



MARMARA UNIVERSITY
INSTITUTE FOR GRADUATE STUDIES
IN PURE AND APPLIED SCIENCES



ASSESSMENT OF GENETIC DIVERSITY
in YELLOW RUST DISEASE RESISTANT
WHEAT (*Triticum aestivum* L.) GENEPOOL
and GENE EXPRESSION ANALYSES

TÜLİN TAŞCIOĞLU

MASTER THESIS

Department of Bioengineering

ADVISOR

Prof. Dr. Ahu ALTINKUT UNCUOĞLU

CO- ADVISOR

Dr. Özge KARAKAŞ METİN

ISTANBUL, 2014



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ÖZET

SARI PAS HASTALIĞINA DAYANIKLI EKMEKLİK BUĞDAY (*Triticum aestivum* L.) GEN HAVUZUNDA GENETİK ÇEŞİTLİLİĞİN BELİRLENMESİ VE GEN ANLATIM ANALİZLERİ

Puccinia striiformis f. sp. *tritici* adlı fungal patojenin neden olduğu sarı pas hastalığı buğdayda (*Triticum aestivum* L.) özellikle epideminin görüldüğü yıllarda önemli verim kayıplarına neden olmaktadır. Bu çalışmada sarı pasa dayanıklı kışlık ekmeklik buğday çeşitlerinin yer aldığı gen havuzunda genetik çeşitlilik ve sarı pas dayanıklılığı ile ilişkili olan *Yr10* geninin anlatım profili ortaya konuldu.

Genetik çeşitlilik çalışmalarında farklı derecelerde sarı pas dayanıklılığı gösteren 30'u Türkiye'den 25 tanesi de Orta Doğu'dan olmak üzere toplam 55 kışlık ekmeklik buğday genotipinde A, B ve D genomlarına dağılmış olan 117 SSR markörünün kullanımıyla "Principal Component Analysis" (PCA) gerçekleştirildi. Her iki populasyon kendi içinde ayrı ayrı değerlendirildiğinde genetik olarak Çetinel-2000 ve Türkmen'in Türkiye için, Behoth 8 ve Douma 4'nin ise Orta Doğu için en yakın çeşitler olduğu belirlendi. Türkiye ve Orta Doğu populasyonlarının birarada değerlendirildiği durumda ise genetik olarak en uzak genotip çiftlerinin Ceyhan-99 – Behoth 6, Gerek 79 – Douma 40989 ve Karahan-99 – Douma 48114 olduğu gösterildi. Tüm populasyonların dağılımını göstermek amacıyla gerçekleştirilen Structure analizi sonucunda gen havuzumuzdaki materyalin beklenildiği gibi Türkiye ve Orta Doğu'nun yanısıra her ikisinde de yer alan populasyonları içeren üçüncü bir ortak dağılım bölgesi oluşturduğu belirlendi.

Sarı pas dayanıklılığı ile ilişkili *Yr10* geninin mevcut gen havuzunda varlığının ortaya konmasını takiben Gerek 79 ve Türkmen çeşitleriyle pozitif kontrol olarak kullanılan Avocet *Yr10*'da sarı pas inokülasyonu gerçekleştirilerek *Yr10* geninin 7 farklı zaman diliminde (0. saat – MOCK, 15.dakika, 12.saat, 24. saat, 48.saat, 72.saat, 96.saat) gerçek zamanlı anlatım profili ortaya konuldu. Sonuçlar değerlendirildiğinde tüm genotipler için *Yr10* geninin anlatımının en fazla 24.saatte düştüğü belirlendi. *Yr10*

geninin anlatımı Gerek 79'da 24.saate kadar azalma gösterirken bu durum Türkmen genotipinde 24.saatten sonra artış yönünde deęişmiştir.

Sonuçta, *Yr10* geninin anlatımının genotip ve patojen etkileşimi açısından zamana baęlı olarak dalgalanma gösterdiği ortaya konuldu. Anlatım profilini tümüyle deęerlendirebilmek için patojen-bitki etkileşiminin daha uzun bir zaman diliminde ve daha fazla sayıda genotip ile ele alınması gereklilięi söz konusudur. Benzer bitki ıslahı çalışmalarında uygun genotiplerin seçilmesinde genetik çeşitlilik açısından mevcut bilgiler oldukça yol göstericidir. Bu nedenle, tez çalışması sonucunda *Yr10* geni taşıdığı belirlenen Gerek79 ve Türkmen genotipleri ile genetik açıdan birbirine en uzak olan genotip çiftleri (Ceyhan-99 – Behoth 6, Gerek 79 – Douma 40989 ve Karahan-99 – Douma 48114) ıslah programlarında kullanılabilecek aday genotiplerdir.

Şubat, 2014

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ABSTRACT

ASSESSMENT OF GENETIC DIVERSITY IN YELLOW RUST DISEASE RESISTANT WHEAT (*Triticum aestivum* L.) GENEPOOL AND GENE EXPRESSION ANALYSES

Yellow rust the destructive fungal disease of wheat (*Triticum aestivum* L.), caused by *Puccinia striiformis* f. sp. *tritici* results in high yield losses especially in the areas of epidemics frequently occurred in wheat. In this study, genetic diversity and expression pattern of a yellow rust disease resistance gene (*Yr10*) in yellow rust resistant winter type bread wheat (*Triticum aestivum* L.) gene pool were investigated.

PCA was conducted by 117 SSR markers scattered throughout A, B, and D genomes in 55 different bread wheat genotypes, showing different resistance to yellow rust, including 30 from Turkey and 25 from Middle East. The closest cultivars were Çetinel-2000 and Türkmen for Turkey and Behoth 8 and Douma 4 for Middle East when the both populations evaluated separately. The most distant three genotype pairs were determined as Ceyhan-99 – Behoth 6, Gerek 79 – Douma 40989 and Karahan-99 – Douma 48114 when Turkey and Middle East populations were evaluated together. Structure analysis showed that distribution of the populations from Turkey and Middle-East were largely separated into different groups as expected and there is evidence for a third group that includes both Turkish and Middle Eastern populations.

Following, observation of *Yr10* gene, related with yellow rust resistance, within this gene pool, real-time gene expression profile of *Yr10* gene was performed in Türkmen, Gerek79 and Avocet *Yr10* as a positive control at 7 different time points (0 hour - MOCK, 15 minutes, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours) after inoculation. It was observed that the most down-regulated time point was 24 hpi for *Yr10* gene expression in all genotypes. Expression of *Yr10* gene was down regulated until 24 hpi in Gerek 79 while up regulation of *Yr10* was observed after 24 hpi in Türkmen.

In conclusion, expression of *Yr10* gene was fluctuated depends on time for plant-pathogen interaction and genotype. The increased time points for expression profiling is required for more effective evaluation of stripe rust resistance response in wheat.

However better conclusions to understand the response of plant to yellow rust attack can be acquired as more genes identified in wheat and presented in future. Available information about genetic diversity is crucial for selection of elite genotypes in plant breeding programs. In the shed light of our results, the most distant genotype pairs (Ceyhan-99 – Behoth 6, Gerek 79 – Douma 40989 and Karahan-99 – Douma 48114) and Gerek 79 and Türkmen genotypes, carrying *Yr10* gene, are good candidates as parents in breeding programs for different traits.

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SYMBOLS

bp	: Base pair
cM	: Centi Morgan
°C	: Degree Celsius
Ha	: Harvested area
Hg	: Hectogram
Kb	: Kilo base
μg	: Microgram
μl	: Microliter
MgCl₂	: Magnesium chloride
ml	: Milliliter
nm	: Nanometer
U	: Unit

ABBREVIATIONS

AFLP	: Amplified Fragment Length Polymorphism
APR	: Adult Plant Resistance
Avr	: Avirulence
CRFIC	: The Central Research Institute for Field Crops
DArT	: Diversity Array Technology
DNA	: Deoxyribonucleic acid
dNTP	: Deoxynucleotide triphosphate
EDTA	: Ethylene Diamine Tetra Acetic Acid
FAO	: Food and Agriculture Organization of the United Nations
HTAP	: High Temperature Adult Plant Resistance
HR	: Hypersensitive Response
Hpi	: Hours post inoculation
ISSR	: Inter Simple Sequence Repeat
LC	: Little Cub
LD	: Linkage Disequilibrium
Lr	: Leaf rust
LRR	: Leucine Rich Repeat
MAS	: Marker Assisted Selection
MMT	: Million Metric Tons
Mpi	: Minutes post inoculation
NBS	: Nucleotide Binding Site
PCR	: Polymerase Chain Reaction
PIC	: Polymorphism Information Content
PCA	: Principle Component Analysis
Pst	: <i>Puccinia striiformis</i> f. sp. <i>tritici</i>
Q-PCR	: Quantitative PCR
QTL	: Quantitative Trait Loci
R	: Virulence gene in host
RAPD	: Random Amplified Polymorphic DNA.
RFLP	: Restriction Fragment Length Polymorphism
RNA	: Ribonucleic acid

RQ : Relative Quantitation
RT-PCR : Reverse Transcriptase PCR
SNP : Single Nucleotide Polymorphism
SSR : Simple Sequence Repeat
TBE : Tris Boric acid EDTA
UV : Ultraviolet
Yr : Yellow rust.

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1. INTRODUCTION

1.1. Aim

Wheat (*Triticum aestivum* L.) is the most important crop for Turkey, which is also one of the gene centers of wheat. Genetic variability is of prime importance for the improvement of many crop species, including wheat, and nearly all crop improvement programs depend on genetic diversity in the available germplasm. Yellow rust caused by *Puccinia striiformis* f. sp. *tritici*, one of the most harmful diseases of bread wheat, results in high yield and quality losses. The best way to fight with this disease would be the use of molecular genetic tools for modern breeding studies.

In this study, two major objectives were (i) to assess genetic diversity within the yellow rust resistant bread wheat (*Triticum aestivum* L.) population consisting of 30 Turkish and 25 Middle-Eastern genotypes, and (ii) to monitorize *Yr10* yellow rust resistance gene expression profile in bread wheat cultivars (Türkmen and Gerek 79) belong to our gene pool at seedling stage.

1.2. General Background

1.2.1. Wheat (*Triticum aestivum* L.)

Wheat is an annual crop species from *Poaceae* family consists of essential elements such as vitamin B, carbohydrates, fibrous nutrients, calcium, iron and zinc which are very crucial for human nourishment. The taxonomy of bread wheat is given in **Figure 1.1**.

Kingdom	Plantae
Phylum	Angiospermae
Class	Monocotyledoneae
Order	Poales
Family	Poaceae
Sub-Family	Pooideae
Tribe	Triticeae
Species	<i>Triticum</i>
Sub-Species	<i>Triticum aestivum</i> L.




Figure 1.1. Taxonomy of *Triticum aestivum* L.

Wheat is one of the major crop species which consumes a healthy human diet up to 20% of calories. To obtain maximum wheat yield, appropriate breeding management and favorable weather are necessary. Wheat seeds germinate in temperature conditions between 3.8°C to 25°C (Herbek and Lee, 2009). The wheat seed production rate and yield quality are important traits for countries having cereal-based daily diet same as Turkey. These traits can be affected by environmental conditions such as climate, biotic or abiotic stress factors and harvesting area. The worldwide wheat production statistics was shown in **Figure 1.2-5** according to the last FAO report for 2012 (Faostat web site: <http://faostat.fao.org/site/339/default.aspx>). Turkey produces 101 million 250 gram loaves of bread wheat every day. Therefore, it is the most important crop for Turkey and has a significant role for the country economics. Generally, wheat is mostly produced in Central Anatolia region of Turkey. Nevertheless, about two million tons of durum wheat and qualified bread wheat has been produced in last year in Turkey (Serttas, 2013).

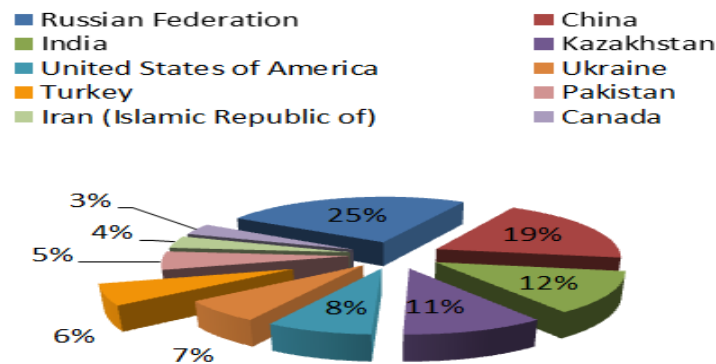


Figure 1.2. Turkey’s place at the graph of planted seeds (tons) when compare to nine other countries.

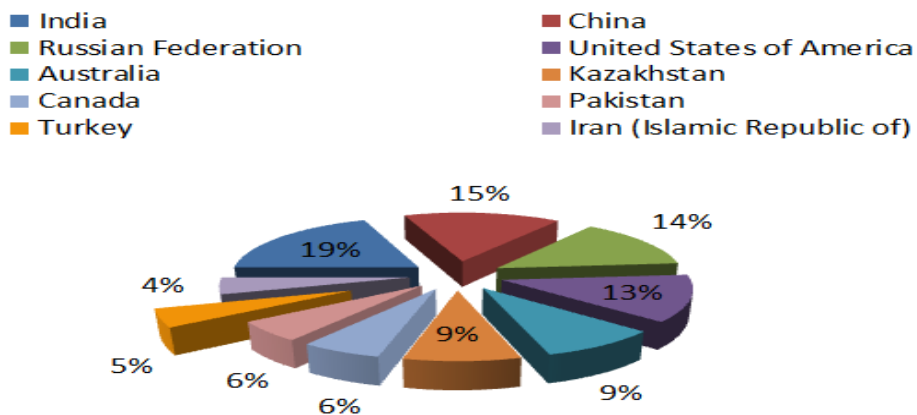


Figure 1.3. Turkey’s place at the graph of harvested area (Ha) when compare to nine other countries.

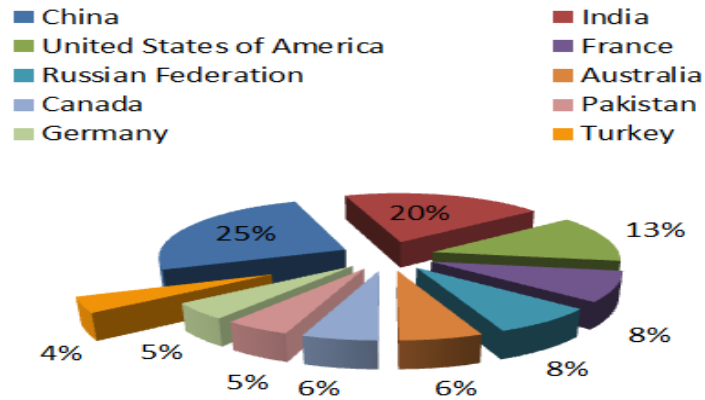


Figure 1.4. Turkey's place at the graph of wheat production (tons) when compare to nine other countries.

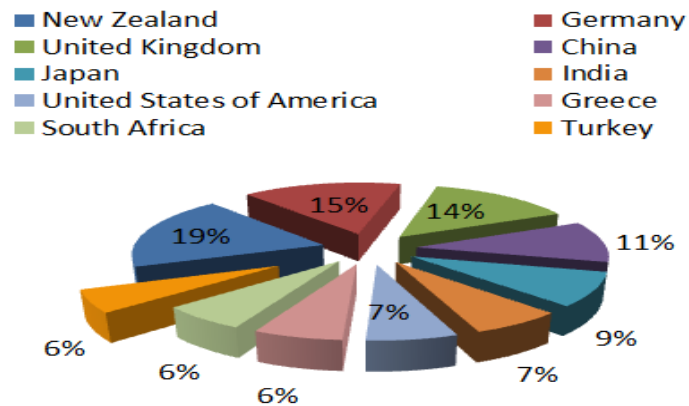


Figure 1.5. Turkey's place at the graph of yield amount (Hg/Ha) when compare to nine other countries.

1.2.1.1. Wheat domestication and genome organization

Fertile Crescent where is the core of West Asia and Mediterranean countries involving Turkey, Syria, Iran, Iraq, Israel, Jordan was an origin for wheat (Jaradat, 2013; Feuillet et al., 2007) (**Figure 1.6.**). In Turkey, Cafer Hoyuk, Cayonu, Can Hasan and Mersin regions were the main origins of wheat domestication (Salamini et al., 2002).

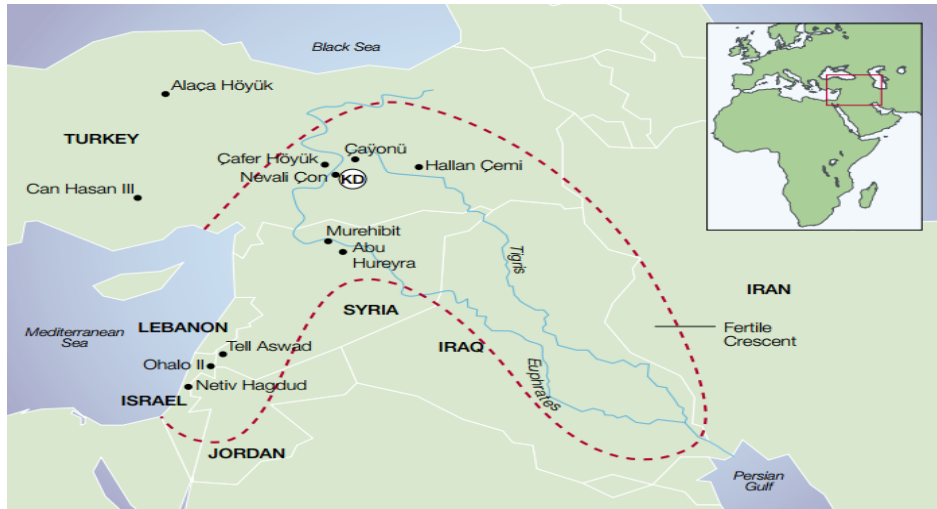


Figure 1.6. Demonstration of the origin of the wheat species: Fertile Crescent (Salamini et al., 2002).

The alteration at spikelet formation shows the importance of domestication of wheat to generate more productive wheat crop for human diet as a food supply (**Figure 1.7.**).

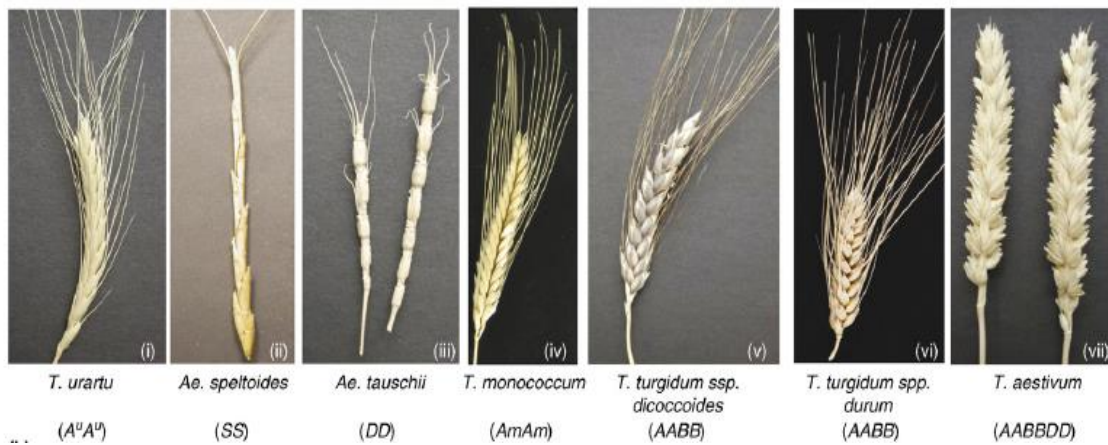


Figure 1.7. The spike forms of wild and domesticated wheat (Feuillet et al., 2007).

Bread wheat (*Triticum aestivum* L.; AABBDD, 2n=42) is an allohexaploid crop species arised from the domestication of wild tetraploid emmer (*T.turgudium dicoccum*; AABB, 2n=28) and wild diploid grass (*Ae. tauschii*; DD, which are progenitors of A, B and D genomes (**Figure 1.8.**).

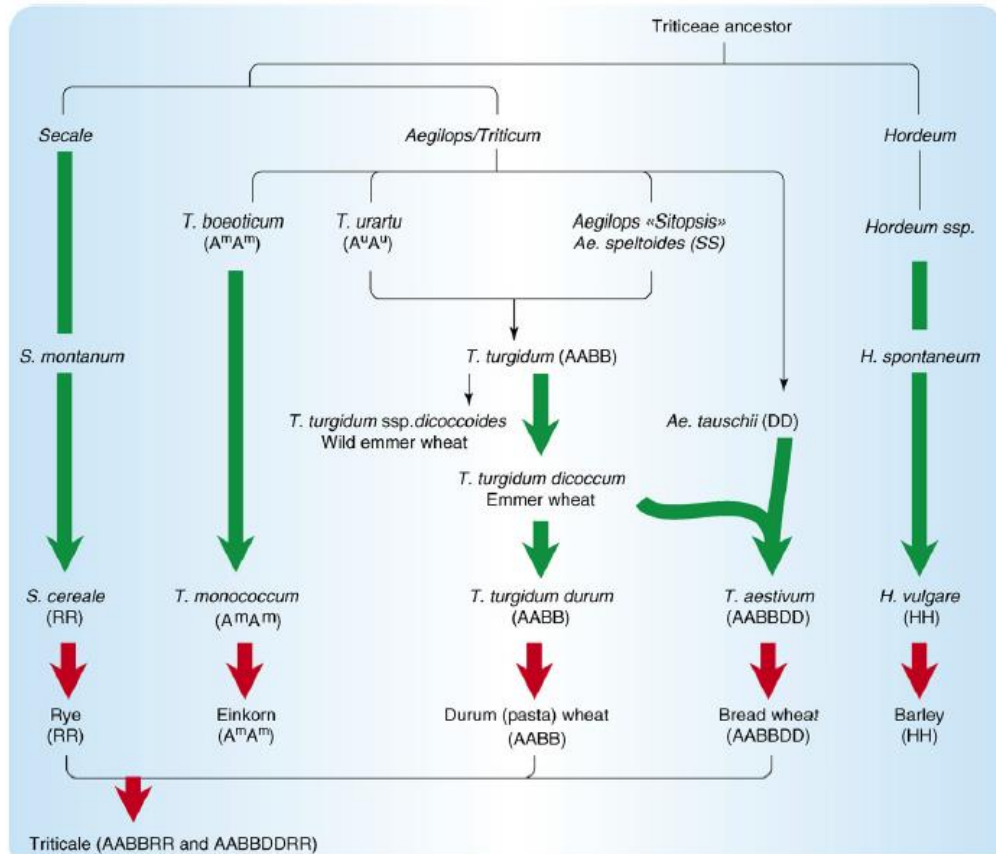


Figure 1.8. The domestication of bread wheat (*Triticum aestivum* L.) species (Feuillet et al., 2007).

The hexaploid bread wheat genome consists of 17 billion bases (Bennett and Smith, 1991) composed of three homoeologous genomes: A, B and D each possess seven metacentric or sub-metacentric chromosomes (Akhunov et al., 2003) and mainly consist of repetitive DNA. Each diploid chromosome of wheat has larger genome than the rice genome (Goff et al., 2002; Varshney et al., 2006) (**Table 1.1**). Wheat genome consist of about 95-99% non-functional genes, hence the functional genes are too less. Besides to this complexity; wheat genome has polyploid nature that makes wheat chromosome difficult to be studied by scientists.

Table 1.1. A comparison of genome size and repetitive structure of major cereals.

Crop (Botanical name)	Polyploidy level and chromosome number	Genome size (bp) (Bennett and Smith, 1991)	Repetitive DNA
Rice (<i>Oryza sativa</i>)	$2n = 2x = 24$	0.4×10^9	35%
Maize (<i>Zea mays</i>)	$2n = 2x = 20$	3.0×10^9	78%
Wheat (<i>Triticum aestivum</i>)	$2n = 6x = 42$	17.9×10^9	83%
Barley (<i>Hordeum vulgare</i>)	$2n = 2x = 14$	5.5×10^9	76%
Sorghum (<i>Sorghum bicolor</i>)	$2n = 2x = 20$	0.8×10^9	-
Rye (<i>Secale cereale</i>)	$2n = 2x = 14$	9.4×10^9	92%

1.2.2. Crop genetic diversity

Crop genetic diversity is essential for sustaining (i) the environment, (ii) the everlasting needs of human-being such as feeding and improvement of sustainable agriculture which provides genetic barriers against biotic and abiotic stress factors (Jiang et al., 2014). The general idea of domestication of plant species based on generating more adapted, tolerant or resistant species at hard conditions such as less harvesting area and increased effects of biotic or abiotic stress factors. In crops such as wheat, it is needed to create new cultivars which have several adaptation requirements (Lenser and Theißen, 2013) to maximize the yield for overall biomass ratio presented in **Figure 1.9.**

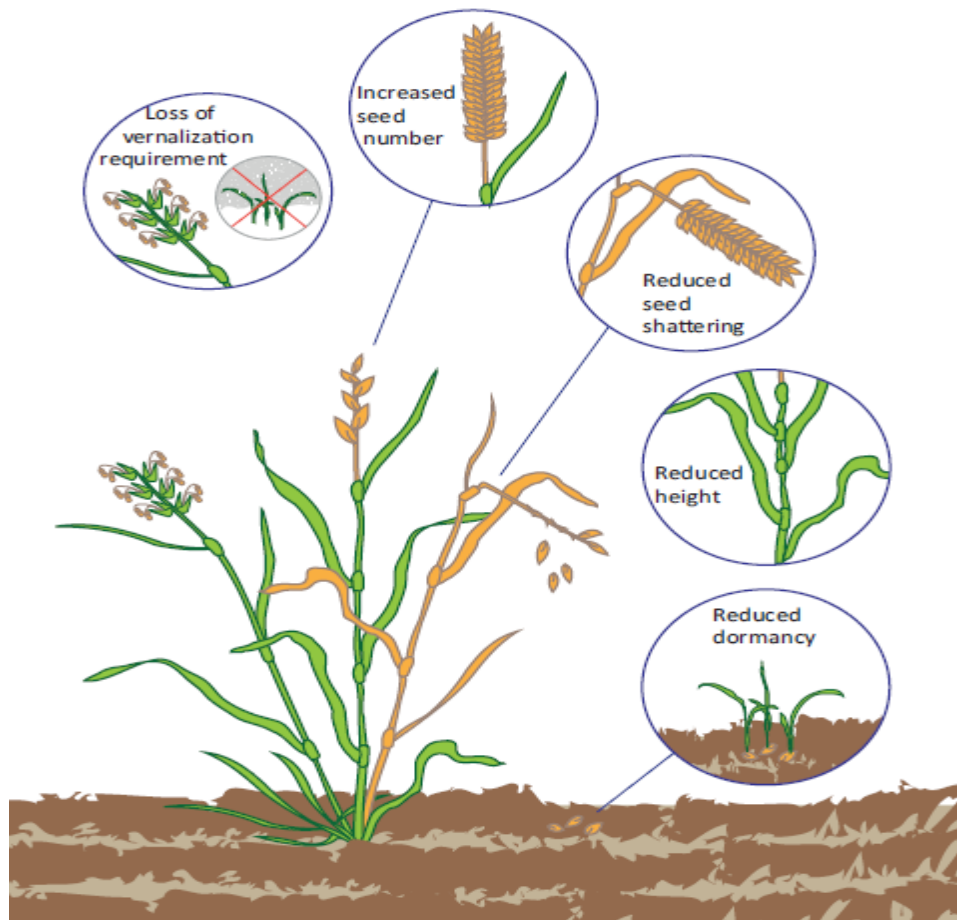


Figure 1.9. Monocot crop adaptation requirements for more effective cultivation and growth conditions.

Genetic basis of crop diversity should be clarified to develop more tolerant or resistant cultivars to decrease the rising levels of vulnerability to pests and diseases (Jiang et al., 2014). The knowledge about germplasm diversity and their genetic relationships could be invaluable for crop improvement studies (Mohammadi and Prasanna, 2003).

Adequate identification and characterization of plant materials is crucial for the successful conservation of crops and their sustainable usage (Arif et al., 2010). Assessment of the levels and patterns of genetic diversity can allow (i) reliable classifications of accessions, (ii) identification of core accessions with possible utility for breeding objectives. A number of methods are being used for genetic diversity analysis based on pedigree data, morphological data, biochemical data and molecular data in germplasm accessions, breeding lines, and populations (Mohammadi and Prasanna, 2003).

1.2.3. Marker systems

Marker systems are being used for the identification and characterization of crop species. A successfully inherited trait or its genetic basis can be assigned by phenotypic or genotypic selection via marker systems. There are different types of marker systems also called as “genetic markers” such as morphological, biochemical and DNA-based markers. DNA-based markers are differentiated in three types according to different types of mutations resulting in the variation (**Figure 1.10.**).

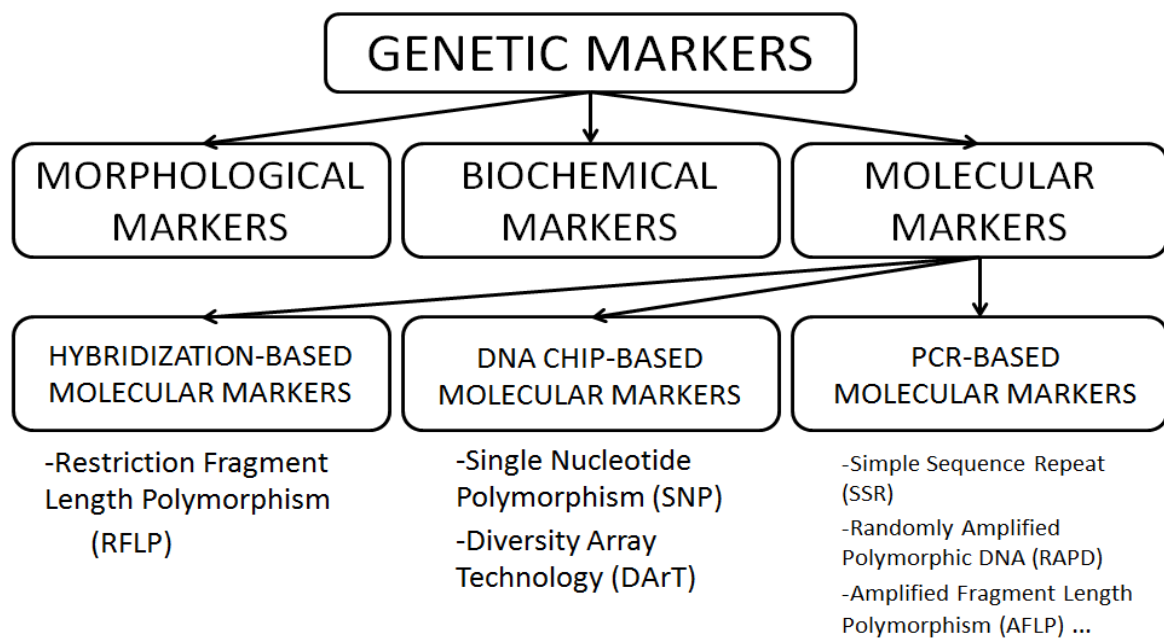


Figure 1.10. A brief summary of marker systems.

1.2.3.1. Morphological markers

Morphological markers also called as “visualizing markers” are depending on phenotypic selection of species. To identify a marker related to a trait can be effected by environmental conditions. If the environment alters the phenotypic feature which was under the control of one locus; it will make it complex to identify the variation resulted from genotypic changes at that locus. Moreover, they won’t allow the determination of heterozygous genotypes. In contrast, they can be identified easily because they are not many and it is easy to separate a specific dominant allele from the recessive one.

1.2.3.2. Biochemical markers

Biochemical markers are protein-based markers mainly consists of enzymes (isoenzyme) and non-enzyme proteins (storage proteins such as gliadin and glutenin). The most important advantages of these markers are having quick, reliable and repeatable results and that they don't affected by environmental conditions. Generally, gliadin and glutenin proteins are the mostly used biochemical markers in wheat. The izoenzymes coded by the different alleles of a gene or coded by different genes are cheap to study. However, they belong to a few numbers of loci, different developmental stages, and different types of tissues.

1.2.3.3. DNA markers

These markers are more effective to determine variations when compare to morphological and biochemical markers. Molecular markers allow the screening of numerous plants and allow analyzing of several resistance genes. DNA markers can occur in all types of tissues. In recent years, there are increases at the use of molecular markers which are unaffected under stress conditions and allow speeding up the cultivation studies. They do not get affected by environmental conditions. Molecular markers are widely used in crop cultivation and breeding studies to investigate the specific loci or genomic regions. The study of natural variation result in great advances for understanding of plant morphology and the response of plants to biotic and abiotic stresses (Fernie et al., 2006).

An ideal polymorphic marker should have highly polymorphic nature, co-dominant inheritance, frequently occurrence in the genome, neutrality to environmental conditions, high reproducible capacity, and should be easy to access and assay, and easy to exchange data between laboratories (Arif et al., 2010).

The complex structure of bread wheat genome resulted in development of new techniques for diversity analyses. The current situation of these methods is now well-established for diversity analyses (El-Assal and Gaber, 2012). It is advantageous to use molecular marker techniques throughout the genome for determining genetic diversity and evolutionary relationships (El-Assal and Gaber, 2012; Karakas et al., 2010; Gostimsky et al., 2005).

RFLP (Restriction Fragment Length Polymorphism) markers are firstly discovered marker system and depend on the hybridization of DNA fragments cut by restriction enzymes called as “probe” with the target DNA (Soller and Bechmann, 1983). The Southern Blotting is used for hybridization of the fragments. The fundamentals of this method are the hybrid occurrence between DNA and DNA or DNA and RNA. It allows identification of desired DNA fragments. RFLP has an advantage by its reproducible and co-dominant nature. PCR-based markers are taken the place of RFLP markers because of its hard experimental conditions.

DNA-chip-base markers take the major advance in molecular biology and are based on the hybridization of samples to immobilized DNA molecules. Microarrays are generally produced by consisting of oligonucleotides at the range of 10 to 25 bases, while chips prepared by micro-decomposition technologies consisting of 0.5 to 2.0 Kb cDNAs (Lemieux et al., 1998). DNA-chip is a spot microarray platform carries plastic or silicon firm surface to monitor the expression of many genes at the same time. To evaluate the gene expression pattern, allele specific nucleotides are used as similar to DNA probs. This technology called “DArT” (Diversity Array Technology) is placed in “High Throughput Technology” by allowing the screening of hundreds of polymorphic loci among the genome at the same time (Jaccoud et al., 2001; Wenzel et al., 2004).

In 1983, Kary Mullis was first discovered the polymerase chain reaction (PCR) and led to development of PCR-based molecular markers (Mullis et al., 1986). PCR reaction amplifies the target gene in a buffer which contains oligonucleotide primer pair, dNTP, Mg^{2+} , and a polymerase enzyme. PCR technique is widely-used for DNA sequencing, DNA map construction, DNA fingerprinting, human genome project, forensic sciences, identification of polymorphism between different species, determination of genetically modified organisms, and cloning studies.

Some of the PCR-based markers are SSR (Simple Sequence Repeat), ISSR (Inter Simple Sequence Repeat), AFLP (Amplified Fragment Length Polymorphism) and RAPD (Random Amplified Polymorphic DNA).

Microsatellites also known as “SSRs” are frequently placed along the genome and the advantageous markers by their reproducible, highly informative and genome specific markers (Morgante and Olivieri, 1993; Rafalski and Tingey, 1993; Powell et

al., 1996) out of other PCR-based markers. SSR repeats are SSRs are the random sequence repeats along eukaryotic genome and the functions of these markers are not well-known. However, You et al. (2002) were described the functions of the SSR markers (**Figure 1.11**) (You et al., 2002).

