GENETIC SCREENING OF TURKISH WHEAT VARIETIES FOR THE DURABLERESISTANCE GENE, LR34.

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ABSTRACT

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Wheat diseases such as rusts and powdery mildews are among the most important and ancient diseases that affect wheat cultivation worldwide. The pathogen race specific resistance genes cannot maintain long lasting resistance. On the other hand, the presence of genes confers the non-race specific resistance last much longer. The durable resistance phenotypes in wheat against various rust and powdery mildew diseases were reported as Lr34, Yr18, and Pm38 separately; nevertheless, they were known to locate very close to each other based on linkage analysis. Recently, it was shown that all of these resistance phenotypes are indeed conferred by the same gene, encoding Adenosine triphosphate–binding cassette transporter (ABC-transporter) type protein. The way with which this transporter is functioning to maintain the durable resistance against different types of pathogens is still unknown.

The presence of LR34 (or ABC-transporter) gene resistant allele can be detected by specially designed markers. In spite of the few DNA sequence differences between the resistant and the susceptible alleles, the easily applicable PCR based markers allow the detection of the presence of this durable resistant allele on any given variety or cultivar. In our study, 62 different Turkish bread wheat varieties were screened by the gene specific molecular markers, developed from those LR34 gene mutation sites. The 11 cultivars determined to contain the resistant allele. This study is the first screening of Turkish cultivars for the presence of LR34 gene.

Keywords: Adult Plant Durable Resistance, Lr34, Leaf Rust, and Stem Rust

ÖZ

TÜRKİYE EKMEKLİK BUĞDAYLARINDA KALICI DİRENÇ GELİŞTİREN LR34 GENİNİN TARANMASI

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Buğdayda bulunan pas hastalıkları (kızıl pas, kahverengi pas, sarı pas), dünyadaki buğday üretimini etkileyen en önemli ve eski hastalıklardır. Patojenin zaman içinde evrimi sonucunda bu özel direnç genlerinin dirençleri etkisiz olmaktadır . Bunun yanında bitkide patojene karşı uzuzn surely direnç sağlayan genler vardır.Daha bitkide çeşitli patojenlere karşı kalıcı direnç sağlıyan çeşitli genlerin (Lr34, Yr18 ve Pm38), genetik bağlantı analizlerine dayanarak birbirlerine çok yakın oldukları biliniyordu. Yeni çalışmaların ışığında , farklı patojenlere karşı direnç oluşturan bu fenotiplerin, aynı gen tarafından sağlandığı belirlenmiştir. Bu önemli direncin ABC-transporter isimli tek bir gene bağlı olduğu bulunmuştur. Genin klonlandığının gösterildiği ve sonrasında sunulan yayınlara dayanılarak herhangi bir buğdaygil bitkide, basit bir kaç PCR reaksiyonu sonucunda dirençli LR34 geninin bulunup bulunmadığı saptanabilir. Dirençli Lr34 geni ile dirençsiz L34 geni birbirlerine büyük ölçüde benzemektedir. Kolaylıkla kullanılabilen Polimeraz Zincir Reaksiyonu temelli markör sistemleri aracılığıyla dirençi LR34 fenotipi , herhangi bir

varyete de tespit edilebilir. .Bu çalışmamızda 62 adet türkiyede yetiştirilen ve kaynaklanan ekmeklik buğday türü, LR34 geninde bulunan mutasyon bölgelerine göre dizayn edilmiş moleküler markörler tarafından taranmıştır. Bu çalışmamızda 11 adet ekmeklik buğday cinsinin dirençli allele sahip olduğu belirlenmiştir. Bu çalışmamız Türkiyede bulunan buğday türleri içinde kendi alanındaki ilk çalışmadır.

Anahtar Kelimeler: Bitkide Kalıcı Direnç, Lr34, Sarı Pas, Kızıl Pas

To my Mother, Father, Sister, Biber

and

My Twin Brother

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INTRODUCTION

1.1) Wheat

Cereals, including maize (*Zea mays* L.), wheat (*Triticum* spp.) and rice (*Oryza sativa* L.) are among the major contributors of the formation of the contemporary human civilization. These cereals are first domesticated 10000 years ago and this domestication process is the one of the most demonstrative example of the human intervention on evolutionary processes. Domestication of plants, especially cereals, deeply changed the human cultural and social network and mostly responsible for the formation of the contemporary world.

After the end of the so-called little ice age, temperature increase probably induced the worldwide expansion of economically crucial grain species. This expansion allowed the transition from hunter-gatherer societies to first plant cultivating societies. The transition into plant cultivating societies induced the spreading of similar agricultural production patterns worldwide, especially temperate regions of the world. This spreading also decreased the genetic diversity of the cereals.

Domestication of grains creates greater amount of food for societies and results in population increase and population increase triggered the demand for more agricultural products. The increasing demand for agricultural products compensated by artificial selection of better yielding cultivars (Darwin 1859).

The first domesticated crop varieties formed in different areas in the world approximately 10000-12000 years ago. The major cereals are originated from different centers of agriculture all around the world: Maize is originated from Central-America, Rice is originated from southern China and Wheat is originated from Middle East region, especially specific area designated as Fertile Crescent (Figure 1.1).

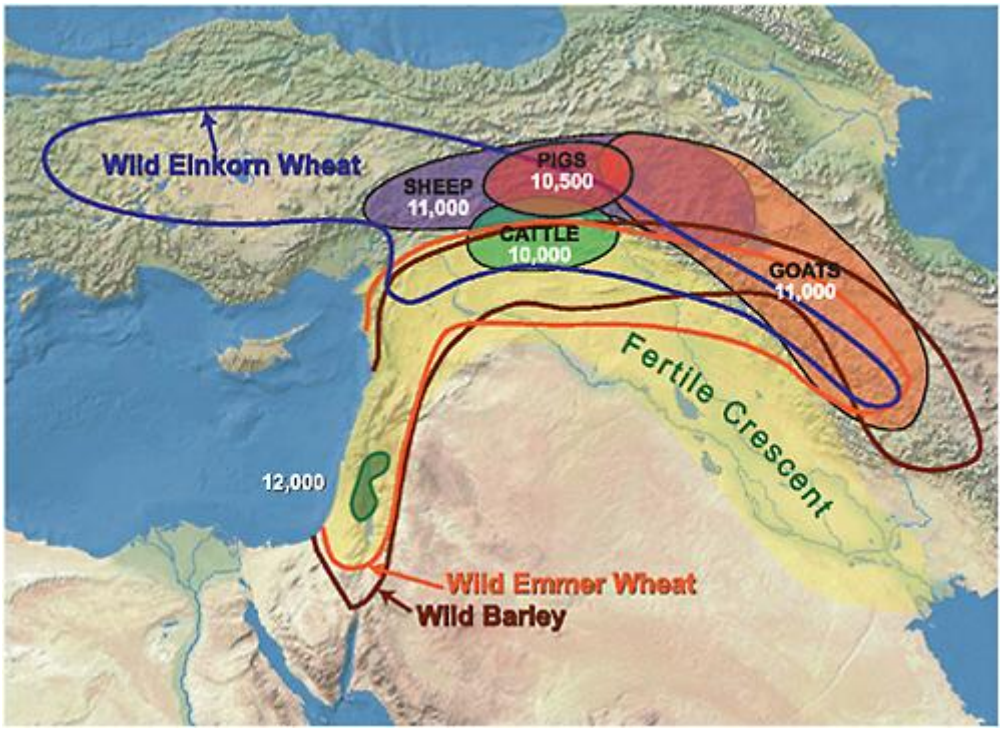


Figure 1.1 Map of the Near East indicating the Fertile Crescent. Shaded areas indicate the approximate areas of domestication of pig, cattle, sheep, and goats with dates of initial domestication in calibrated years. Lines enclose the wild ranges of einkorn wheat, emmer wheat, and barley. The Area Labeled as Fertile Crescent indicates the region where all 3 grains were first domesticated 12,000 years ago (Smith 1994).

It was found that the first wheat domestication happened in the Fertile Crescent. The cradle of domesticated wheat is a small area near the upper reaches of the Euphrates and Tigris rivers, in Turkey now (Lev-Yadun et al. 2000).

The genetic changes needed for wheat domestication are: selection of non-shattering, free-threshing, non-brittle rachis, development of hull-less spikes and better yielding cultivars. Traits that are constantly selected in wheat over generations are relatively high yield, easy to store and harvest (Harlan 1974).

Wild ancestry of wheat is a popular topic in research area and our knowledge for the origin of wheat is consistently improved due to the studies performed on the wild types. According to these studies, in addition to natural polyploid hybridization, artificial (on farmer's fields) selection played an important role on wheat evolution process (Nevo et al. 2002).

During this evolution process, wheat has become the most important food crop in the world. The majority of wheat produced worldwide is consumed by the humans. Annual wheat production is approximately 620 million tons annually (Williams 1993). Wheat is currently grown from Norway to Argentina both in Northern and Southern Hemispheres. The main wheat growing regions in the world are the central plains of the United States, southern regions of Russia, the countries around Mediterranean See, Northern and Central China, Southern Canada, Australia, India and Argentina (Figure 2).

Wheat comprises approximately 30% of the world's cereal production. Apart from carbohydrates, wheat is the one of the most important sources of protein consumed in human diets (Zohary and Hopf 2000). Cultivated wheat is better yielding to other big cereals in their nutritional value (Singh et al. 2000).

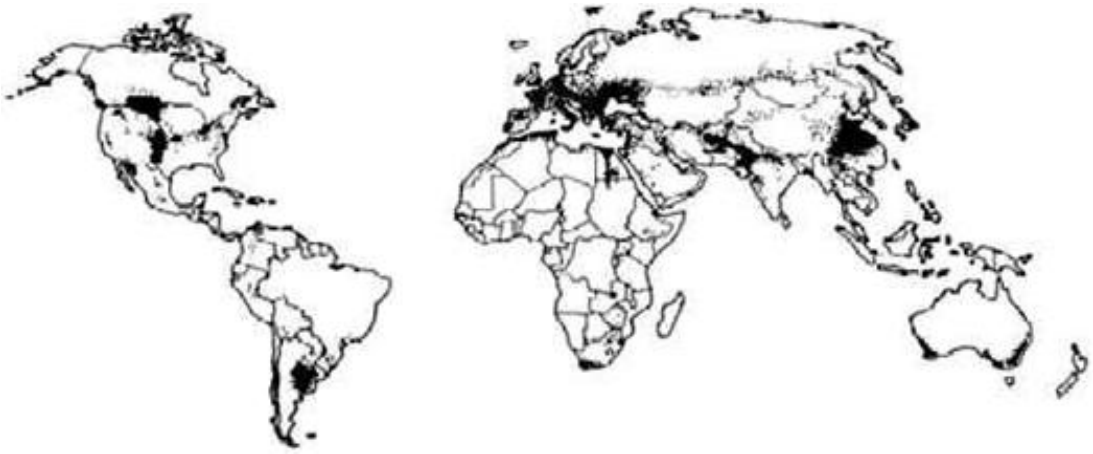


Figure 1.2 Major Wheat growing regions of the world, International Wheat and Maize Improvement Center (CIMMYT 1984).

The grain contains 60% to 80% starch and 7% to 28% storage protein (Avivi 1978, Avivi et al. 1983, Levy and Feldman 1987). The gluten proteins of wheat made wheat the staple for billions of people. Very little amount of produced wheat is used as animal food. Wheat is a crucial dietary component all over the world and moreover, wheat is source of 55% of the worlds' carbohydrates (Morrison 1988).

Efforts to increase the global wheat production and satisfy the increasing demand from both the population increase worldwide and the change of consumption patterns due to increasing living conditions in the developing countries have been relatively successful in maintaining a consistent increase in the wheat yield (Plucknet et al. 1983).

1.1.1) Wheat production in Turkey

Cereals constitute the most important part of the agricultural products, used for food sector. According United Nations Food and Agriculture Organization (FAO) 2002 data, world wheat production is approximately 568 million tones. In the year 2002 wheat was cultivated on 210 million hectares worldwide and mean yield of the wheat cultivation is 270 kg per decare (Table 1.1).

According the FAO statistics, Turkey produces 20 million tons of wheat annually, which means 3.5% of world wheat production and ranking the 8th largest wheat producer in the world (Table 1). In the year 2002, the average yield of wheat in Turkey is 213 kg/decare, which is slightly lower than the world average.

Table 1.1 Worldwide wheat cultivation and production.

Countries	Area Cultivated (hectare)	Production (tons)	Yield (kg/ha)
World	210.785.147	568.108.477	2.695
China	23.631.068	89.330.234	3.780
India	26.200.000	71.470.000	2.728
Russia	22.400.000	50.000.000	2.232
United States	18.541.680	43.992.312	2.373
France	5.243.000	39.031.000	7.444
Ukraine	6.609.000	21.000.000	3.040
Germany	3.017.000	20.786.000	6.890
Turkey	9.400.000	20.000.000	2.128
Pakistan	7.983.000	18.475.000	2.314
United Kingdom	1.989.000	15.814.000	7.951
Canada	8.897.400	15.494.200	1.741
Argentina	6.050.000	13.200.000	2.182
Kazakhstan	11.400.000	12.700.000	1.114
Australia	10.300.000	10.130.000	984

Source: Food and Agriculture Organization (FAO), 2002

Wheat agriculture is performed by 2.9 million small agricultural plants and directly generates income for approximately 15 million people (Bayaner 2005). Wheat production in Turkey is increased from 4 million tons in 1950's to 20 million tons in 2000's (Table 1.2).

Table 1.2 Wheat production levels in Turkey.

Years	Area Cultivated (hectare)	Production (tons)	Yield (kg/ha)
1936-40	3.804.000	4.020.000	1.057
1941-45	3.950.000	3.319.000	840
1945-50	4.206.000	3.630.000	863
1951-55	6.013.000	6.369.000	1.059
1956-60	7.435.000	7.910.000	1.064
1961-65	7.827.000	8.450.000	1.080
1966-70	8.292.000	9.924.000	1.197
1971-75	8.855.000	12.290.000	1.388
1976-80	9.259.000	16.750.000	1.809
1981-86	9.100.000	17.400.000	1.838
1987	9.415.000	18.900.000	2.007
1988	9.435.000	20.500.000	2.173
1989	9.351.000	16.200.000	1.732
1990	9.450.000	20.000.000	2.116
1991	9.630.000	20.400.000	2.118
1992	9.600.000	19.300.000	2.010
1993	9.800.000	21.000.000	2.143
1994	9.800.000	17.500.000	1.786
1995	9.400.000	18.000.000	1.915
1996	9.350.000	18.500.000	1.979
1997	9.340.000	18.650.000	1.997
1998	9.400.000	21.000.000	2.234
1999	9.380.000	18.000.000	1.919
2000	9.400.000	21.000.000	2.234
2001	9.350.000	19.000.000	2.032
2002	9.400.000	20.000.000	2.128
2003	9.360.000	19 000 000	2.031
2004	9.300.000	21 000 000	2.234
2005	9.320.000	21 500 000	2.232
2006	9.200.000	20 010 000	2.174

State Statistics Institute -Devlet İstatistik Enstitüsü (DIE), 2007

In Turkey, wheat production among the cities ranked as: Konya, Adana, Ankara and Tekirdağ respectively.

Quality of the seed is one of the most important factors in wheat yield (Oğuz 1991).Annual demand of wheat seed in Turkey is approximately 1.8 million tones. Since wheat is a self - pollinating variety seed variety must be changed every five years.

