



T.C.

**ÇANAKKALE ONSEKİZ MART UNIVERSITY
SCHOOL OF GRADUATE STUDIES**

DEPARTMENT OF MOLECULAR BIOLOGY AND GENETICS

**EVALUATION OF MOLECULAR MARKERS FOR BIOTIC
STRESS TOLERANCE IN SUNFLOWER (*Helianthus annuus* L.)**

MASTER OF SCIENCE THESIS

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ETHICAL STATEMENT

In this thesis study that I prepared following the Thesis Writing Rules of School of Graduate Studies of Çanakkale Onsekiz Mart University; I declare that I have obtained the data information, and documents I presented in the thesis within the framework of academic and ethical rules, I have presented all information, documents, evaluations, and results following scientific ethics and ethical rules, I cited all the works that I used in my thesis study by making appropriate reference, I did not make any changes in the data used and that the study I presented in this thesis is original. Otherwise, I undertake and declare that I accept all loss of rights that may arise against me.

ETİK BEYAN

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(İmza)

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ABSTRACT
**EVALUATION OF MOLECULAR MARKERS FOR BIOTIC STRESS
TOLERANCE IN SUNFLOWER (*Helianthus annuus* L.)**

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Çanakkale Onsekiz Mart University

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Sunflower (*Helianthus annuus* L.) is widely used in the food industry thanks to its rich nutritional content. The main factors limiting sunflower yield are diseases caused by pathogens. Sunflower downy mildew is a disease caused by the *Plasmopara halstedii* pathogen and can completely prevent yield. At the onset of symptoms, rosette formation on the leaves followed by stunting of the plants is observed. There are many ways to manage disease such as genetic, chemical, cultural methods, and crop rotation. However, these methods usually require a lot of effort and budget. Disease-resistant wild sunflower species have been identified and this wild species is not affected by the disease. Therefore, farming these species and obtaining new generations of wild species will prevent productivity losses. The most effective method of combating this disease is the use of established lines obtained by crossing resistant cultivated plants with sensitive but elite species.

Marker-assisted selection (MAS) is an alternative molecular technique used to ensure the rapid transfer of agronomically and economically important characters controlled by more than one gene and to obtain high-yield, high-quality plant products. Within the scope of the study, sensitive rootstock IMI044B and disease-resistant rootstock H458 sunflower genotypes and F₂ plants (SUN59) obtained by crossing these genotypes were evaluated with 42 SSR markers. Thus, markers thought to be related to endurance were identified. PI genes are race-specific and fully dominant. To date, 37 types of PI genes have been identified in sunflowers. SSR markers associated with PI genes, which were determined to be polymorphic with F₂ individuals, were used in screening studies. This approach aims to identify resistant plants and is environmentally friendly, economical, and sustainable.

Keywords: Molecular Marker, *Helianthus annuus* L, Downy Mildew, MAS

ÖZET

AYÇİÇEĞİNDE BIYOTİK STRES TOLERANSI İÇİN MOLEKÜLER MARKÖRLERİN DEĞERLENDİRİLMESİ

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Çanakkale Onsekiz Mart Üniversitesi

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Ayçiçeği (*Helianthus annuus L.*), endüstriyel olarak en çok kullanılan bitkilerden biridir. Zengin besin içeriği sayesinde gıda sektöründe yaygın olarak kullanılmaktadır. Ayçiçeğinde verimi kısıtlayan başlıca etmenler patojenlerin yol açtığı hastalıklardır. Ayçiçeği mildiyösü, *Plasmopara halstedii* patojeninin yol açtığı ve verim alınmasını tamamen engelleyebilen bir hastalıktır. Semptomların başlangıcında yapraklarda rozetlenmeyi takiben bitkilerde bodurlaşma gözlemlenir. Genetik yöntem, kimyasal yöntem, kültürel yöntem, ürün rotasyonu gibi birçok hastalıkla mücadele yolu vardır. Ancak bu yöntemler genellikle çok fazla emek ve bütçe gerektirir ve başarı oranları çok yüksek değildir. Hastalığa dayanıklı yabani ayçiçeği türleri tespit edilmiştir ve bu yabani tür hastalıktan etkilenmemektedir. Dolayısıyla bu türlerin tarımının yapılması ve yabani türlerin yeni jenerasyonlarının elde edilmesi verim kayıplarının önüne geçecektir. Bu hastalıkla etkili mücadele yöntemi dayanıklı kültür bitkileri ile hassas ama elit türlerin çaprazlanmasıyla elde edilen durulmuş hatların kullanılmasıdır.

Markör destekli seleksiyon (MAS), agronomik ve ekonomik olarak önemli olan ve birden fazla gen tarafından kontrol edilen karakterlerin hızlı bir şekilde aktarımını sağlamak, yüksek verimli, kaliteli bitkisel ürün elde etmek amacıyla kullanılan alternatif bir moleküler tekniktir. Çalışma kapsamında hassas anaç IMI044B ve hastalığa dayanıklı anaç H458 ayçiçeği genotipleri ve bu genotiplerin çaprazlanmasıyla elde edilen F₂ bitkileri SUN59 30 adet SSR markörleriyle değerlendirilmiştir. Böylece dayanıklılıkla ilişkili olabileceği düşünülen markörler belirlenmiştir. PI genleri ırk spesifik ve tam baskındır. Bugüne kadar ayçiçeğinde 37 çeşit PI geni tespit edilmiştir. Bu genler birden fazla patojen ırkına dayanıklılık sağlamaktadır. F₂ bireyleri polimorfik olduğu belirlenen PI genleriyle ilişkili

SSR markörleri tarama çalışmalarında kullanılmıştır. Dayanıklı bitkilerin belirlenmesini amaçlayan bu yaklaşım çevre dostu, ekonomik ve sürdürülebilirdir.

Anahtar Kelimeler: Moleküler Markör, *Helianthus annuus* L, Mildiyö, MAS



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SYMBOLS AND ABBREVIATIONS

%	Percent
µg	Microgram
µl	Microliter
°C	Degrees Celcius
CTAB	Cetyltrimethylammonium Bromide
bp	Base pair
BC	Before Christ
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide Triphosphate
EDTA	Ethylene Diamine Tetra Acetic Acid
kb	Kilo base
km	Kilometer
M	Molar
MAS	Marker Assisted Selection
mg	Miligram
min	Minute
NaCl	Sodium Chloride
NCBI	National Center for Biotechnology Information
ng	Nanogram
PCR	Polymerase Chain Reaction
PVP	Polyvinylpyrrolidone
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
rpm	Revolutions per Minute
sec	Second
SSR	Simple Sequence Repeats
SNP	Single Nucleotide Polymorphism
TAE	Tris-acetate-EDTA

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CHAPTER 1

INTRODUCTION

1.1. Sunflower (*Helianthus annuus* L.)

Sunflower is an agricultural plant that physiologically has a disc-shaped face, carries the seeds in this disc, has flower petals around the disc, and can grow from 1 meter to 5 meters in length (Morris et al., 2019). Its Latin name, *Helianthus annuus*, comes from the combination of the sun and flower words. The feature that causes this is that it is phototropic, that is, the disc-shaped face of the sunflower follows the movements of the sun and orients accordingly. Sunflower belongs to the daisy family, namely the Asteraceae family.

Table 1

Classification of *Helianthus annuus* L.

Kingdom →	Section	→ Class	→ Order	→ Family	→ Subfamily
→ Genus	→ Species				
<i>Plantae</i> → <i>Magnoliophyta</i> → <i>Magnoliopsida</i> → <i>Asterales</i> → <i>Asteraceae</i> → <i>Asteroideae</i> → <i>Helianthus</i> → <i>Helianthus annuus</i>					

Sunflower is grown commercially mainly for its oil and seeds. The reason for this demand for sunflower products is that they are light in taste, rich in nutrients, and quite balanced. It has a diverse content such as water, protein, carbohydrates, fat, and fiber. The minerals it contains calcium, phosphorus, potassium, magnesium, zinc, selenium, and iron. According to medical studies, it has been proven that regular sunflower consumption reduces risky conditions in the cardiovascular system, helps lower cholesterol, supports the immune system, contributes to skin and tissue regeneration, has a high antioxidant content, facilitates digestion, contributes to bone development and structure with its high calcium content, and shows anti-inflammatory properties (Puraikalan & Scott, 2023). Thanks to its beneficial content, sunflower products are frequently added to the diet lists by nutritionists. In addition to its fibrous structure, it is preferred that it does not contain sodium or gluten. Seeds, the fruit of sunflower, can be consumed either roasted or raw. These seeds are consumed not only by humans but also by animals as fodder. Sunflower oil contains the unsaturated fat linoleic acid. Unsaturated fat ratio varies between 48 and 74 percent (Li et al., 2024). The sunflower variety called high oleic sunflower has higher unsaturated oil content and this

variety is more tolerant of high temperature. According to the results of biochemical researches, sunflower oil contains molecules such as flavonoid alkaloids and terpenoids that prevent the formation of fungi, bacteria, microbes, and tumors with biological activities (Huang et al., 2023).

The oilseed plant with the highest cultivation area and production in Türkiye is the sunflower. In Türkiye it is mostly produced in the Marmara Thrace region. It is known that 70% of sunflower cultivation in Türkiye is done in the Thrace Marmara Region, 13% in Central Anatolia, 10,9% in the Black Sea, and the rest in the Eastern and Southeastern Anatolia Region. Despite being grown in a wide variety of climates and regions, the adaptation flexibility of the sunflower plant is quite wide (Süzer, 2010). As a result, production has ramped up, yet it still falls short of meeting the demands of the expanding population. However, properties such as seed yield and oil rate vary according to the sowing time and region. Late sowing in sunflowers reduces the efficiency considerably. The type of seed used in sunflower agriculture is also highly significant; There are three types of sunflower seeds. These can be listed as hybrid, synthetic, and open-pollinated. It is a hybrid variety that is the best among them in terms of quality and yield and gives twice the normal yield (Puttha et al., 2023). Although we pay attention to the features mentioned, sowing time, and variety, the factors that seriously reduce the yield in sunflower agriculture are diseases and pests. Downy mildew diseases are quite common in sunflowers, besides, the plant is quite vulnerable to diseases such as leaf spots and rot. In order to be protected from these diseases, disease-resistant seeds should be preferred. Because the methods of fighting diseases are insufficient. If resistant seeds are not yet available, it is crucial to understand how the chosen variety reacts to diseases and take preventive measures accordingly. In recent studies, resistant varieties against downy mildew, rust, and wilt diseases have been obtained and grown successfully (Sharma et al., 2010).

Sunflower is also selected and grown as a variety according to the purpose to be used. Sunflowers are used in many areas industrially. It acts as a raw material for paint. Sunflower oil is mainly used in the production of environmentally friendly biodiesel. It was sometimes used in soap making and as lamp oil during wartime. According to purposes, there is an ornamental type, oilseed type and confectionary type of sunflowers are present (Miller et al., 1997). In researches, it has been discovered that the by-products obtained from sunflower oil extraction can be used in the food industry due to the phenols and proteins it contains. At

the same time, in the production of sunflower oil, flour, which has high nutritional value, is obtained from the by-products of the seed and oil, and it benefits the concept of zero waste.

1.2. The History of Sunflowers

Sunflower was discovered in the American continent and has been an important cultural plant throughout history. It is known that it was first cultivated by North American Indians around 1000 BC. In the 16th century, sunflower cultivation spread to Europe and became very popular in a short time. Although it was used as a garden and ornamental plant when it was first cultivated, after the discovery of its oil, it started to be used mostly for oil production. In the following years, sunflower cultivation in very large areas in Russia has gained great momentum and has become more productive as a result of genetic studies. Sunflower farming spread all over the world at the end of the 19th century, and it was cultivated in immense areas, after that, its cultivation increased more than the demand (Smith, 2006). Sunflower is a crucial plant as it is resistant to various soil structures and harsh climatic conditions. Additionally, sunflowers are utilized as a rotation crop in agriculture. It enriches the soil content and increases the productivity of the products to be grown in the soil later (Adeleke et al., 2020).

1.3. Significance and Cultivation of Sunflowers

Sunflower is one of the most cultivated plants used as oil raw material. Sunflower farming originated in North America and then spread all over the world (Smith, 2006). The agricultural area of sunflower has become quite widespread due to its adaptation to climatic conditions and resistance to negative factors. Apart from its use in food, it also helped the locals use it as a dye. Although *Helianthus* is generally a perennial plant, it has a total of 51 species, including annual species (Kaya et al., 2012).

Historically, sunflower breeding has evolved through three phases. The first of these is mass selection, where sunflower seeds with productivity potential were selected and grown through quantitative observation. Thus, the divergence of wild species and the domestication process of sunflowers started with this selection (Jocić et al., 2015). It was

proven in the study by Burke et al that the selected seed sizes played a major role in domestication in sunflower breeding and the selection of large seeds. In this way, mass selection contributes to the spread of sunflower cultivation and species and constitutes the beginning of the breeding process. Sunflower species characterized by the first selection began to be grown in Russia in 1967, and the seeds were matched with certain characteristics according to their shape, size, and color. According to the results, round, relatively heavier, and thin-shelled sunflower seeds will be used as oil raw materials because they have a high oil content. Sunflower seeds, which are flatter, lighter, thinner, and darker in color, will be used in animal feed and human snack consumption. Furthermore, mass selection has led to the development of sunflowers that are resistant to pests and diseases that appeared in subsequent years. The second stage is individual selection. This method, which is the most important part of sunflower diversification, has been quite successful. In short, this method is to select the healthiest and most productive individuals from the very beginning of sunflower farming and to harvest and reserve the seeds of these individuals separately. While some of the seeds are stored, some are harvested, ensuring the continuity of the agriculture of elite individuals. Breeding efforts are continued for these elite sunflowers and their hybrids, which have a high number of seeds. Resistance to diseases and pests is also the biggest selection factor in the selection of elite sunflower individuals. Non-resistant individuals are not selected, and those with small seed sizes or light weights are not selected in individual selection. To avoid open pollination, there should be at least 2 km of space in each row so that the breeding work can be more isolated. The biggest effect of the individual selection method in sunflower breeding is that the oil yield is increased from around 30% to 43%. It has also facilitated the emergence of disease-resistant individuals and breeding efforts.

The last method is the Hybrid development method. This approach results in the creation of F_1 and F_2 generations that are more isolated and suitable for exploiting heterosis. An inbred line is the heterozygosity achieved by crossing homozygous individuals in this manner. After heterosis, sunflower hybrids were produced in 1970 after the discovery of the male sterility gene linked to the anthocyanin-pigmented hypocotyl gene (Vranceanu, 1974). However, the disadvantage of this method was that it was necessary to keep the female rows and male rows away from each other to prevent pollination in the production of anthocyanin-rich hybrid plants, which required large space and high costs. Later, such problems were

eliminated with cytoplasmic male sterility, and commercial sunflower hybrids began to be produced.

1.4. Downy Mildew Disease of Sunflower

The most important disease in sunflowers is downy mildew disease. It cannot be treated for a long period. As the infected sunflower grows, the virus continues to infect the newly grown tissue. *Plasmopara halstedii* is also highly contagious; once it infects a sunflower, it spreads rapidly throughout the field. The effects on diseased plants are dwarfism, white-grayish powdery formation, and rosette-like wounds that may appear on the leaves. Types of infection in sunflowers are divided into primary and secondary infections. In primary infections, they are called soil-borne, and the infection is quite lethal and has more severe symptoms. In secondary infections, the infection is airborne and the symptoms are milder than in primary infection. In primary infection, the sunflower is completely infected and the yield is very low, while in secondary infection, only traces are seen on the leaves, which do not significantly affect the yield. Another feature that makes this disease so dangerous is that the oospores of the pathogen survive in the soil for about 10 years, so many control methods are inadequate. *P. halstedii* is a plant pathogenic oomycete that causes devastating diseases in many plants, especially crops. Oomycetes have microstructures called oospores, and they can overwinter in the soil for up to 10 years. Because of their life cycle, oospores germinate within 3 to 5 days and then first infect the roots of plants. Therefore, *P. halstedii* is a soilborne pathogen and causes severe root infections. The germinating oospores give rise to zoosporangia, which release zoospores. The zoospores come into contact with the roots via exudates released by the plants to the roots. The Oomycetes-zoospores-roots cycle is called primary infection. The zoospores act like an inoculant for a season and germinate within a few hours. In the leaves and flowering parts of the plant, the pathogen enters the tissues through the stomata and asexually forms zoosporangiophores. The zoosporangiophores release zoospores and infect that part of the plant by continuing to spread. *P. halstedii* can spread by wind using seedlings of *Helianthus*. The pathogen lands on leaves and other plant parts, and secondary infection occurs. Secondary infection has less negative impact and yield loss than primary infection (Cohen & Sackston, 1973).

Identifying and using cultivars resistant to *P. halstedii* is the most successful and sustainable method of controlling the disease. By crossing susceptible but high quality crops

with disease resistant crops, cross generations with the desired resistance yield can be obtained. To properly control the disease and properly select resistant individuals, it is necessary to study the molecular mechanism of this disease, the spread of infection, the regions where it occurs, and the stages at which it is found. For this purpose, molecular markers are primarily used to identify resistant individuals. Generally, STS (Sequence Tagged Sites), SSR (Simple Sequence Repeat), QTLs (Quantitative Trait Locus) and the like are used. The use of molecular markers is related to downy mildew resistance, and molecular markers that can be successfully used in selecting specific sunflower breeding programs for this resistance allow the detection of genes. They make breeding faster and more reliable and are an important part of the selection process, especially for resistant individuals.

Until the 1980s, only two varieties of downy mildew were known in sunflowers. About 14 other races were identified after they were later reported to Europe from the United States. In all studies conducted to date, a total of 44 races of *P. halstedii* have been identified. *P. halstedii* forms a special sexually produced wall to sustain itself, and it is very difficult to get rid of the pathogen because it forms oospores that can survive in the field and soil for many years after infecting the plant. *P. halstedii* belongs to the Asteraceae family and infects the genera *Helianthus*, *Bidens*, *Artemisia*, and *Xanthium*. *Helianthus* has the highest infection rates, but many other species are also affected (Spring, 2019). Plant sterility is one of the main problems caused by *P. halstedii*. Sunflowers infected with the pathogen are barren because the roots become inflamed and the seeds wither in the flowering stem of the plant. The plant also shows symptoms such as dwarfism, chlorosis, yellow spots on the leaves, and defoliation. The probability of systemic infection is quite high under climatic conditions with abundant rainfall until 15 days after sowing. The suitable temperature for infection is 15-20 °C (Bán et al., 2023).

1.5. Discovery of Downy Mildew Disease in Sunflower

Downy mildew disease in sunflowers is caused by an oomycete called *Plasmopara halstedii*, which causes serious damage to the plant, and the disease generally affects young plants in the early period. For this reason, the yield loss is quite high. Symptoms of the disease include wilting and shedding of the leaves, and in the early stages of the disease, the

formation of a white flour-like powdery coating on the leaf surface, followed by stunting (Gulya et al., 2015).

Downy mildew was first detected in history at the end of the 19th century, when sunflower cultivation was at its peak all over the world. Studies conducted when it was first discovered that the disease agent did not spread when isolated, but that it was a disease that could be transmitted rapidly through air and water. It has been determined that the disease is more common in humid climate conditions and regions with high rainfall. Various methods of combating this disease have been sought for many years. Although some preventive measures were taken, they were not effective enough in eradicating the disease and preventing productivity loss. These measures include disease-free selection of seeds, planting varieties predicted to be resistant, testing various irrigation methods, and implementing crop rotation (Gisi & Sierotzki, 2008). In later years, a chemical called Metalaxyl began to be used.

There are several generas of downy mildew disease that can be identified by its patches. Patches are found generally on down parts of leaves in humid environments and can be gray, blue, white, or violet. The affected leaves of plants will then die. Downy mildew in sunflower caused by *P. halstedii* has typically white powdery patches on the upperside of the leaves. In downy mildew disease, observation is done with the naked eye, if the symptoms point to the disease, it can be looked at with a magnifying glass or microscope to be sure. Although the disease can be understood by looking at the leaves and reproductive organs of an adult plant, there is no symptom to characterize the disease in the seed, then if there is such a suspicion, molecular investigation is used for disease management. Visual or molecular detection of downy mildew in sunflowers is both advantageous and disadvantageous because downy mildew on the leaf can easily fly away or be washed away in windy or rainy weather. This can be misleading in recognizing the disease. Molecular diagnosis may take more time than visual diagnosis. For this reason, although it is necessary to be fast to prevent the spread of the disease, the rate of error with molecular diagnosis is very low. A sample can be taken within a day and a definite result can be reached within a few days. Or, if it is desired to obtain results in a shorter time, the colonized disease can be diagnosed by examining the samples under the microscope. In molecular diagnosis, the presence of the disease can be proved by looking at nucleotides and proteins. Techniques such as serology and isoenzyme comparison are frequently used. DNA-based tests and the

use of molecular markers give the most accurate information about the disease taxonomically (Salcedo et al., 2021).

1.6. Management of Downy Mildew

To manage this devastating pathogen, it is important to detect it in early stages, as it spreads very easily and quickly. After recognizing the symptoms, appropriate control methods must be determined. There are biological, chemical, and cultural methods. Metalaxyl is a fungicide that is effective against downy mildew on sunflowers. This chemical is a very powerful fungus active ingredient, especially effective against plant diseases caused by oomycete class fungi. When used, it provides systemic protection for the plant. This fungicide is rapidly absorbed by the plant and is carried systemically to the roots and leaves of the plant, thus providing complete protection. Seed coating, soil application or spraying on leaves are used as application methods. However, continuous and excessive use of fungicides causes such pathogens to develop resistance to Metalaxyl, which causes the effect of the drug to decrease over time. Also chemical loses its effectiveness over time. Since the soil is washed out by rain, it is very slow to work. In addition, Metalaxyl can harm the environment and spread easily, disrupting the balance of water resources and soil microbiota and negatively affecting beneficial organisms. It can also have negative effects on the health of the person applying it. It is very dangerous if inhaled, swallowed, or in contact with the skin and may cause toxic effects. Additionally, when such chemicals accumulate throughout the food chain, consuming the product is very harmful (Jinyu et al., 1996).

There are also cultural control methods that are also partially or less effective. To increase the yield of sunflowers, crop rotation is the main method used. Crops that cannot be infected by this pathogen are grown, as the pathogen can overwinter in the soil and survive for many years. Other cultivation methods include seed treatment, foliar fungicide spraying, and weed control.

The most effective and successful method is biological management. Here, the pathogen can be controlled before infection using *P. halstedii* resistance genes. The use of resistant plants ensures that not only the plants grown in the field in question, but also the plants and seeds grown in future years are protected from the disease in the long term, leading

to further resistant generations. Today, control of this disease is mainly through resistance development using molecular markers. Resistance genes to downy mildew in *Helianthus* are referred to as PI genes. While some of the PI genes confer race-specific resistance, some PI genes also confer strong resistance to all races. PI-Genes are dominant and race-specific. In wild *Helianthus* plants, 2 dominant resistance genes have been identified, and new resistant strains continue to be found. Resistance type 1 provides resistance in the basal region of the hypocotyl. Type 2 allows the pathogen to penetrate the cotyledons, infecting the hypocotyls. At the hypocotyls, the hypersensitive response (HR) has been activated. HR is a reaction of the infected organism against a pathogen. When HR occurs at this site, the growth of the oomycete is restricted, but it is not killed. Plants have mechanisms for recognizing and responding to biotic stressors. Plants employ inducible defense mechanisms, which are triggered by pathogen attacks, to effectively halt the invasion of pathogens. Systemic acquired resistance (SAR) may also occur in the upper parts of *Helianthus* seedlings. SAR is a response that occurs in plants when a pathogen acts locally on a plant. It acts like an inoculum and results in broad-spectrum and durable resistance throughout the plant. SAR is a signaling mechanism that results in strong resistance, especially to secondary infections. Another defense technique is the production of reactive oxygen species that strengthen the plant cell wall (Radwan et al., 2005).

