T.C.

CANAKKALE ONSEKIZ MART UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF BIOMOLECULAR SCIENCES

MOLECULAR EVALUATION OF SUMMER SQUASH (*Cucurbita pepo* L.) GERMPLASM FOR ZUCCHINI YELLOW MOSAIC VIRUS (ZYMV) RESISTANCE

MASTER OF SCIENCE THESIS

GİZEM GİRGİN

Thesis supervisor

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LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ

Gizem GİRGİN tarafından Doç. Dr. Özge KARAKAŞ METİN yönetiminde hazırlanan ve **22/11/2022** tarihinde aşağıdaki jüri karşısında sunulan "**Yazlık Kabak (*Cucurbita pepo* L.) Germplazmının Kabak Sarı Mozaik Virüs (ZYMV) Dayanıklılığı Açısından Moleküler Olarak Değerlendirilmesi**" başlıklı çalışma, Çanakkale Onsekiz Mart Üniversitesi Lisansüstü Eğitim Enstitüsü **Biyomoleküler Bilimler Anabilim Dalı**'nda **YÜKSEK LİSANS TEZİ** olarak oy birliği ile kabul edilmiştir.

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ETHICAL STATEMENT

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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

Gizem GİRGİN
22/11/2022

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Gizem GİRGİN

Canakkale, November 2022

ÖZET

YAZLIK KABAK (*Cucurbita pepo* L.) GERMPLAZMININ KABAK SARI MOZAİK VİRÜSÜ (ZYMV) DAYANIKLILIĞI AÇISINDAN MOLEKÜLER OLARAK DEĞERLENDİRİLMESİ

Gizem GİRGİN
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Biyomoleküler Bilimler Anabilim Dalı Yüksek Lisans Tezi
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Yaz Kabağı (*Cucurbita pepo* L.) kolay yetişebilen bir çok açıdan özellikle ticari olarak değerli bir bitkidir. Fakat kabağı enfekte eden Kabak Sarı Mozaik Virüsü (ZYMV) sebebiyle önemli derecede ürün kaybı yaşanmaktadır. Çünkü virüs bulaşan kabak da çeşitli semptomlar gözlenir bunlardan biri bitkinin bodur kalması, meyvesinde ve yapraklarında dengeli olmayan yüzey rengi, yumrulu bölgeler ortaya çıkar. Öyleki bu ürün kaybı %95 kadar ulaşılabilir. (ZYMV) Kabak Sarı Mozaik Virüsü, yaprak biti vektörü sayesinde bitkiye taşınır. Yaprak biti vektörlerini denetim altına almak için uygulanan kimyasallar enfeksiyonu engellemekte yetersizdir. Virüsü kontrol edebilmek için seçilen diğer yöntem klasik ıslah. Bu yötemle virüse karşı dayanıklı bitkilerin elit genotiplerini belirlenir, fakat klasik ıslah yöntemi zaman isteyen bir yöntemdir. Klasik ıslah'ın yerini (MAS) markırlar yardımıyla seleksiyon almıştır bunun sebebi tanımlanması gereken karakterlerin ilişkisi olan genleri rahatça ayırt edebilen moleküler markırların kullanılmasına dayanan önemli yöntemdir. Bu çalışma çerçevesinde polimeraz zincir reaksiyona dayalı SSR basit dizi tekrarları markırları önemli özelliklerinden dolayı öncelik tanınmıştır. SSR belirteçleri, maliyet, hız en önemlisi güvenirlik gibi üstünlüklere sahiptir. Bu çalışmada 20 bağlantı grubunun içinden 60 SSR belirteci (Gong et al., 2008) seçilmiştir. Seçilen belirteçler hastalığa karşı dayanıklı bireylerin, hassas bireylerden ayırmak için olanak sağlayacaktır. Çalışmada Trakya Tarımsal Araştırma Enstitüsünde *Cucurbita pepo* L. genotipine sahip anaç bireyler ve dayanıklı hatlarla çaprazlanan F_2 bireyleri elde edilmiştir. Seçilen SSR kullanılarak elimizdeki bireyler ZYMV dayanıklılık açısından genotiplerindeki

polimorfizmler değerlendirildi. Polimorfik belirteçler CMTp131, CMTp142, CMTm66, CMTp190 olarak tespit edildi. Bu belirteçlerin, Kabak Sarı Mozaik Virüsü (ZYMV) direnci sağlayan genlerle ilişkili olabileceği belirlendi. Elde ettiğimiz verilerle belirlediğimiz yazlık kabak (*Cucurbita pepo* L.) genotipleri potansiyel olarak ıslah programları için kullanabilirlik göstermektedir.