1.1.2) Origin, evolution and domestication of wheat

Contemporary wheat as it is known today is a member of two related polyploid species, hexaploid bread wheat (*T. aestivum* (42 chromosomes)) and tetraploid durum wheat (*T. turgidum L.* (28 chromosomes) There is also, *T. monococcum L. einkorn* (14 chromosomes) is rarely found cultivar, which is only found in isolated and hilly regions of Asia Minor (Turkey) and Mesopotamia (Zohary 1969). Wheat is mostly self-pollinating variety and because of this property of wheat, genetic diversity of wheat is preserved in the form of many different clones from different germplasm collections. Primitive wild forms and also the non-domesticated varieties have hulled grains and brittle ears that disintegrate at maturity (Zohary 1969). On the other hand, all domesticated varieties have non-brittle ears which did not disintegrate with maturity and thus needed human assistance on field. The nakedness and non-brittleness phenotype of wheat is coded by Q-locus, located at the chromosome 5 of the A genome of the wheat (Luo et al. 2000).

Evolution of the *Triticum* family is one of the best and successful examples of polyploidy (Soltis and Soltis 1999). Transformation from hunter-gatherer societies to plant cultivating societies is in turn effected and influenced the domestication of wheat. Evolution of contemporary wheat is a result of both artificial and natural selection.

Accumulation of many different resistance genes is available in the genome of polyploid wheat and relative species. With the increasing knowledge of genomic formation of polyploid wheat, accumulated genes are available for scientific research (Carver et al. 2003).

Determination of the formation of polyploid wheat extremely difficult because of the incredible amount of artificial selection pressures in addition to natural selection. Formation of the *Triticeae* family is coupled with the constant hybridization with wild varieties and also gene flow, gene fixation with the progenitor populations (Kellog et al. 1996). DNA sequence changes, convergent and diverging evolution during the evolution of modern wheat varieties explained the complex nature and history of wheat (McIntosh 1988, Feldman and Millet 2001).

Evolutionary studies with many plant species showed that wheat species evolved from different populations like the other polyploid species. Species formed independently started to hybridize with similar species in case of habitat overlapping. This process expands the content of the wheat genome (Soltis and Soltis 1999). The formation process of these species also changes the parent species (Ozkan and Feldman, 2001).

Polyploidy is rather common in Plant kingdom (Wendel 2000). There are two different kind of polyploid species: autopolyploid, which are aroused from chromosome doubling in one species and allopolyploids, which are aroused from hybridization between two different species (Stebbins 1947). Polyploidy is one of the most significant evolutionary events leading to an important amount of variation, thus increasing the adaptability of species. Best characterized example of allopolyploids is the wheat genus (Feldman

2001). It is known that the members of *Triticum* and *Aegilops* species are resulted from several diverging and converging polyploid events.

It is predicted that the *Triticeae* tribe and in it *Triticum* began separating from their ancestors approximately 30 million years ago and 11 million years ago, respectively. It is known that the wheat species have 7 chromosomes; in polyploid wheat species a multiple of 7 chromosomes are present. Several diploid genomes from *Triticea*e group are mostly conserved for the order of genes among the seven pair of chromosomes (Gale and Devos 1998). All chromosomes in different genomes (A, B and D) are evolutionarily related.

1.1.2.1) Origin of A genome

First scientific studies postulated that "A" genome of *T. aestivum* is originated from by *T. monococcum* (Sax 1922, Lilienfeld and Kihara 1934). However, the later studies showed "A" genome is donor is *T. urartu* (Chapman et al. 1976, Yildirim and Akkaya 2006). From the studies on the immunological evaluation of seed proteins, it is determined that "A" genome of *T. turgidum* was contributed by *T. urartu* and the genome of *T. timopheii* is contributed by *T. monococcum* (Konarev et al. 2003). Both, *T. monococum* and *T. turgidum* are grown naturally in a habitat comprised of Iraq, Trans-Caucasus, Syria, Lebanon and South Eastern Turkey (Kimber and Feldman 1987). Sterility of the hybrids formed from these species confirms their separate biological identity (Johnson and Daliwal 1978). Thus, *T. urartu* is determined to be the "A" genome donor of the all polyploid species (Dvorak et al. 1988).

1.1.2.2) Origin of B genome

The origin of the B genomes is controversial. Much evidence suggests that an ancient *Aegilops speltoides* species S genome was the progenitor of the B genome and D genome of the contemporary widely cultivated wheat species (Chapman et al. 1958, Dvorak and Zhang, 1990). S genome of the *A. speltoides* is a significantly diverged form of the B genome (Talbert et al. 1994). Plasmon analysis showed that *A. speltoides* are the B genome donor of the modern wheat varieties (Liu and Tsunewaki 1991), but it remains uncertain whether *A. speltoides is* the direct ancestor of the B genome or there is another donor in formation process of the B genome (Zohary and Feldman 1962). The B genome in *T. turgidum* and the G genome in *T. timopheevii* are related to each other (Dvorak and Appels 1982), but it is suggested that the G genome of *T. timopheevii* is closer to the S genome of *A. speltoides* than to the B genome of *T. turgidum* (Lassner et al. 1989). Results from analysis of organelles shows that B genomes of all polyploid wheat's are stemmed from *A. speltoides* (Tsunewaki 1968).

1.1.2.3) Origin of D genome

Bread wheat includes three different genomes A, B, and D each having 7 pairs. Common wheat is formed from the result of hybridization between the tetraploid wheat *T. turgidum* L. and diploid grass *A. tauschii* (Kihara 1944; McFadden and Sears 1946). The genome of *A. tauschii* and the D genome of bread wheat are highly related and the chromosomes of *A. tauschii* can be easily paired with the D genome of bread wheat (Gill and Raupp 1987).

1.1.2.4) Origin of hexaploid wheat

In world, there are two forms of hexaploid wheat is found. The first variety of hexaploid wheat is *T. zhukovski* Men & Er., which is the result of hybridization between *T. timopheevi* and *T. monococcum.* This variety is unique among its kind according to its genomic composition (Upadya and Swaminthan 1963). The other variety is common hexaploid wheat, *T. aestivum* (Okamoto 1962).

Triticum aestivum, also known as hexaploid wheat or bread wheat, formed approximately 10000 years ago after the domesticated tetraploid wheat hybridization with a *T. tauschii*, also known as *A. squarrosa* (DD) (Dvorak et al. 1998). The first primitive hexaploid wheat variety has hulled grains. The free threshing variant formed as a result of a mutation formed in the Q locus of the A genome (Muramatsu 1986). All polyploid wheat species are disomic.

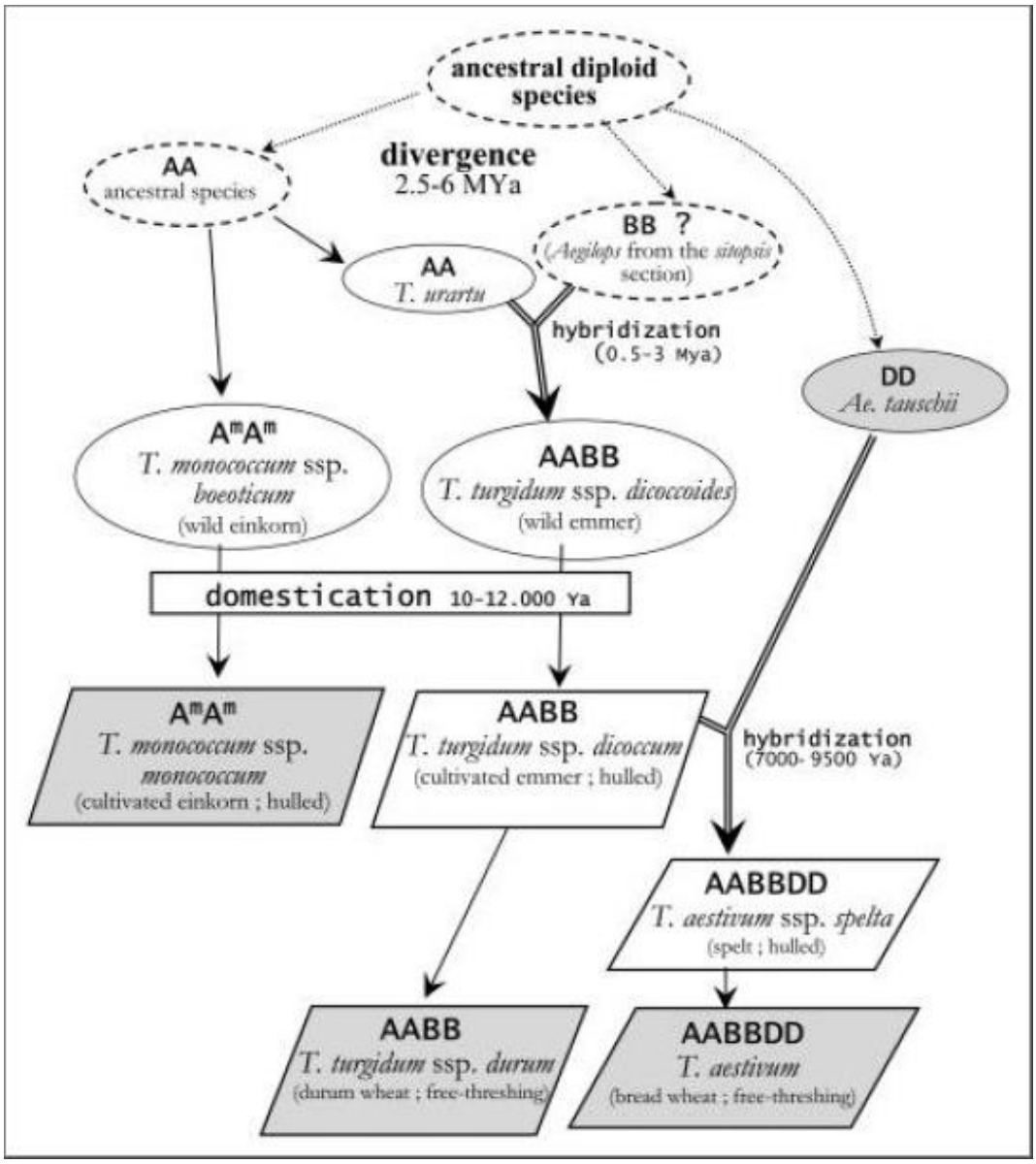


Figure 1.3 Wheat genomes (Vedel and Delseny, 1987).

Hexaploid wheat has little variation than its polyploid progenitors, suggesting that possible formation of a genetic bottleneck effect (Appel and Lagudah 1990). But, latter research suggested that because of the large area of the tetraploid wheat and *A. tauschii* populations shared, *A. tauschii* and tetraploid wheat hybrids may be more common than expected (Feldman 2001). The presence of several similar DNA profiles and molecular markers suggest that bread wheat was domesticated by more than one time (Caldwell et al. 2004, Zhang et al. 2008).

Bread wheat formed and still cultivated in an area where most of its ancestors found and this condition made a relatively higher frequency of intercrossing with its ancestors, mostly because of all polyploid wheat progenitors share at least one common genome (Ohtsuka 1998). Any homologous chromosome pairing in the *Tritieae* tribe allows formation of extra gene exchange and recombination. Homozygosity for any gene exchange favored by natural selection can be reached and available for selection, because hexaploid and tetraploid wheat are self-pollinating species (Zohary and Feldman 1962). The presence of more than one genome in ancient polyploid wheat varieties made possible the successful and rapid expansion of wheat. Polyploid speciation made greater degree of genomic changes and gene flow possible than the ordinary diploid species.

The polyploid wheat is a complete example of convergent evolution in which numerous genomes are combined in the evolutionary process. When combined with inbreeding, polyploid wheat has become a valuable asset for farmers because of its high adaptability to different environmental conditions.

Polyploid wheat species has become successful mostly because of their polyploid nature, which allows the accumulation of mutations. Also, adoption of genetic mechanisms in polyploid wheat, which allow the organization of genome into diploid genomes (A, B, D) was crucial for the selection and stabilization of the species (Sears 1977). Mutation has become an important source of variability in wheat genomes. The three genomes present in the hexaploid wheat allowed to wheat specify to contain

increasing number of mutations in their genomes, which mutations, in time become available for artificial and natural selection.

1.1.2.5) Genomic evolution and modification of wheat

The genome of polyploid wheat is bigger than the relative grass species, such as rice. For comparison Rice genome contains 0.6 pg to 1.0 pg DNA for japonica and indies varieties respectively. On the other hand each genome of wheat single ousts the rice genome: B Genome contains 5.15 pg, A Genome contains 4.93 pg DNA and D Genome contains 5.10 pg DNA respectively (Furuta et al. 1986, Bennet and Smith, 1976, Rees and Walters, 1965, Bennet and Leitch 1995). For phylogenetic point of view, genomes of wheat and genomes of rice varieties (japonica and indies) has a syntenic orientation and also these genomes are similar in their composition (Gale and Davos 1998, Tang et al. 2008).

It has been reported that more than 80% of the wheat genomes includes highly repetitive DNA sequences and retro-transposons (Gu et al. 2004). It is known that the ratio between coded and non-coded content varies with the size of the genome and the non-coding content of all wheat genomes, A, B, D, are bigger than rice separately (Kidwell et al. 2002, Gu et al. 2004). Retro-transposons of the A, B and D genomes are mostly not colinear, and this non-colinearity indicates the occurrence of genomic changes after the combination of these genomes (Gu et al. 2004).

Relative increase or decrease of genetic data can be determined in species with respect to first classified ancestor available. This kind of genetic processes happening in the polyploid wheat also widely occurs in the

several different grasses (Feldman et al. 1997, Shaked et al. 2001, Ma and Gustafson 2005). The frequency of genetic processes in this kind is not universal among all genomes or within the genomes.

The evolution of *Triticum* family is a fertile model for polyploidity (Soltis and Soltis 1999). Large numbers of genes, inherited and saved in the one of three genomes, that confers different types of resistances are present and ready for deployment in the field against potential pathogens in polyploid wheat and also wild wheat varieties. This situation helps the farmers and lately also scientists, to modify and make stronger the wheat, especially commercial wheat.

1.2) Molecular markers and their applications in wheat breeding

With the advent of new technologies, more marker technologies adapted to marker systems. *Triticum aestivum* (bread wheat) is one of the most important targets of marker development studies, because of its importance in human civilization (Rafalski et al. 1996). Every different marker system employed has positive and negative aspects and the choice of the selection of suitable marker is mostly determined by the target species, cost of the system and also information sought after.

Generally molecular markers can be divided into three different groups:

1) First group among these three molecular markers are PCR based molecular markers. Mostly employed PCR based molecular markers are: random amplified polymorphic DNAs (RAPDs), sequence characterized amplified regions (SCARs), simple sequence repeats (SSRs), and microsatellites sequence tagged sites (STS), amplified

fragment length polymorphisms (AFLPs) and cleaved amplified polymorphic sequences (CAPS).

2) Second group composed of Hybridization based molecular markers such as: restriction fragment length polymorphisms (RFLPs) and oligonucleotide fingerprinting technique.
3) Third group is the most recently developed group among them: DNA chip and sequencing based DNA markers such as DNA chips and Microarrays.

First plant molecular marker systems were applied and designed for tomato and maize. After first applications, molecular markers are performed on tomato and maize, these molecular markers rapidly adapted to wheat, barley and rye. Especially wheat species are very important and crucial for the study of polyploidy species. Additionally, Study and understanding of wheat cytogenetics is also important for understanding the Ph system in polyploidity (Arumuganthan and Earle 1991).

Wheat is suitable for cytogenetic studies but on the other hand preparation of molecular maps and development of molecular markers in wheat is extremely difficult chiefly because the polyploidy nature and huge genome size of the wheat. Fortunately, with the development and accumulation of research potential much success is achieved recent years and now many functioning wheat molecular markers is present. Also wheat synteny maps, which visualize the locations of the translocation happened in wheat genomes are prepared.