Breeding sunflowers resistant to downy mildew is possible with several molecular markers. Utilizing a range of molecular markers is crucial for identifying and cultivating superior sunflower genotypes and for conducting breeding studies aimed at achieving resistance to downy mildew. These include STS, SSR, QTL, and others. Molecular markers are associated with downy mildew resistance and can be successfully used in marker-assisted selection for specific sunflower breeding programs for resistance. Molecular markers facilitate the detection of PI genes, make propagation faster and more reliable, and are becoming an important part of the selection process. Employing molecular markers accelerates the selection process compared to traditional breeding techniques. Marker-assisted selection (MAS) is a technique designed to solve the problems of classical plant breeding to allow the rapid transfer of traits controlled by more than one gene, increase yield, and obtain high-quality plant products. These PI genes can be introduced into breeds using either traditional breeding techniques or marker-assisted selection (MAS) methods. The traditional breeding method is more labor-intensive and time-consuming because it requires examining many individuals. Marker-assisted selection (MAS) offers advantages by

accelerating the breeding process and ensuring that the selected markers remain unaffected by environmental conditions. They can be dominantly or co-dominantly inherited. . The inheritance pattern of these markers is relatively straightforward. They are also effective for gene pyramiding, allowing the transfer of multiple traits simultaneously. SSR and SNP markers are frequently employed in genotypic mapping (Brahm et al., 2000).

Host resistance tends to be specific to particular cultivars or accessions and is often less durable. Up to now, breeding efforts have predominantly concentrated on developing host resistance by incorporating single or multiple resistance (R) genes. Another type of resistance is monogenic resistance, which is race-specific and offers robust protection against pathogens (Vincourt et al., 2012). In *Helianthus*, resistance genes are typically dominant and often occur in clusters, in wild types. For instance, PI_6 is found in the wild form of *Helianthus annuus*. Resistance genes discovered to date are PI_5 in *Helianthus tuberosus* and PI_7 in *Helianthus praecox* (Qi et al., 2019). In classical breeding programs, resistant wild types are used as donor plants, but many undesirable lines and traits can also be inherited. With the help of molecular markers that facilitate the molecular mapping of PI genes, this issue is addressed, and the positions of many PI genes are identified (Tan et al., 1992). Molecular markers indicating the presence of PI genes in resistant and F_2 individuals were determined by testing many markers. Some of the molecular markers were monomorphic and did not indicate the distinction between resistant and susceptible genotypes. However, polymorphic primers clearly separated resistant and susceptible genotypes.

1.7. Resistance Against Downy Mildew and Its Importance

Disease resistance in plants is of great importance in agriculture and plant breeding studies. Resistance can be defined as the capacity of the plant to resist or limit infection by a particular pathogen. This feature increases the sustainability and economic efficiency of agricultural production by ensuring healthy growth and high productivity of plants.

Disease resistance can be classified into two primary categories: horizontal and vertical resistance. Vertical resistance is usually linked to one or a few specific genes and provides a highly specific defense mechanism against the pathogen. This type of resistance

usually results in a rapid and strong response, but there is a high probability that the pathogen will overcome this genetic barrier over time. Horizontal resistance, on the other hand, offers a broader spectrum and generally more permanent defense mechanism based on many genes. This type of resistance may be more effective against different strains of the pathogen, but its response is generally less potent (Gontcharov & Goloschapova, 2021).

The defense mechanisms that plants develop against pathogens typically operate at the molecular level. These mechanisms include pathogen recognition proteins, defense response genes, and signal transduction pathways. Pathogen recognition proteins recognize specific molecular structures of pathogens and activate the plant's defense mechanisms. R (resistance) genes, which play a role in this process, are critical components that ensure the resistance of plants to certain pathogens (Pecrix et al., 2019).

Resistance against diseases is vital for the sustainability of agricultural production. Diseases in plants can cause significant yield losses and economic losses. In particular, widespread and devastating diseases such as downy mildew can seriously threaten agricultural production. Incorporating resistance genes into breeding programs minimizes environmental impacts by reducing the use of chemical pesticides and contributes to making agriculture more sustainable.

Modern plant breeding techniques have made great progress in developing disease-resistant plant varieties. In addition to traditional breeding methods, the process of identifying and integrating resistance genes into plants has been accelerated by using molecular biology and genetic engineering tools. Methods such as Marker-Assisted Selection (MAS) are important tools for effectively selecting resistance genes and using them in plant breeding. These methods accelerate plant breeding processes and make the development of resistant plant varieties more effective.

1.8. Identification of Resistance Genes

Resistance genes activate the plant's defense mechanisms against the pathogen. These genes are often called R (resistance) genes and encode proteins that regulate the plant's immune responses. Major genes contributing to downy mildew resistance in sunflowers include Pl genes (Pl₁, Pl₂, Pl₅, etc.). These genes produce specific proteins that recognize the

downy mildew pathogen and prevent infection. Molecular markers identify specific DNA sequences in the plant genome and assist in mapping the regions where resistance genes are located. These markers are used to correlate genotypic variations with phenotypic variations. In Sunflower, molecular markers such as SSR (Simple Sequence Repeat) and SNP (Single Nucleotide Polymorphism) are widely used.

1.9. Genetic Mapping and QTL Analysis

Genetic mapping is a technique used to determine the location of resistance genes on the chromosome. This method detects the location of resistance genes through crossover and genotyping techniques. QTL (Quantitative Trait Loci) analysis is used to examine the genetic control of complex traits such as resistance. QTL mapping determines the effects of resistance genes and the location of these genes in different chromosomal regions. These analyses enable the effective introduction of resistance genes into breeding programs (Neupane et al., 2018).

Isolation and characterization of resistance genes are critical to understanding the mechanisms of functioning of these genes. Gene isolation is performed by cloning and sequencing gene regions identified by genetic mapping and molecular markers. The functions of the isolated genes are characterized by gene expression analyses and biochemical tests.

Identifying downy mildew resistance genes in sunflower and integrating these genes into breeding programs is an important step in combating plant diseases. Modern biotechnology tools such as molecular markers, genetic mapping, QTL analysis and genetic engineering accelerate this process and make it more efficient. In the future, the discovery of new resistance genes and a better understanding of the functions of existing genes will contribute to the development of sunflower varieties that are more resistant to downy mildew.

1.10. Simple Sequence Repeats (SSR)

Simple sequence repeats, SSR primers, are the most suitable molecular markers for the detection of resistance to downy mildew disease in sunflowers. SSR markers show high

polymorphism among genotypes. This variation in the number of repeated units allows the selection of sunflower varieties and the characteristics of resistance genes.

SSR markers are generally obtained from genomic sequences from databases like NCBI or specific genomic databases for the organism of interest. Software tools are used to identify SSRs in sequence data like SSR search. With the use of software tools, appropriate primers must be designed. SSR markers are generally reproducible, once established, the same SSR markers can be used consistently across experiments. The most commonly used method is marker-assisted selection (MAS). This method uses SSR markers to select plants with specific resistance genes. This allows for rapid early selection before disease symptoms appear and without waiting for the plant to grow, develop, and show symptoms. SSR markers are also valuable for genetic mapping and quantitative trait locus (QTL) analysis. They are used to map the locations of resistance genes in the sunflower genome and to identify strains related to disease resistance. SSR markers are also used to monitor whether resistance genes remain effective against evolving pathogen strains by their stability.

1.11. Marker Assisted Selection (MAS)

Marker-assisted selection (MAS) has become a promising technique for achieving desired outcomes in plants by utilizing molecular markers, allowing for rapid improvement of target traits. It enables rapid and precise selection of desired traits within plant populations. Molecular Markers can be associated with genetic variations or phenotypic characteristics. The MAS helps determine the desired genetic traits in plants. Genetic markers suitable for the application stages of MAS must first be determined. Marks associated with the target's trait or disease resistance need to be identified. This is done through genetic mapping projects. The marker located at the locus closest to the desired gene in the gene map is selected. In the next stage, the markers must be verified. PCR is performed to verify whether the identified brands are actually associated with the target features. If they are related, the distinction between the disease-resistant individual and the disease-susceptible individual is observed in gel electrophoresis. Finally, the population is scanned, a large plant population is scanned using genetic markers, and this scan helps identify individuals with target characteristics. Individuals with the desired characteristics are

selected from the screened population and breeding studies are carried out on these individuals.

Downy mildew disease poses a significant threat to sunflower plants. In the application of MAS system in sunflower, first the resistance genes are identified and the genes that provide resistance against this disease are determined as a result of genetic research and by examining the genomes. Once genetic markers associated with resistance genes are developed, these markers are used to identify plants resistant to downy mildew. Using these developed markers, a large sunflower population is screened and as a result, resistant individuals are identified. Individuals resistant to downy mildew are selected from the screened population and breeding studies are carried out using these individuals so that the development of resistant sunflower varieties is accelerated with this method.

MAS method offers a faster and more effective selection process compared to traditional culinary methods, and the identification of resistant plants takes less time and costs less.

1.12. Choosing the Appropriate Molecular Marker

There are three molecular marker groups have been used in common sunflower; RFLP (Restriction Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), and AFLP (Amplified Fragment Length Polymorphism). By using RFLP in sunflower, the diversity of sunflower and the family tree of the genus were determined and the wild species were examined (Bouzidi et al., 2002). Then, random regions of DNA were amplified using random primers with the RAPD marker technique. RAPD marker was used in genetic diversity studies in sunflowers, especially it played a major role in genome mapping and identification of rust disease (Şahin et al., 2018). However, this technique is not genome-specific and its reproducibility is very low. For this reason, it is not a sufficient technique on its own and has been a guide in subsequent studies. AFLP is a technique based on PCR and this technique is generated by the influence of RFLP. Likewise, this marker has been used in fingerprinting and genetic mapping in many plants. It has also been used successfully in DNA fingerprinting analysis in sunflowers (Brahm et al., 2000b). SSR (Simple Sequence Repeat) is a highly polymorphic genomic marker. A small piece of DNA containing repeats is amplified by PCR, and the results are sized in gel electrophoresis.

The position of the bands and the differences between sensitive and resistant individuals are evaluated according to the electrophoresis gel result. This technique was first used on soybeans, and later it gave successful results on various crops. SSR markers help creating genetic maps, and also determines genotypes, by evaluating the elite quality of seeds, and in many other breeding studies, as it facilitates selection and is reliable. At the same time, these microsatellites can be transferred very easily and are not affected by environmental factors (Solodenko, 2018). When examining the gene maps with molecular markers in sunflowers, primers located in proximity to the resistance genes are observed. The primer closest to the resistance gene region may be the most suitable for use in marker-assisted selection in resistance studies. However, it is essential to consider the various characteristics of primers to select the appropriate marker. Given that the study will not utilize a variety of species and that the specific resistance gene of interest has been identified, SSR markers will be employed. SSR markers are particularly suitable for plant breeding and molecular resistance studies. The selected primer must be polymorphic and capable of distinguishing between resistant and sensitive individuals. The appropriate marker must also be inherited codominant and be frequently present in the genome. Sequence information of SSR primers was obtained from the NCBI database.

CHAPTER 2

PREVIOUS STUDIES

The Pl gene family is one of the genes that provide resistance against downy mildew. These genes activate plants' defense mechanisms against the pathogen and provide resistance to downy mildew infection. The most researched genes within the Pl gene family include Pl₁, Pl₂, Pl₅, and Pl₈. Pl₁₁ and Pl₁₂ genes are early identified resistance genes against downy mildew (Molinero-Ruiz et al., 2003). These genes recognize the pathogen at the initial stage of infection and activate defense mechanisms. Studies have found that these genes provide high levels of resistance in sunflower plants. Pl₅ and Pl₈ genes have an important place in modern breeding programs. These genes encode defense proteins that prevent the pathogen from entering the plant. The Pl₅ gene is effective against downy mildew pathogenic races that are particularly common in Europe. It has been determined that the Pl₈ gene provides a strong defense against pathogenic strains common in the American continent (Tourvieille de Labrouhe et al., 2010).

R (resistance) genes are important components of plants' resistance mechanisms against diseases. In sunflowers, R-genes trigger a defense response by recognizing specific effector proteins of the downy mildew pathogen. R-genes detected in sunflower using genetic mapping and molecular markers. Among these genes, those classified within the Resistance Gene Candidate (RGC) family are especially prominent. RGC genes encode NLR (Nucleotide-binding Leucine-rich Repeat) proteins, which are pathogen recognition proteins, and these proteins initiate the plant's defense mechanism by recognizing pathogen effectors. Studies on the mechanisms of action of R-genes have revealed how these genes play a role in the plant immune system. For example, certain effector proteins of *Plasmopara halstedii* are recognized by R-genes upon entry into the sunflower plant, and this recognition results in local cell death known as the hypersensitive response (HR). This process protects the plant from infection by limiting the spread of the pathogen (Goloschapova, 2023).

QTL regions affecting resistance to downy mildew in sunflower were determined by genetic mapping studies. In these studies, QTL regions associated with resistance were identified, especially on chromosomes 10 and 13. Genes located in these regions provide a strong defense response against the pathogen and increase the resistance of plants to downy

mildew infection (Pecrix et al., 2018). Resistance genes determined by QTL analyzes are used in plant breeding with the Marker-Assisted Selection (MAS) method.



CHAPTER 3

MATERIAL AND METHOD

3.1. Plant Material

To be used in the study, sunflower (*Helianthus annuus* L.) genotypes belonging to the Asteraceae family were obtained from the Thrace Agricultural Research Institute. 15 genotypes of parental H458, which is resistant to downy mildew, and 15 genotypes of IMI044 B, which is known to be susceptible to downy mildew, and 100 individuals of the F₂ generation were taken from the Trakya Agricultural Research Institute.



Figure 2. H458B Resistant parental and IMI044B Sensitive Parental Sunflowers

Table 2

Commercial Sunflower Genotypes used in this study

Genotype	Number of Individuals	Characteristics
H458	15	Resistant parent
IMI044B	15	Susceptible parent
SUN59	100	Hybrid

3.2. Genomic DNA Isolation

Leaf samples were cut from the junction of the leaf and stem of the plant, each sample was numbered, packaged, and stored at -80 °C. Approximately 80 mg of leaf sample was used for each Eppendorf tube. Leaf samples were ground into powder with the help of ceramic beads. After the ceramic beads were removed, 500 µl of CTAB (Cetyltrimethylammonium Bromide) solution heated to 60 °C was added to each sample. 10 mg PVP (Polyvinylpyrrolidone) was added to remove phenolic compounds. The samples were incubated in a water bath at 60 °C for 30 minutes. After incubation, 750 µl of chloroform-octanol was added to the samples. The samples are gently inverted 20-25 times, then centrifuged at 13,000 rpm for 15 minutes to separate the phases. Each sample has a clear supernatant phase (light yellow or transparent) and a green pellet phase. Approximately 300 µl of supernatant is obtained and transferred to a new Eppendorf tube. If needed the chloroform-octanol step could be repeated. 5 M NaCl solution is added as 1/2 of the sample volume (in this case, 150 µl solution was added for 300 µl sample). 450 µl of cooled 95% ethanol is added and the samples are kept at -80 C for 1 hour for the DNA to precipitate. At the end of the period, the samples are left at room temperature for 10 minutes and then centrifuged at 13,000 rpm for 10 minutes. DNA that has settled to the bottom and stuck to the tube is bounced by pipetting with 75% cold ethanol. 1 ml of ethanol is used in this process. After centrifugation at 13,000 rpm for 5 minutes, the liquid in the tubes is evacuated by decantation. At this stage, the DNA has a highly visible white structure and has stuck to the bottom of the tube. The samples are left with the tubes open for approximately 12 hours to dry. 100 µl of nuclease-free water is added to the dried DNA samples. To prevent RNA activity, 2 µl RNase is added to each tube and incubated in a water bath at 37 °C for 20 minutes for the enzyme to work.

Table 3

CTAB isolation buffers components

CTAB Components	Concentration
Tris-HCl (pH:8.0)	100 mM
EDTA (pH:8.0)	20 mM
NaCl	1.4 M
CTAB(cetyltrimethylammonium bromide)	2%

Chloroform octanol used 24:1 ratio and 2-Mercaptoethanol 0.01M.

3.3. Agarose Gel Electrophoresis

To visualize isolated DNA strands, the samples were visualized in agarose gel electrophoresis. To prepare agarose gel, 50 mL TAE (Tris Acetic acid EDTA) buffer and 500 mg agarose are mixed in an Erlenmeyer and microwaved until the agarose is completely dissolved in the buffer. For much more samples; 4 g agarose and 200 ml TAE buffer were mixed and heated. When the solution cools to approximately 55 °C, 3 µl - 12 µl for the ethidium bromide is added, mixed, and poured onto the gel disk. Combs are attached and the gel is waited to set. When the gel is ready, the combs are removed and 5 µl of sample for each well is mixed with loading dye and loaded into the wells. Samples were run in gel electrophoresis at 100 to 120 volts for 60 minutes. The gel was examined under UV light using the UVP PhotoDoc-It Imaging System model imaging device.

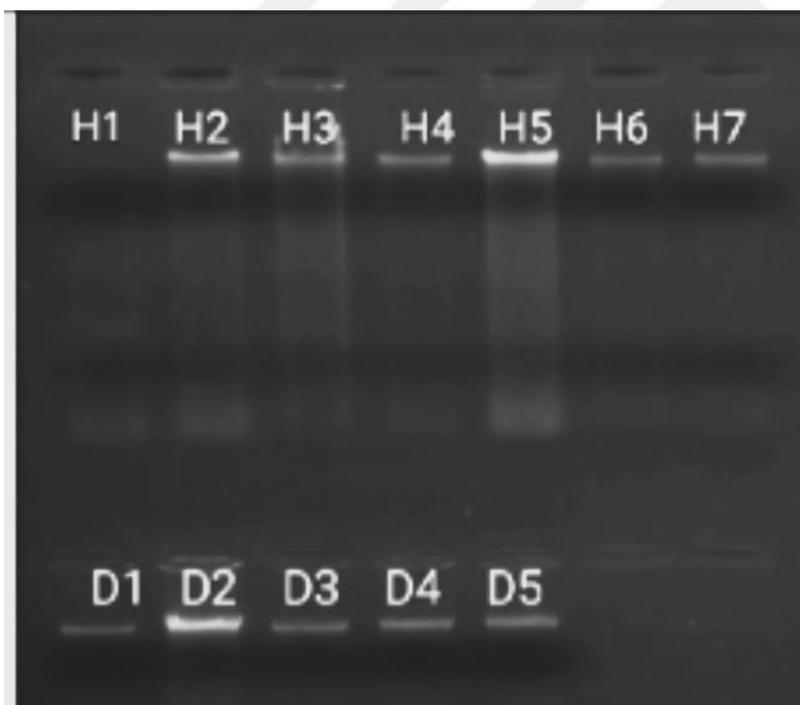


Figure 3. 1% Agarose Gel electrophoresis screen with sensitive and resistant parental samples. H1, H2, H3, H4, H5, H6, H7 are sensitive DNA samples. D1, D2, D3, D4, D5 are resistant DNA samples.

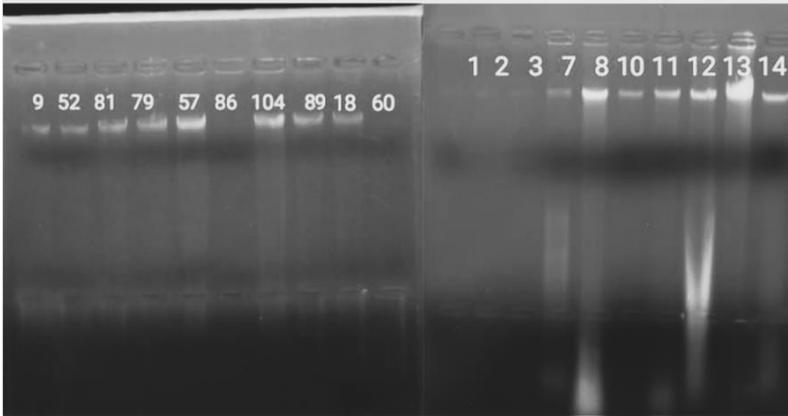


Figure 4. F₂ Individuals DNA samples in 1% Agarose Gel Electrophoresis

3.4. Quantification of DNA through the NanoDrop Spectrophotometer

Isolated DNAs were analyzed with a nanodrop (IMPLEN-Nanophotometer P330) device. DNA concentration and quality A₂₆₀/A₂₈₀ and A₂₆₀/A₂₃₀ were measured. These values showed us the success of insulation. Non-optimal ratios may be due to phenolic compound residue.

Table 4

Quantification of Sunflowers DNA through the NanoDrop Spectrophotometer

Sample Name	Concentration (ng/μl)	Sample Name	Concentration (ng/μl)
R6	323	H12	124
R11	232	H13	143
H6	24	H11	96
H8	45	R7	1220
H10	72,5	R8	276

Continuation of Table 4

Sample Name	Concentration (ng\µl)	Sample Name	Concentration (ng\µl)
H12	124	H4	360
H13	143	H8	203
H11	96	H22	810
R7	1220	R5	467
R8	276	R4	715
R10	114	H6	240
R11	1380	F2-85	100
R12	392	F2-51	63,5
R2	680	F2-101	238
R3	411	F2-19	124
H5	1430	F2-21	144
R1	180	F2-89	176
H32	725	F2-37	77,5
R6	124	F2-25	130
H13	371	F2-14	185
R23	72	F2-22	168
H7	454	F2-91	137
H1	432	F2-40	132

Continuation of Table 4

Sample Name	Concentration (ng\µl)	Sample Name	Concentration (ng\µl)
F2-47	69,5	55	254
F2-3	100	4	185
F2-43	83,5	26	95
F2-95	139	35	79,5
F2-94	304	51	48,5
F2-12	63,5	24	460
F2-52	133	17	212
F2-44	212	9	25,5
H33	345	27	266
R34	338	87	168
H35	407	6	198
50	148	73	97,5
89	158	21	99,5
68	99,5	69	125
92	169	93	144
59	240	107	107
82	237	90	282
53	209	8	286

Continuation of Table 4

Sample Name	Concentration (ng\µl)	Sample Name	Concentration (ng\µl)
104	359	80	150
45	31,5	49	210
98	148	63	90,5
67	130	42	75,5
65	94,5	70	153
13	116	78	274
77	93,5	64	212
10	178	16	110
105	270	47	67
7	223	34	113
54	250	83	703
31	80	62	122
23	125	96	226
84	153	75	208
53	258	60	101
79	220	66	251
15	113	81	190
100	110	5	160

Continuation of Table 4

Sample Name	Concentration (ng\μl)	Sample Name	Concentration (ng\μl)
86	157	2-	150
38	52,5	46	110
36	156	61	114
71	140	97	246
107	60,5	18	248
88	306	76	66,5
56	24,5	72	122
108	231	39	180
57	332	11	20,1
102	413	20	502
1-	142		

Nanodrops measures DNA quality and concentration by using absorbance of nucleic acids. Nucleic acids absorb UV at specific wavelengths, DNA absorbs UV at 260 nm and RNA absorbs UV at also 260 nm. However, they have a different absorbance pattern due to the ribose sugars. According to absorbance at 260 nm, concentration analysis was performed. Between 280 and 230 nm nanodrop evaluates the purity of nucleic acids. This mechanism is based on Beer-Lambert law(Wilfinger et al., 1997).

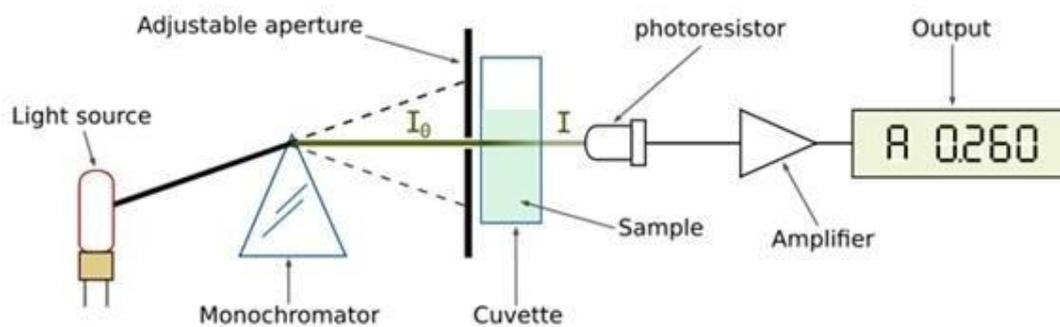


Figure 5. Nanodrop Analysis Mechanism

3.5. Bulk Segregant Analysis (BSA)

Bulk Segregant analysis helps form a gene pool of interest. BSA allows the appropriate primer to be easily detected without screening individuals one by one. While bulk tubes are being prepared, 14 maternal DNA samples are taken equally and collected in a single tube. In disease-resistant bulk tubes, 5 microliters of each of the 14 resistant rootstock isolated DNA samples are taken to create a resistant bulk tube. The same procedure was applied to 14 disease-sensitive parental DNA samples and a sensitive bulk tube was developed. The importance of using bulk markers is first tested in this large pool of sensitive and resistant individuals, and when a difference such as polymorphism is observed, this marker is tested one by one in the rootstock and spring individuals. Bulk tubes are used in genetic studies to increase genetic diversity and benefit from a wide range of genes. Another advantage of bulk tubes is that they make it easier to distinguish individuals with two different phenotypes or genotypes. Thus, it allows to select the right individuals from a large population quickly and more easily in studies carried out using these samples, and is also widely used in breeding programs. In addition, since bulk tubes contain a combination of different resistance genes, it helps to develop a stronger resistance mechanism. Another benefit of preparing honey tubes in selecting the right mercury at the beginning of the study is that genetic differences can be observed in some plant individuals even if they grow in the same environmental conditions. To avoid misleading this result, these mixture tubes are created from all resistant or sensitive individuals.

