# Molecular Analysis of Resistance Genes to Brown Rust *Lr9, Lr19, Lr34, Lr35* and Yellow Rust *Yr9, Yr18* in Azerbaijan Wheat Germplasm

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**Rust is the most devastating fungal disease causing significant losses of grain production. Growing rust resistant varieties is the most sustainable, cost-effective and environmentally friendly approach for controlling rust diseases. To date, 81 brown and 53 yellow rust resistance genes have been detected in bread and durum wheat genotypes and their wild species using different molecular methods. The primary goal of the research was to establish the presence of *Lr* ans *Yr* genes in wheat samples collected in Gene Pool of the Reserch Institute of Crop Husbandry (Baku, Azerbaijan). Four *Lr* genes, *Lr9, Lr19, Lr34, Lr35* and two *Yr* genes, *Yr9 u Yr18* were analysed using various molecular markers. 1100 bp specific fragments in PCR profiles indicate the presence of *Lr9* gene on 6B chromosome of 60% of the studied 78 genotypes. Positive results were obtained in 45 genotypes for identification of *Lr19* gene using SCAR markers SCS123 and SCS253. Allelic state of *Lr34* gene was studied using Lr34/csLV34a and Lr34/csLV34b markers. Molecular analysis showed the presence of allele *a* of *Lr34* in 21 and allele *b* in 9 genotypes. Two genotypes were found to carry both alleles of *Lr34*. 54% of the studied wheat genotypes had no allele of *Lr34* gene. Fragments characteristic of *Lr35* gene were not visualized in PCR profiles of 61 genotypes. Fragments of 250 bp diagnostic for *Yr18* gene were visualized in electrophoretic profiles of 40 genotypes. 150 bp fragments characteristic of *Yr9* gene were amplified in all genotypes with the exception of four samples. These results will serve as a base for plant breeders to develop durable rust-resistant wheat varieties and to control wheat leaf rust diseases in Azerbaijan.**

***Keywords**: Wheat, brown rust, yellow rust, Lr9, Lr19, Lr34, Lr35, Yr9, Yr18, PCR, STS, SSR, SCAR*

## INTRODUCTION

Rust diseases are the main factors decreasing productivity and quality of cereals all over the world (Aktar-Uz-Zaman et al., 2017), including Azerbaijan. Three rust diseases, namely, leaf or brown rust caused by *Puccinia triticina Eriks*, stem or black rust caused by *Puccinia graminis f. sp. Tritici West*, and stripe or yellow rust caused by *Puccinia striiformis* f. *tritici* Eriks, are the most economically significant and common diseases among global wheat cultivars. These diseases can cause the production loss up to 40-50% depending on the infection degree and duration. Food security is a strategy of our government and it is necessary to develop highly productive and resistant to biotic stress plants for providing the population with qualitative and ecologically pure food products. Despite the successes of the practical selection achieved on the issue of resistance, rust diseases still remain deleterious for cereals all over the world (Jighly et al., 2015; Periyannan et al., 2017). A resistant variety is considered as an important element in the integrated protective system of plants against diseases and pests. Therefore, the development of varieties resistant to infections is more effective and ecologically safe method of struggling against rust diseases. Genotypes with a high genetic potential for the rust diseases resistance are of great practical importance. Thus, these genotypes are sources of the disease resistance genes and in the future they can be successfully used in molecular selection programs as parental forms. The permanent search for new donors, protected with genes, which are new for selection, and easily transfer properties at crossing is necessary for the development of resistant varieties (Riar et al., 2012; Abou-Elseoud et al., 2014; Hubbard et al., 2015).

Since the beginning of the XX century in various countries the investigations have been carried out to develop productive varieties, tolerant to adverse climatic conditions and diseases. Hybridological analysis revealed that genes for rust diseases resistance can function independently from each other or they can manifest complimentary or duplicate effects (Abdelbacki et al., 2013; Maccaferri et al., 2015). Moreover, an additional interaction, eliminating the effectiveness of the major gene, can occur between resistance and parental genes. In some cases additional resistance genes stimulate the

effectiveness of the major gene in varieties sensitive to diseases. Various genes can interact showing cumulative effect. The rust resistance genes are divided into two categories in the scientific literature: major genes or oligogenes and secondary or minor genes. The latter includes modificator genes, additional genes, non-specific resistance genes etc.

Currently, over 81 resistance genes against brown rust disease have been identified in wheat genotypes by various genetic and biochemical approaches (McIntosh et al., 2008; McIntosh et al., 2011, Aktar-Uz-Zaman et al., 2017). The majority of them are juvenile genes (expressing in youth period of the plant). *Lr12, Lr13, Lr21, Lr22, Lr34, Lr35, Lr37* are mature plant genes. The number of *Lr* genes effective against rust causatives is getting less every year (Ittu, 2000). Sexual hybridization and other processes occurred in pathogen cause the formation of virulent biotypes and forms, overcoming existing resistance. Therefore, a permanent search for new genes is necessary. Such an approach is important and actual for selection. Recently, using molecular biological methods DNA markers have been developed, which in combination with the respective resistance genes are powerful tools in the identification of genes (Cheikowski and Stepien, 2001).

Extensive investigations have been carried out on identification of resistance genes against wheat rust diseases and determination of their effectivenes in different geographical regions of the world (Mesterhazy et al., 2000, Abou-Elseoud et al., 2015, Imbaby et al., 2014). For example, using molecular markers and standard genetic methods *Lr3* gene 42%, *Lr26* - 28% , *Lr13* - 13%, *Lr37* - 9%, *Lr10* - 4%, *Lr1, Lr14a, Lr17b* - 1% were found in genotypes in Greece and Czechia. The gene *Lr37* appeared to be more effective against *P. triticina*, while the genes *Lr1, Lr10, Lr13* were effective only in combination with other genes. Australian wheat genotypes were shown to be protected from brown rust by the genes *Lrl3, Lr24, Lr34, Lr37* and from yellow rust by the genes *Yr17* and *Yr18*. It is very interesting that the genes *Lr34* and *Yr18* are linked and they both are widely distribited in selection materials of CIMMYT, South and North America and China (Li et al., 2010). The gene *Lr13* has been providing durable protection in the Australian continent for more than 20 years (Huerta-Espino et al., 2011). As in Australia this gene is protecting more than a half of the varieties cultivated in England. *Lr26* (22%), *Lr37* (20%), *Lr10* (17%), *Lr17b* (*LrH*) (10%), *Lrl* (7%), *Lr3a* (6%) and *Lr20* (4%) are less frequent genes (Powell et al., 2013).