The newly developed molecular markers are extensively used in wheat systems for gene isolation, evolutionary relationship determination and most importantly marker assisted selection studies.

1.3) Diseases of wheat

1.3.1) Rust diseases

Rusts are diseases of plant species caused by fungal pathogens. Approximately 7800 rust species are known today. The rusts that effect wheat and barley are members of the *Pucciniales* family. Characterization of the rust species is a hard task and generally rusts are mistaken with smuts. All known rust species are obligate parasites and needed a host species in order to complete their life cycle. Treatment of rust diseases is extremely hard. Several chemical fungicides such as Triforine and Mancozeb are used for treatment, but after the infection starts, these fungicides have little ability to protect crop yield. Sulphur is also employed in farmers' field to stop the germination process of fungi. The wheat rust is one of the most important and widespread diseases of wheat.

1.3.1.1) Leaf rust

Leaf rust, whose causative agent is *Puccinia triticina Erics.*, is the most important and widespread rust disease among the three major rust diseases: Leaf rust, stripe rust and stem rust. During the life cycle of the *Puccinia triticina* Erics., five different kinds of spores are formed. First uredospores formed and then teleutospores and basidiospores and last steps of the fungal life cycle are pycniaspores and aeciospores on the hosts (Singh 2004). The germination occurs under humid conditions. Optimum temperatures for germination are between 15 °C and 20 °C.

Life cycle of *P. triticina* includes alternation of generations and both sexual and asexual reproduction is observed. During its life cycle, fungus needs

an alternative host, *Thalitactrum spp.* (Mains 1932). The optimum temperature range for the growth and germination of fungi is between approximately 15 °C to 20 °C. First the infection starts and during the progress of the infection , fungi started to sporulation and symptoms of leaf rust appears on the leaf of the plant in leaf rust on wheat (Singh et al. 1992).

Leaf rust can cause yield losses from 25% to 90% (Mains 1930). Even in the most developed countries with a developed and effective agriculture policies such as United States of America, leaf rust continue to cause significant and chronic yield losses annually (Kansas Department of Agriculture, Topeco, Kansas).

Maximum range of yield loss caused by leaf rust can increase up to 90%. Leaf can cause up to 75% because reduced kernel number per head Caldwell et al. 1934). Leaf rust reduced the kernel number per head and also lowers the grain quality if the infection happens at the grain filling stage of the wheat (Martin et al. 2003).

For each LR gene (Leaf Rust Resistance Gene) in wheat there is a corresponding locus in *P. triticina* with alleles that confers race specific resistance specific for the pathogen species infected the plant (Kolmer and Dyck 1994).

There is an incredible amount of genetic diversity in *P. triticina* populations and also potential to evolve. Because of these properties of *P. triticina*, breeding a stable race specific resistance in wheat is extremely hard. If only one race specific resistance gene is present in the cultivar, approximately in five years' time after deployment on the field,

single gene lose all of its effect like LR21. LR 21 is once a very popular and powerful leaf rust resistance gene but after twenty years on field it lost practically all of its effect. Up to date more than sixty leaf rust resistance genes are determined, among them majority of the resistance genes area race specific resistance genes and minority is durable resistance genes (McIntosh et al. 2007).

1.3.1.2) Stem rust

The stem rust, also called black rust, whose causative agent is *Puccinia graminis*, second important major wheat rust after leaf rust. Stem rust regularly cause yield losses in temperate wheat growing regions annually (Leonard 2001). Life cycle of *P. graminis* includes alternation of generations like leaf rust. Moreover *Puccinia graminis* requires alternative host species in its different life cycle stages (Roelfs et al. 1992) (Figure 1.4).

In the spring and summer, urediniospores of stem rust dispersed by the wind and as a result of this dispersion, infection occurs. Uredinospores initiates the infection via penetrate into the plant via stoma. After the penetration process, rusts starts to produce teliospores. After the production of teliospores rust stays dormant during the winter. With the start of spring newly formed basidiopores formed and by wind spread and infect alternative host species such as: *Berberis vulgaris* or other susceptible *Berberis*, *Mahonia*, or *Mahoberberis* species. In *Berberis vulgaris* (barberry) or another alternative host, basidiospore penetrates the leaf epidermis directly, and after these process fungi starts to produce pycnia (Roelfs 1985).

With the formation of pycnia, sexual phase of life cycle initiates. Compatible pycnias mate and formed into diploid aecium. After this produced diploid aeciospores spread by wind and this time infects wheat and lastly after aeciospores reaches wind and penetrate it, uredia forms and the cycle begins again (Roelfs 1985).

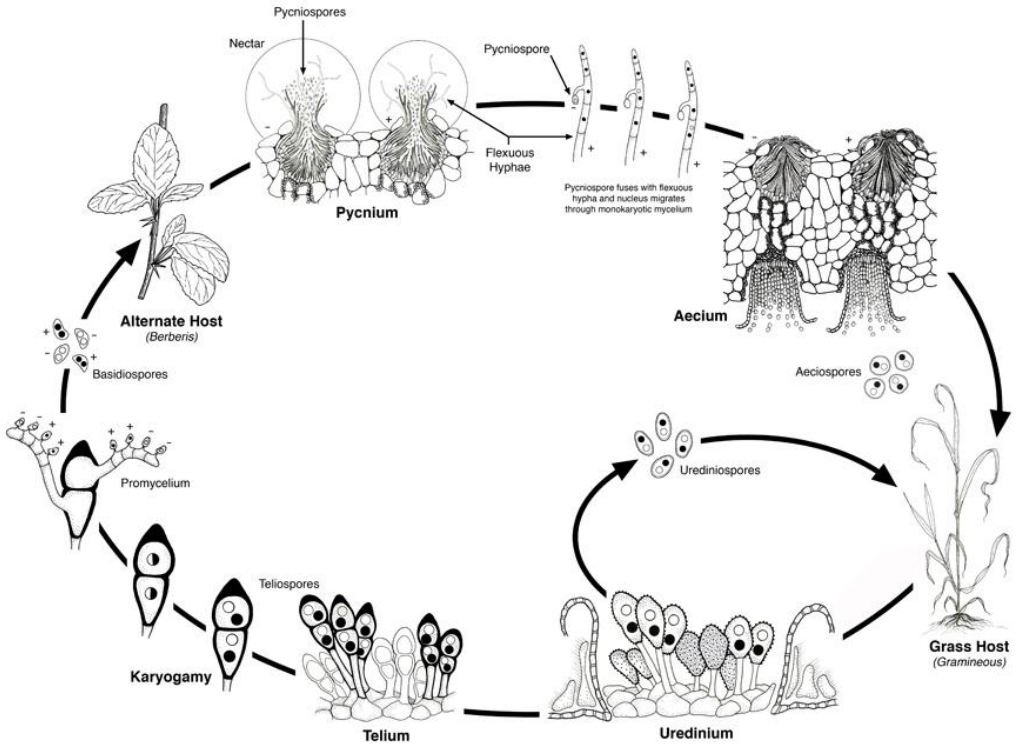


Figure 1.4 Life Cycle of *P. graminis* (Leonard 2001).

1.3.1.3) Stripe rust

Puccinia striiformis also known as Stripe rust or Yellow rust, is the third member of major wheat rusts. The unique feature of stripe rust is that while the other major wheat rusts effect wheat grown in the temperate climates, stripe rust predominantly effect cooler climates (Hylander 1953).

Infection of stripe rust chiefly depends on the presence of green tissue and with the presence of green tissue in plant it can occurs any growth stage. After the infection initiates yellow to orange spots began to appear and also because the color of these spots, stripe rust is also known as yellow rust (Kuraparthy et al. 2007).

Up to date more than forty stripe rust resistance genes are listed (McIntosh et al. 2007). Currently, like the fate of race specific Leaf Rust resistance genes, majority of resistance genes lost their effectiveness in a decade approximately. Only few exceptions remain effective in the United States of America such as: Yr5, Yr 15, Yr 26 and Yr 40 (Kuraparthy et al. 2007).

1.3.2) Powdery mildew

The fungus that caused powdery mildew is the heterothallic ascomycete, *Blumeria graminis* sp. *tritici*. The presence of powdery mildew infection can be determined by small white pustules from fungi spotted on the plant (Carver et al. 2003). Powdery mildew has a temperature range of 15 to 22 °C. Also, the powdery mildew infection under the humid conditions and dense cultivation increase the prevalence of powdery mildew infection. An interesting aspect of powdery mildew is that high humidity promote the infection, however presence of water on the leaves of plant limits the infection by inhibiting the germination of fungus. Infected leaves lose their color and die prematurely.

In the absence of effective resistance genes powdery mildew infection resulted in significant yield loss Reductions in grain yield can be varied

from 5% to 45 % in the USA (Coakley et al. 1984). The disease also reduces softness of kernel, content of protein and flour yield (Johnson 1956).

1.4) Adult plant or durable resistance

Selection of wheat species and resistance genes with efficient and durable rust resistance is a crucially important point in wheat research (*Triticum aestivum*). Race specific resistance genes, which comprise the majority of resistance genes, are effective only for a limited amount of time because of adaptation potential of the pathogen (Lagudah et al. 2009).

The development of wheat species with effective resistance requires pooling these genes in the cultivars. Thus, increasing global wheat production yield is chiefly based on the determination of durable resistance genes. The cultivar Frontana has been first recorded cultivar which confers durable resistance to leaf rust (Dycke 1966). It is recorded that Frontana cultivar possesses several durable resistance genes among which the most important is LR34 (Sing and Rajaram 1992). LR34 continue to confer resistance after cultivation history of more than 40 years and which is crucially different than the race specific resistance genes (Kolmer et al. 2003). There are examples of expression of durable resistance of LR34 in cooler climates (Sing 1992). Durable resistance coded by LR34 also known to increase the effects of other resistance genes already presents (German and Kolmer 1992). Wheat cultivars, which possess LR13 and LR16 in addition to LR34 were reported to be highly resistant (Sanborski and Dyck 1982, Liu and Kolmer 1997).

The LR34 gene also confers moderate resistance to stripe rust thus the locus also named as Yr18 (McIntosh 1992). LR34 gene also confers resistance to powdery mildew and categorized among the powdery mildew resistance genes as Pm38 (Spielmeyer et al. 2005). There are also reports about the resistance of LR34 against barley yellow dwarf virus, Bdv1 (Singh 1993) and stem rust (Dyck 1994).

The *LR34* gene has become a very important gene in the development of rust resistant wheat development research studies. By pooling of LR34 with other adult plant resistance genes, higher levels of resistance comparable or even higher than race-specific genes can be achieved (Singh and Trethowan 2007).

Presence of LR34 can be deduced from development of a necrotic leaf tip. Still presence leaf tip necrosis can be varied among environment and is not a definitive and efficient marker (Singh 1992). Diagnostic molecular markers which are located very close to LR34 gene produced and more markers are developed (Bossolini et al. 2006; Lagudah et al. 2006). The presence of molecular markers made it rather simple to determine the presence of LR34 in a cultivar. However, recombination events between these molecular marker containing chromosome regions lowers the specificity and detection potentials (Kolmer et al. 2008, McCallum et al. 2006).

After the isolation of LR34 gene, it is determined that it encodes pleiotropic drug resistance (PDR)-like ATP binding cassette (ABC) transporter (Krattinger et al. 2009). Recently, it is revealed that separate resistance genes for leaf rust, stripe rust and powdery mildew actually are the same gene, LR34. The nucleotide sequence of LR34 is 11,805 bp long and

includes 24 exons. Up to date, only two different LR34 alleles have been designated: resistance cultivar firstly cloned from Chinese Spring and the susceptible allele which are cloned from Renan cultivar. (Lagudah et al. 2009). The two alleles differed in only three nucleotide polymorphisms. One single nucleotide polymorphism is located in the 4th intron, another one as the 3 bp deletion located in 11th exon and a second single nucleotide polymorphism (SNP) is in 12th exon of the *LR34* gene. Resistant LR34 allele can be found in similar sequence all over the world and this indicates the resistance of LR34 is originates in a single spot and single population of wheat (Lagudah et al. 2009).

Pooling of durable resistance genes and race specific resistance genes both increase the life span of race specific resistance genes and also by pooling effect increase the overall resistance of the cultivar. This combination also increases the resistance of the seedling plants in which durable resistance generally do not function. It is important to note that cultivars that contain LR34 also appear to confer resistance against newly emerged and extremely virulent Ug99 (Jin and Singh 2006). Up to date, very little number of cultivars possess different set of resistance genes have resistance against Ug99.Thus the resistance capability of LR34 against Ug99 potentially extremely valuable.

CHAPTER 2

MATERIALS AND METHODS

2.1) Materials

2.1.1) Chemicals

Table 2.1 Materials and suppliers.

Chemicals	Suppliers
Agarose	Sigma
DNA Size Marker	Fermentas
dNTP	Metis
Ethidium Bromide	Sigma
Primers	Iontek
MgCl2	Metis
Taq DNA Polymerase	Metis

2.1.2) Equipment

Table 2.2 Equipment and Suppliers.

Equipment	Suppliers
Centrifuge	Eppendorf
Deepfreeze	Bosch
Electrophoretic Gel System	KODAK Gel Logic
UV Transilluminator	KODAK Gel Logic
Power Supply	Gibco BRL
Thermal Cycler	Eppendorf
Micro Pipettes	Eppendorf
Vortex	Heidolph
Spectrophotometer	NanoDrop ND-1000

2.1.3) Plant materials

Table 2.3 Turkish wheat cultivars used in our study.

Cultivar No	Name of Cultivar	Origin	Year
1	Kınaca 97	B.D.M. Kışlık Hububat Arş. Ens./Konya	1997
2	Altay 2000	Anadolu Tarımsal Arş. Ens./Eskişehir	2000
3	Yıldız 98	Anadolu Tarımsal Arş. Ens./Eskişehir	1998
4	Orso		
5	İkizce 96	Tarla Bitkileri Mrk. Arş. Ens./Ankara	1996
6	Gerek 79	Anadolu Tarımsal Arş. Ens./Eskişehir	1979
7	Bolal 2973	Anadolu Tarımsal Arş. Ens./Eskişehir	1970
8	Göksu 99	B.D.M. Kışlık Hububat Arş. Ens./Konya	1999
9	Bezostaya	Anadolu Tarımsal Arş. Ens./Eskişehir	1968
10	Sofu		
11	ES 97 KE 10		
12	Kırkpınar 79	Trakya Tarımsal Arş. Ens./Edirne	1979
13	Çakmak 79		
14	Kırgız 95	Anadolu Tarımsal Arş. Ens./Eskişehir	1995
15	Topbaş-Ekmeklik		
16	Orzo-96		
17	Kalkıç 88		
18	Berbinen 469		
19	4875		
20	Gün91	Tarla Bitkileri Mrk. Arş. Ens./Ankara	1991
21	Kutluk 94		
22	Dağdaş 94	B.D.M. Kışlık Hububat Arş. Ens./Konya	1994
23	Kate A1	Trakya Tarımsal Arş. Ens./Edirne	1988
24	Sultan 95		
25	Flamura 85		
26	Basribey 95	Ege Tarımsal Arş. Ens./İzmir	1995
27	Pehlivan	Trakya Tarımsal Arş. Ens./Edirne	1998
28	Tekirdağ		
29	EBDV 2		
30	Saraybosna	Trakya Tarımsal Arş. Ens./Edirne	2001
31	Guadalupe		
32	Gelibolu		
33	BBVD 2		
34	BBVD 15		
35	Mirzabey 42		
36	Tosunbey		
37	Zencirli	Tarla Bitkileri Mrk. Arş. Ens./Ankara	2002
38	Atlı		
39	Seval		
40	Altın		
41	Demir		
42	Yılmaz		
43	UzunYayla		
44	İkizce	Tarla Bitkileri Mrk. Arş. Ens./Ankara	1996
45	Dağdaş Bread		
46	Prostar	Trakya Tarımsal Arş. Ens./Edirne	1999
47	Atilla-12	Trakya Tarımsal Arş. Ens./Edirne	2001
48	Aldane		
49	Selimiye		
50	Dropia		
51	Golia	TİGEM /Ankara	1999
52	Tima		
53	Nina		
54	Sagittario	TASACO Tar. San.ve Tic. Ltd .Şti./İstanbul	2001

Table 2.3 Continued

55	Yunak
56	Syrene Odeskaja
57	Albatros Odeskaya
58	Krasunta Odeskaya
59	Esperia
60	Saroz 99
61	Bezostaya
62	ES 96

2.2) Methods

2.2.1) DNA isolation

Genomic DNA isolations of the wheat cultivars and Aegilops samples are performed using CTAB method.

