GENETIC DIVERSITY OF SCALD (RHYNCHOSPORIUM SECALIS) DISEASE RESISTANT AND SENSITIVE TURKISH BARLEY SEED SOURCES AS DETERMINED WITH SIMPLE SEQUENCE REPEATS

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

AYTEN DİZKIRICI

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
BIOLOGY

AUGUST 2006

Approval of the Graduate School of Natural and Applied Sciences

_____

Prof. Dr. Canan ÖZGEN
Director

I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science.

_____

Prof. Dr. Semra KOCABIYIK
Head of the Department

This is to certify that we have read this thesis and that in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

_____

Prof. Dr. Zeki KAYA
Supervisor

Examining Committee Members

Prof. Dr. Zeki KAYA	(METU, BIO)	_____
Prof. Dr. İnci TOGAN	(METU, BIO)	_____
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Assoc. Prof. Dr. İrfan KANDEMİR	(Z. Karaelmas Unv.BIO)	_____

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Name, Last Name: Ayten DİZKIRICI

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ABSTRACT

GENETIC DIVERSITY OF SCALD (RHYNCHOSPORIUM SECALIS) DISEASE RESISTANT AND SENSITIVE TURKISH BARLEY SEED SOURCES AS DETERMINED WITH SIMPLE SEQUENCE REPEATS

Dizkırıcı, Ayten

M.S., Department of Biology

Supervisor: Prof. Dr. Zeki Kaya

August 2006, 91 pages

Scald disease (*Rhynchosporium secalis*) is one of the major plant diseases causing considerable yield loss in barley (*Hordeum vulgare*) plantations in Turkey. To develop, scald resistant barley varieties, C.R.I.F.C. of Turkey has a large accumulated collection of barley seed sources in hand, but these samples are difficult to be followed and used in the breeding programs due to lack of genetic studies on them. Thus, the objective of this study was to characterize and fingerprint of eighty barley seed sources, and assess the magnitude and pattern of genetic diversity that could be used to have more efficient scald disease resistant breeding programs in the future.

Forty scald disease resistant and 40 scald sensitive Turkish barley seed sources were screened using 6 simple sequence repeats (SSR) primers. Each of barley seed source were represented with four seeds, assuming they are genetically uniform since barley is a self-pollinated crop. Estimated genetic parameters indicated that scald disease resistant and sensitive barley seed sources still maintain large amount of genetic diversity. For example, expected heterozygosity was 0.62±0.01 and 0.64±0.01 for resistant and sensitive Turkish barley seed sources, respectively.

Thirty-nine percent of total genetic variation was between populations for resistant and 46% for sensitive group, while 61% of total variation was within populations for resistant group and 54% for sensitive group.

When overall Turkish barley seed sources were considered, genetic distances between scald sensitive seed source S18 and resistant R1 as well as between sensitive S28 and resistant R1 were large. Scald resistant and sensitive barley seed sources were generally located in different clusters in dendrogram.

The presence of R25, R39 and S16 barley seed sources with high genetic diversity parameters among studied seed sources, suggests that this diversity could be important drive in future barley breeding program in Turkey. However, further study is needed to illustrate genetic divergence of Turkish barley seed sources with use of more molecular markers.

Key Words: *Hordeum vulgare*, SSR markers, genetic diversity, barley accessions, scald disease.

ÖZ

YAPRAK LEKESİ HASTALIĞINA (RHYNCHOSPORIUM SECALIS) KARŞI DİRENÇLİ VE HASSAS OLAN TÜRK ARPA TOHUM KAYNAKLARINDA, BASİT DİZİ TEKRARLARI KULLANILARAK GENETİK ÇEŞİTLİLİĞİN BELİRLENMESİ

Dizkırıcı Ayten

Yüksek lisans, Biyoloji Bölümü

Tez Yöneticisi: Prof. Dr. Zeki Kaya

Ağustos 2006, 91 sayfa

Yaprak lekesi hastalığı (*Ryhnchosporium secalis*) Türkiye'de önemli arpa (*Hordeum vulgare*) ürün kayıplarına neden olan ciddi hastalıklardan bir tanesidir. Türkiye'deki Tarla Bitkileri Merkez Araştırma Enstitüsü, yaprak lekesi hastalığına karşı dayanıklı olan arpa çeşitleri geliştirmek için çok sayıda arpa tohum kaynaklarını elinde bulundurmaktadır. Fakat genetik çalışmaların yetersizliğinden dolayı bu örnekleri üretim programlarında takip etmek oldukça zordur. Bu çalışmanın amacı arpa tohum kaynaklarını genetik olarak karakterize etmek, izlemek ve yaprak lekesi hastalığına dayanıklılık ıslah programlarında daha etkili kullanabilmek için tohum kaynaklarında genetik çeşitliliğin boyutunu ve yapılaşmasını belirlemektir.

Yaprak lekesi hastalığına karşı 40 adet dirençli ve 40 adet hassas Türk arpa tohum kaynağı 6 tekrarlanan basit dizi (SSR) belirteçleriyle taranmıştır. Arpa kendilenen bir bitki olduğu için, herbir arpa tohum kaynağını temsilen genetik olarak aynı olduğu varsayıln dört tohum kullanılmıştır. Tahmin edilen genetik parametreleri yaprak lekesi hastalığına dirençli ve hassas arpa tohum kaynaklarının hala yüksek oranda genetik çeşitliliğe sahip olduklarını göstermiştir. Örneğin beklenen heterozigotluk dirençli arpa tohum kaynaklarında 0.62±0.01 iken hassas thum kaynaklarında 0.64±0.01'dir.

Toplam genetik çeşitliliğin %39'unun populasyonlar arasında, %61'inin populasyon içinde, hassas örnekler için % 46'sının populasyonlar arasında, % 54'ünün populasyon içinde olduğunu göstermektedir.

Bütün Türk arpa tohum kaynakları düşünüldüğünde yaprak lekesi hastalığına karşı hassas S28 nolu ve dirençli R1 nolu tohum kaynakları yanında hassas S18 nolu ve dirençli R1 nolu tohum kaynakları arasında ki genetik mesafe en yüksektir. Yapılan küme analizinde yaprak lekesi hastalığına karşı dirençli ve hassas olan tohum kaynakları genellikle farklı kümelere yerleşmişlerdir.

Yüksek genetik çeşitliliğe sahip olan R25, R39 ve S16 arpa tohum kaynakları, Türkiye'de gelecekte yapılacak olan arpada yaprak lekesi hastalığına dayanıklılık ıslah çalışmalarına önemli ivme kazandırabilir. Bunun yanında arpa tohum kaynaklarındaki genetik farklılaşmaları daha iyi tesbit edebilmek için, çok sayıda molekuler belirteçlerin kullanıldığı yeni çalışmalara gereksinim duyulmaktadır.

Anahtar Kelimeler: *Hordeum vulgare*, SSR belirteçleri, genetik çeşitlilik, Arpa,Yaprak lekesi hastalığı.

to my mother and father…

ACKNOWLEDGEMENTS

I am greatly indebted to my supervisor, Prof. Dr. Zeki KAYA for his guidance, supervision and endless patience throughout the study.

I would like to express my thanks to all jury members for their comments and criticism.

I would like to thank you Dr. Taner AKAR from the Central Research Institute for Field Crops (Ankara, Turkey) for providing seed sources.

I wish to express my deep gratitude to Assoc. Prof. Dr. İrfan KANDEMİR, Dr. Gaye KANDEMİR, Dr. Burcu ÇENGEL and Dr. Yasemin İÇGEN for their continuous help, advice, collaboration and encouragement in every step of this study.

I would like to thank you all colleagues from Department of Biology, Plant Genetics and Tissue Culture Laboratory; especially, Elif GÜREN, Zeynep ÖZKURT and Özge TOZKAR for their support.

I owe special thanks to my dear family: to my parents, Şaban and Firdes DİZKIRICI, my sister Aysel MİLANLIOĞLU, and my brother Ali DİZKIRICI, for their continuous love, support, understanding and encouragement through all my life. Their support is so precious to me.

Also, I would like to express my gratitude to Yunus DÜMEN, Ümmühan EREN and Şükrü-Sıdıka ATALAY and their children Büşra, Orhan, Hakan for their support during my life in Ankara.

And very special thanks to my brother in law Koray MİLANLIOĞLU for his faith in me and endless love, help, support, encouragement, understanding and patience.

This study was funded by METU Research Foundation (METU Research Fund Project: 2006-0702-0001) and TUBITAK for providing fellowship from the TUBITAK (TBAG-2357-103T178) project.

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LIST OF ABBREVIATIONS

APS	Ammonium Per Sulphate
AFLP	Amplified Fragment Length Polymorphism
Avr	Avirulence
β-ME	Beta Mercapto Ethanol
CRIFC	Central Research Institute for Field Crops
CTAB	Cetyl Trimethyl Ammonium Bromide
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribose triphosphate
EDTA	Ethylene Diamine Tetra Acetic Acid
GDA	Genetic Data Analysis
HR	Hypersensitive Response
LRR	Leucine Rich Repeats
MAS	Marker Assisted Selection
NBS	Nucleotide Binding Site
PAGE	Polyacrilamide Gel Electrophoresis
PCR	Polymerase Chain Reaction
QRL	Quantitative Resistance Loci
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorfic DNA
RFLP	Restriction Fragment Length Polymorphism
STS	Sequence Tagged Site
SSR	Simple Sequence Repeat
TBE	Tris Boric acid EDTA (Ethylene diamine tetra aceticacid disodium salt)
TE	Tris EDTA Buffer
UPGMA	Unweighted Pair Group Method using Arithmetic Averaging

CHAPTER I

INTRODUCTION

Barley is the fourth most important cereal in the world in terms of world production after wheat, rice, and corn. It is a cereal adapted to and produced over a wider range of environmental conditions than any other cereal. Barley has a significant place in the world's food supply as human food, malt products, and livestock feed. Also it serves as an important experimental or model plant species for numerous studies in malting and brewing chemistry, biochemistry, biology, and biotechnology (Duke, 1983).

1.7. Description and Morphology of Barley (*Hordeum sp*.)

Hordeum is characterized by having one-flowered spikelets and usually a long, subulate, and hairy rachilla. The second (sometimes even a third) sterile flower may rarely be developed at the tip of the rachilla, both on the central and lateral spikelets. The one-flowered spikelets (a triplet) are found on oppositely alternating sides at each node of the flat rachis. In the triplet the central spikelets are bisexual, and the lateral spikelets are either sterile or rudimentary (for example, two-rowed cultivated barley), male fertile or rarely perfect. This character may be rather variable in the wild species, as it is genetically as well as environmentally determined.

Head — Barley heads may have either six rows or two rows of kernels. In 6-row barley there are three kernels at each joint (node) on alternate sides of the head, resulting in six rows of kernels. In 2-row barley only one kernel develops at each joint on alternate sides of the head, resulting in two rows of kernels.

There are some differences in the appearance of kernel between 6-row and 2- row barley varieties. This situation is useful in variety identification. In kernel of 6-row varieties, two-thirds are twisted in appearance. These are the lateral or side kernels as they grow alongside and overlap the central kernels. However in kernel of 2- row varieties, all of them are straight and symmetrical because there are no lateral kernels. The density of a head of barley depends on the length of the rachis internodes. If these internodes are short, the kernels are crowded and the head is dense. If the internodes are long, the head is lax as the kernels are spaced farther apart.

Grain structure — Barley grain is comprised of the caryopsis and the enclosing hull (husk) formed from the lemma and palea (Figure 1.1). The caryopsis consists of the pericarp, aleurone layer, endosperm, and germ or embryo. The pericarp is developed from ovary walls and acts as a protective cover for kernel. The endosperm is a starchy mass and is a source of nutrients for the developing embryo. The aleurone is the outer layer of the endosperm cells. The embryo is located at the end of the caryopsis on its dorsal side.

Hull — Barley varieties have either covered or naked kernels, generally referred to as hulled or hull-less. This character is stable and therefore very useful. In hulled varieties there are differences in the tightness of adherence of the hull. The hull is the inedible outer coating of the kernel. The degree of adherence of the hull is subject to variation due to environment but is a useful character in some varieties. Hulless barley is convenient and is becoming increasingly popular both for human nutrition and as feed for livestock. In hulless barley, unlike covered barley, the hull is removed during harvesting. If completely removed, there is generally considerable damage to the kernel, that is, cracked and broken kernel and (or) germ removed (Kling, 2004).

Glume — Glume may have different lengths. Sometimes it is completely covered with hairs. In some varieties this character is constant while in other

varieties it is variable. Most barley has two glumes that are equal in size and shape (Wiebe and Reid, 1961).

Awn — The lemma of barley usually terminates in an awn that varies in length from 5 cm to 3 cm. Also sometimes, instead of an awn, it may terminate in a three-lobed appendage known as a hood. The long-awned barleys have awns which are longer than spike. The length of these awns is somewhat influenced by environment. There is not any barley strain in which the awns on the lateral kernels are consistently longer than the awn on the central floret (Reid and Wiebe, 1979). In some barleys the awn is deciduous, in other words it drops when the kernel is near-ripe stage.

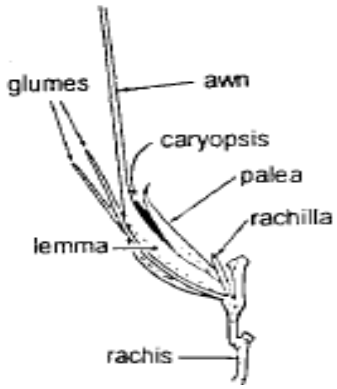


Figure 1.1. Structural details of small barley grain inflorescences (University of California Statewide IPM project, Publication no: 3333)

1.2. Taxonomy of Barley

Barley is one of the oldest and widely grown cereal crops in the world. It belongs to the genus *Hordeum*, tribe *Triticeae*, family *Poaceae* (*Gramineae*) that is the largest family of monocotyledonous plants. Besides cultivated barley, *H. vulgare,* there are 31 species in this genus (Bothmer *et al.*, 1995). With about 10 000 species, the *Poaceae* family hold the fourth place in the list of the largest plant families, behind the Daisy family, the Orchids, and the Pea family. This

family includes wheat, barley, rice, rye, oat and corn, providing the grain that is the staple food of most of mankind and the major type of feed. Compared with wheat, the taxonomy and evolution of barley is relatively straight forward, with the term barley only used to describe one species, *Hordeum vulgare*. However, a number of different subspecies have been identified growing wild in and around the Fertile Crescent (Figure 1.2).

1.3. Origin of Cultivated Barley

Barley was the first domesticated cereal, most likely originating in the Fertile Crescent area (Figure 1.2). It was probably first used in agriculture in Western Asia, perhaps as early as 7000 BC. Since the first description of the wild barley (Figure 1.3), *Hordeum spontaneum* (a two-row form also known as *Hordeum vulgare* var. *spontaneum*), the opinions and disagreements about the common ancestor and the origin of cultivated barley (Figure 1.4) arose more than one hundred years ago.

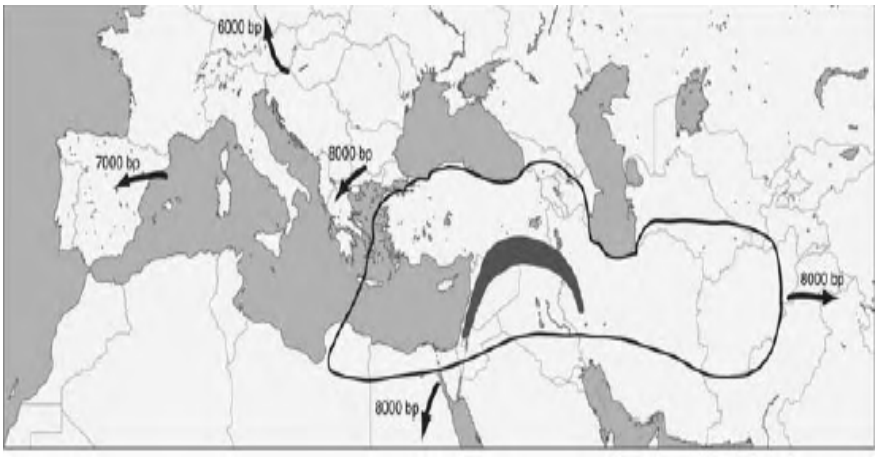


Figure 1.2. The Fertile Crescent, the area of early domestication of cultivated barley (*H. vulgare* L. ssp. *vulgare*) in the Middle East, distribution of the wild progenitor of barley (*H. vulgare* L. ssp. *spontaneum*) (within solid line) and approximate time, year before present (BP) for cultivated barley to reach different areas (Bothmer *et al.*, 2003).

Most of the cultivated barleys have been classified into the two groups, *H. vulgare* L., the 6-rowed barleys, and *H.distichum* L., the 2-rowed types. The most likely progenitor of cultivated varieties is a wild species of the genus *Hordeum* found wild in areas of southwestern Asia. Barley and other cereals such as wheat, and rye are defined as domesticated when they possess a tough rachis, which is a prerequisite for an effective harvest. The establishment of non-brittle mutants was probably the first selection criterion in early agriculture. *Hordeum spontaneum* C. Koch is a 2-rowed type with brittle rachis and was first described in 1848. It has great potential for breeding purposes. It is highly variable and belongs to the primary gene pool of barley as there are no biological sterility barriers in crosses with the crop. All other *Hordeum* species, except *H. bulbosum* (secondary gene pool) belong to the tertiary gene pool (Figure 1.5) *H. bulbosum* shares the basic H genome with cultivated barley, but crosses with some difficulty to the crop (Bothmer *et al,.*2003). A wild 6-rowed barley *Hordeum agriocrithon* was found by Aberg in 1938 and was previously considered to be the progenitor of cultivated 6-rowed barleys.

Figure 1.3. Wild Barley (Mattana, 2004)

Studies have shown that the accidental crossing between a wild two-row form (*Hordeum spontaneum*) and a six-row form (*Hordeum vulgare*) produces a six-row form (*Hordeum agriocrithon*). However, if left alone for several generations, this six-row form gradually reverts to the wild two-row species. Six-

row types of barley have poor dispersal as the seeds are held within the seed heads at maturity, falling out only with mechanical threshing by humans. These barleys are termed non-brittle (Wilson, 1955).

Figure 1.4. Cultivated Barley (www.hgca.com/images/upload/barley.jpg)

The wild two-row progenitors are brittle, and the seeds readily drop from the heads when mature. This allows wild barleys to be more successful at colonizing new habitats away from human influence, while the cultivated six-row forms are dependent upon humans for their dispersal.

1.4. Distribution and Economic Importance of Barley in the World

Barley is grown in a range of extreme environments that vary from northern Scandinavia to the Himalayan Mountains. Very few other plant groups show such a wide adaptation as does the barley. Barley is noted for its tolerance to cold, drought, alkali, and salinity. Egyptian scripts suggest that barley is more important than wheat for human food because of its tolerance against salt. It requires less water than many other cereal crops and much of the world's production is in sub-humid or semi-arid regions. Barley grows well on well-drained soils, which need

not be as fertile as those required for wheat. Its rapid growth enables it to compete well with weeds and other grasses (Nilan and Ullrich, 1993).