The genes *Lr23, Lr26, Lr34* and *Lrl*3 have been identified in Indian varieties. In Iraq mainly the genes *Lr3, Lrl0, Lrl6, Lrl7, Lr23, Lr26, Lrl3* and *Lrl* and their combinations provide resistance.

Scientists from Russia and CIS countries also begin performing studies in this direction (Sibikeyev et al., 1996; Tirishkin, 2006; Karelov et al., 2001). The genes *Lr9, Lr19, Lr24* and *Lr38* were found to be effective against *P. triticina* population in the Russian territory.

To date 53 stripe rust resistance genes (*Yr1–Yr53*) and numerous temporarily designated genes have been reported in wheat (http://wheat.pw.usda.gov/cgi-bin/graingenes).

Most of these genes have been mapped on chromosomes and/or specific chromosomal regions, and many of them have been used in wheat breeding programs worldwide (Zhang et al., 2013). At first, eight resistance genes against yellow rust disease were detected and denoted as *Yr1, Yr2, Yr3* etc. Then the genes *Yr3* and *Yr4* were divided into other genes. Currently a lot of yellow rust resistance genes have been identified. Among these genes *Yr11, Yr12, Yr13, Yr14, Yr16* are mature plant genes. Yellow rust resistance genes were detected mainly in bread wheat (*Triticum aestivum*) (McGrann et al., 2014; Basnet et al., 2014). However, most genes were transferred from other species and wild cereals by introgression. For example, the gene *Yr8* was transferred to bread wheat from *Aegilops comosa*, *Yr9* from rye, *Yr24* and *Yr28* from *Aegilops tauschii*, and *Yr26* from *Haynaldia villosa*. Until now a lot of useful alien genes have been transferred to wheat plants. But it is not possible to use all of them in the selection of commercial varieties, as alien chromosom segments are not able to compensate losses of wheat native chromosomes or they have undesirable genes decreasing grain yield and quality (Powell et al., 2013; Luo et al., 2008).

During the last decade the number of named and mapped resistance genes in wheat increased pronouncedly (McIntosh et al., 1998; McIntosh et al, 2005; McIntosh et al., 2007). However, the wheat genome (17.3 pg per cell) belongs to the most numerous cultivated species and contains nearly 17000 Mbp per haploid nucleus. Because of the size and high percentage (over 90%) of non-coding sequences and A, B and D genomes with 7 homologous chromosomes it is difficult to perform molecular identification and cloning of wheat resistance genes. The average size of each (in total 42) hexaploid wheat chromosome is 800 Mbp. Physical distance between crossing-overs (=1 cM) varies from 0.3 to 3.0 Mbp (Feuillet et al., 1997). Wild relatives of wheat usually have one common genome, which is vey useful for searching and mapping new resistance genes. Since wheat-related species carry different genomes (*Triticum* sp., ge-

nome B; *Aegilops speltoides*, genome S similar to B; *Triticum boeoticum*, genome A; and *Aegilops squarrosa*, genome D) they were and still are used as sources of resistance genes in plant breeding. A usual way of transferring the resistance genes is using wheat lines with translocation of a chromosome fragment carrying a wild species gene. This transfer was performed for the genes *Lr19, Lr24* and *Lr*29 derived from *Agropyronelongatum* (Schachermayr et al., 1995, Prins et al., 1996). RFLP, RAPD, CAPS, SCAR, STS markers have been obtained using new DNA-based methods to identify individual resistance genes in wheat (Cheikowski and Stepien, 2001).

Revealing the genes by markers for detection of protein encoding sequences of nucleotide-binding sites and leucine-rich repeats (NBS/LRR) seems to be a perspective method for identification of the resistance genes. The mentioned sequences are available in some resistance genes and have been identified in various crops (tomato, potato, wheat, rice, flax) and also in model species such as *Arabidopsis* and *Nicotiana* (Leister et al., 1999, Salamini, 1999, Salman et al., 2000). Using RFLP probes from *Aegilops squarrosa* (*Triticum tauschii*) having resistance genes against cyst nemotade of cereals, 29 loci that presumably are homological to the analogs of the resistanse genes were identified. These loci are identical to the amino-acid sequence of cyst nemotade cre locus in *T. Tauschii*by 30-70%.

Using RFLP molecular markers *Lr1, Lr9, Lr10, Lr13, Lr19, Lr23, Lr24, Lr25, Lr27, Lr28, Lr29, Lr31, Lr34, Lr35, Lr37* and *Lr47*, which are the rust resistance genes of leaves were mapped on chromosomes. Despite the reliability of RFLP markers, they are very expensive and labor-intensive. DNA of high purity is required for these markers. Therefore, they are not suitable for the marker assisted selection. For practical purposes RFLP markers related to a corresponding resistance gene were converted to specific PVR markers-STS and CAPS. RAPD markers can be converted to SCAR ones (Cheikowski and Stepien, 2001).

Until now STS or SCAR and CAPS markers for the genes *Lr1, Lr9, Lr10, Lr24, Lr28, Lr35, Lr37* and *L47* have been reported. The enzymatic marker (endopeptidase Ep-D1c) for the gene *Lr19* was also developed and used. (Winzeler et al., 1995). Furthermore, microsatellite markers (simple sequence repeats-SSR) for the resistance genes *Lr3b*g and *Lr18* were also developed.

Moreover, molecular markers can be used in the pyramiding of various resistance genes in classical breeding process to achieve durable resistance. The durable resistance in one variety can be achieved by combination of several genesrather than by a single gene, partly encoding resistance (Cheikowski and Stepien, 2001). The development of PCR-based allel-spesific markers in poliploid species is more complicated than in diploid species. Because, PCR can cause an amplification of multiple, similar sized fragments from more than one genome (Helguera et al., 2000). Therefore, false-positive response can be obtained when the presence of the markers designed for varieties and lines described in the earlier papers (not used in experiments yet) is examined in lines and cultivars with different genetic background. Markers mentioned in the literature must be examined before using them in genetic and breeding programmes.

Therefore, the purpose of the present study was to detect brown (*Lr*) and yellow rust (*Yr*) resistance genes in Azerbaijan wheat germplasm using STS, SSR və SCAR markers.

## MATERIALS AND METHODS

***Plants materials.*** Wheat genotypes differing in resistance to diseases, productivity, architectonics and other physiological traits, collected in the Gene Pool of the Research Institute of Crop Husbandry (Baku) were used as research objects. Plants were cultivated under field conditions.

***Extraction of plant DNA.*** DNA extraction was carried out using the CTAB method with some modifications (Murray & Thompson, 1980).