Table 2.4 The *Aegilops* species used in the study.

Number of Sample	Species	Accession Number	Location
4	Aegilops caudate	TR55060	Yozgat
7	Aegilops crassa	X	X
8	Aegilops crassa	TR48147	X
9	Aegilops cylindirica	TR51865	Balıkesir
11	Aegilops cylindirica	TR46750	Bingöl
12	Aegilops cylindirica	TR51873	Bursa
14	Aegilops cylindirica	TR46766	Hakkari
16	Aegilops cylindirica	TR52018	Konya
17	Aegilops cylindirica	TR49055	ŞanlıUrfa
25	Aegilops sqourossa	TR57150	X
26	Aegilops ligustica	TR39693	X
27	Aegilops speltoides	TR52069	Adıyaman
28	Aegilops tauschii	TQ18	ŞanlıUrfa
29	Aegilops tauschii	TQ36	ŞanlıUrfa
30	Aegilops tauschii	TQ40	ŞanlıUrfa
31	Aegilops tauschii	TQ33	ŞanlıUrfa
32	Aegilops tauschii	TQ16	ŞanlıUrfa

Freeze dried 200 mg of individual plant tissue was powdered in liquid nitrogen on mortar. Autoclaved spatulas are used for transfer of crushed plant tissue to 1.5 mL eppendorfs.

The plant tissue is dispersed in 0.5 mL extraction buffer (50 mM Tris-Cl, pH 8.0, 10 mM EDTA, pH 8.0, 0.7 M NaCl, %1 CTAB v/w (Hexa-decyltrimethylammonium bromide]) and 5 μL B-mercaptoethanol and 5 μL Proteinase K(10mg/ml) is added into the tubes simultaneously. The mixture was incubated in 65 ^{o}C for 1 hour by inverting the tubes gently several times. 0.5 mL of Chloroform was added and the solution was mixed to form an emulsion which was centrifuged at 13000 rpm for 20 minutes at 18 ^{o}C to perform phase separation.

Supernatant is transferred to a new centrifuge tube. 1 μL RNAase A (10 mg/ml) is added to the supernatant and the tube is incubated at 37 ^{o}C.

After the incubation period, 500 μL of Isopropanol (-20 ^{o}C) is added to tubes. The tubes containing the mixture are mixed gently. The mixture is placed on ice and incubated for 5 minutes. Then the mixture is centrifuged at 130000 rpm at 4 ^{o}C for 30 minutes.

The remaining pellet after the centrifugation step is DNA. Supernatant is discarded. Pellet is washed with 500 μL 70 % Ethanol. The Ethanol-DNA mixture is centrifuged at 15000 rpm for 5 minutes at 4 ^{o}C. After this step supernatant is discarded and tube is again centrifuged at 15000 rpm for 5 minutes at 4 ^{o}C. Double distilled water of 40 μL is added and pellet is dissolved in water. The DNA samples were stored at -80 ^{o}C.

Nanodrop was used to measure the concentration of isolated genomic DNA in the samples.

2.2.2) Design of the PCR primers for screening

The primers used in our study were directly from Lagudah et al. 2009, which were designed for amplification of mutation site of the LR34 genes resistant and susceptible alleles (Figure 2.1 and Figure 2.2).

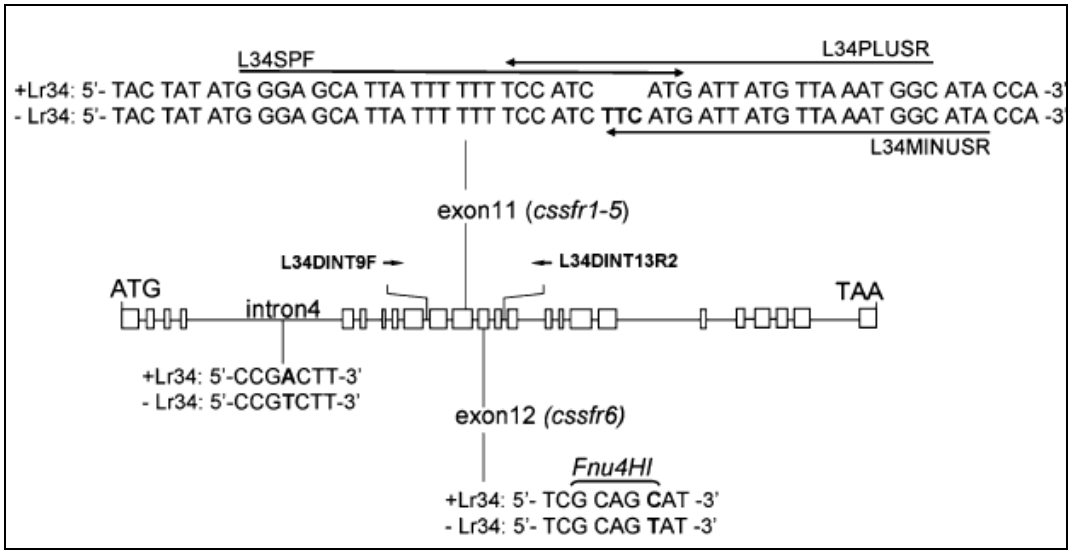
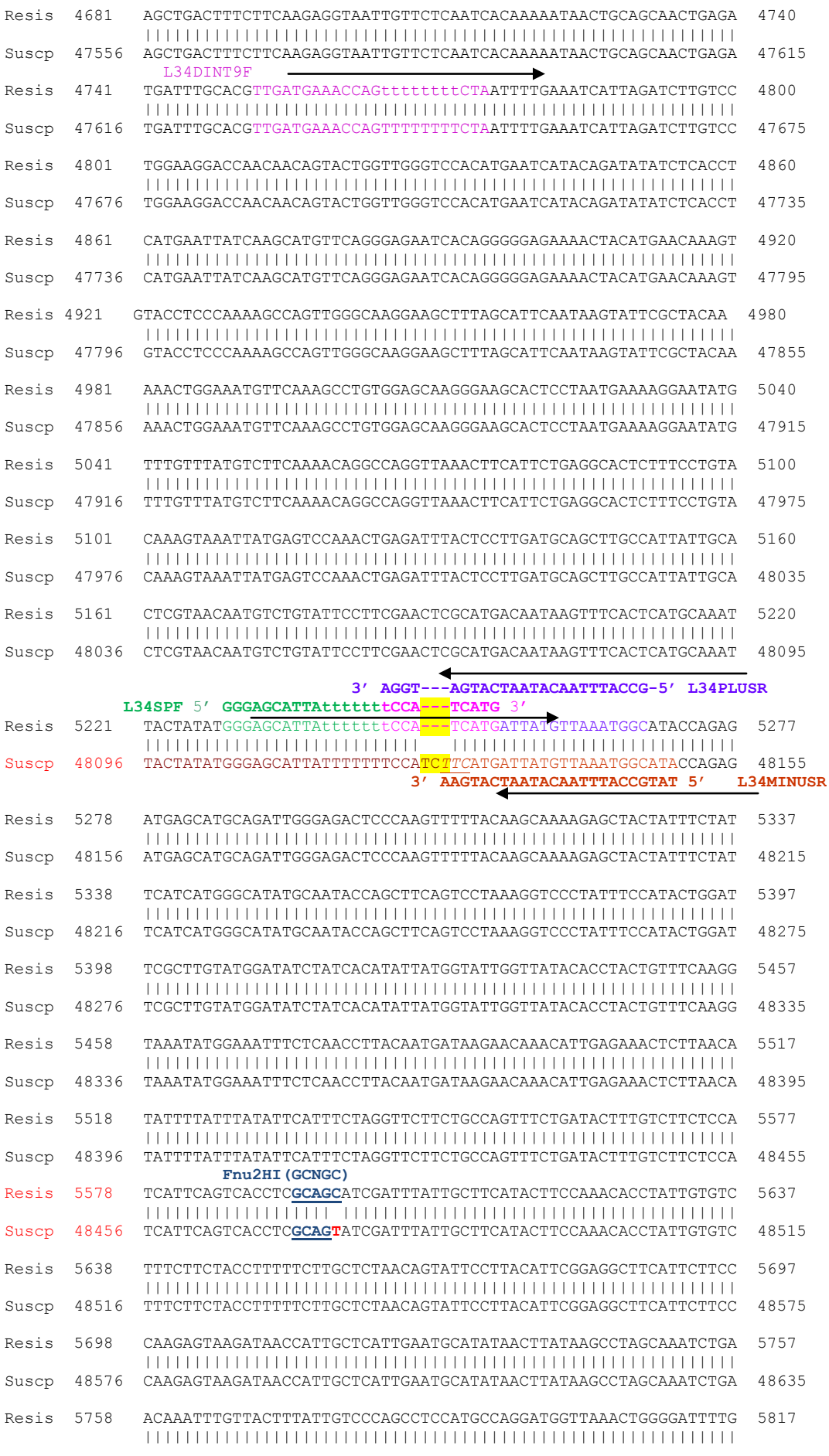


Figure 2.1 Genomic orientation of the primers according to the resistant and susceptible allele of the LR34 (Lagudah et al. 2009).


Resis 4681 AGCTGACTTTCTTCAAGAGGTAATTGTTCTCAATCACAAAAATAACTGCAGCAACTGAGA 4740
Suscp 47556 AGCTGACTTTCTTCAAGAGGTAATTGTTCTCAATCACAAAAATAACTGCAGCAACTGAGA 47615
L34DINT9F
Resis 4741 TGATTTGCACGTTGATGAAACCAGttttttttCTAATTTTGAAATCATTAGATCTTGTCC 4800
Suscp 47616 TGATTTGCACGTTGATGAAACCAGTTTTTTTTCTAATTTTGAAATCATTAGATCTTGTCC 47675
Resis 4801 TGGAAGGACCAACAACAGTACTGGTTGGGTCCACATGAATCATACAGATATATCTCACCT 4860
Suscp 47676 TGGAAGGACCAACAACAGTACTGGTTGGGTCCACATGAATCATACAGATATATCTCACCT 47735
Resis 4861 CATGAATTATCAAGCATGTTCAGGGAGAATCACAGGGGGAGAAAACTACATGAACAAAGT 4920
Suscp 47736 CATGAATTATCAAGCATGTTCAGGGAGAATCACAGGGGGAGAAAACTACATGAACAAAGT 47795
Resis 4921 GTACCTCCCAAAAGCCAGTTGGGCAAGGAAGCTTTAGCATTCAATAAGTATTCGCTACAA 4980
Suscp 47796 GTACCTCCCAAAAGCCAGTTGGGCAAGGAAGCTTTAGCATTCAATAAGTATTCGCTACAA 47855
Resis 4981 AAACTGGAAATGTTCAAAGCCTGTGGAGCAAGGGAAGCACTCCTAATGAAAAGGAATATG 5040
Suscp 47856 AAACTGGAAATGTTCAAAGCCTGTGGAGCAAGGGAAGCACTCCTAATGAAAAGGAATATG 47915
Resis 5041 TTTGTTTATGTCTTCAAAACAGGCCAGGTTAAACTTCATTCTGAGGCACTCTTTCCTGTA 5100
Suscp 47916 TTTGTTTATGTCTTCAAAACAGGCCAGGTTAAACTTCATTCTGAGGCACTCTTTCCTGTA 47975
Resis 5101 CAAAGTAAATTATGAGTCCAAACTGAGATTTACTCCTTGATGCAGCTTGCCATTATTGCA 5160
Suscp 47976 CAAAGTAAATTATGAGTCCAAACTGAGATTTACTCCTTGATGCAGCTTGCCATTATTGCA 48035
Resis 5161 CTCGTAACAATGTCTGTATTCCTTCGAACTCGCATGACAATAAGTTTCACTCATGCAAAT 5220
Suscp 48036 CTCGTAACAATGTCTGTATTCCTTCGAACTCGCATGACAATAAGTTTCACTCATGCAAAT 48095
3′ AGGT---AGTACTAATACAATTTACCG-5′ L34PLUSR
L34SPF 5′ GGGAGCATTATttttttCCA---TCATG 3′
Resis 5221 TACTATATGGGAGCATTATttttttCCA---TCATGATTATGTTAAATGGCATACCAGAG 5277
Suscp 48096 TACTATATGGGAGCATTATTTTTTTCCATCTTCATGATTATGTTAAATGGCATACCAGAG 48155
3′ AAGTACTAATACAATTTACCGTAT 5′ L34MINUSR
Resis 5278 ATGAGCATGCAGATTGGGAGACTCCCAAGTTTTTACAAGCAAAAGAGCTACTATTTCTAT 5337
Suscp 48156 ATGAGCATGCAGATTGGGAGACTCCCAAGTTTTTACAAGCAAAAGAGCTACTATTTCTAT 48215
Resis 5338 TCATCATGGGCATATGCAATACCAGCTTCAGTCCTAAAGGTCCCTATTTCCATACTGGAT 5397
Suscp 48216 TCATCATGGGCATATGCAATACCAGCTTCAGTCCTAAAGGTCCCTATTTCCATACTGGAT 48275
Resis 5398 TCGCTTGTATGGATATCTATCACATATTATGGTATTGGTTATACACCTACTGTTTCAAGG 5457
Suscp 48276 TCGCTTGTATGGATATCTATCACATATTATGGTATTGGTTATACACCTACTGTTTCAAGG 48335
Resis 5458 TAAATATGGAAATTTCTCAACCTTACAATGATAAGAACAAACATTGAGAAACTCTTAACA 5517
Suscp 48336 TAAATATGGAAATTTCTCAACCTTACAATGATAAGAACAAACATTGAGAAACTCTTAACA 48395
Resis 5518 TATTTTATTTATATTCATTTCTAGGTTCTTCTGCCAGTTTCTGATACTTTGTCTTCTCCA 5577
Suscp 48396 TATTTTATTTATATTCATTTCTAGGTTCTTCTGCCAGTTTCTGATACTTTGTCTTCTCCA 48455
Fnu2HI (GCNGC)
Resis 5578 TCATTCAGTCACCTCGCAGCATCGATTTATTGCTTCATACTTCCAAACACCTATTGTGTC 5637
Suscp 48456 TCATTCAGTCACCTCGCAGTATCGATTTATTGCTTCATACTTCCAAACACCTATTGTGTC 48515
Resis 5638 TTTCTTCTACCTTTTTCTTGCTCTAACAGTATTCCTTACATTCGGAGGCTTCATTCTTCC 5697
Suscp 48516 TTTCTTCTACCTTTTTCTTGCTCTAACAGTATTCCTTACATTCGGAGGCTTCATTCTTCC 48575
Resis 5698 CAAGAGTAAGATAACCATTGCTCATTGAATGCATATAACTTATAAGCCTAGCAAATCTGA 5757
Suscp 48576 CAAGAGTAAGATAACCATTGCTCATTGAATGCATATAACTTATAAGCCTAGCAAATCTGA 48635
Resis 5758 ACAAATTTGTTACTTTATTGTCCCAGCCTCCATGCCAGGATGGTTAAACTGGGGATTTTG 5817

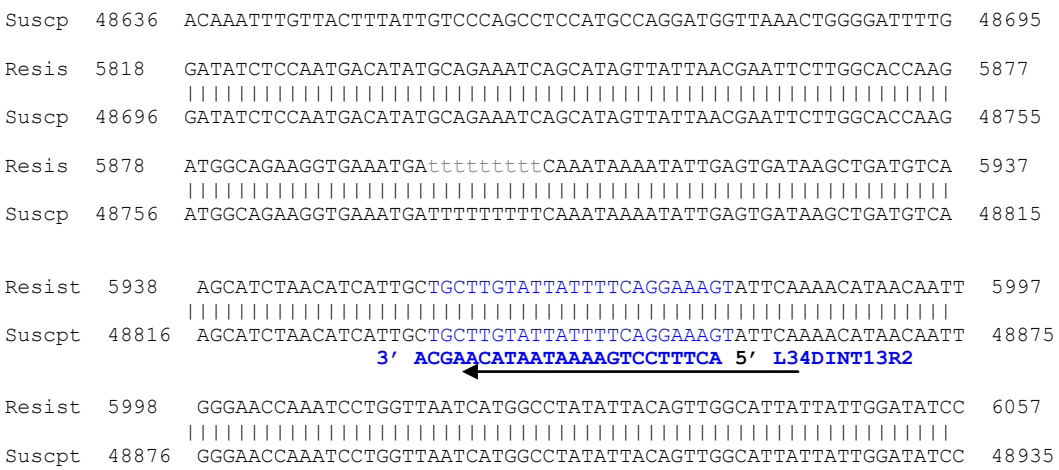


Figure 2.2 Primer positions on the susceptible and resistant LR34 alleles (Resis is denoted for resistant LR34 allele and Suscp is denoted for susceptible LR34 allele).