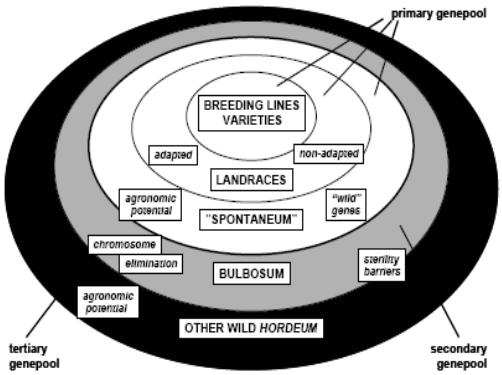


Figure 1.5. Gene pools in cultivated barley (Bothmer *et al*., 2003).

Barley is the major grain crop for feed and food in Northern area of the world, or at high elevations where its short growing season makes it more dependable than wheat or oats. It is the most important cereal grain for malting because of special physical and chemical properties. While most of the annual production is used for this purpose annually, small quantities of unmalted barley are used for food products (Nilan and Ullrich, 1993).

1.5. Barley Genome

Barley is not only an important crop worldwide, but also an excellent system for genome mapping and map-based analysis (Costa *et al*., 2001). The three main advantages of barley for genetic studies are that: (1) barley is a diploid (2n=14) with seven cytological distinct chromosomes containing approximately 5.3×10^9 bp DNA (Bennett and Smith, 1976); (2) barley chromosomes are homologous to those of common wheat (Moore *et al.,* 1995), which allows barley to serve as a model system for the more complex polyploid cereals; and (3) barley doubled-

haploid (DH) reference populations can be produced with relative ease, allowing repeated phenotyping and genotyping to generate genetic maps of qualitative and quantitative trait loci (Chen and Hayes, 1989).

In barley, as in other cereals, the genome consists of a complex mixture of unique and repeated nucleotide sequences (Flavell, 1980). Approximately 10-20 % of the barley genome is tandemly arranged repeated sequences while 50-60 % is repeated sequences interspersed among one another or among unique nucleotide sequences (Rimpau *et al.,* 1980).

1.6. Cultivars of Barley

Barley can be divided by the number of kernel rows in the head. Two forms have been cultivated; two-row barley (traditionally known as *Hordeum distichum*), and six-row barley *(Hordeum vulgare*). In both two- and six-row barley, each individual node of the rachis has three spikelets, but the fertility (or sterility) of the florets differs in each type. In six-row barley, all three spikelets (per rachis node) contain a fertile floret. These florets develop into kernels and thus each rachis node in the mature spike of six-row barley has three kernels. When the rachis is viewed from one side, there appears to be three rows of kernels (Figure 1.6). Barley has multiple stems (tillers) per plant, with many of the stems producing spikes. Two-row barley plants generally have more spikes per plant, but the number of tillers is greatly influenced by environmental conditions.

1.7. Chemical composition and significance

Barley grain composition and quality are influenced by environmental conditions. These are temperature, day length, water condition, and some minerals in the soil. A proximate composition of barley grain as reported by MacGregor (1993) is presented in Table 1.1.

Figure 1.6. Two and six - row of Barley (Mueller, 2006)

Table 1.1. Barley's grain composition (MacGregor, 1993)

Component	**Content (% dry weight)**
Starch	60–64
Arabinoxylans	4.4–7.8
β-Glucans	3.6–6.1
Cellulose	1.4–5.0
Simple carbohydrates (glucose, fructose, sucrose, maltose)	0.41–2.9
Oligosaccharides (raffinose, fructosans)	0.16–1.8
Proteins	8–15
Lipids	2–3
Minerals	3

Barley also contains small quantities of the B-complex vitamins, including thiamin (B1), riboflavin (B2), nicotinic acid, pyridoxine (B6), and pantothenic acid, biotin, folic acid, and vitamin E.

1.8. Uses of Barley

In many countries, 80 to 90% of barley production is used for animal feeds and malt. Today, the use of barley in human foods is very limited. Recently, Japan and Korea have been increasing their use of barley for human consumption. Although small quantities of pot or pearled barley are used in the Western countries in breakfast cereals, soups, stews, bakery blends, and for baby foods, efforts are being made in order to increase utilization of barley grain for human foods (Jadhav e*t al.,* 1998).

If barley can be grown more easily than corn in an area, it has greatest importance as a feed crop. It is usually used for the feeding of hogs or cattle but is satisfactory for other classes of livestock. Low protein barleys are favored for malting and brewing, but barley of high protein content is more desirable for animal feed.

Apart from its use in feeding, the next most important use of barley is malting. Barley is used for malt, most of which is used for brewing alcoholic beverages. The brewing industry uses a mixture of two-row (*Hordeum distichon* L.) and six-row barley (*Hordeum vulgare L.)* in the production of malt beverages. Malting barley is high quality barley that has the special characteristics necessary to produce malt. Certain varieties are recommended for malting as they meet quality requirements better than nonmalting varieties (Schwartz *et al*., 1997).

Barley has normal or high lysine and amylase starch levels, and low or high levels of β-glucan that could provide excellent opportunities for enhancing barley food. Moreover, it does not contain any known antinutritional factors. In spite of these interesting characteristics, barley remains an underutilized cereal in human foods, pharmaceutical and industrial products, except for the malting, brewing (Jin *et al.*, 2004).

1.9. Barley Breeding

Breeding new barley varieties is based on creating new allele combinations and subsequent testing and selection of the desirable phenotypes during the selfing generations. Heritable variation is created mainly by controlled crosses between adapted high yielding cultivars and breeding lines. Although variety breeding is based on elite germplasm, specific traits may be introgressed from wild barley and landraces in backcrossing programs (Nevo, 1992). Spontaneous mutations, as well as mutations induced by radiation or chemical treatments, have also been used (Briggs, 1978).

Plant breeding programs require a genetic diagnostic assay that is relatively inexpensive and can be performed on thousands of individuals. All steps in the genetic diagnostic assay including DNA extraction, DNA quantification, amplification reaction, allele analyses and data read out, should be automated for fast output (Rafalski and Tingey, 1993). Selection for desirable traits is made both in the field and in the laboratory. In the field agronomical characters including earliness, straw length, lodging resistance and disease resistance are monitored. After harvest yield, thousand grain weights, hectoliter weight and grading are measured as well as the protein content of the grain. Also malting properties including extract yield, viscosity of grain and malt and milling energy may be tested. Selection for specific traits is done during the selfing generations starting from the F2 generation. In a breeding program several traits have to be considered simultaneously to reach the desired agronomical type.

The effective way to select specific traits such as disease resistance is based on DNA markers. There are a lot of DNA markers which are link to genes in barley that confer resistance to the fungal pathogens causing scald. Disease resistance genes in a population can be tracked by using marker – assisted selection (MAS). Through this method, disease resistant offsprings are selected and different disease resistance genes can be accumulated into various barley variety backgrounds.

1.10. Diseases of Barley

Diseases occur when a susceptible host is exposed to a virulent pathogen such as fungi, bacteria, and virus under favorable environmental conditions. To control of diseases, the pathogens must be known and interacting factors must be manipulated. Little can be done to modify the environment but growers can minimize the risk of diseases by sowing resistant varieties and adopting practices to reduce inoculums. Resistant varieties provide the easiest and most effective option (Krupinsky *et al*., 2002).

Diseases of barley are classified as fungal diseases such as Leaf Blotch (scald), Spot Blotch, Leaf Stripe and Powdery Mildew, bacterial diseases such as Bacterial Kernel Blight, Bacterial Leaf Blight, Bacterial Stripe, viral diseases such as Barley Mild Mosaic, Barley Yellow Mosaic, Barley Yellow Dwarf and nematode diseases such as Cereal Root Knot Nematode (Mathre, 2000).

1.10.1. Scald Disease of Barley

One of the most important and the most common fungal diseases of barley is Scald, also known as Leaf Blotch. Scald is a fungal disease caused by *Rhynchosporium secalis* that attacks the leaves and heads of plant and may cause significant yield losses if it spreads to the upper parts of plant. The pathogen is spread from plant to plant primarily by water-splash dispersion of spores, and can persist from season to season in crop residues. Perhaps the most significant feature of *R. secalis* is the high level of pathogenic variability encountered in natural populations, which has repeatedly been demonstrated in different regions of the world where the disease is a problem. It is highly variable in pathogenicity, specificity, morphology, color, isozymes and DNA molecular markers (Garvin *et al*., 1997).

The primary loss from scald is reduced yield, which can reach or exceed 25% (Schaller, 1951). In addition, scald affects the quality of barley grain, which

is directly related to malting quality. Most of the production of barley is used for malting, so this disease is a major problem for barley producers.

One approach for reducing the severity of Scald in the field is through the use of fungicides. This enables a measure of control in some situations, but the effectiveness of fungicides in controlling scald can vary from location to location (Kendall *et al*., 1993). Furthermore, pathotypes of *R. secalis* that are resistant to commonly used fungicides now have been identified in field populations, reducing fungicide effectiveness in some instances (Kendall *et al.,* 1993 and, Locke and Phillips, 1995).

An alternative approach to scald control is through the use of naturally occurring scald-resistance genes, of which thirteen have so far been named (Jørgensen, 1992 and, Wettstein-Knowles, 1993). The incorporation of these genes into barley cultivars has been useful in combating scald in many instances. However, the different resistance genes work with varying degrees of effectiveness against pathotypes of *R. secalis* from different continents as well as from different geographic areas within the same country (Ali and Boyd, 1974 and Tekauz, 1991). Furthermore, even if resistance genes are initially found to be effective under field conditions, the highly variable nature of *R. secalis* may result in the selection of new pathotypes that can overcome them over time.

Progress toward characterizing new resistance genes to Scald disease has not proceeded as rapidly as might be expected, particularly given the seriousness of the disease in many parts of the world (Shipton *et al.,* 1974). This may be due to the fact that some currently available resistance genes are sufficiently effective for controlling the disease, and it may also reflect the general difficulty of conducting accurate genetic studies for scald resistance, particularly under field conditions. Nonetheless, it is clear that existing scald resistance genes are under constant threat of being overcome by new virulent pathotypes of the scald pathogen (Brown, 1990). Thus, novel scald resistance genes may serve two useful purposes. First, they may be of value in situations where existing scald resistance genes do not confer adequate protection against the disease. Second, they can provide a

measure of security to breeding programs if resistance genes that are currently in use fail over time.

Scald is easily identified from the oval to lens-shaped or elongated spots (lesions) which develop mostly on the leaves and leaf sheaths. At first, the lesions are water-soaked, with a dark green to pale grayish green color. Later they dry out; the centers become light tan or straw brown to grayish white, and are surrounded by prominent, dark brown to reddish brown borders that are often wavy (Figure 1.7).

With time the lesions enlarge, merge, and form elongated, irregular blotches of various shapes and sizes on the leaves. The tip of the leaf beyond the lesions collapses and dies. Sometimes older lesions have a "zoned" appearance. The scald symptoms are generally similar on all cereals and forage grasses. Under severe conditions, medium brown lesions may appear on the awns and tips of barley glumes. Lens-shaped lesions, which are dark blue to pale grayish green, may appear at the base of the kernels.

Figure 1.7. Scald appears first as water-soaked, grayish-green spots turning to tan sots with brown margins (Davis, 2003)

Yield loss from scald disease is determined by the amount of leaf area infected and the time of infection. If infection of barley is occurred during late

stages of plant development, it will result in less loss. Under conditions of high relative humidity, disease development will be most severe. Yield losses can be serious under these circumstances.

Rhynchosporium secalis is an obligate parasite so it can not complete its life cycle in the absence of a living plant host. The *Rhynchosporium* fungi over season as mycelia on dead or living leaves of infected plants and on other crop debris. During prolonged periods of cool, moist weather in the spring, the scald fungi resume growth on fall-infected tissues and produce large numbers of colorless, two-celled, microscopic spores called conidia, which develop in a thin layer of slime on the surface of the lesions from a stroma of spore-producing mycelia (Figure 1.8). The conidia are carried by splashing rains and air currents to new growth, where the leaves, leaf sheaths, and seedlings become infected (Caldwell, 1937).

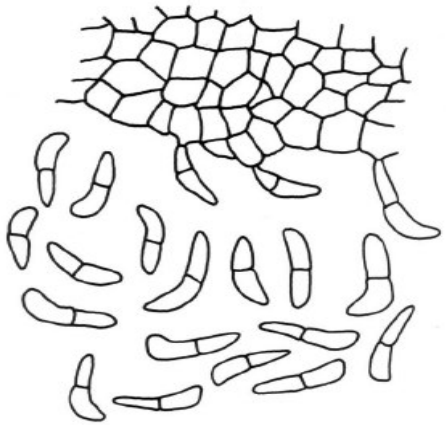


Figure 1.8. Two-celled, colorless conidia borne on a stroma at the surface of a lesion (Gray, 1988).

R. secalis is not a very specialized pathogen; also it can use a range of hosts. Barley (*Hordeum vulgare*) is a major host species, the pathogen may also be found on a large range of related genera of grasses.

The production of spore and infection occur repeatedly during cool, moist, humid periods and continue until the crop ripens. Scald is checked during hot and dry summer weather. New infections occur in the fall when cool, damp weather returns. The scald fungi are not carried within seed, but can be carried on the seed. Large numbers of conidia are produced on the seed and may infect seedlings when the soil temperature is around 16 °C. The *Rhynchosporium* fungi can survive on grass for up to a year (Greifenkamp, 2002).

1.11 Genetic Control of Plant Diseases

Plants utilize a variety of strategies to defend against pathogen attack. One strategy is to strengthen the cell wall, thereby making a barrier between the plant cell and the pathogen. A second strategy that the plant utilizes is the production of antimicrobial compounds, such as toxic secondary metabolites and hydrolytic enzymes. The predominant strategy of plants to defend against pathogen, however, is the hypersensitive response (HR). The first insight into the genetics of plant disease resistance involving the HR was the pioneering work done by Flor (Flor, 1956). Flor (1956) proposed a gene-for-gene model for the genetic interaction between plant and pathogen. This model states that a dominant gene from the host interacts with a corresponding dominant avirulence gene from the pathogen. Disease resistance genes permit the plant to detect and resist pathogen strains that express genetically complementary or matching genes called avirulence genes. In gene-for- gene interactions between plants and pathogens, requires dominant or semidominant resistance genes in the plant, and a corresponding avirulence (Avr) gene in pathogen. Resistance (R) genes are presumed to enable plants to detect Avr-gene.

The majority of plant disease resistance genes are member of very large multigene families. They encode structurally related proteins containing nucleotide binding site domains (NBS) and C-terminal leucine rich repeats (LRR). Only a few plant resistance genes have been functionally analyzed and the origin and evolution of plant resistance genes remain obscure.

The presence of a single dominant or semidominant resistance (R) gene allele can determine resistance to a specific plant pathogen. The products of such genes have been suggested to act as receptors that specifically bind ligands encoded by the corresponding pathogen avirulence factors in a gene-for-gene recognition process (Baker *et al*., 1997 and Hammond and Jones, 1997). The R gene/avirulence factor complex is thought to initiate a series of signaling situations leading to disease resistance.

Cloned R genes or linked marker DNAs of desired resistance genes now provide novel tools for plant breeders to improve the efficiency of plant breeding strategies, via marker assisted breeding (selection) and by using transformation for accelerating the introgression of useful R genes from related species (Özgen and Kınacı, 1984 and Jiang *et al*., 1994). Furthermore, plant breeders developed near-isogenic inbred lines (NILs) in numerous crop species (barley, wheat and tomato) in which resistance genes have been introgressed from wild species by recurrent backcrossing and selection for resistance as a single difference between resistant NILs and recurrent (susceptible) parent.

1.11. 1. Marker Assisted Selection (MAS) in Crop Plants

Marker-assisted selection involves selecting individuals based on their marker pattern (genotype) rather than their observable traits (phenotype). Many of the complications of a phenotype-based assay can be reduced through direct identification of genotype with a DNA-based diagnostic assay. For this reason, DNA-based genetic markers are being integrated into several genetic systems, and are expected to play an important role in the future of breeding. Molecular assisted selection is especially advantageous for agronomic traits that have closely linked molecular markers, such as resistance to pathogens.

Availability of tightly linked genetic markers for resistance genes will help in identifying plants carrying these genes simultaneously without subjecting them to the pathogen attack in early generations. The breeder would require little

amounts of DNA from each of the individual plants to be tested without destroying the plants using the known resistance gene markers. The genotype of the individual plant for resistance or susceptibility could be directly analyzed by the presence or absence of the marker (Paterson *et al*., 1991)

Pathogens are known to overcome resistance provided by a single gene. Durability of resistance has been increased in several crops by pyramiding of resistance genes into the same plant genotype. Hence the pyramiding of major resistance genes increases the chance of resistance trait to survive for longer periods in the life span of the desired plant.

1.11. 2. Molecular Marker Technologies

Conventional cereal breeding is time consuming and very depended on environmental conditions. Breeding a new variety takes between eight and twelve years and even then the release of an improved variety can not be guaranteed. Hence, breeders are extremely interested in new technologies that could make this procedure more efficient. Molecular marker technology offers such a possibility by adopting a wide range of novel approaches to improving the selection strategies in cereal breeding (Korzun, 2003)

Molecular markers (DNA markers) reveal neutral sites of variation at the DNA sequence level. By 'neutral' is meant that, unlike morphological markers, these variations do not show themselves in the phenotype, and each might be nothing more than a single nucleotide difference in a gene or a piece of repetitive DNA. They have the big advantage that they are much more numerous than morphological markers, and they do not disturb the physiology of the organism. There are a lot of DNA markers such as amplified fragment length polymorphism (AFLP), Random amplified polymorphic DNA (RAPD), Simple sequence repeat (SSR), restriction fragment length polymorphism (RFLP). All of them have some

advantages and disadvantages in plant population researches. One of the most used DNA marker is microsatellite or simple sequence repeats (SSRs).

Genomic microsatellite (simple sequence repeats; SSRs), repeats of 1-6 bp nucleotide motifs, have been detected in the genomes of every organism analyzed so far, and are often found at frequencies much higher than would be predicted purely on the grounds of base composition (Tautz and Renz, 1984)

Plant genomes contain large numbers of simple sequence repeats (SSRs) which are tandemly repeated and widely scattered at many hundreds of loci throughout the chromosome complement. Typically they may be dinucleotides (AC)*n*, (AG)*n*, (*AT*)*n*; trinucleotides (TCT)*n*, (TTG)*n*; tetranucleotides (TATG)*n* and so on, where *n* is the number of repeating units within the microsatellite locus. In addition to occurring at many different loci, they can also be polyallelic. (AT)*n* dinucleotides are the most abundant type of SSR in plants (Ma *et al.*, 1996).

Sequence information for SSR amplification is obtained either from gene bank data or by sequencing positive clones probed from DNA libraries with simple sequence repeats. Currently, specific primer sequences for over 600 barley SSR loci are available. Recently, primers based on the conserved regions of sequenced resistance genes have been used for amplifying resistance gene analogs (RGA) in many crop species, including barley (Leister *et al.,* 1996)

Polymerase chain reaction (PCR) amplification is used to generate DNA banding patterns on a gel and to reveal the polymorphism based on different numbers of repeats at the two alleles of a locus. The marker thus has the advantage of being codominant. In addition they are simple, PCR-based and extremely polymorphic, and highly informative due to the number and frequency of alleles detected and to their ability to distinguish between closely-related individuals. They find application as markers for mapping, cultivar identification, protecting germplasm, determination of hybridity, analysis of gene pool variation, and as diagnostic markers for traits of economic value (Powell *et al*., 1996).

