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Evaluation of Winter Wheat Varieties For Resistance to Yellow and Brown Leaf Rust in Azerbaijan

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ABSTRACT

The objective of work was to identify effective yellow and brown leaf rust resistance genes for the improvement of wheat quality in Azerbaijan. 61 bread wheat (Triticum aestivum L.) genotipes were taken from Research Institute of Crop Husbandry (Baku, Azerbaijan). PCR amplification of the SSR marker located on the chromosome 7D Xgwm295 linked to the Yr18 gene showed a fragment of 250 bp only in 40 genotypes. SSR marker XGWM582 in the chromosome 1B, associated with the gene Yr9 amplified a band of 150 bp in 93% of genotypes. The STS markers Lr34/csLV34a and Lr34/csLV34b were proved as effective markers for the identification and selection of Lr 34 gene. The csLV34a allele (229-bp) was linked in repulsion with Lr34 presented in 21 genotypes, whereas the csLV34b allele (150-bp) was linked in coupling with Lr34 in only 9 genotypes. 2 genotypes contain both alleles linked to the Lr34 .

KEYWORDS : yellow rust, Yr 18, Yr9, brown leaf rust, Lr 34, Triticum aestivum L.

Introduction

Wheat (*Triticum aestivum* L.) is an important crop and a primary food source for humans. Demand for wheat is growing due to the rapid growth of the world population. To ensure food security of the increasing world population breeding and introduction of new high yielding varieties resistant to abiotic and biotic stresses has been and remains the most important task facing breeders and wheat producers.

Yellow and brown rust diseases are currently one of the main factors decreasing wheat productivity and quality all over the world including Azerbaijan. Depending on the severity and duration of infection, yield losses may reach 40-50% (McIntosh et al., 1995; McIntosh et al., 2008). Yellow rust of wheat is the most common and harmful fungal disease caused by the fungal pathogen Puccinia striiformis f. sp. tritici. In most regions of the wheat cultivation crop losses caused by yellow rust, vary from 10% to 70% depending on the susceptibility of varieties, the rate of development of the disease and its duration (Chen, 2005). During the last decades significant advancements have been reached in the struggle against these diseases.

The most effective, environmentally and economically feasible way to protect wheat from the rust disease is the use of resistant varieties. However, the regular evolution of pathogens and appearance of their virulent races require a continuous search for new donors of sustainability.

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). Brown rust caused by the fungus *Puccinia triticina Eriks*, also remains one of the most damaging diseases of wheat worldwide. To date, more than 70 Lr genes controlling resistance to leaf rust, located on different chromosomes of wheat and related species differing in their effectiveness have been described.

Genes of resistance were primarily observed in bread wheat (*Triticum aestivum)*. However, majority of the genes were introduced through introgression from other species and wild cereals (Riar et al., 2012). For instance, Yr8 was introduced into bread wheat from *Aegilops comosa,* Yr9 from rye, Yr24 and Yr28 from *Aegilops tauschii,* Yr26 from *Haynaldia villosa.* Various useful foreign genes have been introduced into wheat (Sibikeyev, 2002). Although efficiency of various genes differs depending on regions, on the whole there are not genes which are not overcome. For example, genes Yr1, Yr2, Yr3, Yr9 və Yr1+Yr3, Yr2+Yr3, YrH-Yr9 that occur more frequently in Holland were reported to be gene combinations. Wheat genotypes in Australia were shown to be protected from brown rust by Lrl3, Lr24, Lr34, Lr37 and from yellow rust by Yrl7, Yrl8 genes (Gaynullin, 2008, Park et al., 2007, Sibikeyev, 2002). It is interesting that genes Yr18 and Lr34 are liked and both of them are widely spread in selection materials of CIMMYT, Southern and Nothern America and China (Kolmer et al., 2008).

The gene Lr34, confers durable, adult plant resistance (APR) to leaf rust caused by *Puccinia triticina*, stripe rust caused by *Puccinia striiformis* f.sp. *tritici* and powdery mildew caused by *Blumeria graminis*. The Lr34/Yr18 gene has been used in agriculture for more than 100 years. The gene Lr34, which is localized on chromosome 7D is among the most highly effective genes of resistance to brown rust. (Karelov et al., 2011). Unlike other Lr-genes, it is not race-specific and provides overall stability for wheat throughout the vegetation period (Singh & Rajaram, 1992). In this respect, the gene is of great value. Furthermore, it was found that it is genetically inseparable from the gene Yr18, providing moderate resistance to yellow rust (Kolmer et al., 2008). Lr34/Yr18, gene for resistance to brown rust is also known as «slow rusting gene» (Lagudah et al., 2006). This makes Lr34 a unique and highly valuable resource for the rust resistance breeding.

Amounts of R genes, which are effective against rust disease causatives decrease from year to year. Because, sexual hybridization and other processes occurring in pathogen lead to creating of virulent biotypes and forms, which overcome the existing resistance. Therefore the search of the new genes of this kind is always inevitable.