2.2.3) Primer preparation

The primers were acquired in the powder form from Iontek (Istanbul, Turkey). First 100 μM primer stocks were prepared with addition of PCR grade distilled water. Pipetting is performed and diluted primers are incubated at room temperature for 5 minutes. A 1:10 diluted primers are prepared and finally total 50μL aliquots were stored at -20◦C.

2.2.4) Polymerase chain reaction (PCR)

The amplification reactions were performed in thermocycler (Eppendorf, Mastercycler®, 230 V, 50 Hz), the total reaction volume was 20 µl for every tube used. The $MgCl_2$ content and primer concentrations are optimized.

2.2.5) PCR amplifications

In a 200 µL sterile PCR tube, the PCR components are mixed according to following conditions: 1X PCR Buffer (75 mM Tris-HCl with pH 8.8 at 25 °C, 20 mM $(NH_4)_2SO_4$, 0.01% (v/v) Tween20), 0.25 mM dNTP mix, 1.5 mM $MgCl_2$, 1U of *Taq* DNA polymerase (5u/µL), 10 pmol forward primer, 10 pmol reverse primer and sterile distilled water up to 20 µL volume in a 200 µL PCR tube. PCR cycling conditions were 94 °C for 2 min as initial denaturation, 35 cycles of three steps as; denaturation at 94 °C for 1 min, annealing at 58°C for 1 min and extension at 72 °C for 1 min and 1 cycle of 5 min extension at 72 °C. Sequences of primers that were used in PCR amplifications are shown in Table 2.1.

PCR amplification for markers *cssfr1* to *cssf4* was performed using various primer combinations. PCR conditions were as described by Lagudah et al. (2009) with optimized annealing temperatures.

Table 2.5 Primers used in the study.

Name	Sequence (5′ to 3′)
LR34DINT9F	TTGATGAAACCAGTTTTTTTTCTA
LR34PLUSR	GCCATTTAACATAATCATGATGGA
LR34MINUSR	TATGCCATTTAACATAATCATGAA
CSLV34F	GTTGGTTAAGACTGGTGATGG
CSLV34R	TGCTTGCTATTGCTGAATAGT®
Actin F	ATGGAAGCTGCTGGAATCCAT
Actin R	CCTTGCTCATACGGTCAGCAATAC

2.2.6) Preparation of DNA gels

To prepare 1 % gel, 0.5 g of Agarose (Applichem) was melted by boiling in the 50 mL, 1 X TBE buffer (for 10 X buffer; 107.78 g Tris, 7.44 gr EDTA-Na_2 salt and 55 g boric acid was dissolved in 1 L distilled water). When cooled down to about 60 °C, 13 μL of ethidium bromide was added (final concentration should be 0.5 mg/ml) and it was poured off in to the gel tray.

2.2.7) PCR amplification with actin primers

DNA samples were tested for the presence of actin gene by using actin gene specific primers. For PCR reaction following components were combined in a 200 mL sterile PCR tube; 0.5 μL cDNA template, 4 μL 10 X PCR buffer, 2.4 μL 25mM $MgCl_2$, 0.5 μL dNTP, 1 μL forward primer, 1 μL reverse primer 1 μL Taq DNA polymerase (5 U/μL) and the volume was completed to 20 μL with sterile PCR water. PCR cycling conditions were; initial denaturation at 94 °C for 3 minutes, 35 cycles of three steps as denaturation at 94 °C for 1 minute, annealing at 53 °C for 1 minute, and extension at 72 °C for 1 minute and for the last step 5 min for 72 °C. PCR product was visualized on 1% agarose gel with ethidium bromide staining prepared as in section 2.5.3.

2.2.8) Agarose gel electrophoresis

The results of PCR reaction is observed under UV light, by employing KODAK Gel Logic visualization system. The concentration of agarose in the gel was optimized to 1 % from the literature cited and previous

laboratory experience. The agarose gels were run at 100 V (BioRad) and agarose gels are prepared with the addition of ethidium bromide in order to visualize gels under UV light (Kodak Gel Logic UV Transilluminator).

CHAPTER 3

RESULTS

3.1) Experimental strategies

In this study, screening of the LR34 gene is performed on samples of Turkish wheat varieties and on some *Aegilops* species. The main steps that were followed included: DNA isolation, PCR amplification and verification of the presence the LR34 gene resistant allele by detecting presence or absence and/or by the sizes of the products using allele specific primers. The samples that we analyzed were from wheat leaves, which were obtained from the seedlings grown for 10-15 days in the lab conditions. The leaves were collected according to Table 2.1.

3.2) DNA isolation

The concentration and quality of isolated DNA was determined at 260 nm using the Nanodrop 2000 spectrophotometer (Nano-drop Technologies, Wilmington, DE) (Table 3.1 and 3.2).

Table 3.1 DNA **c**oncentrations of the *Aegilops* species after DNA isolation *via* CTAB method.

Name of the sample	Number assigned	Concentration (ng/μL)
Aegilops caudate	4	60.4
Aegilops crassa	7	307.0
Aegilops crassa	8	306.7
Aegilops cylindirica	9	1333.4
Aegilops cylindirica	11	622.1
Aegilops cylindirica	12	777.8
Aegilops cylindirica	14	3938.9
Aegilops cylindirica	16	405.3
Aegilops cylindirica	17	634.9
Aegilops sqourossa	25	2686.9
Aegilops ligustica	26	767.7
Aegilops speltoides	27	1486.9
Aegilops tauschii	28	551.3
Aegilops tauschii	29	492.6
Aegilops tauschii	30	2229.0
Aegilops tauschii	31	488.5
Aegilops tauschii	32	202.1

Table 3.2 DNA concentrations of the domesticated wheat varieties after DNA isolation *via* CTAB method.

Name of the sample	Number assigned	Concentration (ng/µl)
Yıldız 98	3	72.8
Göksu 99	8	54.3
Bezostaya	9	72.3
Kırgız 95	14	60.3
Topbaş-Ekmeklik	15	61.8
Gün91	20	55.0
Dağdaş 94	22	50.1
Saraybosna	30	84.1
Zencirli	37	89.0
Atlı	38	126.2
Altın	40	21.9
İkizce	44	83.9
Prostar	46	83.4
Atilla-12	47	32.8
Aldane	48	51.8
Selimiye	49	62.6
Dropia	50	63.6
Golia	51	90.5
Tima	52	183.3
Nina	53	82.9
Sagittario	54	46.6
Yunak	55	11.1
Syrene Odeskaja	56	54.8
Albatros Odeskaya	57	57.2
Name of Cultivar	58	49.7
Krasunta Odeskaya	59	85.7
Esperia	60	49.0
Saroz 99	61	54.2
Bezostaya	62	51.6

Remaining samples are kindly isolated by my fellow Kubilay Yıldıray before the beginning of my thesis study. In addition to this, the samples were visualized in agarose gel electrophoresis (Figure 3.1).

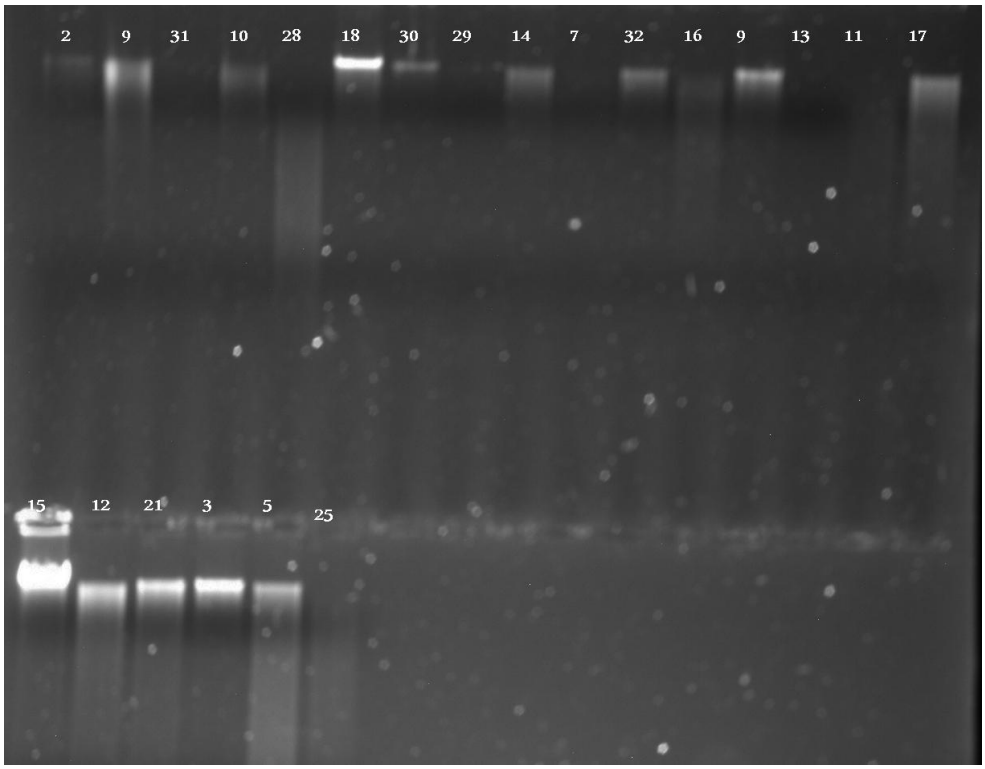


Figure 3.1 CTAB DNA isolation results of domesticated wheat samples. The numbers are the same as in Table 2.3.

2.3) Genetic screening

In this study, a total of 62 domesticated wheat varieties and 17 *Aegilops* species subjected to genetic screening. The results are shown below on the Tables 3.3 and 3.4.

Table 3.3 Results of genetic screening on domestic wheat varieties.

		Cssfr1	**Cssfr2**	**Cssfr1/2**	**Cssfr3**	**Cssfr4**	**Cssfr3/4**	**all**
		R-allele+	**S-allele+**		**R-allele++**	**S-allele+**		
1	Kınacı 97	-	+	S	+	+	H	I
2	Altay2000	-	+	S	+	+	H	I
3	Yıldız98	+	+	H	++	++	H	H
4	Orso	-	+	S	+	+	H	I
5	İkizce 96	-	+	S	+	+	H	I
6	Gerek 79	-	+	S	+	+	H	I
7	Bolal 2973	-	+	S	+	++	S	S
8	Göksu 99	+	-	R	++	+	R	R
9	Bezostaya	+	-	R	++	+	R	R
10	Sofu	-	-	N	-	-	N	N
11	ES 97 KE 10			I			I	I
12	Kırkpınar 79	-	+	S	+	+	I	I
13	Çakmak 79	-	-	N	+	-	I	I
14	Kırgız 95	-	-	N	+	-	I	I
15	Topbaş Ekmeklik	-	-	N	+	++	S	I
16	Orza 96/Hv	-	-	N	-	-	N	N
17	Kalkıç 88	-	+	S	+	++	S	S
18	Berbinen 469	-	+	S	+	++	S	S
19	4875/Hv	-	-	S	-	-	N	N
20	Gün 91	-	+	S	+	++	S	S
21	Kutluk 94	-	+	S	+	++	S	S
22	Dağdaş 94	-	+	S	+	++	S	S
23	Kate A1	-	+	S	+	++	S	S
24	Sultan 95	+	-	R	++	+	R	R
25	Flamura 85	-	+	S	+	++	S	S
26	Basribey 95	-	-	I	+		I	I
27	Pehlivan	-	+	S	+	++	S	S
28	Tekirdağ	+	-	R	++	+	R	R
29	EBDV 2	-	+	S	+	++	S	S
30	Saraybosna	+	+	H	++	++	H	H
31	Guadalupe	-	+	S	+	+	I	I
32	Gelibolu	+	-	R	++	+	R	R
33	BBVD 2	-	+	S	+	++	S	S
34	BBVD 15	-	+	S	+	+	I	I
35	Mirzabey 42	-	-	N			I	N
36	Tosunbey	-	+	S	+	++	S	S
37	Zencirli	+	+	H	++	++	H	H
38	Atlı	-	-/+	I	+	++	S	I
39	Seval	-	+	S	+	++	S	S
40	Altın	-	+	S	+	-	I	I

Table 3.3 Continued

		Cssfr1	Cssfr2	Cssfr1/2	Cssfr3	Cssfr4	Cssfr3/4	all
		R-allele+	S-allele+		R-allele++	S-allele+		
41	Demiş	-	+	S	+	+	U	U
42	Yılmaz	-	-	N	-	-	N	N
43	Uzunyayla	-	+	S	+	++	S	S
44	İkizce	-	+	S	+	+	U	U
45	Dağdaş Bread	-	+	S	+	++	S	S
46	Prootar	+	_	R		+	U	U
47	Atilla 12	-	+	S		?	U	U
48	Aldane	-	-	N		++	U	U
49	Selimiye	-	+	S			U	U
50	Dropia	+	-	R		+	U	U
51	Golia	-	+	S		?	U	U
52	Tima	-	+	S		++	U	U
53	Nina	-	+	S		++	U	U
54	Sagittarius	-	+	S		++	U	U
55	Yunak	-	+	S		++	U	U
56	Syrene Odeskaya	+	-	R		+	U	U
57	Albatros Odeskaya	-	-	N		+	U	U
58	Krasunta Odeskaya	+	-	R		+	U	U
59	Esperia	+	-	R		+	U	U
60	Serez 95	-	+	S		?	U	U
61	Bezostaya	-	+	S		+	U	U
62	ES 96	-	-	N			U	U

R denotes resistant allele. S denotes susceptible allele, H denotes heterozygous allele, and I denotes unclassified allele.

Table 3.4 Overall proportion of the samples found to be resistant susceptible and null for LR34 locus.