Anahtar Kelimeler: *Cucurbita pepo* L., ZYMV, SSR

ABSTRACT

MOLECULAR EVALUATION OF SUMMER SQUASH (*Cucurbita pepo* L.) GERMPLASM FOR ZUCCHINI YELLOW MOSAIC VIRUS (ZYMV) RESISTANCE

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Çanakkale Onsekiz Mart University

School of Graduate Studies

Master of Science Thesis in Biomolecular Sciences

Advisor: Assoc. Prof. Özge KARAKAŞ METİN

22/11/2022, 49

Summer Squash (*Cucurbita pepo* L.) is an easily grown commercially valuable plant in many respects. However, due to the Zucchini Yellow Mosaic Virus, which infects the pumpkin, there is a significant loss of product. Because various symptoms are observed in the infected zucchini, one of which is the stunting of the plant, unbalanced surface color on the fruit and leaves, and tuberous areas. So that this product loss can be reached up to 95%. ZYMV is carried to the plant by the aphid vector. Chemicals applied to check aphid vectors are insufficient to prevent infection. The other method chosen to control the virus is classical breeding. Therefore this method, elite genotypes of virus-resistant plants are determined, but the classical breeding method is time-consuming. Classical breeding (MAS) has been replaced by selection with the help of markers because it is a crucial method based on the application of molecular markers that can easily distinguish the genes related to the characters to be defined. In this study, SSR simple sequence repeat markers based on polymerase chain reaction were given priority because of their important properties. SSR markers have advantages such as cost, speed, and most importantly, reliability. In this study, 60 SSR markers (Gong et al., 2008) were selected from 20 linkage groups. Selected markers will allow distinguish resistant individuals from susceptible individuals. In the frame of this proposal parental individuals with *Cucurbita pepo* L. genotype and F_2 individuals crossed with resistant lines were obtained in Thrace Agricultural Research Institute. By using the selected SSR, polymorphisms in the genotypes of the individuals were evaluated in terms of ZYMV resistance. Polymorphic markers were detected as CMTp131, CMTp142, CMTm66, and CMTp190. It was determined that these markers may be related to the genes that provide

the ZYMV resistance genotype. *Cucurbita pepo* L. genotypes that are determined with the data we have obtained can potentially be used for breeding programs.

Keywords: *Cucurbita pepo* L. ZYMV, SSR

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SYMBOLS AND ABBREVIATIONS

℃	Degrees Celsius
%	Percent
µl	Microliter
µg	Microgram
AGE	Agarose gel electrophoresis
AFLP	Amplified Fragment Length Polymorphism
CP	Coat protein
CI	Cylindrical inclusion protein
CTAB	Hexadecltrimethylammonium bromide
DNA	Deoxyribonucleic acid
FAO	Food And Agriculture
g	Gram
HC-Pro	Helper component protein
ISSR	Inter Simple Sequence Repeat
RAPD	Random Amplified Polymorphic DNA
RNA	Ribonucleic acid
NaCl	Sodium chloride
PBT	Polybutylene terephthalate
SNP	Single nucleotide polymorphism
SSLP	Simple Sequence Length Polymorphism
SSR	Simple Sequence Repeat

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CHAPTER 1
INTRODUCTION

1.1. Introduction

In our country, *C. pepo* (squash) is one of the most easily grown vegetables that contain high nutritional and medicinal values. In addition to its use in the food industry, it is one of the vegetables that have an important added value. Food production is becoming more difficult every day, in parallel with the population growth in the world. It is known that there is a decrease in the amount of vegetable and animal products per person due to factors such as diseases caused by viruses, climatic changes, inefficient use of the soil, and natural disasters, especially in limited planting areas. Plants are consumed more due to the low price and low production of animal products.

Figure 1. *Cucurbita pepo* L. (Kennedy, 2020).

Vegetables from herbal products support animal food consumption with their protein-rich properties. Moreover, the seeds of *Cucurbita pepo* are the most preferred dried nuts in our country and consumes all year round. Its seeds are rich in protein and fat (Yanmaz, 2015). *Cucurbita pepo* seeds contain vitamins, minerals, and antioxidants that the human body needs. Also, *C. pepo* production in 2017 in the world was 27 million tons, while in Turkey It reached 393.795 tons (FAO, 2017).

ZYMV contains significantly harmful viruses (Blua and Perring, 1989). ZYMV symptoms appear in both the leaf and fruit of summer squashes symptoms include severe mosaic, deformation, blistered, and distinctive yellow mottling of leaves. Particularly fruit contains severe symptoms such as knobbly areas, unequal surface colouring, and Infected plants that are stunted (reduced size of fruit) that causes yield losses of up to 95% (Desbiez and Lecoq, 1997). As far as is known chemical and insecticide usage is ineffective against ZYMV (Nameth et al., 1986).

Cucurbita pepo L. contains elite germplasms tolerant to the Yellow Mosaic Virus. For the selection of the summer squash resistant to ZYMV via breeding is the most effective method (Whitaker & Robinson, 1986; Paris, 2008). This method increases efficiency and reliability by using molecular markers compared to traditional breeding (Francia et al., 2005). A molecular marker is used to determine the parents to be used in plant breeding and to obtain information about the genome structure. In the frame of this thesis, we used SSR markers. Gong determined 20 linkage groups. 40-60 SSRs primers which cover 20 linkage groups were used to investigate ZYMV resistance parental genotypes. Because SSRs are the most useful molecular markers in plant biology so in the same population high levels of polymorphism can be easily observed (Vieira et al., 2016).

1.2. Taxonomy And Cultivation

Table 1

Taxonomic hierarchy of cultivated summer squash

Kingdom:	Plantae
Subkingdom:	Viridiplantae
Infrankingdom:	Streptophyta
Superdivision:	Embryophyta
Division:	Tracheophyta
Subdivision:	Spermatophytina
Class:	Magnoliopsida
Superorder:	Rosanae
Order:	Cucurbitales
Family:	Cucurbitaceae
Genus:	*Cucurbita* L.
Species:	*Cucurbita pepo* L.