3.6. PCR

In the PCR study, different primers were first tested on bulk samples prepared from sensitive rootstocks and resistant rootstocks. Thus, while monomorphic primers could not differentiate between sensitive and resistant individuals, polymorphic primers showed significant differences between resistant and sensitive individuals at the optimum annealing temperature. In the next step, PCR was performed by binding the bulk samples with primers one by one. In the last stage, PCR was performed on offspring F_2 individuals with polymorphic SSR primers. All experimental stages took place on ice. The primers used were diluted to 90 μl water and 10 μl primer. 2 μl of 10x PCR buffer was prepared for each sample. Then, 1 μl of 50 mM MgCl_2 , the catalyst, was added into the buffer. 0.4 μl of dNTP diluted to 2.5 mM was added to each tube. 1 μl of the forward and 1 μl of the reverse diluted SSR primers were used. 12 μl of nuclease-free water was added, but since DNA was not added to the negative control, 14 μl was added. 2 μl of isolated and diluted DNA was used. 0.15 μl of Taq polymerase enzyme was used for each sample. After mini-spin centrifugation, PCR was run at the appropriate annealing temperature.

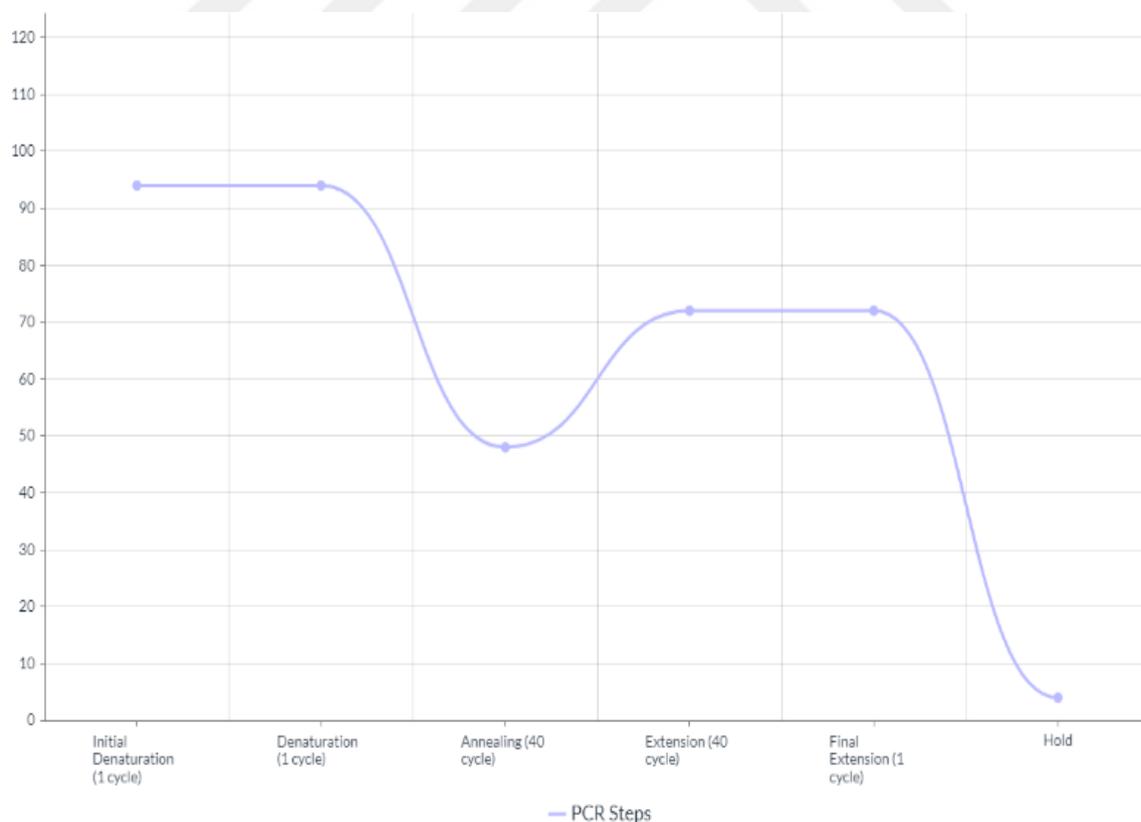


Figure 6. PCR steps with Cycle Temperatures

Table 5

PCR components with Concentrations

PCR Components	Concentration	Volume
10 X PCR Buffer	10 X	2 μ l
MgCl₂	50 mM	1 μ l
dNTP	2.5 mM	0,4 μ l
SSR Forward Primer	10 μ M	1 μ l
SSR Reverse Primer	10 μ M	1 μ l
Taq DNA Polymerase	5 U/ μ l	0,15 μ l
Genomic DNA	50 ng/ μ l	2 μ l
dH₂O	-	12,5 μ l

CHAPTER 4

RESEARCH FINDINGS

4.1. Genomic DNA Isolation

The DNA of a total of 130 plant samples, 15 of which were sensitive, 15 were resistant and 100 were F₂ generation, were successfully isolated. CTAB-based isolation protocol according to Doyle and Doyle's (1987) was used. After isolation, the success of the isolation was measured with 2% Agarose electrophoresis and also the concentration and quality of DNA was measured with NanoDrop Spectrophotometer (IMPLEN-Nanophotometer P330).

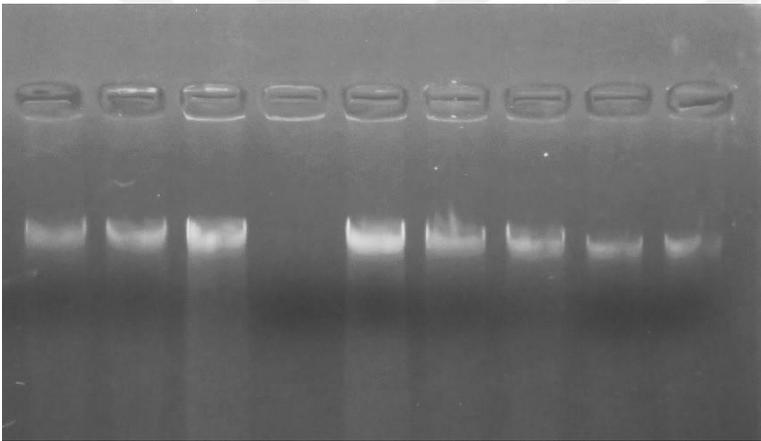


Figure 7. Screening of DNA isolation in 1% Agarose gel

4.2. Markers Tested on Bulks

To detect polymorphism, the markers were first tested on prepared durable and sensitive bulk tubes. According to the results obtained from these samples and the Agarose gel electrophoresis image, 19 of the 36 markers tested showed monomorphic features and 17 of them showed polymorphic features and it turned out that they were specific for resistance genes. The names of monomorphic and polymorphic SSR primers are listed in the table below.

Table 6

SSR markers that are used and Polymorphisms

SSR Marker	Polymorphism
ORS 166	✓
ORS 1008	✓
ORS 965	✓
ORS 552	✓
ORS 172	X
ORS 662	✓
ORS 665	X
ORS 630	✓
ORS 317	✓
ORS 511	✓
ORS 728	✓
ORS 995	✓
ORS 721	X
ORS 1039	X
ORS 1125	X
ORS 1197	✓
ORS 581	X
ORS 203	✓
ORS 1065	✓
ORS 1070	X
ORS 936	✓
ORS 1011	X
ORS 333	✓
ORS 966	✓
ORS 822	X
ORS 928	X

Continuation of Table 6

ORS 331	X
ORS 45	X
ORS 13	X
ORS 191	X
ORS 849	X
ORS 328	X
ORS 1030	X
ORS 1114	X
ORS 1092	✓
ORS 224	X

The figure 7 is the agarose gel electrophoresis image of 4 different SSR primers tested on sensitive and durable bulk samples. In the image, there is a 150 bp ladder in the first well. The first marker, ORS 1115, did not reveal any difference in disease-resistant and susceptible bulk samples and was determined to be monomorphic. The band profile of durable and delicate bulks looks exactly the same. For this reason, ORS 1115 has not been tested on broodstock and F₂ individuals. However, in the image, the ORS 203 primer showed a clear difference between the resistant and sensitive bulk samples and was determined to be polymorphic. Resistant bulk shows a different band from sensitive bulk and thus ORS 203 has a high potential to distinguish between resistant and sensitive individuals, in 530 length. Another polymorphic ORS 1065 primer, which was detected in the image, also showed the same difference and had 420 bp band length.

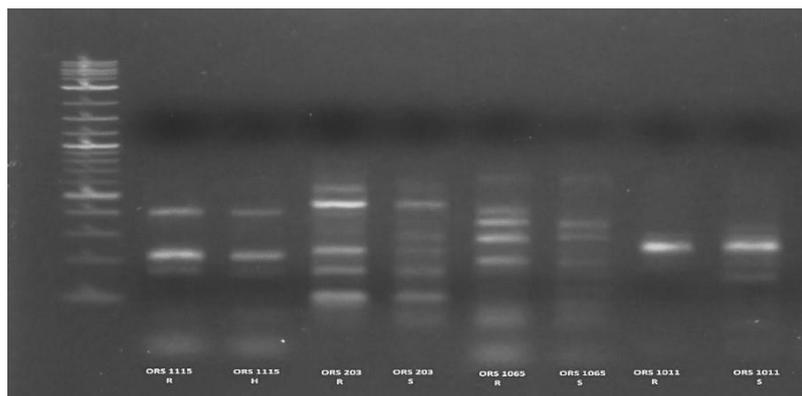


Figure 8. Screening of Resistant and Sensitive bulks with the markers ORS 1125, ORS 203, ORS 1065 and ORS 1011.

4.3. Screening of Polymorphic Markers on Individuals

4.3.1 ORS 166

ORS 166 is a SSR primer that was mapped to LG8 as being associated with PI genes, was 344 bp. ORS 166 has been tested on resistant and susceptible bulks, polymorphism detected between resistant and susceptible bulk tubes. Resistant and susceptible parental genotypes which form bulk tubes also used in PCR analysis individually, shown in the Figure 9. According to agarose gel electrophoresis, band profile differences were detected between resistant and susceptible parental individuals. In Figure 9, Resistant bulk gave a 500 bp band length.

Table 7

ORS 166 Reverse and Forward Primers

Primer	Reverse	Forward
ORS 166	TGTTAAGAACCGCGACAACACTGC	CAGCCACATGCCCTCTGAC

ORS 166 was also tested on hybrid F₂ individuals and the similar band profile as the resistant parents forming resistant bulk was observed in 32 out of 100 samples (Figure 10). It can be said that these hybrids could be resistant individuals.

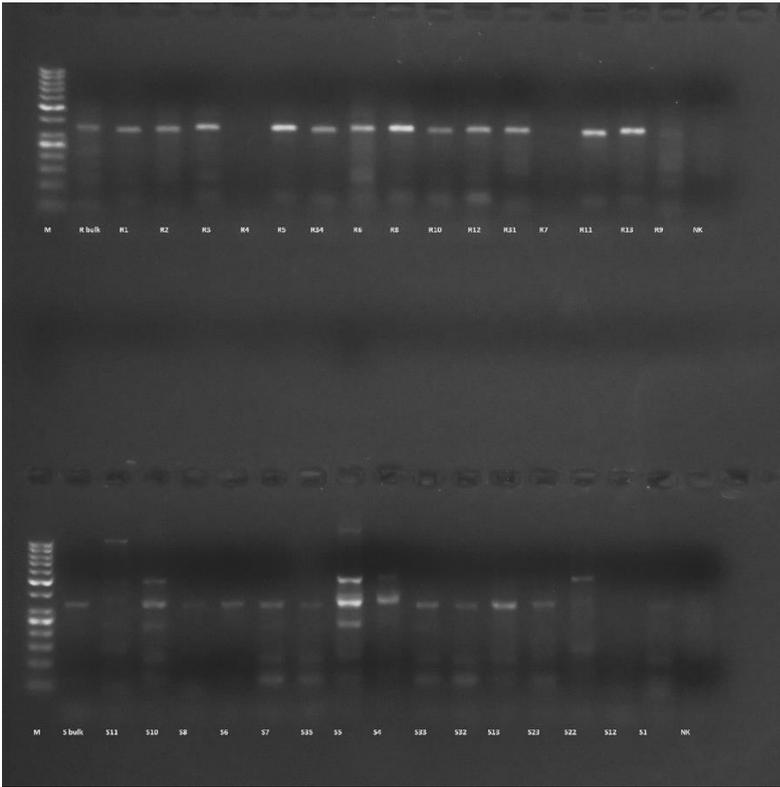
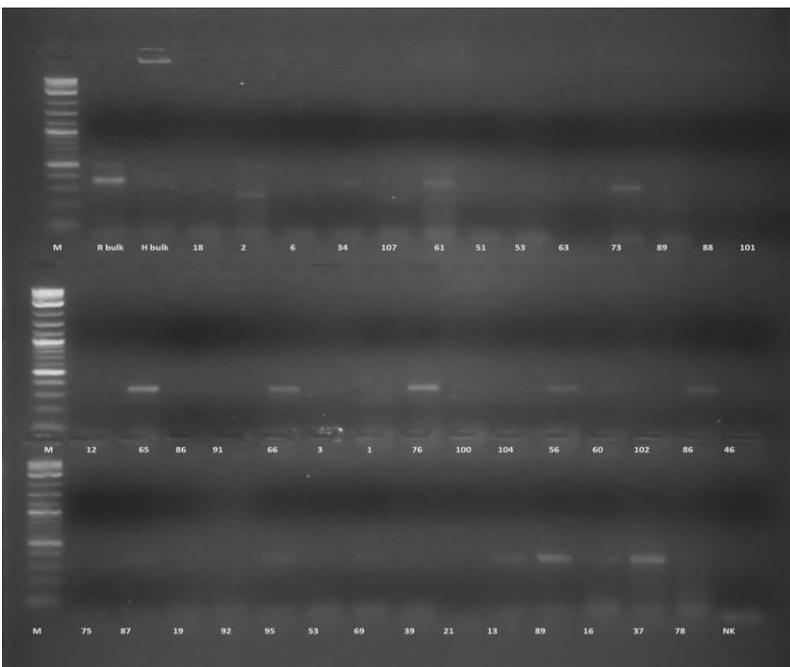


Figure 9. This result shows the PCR products of resistant and susceptible bulks and individuals from bulk pools with ORS 166 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.



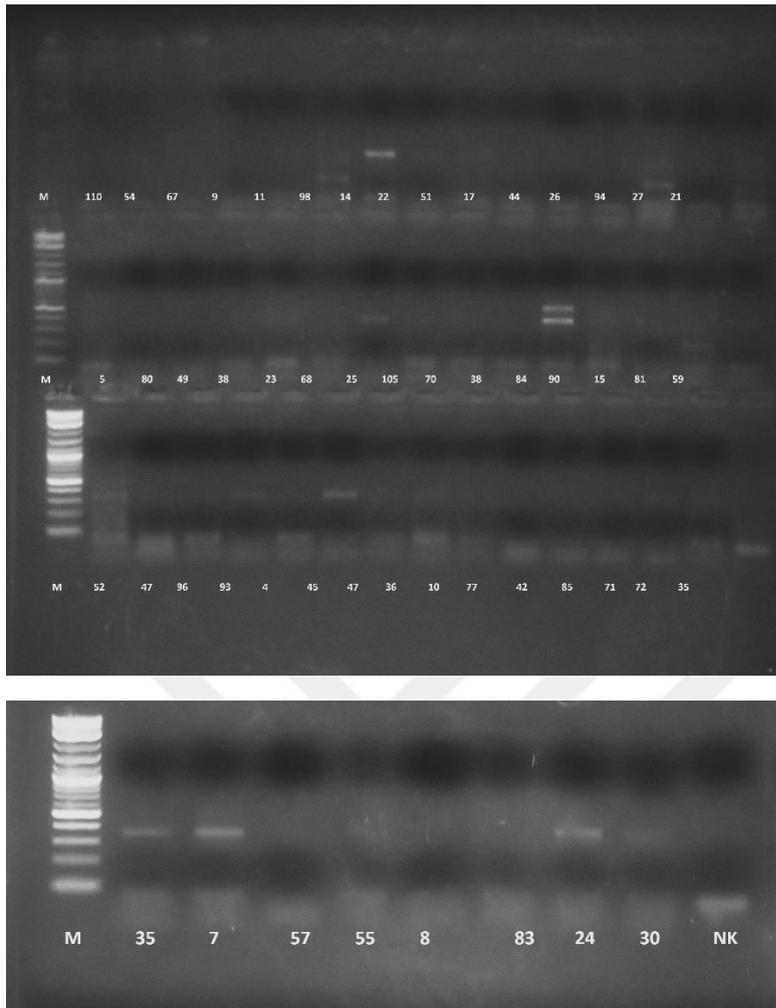


Figure 10. This result shows the PCR products of resistant and susceptible bulks and F₂ individuals (SUN59) formed by crossing IMI044B x H458B genotypes with ORS 166 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.

4.3.2. ORS 1008

ORS 1008 is a SSR primer that was mapped to LG1 as being associated with Pl genes, was 349 bp. ORS 1008 has been tested on resistant and susceptible bulks and polymorphism detected between them (Figure 11). Resistant bulk gave 400bp band length. Resistant and susceptible parental genotypes that form bulk pools were also tested in PCR analysis individually, as shown in Figure 12. According to agarose gel electrophoresis, band profile

differences were not detected between resistant and susceptible parental individuals which forms bulk tubes.

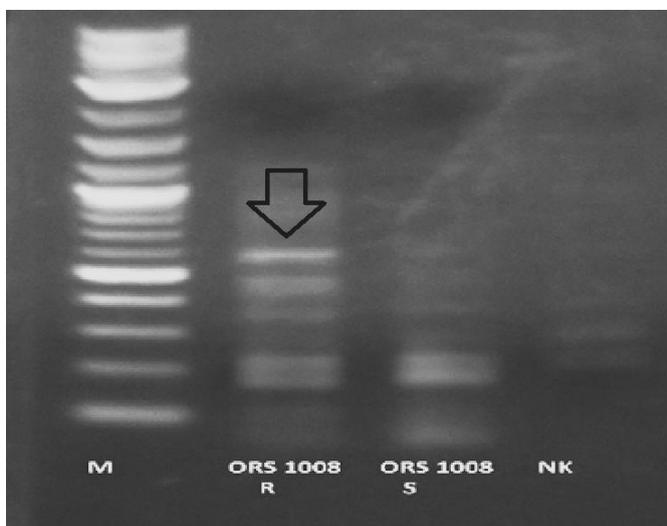


Figure 11. This result shows the PCR products of resistant and susceptible bulks in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.

Table 8

ORS 1008 Reverse and Forward Primers

Primer	Reverse	Forward
ORS 1008	CATGAGGGCATTCTTGTCATT	GATCACCTTCACTATCCACAACC

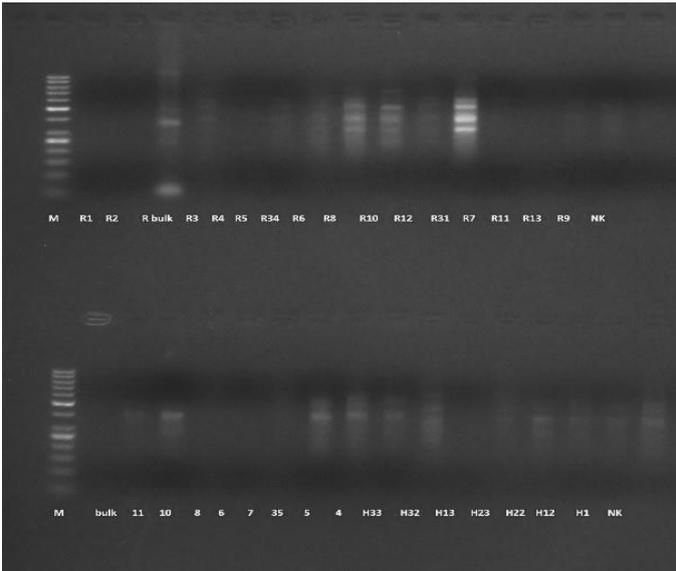
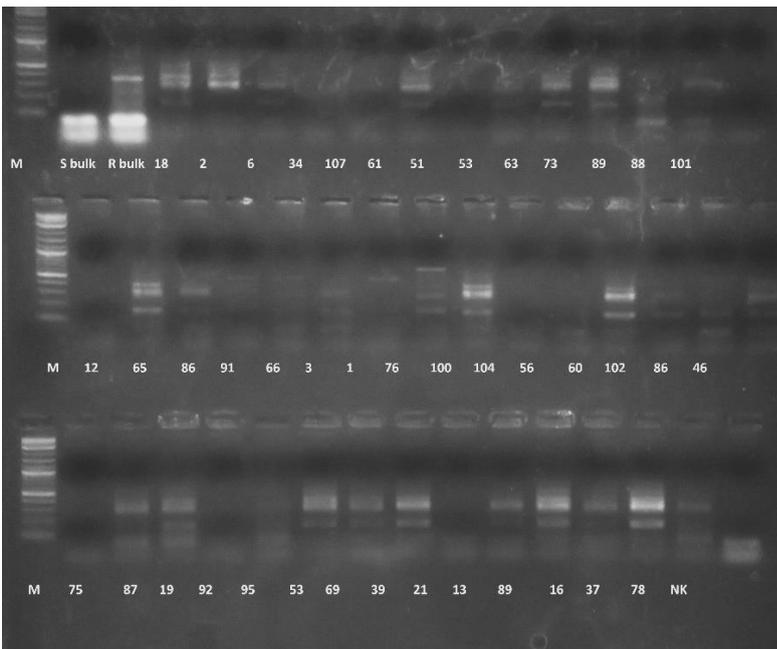


Figure 12. This result shows the PCR products of resistant and susceptible bulks and individuals from bulk pools with ORS 1008 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.

Figure 13 shows the agarose gel electrophoresis results of 100 F₂ individuals with ORS 1008. A similar band profile as the resistant bulk was observed in 40 F₂ individuals out of 100.



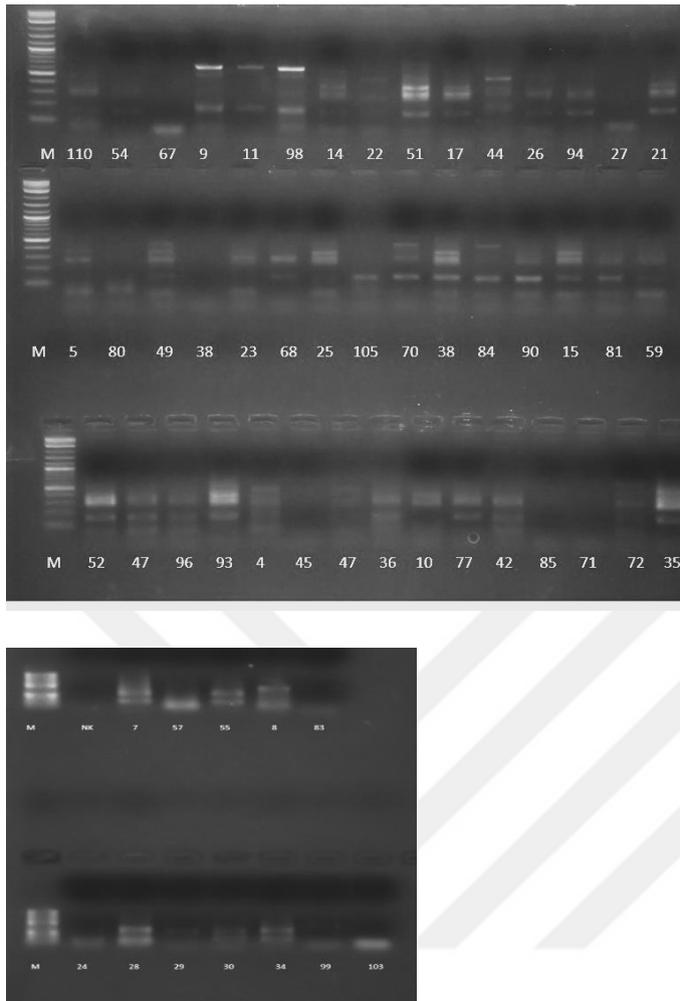


Figure 13. This result shows the PCR products of resistant and susceptible bulks and F₂ individuals (SUN59) formed by crossing IMI044B x H458B genotypes with ORS 1008 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.