	Csffr1/2	Csffr3/4	Csffr1 and Cssfr2	Csffr3 and Cssfr4
No of null	-/-	-/-	11	5
No of resistant	+/-	++/+	10	5
No of susceptible	-/+	+/++	36	17
No of heterozygote	+/+	++/++	3	10
No of Indecisive			2	25

In addition to this, the samples were visualized in agarose gel electrophoresis (Figure 3.3, Figure 3.4, Figure 3.5, and Figure 3.6.).

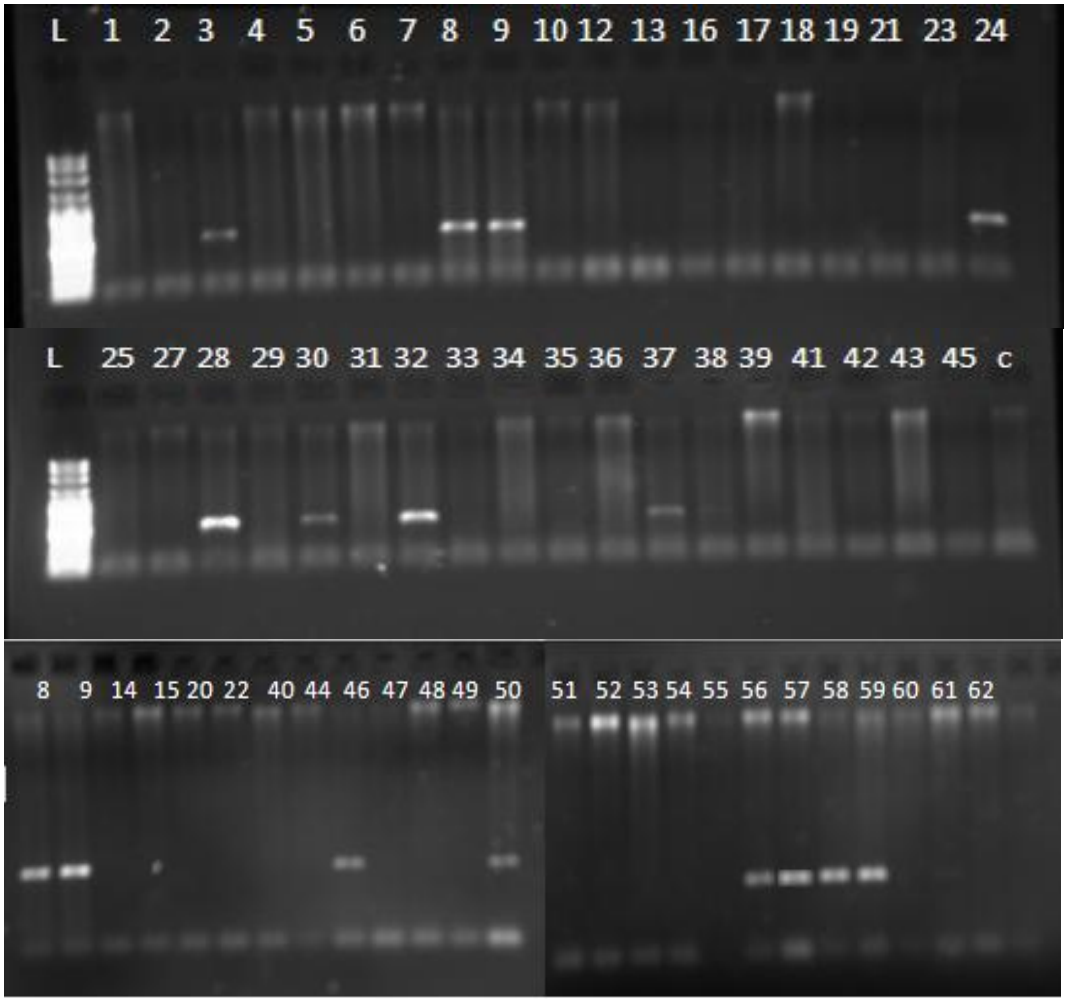


Figure 3.2 Agarose gel visualization of *Cssfr1* for domestic wheat varieties. The numbers are the same as in Table 2.3.

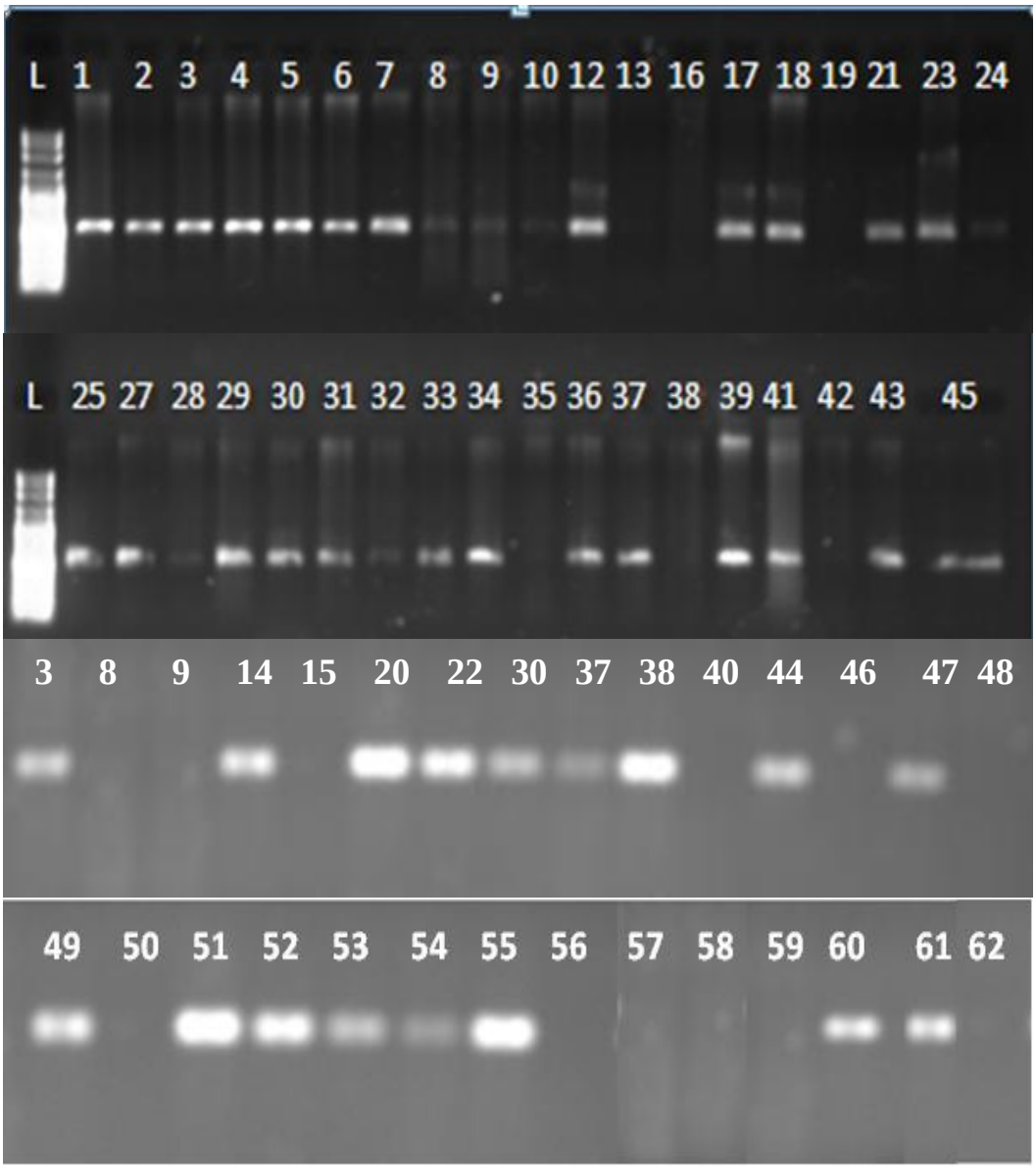


Figure 3.3 Agarose gel visualization of Cssfr2 for domestic wheat varieties. The numbers are the same as in Table 2.3.

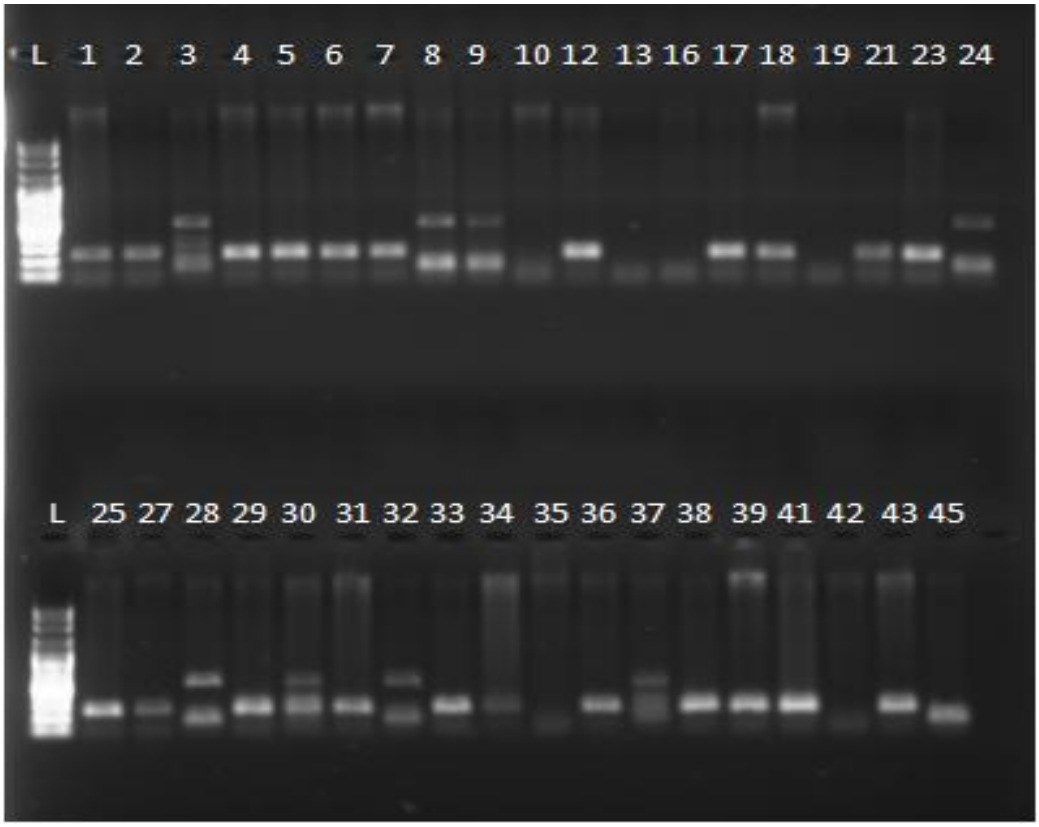


Figure 3.4 Agarose gel visualization of Cssfr3 for domestic wheat varieties. The numbers are the same as in Table 2.3.

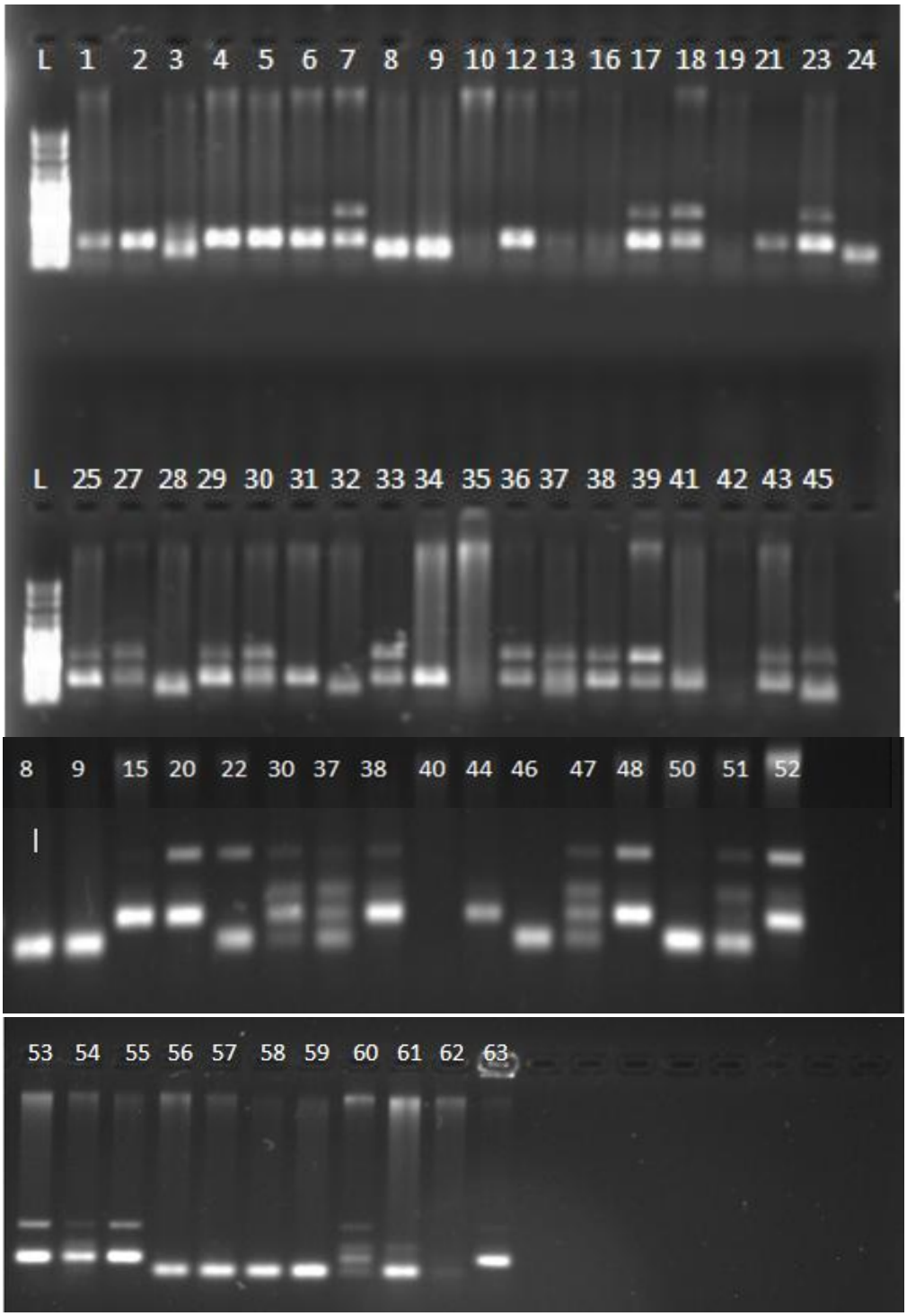


Figure 3.5 Agarose gel visualization of Cssfr4 for domestic wheat varieties. The numbers are the same as in Table 2.3.

In our study, we start with 17 Aegilops species, in order to determine the possible donor of the LR34 gene. Cssfr markers do not work in the *Aegilops* samples. In order to understand, whether there is no LR34 into *Aegilops* samples or whether there is a PCR problem, we performed Actin PCR for the *Aegilops* samples as positive controls (Table3.5, Figure 3.6).

Table 3.5 PCR results with the *Aegilops* samples.

No	Name	Cssfr1/2	Cssfr3/4	Actin
4	Aegilops cudata	-	-	+
7	Aegilops crassa	-	-	+
8	Aegilops crassa	-	-	+
9	Aegilops cylindirica	-	-	+
11	Aegilops cylindirica	-	-	+
12	Aegilops cylindirica	-	-	+
14	Aegilops cylindirica	-	-	+
16	Aegilops cylindirica	-	-	+
17	Aegilops cylindirica	-	-	+
25	Aegilops sqourossa	-	-	+
26	Aegilops ligustica	-	-	+
27	Aegilops speltoides	-	-	+
28	Aegilops tauschii	-	-	+
29	Aegilops tauschii	-	-	+
30	Aegilops tauschii	-	-	+
31	Aegilops tauschii	-	-	+
32	Aegilops tauschii	-	-	+

The numbers are the same as in Table 2.4.