1.12 Quantitative Trait Loci (QTL)

Many traits of agronomic interest are controlled by a single gene and a few distinct phenotypic classes are seen. These classes can be used to predict the genotypes of the individuals. For example, if a tall and short pea plant is crossed, the genotype of short plant can be known. Thus, generalized genotype for the tall plant phenotype could be assigned. These types of phenotypes are called discontinuous traits.

Some traits are not seen distinct phenotypic classes. Rather, when a segregating population is analyzed for these traits, a continuous distribution is found such as ear length in corn. These types of traits are called continuous traits and cannot be analyzed in the same manner as discontinuous traits. Because continuous traits are often given a quantitative value, they are often referred to as quantitative traits, and the area of genetics that studies their mode of inheritance is called quantitative genetics. Furthermore, the loci controlling these traits are called quantitative trait loci or QTL. These traits are controlled by multiple genes and also can be affected by the environment to varying degrees (Mauricio, 2001).

Quantitative trait locus (QTL) mapping, which is based on the use of DNA markers, is a highly effective approach for studying genetically complex forms of plant disease resistance. With QTL mapping, the roles of specific resistance loci can be described, and interactions between resistance genes, plant development, and the environment can be analyzed. Sometimes plant disease resistance is genetically simple and has been analyzed by traditional methods of plant pathology, breeding, and genetics. However, genetically complex forms of disease resistance are more poorly understood (Young, 1996).

Most complex resistance traits are controlled by multiple loci. These resistance phenotypes tend to be measured quantitatively, so they are known as quantitative resistance characters, and the genetic loci associated with them are called quantitative resistance loci (QRLs).

Quantitatively inherited traits are those that have a strong genetic component but which, under normal conditions of measurement, can not be shown to be controlled by individually recognizable loci. There are many reasons for the inability to recognize individual loci. Some disease reactions are difficult to score reliably and others are highly sensitive to environment (Bai and Shaner, 1994). A crop cultivar with adequate resistance in one location may be unacceptably susceptible in another. Environmentally sensitive traits are difficult to measure accurately, resulting in lowered estimates of heritability and a reduced likelihood of appearing Mendelian unless special experimental precautions are taken (Young, 1996).

QTL mapping involves testing DNA markers throughout a genome for the likelihood that they are associated with a QTL. Individuals in a suitable mapping population (F_2, backcross, recombinant inbred) are analyzed in terms of DNA marker genotypes and the phenotype of interest. For each DNA marker, the individuals are split into classes according to marker genotype. Mean and variance parameters are calculated and compared among the classes. A significant difference between classes suggests there is a relationship between the DNA marker and the trait of interest—in other words, the DNA marker is probably linked to a QTL (Young, 1996).

CHAPTER II

JUSTIFICATION OF THE STUDY

Barley (*Hordeum vulgare* L.) is cultivated worldwide on an estimated 57.2 million ha with total and mean production of 132 million tones and 2308 kg/ha, respectively. In Turkey, it is cultivated on 3.6 million ha with a production of 8 million tones and means of 2204 kg/ha (www.FAO.org, 2000).

Turkish barley plantations have been under the threat of Scald (*Rhynchosporium secalis*) disease with significant damage to yield during suitable environmental condition for scald. In a survey in central Anatolia, Mamluk *et al.* (1997) observed that up to 20% plants were infected in more than half of the fields that were inspected. Scald affects the quality of barley and causes yield losses. In Turkey, Kavak (1998) reported yield losses of 8.9, 19.6 and 30.5% with the infection levels 46.7%, 68%, 80.1%, respectively.

The cultivation of resistant barley varieties is considered to be the most economically and environmentally safe method to reduce the yield losses caused by scald disease. Until recently, virtually all progress in resistant cultivar breeding has relied on phenotypic selection depend on many environmental factors. Various procedures of phenotypic selection are time consuming and laborious; today, direct identification of genotypes with DNA based diagnostic assays are widely used in the world. Marker assisted selection (MAS) provides a valuable potential for increasing selection efficiencies.

In the current study, eighty Turkish barley seed sources were selected from the barley-seed collections of Central Research Institute for Field Crops, Ankara. These seed sources were phenotypically preclassified whether they are resistant or

sensitive to scald disease without much of experimental testing. Thus, some of 80 seed sources were phenotypically considered to be resistant and others were considered to be sensitive to scald disease. Choosing suitable seed sources for scald resistance breeding by phenotypically, is inefficient due to environmental variation, as well as expensive and labor intensive. However, marker assisted selection could improve the efficiency of Turkish barley breeding dealing with scald disease and reliance, also helps to decrease time for selection of interested seed sources. With the present study using microsatellite (SSR) markers, it was aimed to screen available barley seed collection to assess genetic diversity parameters for scald disease resistance and sensitive Turkish barley seed sources that could help to selection, monitor, fingerprint, and use more efficiently these seed sources in scald disease resistant breeding program of barley.

CHAPTER III

OBJECTIVES OF THE STUDY

The general objective of this study was to characterize Turkish barley seed sources genetically with the use of molecular markers. These seed sources were previously grouped with respect to their resistance or sensitivity to the Scald *(Rhynchosporium secalis)* disease by the Central Research Institute for Field Crops (Ankara, Turkey) and used in Scald resistant barley breeding programs.

Specifically the following objectives were also set for the study, but due to limited number of markers used in the study, these objectives could not be fully addressed.

1- To determine the magnitude and pattern of genetic variation existing in Turkish barley seed sources by means of SSR primers

2- To reveal the extend of genetic diversity within and between scald disease resistant and sensitive Turkish barley seed sources, by employing genetic diversity measures such as polymorphism, mean number of alleles per locus and heterozygosity.

3- To group and fingerprint the Turkish barley seed sources for future use of material transfer and monitoring by use of molecular marker data.

CHAPTER IV

MATERIAL AND METHODS

4.1. Plant materials

Eighty Turkish barley seed sources were obtained from the Central Research Institute for Field Crops (Ankara, Turkey). Forty of them are considered to be resistant to scald disease (Table 4.1), whereas the remaining forty seed sources are sensitive to this disease (Table 4.2).

Barley seed sources which are represented with numbers are not proprietary, so they are called lines and available to breeders. Approximately, 50% of resistant Turkish barley seed source are lines and the other 50 % are proprietary barley accessions. All of these proprietary barley seed sources are employed in Turkey. On the other hand, in sensitive Turkish barley seed sources, about 10% are lines and remaining barley accessions are proprietary.

4.1.1. DNA Extraction from Barley Leaves

Four seeds of each seed sources were planted into plastic pots and they were irrigated once every three days. Pots were maintained in growth room at 24°C until seeds were germinated. Genomic DNA was isolated from leaves of approximately two weeks-old barley seedlings. For each barley seed source, four seedlings were used to obtain DNA. About 100 mg of fresh leaves were ground in 700 µl CTAB (Cetyl Trimethyl Ammonium Bromide, Chemical composition is in Appendix A) buffer, containing autoclaved sand, using a pestle and a mortar. After getting a homogenous green liquid, it was transferred in a 1.5 ml Eppondorf tube and 50 µl β-ME (Beta Mercapto Ethanol) was added. Each tube was incubated in a water bath, at 65°C, for 1 hour. Then, 500 µl chloroform-

isoamylalcohol (24:1) was added to each tube and cetrifugated at 13.000 rpm for 15 minutes at 4°C. Supernatant was transferred to a fresh Eppendorf tube and is added 500 µl ice cold pure isopropanol. Tubes were incubated at -80°C for 30 minutes. Then, centrifugation was repeated for another 10 minutes at 4°C. The top aqeous part was poured off and pellet was washed with 200 µl of 70% Ethanol twice. After drying for about 30 minutes, the pellet was dissolved in 50 µl TE (Tris EDTA) buffer (Modified from Doyle & Doyle CTAB Procedure, 1987).

Table 4.1. Scald Disease (*Rhynchosporium secalis*) Resistant Turkish Barley Seed Sources (0= Very Resistant, 1= Resistant, 2=Moderately Resistant)

Seed Source Codes	Seed Sources (registered cultivars or parental materials)	Scald Disease Resistant Scores
R1	Rabur / Luther	1
R2	NE 76148 WBCBPI 388643	0
R3	YEA 761 - 3 / YEA 741 - 2	0
R4	324 P.K - 5 / Tuil 10	0
R5	YEA 171 - 3 / YEA 50.1	1
R6	3896 / 1 - 15 / 3 / 3896 / 28 // 284 / 28 / 4 / EinbuII CI 7321	1
R7	3896 / 1 - 15 / 3 / 3896 / 28 // 284 / 28 / 4 / Einbull CI 7321	0
R8	YEA 1139 /YEA 605.5	2
R9	132TH / 22 / 3 / TokakP386 / P49 -10	2
R10	3896 / 1 - 15 / 3 / 3896 / 28 // 284 / 28 / 4 / Einbull CI 7321	0
R11	YEA 324/ YEA 68.3	1
R12	YEA 762 - 2 / Tokak	1
R13	YEA1727 / YEA 6055	0
R14	3896 / 1 - 15 / 3 / 3896 / 28 // 284 / 28 / 4 / Einbull // CI 7321	2
R15	3896 / 1 - 15 / 3 / 3896 / 28 // 284 / 28 / 4 / Einbull // CI 7321	0

Table 4.1. (*Continued*) Scald Disease (*Rhynchosporium secalis*) Resistant Turkish Barley Seed Sources (0= Very Resistant, 1= Resistant, 2=Moderately Resistant)

Seed Source Codes	Seed Sources (registered cultivars or parental materials)	Scald Disease Resistant Scores
R16	3896 / 1 - 15 / 3 / 3896 / 28 // 284 / 28 / 4 / Einbull // CI 7321	0
R17	3896 / 1 - 15 / 3 / 3896 / 28 // 284 / 28 / 4 / Einbull // CI 7321	0
R18	3896 - 1 - 15 / 3 / 3896 / 28 // 284 / 28 / 4 / Einbull // CI 7321	0
R19	3896 / 1 - 15 / 3 / 3896 / 28 // 284 / 4 / Edynbul // CI 7321	0
R20	3896 / 1 - 15 / 3 / 3896 / 28 // 284 / 4 / Edynbul // CI 7321	0
R21	3896 / 1 - 15 / 3 / 3896 / 28 // 284 / 28 / 4 / Einbul // CI 7321	0
R22	YEA 454 - 1 / 5054	0
R23	3896 / 1 - 15 / 3 / 3896 / 28 // 284 / 28 / 4 / Einbul //CI 7321	0
R24	3896 / 1 - 15 / 3 / 3896 / 28 // 284 / 28 / 4 / Einbul// CI 7321	0
R25	YEA 762 - 2 / YEA 605 - 5	1
R26	3896 / 1-15 / 3 / 3896 / 28 //284 / 28 / 4 / Einbul // CI 7321	1
R27	3896 / 1-15 / 3 / 3896 / 28 //284 / 28 / 4 / Einbul // CI 7321	1
R28	YEA 762.2 / Tokak	1
R29	Flamenco / WM	0
R30	H272/Bgs/3/Mzg/Gva//PI 002917/4/Deir Alla10 //Mzg/DL71	0
R31	H272/Bgs/3/Mzg/Gva//PI002917/4/Deir Alla106//Mzg/DL71	0
R32	CWB22 - 6 - 13 / ICB - 102411	0
R33	Tryll / Hudson // Obruk - 86	0
R34	Bastion M	0
R35	AVD-121/Bülbül-89	0
R36	AVD-121/Bülbül-89	0
R37	4814/3/3896/Gzk//132TH	0
R38	4814/3/3896/Gzk//132TH	1
R39	WBelt-39/Tokak	0
R40	Wysor	0

Table 4.2. Scald Disease (*Rhynchosporium secalis*) Sensitive Turkish Barley Seed Sources (3= Sensitive, 4=Very Sensitive)

Seed Source Codes	Seed Sources (registered cultivars or parental materials)	Scald Disease Sensitive Scores
S1	73TH/105//E10BULKCI7321/3/CWB117-5-9-5	4
S2	Roho//Alger/Ceres362-1-1/ 3'Alpha/Durra	4
S3	CWB117-9-7/3/ROHO //ALGER /CERES362-1-1	4
S4	VIRINGA'S'/3/4679/105//132TH	3
S5	ANTERES/KY63-1294//CWB117-77-9-7	3
S6	Roho//Alger/Ceres362-1-1/3/Alpha/Durra	4
S7	CWB117-5-9-5/3/ROHO/MASURKA//ICB-103020	4
S8	CWB117-5-9-5//RHN-03/LİGNEE640	4
S9	CHİCM/AN57//ALBERT/3/ICB-102379/4/GKOMEGA	4
S10	RADİCAL/BİRGİT//PAMİR-163	3
S11	TOK/4857//YEA2049-3-1-1	4
S12	ALPHA/2104	4
S13	1993-94(IWFBSP)	4
S14	ESKİŞEHİRÖVD ST 3760	4
S15	YEA557.6/YEA422.1//80-5042	3
S16	YEA605.20	3
S17	TOKAK/3/ALPHA//SUL/NACTA	3
S18	4857/VİRİNGA"S"	4
S19	111TH/P12-119//4857	4
S20	PLATEN672//353TH/P12-119	4
S21	ANTARES/KY63-1294/3/ROHO //ALGER/CERES362-1-1	4
S22	ROHO//ALGER/CERES362-1-1/3' ALPHA /DURRA	4
S23	TOKAK/PAMİR-175	4
S24	PAMİR-159/WKN185-82	4
S25	PAMİR-010/PAMİR-159	3
S26	ROHO7MASURKA//OBRUK	4
S27	ANTARES/KY63-1294/3/ROHO //ALGER/CERES 362-1-1	4
S28	274 ESK.ÖVD	4
S29	ANTARES/KY63-1264//LİGNEE 131	4
S30	YEA1276/132TH//5053/3/COSS/OWB71080-44-1H	3

Table 4.2 (*Continued*) Scald Disease (*Rhynchosporium secalis*) Sensitive Turkish Barley Seed Sources (3= Sensitive, 4=Very Sensitive)

Seed Source Codes	Seed Sources (registered cultivars or parental materials)	Scald Disease Resistant Scores
S31	2925/1//1246/78/3/YEA475-4/4/TARM-92	3
S32	5807/4857	3
S33	TOKAK/CWB117-77-9-7	3
S34	SONJA/MS//P12222/SCIO/4/TOKAK	3
S35	CUM-50/700.1	3
S36	11ESK.ÖVD	3
S37	PAMİR-42/BÜLBÜL	4
S38	PAMİR-42/BÜLBÜL	4
S39	PAMİR-42/BÜLBÜL	4
S40	BELTS-60-1807/HENRY//SUSSEX/ 3/2/ BARSOY/4/B/A/5/K-247/2401-13/VAVİLON	4

4.2. Chemicals

All chemicals used in this study were in molecular grade. The list of these chemicals and their suppliers were provided in Appendix A.

4.3. Gel Electrophoresis

4.3.1. Agarose Gel Electrophoresis

Agorose gels were prepared by dissolving and boiling of agarose in 1XTBE (Tris Boric acid EDTA) buffer in a microwave oven. The solution was poured into horizontal gel tray that had inserted combs and was let to polymerize. After polymerization, 1XTBE buffer was poured into electrophoresis apparatus and combs were gently removed from the gel. The samples were mixed with formamide loading dye and loaded into wells of the gel by using a micropipette. Gels were run at 80-100 volts for 1 hour. When electrophoresis was completed, DNA fragments were stained with 5 µg/ml ethidium bromide. After staining, the bands were visualized by direct examination of the gel under UV light.

4.3.2. Polyacrylamide Gel Electrophoresis

During the experiment, 7.5% polyacrylamide gels were used. To make a mixture of desired polyacrylamide percentage, 10 ml Buffer Solution B (Appendix A), 6 ml dH_2O, 7.5 ml Acrylamide-Bisacrylamide (19:1), 10 ml dH_2O, and 6.5 ml Ammonium Per Sulphate (APS) were put in a glass beaker, with the given order. Immediately, the mixture was poured into the glass plates and sticked a comb into the gel solution. The excess gel solution was removed. Polymerization of the acrylamide needs for 30-60 minutes at room temperature. After polymerization, the comb was removed and gel was inserted into Hoefer gel box. The same electrophoresis buffer in both of the reservoirs and in the gel was used. Approximately 12 µl DNA with the appropriate amount of gel loading buffer was loaded in the well via a Hamilton syringe. After running at 200 volts for 1.5 hours (until the marker dyes migrated the desired distance), one of the glass plate was separated carefully with a razor blade and the gel was notched to ensure the orientation of the gel. The gel was placed approximately 15 minutes in a staining tray containing 5 µg/ml Ethidium Bromide. After staining, the bands were visualized by direct examination of the gel under UV light.

4.4. Polymerase Chain Reaction (PCR)

Simple Sequence Repeat (SSR) primers to screen 80 Turkish barley seed sources were selected from previous studies (Williams *et al*., 2001, Macaulay *et al.,* 2001) based on their polymorphism. Selected primer-sequences were sent to a commercial firm for synthesis (Elips Health Products, Turkey). The list of these primers and their sequences were provided in Table 4.3.

Total of 6 primer-pairs (*Bmag603, Bmag6, Bmac67, Bmag225, Bmac209, Bmag206*) were tested to screen DNA of 80 barley seed sources (cultivars and parental materials). Four of these primers were obtained from a study which is about mapping of a gene for leaf scald resistance in barley (Williams *et al*., 2001).

Table 4.3. The list of SSR Primers used in the study (* Williams *et al*., 2001, Macaulay *et al*., 2001)

Primer	Forward/Reverse	Repeat type
***Bmag603**	5^1 ATACCATGATACATCACATCG 3^1 5^1 GGGGGTATGTACGACTAACTA 3^1	$(AG)_{24}$
***Bmag6**	5^1 TTAAACCCCCCCCCTCTAG 3^1 5^1 TGCAGTTACTATCGCTGATTTAGC 3^1	$(AG)_{17}$
***Bmac67**	5^1 CTGCAGGTTTCAGGAAGG 3^1 5^1 AGATGCCCGCAAAGAGTT 3^1	$(AC)_{21}$
***Bmag225**	5^1 AACACACCAAAAATATTACATCA 3^1 5^1 CGAGTAGTTCCCATGTGAC 3^1	$(AG)_{26}$
Bmac209	5^1 CTAGCAACTTCCCAACCGAC 3^1 5^1 ATGCCTGTGTGTGGACCAT 3^1	$(AC)_{13}$
Bmag206	5^1 TTTTCCCCTATTATAGTGACG 3^1 5^1 TAGAACTGGGTATTTCCTTGA 3^1	$(GT)_5(AG)_{14}$

PCR amplifications were performed in a 25 µl reaction mixture containing about 3ng/µl genomic DNA, 10X PCR Buffer (Mg_2Cl free), 25mM Mg_2Cl, 5 mM dNTPs, 10 µM each of the primer and 5 Unit *Taq* polymerase. For each primer-pair, different PCR reaction mixtures were determined after optimization experiments. The optimized reaction mixtures for each primer were provided in Table 4.4. The reaction mixtures were prepared in thin-walled 0.2 ml Eppendorf tubes and run on a thermocycler Eppendorf-Mastercycler, Eppendorf, Canada, and Techne-genius Thermocycler, Techne, USA.