Higher effectiveness of programs for improving the yield and grain quality can be achieved by using selection with molecular markers (MAS, Marker Assisted Selection). One of the advantages of these markers is their relative stability and independence from environmental changes. Such an approach is important and relevant for selection (Sehgal, 2012). Using molecular biology techniques DNA-markers have been elaborated recently. Binding to relative resistance genes these markers provide reliable means for the identification of the genes.

Several types of markers have been proposed for the identification of the genes Yr and Lr, but the microsatellite markers are the most widely used. Microsatellites show a much higher level of polymorphism and informativeness in hexaploid bread wheat than any other marker system. The efficiency of microsatellite marker development depends on the abundance of repeats in the target species and the ease with which these repeats can be developed into informative markers. The genomic abundance of microsatellites and their ability to be associated with many phenotypes, make this class of molecular markers a powerful tool for diverse application in plant genetics. Since STS markers are used in such experiments as genome mapping or revelation of tested sequences in recombinant clones, when polymorphism is not required, STS markers can be considered as monomorph DNA-markers.

On this basis, the objective of this study was to determine the presence of the Yr18, Yr9 and Lr34 genes in different wheat genotypes using microsatellite and STS markers.

Materials and methods

61 soft wheat genotypes (*Triticum aestivum L.*) collected in the Gene Pool of the Research Institute of Agriculture acted as a research object. Plants were cultivated in field conditions.

Extraction of plant DNA

DNA extraction was carried out using the CTAB method with some modifications (Murray & Thompson, 1980). Fresh plant tissue as a fragment of leaf was minced in liquid nitrogen, suspended in 1000 µl of CTAB extraction buffer (100 mM Tris-HCl, pH 8.0; 20 mM EDTA, pH 8.0; 1.4 mM NaCl; 40 mM β-mercaptoethanol), and pre-warmed in a water bath at 60°C. Homogenization was completed by intense Vortex shaking. Then 400 ml of chloroform (99.8%) was added into each tube and the tubes were gently mixed. Next the tubes were placed in a water bath and incubated for 10 min at 60 °C. After incubation, the tubes were centrifuged in an Eppendorf type benchtop centrifuge (15,000 g) for 10 min at room temperature. After centrifugation the supernatant was carefully selected (taking care not to capture sediment particles) and transferred to clean 1,5 ml Eppendorf type tubes and 600 ml of cold isopropanol was added, mixed well and left at room temperature for 3-5 minutes. At this stage we can observe the dispersed DNA precipitate. The tube contents were centrifuged at room temperature in the Eppendorf type benchtop centrifuge (15,000 g) for 10 min.

The precipitate was washed several times with 70% ethanol, dried in a thermostat at 56 °C for 5 minutes and dissolved in TE buffer (10 mM Tris-HCl, pH 8; 1 mM EDTA). Samples were left in a refrigerator at 4°C for the complete dissolution of the DNA in a buffer.

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. Two SCAR primers - SCS123 and SCS253 (Eurofins mwg operon) – were used for the test (Table 1). PCR was performed in the "Applied Biosystems 2720 Thermal Cycler" (Singapore) thermocycler under the following conditions: 1 cycle - 3 minutes at

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

Primer description	Gene	Sequence 5′→3′	Annealing temperature	Product size
F:XGWM582	Yr9	AAGCACTACGAAAATATGAC	60	150
R: XGWM582		TCTTAAGGGGTGTTATCATA		
F: XGM295	Yr18	GTGAAGCAGACCCACAACAC	60	250
R: XGM295		GACGGCTGCGACGTAGAG		
F: csLV34	Lr34	GTTGGTTAAGACTGGTGATGG	56	229 150
R: csLV34		TGCTTGCTATTGCTGAATAGT		

94 ° C; 38 cycles - 1 min at 94 ° C, an annealing step at variable annealing temperatures depending on the primer pairs for 1 min, 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 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 and discussion

Screening of the gene Yr18 was performed using a marker XGWM 295 (3′ GTGAAGCAGACCCACAACAC5′/3′GACGGCTGCGACGTAGAG5′).Electrophoretic profiles in figure 1 showed the responsibility of the used marker for the synthesis of fragments in 250 bp region. In 66% of the fragments amplification of the expected fragment for the gene Yr 18 was successful (Table 2). Microcatellite marker XGWM582 (3′ AAGCACTACGAAAATATGAC 5′/3′ TCTTAAGGGGTGTTATCATA 5′) was used for the identification of the yellow rust resistance gene Yr9. Amplifications in 150 bp region are characteristics for this marker (Fig.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 2).

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. Therefore we performed also screening to test the given locus via the gene Lr34. 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 (Langudah et al. 2006). Therefore, we used markers Lr34/

csLV34a (3'GTTGGTTAAGACTGGTGATGG5') and Lr34/csLV34b (3' TGCTTGCTATTGCTGAATAGT 5') to identify a and b alleles of the gene Lr34. The spesific marker for the allele Lr34/csLV34a have to lead tothe amplification of 229 bp fragments (Fig. 3). PCR analysis using this primer revealed corresponding locus only in 21 genotypes (Table 2). 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 Lr 34 , 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 (Guneshli, Dagdash, S5, Zirve-80,Girmizigul-1, Tale-38, Tigre, Bezostaya-1, 29 ES WVT (7)) among 61 genotypes. The analysis of the documented electrophoresis gels of amplified PCR products when using this marker, showed that the existence of allele *b* of the gene *Lr34* was not confirmed in approximately 85% of our wheat genotypes

Figure 1. PCR - profiles of Triticum aestivum L. plants, for Yr18. Arrow indicates the 250 bp. Molecular weight marker - 100 bp.