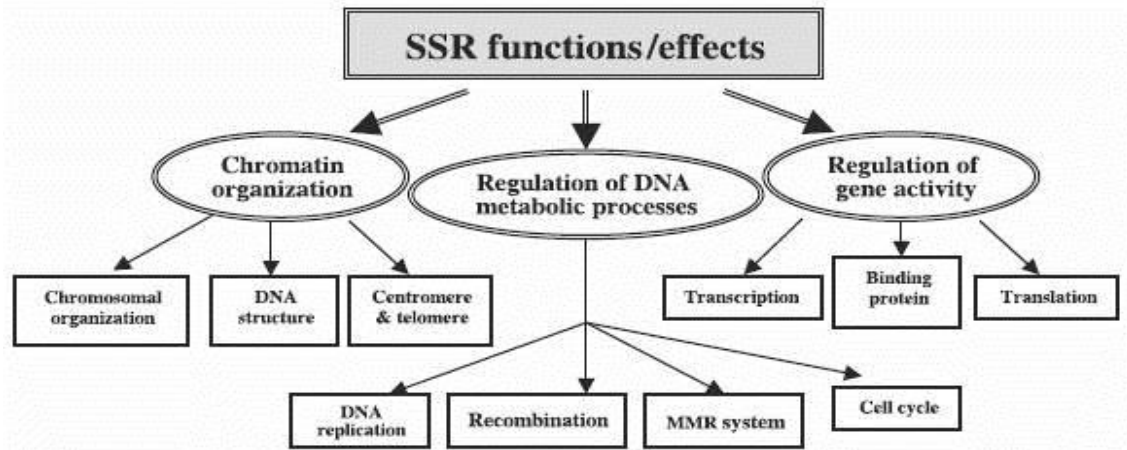


Figure 1.11. Functions of SSRs throughout the genome.

SSR repeats are ranging from mono- to hexa- nucleotide repeats and the most common SSR repeats are $(CA)_n$, $(AAT)_n$, and $(GATA)_n$ in plants and animals as well (Jarne and Lagoda, 1996). Variation comes from the changes at the number of repeats. There are mainly six types of SSR markers named as BARC (Song et al., 2005), CFA and CFD (Sourdille et al., 2004; Guyomarc'h et al., 2002), GDM (Pestsova et al, 2000), GWM (Röder et al. 1998; Ganal and Röder, 2007) and WMC (Gupta et al., 2002) to assess genetic diversity in plants. In addition, Ezgi et al. was evaluated the di- and tetra-nucleotide SSR repeats as $(AC)_n$, $(AG)_n$, $(AT)_n$, $(CA)_n$, $(GA)_n$, $(TA)_n$ and $(TAGA)_n$ by screening with SSR markers related to yellow rust resistance genes in Turkish wheat germplasm (Cabuk Sahin, 2009). The most common SSR motif was determined as $(GA)_n$ and the less common SSR motif was $(TAGA)_n$ tetra nucleotide repeat in that wheat gene pool (Cabuk et al., 2011).

Because of the hexaploid structure of the bread wheat genome, the microsatellite markers are better tools for assessing their complex structure with the highly polymorphic informative structures of these markers when compare to other molecular marker systems. For instance, over thousands of mapped microsatellite markers for wheat are commonly used for genetic analyses such as genetic mapping, gene tagging, and genetic diversity analyses (Ehtemam et al., 2010).

1.2.4. Statistical tools

To evaluate the informativeness of molecular markers, to determine linkage between loci, to assess genetic diversity and to estimate structure of a given population can be measured by statistical tools in the same order: Polymorphism Information Content (PIC) value calculation, Linkage Disequilibrium (LD) analysis, Principle Component Analysis (PCA) and STRUCTURE analysis.

The informativeness of a co-dominant or dominant marker system such as biochemical markers or DNA markers, can be measured by calculation of polymorphism information content (PIC) value given by a formula (Botstein et al., 1980; Roychoudhury and Nei; 1988). Each offspring of species has different amount of polymorphic information (Hildebrand et al., 1992).

Linking DNA polymorphism is getting more important tool to identify phenotypic variation for breeding programs (Sakiroglu et al., 2012; Lande and Thompson, 1990). Linkage disequilibrium (LD) can be defined as association between a pair of markers located on the same chromosome (Morton, 2005). LD mapping in plants allows to detect and to locate quantitative trait loci (QTL) by the strength of the relative correlation of a trait and a marker (Mackay and Powell, 2007). The use of “unrelated genotypes” in other words “natural populations” can provide greater resolution for linkage-based association studies (Varshney et al., 2005) and genetic diversity analyses.

GenAlEx plugin which run on an excel file allows to analyze co-dominant, binary and haploidic data and provides graphical population by population summaries of various allelic indices. Besides, co-dominant genetic distances options genotypic, allelic and microsatellite individual x individual options. Moreover, analyses for a single or multiple populations and multiple loci are provided. It allows converting co-dominant genotypic data to be exported as genetic distance matrix (Peakall and Smouse, 2006). Following acquiring of genetic distance matrix, this plugin allows creating a principle component analysis (PCA) graph. The main principle of PCA is to decrease multiple variants into two variants in a spatial space.

Population genetics interests in the variations of allele frequencies between and within populations (Evanno et al., 2005). There is a model-based Bayesian clustering method called “Structure” analysis. The program “STRUCTURE” assigns individuals

to subpopulations and uses that information to test marker–trait associations (Varshney et al., 2005; Pritchard et al., 2000). This software delineates the clusters of individuals related to their genotypes at multiple loci (Evanno et al., 2005).

1.2.5. Yellow rust disease of wheat

There are two major types of stress factors effecting plant growth and development: (i) stress factors which are caused by the mutations occurring randomly within the genome and uncontrolled cell divisions caused by some diseases, (ii) biotic and abiotic stress factors. The main causes of both biotic and abiotic stresses were given in **Table 1.2.** below (Agrios, 2005).

Table 1.2. Biotic and abiotic stress factors of plants.

Stress Factors of Infectious / Biotic Plant Diseases	Fungi
	Prokaryotes
	Parasitic higher plants and green algae
	Viruses and viroids
	Nematodes
	Protozoa
Stress Factors of Noninfectious / Abiotic Plant Diseases	Lack/ excess light
	Lack of oxygen
	Air pollution
	Nutrient deficiencies
	Mineral toxicities
	Soil acidity or alkalinity (pH)
	Toxicity of pesticides
	Improper cultural practices

For biotic stress factors such as fungi, the plant response is controlled by serials of signal transductions by chemicals secreted by the expression of genes which are responsible for this response. For example, in any case of pathogen interaction with host cells, there will be some plant disease resistance responses/signals secreted. The resistance at early recognition of pathogen to host cell is important for plants. The defense mechanism can include cell wall structural defenses (such as the production of phenolic, cellulose or cell wall proteins) or biochemical wall, membrane, cytoplasm, and nucleus defense reactions. Nucleus defense reactions may involve oxidative reactions, production of elicitors, hypersensitive response, chemicals like ethylene, pathogenesis-related proteins such as hydrolytic enzymes and inhibitors (**Figure 1.12.**) (Agrios, 2005).

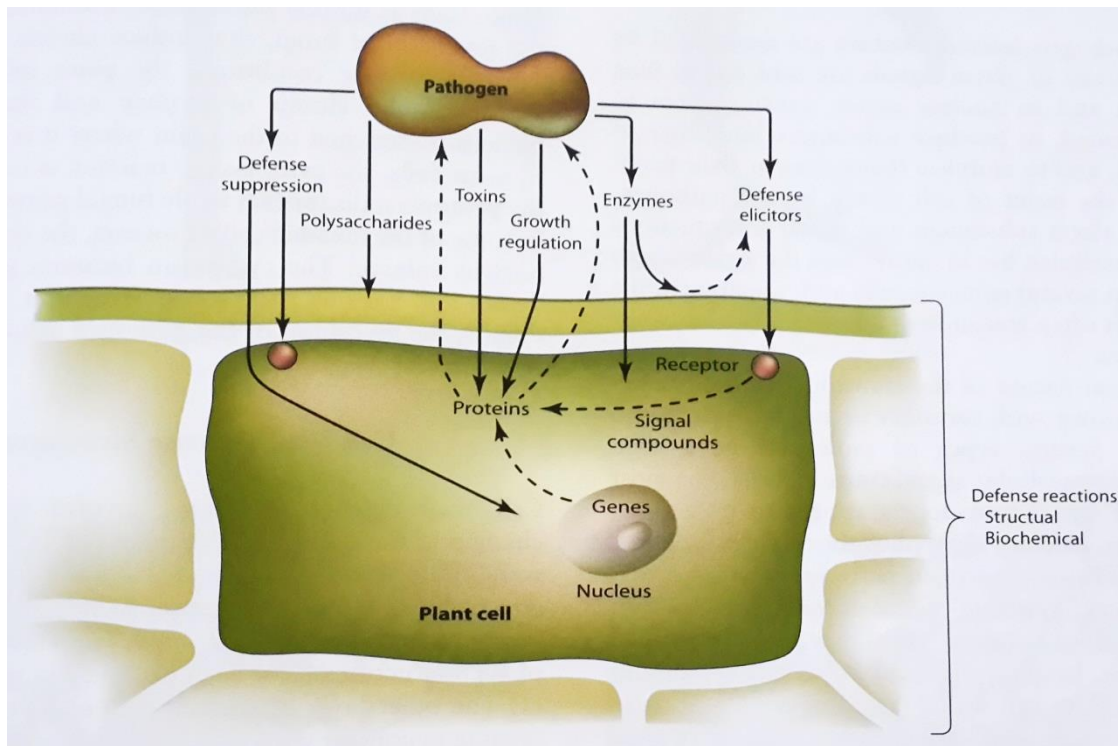


Figure 1.12. Schematic illustration of pathogen-host interaction (Agrios, 2005).

There are limitations of high quality wheat production due to biotic stresses and the fungal rust diseases are the main cause of these limitations. Rust diseases; leaf rust (*Puccinia triticina* (*Pt*), stem rust (*Puccinia graminis* f. sp. *tritici* (*Pgt*)) and stripe/yellow rust (*Puccinia striiformis* f. sp. *tritici* (*Pst*)) cause significant yield loss in wheat production. Yellow rust is most frequently occurring rust disease rather than other rust diseases.

In our country, inefficient seed production may cause yield loss up to 100%. Nevertheless, the yield loss caused by all types of rust diseases varying from 12-80%, while the yield loss caused by yellow rust reaches up to 100% in conditions where susceptible lines were cultivated. However, yield loss varies from year to year depending on the susceptibilities of cultivars, environmental conditions or the race of the pathogen (Zeybek and Yiğit, 2004; Chen, 2005). For instance, as a result of the epidemic occurred in 1978, and 1990; the bread wheat cultivars Köse 220/39, Sürak 1593/51, Sivas 111/33 and Seri 81 were discontinued. The epidemics occurred in 1994, 1997 and 1998 are still threatening the Gerek 79 bread wheat cultivar.

Puccinia striiformis f. sp. *tritici* is the fungal pathogen of wheat results in yellow rust disease. This pathogen also effects barley, rye and other types of cereals. Moreover, *Pst* is the most virulent rust pathogen regarding of virulence (Kolmer et al., 2009). This high level of variance probably caused by the mutations and somaclonal variations (Stubbs, 1985). *Puccinia striiformis* f. sp. *tritici* needs the lower temperature to be grown on leaves of cereals when compare to other rust diseases. The optimal temperatures for *Pst* pathogen to grow are between 0°C and 23°C (optimal at 11°C). Its life cycle mainly consists of two major steps as being uredinial and telial. Yellow/ stripe rust disease takes its name from the yellow, narrow and linear stripe-like spores placed on leaves or spikelets of cereals especially on wheat (**Figure 1.13**). The inoculum source for wheat is only known as urediniaspores. Every uredium has thousands of urediniaspores. When proper conditions supplied, infection will be completed in 6-8 hours and the urediniaspores which are responsible to spread of the disease will be grown in 12-14 days. The life cycle of the pathogen is given in **Figure 1.14**. The pathogen utilizes from water and nutrients of host plants and makes the plants weaker. As a result of this weakness, the amount of yield and quality of the products gets lower.



Figure 1.13. *Puccinia striiformis* f. sp. *tritici* spores on bread wheat genotypes. (Photo taken at The Central Research Institute for Field Crops, Ankara)

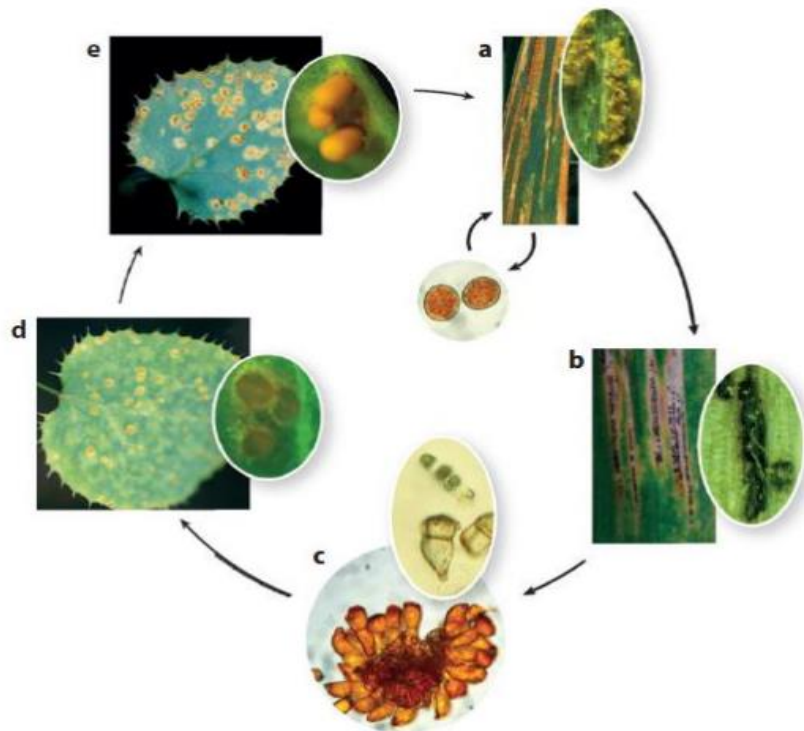


Figure 1.14. The life cycle of *Puccinia striiformis*. (a) uredinia on the primary host: wheat (b) teliospores during the telia stage germination of a basidium (c) four basidiospores (d) aecidiospores on Barberry (e) aecidiopores going to infect the primary host.(Figure taken from PhD thesis of Sorensen, C.K., 2012; Hovmøller et al., 2011).

1.2.5.1. Prevention strategies

Plant and pathogen co-evolution is a complex, continuing process that involves layers of host recognition and pathogen avoidance. Modern agricultural crops, and wild plant populations, face constantly evolving pathogen populations, necessitating the generation of new resistant germplasm (Ayliffe et al., 2008).

Fungicides are being used by most of the farmers to prevent from yield loss in wheat production. However, usage of fungicides is not an effective way to fight with the yellow rust disease because of the sharp changes at the virulence for yellow rust pathogen. Also, fungicide applications resulted in economic losses up to multimillion dollars and it is dangerous to apply for users because of its toxic nature. All of these resulted in the requirement of development of new strategies rather than fungicides to fight with the yellow rust disease. The best way to fight with yellow rust will be the ensurance of the production of wheat cultivars which has adequate level of durable resistance (Chen, 2005).

Resistance to yellow rust disease was correlated with both qualitative and quantitative genetic mechanisms. For qualitative genetic resistance, a British scientist, Sir Roland Biffen, conducted a study which recommends that the yellow rust resistance could be inherited by Mendelian laws with an effect of a single gene and would resulted in full plant resistance caused by a dominant allele (Biffen et al., 1905). For quantitative genetic resistance, Vanderplank suggested that there are two types of plant resistance mechanisms consists of (1) vertical resistance controlled by a few ‘major’ resistance genes is strong but effective for a few race of pathogen, (2) horizontal resistance controlled by many ‘minor’ resistance genes is weaker but effective for all races of pathogen. Although, some of the plant response to pathogens begins before the infection, yet, the most effective plant biotic stress resistance occurs when there is a real infection caused by a pathogen (Agrios, 2005).

In 1971, Flor took the mystery behind the resistance mechanism into light with his study on flax rust (*Melansora lini*). He claimed that the pathogen resistance is obtained from the interaction between a virulence gene (R) in plant and an avirulence (Avr) gene in pathogen (Flor, 1971). This resistance called as “gene-for-gene” plant resistance. The R genes has two subunits consists of “NBS” and “LRR” (“Nucleotide Binding Site” –

“Leucine Rich Repeat”). The NBS subunit is responsible for the GTP/ATP binding, while LRR subunit is responsible for the ligand binding (McHale et al., 2006). NBS-LRR-mediated disease resistance is effective only under the biotrophic pathogen attack (Jones and Dangl, 2006).

The importance of the production of yellow rust resistant cultivars led scientists to determine the disease resistance genes. There are *Yr* genes 53 officially named yellow rust resistance genes (*Yr*) identified, mapped and catalogued; *Yr1* to *Yr53* (Zhang et al., 2013). There are three major *Yr* gene clusters consists of (1) seedling resistance or major genes or all-stage resistance genes, (2) adult plant resistance genes (APR) and (3) high temperature adult plant resistance genes (HTAP). For seedling resistance mechanism, the resistance is under the control of the genes; *Yr1–10*, *Yr15*, *Yr17*, *Yr19–Yr28*, *Yr31–Yr35*, *Yr37–38*, *Yr40–45*, *Yr47*. For adult plant resistance mechanism, resistance is under the control of the genes; *Yr18*, *Yr29*, *Yr30*, *Yr46*, *Yr48*, *Yr49*. Also for high temperature adult plant resistance mechanism, the resistance is under the control of the genes; *Yr11–Yr14*, *Yr16*, *Yr36*, *Yr39* (Asad et al., 2012). However, only three of them were cloned: *Yr10*, *Lr34/Yr18* and *Yr36* (Yuan et al., 2012). *Yr10* out of three genes is the first race specific yellow rust resistance gene whose presence was determined in PI178383 bread wheat genotype (Wang et al., 2002). The product of this gene is known as a protein which has a structure like NBS-LRR protein (*R* gene product) (<http://www.ncbi.nlm.nih.gov/>).

In 1902, firstly H.M. Ward mentioned about the necrotic defense reaction of plants against to pathogens by another type of resistance activated by signal transduction (Ward, 1902). In 1915, E.C. Stakman working on cereals called this response as “**Hypersensitive Response**” (HR) (Stakman, 1915). In hypersensitive response, there is a complex signal transmission as a respond to stress caused by pathogen infection with the secretion of some hormones like “ethylene”, some antimicrobial chemicals and numerous enzymes as defense responses. By the transfer of signals from cell to cell; the infected cells and the cells nearby will be go through a necrotic process to prevent from the spread of the infection.

1.2.5.2. Investigation of yellow rust disease resistance at RNA level

The release of chemicals induced by pathogens is generally under the control of many serial reactions within the cell. The host cell reacts as susceptible or resistant belonging to the presence or absence of a resistance gene in their genetic diversity. For plants such as wheat, the gene expression analysis of specific disease resistance genes has been done for many years and so on. If the genes which are responsible for plant defense mechanism to pathogens could be defined, further the gene targeting studies can be done. For allohexaploid bread wheat, because of its large and complex genome, the genome-wide transcriptome studies take an important place to understand basis of host-pathogen interactions at genome level. PCR-based quantitative analysis of gene expression profiles of genes responsible for wheat biotic or abiotic stress tolerance or resistance is widely studied. To investigate resistance mechanism at RNA level allows understanding the expression profile of desired gene which has known as resistance gene for yellow rust in a given germplasm.

1.2.5.2.1. Gene-specific expression analysis

The main principle of the gene expression analysis is to evaluate the variations between different transcriptomes consists of different types and amounts of mRNA transcripts (Yu, 2012). By gene expression analysis, the difference at the gene regulations for a specific trait will result in the estimation of the role of known or unknown gene of interest. Therefore, the precise estimation of the regulation of gene expression in a living cell will allow understanding the plant-pathogen interactions and the defense mechanism (Aceituno et al., 2008). With this reason, gene expression analyses are important tools for leading to determine plant disease resistance mechanisms for further breeding studies. The gain of durable disease resistance to plants should be the main objective of gene expression studies in pathogen-host interactions (Lowe et al., 2011). The three main questions are being asked to understand the aims of expression profiling analysis are how active are different genes in different cells?, how does the genes regulated under various conditions? (stages of a cell cycle, different environments, disease states, knockout experiments), and which genes can be regulated together in which states of conditions? (Craven, 2011).

The applications of domestication of a plant species from their wild progenitor are many, yet molecular analysis is bringing key changes into light that are responsible for encoding transcription factors and proteins which regulates the expression of many other genes (Fedoroff, N., 2010).

There is a technology called “Real-time PCR analysis” which allows quantification of nucleic acids. This technology is getting invaluable tool for scientists working in different disciplines (Klein, 2002). For gene expression analysis, following total RNA isolation, cDNA template should be obtained for real-time PCR analysis by reverse transcription reaction. By real-time PCR analysis, scientist can quantify the amplification pattern of the specific gene or genes at the same time with polymerase chain reaction by the help of fluorescent dyes such as TaqMan probes or Sybr Green dyes. The difference between these fluorescent dyes is the binding and luminescence behaviors. TaqMan probes are carrying two dyes (Reporter and Quencher) and are giving luminescence by the release of reporter dye when the amplification starts, whereas the Sybr Green dye randomly binds to double stranded DNA molecules and gives green luminescence. However, gene-specific studies won't affected by the use of Sybr Green dye (**Figure 1.15.**). This technology allows investigating expression pattern of a single gene or multiple genes in same reaction.

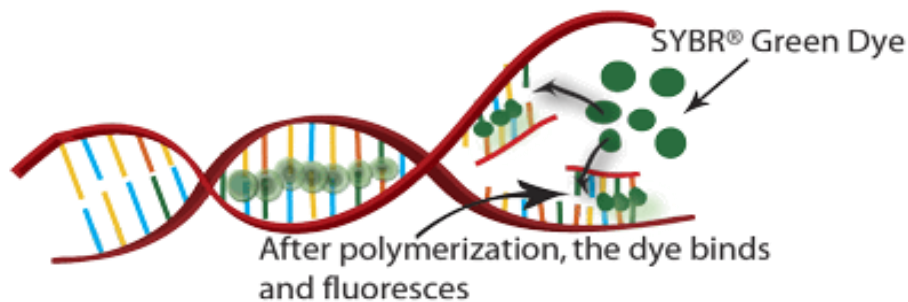


Figure 1.15. Schematic illustration of Sbyr Green function.

In this study, two major objectives were (i) to assess genetic diversity within the yellow rust resistant bread wheat (*Triticum aestivum* L.) population consisting of 30 Turkish and 25 Middle-Eastern genotypes, and (ii) to monitorize *Yr10* yellow rust resistance gene expression profile in bread wheat cultivars (Türkmen and Gerek 79) belong to our gene pool at seedling stage.

2. MATERIALS and METHODS

2.1. Assessment of Genetic Diversity in Yellow Rust Resistant Wheat Gene Pool

Genetic diversity analyses by Simple Sequence Repeat (SSR) markers using 117 primers were conducted in fifty five bread wheat (*Triticum aestivum* L.) genotypes.

2.1.1. Plant material

Yellow rust resistant winter type bread wheat (*Triticum aestivum* L.) gene pool was constructed by obtaining 30 of Turkish genotypes from Turkish Ministry of Food, Agriculture and Livestock, The Central Research Institute for Field Crops (CRIFC), Ankara and 25 of Middle-Eastern genotypes from National Commission for Biotechnology (NCBT) (Table 2.1).

Table 2.1. Bread wheat (*Triticum aestivum* L.) genotypes used in this study.

TURKEY			MIDDLE-EAST		
ID	Name	Characteristics	ID	Name	Characteristics
1	Pamukova97	C	31	Behoth 8	C
2	Cemre	C	32	Jaolan 2	C
3	Tahirova	C	33	Douma 4	C
4	Hanlı	C	34	Sham 10	C
5	Ceyhan-99	C	35	Douma 2	C
6	Pandas (Panda)	C	36	Sham 4	C
7	Karatopak	C	37	Behoth 4	C
8	Osmaniyem	C	38	Behoth 6	C
9	Carisma	C	39	Sham 6	C
10	Yakar-99	C	40	Sham 8	C
11	Aksel 2000	C	41	Acsad 1139	AL
12	Bayraktar 2000	C	42	Acsad 1133	AL

Table 2.1. Continued.

13	Demir 2000	C	43	Acsad 1115	AL
14	Atlı-2002	C	44	Acsad 1159	AL
15	Çetinel-2000	C	45	Acsad 1071	AL
16	Alpu 2001	C	46	Douma 40860	AL
17	Tekirdağ	C	47	Douma 40863	AL
18	Lancer	C	48	Douma 40855	AL
19	Gün-91	C	49	Douma 40856	AL
20	Türkmen	C	50	Douma 40992	AL
21	Gerek 79	C	51	Douma 40988	AL
22	Aytın 98	C	52	Douma 40989	AL
23	Altay 2000	C	53	Douma 40444	AL
24	Karahan-99	C	54	Douma 48114	AL
25	Konya-2002	C	55	Douma 40765	AL
26	Aldane	C			
27	Nurkent	C			
28	Kaşif Bey 95	C			
29	İzgi 2001	C			
30	Sönmez 2001	C			

C: Cultivar, AL: Advanced Line.

2.1.2. Genomic DNA isolation

Genomic DNA isolation was performed according to the method of Weining and Langridge (1991) by using stock solutions given in **Table 2.2**. The quality of DNA was assessed by using Nanodrop1000 spectrophotometer at 260/280 nm absorbance value and stock DNAs were diluted to 50 ng/μl for PCR reactions.

Table 2.2. Stock solutions used in DNA isolation.

Chemical Agent	Components	Concentration	Catalog No
Squash buffer	N-Lauroylsarcosine sodium salt	2%	Sigma – L-9150
	Tris-HCl (pH 8.0)	0.1 M	Sigma – T-5941
	EDTA (pH 8.0)	10 mM	Sigma - SIE6635
NaAc	Cold NaAc	3M	Sigma - S-58750
	Glacial acetic acid (Adjust pH to 4.8)		
EDTA	EDTA	10 mM	Sigma - SIE6635
RNase	RNase	2U	Promega M4261

2.1.3. Agarose gel electrophoresis

Genomic DNAs were separated on 0.8 % agarose gels by using solutions given in **Table 2.3**. GeneRuler™ 50bp, 100 bp and 1kb DNA Ladder (Fermentas), were used to estimate amplicon sizes. Electrophoreses were performed with 0.5 X TBE for two hours at 200V. MiniBIS Pro Visualizing System (DNr Bio-Imaging Systems) was used to visualize band patterns on agarose gel.

Table 2.3. Solutions used for agarose gel electrophoresis.

Chemical Agent	Ingredients	Concentration
TBE Buffer (10X)	Trisma Base	890 mM
	EDTA	20 mM
	Boric Acid	890 mM
Loading Dye (6X)	Bromophenol blue	0.25% (w/v)
	Sucrose	40% (w/v)
Ethidium Bromide	Ethidium Bromide	10 mg/ml

2.1.4. Polymerase Chain Reaction (PCR)

117 genome-wide SSR markers (**Appendix 1**) consists of 35, 38 and 44 markers specific to A, B, and D genomes of bread wheat (Somers et al., 2004; Röder et al., 1998) were used to assess genetic diversity within bread wheat gene pool. A total of 41 *barc*, 40 *wmc*, 20 *cfb*, 9 *cfa*, 6 *gwm* and 1 *gdm* markers were chosen for SSR analysis (**Table 2.4**). Polymerase chain reactions were conducted at 25 µl volume in an Applied Biosystem GeneAmp® 9700 PCR and final concentrations of components were given in **Table 2.5**.

Table 2.4. Distribution of SSR markers belong to A, B and D genome of bread wheat used in this study.

SSR MARKER CODE	Bread Wheat (<i>Triticum aestivum</i> L.) Genome											
	A Genome				B Genome				D Genome			
	SSR Markers with One Polymorphic Band	SSR Markers with More Than One Polymorphic Band	Monomorphic Band Pattern	Total	SSR Markers with One Polymorphic Band	SSR Markers with More Than One Polymorphic Band	Monomorphic Band Pattern	Total	SSR Markers with One Polymorphic Band	SSR Markers with More Than One Polymorphic Band	Monomorphic Band Pattern	Total
<i>barc</i>	8	1	1	10	5	6	4	15	7	4	5	16
<i>cfa</i>	4	1	2	7	-	2	-	2	-	-	-	-
<i>efd</i>	-	-	1	1	-	-	1	1	6	5	7	18
<i>gdm</i>	-	-	-	-	-	-	-	-	-	1	-	1
<i>gwm</i>	1	1	-	2	2	-	-	2	1	-	1	2
<i>wmc</i>	9	5	1	15	12	3	3	18	5	1	1	7
Total	22	8	5	35	19	11	8	38	19	11	14	44
117												

Table 2.5. Components of the PCR reaction.

PCR Components	Initial Concentration	Volume (μ l)	Final Concentration
10 X <i>Taq</i> Buffer	10 X	2.5	1 X
MgCl₂	50 mM	1.5	3 mM
dNTP	2.5 mM	2	0.2 mM
SSR Forward Primer	100 μ M	1	4 μ M
SSR Reverse Primer	100 μ M	1	4 μ M
<i>Taq</i> DNA Polymerase	5 U/ μ l	0.2	0.04 U/ μ l
Genomic DNA	50 ng/ μ l	2	4 ng/ μ l
dH₂O	-	14.8	-

PCR steps and cycles were presented in **Table 2.6**.

Table 2.6. PCR program for SSR primers.

Reaction Steps	Number of Cycle	Temperature (°C)	Time (min)
<i>Initial Denaturation</i>	1	94	03:00
<i>Denaturation</i>	40	94	01:00
<i>Annealing Temperature</i>		48-69.5	01:00
<i>Extension</i>		72	01:00
<i>Final Extension</i>	1	72	10:00
<i>Hold</i>	1	4	∞

2.1.5. Data analyses

60 SSR markers giving one allele per each locus were used to assess genetic diversity within our gene pool. The allelic sizes among polymorphic SSRs that giving one allele per locus were detected via using Genosoft 3.8.2 software from VWR (<https://www.vwr.com/>) based on a given molecular weight standard (MWS) by comparing the band positions. The highly polymorphic information that every SSR carried within this gene pool were identified by the calculation of Polymorphism information content (PIC) value. Polymorphism Information Content (PIC) values were estimated by the formula (Botstein et al., 1980). In order to estimate the PIC values for each marker three different matrixes were created. The squared allele frequency estimates the all different alleles of a given marker were summed across all loci and subtracted from 1 to obtain the PIC values.

$$PIC = 1 - \sum (P_{ij}^*)^2$$

*: P_{ij} is the proportion of the i^{th} allele frequency at j^{th} loci.

The linkage between SSR markers used in genetic diversity analysis which was located on the same chromosome was determined by Linkage Disequilibrium (LD) analysis. A linkage disequilibrium analysis was conducted by GENEPOP 4.0 software

between the SSR markers known to be located on the same chromosome. The program results a p -value for each of the two locus pairs based on the non-random association between different alleles of the different loci. Since we used a high number of tests, the number of false positive arising from multiple testing poses a serious concern about the validity of the results. In order to control such false positives, a false discovery adjustment procedure was used to control. The software program Q-value used to correct the p -values of linkage analyses. If the resulting re-adjusted p -value (called q -value in the software program) is lower than 0.05 between the SSR markers, then the two markers were declared to be linked. We estimated LD only between the markers pairs that are known to be on the same chromosome or linkage groups based on the previous studies (Grain Genes: <http://wheat.pw.usda.gov/GG2/index.shtml>; Somers et al., 2004; Röder et al., 1998).

Diversity determination among 55 genotypes was conducted by Principle Component Analysis (PCA). SSR data was employed to conduct a PCA using software program GenAEx 6.5 (Peakall and Smouse 2001) (missing data was coded as “-9” in the matrix for statistical analyses). After estimation of genetic distance, PCA was conducted to visualize the distribution of the genotypes on a PCA graph based on the first two principal components.

Regarding population structure, Turkey and Middle-East populations were evaluated by Model-based clustering via STRUCTURE software. Genotypic data was uploaded to STRUCTURE software and simulation parameters were determined. In order to deduce the optimal value of K , we evaluated $K = 1-10$. In the model set in this study, the admixture and correlated alleles were assumed. The data was collected from a 100000 Markov Chain Monte Carlo (MCMC) replications after discarding 10000 burn-in replications. This level of replication and burn-ins were demonstrated to be sufficient in the literature (Sakiroglu et al., 2010). Structure Harvester v0.6.93 is a webserver (Earl et al., 2012) that processes the structure results to allow the deduction of the optimal number of clusters “ K ”. The results from STRUCTURE program was loaded to the Structure Harvester web server in “.zip” format and the best number of clusters were determined based on ad-hoc method described by Pritchard et al. (2000) in the user manual as well as Evanno’s method (Evanno et al., 2005).

2.2. Gene Expression Analyses

2.2.1. Presence of *Yr10* gene in wheat genepool

To monitorize the expression pattern of seedling-stage yellow rust resistance gene *Yr10*, initially its presence in bread wheat gene pool was investigated by PCR analysis. The isolated DNAs from fifty five bread wheat genotypes were screened by specific *Yr10* gene's exon 1 and exon 2 primers (**Figure 2.1.**) (Temel et al., 2008).

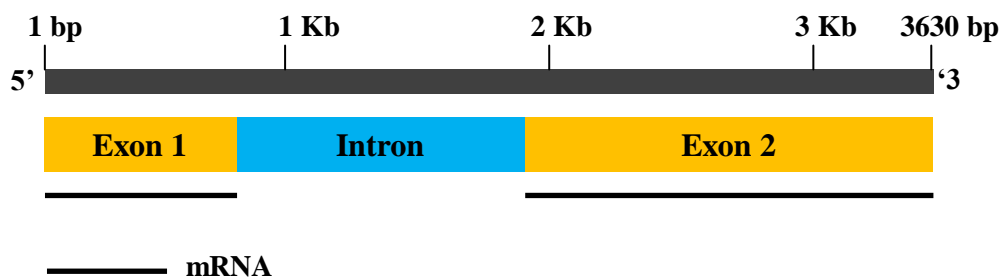


Figure 2.1. Schematic illustration of *Yr10* gene.

Primer pairs for exon1 and exon 2 (Temel et. al., 2008) were used for PCR amplifications (**Table 2.7.**).

Table 2.7. Primers use in this study specific to *Yr10* gene's exon 1 and exon 2.

Primers		Sequence	T _m (°C)	T _a (°C)
Exon 1	F	5' CTT gCT ggC gAC CTg CTT A 3'	70	64,5
	R	5' TgT TTC gCT CCA CgC TgA CT 3'	70	
Exon 2	F	5' Tgg TAg TAg AgT AAT CgC AAC A 3'	68	62
	R	5' Tgg TAg TAg AgT AAT CgC AAC A 3'	66	

PCRs were conducted at 25 µl volume in an Applied Biosystem GeneAmp® 9700 PCR System. Final concentrations of components were given in **Table 2.8** and PCR program for the reaction was given in **Table 2.9.**

Table 2.8. Components of the PCR reaction by *Yr10* gene specific primers.

PCR Components	Initial Concentration	Volume (μ l)	Final Concentration
10XBuffer	10 X	2,5 μ l	1 X
MgCl₂	50 mM	1,5 μ l	3 mM
dNTP	2.5 mM	2 μ l	0.2 mM
Primer F	100 μ M	1 μ l	4 mM
Primer R	100 μ M	1 μ l	4 mM
Taq DNA Polymerase	5 U/ μ l	0,2 μ l	0.04 U/ μ l
dH₂O	-	14,8 μ l	-
Sample DNA	50 ng/ μ l	2 μ l	4 ng/ μ l

Table 2.9. PCR program for exon1 and exon 2 of *Yr10* gene.