Table 4.4. Optimized PCR conditions for different SSR primer-pairs

Primer names	Reaction Mixture Componenets							
	H_2O	**dNTP (5mM)**	**10X Buffer**	**Primer-pairs (10µM)**	**$MgCl_2$ (25mM)**	**Taq (5u/µl)**	**DNA (3ng/µl)**	**Total vol.**
Bmag603	14.75µl	2 µl	2 µl	1+1 µl	2 µl	0.25 µl	2 µl	25 µl
Bmag6	10.75 µl	3 µl	2 µl	2+2 µl	2 µl	0.25 µl	3 µl	25 µl
Bmac67	12.75 µl	2 µl	2 µl	2+2 µl	2 µl	0.25 µl	2 µl	25 µl
Bmag225	10.75 µl	2 µl	2 µl	2+2 µl	3 µl	0.25 µl	3 µl	25 µl
Bmac209	12.25 µl	2 µl	2.5 µl	2+2 µl	2 µl	0.25 µl	2 µl	25 µl
Bmag206	11.75 µl	2 µl	2 µl	2+2 µl	2 µl	0.25 µl	3 µl	25 µl

The steps of PCR conditions for studied primer-pairs are presented in Table 4.5a and 4.5b. For Bmag206, Bmag603, Bmac67 primer-pairs, after 3 minutes at 94°C, the PCR involved 30 cycles of amplification, including 1 minute at 94°C, 1 minute at 55°C and 1 minute at 72°C, and a final extension step of 5 minutes at 72°C were applied.

For Bmac209, Bmag225, Bmag6 primer-pairs, after 3 minutes at 95°C, the PCR involved 30 cycles of amplification, with 1 minute at 94°C, 1 minute at 56°C, 1 minute at 72°C, and a final extension step of 5 minutes at 72°C steps applied.

Table 4.5a. PCR Cycling Steps and conditions for Bmag206, Bmag603, Bmac67 primer-pairs

Step	Temperature	Time	Cycle #	Description
1	94°C	3 minutes	1	Initial denaturation
2	94°C	1 minute	30	Denaturation
	55°C	1 minute		Annealing
	72°C	1 minute		Extension
3	72°C	5 minutes	1	Final extension
4	4°C	-	-	Hold

Table 4.5b. PCR Cycling Steps and conditions for Bmac209, Bmag225, Bmag6 primer-pairs

Step	Temperature	Time	Cycle #	Description
1	95°C	3 minutes	1	Initial denaturation
2	94°C	1 minute	30	Denaturation
	56°C	1 minute		Annealing
	72°C	1 minute		Extension
3	72°C	5 minutes	1	Final extension
4	4°C	-	-	Hold

4.5. Data collection

Amplification products were scored visually after they were photographed. pUC19 DNA/MspI (Hpall) DNA ladder (MBI Fermantas) was used to determine the size of SSR bands. The range of ladder was between 34-501 base pairs with intervals of 34, 67, 110, 111, 147, 190, 242, 331, 404, 489, 501 base pairs.

Different band patterns were observed in SSR primers as expected. For each primer pair, product was treated as loci with different alleles (bands with different size) and scored as 1 to 5. While some primer-pairs yielded only one band combination, the others produced more than single bands. They are treated as loci with multiple alleles. After allelic designations in each primer pairs, genotypes of individuals from each of 80 Turkish barley seed sources were determined and recorded. Alleles and genotypes produced by SSR primer-pairs were listed in Table 4.6.

Table 4.6. Observed number of alleles, Size of Alleles (base-pair) and observed genotypes of SSR loci in Turkish barley seed sources

SSR loci	Number of alleles	Size of Alleles (bp)	Observed genotypes
Bmag603	5	111, 120, 132, 140, 147	11, 12, 22, 23, 44,45
Bmag6	3	170, 175, 180	11, 22, 33
Bmac67	5	145, 160, 165, 170, 175	12, 24, 35
Bmag225	5	140, 147, 161, 171, 181	12, 13, 23, 34, 45
Bmac209	4	175, 185, 190, 200	11, 12, 13, 23, 34
Bmag206	2	250, 999	11, 00

4.6. Analysis of Data

The data of all SSR loci were collected and organized as in Appendix B so that it could be analyzed with POPGENE programme (version 1.31, Microsoft Windows-Based Freeware for Population Genetics Analysis) (Yeh *et al*., 1999). The following parameters were estimated using this programme: observed number of alleles per locus (n_a), effective number of alleles per locus (n_e) locus (Kimura and Crow, 1964), the proportion of polymorphic loci (P), observed (H_o), and expected heterozygosities (H_e) (Nei, 1987), and F- statistics (F_{IT}, F_{IS}, F_{ST}). Also, GDA Software (Genetic Data Analysis, Lewis and Zaykin, 2002) was used to construct UPGMA (Unweighted Pair Group Method using Arithmetic Averaging) trees for Nei's (1978) and coancestry genetic distance between populations. This program uses the data file in NEXUS file format (Maddison *et al.*, 1997) and part of the data was given in Appendix C. These parameters were also calculated for barley seed sources grouped as scald resistant and sensitive groups as well as for groups based on source origins.

4.6.1. Allele frequencies

Following the determination of the allelic designations of SSR loci, the estimation of the allele frequencies was carried out by the following equation:

$$f(A_i) = \hat{x}_i = \frac{(2N_{ii} + \sum_{j=1}^{m} N_{ij})}{2N}$$

where $f(A_i)$ is the frequency of any allele, N represents the number of the individuals in the population, N_{ii} and N_{ij} represent the number of A_{ii} and A_{ij} genotypes, respectively and m represent the number of alleles in a locus (Nei, 1987)

4.6.2. Measures of Genetic Variation

In order to determine the amount of genetic variation, the following parameters were estimated.

a- Observed number of alleles at a locus

One component of the genetic variation is the mean number of alleles per locus (n_a). It is also called as the allelic richness. The formula used to calculate this parameter is as follows:

$$Mean(n_a) = \frac{\sum_i n_{a_i}}{r}$$

where (n_{a_i}) is the number of alleles at the i^{th} locus and r is the number of loci (Nei, 1987)

b- Effective number of alleles at a locus

Mean number of alleles gives an estimate that is inflated by deleterious genes of which the contribution to genetic variability is small. Kimura and Crow (1964) introduced the concept of effective number of alleles. This number is defined as the reciprocal of homozygosity.

$$\hat{n}_e = 1/\sum x_i^2$$

where $\hat{n}_e$ is the effective number of alleles and x_i is the frequency of i^{th} allele.

c- Proportion of Polymorphic Loci

If the sample size and the number of polymorphic loci involved in the study are large enough, genetic variation can be studied by measuring the proportion of polymorphic loci and average heterozygosity per locus. To be called polymorphic,

the most common allele *(x_i)* should have a frequency of equal to or smaller than 0.99 or 0.95. In this study, 0.99 criterion was used. The proportion of polymorphic loci was calculated by the following equation:

$$\hat{p} = \frac{n_p}{r}$$

where n_p is the number of polymorphic loci in r number of loci (Nei, 1987).

d- Heterozygosity

The most widespread measure of genetic diversity in a population is the amount of heterozygosity.

The unbiased estimate of heterozygosity at a locus was calculated by the following formula:

$$\hat{h} = \frac{2N\left(1 - \sum \hat{x}_i^2\right)}{2N - 1}$$

where N is the number of individuals and x_i is the frequency of an allele in a SSR loci (Nei, 1987).

4.6.3. F-Statistic

These measures of heterozygosity can be used to define three levels of inbreeding (Nei, 1987).

F_{IS} measures the fixation index or inbreeding coefficient within subpopulations; the degree to which the actual heterozygosity within subpopulations deviates from Hardy-Weinberg equilibrium. F_{IS} was estimated with the following equation:

$$F_{IS} = \frac{H_S - H_I}{H_S} = 1 - \frac{H_I}{H_S}$$

F_{IT} used for measuring the fixation index over the total population (inbreeding coefficient). That is the degree of deviation from Hardy-Weinberg expectations in heterozygosity. It was estimated by the following equation:

$$F_{IT} = \frac{H_T - H_I}{H_T} = 1 - \frac{H_I}{H_T}$$

F_{ST} is the reduction in fixation index due to differences among subpopulations in allele frequencies. It was estimated by the equation:

$$F_{ST} = \frac{H_T - H_S}{H_T} = 1 - \frac{H_S}{H_T}$$

Where,

H_I = observed heterozygosity of an individual in any subpopulation

H_S = expected heterozygosity of an individual in any subpopulation

H_T = expected heterozygosity of an individual in the total population.

H_I was estimated by the following equation (Nei, 1987):

$$H_I = \frac{\sum_{j=1}^{s} \hat{h}_{o_j}}{s}$$

where *s* is the number of subpopulations and ($\hat{h}_{o_j}$) is the observed heterozygosity in subpopulation *j*.

H_S was calculated by the following equation (Nei, 1987):

$$H_s = \frac{\sum_{j=1}^{s} \hat{h}_j}{s}$$

where ($\hat{h}_j$) is the expected heterozygosity in subpopulation j

H_T was estimated by the following formula (Nei, 1987):

$$H_T = 1 - \sum_i x_{ia}^2$$

where (x_{ia}) is the frequency of the i^{th} allele averaged over all subpopulations.

The three types of fixation indices are related to each other in the following way, so for example one can estimate one of the indices if other two are known.

$$1\text{-}F_{IT} = (1\text{-}F_{IS})\ (1\text{-}F_{ST})$$

Finally, by using the reduction in fixation index, gene flow between subpopulations (N_m) can be estimated. It was calculated by the following formula:

$$N_m = 0.25\ (1\text{-}F_{ST}) / F_{ST}$$

4.6.4. Genetic Distance

Genetic distance is the extent of gene differences between pairs of populations. Distance measures are generally analogous to geometric distances; for example, zero distance is equivalent to no difference between groups.

The most widely used genetic distance measure is that of Nei's Genetic Distance (Nei 1978) and calculated as follows:

I is the identity between two populations *x* and *y*

$$I = \frac{J_{xy}}{\left(J_x J_y\right)^{1/2}}, \text{ where}$$

$$J_{xy} = \sum_i^m x_i y_i \qquad J_x = \sum_i^m x_i^2 \qquad J_y = \sum_i^m y_i^2$$

and x_i and y_i represent the frequencies of the i^{th} allele in the *x* and *y* populations.

For multiple loci, J_{xy}, J_x and J_y were calculated by summing over all alleles at all loci studied. Then the average value was calculated by dividing these sums by the number of loci. The average values J'_{xy}, J'_x and J'_y were used to calculate the genetic identity and distance, D'.

$$I' = \frac{J'_{xy}}{\left(J'_x J'_y\right)^{1/2}} \qquad \text{and} \qquad D' = -\ln I'$$

Nei's distance is appropriate for long term evolution when populations diverge because of genetic drift and genetic mutation. The other genetic distance model is Reynolds' distance or "coancestry" distance (Reynolds *et al*, 1983). This distance is appropriate divergence due to drift only and no assumptions need to be made about the ancestral population. Reynolds' distance, its neglect of the importance of mutation, may work better in small population size (=high potential for drift). Therefore, coancestry distance model can be appropriate for this study to get differences between Turkish barley seed sources.

Reynolds' or coancestry distance can be estimated by the following formula

$$D' = -\ln\left(I - \theta\right)$$

In this formula coancestry coefficient is donated as θ and D' is genetic distance. In the drift situation, in which mutation is excluded, the weighted estimator of D' appears to be a better measure of distance than others that have appeared in the literature (Reynolds *et al*, 1983).

Genetic distances (Nei, 1978 and Reynolds *et al.*, 1983) between barley seed sources as grouped (scald disease resistant and sensitive, seed source origins) were calculated separately.

4.6.5. Phylogenetic Trees

Clustering method is employed to obtain a phylogenetic tree or dendrogram by considering pairwise similarities or distances among populations. Therefore related populations are organized in a biologically meaningful way. In this study, dendrograms were constructed by using UPGMA method (Nei, 1978 and Reynolds *et al.*, 1983) to reveal the genetic distance of populations. Phylogenetic trees were constructed for barley seed sources and grouped barley seed sources based on their origins.

CHAPTER V

RESULTS

5.1. Optimization of PCR conditions for Turkish Barley Cultivars

In order to obtain the best banding patterns, all of the PCR components such as $MgCl_2$, PCR Buffer, *Taq* Polymerase and dNTP mixture in different concentration were tested for Turkish barley seed sources. For instance, among the tested PCR conditions, mixture 6 and mixture 10 were found to yield the best PCR products for Bmac209 and Bmag206 primers, respectively (Table 5.1). The photo of Bmac209, Bmag206 primer-pair optimizations can be seen at Figure 5.1.

Table 5.1. PCR mixture combinations in PCR optimization experiments for Bmac209, Bmag206 primer-pairs.

mix.	H_2O	dNTP (5mM)	10X Buffer	Primer-pairs (10µM)	$MgCl_2$ (25mM)	*Taq* (5u/µl)	DNA (3ng/µl)
1	12.75 µl	2 µl	2 µl	2+2 µl	2 µl	0.25 µl	2 µl
2	13.75 µl	1 µl	2 µl	2+2 µl	2 µl	0.25 µl	2 µl
3	14.75 µl	1 µl	1 µl	2+2 µl	2 µl	0.25 µl	2 µl
4	16.75 µl	1 µl	1 µl	1+1 µl	2 µl	0.25 µl	2 µl
5	17.75 µl	1 µl	1 µl	1+1 µl	1 µl	0.25 µl	2 µl
6	**12,25 µl**	**2 µl**	**2,5 µl**	**2+2 µl**	**2 µl**	**0,25 µl**	**2 µl**
7	11.75 µl	2 µl	2.5 µl	2+2 µl	2.5 µl	0.25 µl	2 µl
8	9.75 µl	2 µl	2.5 µl	3+3 µl	2.5 µl	0.25 µl	2 µl
9	11.25 µl	2 µl	2.5 µl	3+3 µl	1 µl	0.25 µl	2 µl
10	**11,75 µl**	**2 µl**	**2 µl**	**2+2 µl**	**2 µl**	**0,25 µl**	**3 µl**

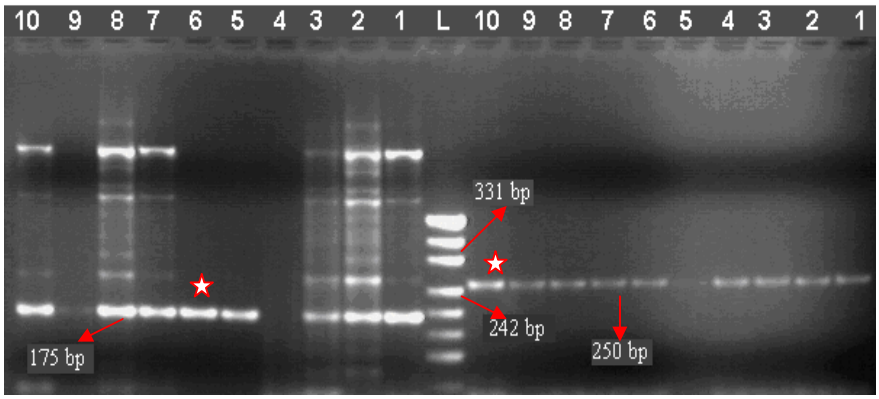


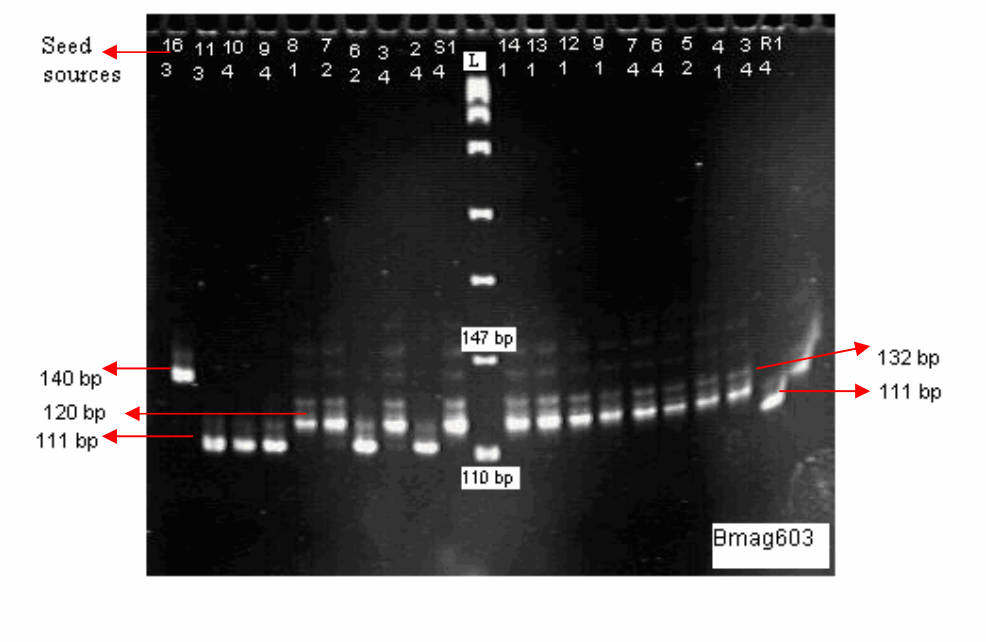
Figure 5.1. Banding patterns of Bmac209 (Left side) and Bmag206 (Right side) primers on agarose gel. L= DNA size marker

The banding patterns created by different PCR mixture combinations of Bmac209 primer are seen at the left side of the ladder in Figure 5.1. Within 10 different PCR mixture combinations, sixth one was preferred. Moreover, right side of the ladder shows banding patterns of Bmag206 primer and tenth PCR mixture combination was used for Bmag206 primer. Stars indicate the best bands with expected size for Bmac209 at line 6 and Bmag206 at line 10.

After optimization of PCR reactions, 6 best yielding primer- pairs were used to screen 320 DNA (80 populations x 4 samples) samples and the marker data were collected. For screening 320 DNA samples, a 16x18 and 16x20 PAGE systems were used. DNA banding patterns of selected primer-pairs in PAGE system have been provided in Figure 5.2.

In DNA screening studies with Bmag603, Bmag206, and Bmac67 markers, alleles of scald disease resistant and sensitive barley seed sources are seen on the right and left side of the ladder, respectively (Figure 5.2). Some differences between size of alleles of Scald disease resistant and sensitive barley seed sources were observed. For each primer, observed number of alleles was indicated with arrows. In photo of Bmag206 primer all samples were scald disease resistant seed sources and only one allele indicated with arrow was observed.