4.3.3 ORS 552

ORS 552 is a SSR primer that was mapped to LG1 as being associated with PI genes, was 410 bp. ORS 552 has been tested on resistant and susceptible bulks and polymorphism detected between them (Figure 14 and Figure 15). Resistant bulk gave 270 bp band length. Resistant and susceptible parental genotypes that form bulk pools were also tested in PCR analysis individually, as shown in Figure 14 and Figure 15. According to agarose gel electrophoresis, band profile differences were detected between resistant and susceptible

parental individuals which forms bulk tubes. ORS 552 was also tested on hybrid F₂ individuals and a similar band profile as the resistant parents forming resistant bulk was observed in 62 out of 100 samples (Figure 16). It can be said that these hybrids could be resistant individuals.

Table 9

ORS 552 Reverse and Forward Primers

Primer	Reverse	Forward
ORS 552	CCATCCCTTCCCTCTCTTTC	GTGGCTGGAATCTCATCACC

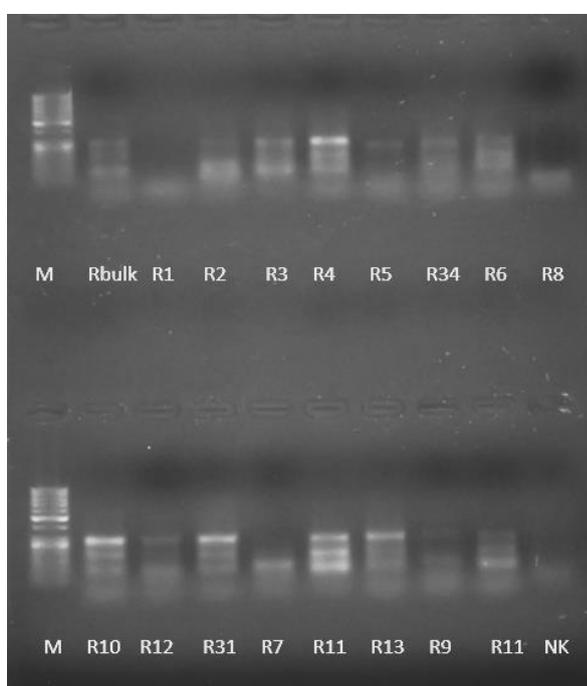


Figure 14. This result shows the PCR products of resistant bulks and individuals from bulk pools with ORS 552 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.

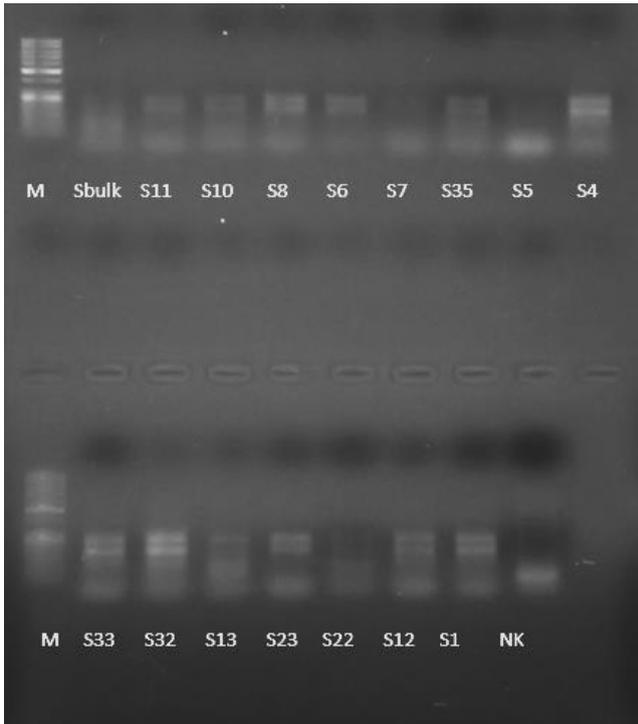
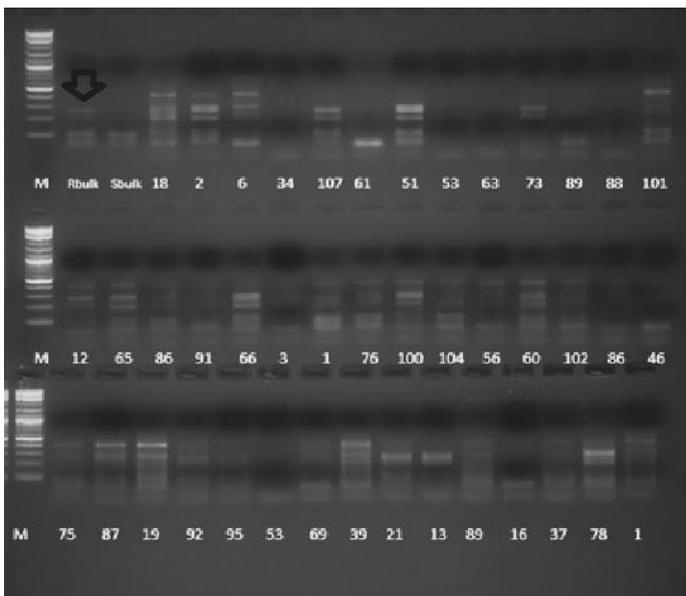


Figure 15. These results show the PCR products susceptible bulks and individuals from bulk pools with ORS 552 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.



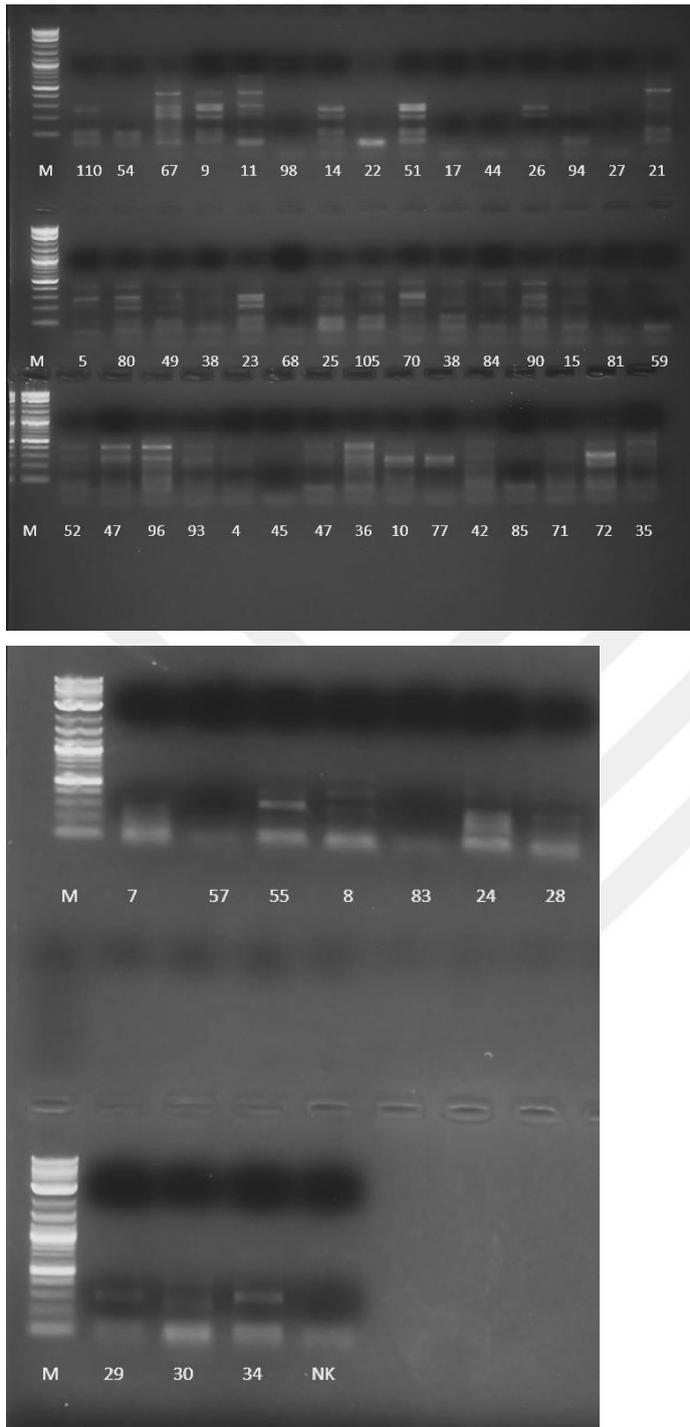


Figure 16. These results show the PCR products of resistant and susceptible bulks and F₂ individuals (SUN59) formed by crossing IMI044B x H458B genotypes with ORS 552 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.

4.3.4. ORS 333

ORS 333 is a SSR primer that was mapped to LG4 as being associated with PI genes, was 567 bp. ORS 333 has been tested on resistant and susceptible bulks and polymorphism detected between them (Figure 17). Resistant bulk gave 350 bp band length. Resistant and susceptible parental genotypes that form bulk pools were also tested in PCR analysis individually, as shown in Figure 18. According to agarose gel electrophoresis, band profile differences were detected between resistant and susceptible parental individuals which forms bulk tubes. ORS 333 was also tested on hybrid F₂ individuals and a similar band profile as the resistant parents forming resistant bulk.

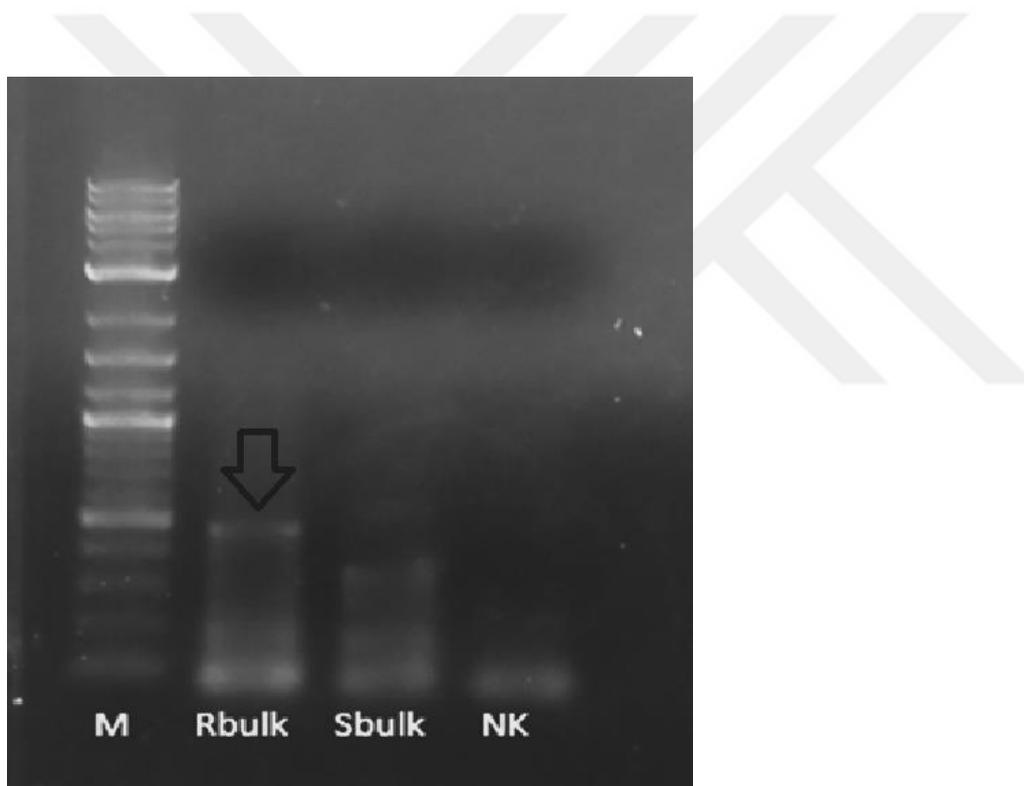


Figure 17. This result shows the PCR products of resistant and susceptible bulks in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.

Table 10

ORS 333 Reverse and Forward Primers

Primer	Reverse	Forward
ORS 333	ATATTAAGTTTTGGTTTTAGCCAGAA	CGGTTAAGATGGTTCAGTTGG

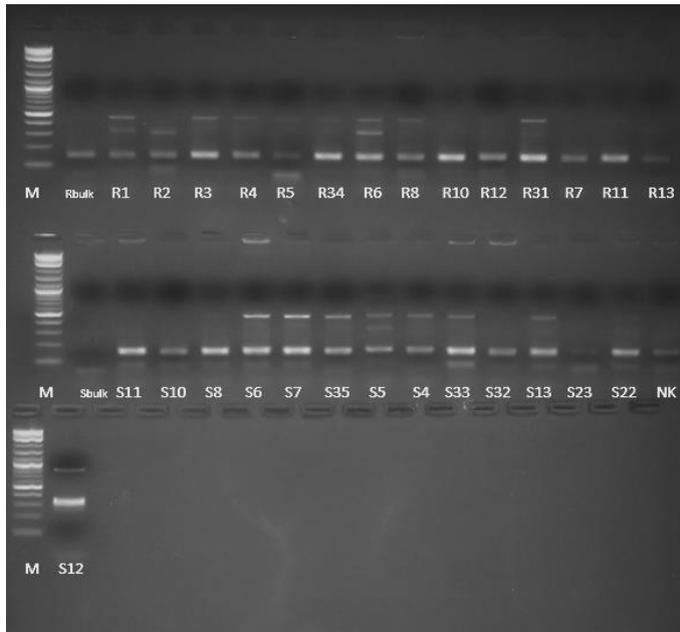
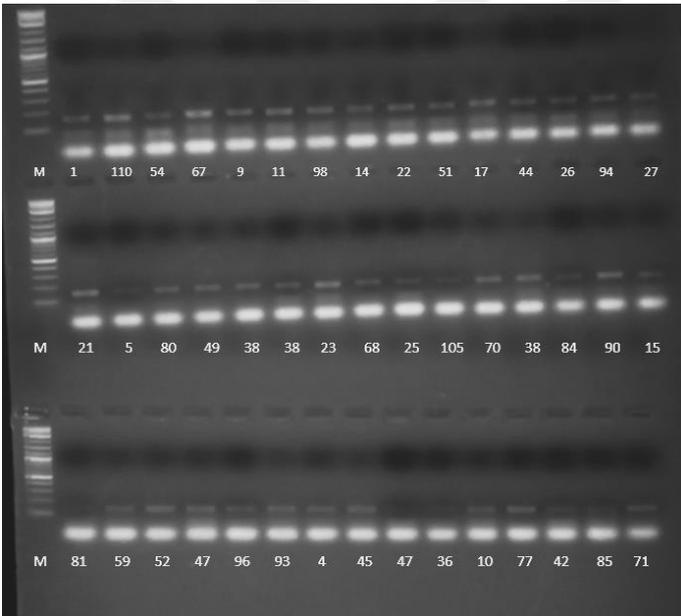
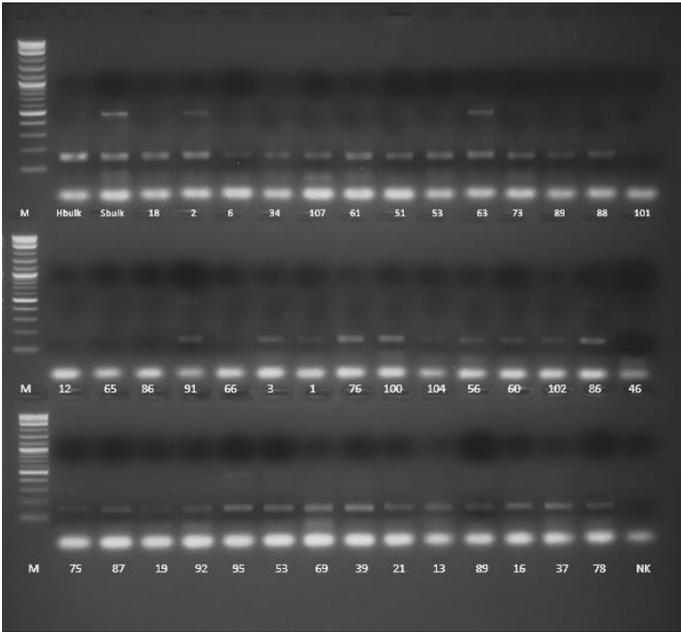


Figure 18. These results show the PCR products of resistant and susceptible bulks and individuals from bulk pools with ORS 333 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control



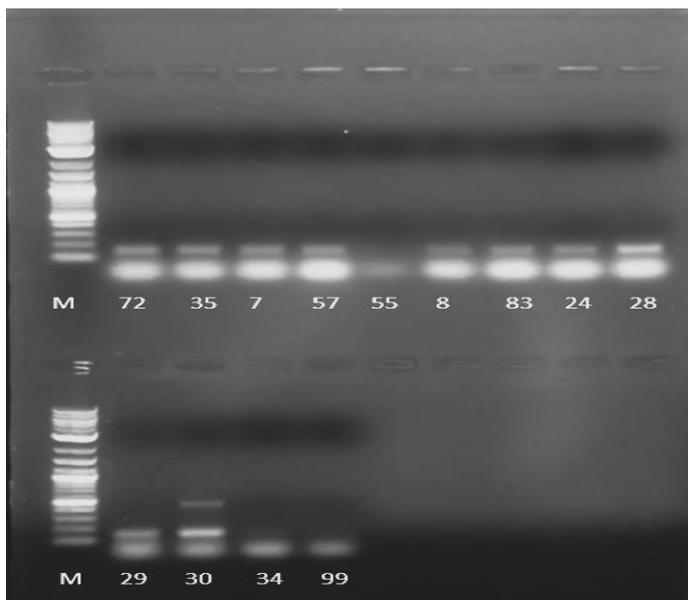


Figure 19. These results show the PCR products of resistant and susceptible bulks and F₂ individuals (SUN59) formed by crossing IMI044B x H458B genotypes with ORS 333 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.

4.3.5 ORS 662

ORS 662 is a SSR primer that was mapped to LG1 as being associated with PI genes, was 491 bp. ORS 662 has been tested on resistant and susceptible bulks and polymorphism detected between them (Figure 21). Resistant bulk gave 250 bp band length. Resistant and susceptible parental genotypes that form bulk pools were also tested in PCR analysis individually, as shown in Figure 20. According to agarose gel electrophoresis, band profile differences were not detected between resistant and susceptible parental individuals which forms bulk tubes. ORS 662 was also tested on hybrid F₂ individuals and a similar band profile as the resistant parents forming resistant bulk was observed in 23 out of 100 samples (Figure 21). It can be said that these hybrids could be resistant individuals.

Table 11

ORS 662 Reverse and Forward Primers

Primer	Reverse	Forward
ORS 662	CCTTTACAAACGAAGCACAATTC	CGGGTTGGATATGGAGTCAA

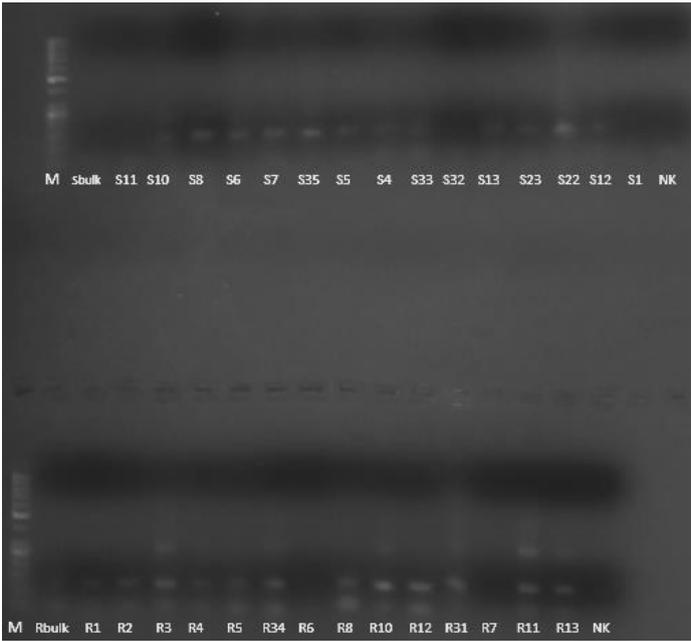
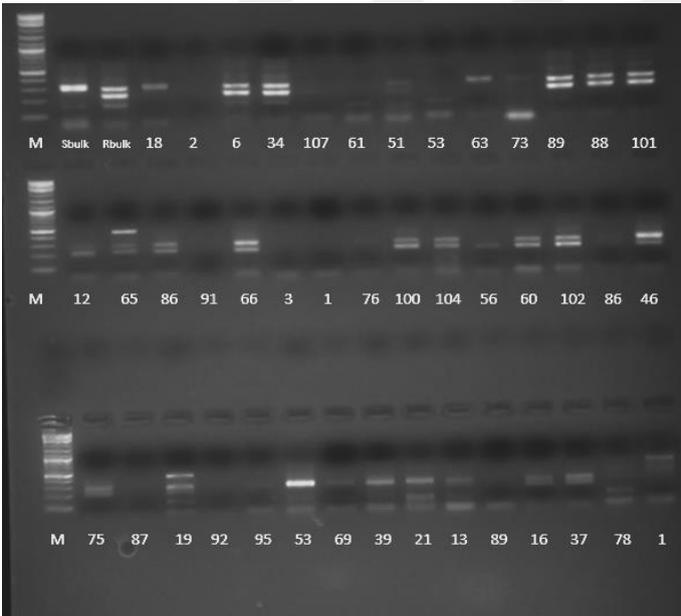


Figure 20. These results show the PCR products of resistant and susceptible bulks and individuals from bulk pools with ORS 662 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.



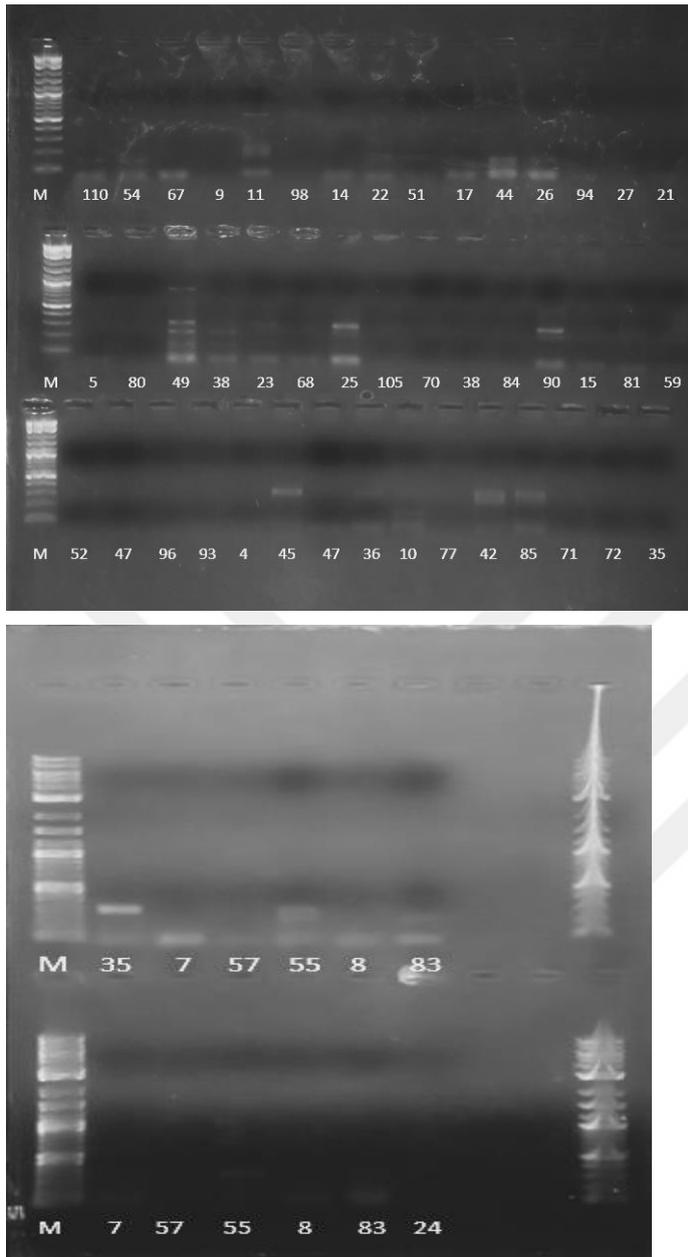


Figure 21. This result shows the PCR products of resistant and susceptible bulks and F₂ individuals (SUN59) formed by crossing IMI044B x H458B genotypes with ORS 662 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.