	Reaction Steps	# Cycle	Temperature ($^{\circ}$ C)	Time (min)		Reaction Steps	# Cycle	Temperature ($^{\circ}$ C)	Time (min)
EXON1	<i>Initial Denaturation</i>	1	94	02:00	EXON2	<i>Initial Denaturation</i>	1	94	03:00
	<i>Denaturation</i>	35	94	00:45		<i>Denaturation</i>	35	94	01:00
	<i>Annealing Temperature</i>		64,5	00:45		<i>Annealing Temperature</i>		62	01:00
	<i>Extension</i>		72	03:30		<i>Extension</i>		72	01:00
	<i>Final Extension</i>	1	72	10:00		<i>Final Extension</i>	1	72	10:00
<i>Hold</i>	1	4	∞	<i>Hold</i>	1	4	∞		

Exon 1 and exon 2 amplicons of *Yr10* gene were separated on 2% agarose gel for analysis of PCR products. GeneRuler™ 1 kb DNA ladder (Fermentas) was used to estimate the amplicon size. Electrophoresis was performed for two hours at 200V. UViproMW Software (Version 11.02) belong to Uvitec Capturing System was used to visualize band pattern on agarose gel.

2.2.2. Pathogen inoculation and sampling

Puccinia striiformis f. sp. *tritici* was used as pathogen material for spray inoculation. Growing of plant material and spreading the inoculum of yellow rust pathogen (*Puccinia striiformis* f. sp. *tritici*) was performed at the greenhouse of the Central Research Institute for Field Crops (CRIFC), Republic of Turkey Ministry of Food, Agriculture and Livestock, Ankara. Genotypes carrying *Yr10* gene confirmed by the analyses stated in section 2.2.1 (Presence of *Yr10* gene in wheat gene pool) were planted in soil and grown up till they became two weeks old. A yellow rust resistant

genotype that carries *Yr10* yellow rust resistance gene, 'Avocet *Yr10*', was used as a positive control of the experiment and Little Cub genotype which is susceptible for yellow rust used as negative control to verify the success of the inoculations. Uredospores were grown on a winter wheat at its early life stage by covering with a nylon bag to avoid from any contamination of other isolates of rust fungus. The uredospores collected from the experimental research sites of CRIFC mixed with the simple oil used for spray inoculation (**Figure 2.2.**). As a negative control, mock samples were sprayed with only oil. Mock and pathogen inoculated samples were collected at 7 different time points: 0 Hpi – mock, fifteen mpi, 12 hpi, 24 hpi, 48 hpi, 72 hpi, 96 hpi (**mpi:** minutes post inoculation; **hpi:** hours post inoculation). The first leaves of all tagged samples were collected and stored at -86°C freezer following treatment with liquid nitrogen (-196°C).



Figure 2.2. Inoculation of yellow rust uredospores and sampling. A: Plant material used for gene expression analysis of *Yr10*; B: Collected uredospores mixed with the simple oil used for spray inoculation; C, D: Negative control (Mock samples sprayed with only oil) and yellow rust inoculated plant material; E: All inoculated samples were kept at greenhouse; F, G: Primary leaf tissues from two leaf stage plants were taken to aluminum foils and kept at liquid nitrogen; H: All samples per each time point were kept at -86°C for further expressional studies.

2.2.2.1. Validation of inoculation

Before beginning to total RNA isolations from samples, the success of inoculation was proved by phenotypic selection according to 0-9 scale given in **Table 2.10.** (McNeal et al., 1971).

Table 2.10. Resistance to susceptibility scale for pathogen infection.

Infection type		Host response	Symptoms	
Resistance	0-6	0	Immune	No visible uredia
		1	Very resistant	Necrotic flecks
		2	Resistant	Necrotic areas without sporulation
		3-4	Resistant	Necrotic and chlorotic areas with restricted sporulation
		5-6	Moderately resistant	Moderate sporulation with necrosis and chlorosis
Susceptibility	7-9	7-8	Moderately susceptible	Sporulation with chlorosis
		9	Susceptible	Abundant sporulation without chlorosis

2.2.3. Total RNA isolation for *Yr10* expression

The collected leaf samples were isolated following Roche's Protocol by using "Trizol Reagent". Total RNAs were separated on 1% agarose gel (**Table 2.11.**), stained by RedSafe (Intron Biotechnology, Catalog No: 21141) and visualized under UV light by MiniBIS Pro Visualizing System (DNr Bio-Imaging Systems).

Table 2.11. Solutions used for DEPC-treated agarose gel electrophoresis.

Chemical Agent	Ingredients	Concentration
TBE Buffer (10X)	Trisma Base	890 mM
	EDTA	20 mM
	Boric Acid	890 mM
Loading Dye (6X) (DEPC Treated)	Bromophenol blue	0.025% (w/v)
	Xcylene cyanol	0.025% (w/v)
	Glycerol	92.10 g/mol
	DEPC-treated distilled water	-
Safe Red	Safe Red	20000X

RNA samples were treated with DNase to avoid from DNA contamination. RNA dilutions were prepared for each sample in total volume of 15.5 μ l for final 10 μ g/ μ l RNA concentration. 1.5 μ l of DNase (Promega) and 3 μ l of DNase Reaction Buffer were dissolved in 10 μ l dH₂O. Following, 10 μ g/ μ l RNA samples were combined with DNase; then the mixture was incubated at 37°C for 10 minutes for DNase activation (**Table 2.12**).

Table 2.12. Solutions used for DNase treatment (A and B solutions were mixed together and incubated at 37°C to avoid from DNA contamination).

A Solution	RNA+dH ₂ O	15,5 μ l
B Solution	1,5 μ l DNase I (1u/ μ l)	14,5 μ l
	3 μ l DNase Reaction Buffer (10X)	
	10 μ l dH ₂ O	

2.2.4. Verification of avoidance from DNA contamination: 18S rDNA amplification

RNA samples were used as template for 18S ribosomal DNA PCR to evaluate if there is any DNA contamination or not. With this aim; a PCR reaction was conducted at 25 μ l volume in an Applied Biosystem GeneAmp® 9700 PCR System. Final concentrations of components were given in **Table 2.13**.

Table 2.13. PCR components and their final concentrations for 18S rDNA amplification.

PCR Components	Initial Concentration	Volume (μ l)	Final Concentration
10XBuffer	10 X	2,5	1X
MgCl₂	50 mM	2	4 mM
dNTP	2.5 mM	2	0.2 mM
Primer F	100 μ M	1	4 μ M
Primer R	100 μ M	1	4 μ M
Taq DNA Polymerase	5 U/ μ l	0.2	0.04 U/ μ l
dH₂O	-	12.3	-
RNA	10 μ g/ μ l	4	1.6 μ g/ μ l

PCR program for 18S rDNA was given in **Table 2.14**.

Table 2.14. PCR program for 18S rDNA gene.

PCR Program	Reaction Steps	Number of Cycle	Temperature ($^{\circ}$ C)	Time (min)
	<i>Initial Denaturation</i>	1	94	02:00
	<i>Denaturation</i>	30	94	00:30
	<i>Annealing Temperature</i>		50	00:30
	<i>Extension</i>		72	01:00
	<i>Final Extension</i>	1	72	07:00
	<i>Hold</i>	1	4	∞

18S rDNA PCR products were loaded to 1.5% agarose gel and electrophoresis was run at 150V and a 100 bp marker (New England Biolabs) was used as marker.

2.2.5. cDNA synthesis

RNA samples (10 μ g/ μ l) were diluted (1/5) and RNA template (2 μ g/ μ l) were used for first chain synthesis as complementary DNA. As biological control; three replicates of RNA samples per each genotype for each time point was pooled. The RT-PCR (cDNA synthesis) was performed using 'High Capacity cDNA Reverse

Transcription Kit, ABI' (Catalog No: 4368814). 10 µl of 2X master mix and 10 µl of diluted RNA were mixed to total volume at 20 µl at reverse transcriptase PCR. The 2X master mix preparation was given in **Table 2.15**.

Table 2.15. PCR components for cDNA synthesis.

PCR Components	Initial Concentration	Volume (µl)	Final Concentration
RT-PCR Buffer	10X	2	1X
25X dNTP Mix	100 mM	0.8	4 mM
Random Primers	10X	2	1X
MultiScribe Reverse Transcriptase	50 U/ µl	1	50 U
NF-H ₂ O	-	4.2	-

PCR reaction was performed at Applied Biosystem GeneAmp® 9700 PCR System in the program given below at **Table 2.16**.

Table 2.16. RT-PCR Program for cDNA synthesis.

Program	Step1	Step2	Step3	Step4	Step5
Temperature (°C)	25	37	37	85	4
Time (min)	10	60	60	5	∞

2.2.6. Real-time PCR (Q-PCR) analysis

The RNAs which converted into cDNA by previous RT-PCR analyses were used as template for the *Yr10* gene expression analyses. The analyses were performed at StepOne™ Software (v2.2.2) at Real-Time PCR System, ABI (Catalog No: 4376600). *Actin Beta* and *GAPDH* (Glyceraldehyde-3-Phosphate Dehydrogenase) genes were used as multiple controls. Power Sybr® Green Master Mix, ABI (Catalog No: 4367659) was used for the detection of the amplification. Real-Time PCR reaction mix was prepared at the concentrations shown at **Table 2.17**.

Table 2.17. Real-Time PCR components used in this study.

PCR Components	Initial Concentration	Volume (μl)	Final Concentration
Power Sybr Green Master Mix	2X	25	1X
Primer F	10 pmol	4.5	0.9 pmol
Primer R	10 pmol	4.5	0.9 pmol
NF-H₂O	-	14	-

The Q-PCR program was given at **Table 2.18.**

Table 2.18. Real-Time PCR Program.

Reaction Steps	Number of Cycle	Temperature ($^{\circ}$C)	Time (min)
Initial Denaturation	1	95	10
Denaturation	40	95	00:15
Annealing		60	1

3. RESULTS and DISCUSSION

3.1. Assessment of Genetic Diversity in Yellow Rust Resistant Wheat Gene Pool

3.1.1. Genomic DNA isolation

The genomic DNA concentrations and dilution ratios of 55 bread wheat (*Triticum aestivum* L.) genotypes were given at **Appendix 2**. The genomic DNAs of 25 bread wheat genotypes out of 55 were shown in **Figure 3.1**.

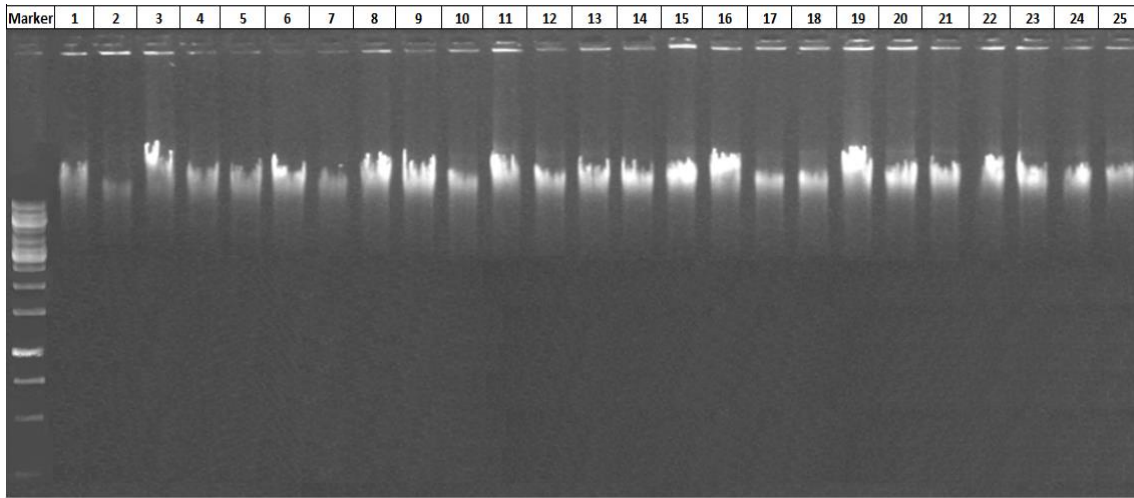


Figure 3.1. The genomic DNA band profiles of 25 bread wheat genotypes. **Marker:** GeneRuler™ 1 kb DNA Ladder (Fermentas). **1:**Alpu2001, **2:** Tekirdağ, **3:** Lancer, **4:** Gün-91, **5:** Türkmen, **6:** Gerek 79, **7:** Aytın 98, **8:** Altay 2000, **9:** Karahan-99, **10:** Konya 2002, **11:** Aldane, **12:** Nurkent, **13:** Kaşif Bey 95, **14:** İzgi2001, **15:** Sönmez 2001, **16:** Behoth 8, **17:** Jaolan 2, **18:** Douma 4, **19:** Sham 10, **20:** Douma 2, **21:** Sham 4, **22:** Behoth 4, **23:** Behoth 6, **24:** Sham 6, **25:** Sham 8.

3.1.2. Polymerase Chain Reaction (PCR)

As a result of screening of 55 bread wheat genotypes with 117 genome-wide SSR markers by polymerase chain reaction (PCR), 27 SSR markers were given 37 monomorphic alleles (**Figure 3.2**), while 90 SSR markers were giving 1620 polymorphic alleles (**Figure 3.3**). Thirty polymorphic markers were given more than one allele per genotype and they yielded 666 polymorphic alleles (**Figure 3.4**). Meanwhile, the remaining 60 polymorphic markers produced 954 polymorphic alleles.

A monomorphic SSR band profile was given in **Figure 3.2**.

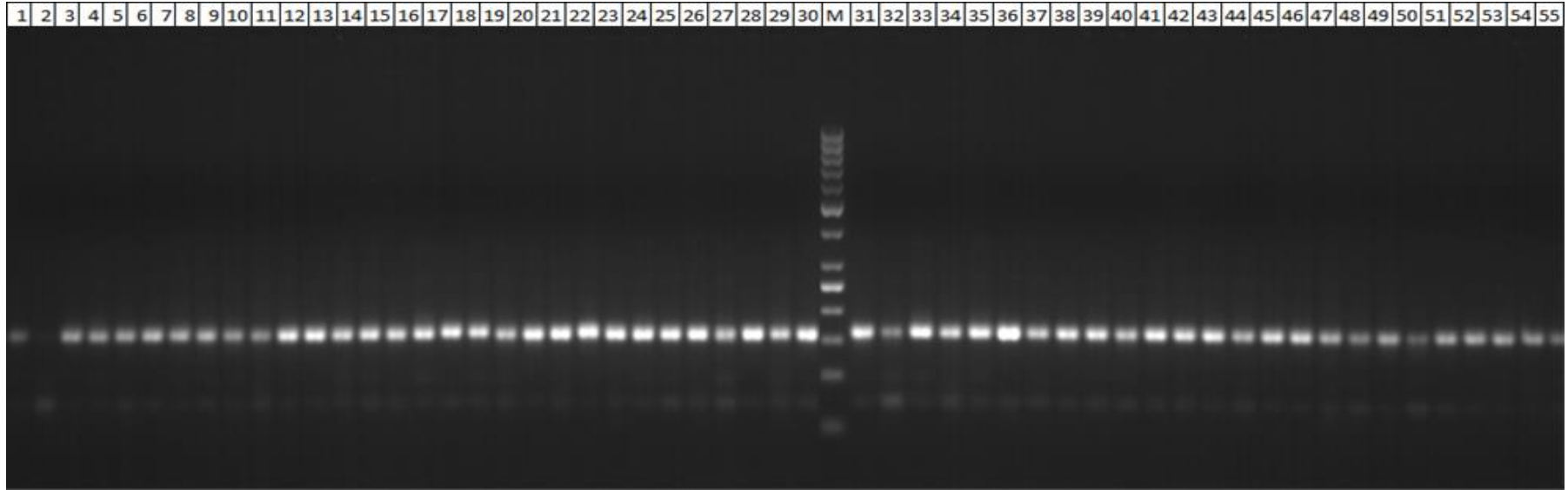


Figure 3.2. Monomorphic band profile of PCR products obtained by BARC196 primer pair at 2% agarose gel.

1: Pamukova97, **2:** Cemre, **3:** Tahirova, **4:** Hanlı, **5:** Ceyhan-99, **6:** Pandas, **7:** Karatopak, **8:** Osmaniyem, **9:** Carisma, **10:** Yakar-99, **11:** Aksel 2000, **12:** Bayraktar 2000, **13:** Demir 2000, **14:** Atlı-2002, **15:** Çetinel-2000, **16:** Alpu 2001, **17:** Tekirdağ, **18:** Lancer, **19:** Gün-91, **20:** Türkmen, **21:** Gerek 79, **22:** Aytın 98, **23:** Altay 2000, **24:** Karahan-99, **25:** Konya-2002, **26:** Aldane, **27:** Nurkent, **28:** Kaşif Bey 95, **29:** İzgi 2001, **30:** Sönmez 2001, **M:** GeneRuler™ 50 bp DNA Ladder (Fermentas), **31:** Behoth 8, **32:** Jaolan 2, **33:** Douma 4, **34:** Sham 10, **35:** Douma 2, **36:** Sham 4, **37:** Behoth 4, **38:** Behoth 6, **39:** Sham 6, **40:** Sham 8, **41:** Acsad 1139, **42:** Acsad 1133, **43:** Acsad 1115, **44:** Acsad 1159, **45:** Acsad 1071, **46:** Douma 40860, **47:** Douma 40863, **48:** Douma 40855, **49:** Douma 40856, **50:** Douma 40992, **51:** Douma 40988, **52:** Douma 40989, **53:** Douma 40444, **54:** Douma 48114, **55:** Douma 40765.

A polymorphic band profile resulted one allele per each genotype was given in **Figure 3.3**.

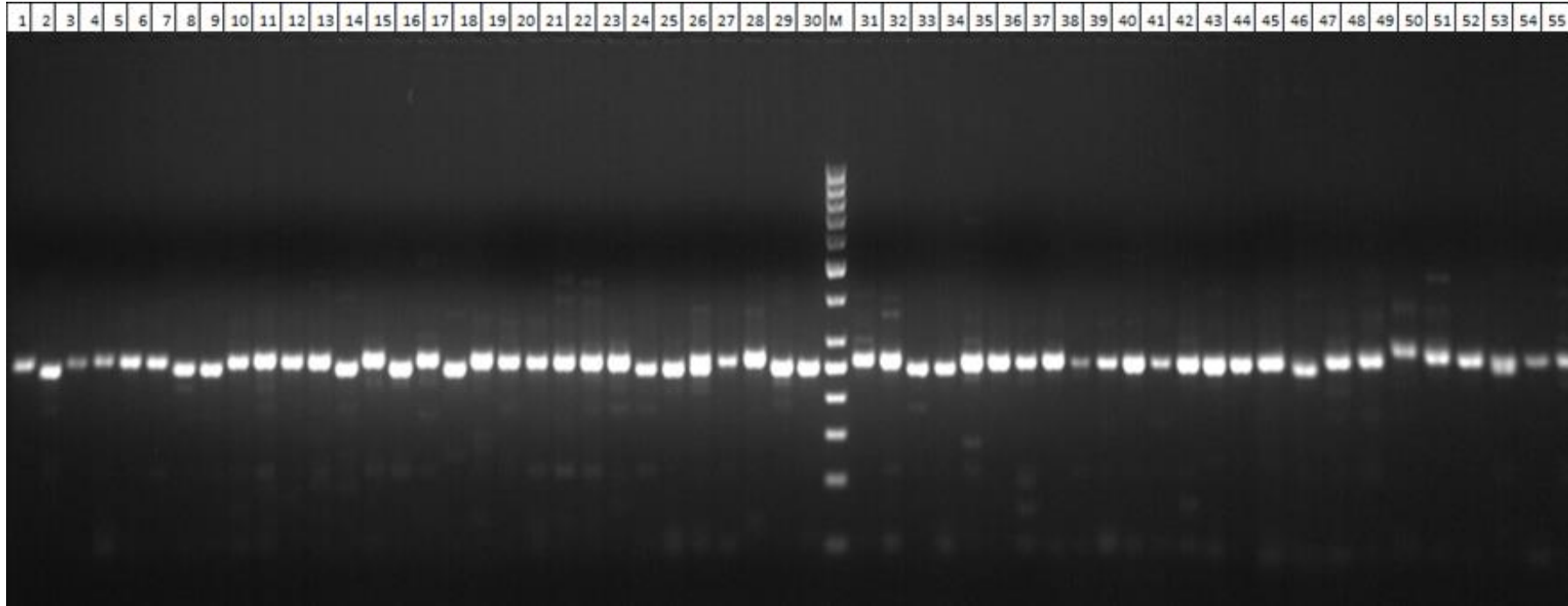


Figure 3.3. The single polymorphic band profile of PCR products obtained by BARC286 primer pair at 2% agarose gel.

1: Pamukova97, **2:** Cemre, **3:** Tahirova, **4:** Hanlı, **5:** Ceyhan-99, **6:** Pandas, **7:** Karatopak, **8:** Osmaniyem, **9:** Carisma, **10:** Yakar-99, **11:** Aksel 2000, **12:** Bayraktar 2000, **13:** Demir 2000, **14:** Atlı-2002, **15:** Çetinel-2000, **16:** Alpu 2001, **17:** Tekirdağ, **18:** Lancer, **19:** Gün-91, **20:** Türkmen, **21:** Gerek 79, **22:** Aytın 98, **23:** Altay 2000, **24:** Karahan-99, **25:** Konya-2002, **26:** Aldane, **27:** Nurkent, **28:** Kaşif Bey 95, **29:** İzgi 2001, **30:** Sönmez 2001, **M:** GeneRuler™ 50 bp DNA Ladder (Fermentas), **31:** Behoth 8, **32:** Jaolan 2, **33:** Douma 4, **34:** Sham 10, **35:** Douma 2, **36:** Sham 4, **37:** Behoth 4, **38:** Behoth 6, **39:** Sham 6, **40:** Sham 8, **41:** Acsad 1139, **42:** Acsad 1133, **43:** Acsad 1115, **44:** Acsad 1159, **45:** Acsad 1071, **46:** Douma 40860, **47:** Douma 40863, **48:** Douma 40855, **49:** Douma 40856, **50:** Douma 40992, **51:** Douma 40988, **52:** Douma 40989, **53:** Douma 40444, **54:** Douma 48114, **55:** Douma 40765.

A polymorphic band profile resulted multiple alleles per each genotype was given in **Figure 3.4**.

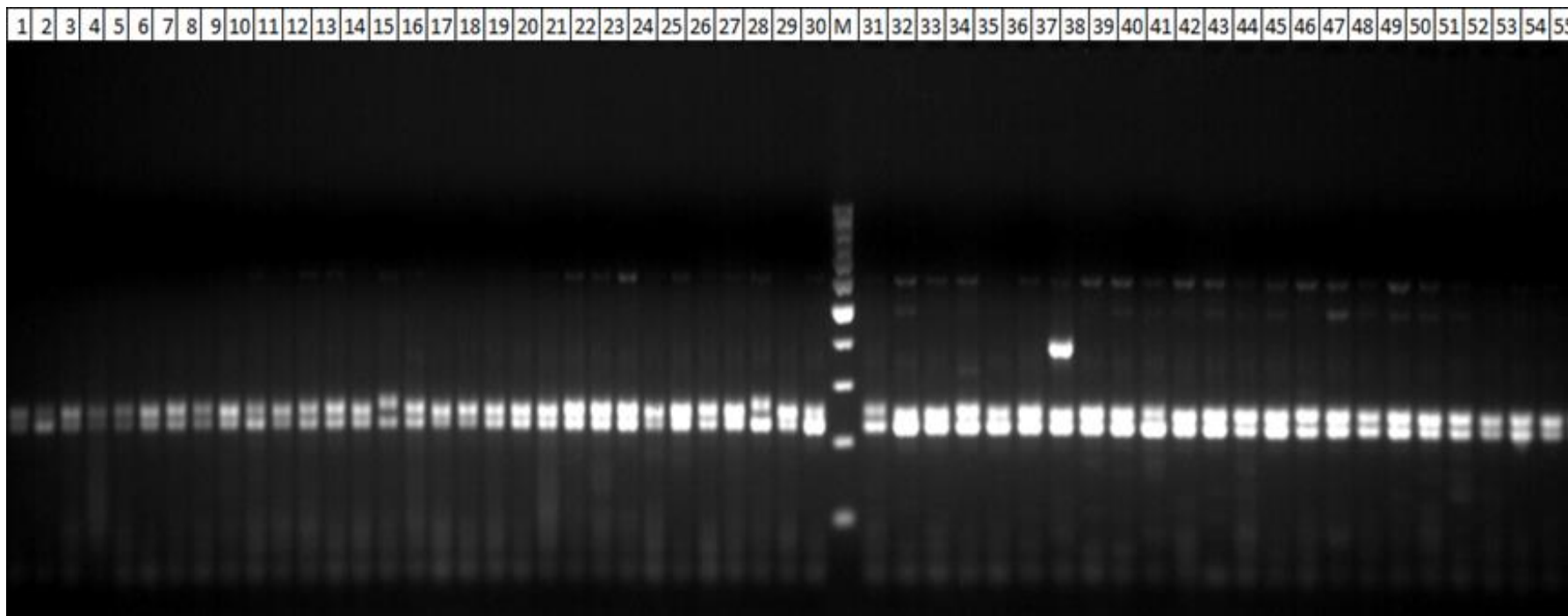


Figure 3.4. The multiple polymorphic band profile of PCR products obtained by BARC135 primer pair at 2% agarose gel. **1:** Pamukova97, **2:** Cemre, **3:** Tahirova, **4:** Hanlı, **5:** Ceyhan-99, **6:** Pandas, **7:** Karatopak, **8:** Osmaniyem, **9:** Carisma, **10:** Yakar-99, **11:** Aksel 2000, **12:** Bayraktar 2000, **13:** Demir 2000, **14:** Athı-2002, **15:** Çetinel-2000, **16:** Alpu 2001, **17:** Tekirdağ, **18:** Lancer, **19:** Gün-91, **20:** Türkmen, **21:** Gerek 79, **22:** Aytın 98, **23:** Altay 2000, **24:** Karahan-99, **25:** Konya-2002, **26:** Aldane, **27:** Nurkent, **28:** Kaşif Bey 95, **29:** İzgi 2001, **30:** Sönmez 2001, **M:** GeneRuler™ 50 bp DNA Ladder (Fermentas), **31:** Behoth 8, **32:** Jaolan 2, **33:** Douma 4, **34:** Sham 10, **35:** Douma 2, **36:** Sham 4, **37:** Behoth 4, **38:** Behoth 6, **39:** Sham 6, **40:** Sham 8, **41:** Acsad 1139, **42:** Acsad 1133, **43:** Acsad 1115, **44:** Acsad 1159, **45:** Acsad 1071, **46:** Douma 40860, **47:** Douma 40863, **48:** Douma 40855, **49:** Douma 40856, **50:** Douma 40992, **51:** Douma 40988, **52:** Douma 40989, **53:** Douma 40444, **54:** Douma 48114, **55:** Douma 40765.

The data obtained by 60 markers was used for statistical analyses (except the multiple alleles per genotype obtained by 30 polymorphic SSR marker analyses) to prevent possible increase at the error rate at statistical analyses. The percentage of polymorphic locus for A genome was 0.857, for B genome was 0.789, and for D genome was 0.681. The mean number of alleles per locus was 14.162 and allele percentage per A, B, and D genome was 34.03, 35.06 and 30.89, respectively. A summary of the results of the SSR analyses was given below in **Table 3.1**.

Table 3.1. A brief summary of SSR results.

PARAMETER	AMOUNT
Primers used in this study	117
Primers amplified polymorphic alleles	90
Primers amplified monomorphic alleles	27
Polymorphic primers used in PCA	60
Polymorphic primers not used in PCA	30
Total number of alleles	1657
Number of monomorphic alleles	37
Number of polymorphic alleles given by markers not used in statistical analyses	666
Number of polymorphic alleles given by markers used in statistical analyses	954
Number of polymorphic alleles used in statistical analyses for A genome	360
Number of polymorphic alleles used in statistical analyses for B genome	321
Number of polymorphic alleles used in statistical analyses for D genome	273
Percentage of allele numbers obtained from A genome for statistical analyses	37.7
Percentage of allele numbers obtained from B genome for statistical analyses	33.6
Percentage of allele numbers obtained from D genome for statistical analyses	28.6
Percentage of polymorphic alleles	97.76
Percentage of monomorphic alleles	2.24
Percentage of polymorphic alleles given by markers used in statistical analyses	51.2
Percentage of polymorphic alleles given by markers not used in statistical analyses	25.60
Percentage of polymorphic locus ratio related to A genome	0.857
Percentage of polymorphic locus ratio related to B genome	0.789
Percentage of polymorphic locus ratio related to D genome	0.681
Mean number of allele per locus	14.162
Total allele percentages per A genome	34.03
Total allele percentages per B genome	35.06
Total allele percentages per D genome	30.89

SSR markers are one of the most powerful DNA marker system due to their chromosome specific, highly polymorphic (Plaschke et al., 1995; Huang et al., 2002), highly reproducible and co-dominant nature (Röder et al., 1998) in eukaryotic genomes (Zhang et al., 2010). The SSR markers used in this study were placed on 7 chromosomes of A, B and D genomes related to their PCR amplification types in **Table 3.2**. Raw data obtained a total of 60 SSRs consisting 22, 19 and 19 SSRs belong to A, B and D genome respectively. They were used in genetic distance and population structure determination analyses. In a study by Ahmad (2002), 13 wheat cultivars of diverse origins were evaluated using 43 SSR markers, selected on the basis of their known genetic locations to give uniform coverage for all three wheat genomes (A, B, and D). The study detected 156 polymorphic alleles at 43 loci, with a wide range of allelic variants for each locus; the range of alleles per locus was 2–8 (average 3.6). Another study (Prasad et al., 2000) examined the utility of a set of 20 wheat SSR markers to detect DNA polymorphism, identify genotypes, and estimate genetic diversity among 55 elite wheat genotypes. As a result of the study, they detected 155 alleles at 21 loci using microsatellite primer pairs. Their 20 primers amplifying 21 loci, 17 of the primers and their corresponding 18 loci were assigned to different chromosomes among A, B and D genomes. The number of alleles was ranged from 1 to 13, with an average of 7.4 alleles per locus. The mean value of polymorphic information content (PIC) was estimated as 0.71 (Prasad et al., 2000). Fufa et al. (2005) have reported that 68 wheat SSR markers screened for amplification products and polymorphism information produced 141 bands (monomorphic and polymorphic) across 30 hard red winter wheat cultivars, with a range of 1–5 and average of 3 bands per locus. Genetic diversity per locus was 0.289–0.958, and the average genetic distance across all loci in 30 cultivars was 0.623. The average genetic distance from all 68 markers was 0.427. Fufa et al. (2005) suggest that the higher SSR-based distance could be due to more complete coverage of the genomes by the markers or to the diversity of the lines used in their study. Using a more diverse set of cultivars, Almanza-Pinzon et al. (2003) found higher levels of diversity; their SSR markers were more polymorphic than those in previous studies (Plaschke et al., 1995; Bohn et al., 1999).

Table 3.2. Amplification pattern and chromosomal position of SSR markers used in this study.

Chromosome	Amplification Type								
	One Polymorphic Band*			Multiple Polymorphic Bands			Monomorphic Band/Bands		
	A	B	D	A	B	D	A	B	D
1	<i>barc17</i> <i>barc83</i> <i>barc148</i> <i>barc158</i> <i>cfa2135</i> <i>cfa2153</i> <i>wmc278</i>	<i>barc131</i> <i>wmc44</i> <i>wmc416</i>	<i>cfid27</i> <i>cfid63</i> <i>cfid83</i> <i>wmc147</i>	<i>wmc716</i>	<i>barc80</i> <i>barc137</i> <i>barc181</i>	-	-	-	<i>barc271</i>
2	<i>cfa2263</i> <i>gwm636</i> <i>wmc177</i> <i>wmc407</i> <i>wmc522</i>	<i>wmc154</i> <i>wmc361</i> <i>wmc477</i> <i>wmc770</i>	<i>cfid53</i> <i>wmc503</i>	<i>cfa2201</i> <i>gwm558</i>	<i>cfa2278</i>	-	-	<i>cfid238</i>	<i>cfid56</i> <i>cfid116</i>
3	<i>barc314</i> <i>cfa2193</i> <i>wmc532</i> <i>wmc559</i>	<i>barc75</i> <i>barc164</i>	-	<i>wmc169</i> <i>wmc428</i>	<i>barc84</i>	<i>barc125</i> <i>barc135</i> <i>cfid9</i> <i>cfid55</i> <i>cfid152</i>	<i>wmc153</i>	-	<i>wmc656</i> <i>cfid223</i>
4	<i>barc343</i> <i>wmc262</i>	<i>gwm375</i> <i>wmc710</i>	<i>barc1183</i> <i>wmc720</i>	<i>wmc219</i>	<i>cfa2149</i>	<i>wmc285</i>	<i>cfid257</i>	<i>barc227</i> <i>barc1045</i> <i>wmc413</i>	-
5	<i>barc117</i>	<i>barc243</i> <i>wmc75</i>	<i>barc143</i> <i>barc177</i> <i>barc286</i> <i>cfid57</i> <i>gwm174</i> <i>wmc765</i>	-	-	<i>cfid29</i>	<i>cfa2250</i>	-	<i>barc130</i> <i>gwm212</i> <i>cfid12</i>
6	<i>wmc201</i>	<i>barc178</i> <i>wmc397</i> <i>wmc494</i>	<i>barc273</i>	<i>barc1165</i> <i>wmc256</i>	<i>barc79</i> <i>barc354</i>	<i>cfid76</i> <i>gdm127</i>	<i>barc107</i>	-	<i>barc96</i> <i>cfid47</i> <i>barc175</i> <i>cfid33</i> <i>barc196</i>
7	<i>barc222</i> <i>wmc525</i>	<i>gwm537</i> <i>wmc396</i> <i>wmc517</i>	<i>barc214</i> <i>barc235</i> <i>cfid69</i> <i>wmc463</i>	-	<i>wmc276</i> <i>wmc335</i> <i>wmc476</i>	<i>barc172</i> <i>barc184</i>	<i>cfa2257</i>	<i>wmc311</i> <i>barc72</i> <i>barc255</i> <i>wmc426</i>	<i>cfid14</i>

*: Raw data obtained from these markers were used as template for statistical analyses.

3.1.3. Data analyses

Ninety SSR markers were resulted in polymorphic band pattern, whereas twenty-seven SSR markers were resulted in monomorphic band pattern out of 117 SSR markers scattered throughout A, B and D genomes of 55 bread wheat genotypes. Band sizes of polymorphic SSRs were given in **Appendix 3**.

Since, recent genetic diversity analysis methods are available to use multiallelic molecular marker data and providing scientist for an easier way to obtain rich graphical outputs by software such as genetic analysis on excel GenAlEx 6.5. (Peakall and Smouse, 2012). Due to the allowance of using multiallelic data for genetic diversity analysis, we used raw multiallelic data obtained from SSR analyses to determine genetic distance within our gene pool. Multiallelic SSR data was used for all kinds of statistical analysis that conducted in this study. As a result of PIC value calculation, the SSR locus *wmc262* located on the chromosome 4A had the highest PIC of 0.960, whereas the SSR locus *barc314* located on chromosome 3A had the lowest PIC of 0.759. *Wmc262* was resulted in the highest number of alleles of 33, whereas *barc314* was resulted in the lowest number of alleles of 7. The all diversity measure of PIC values were shown in **Table 3.3** and **Table 3.4**. The comparison of PIC values within A, B and D genomes were given in **Figure 3.5, 3.6** and **3.7**. The highest PIC value that A genome was carried at *wmc262* marker located on 4A chromosome with the PIC value of 0.960. The highest PIC value (0.954) that B genome was carried at *wmc44* marker located on 1B chromosome. The highest PIC value that D genome was carried at *gwm174* marker located on 5D chromosome with the PIC value of 0.948.

Table 3.3. Allele sizes and PIC values of polymorphic SSR markers giving single band.