A)



B)

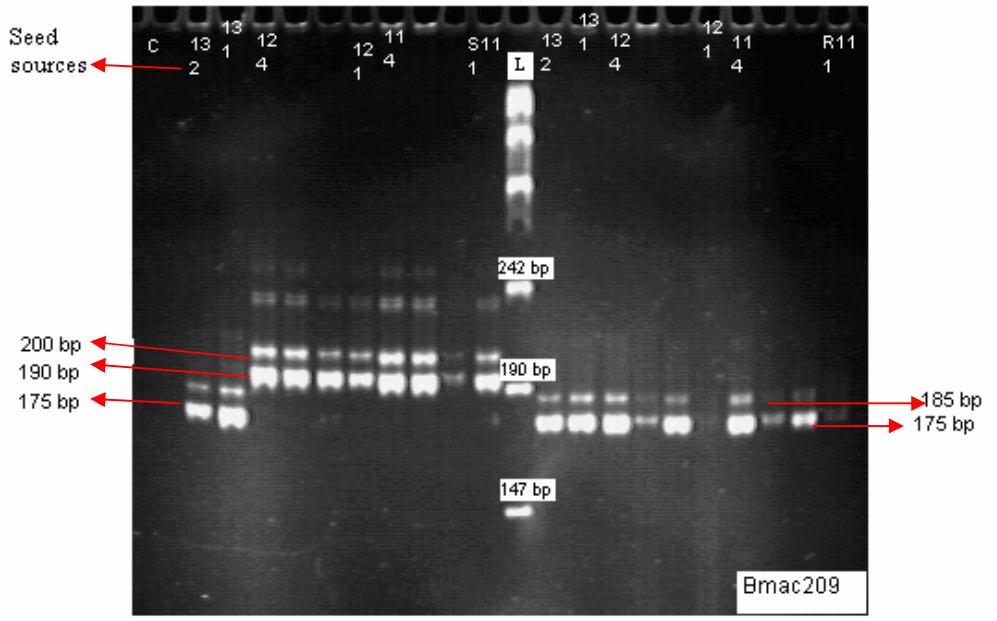
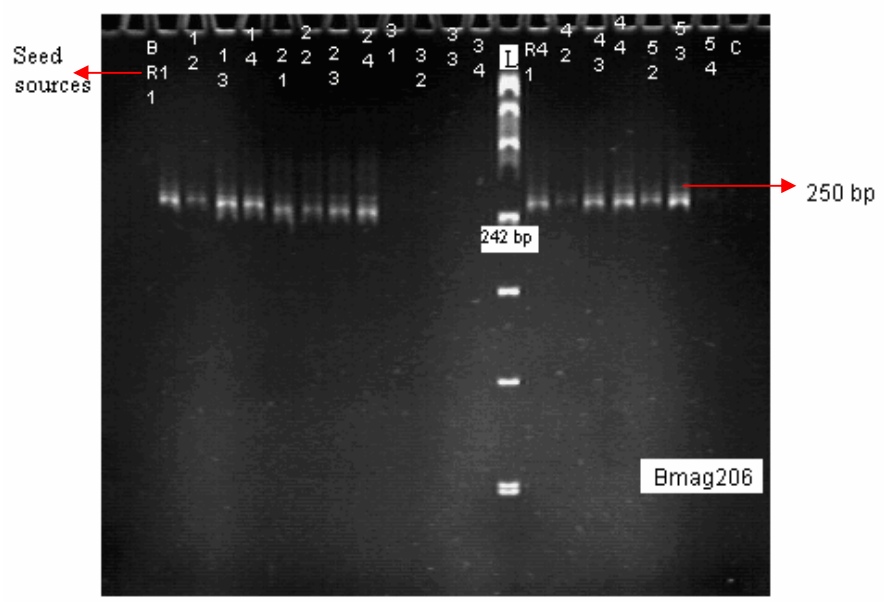


Figure 5.2. The PAGE gels of SSRs primers. A) Bmag603, B) Bmac209, C) Bmag206, and D) Bmac67 .The segregating bands for each primer were indicated with red arrows R: scald disease resistant, S: scald disease sensitive.

C)



D)

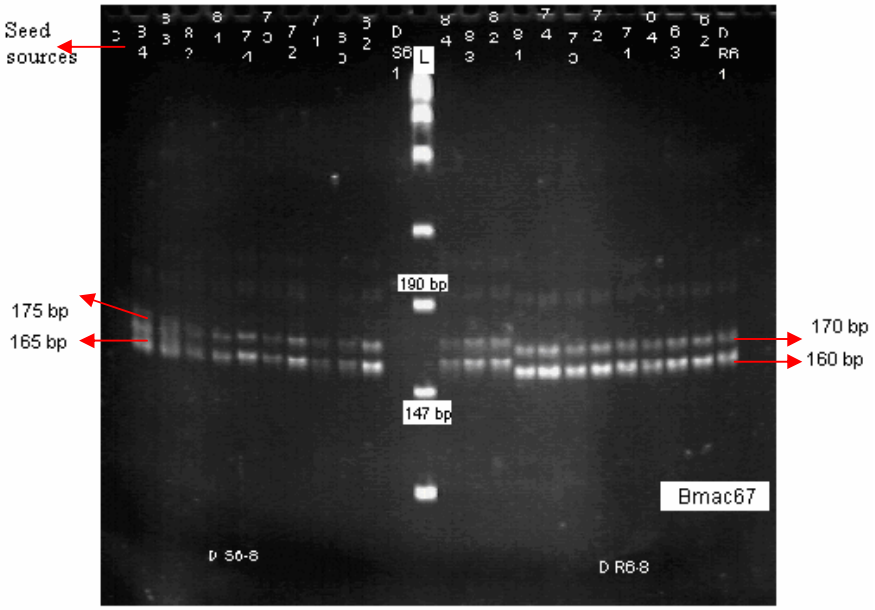


Figure 5.2. ***(Continued).*** **The PAGE gels of SSRs primers. A) Bmag603, B) Bmac209, C) Bmag206, and D) Bmac67. The segregating bands for each primer were indicated with red arrows R: scald disease resistant, S: scald disease sensitive.**

5.2. Population Genetic Structure

5.2.1 Genetic Diversity and F-Statistics of Turkish Barley Seed Sources Grouped as Resistant or Sensitive to Scald Disease

Eighty Turkish barley seed sources were grouped as resistant or sensitive to scald disease and POPGENE (Yeh *et al*., 1999) program was used for calculating the genetic variation statistics of each group (Table 5.2). The same statistics were also calculated for multi-populations and the results were given (Table 5.3). Moreover private (unique) alleles are another measure of genetic diversity. Barley seed sources having private alleles were indicated at Table 5.4.

Table 5.2. Estimated population genetic diversity parameters for scald disease resistant and sensitive Turkish barley seed sources (*Hordeum vulgare*). n= sample size, *P* = the proportion of polymorphic loci, n_a = observed number of alleles, n_e = effective number of alleles (Kimura and Crow, 1964) Ho = the observed heterozygosity, and H_e = the expected heterozygosity (Levene, 1949)

R/S	n	(n_a)	(n_e)	Polymorphic loci (P)		Mean Heterozygosity	
				number	%	H_o	H_e
R	319	3.7±0.06	2.7±0.03	6	100.0	0.63±0.02	0.62±0.01
S	319	3.8±0.06	2.9±0.04	6	100.0	0.61±0.02	0.64±0.01
MEAN	637	4.0±0.05	2.9±0.01	6	100.0	0.62±0.01	0.65±0.01

Scald disease resistant and sensitive barley seed sources did not differ greatly in the mean number of observed alleles (n_a) per locus, number of effective allele (n_e) per locus (Kimura and Crow, 1964), proportion of polymorphic loci (P), and mean expected and observed heterozygosity. However, the mean observed heterozygosity in resistant group is little higher than sensitive ones. Moreover, the difference between expected and observed heterozygosity is high in sensitive group, suggesting that there are some factors lowering the observed heterozygosity in this group of seed sources.

Table 5.3. Multi-populations statistics and summary of heterozygosity statistics for all loci. n = sample size, P = the proportion of polymorphic loci, n_a = observed number of alleles, n_e= effective number of alleles (Kimura and Crow, 1964) H_o = the observed heterozygosity, and H_e = the expected heterozygosity (Levene, 1949)

	n	n_a	n_e	# of P	% of P	H_o	H_e
Mean	637	4.0	2.9	6	100.0	0.62	0.65
St.error		0.05	0.02			0.01	0.01

Table 5.4. Unique (Private) alleles in Turkish barley seed sources

Locus	Allele	Frequency	Observed population
Bmac67	1	0.38	R9
Bmag225	1	0.38	R22
Bmag225	1	0.50	S7
Bmag603	5	0.25	S16

In order to explore the pattern of genetic variation in resistant and sensitive Turkish barley seed sources and to compare them with each other, Wright's F-statistics (Wright, 1978 and Nei, 1987) were employed. F-statistics is needed to detect if any deviation from Hardy-Weinberg expectations in gene frequencies exists.

F_{IS} values of resistant and sensitive group were -0.67 and -0.76 respectively. These results indicate that within the subpopulations, heterozygotes were 67% (resistant group) and 76% (sensitive group) higher than they were expected (Table 5.5 and 5.6). Bmag225 locus had the highest heterozygotes (-0.93) within the scald disease resistant Turkish barley seed sources (Table 5.5) while Bmac67 locus had the highest rate of heterozygotes (-0.95) within the scald disease sensitive Turkish barley seed sources (Table 5.6).

$\mathbf{F_{IT}}$ giving the total deviation of populations was -0.01 in resistant group (Table 5.5), indicating that 0.1% excess heterozygotes. However in sensitive group this value was 0.04 showing 0.4% more homozygotes than it was expected (Table 5.6).

$\mathbf{F_{ST}}$ values were estimated to be 0.39 for scald disease resistant group and 0.46 for sensitive group. This shows that 39% of total genetic variation was between seed sources of resistant group and 46% in between sensitive seed sources. About 61% of total genetic variation was within seed sources of resistant group and 54% in sensitive seed sources. It appears that there is high genetic differentiation within seed sources in general. Genetic differentiation among sensitive seed sources is higher (7%) than that of scald disease resistant barley seed sources. In both of scald disease resistant and sensitive barley seed sources, Bmag6 locus had the highest contribution to differentiation between barley seed sources (F_{ST} = 0.87 for resistant, F_{ST} = 0.94 for sensitive barley seed sources). The gene flow (N_m) estimated for this study was 0.38 for resistant group and 0.30 for sensitive group (Table 5.5 and 5.6).

Table 5.5. Summary of F-Statistics and gene glow for the 6 polymorphic loci in 40 resistant Turkish seed sources (accessions) of *Hordeum vulgare*
F_{IS} = the fixation index within subpopulations
F_{IT} = the fixation index over total population
F_{ST} = the reduction in fixation index due to differences among subpopulations (Nei (1987)
Nm = Gene flow estimated from $F_{ST} = 0.25(1 - F_{ST})/F_{ST}$.
CI = Bootstrap confidence intervals for the parameters (Number of permutations in breeding analysis; 1000) (Weir, 1996).
*** = Statistically significant at P<0.05**

Locus	Sample size	F_{IS}	F_{IT}	F_{ST}	Nm
Bmag603	320	-0.75±0.08	-0.28±0.12	0.26±0.06	0.69
Bmac67	318	-0.87±0.05	-0.63±0.09	0.13±0.04	1.72
Bmag206	320	1.00±0.00	1.00±0.00	0.69±0.09	0.11
Bmac209	318	-0.88±0.04	-0.43±0.07	0.24±0.03	0.79
Bmag6	318	1.00±0.00	1.00±0.00	**0.87**±0.05	0.04
Bmag225	318	**-0.93**±0.02	-0.38±0.03	0.29±0.02	0.63
Mean	319	**-0.67***	**-0.01**	**0.39***	**0.38**
CI (95%)		-0.82-(-0.04)	-0.45-0.50	0.20-0.57	

Table 5.6. Summary of F-Statistics and gene flow for the 6 polymorphic loci in 40 sensitive Turkish seed sources (accessions) of *Hordeum vulgare*
F_{IS} = the fixation index within subpopulations
F_{IT} = the fixation index over total population
F_{ST} = the reduction in fixation index due to differences among subpopulations (Nei (1987)
Nm = Gene flow estimated from $F_{ST} = 0.25(1 - F_{ST})/F_{ST}$.
CI = Bootstrap confidence intervals for the parameters (Number of permutations in breeding analysis; 1000) (Weir, 1996).
*** = Statistically significant at P<0.05**

Locus	Sample size	F_{IS}	F_{IT}	F_{ST}	Nm
Bmag603	320	-0.70±0.09	-0.01± 0.11	0.41± 0.05	0.36
Bmac67	314	**-0.95**±0.04	-0.34± 0.02	0.31± 0.02	0.55
Bmag206	320	1.00±0.00	1.00± 0.00	0.81± 0.08	0.06
Bmac209	320	-0.87±0.05	-0.37± 0.05	0.27± 0.03	0.68
Bmag6	320	1.00 ± 0.00	1.00 ± 0.00	**0.94**± 0.05	0.02
Bmag225	318	-0.91± 0.03	-0.55 ± 0.04	0.19 ±0.03	1.08
Mean	319	**-0.76***	**0.04**	**0.46***	**0.30**
CI (95%)		-0.86-(-0.25)	-0.34-0.55	0.27-0.66	

5.2.2. Genetic Diversity and F-Statistics of Turkish Barley Seed Sources Grouped with Respect to the Common Parents

Population genetic diversity parameters were also calculated for thirty two groups that were arranged according their common parental origins (Table 5.7). Fifteen barley seed sources grouped with respect to the common parental origins were resistant to scald disease, while seventeen barley seed sources were sensitive to the disease. All of the parameters were also calculated for these thirty two groups of Turkish barley seed sources (Table 5.8).

Table 5.7. Grouping of Turkish barley seed sources according to the common parental origins. R: resistant, and S: sensitive barley seed sources to scald disease.

Codes	Common parents of seed sources	
P1	R	Rabur/Luther
P2	R	NE76148/WBCBPI/388643
P3	R	YEA
P4	R	324P.K-5/Tuil10
P5	R	3896/1-15/3/3896/28//284/28/4/EinbuIICI 7321
P6	R	TOKAK
P7	R	YEA 762-2/Tokak
P8	R	Flamenco/WM
P9	R	H272/Bgs/3/Mzg/Gva//PI002917/4/DeirAlla10//Mzg/DL71
P10	R	CWB22-6-13/ICB-102411
P11	R	Tryll/Hudson//Obruk-86
P12	R	BastionM
P13	R	AVD-121/Bülbül-89
P14	R	4814/3/3896/Gzk//132TH
P15	R	Wysor
P16	S	CWB117
P17	S	Roho//Alger/Ceres362-1-1/ 3'Alpha/Durra
P18	S	ROHO
P19	S	CHİCM/AN57//ALBERT/3/ICB-102379/4/GKOMEGA
P20	S	PAMIR
P21	S	ALPHA/2104
P22	S	1993-94(IWFBSP)
P23	S	ESKISEHIR
P24	S	YEA
P25	S	VIRINGA
P26	S	4857
P27	S	PLATEN672//353TH/P12-119
P28	S	ANTARES/KY63-1264//LİGNEE 131
P29	S	TOKAK
P30	S	CUM-50/700.1
P31	S	PAMİR-42/BÜLBÜL
P32	S	BELTS-60-1807/HENRY//SUSSEX/3/2/BARSOY/4/B/A/5/K-247/2401-13/VAVILON

Table 5.8. Estimated genetic diversity parameters of Turkish barley seed sources, grouped according to the common parental origins. n = sample size n_a= observed number of alleles, n_e = effective number of alleles (Kimura and Crow, 1964), *P* = the proportion of polymorphic loci, # = Number of polymorphic loci, H_o = observed heterozygosity, and H_e = expected heterozygosity (Levene, 1949)

Seed Source Codes	n	n_a	n_e	P		Mean Heterozygosity	
				#	%	H_o	H_e
P1*	8	1.7±0.25	**1.5**±0.25	4	66.7	0.54±0.25	0.33±0.14
P2*	8	1.8±0.58	1.7±0.44	3	**50.0**	**0.50**±0.27	**0.32**±0.18
P3*	56	**3.5**±0.20	**2.7**±0.09	6	**100.0**	0.62±0.09	0.63±0.01
P4*	8	1.7±0.25	1.7±0.25	4	66.7	**0.67**±0.25	0.38±0.14
P5*	119	2.7±0.10	1.9±0.06	6	**100.0**	0.66±0.06	0.45±0.01
P6*	16	2.8±0.26	2.4±0.25	6	**100.0**	**0.67**±0.18	0.58±0.05
P7*	16	1.7±0.18	1.7±0.18	4	66.7	**0.67**±0.18	0.36±0.09
P8*	8	1.8±0.20	1.7±0.19	5	83.3	0.62±0.24	0.45±0.11
P9*	16	3.0±0.31	2.5±0.23	6	**100.0**	0.58±0.16	**0.63**±0.03
P10*	8	1.7±0.25	1.7±0.25	4	66.7	**0.67**±0.25	0.38±0.14
P11*	8	1.7±0.25	1.7±0.18	4	66.7	**0.67**±0.25	0.38±0.14
P12*	8	1.7±0.25	1.7±0.25	4	66.7	**0.67**±0.25	0.38±0.14
P13*	16	2.7±0.36	2.0±0.18	5	83.3	0.52±0.18	0.45±0.09
P14*	16	2.2±0.18	1.9±0.17	5	83.3	0.56±0.16	0.45±0.08
P15*	8	2.2±0.40	1.8±0.25	5	83.3	0.58±0.24	0.45±0.11
P16**	23	2.7±0.25	2.4±0.21	6	**100.0**	0.62±0.14	0.57±0.03
P17**	24	2.7±0.24	2.0±0.18	6	**100.0**	0.53±0.15	0.47±0.05
P18**	40	3.3±0.14	**2.9**±0.12	6	**100.0**	0.59±0.11	**0.65**±0.03
P19**	8	2.0±0.31	1.9±0.27	5	83.3	0.58±0.24	0.48±0.12
P20**	24	2.8±0.34	2.5±0.29	5	83.3	0.61±0.14	0.54±0.08
P21**	8	1.7±0.25	1.6±0.25	4	66.7	0.63±0.24	0.38±0.14
P22**	8	1.7±0.25	1.7±0.25	4	66.7	**0.67**±0.25	0.38±0.14
P23**	24	3.0±0.37	2.6±0.31	5	83.3	0.61±0.14	0.54±0.08
P24**	32	**3.5**±0.26	2.2±0.17	6	**100.0**	0.66±0.12	0.53±0.04
P25**	16	2.3±0.29	2.1±0.23	5	83.3	0.56±0.17	0.50±0.09
P26**	16	1.8±0.26	1.8±0.23	4	66.7	0.63±0.17	0.37±0.09
P27**	8	**1.5**±0.27	**1.5**±0.27	3	**50.0**	**0.50**±0.27	**0.29**±0.15
P28**	8	1.7±0.25	1.6±0.25	4	66.7	**0.50**±0.27	0.36±0.14
P29**	40	2.8±0.25	2.2±0.17	5	83.3	0.63±0.11	0.48±0.06
P30**	8	1.7±0.25	1.7±0.25	4	66.7	**0.67**±0.25	0.38±0.14
P31**	24	1.7±0.15	1.7±0.14	4	66.7	0.64±0.14	0.35±0.07
P32**	8	1.7±0.25	1.7±0.25	4	66.7	**0.67**±0.25	0.38±0.14
MEAN	637	4.0±0.05	2.9±0.02	6	100.0	0.62±0.01	0.65±0.01

* = Scald disease resistant Turkish barley seed source groups, formed with common origins

** = Scald disease sensitive Turkish barley seed source groups, formed with common origins

According to the multi-populations descriptive statistics, it was found that **observed number of alleles (n_a)** was 4.0 for all loci. The mean number of observed alleles within the populations grouped according to the common parental origins differed between 1.7 and 3.5 [P3, (R YEA)] for scald disease resistant, 1.5 [P27, (S PLATEN672//353TH/P12-119)] and 3.5 [P24, (S YEA)] for scald disease sensitive groups. Moreover **effective number of alleles (n_e)** was 2.9 when all barley seed sources considered. It ranged between 1.5 [P1, (R Rabur/Luther)] and 2.7 [P3, (R YEA)] for scald disease resistant seed sources, 1.5 [P27, (S PLATEN672 // 353TH/P12-119)] and 2.9 [P18, (S ROHO)] for scald disease sensitive seed sources. All of the values of these seed source-groups were given in Table 5.8.