Cucurbita L. has 20 pairs of chromosomes and includes important 13 species. The diverse genus *Cucurbita pepo* was domesticated for years (Decker, 1988). As known in Oaxaca, Mexico, there is a cultivated zucchini dating back about 10,000 years. *C. pepo* has a polymorphic variant in the plant kingdom (Zraidi et al., 2007). *C. pepo* genomic variants related to horticulturally and morphology traits that include fruit shapes such as marrow, cocozelle, scallop, crookneck, straight neck, and zucchini.

Five domesticated species and 22 wild species are found within the genus and are native to the Western hemisphere. Summer squash and variants are currently grown in a range of cool temperate to tropical climates.

The Cucurbitaceae family is one of the most consumed vegetable groups in the world such as pumpkin, melon, watermelon, cucumber, honey melon, and summer squash. Anatolian and Caucasian countries, which are large in number of Turks, contain most of the genetic characteristics of vegetables grown and consumed. Even some of the cucurbits gene is known to be the center of Turkey.

1.3. *Cucurbita pepo* Contents

Minerals in *C. pepo* seeds; magnesium (0.1%), potassium (0.03%), calcium (0.02%), and phosphorus (0.01%), vitamins; plenty of vitamins A, B, C, and K. Fat ratios are approximately 1% in fruit and 35-40% in seed (Günay, 2005). Thanks to these nutrients and high fiber in *C. pepo* seeds, it is known to be effective in preventing constipation, diabetes, and cancer (Ermiş, 2010). All these diseases are very famous in our country and cause a rising death rate. Additionally, the seed of the pumpkin contains piperazine substance that helps to reduce stomach discomfort, kidney stones, and tapeworm living in the intestine (Günay, 2005).

1.4. FAO

Summer squash grows easily and immediately. After planting, fruits are collected for about 50 days. Also, zucchini in our country is 107.8% (TurkStat, "Vegetables", 2017-2018 report) given percentage to meet the domestic consumption of domestic production is sufficient and *C. pepo* production in 2017 in the world 27 million tons, while Turkey reached 393.795 tons (FAO, 2017). Therefore, *C. pepo* L. has added value for our country, although most of the produce is consumed in the country, it has a small amount of exports.

Although *Cucurbita pepo* L. is the homeland of America that is grown mostly in our country, especially in the Thrace, Konya, and Nevşehir regions. Pumpkin is a vegetable consumed in our country as summer squash and in the winter as a pumpkin. It is a product with added value as well as the fruit of the pumpkin. For example, pumpkin seeds are widely used, such as nuts, and animal feed, due to their high nutritional value. Therefore, the total annual amount of production varies between 400 and 500 thousand tons, but 400 thousand

tons of production consists of summer squash (*Cucurbita pepo* L.). Some of them are even exported.

1.5. ZYMV Physiological and Cytological Characteristics

Cucurbita pepo L., among Cucurbitaceae, is a plant that is produced and consumed in our country. However, some abiotic (climate, temperature, light, soil, water, mineral, pH) and biotic (bacteria, virus) factors cause diseases during its production. The most important of these diseases are about 200 viral diseases caused by viruses. Due to these diseases, significant yield and product losses occur in zucchini (Zitter, 1996; Yılmaz & Çığşar, 2006).

Figure 2. Infected *Cucurbita pepo* L. leaf by ZYMV (Nelson, 2015).

Some of the viruses damage and prevent the growth of the Cucurbita which results in the abnormal formation of stunted fruits. Some different virus species infect the Cucurbitaceae family (Lisa and Lecoq, 1984; Purcifull, 1984).

Potyviridae contain a single-stranded RNA of Zucchini Yellow Mosaic Virus that is a widely harmful pathogen for plant areas. This virus naturally transmits to associate with the Cucurbitaceae (Desbiez and Lecoq, 1997; Berger, 2001). Potyviridae includes approximately 30% of known plant viruses that is a widespread plant pathogens (Ward and Shukla, 1991). ZYMV, the *C. pepo*, was effective for the first time in Italy and was reported by Lisa (1981), but the ZYMV virus in Australia, France, Egypt, Germany, Israel, Japan, Jordan, and Spain was also affected. Zucchini yellow mosaic virus was found to be common in Morocco and Turkey as countries of 60% of the world production areas were found affected.