GENOME		MARKER NAME	SIZE RANGES of ALLELES (bp)	NUMBER of ALLELES	PIC VALUES
A	1A	<i>barc17</i>	308-269	12	0.879
		<i>barc83</i>	295-256	18	0.912
		<i>barc148</i>	221-192	16	0.905
		<i>barc158</i>	269-239	17	0.909
		<i>cfa2135</i>	194-173	10	0.881
		<i>ca2153</i>	224-163	22	0.934
		<i>wmc278</i>	226-205	17	0.916
	2A	<i>cfa2263</i>	163-116	16	0.907
		<i>gwm636</i>	119-91	12	0.778
		<i>wmc177</i>	213-187	16	0.907
		<i>wmc407</i>	150-112	12	0.844
		<i>wmc522</i>	235-175	29	0.956
	3A	<i>barc314</i>	297-259	7	0.759
		<i>cfa2193</i>	285-247	11	0.825
		<i>wmc532</i>	198-153	21	0.936
		<i>wmc559</i>	346-261	16	0.849
	4A	<i>barc343</i>	184-155	16	0.922
		<i>wmc262</i>	247-157	33	0.960
	5A	<i>barc117</i>	244-210	12	0.885
	6A	<i>wmc201</i>	283-233	15	0.874
		<i>barc222</i>	200-182	8	0.764
7A	<i>wmc525</i>	280-190	24	0.943	
	1B	<i>barc131</i>	250-216	15	0.903
<i>wmc44</i>		292-206	31	0.954	
<i>wmc416</i>		269-224	19	0.922	
2B	<i>wmc154</i>	259-125	18	0.926	
	<i>wmc361</i>	240-221	10	0.792	
	<i>wmc477</i>	191-170	15	0.913	
	<i>wmc770</i>	194-91	28	0.944	
	3B	<i>barc75</i>	140-102	9	0.833
<i>barc164</i>		213-171	15	0.907	
4B	<i>gwm375</i>	179-137	13	0.876	
	<i>wmc710</i>	141-97	13	0.876	
5B	<i>barc243</i>	247-199	21	0.928	
	<i>wmc75</i>	274-237	15	0.893	
6B	<i>barc178</i>	344-262	16	0.907	
	<i>wmc397</i>	200-160	17	0.919	
	<i>wmc494</i>	256-214	19	0.922	

Table 3.3. Continued.

	7B	<i>gwm537</i>	244-210	13	0.878
		<i>wmc396</i>	200-150	14	0.880
		<i>wmc517</i>	225-173	20	0.926
D	1D	<i>cfid27</i>	160-139	11	0.846
		<i>cfid63</i>	306-263	15	0.905
		<i>cfid83</i>	258-230	17	0.915
		<i>wmc147</i>	173-150	10	0.842
	2D	<i>cfid53</i>	284-221	17	0.838
		<i>wmc503</i>	329-236	23	0.919
	3D		-		
	4D	<i>barc1183</i>	277-250	10	0.825
		<i>wmc720</i>	161-112	18	0.932
	5D	<i>barc143</i>	305-274	11	0.853
		<i>barc177</i>	160-116	16	0.881
		<i>barc286</i>	276-241	11	0.849
		<i>cfid57</i>	317-263	13	0.869
		<i>gwm174</i>	276-147	26	0.948
		<i>wmc765</i>	200-150	14	0.899
	6D	<i>barc273</i>	254-221	10	0.852
	7D	<i>barc214</i>	232-208	10	0.838
		<i>barc235</i>	310-274	13	0.862
		<i>cfid69</i>	262-206	16	0.862
		<i>wmc463</i>	168-140	12	0.868

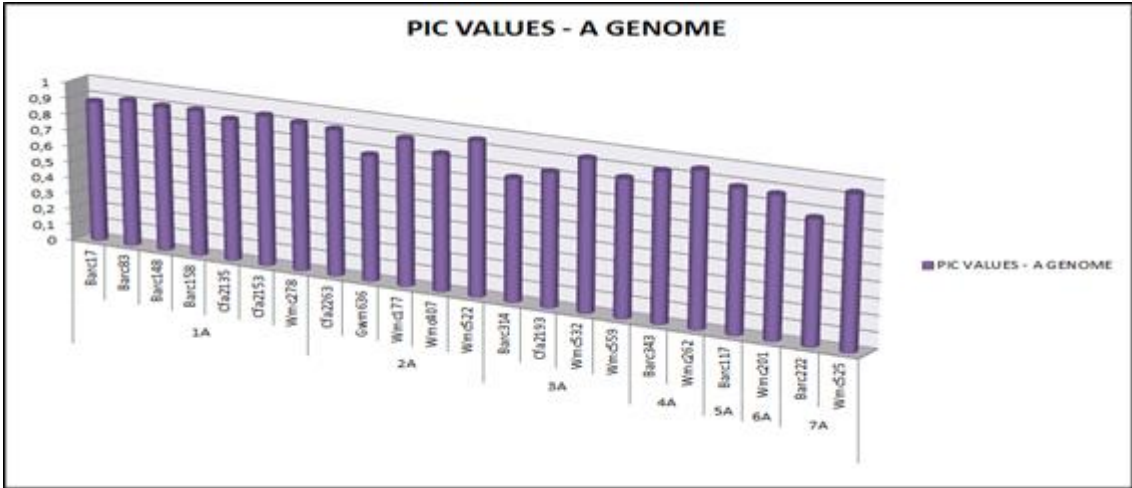


Figure 3.5. PIC values of each SSR marker in A genome.

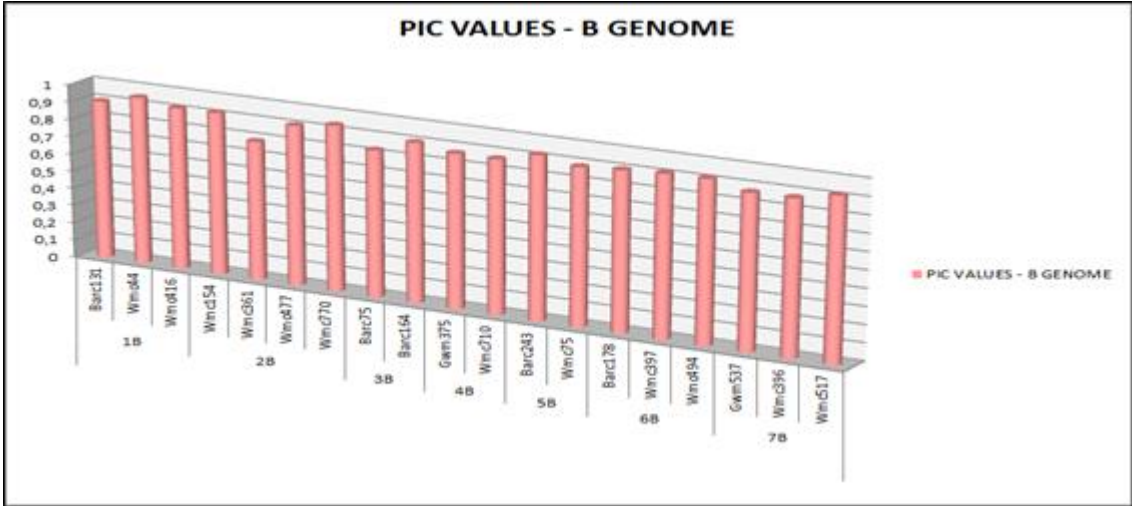


Figure 3.6. PIC values of each SSR marker in B genome.

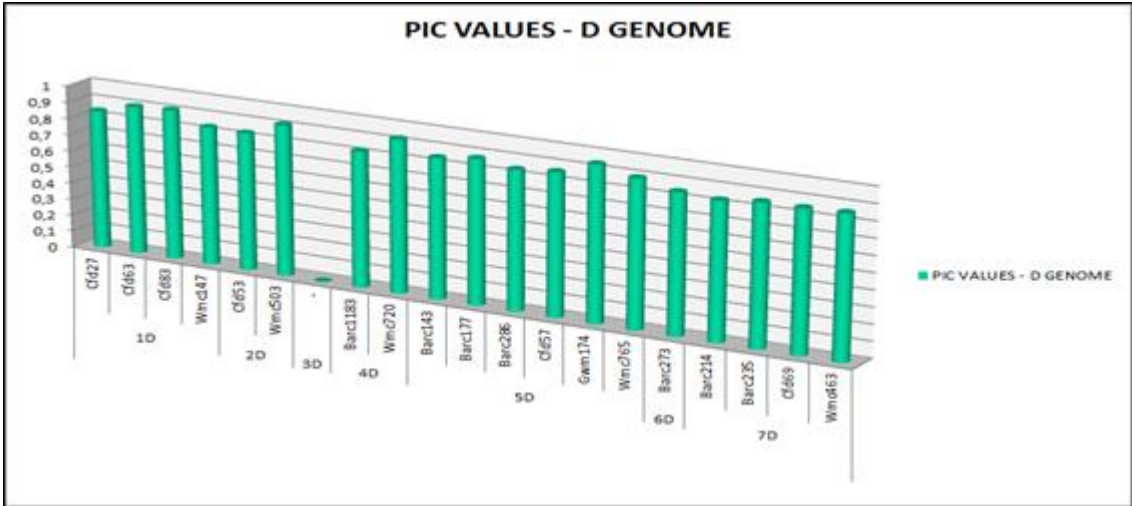


Figure 3.7. PIC values of each SSR marker in D genome.

PIC value graphs showed the high level of polymorphic information for each SSR.

Table 3.4. Distribution, number of alleles and PIC values of SSR markers per each chromosome and genome.

Chromosome		1	2	3	4	5	6	7	
A	# Markers	7	5	4	2	1	1	2	PIC Values (Mean) 0.883
	Markers	<i>barc17</i> <i>barc83</i> <i>barc148</i> <i>barc158</i> <i>cfa2135</i> <i>cfa2153</i> <i>wmc278</i>	<i>cfa2263</i> <i>gwm636</i> <i>wmc177</i> <i>wmc407</i> <i>wmc522</i>	<i>barc314</i> <i>cfa2193</i> <i>wmc532</i> <i>wmc559</i>	<i>barc343</i> <i>wmc262</i>	<i>barc117</i>	<i>wmc201</i>	<i>barc222</i> <i>wmc525</i>	
	Total # Alleles	112	85	55	49	12	15	32	
	PIC Values (Mean)	0.906	0.879	0.843	0.941	0.886	0.875	0.854	
Chromosome		1	2	3	4	5	6	7	
B	# Markers	3	4	2	2	2	3	3	PIC Values (Mean) 0.899
	Markers	<i>barc131</i> <i>wmc44</i> <i>wmc416</i>	<i>wmc154</i> <i>wmc361</i> <i>wmc477</i> <i>wmc770</i>	<i>barc75</i> <i>barc164</i>	<i>gwm375</i> <i>wmc710</i>	<i>barc243</i> <i>wmc75</i>	<i>barc178</i> <i>wmc397</i> <i>wmc494</i>	<i>gwm537</i> <i>wmc396</i> <i>wmc517</i>	
	Total # Alleles	65	71	24	26	36	52	47	
	PIC Values (Mean)	0.927	0.894	0.870	0.876	0.911	0.917	0.895	
Chromosome		1	2	3	4	5	6	7	
D	# Markers	4	2	-	2	6	1	4	PIC Values (Mean) 0.872
	Markers	<i>cfid27</i> <i>cfid63</i> <i>cfid83</i> <i>wmc147</i>	<i>cfid53</i> <i>wmc503</i>	-	<i>barc1183</i> <i>wmc720</i>	<i>barc143</i> <i>barc177</i> <i>barc286</i> <i>cfid57</i> <i>gwm174</i> <i>wmc765</i>	<i>barc273</i>	<i>barc214</i> <i>barc235</i> <i>cfid69</i> <i>wmc463</i>	
	Total # Alleles	53	40	-	28	91	10	51	
	PIC Values (Mean)	0.877	0.879	-	0.879	0.884	0.853	0.858	

A total of 1657 alleles were generated from 55 bread wheat cultivars using 117 SSR markers. 954 polymorphic alleles of 1657 were used for statistical analysis to determine genetic variability of the gene pool. The mean number of alleles per locus was 14.162 and the average PIC value was high with the value of 0.884. When these results compared with others, the level of polymorphism was higher in this study. It was

found that the mean number of alleles per locus was 7.97 and the mean PIC value was 0.65 for narrow mixed population consists of 49 durum and bread wheat varieties (Achtar et al., 2010). In other recent study, Hao et al. (2011) were found the mean PIC value at 0.650; ranging from 0 to 0.965 by the amount of number of alleles at 6724 within 250 bread wheat lines (Hao et al., 2011). Although our population size was 55, we had the number of alleles at 1657 and the mean value of PIC was higher than these studies.

As a result of SSR analyses with the polymorphic markers given one allele per one genotype; the mean numbers of alleles and PIC values were changed in different markers along seven sets of chromosomes among A, B and D genome of bread wheat which used in statistical analyses. Our study showed that the more number of alleles does not mean that the more average number of PIC values in any case of SSR marker analysis. The frequency of different alleles at different size is more important parameter than the number of alleles amplified by a marker. With respect to our SSR analyses results, the mean number of alleles obtained by the PCR amplifications was compared with the mean number of PIC values according to the number of markers used in statistical analyses per each chromosome of wheat. Consequently, the number of markers used for statistical analyses per each chromosome was varied from zero to seven; the total numbers of alleles was varied from 10 to 112; and the mean number of PIC values was varied from 0.843 to 0.941. The distribution of number of alleles and PIC values per each chromosome were given in **Table 3.4**. Correspondingly, *barc117* marker placed on 5A chromosome was more polymorphic than *wmc201* placed on 6A with the mean number of PIC value of 0.886 compared to 0.875 with the number of alleles of 12 and 15, respectively. Following, the mean number of PIC values obtained from two markers; *barc243* and *wmc75*; placed on 5B chromosome was much higher than the mean number of PIC values obtained from different pair of two markers; *cf53* and *wmc503*; placed on 2D chromosome with the mean number of PIC value of 0.911 compared to 0.879 with the total number of alleles from 36 to 40. The significant mean number of PIC values obtained from four markers per chromosomes was ranged at chromosome 3A when compared to other chromosomes 7D and 1D. Their mean number of PIC values was 0.843, 0.858 and 0.877 and the total number of alleles was ranged from 55, 51 and 53 in the same order. Yet, the total number of alleles was increased

proportionally in the calculation of averages with five markers per chromosome: 85; six markers: 91 and seven markers: 112 and following PIC values of 0.879, 0.884 and 0.906 (**Table 3.4**). The mean number of alleles per each A, B and D genomes were ranged from 16.32, 16.48 and 12.16. According to all these data obtained from genetic diversity analyses are shown the B genome was carrying more number of alleles than A and D genomes. However, the lack of markers on chromosome 3D may affected the number of alleles and polymorphism ratios (the number of alleles on chromosome 3D were assumed as “0” for statistical analysis).

Our results were differed from another study on 998 wheat accessions of bread wheat by 24 genome-wide SSR marker analysis conducted by Huang et al. (2002). They claimed that there is a correlation between the number of alleles and the value of gene diversity (PIC) and they reported that a study conducted by Prasad et al (2000) was not agree with this deduction. According to our study, nearly all polymorphism information content values were increased by the increase at the number of alleles. Yet, there were deviations from this positive correlation. The results were proved that the allele frequency is affecting the polymorphism information content values more than the number of alleles. It is also correlated with the number of accessions used in a genetic diversity analysis. Huang et al (2002) also recommended using larger number of genotypes will positively affect the precise distribution of correlation coefficient. In the same study, 24 genome-wide SSR markers were used to evaluate genetic diversity between 998 wheat accessions. The number of alleles per locus was range from 4 to 46 with a mean number of 18.1 alleles per locus. Our number of alleles was ranging from 7 with the lowest PIC value of 0.759 on chromosome 3A at the ranges of alleles of 259-297 to 33 with the highest PIC value of 0.96 on chromosome 4A at the ranges of alleles of 157-247. Besides, the mean number of alleles was lower than theirs with the value of 14.98.

In our study, the highest number of allele was 24.5 on chromosome 4A and the lowest number of allele was 10 on chromosome 6D. The PIC values among these chromosomes were 0.941 and 0.853. Even the highest PIC value out of 21 chromosomes was on chromosome 4A, the lowest PIC value was not on chromosome 6D. The lowest PIC value of 0.843 was on chromosome 3A.

SSR loci in wheat were shown random and highest proportion of microsatellites was occurred in B genome according to the study of Gupta et al. (1999). In our study, the mean PIC value for B genome was also highest by the number of 0.899, when compare to A genome 0.883 and D genome 0.872.

Linkage disequilibrium analysis showed patterns of LD and confirmed that three pairs of markers were located on the same chromosomes (**Figure 3.8**).

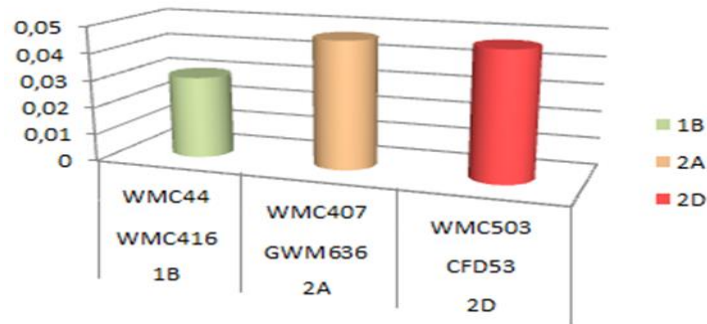


Figure 3.8. Chromosomal linkage between three SSR marker pairs which belong to A, B and D genome.

Linkage disequilibrium (LD) is one of the most recent core fields of plant genomics (Wang et al., 2007). LD which can be defined as the nonrandom association of alleles at different loci has a supplementary role in association mapping as allowing scientists to determine the resolution of an association study. In order to better understanding of LD, more species should be analyzed (Flint-Garcia et al., 2003). LD was conducted based on the concept of false discovery rate (FDR). This method allows precise estimation of balance between true and false positives in a genome-wide scan for linkage (Storey and Tibshirani, 2003). In this approach, q -value is similar to the traditional p -value, whereas the p -value is a measure of significance in terms of the false positive rate, the q -value is a measure in terms of the FDR (Storey and Tibshirani, 2003). Features with q -values 5% are supposed as significant in some genome-wide test of significance. Due to its feature as ≤ 0.05 , the q -values directly provide a meaningful measure called significant (Storey and Tibshirani, 2003). According to our LD results within our gene pool, even though 81 SSR marker pairs located on the same chromosomes, they were not linked to each other considering their q -values were higher than 0.05 (**Appendix 4**). Moreover, there were 1751 SSR marker pairs which were not located on the same chromosomes as a result of manipulation of iterations at software.

In the study of Chen et al. (2012), they used 269 SSR loci and they obtained 36.046 locus pairs in Chinese winter wheat collection. 1340 of them were revealed at $p < 0.001$ level, and 79 of them found at $r^2 > 0.1$ and $p < 0.001$. 1692 of locus pairs were detected as linked. Among linked locus pairs, only 162 of them were at $p < 0.001$ and of all significant pairs, 21 primer pairs were shown an r^2 value greater than 0.1 (Chen et al., 2012). On the other hand, only three SSR marker pairs: *wmc416* and *wmc44* on chromosome 1B, *wmc407* and *gwm636* on chromosome 2A, and *wmc503* and *cf53* on chromosome 2D were declared to be linked by q -values of 0.0302, 0.0469 and 0.0469 (**Figure 3.8**). These markers were located by genetic distance of 34 cM, 18 cM and 4 cM according to Somers et al. (2004) and Fox et al. (2013) (Fox et al., 20013; Somers et al., 2004). Zhang et al. (2010) were determined long-distance LD blocks on 1D, 2A, and 6A chromosomes of wheat and the longest one (>40 cM) was on chromosome 6A (Zhang et al., 2010). The LD distance between loci on wheat chromosomes range from 0.5 to 50 cM (Zhang et al., 2010; Chao et al., 2007; Somers et al., 2007; Breseghello and Sorrells, 2006; Maccaferri et al., 2006) is relatively high when compare to maize (200-2000 bp, Tenailon et al., 2001) and Arabidopsis (less than 10 Kb, Song et al., 2009; Kim et al., 2007) LD blocks (Zhang et al., 2010). Our results were correlated with these previous results by the LD from 4 to 34 cM.

Allelic data obtained from SSR analysis giving a single polymorphic band pattern per each genotype was used for the construction of the genotypic distance matrix given in **Appendix 5**. The genetic distance matrix showed us the genotype pairs; ‘Ceyhan-99 – Behoth 6’; ‘Ceyhan-99 – Douma 2’; ‘Ceyhan-99 – Sham 8’; ‘Gerek 79 – Douma 40989’; ‘Karahana-99 – Douma 40989’; ‘Aytın 98 – Douma 40989’; ‘Gerek 79 – Douma 48114’; ‘Hanlı – Behoth 6’; ‘Karahana-99 - Douma 48114’ were the most distant genotype pairs according to their genetic distance values given in the same order: ‘207.5’, ‘203.7’, ‘198.2’, ‘197.8’, ‘197.6’, ‘197.4’, ‘195.6’, ‘195.6’, and ‘195.4’. While the most distant two cultivars were Ceyhan-99 (ID number: 5) and Behoth 6 (ID number: 38) by the distance of ‘207.53’; the most distant cultivar and advanced line were Gerek 79 (ID number: 21) and Douma 40989 (ID number: 52) by the distance of ‘197.87’ between Turkey and Middle-East populations. The most distant two advanced lines within Middle-East population were Acsad 1133 (ID number: 42) and Douma 40444 (ID number: 53) by the distance of ‘154.45’. The most distant two cultivars from

Turkey population were Ceyhan-99 (ID number: 5) and Kaşif Bey 95 (ID number: 28) by the distance of '152.47'. When compare to PCA graph results, it can be verified that Kaşif Bey 95 Turkish cultivar line was separated from other Turkish cultivars. The closest cultivar lines were Turkish cultivars Çetinel-2000 (ID number: 15) and Türkmen (ID number: 20) by the kinship of '2.47'. The closest Middle-Eastern cultivars were Behoth 8 (ID number: 31) and Douma 4 (ID number: 33) by the kinship of '3.40'. The closest advanced breeding lines were Douma 40856 (ID number: 49) and Douma 40989 (ID number: 52) by the kinship of '15.50'. The closest Turkish and Middle-Eastern cultivars were Kaşif Bey 95 (ID number: 28) and Behoth 8 (ID number: 31) by the kinship of '38.99', respectively. All these results can be verified by comparing PCA graph. The PCA graph showing the arrangements of the genotypes belonging to their distance from themselves at the spatial level was given below in **Figure 3.9**. Respectively, the Middle-East genotypes were placed against to Turkish genotypes. Consequently, the genotypes per A, B, C and D subgroups of PCA graph are given below in that order: their names, characteristics (C:cultivar/ AL:advanced line) and sample IDs.

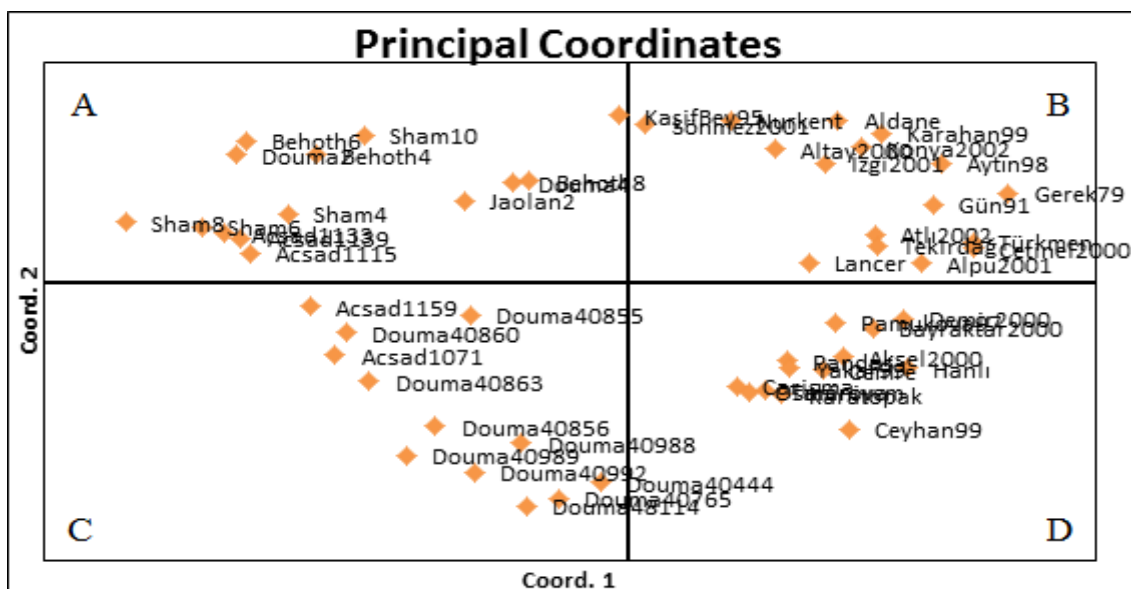


Figure 3.9. A, B, C and D are four sub-groups within our gene pool obtained by PCA analysis. **Group A:** Behoth 8, C, (31); Jaolan 2, C, (32); Douma 4, C, (33); Sham 10, C, (34); Douma 2, C, (35); Sham 4, C, (36); Behoth 4, C, (37); Behoth 6, C, (38); Sham 6, C, (39); Sham 8, C, (40); Acsad 1139, AL, (41); Acsad 1133, AL, (42); Acsad 1115, AL, (43); Kaşif Bey 95, C, (28). **Group B:** Atlı-2002, C, (14); Çetinel-2000, C, (15); Alpu 2001, C, (16); Tekirdağ, C, (17); Lancer, C, (18); Gün-91, C, (19); Türkmen, C, (20); Gerek 79, C, (21); Aytn 98, C, (22); Altay 2000, C, (23); Karahan-99, C, (24); Konya-2002, C, (25); Aldane, C, (26); Nurkent, C, (27); İzgi 2001, C, (29); Sönmez 2001, C, (30). **Group C:** Acsad 1159, AL, (44); Acsad 1071, AL, (45); Douma 40860, AL, (46); Douma 40863, AL, (47); Douma 40855, AL, (48); Douma 40856, AL, (49); Douma 40992, AL, (50); Douma 40988, AL, (51); Douma 40989, AL, (52); Douma 40444, AL, (53); Douma 48114, AL, (54); Douma 40765, AL, (55). **Group D:** Pamukova 97, C, (1); Cemre, C, (2); Tahirova, C, (3); Hanlı, C, (4); Ceyhan-99, C, (5); Pandas, C, (6); Karatopak, C, (7); Osmaniye, C, (8); Carisma, C, (9); Yakar-99, C, (10); Aksel 2000, C, (11); Bayraktar 2000, C, (12); Demir 2000, C, (13).

The output of the Bayesian approach was analyzed with the structure the Structure Harvester program (**Figure 3.10**) and results suggests that optimal number of $K=3$ implying the existence of three main populations. Clustering pattern obtained from Structure Software based on Bayesian approach is visualized in a graph to demonstrate the population structure. The structure of our gene pool was shown in **Figure 3.11**. Analyses showed that the populations from Turkey and Middle-East were largely separating into different groups and there is evidence for a third group that includes both Turkish and Middle Eastern populations. In another study, there were four subpopulations obtained by PCoA analysis within U.S. soft and hard elite winter wheat accessions (Zhang et al., 2010). Also, Chen et al. (2012) determined three subpopulations distinctly separated according to their geographical eco-types of accessions of 90 Chinese winter wheat based on 269 SSR markers by principle coordinate analysis (Chen et al., 2012).

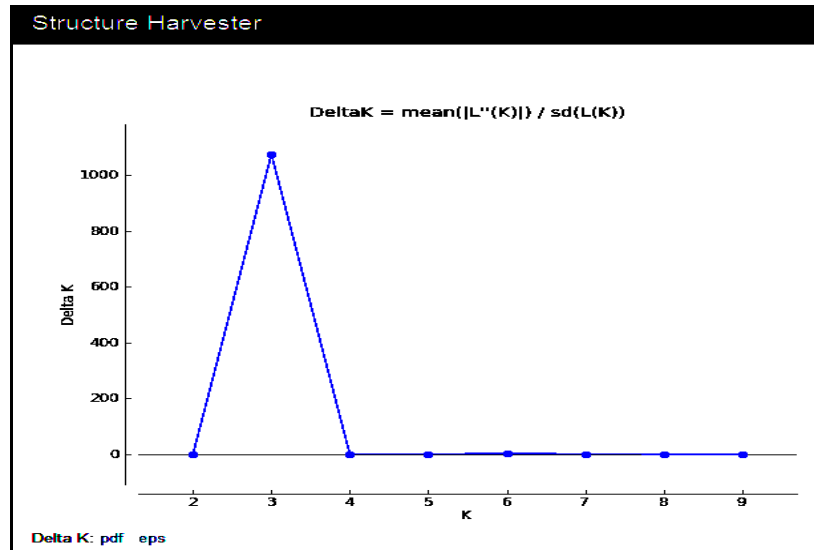


Figure 3.10. The graph to determine the optimal number of K obtained from Structure Harvester.

In the study of Chen et al. (2012), they evaluated the population structure of the 90 accessions using STRUCTURE V2.3.3 software based on 269 genome-wide SSR markers. The optimal number of subpopulations (K) was identified dividing the population into three subgroups (Chen et al., 2012). In another study, the optimal K value was evaluated as consisting of four subgroups (Zhang et al., 2010).

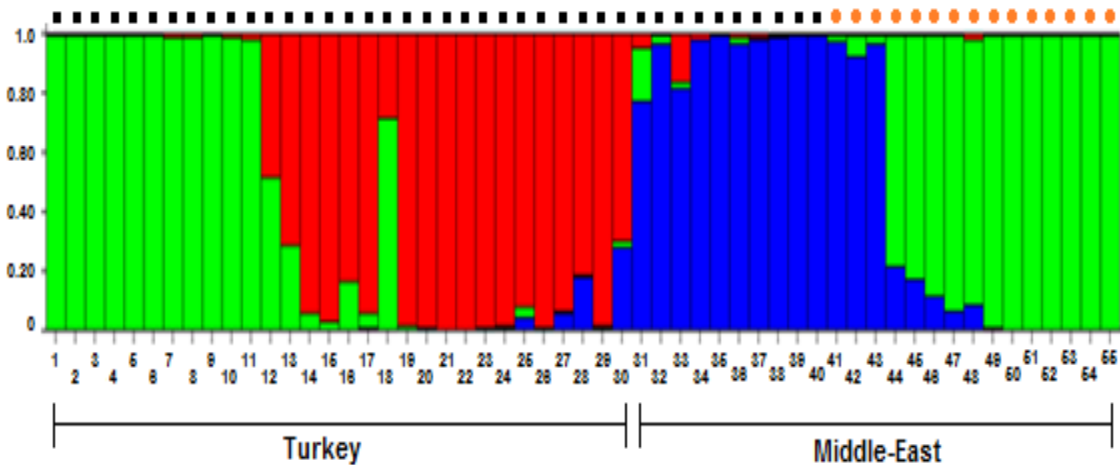


Figure 3.11. Population Structure Graph at K=3.

The numbers on the horizontal coordinate represent the genotypes corresponding to Table 2.1.

■: "cultivar", ■: "advanced line".

According to Structure graph, genotypes 1–11 and 49–55 took place in the same subpopulation; whereas the 19–24-26-29 and 32–43 genotypes were completely appeared in two distinct populations. According to the green subpopulation; Turkish cultivars and Middle-Eastern advanced lines has a common feature.

Evaluation of genetic diversity may lead for more effective selection of significant genotypes for improving yield capacity in narrowed diversity for the world increasing populations. PCA analysis can be more informative indicator of differences among wheat genotypes towards to other cluster analysis (Khodadadi et al., 2011). It is possible to use co-dominant markers in PCA. For instance, Sakiroglu et al. (2010) were investigated wild diploid alfalfa accession's diversity using SSRs in PCA analysis (Sakiroglu et al., 2010). According to our genetic distance matrix, PCA analysis was given an abundant amount of possibilities to think about structure of our gene pool.

3.2. Gene Expression Analyses

3.2.1. Presence of *Yr10* gene in wheat gene pool

PCR analyses by *Yr10* gene-specific primers showed that there were exon 1 and exon 2 amplifications only in Türkmen and Gerek 79 genotypes from our gene pool as shown in **Figure 3.12** and **Figure 3.13**.

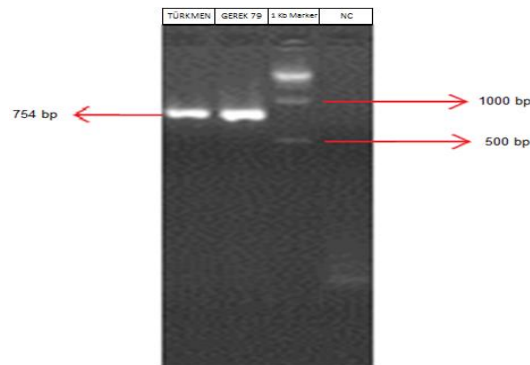


Figure 3.12. A single band at the band size of 754 (bp) obtained from PCR reaction by using exon 1 primer pair of *Yr10* gene at 2% agarose gel. Türkmen; Gerek 79; **M**: GeneRuler™ 1 Kb DNA Ladder (Fermentas); **NC**: Negative control.

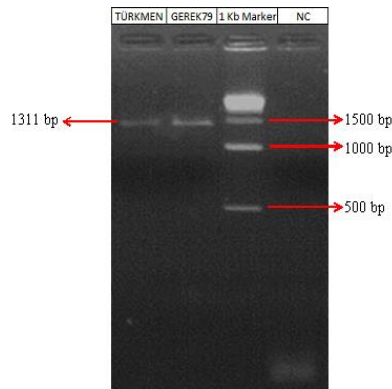


Figure 3.13. A single band obtained from PCR reaction by using exon 2 primer pairs of *Yr10* gene at 2% agarose gel. Türkmen; Gerek 79; **M**: GeneRuler™ 1 Kb DNA Ladder (Fermentas); **NC**: Negative control.

Türkmen, Gerek 79 and ‘Avocet *Yr10*’ genotypes were used as plant material both for validation and real-time PCR studies (**Figure 3.14**).

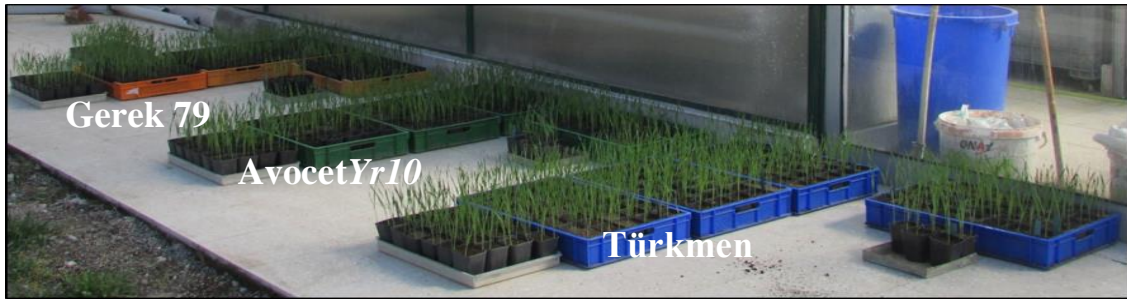


Figure 3.14. Plant material used for gene expression analysis of *Yr10*.

3.2.2. Validation of inoculation

After sampling, the accuracy of the infections were monitored and scored according to 0-9 scale given in Table 2.10. The scoring results were given in Table 3.5, 3.6 and 3.7. ‘Little Cub’ genotype were used a positive control of the infections because of its susceptible nature to yellow rust. The infected plant leaves were screened and shown in Figure 3.15.

Table 3.5. Inoculation scores of Türkmen genotype.

TÜRKMEN							
No	0 hpi - Mock	00:15 mpi	12 hpi	24 hpi	48 hpi	72 hpi	96 hpi
1	0	7	8	8	8	8	7
2	0	7	8	8	8	8	7
3	0	7	8	8	8	7	8

Table 3.6. Inoculation scores of Gerek 79 genotype.