The proportion of polymorphic loci was ranged between 50% and 100% for both scald disease resistant and sensitive seed source-groups. For scald disease resistant groups, P2 (R NE76148/WBCBPI/388643) had the lowest (50%) value and P3 (R YEA), P5 (R 3896/1-15/3/3896/28//284/28/4/EinbuIICI7321), P6 (R TOKAK), P9 (RH272/Bgs/3/Mzg/Gva// PI002917/4/DeirAlla10 //Mzg/DL71), P16 (S CWB117) had the highest values. For scald disease sensitive groups, P27 (S PLATEN672//353TH/P12-119 P17) had the lowest value and P16 (S CWB117), P17 (S Roho//Alger/Ceres362-1-1/ 3'Alpha/Durra), P18 (S ROHO) and P24 (S YEA) had the highest values (100%) (Table 5.8).

Observed heterozygosity values varied between 0.50 and 0.67 for both scald disease resistant and sensitive seed source-groups. In resistant barley seed sources, P2 (R NE76148/WBCBPI/388643) had the lowest value; on the other hand P4 (R 324P.K-5/Tuil10), P6 (R TOKAK) , P7 (R YEA 762-2/Tokak), P10 (R CWB22-6-13/ICB-102411) , P11(R Tryll/Hudson //Obruk-86), P12 (R BastionM) had the highest values. In sensitive barley seed sources P27 (S PLATEN672//353TH/P12-119) and P28 (S ANTARES/KY63-1264//LİGNEE 131) had the lowest values, and P22 (S 1993-94 (IWFBSP)), P30 (S CUM-50/700.1) and P32 (S BELTS-60-1807/HENRY//SUSSEX/3/2 *BARSOY/4/B/A/5/K-247/2401-13/VAVİLON) had the highest observed heterozygosity values (Table 5.8).

Expected heterozygosity values differed from 0.32 and 0.63 for resistant barley seed source-groups and 0.29 and 0.65 for scald sensitive barley seed source-groups. For resistant groups P2 (R NE76148/WBCBPI/388643) had the lowest, and P9 (R H272/Bgs/3/Mzg/Gva//PI002917/4/DeirAlla10//Mzg/DL71) had the highest value. For sensitive barley seed sources P27 (S PLATEN672//353TH/P12-119) had the lowest, P18 (S ROHO) had the highest value (Table 5.8).

F-statistics were also calculated according to the common parental origins of Turkish barley seed sources. These values were calculated for both scald disease resistant (Table 5.9) and sensitive Turkish barley seed source-groups (Table 5.10), separately.

Table 5.9. Summary of F-Statistics calculated separately for the 6 polymorphic loci in scald disease resistant Turkish barley seed sources grouped with respect to their common parental origins.
F_{IS} = the fixation index within subpopulations
F_{IT} = the fixation index over total population
F_{ST} = the reduction in fixation index due to differences among subpopulations (Nei 1987, Molecular Evolutionary Genetics)
CI = Bootstrap confidence intervals for the parameters (Number of permutations in breeding analysis; 1000) (Weir, 1996).
*** = Statistically significant at P<0.05**

Locus	Sample size	F_{IS}	F_{IT}	F_{ST}
Bmag603	320	-0.59±0.24	-0.13±0.20	0.29±0.08
Bmac67	318	-0.82±0.08	-0.77±0.08	0.02±0.01
Bmag206	320	1.00±0.00	1.00±0.00	0.66±0.12
Bmac209	318	-0.75± 0.14	-0.29± 0.13	0.26±0.06
Bmag6	318	1.00±0.00	1.00±0.00	0.73±0.12
Bmag225	318	-0.86± 0.07	-0.46±0.14	0.22±0.11
MEAN	319	**-0.53**	**-0.01**	**0.35***
CI (95%)		-0.67-0.25	-0.42-0.53	0.14-0.38

Table 5.10. Summary of F-Statistics calculated separately for the 6 polymorphic loci in scald disease sensitive Turkish barley seed sources grouped with respect to their common parental origins.

F_{IS} = the fixation index within subpopulations
F_{IT} = the fixation index over total population
F_{ST} = the reduction in fixation index due to differences among subpopulations (Nei 1987, Molecular Evolutionary Genetics)
CI = Bootstrap confidence intervals for the parameters (Number of permutations in breeding analysis; 1000) (Weir, 1996).
*** = Statistically significant at P<0.05**

Locus	Sample size	F_{IS}	F_{IT}	F_{ST}
Bmag603	320	-0.39±0.13	0.04±0.12	0.31±0.08
Bmac67	314	-0.72±0.06	-0.35±0.03	0.22±0.04
Bmag206	320	1.00±0.00	1.00±0.00	0.59±0.09
Bmac209	320	-0.72±0.07	-0.34±0.06	0.22±0.05
Bmag6	320	1.00±0.00	1.00±0.00	0.74±0.13
Bmag225	318	-0.84±0.07	-0.53±0.05	0.17±0.03
MEAN	319	**-0.45**	**0.06**	**0.35***
CI (95%)		-0.56-0.26	-0.34-0.55	0.15-0.39

When fixation index (F_{IS}) within subpopulations for all loci were considered; the mean value was -0.53 for resistant (Table 5.9) and -0.45 for sensitive barley seed source-groups (Table 5.10). It means that within scald resistant and sensitive subpopulations heterozygotes were 53% and 45% higher than expected, respectively. However, when all 32 Turkish barley accessions were considered this value was -0.48. This result indicates that within 32 barley seed sources, heterozygotes were 48% higher than expected (Table 5.11).

F_{IT} which gives the total deviation of populations had the mean value of -0.01 (Table 5.9) for resistant barley seed source-groups indicating that there was very little excess heterozygotes within resistant populations. This value was 0.06 (Table 5.10) for sensitive barley seed source-groups. Therefore we can say that 0.6% excess homozygotes were observed within scald disease sensitive populations.

The reduction in fixation index due to genetic differentiation among subpopulations ($\mathbf{F_{ST}}$) was also estimated. Mean F_{ST} value was 0.35 for both scald disease resistant and sensitive barley seed source-groups indicating that 35% of total genetic variation was between subpopulations, and 65% of the total variation observed within populations for both resistant and sensitive seed source-groups. When overall 32 Turkish barley accessions were considered, this value was 0.37 (Table 5.11) indicating that 37% of total variation was between subpopulations, and 63% of the total variation observed within populations.

Table 5.11. Summary of F-Statistics calculated separately for the 6 polymorphic loci in 32 Turkish barley accessions of *Hordeum vulgare* which were grouped according to the common parental origins

F_{IS} = the fixation index within subpopulations
F_{IT} = the fixation index over total population
F_{ST} = the reduction in fixation index due to differences among subpopulations (Nei 1987, Molecular Evolutionary Genetics)
CI = Bootstrap confidence intervals for the parameters (Number of permutations in breeding analysis; 1000) (Weir, 1996).
*** = Statistically significant at P<0.05**

Locus	Sample size	F_{IS}	F_{IT}	F_{ST}
Bmag603	640	-0.48±0.14	-0.03±0.12	0.30±0.06
Bmac67	632	-0.76±0.04	-0.45±0.06	0.18±0.04
Bmag206	640	1.00±0.000	1.00±0.00	0.64±0.09
Bmac209	638	-0.73±0.08	-0.31±0.07	0.24±0.04
Bmag6	638	1.00±0.00	1.00±0.00	0.75±0.08
Bmag225	636	-0.85±0.05	-0.49±0.10	0.19±0.06
MEAN	637	**-0.48**	**0.06**	**0.37***
CI (95%)		-0.63-0.32	-0.36-0.58	0.16-0.40

5.2.3 Genetic Diversity of 80 Turkish Barley Seed Sources

Also all genetic diversity parameters were estimated for 80 Turkish barley seed sources individually. Genetic diversity statistics were provided at Tables 5.12 and 5.13 for resistant and sensitive Turkish barley seed sources, respectively.

Table 5.12. The population genetic diversity parameters for 40 resistant Turkish seed sources of *Hordeum vulgare*. n = Sample size per locus, n_a = observed number of alleles per locus, n_e = Effective number of alleless (Kimura and Crow, 1964), *P* = the proportion of polymorphic loci H_o = the observed heterozygosity, and *He* = the expected heterozygosity (Levene, 1949)

Codes	Sample size (n)	n_a	n_e	Polymorphic loci (P)		Mean Heterozygosity	
				#	% P	H_o	H_e
R1	8	1.7±0.25	**1.5**±0.25	4	66.7	0.54±0.25	0.33±0.14
R2	8	1.8±0.58	1.7±0.44	3	**50.0**	**0.50**±0.27	0.32±0.18
R3	8	**1.5**±0.27	**1.5**±0.27	3	**50.0**	**0.50**±0.27	**0.29**±0.15
R4	8	1.7±0.25	1.7±0.25	4	66.7	0.67±0.25	0.38±0.14
R5	8	1.8±0.20	1.8±0.20	5	83.3	0.67±0.25	0.48±0.11
R6	8	1.7±0.25	1.7±0.25	4	66.7	0.67±0.25	0.38±0.14
R7	8	1.7±0.25	1.7±0.25	4	66.7	0.67±0.25	0.38±0.14
R8	8	2.1±0.49	2.0 ±0.36	5	83.3	0.67±0.25	0.49±0.13
R9	8	2.5±0.52	2.1±0.38	5	83.3	0.67±0.25	0.52±0.14
R10	8	2.2±0.20	1.9±0.16	6	**100.0**	0.67±0.25	0.54±0.04
R11	8	2.0±0.54	1.9±0.38	4	66.7	0.67±0.25	0.42±0.16
R12	8	1.7±0.25	1.7±0.25	4	66.7	0.67±0.25	0.38±0.14
R13	8	1.7±0.25	1.7±0.25	4	66.7	0.67±0.25	0.38±0.14
R14	8	1.8±0.20	1.7±0.19	5	83.3	0.58±0.23	0.44±0.11
R15	8	2.2±0.49	2.0±0.36	5	83.3	0.67±0.25	0.49±0.13
R16	8	1.8±0.20	1.8±0.20	5	83.3	0.67±0.25	0.45±0.11
R17	8	1.7±0.25	1.7±0.25	4	66.7	0.67±0.25	0.38±0.14
R18	8	1.7±0.25	1.7±0.25	4	66.7	0.67±0.25	0.38±0.14
R19	8	1.7±0.25	1.7±0.25	4	66.7	0.67±0.25	0.38±0.14
R20	8	1.7±0.25	1.7±0.25	4	66.7	0.67±0.25	0.38±0.14
R21	8	2.0±0.31	1.8±0.24	5	83.3	0.67±0.25	0.47±0.12
R22	8	2.2±0.58	1.9±0.42	4	66.7	0.63±0.24	0.43±0.16
R23	8	1.8±0.20	1.7±0.19	5	83.3	0.63±0.24	0.45±0.11
R24	8	1.7±0.25	1.7±0.25	4	66.7	0.67±0.25	0.38±0.14
R25	8	2.7±0.40	**2.2**±0.27	6	**100.0**	0.54±0.25	**0.61**±0.05
R26	7	1.8±0.24	1.8±0.24	5	83.3	0.67±0.27	0.49±0.14
R27	8	1.8±0.37	1.7±0.30	4	66.7	0.67±0.25	0.40±0.15
R28	8	1.7±0.25	1.7±0.25	4	66.7	0.67±0.25	0.38±0.14
R29	8	1.8±0.20	1.7±0.19	5	83.3	0.63±0.24	0.45±0.11
R30	8	**2.8**±0.58	**2.2**±0.45	5	83.3	0.54±0.25	0.52±0.11
R31	8	2.2±0.37	1.9±0.24	5	83.3	0.62±0.24	0.50±0.12
R32	8	1.7±0.25	1.7±0.25	4	66.7	0.67±0.25	0.38±0.14
R33	8	1.7±0.25	1.7±0.25	4	66.7	0.67±0.25	0.38±0.14
R34	8	1.7±0.25	1.7±0.25	4	66.7	0.67±0.25	0.38±0.14
R35	8	2.5±0.52	2.0±0.48	5	83.3	0.54±0.25	0.48±0.13
R36	8	**1.5**±0.27	**1.5**±0.27	3	**50.0**	**0.50**±0.27	**0.29**±0.15
R37	8	2.0±0.31	1.8±0.24	5	83.3	0.58±0.24	0.47±0.12
R38	8	2.2±0.37	1.9±0.34	5	83.3	0.54±0.22	0.48±0.13
R39	8	2.5±0.27	**2.2**±0.17	6	**100.0**	0.67±0.25	0.60±0.04
R40	8	2.2±0.37	1.8±0.24	5	83.3	0.58±0.24	0.45±0.12
MEAN	319	3.7±0.06	2.7±0.03	6	100.0	0.63±0.02	0.62±0.01

Table 5.13. The population genetic diversity parameters for 40 sensitive Turkish seed sources of *Hordeum vulgare*. n = Sample size per locus, n_a = observed number of alleles per locus, n_e = Effective number of alleles (Kimura and Crow, 1964), *P* = the proportion of polymorphic loci, H_o = the observed heterozygosity, and *He* = the expected heterozygosity (Levene, 1949)

Codes	Sample size (n)	n_a	n_e	Polymorphic loci (P)		Mean Heterozygosity	
				#	% P	H_o	H_e
S1	7	2.2±0.44	2.1±0.35	5	83.3	0.58±0.24	0.54±0.16
S2	8	1.8±0.58	1.7±0.44	3	**50.0**	**0.50**±0.27	0.32±0.18
S3	8	2.2±0.58	1.9±0.44	4	66.7	0.58±0.25	0.42±0.16
S4	8	1.8±0.37	1.7±0.29	4	66.7	0.63±0.25	0.39±0.15
S5	8	1.8±0.37	1.7±0.29	4	66.7	0.63±0.25	0.39±0.15
S6	8	2.0±0.31	1.8±0.24	5	83.3	0.58±0.25	0.45±0.12
S7	8	1.7±0.25	1.7±0.25	4	66.7	0.67±0.25	0.39±0.15
S8	8	1.8±0.20	1.8±0.20	5	83.3	0.67±0.25	0.45±0.11
S9	8	2.0±0.31	1.9±0.25	5	83.3	0.58±0.25	0.48±0.11
S10	8	1.7±0.40	1.6±0.32	3	**50.0**	**0.50**±0.27	0.30±0.16
S11	8	1.7±0.25	1.6±0.25	4	66.7	0.63±0.25	0.38±0.14
S12	8	1.7±0.25	1.6±0.25	4	66.7	0.63±0.25	0.38±0.14
S13	8	1.7±0.25	1.7±0.25	4	66.7	0.67±0.25	0.38±0.14
S14	8	1.8±0.37	1.7±0.30	4	66.7	0.67±0.25	0.40±0.15
S15	8	1.7±0.25	1.7±0.25	4	66.7	0.67±0.25	0.38±0.14
S16	8	**2.8**±0.58	**2.6**±0.53	5	83.3	0.63±0.25	**0.61**±0.15
S17	8	1.7±0.25	1.6±0.24	4	66.7	0.58±0.23	0.37±0.14
S18	8	1.7±0.25	1.6±0.24	4	66.7	**0.50**±0.27	0.36±0.14
S19	8	1.7±0.25	1.6±0.24	4	66.7	0.58±0.25	0.36±0.14
S20	8	**1.5**±0.27	**1.5**±0.27	3	**50.0**	**0.50**±0.27	**0.29**±0.15
S21	8	**1.5**±0.27	**1.5**±0.27	3	**50.0**	**0.50**±0.27	**0.29**±0.15
S22	8	1.8±0.37	1.7±0.30	4	66.7	**0.50**±0.27	0.40±0.15
S23	8	1.7±0.25	1.7±0.25	4	66.7	0.67±0.25	0.38±0.14
S24	8	1.7±0.25	1.7±0.25	4	66.7	0.67±0.25	0.38±0.14
S25	8	1.8±0.37	1.7±0.30	4	66.7	0.67±0.25	0.40±0.15
S26	8	1.7±0.25	1.7±0.25	4	66.7	0.67±0.25	0.38±0.14
S27	8	**2.8**±0.37	2.2±0.30	6	**100.0**	0.54±0.25	0.60±0.06
S28	8	1.8±0.37	1.7 ±0.30	4	66.7	**0.50**±0.27	0.38±0.15
S29	8	1.7±0.25	1.6±0.24	4	66.7	**0.50**±0.27	0.36±0.14
S30	8	2.0±0.31	1.8±0.24	5	83.3	0.67±0.25	0.47±0.14
S31	8	1.7±0.25	1.7±0.25	4	66.7	0.67±0.25	0.38±0.14
S32	8	1.7±0.25	1.7±0.25	4	66.7	0.67±0.25	0.38±0.14
S33	8	1.8±0.37	1.7±0.32	4	66.7	0.58±0.25	0.38±0.15
S34	8	1.7±0.25	1.7±0.25	4	66.7	0.67±0.25	0.38±0.14
S35	8	1.7±0.25	1.7±0.25	4	66.7	0.67±0.25	0.38±0.14
S36	8	1.7±0.25	1.7±0.25	4	66.7	0.67±0.25	0.38±0.14
S37	8	1.7±0.25	1.6±0.25	4	66.7	0.63±0.25	0.38±0.14
S38	8	1.7±0.25	1.6±0.25	4	66.7	0.63±0.25	0.38±0.14
S39	8	1.7±0.25	1.7±0.25	4	66.7	0.67±0.25	0.38±0.14
S40	8	1.7±0.25	1.7±0.25	4	66.7	0.67±0.25	0.38±0.14
MEAN	319	3.8±0.06	2.9±0.04	6	100.0	0.61±0.02	0.64±0.01

5.2.3.1 Allelic Richness of 80 Turkish Barley Seed Sources

Although in multi-populations descriptive statistics, it was found that observed number of alleles (n_a) was 4.0 (Table 5.3) for all loci, the mean number of observed alleles per locus (n_a) ranged from1.5 to 2.8 in both scald disease resistant and sensitive Turkish barley seed sources (Table 5.12 and 5.13). Moreover, in multi-populations descriptive statistics, while effective number of alleles (n_e) value was 2.9 (Table 5.3) for all loci, n_e varied between 1.5 and 2.2 for resistant (Table 5.12) and between 1.5 and 2.6 for sensitive Turkish barley seed sources (Table 5.13).