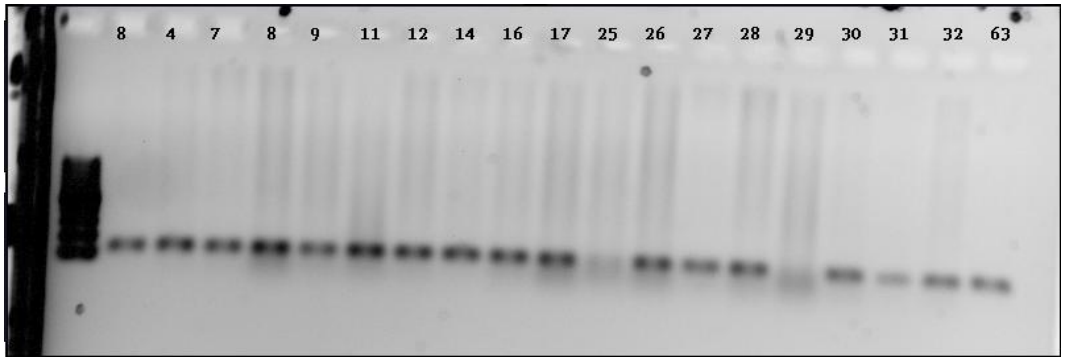


Figure 3.6 Actin PCR results with the *Aegilops* samples. The numbers are the same as in Table 2.4.

CHAPTER 4

DISCUSSION

4.1) DNA isolation

One of the most important points of DNA isolation process is the quality of DNA. The quality of the DNA isolated is a prerequisite for the experimental success. The major purpose to perform CTAB method based DNA isolation is to obtain pure DNA from the samples and use this DNA in PCR.

DNA was isolated from the leaves of the plant samples that are grown in the growth chamber of the laboratory. CTAB method is chiefly employed because of the method is suitable for isolation of plant DNA and its role of elimination of potentially contaminating poly-phenolic compound and polysaccharides. Basically, Plant DNA isolation via CTAB method separated three basic processes: incubation with CTAB, Separation with organic chloroform and acquiring DNA with polar ethanol and isopropanol (Steward et al. 1993). During the isolation procedure, the frozen material must remain frozen during the process. If not, hydration of sample allows the formation of the enzymatic reactions which may harm DNA and also formation of poly-phenolic compounds (Scarafoni et al. 2009).

During the isolation procedure, handling of the samples should be performed very carefully (Scarafoni and Duranti 2001). Grinding, handling and pipetting errors may result in sheering of the DNA strands and formation of unwanted chemicals. Moreover the chemicals employed during the isolation such as: chloroform, ethanol and isopropanol maybe the possible sources of contaminants and effect the success of the PCR reaction.

4.2) Screening for LR34 (R) allele

The wheat rusts are among the most effective and widespread wheat diseases. Rust diseases are ancient diseases but still they cause regular yield losses in even developed countries. Against deployment of resistance genes on the field against diseases, continuous evolution of rust diseases still possesses a great threat. Even, evolutionary potential of the rust species outpaced the race specific resistance genes in less than a decade.

Rust resistance genes become more effective when together. Presence of a only one resistance gene in a cultivar creates 22% difference of yield against an infection (Dyck and Lukow 1988).Wheat cultivars which possess resistance genes LR41 and LR42 reported to increase the yield 63% and %26 percent respectively (Martin et al., 2006). Today, leaf rust is among the one of the most important causes of yield reduction in wheat. Research of leaf rust resistance and search for new leaf rust resistance genes one of the major goals of the International Maize and Wheat Improvement Center (CIMMYT). Leaf rust infections all over the world and the resistances of newly developed cultivars are observed from the agricultural research stations of CIMMYT (Marasas et al. 2004).

Leaf rust, caused by *P. triticina* affects the hexaploid wheat but also effects tetraploid durum wheat, emmer wheat and *A. speltoides* and *A. cylindirica.*

Annual leaf rust infections, the yield loss that are caused and also the race phenotypes are recorded in Canada, since 1931, and in United States, since 1926 (Johnson et al. 1968). For each race specific resistance gene present in the wheat, there is a complementary virulence locus located in the genome of the pathogen (Kolmer and Dyck 1994).

In nature *P. triticina* populations are highly variable, chiefly because the evolutionary pressure of the presence of the race specific resistance genes. The variation in the pathogen populations is so important that even a single base mutation in an avirulent pathogen is enough for the pathogen to gain virulence against a resistance gene (Samborski and Dyck 1968. Kolmer 1992).

Molecular markers are employed in studies for the determination of the variation in the *P. triticina* populations. With the advent and employment of molecular markers on pathogen population's genetic relationships between *P. triticina* populations can be determined (Kolmer and Liu. 2000).

For additional purposes, quantitative Real Time PCR (qRT-PCR) should be performed on our samples, since it allows us to detect the gene expression differences happened in the presence of infection. And also allow us to understand the expression patterns and the expression sites of the LR34 gene.

4.3) LR34: This Study

In our study, we used recently developed markers by Lagudah et al. (2009), they target the interval between intron 9 to intron 13 of the LR34 gene located in the D genome. The primer combinations named as Cssfr1 and Cssfr2 were used in allele specific amplification of LR34 gene. These molecular markers are based on indel polymorphism in exon 11. In Cssfr1 and Cssfr2 marker system uses the same forward primer, but the Cssfr1 reverse primer is designed to align with the resistant allele and the Cssfr2 reverse primer is designed to align with the susceptible allele. Amplification of the resistant allele results in 517 bp product. On the other hand, amplification of the susceptible allele generates 523 bp product (Lagudah et al. 2009).

Cssfr3 and Cssfr4 marker system is the combination of Cssfr1 and Cssfr2 marker systems with the older csLV34 co-dominant STM (sequence tagged marker). When the LR34 is present, primers of csLV34 marker gives a product of 150 bp regardless of resistant and susceptible allele (Mc. Callum et al. 2008, Lagudah et al. 2009). Regardless of the breeding lineage, all LR34 containing genotypes give the same allele specific product generated by Cssfr1.

Determining the presence of LR34 in wheat cultivars and breeding lines is important for leaf rust resistance but also important for stripe rust, stem rust and powdery mildew resistance because resistance to these diseases is completely linked to LR34 (Spielmeyer et al. 2005).

CHAPTER 5

CONCLUSION

The main aim of this study was to demonstrate the presence of crucially important durable resistance conferring allele LR34 in Turkish wheat germplasm. For this purpose Turkish wheat varieties that we could found, were screened for the presence of resistant LR34 allele with the application of diagnostic markers.

Additionally, 18 *Aegilops* type species were also screened for the presence of resistant and susceptible LR34 allele in order to determine the possible evolutionary origin of resistant LR34 allele.

We used cultivar Bezostaja (#9) as a positive control since it is known to possess the resistant LR34 allele. For the screening with compatible Cssfr1/Cssfr2 molecular marker systems we found that among the cultivars screened, ten of the them and Bezostaja were found to be containing resistant LR34 allele: Göksu 99 (#8), Sultan 95 (#24), Tekirdağ (#28), Gelibolu (#32), Prootar (#46), Dropia (#50). Syrene Odeskaja (#56). Albatros Odeskaja (#57). Krasunta Odeskaja (#58) and Esperia (#59). Also among the cultivars we screened, we found 11 null alleles suggesting the likelihood of the absence of the LR34 locus in those genomes: Sofu (#10), Çakmak 79 (#13), Kırgız 95 (#14), Topbaş Ekmeklik (#15), Orza 98/Hv (#16), 4875/Hv (#19), MirzaBey 42 (#35), Yılmaz (#42), Aldane (#48), ES96 (#62). Among our results, there were heterozygous individuals that contain both

the resistant and susceptible alleles: Yıldız 98 (#3). Saraybosna (#30), Zencirli (#37). Remaining 35 wheat cultivars are determined to be susceptible (Table 3.3). The cultivars ES97 KE 10 (#11) and BasriBey (#26) seeds did not germinated thus, we simply did not produced data for them.

For the screening with the co-dominant marker systems Cssfr3/Cssfr4 the results were generally compatible with the results of Cssfr1/Cssfr2 marker system.

Using the Cssfr3/Cssfr4 marker system alone, we determined 4 resistant cultivars and Bezostaja(#9) which possess the resistant LR34 allele: Göksu 99(#8), Sultan 95 (#24). Tekirdağ (#28), Gelibolu (#32).

For the *Aegilops* species, which are the relatives of *Triticum aestivum*, we can conclude that their genomes do not possess the LR34 gene, at least in the form of the LR34 of *Triticum aestivum*.

In conclusion, with this study, we screened the Turkish wheat cultivars available, for the presence of LR34 resistant allele with two set of markers: Cssfr1/Cssfr2 and Cssfr3/Cssfr4 confirming each other.

To our knowledge, there are no reports of screening of LR34 in Turkey. Moreover, with the screening over *Aegilops* species, we can conclude that they do not possess LR34 allele in the form of LR34 from bread wheat. Although, the routine genetic screening for the detection of resistance alleles, especially for the long lasting durable resistance is carried out worldwide, to our knowledge, our study is the first of its kind that had been conducted on Turkish wheat cultivars possess. Thus, the cultivars

having the LR34 resistant allele verified can be used in breeding studies for crop improvement.

REFERENCES

Appels R. and Lagudah E. S., 1990, *Manipulations of chromosomal segments from wild wheat for improvement for bread wheat*., Aust. J. Plant. Physiol., 17: 253-366.

Arumuganthan K. and Earle E. D., 1991, *Nuclear DNA content of some important plant species.,* Plant Mol. Biol. Reptr., 9: 208-219.

Avivi L., 1978, *High grain protein content of the wild tetraploid wheat. Triticum dicocoides.,* p. 373-380. In S. Ramanajuam (ed.) Proc. Int. Wheat Genetic Symp., 5th. New Delhi. India . 23-28 February 1987. Indian Agric. Res. Inst., New Delhi. India.

Avivi L., Levy A. A. And Feldman M., 1983, *Studies oh high protein durum wheat derived from crosses with the wild tetraploid wheat. Triticum turgidum var. diccocoides*., p. 199-204. In S. Sakamato Proc. Int. Wheat. Genet Symp., 6th Kyoto . Japan. 28 Nov-3 Dec. 1983. Plant GermPlasm Inst., Fac. Agric., Kyoto Univ., Kyoto. Japan.

Bayaner. A., 2005, *Institutional structure of agriculture in Turkey*., The Ministry of Agriculture and Rural Affairs Strategy Development Presidency Publication. Ankara. Turkey.

Bennett J., 1981, *Biosynthesis of the light-harvesting chlorophyll a/b protein. polypeptide turnover in darkness.,* Eur. J. Biochem., 118: 61-70.

Bennett M. D. and Leitch I. J., 1995, *Nuclear DNA amounts in angiosperms.*, Annals of Botany., 76: 113–176.

Bennet M. D. and Smith J. B., 1976, *Nuclear DNA amounts in angiosperms*., Philosophical Transactions of Royal Society of London. B 274: 227-274.

Bossolini E., Krattinger S. G. And Keller B., 2006, *Development of simple sequence repeat markers specific for the LR34 resistance region of wheat using sequence information from rice and Aegilops tauschii.,* TAG. 113:1049-1062.

Caldwell R. M., Kraybill H. R., Sullivan J. T. and Compton J. E., 1934, *Effects of leaf rust(Puccinia triticina) on yield. physical characters and composition of winter wheats*., J. Agric. Res., 48:1049-1071.

Carleton M. A., 1915, *Hard wheats winning their way*., USDA Year-book 1914. U.S. Gov. Print Office. Washington. DC. p: 319–420.

Carver B. F., Martin J. N., Hunger R. M. and Cox T. S., 2003, *Contributions of leaf rust resistance and awns to agronomic and grain quality in winter wheat*., Crop. Sci. 43: 1712-1717.

Chapman B., Riley R. and Unrau J., 1958, *Evidence of the origin of the B genome of wheat*., The Journal of Heredity, 45: 92-98.

Chapman V., Miller T. E. and Riley R., 1976, *Equivalence of the A genome of bread wheat and that of Triticum urartu*., Genet. Res., 27: 69-76.

Coakley S. M., Boyd W. S. and Line R. F., 1984, *Statistical models for predicting stripe rust on winter wheat in the Pacific Northwest.,* Phytopathology. 72: 1539-1542.

Darwin C., 1859, *On the origin of species by means of natural selection or the preservation of favoured races in the struggle for life.,* p:18-24 London. J. Murray.

Dvorak J. and Appels P., 1982, *Chromosome and nucleotide sequence differentiation in genomes of polyploid Triticum species.,* TAG 63: 349-360.

Dvorak J. Di Terlizzi P. Zhang H. Resta P., 1992, *The evolution of polyploid wheats: identification of the A genome donor species.*, Genome, 36: 21–31.

Dvorak J. and Zhang H.B., 1990, *Variation in repeated nucleotide sequences sheds light on the phylogeny of the wheat B and G genomes.,* Proc. of the Nat. Aca. of Sci., 87: 9640-9644.

Dvorak J., Luo M. C., Yang Z. L. and Zhang H. B., 1998, *The structure of the Aegilops tauschii genepool and the evolution of hexaploid wheat.,* TAG, 97: 657–670.

Dyck P. L., 1994, *The transfer of leaf rust resistance from Triticum turgidum spp. Diccocoides to hexaploid wheat.*, Can. J. Plant Sci., 74: 671-673.

Dyck P. L. and Lukow O. M., 1988, *The genetic analysis of two interspecific sources of leaf rust resistance and their effect on the quality of common wheat.,* Can. J. Plant Sci., 68: 633–639.

Eastwood R. F., Lagudah E. S. and Appels R., 1994, *A direct search for DNA sequences tightly lined to cereal cyst nematode resistance genes in Triticum tauschii.,* Genome, 37: 311-319.

Feldman M., 2001, *The origin of cultivated wheat*., pp. 1–56 in The Wheat Book. edited by A. P. Bonjean and W. J. Angus. Lavoisier Publishing. Paris.

Feldman M. And Millet E., 2000, *The contribution of the discovery of wild emmer to an understanding of wheat evolution and domestication and to wheat improvement.,* Israel Journal of Plant Sciences, 49: S25-S35.

Feldman M., Liu G. And Segal G., 1997, *Rapid elimination of low-copy DNA sequences in polyploid wheat: A possible mechanism for differentiation of homoeologous chromosomes*., Genetics, 1381-1387

Furuta Y., Nishikawa K., and Yamaguchi S., 1986, *Nuclear DNA content in diploid wheat and its relatives in relation to the phylogeny of tetraploid wheat.,* Jpn. J. Genet., 61: 97–105.

Gale M. D. and Devos K. M., 1998, *Plant comparative genetics after 10 years.* Science, 282**:** 656–659.

Gill B. S. and Raupp W. J., 1987, *Direct genetic transfers from Aegilops squarrosa L. to hexaploid wheat.,* Crop Sci., 27: 445–450.

Gu Y., Anderson O. D., Londeore C., Kong X., Chibbar R. N. and Lazo G. R., 2004, *Structural organization of the barley D-hordein locus in comparison with its orthologous regions of wheat genomes.,* Genome. 46: 1084–1097.

Gupta M. J., Roy J. K. and Prasad M., 1999, *DNA chips. microarrays and genomics.,* Curr. Sci. in press.

Harlan J. R. and Dewet M. J., 1974, *Origin of cultivated plants: sympatric evolution in sorghum.,* Symposium on Origin of Cultivated Plants: XI. International Congress of Genetics.