GEREK 79							
No	0 hpi - Mock	00:15 mpi	12 hpi	24 hpi	48 hpi	72 hpi	96 hpi
1	0	8	8	7	8	7	7
2	0	8	8	7	7	8	7
3	0	8	7	8	7	7	7

Table 3.7. Inoculation scores of Avocet *Yr10* genotype.

AVOCET ' <i>Yr10</i> '							
No	0 hpi - Mock	00:15 mpi	12 hpi	24 hpi	48 hpi	72 hpi	96 hpi
1	0	1	1	1	1	1	1
2	0	0	1	1	1	1	1
3	0	1	1	1	1	1	1

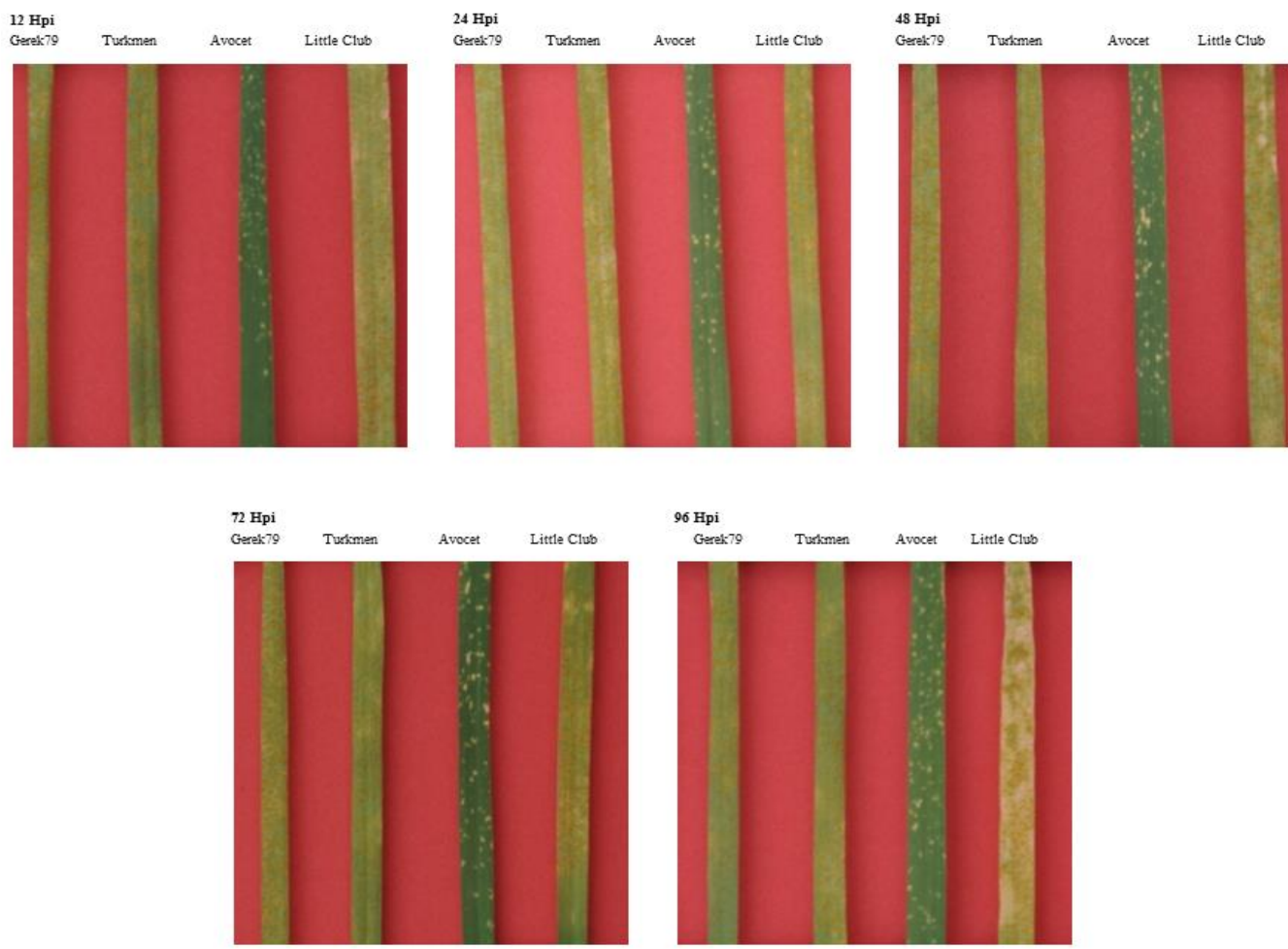


Figure 3.15. The inoculation results on infected leaves at 12, 24, 48, 72 and 96 hpi.

3.2.3. Total RNA isolation

Total RNA concentrations belong to infected samples were given in **Appendix 6**.

DNase-treated RNAs of samples were loaded on 1.5% DEPC-treated agarose gel and shown in **Figure 3.16**.

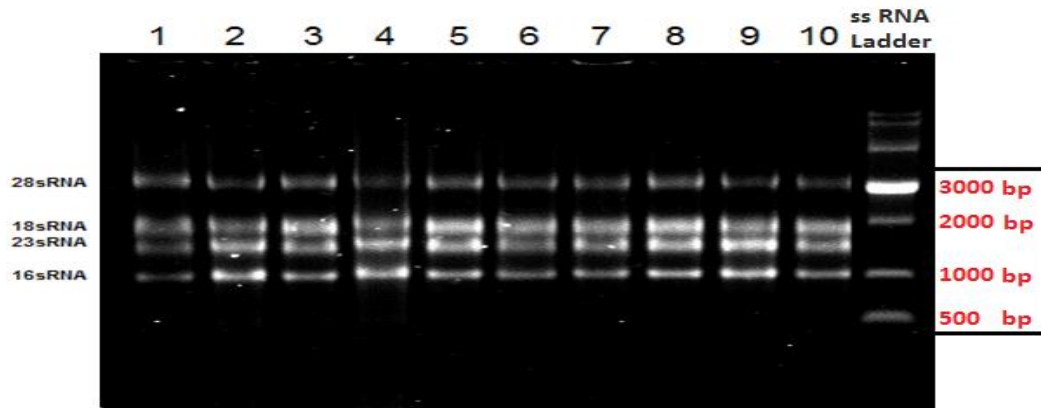


Figure 3.16. RNA samples after DNase treatment on 1.5% agarose gel. **1:** Türkmen-0 hpi - mock, **2:** Türkmen-15 mpi, **3:**Türkmen-12 hpi, **4:** Gerek 79-0 hpi mock, **5:** Gerek 79-15 mpi, **6:** Gerek 79-12 hpi, **7:** Avocet *Yr10*-0 hpi - mock, **8:** Avocet *Yr10*-15 mpi, **9:** Avocet *Yr10*-12 hpi, **10:** Türkmen-24 hpi.

3.2.4. Verification of avoidance from DNA contamination: 18S rDNA amplification

Total RNAs of infected samples with their 3 biological replicates (63 in total) and were used as template for 18S rDNA PCR reaction. 18S rDNA PCR products were loaded on 1.5% agarose gel and shown in **Figure 3.17**. As a result of PCR amplifications, there was no amplification at RNA samples and negative control; except positive control (PC). Real-time PCR study was conducted without any DNA contamination.

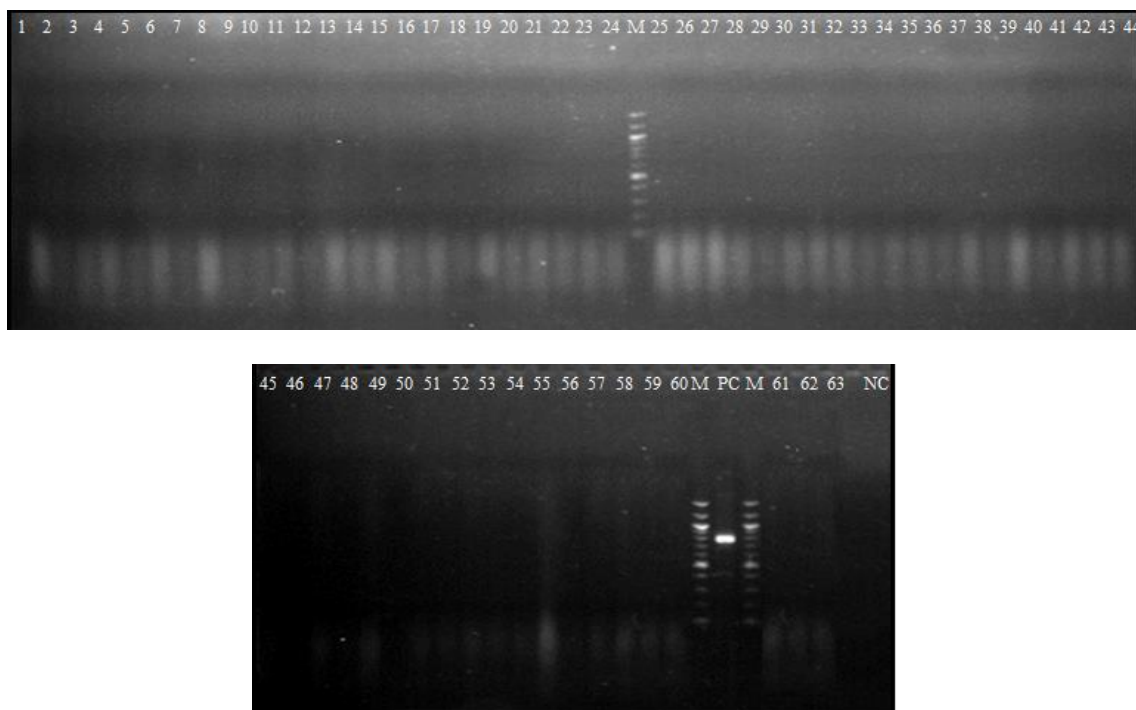


Figure 3.17. 18S rDNA amplification products on 1.5% agarose gel.

1: Gerek 79- 0 hpi - mock 1, **2:** Gerek 79- 0 hpi - mock 2, **3:** Gerek 79- 0 hpi - mock 3, **4:** Türkmen- 0 hpi - mock 1, **5:** Türkmen- 0 hpi - mock 2, **6:** Türkmen- 0 hpi - mock 3, **7:** Avocet *Yr10*- 0 hpi - mock 1, **8:** Avocet *Yr10*- 0 hpi - mock 2, **9:** Avocet *Yr10*- 0 hpi - mock 3, **10:** Gerek 79- 15 mpi - 1, **11:** Gerek 79- 15 mpi - 2, **12:** Gerek 79- 15 mpi - 3, **13:** Türkmen- 15 mpi - 1, **14:** Türkmen- 15 mpi - 2, **15:** Türkmen- 15 mpi - 3, **16:** Avocet *Yr10*- 15 mpi - 1, **17:** Avocet *Yr10*- 15 mpi - 2, **18:** Avocet *Yr10*- 15 mpi - 3, **19:** Gerek 79- 12 hpi - 1, **20:** Gerek 79- 12 hpi - 2, **21:** Gerek 79- 12 hpi - 3, **22:** Türkmen- 12 hpi - 1, **23:** Türkmen- 12 hpi - 2, **24:** Türkmen- 12 hpi - 3, **M:** 100 bp marker (New England Biolabs) **25:** Avocet *Yr10*- 12 hpi - 1, **26:** Avocet *Yr10*- 12 hpi - 2, **27:** Avocet *Yr10*- 12 hpi - 3, **28:** Gerek 79- 24 hpi - 1, **29:** Gerek 79- 24 hpi - 2, **30:** Gerek 79- 24 hpi - 3, **31:** Türkmen- 24 hpi - 1, **32:** Türkmen- 24 hpi - 2, **33:** Türkmen- 24 hpi - 3, **34:** Avocet *Yr10*- 24 hpi - 1, **35:** Avocet *Yr10*- 24 hpi - 2, **36:** Avocet *Yr10*- 24 hpi - 3, **37:** Gerek 79- 48 hpi - 1, **38:** Gerek 79- 48 hpi - 2, **39:** Gerek 79- 48 hpi - 3, **40:** Türkmen- 48 hpi - 1, **41:** Türkmen- 48 hpi - 2, **42:** Türkmen- 48 hpi - 3, **43:** Avocet *Yr10*- 48 hpi - 1, **44:** Avocet *Yr10*- 48 hpi - 2, **45:** Avocet *Yr10*- 48 hpi - 3, **46:** Gerek 79- 72 hpi - 1, **47:** Gerek 79- 72 hpi - 2, **48:** Gerek 79- 72 hpi - 3, **49:** Türkmen- 72 hpi - 1, **50:** Türkmen- 72 hpi - 2, **51:** Türkmen- 72 hpi - 3, **52:** Avocet *Yr10*- 72 hpi - 1, **53:** Avocet *Yr10*- 72 hpi - 2, **54:** Avocet *Yr10*- 72 hpi - 3, **55:** Gerek 79- 96 hpi - 1, **56:** Gerek 79- 96 hpi - 2, **57:** Gerek 79- 96 hpi - 3, **58:** Türkmen- 96 hpi - 1, **59:** Türkmen- 96 hpi - 2, **60:** Türkmen- 96 hpi - 3, **M:** 100 bp marker (New England Biolabs), **PC:** Positive control - DNA of Türkmen genotype, **M:** 100 bp marker (New England Biolabs), **61:** Avocet *Yr10*- 96 hpi - 1, **62:** Avocet *Yr10*- 96 hpi - 2, **63:** Avocet *Yr10*- 96 hpi - 3, **NC:** Negative control.

3.2.5. Real-time PCR (Q-PCR) analysis

Gene expression analyses were conducted using *Yr10* gene-specific primers in Türkmen, Gerek 79 and Avocet *Yr10* genotypes at 7 different time points (0 hpi - mock, 15 mpi, 12 hpi, 24 hpi, 48 hpi, 72 hpi, 96 hpi). Three biological replicates of each genotype at each time point were pooled. These pooled ‘cDNA’s of each Türkmen, Gerek 79 and Avocet *Yr10* genotypes were used as template for cDNA synthesis for real-time PCR reaction. As a result of real-time PCR reactions, *Yr10* gene expression graphs of each genotype were obtained and given in **Figure 3. 18-20**. ‘Mock’ samples

for Türkmen, Gerek 79 and Avocet *Yr10* genotypes were assumed as biological control of the reaction. Amplifications obtained by ACBT and GAPDH primer pairs were used as multiplex endogenous control for the normalization of the *Yr10* gene expression for each reaction.

The *Yr10* gene expression of Türkmen genotype was down-regulated at 15 mpi, and then there were an increase at down-regulation at 12 hpi. Following, the top level of down-regulation was at 24 hpi. In contrast to this sharp down-regulation, there was an up-regulation at 48, 72 and 96 hpi.

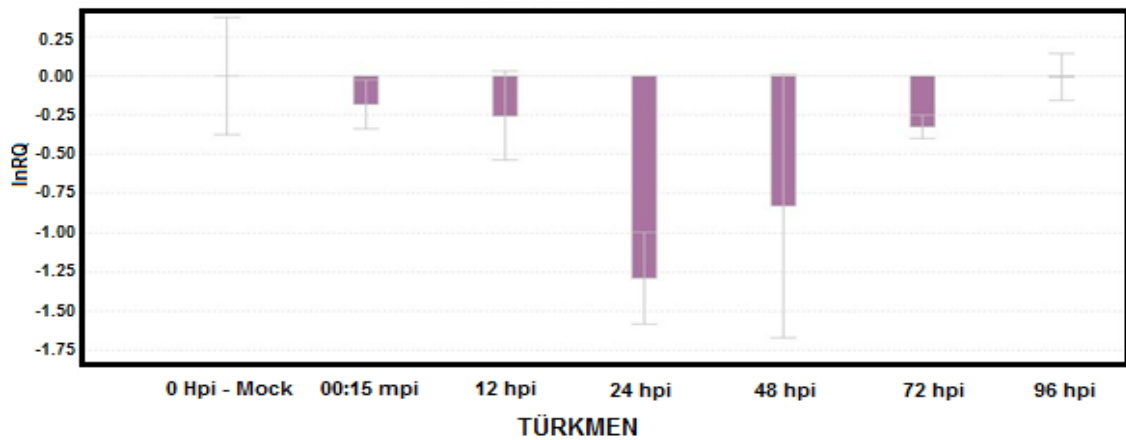


Figure 3.18. *Yr10* gene expression in Türkmen genotype in seven time points: 0 hpi - mock, 00:15 mpi, 12 hpi, 24 hpi, 48 hpi, 72 hpi, 96 hpi (lnRQ: logarithmic Relative Quantification).

The *Yr10* gene expression at Gerek 79 genotype was slightly up-regulated. Following this increase at gene expression, there was a significant increase at up-regulation of *Yr10* gene at 12 hpi. Conversely, there was a sharp down-regulation at 24 hpi. At 48 hpi, there was an increase at up-regulation of *Yr10* gene. The up-regulation was continued to increase at 72 and 96 hpi.

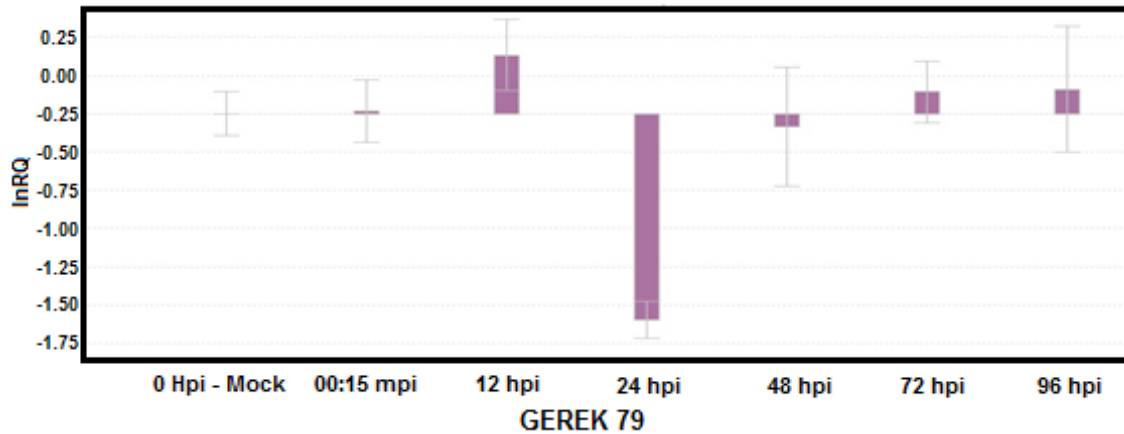


Figure 3.19. *Yr10* gene expression in Gerek 79 genotype in seven time points: 0 hpi - mock, 00:15 mpi, 12 hpi, 24 hpi, 48 hpi, 72 hpi, 96 hpi. (lnRQ: logarithmic Relative Quantification).

The *Yr10* gene expression of Avocet *Yr10* genotype was down-regulated at 15 mpi. Following, the down-regulation was increased at 12 hpi. Similar to other 24 hpi gene expression profiles of Türkmen and Gerek 79 genotypes; the down-regulation of *Yr10* gene at Avocet *Yr10* genotype was sharply increased at 24 hpi. But, the *Yr10* gene expression was up-regulated at 48 hpi. In contrast; there was an increase at the down-regulation of *Yr10* gene at 72 and 96 hpi.

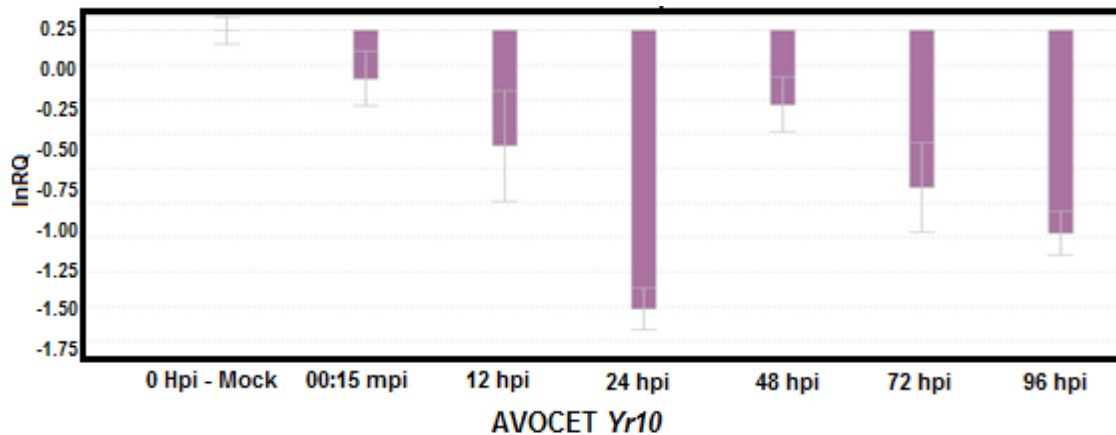


Figure 3.20. *Yr10* gene expression in Avocet *Yr10* genotype in seven time points: 0 hpi - mock, 00:15 mpi, 12 hpi, 24 hpi, 48 hpi, 72 hpi, 96 hpi. (lnRQ: logarithmic Relative Quantification).

Utilization of disease resistant varieties has proved to be the safest, most economical, and effective method to fight with yellow rust disease (Jiang et al., 2013). The danger caused by the use of limited resistance sources is becoming a great concern for breeders or pathologists (Li and Xia, 2006). The key problem is that there are breakdown of widely-used resistance genes occurs caused by the rapid improvement of

Pst pathogen isolates (Jiang et al., 2013). There are only three yellow rust disease resistance genes were cloned: *Yr10* (Laroche et al., 2000), *Yr18* (Krattinger et al., 2009) and *Yr36* (Fu et al., 2009). *Yr10* gene, responsible for yellow rust resistance at seedling-stage, was selected and used to investigate gene expression pattern by real-time PCR in this study. Expression profiling of *Yr10* in Türkmen and Gerek 79 genotypes after yellow rust attack at six different time points (15 min., 12 hours, 24 hours, 48 hours, 72 hours, 96 hours) at seedling stage was investigated by real time PCR which is one of the most widely-used techniques for gene expression profiling (Long et al., 2011).

Real-time PCR results (**Figure 3.18-20**) showed that *Yr10* gene was not expressed at basal level before *Pst* infection (mock inoculated plants) in all genotypes. In contrast to our results Xingquan et al. (2010) reported the expression of eight candidate genes related with yellow rust resistance was observed at basal levels before infection of wheat plants. Expression profile of *Yr10* was down regulated at all six time points in Türkmen while Gerek 79 showed up-regulation at 15 mpi and 12 hpi in our study. The 24 hpi was critical for all genotypes because of the highest down regulation of *Yr10*. The lnRQ values for 24 hpi were ranged between (-1.30) – (-1.60). After 24 hpi, it was observed that lnRQ values were fluctuated and reached to almost basal level significantly in Türkmen and Gerek 79 suggesting that this gene may participate in plant defense response through different regulatory mechanisms. The fluctuations of expression levels of *Yr10* in Türkmen and Gerek 79 as well as positive control Avocet *Yr10* between different time points can be attributed short term effects of pathogen on plant after inoculation. It is also obvious that genes involved in stress response and signaling pathways are also down regulated in virulent infections. On the other hand, Yu et al. (2010) investigated the expression patterns of twelve genes involved in the incompatible interaction between wheat and *Puccinia striiformis* f. sp. *tritici* at 12, 18, 24, 48, 72 and 120 hpi. According to their study, the most of defense-related genes were reached their maximum level at 24 hpi (Yu et al., 2010). Similarly, Xingquan et al. (2010) were used wheat (*Triticum aestivum* L.) - *Secale cereale* alien disomic substitution NR1121 line as plant material and used CYR32 as pathogen for yellow rust disease inoculation at 0, 24, 48, 72, 96, 168, 240, and 336 hpi. As a result of their study, expression of six genes (AcsA, GST, LTP2, UPL2, CP450, and SPKSNT7) transcripts

were stimulated and up-regulated to their highest levels at 24 hpi, while that of two genes (SHMT and SAMDC) were significantly expressed at 48 hpi.

In our study, we investigated *Yr10* gene expression pattern assuming as responsible for seedling-stage yellow rust resistance as a major gene. Yet, to obtain more precise pattern of gene expression for yellow rust resistance at early stage of the infection, there should be additional study including various time points and different cultivars to elucidate this complex response (Maytalman et al., 2013). Furthermore, our results demonstrated that *Yr10* gene was induced after 24 hpi in Türkmen and Gerek 79, suggesting it is transcriptionally activated for the host defense response because the pathogen tries to mediate host cell expression by down regulating some genes so that plant can feed itself or suppress host defense mechanism thereby it can propagate.

4. CONCLUSIONS

In this study, genetic diversity and expression pattern of a yellow rust disease resistance gene (*Yr10*) in yellow rust resistant winter type bread wheat (*Triticum aestivum* L.) gene pool were investigated.

The use of molecular markers distributed on all genomes (A, B, D) of allohexaploid wheat is important to assess genetic diversity. In this study, fifty five bread wheat genotypes were screened by 117 microsatellites scattered throughout A, B and D genomes and the multi-allelic data was used for genetic diversity analysis. The Ceyhan-99 Turkish genotype which was the most distant cultivar to Middle-Eastern cultivar, Behoth 6, had the highest distance value with another Turkish cultivar, Kaşif Bey 95 which was placed on Middle-Eastern subgroup at PCA analysis. This result shows that Kaşif Bey 95 cultivar is closer to Middle-Eastern genotypes than Turkish cultivars. The other most distant two genotype pairs were determined as Gerek 79 – Douma 40989 and Karahan-99 – Douma48114. When compare to PCA graph results, it can be verified that Kaşif Bey 95 Turkish cultivar was separated from other Turkish cultivars. The closest Turkish cultivars were Çetinel-2000 and Türkmen and the closest Middle-Eastern cultivars were Behoth 8 and Douma 4. Furthermore, the Middle-East genotypes were placed against to Turkish genotypes in PCA graph. Structure analysis showed that the populations from Turkey and Middle-East were largely separating into different groups and there is evidence for a third group that includes both Turkish and Middle Eastern populations. According to the third subpopulation; Turkish cultivars and Middle-Eastern advanced lines have common features.

There is pressing need for all those who are interested in plant genetic resources conservation and use to be more involved in all the aspects of genetic diversity – to study, understand, enhance, conserve and use it. To do so, we need to understand the extent and distribution of diversity in species. There remain many unresolved questions about the extent and distribution of genetic diversity in useful plant species. What is the most useful combination of molecular, biochemical and agromorphological characters for the required understanding of the patterns of diversity? It is important that these are tackled in a systematic way and not through the continued accumulation of data in an almost random fashion that is often is the case. This will require cooperation between

investigators, research centers and countries. In the light of increased use of molecular methods for studying plant genetic diversity, there is also the need to link the information on molecular variation to plant genetic resources management in a more meaningful way than it is presently done and this could be done on particular crop gene pools. In the shed light of our results, the most distant genotype pairs (Ceyhan-99 – Behoth 6, Gerek79 – Douma 40989 and Karahan-99 – Douma 48114) could be eligible to use as candidate genotypes in breeding programs for different traits.

Identification of expression patterns of gene/s responsible for interested traits is another issue for plant breeding programs. In this study, we investigated the expression profile of yellow rust resistant gene, *Yr10* in wheat gene pool. It was observed that the most down-regulated time point was 24 hpi for *Yr10* gene expression in all genotypes and expression levels were fluctuated depends on the genotype and time points that we used. Expression of *Yr10* gene was down regulated until 24 hpi in Gerek79 while up regulation of *Yr10* was observed after 24 hpi in Türkmen. The increased time points for expression profiling is required for more effective evaluation of stripe rust resistance response in wheat. However better conclusions to understand the response of plant to yellow rust attack can be acquired as more genes identified in wheat and presented in future. In the frame of our results, Gerek 79 and Türkmen genotypes, carrying *Yr10* gene, can be used as material for wheat breeding programs focused on yellow rust resistance.

Further studies on genetic diversity and gene expression profiling can lead to develop novel breeding lines which are resistant or tolerant with higher genetic infrastructures as environmentally friendly and consuming the everlasting needs of human-being. For yellow rust epidemics, owing to the requirements for inoculation such as appropriate climatic conditions or humidity; differ from region to region. With respect to these requirements, breeders should take the data obtained from geographical regions into account for wheat improvement studies against yellow rust. It is needed breeders to monitorize their fields consistently for yellow rust resistance due to the unstable yellow rust epidemics. The combination of recent studies about cultivation features of same genotypes from our gene pool and recent genetic diversity analyses may lead breeders to estimate the further selection of cultivars to develop elite breeding lines resistant from our gene pool to fungal epidemics.

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Electronical Resources

Food And Agriculture Organization Of The United Nations

<http://faostat.fao.org/site/339/default.aspx>

Grain Genes

<http://wheat.pw.usda.gov/GG2/index.shtml>

National Center for Biotechnology Information

<http://www.ncbi.nlm.nih.gov/>

VWR International, LLC

<https://www.vwr.com/>

APPENDICES

APPENDIX 1. SSR markers used for assessing genetic diversity.

SSR MARKER	GENOME	PRIMER SEQUENCE		Tm (°C)	Ta (°C)
			5' '3		
<i>barc17</i>	1A	F:	GCG CAA CAT ATT CAG CTC AAC A	69	60
		R:	TCC ACA TCT CGT CCC TCA TAG TTT G	74	
<i>barc83</i>		F:	AAG CAA GGA ACG AGC AAG AGC AGT AG	76	69
		R:	TGG ATT TAC GAC GAC GAT GAA GAT GA	73	
<i>barc148</i>		F:	GCG CAA CCA CAA TGT ATG CT	68	57
		R:	GGG GTG TTT TCC TAT TTC TT	64	
<i>barc158</i>		F:	TGT GTG GGA AGA AAC TGA GTC ATC	72	60
		R:	AGG AAT ACC AAA AGA AGC AAA CCA AC	71	
<i>cfa2135</i>		F:	TGC CTA AAT CTA AAT GCC CG	66	63
		R:	GGA TAA TGT GCA TGT TCA CCG	69	
<i>cfa2153</i>		F:	TTG TGC ATG ATG GCT TCA AT	64	57
		R:	CCA ATC CTA ATG ATC CGC TG	68	
<i>wmc278</i>		F:	AAA CGA TAG TAA AAT TAC CTC GGA T	68	60
		R:	TCA AAA AAT AGC AAC TTG AAG ACA T	66	
<i>wmc716</i>	F:	CAT TTA TGT GCA CGC CGA AG	68	61	
	R:	CCA TAA GCA TCG TCA CCC TG	70		
<i>cfa2263</i>	2A	F:	GGC CAT GTA ATT AAG GCA CA	66	63
		R:	CTC CCA GGA GTA CAG AAG AGG A	73	
<i>gwm636</i>		F:	CGG TAG TTT TTA GCA AAG AG	64	56,5

APPENDIX 1. Continued.

		R:	CCT TAC AGT TCT TGG CAG AA	66	
<i>wmc177</i>		F:	AGG GCT CTC TTT AAT TCT TGC T	68	66
		R:	GGT CTA TCG TAA TCC ACC TGT A	69	
<i>wmc407</i>		F:	GGT AAT TCT AGG CTG ACA TAT GCT C	73	64
		R:	CAT ATT TCC AAA TCC CCA ACT C	68	
<i>wmc522</i>		F:	AAA AAT CTC ACG AGT CGG GC	68	64
		R:	CCC GAG CAG GAG CTA CAA AT	70	
<i>cfa2201</i>		F:	CAA ACC AAC CTC ATT GAC CC	68	66
		R:	CCA CCA GAA CTT CAA CCT GG	70	
<i>gwm558</i>		F:	GGG ATT GCA TAT GAG ACA ACG	69	63
		R:	TGC CAT GGT TGT AGT AGC CA	68	
<i>barc314</i>	3A	F:	CTG TGG AAA CCA ATA AAA ACA A	64	60,5
		R:	GTG CGC GAA TAA CTA CAA GAA A	68	
<i>cfa2193</i>		F:	ACA TGT GAT GTG CGG TCA TT	66	62,5
		R:	TCC TCA GAA CCC CAT TCT TG	68	
<i>wmc532</i>		F:	GATACATCAAGATCGTGCCAAA	68	62,5
		R:	GGGAGAAATCATTAAACGAAGGG	69	
<i>wmc559</i>		F:	ACA CCA CGA ATG ATG TGC CA	68	63
		R:	ACG ACG CCA TGT ATG CAG AA	68	
<i>wmc169</i>		F:	TAC CCG AAT CTG GAA AAT CAA T	66	59,5
		R:	TGG AAG CTT GCT AAC TTT GGA G	69	
<i>wmc428</i>		F:	TTA ATC CTA GCC GTC CCT TTT T	68	63,5
		R:	CGA CCT TCG TTG GTT ATT TGT G	69	
<i>wmc153</i>		F:	ATG AGG ACT CGA AGC TTG GC	70	61,5
		R:	CTG AGC TTT TGC GCG TTG AC	70	

APPENDIX 1. Continued.

<i>barc343</i>	4A	F:	GGC CTA ATT ACA AGT CCA AAA G	68	61,5	
		R:	GCT CAA AGT AAA GTT CAC GAA TAT	67		
<i>wmc262</i>		F:	GCT TTA ACA AAG ATC CAA GTG GCA T	71	61	
		R:	GTA AAC ATC CAA ACA AAG TCG AAC G	71		
<i>wmc219</i>		F:	TGC TAG TTT GTC ATC CGG GCG A	73	63	
		R:	CAA TCC CGT TCT ACA AGT TCC A	69		
<i>cfid257</i>		F:	TCT CAA CTT GCA ACT GCC AC	68	62,5	
		R:	CCC TCC ATG GAT TCT TGC TA	68		
<i>barc117</i>		5A	F:	TCA TGC GTG CTA AGT GCT AA	66	61,5
			R:	GAG GGC AGG AAA AAG TGA CT	68	
<i>cfa2250</i>	F:		AGC CAT AGA TGG CCC TAC CT	70	63,5	
	R:		CAC TCA ATG GCA GGT CCT TT	68		
<i>wmc201</i>	6A	F:	CAT GCT CTT TCA CTT GGG TTC G	71	64	
		R:	GCG CTT GCA GGA ATT CAA CAC T	71		
<i>barc1165</i>		F:	GCG CCA TCA AGC CTC AAA ACT CTG TA	76	66,5	
		R:	CGC AAC GTT TTC CTT TCC CAT AAT ACT	73		
<i>wmc256</i>		F:	CCA AAT CTT CGA ACA AGA ACC C	69	62,5	
		R:	ACC GAT CGA TGG TGT ATA CTG A	69		
<i>barc107</i>		F:	CGA ACT GAA ACT GAA AAC ACA GAC ATC	73	65	
		R:	AGG GAG AGT GGA CGT CAT AAT TTG TG	74		
<i>barc222</i>		7A	F:	AAA TCC GGC ATC TGC TGT ATC CAT A	73	65
			R:	GTC CGG CCG CTG AAT ACT GTT	73	
<i>wmc525</i>	F:		GTT TGA CGT GTT TGC TGC TTA C	69	61,5	
	R:		CTA CGG ATA ATG ATT GCT GGC T	69		
<i>cfa2257</i>	F:		GAT ACA ATA GGT GCC TCC GC	70	59,5	

APPENDIX 1. Continued.