5.2.3.2 Proportion of Polymorphic Loci of 80 Turkish Barley Seed Sources

The proportion of polymorphic loci (0.99 criterion) varied between 50 and 100%. Resistant seed sources of R2, R3, R36 and sensitive seed sources of S2, S10, S20 and S21 had the lowest proportion of polymorphic loci. Moreover resistant seed sources of R10, R25, R39 and sensitive seed sources of S27 had the highest values of proportion of polymorphic loci. (Table 5.12 and Table 5.13).

5.2.3.3 Heterozygosity of 80 Turkish Barley Seed Sources

Observed heterozygosity (H_o) values varied between 0.50 and 0.67 in both resistant and sensitive Turkish barley seed sources (Table 5.12 and Table 5.13). Scald disease resistant seed sources R2, R3, R36 and sensitive seed sources S2, S10, S18, S20, S21, S22, S28 and S29 had the lowest heterozygosity compared to the others (Table 5.12 and Table 5.13).

Expected heterozygosity (He) values varied between 0.29 and 0.61. Resistant seed sources of R3, R36, and sensitive seed sources of S20 and S21 had the lowest expected heterozygosity. R25 for resistant seed sources and S16 for sensitive seed sources had the highest values (Table 5.12 and Table 5.13).

5.2.3.4 F-Statistic of 80 Turkish Barley Seed Sources

The mean value of overall fixation index within subpopulations ($\mathbf{F_{IS}}$) for all loci was -0.71 for this study. This result indicates that within the subpopulations, heterozygotes were 71% higher than expected (Table 5.14).

The total deviation of populations ($\mathbf{F_{IT}}$) had the mean value of 0.05 indicating that 0.5% excess homozygotes were observed within populations (Table 5.14).

Mean F_{ST} value was 0.44 indicating that 44% of total genetic variation was between populations, and 66% of the total genetic variation observed was within populations (Table 5.14). This value showed that there was a very high genetic differentiation within Turkish barley seed sources.

The gene flow, **Nm**, was estimated by means of F_{ST} values. Nm values below 1.0 are critical, meaning that the population begins to differentiate because of genetic drift and Nm value of 0.5 is the most critical value (Wright 1969). When it is below 0.5, populations will diverge extensively as a result of genetic drift. Gene flow value estimated for this study was 0.31 indicating that our populations in a critical state and there is a threat of genetic drift (Table 5.14).

Table 5.14. Summary of F-Statistics for the 6 polymorphic loci in 80 Turkish accessions of *Hordeum vulgare*
F_{IS} = the fixation index within subpopulations
F_{IT} = the fixation index over total population
F_{ST} = the reduction in fixation index due to differences among subpopulations (Nei (1987)
Nm = Gene flow estimated from $F_{ST} = 0.25(1 - F_{ST})/F_{ST}$.
CI = Bootstrap confidence intervals for the parameters (Number of permutations in breeding analysis; 1000) (Weir, 1996).
*** = Statistically significant at P<0.05**

Locus	Sample size	F_{IS}	F_{IT}	F_{ST}	N_m
Bmag603	640	-0.72±0.06	-0.13±0.08	0.34±0.04	0.48
Bmac67	632	-0.91±0.03	-0.42±0.04	0.25±0.02	0.73
Bmag206	640	1.00±0.00	1.00±0.00	0.77±0.06	0.08
Bmac209	638	-0.87±0.03	-0.39±0.04	0.26±0.02	0.72
Bmag6	638	1.00±0.00	1.00±0.00	0.91±0.03	0.03
Bmag225	636	-0.92±0.02	-0.43±0.03	0.26±0.02	0.73
MEAN	637	**-0.71***	**0.05**	**0.44***	**0.31**
CI (95%)		-0.84-(-0.14)	-0.37-0.54	0.26-0.62	

5.2.4. Genetic Distance of Turkish Barley Seed Sources

In order to estimate the genetic relationship between Turkish barley seed sources, they were grouped according to the common parental origins, Nei's unbiased genetic distance values were calculated among these barley seed sources (Nei, 1978) via GDA software (Lewis and Zaykin, 2002). Genetic distance value ranges between "0 and 1", 0 distance is equivalent to no difference. Genetic distance values were calculated for 32 Turkish barley seed sources grouped according to the common parental origins, some of these are resistant to scald disease of barley and others are sensitive. Dendrogram was constructed by using UPGMA (Unweighted Pair Group Method using Arithmetic Averaging) method to see the grouping among the seed sources (Figure 5.3).

When the 32 Turkish barley seed sources were considered genetic distance varied between 0.00 and 1.00. According to the results P4-P5, P6-P9, P7-P22, P7-P24, P7-P26, P7-P29, P7-P30, P29-P30, P10-P12, P22-P24, P22-P26, P22-P29, P22-P30,

P23-P29, P24-P29, P24-P30, P26-P29, P26-P30, P27-P28, P29-P30 were the most closely related seed sources with 0.00 genetic distance values. On the other hand, there were many distance related seed sources with the genetic distance value of 1.00.

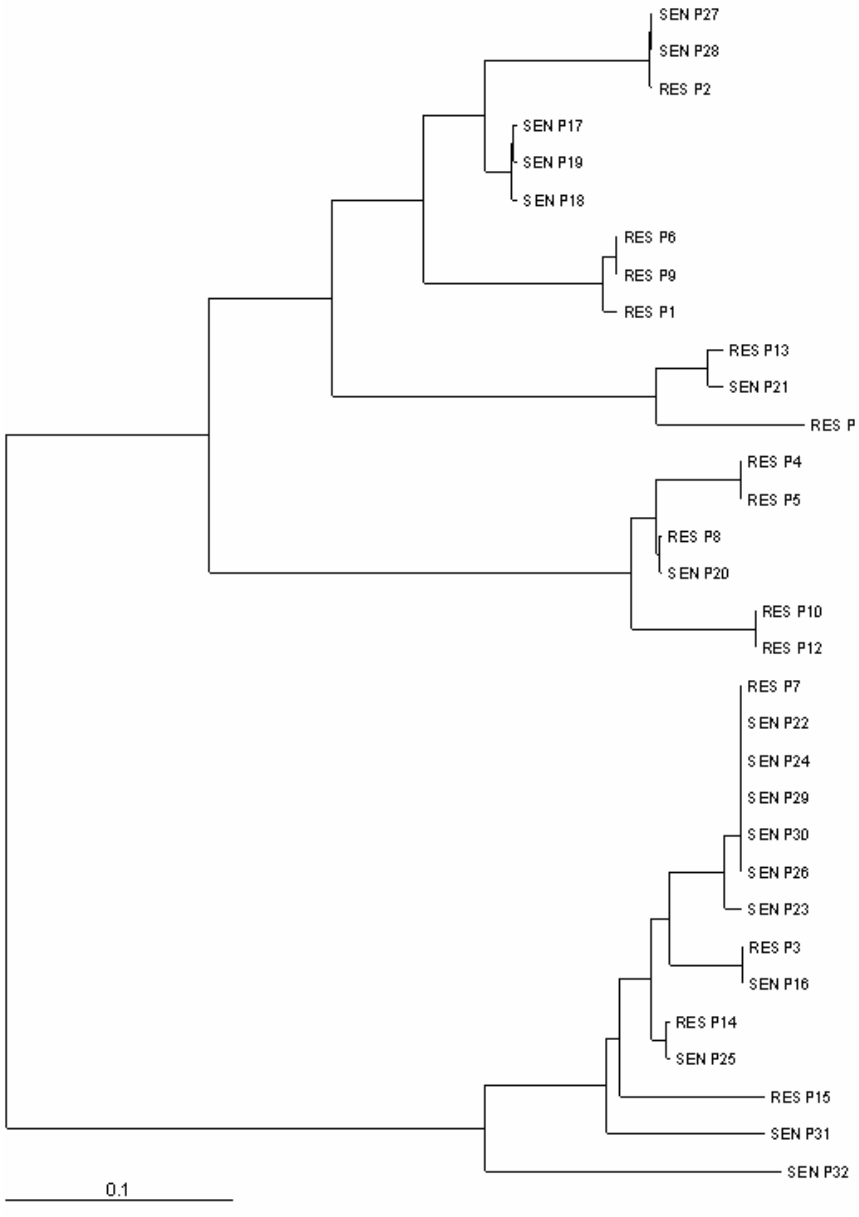


Figure 5.3. Dendrogram based on Nei's (1978) genetic distance for 32 Turkish barley seed sources with UPGMA method.

In the dendrogram two major groups were observed. In these groups both of resistant and sensitive seed sources can be identified. However, especially in the second group (lower branch) of dendrogram, sensitive barley seed sources constituted a cluster (P22, P24, P26, P29, P30, and P23). There were also small clusters in both of the two major groups. While some of these small clusters contained sensitive or resistant seed sources only, others were formed by both resistant and sensitive barley seed sources. For example; P1, P6, P9 scald resistant barley seed sources constituted one cluster, P17, P18, P19 scald sensitive barley seed sources formed another cluster, but P27, P28 scald sensitive barley seed sources grouped with P2 scald resistant barley seed sources. The most distantly grouped Turkish barley seed source was P32 that was located in the second major cluster group.

In addition to the Nei's (1978) genetic distance, coancestry (Reynolds') distance was used to estimate the genetic distance between 80 Turkish barley seed sources. The dendrogram was constructed by using UPGMA method for coancestry distance was given at Figure 5.4.

When overall Turkish barley seed sources were considered, genetic distance between sensitive 18 (4857/VIRINGA"S") and resistant 1 (Rabur / Luther) as well as between sensitive 28 (274 ESKISEHIR-.ÖVD) and resistant 1 (Rabur / Luther) were large. As seen in Figure 5.4, generally resistant and sensitive Turkish barley seed sources were grouped separately. In the study, fifteen resistant barley seed sources which had the same parental origins (R6, 7, 10, 14, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, and 27) were located in the same cluster group.

Turkish barley seed sources R11, R12, R28, R5, R10, R37, R38, S4, S5, S13, S14, S15, S17, S31, S34, S35, S36, S19, S33 were located in the same cluster. When their parental origins were considered, it was observed that these populations were generally proprietary barley seed sources such as YEA, ESKISEHIR, TOKAK and ANTARES.

Three PAMİR-42/BULBUL sensitive barley seed sources (S37, S38, S39) were grouped in the same cluster with the S23 (TOKAK/PAMİR-175) sensitive seed source.

S6, S9, and S2 seed sources formed another cluster in which S6 and S2 had the same parental origin (Roho//Alger/Ceres362-1-1/3/Alpha/Dura). Moreover, S20, S21, S29, and S22 seed sources were located in the same cluster, these scald disease sensitive barley seed sources had PLATEN, ANTARES and ROHO common parental origins.

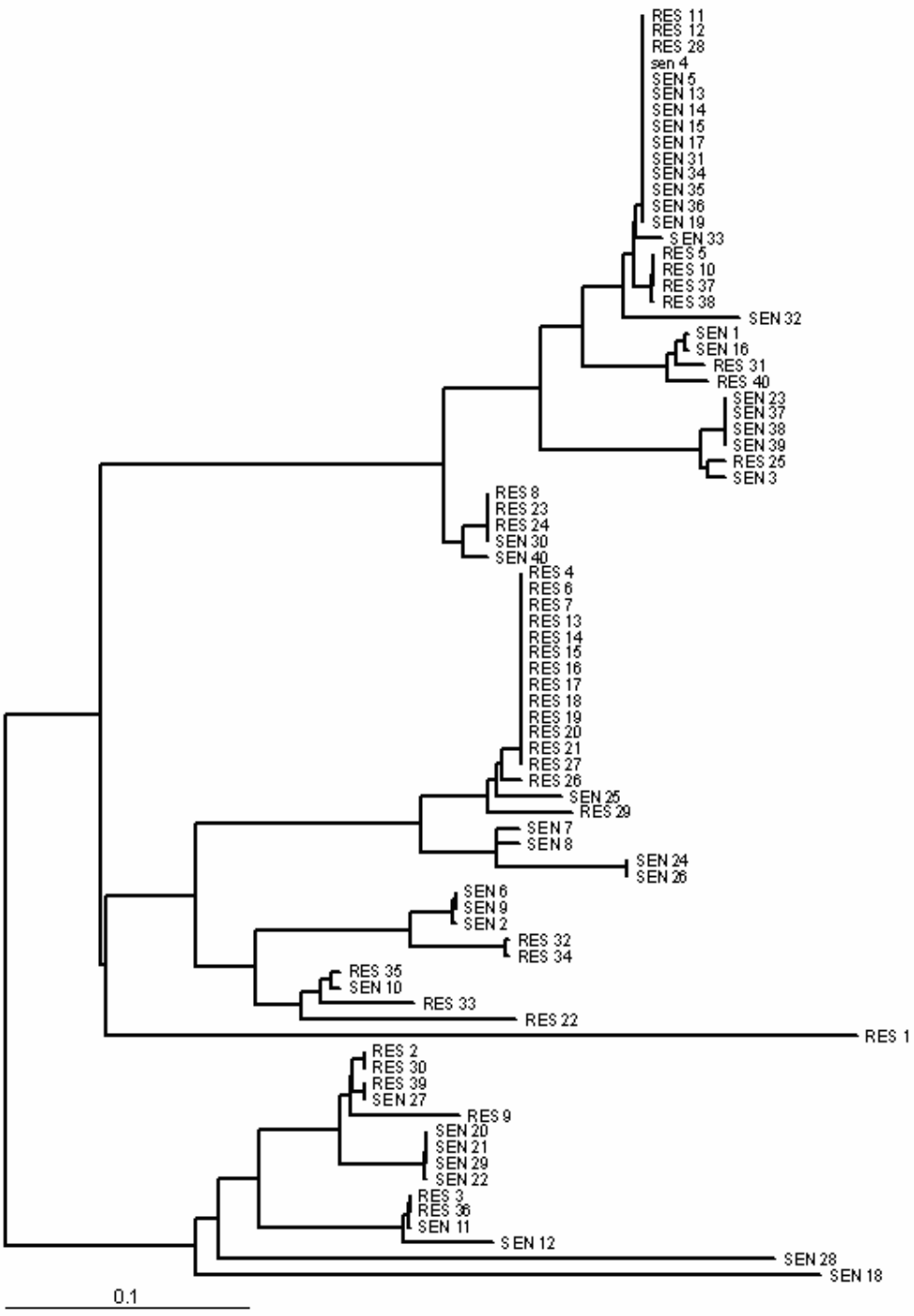


Figure 5.4. Dendrogram based on coancestry (Reynolds') distance for 80 Turkish barley seed sources with UPGMA method.

CHAPTER VI

DISCUSSION

6.1. Genetic Structure of Turkish Barley Seed sources

Eighty Turkish barley seed sources obtained from the Central Research Institute for Field Crops (Ankara, Turkey) were used during the experiment. Leaf tissues of 4 seeds for each 80 population were used for DNA extraction.

Genetic characterization of Turkish barley seed sources was accomplished by using SSR primers. Observed number of alleles per locus, effective number of alleles per locus, observed and expected heterozygosity and proportion of polymorphic loci F-statistics and genetic distance parameters were estimated for all populations of Turkish barley seed sources.

6.1.1. Genetic Diversity of Scald Disease Resistant and Sensitive Turkish Barley Seed Sources

Observed number of alleles per locus is one of the components of the genetic variation. There was no significant difference between observed number of alleles per locus between scald sensitive Turkish barley seed sources (3.8) and scald resistant Turkish barley seed sources (3.7).

When 80 Turkish barley seed sources were grouped according to their common parents, values ranged from 1.7 to 3.5 for resistant, and 1.5 to 3.5 for sensitive barley seed sources. The highest value, 3.5, was observed in P3 (R YEA) and P24 seed sources (S YEA).

Effective number of alleles (n_e) was calculated as 2.7 and 2.9 for resistant (R) and sensitive (S) groups, respectively. There were no significant differences between R and S groups. When grouping was performed according to common parental origins of barley seed sources, the value of effective number of alleles was computed between 1.5 and 2.7 for resistant, 1.5 and 2.9 for sensitive Turkish barley seed sources.

The actual or observed number of alleles is equal to effective number of alleles when all alleles have the same frequency (Kimura and crow, 1964). Otherwise effective number of alleles is always smaller than observed number of alleles since deleterious genes are not included in effective number of alleles. In this study, mean effective number of alleles was not always lower than observed number of alleles. Approximately in the half of the population, observed number of alleles was equal to effective number of alleles.

Chen *et al.* (2006) reported that the mean number of alleles per locus was 2.8 varying from 2.7 to 3.0 in Chinese cultivated barley by Sequence tagged site (STS) markers. In our study, the value of effective number of alleles for all loci was observed 2.9 for sensitive and 2.7 for resistant Turkish barley seed sources. These results are similar with finding of Chen *et al.* (2006). Material and methods used for both studies were different, so these little differences may be expected.

Allelic variation is parallel with the trends of genetic variation. Briefly, it can be said that sensitive group (S) has more genetic variation when observed and effective number of alleles were considered. Because, both observed and effective numbers of alleles in S group were higher than those of R group.

Proportion of polymorphic loci, which is another measure of genetic variation, were calculated as 100.0 % for both sensitive and resistant Turkish barley seed sources. This value ranged from 50% to 100% when Turkish barley seed sources were considered individually.

Comparisons between molecular markers in different species have generally demonstrated that SSR loci presented the highest levels of polymorphism per locus (Maughan *et al*., 1995, Powell *et al*., 1996 and Russell *et al.,* 1997a)

Chen *et al.* (2006) found that the proportion of polymorphic loci per population ranged 71% to 100% with an average of 84%. These results are close to the results of our study.

Unique (private) alleles are important because they may be diagnostic for particular inbred lines or for regions of the genome specific to a particular type of genotype (Senior *et al*., 1998). The number of unique alleles in a population is an indication of the diversity present in the germplasm and its potential as a reservoir of novel alleles for crop improvement. In our study, the first allele of Bmac67 locus with the frequency of 0.38 in R9 seed source, again the first allele of Bmag225 locus with the frequencies of 0.38 and 0.50 in R22 and S7 seed sources, respectively, and fifth allele of Bmag603 locus with the frequency of 0.25 in S16 barley seed source were observed as private or unique alleles.

Ivandic *et al.* (2002) used SSR markers to analyse wild barley collected from Iran, Turkey and Israel. They found one private allele for Bmag225 primer and eight private alleles for Bmac67 primer in barley cultivars collected from Turkey. As Ivandic *et al.* (2002) studied with wild forms of barley; high number of private alleles is expected in the study of Ivandic et al. (2002) than those in the current study in which breeding materials are used.

Heterozygosity measures are widely used descriptors of genetic diversity. One of the advantages of a highly variable marker system like SSRs which detect codominant single locus variation is the ability to directly detect the level of heterozygosity in populations. Although, each barley seed source was represented by four seeds, presence of high level of heterozygosity was evidence from results.