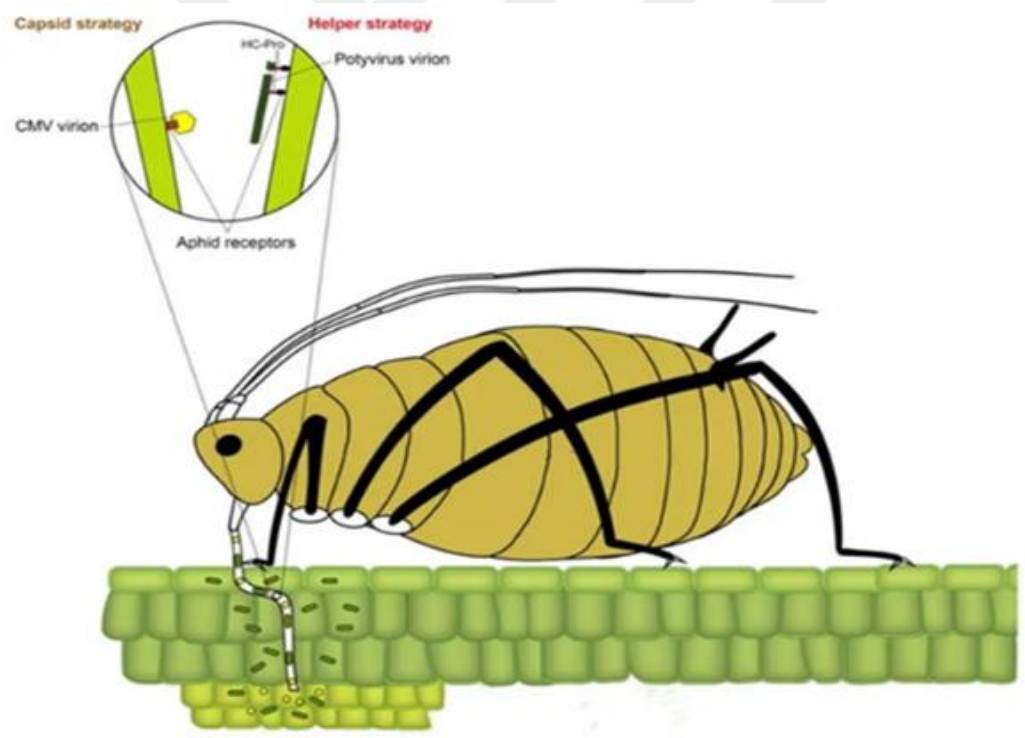


Figure 3. Schematic illustration of pathogen-host interaction by aphid vectors (Gadhave K. R., 2020).

ZYMV is carried mechanically and horizontally by diverse aphid vectors (Katis et al., 2006). The virus infects permanently. As known for viral movement these viruses have at least five proteins that are coat protein, cylindrical inclusion protein, helper component protein, and viral genome-linked protein. Particularly the coat protein assists far away spread and easily cell-to-cell movement by altering the path of plasmodesmata and linking to the viral RNA.

1.6. Marker Systems

Marker systems are significant for using to select phenotypes and genotypes of reliably inherited traits for all crops. In this way, they are identified and characterized them. This marker system is separated into three parts such as morphological, biochemical, and molecular markers (Figure 4).

1.6.1. Morphological Markers

Morphological markers are called 'visualizing markers' that are determined the phenotype of crop species. Moreover, with this marker, the difference between recessive and dominant characters can be readily determined, ich not identified heterozygous characters or homozygous dominant genes. Also, some environmental conditions can be changed by one locus that controls some characters.

1.6.2. Biochemical Markers

Biochemical markers have based on especially proteins, secondary metabolites, fatty acids, alkaloids, and isoenzymes. Biochemical markers are reliable and quick processes that also have repeatable results.

1.6.3. Molecular Markers

A great number of aphids carry Zucchini yellow mosaic viruses to impact plants as far as is known chemical and insecticides usage is ineffective against ZYMV (Nameth et al., 1986; GalOn, 2007). *Cucurbita pepo* L. contains elite germplasms tolerant to the Yellow Mosaic Virus. Producing summer squash resistant to ZYMV with the breeding method is the most effective method (Whitaker & Robinson, 1986; Paris, 2008). Compared to traditional breeding, molecular markers increase efficiency and reliability (Francia et al., 2005).

Molecular markers are defined and correlated genetic kinship (Lowe et al., 1996), determination of culture types, gene characterization, duplication, deletion, determination of mutated genotypes, and determination of gene source (Rafalski et al., 1996). It is used to determine the parents to be used in plant breeding and to obtain information about the genome structure.

Molecular markers provide for the studying and screening of plants. Although the result of plant genetic diversity is obtained rapidly and effectively (Plaschke et al., 1995). Several molecular marker techniques play a role in genetic diversity. Microsatellite markers in the other words SSRs (Simple Sequence Repeats) are powerful and significant DNA marker systems because they have significant properties highly polymorphic, reproducible, co-dominant, and chromosome specific in eukaryotic genomes. Moreover, all plant species' genomes are easily and reliably detected by using microsatellites (Huang et al., 2002; Zhang et al., 2010).

Improvement of economically valuable plants, and creation of plant varieties resistant to various stress conditions, and high yield; It is an important area that has been studied for ages. Moreover, labor-intensive also long-term breeding techniques, the development of molecular techniques has accelerated breeding studies and facilitated the acquisition of special varieties. Molecular marker techniques have been obtained as a result of long efforts. It gives much more reliable results besides the morphological and biochemical markers used in cases such as distinguishing and protecting the varieties grown, and detecting and choosing genotypes to be used in breeding studies. They are divided into three as morphological, biochemical, and molecular (Figure 4).