***DNA quantification.*** After dissolution of the DNA the quantity was determined by optical density (OD) at λ=260 using the ULTROSPEC 3300 PRO spectrophotometer (“AMERSHAM”, USA). Purity of the genomic DNA was determined by the ratio of absorptions at A260/A280. Quality of the DNA was checked on the basis of performance of the extracted DNA samples in 0.8% agarose gel stained with 10 mg/mL of ethidium bromide in 1 × TBE (Tris base, Boric acid, EDTA) buffer. The gel was developed and photographed under ultraviolet light using “Gel Documentation System UVITEK” (UK).

***DNA amplification.*** Polymerase chain reaction was performed by Williams (1990). DNA amplification was performed in a 25 µl reaction mixture volume, 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 $MgCl_2$, and 0.2 units of Taq-polymerase in the incubation buffer. Different primers were used for the test (Table 1).

PCR was performed in the “Applied Biosystems 2720 Thermal Cycler” under the following conditions: 1 cycle - 3 minutes 94 º C; 38 cycles - 1 min at 94 º C, an annealing step at variable annealing temperatures depending on the primer

pairs for 1 min, 2 minutes at 72 º C; the final elongation cycle was performed at 72 ° C for 10 min, then kept at 4 ° C.

PCR products were analyzed by electrophoresis in a 1.2-2% agarose gel in the HR-2025-High Resolution («IBI SCIENTIFIC» U.S.) horizontal electrophoresis machine with addition of ethidium bromide and documented using «Gel Documentation System UVITEK». Dimensions of amplified fragments were determined with respect to 1kb DNA marker. Statistical analysis included binary matrix compilation for each of the primers, in which "presence" (1) or "absence" (0) of fragments with equal molecular weight on the electrophoregram were noted.

## RESULTS & DISCUSSION

***Detection of brown leaf rust resistance gene Lr9 in durum and bread wheat genotypes using STS marker.*** STS markers J13/1 and J13/2 were used for *Lr9* gene screening. The objects of the screening for this gene were 78 genotypes (26 genotypes of bread (*Triticum aestivum* L.) and 52 genotypes of durum (*Triticum durum* Desf.) wheat) (Table 2). In PCR profiles of 60% genotypes (16 of them are durum and 31 bread wheat genotypes) 1100 bp fragments were detected, suggesting the presence of the gene *Lr9* on 6B chromosomes of these genotypes. In 40% of genotypes (10 samples of durum and 21 of bread wheat genotypes) the expected fragment was not amplified (Figure 1).

**Table 1.** Nucleotide sequence of the primers used for the DNA amplification.

| Primer description | Gene | Nucleotide sequence | Annealing temperature | Product size, bp |
|---|---|---|---|---|
| J13/1 | *Lr9* | TCCTTTTATTCCGCACGCCGG | | |
| J13/2 | | CCACACTACCCCAAAGAGACG | 62 | 1100 |
| SCS123F | *Lr19* | CCTGATCACCAATGACGATT | 60 | 688 |
| SCS123R | | CCTGATCACCTTGCTACAGA | | |
| SCS253F | | GCTGGTTCCACAAAGCAAA | 63 | 737 |
| SCS253R | | GGCTGGTTCCTTAGATAGGTG | | |
| csLV34a | *Lr34* | GTTGGTTAAGACTGGTGATGG | 56 | 229 |
| csLV34b | | TGCTTGCTATTGCTGAATAGT | | 150 |
| Lr35F | *Lr35* | AGAGAGAGTAGAAGAGCTGC | 55 | 900 |
| Lr35R | | AGAGAGAGAGCATCCACC | | |
| XGWM582F | *Yr9* | AAGCACTACGAAAATATGAC | 60 | 150 |
| XGWM582R | | TCTTAAGGGGTGTTATCATA | | |
| XGM295F | *Yr18* | GTGAAGCAGACCCACAACAC | 60 | 250 |
| XGM295R | | GACGGCTGCGACGTAGAG | | |

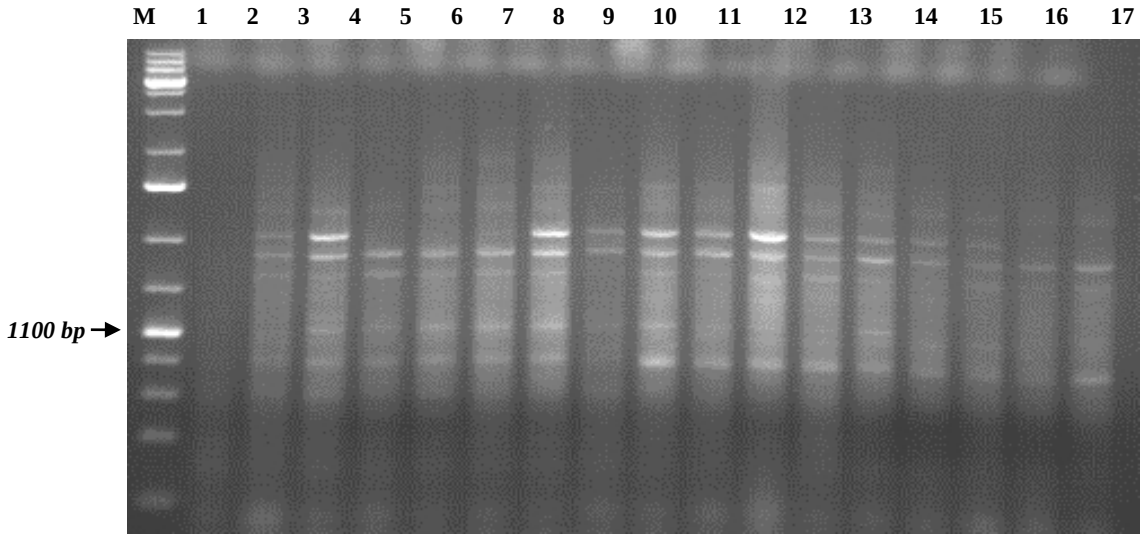


**Figure 1**. PCR - profiles of wheat plants, for *Lr9*. Arrow indicates the 1100 bp. M - 1kb Plus DNA Ladder. 1 - Vugar, 2 – Alinca 84, 3 - Tartar, 4 - Sharg, 5 – Tartar 2, 6 – Shirvan 5, 7 - Gyzyl bugda, 8- Garabag, 9 - Yagut, 10 - Turan, 11 – Mirbashir 50, 12 – Shirvan 3, 13 - Mugan, 14 - Ag bugda, 15 – Barakatli 95, 16 - Gioaza, 17 – Beltago.