		R:	CCA TTA TGT AAA TGC TTC TGT TTG A	68	
<i>barc131</i>	1B	F:	GCG AAT TCG GAA AAA CCA GAA AAT TTG A	72	67
		R:	GCG CTT TGA TAT GCA TGT CAC CTT AAA A	74	
<i>wmc44</i>		F:	GGT CTT CTG GGC TTT GAT CCT G	73	67
		R:	TGT TGC TAG GGA CCC GTA GTG G	75	
<i>wmc416</i>		F:	AGC CCT TTC TAC CGT GTT TCT T	69	64,5
		R:	TAT GGT CGA TGG ACT GTC CCT A	71	
<i>barc80</i>		F:	GCG AAT TAG CAT CTG CAT CTG TTT GAG	75	69
		R:	CGG TCA ACC AAC TAC TGC ACA AC	74	
<i>barc137</i>		F:	GGC CCA TTT CCC ACT TTC CA	70	65
		R:	CCA GCC CCT CTA CAC ATT TT	68	
<i>barc181</i>	F:	CGC TGG AGG GGG TAA GTC ATC AC	77	69	
	R:	CGC AAA TCA AGA ACA CGG GAG AAA GAA	75		
<i>wmc154</i>	2B	F:	ATG CTC GTC AGT GTC ATG TTT G	69	67
		R:	AAA CGG AAC CTA CCT CAC TCT T	69	
<i>wmc361</i>		F:	AAT GAA GAT GCA AAT CGA CGG C	69	65
		R:	ATT CTC GCA CTG AAA ACA GGG G	71	
<i>wmc477</i>		F:	CGT CGA AAA CCG TAC ACT CTC C	73	67
		R:	GCG AAA CAG AAT AGC CCT GAT G	71	
<i>wmc770</i>		F:	TGT CAG ACT TCC TTT GAT CCC C	71	64
		R:	AAG ACC ATG TGA CGT CCA GC	70	
<i>cfa2278</i>		F:	GCC TCT GCA AGT CTT TAC CG	70	65
		R:	AAG TCG GCC ATC TTC TTC CT	68	
<i>cf2238</i>		F:	GTT GAG GAG GAC AAA GAG GC	70	64
		R:	GAT ACG AGC GAG CCC ATA AA	68	

APPENDIX 1. Continued.

<i>barc75</i>	3B	F:	AGG GTT ACA GTT TGC TCT TTT AC	68	63,5
		R:	CCC GAC GAC CTA TCT ATA CTT CTC TA	74	
<i>barc164</i>		F:	TGC AAA CTA ATC ACC AGC GTA A	68	59
		R:	CGC TTT CTA AAA CTG TTC GGG ATT TCT AA	74	
<i>barc84</i>		F:	CGC ATA ACC GTT GGG AAG ACA TCT G	76	65,5
		R:	GGT GCA ACT AGA ACG TAC TTC CAG TC	76	
<i>gwm375</i>		F:	ATT GGC GAC TCT AGC ATA TAC G	69	64
		R:	GGG ATG TCT GTT CCA TCT TAG C	71	
<i>wmc710</i>		F:	GTA AGA AGG CAG CAC GTA TGA A	69	59
		R:	TAA GCA TTC CCA ATC ACT CTC A	68	
<i>cfa2149</i>	F:	CTT GGA GCT CGG GTA GTA GC	72	61,5	
	R:	AAG GCA GCT CAA TCG GAG TA	68		
<i>barc227</i>	F:	GCA CCG AAGC TCT AAT ACC AAA TGT	73	67	
	R:	GCG TTG AGG AGT GTT CTC TGT TCT GGA	78		
<i>barc1045</i>	F:	GCG TGT AAT AAA ACT GGT TGG ATA	69	63	
	R:	GCG AGT ATG GTA ATT TCT AGG GAG TC	74		
<i>wmc413</i>	F:	CAC TGG AAA CAT CTC TTC AAC T	68	62,5	
	R:	ACA GGA AAG GAT GAT GTT CTC T	68		
<i>barc243</i>	F:	CGC AAA ATC GAA ATT AAA AAT GGA AA	67	57	
	R:	GAT CCT CCT TTC AGC TGG CCT ATT A	74		
<i>wmc75</i>	F:	GTC CGC CGC ACA CAT CTT ACT A	73	61,5	
	R:	GTT TGA TCC TGC GAC TCC CTT G	73		
<i>barc178</i>	F:	GCG TAT TAG CAA AAC AGA AGT GAG	70	65	
	R:	GCG ACT AGT ACG AAC ACC ACA AAA	72		
<i>wmc397</i>	F:	AGT CGT GCA CCT CCA TTT TG	68	63,5	

APPENDIX 1. Continued.

		R:	CAT TGG ACA TCG GAG ACC TG	70	
<i>wmc494</i>		F:	GGA TCG AGT CTC AAG TCT ACA A	69	60,5
		R:	AGA AGG AAC AAG CAA CAT CAT A	66	
<i>barc79</i>		F:	GCG TTG GAA AGG AGG TAA TGT TAG ATA G	75	66,5
		R:	TCG TGG GTT ACA AGT TTG GGA GGT CA	76	
<i>barc354</i>		F:	CGT TGT TTG CGT AGA AGG AGG TT	72	67
		R:	GCG AAT GCG GGC GAT AAA GTG G	75	
<i>gwm537</i>	7B	F:	ACA TAA TGC TTC CTG TGC ACC	69	61
		R:	GCC ACT TTT GTG TCG TTC CT	68	
<i>wmc396</i>		F:	TGC ACT GTT TTA CCT TCA CGG A	69	61,5
		R:	CAA AGC AAG AAC CAG AGC CAC T	71	
<i>wmc517</i>		F:	ATC CTG ACG TTA CAC GCA CC	70	60,5
		R:	ACC TGG AAC ACC ACG ACA AA	68	
<i>wmc276</i>		F:	GAC ATG TGC ACC AGA ATA GC	68	56
		R:	AGA AGA ACT ATT CGA CTC CT	64	
<i>wmc335</i>		F:	TGC GGA GTA GTT CTT CCC CC	72	61,5
		R:	ACA TCT TGG TGA GAT GCC CT	68	
<i>wmc476</i>		F:	TAC CAA CCA CAC CTG CGA GT	70	60
		R:	CTA GAT GAA CCT TCG TGC GG	70	
<i>wmc311</i>		F:	GGG CCT GCA TTT CTC CTT TCT T	71	61,5
		R:	CTG AAC TTG CTA GAC GTT CCG A	71	
<i>barc72</i>		F:	CGT CCT CCC CCT CTC AAT CTA CTC TC	79	63,5
		R:	CGT CCC TCC ATC GTC TCA TCA	73	
<i>barc255</i>		F:	GTG GCG GCT TGC GGG TGG CTG AGT A	82	65
		R:	GGG TCG GCT AGC CTT CTT TTA TGT T	74	

APPENDIX 1. Continued.

<i>wmc426</i>		F:	GAC GAT CGT TTC TCC TAC TTT A	68	60,5	
		R:	ACT ACA CAA ATG ACT GCT GCT A	68		
<i>cfid27</i>	1D	F:	GCA GCA AGA TCA AAT CGA CA	66	63	
		R:	ACT GAG GAC TTG GTG CCA TC	70		
<i>cfid63</i>		F:	TCC TGA GGA TGT TGA GGA CC	70	65	
		R:	GAG AGA GGC GAA ACA TGG AC	70		
<i>cfid83</i>		F:	AAG GAT GGA GAG GAC CCC TA	70	64	
		R:	GGA GGT GGA GCA ACC TAT CA	70		
<i>wmc147</i>		F:	AGA ACG AAA GAA GCG CGC TGA G	73	66	
		R:	ATG TGT TTC TTA TCC TGC GGG C	71		
<i>barc271</i>		F:	CGC ACC TAA TAT CGT AAA ACA ATG TA	70	69,5	
		R:	CGC TTT CCC AGA ATA TTA TTT GTA TTG T	71		
<i>cfid53</i>	2D	F:	CCC TAT TTC CCC CAT GTC TT	68	64	
		R:	AAG GAG GGC ACA TAT CGT TG	68		
<i>wmc503</i>		F:	GCA ATA GTT CCC GCA AGA AAA G	69	66,5	
		R:	ATC AAC TAC CTC CAG ATC CCG T	71		
<i>cfid56</i>		F:	TTG CAT AAT TAC TTG CCC TCC	67	61,5	
		R:	CTG GTC CAA CTT CCA TCC AT	68		
<i>cfid116</i>		F:	TTT GCC CAT TACAACAAGCA	64	60	
		R:	CAAGCAGCACCTCATGACAG	70		
<i>barc125</i>		3D	F:	GCG TCG AGG GTA AAA CAA CAT AT	70	64,5
			R:	GTA GCG TCA GTG CTC ACA CAA TGA	74	
<i>barc135</i>	F:		ATC GCC ATC TCC TCT ACC A	68	63	
	R:		GCG AAC CCA TGT GCT AAG T	68		
<i>cfid9</i>	F:		TTG CAC GCA CCT AAA CTC TG	68	59,5	

APPENDIX 1. Continued.

		R:	CAA GTG TGA GCG TCG G	65	
<i>cfid55</i>		F:	CCA GTA GCC GGC CCT ACT AT	72	62,5
		R:	GCA CGA GAT ACG GAC AAT CA	68	
<i>cfid152</i>		F:	TGG AAG TCT GGA ACC ACT CC	70	64
		R:	GCA ACC AGA CCA CAC TCT CA	70	
<i>wmc656</i>		F:	AAG TAG GCG AGC GTT GT	64	56,5
		R:	TTT CCC TGG CGA GAT G	62	
<i>cfid223</i>		F:	AAG AGC TAC AAT GAC CAG CAG A	69	64
		R:	GCA GTG TAT GTC AGG AGA AGC A	71	
<i>barc1183</i>	4D	F:	TCC CGC CCT CTC TAG ACG AAA CTG T	77	69
		R:	CGG GAA AGG AAA GGA GCT TAC GGT	76	
<i>wmc720</i>	4D	F:	CAC CAT GGT TGG CAA GAG A	68	62
		R:	CTG GTG ATA CTG CCG TGA CA	70	
<i>wmc285</i>	4D	F:	TGT GGT TGT ATT TGC GGT ATG G	69	63
		R:	TTG TGG TGC TGA GTT AGC TTG T	69	
<i>barc143</i>	5D	F:	TTG TGC CAA ATC AAG AAC AT	62	58
		R:	GGT TGG GCT AGG ATG AAA AT	66	
<i>barc177</i>	5D	F:	GCG ATC CTG TTG TTG AGC GTT TGC ATA A	76	63,5
		R:	TCC CGT TTT CCC GTG TGT TAG TCT A	74	
<i>barc286</i>	5D	F:	GCG AAG AAA ACA TTA GAC CAA AA	66	61,5
		R:	GCG ATA TGT TTC CCG ACA ACT A	69	
<i>cfid57</i>	5D	F:	ATC GCC GTT AAC ATA GGC AG	68	60
		R:	TCA CTG CTG TAT TTG CTC CG	68	
<i>gwm174</i>	5D	F:	GGG TTC CTA TCT GGT AAA TCC C	71	61,5
		R:	GAC ACA CAT GTT CCT GCC AC	70	

APPENDIX 1. Continued.

<i>wmc765</i>		F:	GGG ATC AGA CTG GGA CTG GAG	75	62
		R:	GGG TTG GCT TGG CAG AGA A	70	
<i>cfid29</i>		F:	GGT TGT CAG GCA GGA TAT TTG	69	59
		R:	TAT TGA TAG ATC AGG GCG CA	66	
<i>barc130</i>		F:	CGG CTA GTA GTT GGA GTG TTG G	73	63
		R:	ACC GCC TCT AGT TAT TGC TCT C	71	
<i>gwm212</i>		F:	AAG CAA CAT TTG CTG CAA TG	64	58
		R:	TGC AGT TAA CTT GTT GAA AGG A	66	
<i>cfid12</i>		F:	GTT ACC CAA ACC TGC CCT TT	68	60
		R:	CTA CGA GTC GGG ATC AGC AT	70	
<i>barc273</i>		F:	AAT TCA GAG AAA CAC ACC TCC CTT TTA	72	62,5
		R:	ACT CCA TCA ACC CCG TTC ATT A	69	
<i>cfid76</i>		F:	GCA ATT TCA CAC GCG ACT TA	66	61,5
		R:	CGC TCG ACA ACA TGA CAC TT	68	
<i>gdm127</i>		F:	ACG GGG AAA TTA AAA CGA CC	55	48
		R:	TGA GAT GGA ATC GAC AGA AA	55	
<i>barc96</i>	6D	F:	AAG CCT TGT TGT TCC GTA TTA TT	66	62
		R:	GCG GTT TAT ATT TTG TGG TTG AGC ATT TT	72	
<i>cfid47</i>		F:	TGA CCA TGT CAT GTT TTA TAC CAC T	69	60
		R:	TGG ACT ACA TGT CAA GCA CAA A	68	
<i>barc175</i>		F:	GCG TAA CAG AAG CGG AGA AAG C	73	66
		R:	GCG AAT CAT TTA GTG TTA GGT GGC AGT G	76	
<i>cfid33</i>		F:	TAC CGC AAT AAT CAC ACC CA	66	58
		R:	GGT CGA TGG ACT GTC CCT AA	70	
<i>barc196</i>		F:	GGT GGG TTT TAT CGA ATA GAT TTG CT	71	63

APPENDIX 1. Continued.

		R:	GCG TTT CGT CAA GAT TAA TGC AGG TTT	73	
<i>barc214</i>	7D	F:	CGC TTT CGG GAC AGT GAA GGT GTA T	76	66
		R:	CGG TAC GCG CGA GGA GGA AGA AGG	81	
<i>barc235</i>		F:	GCG CTC ACC CTC CTA CAC TTC CTA	77	63,5
		R:	GCG CAA GTC TGT CAA AGC CTA A	71	
<i>cf69</i>		F:	AAA TAC CTT GAA TTG TGA GCT GC	68	60,5
		R:	R: 5' TCT GTT CAT CCC CAA AGT CC	68	
<i>wmc463</i>		F:	F: 5' GAT TGT ATA GTC GGT TAC CCC T	69	60,5
		R:	ATT AGT GCC CTC CAT AAT TGT G	68	
<i>barc172</i>		F:	GCG AAA TGT GAT GGG GTT TAT CTA	70	61,5
		R:	GCG ATT TGA TTT AAC TTT AGC AGT GAG	72	
<i>barc184</i>	F:	TTC GGT GAT ATC TTT TCC CCT TGA	70	61	
	R:	CCG AGT TGA CTG TGT GGG CTT GCT G	79		
<i>cf14</i>	F:	CCA CCG GCC AGA GTA GTA TT	70	61	
	R:	TCC TGG TCT AAC AAC GAG AAG A	69		

APPENDIX 2. *q*-values of unlinked SSR markers located on the same chromosomes.

Locus pairs			Chi ²	df	<i>q</i> -value
1A	Barc158	Barc17	15,09124	4	0,106543
2A	Wmc177	Wmc407	14,45285	4	0,110142
1A	Barc158	Wmc278	13,7267	4	0,125423
5D	Barc286	Wmc765	11,91659	4	0,162275
3A	Barc314	Cfa2193	11,16656	4	0,192165
1A	Cfa2153	Wmc278	10,71076	4	0,206172
2A	Gwm636	Wmc177	10,49379	4	0,214929
1A	Barc17	Barc83	10,30463	4	0,225447
3A	Wmc532	Wmc559	10,07077	4	0,235351
4B	Gwm375	Wmc710	10,05938	4	0,235351
5D	Barc286	Cfd47	10,0048	4	0,236024
6B	Barc178	Wmc397	9,846114	4	0,246985
2D	Barc214	Cfd53	9,754912	4	0,248982
2A	Cfa2263	Gwm636	9,373531	4	0,259927
1A	Barc148	Wmc278	9,143431	4	0,273413
1A	Barc158	Barc83	9,077555	4	0,273413
2B	Wmc477	Wmc770	8,78613	4	0,288034
1A	Barc83	Cfa2153	8,30188	4	0,308273
1A	Cfa2135	Wmc278	8,172328	4	0,314694
7D	Barc235	Cfd69	8,054406	4	0,319018
1A	Barc148	Cfa2135	7,771133	4	0,324579
5B	Barc243	Wmc75	7,916972	4	0,324579
3A	Cfa2193	Wmc559	7,467427	4	0,337939
6B	Barc178	Wmc494	7,464253	4	0,337939
3A	Cfa2193	Wmc532	7,365	4	0,34198
5D	Barc143	Cfd57	7,315917	4	0,342916
1A	Barc148	Cfa2153	7,152522	4	0,35546
5D	Barc177	Cfd57	6,787411	4	0,377987
7B	Gwm537	Wmc396	6,713759	4	0,381086
2A	Wmc177	Wmc522	6,531516	4	0,393625
3A	Barc314	Wmc559	6,481872	4	0,393625
1B	Barc131	Wmc416	6,30382	4	0,404668
1B	Barc131	Wmc44	6,279446	4	0,404809
1A	Barc17	Cfa2135	6,149891	4	0,415206
2A	Cfa2263	Wmc407	6,089776	4	0,41648
5D	Barc143	Barc177	6,013406	4	0,418917
5D	Cfd57	Gwm174	5,955706	4	0,422411
7D	Cfd69	Wmc463	5,832612	4	0,432553
5D	Barc143	Barc286	5,763559	4	0,438856
1D	Cfd27	Cfd83	5,661231	4	0,438929
3B	Barc164	Barc75	5,703885	4	0,438929
2B	Wmc154	Wmc477	5,555156	4	0,442951

APPENDIX 2. Continued.

1A	Barc83	Cfa2135	5,534225	4	0,445173
4A	Barc343	Wmc262	5,49164	4	0,447737
5D	Barc143	Gwm174	5,480444	4	0,448503
1D	Cfd27	Wmc147	5,446528	4	0,451588
2A	Wmc407	Wmc522	5,411685	4	0,454504
2A	Gwm636	Wmc522	5,351938	4	0,461041
7A	Barc222	Wmc525	5,216604	4	0,472759
5D	Barc286	Gwm174	5,088549	4	0,478589
1A	Barc17	Wmc278	4,850175	4	0,49496
1A	Barc158	Cfa2153	4,733217	4	0,504855
1A	Barc83	Wmc278	4,194437	4	0,56177
5D	Barc177	Wmc765	4,148931	4	0,565417
2D	Barc214	Wmc503	4,016685	4	0,573452
2A	Cfa2263	Wmc522	3,909249	4	0,582715
2B	Wmc361	Wmc477	3,835294	4	0,586708
4D	Barc1183	Wmc720	3,741522	4	0,594519
2B	Wmc154	Wmc361	3,613926	4	0,610045
7D	Barc235	Wmc463	3,450258	4	0,625226
1A	Barc148	Barc158	3,358777	4	0,637331
5D	Barc143	Wmc765	3,244114	4	0,646959
3A	Barc314	Wmc532	3,202467	4	0,64952
2B	Wmc361	Wmc770	3,067108	4	0,664251
1A	Barc158	Cfa2135	3,0285	4	0,666362
5D	Gwm174	Wmc765	2,488507	4	0,715623
5D	Cfd57	Wmc765	1,946061	4	0,765719
1D	Cfd63	Wmc147	1,894042	4	0,769608
7B	Wmc396	Wmc517	1,81505	4	0,775299
1A	Barc148	Barc83	1,596402	4	0,794194
1A	Cfa2135	Cfa2153	1,542867	4	0,794529
2A	Cfa2263	Wmc177	1,370181	4	0,798592
1D	Cfd27	Cfd63	1,291225	4	0,80272
1D	Cfd63	Cfd83	1,166191	4	0,809506
1A	Barc17	Cfa2153	1,069663	4	0,816456
1A	Barc148	Barc17	0,560788	4	0,826549
2B	Wmc154	Wmc770	0,351547	4	0,826549
5D	Barc177	Barc286	0,748479	4	0,826549
5D	Barc177	Gwm174	0,058162	4	0,826549
6B	Wmc397	Wmc494	Infinity	4	0,826549
7B	Gwm537	Wmc517	0,861061	4	0,826549

APPENDIX 3. Genomic DNA Concentrations belongs to 55 bread wheat genotypes.

No	Sample Name	A₂₆₀	A₂₈₀	260/280	260/230	Stock ng/µl
1	Pamukova97	18.866	9.49	1.99	2.09	943.2
2	Cemre	21.586	10.837	1.99	2.13	1079.3
3	Tahirova	17.245	8.708	1.98	2.05	862.2
4	Hanlı	17.459	8.675	2.01	2.08	872.9
5	Ceyhan99	16.065	8.114	1.98	2.01	803.2
6	Pandas	20.509	10.287	1.99	2.07	1025.4
7	Karatopak	32.134	15.867	2.03	2.13	1606.7
8	Osmaniyem	25.421	12.662	2.01	2.11	1271
9	Carisma	20.873	10.281	2.03	2.1	1043.6
10	Yakar99	28.679	14.352	2	2.18	1433.9
11	Aksel2000	18.816	9.307	2.02	2.1	940.7
12	Bayraktar2000	31.132	15.559	2	2.21	1556.6
13	Demir2000	35.632	17.552	2.03	2.21	1781.6
14	Atlı2002	19.958	9.912	2.01	2.1	997.9
15	Çetinel 2000	26.267	13.152	2	2.03	1313.3
16	Alpu2001	36.13	17.792	2.03	2.21	1806.5
17	Tekirdağ	33.72	16.842	2	2.19	1685.9
18	Lancer	18.227	9.157	1.99	2.08	911.3
19	Gün91	23.213	11.689	1.99	2.22	1160.6
20	Türkmen	23.485	11.685	2.01	2.15	1174.2
21	Gerek79	25.728	12.703	2.03	2.18	1286.3
22	Aytın98	21.234	10.597	2	2.12	1061.6
23	Altay2000	28.262	13.782	2.05	2.13	1413.1
24	Karahan99	30.709	15.236	2.02	2.17	1535.4
25	Konya2002	27.422	13.81	1.99	2.12	1371.1
26	Aldane	25.244	12.393	2.04	2.1	1262.1
27	Nurkent	21.367	10.739	1.99	2.06	1068.3
28	KaşifBey 95	14.139	7.095	1.99	1.9	706.9
29	İzgi2001	21.402	10.675	2	1.94	1070.1
30	Sönmez 2001	20.177	10.076	2	1.99	1008.8
31	Behoth 8	10.232	5.045	2.03	1.89	511.6
32	Jaolan 2	9.238	4.886	1.89	1.9	461.9
33	Douma 4	13.808	6.766	2.04	2.12	690.38
34	Sham 10	6.358	3.096	2.05	1.84	317.9
35	Douma 2	10.36	5.016	2.07	1.97	518
36	Sham 4	8.188	4.027	2.03	1	409.4
37	Behoth 4	11.583	5.658	2.05	1.86	579.1
38	Behoth 6	11.323	5.548	2.04	1.83	566.1
39	Sham 6	8.135	3.954	2.06	1.92	406.7
40	Sham 8	13.543	6.938	1.95	1.9	677.1
41	Acsad 1139	10.981	5.399	2.03	1.94	549

APPENDIX 3. Continued.

42	Acsad 1133	6.813	3.349	2.03	1.78	340.6
43	Acsad 1115	8.061	3.938	2.05	1.95	403
44	Acsad 1159	8.824	4.289	2.06	1.95	441.2
45	Acsad 1071	9.291	4.532	2.05	1.92	464.5
46	Douma 40860	11.685	5.688	2.05	1.9	584.2
47	Douma 40863	10.384	5.352	1.94	1.73	519.2
48	Douma 40855	10.057	5.11	1.97	1.76	502.8
49	Douma 40856	10.709	5.249	2.04	2	535.4
50	Douma 40992	11.974	5.867	2.04	2.09	598.7
51	Douma 40988	13.134	6.458	2.03	2.01	656.6
52	Douma 40989	12.843	6.31	2.04	1.87	642.1
53	Douma 40444	11.866	5.834	2.03	1.84	593.3
54	Douma 48114	10.558	5.148	2.05	1.92	527.9
55	Douma 40765	15.606	7.834	1.99	2.05	780.2

APPENDIX 4. Allele sizes per each genotype in one locus.

Genotype ID	Chromosome	1A	1A	1A	1A	1A	1A	1A	1A	1A	1A
	Marker	BARC17	BARC83	BARC148	BARC158	CFA2135	CFA2153	WMC278	WMC716-A	WMC716-B	WMC716-C
1	Pamukova97	269	258	207	269	188	215	214	155	137	0
2	Cemre	279	267	204	264	185	211	212	166	134	0
3	Tahirova	274	256	198	264	184	195	211	166	134	0
4	Hanlı	292	265	200	259	183	218	213	146	132	0
5	Ceyhan-99	279	267	0	266	183	195	207	152	134	0
6	Pandas (Panda)	308	265	200	250	183	195	209	202	132	0
7	Karatopak	292	267	196	262	184	163	205	152	134	0
8	Osmaniyem	284	265	196	262	184	202	206	200	159	134
9	Carisma	284	267	202	262	183	213	208	163	134	0
10	Yakar-99	279	267	198	259	185	187	207	161	132	0
11	Aksel 2000	271	269	200	264	173	195	209	150	132	0
12	Bayraktar 2000	279	274	194	250	185	193	208	152	132	0
13	Demir 2000	279	272	198	259	185	208	211	161	132	0
14	Atlı-2002	292	261	198	259	187	196	211	166	132	0
15	Çetinel-2000	279	274	196	259	185	210	211	159	132	0
16	Alpu 2001	281	276	194	257	187	198	209	161	130	0
17	Tekirdağ	308	263	196	257	185	213	211	159	130	0
18	Lancer	292	263	192	257	180	211	212	189	129	0
19	Gün-91	292	274	196	257	187	210	209	146	129	0
20	Türkmen	279	274	196	247	184	203	209	198	127	0
21	Gerek 79	295	274	196	255	183	202	209	195	127	0
22	Aytın 98	295	261	196	255	183	203	209	191	126	0
23	Altay 2000	295	263	198	252	184	205	209	144	127	0
24	Karahan-99	295	274	204	250	185	224	212	144	127	0
25	Konya-2002	284	276	200	250	188	224	212	155	126	0

APPENDIX 4. Continued.

26	Aldane	284	276	204	250	187	215	212	157	126	0
27	Nurkent	295	263	196	250	187	210	212	144	127	0
28	Kaşif Bey 95	281	263	198	252	187	202	212	143	126	0
29	İzgi 2001	279	274	200	250	187	213	213	152	124	0
30	Sönmez 2001	284	276	205	250	0	200	214	161	124	0
31	Behoth 8	279	276	202	247	185	198	213	153	124	0
32	Jaolan 2	284	265	198	247	185	196	214	152	124	0
33	Douma 4	281	278	200	247	187	191	213	143	124	0
34	Sham 10	281	276	204	247	191	196	216	143	124	0
35	Douma 2	287	278	202	252	191	196	214	153	124	0
36	Sham 4	289	278	198	251	191	196	216	141	124	0
37	Behoth 4	0	283	205	250	193	202	218	141	124	0
38	Behoth 6	281	281	205	247	193	195	219	143	124	0
39	Sham 6	289	281	204	247	193	195	219	150	124	0
40	Sham 8	0	283	204	247	193	193	220	150	124	0
41	Acsad 1139	289	272	209	245	194	202	218	152	122	0
42	Acsad 1133	284	272	211	247	193	202	220	143	124	0
43	Acsad 1115	289	285	211	245	191	202	218	143	124	0
44	Acsad 1159	281	272	213	245	193	206	217	157	122	0
45	Acsad 1071	287	274	213	243	0	203	216	148	122	0
46	Douma 40860	284	274	215	243	191	203	216	141	122	0
47	Douma 40863	284	288	217	243	193	217	216	139	122	0
48	Douma 40855	271	276	211	240	0	166	219	139	122	0
49	Douma 40856	281	293	215	240	0	205	218	153	122	0
50	Douma 40992	271	278	219	244	194	208	220	146	122	0
51	Douma 40988	0	295	219	243	194	210	220	137	121	0
52	Douma 40989	284	278	221	239	193	205	222	143	121	0

APPENDIX 4. Continued.

53	Douma 40444	279	280	219	0	194	214	223	137	121	0
54	Douma 48114	279	283	219	248	193	217	226	142	124	0
55	Douma 40765	280	285	219	0	194	0	226	142	126	0
Genotype ID	Chromosome	2A	2A	2A	2A	2A	3A	3A	3A	3A	3A
	Marker	CFA2263	GWM636	WMC177	WMC407	WMC522	BARC314	CFA2193	WMC169-A	WMC169-B	WMC169-C
1	Pamukova97	121	108	202	125	224	262	257	145	0	119
2	Cemre	132	108	211	125	224	262	260	145	0	106
3	Tahirova	129	108	211	125	233	265	263	150	0	110
4	Hanlı	132	105	197	128	233	265	274	0	128	112
5	Ceyhan-99	116	112	203	128	207	268	267	145	130	112
6	Pandas (Panda)	138	107	203	0	209	265	274	159	0	115
7	Karatopak	126	105	206	112	225	268	274	150	134	117
8	Osmaniyem	141	104	206	0	209	268	274	161	0	117
9	Carisma	132	110	203	133	188	268	285	161	0	119
10	Yakar-99	144	110	203	150	235	268	277	161	0	119
11	Aksel 2000	138	116	193	133	210	265	257	0	139	119
12	Bayraktar 2000	135	116	193	135	211	268	277	0	140	122
13	Demir 2000	141	118	203	0	209	268	277	159	139	119
14	Atlı-2002	141	118	209	133	200	265	277	0	141	122
15	Çetinel-2000	144	108	208	115	188	265	281	171	0	122
16	Alpu 2001	150	118	202	142	207	268	281	0	141	124
17	Tekirdağ	132	112	206	137	207	265	281	171	0	124
18	Lancer	138	119	187	135	191	297	277	176	0	124
19	Gün-91	132	118	203	137	222	265	281	0	145	122
20	Türkmen	144	116	205	137	228	265	277	0	142	126
21	Gerek 79	144	114	205	135	227	265	277	0	144	126
22	Aytın 98	132	116	205	135	227	265	277	0	142	126

APPENDIX 4. Continued.

23	Altay 2000	132	116	208	133	194	259	281	164	145	124
24	Karahan-99	144	114	188	0	224	262	270	171	0	122
25	Konya-2002	141	91	200	132	225	262	274	164	150	126
26	Aldane	141	116	209	135	179	262	274	171	0	126
27	Nurkent	150	118	203	133	184	262	274	171	0	128
28	Kaşif Bey 95	141	116	213	132	194	262	274	166	148	128
29	İzgi 2001	0	107	205	115	177	262	277	173	0	128
30	Sönmez 2001	144	116	209	135	177	262	253	148	0	128
31	Behoth 8	138	116	202	135	211	262	270	166	150	130
32	Jaolan 2	141	116	202	135	193	265	270	0	150	130
33	Douma 4	156	114	190	135	210	265	274	176	0	130
34	Sham 10	153	118	191	135	219	268	274	0	150	130
35	Douma 2	153	116	191	133	190	268	274	168	152	132
36	Sham 4	150	116	200	132	190	268	277	168	154	132
37	Behoth 4	160	118	190	133	210	268	274	178	0	132
38	Behoth 6	156	118	191	137	217	268	274	168	154	134
39	Sham 6	147	114	200	133	190	268	277	154	0	134
40	Sham 8	147	116	198	133	190	268	274	173	154	134
41	Acsad 1139	156	118	190	135	188	268	274	171	154	134
42	Acsad 1133	156	116	198	133	206	271	277	178	0	134
43	Acsad 1115	147	116	203	133	204	271	270	171	152	134
44	Acsad 1159	0	116	202	132	203	268	274	178	0	134
45	Acsad 1071	147	116	202	133	187	268	277	169	154	134
46	Douma 40860	156	116	203	133	201	268	263	181	0	134
47	Douma 40863	156	116	188	137	201	268	263	176	0	132
48	Douma 40855	156	116	203	133	200	271	267	171	152	132
49	Douma 40856	150	116	205	0	184	274	263	176	0	132

APPENDIX 4. Continued.

50	Douma 40992	160	118	206	139	209	274	263	152	0	128
51	Douma 40988	147	116	205	135	207	274	263	164	148	128
52	Douma 40989	150	114	193	137	176	274	253	171	0	126
53	Douma 40444	154	114	205	135	175	274	247	166	0	124
54	Douma 48114	163	111	206	130	193	274	253	164	0	122
55	Douma 40765	144	114	208	128	179	274	253	164	0	122
Genotype ID	Chromosome	3A	3A	3A	3A	4A	4A	4A	4A	4A	4A
	Marker	WMC428-A	WMC428-B	WMC532	WMC559	BARC343	Wmc219-A	Wmc219-B	Wmc219-C	Wmc219-D	WMC262
1	Pamukova97	275	239	174	280	159	0	0	0	0	243
2	Cemre	275	239	172	284	172	214	0	154	0	184
3	Tahirova	275	234	176	261	0	0	0	154	134	238
4	Hanlı	258	234	170	296	174	0	0	150	130	184
5	Ceyhan-99	272	226	153	300	166	0	0	154	0	238
6	Pandas (Panda)	272	226	153	276	158	205	0	154	0	210
7	Karatopak	278	231	178	307	158	0	0	0	0	0
8	Osmaniyem	278	231	153	315	161	0	0	158	138	238
9	Carisma	275	236	155	315	166	0	0	158	138	193
10	Yakar-99	275	231	178	315	159	222	200	156	138	200
11	Aksel 2000	275	229	174	330	174	0	0	0	0	182
12	Bayraktar 2000	278	229	180	322	0	0	0	158	141	247
13	Demir 2000	275	234	158	322	171	0	0	163	141	182
14	Atlı-2002	281	234	158	338	0	225	203		0	245
15	Çetinel-2000	278	234	160	292	158	0	0	161	0	200
16	Alpu 2001	275	234	160	330	166	0	0	163	143	238
17	Tekirdağ	275	234	182	292	171	0	0	165	145	180
18	Lancer	275	234	178	330	162	0	0	161	141	233
19	Gün-91	275	231	160	330	155	0	0	165	145	206

APPENDIX 4. Continued.

20	Türkmen	281	231	184	330	162	0	0	163	145	219
21	Gerek 79	281	231	184	330	162	0	0	163	145	217
22	Aytın 98	272	231	182	330	167	0	0	163	143	202
23	Altay 2000	272	231	184	338	155	231	205	163	145	202
24	Karahan-99	275	231	160	330	161	0	0	0	0	180
25	Konya-2002	275	234	178	330	0	0	0	165	143	175
26	Aldane	272	231	160	330	167	0	165	145	0	173
27	Nurkent	272	231	180	338	162	228	203	163	143	226
28	Kaşif Bey 95	272	231	186	338	164	228	200		0	226
29	İzgi 2001	275	231	163	300	155	0	0	0	0	191
30	Sönmez 2001	272	231	163	346	167	0	0	165	145	169
31	Behoth 8	269	226	186	338	167	0	0	165	145	167
32	Jaolan 2	269	229	176	338	169	0	172	152	0	167
33	Douma 4	269	229	184	346	155	228	203		0	221
34	Sham 10	272	226	188	338	169	0	0	165	148	167
35	Douma 2	269	231	182	338	156	0	174	154	0	204
36	Sham 4	269	231	178	338	156	0	0	165	0	198
37	Behoth 4	272	229	184	338	0	216	192		0	228
38	Behoth 6	269	231	192	338	0	0	182	167	0	210
39	Sham 6	272	229	182	338	156	231	0	167	0	195
40	Sham 8	272	231	182	338	156	234	211	167	0	193
41	Acsad 1139	272	231	186	338	155	0	174	154	0	200
42	Acsad 1133	275	231	182	338	164	231	211	167	0	215
43	Acsad 1115	272	234	188	330	171	0	174	154	0	159
44	Acsad 1159	272	231	192	322	171	0	0	165	0	213
45	Acsad 1071	272	229	180	322	164	231	208	165	150	0
46	Douma 40860	278	226	169	322	169	0	0	165	148	208

APPENDIX 4. Continued.