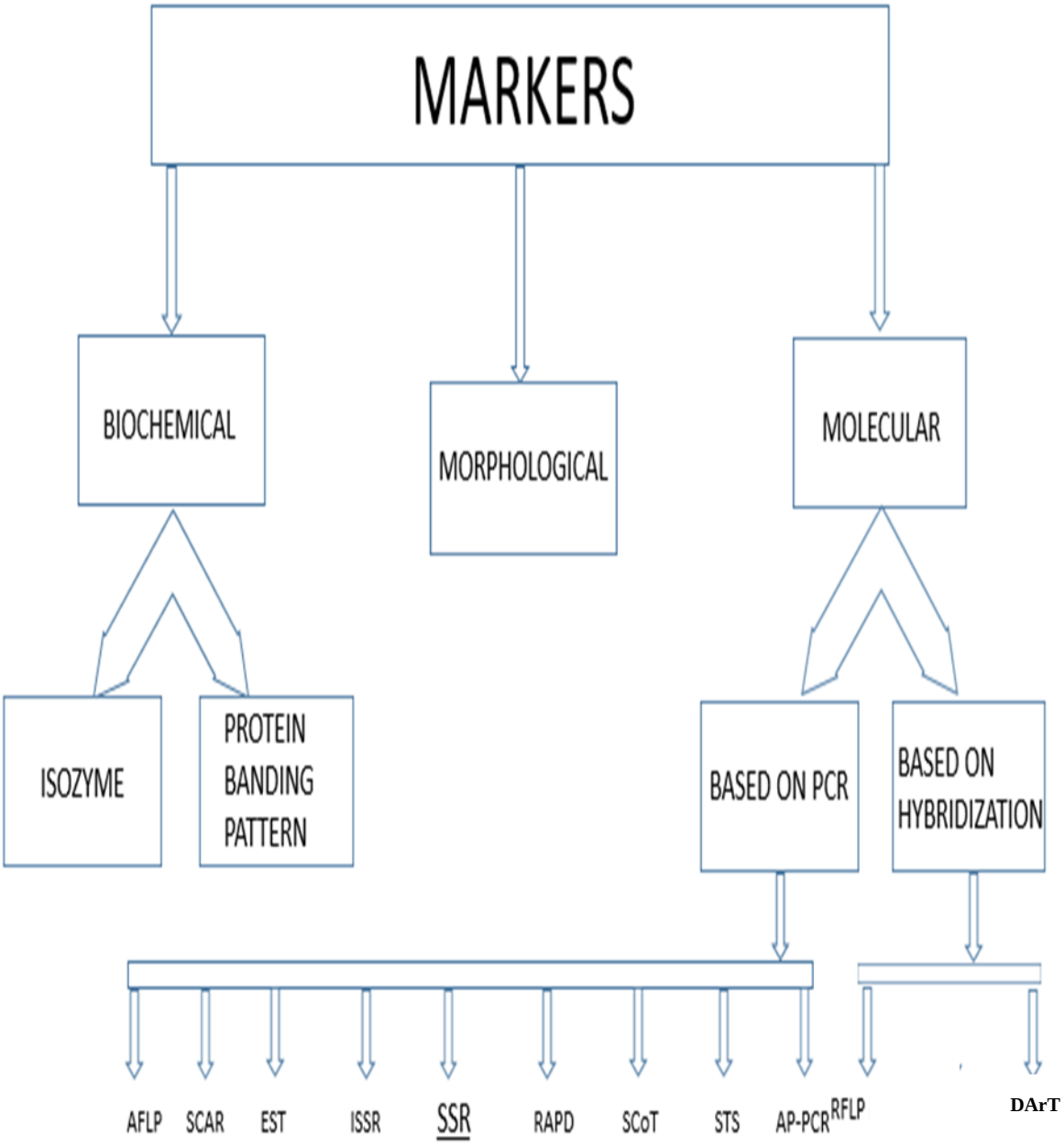


Figure 4. A brief summary of marker systems.

SSR

Microsatellites are short nucleotide motifs with tandem repeats that have one to six base pairs. SSR can affect gene expression and chromatin organization, DNA metabolic processes, and cell cycle (You et al., 2002) that lead to showed resistance or susceptible plants. SSRs are highly reproducible and polymorphic so SSR markers are defined as markers of selection in the genome of a plant. Therefore SSR markers have spread in molecular genetics and plant breeding (W. Powell et al., 1996; S. Ghosh et al., 2002).

Microsatellites have many variations such as SSR, STR, and SSLP. They are detected widely in prokaryotes and euchromatin of eukaryotes that are found in cell components.SSRs have different properties such as; codominant, multi-allele genetic markers highly informative, and easily transferable among related species therefore they have been widely used markers to investigate plants genotype for the last twenty years (Pérez-Jiménez et al., 2013; Mason, 2015; Phumichai et al., 2015). SSR markers have important functions that play role in breeding easily and genetic variation to link genotype and phenotype (Hayward et al., 2015).

SSR marker loci have some advantages that are produced with two primer PCR reactions but ISSRs and RAPDs assay with single primers. These primers are a forward and reverse direction for annealing DNA template thus both primers increase the specificity (Mei et al., 2015).

Large amounts of SSRs variations are found intensely in 5'-UTR regions in plants that operated gene expression. Because these SSRs affect firstly transcription and then the translation process. Moreover, intronic SSRs can play the role of inducing mRNA splicing and heterochromatin gene silencing (Li et al., 2004). Finally, all changes can cause phenotypic alteration (Nalavade et al., 2013**)** Figure 5 (Z Dang, 2020).

Marker	PCR-Based	Mode of inheritance	Locus specificity	Level of polymorphism	Reproducibility
RFLP	No	Co-dominant	Yes	Low-Medium	High
RAPD	Yes	Dominant	No	Medium-High	Low
SCAR	Yes	Co-dominant	Yes	High	High
AFLP	Yes	Dominant	No	High	Medium
SSR	Yes	Co-dominant	Yes	High	High
ISSR	Yes	Dominant	Yes	High	Medium
CpSSR	Yes	Co-dominant	Yes	Low	High
SAMPL	Yes	Co-dominant	Yes	High	High
SRAP	Yes	Dominant	No	Medium-High	Low
SSCP	Yes	Co-dominant	Yes	High	High
CAPS	Yes	Co-dominant	Yes	High	High
SNP	Yes	Co-dominant	Yes	Extremely High	High
DArT	No	Dominant	Yes	High	High
EST	Yes	Dominant	Yes	High	High

Figure 5. Compared to different types of molecular markers (Nadeem et al. 2018).