**Table 2.** Results of the PCR analysis for the gene *Lr9.*

| | | | |
|---|---|---|---|
| ***Triticum durum* Desf.** | | | |
| Vugar | - | Mugan | + |
| Shiraslan 23 | - | Ag bugda | + |
| Alinca 84 | + | Kakhraba | - |
| Tartar | + | Mirvari | - |
| Sharg | - | Salt tolerant Mapping pop (F7) | + |
| Gyzyl bugda | + | ID VT 06-DTA (6) | + |
| Tartar 2 | - | S2 | + |
| Garabag | + | Barakatli 95 | + |
| Yagut | + | Kollektivnaya 77 | + |
| Turan | + | Fadda 98 | + |
| Mirbashir 50 | + | Gioaza | - |
| Shirvan 3 | + | Beltago | - |
| Shirvan 5 | - | Polonicum | - |
| ***Triticum aestivum* L.** | | | |
| Akinchi 84 | + | rsi-13 (Shafag 2) | + |
| Pirshahin 1 | - | Pirshahin | + |
| Gunashli | + | Ugur | + |
| Dagdaş | - | Parzivan 1 | - |
| FARAN Dolc | + | Parzivan 2 | + |
| Renan | + | Shaki 1 | - |
| Avreka | + | S1 | - |
| Pactole | - | Nurlu 99 | + |
| S5 | + | Gyrmyzy gul 1 | + |
| TH1 | - | Azamatli 95 | + |
| D8 sechme №5 | + | Tale 38 | + |
| S4 | + | Ruzi 84 | + |
| S3 | + | 12nd FAWWON №97 (130/21) | - |
| Sechme SS | - | 4th FEFWSN №50 (130/32) | - |
| Mirbashir 128 | + | FO2 N7-A (karlik) | + |
| Yegana | - | Tigre | + |
| Zirva 80 | + | Bezostaya 1 | - |
| Fatima | + | Kanada 2 | + |
| Azan | + | Sechme sunbul giz | + |
| Azeri | - | S9 | + |
| Murov | + | Sechme sunbul ag | + |
| Murov 2 | - | Bogdanka | + |
| Səba | + | Kripsinka | + |
| Taraggi | + | Polşa | + |
| Bayaz | + | Podolyanka | - |
| Shafag | - | Miranovka | - |

*Note: [+] – presence of the expected locus, [-] – absence of the locus.

***Detection of yellow rust resistance genes Yr9 and Yr18 using SSR markers.*** Screening of the gene *Yr9* was performed using a microcatellite marker XGWM582. Amplifications in 150 bp region are characteristics for this marker (Figure 2). It is interesting that, amplification was successful in 93% of the genotypes, indicating the presence of the gene *Yr* on 1BL chromosome of these genotypes. The exceptions are 4 samples-Azeri, 16th FAWWON-IR (46), 16th FAWWON-IR (90), 16th FAWWON-IR (47). Characteristics fragments for this gene were not synthesized in these genotypes (Table 3).

Molecular marker XGWM 295 was used for the identification of the yellow rust resistance gene *Yr18*. Electrophoretic profiles in figure 5 showed the responsibility of the used marker for the synthesis of fragments in 250 bp region. In 66% of the genotypes, amplification of the expected fragment for the gene *Yr18* was successful (Table 3).

It is known that the *gene Yr18* is genetically inseparable from the leaf rust resistance gene- *Lr34.* These genes are located on the same segment of the chromosome 7D. The locus *Lr34/Yr18* is of great practical interest for solving the discussed problem in bread wheat (Kolmer et al., 2008). Therefore, we performed also screening to test the given locus via the gene Lr34.

***Assessment of Lr34 allele status, using the codominant STS Lr34/Cslv34 marker.*** Several SSR, STS and CAPS-markers were proposed to identify the gene *Lr34.* However, codominant STS-marker csLV34, closely associated with the locus *Lr34* (0.4 cM), which is a biallelic 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 spesific marker for the allele *Lr34/csLV34a* have to lead to the amplification of 229 bp fragments (Figure 4). PCR analysis using this primer revealed corresponding locus only in 21 genotypes (Table 3). This represents approximately 34% of all tested genotypes. №50 (130/32), FO2 N7-A (dwarf) have allele *a* of the brown rust resistance gene - *Lr34.* This allele was not identified in the rest of the genotypes (66%).

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, Zirve 80, Gyrmyzy gul 1, Tale 38, Tigre, Bezostaya 1, 29 ES WVT (7)) among 61 genotypes. The electrophoretic analysis of amplified PCR products showed that the existence of allele *b* of the gene *Lr34* was not confirmed in approximately 85% of our wheat genotypes.

Interestingly that in two genotypes – Zirve 80 and Gyrmyzy gul 1 both alleles of the gene *Lr34* were identified. According to the general analysis of the results related to the both markers, 54% of the wheat genotypes do not possess any allele of the gene *Lr34.* The presence of allele 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).

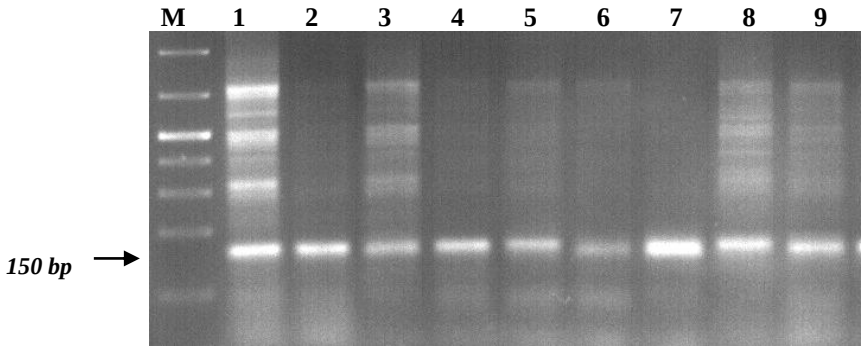


**Figure 2.** PCR - profiles of *Triticum aestivum* L. plants, for *Yr9*. Arrow indicates the 150 bp. M - molecular weight marker 100 bp. 1 - Pirshahin-1, 2 - Gunashli, 3 - Dagdash, 4 - FARAN Dolc, 5 - Renan, 6 - Avreka, 7 - Pactole, 8 - S5, 9 - TH1.