47	Douma 40863	275	234	170	322	171	231	208	165	150	208	
48	Douma 40855	272	0	169	322	0	0	0	163	0	217	
49	Douma 40856	275	231	184	322	162	228	0	163	0	188	
50	Douma 40992	272	0	196	315	162	228	0	163	0	210	
51	Douma 40988	261	239	192	300	162	0	0	163	145	210	
52	Douma 40989	281	0	196	272	179	0	0	163	145	157	
53	Douma 40444	281	234	198	272	172	0	0	163	145	208	
54	Douma 48114	284	0	190	265	176	228	0	163	0	210	
55	Douma 40765	281	0	181	262	184	0	165	150	0	160	
Genotype ID	Chromosome	5A	6A	6A	6A	6A		6A	6A	6A	7A	7A
	Marker	BARC117	BARC1165-A	BARC1165-B	BARC1165-C	BARC1165-D		WMC201	WMC256-A	WMC256-B	BARC222	WMC525
1	Pamukova97	210	0	0	134	102		0	126	106	197	209
2	Cemre	210	0	154	0	100		0	112	0	192	0
3	Tahirova	210	0	150	132	102		262	112	0	195	280
4	Hanlı	215	0	154	132	102		283	131	110	197	280
5	Ceyhan-99	215	0	152	134	102		277	117	0	197	209
6	Pandas (Panda)	215	0	0	137	103		283	134	112	195	236
7	Karatopak	226	187	158	141	106		283	139	112	195	216
8	Osmaniyem	215	0	158	141	106		283	139	112	195	216
9	Carisma	218	0	158	143	106		0	134	112	195	274
10	Yakar-99	215	0	161	141	106		259	119	0	184	236
11	Aksel 2000	215	0	158	141	106		262	134	110	197	226
12	Bayraktar 2000	215	0	158	143	106		262	119	0	197	226
13	Demir 2000	218	0	158	141	106		283	121	0	195	229
14	Atlı-2002	221	0	0	143	106		262	121	0	200	229
15	Çetinel-2000	218	0	161	141	106		268	121	0	195	242
16	Alpu 2001	221	0	158	141	106		280	136	117	197	274

APPENDIX 4. Continued.

17	Tekirdağ	218	0	0	143	106	274	124	0	197	229
18	Lancer	226	0	161	141	106	274	136	117	187	229
19	Gün-91	232	0	158	141	106	265	124	0	197	219
20	Türkmen	0	0	158	143	106	280	136	114	197	224
21	Gerek 79	226	0	0	143	106	271	139	114	197	224
22	Aytın 98	226	0	158	141	106	253	121	0	197	231
23	Altay 2000	221	0	158	141	106	256	121	0	195	229
24	Karahan-99	221	0	158	141	106	259	121	0	195	231
25	Konya-2002	224	0	0	143	106	265	119	0	197	224
26	Aldane	221	0	158	143	106	262	121	0	195	234
27	Nurkent	218	0	161	141	106	256	136	90	195	226
28	Kaşif Bey 95	218	0	158	141	106	265	119	0	189	205
29	İzgi 2001	218	0	0	141	108	250	119	0	192	0
30	Sönmez 2001	224	0	0	143	106	233	121	0	195	0
31	Behoth 8	224	0	0	141	106	265	136	0	195	200
32	Jaolan 2	224	0	158	141	106	250	134	112	195	200
33	Douma 4	224	0	163	145	110	250	121	0	192	262
34	Sham 10	224	0	163	145	110	262	121	0	187	198
35	Douma 2	224	0	163	145	110	265	139	0	189	262
36	Sham 4	224	0	145		110	265	139	0	192	221
37	Behoth 4	224	0	163	143	108	239	124	0	192	198
38	Behoth 6	224	0	165	143	108	265	139	117	187	198
39	Sham 6	0	0	0	145	108	265	139	117	192	195
40	Sham 8	224	0	0	145	108	265	139	117	195	195
41	Acsad 1139	224	0	0	143	108	265	139	117	195	259
42	Acsad 1133	232	0	161	141	108	253	139	117	195	248
43	Acsad 1115	229	0	0	141	106	250	124	0	195	216

APPENDIX 4. Continued.

44	Acsad 1159	229	0	0	141	106	265	126	0	192	216
45	Acsad 1071	229	0	161	141	106	265	124	0	192	216
46	Douma 40860	241	0	161	143	108	262	126	0	187	193
47	Douma 40863	232	0	170	143	106	262	126	0	189	193
48	Douma 40855	232	0	161	141	106	262	142	119	192	214
49	Douma 40856	232	0	0	145	108	262	142	119	192	0
50	Douma 40992	235	0	0	141	106	262	136	0	187	190
51	Douma 40988	235	0	0	141	106	244	126	0	182	202
52	Douma 40989	235	0	170	143	106	265	139	0	192	193
53	Douma 40444	238	0	0	141	106	268	125	0	192	0
54	Douma 48114	244	0	141	0	106	256	139	0	195	0
55	Douma 40765	238	0	0	143	108	268	120	0	192	193
Genotype ID	Chromosome	1B	1B	1B	1B	1B	1B	1B	1B	1B	1B
	Marker	BARC80-A	BARC80-B	BARC131	BARC137-A	BARC137-B	BARC137-C	BARC181-A	BARC181-B	WMC44	WMC416
1	Pamukova97	120	99	223	0	204	0	106	0	211	237
2	Cemre	111	0	220	0	180	0	142	104	211	237
3	Tahirova	110	106	218	0	210	175	142	102	209	226
4	Hanlı	108	0	220	0	181	0	147	115	207	227
5	Ceyhan-99	107	0	245	0	203	0	148	107	207	231
6	Pandas (Panda)	118	0	245	0	186	0	153	108	206	231
7	Karatopak	109	0	225	0	206	0	157	120	207	231
8	Osmaniyem	109	0	227	0	174	0	112	0	206	229
9	Carisma	111	0	250	0	183	0	164	112	206	232
10	Yakar-99	120	96	236	0	204	0	164	112	266	224
11	Aksel 2000	108	0	0	210	0	0	157	120	225	231
12	Bayraktar 2000	108	0	227	219	0	0	157	119	243	229
13	Demir 2000	106	0	250	0	216	174	164	112	206	227

APPENDIX 4. Continued.

14	Atlı-2002	106	0	250	230	195	0	171	115	264	227
15	Çetinel-2000	106	0	225	0	187	0	135	0	238	229
16	Alpu 2001	106	0	225	0	0	170	171	116	207	226
17	Tekirdağ	106	0	231	0	178	160	164	137	236	231
18	Lancer	125	92	225	0	204	0	134	0	247	227
19	Gün-91	114	0	248	0	0	0	171	115	207	229
20	Türkmen	105	0	223	0	198	178	168	115	245	229
21	Gerek 79	105	0	223	0	198	178	164	114	247	231
22	Aytın 98	105	0	225	0	200	177	164	115	267	229
23	Altay 2000	105	0	223	0	209	187	171	116	236	227
24	Karahan-99	105	0	236	0	0	190	171	116	271	227
25	Konya-2002	106	0	225	0	0	190	171	126	207	229
26	Aldane	109	0	236	0	211	178	171	118	267	231
27	Nurkent	115	0	223	0	186	0	175	118	210	232
28	Kaşif Bey 95	106	0	248	0	211	0	175	118	210	232
29	İzgi 2001	107	0	223	0	0	189	175	138	241	232
30	Sönmez 2001	106	0	234	0	214	195	120	0	210	232
31	Behoth 8	115	0	220	0	0	0	191	125	213	234
32	Jaolan 2	106	0	220	0	207	0	196	125	211	237
33	Douma 4	93	0	220	0	194	171	196	125	214	238
34	Sham 10	105	0	223	225	0	192	196	126	271	238
35	Douma 2	106	0	223	0	0	0	196	125	271	238
36	Sham 4	106	0	245	238	216	0	196	126	273	237
37	Behoth 4	107	0	223	0	0	0	196	125	215	240
38	Behoth 6	115	0	223	0	197	0	196	125	215	240
39	Sham 6	107	0	245	0	198	178	196	135	277	238
40	Sham 8	108	0	243	247	0	217	196	135	275	240

APPENDIX 4. Continued.

41	Acsad 1139	108	0	220	228	0	203	196	126	279	247
42	Acsad 1133	108	0	220	0	217	0	0	0	279	247
43	Acsad 1115	108	0	218	0	206	189	196	137	218	245
44	Acsad 1159	109	0	229	0	217	200	191	125	218	253
45	Acsad 1071	110	0	216	0	217	197	191	134	279	248
46	Douma 40860	110	0	231	230	219	200	191	125	280	245
47	Douma 40863	111	0	231	0	207	184	196	126	221	245
48	Douma 40855	111	0	227	222	214	198	203	138	282	247
49	Douma 40856	112	0	240	230	0	0	128	0	224	0
50	Douma 40992	113	0	227	230	0	0	200	126	264	257
51	Douma 40988	113	0	227	221	0	0	191	126	224	260
52	Douma 40989	114	101	216	224	209	194	191	122	288	264
53	Douma 40444	112	100	227	0	0	184	178	118	229	267
54	Douma 48114	126	101	216	0	0	0	178	118	292	269
55	Douma 40765	101	0	218	227	209	0	182	119	292	269
Genotype ID	Chromosome	2B	2B	2B	2B	3B	3B	4B	4B	4B	4B
	Marker	WMC154	WMC361	WMC477	WMC770	BARC75	BARC164	CFA2149	CFA2149	GWM375	WMC710
1	Pamukova97	160	238	181	111	0	0	218	205	150	135
2	Cemre	155	235	180	140	0	178	218	205	152	114
3	Tahirova	158	235	180	113	0	0	218	205	160	132
4	Hanlı	155	240	176	126	102	171	218	202	165	100
5	Ceyhan-99	160	231	178	93	104	171	221	205	165	135
6	Pandas (Panda)	155	226	176	162	140	0	221	200	165	109
7	Karatopak	157	226	181	147	104	183	221	208	137	141
8	Osmaniyem	157	231	0	147	107	180	221	205	160	0
9	Carisma	0	226	176	150	107	171	221	205	176	116
10	Yakar-99	126	226	176	135	107	173	218	0	176	111

APPENDIX 4. Continued.

11	Aksel 2000	146	226	178	170	109	173	224	210	162	116
12	Bayraktar 2000	162	226	178	152	0	173	226	210	179	97
13	Demir 2000	163	0	173	140	105	173	226	210	179	100
14	Atlı-2002	125	0	178	170	111	178	229	210	179	100
15	Çetinel-2000	162	226	176	137	111	173	232	213	173	119
16	Alpu 2001	160	231	173	160	113	185	232	210	142	138
17	Tekirdağ	126	228	176	115	107	180	232	213	176	114
18	Lancer	259	228	176	150	113	208	235	213	170	135
19	Gün-91	158	226	173	128	0	178	221	0	179	119
20	Türkmen	158	224	172	170	0	192	232	213	179	100
21	Gerek 79	157	224	172	170	0	0	235	215	179	100
22	Aytın 98	145	224	170	173	113	192	235	210	155	100
23	Altay 2000	155	224	173	145	107	175	226	0	173	97
24	Karahan-99	160	224	173	173	113	178	235	213	176	119
25	Konya-2002	153	226	170	147	0	178	232	215	179	135
26	Aldane	163	224	172	91	0	180	221	0	179	100
27	Nurkent	158	226	172	133	113	178	235	215	173	135
28	Kaşif Bey 95	128	226	172	133	113	178	235	213	173	138
29	İzgi 2001	158	226	172	142	113	175	232	218	173	119
30	Sönmez 2001	160	224	175	162	117	180	229	0	176	100
31	Behoth 8	158	233	170	130	113	190	229	215	176	116
32	Jaolan 2	160	233	170	133	105	192	229	215	176	114
33	Douma 4	128	226	173	111	107	192	229	215	162	132
34	Sham 10	153	233	173	133	113	183	229	215	173	114
35	Douma 2	170	224	175	135	111	180	226	0	173	132
36	Sham 4	163	224	178	165	107	180	0	0	176	114
37	Behoth 4	169	233	178	137	107	180	229	215	165	129

APPENDIX 4. Continued.

38	Behoth 6	165	224	176	137	113	192	232	215	173	114
39	Sham 6	165	226	180	162	107	213	232	215	173	114
40	Sham 8	167	221	181	162	107	213	232	213	173	114
41	Acsad 1139	172	226	183	137	111	178	226	0	170	135
42	Acsad 1133	165	221	184	168	113	175	226	0	170	132
43	Acsad 1115	167	226	183	137	113	210	226	213	173	114
44	Acsad 1159	169	221	0	194	107	200	229	208	173	100
45	Acsad 1071	169	221	188	137	113	175	226	213	170	126
46	Douma 40860	167	224	191	140	0	202	224	210	170	129
47	Douma 40863	158	226	0	179	111	173	226	210	170	129
48	Douma 40855	165	224	191	170	107	208	226	213	162	114
49	Douma 40856	162	221	191	142	105	208	226	213	162	114
50	Douma 40992	167	226	191	142	109	183	226	213	160	126
51	Douma 40988	172	226	189	182	109	183	226	213	157	114
52	Douma 40989	169	233	188	142	107	185	226	213	160	132
53	Douma 40444	165	221	179	147	104	201	226	213	150	132
54	Douma 48114	165	223	184	174	104	173	224	0	152	132
55	Douma 40765	172	226	182	122	102	173	224	213	150	132
Genotype ID	Chromosome	5B	5B	6B	6B	6B	6B	6B	6B	6B	7B
	Marker	BARC243	WMC75	BARC79-A	BARC79-B	BARC178	BARC354-A	BARC354-B	WMC397	WMC494	GWM537
1	Pamukova97	247	268	157	0	0	374	0	183	256	215
2	Cemre	209	263	0	0	300	374	0	190	242	217
3	Tahirova	218	242	164	0	0	366	0	192	242	212
4	Hanlı	226	266	164	0	297	383	0	192	242	217
5	Ceyhan-99	209	263	157	0	297	391	0	192	244	210
6	Pandas (Panda)	221	240	167	0	297	391	0	190	250	217
7	Karatopak	213	263	150	0	297	383	0	190	250	212

APPENDIX 4. Continued.

8	Osmaniyem	213	263	159	0	300	391	0	192	242	212
9	Carisma	202	240	167	0	0	391	0	200	242	228
10	Yakar-99	213	260	167	0	300	391	0	187	247	217
11	Aksel 2000	213	263	162	0	300	366	0	190	236	222
12	Bayraktar 2000	215	266	145	0	297	328	0	187	244	217
13	Demir 2000	215	266	159	0	297	391	0	190	236	230
14	Atlı-2002	215	268	164	0	340	400	0	187	236	230
15	Çetinel-2000	217	268	167	0	300	366	0	185	247	220
16	Alpu 2001	217	268	159	0	300	400	0	185	236	215
17	Tekirdağ	217	271	164	0	303	400	0	185	234	220
18	Lancer	199	274	164	0	287	374	0	180	234	236
19	Gün-91	205	271	172	152	262	400	0	185	231	233
20	Türkmen	228	271	147	0	297	321	0	183	239	220
21	Gerek 79	0	271	147	0	293	321	0	180	247	228
22	Aytın 98	226	268	147	0	297	307	0	180	247	222
23	Altay 2000	214	268	147	0	321	307	0	187	239	222
24	Karahan-99	214	268	172	0	321	366	0	180	229	225
25	Konya-2002	234	268	172	0	293	366	0	180	229	222
26	Aldane	215	268	162	0	293	366	0	178	226	233
27	Nurkent	204	268	172	0	314	358	0	178	224	225
28	Kaşif Bey 95	217	266	172	152	344	343	0	178	224	217
29	İzgi 2001	214	268	167	0	293	335	0	175	234	225
30	Sönmez 2001	214	266	147	0	290	335	0	173	224	233
31	Behoth 8	221	263	164	0	290	350	0	171	221	217
32	Jaolan 2	211	263	164	0	307	343	0	173	221	241
33	Douma 4	211	237	172	0	287	343	0	173	219	225
34	Sham 10	218	260	162	0	0	293	0	173	219	217

APPENDIX 4. Continued.

35	Douma 2	221	237	164	0	287	343	0	171	221	222
36	Sham 4	213	260	162	0	293	328	0	164	234	241
37	Behoth 4	221	266	172	0	287	328	0	173	219	222
38	Behoth 6	201	240	172	0	290	335	0	173	219	222
39	Sham 6	213	258	162	0	290	321	0	164	234	244
40	Sham 8	211	260	159	0	290	321	0	164	234	244
41	Acsad 1139	221	237	162	0	283	335	0	173	216	225
42	Acsad 1133	213	255	162	0	287	335	0	173	219	217
43	Acsad 1115	221	253	157	0	290	335	0	164	219	225
44	Acsad 1159	222	253	167	0	287	328	0	171	214	217
45	Acsad 1071	201	250	167	0	0	335	0	171	216	220
46	Douma 40860	202	247	140	0	321	287	0	171	219	233
47	Douma 40863	208	247	155	0	280	290	0	171	221	220
48	Douma 40855	214	245	155	0	283	328	0	162	236	222
49	Douma 40856	204	245	164	0	0	343	0	160	216	220
50	Douma 40992	213	245	157	0	283	350	0	169	216	220
51	Douma 40988	226	245	0	0	280	343	0	169	216	217
52	Douma 40989	209	242	160	0	0	343	0	167	216	230
53	Douma 40444	212	245	160	138	277	343	316	167	230	220
54	Douma 48114	219	245	152	0	284	347	0	165	222	220
55	Douma 40765	218	245	152	0	284	316	0	165	233	225
Genotype ID	Chromosome	7B	7B	7B	7B	7B	7B	7B	7B	1D	1D
	Marker	WMC276-A	WMC276-B	WMC335-A	WMC335-B	WMC396	WMC476-A	WMC476-B	WMC517	CFD27	CFD63
1	Pamukova97	287	0	0	0	175	232	0	209	154	279
2	Cemre	290	0	124	0	175	225	0	198	148	285
3	Tahirova	304	0	121	82	175	236	180	215	141	282
4	Hanlı	293	0	124	84	200	225	183	196	150	279

APPENDIX 4. Continued.

5	Ceyhan-99	304	0	124	84	175	236	0	198	150	266
6	Pandas (Panda)	311	0	126	84	175	222	0	200	150	282
7	Karatopak	300	0	128	86	175	229	0	220	150	277
8	Osmaniyem	304	0	126	86	175	239	0	211	150	263
9	Carisma	307	0	128	86	175	222	0	213	150	271
10	Yakar-99	256	0	128	88	175	229	186	213	152	279
11	Aksel 2000	326	0	128	86	172	236	0	225	139	271
12	Bayraktar 2000	307	0	126	86	172	229	186	196	143	277
13	Demir 2000	319	0	126	86	178	229	183	225	154	268
14	Atlı-2002	315	0	128	88	172	232	183	220	154	291
15	Çetinel-2000	300	274	115	88	172	239	183	222	154	285
16	Alpu 2001	322	0	128	88	172	236	183	213	145	285
17	Tekirdağ	290	241	130	90	172	232	183	202	143	277
18	Lancer	319	0	128	90	172	222	183	204	152	285
19	Gün-91	0	0	130	90	172	229	183	204	143	279
20	Türkmen	319	0	128	88	169	229	180	196	150	279
21	Gerek 79	319	0	79	0	166	225	0	198	154	282
22	Aytın 98	319	0	128	88	169	225	180	196	154	282
23	Altay 2000	265	0	115	89	166	236	180	204	154	282
24	Karahan-99	265	0	130	89	166	232	176	206	152	277
25	Konya-2002	322	0	126	90	187	225	0	198	152	288
26	Aldane	326	0	130	88	178	219	176	209	143	288
27	Nurkent	319	0	0	0	163	222	0	200	152	297
28	Kaşif Bey 95	265	0	126	86	166	229	173	211	152	300
29	İzgi 2001	304	274	113	86	163	229	173	220	152	279
30	Sönmez 2001	315	0	126	86	184	209	173	206	154	297
31	Behoth 8	265	0	126	88	161	225	0	198	154	294

APPENDIX 4. Continued.

32	Jaolan 2	307	0	126	84	158	222	0	206	150	297
33	Douma 4	307	0	126	86	153	225	170	198	152	297
34	Sham 10	311	0	126	90	158	222	170	200	154	300
35	Douma 2	311	0	124	86	158	222	170	209	154	300
36	Sham 4	307	262	124	86	155	219	170	215	158	303
37	Behoth 4	311	0	128	86	158	222	170	206	158	297
38	Behoth 6	259	0	126	86	155	219	170	194	160	297
39	Sham 6	259	0	124	84	155	219	167	211	160	297
40	Sham 8	259	0	107	88	153	219	0	209	160	300
41	Acsad 1139	307	0	124	86	153	219	167	202	158	297
42	Acsad 1133	300	0	124	82	153	215	164	200	150	297
43	Acsad 1115	304	0	124	86	153	212	164	200	158	282
44	Acsad 1159	304	0	121	84	153	215	164	190	156	288
45	Acsad 1071	300	0	121	82	150	212	0	196	156	285
46	Douma 40860	300	0	119	82	150	206	164	192	158	291
47	Douma 40863	297	0	121	82	150	209	164	200	156	306
48	Douma 40855	250	0	124	82	172	206	164	198	156	300
49	Douma 40856	293	0	121	84	153	219	0	180	154	306
50	Douma 40992	297	0	124	82	150	219	167	179	154	303
51	Douma 40988	293	0	111	84	169	186	0	173	150	297
52	Douma 40989	290	0	124	84	153	229	0	0	156	291
53	Douma 40444	286	0	126	86	153	226	0	196	154	291
54	Douma 48114	276	0	124	84	153	226	167	189	141	294
55	Douma 40765	272	0	126	83	153	0	0	196	150	294
Genotype ID	Chromosome	1D	1D	2D	2D	3D	3D	3D	3D	3D	3D
	Marker	CFD83	WMC147	CFD53	WMC503	BARC125-A	BARC125-B	BARC125-C	BARC135-A	BARC135-B	BARC135-C
1	Pamukova97	258	172	221	300	0	140	0	0	245	226

APPENDIX 4. Continued.

2	Cemre	256	169	228	238	0	138	106	0	245	229
3	Tahirova	256	172	234	297	0	144	108	0	245	226
4	Hanlı	255	167	244	294	0	144	106	0	248	226
5	Ceyhan-99	245	167	249	294	0	144	108	0	248	229
6	Pandas (Panda)	243	164	264	236	152	0	109	0	252	229
7	Karatopak	245	162	254	300	148	0	111	0	252	229
8	Osmaniye	239	162	244	297	154	0	112	0	248	229
9	Carisma	240	162	244	236	0	0	111	0	252	229
10	Yakar-99	238	164	257	273	146	0	112	0	248	232
11	Aksel 2000	235	162	254	238	150	0	112	0	252	229
12	Bayraktar 2000	237	161	254	300	162	140	114	0	255	229
13	Demir 2000	238	162	254	300	156	0	114	0	252	232
14	Atlı-2002	235	162	247	300	156	0	114	0	262	232
15	Çetinel-2000	233	161	254	243	150	0	116	0	255	232
16	Alpu 2001	234	162	257	303	152	0	114	0	252	232
17	Tekirdağ	235	161	268	245	154	0	116	0	252	232
18	Lancer	237	164	250	275	164	0	114	0	252	229
19	Gün-91	233	164	247	248	0	142	114	0	252	229
20	Türkmen	232	161	254	303	167	142	114	0	252	229
21	Gerek 79	232	161	257	306	162	140	114	0	252	229
22	Aytın 98	230	159	257	306	160	0	114	0	252	229
23	Altay 2000	235	159	254	278	150	0	112	0	252	229
24	Karahan-99	230	159	261	306	167	140	114	0	245	229
25	Konya-2002	230	159	261	308	152	0	116	0	248	229
26	Aldane	230	150	280	245	162	0	114	0	252	229
27	Nurkent	232	162	254	245	150	0	114	0	248	229
28	Kaşif Bey 95	235	162	284	248	167	0	116	0	259	229

APPENDIX 4. Continued.

29	Izgi 2001	232	161	261	248	0	144	114	0	248	226
30	Sönmez 2001	0	159	284	248	164	0	117	0	245	226
31	Behoth 8	232	162	257	250	150	0	117	0	252	226
32	Jaolan 2	231	162	268	250	152	0	116	0	245	226
33	Douma 4	235	161	268	311	160	0	117	0	245	226
34	Sham 10	233	164	284	252	158	117	0	0	248	226
35	Douma 2	236	162	268	314	162	0	117	0	245	226
36	Sham 4	232	164	268	314	158	0	0	0	248	226
37	Behoth 4	235	164	266	314	164	142	117	389	245	223
38	Behoth 6	233	164	284	252	162	0	119	0	245	223
39	Sham 6	231	166	272	314	158	0	117	0	245	223
40	Sham 8	231	166	272	314	158	0	119	0	245	223
41	Acsad 1139	236	166	268	314	167	0	119	0	242	220
42	Acsad 1133	231	164	276	252	160	0	119	0	242	220
43	Acsad 1115	231	164	272	314	156	0	119	0	242	220
44	Acsad 1159	233	164	257	252	156	0	121	0	242	220
45	Acsad 1071	233	164	257	252	158	0	117	0	242	217
46	Douma 40860	231	164	280	252	158	0	116	0	242	220
47	Douma 40863	232	167	257	252	156	0	117	0	242	217
48	Douma 40855	233	166	268	314	156	0	116	0	238	217
49	Douma 40856	234	162	268	314	158	0	117	0	235	217
50	Douma 40992	239	166	264	314	0	144	116	0	235	214
51	Douma 40988	238	167	264	317	156	0	116	0	235	212
52	Douma 40989	238	166	250	326	156	0	116	0	235	214
53	Douma 40444	241	169	254	261	156	0	116	0	235	214
54	Douma 48114	238	173	257	264	158	0	116	0	235	214
55	Douma 40765	0	173	247	329	166	0	116	0	235	212

APPENDIX 4. Continued.

Genotype ID	Chromosome	3D	3D	3D	3D	3D	3D	3D	4D	4D	5D
	Marker	CFD9-A	CFD9-B	CFD9-C	CFD55-A	CFD55-B	CFD152-A	CFD152-B	BARC1183	WMC720	BARC143
1	Pamukova97	218	0	140	281	0	274	0	250	125	277
2	Cemre	210	0	140	281	0	277	0	250	137	279
3	Tahirova	184	0	140	273	0	280	0	260	134	277
4	Hanlı	210	0	143	278	0	297	0	253	120	282
5	Ceyhan-99	205	0	145	262	0	329	0	257	122	279
6	Pandas (Panda)	226	0	147	262	0	303	0	257	125	285
7	Karatopak	192	0	147	267	0	287	0	260	143	282
8	Osmaniyem	229	0	147	281	252	307	0	263	128	277
9	Carisma	221	0	150	270	0	303	0	263	155	279
10	Yakar-99	232	0	150	267	228	311	0	263	122	274
11	Aksel 2000	208	0	0	243	0	307	0	267	158	279
12	Bayraktar 2000	187	0	152	265	0	311	0	267	143	279
13	Demir 2000	197	0	152	281	0	321	0	263	125	285
14	Atlı-2002	229	0	154	267	0	303	0	263	125	285
15	Çetinel-2000	210	0	154	273	0	307	0	263	143	285
16	Alpu 2001	197	0	154	275	0	333	0	263	128	285
17	Tekirdağ	226	0	154	275	0	303	0	267	140	288
18	Lancer	221	0	154	273	0	311	0	267	140	285
19	Gün-91	235	0	154	275	0	300	0	263	161	291
20	Türkmen	229	0	156	273	0	303	0	263	147	294
21	Gerek 79	229	189	154	273	0	303	0	263	147	305
22	Aytın 98	197	189	154	273	0	303	0	260	143	297
23	Altay 2000	226	0	154	0	0	283	0	263	143	297
24	Karahan-99	238	0	154	275	0	303	0	253	158	300
25	Konya-2002	221	0	156	289	0	300	0	260	153	297

APPENDIX 4. Continued.

26	Aldane	221	0	156	289	0	297	0	270	147	297
27	Nurkent	218	0	156	278	0	280	0	267	122	291
28	Kaşif Bey 95	218	0	156	278	0	300	0	270	128	288
29	İzgi 2001	215	0	156	278	0	300	0	277	140	294
30	Sönmez 2001	224	0	156	289	0	297	0	270	150	294
31	Behoth 8	221	0	156	275	0	316	0	277	137	282
32	Jaolan 2	229	0	156	275	0	277	0	274	155	282
33	Douma 4	202	0	156	289	0	293	0	277	122	285
34	Sham 10	202	0	158	278	0	321	293	267	137	285
35	Douma 2	202	0	158	275	0	297	0	260	153	282
36	Sham 4	241	0	158	275	0	321	0	267	155	288
37	Behoth 4	221	0	158	278	0	297	0	263	155	291
38	Behoth 6	241	0	158	275	0	293	0	260	137	288
39	Sham 6	241	0	158	275	0	318	0	260	155	294
40	Sham 8	241	0	158	273	0	318	0	257	153	294
41	Acsad 1139	202	0	158	275	0	293	0	257	153	285
42	Acsad 1133	247	0	158	273	0	274	0	253	117	282
43	Acsad 1115	202	0	161	273	0	274	0	260	153	285
44	Acsad 1159	202	0	158	283	0	290	0	260	131	282
45	Acsad 1071	247	0	158	270	0	311	0	257	122	282
46	Douma 40860	238	0	156	273	0	311	0	260	120	285
47	Douma 40863	235	0	156	273	0	287	0	260	120	282
48	Douma 40855	221	0	156	273	0	307	0	263	131	291
49	Douma 40856	238	0	156	273	0	307	0	267	114	285
50	Douma 40992	221	0	156	278	0	277	0	267	112	285
51	Douma 40988	215	189	154	278	0	265	0	263	112	285
52	Douma 40989	200	0	154	281	0	303	0	263	117	279

APPENDIX 4. Continued.

53	Douma 40444	193	0	152	281	0	297	0	263	147	279	
54	Douma 48114	238	0	0	281	0	253	0	257	114	279	
55	Douma 40765	193	0	152	283	0	270	0	254	131	279	
Genotype ID	Chromosome	5D	5D	5D	5D	5D	5D	5D	6D	6D	6D	6D
	Marker	BARC177	BARC286	CFD29-A	CFD29-B	CFD57	GWM174	WMC765	BARC273	CFD76-A	CFD76-B	CFD76-C
1	Pamukova97	150	250	208	0	267	194	180	226	150	0	0
2	Cemre	0	241	0	0	0	0	0	224	0	0	0
3	Tahirova	150	256	164	0	263	272	190	221	0	0	0
4	Hanlı	152	259	164	0	270	250	185	224	145	0	0
5	Ceyhan-99	154	259	164	0	270	276	180	221	147	0	0
6	Pandas (Panda)	129	256	167	0	274	227	158	224	147	0	0
7	Karatopak	131	244	167	0	277	203	178	229	154	0	0
8	Osmaniye	154	247	164	0	281	206	190	232	152	0	0
9	Carisma	154	250	177	0	277	230	185	229	161	0	0
10	Yakar-99	156	259	194	0	277	254	192	226	182	0	150
11	Aksel 2000	154	262	169	0	277	0	180	232	187	0	140
12	Bayraktar 2000	158	259	164	0	281	206	187	232	192	167	0
13	Demir 2000	158	247	175	0	285	214	169	235	187	0	150
14	Atlı-2002	156	266	180	0	285	224	195	235	187	0	150
15	Çetinel-2000	156	247	175	0	289	203	171	235	187	0	158
16	Alpu 2001	158	266	183	0	285	250	195	235	158	0	0
17	Tekirdağ	156	247	183	0	285	214	195	235	187	0	154
18	Lancer	158	262	191	0	277	214	180	232	152	0	0
19	Gün-91	158	259	180	0	281	209	197	235	192	0	154
20	Türkmen	158	259	205	0	285	200	190	229	192	172	0
21	Gerek 79	156	259	203	0	289	200	192	0	192	172	0
22	Aytn 98	156	256	185	0	289	217	190	235	195	0	154

APPENDIX 4. Continued.

23	Altay 2000	154	256	0	0	270	217	197	232	192	161	0
24	Karahan-99	160	247	194	0	289	194	150	224	192	0	152
25	Konya-2002	152	244	183	0	270	217	195	235	192	0	154
26	Aldane	154	247	183	0	289	247	195	235	163	0	0
27	Nurkent	156	259	183	0	289	211	190	238	187	161	0
28	Kaşif Bey 95	156	266	172	0	285	264	197	241	192	163	0
29	İzgi 2001	156	250	175	0	292	192	171	232	195	165	0
30	Sönmez 2001	160	250	169	0	289	200	195	232	192	0	156
31	Behoth 8	156	262	172	0	281	261	171	232	192	161	0
32	Jaolan 2	154	262	169	0	281	257	171	241	189	0	156
33	Douma 4	152	247	194	0	289	209	197	232	192	0	158
34	Sham 10	154	247	205	172	289	211	190	235	192	161	0
35	Douma 2	154	259	172	0	292	200	197	238	192	161	0
36	Sham 4	154	259	214	0	274	209	197	241	192	0	156
37	Behoth 4	156	259	203	0	289	254	197	238	192	161	0
38	Behoth 6	156	262	172	0	292	230	171	244	187	161	0
39	Sham 6	152	259	214	0	277	203	197	254	195	0	158
40	Sham 8	150	256	214	0	274	206	200	244	195	0	158
41	Acsad 1139	152	256	169	0	296	194	200	241	192	163	0
42	Acsad 1133	127	256	0	0	289	186	187	238	195	0	158
43	Acsad 1115	152	253	169	0	277	200	187	241	195	0	158
44	Acsad 1159	123	253	191	0	285	247	187	235	192	163	0
45	Acsad 1071	148	253	175	0	285	178	192	238	195	158	0
46	Douma 40860	148	256	177	0	289	176	195	241	195	172	0
47	Douma 40863	123	244	200	0	292	192	187	244	195	172	0
48	Douma 40855	143	256	194	0	277	192	200	235	195	0	158
49	Douma 40856	122	259	208	0	281	189	190	241	192	161	0

APPENDIX 4. Continued.

50	Douma 40992	143	276	211	0	317	209	178	244	192	161	0
51	Douma 40988	146	266	175	0	300	206	192	241	192	0	158
52	Douma 40989	143	260	172	0	304	206	197	241	189	161	0
53	Douma 40444	141	253	177	0	304	162	190	241	189	172	157
54	Douma 48114	116	259	170	0	277	147	185	241	189	0	157
55	Douma 40765	141	259	197	0	277	209	193	241	192	0	159
Genotype ID	Chromosome	6D	6D	6D	7D	7D	7D	7D	7D	7D	7D	7D
	Marker	GDM127-A	GDM127-B	GDM127-C	BARC172-A	BARC172-B	BARC184-A	BARC184-B	BARC214	BARC235	CFD69	WMC463
1	Pamukova97	187	0	0	263	0	234	0	221	280	215	162
2	Cemre	185	0	0	271	247	234	0	213	277	224	158
3	Tahirova	185	0	0	274	0	211	191	208	274	221	164
4	Hanlı	182	0	0	274	0	231	0	208	283	224	158
5	Ceyhan-99	185	0	0	277	0	209	0	215	283	229	160
6	Pandas (Panda)	203	187	0	271	0	224	186	215	283	232	156
7	Karatopak	185	0	0	279	253	226	0	218	280	235	160
8	Osmaniyem	0	0	0	271	0	205	0	221	290	235	162
9	Carisma	202	185	0	268	0	226	0	218	287	235	158
10	Yakar-99	187	0	0	277	253	226	0	221	290	235	164
11	Aksel 2000	200	185	0	288	253	221	0	221	293	235	164
12	Bayraktar 2000	202	183	0	288	255	229	0	224	293	241	160
13	Demir 2000	185	0	0	274	253	221	0	221	290	244	164
14	Atlı-2002	187	0	0	277	0	219	0	224	297	241	166
15	Çetinel-2000	189	0	0	279	0	219	0	229	293	241	142
16	Alpu 2001	187	0	0	0	0	202	0	224	297	241	164
17	Tekirdağ	187	0	0	282	0	224	0	232	300	247	160
18	Lancer	189	0	0	282	0	200	0	224	283	241	166
19	Gün-91	187	0	0	282	255	219	0	229	307	241	156

APPENDIX 4. Continued.