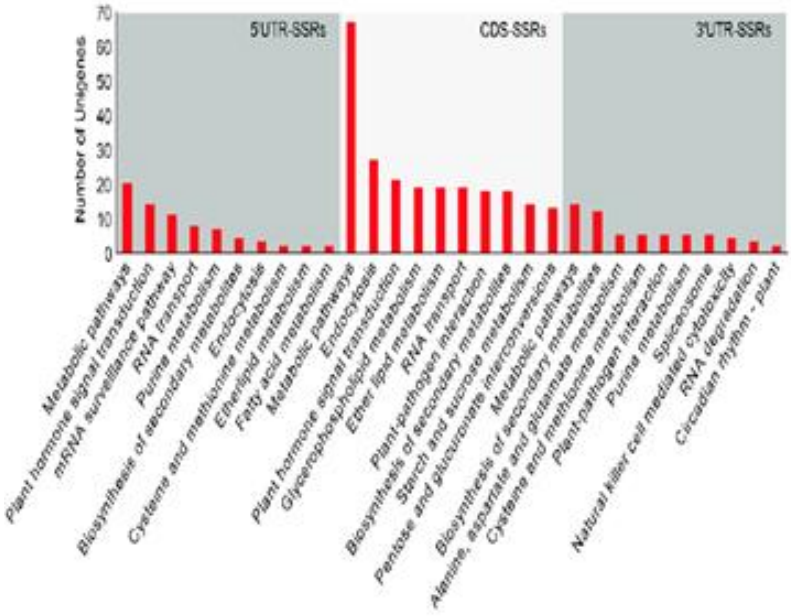


Figure 6. Functional classification of the SSR-containing sequences (Dang, 2020).

CHAPTER 2
PREVIOUS STUDIES

2.1.Previous studies on summer squash with ZYMV disease

Zucchini yellow mosaic virus leads to great harm to *Cucurbita pepo* L. crops (Lisa et al. 1981) primarily described (Lecoq et al. 1981; Lisa and Lecoq 1984; Desbiez and Lecoq 1997). Also, Single-stranded RNA potyvirus that spread on seed or fruits of *Cucurbita pepo* L. by many aphid species and migrating birds (Johansen et al., 2001, 2003; Gal-On, 2007; Eagles et al., 2013, 2014; Boyle et al., 2014; Firth et al., 2017).

Additionally, ZYMV can be carried widely among members of the family Cucurbitaceae (Pachner and Lelley, 2004). When Zucchini Yellow Mosaic Virus infects summer squash yellow mosaic, malformation symptoms are observed. Chemical application is ineffective in avoiding infection. (Nameth et al., 1986). Another strategy is breeding for producing resistance summer squash against ZYMV that is effective to control infection (Whitaker and Robinson, 1986; Paris, 2008). Compared to conventional breeding, applied molecular markers increase the efficiency of cross fertile breeding. Several molecular markers are more advantageous than classical breeding especially the reliability of detection resistance crops (Francia et al., 2005).

Molecular markers include different types of markers such as AFLP, RAPD, and SSR to link within *C. pepo* also these markers have several significant properties (Lee et al., 1995; Brown and Myers, 2002; Zraidi and Lelley, 2004, 2007). Moreover, the heredity of genes of ZYMV resistance were identified by using SNP markers (Capuozzo et al., 2017). Transferability traits of SSR between species within a genus are an important characteristic of Cucurbita SSR markers (Gong et al., 2008). They also updated the *C. pepo* map. Moreover, high-density SNP-based genetic maps are developed (Montero-Pau et al., 2017).

CHAPTER 3
MATERIAL METHOD

3.1 Plant Material

Firstly Twenty summer squash were cultured at Thrace Agricultural Institute. All rootstocks (Angellina, Regina, K10 Kabuklu, and Sulu Saray) were grown in the greenhouse by Thrace Agricultural Institute. Two lines of Angellina and Regina are resistant to ZYMV cultivars despite that two lines of K10 Kabuklu and Sulu Saray are zucchini yellow mosaic virus (ZYMV) susceptible cultivars.

Table 2

Lines of resistance or susceptible cultivars

Resistance to Zucchini Yellow Mozaic Virus	Varieties
Susceptible	Sulu Saray
Resistant	Angelina
Resistant	Regina
Susceptible	Kabuklu K-10

Figure 7. Steps of cultivation *Cucurbita pepo* L.

Figure 8. *Cucurbita pepo* L. sample for DNA isolation.