Observed heterozygosity values were 0.63 and 0.61 for resistant and sensitive Turkish barley seed sources, respectively. On the other hand, expected heterozygosity was 0.62 for resistant and 0.64 for sensitive barley accessions. Moreover this value was observed between 0.33 and 0.65 in groups which were made according to common parents of barley accessions. P18 (S ROHO) had the highest value of expected heterozygosity in addition to the highest effective number of alleles. The ability of SSRs to detect high levels of polymorphism clearly reveals that higher outcrossing rates are operating in Turkish barley seed sources and selection acts in these populations that favor heterozygotes.

Struss and Plieske (1998) reported expected heterozygosity value as 0.30-0.86 for different barley samples with a mean of 0.72. In our experiment, overall mean of expected heterozygosity value was found as about 0.65. This difference may be caused by different sample size of populations and primer-pairs which were used in the present study. Struss and Plieske (1998) used more samples for each barley seed source and primer-pairs, compared to present experiment so this differentiation could be expected. Also, Struss and Plieske (1998) used wild form of barley and barley landraces in addition to the barley cultivars, so higher heterozygosity values are again expected to be observed.

Chen *et al* (2006) reported that the value of gene diversity index (expected heterozygosity) was between 0.28 and 0.49 in Chinese cultivated barley sources. In our study, the value of expected heterozygosity was found between 0.29 and 0.61 when all Turkish barley seed sources were considered together regardless of their resistance and sensitivity to scald disease. Therefore, it can be safe to say that Turkish barley seed sources still maintain high genetic diversity despite of ongoing breeding practices for scald disease resistance. This means that great improvement for scald disease resistance as well as other traits could be still made with the use of these Turkish barley seed sources.

Ivandic *et al.* (2002) used SSR to analyse 39 wild barley genotypes originating from Iran (10 genotypes), Turkey (10 genotypes) and Israel (19

genotypes). They estimated expected heterozygosity between 0.18 and 0.91 with the mean 0.67 in wild form of Turkish barley. Our results support these findings. Ivandic *et al.* (2002)'s results report a slightly higher diversity than those found in our study. This could be expected since in Ivandic *et al.* (2002) wild forms of Turkish barley were used. Genetic diversity value of wild form is expected to be larger than those in cultivated form since wild barley is known to exhibit rich phenotypic and genotypic diversity (Nevo *et al*., 1979, 1984)

6.1.2 Genetic Differentiation of Scald Disease Resistant and Sensitive Turkish Barley Seed Sources

F- Statistics

Although the pattern and magnitude of genetic diversity is important, determination of deviation from Hardy-Weinberg expectations is also important. Because, these deviations indicate that there may be some forces operating to violate Hardy-Weinberg expectations in heterozygosities. In order to estimate these deviations from Hardy-Weinberg expectations, Wright's F-Statistics were used.

The inbreeding coefficients (F_{IS}, F_{IT}) or fixation indices are direct measure of increase in homozygosity due to inbreeding. F_{IS} measures the degree of inbreeding within a subpopulation; F_{IT} measures the degree of inbreeding when the subpopulations are assembled into a single population. These measures also demonstrate any deviation from the Hardy-Weinberg expectations in heterozygosity (Wright, 1969).

Estimated F_{IS} values were generally negative. In the current study the mean value was estimated as -0.71, stating that in Turkish barley seed sources, heterozygosity was observed higher than expected. Also, F_{IS} value was also negative when groups (according to the resistant and sensitive to scald disease of barley and common parents) were considered. F_{IS} value was -0.67 for resistant (R)

group and -0.76 for sensitive (S) group. These results revealed that scald disease sensitive group had more heterozygosity than expected than that of scald disease resistant group. This suggests that selection pressure among scald disease resistant Turkish barley seed sources may be higher than those in sensitive ones since there is less selection activity with sensitive barley seed sources. Excess heterozygosity can be caused by negative assortative mating and selection against homozygotes (El-Kassaby *et al*., 1987; Fady and Conkle, 1993).

F_{ST} is equal to Nei's G_{ST} so, it can be used for the same purpose. Mean F_{ST} value was calculated to be as 0.39 for resistant and 0.46 for sensitive Turkish barley seed sources. These results indicate that while 39% of total variation was between seed sources, and 61% of the total variation was within scald disease resistant seed sources, while 46% of total variation was between, and 54% of the total variation was within scald disease sensitive seed sources. According to these results, it can be said that more differentiation among sensitive Turkish barley seed sources may be occurring than those between scald disease resistant seed sources.

Chen *et al.,* (2006) calculated G_{ST} value in Chinese cultivated barley populations as 0.17, indicating 17% of the total variation was between eco-geographic zones and 83% was within the zones. The low genetic variation among zones (17%) is indicative of the high rates of gene flow between zones from both human migration and agricultural trade (Aldrich *et al.*, 1992).

Turpeinen *et al*., (2003) estimated G_{ST} value to be 0.31 indicating that 31% of the total variation was between populations and 69% was within in wild form of barley populations. Estimated F_{ST} (G_{ST}) value over all loci showed that the degree of diversity (0.44) is greater within than between populations. This is in contrast to expectations under conditions of limited migration between populations and the high selfing rate within the population. Our results are in agreement with other barley studies where a higher degree of genetic diversity is

partitioned within, rather than between, populations (Nevo *et al*., 1979, 1986; Dawson *et al*., 1993; Baum *et al*., 1997, Turpeinen *et al*., 2001)

The distribution of genetic variation among populations can be used to estimate amounts of gene flow (*Nm*) among populations that is the number of immigrants per generation. When Nm<1, populations begin to differentiate due to genetic drift (Wright, 1969). It can not be ignored, since low levels of gene flow allow higher levels of differentiation among populations. In this study, populations had low amount of gene flow. All mean values were smaller than 0.5 so 80 Turkish barley seed sources could diverge as a result of genetic drift. These barley sources are not brought together via breeding practices.

Genetic Distance

Nei's unbiased measures of genetic distance (1978) were estimated using GDA software (Lewis and Zaykin, 2002). Dendrogram with UPGMA method was constructed to see differentiation of Turkish barley seed sources which were grouped according to the common parental origins.

In cluster analysis, two large branches were observed (Figure 5.3). While the first branch had more scald disease resistant barley seed sources, the second branch had more sensitive barley seed sources. Especially in the second branch, sensitive P22, P24, P29, P30, P26 and P23 seed sources were observed at the same cluster, showing that they were the closest barley seed sources. The first branch covered five groups, one of these groups had only resistant seed sources, the other one had sensitive seed sources and the remaining three groups had both sensitive and resistant barley seed sources showing that these barley seed sources may be products of hybrids carried out in early generations of breeding.

Sensitive P32 barley seed source was the most distantly clustered barley seed sources in the second branch. This result was expected because there was no common parent between this and other barley seed sources.

6.1.3. Common Origin of Turkish Barley Coancestry Used in Scald Disease Resistant Breeding

When coancestry distance was used, the grouping of sensitive and resistant Turkish barley seed sources was clearly revealed. 3896 / 1 - 15 / 3 / 3896 / 28 // 284 / 28 / 4 / Einbull // CI 7321 scald disease resistant seed sources were generally grouped at the same cluster. Also, this cluster included R4 and R13 seed sources. Although these two seed sources had no common parents with 3896 / 1 - 15 / 3 / 3896 / 28 // 284 / 28 / 4 / Einbull // CI 7321 seed sources, they may have same or related coancestry of past that was not known to Turkish barley breeders.

The other large cluster included both of sensitive and resistant Turkish barley seed sources. Most of these seed sources had the same common parents of YEA and TOKAK. Also, in this cluster, seed sources having ESKISEHIR, VIRINGA and ANTERES were present. These results could be used to determine the origins of parental barley seed sources which will be used in future scald disease breeding or other barley breeding programs in Turkey.

6.1.4. Fingerprinting and Genetic Diversity Status of Turkish Barley Seed Sources used in Scald Disease Resistant Breeding

Primer-pairs of Bmac67, Bmag225 and Bmag603 had exclusive alleles, meaning that they were unique to a single barley accession. These unique alleles were seen in both sensitive and resistant barley seed sources. The unique allele of Bmac67 and Bmag603 were observed only resistant in (R9) and sensitive (S16) barley seed sources, respectively. Unique allele of Bmag225 primer was also seen in both sensitive (S7) and resistant (R22) barley seed sources. Thus, these primer-pairs can be used to select certain barley seed sources for future germplasm selection and marker assisted breeding.

R3 and R36 scald disease resistant barley seed sources as well as S20 and S21 scald disease sensitive barley seed sources had the lowest effective and

observed number of alleles, proportion of polymorphic loci, observed and expected heterozygosity. Therefore, it seems that these barley accessions are genetically more uniform than others. It is expected result since all of these seed sources are used as proprietary barley in Turkey. If these barley seed sources have desirable features such as disease resistance, good grain quality and high yield production, they could be preferred as a parental stock due to their genetic purity.

The microsatellite markers seem to be able to differentiate Turkish barley seed sources. The high degree of polymorphism of microsatellite markers allowed a rapid and efficient identification of barley genotypes. The high heterozygosity value of barley microsatellite markers (with an average of over 0.60) makes them ideal markers for differentiating between barley samples. However, for future studies, more samples per barley seed source and more SSR markers should be considered to have firm groups and genetic parameters with lower standard errors.

CHAPTER VII

CONCLUSION

To determine the genetic distance, and genetic diversity within and between the 80 Turkish barley seed sources, 6 SSR primer-pairs were studied.

The range of the genetic diversity parameters calculated in the present study are, 1.5-2.6 for effective number of alleles, 50.0-100.0 for percent polymorphic loci, 0.29-0.61 for expected heterozygosity and 0.50-0.67 for observed heterozygosity.

The observed and effective numbers of alleles were paralleled with percent of polymorphic loci. R25, R39 and S16 barley seed sources had generally the highest values for genetic diversity parameters and they were also genetically the richest accessions among studied 80 barley seed sources. All of these seed sources are proprietary barley seed sources that are used in Turkey. On the other hand, R3, R36, S20 and S21 had the lowest number of observed and effective number of alleles, heterozygosity and polymorphic loci indicating that they are genetically close to be called pure lines.

Private alleles can be used to select a particular seed source. These alleles are indication of genetic diversity.

Results of F-Statistics revealed that there were some deviations from expected heterozygosities. Fixation index within subpopulations for all loci (F_{IS}) was -0.71, indicating that 71% more heterozygotes were observed than it was expected. Moreover, 55-60% of total variation (F_{ST}) of Turkish barley seed

sources was within seed sources while 40-45% of total variation between barley seed sources.

Despite of considerable differentiation among especially in scald disease resistant seed sources, there is still large amount of genetic diversity existing in Turkish barley seed sources collected for scald disease resistance breeding. This large genetic variation within seed sources could be important drive in future barley breeding program in Turkey.

Based on the dendrogram constructed with genetic distance data, there were 3 major branches in dendrogram. In general, sensitive barley seed sources were grouped at the first cluster, and resistant barley accessions were grouped at the second cluster indicating that selection activities for scald disease resistant barley and sensitivity have lead to some genetic changes.

In future studies, to obtain more reliable results, more samples per Turkish barley seed source, more simple sequence repeat (SSR) markers and different type of molecular markers should be used.

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APPENDIX A

BUFFERS, CHEMICALS and EQUIPMENTS

Buffers and Solutions for DNA Extraction from barley leaves

2X CTAB: 2 gr CTAB (Cetyl Trimethyl Ammonium Bromide), (SIGMA)
10 ml (pH: 8.0) Tris HCl, (SIGMA)
4 ml (pH: 8.0) 0.5M EDTA, (FLUKA)
28 ml 5M NaCl is completed with 100 ml with dH_2O

Chloroform-Isoamilalcohol, (FLUKA)**:** (24:1)

β-Mercaptoethanol, (SIGMA)**:** 17.5 ml β-Mercaptoethanol is completed with 250 ml with dH_2O

Isopropanol, (FLUKA)**:** Pure isopropanol, ice cold

Ethanol: 70% in dH_2O

TE buffer: 10 mM Tris HCl (pH: 7)
10 mM Ethylenediaminetetraaceticacid disodium salt (EDTA)

Buffers and Solutions for Polymerase Chain Reaction (PCR)

10X PCR Buffer ($MgCl_2$ free) (BIORON)**:**

$MgCl_2$ Stock Solution (BIORON)**:** 25 mM $MgCl_2$

dNTPs (LAROVA) **:** 5 mM

***Taq* DNA polymerase** (BIORON)**:** 5U/µl

Sterile Water: dH_2O

Primer-pairs: 10 µM

Electrophoresis Buffers and Gel Systems

***Agarose Gel Electrophoresis**

10X TBE Buffers: 108 gr Trizma Base, (SIGMA)
55 gr Boric Acid, (SIGMA)

40 ml EDTA, (FLUKA) (0.5 M, pH: 8) is completed with 1000 ml with dH_2O

Running Buffers: X TBE prepared in distilled H_2O

Agarose, (SIGMA)**:** 1,5 or 2 percent (w/v) Agarose gel

Ethyidium Bromide, (SIGMA)**:** 4mg/ml

Loading Buffer: 9.5 ml Formamide, (SIGMA)

500 µl EDTA (0.5 M)

15 mg Bromophenolblue, (SIGMA)

15 mg Xylene cyanol, (SIGMA)

***Polyacrylamide Gel Electrophoresis**

10X TBE Buffers: 108 gr Trizma Base

55 gr Boric Acid, (SIGMA)

40 ml EDTA (0.5 M, pH: 8) dissolve in 800 mL deionized water, then bring total volume to 1 L

APS (Ammonium Per Sulphate)**:** 0.74 gr is completed with 100 ml with dH_2O

Acrylamide-Bisacrylamide, (APPLICHEM)**:** 40% Stock Solution Mixture (19:1)

Solution B: 20 ml 10X TBE

30 ml H_2O

120 µl TEMED, (APPLICHEM)

Equipments

Autoclave: Kermanlar – İSTANBUL

Centrifuge: Sigma 113

Deepfreezer: Sanyo – Medical Freezer

Vertical Electrophoresis System: Hoefer SE 600 Series Elect. Unit

Horizontal Electrophoresis System: Maxicell EC360M Elect. Unit

Thermocyclers: Eppendorf- Mastercycler, Techne-genius

Magnetic Stirrer: Labor Brand/Hotplate L-81

Ovens: Dedeoğlu

PH meter: Hanna Inst.

Power Supplies: EC135-90 E-C

Refrigerator: AEG

UV Transilluminator: Vilbor Lourmant

Vortex: Nüve NM110

Water Bath: Memmert

Micropipettes: GILSON

Hamilton Syringe: Microliter syringe

APPENDIX B

A PART OF THE POPGENE DATA FILE

/*data set for Turkish barley seed sources*/

Number of populations = 10

Number of loci = 6

Locus name:

Bmag603 Bmac67 Bmag206 Bmac209 Bmag6 Bmag225

name = resis1

22	24	11	12	11	34
22	24	11	12	11	34
22	24	11	12	11	34
23	24	11	12	11	34

name = resist2

11	24	11	34	11	23
11	24	11	34	11	23
11	24	11	34	11	23
11	35	11	34	11	23

name = resist3

11	24	22	34	11	23
11	24	22	34	11	23
11	24	22	34	11	23
11	24	22	34	11	23

name = resist4

23	24	11	12	22	45
23	24	11	12	22	45
23	24	11	12	22	45
23	24	11	12	22	45

name = resist5

23	24	22	12	33	34
23	24	11	12	33	34
23	24	11	12	33	34
23	24	22	12	33	34

name = resist6

23	24	11	12	22	45
23	24	11	12	22	45
23	24	11	12	22	45
23	24	11	12	22	45

name = resist7

23	24	11	12	22	45
23	24	11	12	22	45
23	24	11	12	22	45
23	24	11	12	22	45

name = resis8

23	24	11	12	33	34
23	35	11	12	33	34
23	35	11	12	33	34
23	35	22	12	33	34

name = resist9

23	24	11	12	33	34
12	12	11	34	11	34
12	12	11	34	11	34
12	12	11	34	11	34

name = resist10

23	24	22	12	33	34
23	24	22	12	33	34
23	24	22	12	33	34
23	24	11	12	22	45

APPENDIX C

A PART OF THE GDA DATA FILE

```
#nexus
   [! Data from the scald disease of barle ]
      begin gdadata;
     dimensions nloci=6 npops=8;
      format tokens missing=? datapoint=standard;
                  1 'Bmag603' [/ 1 2 3 4 5],
                  2 'Bmac067' [/ 1 2 3 4 5],
                  3 'Bmag206' [/ 1 2],
                  4 'Bmac209' [/ 1 2 3 4],
                 5 'Bmag006' [/ 1 2 3],
                  6 'Bmag225' [/ 1 2 3 4 5],
        ;
        matrix
        RES_1:
                  _1_   2/2  2/4  1/1  1/2  1/1  3/4
                  _2_   2/2  2/4  1/1  1/2  1/1  3/4
                  _3_   2/2  2/4  1/1  1/2  1/1  3/4
                  _4_   2/3  2/4  1/1  1/2  1/1  3/4,
        RES_2:
                  _1_   1/1  2/4  1/1  3/4  1/1  2/3
                  _2_   1/1  2/4  1/1  3/4  1/1  2/3
                  _3_   1/1  2/4  1/1  3/4  1/1  2/3
                  _4_   1/1  3/5  1/1  3/4  1/1  2/3,
        RES_3:
                  _1_   1/1  2/4  2/2  3/4  1/1  2/3
                  _2_   1/1  2/4  2/2  3/4  1/1  2/3
                  _3_   1/1  2/4  2/2  3/4  1/1  2/3
                  _4_   1/1  2/4  2/2  3/4  1/1  2/3,
```

```
        RES_4:
                _1_  2/3  2/4  1/1  1/2  2/2  4/5
                _2_  2/3  2/4  1/1  1/2  2/2  4/5
                _3_  2/3  2/4  1/1  1/2  2/2  4/5
                _4_  2/3  2/4  1/1  1/2  2/2  4/5,
        RES_5:
                _1_  2/3  2/4  2/2  1/2  3/3  3/4
                _2_  2/3  2/4  1/1  1/2  3/3  3/4
                _3_  2/3  2/4  1/1  1/2  3/3  3/4
                _4_  2/3  2/4  2/2  1/2  3/3  3/4,
        RES_6:
                _1_  2/3  2/4  1/1  1/2  2/2  4/5
                _2_  2/3  2/4  1/1  1/2  2/2  4/5
                _3_  2/3  2/4  1/1  1/2  2/2  4/5
                _4_  2/3  2/4  1/1  1/2  2/2  4/5,
        RES_7:
                _1_  2/3  2/4  1/1  1/2  2/2  4/5
                _2_  2/3  2/4  1/1  1/2  2/2  4/5
                _3_  2/3  2/4  1/1  1/2  2/2  4/5
                _4_  2/3  2/4  1/1  1/2  2/2  4/5,
        RES_8:
                _1_  2/3  2/4  1/1  1/2  3/3  3/4
                _2_  2/3  3/5  1/1  1/2  3/3  3/4
                _3_  2/3  3/5  1/1  1/2  3/3  3/4
                _4_  2/3  3/5  2/2  1/2  3/3  3/4

        ;
END;
```