20	Türkmen	205	187	0	277	255	229	0	226	303	218	158
21	Gerek 79	189	0	0	277	255	226	0	229	303	262	158
22	Aytın 98	189	0	0	277	255	216	0	226	303	221	158
23	Altay 2000	189	0	0	282	258	224	0	229	303	244	140
24	Karahan-99	189	0	0	268	0	216	0	232	307	244	156
25	Konya-2002	187	0	0	282	0	221	0	224	303	241	158
26	Aldane	187	0	0	282	0	221	0	226	307	244	158
27	Nurkent	189	0	0	282	0	221	0	229	303	238	164
28	Kaşif Bey 95	189	0	0	285	258	221	0	229	303	238	164
29	İzgi 2001	187	0	0	279	258	219	0	232	303	241	140
30	Sönmez 2001	187	0	0	282	260	200	0	226	303	241	160
31	Behoth 8	189	0	0	277	0	229	198	226	300	238	162
32	Jaolan 2	189	0	0	282	0	219	193	224	303	241	162
33	Douma 4	189	0	0	279	255	224	0	224	303	241	158
34	Sham 10	191	0	0	279	0	224	0	226	307	241	156
35	Douma 2	189	0	0	279	0	202	0	224	307	238	160
36	Sham 4	189	0	0	274	0	224	0	226	307	247	160
37	Behoth 4	189	0	0	279	0	202	0	229	307	244	164
38	Behoth 6	191	0	0	279	0	221	0	229	307	238	160
39	Sham 6	189	0	0	274	0	219	0	229	307	244	162
40	Sham 8	189	0	0	277	0	221	0	226	310	244	162
41	Acsad 1139	189	0	0	279	0	198	0	224	307	238	166
42	Acsad 1133	192	0	0	279	253	216	0	226	307	244	168
43	Acsad 1115	191	0	0	274	0	214	0	226	303	235	162
44	Acsad 1159	207	205	202	277	0	211	0	221	300	235	162
45	Acsad 1071	192	0	0	277	0	211	0	224	297	235	160
46	Douma 40860	191	0	0	277	0	211	0	218	297	235	162

APPENDIX 4. Continued.

47	Douma 40863	192	0	0	277	0	211	0	218	290	235	162
48	Douma 40855	194	0	0	266	0	209	0	224	293	241	158
49	Douma 40856	194	0	0	277	0	207	0	224	297	235	154
50	Douma 40992	196	0	0	277	0	211	0	224	297	229	152
51	Douma 40988	200	0	0	274	0	200	0	224	274	232	152
52	Douma 40989	200	0	0	268	0	186	0	218	297	226	150
53	Douma 40444	200	0	0	268	0	225	191	227	284	211	154
54	Douma 48114	200	0	0	279	0	217	0	227	297	209	154
55	Douma 40765	200	0	0	282	0	215	0	224	297	206	154

APPENDIX 5. Genetic distance within our gene pool.

1 st Genotype	2 nd Genotype	Linear Genetic Distance	1 st Genotype	2 nd Genotype	Linear Genetic Distance	1 st Genotype	2 nd Genotype	Linear Genetic Distance
5	38	207,53	2	35	178,33	29	50	171,85
5	35	203,79	29	52	177,41	23	54	171,70
5	40	198,28	5	42	177,34	12	35	171,67
21	52	197,87	4	34	177,29	27	50	171,39
24	52	197,68	21	47	177,29	40	54	171,36
22	52	197,43	24	47	177,10	11	40	171,34
21	54	195,63	13	38	177,04	26	51	171,29
4	38	195,63	27	52	176,94	26	45	171,27
24	54	195,44	8	38	176,89	19	55	170,54
26	52	195,43	35	54	176,87	3	40	170,16
22	54	195,20	11	35	176,85	15	54	169,69
26	54	193,19	22	47	176,85	9	35	169,28
21	50	192,31	10	38	175,72	6	35	168,77
24	50	192,12	3	35	175,67	20	50	168,67
4	35	191,90	19	50	175,55	25	47	168,67
22	50	191,88	12	38	175,41	23	50	168,38
5	37	190,33	40	53	175,39	13	40	167,79
26	50	189,87	29	54	175,17	8	40	167,65
25	52	189,26	26	47	174,84	37	53	167,44
5	34	189,18	27	54	174,70	29	55	166,84
21	55	187,30	7	40	174,49	1	38	166,78
24	55	187,11	21	53	174,33	7	37	166,54
25	54	187,02	20	52	174,23	10	40	166,48
22	55	186,87	24	53	174,14	27	55	166,37
4	40	186,39	23	52	173,94	15	50	166,37
26	55	184,86	22	53	173,89	34	53	166,30
38	53	184,64	25	49	173,76	12	40	166,16
5	39	183,84	5	36	173,73	5	43	165,95
7	38	183,74	21	51	173,73	25	53	165,71
25	50	183,70	21	45	173,71	19	49	165,61
38	55	182,80	40	55	173,56	37	55	165,61
21	49	182,38	24	51	173,54	4	42	165,44
24	49	182,19	24	45	173,52	7	34	165,39
2	38	182,06	13	35	173,30	25	51	165,11
22	49	181,94	22	51	173,29	25	45	165,10
19	52	181,11	22	45	173,28	2	37	164,87
35	53	180,90	8	35	173,16	34	55	164,46
38	54	180,60	9	38	173,01	21	46	163,78

APPENDIX 5. Continued.

11	38	180,59	5	41	172,99	9	40	163,77
7	35	180,00	2	40	172,82	15	38	163,74
26	49	179,93	6	38	172,50	2	34	163,72
3	38	179,40	20	54	171,99	20	55	163,66
35	55	179,07	10	35	171,99	24	46	163,59
19	54	178,87	4	39	171,95	37	54	163,41
25	55	178,69	15	52	171,92	11	37	163,39
4	37	178,44	26	53	171,89	23	55	163,37
22	46	163,34	19	51	156,97	36	53	150,85
6	40	163,26	19	45	156,95	16	40	150,73
1	35	163,05	39	54	156,92	17	50	150,73
34	54	162,26	11	39	156,90	20	53	150,69
11	34	162,24	29	47	156,82	42	54	150,41
3	37	162,21	38	51	156,79	11	42	150,39
29	49	161,91	15	49	156,43	23	53	150,39
4	36	161,84	27	47	156,36	21	39	150,27
21	40	161,49	17	52	156,28	41	53	150,10
27	49	161,45	16	35	156,24	20	51	150,09
15	55	161,36	9	37	155,82	24	39	150,07
26	46	161,33	3	39	155,72	20	45	150,07
24	40	161,30	16	54	155,45	30	55	149,97
20	38	161,27	6	37	155,31	7	36	149,94
4	41	161,10	35	50	155,29	22	39	149,83
3	34	161,06	25	46	155,16	21	38	149,80
22	40	161,05	30	50	154,98	23	51	149,80
39	53	160,95	14	50	154,74	23	45	149,78
30	52	160,54	9	34	154,67	40	50	149,78
19	47	160,52	15	40	154,49	14	55	149,73
14	52	160,30	42	53	154,45	1	37	149,59
21	44	160,29	6	34	154,16	9	39	149,33
24	44	160,10	4	43	154,06	3	42	149,21
7	39	160,05	17	54	154,05	7	41	149,20
15	35	160,00	29	53	153,87	26	43	149,15
16	38	159,97	28	50	153,78	36	55	149,01
22	44	159,85	20	47	153,64	6	39	148,82
13	37	159,84	7	42	153,55	28	55	148,77
8	37	159,70	27	53	153,40	21	42	148,62
28	52	159,34	13	39	153,35	1	34	148,44
39	55	159,12	23	47	153,35	24	42	148,43
26	40	159,05	29	51	153,27	15	53	148,38
38	50	159,02	29	45	153,25	2	36	148,27
20	49	158,73	8	39	153,21	41	55	148,26
13	34	158,70	35	51	153,06	5	32	148,19

APPENDIX 5. Continued.

8	34	158,55	25	40	152,88	22	42	148,18
10	37	158,53	27	51	152,80	26	39	147,82
23	49	158,44	27	45	152,78	15	51	147,78
2	39	158,38	42	55	152,61	15	45	147,77
30	54	158,30	5	28	152,47	40	51	147,55
12	37	158,21	16	50	152,13	2	41	147,52
14	54	158,06	10	39	152,04	21	41	147,49
26	44	157,84	20	40	152,02	24	41	147,30
16	52	157,69	2	42	151,87	16	55	147,12
19	53	157,57	12	39	151,72	22	41	147,05
1	40	157,54	25	44	151,67	19	46	147,01
20	35	157,53	21	43	151,59	13	42	146,85
10	34	157,38	24	43	151,40	36	54	146,81
28	54	157,10	15	47	151,34	11	36	146,79
12	34	157,06	22	43	151,15	8	42	146,70
15	37	146,54	19	38	140,69	24	48	135,92
5	33	146,43	34	50	140,68	28	53	135,80
17	38	146,21	18	38	140,65	17	47	135,70
26	42	146,17	4	28	140,58	22	48	135,67
41	54	146,07	27	40	140,56	13	43	135,47
21	35	146,06	2	43	140,49	39	50	135,34
11	41	146,05	20	46	140,14	8	43	135,32
17	55	145,72	15	39	140,05	28	51	135,20
3	36	145,61	25	42	140,00	28	45	135,18
10	42	145,53	30	47	139,95	19	43	134,83
15	34	145,39	23	46	139,84	13	52	134,72
12	42	145,21	29	44	139,82	4	33	134,54
26	41	145,04	14	47	139,71	15	44	134,34
30	49	145,04	37	51	139,60	10	43	134,15
3	41	144,87	27	44	139,36	16	53	134,15
14	49	144,80	9	36	139,22	12	43	133,83
19	40	144,73	43	54	139,03	26	48	133,66
5	30	144,59	11	43	139,01	16	51	133,55
5	31	144,20	18	52	138,98	15	42	133,55
20	37	144,07	25	41	138,87	16	45	133,53
28	49	143,85	28	47	138,76	19	39	133,50
19	44	143,52	6	36	138,71	18	50	133,42
29	46	143,32	9	41	138,47	39	51	133,11
13	36	143,25	34	51	138,45	1	36	132,99
8	36	143,10	14	35	138,03	17	53	132,74
1	39	143,10	35	52	138,02	4	30	132,70
43	53	143,07	6	41	137,96	21	37	132,60
25	43	142,98	15	46	137,83	14	40	132,52

APPENDIX 5. Continued.

20	34	142,93	3	43	137,83	40	52	132,51
27	46	142,85	20	39	137,58	13	54	132,48
9	42	142,82	23	40	137,56	4	31	132,31
16	37	142,78	16	47	137,10	35	49	132,26
13	41	142,50	30	53	136,99	1	41	132,25
17	35	142,47	17	40	136,96	17	51	132,14
8	41	142,35	19	35	136,96	17	45	132,13
6	42	142,31	18	35	136,92	5	27	131,98
16	49	142,19	14	53	136,76	19	42	131,85
7	43	142,17	18	54	136,74	21	34	131,46
10	36	141,93	20	44	136,64	9	43	131,44
37	50	141,83	1	42	136,59	18	40	131,41
14	38	141,77	5	44	136,52	29	43	131,13
38	52	141,76	30	51	136,40	20	42	131,08
25	39	141,65	30	45	136,38	6	43	130,93
16	34	141,63	23	44	136,35	19	41	130,72
12	36	141,61	4	32	136,29	27	43	130,66
43	55	141,23	16	39	136,29	21	36	130,62
10	41	141,19	14	51	136,16	24	36	130,43
29	40	141,03	14	45	136,14	22	36	130,19
12	41	140,87	21	48	136,11	15	36	129,94
17	49	140,79	38	49	135,99	29	39	129,80
16	42	129,78	18	49	123,48	24	35	118,11
27	39	129,34	32	55	123,46	14	39	118,08
15	41	129,20	18	37	123,46	39	52	118,07
13	50	129,16	14	34	123,42	9	28	117,96
17	37	129,01	34	52	123,41	13	32	117,70
42	50	128,83	36	51	123,00	11	30	117,65
7	28	128,68	28	40	122,96	34	49	117,65
18	55	128,41	30	44	122,95	8	32	117,55
26	36	128,18	2	32	122,72	6	28	117,45
29	42	128,16	14	44	122,72	43	50	117,45
20	43	127,95	7	33	122,64	4	52	117,33
17	34	127,87	17	39	122,52	11	31	117,26
27	42	127,69	19	34	122,35	18	39	116,97
23	43	127,66	18	34	122,31	33	52	116,96
25	48	127,49	41	51	122,25	3	30	116,47
20	36	127,48	17	46	122,19	10	32	116,38
22	38	127,45	25	36	122,01	1	50	116,20
29	41	127,03	13	28	121,99	9	21	116,19
2	28	127,01	8	28	121,84	3	31	116,08
40	49	126,75	28	44	121,76	12	32	116,07
20	41	126,73	1	52	121,76	8	21	116,04

APPENDIX 5. Continued.

42	51	126,60	33	55	121,71	17	42	116,02
27	41	126,56	31	53	121,31	9	24	116,00
30	46	126,44	32	54	121,26	13	33	115,95
23	39	126,33	11	32	121,25	26	35	115,86
14	46	126,21	2	33	120,97	8	24	115,85
16	36	126,18	7	30	120,80	8	33	115,80
12	52	125,93	5	46	120,76	9	22	115,75
15	43	125,64	10	28	120,67	29	48	115,65
11	28	125,53	7	31	120,41	8	22	115,60
16	41	125,44	12	50	120,37	18	53	115,44
32	53	125,30	31	52	120,35	12	55	115,36
28	46	125,25	12	28	120,35	43	51	115,22
36	50	125,23	16	44	120,10	27	48	115,18
1	43	125,21	4	27	120,09	4	54	115,10
23	42	124,68	3	32	120,06	18	51	114,84
4	44	124,63	1	54	119,52	18	45	114,82
14	37	124,57	33	54	119,51	31	50	114,80
37	52	124,56	11	33	119,49	10	33	114,63
41	50	124,48	31	55	119,48	5	45	114,60
7	32	124,40	19	48	119,34	12	33	114,31
3	28	124,35	13	49	119,22	30	43	114,26
30	40	124,16	2	30	119,13	13	47	114,13
13	55	124,15	37	49	118,80	13	30	114,11
22	35	123,71	2	31	118,74	14	43	114,02
12	54	123,69	17	44	118,70	8	30	113,96
16	46	123,60	16	43	118,40	19	36	113,86
23	41	123,55	18	47	118,39	9	26	113,74
33	53	123,54	3	33	118,31	13	31	113,71
19	37	123,50	31	54	118,12	5	23	113,71
9	32	113,67	30	41	110,15	35	47	104,48
5	26	113,64	28	42	110,09	12	44	104,40
44	53	113,63	9	30	110,08	15	32	104,40
8	26	113,60	17	43	110,00	24	37	104,28
8	31	113,56	14	41	109,92	3	27	103,86
3	21	113,21	27	36	109,70	1	30	103,85
6	32	113,16	25	35	109,69	5	25	103,82
28	43	113,06	9	31	109,69	29	35	103,80
3	24	113,02	44	54	109,60	1	31	103,46
30	39	112,93	11	44	109,58	7	25	102,81
10	30	112,79	6	30	109,57	4	45	102,70
3	22	112,77	6	31	109,18	15	33	102,64
7	44	112,73	22	34	109,11	12	53	102,39
12	30	112,47	7	26	108,99	2	52	102,35

APPENDIX 5. Continued.

20	48	112,47	28	41	108,96	36	49	102,20
5	21	112,44	4	46	108,86	32	52	102,07
17	36	112,42	26	38	108,80	26	37	102,03
10	31	112,40	15	28	108,68	9	44	102,01
39	49	112,31	3	44	108,40	20	32	101,93
5	24	112,25	38	47	108,21	4	49	101,84
23	48	112,17	7	27	108,20	38	48	101,82
12	31	112,08	11	54	108,15	4	23	101,81
5	22	112,00	14	36	107,97	12	51	101,79
9	33	111,92	36	52	107,96	12	45	101,77
44	55	111,80	25	38	107,93	4	26	101,75
4	50	111,78	9	25	107,57	13	27	101,50
28	39	111,73	29	38	107,54	6	44	101,50
1	28	111,73	1	32	107,44	33	49	101,46
17	41	111,67	8	25	107,42	41	49	101,45
14	42	111,58	41	52	107,22	18	44	101,40
42	52	111,57	18	36	106,86	8	27	101,35
7	21	111,43	4	55	106,77	1	47	101,17
6	33	111,41	23	36	106,69	15	30	100,80
33	50	111,40	2	27	106,52	10	21	100,67
30	42	111,28	1	49	106,26	16	32	100,63
7	24	111,24	20	28	106,22	13	46	100,63
1	55	111,19	18	41	106,11	10	24	100,48
13	53	111,18	13	44	106,03	15	31	100,41
2	44	111,06	8	44	105,89	10	22	100,23
24	38	111,05	42	49	105,80	43	52	100,18
7	22	110,99	5	48	105,71	10	27	100,18
3	26	110,76	1	33	105,69	20	33	100,17
13	51	110,58	12	47	105,34	2	54	100,11
13	45	110,56	11	27	105,04	5	29	99,99
18	42	110,46	16	28	104,92	12	27	99,86
12	49	110,43	18	46	104,89	11	55	99,82
11	52	110,39	31	49	104,86	31	47	99,77
22	37	110,25	11	50	104,83	32	50	99,68
15	48	110,16	10	44	104,72	6	52	99,64
29	36	110,16	3	25	104,59	9	19	99,42
5	47	99,31	10	54	94,97	4	24	89,27
8	19	99,28	11	49	94,90	29	34	89,20
18	43	99,08	21	28	94,75	35	45	89,19
40	47	98,97	7	19	94,67	2	45	89,13
16	33	98,88	17	48	94,52	6	55	89,07
30	48	98,77	43	49	94,42	10	46	88,95
14	48	98,54	23	35	94,37	24	34	88,81

APPENDIX 5. Continued.

20	30	98,34	6	50	94,08	5	20	88,79
6	21	98,24	46	54	93,83	21	33	88,70
10	26	98,22	23	38	93,82	2	23	88,24
1	53	98,21	4	48	93,82	15	27	88,19
35	48	98,08	11	46	93,81	4	29	88,09
6	24	98,04	4	53	93,79	44	50	88,02
20	31	97,94	26	32	93,36	7	20	87,79
46	53	97,87	30	36	93,29	4	25	87,70
6	22	97,80	4	51	93,19	45	54	87,68
1	51	97,61	2	26	93,08	1	46	87,66
1	45	97,60	38	45	92,93	11	45	87,66
28	48	97,58	33	45	92,80	11	21	87,48
9	27	97,47	3	29	92,74	11	24	87,29
32	51	97,45	3	46	92,63	3	15	87,26
6	54	97,40	40	48	92,57	25	32	87,19
27	35	97,38	9	20	92,55	11	22	87,04
10	52	97,20	8	20	92,40	2	25	86,91
16	30	97,04	9	23	92,25	17	32	86,87
7	46	96,97	8	23	92,11	21	30	86,87
6	27	96,96	28	36	92,09	11	53	86,85
2	50	96,79	10	25	92,05	2	49	86,85
4	47	96,75	12	46	91,84	38	46	86,77
16	31	96,65	2	55	91,78	11	23	86,77
3	19	96,44	45	53	91,71	14	28	86,71
33	47	96,37	10	50	91,65	11	26	86,70
31	51	96,21	1	27	91,24	10	55	86,64
31	45	96,20	17	28	91,16	32	49	86,57
46	55	96,03	37	47	91,02	26	34	86,55
16	48	95,93	7	29	90,97	5	15	86,49
25	37	95,86	7	45	90,81	3	45	86,47
21	32	95,80	29	37	90,34	21	31	86,47
6	26	95,79	27	38	90,31	7	52	86,44
1	44	95,78	9	15	90,24	31	46	86,26
9	29	95,73	8	46	90,12	11	51	86,25
33	51	95,70	8	15	90,09	9	46	86,24
5	19	95,67	7	23	89,92	44	51	85,79
24	32	95,61	45	55	89,87	6	46	85,73
8	29	95,58	34	47	89,87	20	27	85,73
2	21	95,53	11	47	89,81	19	28	85,64
22	32	95,37	6	25	89,62	18	28	85,60
2	24	95,34	25	34	89,59	7	15	85,48
2	46	95,29	3	20	89,56	5	52	85,43
2	22	95,09	3	23	89,27	17	33	85,11

APPENDIX 5. Continued.

34	45	84,72	5	50	79,88	6	51	75,50
3	52	84,67	28	35	79,78	23	34	75,48
37	48	84,62	19	33	79,60	29	32	75,34
39	47	84,53	8	54	79,59	13	48	75,22
16	27	84,43	6	45	79,57	8	48	75,07
7	54	84,20	18	33	79,56	26	31	75,07
6	49	84,14	9	54	79,44	2	29	75,06
31	40	83,97	33	44	79,37	27	32	74,87
8	45	83,96	3	50	79,11	5	55	74,87
10	19	83,90	6	47	79,05	5	14	74,87
40	45	83,68	11	25	78,86	34	46	74,78
27	37	83,54	14	30	78,83	10	15	74,72
34	48	83,48	2	53	78,80	9	17	74,60
17	30	83,28	48	54	78,79	6	20	74,59
13	23	83,22	11	48	78,77	47	55	74,59
5	54	83,20	2	19	78,76	8	17	74,45
13	26	83,15	6	23	78,68	36	47	74,42
35	46	83,04	9	14	78,62	21	27	74,26
17	31	82,88	8	14	78,47	3	55	74,10
33	46	82,87	26	33	78,47	31	43	74,07
48	53	82,82	14	31	78,44	30	38	73,91
10	45	82,80	2	51	78,21	10	48	73,91
31	44	82,77	39	48	78,13	7	14	73,86
3	54	82,43	42	47	78,02	41	47	73,68
14	32	82,43	32	45	77,91	1	26	73,67
7	48	81,92	6	29	77,77	10	53	73,66
10	23	81,90	19	30	77,76	12	48	73,59
8	52	81,83	18	30	77,72	10	51	73,06
2	47	81,76	3	48	77,59	11	29	73,05
10	49	81,71	40	46	77,53	3	16	73,02
9	52	81,68	19	31	77,37	1	23	72,96
12	23	81,59	24	31	77,33	31	39	72,75
12	26	81,52	18	31	77,32	28	38	72,71
32	47	81,48	18	48	77,22	34	40	72,50
6	19	81,47	22	31	77,08	22	28	72,40
19	32	81,35	10	20	77,02	47	54	72,39
18	32	81,31	10	47	76,62	25	33	72,30
48	55	80,98	47	53	76,42	6	15	72,29
30	35	80,97	8	50	76,27	5	16	72,25
7	50	80,88	9	50	76,12	46	50	72,25
24	33	80,72	1	21	76,12	12	21	71,94
14	33	80,67	6	53	76,10	2	20	71,88
33	40	80,58	9	16	76,01	23	32	71,87

APPENDIX 5. Continued.

4	21	80,54	1	24	75,93	12	24	71,75
23	37	80,54	7	55	75,87	42	48	71,63
22	33	80,48	8	16	75,86	3	17	71,62
2	48	80,24	37	45	75,73	5	49	71,53
10	29	80,20	1	22	75,68	12	22	71,51
4	22	80,10	3	14	75,63	34	44	71,29
9	45	80,08	7	47	75,52	8	55	71,26
7	16	71,25	32	40	65,69	6	16	58,05
9	48	71,19	19	27	65,15	2	14	57,95
3	47	71,19	18	27	65,11	8	51	57,69
9	55	71,11	44	49	64,99	9	51	57,54
31	42	71,10	1	48	64,97	15	24	57,37
38	44	71,01	9	47	64,80	33	35	57,36
7	49	70,94	22	30	64,52	9	18	57,30
5	17	70,85	32	44	64,49	28	32	57,27
44	52	70,75	45	51	63,86	48	50	57,20
11	19	70,71	11	20	63,84	8	18	57,15
6	48	70,68	4	19	63,77	29	31	57,06
33	43	70,68	31	38	63,33	37	40	57,02
13	24	70,67	13	21	63,15	23	33	56,98
17	27	70,67	10	14	63,09	4	20	56,89
46	51	70,02	39	46	63,09	6	17	56,65
31	41	69,97	7	53	62,90	27	31	56,59
15	23	69,92	42	45	62,74	42	46	56,58
15	26	69,85	13	22	62,72	18	26	56,45
7	17	69,84	34	43	62,60	15	29	56,20
2	15	69,58	7	51	62,30	21	23	55,98
37	46	69,58	5	53	61,89	21	26	55,91
13	29	69,50	48	52	61,76	37	44	55,81
33	39	69,35	40	44	61,76	15	25	55,80
39	45	69,24	11	15	61,53	32	43	55,79
3	49	69,17	5	51	61,29	32	35	55,60
13	25	69,11	34	39	61,27	2	16	55,34
25	31	68,90	3	53	61,12	33	48	55,20
8	47	68,68	33	38	61,09	12	19	55,18
27	34	68,07	6	14	60,66	46	52	54,99
36	48	68,03	3	51	60,52	4	18	54,98
32	46	67,98	10	16	60,48	48	51	54,97
12	29	67,87	29	33	60,45	20	24	54,90
33	42	67,71	43	48	60,25	4	15	54,59
1	25	67,50	27	33	59,98	32	39	54,46
12	25	67,47	34	42	59,62	3	18	54,31
20	23	67,45	31	35	59,59	2	17	53,94

APPENDIX 5. Continued.

20	26	67,38	1	19	59,35	4	14	53,87
41	48	67,28	32	38	59,34	20	29	53,73
35	44	67,27	1	29	59,24	16	24	53,61
30	37	67,14	36	45	59,14	23	31	53,58
5	18	66,87	10	17	59,08	20	25	53,33
43	47	66,64	18	21	58,89	31	36	53,11
33	41	66,57	18	24	58,70	9	13	53,04
8	49	66,34	31	48	58,59	36	46	52,98
14	27	66,22	34	41	58,49	8	13	52,89
9	49	66,19	30	32	58,47	25	28	52,88
16	23	66,15	18	22	58,46	32	42	52,82
45	50	66,09	41	45	58,39	7	18	52,54
16	26	66,09	8	53	58,29	28	29	52,49
28	37	65,94	9	53	58,14	1	20	52,47
16	29	52,43	30	33	43,58	10	13	37,51
17	23	52,39	24	30	43,43	14	24	37,38
17	26	52,32	21	24	43,43	38	42	37,38
41	46	52,23	35	40	43,19	36	44	37,21
16	25	52,04	45	49	43,06	44	47	37,21
22	27	51,91	37	41	43,01	13	15	37,21
32	41	51,69	52	53	42,88	14	22	37,14
30	34	51,66	33	34	42,75	1	3	37,09
43	45	51,36	32	48	42,48	41	44	36,47
24	28	51,31	28	33	42,38	13	18	36,39
47	50	50,81	21	29	42,26	15	22	36,29
28	34	50,47	32	37	42,14	38	41	36,24
18	25	50,28	18	19	42,13	1	16	35,93
38	40	50,25	21	25	41,87	4	9	35,65
1	15	50,17	10	18	41,78	4	8	35,50
3	13	50,05	34	36	41,63	1	7	35,31
11	14	49,91	17	21	41,59	13	14	35,27
33	36	49,71	38	43	41,57	18	20	35,25
4	17	49,43	2	18	41,41	6	13	35,08
5	13	49,28	17	24	41,40	5	6	35,02
46	49	49,22	3	12	41,26	26	30	34,89
45	52	48,83	47	48	41,18	32	36	34,82
49	53	48,65	17	22	41,15	12	18	34,75
47	51	48,58	52	55	41,05	1	17	34,53
12	20	48,30	32	34	41,00	5	9	34,51
7	13	48,28	42	44	40,81	12	14	34,37
14	23	47,95	1	5	40,74	19	24	34,33
14	26	47,88	5	12	40,49	14	29	34,23
39	44	47,32	4	16	40,35	14	25	33,83

APPENDIX 5. Continued.

11	16	47,30	16	21	40,18	20	22	33,82
37	43	47,12	30	31	40,18	36	38	33,79
19	23	46,87	1	9	40,07	22	23	33,63
18	23	46,83	11	18	39,93	22	26	33,56
49	55	46,81	1	8	39,92	47	52	33,54
19	26	46,81	16	22	39,75	19	29	33,15
13	19	46,39	13	20	39,51	15	18	32,95
48	49	46,27	7	12	39,49	19	25	32,76
31	37	46,13	6	18	39,34	3	4	32,67
12	15	46,00	38	39	39,02	25	27	32,39
11	17	45,89	28	31	38,99	2	13	32,37
37	39	45,79	52	54	38,85	40	43	32,33
43	46	45,20	26	28	38,84	27	29	32,00
25	30	45,00	23	28	38,77	35	39	31,96
31	34	44,98	17	29	38,67	4	5	31,90
49	54	44,61	1	14	38,54	5	10	31,80
29	30	44,61	18	29	38,43	12	16	31,76
9	12	44,25	17	25	38,28	4	7	30,89
37	42	44,15	35	43	37,84	23	30	30,89
8	12	44,10	45	48	37,60	36	40	30,87
33	37	43,90	14	21	37,57	13	17	30,83
24	27	30,83	46	47	21,44	23	25	15,32
44	48	30,81	14	18	21,32	45	47	15,28
35	41	30,80	53	54	21,30	4	11	15,05
5	8	30,63	22	24	21,08	2	4	14,99
12	17	30,36	36	43	20,97	3	6	14,97
35	42	30,31	40	42	20,95	32	33	14,89
35	36	30,06	14	19	20,81	39	40	14,44
43	44	29,43	49	51	20,80	15	16	14,24
34	35	29,31	2	9	20,66	25	26	14,05
1	4	28,85	19	20	20,57	35	37	13,83
10	12	28,72	2	8	20,51	1	11	13,80
9	11	28,71	27	28	20,49	16	17	13,77
8	11	28,56	24	29	20,27	23	29	13,72
3	5	28,12	4	12	20,23	6	7	13,20
51	53	27,85	4	10	20,13	10	11	13,19
47	49	27,78	22	29	20,03	53	55	12,97
46	48	27,67	36	39	19,64	1	13	12,96
5	11	26,94	22	25	19,52	3	10	12,54
6	12	26,29	14	20	19,50	24	26	12,48
36	37	26,15	1	2	19,41	25	29	11,85
1	18	26,13	16	18	19,32	42	43	11,38
51	55	26,01	4	13	18,59	39	41	10,85

APPENDIX 5. Continued.

15	21	25,95	50	51	18,58	7	10	10,76
3	11	25,73	26	27	18,48	6	11	10,75
50	53	25,62	31	32	18,28	7	9	10,72
2	5	25,46	23	27	18,28	45	46	9,93
40	41	25,29	14	16	18,21	2	6	9,56
17	19	24,82	26	29	18,02	12	13	8,79
1	10	24,55	36	42	18,00	1	12	8,62
11	13	24,33	6	9	17,96	24	25	8,43
51	52	24,14	17	20	17,94	54	55	8,33
7	11	23,95	39	43	17,89	2	11	8,05
51	54	23,81	6	8	17,81	28	30	7,88
5	7	23,79	2	3	17,68	35	38	7,06
50	55	23,78	15	17	17,53	41	43	7,03
23	24	23,75	17	18	17,30	7	8	6,84
20	21	23,64	50	52	17,27	39	42	6,51
2	12	23,58	37	38	17,20	3	9	6,39
16	19	23,42	36	41	16,86	2	10	6,34
4	6	23,13	19	21	16,77	14	17	4,44
15	19	23,04	16	20	16,54	41	42	4,35
49	50	23,03	27	30	16,41	3	7	4,33
13	16	22,97	19	22	16,33	8	9	3,88
21	22	22,35	2	7	15,91	31	33	3,40
34	38	22,25	44	46	15,77	6	10	3,22
1	6	22,12	11	12	15,54	3	8	2,83
14	15	21,97	9	10	15,52	15	20	2,47
44	45	21,92	49	52	15,50			
50	54	21,58	34	37	15,48			
23	26	21,49	8	10	15,37			

APPENDIX 6. Total RNA concentrations measured by Qubit® 2.0 Fluorometer (Invitrogen-Life technologies; Cat. No: Q32866).

Sample No	Genotype	Biological Replicates	Total RNA Concentration (ng/μl)	Sample No	Genotype	Biological Replicates	Total RNA Concentration (ng/μl)	Sample No	Genotype	Biological Replicates	Total RNA Concentration (ng/μl)
1	Gerek79	0h Mock 1	1068	22	Türkmen	12h - 1	3720	43	Avocet 'Yr10'	48h - 1	1740
2	Gerek79	0h Mock 2	3900	23	Türkmen	12h - 2	4760	44	Avocet 'Yr10'	48h - 2	3120
3	Gerek79	0h Mock 3	3660	24	Türkmen	12h - 3	3360	45	Avocet 'Yr10'	48h - 3	1920
4	Türkmen	0h Mock 1	2000	25	Avocet 'Yr10'	12h - 1	2440	46	Gerek79	72h - 1	1278
5	Türkmen	0h Mock 2	4020	26	Avocet 'Yr10'	12h - 2	1852	47	Gerek79	72h - 2	5160
6	Türkmen	0h Mock 3	2360	27	Avocet 'Yr10'	12h - 3	4120	48	Gerek79	72h - 3	1488
7	Avocet 'Yr10'	0h Mock 1	6120	28	Gerek79	24h - 1	1940	49	Türkmen	72h - 1	3960
8	Avocet 'Yr10'	0h Mock 2	2060	29	Gerek79	24h - 2	2340	50	Türkmen	72h - 2	1328
9	Avocet 'Yr10'	0h Mock 3	6700	30	Gerek79	24h - 3	2940	51	Türkmen	72h - 3	3660
10	Gerek79	15 min - 1	4520	31	Türkmen	24h - 1	2520	52	Avocet 'Yr10'	72h - 1	1392
11	Gerek79	15 min - 2	4020	32	Türkmen	24h - 2	2740	53	Avocet 'Yr10'	72h - 2	1586
12	Gerek79	15 min - 3	3180	33	Türkmen	24h - 3	3680	54	Avocet 'Yr10'	72h - 3	2560
13	Türkmen	15 min - 1	970	34	Avocet 'Yr10'	24h - 1	5180	55	Gerek79	96h - 1	1012
14	Türkmen	15 min - 2	3200	35	Avocet 'Yr10'	24h - 2	2800	56	Gerek79	96h - 2	2800
15	Türkmen	15 min - 3	2480	36	Avocet 'Yr10'	24h - 3	5080	57	Gerek79	96h - 3	882
16	Avocet 'Yr10'	15 min - 1	5580	37	Gerek79	48h - 1	1984	58	Türkmen	96h - 1	4860
17	Avocet 'Yr10'	15 min - 2	5800	38	Gerek79	48h - 2	7320	59	Türkmen	96h - 2	2240
18	Avocet 'Yr10'	15 min - 3	4100	39	Gerek79	48h - 3	3380	60	Türkmen	96h - 3	1170
19	Gerek79	12h - 1	4220	40	Türkmen	48h - 1	6220	61	Avocet 'Yr10'	96h - 1	1670
20	Gerek79	12h - 2	7140	41	Türkmen	48h - 2	1642	62	Avocet 'Yr10'	96h - 2	2400
21	Gerek79	12h - 3	3740	42	Türkmen	48h - 3	3260	63	Avocet 'Yr10'	96h - 3	2780

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Education Statue

Degree	Section/Program	University/ Highschool	Graduation Year
Highschool	-	Yenilevent Highschool	2005
University	Molecular Biology and Genetics	Haliç University	2009

Scientific Works

1. Tascioglu, T., Karakas Metin, O., Sakiroglu, M., Aydin, Y., Akan, K., Altinkut Uncuoglu, A. (Abstract Book, p331). Assessing Population Structure and Genetic Diversity in Wheat Gene Pool for Yellow Rust Resistance. Poster presentation at 7th EPSO Conference, Porto Heli, Greece.
2. Tascioglu, T., Altinkut Uncuoglu, A., Karakas Metin, O. (New Biotechnology, Volume 29, Supplement 23–26 September 2012, Pages S140-S141). Structural and functional assessments of wheat (*Triticum aestivum* L.) gene pool for yellow rust resistance. Poster presentation at 15th European Congress on Biotechnology, Istanbul, Turkey.

Project Assignment

1. Scholarship, Funded by TUBITAK MAM-GMBE, 2011, TOVAG Project No: 1100539.

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