3.2 DNA Isolation

The genomic DNA isolation method was performed according to Doyle and Doyle's (1987) CTAB-based genomic DNA isolation protocol making some changes. All leaves of summer squash were taken for DNA isolation and transferred into liquid nitrogen. All tubes were labeled. Beams were put into labeled tubes (Angellina, Regina, K10 Kabuklu, and Sulu Saray) by tweezer. Leave samples were measured from 50 mg to 100 mg in the tube. Dried and small amounts of leaves sample in the tube were ground via Retsch Mixer Mill MM 400 which obtains to mix and homogenize powders for 1 min at 22.5 frequency. Homogenized samples were put at -80℃. Preparation solution was carried out in 600 µl CTAB and 8 mg PBT(PVP) was added into homogenized samples in each tube which was incubated for 25 min at 60℃. Beta-mercaptoethanol was added in a ratio of 0.2 % CTAB solution into each tube for 10 min at 60℃ and then all samples were kept at room temperature for 5 min. 24:1 Chloroform: octanol solution was added into each tube that centrifuged for 15 min at 13000 rpm. 500 µl supernatant was taken from tubes. Half of the final volume of 5M NaCl and a duplicate of the first volume of 95% ethanol were added to the supernatant. Samples were incubated at -80℃ for one hour. Samples were centrifuged for 10 min at 13000 rpm. Samples were washed with 1000 µl 75% ethanol. 100µl nuclease free water and 2 µl RNAase were added into tubes and which were incubated at 37℃ for 30 min.

Table 3

Chemicals used for DNA isolation

Chemical Agents	**Components**	**Concentrations of Solution**
CTAB Isolation Buffer	Tris-HCl	100 mM
	EDTA	20 mM
	CTAB	2%
	NaCl	1,4 M
Chloroform:Octanol	Chloroform Octanol	24:1
Sodium Chloride	NaCl	5M

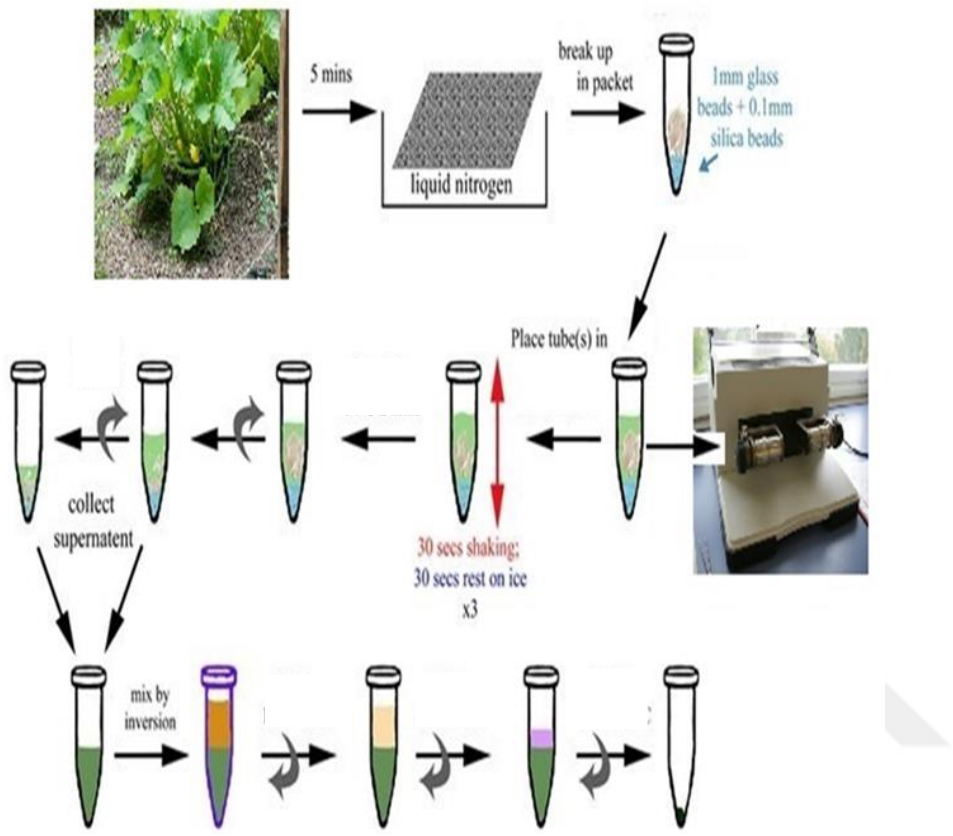


Figure 9. The first step of DNA isolation is homogenization.