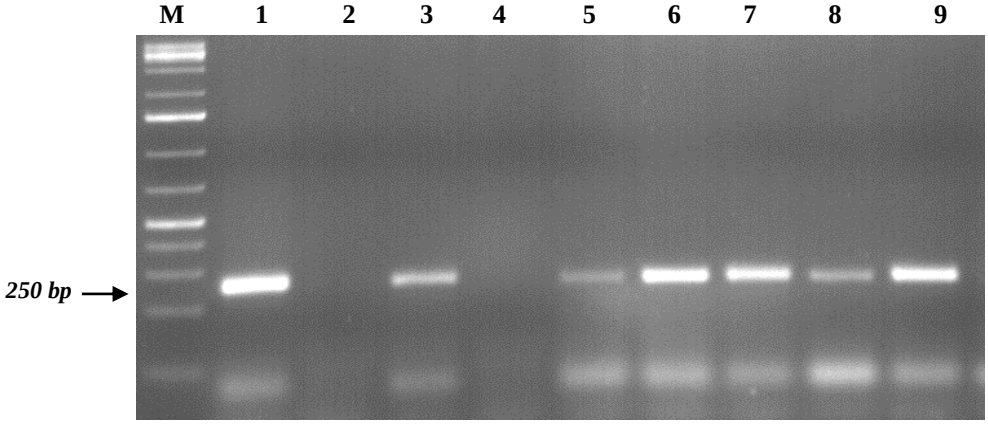


**Figure 3.** PCR - profiles of Triticum aestivum L. plants, for *Yr18*. Arrow indicates the 250 bp. M - molecular weight marker - 100 bp. 1 - Pirshahin-1, 2 - Saba, 3 - Guneshli, 4 - Bayaz, 5 - Dagdash, 6 - FARAN Dole, 7 - Renan, 8 - Avreka, 9 – Pactole.

**Table 3.** Results of the PCR analysis for the genes *Yr18*, *Yr9*, and *Lr34*.

| № | Genotypes | *Yr18* - **250bp** | *Yr9* - **150bp** | *Lr34/ csLV34a* - **229bp** | *Lr34/ csLV34b* - **150bp** |
|---|---|---|---|---|---|
| **1** | **2** | **3** | **4** | **5** | **6** |
| 1. | Pirshahin 1 | + | + | + | - |
| 2. | Gunashli | + | + | - | + |
| 3. | Dagdash | + | + | - | + |
| 4. | FARAN Dolc | + | + | + | - |
| 5. | Renan | + | + | + | - |
| 6. | Avreka | + | + | + | - |
| 7. | Pactole | + | + | + | - |
| 8. | S5 | + | + | - | + |
| 9. | TH1 | + | + | + | - |
| 10. | D8 sechme №5 | + | + | + | - |
| 11. | S4 | + | + | + | - |
| 12. | S3 | + | + | + | - |
| 13. | Sechme SS | + | + | - | - |
| 14. | Mirbashir 128 | + | + | - | - |
| 15. | Yegane | + | + | - | - |
| 16. | Zirve 80 | + | + | + | + |
| 17. | Fatima | + | + | - | - |
| 18. | Azan | + | + | + | - |
| 19. | Azeri | + | + | - | - |
| 20. | Murov | + | + | - | - |
| 21. | Murov 2 | + | + | + | - |
| 22. | Saba | - | + | + | - |
| 23. | Taraggi | + | + | + | - |
| 24. | Beyaz | - | + | - | - |

*Continued Table 3*

| 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|
| 25. | Shafag | + | + | + | - |
| 26. | rsi-13 (Shafaq-2) | + | + | - | - |
| 27. | Pirshahin | + | + | - | - |
| 28. | Ugur | + | + | - | - |
| 29. | Perzivan 1 | + | + | - | - |
| 30. | Perzivan 2 | - | + | - | - |
| 31. | Shaki 1 | - | + | + | - |
| 32. | S1 | - | + | - | - |
| 33. | Nurlu 99 | - | + | - | - |
| 34. | Gyrmyzy gul 1 | - | + | + | + |
| 35. | Azamatli 95 | - | + | + | - |
| 36. | Tale 38 | + | + | - | + |
| 37. | Ruzi 84 | + | + | - | - |
| 38. | 12nd FAWWON №97 (130/21) | + | + | + | - |
| 39. | 4th FEFWSN №50 (130/32) | + | + | + | - |
| 40. | FO2 N7-A (karlik) | + | + | + | - |
| 41. | Tigre | + | + | - | + |
| 42. | Bezostaya 1 | + | + | - | + |
| 43. | Kanada 2 | - | + | - | - |
| 44. | Sechme sunbul giz | - | + | - | - |
| 45. | S9 | + | + | - | - |
| 46. | Sechme sunbul ag | - | + | - | - |
| 47. | Bogdanka | + | + | - | - |
| 48. | Kripsinka | + | + | - | - |
| 49. | 16th FAWWON-IR (61) | - | + | - | - |
| 50. | 16th FAWWON-IR (46) | + | - | - | - |
| 51. | 16th FAWWON-IR (52) | - | + | - | - |
| 52. | 16th FAWWON-IR (90) | + | - | - | - |
| 53. | 16th FAWWON-IR (47) | + | - | - | - |
| 54. | 29 ES WVT (7) | - | + | - | + |
| 55. | 29 ES WVT (26) | - | + | - | - |
| 56. | 29 ES WVT (38) | - | + | - | - |
| 57. | 29 ES WVT (30) | - | + | - | - |
| 58. | 16 SAWWVT (29) | - | + | - | - |
| 59. | 16 SAWWVT (34) | - | + | - | - |
| 60. | 39 IBWSN (97 №) | - | + | - | - |
| 61. | 11st IWWYT-R (9816 №) | - | + | - | - |

*Note: [+] – presence of the expected locus, [-] – absence of the locus.

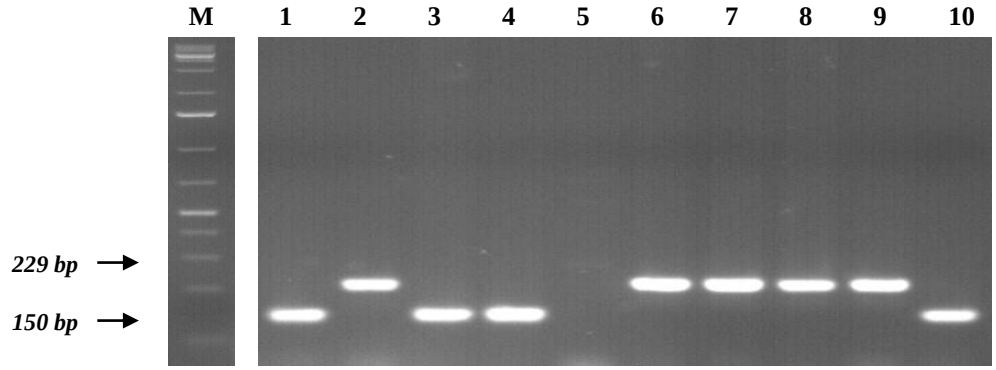


**Figure 4.** PCR - profiles of *Triticum aestivum* L. plants for *Lr 3*4. Arrow indicates the 150 bp and 229 bp. molecular weight marker - 100 bp. 1 - Dagdash, 2 – Pirshahin 1, 3 - Guneshli, 4 - S5, 5 - Saba, 6 - FARAN Dole, 7 - Renan, 8 - Avreka, 9 - Pactole, 10 – Tale 38.