4.3.6 ORS 728

ORS 728 is a SSR primer that was mapped to LG8 as being associated with PI genes, was 403 bp. ORS 728 has been tested on resistant and susceptible bulks and polymorphism

was detected between them (Figure 22). Resistant bulk gave 400 bp band length. Resistant and susceptible parental genotypes that form bulk pools were also tested in PCR analysis individually, as shown in Figure 23. According to agarose gel electrophoresis, band profile differences did not detected between resistant and susceptible parental individuals which forms bulk tubes and in hybrid F₂ individuals.

Table 12

ORS 728 Reverse and Forward Primers

Primer	Reverse	Forward
ORS 728	CCAAACTCTGAATGATACTTGTGAC	CTCCATAGCAACCACCTGAAA

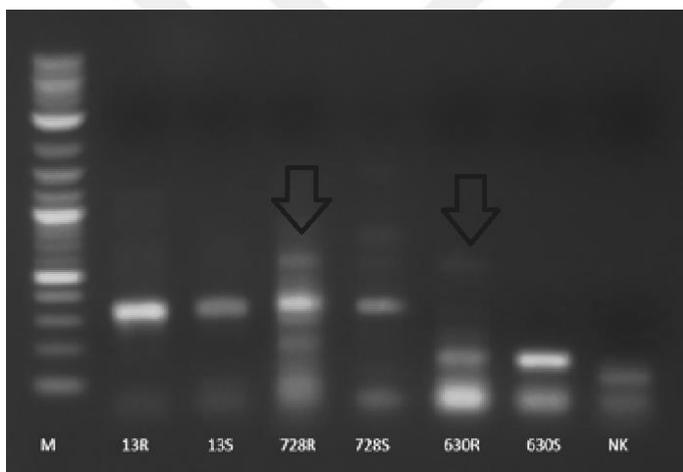


Figure 22. These results show the PCR products of resistant and susceptible bulks in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.

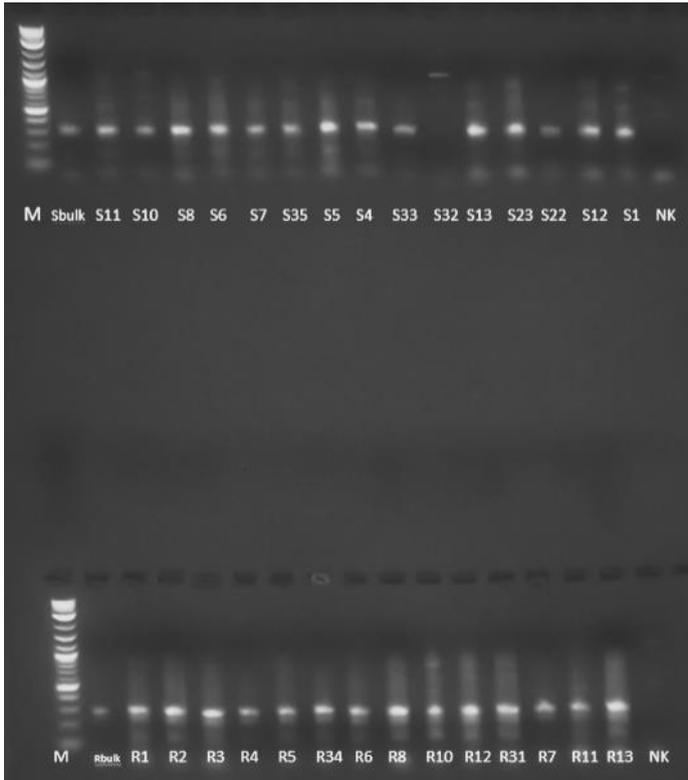
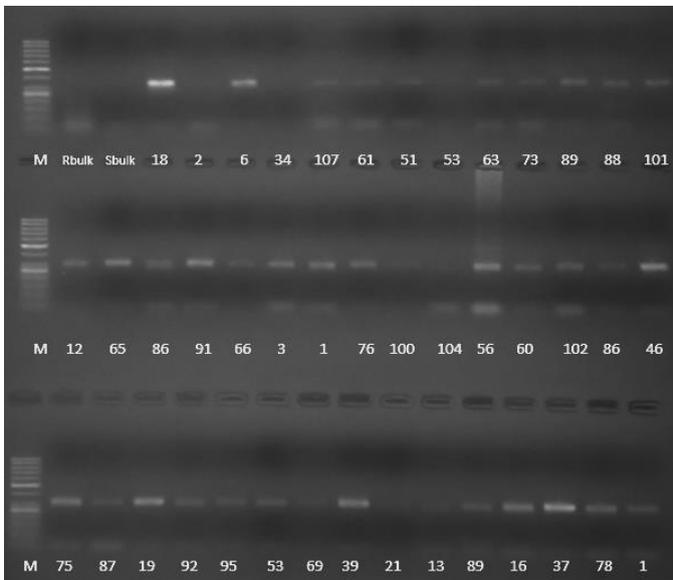


Figure 23. These results show the PCR products of resistant and susceptible bulks and individuals from bulk pools with ORS 728 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant, S: susceptible genotypes, NK: negative control



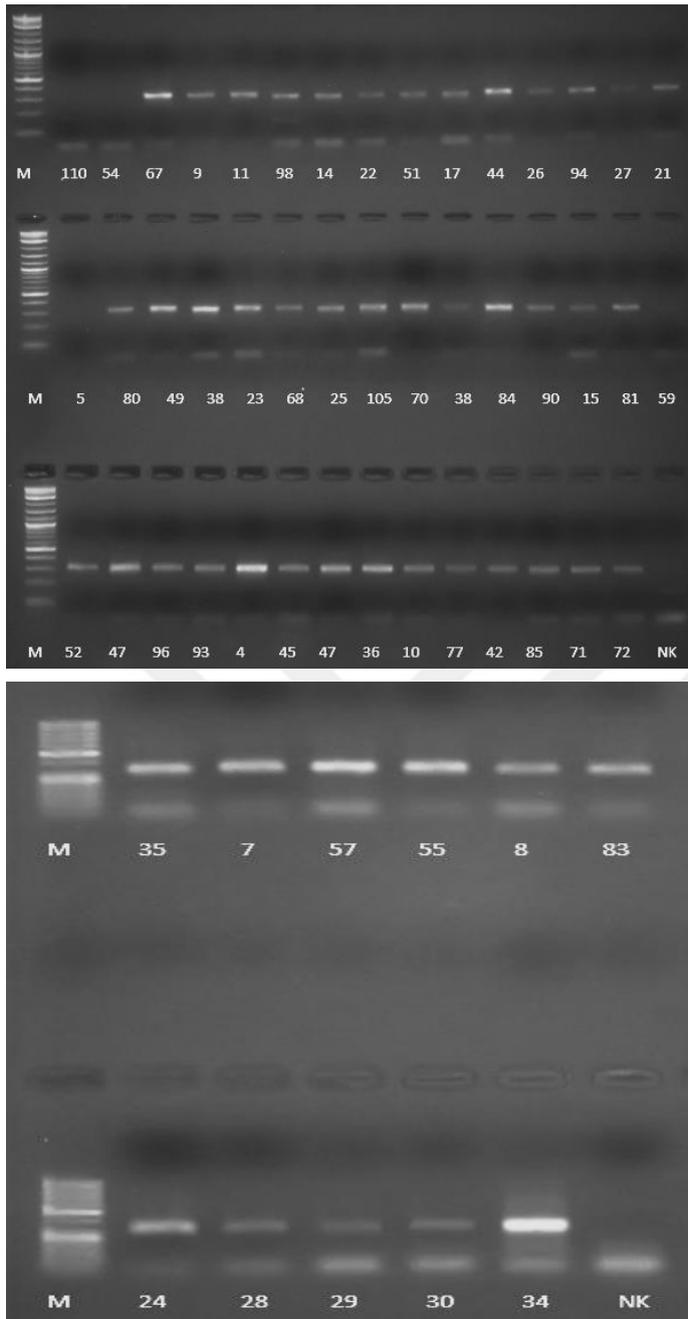


Figure 24. These results show the PCR products of resistant and susceptible bulks and F2 individuals (SUN59) formed by crossing IMI044B x H458B genotypes with ORS 728 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.

4.3.7. ORS 965

ORS 965 is a SSR primer that was mapped to LG1 as being associated with Pl genes, was 487 bp. ORS 965 has been tested on resistant and susceptible bulks and polymorphism detected between them(Figure 25). Resistant bulk gave 600 bp band length. Resistant and susceptible parental genotypes that form bulk pools were also tested in PCR analysis individually, as shown in Figure 26. According to agarose gel electrophoresis, band profile differences were not detected between resistant and susceptible parental individuals which forms bulk tubes. ORS 965 was also tested on hybrid F₂ individuals and did not give a distinctive band profile(Figure 27).

Table 13

ORS 965 Reverse and Forward Primers

Primer	Reverse	Forward
ORS 965	CTTACCCTCCTCAGACCCTACCT	TTGGATTACCTTGGATAGTCAGC

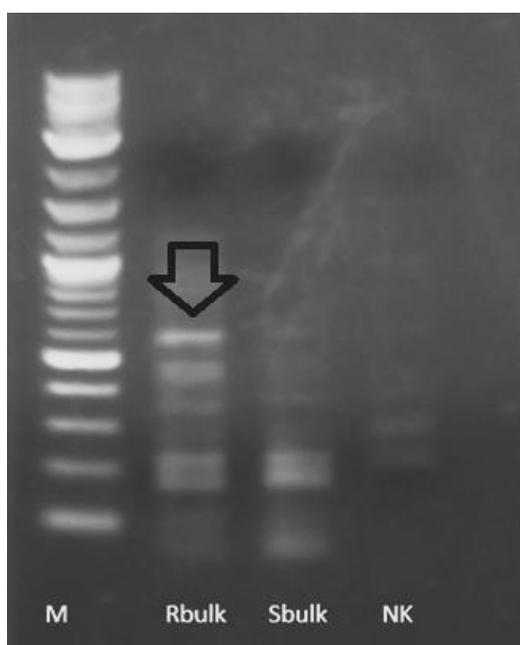


Figure 25. These results show the PCR products of resistant and susceptible bulks in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.

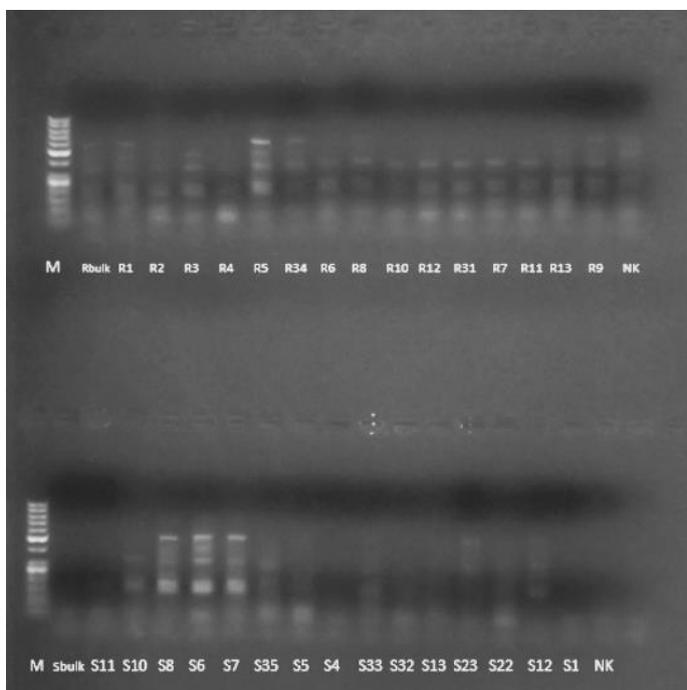
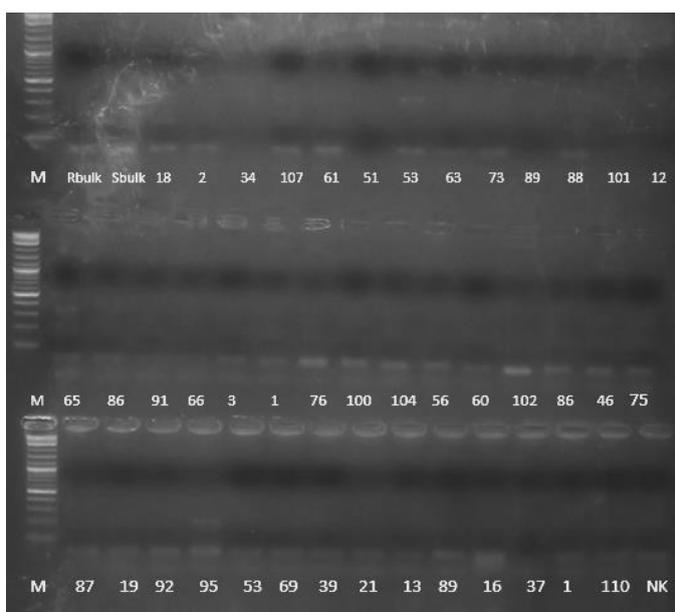


Figure 26. These results show the PCR products of resistant and susceptible bulks and individuals from bulk pools with ORS 965 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control



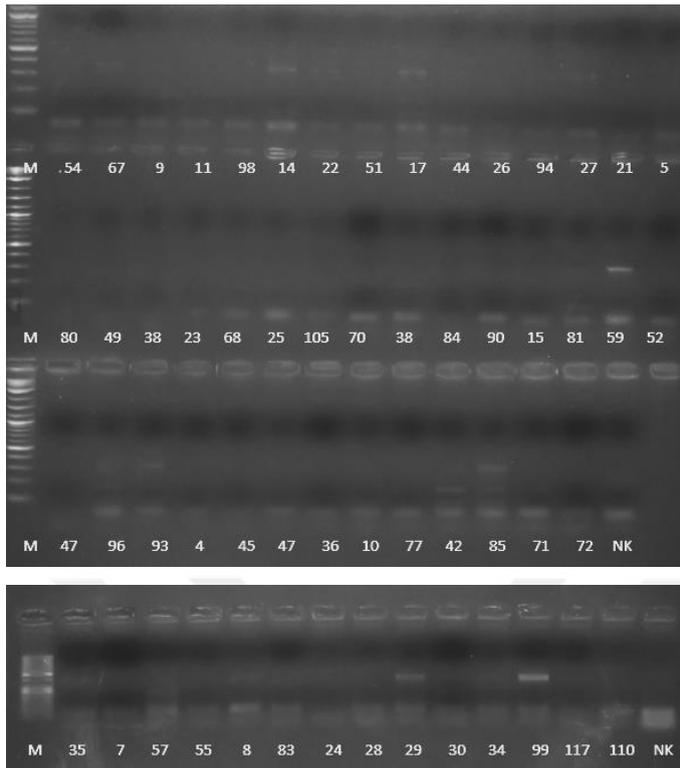


Figure 27. These results show the PCR products of resistant and susceptible bulks and F₂ individuals (SUN59) formed by crossing IMI044B x H458B genotypes with ORS 965 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.

4.3.8. ORS 966

ORS 966 is a SSR primer that was mapped to LG8 as being associated with PI genes, was 435 bp. ORS 966 has been tested on resistant and susceptible bulks and polymorphism detected between them (Figure 28). Resistant and susceptible parental genotypes that form bulk pools were also tested in PCR analysis individually, as shown in Figure 28. According to agarose gel electrophoresis, band profile differences are detected between resistant and susceptible parental individuals which forms bulk tubes.

Table 14

ORS 966 Reverse and Forward Primers

Primer	Reverse	Forward
ORS 966	ATTTGCTGAGACCATGAGCATC	TCAAAGATGTCACCATAGGAAAGA

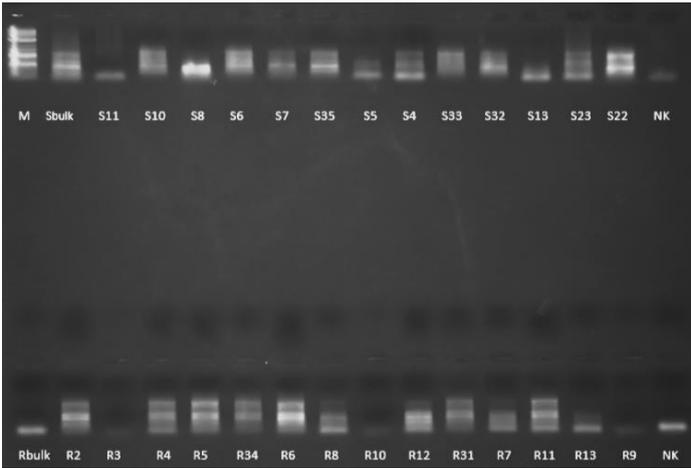
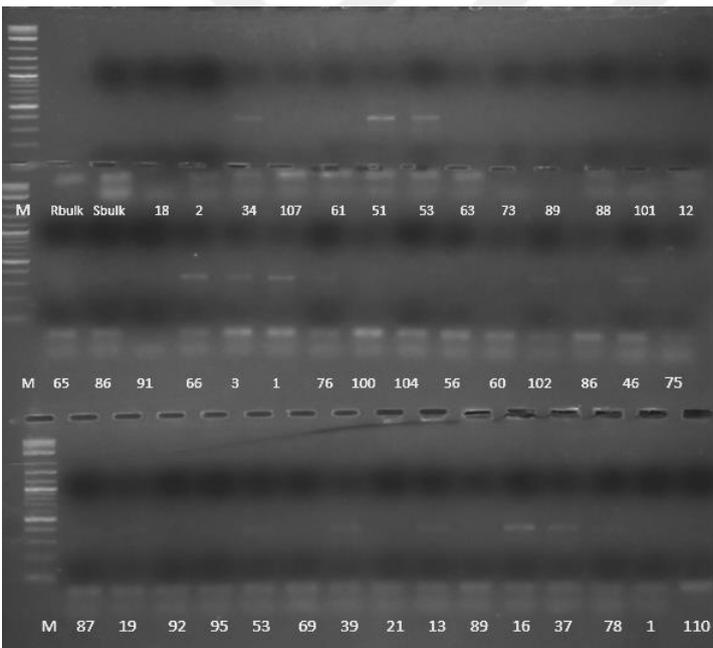


Figure 28. This results shows the PCR products of resistant and susceptible bulks and individuals from bulk pools with ORS 966 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.



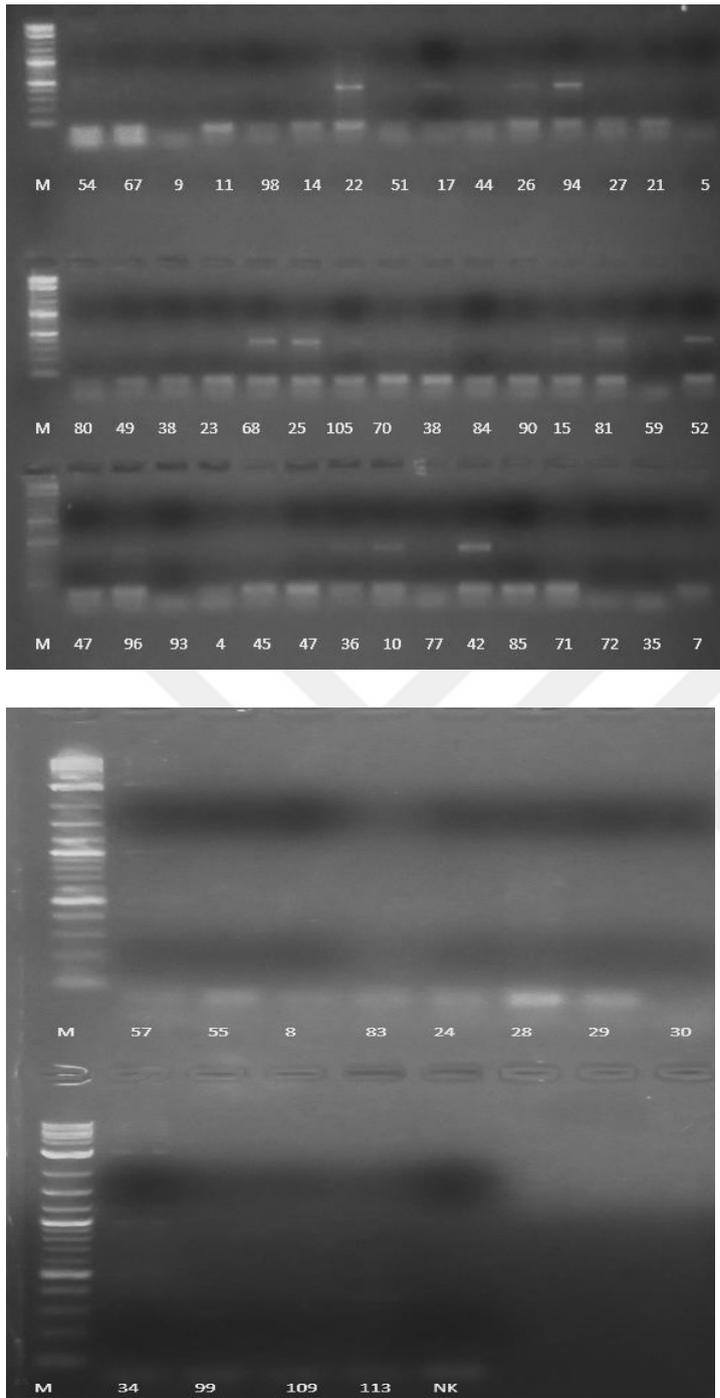


Figure 29. These results show the PCR products of resistant and susceptible bulks and F2 individuals (SUN59) formed by crossing IMI044B x H458B genotypes with ORS 966 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.

4.3.9. ORS 995

ORS 995 is a SSR primer that was mapped to LG8 as being associated with PI genes, was 375 bp. ORS 995 has been tested on resistant and susceptible bulks and polymorphism detected between them(Figure 30). Resistant bulk gave 580 bp band length. Resistant and susceptible parental genotypes that form bulk pools were also tested in PCR analysis individually, as shown in Figure 31. According to agarose gel electrophoresis, band profile differences were detected between resistant and susceptible parental individuals which forms bulk tubes. ORS 995 was also tested on hybrid F₂ individuals and a similar band profile as the resistant parents forming resistant bulk was observed in 83 out of 100 samples (Figure 32). It can be said that these hybrids could be resistant individuals.

Table 15

ORS 995 Reverse and Forward Primers

Primer	Reverse	Forward
ORS 995	TGTATGTGGAGGCCAACAAGTAT	CATGCTTTCTAGGATGGTCAGTT

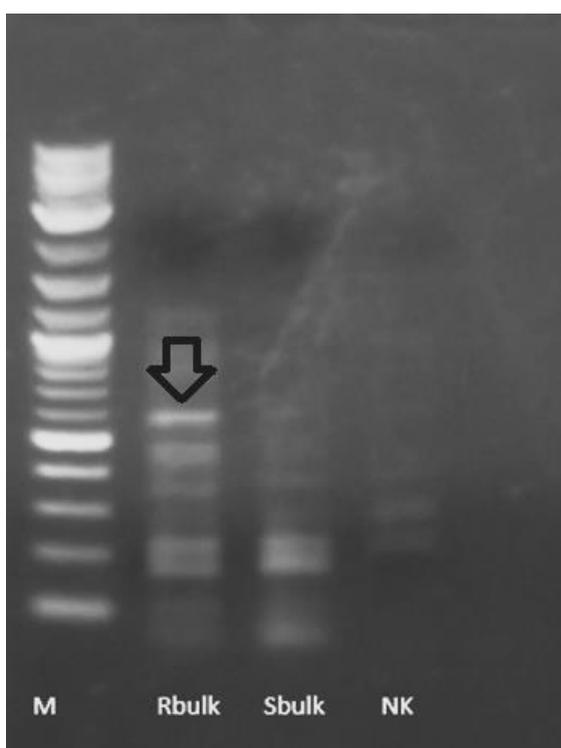


Figure 30. These results show the PCR products of resistant and susceptible bulks in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.