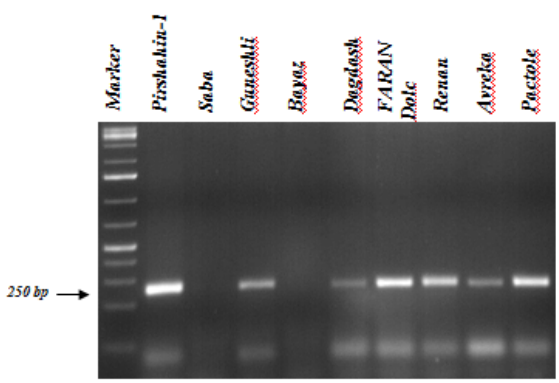


Figure 2. PCR - profiles of Triticum aestivum L. plants, for Yr9. Arrow indicates the 150 bp. Molecular weight marker - 100 bp.

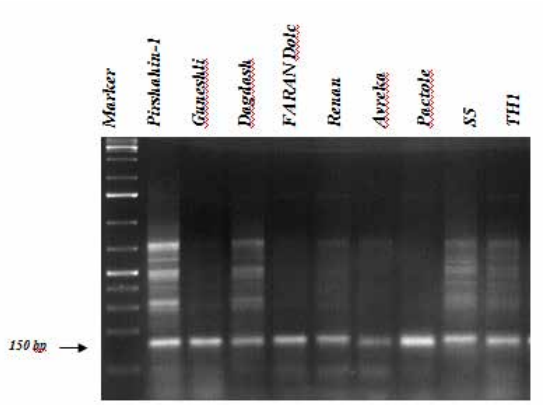
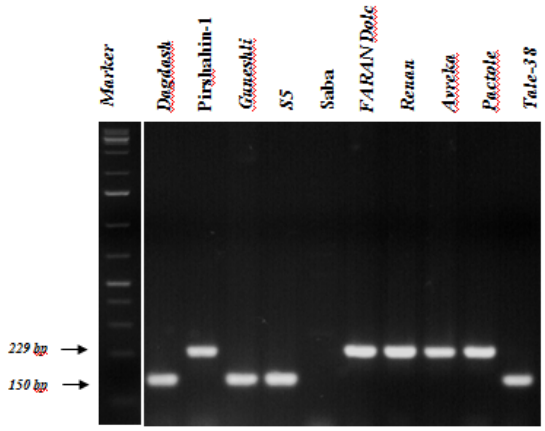


Figure 3. PCR - profiles of Triticum aestivum L. plants, for Lr 34. Arrow indicates the 150 bp and 229 bp. Molecular weight marker - 100 bp.



Interestingly, that in two genotypes –Zirve-80 and Gyrmyzygul-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).

Table 2. Results of the PCR analysis for the genes Yr18, Yr9 and Lr34

№	Genotypes	Yr18, 250bp	Yr9, 150bp	Lr34/ csLV34a, 229bp	Lr34/ csLV34b, 150bp
1	Pirshahin-1	+	+	+	-
2	Guneshli	+	+	-	+
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	Taragi	+	+	+	-
24	Beyaz	-	+	-	-
25	Shafaq	+	+	+	-
26	rsi-13 (shafaq-2)	+	+	-	-
27	Pirshahin	+	+	-	-
28	Ugur	+	+	-	-
29	Perzivan-1	+	+	-	-
30	Perzivan-2	-	+	-	-
31	Sheki-1	-	+	+	-
32	S1	-	+	-	-
33	Nurlu-99	-	+	-	-
34	Gyrmyzyi 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.**

It would be interesting to compare our 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, we performed the comparison according to this allele. Only 7 genotypes from the tested 61 showed a positive result for the both genes (Table 2). Therefore we can confidently say that the locus Lr34/Yr18 is present on 7D chromosomes of the genotypes Guneshli, Tigre, Tale-38, S5, Dagdash, Zirve-80, Bezostaya-1. Negative results were obtained in 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 Lr 34 and negative results for the gene Yr 18 were obtained only in two genotypes. The amplification of the characteristic fragments for the gene Yr 18 was successful in 54% of the genotypes and specific fragments for b allele of the gene Lr 34 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.) in 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 non-specific 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.

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.

Carrying out further researches is considered relevant. Moreover, the material under study is a valuable resource for wheat breeding for resistance to leaf rust. Thus, knowing better adapted varieties present in effective Yr and Lr genes which may be used as donors of resistance in wheat breeding programs in Azerbaijan and using these genes or by pyramiding of different resistance genes in this genotype can significantly improve the efficiency of resistant variety breeding (Mesterhazy et al., 2000), thus helping to avoid the creation of varieties that are genetically homogeneous (Mebrate et al., 2008).

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