He S., Ohm H. and Mackenzie S., 1992, *Detection of DNA sequence polymorphisms among wheat varieties.,* TAG, 84: 573-578.

Helentjaris T., Slocum M., Wright S., Schaefer A. and Nienhuis J., 1985, *Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms.,* TAG, 72. 6: 761-769.

Hofte H., Desprez T., Amselem J., Chiapello H., Rouze P., Caboche M., Moisan A., Jourjon M. F., Charpenteau J.L., Bertho P., Marcillac G., Gigot G. C., Fleck J.,Phillips J. G., Axelos M.,Bardet C. and Lescure B.,1993, *An inventory of expressed sequence tags obtained by partial sequencing of cDNAs from Arabidopsis thaliana.*, Plant., 4: 1051-1061.

Hylander N., Jorstad I. and Nannfeld N. A., 1953, *Enumeratio uredionearum Scandinavacarum.,* Opera Bot., 1.1-102.

Jin Y. and Singh R. P., 2006, *Resistance in U.S. wheat to recent African isolates of Puccinia graminis f. sp. tritici with virulence to resistance gene Sr31.,* Plant Dis., 476-480.

Johnson T., 1956, *Physiologic races of leaf rust of wheat in Canada.,* Can. J. Agric. Sc., 36: 323-332.

Johnson B. L. and Dhaliwal H. S., 1978, *Triticum urartu and genome evolution in the tetraploid wheats.,* Am. J. Bot., 65: 907–918.

Kellogg E. A., Appels R. and Mason-Gamer R. J., 1996, *When genes tell different stories: The diploid genera of Triticeae (Gramineae).,* Syst. Bot. 21: 321-347.

Kidwell K., Barret B. and Bayram M., 2002, *Identifying AFLP and microsatellite markers for vernalization response gene Vrn-B1 in hexaploid wheat using reciprocal mapping populations.,* Plant Breeding. 121:410-406.

Kihara H., 1944, *Discovery of the DD-analyser. one of the ancestral nature of polyploidy,.* Crit. Rev. Plant Sci., 12: 243–273.

Kimber G. and Feldman M., 1987, *Wild wheat: An introduction to special report no. 353. columbia*., College of Agric. Univ. of Missouri.

Koebner R. M. D., Donini P., Law J. R., Reeves J. C. and Cooke R. J., 1998, *Temporal trends in the diversity of UK wheat*., TAG, 100: 912-917.

Kolmer J. A. and Dyck P. L., 1994, *Gene expression in the Triticum aestivum-Puccinia recondita f sp. tritici gene-for gene system*., Phytopathology, 84: 437-440.

Kolmer J. A., Singh R. P., Garvin D. F., Viccars L., Williams H. M., Huerta-Espino J. Ogbonnaya F. C. and Lagudah E. S., 2008, *Analysis of the LR34/Yr18 rust resistance region in wheat germplasm*., Crop. Sci., 48: 1841-1852.

Konarev P. V., Volkov V. V., Sokolova A. V., Koch M. H. and Svergun D. I., 2003, *PRIMUS: a windows pc-based system for small-angle scattering data analysis*., J. Appl. Cryst. 36: 1277–1282.

Krattinger S. G., Lagudah E. S., Spielmeyer W., Singh R. P., Huerta-Espino J., McFadden H., Bossolini E., Selter L. L. and Keller B., 2009, *A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat.* Science, 323:1360–1363.

Kuraparthy V. P., Chhuneja P., Dhaliwal H. S., Kaur S., Bowden R. L. and Gill B. S., 2007, *Characterization and mapping of cryptic alien introgression from Aegilops geniculata with new leaf rust and stripe rust resistance genes.*, TAG, 114: 1379-1389.

Lagudah E. S., 1991, *The molecular-genetic analysis of Triticum tauschii: the D-genom.*, Genome, 34(3): 387–395.

Lagudah E. S., Mc. Fadden H., Singh R. P., Huerto-Espino J., Bariana H. S. and Spielmeyer B., 2006, *Molecular characterization of the LR34 /Yr18 slow rusting resistance gene region in wheat.*, TAG, 114:21-30.

Lagudah E. S., Krattinger S. G., Herrera-Foessel S., Singh R. P., Huerta-Espino J., Spielmeyer W., Brown- Guedira G., Selter L. L. and Keller B., *2009, Gene-specific markers for the wheat gene LR34/Yr18/Pm38 which confers resistance to multiple fungal pathogens.*, TAG, 119:889-898

Lassner W. M., Peterson P. and Yoder I. J., 1989, *Simultaneous amplification of multiple DNA fragments by polymerase chain reaction in the analysis of transgenic plants and their progeny.*, Plant. Mol. Biol. Reptr., 7: 116–128.

Leonard K. J., 2001, *Stem rust-future enemy*?. p 119-146. In P. D. Peterson(ed.) *Stem rust of wheat : From ancient enemy to a modern foe.*, Aps. press. St. Paul MN.

Lev-Yadun S.and Gopher A., and Abbo S., 2000, *The cradle of agriculture*., Science, 288:1602–2603.

Levy A. A. and Feldman M., 1987, *Increase in grain protein percentage in high-yielding common wheat breeding lines by genes from wild tetraploid wheat*. Euphytica. 26: 353-359.

Lilienfeld F.A. and Kihara H., 1934, *Genomeanalyse bei Triticum und Aegilops. V. Triticum timopheevi Zhuk.,* Cytologia., 6: 87-122.

Liu Y. G. and Tsunewaki K.,1991, *Restriction Fragment Length Polymorphism Analysis in Wheat*., Jpn. J. Genet., 66: 617-633.

Luo M. C., Yang Z. L. . Kota R. S., and Dvorak J., 2000, *Recombination of chromosomes 3Am and 5Am of Triticum monococcum with homeologous chromosomes 3A and 5A of wheat: The distribution of recombination across chromosomes.*, Genetics, 154: 1301–1308.

Mains E. B., 1930, *Effect of leaf rust (Puccinia triticina Erics.) on yield of wheat*., J. Agric. Res., 40:417-446.

Mains E. B., 1933, *Studies concernin heteroecious rusts*., Mycologia, 25:407-414.

Marasas C. N., Smale M. and Singh R. P.,2004, *The economic impact in developing countries of leaf rust resistance breeding in CIMMYT-related spring bread wheat.,* Economics Program Paper 04-01. CIMMYT. Mexico D.F., 36.

Martin J. A., Murphy R. A. and Power R. F. G.,2003, *Cloning and expression of fungal phytases in genetically modified strains of Aspergillus awamori.,* J. Ind. Microbiol. Biotech., 30: 568–576.

Martin J. M., Meyer F. D., Smidansky E. D., Wanjugi H. Blechl A. E. and Giroux M. J., 2006, *Complementation of the Pina (Null) allele with the wild type Pina sequence restores a soft phenotype in transgenic wheat.,* TAG, 113: 1563–1570.

Ma Z. Q. and Lapitan N. L. V., 1998, *A comparison of amplified and restriction fragment length polymorphism in wheat*., Cereal Res. Commun., 26: 7-13.

Ma X. P. and Gustafson J. P., 2005, *Genome evolution of allopolyploids.,* Cytogenet. Genome Research, 109: 236-249.

Mc. Callum B. D. and Seto-Goh P., 2006, *Physiological specialization of Puccinia triticina. the casual agent of wheat leaf rust in Canada in 2004*., Can. J. Plant Pathol., 28:566-576.

McIntosh R. A., 1992, *Close genetic linkage of genes conferring adult plant resistance to leaf and stripe rust of wheat.,* Plant Pathology, 41: 523-527.

McIntosh R. A., Yamazaki Y., Devos K. M., Dubcocski J., Rogers J. and Appels R., 2007, *Catalogue of gene symbols for wheat*., verified at komugi intergrated wheat science database.

McIntosh R. A., 1988, *A catalogue for gene symbols for wheat.,* Inst. Of Plant Science Research, Cambridge.

Morrison W. R., 1988, *Lipids in cereal starch : A review*. J. Cereal Sci., 8: 1-15.

Muramatsu M., 1986, *The vulgare super gene. Q: its universality in durum wheat and its phenotypic effects in tetraploid and hexaploid wheats.* Can. J. Genet. Cytol., 28: 30–41.

Nevo E., Korol A. B., Beiles A. and Fahima T., 2002, *Evolution of wild emmer and wheat improvement. Population genetics. genetic resources. and genome organization of wheat progenitor:Triticum dicoccoides.*, Springer Verlag,Heidelberg.

Oğuz C., 1991, *Konya ilinde kuru koşullarda tahıl mercimek yetiştiren tarım işletmelerinin ekonomik faaliyet sonuçlarının değerlendirilmesi*(Basılmamış Doktora Tezi)., Ç.Ü.Fen Bilimleri Enstitüsü. Adana.

Ohtsuka I., 1998, *Origin of the central European spelt wheat.,* In: Slinkard AE (ed) Proc 9th Int Wheat Genet Symposium. University Extension Press. University of Saskatchewan. Canada. p: 303–305.

Okamoto M., 1962, *Identification of the chromosomes of common wheat belonging to the A and B genomes*., Can. J. Genet. Cytol., 4: 31-37.

Ozkan H. and Feldman M., 2001,*Genotypic variation in tetraploid wheat affecting homoeologous pairing in hybrids with Aegilops peregrina*. Genome, 1000-1006.

Plucknett D. L., Smith N. J. H., Williams J. T., and Anishetty N. M., 1983, *Crop germplasm conservation and developing countries*., Science, 220: 163-169.

Rafalski J. A., Vogel J. M., Morgante M., Powell W., Andre C.and Tingey S., 1996, *In: Birren B. Lai E (eds) Non-mammalian genome analysis: a practical guide.,*Academic Press. New York. p: 75–134.

Rees H. and Walters M. R., 1965, *Nuclear DNA and the evolution of wheat.,* Heredity, 20:73–82.

Reiter R., Williams S. G. K., Feldmann K. A., Rafalski A., Tingey S. V. and Scolnik P. A., 1991, *Global and local genome mapping in Arabidopsis thaliana by using recombinant inbred lines and random amplified polymorphic DNAs.,* Proc.Natl. Acad. Sci., 89: 1477-1481.

Roelfs A. P., 1985, *Wheat and rye stem rust*. p 4-38. In A. P. Roelfs and W. R. Busnell edition. The Cereal Rusts. Academic Press Orlanda FL.

Roelfs A. P., Singh R. P. and Saari E. E., 1992, *Rust diseases of wheat: Concepts. methods and disease management*., CIMMYT D.F. Mexico.

Sax K.,1922, *Sterility in wheat hybrids: 11. Chromosome behavior in partially sterile hybrids.,* Genetics, 7**:** 513-552.

Scarafoni A., Ronchi A. and Duranti M., 2009, *A real-time PCR method for the detection and quantification of lupin flour in wheat flour-based matrices.,* Food Chemistry, 115: 1088-1093.

Sears E.R., 1977, *An induced mutant with homoeologous pairing in common wheat.,* Can. J. Genet. Cytol., 19:585–593.

Shaked H., Kashkush K., Ozkan H., Feldman M., and Levy A.A., 2001, *Sequence elimination and cytosine methylation are rapid and reproducible responses of the genome to wide hybridization and allopolyploidy in wheat.,* Plant Cell, 13: 1749–1759.

Singh R. P. and Rajaram S., 1992, *Genetics of adult plant resistance in Frontana and three CIMMYT wheat*., Genome, 35: 24-31.

Singh R. P., Nelson J. C. and Sorrells M. E., 2000, *Mapping Yr28 and other genes for resistance to stripe rust in wheat.,* Crop. Sci., 40: 1148-1155.

Singh R. P., Huerta-Espino J., Pfeiffer W. and Figueroa-Lopez P., 2004, *Occurrence and impact of new rust race on durum wheat in north-western Mexico from 2001 to 2003.,* Plant Dis., 88: 703-708.

Smith B. D., 1995, *The emergence of agriculture. Scientific American Library*. New York.

Soltis D. E. and Soltis P. S., 1999, *Polyploidy: Recurrent formation and genome evolution.* Trends. Ecol. Evol., 14: 348–352.

Singh R. P.,Trethowan P. M., 2007, *Breeding spring bread wheat for irrigated and rainfed production systems of developing world*.,. In: M. Kang and P.M. Priyadarshan (eds.). Breeding Major Food Staples. Blackwell Publishing. p: 109-140.

Spielmeyer W., McIntosh R. A., Kolmer J. A. and Lagudah E. S., 2005,*Powdery-mildew resistance and LR34/Yr18 genes for durable resistance to leaf and stripe rust cosegragate ata a locus on the short arm of the chromosome 7D of wheat*., TAG, 111: 731-735.

Spielmeyer W., Singh R. P., McFadden H., Wellings C. R., Huerta-Espino J., Kong X., Appels R. and Lagudah E. S., 2008, *Fine scale genetic and physical mapping using interstitial deletion mutants ofLR34/Yr18: a disease resistance locus effective against multiple pathogens in wheat.,* TAG, 4: *481-490*

Stebbins G. L., 1947, *The origin of the complex Bromus carinatus and its phytogeographic implications.,* Contr. Gray Herb., 165: 42-55.

Steward G. F., Wikner J., Cochlan W. P., Smith D.C. and Azam F., 1993, *Measurement of virus production in the sea. 11. Field results.,* Mar. Microb. Food Webs, 6: 79-90.

Stubbs R. W., 1985, *Stripe rust.* In: Roelfs AP. Bushnell WR (eds) The cereal rusts. Academic Press. New York. Vol II. p: 61–101.

Talbert L.E., Blake N. K., Chee P. W., Blake T. K. and Magyar G. M., 1994, *Evaluation of sequence tagged site facilitated PCR products as molecular markers in wheat* ., TAG, 87: 789-784.

Tang H., Yamada K., Lim J., Shinn P., Banno F. and Ecker J. R., 2008, *Syntenyandcolinearityinplantgenomes.,* Science, 320: 486–488.

Tsunewaki K., 1968, *Origin and phylogenetic diffrentiation of common wheat revealed by comparative analysis*., In: proc. 3rd Int. wheat. genet. synp. Canberra . p:71-78.

Upadhya M. D. and Swaminthan S., 1963, *Genome analysis in Triticum zhukovskyi. a new hexaploid wheat*., Chromosoma, 14: 589-600.

Vaccino P., Becker h., Brandolini a., Salamini F. and Kilian B., 2009, *A catalogue of triticum monococcum genes encoding toxic and immunogenic peptides for celiac disease patients*., Mol. Genet. Genomics, 281: 289–300.

Vedel F. and Delseny M., 1987, *Repetitivity and variability of higher plant genomes*., Plant Physiol. Biochem., 25: 191 -210.

Wendel J. F., 2000, *Genome evolution in polyploids*., Plant Molecular Biology, 42: 225-249.

Williams M. E., 1993, *In: Sheridan WF (ed) Abstr 35th Annu Maize Genet Conf*. University of North Dakota. p: 46.

Yildirim F. and Akkaya M. S., 2006, *DNA fingerprinting and genetic characterization of Anatolian Triticum spp. using AFLP markers*., Genet. Resour. Crop. Evol., 53: 1033-1042.

Zohary D., 1969, *The progenitors of wheat and barley in relation to domestication and agricultural dispersal in the Old World*., in P. J. Ucko and G. W. Dimbleby. eds., The domestication and exploitation of plants and animals. Duckworth. London. UK. p: 47-66.

Zohary D. and Feldman M., 1962, *Hybridization between amphiploids and the evolution of polyploids in the wheat (Aegilops-Triticum) group*., Evolution, 16: 44–61

Zohary D. and M. Hopf., 2000, *Domestication of plants in the Old World.* 3rd ed. Oxford University Press, Oxford. UK.