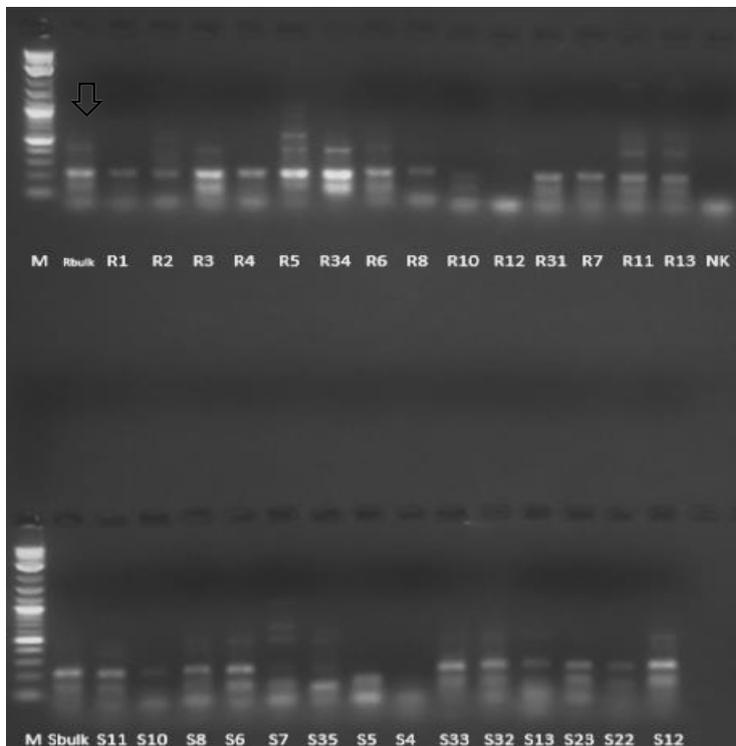
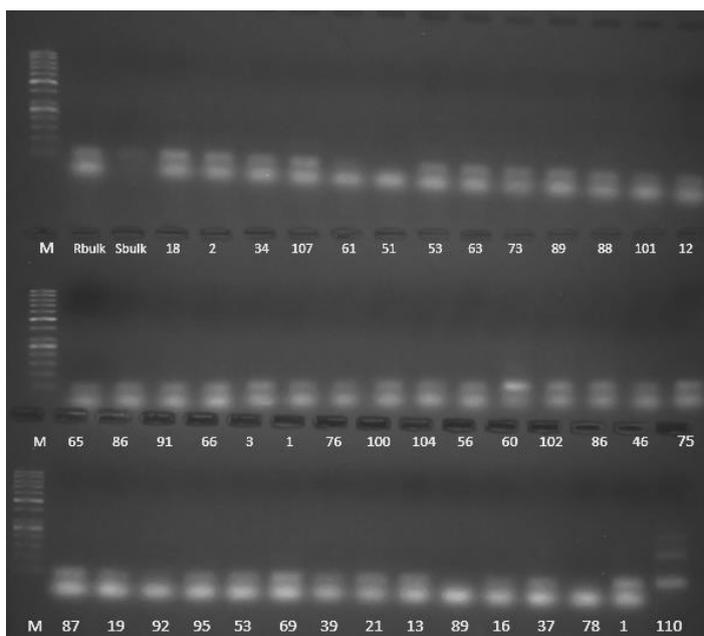


Figure 31. These results show the PCR products of resistant and susceptible bulks and individuals from bulk pools with ORS 995 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control



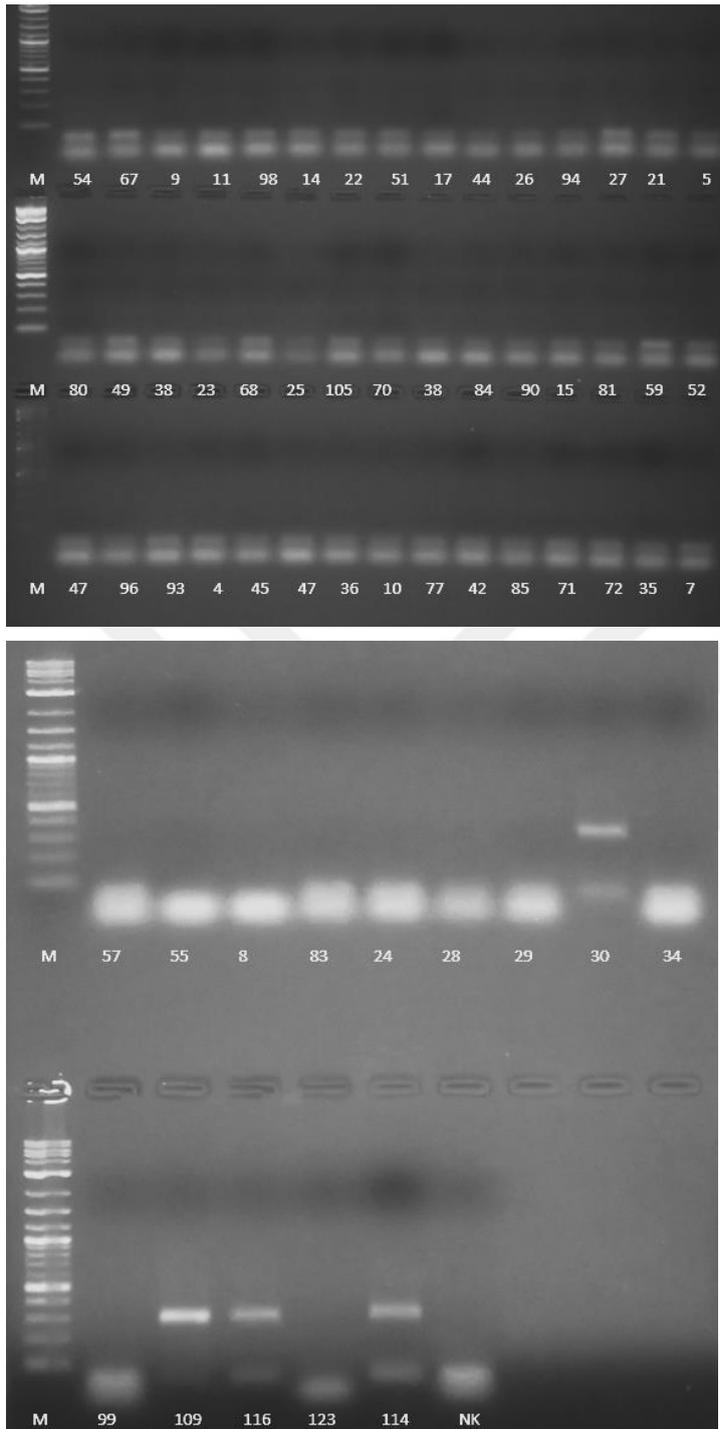


Figure 32. These results show the PCR products of resistant and susceptible bulks and F₂ individuals (SUN59) formed by crossing IMI044B x H458B genotypes with ORS 995 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.

4.3.10. ORS 1197

ORS 1197 is a SSR primer that was mapped to LG2 as being associated with PI genes and was 476 bp. ORS 1197 has been tested on resistant and susceptible bulks and polymorphism detected between them(Figure 33). Resistant bulk gave 900 bp band length. Resistant and susceptible parental genotypes that form bulk pools were also tested in PCR analysis individually, as shown in Figure 33. According to agarose gel electrophoresis, band profile differences are detected between resistant and susceptible parental individuals which forms bulk tubes. ORS 1197 was also tested on hybrid F₂ individuals. However, similar band profile as the resistant parents forming resistant bulk did not observed in 100 samples(Figure 34).

Table 16

ORS 1197 Reverse and Forward Primers

Primer	Reverse	Forward
ORS 1197	CAAACAATCACGCAAGGGTTTA	CCCAGTACGTTACAGTCGTGTGTT

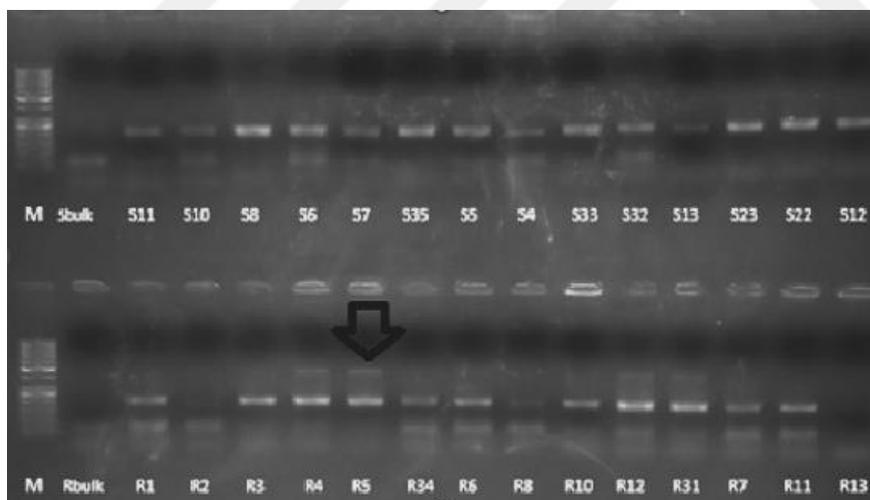
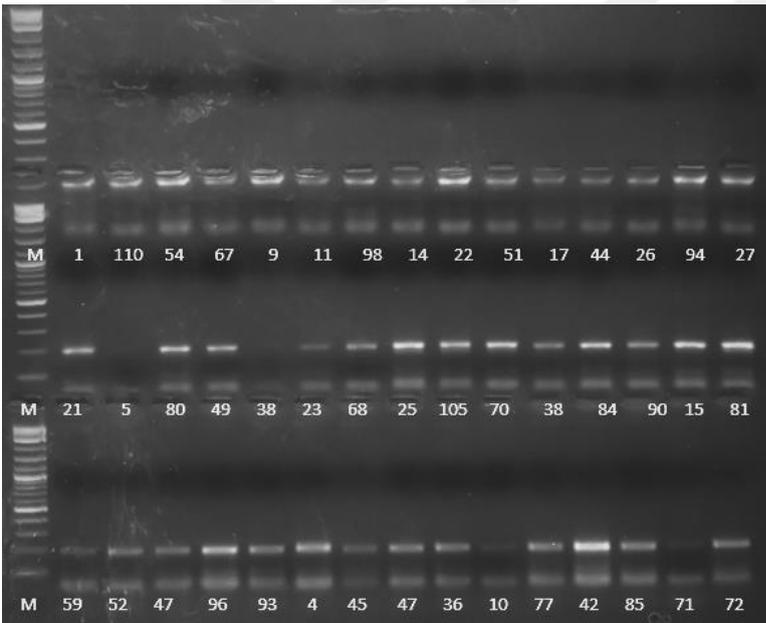
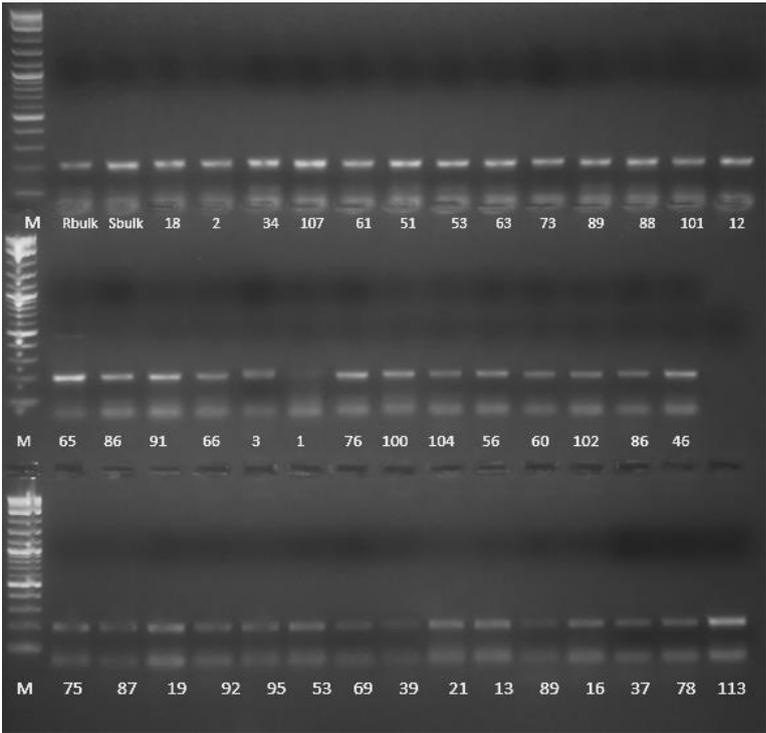


Figure 33. These results show the PCR products of resistant and susceptible bulks and individuals from bulk pools with ORS 1197 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.



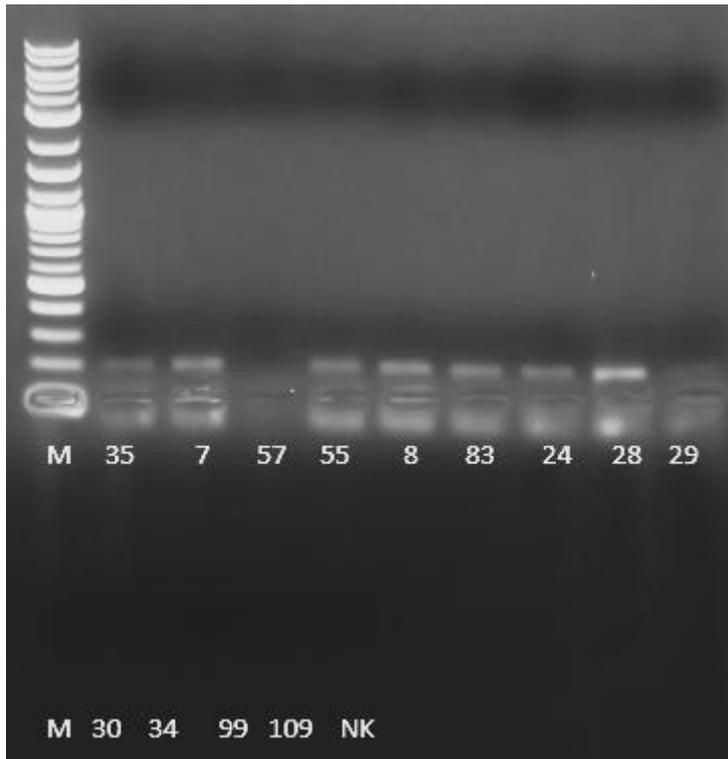


Figure 34. These results show the PCR products of resistant and susceptible bulks and F₂ individuals (SUN59) formed by crossing IMI044B x H458B genotypes with ORS 1197 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.

4.3.11. ORS 1065

ORS 1065 is a SSR primer that was mapped to LG2 as being associated with Pl genes, was 449 bp. ORS 1065 has been tested on resistant and susceptible bulks and polymorphism detected between them(Figure 35). Resistant bulk gave 350 bp band length. Resistant and susceptible parental genotypes that form bulk pools were also tested in PCR analysis individually, as shown in Figure 35. According to agarose gel electrophoresis, band profile differences are detected between resistant and susceptible parental individuals which forms bulk tubes. ORS 1065 was also tested on hybrid F₂ individuals and a similar band profile as the resistant parents forming resistant bulk was observed in 8 out of 100 samples(Figure 36).

Table 17

ORS 1065 Reverse and Forward Primers

Primer	Reverse	Forward
ORS 1065	GGCTGGGAATCAACTGCTACTAC	ACCGCTGTCAACACCTTAAACTC

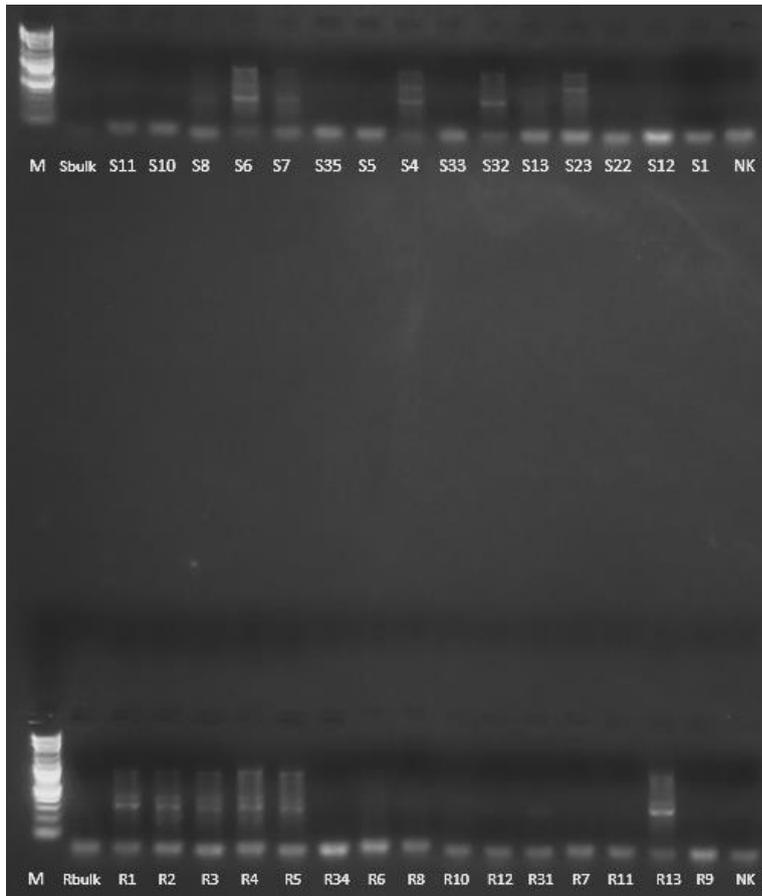
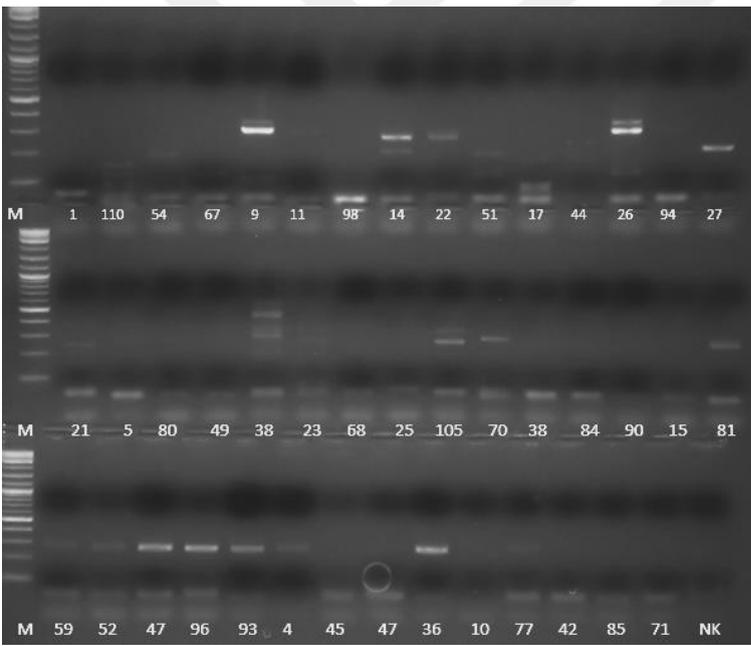
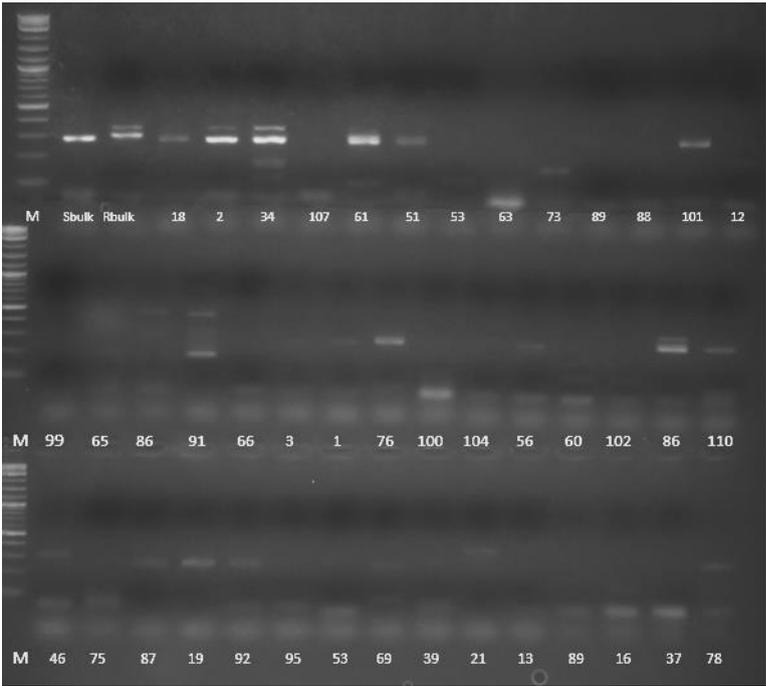


Figure 35. This results shows the PCR products of resistant and susceptible bulks and individuals from bulk pools with ORS 1065 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.



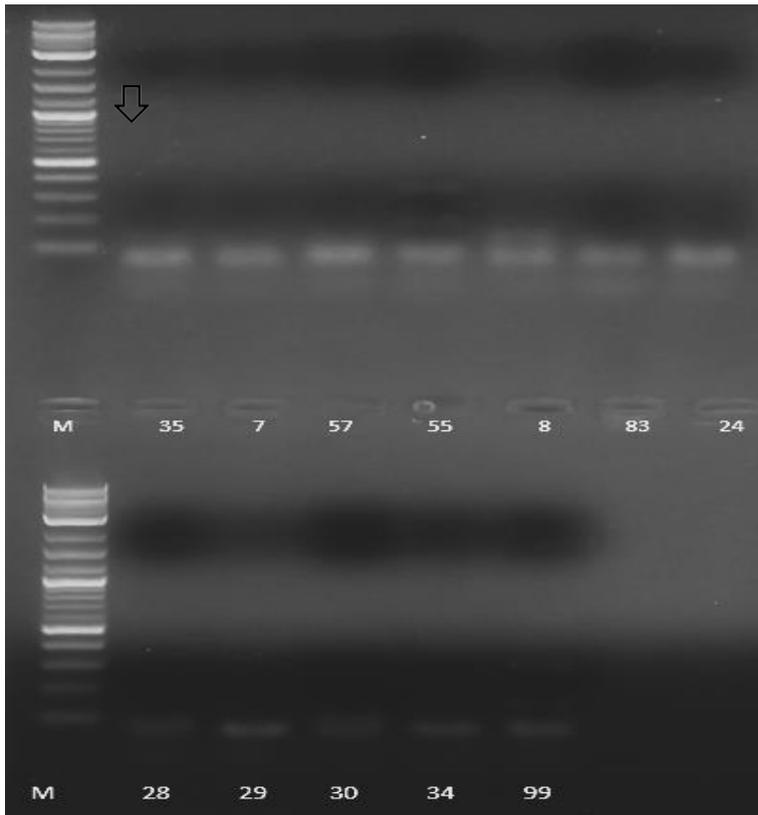


Figure 36. These results show the PCR products of resistant and susceptible bulks and F₂ individuals (SUN59) formed by crossing IMI044B x H458B genotypes with ORS 1065 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.

4.3.12. ORS 936

ORS 936 is a 384 bp SSR marker used in sunflower genetic research, consisting of short, repetitive DNA sequences that are highly variable among individuals. This marker enhances the efficiency of marker-assisted selection, facilitating targeted improvements in sunflower breeding programs. ORS 936 successfully differed R bulk with the 480 bp band length and S bulk samples which means ORS 936 is polymorphic. However, in parent and F₂ samples there is no difference between resistant and sensitives on agarose gel electrophoresis results. R bulk has a 480 bp band length.