We have compared the results obtained for the brown rust resistance gene *Lr34* as well as for the yellow rust resistance gene *Yr18*. As the gene imparting resistance has *b* allele, comparison was performed according to this allele. Only 7 genotypes from the tested 61 showed a positive result for the both genes. Therefore, we can confidently say that the locus *Lr34/Yr18* is present on 7D chromosomes of the genotypes Gunashli, Tigre, Tale 38, S5, Dagdash, Zirve 80, Bezostaya-1. Negative results were obtained for 19 genotypes (approximately, 33%) for the both genes. In other words, the locus *Lr34/Yr18* related to resistance to both brown and yellow rust is absent in these genotypes. Positive

results for *b* allele of the gene *Lr34* and negative results for the gene *Yr18* were obtained only for two genotypes. The amplification of the characteristic fragments for the gene *Yr18* was successful in 54% of the genotypes and specific fragments for b allele of the gene *Lr34* were not synthesized. It should be noted that, some of these genotypes, lacking this locus are resistant to deleterious diseases in field conditions. Apparently, the resistance of such genotypes is determined by other genes.

Genetic diversity of modern bread wheat varieties (*Triticum aestivum* L.) based on the genes of resistance to yellow rust (P. *stiiformis* Westend. f. sp. *tritici*) and brown rust (Puccinia tritici Erikss.) is small. At best 1-2 and sometimes 3 genes are identified in these varieties (Gaynullin, 2008, Sibikeyev, 2002, Mesterhazy et al., 2000). The protection strategy of bread wheat from rust, which is the most common and harmful disease, includes several directions having their pros and cons. From a genetic point of view, the search for the new resistance genes *Lr* and *Yr*, as well as creation of varieties that combine race-specific and nonspecific resistance, provide effective and long-term resistance to these infections (Sehgal et al., 2012). Therefore, finding sources and donors of bread wheat to this disease, as well as identifying combinatory of the genes *Lr* and *Yr*, providing resistance to brown and yellow rust, remains an urgent problem in the selection of bread wheat.

***Determination of brown leaf rust resistance genes Lr19 and Lr35 using SCAR markers.*** *Lr19* localized on the 7D chromosome is one of the few widely effective genes conferring resistance against brown leaf rust in wheat. (Gupta et. al., 2006) Foreign *Lr19* gene demonstrated efficacy against all pathotypes of leaf rust in South Africa (Prins et al., 1997) India (Tomar and Menon, 1998), Europe (Mesterhazy et al., 2000) and Canada (McCallum and Seto-Goh, 2003). The *Lr19* translocation is associated with deleterious agronomic effects and as a result modified forms of the translocation have been derived by different researchers in an attempt to remove the genes responsible. It was reported that *Lr19* was associated with increases in grain yield. Aerial biomass was also increased when *Lr19* was introgressed, although differences were not ssociated with improved light interception (indirectly measured) or radiation use efficiency (RUE). The physiological basis of the increased biomass and the mechanisms causing increased number of grains per spike, in terms of dynamic of floret development, are not completely understood.

*Lr-19* translocation originally produce by Sharma and Knott (1966) when they transform leaf rust resistance genes 7e l1 chromosome of Thinopyrum ponticum a long arm of chromosome 7 D of common wheat. Heurta-Espino and Singh (1994) reported first virulence in Puccinia Triticinan to *Lr-19* and it is an effective source of leaf rust resistance worldwide. The cut-of point of *Lr-19* translocation is located in the middle of long arm of chromosome 7D and find that the distal half of 7 D was replaced by Thinopyrum Chromatinv. During meiosis Thinopyrum segment 7DL does not pair with homologous wheat segment, complicating attempts to study linkage relationship or to recombine its genes (Sehgal, 2012).

Despite the virulence for the *Lr19* gene, there are reports that in the last decade it has demonstrated high efficacy in wheat cultivation areas (Huerta-Espino and Singh, 1994; Sibikeev et al., 1997). High efficacy of the *Lr19* gene in Asia, Australia and Europe indicates that this gene can be used in combination with other Lr genes for long-term resistance to leaf rust all over the world (Roelfs, 1988; Pink, 2002)

On this basis, the objective of this study was to determine the presence of the *Lr19* gene in different wheat genotypes using SCAR markers. DNA samples were screened using two SCAR molecular markers bound to a known *Lr19* gene of resistance to brown leaf rust. SCAR markers are polymorphic and amplified unique bands linked to the *Lr19* gene (Gupta et al., 2006). This gives a possibility of using these markers in marker-assisted breeding for *Lr19* gene.

Figure 5 reflects the PCR profiles performed using SCS123 F/R molecular marker. This marker must lead to the amplification of fragments of 688 bp in size. As a result of PCR test with this primer the locus of the 688 bp region was detected only in 48 genotypes. This is approximately 79% of all investigated genotypes.

Fragment linked to the SCS123F/R marker was not synthesized in the following genotypes - Pirshahin-1, Pactole, 8th WWEERYT (32 №), 3 RBWYT (521 №), 3 RBWYT (536 №), 11st IWWYT-R (9816 №), S5, 16th FAWWON-IR (90), 16th FAWWON-IR (47), S1, Nurlu-99 Kyrmyzygyul-1, 12nd FAWWON № 97 (130/21).

The second SCAR marker linked to the studied *Lr19* gene of resistance to brown leaf rust was SCS253 F/R. Amplification products with the use of this marker are detected in the 737 bp region. As can be seen from Figure 6, the expected fragment in the 737 bp region was synthesized in only in 53 of 61 genotypes, in other words, in approximately 87% of all investigated genotypes. Fragments specific for the SCS 253F/R SCAR marker were not amplified in the following genotypes - 3 RBWYT (521 №), Zirve 80, Gyrmyzy gul 1, S1, Azamatli 95, Tale 38, Ruzi 84 and 12nd FAWWON № 97 (130/21).

