (9816 №)	-	-
Sheki-1	+	-			

According to the PCR profiles obtained with the marker *Lr34/csLV34b*, used for the identification of the *b* allele of *Lr34*, characteristic fragments in 150 bp region were synthesized only in 15% genotypes. In other words, allele *b* of the gene *Lr34* was identified only in 9 (Gunashli, Dagdash, S5, Zirva-80, Gyrmyzy gul-1, Tale-38, Tigra, Bezostaya-1, 29ESWVT(7)) among 61 genotypes. It is interesting to note that both alleles of the gene *Lr34* were identified in two genotypes, Zirva-80 and Gyrmyzy gul-1. Such a result could be obtained due to crossing over. (Kolmer et al., 2008). According to the general analysis of the results related to both markers, 54% of the wheat genotypes do not

possess any allele of the gene *Lr34*. The presence of allelic state *csLV34b* indicates wheat tolerance to the causative of brown rust, associated with the gene *Lr34*, whereas *csLV34a* indicates the absence of such a tolerance (Karelov et al., 2011). It should be also noted that resistance associated with this locus is a “slow rusting” type, i.e. slowly developing rust at typical reactions of susceptibility. This resistance type cannot be identified by phenotype at high levels of infections (epiphytotic years or artificial infectious backgrounds). The wheat cultivars are of different types and become susceptible to different types of rust because of narrow genetic bases for resistance.

The evolution rates of pathogens are very fast and rapid. So, it is necessary to find out new and better sources for resistance. The genetic resistance is important to control many phytopathogenic epidemics. Cultivars having only *Lr34* do not possess high levels of resistance. To get high resistance, additional effective resistance genes are required for wheat genotypes. For long lasting resistance certain combinations of genes are needed. Moderate to high levels of resistance have been observed for over 30 years in spring wheat cultivars with combinations of *Lr13*, *Lr16*, *Lr23* and *Lr34* (Kolmer and Jin, 2007). Long lasting resistance has been detected in wheat genotypes developed at CIMMYT with combinations of adult plant genes *Lr34*, *Lr46*, and *Lr68*. To maintain rust resistance, new sources of effective rust resistance genes must be added to breeding germplasm regularly, because of the continual evolution and selection of rust races. The obtained results can be used in breeding and genetic programs on creation of forms resistant to leaf rust pathogen populations in Azerbaijan.

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*Institute of Molecular Biology and Biotechnology
huseynova-i@botany-az.org*

AZƏRBAYCANDA YUMŞAQ BUĞDANIN RÜŞEYM PLAZMASINDA *Lr34* YARPAQ PASINA DAVAMLILIQ GENİNİN ALLEL VƏZİYYƏTİNİN MOLEKULAR QIYMƏTLƏNDİRİLMƏSİ

İ.M. Hüseynova, S.M. Rüstəmov, F.B. Quliyeva, C.Ə. Əliyev

Tədqiqat işi Elmi-Tədqiqat Əkinçilik İnstitutunun (Azərbaycan) 61 sort və aparıcı xətlərdən ibarət yumşaq (*Triticum aestivum* L.) buğda kolleksiyasında yarpaq pasına davamlılıqla əlaqəli *Lr34* lokusunun allel vəziyyətini təyin etmək məqsədilə aparılmışdır. Molekulyar skrining üçün kodominant *csLV34STS* markerdən istifadə olunmuşdur. *Lr 34* ilə əlaqəli *csLV34b* alleli ilə ilişikli 150 bp PZR fraqmentlər tədqiq edilmiş genotiplərin 15%-də müşahidə edilmişdir. *csLV34a* allelinə məxsus 229 bp bəndlər genotiplərin 34%-də amplifikasiya olunmuşdur ki, bu da davamlılığın

olmamasını göstərir. Amma Zirvə-80 və Qırmızı Gül-1 genotiplərində Lr34/Yr18 gen kompleksinin hər iki alleli müşahidə edilmişdir. Hər iki markerlə ümumi analizlərə görə tədqiq olunan genotiplərin 54%-də heç bir bənd sintez olunmamışdır.

Açar sözlər: *Triticum aestivum L.*, yarpaq pası, Lr34 geni, allel vəziyyət

МОЛЕКУЛЯРНАЯ ОЦЕНКА АЛЛЕЛЬНОГО СОСТОЯНИЯ ГЕНА УСТОЙЧИВОСТИ К ЛИСТОВОЙ РЖАВЧИНЕ *Lr34* В ЗАРОДЫШЕВОЙ ПЛАЗМЕ МЯГКОЙ ПШЕНИЦЫ В АЗЕРБАЙДЖАНЕ

И.М. Гусейнова, С.М. Рустамова, Ф.Б. Гулиева, Дж.А. Алиев

Данное исследование было проведено с целью определения аллельного состояния локуса Lr34, связанного с устойчивостью к листовой ржавчине, в коллекции мягкой пшеницы (*Triticum aestivum L.*) из 61 сорта и передовых линий из Научно-исследовательского института земледелия (Азербайджан). Для молекулярного скрининга был использован кодоминантный STS маркер csLV34. Наличие 150 бп ПЦР фрагментов, сопряженных с аллелем csLV34b, связанным с Lr34, было обнаружено у 15% исследованных образцов. 229 бп полосы, принадлежащие csLV34a аллелю, амплифицировались у 34% генотипов, что указывает на отсутствие такой устойчивости. Однако, у сортов Зирва-80 и Гырмызы Гюл-1 присутствуют оба аллеля генного комплекса Lr34/Yr18. По общему анализу с обоими маркерами, у 54% исследуемых генотипов пшеницы не выявляются ожидаемые полосы.

Ключевые слова: *Triticum aestivum L.*, листовая ржавчина, ген Lr34, аллельное состояние