Table 18

ORS 936 Reverse and Forward Primers

Primer	Reverse	Forward
ORS 936	TGCCAGACGAGCCATAGATAAC	ACAAGGAAAGACACTCTTGCTCA

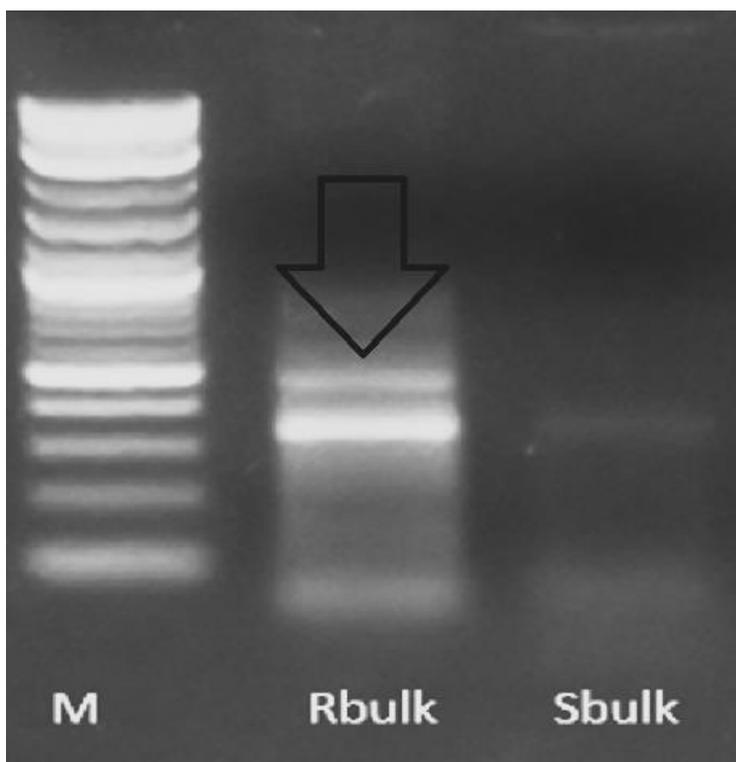


Figure 37. This results show the PCR products of resistant and susceptible bulks in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.

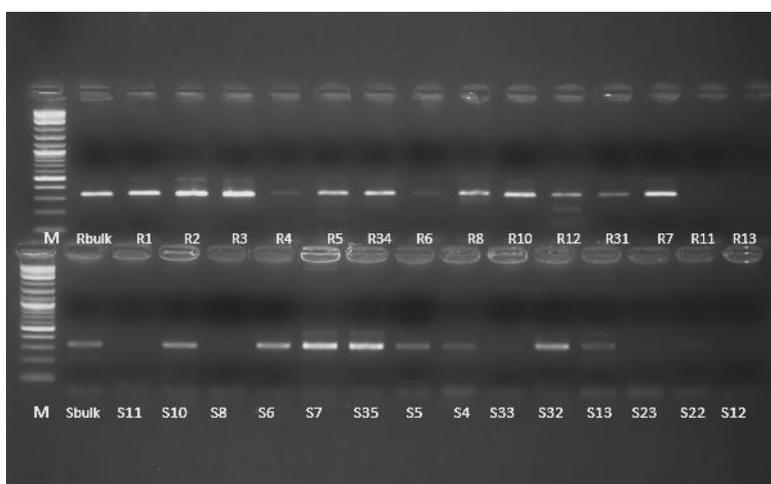
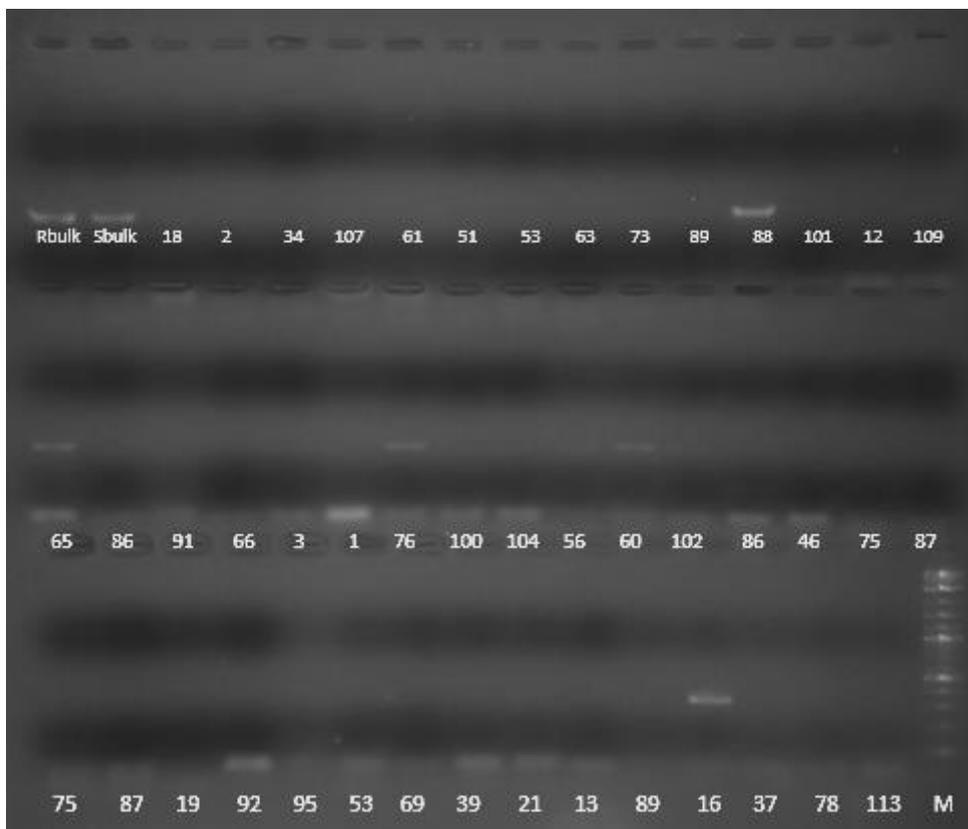


Figure 38. This results shows the PCR products of resistant and susceptible bulks and individuals from bulk pools with ORS 936 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.



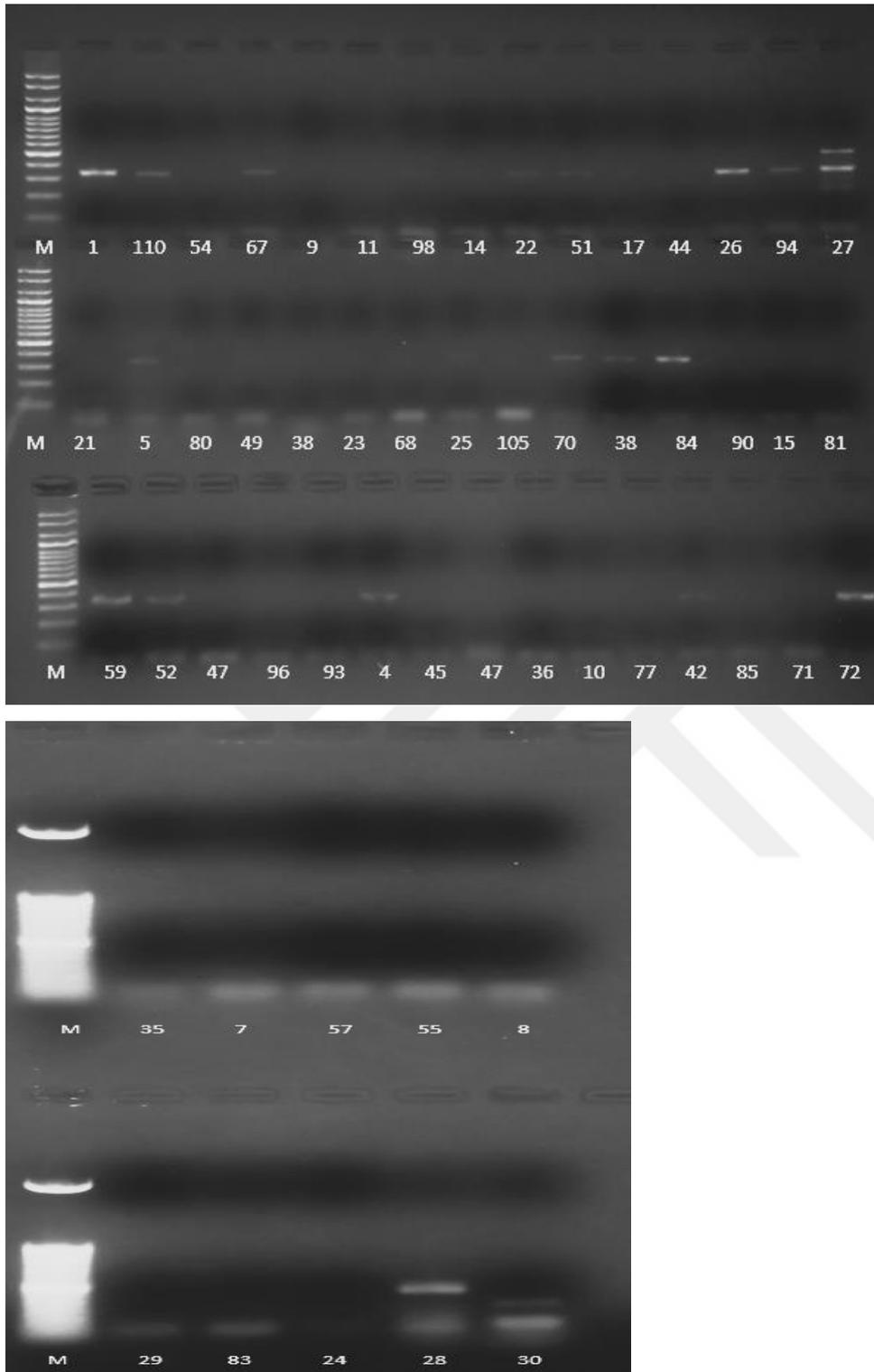


Figure 39. These results show the PCR products of resistant and susceptible bulks and F₂ individuals (SUN59) formed by crossing IMI044B x H458B genotypes with ORS 936 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.

4.3.13. ORS 630

ORS 630 is a SSR primer that was mapped to LG8 as being associated with PI genes, was 405 bp. ORS 630 has been tested on resistant and susceptible bulks and polymorphism detected between them (Figure 40). Resistant and susceptible parental genotypes that form bulk pools were also tested in PCR analysis individually, as shown in Figure 14 and Figure 15. According to agarose gel electrophoresis, band profile differences were detected between resistant and susceptible parental individuals which forms bulk tubes. ORS 630 was also tested on hybrid F₂ (Figure 41).

Table 19

ORS 630 Reverse and Forward Primers

Primer	Reverse	Forward
ORS 630	TGTGCTGAGGATGATATGCAG	GCACGACCCGGATATGTAAC

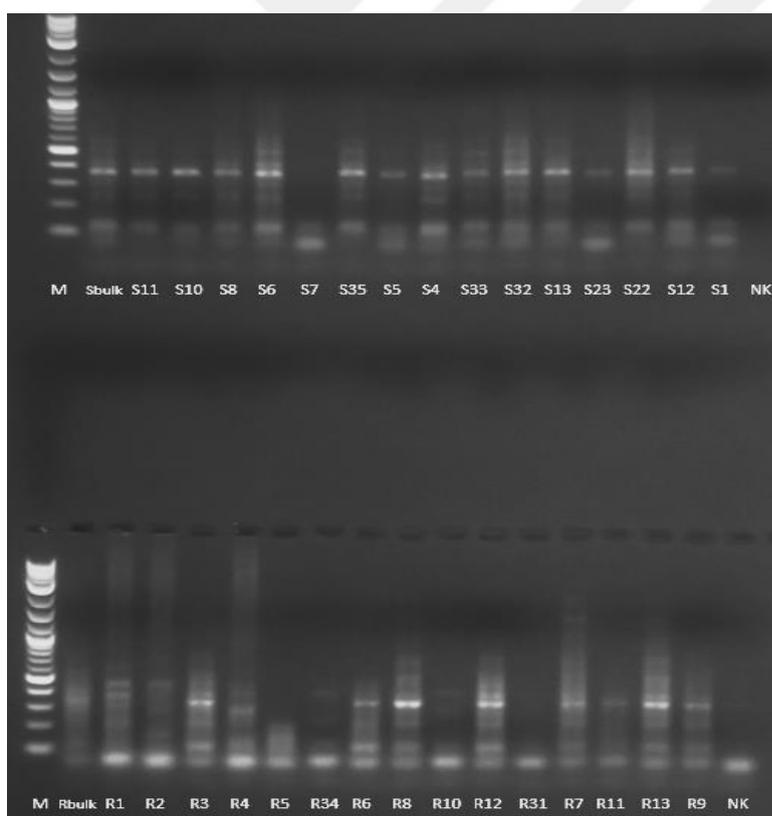
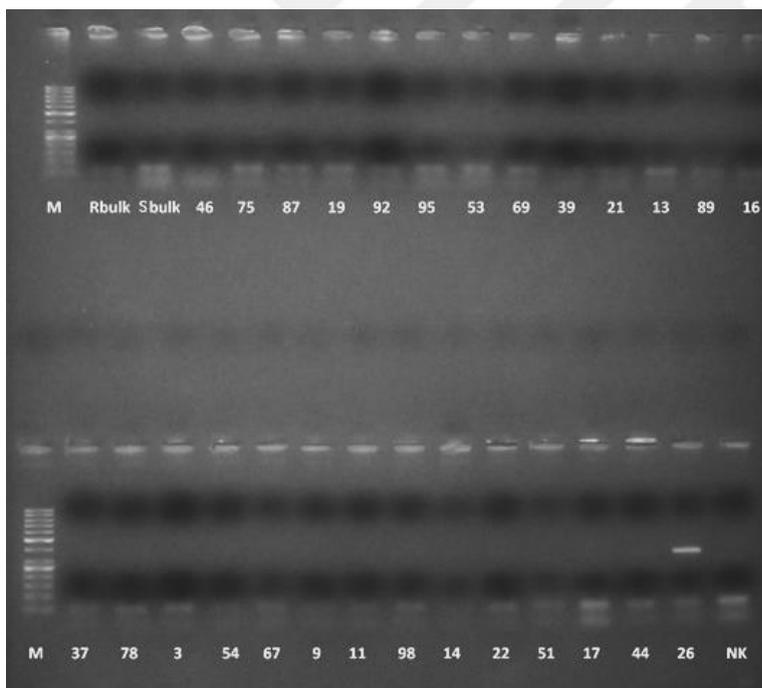
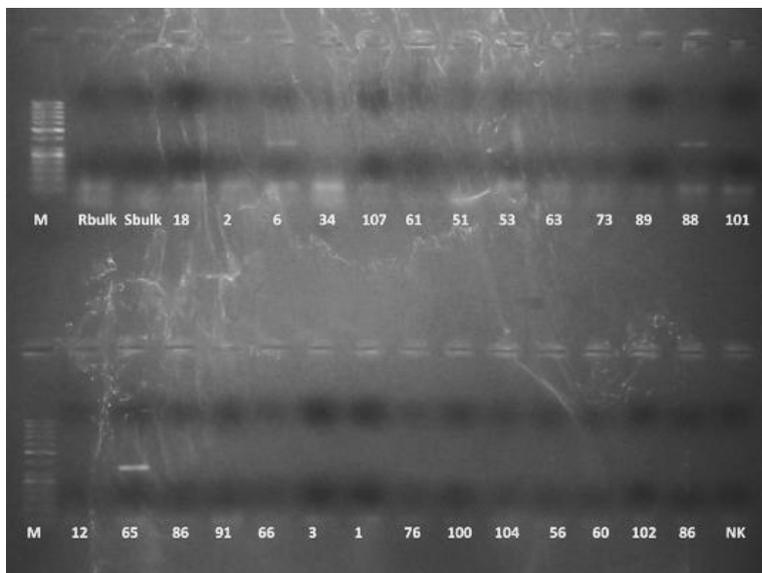


Figure 40. These results show the PCR products of resistant and susceptible bulks and individuals from bulk pools with ORS 630 primer in 2% Agarose Gel. M: Marker,

GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.



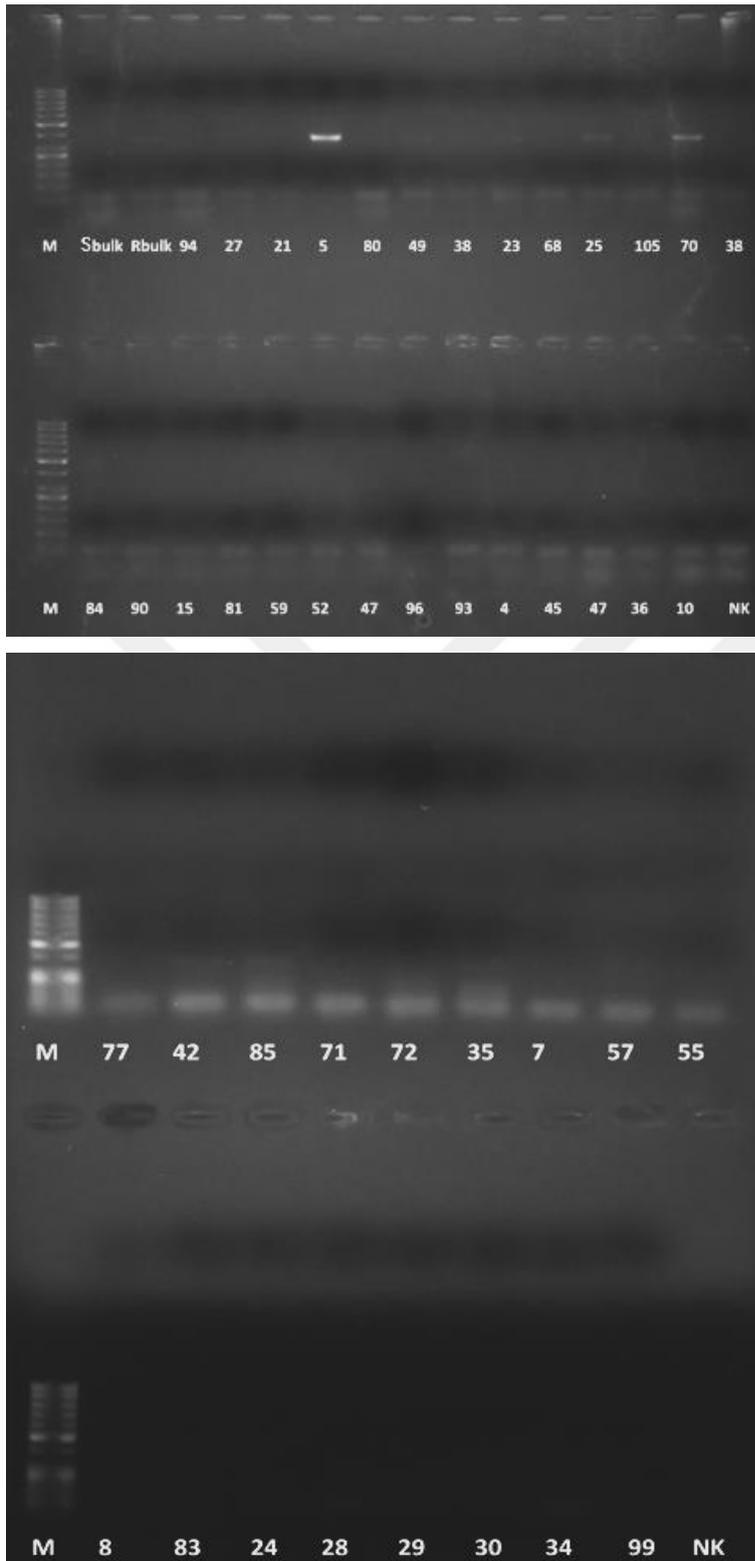


Figure 41. These results show the PCR products of resistant and susceptible bulks and F₂ individuals (SUN59) formed by crossing IMI044B x H458B genotypes with ORS 630 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.

4.3.14. ORS 317

ORS 317 is a SSR primer that was mapped to LG4 as being associated with PI genes, was 379 bp. ORS 317 has been tested on resistant and susceptible bulks and polymorphism detected between them (Figure 42). Resistant bulk gave 450 bp band length. Resistant and susceptible parental genotypes that form bulk pools were also tested in PCR analysis individually, as shown in Figure 42. According to agarose gel electrophoresis, band profile differences were detected between resistant and susceptible parental individuals which forms bulk tubes. ORS 317 was also tested on hybrid F₂ individuals (Figure 43).

Table 20

ORS 317 Reverse and Forward Primers

Primer	Reverse	Forward
ORS 317	GGTCGTATGCTTAATTCTTTCTCT	TTTGGCAGTTTGGTGGCTTA

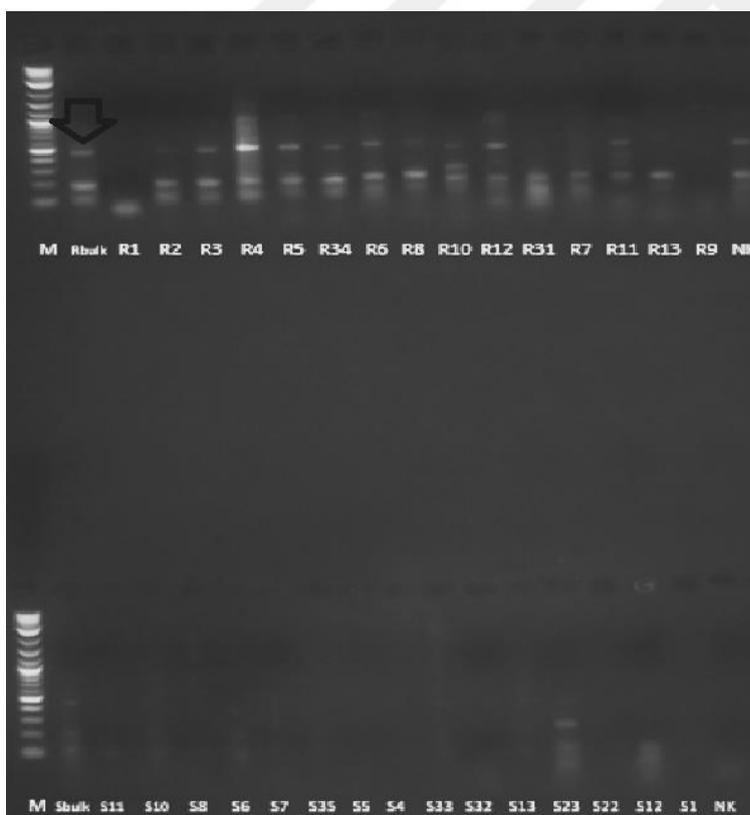
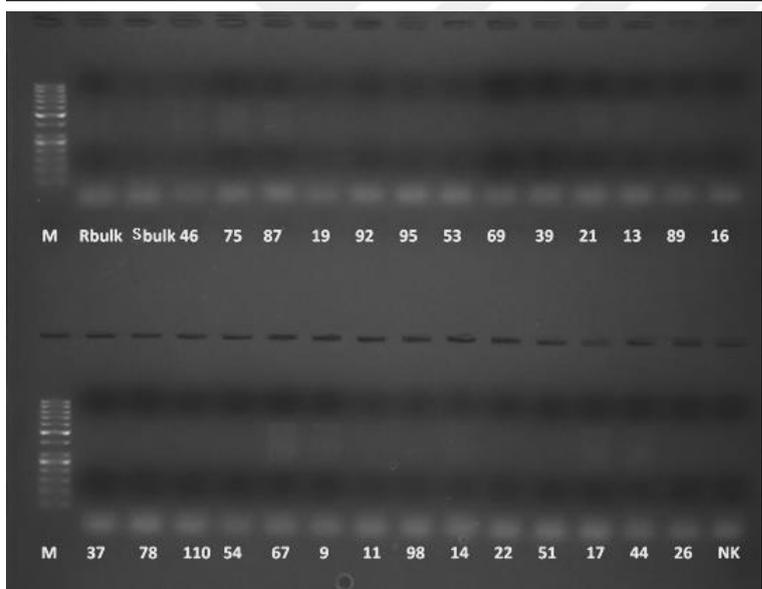
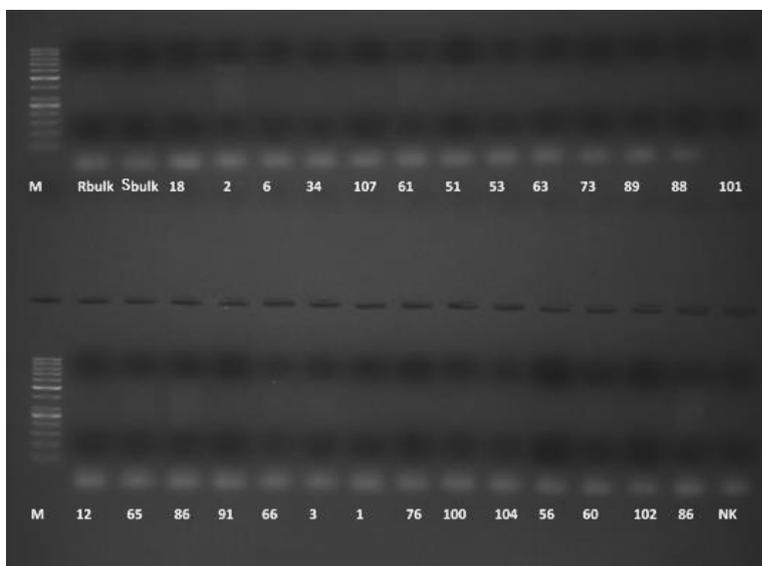