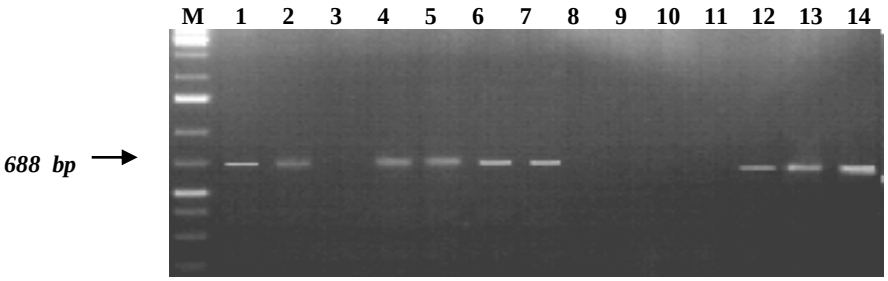


**Figure 5.** PCR-profiles of *Triticum aestivum* L. plants induced by SCS123F/R primer. Arrow indicates the 688 bp. M- 1 kb DNA ladder. 1 – 10 SAWVT (11 №), 2 – 3th FAWWON (117 №), 3 – 8th WWEERYT (32 №), 4 – 4th RWVT-LRCA (89 №), 5 – 14th FAWWON (86 №), 6 – 8th WONSA (65 №), 7 – 9th WON-SA (27 №), 8 – 3 RBWYT (521 №), 9 – 12nd FAWWON №97 (130/21), 10 – 3 RBWYT (536 №), 11 – 3 RBWYT (518 №), 12 – 39 IBWSN (113 №), 13 – 14 SAWYT (49 №).

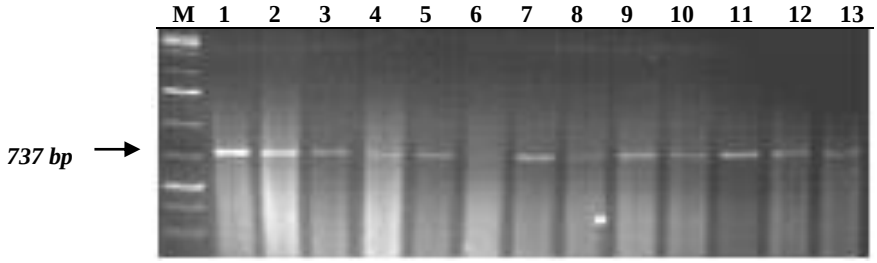


**Figure 6**. PCR-profiles of *Triticum aestivum* L. plants induced by the SCS253F/R primer. Arrow indicates the 737 bp. M – 1kb DNA ladder. **1** - 39 IBWSN (97 №), 2 - 11st IWWYT-R (9816 №), 3 – S5, 4 – Mirbashir 128, 5– Yegane, 6 – Zirve 80, 7 – Fatima, 8 – Aran, 9 – Azeri, 10 – Murov, 11 – Murov-2, 12 – Saba, 13 – Taraggi.

Comparative analysis of PCR profiles obtained with the use of both SCAR markers demonstrates, that the results are the same in 82% of genotypes: specific amplification fragments were identified in 45 genotypes with the use of both SCS123F/R and SCS253F/R markers, which indicates that the *Lr19* gene of resistance to brown leaf rust is present on 7D chromosomes of these genotypes. The existence of the *Lr19* gene has not been proven in 5 of 61 genotypes used, because specific fragments amplified with any of the applied markers were not identified in these genotypes.

The results obtained with different markers did not match in 18% of genotypes. After using the SCS123F/R marker, nine genotypes (Pirshahin-1, Pactole, 8th WWEERYT (32 №), 3 RBWYT (536 №), 11st IWWYT-R (9816 №), S5, 16th FAWWON-IR (90), 16th FAWWON-IR (47), Nurlu 99) did not match, i.e. fragments in the 688 bp region specific for the SCS123F/R marker were not synthesized in these genotypes, on the contrary, the 737 bp fragments, linked with the SCS253F/R marker, were amplified. And this kind of mismatch was detected in three genotypes (Zirve 80, Azamatli 95, Ruzi 84) with the use of the SCS253F/R marker, in other words, amplification products specific for the SCS253F/R marker were absent in these genotypes, on the contrary, synthesis of PCR profiles specific for the SCS123F/R marker has successfully been performed.

The absence of marker components with the Lr19 gene in these samples may be due to an incomplete linkage of the marker and the gene (Tirishkin, 2006).

Attention is drawn to the fact that resistance and high sensitivity to brown leaf rust are observed among the genotypes in which amplification products have not been revealed, thus indicating the absence of the *Lr19* gene.

Gyrmyzy gul-1 wheat genotype in field conditions also demonstrates high susceptibility to the brown rust pathogen and is completely affected by the *Puccinia recondite* f. sp. *tritici* fungus. The genotypes called 3 RBWYT (521 №), S1 and Tale-38 in field conditions are estimated as moderately resistant to this disease. It is interesting that the 12nd FAWWON № 97 (130/21) genotype actually demonstrates high resistance to this harmful disease, despite the absence of the Lr19 gene. Apparently, the resistance of this genotype may be caused by other *Lr*-genes.

The marker Lr35F/Lr35R was used to identify the gene *Lr35*. The analysis was performed on 61 bread (*Triticum aestivum L.)* wheat genotypes.

When using the marker *Lr35F/Lr35R*, specific fragments had to be synthesized at 900 bp region (the figure is not presented). Fragments were not visualized at this region in the obtained electrophoretic profiles. In other words, using this marker, the existence of the gene *Lr35* on 2B chromosomes have not been proven.

## CONCLUSION

The study of the genetic basis of plant resistance, the search for effective genes and their introduction into the culture of wheat significantly prevent the spread of the epiphytotic disease and stabilize the grain yield capacity. Development and deployment of cultivars with host genetic resistance is the most ecofriendly way to reduce the losses. The use of modern molecular-genetic techniques greatly accelerates the process of the identification of genotypes resistant to diseases and the creation of disease-resistant varieties. Molecular markers are widely used for the investigation of the bread wheat genome structure, identifying and mapping genes responsible for expression of the useful properties, as well as for the isolation and cloning of genes for studying their controlled properties and transmission them to other varieties (i.e. for genetic transformation). Thus, the use of molecular markers in breeding allows us to obtain information on the sign at the early stages of development, without waiting for the phenotypic expression of feature, simplifies testing resistance to various diseases, requiring thorough assessment by traditional research methods.