Figure 42. These results show the PCR products of resistant and susceptible bulks and individuals from bulk pools with ORS 317 primer in 2% Agarose Gel. M: Marker,

GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control



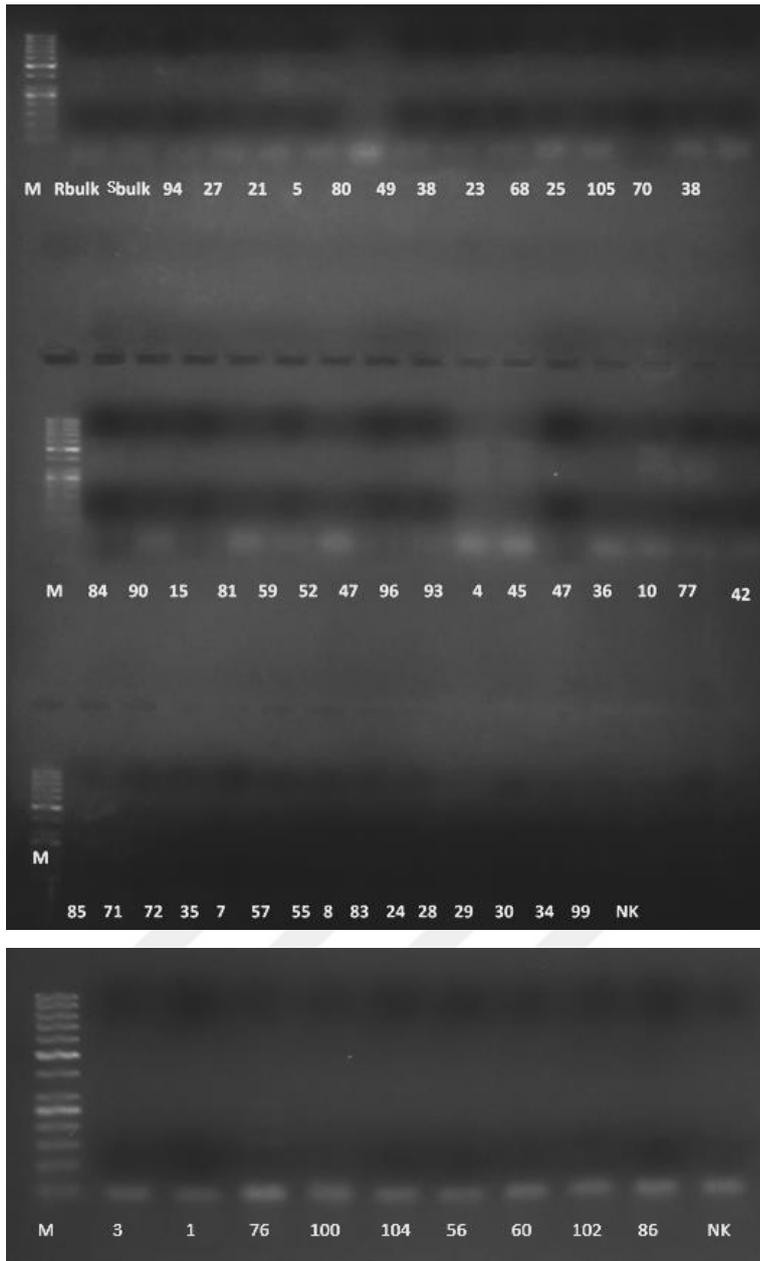


Figure 43. These results show the PCR products of resistant and susceptible bulks and F₂ individuals (SUN59) formed by crossing IMI044B x H458B genotypes with ORS 317 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.

4.3.15. ORS 511

ORS 511 is a SSR primer that was mapped to LG8 as being associated with Pl genes, was 577 bp. ORS 511 has been tested on resistant and susceptible bulks and polymorphism

detected between them (Figure 44). Resistant and susceptible parental genotypes that form bulk pools were also tested in PCR analysis individually, as shown in Figure 44. According to agarose gel electrophoresis, band profile differences were detected between resistant and susceptible parental individuals which forms bulk tubes. ORS 511 was also tested on hybrid F₂ individuals (Figure 45).

Table 21
ORS 511 Reverse and Forward Primers

Primer	Reverse	Forward
ORS 511	CGGGTTGCGAGTAACAGGTA	TGGCTCAGATTAAGTTCACACAG

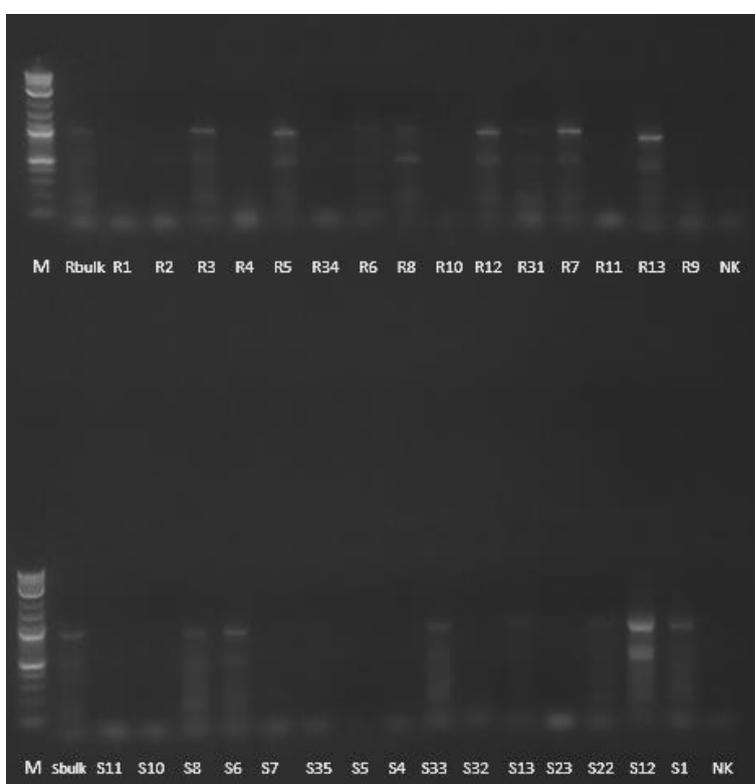


Figure 44. These results show the PCR products of resistant and susceptible bulks and individuals from bulk pools with ORS 511 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.

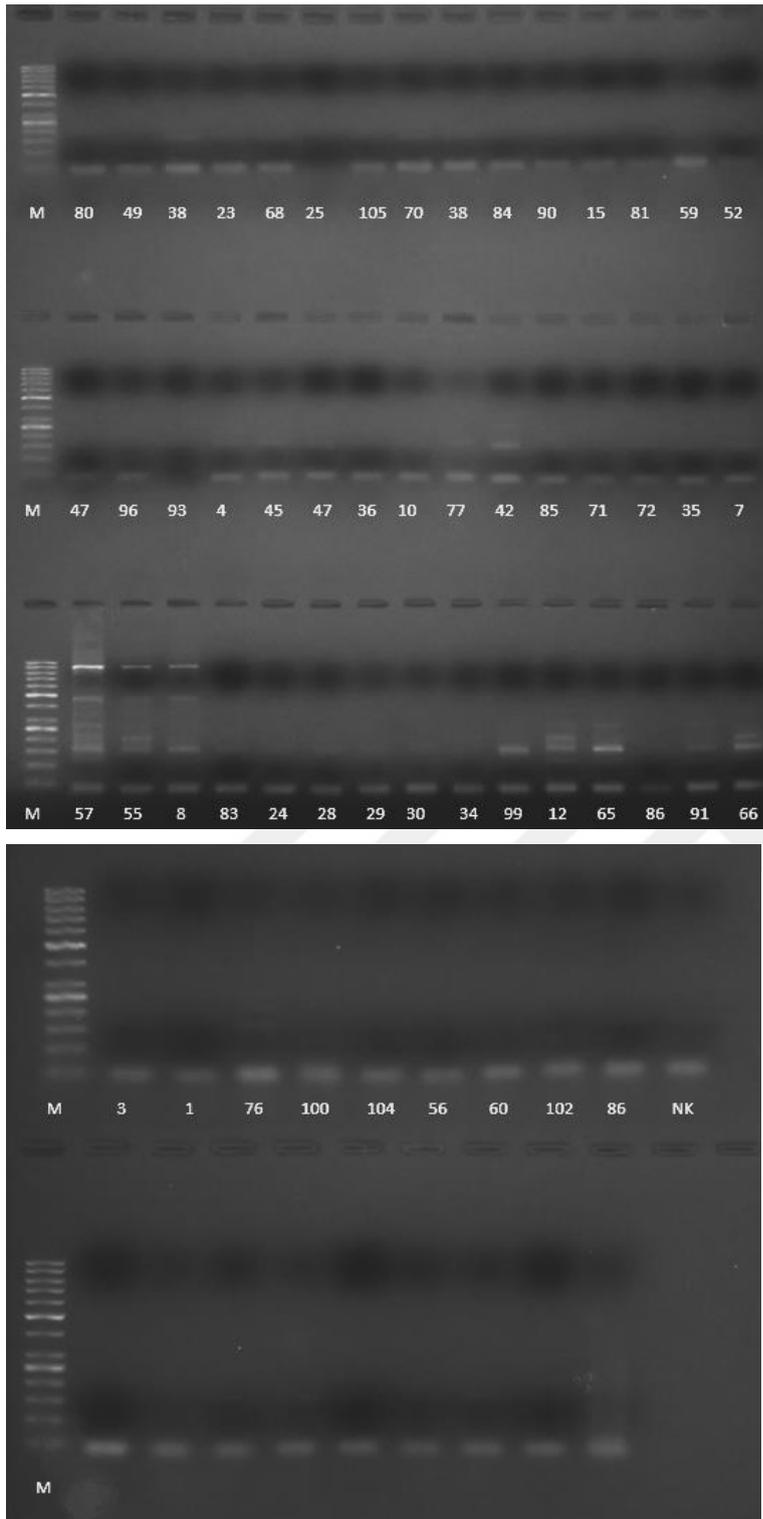


Figure 45. These results show the PCR products of resistant and susceptible bulks and F₂ individuals (SUN59) formed by crossing IMI044B x H458B genotypes with ORS 511 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.

4.3.16. ORS 1092

ORS 1092 is a SSR primer that was mapped to LG8 as being associated with PI genes, was 440 bp. ORS 1092 has been tested on resistant and susceptible bulks and polymorphism detected between them (Figure 46). Resistant bulk gave 250 bp band length. Resistant and susceptible parental genotypes that form bulk pools were also tested in PCR analysis individually, as shown in Figure 46. According to agarose gel electrophoresis, band profile differences were detected between resistant and susceptible parental individuals which forms bulk tubes. ORS 1092 was also tested on hybrid F₂ individuals and a similar band profile as the resistant parents forming resistant bulk was observed in 28 out of 100 samples (Figure 47).

Table 22

ORS 1092 Reverse and Forward Primers

Primer	Reverse	Forward
ORS 1092	GAGAACGGTAAACAGTGAGAAAGG	CCACGTCAGCATACCCAAATACT

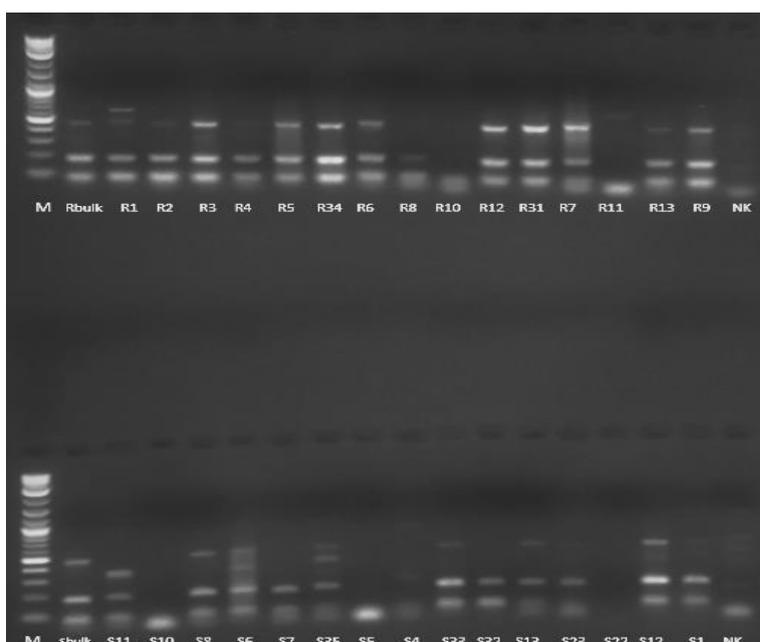


Figure 46. These results show the PCR products of resistant and susceptible bulks and individuals from bulk pools with ORS 1092 primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control

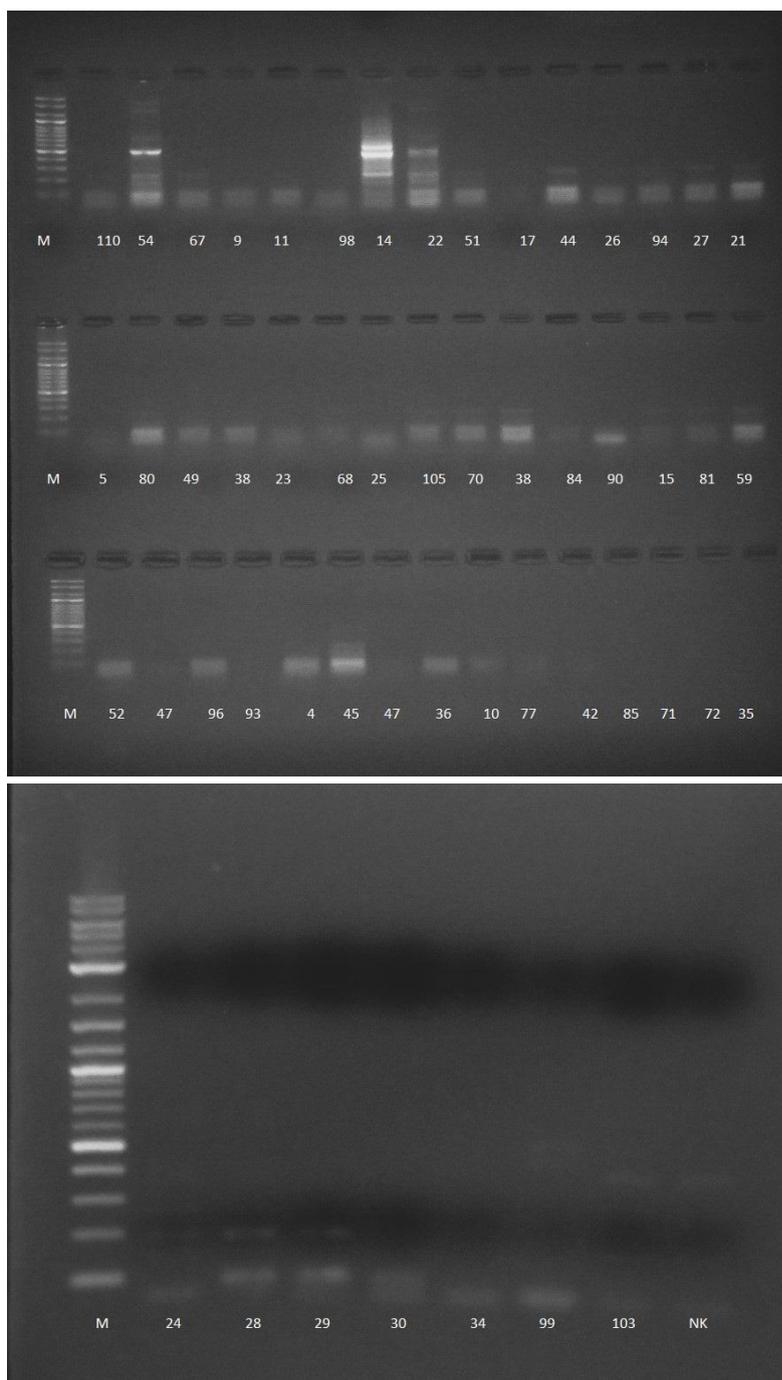


Figure 47. These results show the PCR products of resistant and susceptible bulks and F₂ individuals (SUN59) formed by crossing IMI044B x H458B genotypes with ORS 1092

primer in 2% Agarose Gel. M: Marker, GeneRuler 1Kb DNA ladder, R: resistant genotypes, S: susceptible genotypes, NK: negative control.



CHAPTER 5

RESULTS AND RECOMMENDATIONS

5.1. Results

Resistance genes in sunflowers are of great importance in terms of plant breeding and preventing yield loss. One of the most important factors limiting the yield of sunflowers is the downy mildew disease caused by the oomycete pathogen called *P. halstedii*. In combating downy mildew in sunflowers, alternative approaches to biological control prove inadequate and significantly restrict yield production. The best method of combating the disease is the development of disease-resistant plants and their use in breeding programs. Breeding research utilizing various resistance genes identified in wild sunflower species and subsequently detected through molecular marker-assisted selection is expected to yield the most successful outcomes in disease control.

Within the scope of this study, DNA concentration and quality were assessed using a Nanodrop spectrophotometer and 1% agarose gel electrophoresis. SSR molecular markers, which are mapped closest to the resistance genes in the linkage groups revealed in previous studies, were first tested in resistant and susceptible bulk pools, and 16 of the 42 SSR primers detected polymorphism. Band profiles differed between resistant and susceptible bulks. Upon examining the band profiles resulting from PCR analyses, some primer groups revealed an extra band in the resistant sample that was absent in the sensitive sample, suggesting a potential marker for disease resistance. Conversely, certain primer combinations displayed a distinctive band in the sensitive individuals. Sixteen SSR molecular markers demonstrating polymorphism revealed distinctive band profiles in 14 sensitive and 14 resistant parental individuals, mirroring those previously observed in bulk pools. Based on the results from agarose gel electrophoresis, we successfully identified the characteristic band profiles of both resistant and sensitive individuals. One hundred distinct F₂ individuals previously isolated from the F₂ generation (SUN59) were tested with SSR molecular markers exhibiting polymorphism. It was observed that the downy mildew resistance gene was inherited in some F₂ individuals. Based on the 2% agarose gel electrophoresis results, a band profile indicative of resistance was observed in numerous F₂ individuals.

In this research, rather than developing new molecular markers closely associated with the Pl genes in sunflower, we conducted a screening of commercially significant sunflower genotypes in Turkey. To achieve this, we utilized existing molecular markers previously identified as being closely linked to the Pl genes for our screening efforts. However, the usage of SSR primers is not limited. Duca et al. (2013) evaluated ten microsatellite markers to determine the genetic distances among ten parental sunflower genotypes. Their analysis revealed that the SSR primers ORS78, ORS237, ORS349, ORS366, ORS509, ORS811, and ORS836 produced polymorphic profiles. These markers were subsequently recommended for use in sunflower fingerprinting. Liu et al., 2012, mapped Pl₁₆ in a sunflower downy mildew differential line, HA-R4 of the LG1. For this purpose, similar to this thesis, 246 SSR primers screened between the resistant and susceptible bulks, and six primers produced weak bands or no product. Seven primers showed polymorphisms three from LG 1 (ORS53, ORS552, and ORS837), two from LG 10 (ORS908 and ORS1008), one from LG 8 (ORS243), and one from LG 15 (ORS344). Similarly, in this thesis, sixteen primers showed polymorphisms four from LG1(ORS 552, ORS 1008, ORS 662, ORS 965), five from LG13(ORS 995, ORS 511, ORS 728, ORS 1092, ORS 966), three from LG2(ORS 1197, ORS 203, ORS 1065), two from LG8(ORS 166, ORS 630) and two from LG4(ORS 317, ORS 191). ORS 1008 is located at the LG1 group of the Western Sunflower *H. anomalus*, Desert Sunflower *H. deserticola* (Lai et al. 2005) and also found assigned to LG8(Tang et al. 2003). The ORS1008 marker was previously reported as the closest marker linked to the Pl₁₃ gene at a distance of 0.9 cM on LG1 (Liu et al.2012). In this study, the ORS 1008 marker was shown on the LG1 SSR map.

Ahmed et al. (2021) conducted a study on the use of SSR markers to map resistance genes against downy mildew in sunflowers and in 2022 they used SSR markers to check the genetic purity of 23 parents and their 60 F₂ generation in sunflowers. 92 of the 110 markers showed polymorphism. Liu et al. (2019) explored the genetic diversity and structure of sunflower populations using SSR markers to understand the distribution of downy mildew resistance. They analyzed a large collection of sunflower accessions from different geographic regions, identifying specific SSR markers associated with resistance to various races of *Plasmopara halstedii*.

As a result of BSA screening with ORS 552 SSR primer that was mapped to LG1, gave 270 bp in length with the resistant bulk and individuals forming bulk, H458B and

IMI044B genotypes were observed. In the SUN59 genotype, a single band of 270 bp in length was observed in 62 individuals out of 100. H458B, and SUN59 genotypes were given the same bands. In the screening of F₂ individuals that were created using the IMI044B and H458B genotypes, only a single band with a length of 270 bp differed between bulk pools. Although the ORS 522 SSR primer was polymorphic for the genotype H458B, the F₂ generation result was not totally selective. For this reason, further studies will require the ORS 522 SSR primer to be used in future studies. This study collectively demonstrates that SSR markers facilitate the identification of resistance genes but also enhance the efficiency and precision of breeding programs. The integration of SSR markers into sunflower breeding strategies holds great promise for developing resistant cultivars and ensuring sustainable disease management practices.

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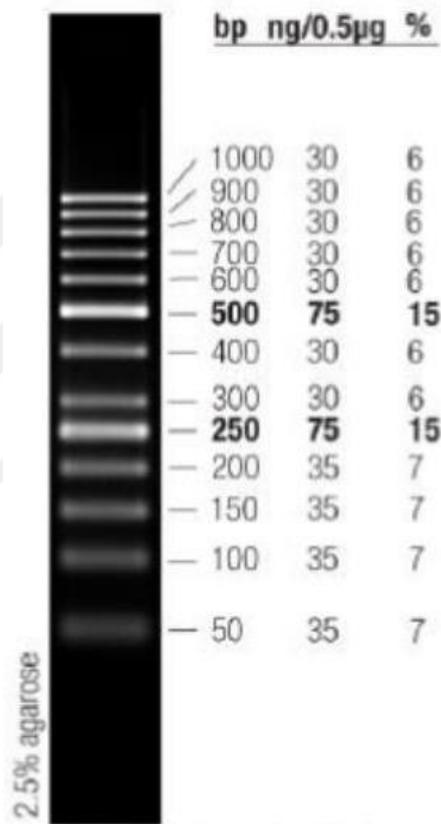
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APPENDICES

APPENDIX 1

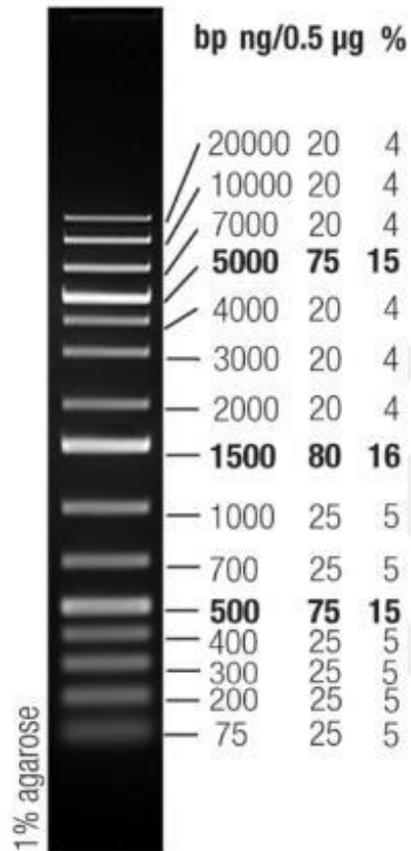
GENERULER 50 BP DNA LADDER



0.5 µg/lane, 8 cm length gel,
1X TBE, 5 V/cm, 1 h

APPENDIX 2

GENERULER 1 KB DNA LADDER



0.5 µg/lane, 8 cm length gel,
1X TAE, 7 V/cm, 45 min