The wheat cultivars are of different types and become susceptible to different types of rust because it has 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. The wheat production has been dependent on the use and development of rust resistance genotypes having well characterized and diverse genes. It is also concluded that in wheat certain and different combinations of genes give long lasting and better resistance for rust diseases than given by any individual genes.

The obtained results can be used in breeding and genetic programs on creation of forms resistant to leaf rust pathogen populations in Azerbaijan. Thus, information about the existence of effective *Lr* and *Yr* genes in adapted varieties that can be used as donors for resistance, and usage of these distinct genes or by pyramiding of different resistance genes in the genotype can significantly improve the efficiency of breeding of resistant varieties, thus assisting to avoid the creation of varieties that are genetically homogeneous. This information will serve as a foundation for plant breeders and geneticists to develop durable rust-resistant wheat varieties through marker-assisted breeding or gene pyramiding.

## ACKNOWLEDGEMENT

This work was financially supported by the Science Development Foundation under the President of the Republic of Azerbaijan **(EIF/GAM-2-2013-2(8)-25/16/3).**

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## Azərbaycan Buğda Germplazmasında Qonur Pasa Qarşı *Lr9, Lr19, Lr34, Lr35* və Sarı Pasa Qarşı *Yr9, Yr18* Davamlılıq Genlərinin Molekulyar Analizi

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Pas dənin məhsuldarlığını aşağı salan təhlükəli göbələk xəstəliyidir. Pas xəstəliklərinə nəzarət üçün pasa davamlı sortların becərilməsi səmərəli və ekoloji baxımdan daha təhlükəsiz yanaşmadır. Müxtəlif molekulyar metodlardan istifadə etməklə, yumşaq və bərk buğda genotiplərində və onların yabanı növlərində hələlik qonur pasa qarşı 81 və sarı pasa qarşı 53 davamlılıq geni aşkar edilmişdir. Tədqiqat işinin əsas məqsədi Əkinçilik ET İnstitutunun genofondunda toplanmış buğda nümunələrində *Lr* və *Yr* genlərinin mövcudluğunu müəyyənləşdirmək olmuşdur. *Lr9, Lr19, Lr34, Lr35* və *Yr9* və *Yr18* genləri müxtəlif molekulyar markerlərlərdən istifadə etməklə analiz olunmuşdur. PZR profillərdə 1100 bp uzunluğunda spesifik fraqmentlərin olması ilə tədqiq edilən 78 genotipdən 60%-nin 6B xromosomlarında *Lr9* geninin mövcudluğu müəyyən edilmişdir. SCS123 və SCS253 SCAR markerlərin köməyilə *Lr19* geninin identifikasiyası zamanı 45 genotipdə müsbət nəticə müşahidə edilmişdir. *Lr43* geninin allel vəziyyəti Lr34/csLV34a və Lr34/csLV34b markerlərin köməyilə tədqiq edilmişdir. Molekulyar analiz 21 genotipdə *Lr34* geninin *a* allelini, 9 genotipdə isə *b* allelini üzə çıxarmışdır. İki genotipdə isə *Lr34* geninin hər iki alleli müşahidə edilmişdir. Genotiplərin 54%-də Lr43 geninin heç bir alleli müəyyən edilməmişdir. 61 genotipin PZR profillərində *Lr35* geni üçün xarakterik fraqmentlər aşkar edilməmişdir. 40 genotipin elektroforetik profillərində *Yr18* geni üçün diaqnostik 250 bp fraqmentlər müşahidə edilmişdir. 4 genotip istisna olmaqla, digər genotiplərin hamısında *Yr9* geni üçün xarakterik 150 bp sahədə fraqmentlər amplifikasiya olunmuşdur. Bu nəticələr Azərbaycanda buğdanın pas xəstəliklərininə nəzarət edilməsi və pasa davamlı buğda sortlarının yaradılmasında əsas kimi istifadə oluna bilər.

***Açar sözlər:*** *Buğda, qonur pas, sarı pas, Lr9, Lr19, Lr34, Lr35, Yr9, Yr18, PCR, STS, SSR, SCAR*

## Молекулярный Анализ Генов Устойчивости *Lr9, Lr19, Lr34, Lr35* к Бурой Ржавчине И Yr9, Yr18 к Желтой Ржавчине в Гермплазме Пшеницы Азербайджана

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Ржавчина – это опасное грибковое заболевание, понижающее урожайность зерна. С целью усиления контроля над заболеванием ржавчиной наиболее выгодным и безопасным с экологической точки зрения подходом является выращивание устойчивых к этому заболеванию сортов. С помощью различных молекулярных методов на сегодняшний день в генотипах мягкой и твердой пшеницы и их диких видов выявлены 81 ген, устойчивый к бурой ржавчине, и 53 гена, ответственных за устойчивость к желтой ржавчине. Основная цель работы заключалась в установлении наличия генов *Lr* и *Yr* в образцах пшеницы, взятых из генофонда НИ Института земледелия. С использованием различных молекулярных маркеров были изучены четыре *Lr* гена – *Lr9, Lr19, Lr34, Lr35* и два *Yr* гена – *Yr9* и *Yr18*. Благодаря обнаружению в ПЦР профилях специфических фрагментов длиною 1100bp, у 60%-ов из исследованных 78 генотипов было выявлено наличие в 6B хромосоме гена *Lr9*. При идентификации гена *Lr19* с помощью SCAR маркеров SCS123 и SCS253 был получен положительный результат в 45 генотипах. Аллельное состояние гена *Lr43* было исследовано с помощью маркеров Lr34/csLV34a и Lr34/csLV34b. Молекулярным анализом в 21 генотипе была выявлена *a* аллель, а в 9 генотипах – *в* аллель гена *Lr34*. В двух же генотипах наблюдались обе аллели гена *Lr34*. У 54%-ов генотипов алели гена Lr43 не выявлялись. В ПЦР профилях 61 генотипа не обнаруживались характерные для гена *Lr35* фрагменты. На электрофоретических профилях 40 генотипов наблюдались диагностические для гена *Yr18* фрагменты длиной 250 bp. Во всех генотипах, за исключением четырех, амплифицировались характерные для гена *Yr9* фрагменты длиной 150 bp. Наши результаты могут служить основанием для выведения продолжительно устойчивых к ржавчине сортов пшеницы и усиления, тем самым, контроля над заболеванием ржавчиной пшеницы в Азербайджане.

***Ключевые слова:*** *Пшеница, бурая ржавчина, желтая ржавчина, Lr9, Lr19, Lr34, Lr35, Yr9, Yr18, ПЦР, STS, SSR, SCAR*