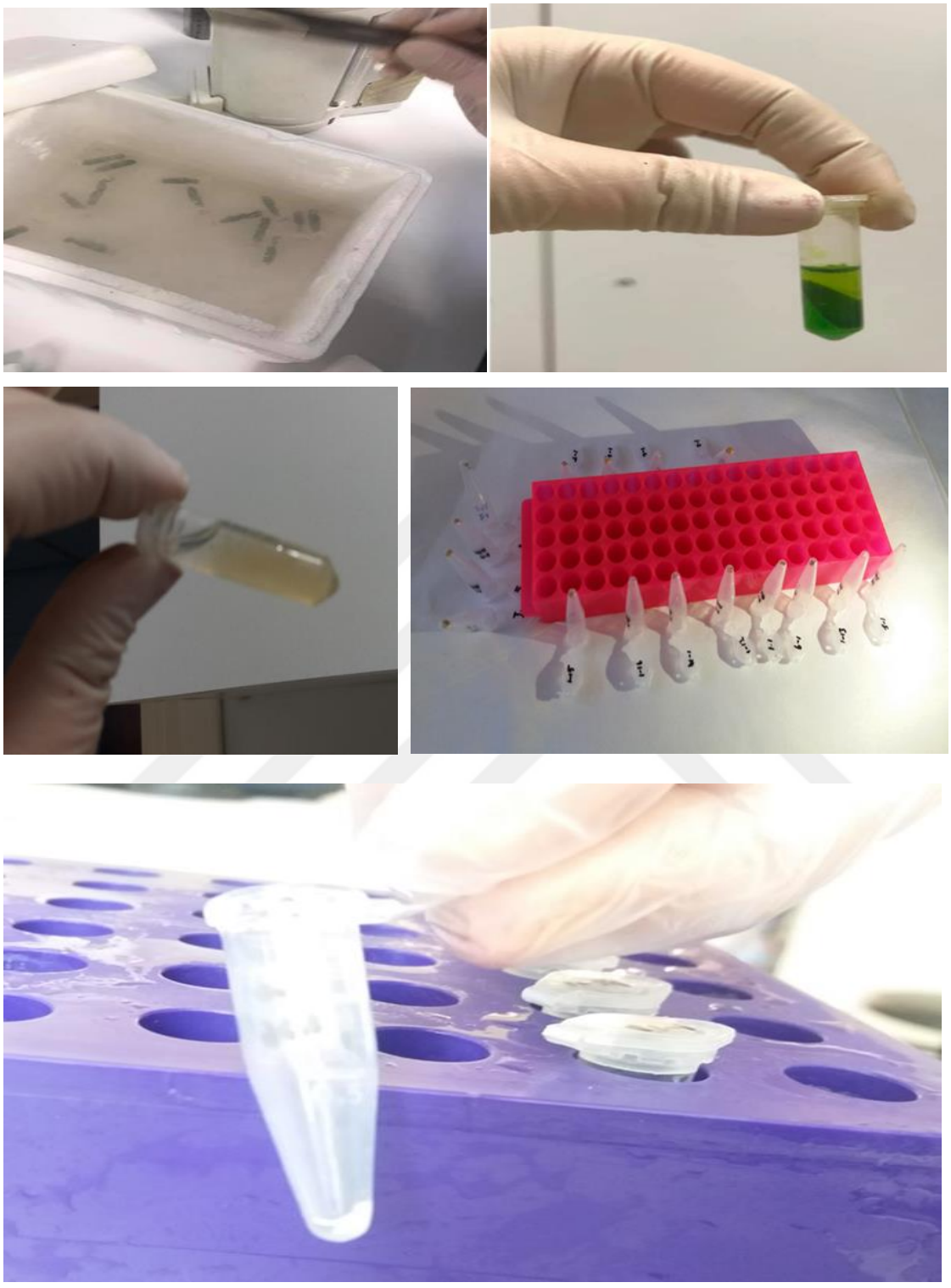


Figure 10. Stage of DNA isolation from plant material.

3.3. Molecular Analysis

A total of DNA was extracted from *Cucurbita pepo* L. via this process (Fulton et al. 1995). Obtained DNA was measured via Qubit® dsDNA HS Assay Kits then read the concentration of all assay tubes(from 1–20 μL) via Qubit® 3.0 Fluorometer. The quality of samples was examined via electrophores with a 1.2% agarose gel.

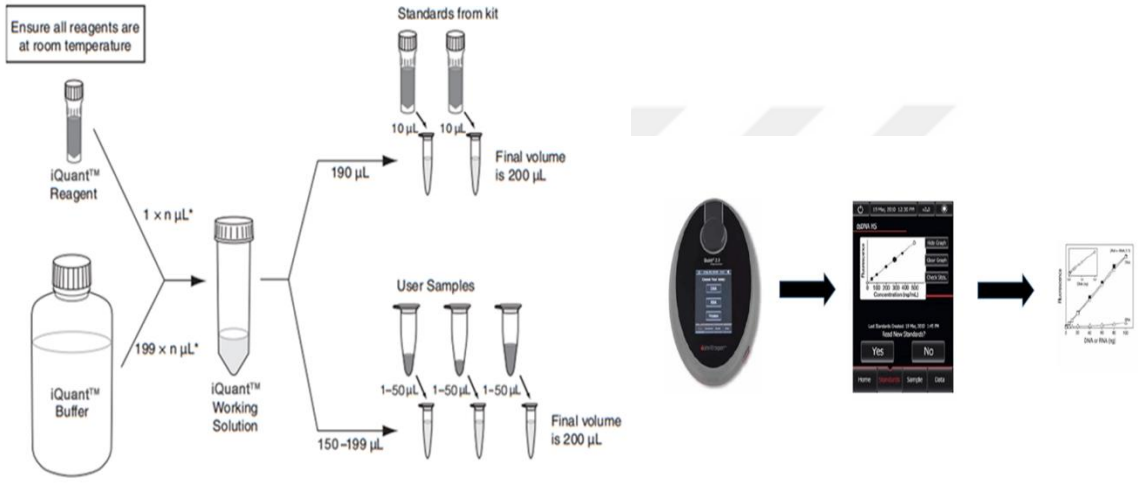


Figure 11. Step of preparing Qubit® solution for measuring DNA concentration of samples.

3.4. SSR Design

SSR primers were selected from the summer squash genetic linkage map (Gong et al., 2008). This map shows 20 linkage groups of *Cucurbita pepo* L. SSR markers are selected via 2-3 markers for one linkage group. First of all, 61 SSR primers **(Appendix 1)** which cover 20 linkage groups were used to investigate ZYMV resistant parental genotypes. Separating resistant and susceptible genotypes in parental and F_2 populations were investigated by using polymorphic SSRs.

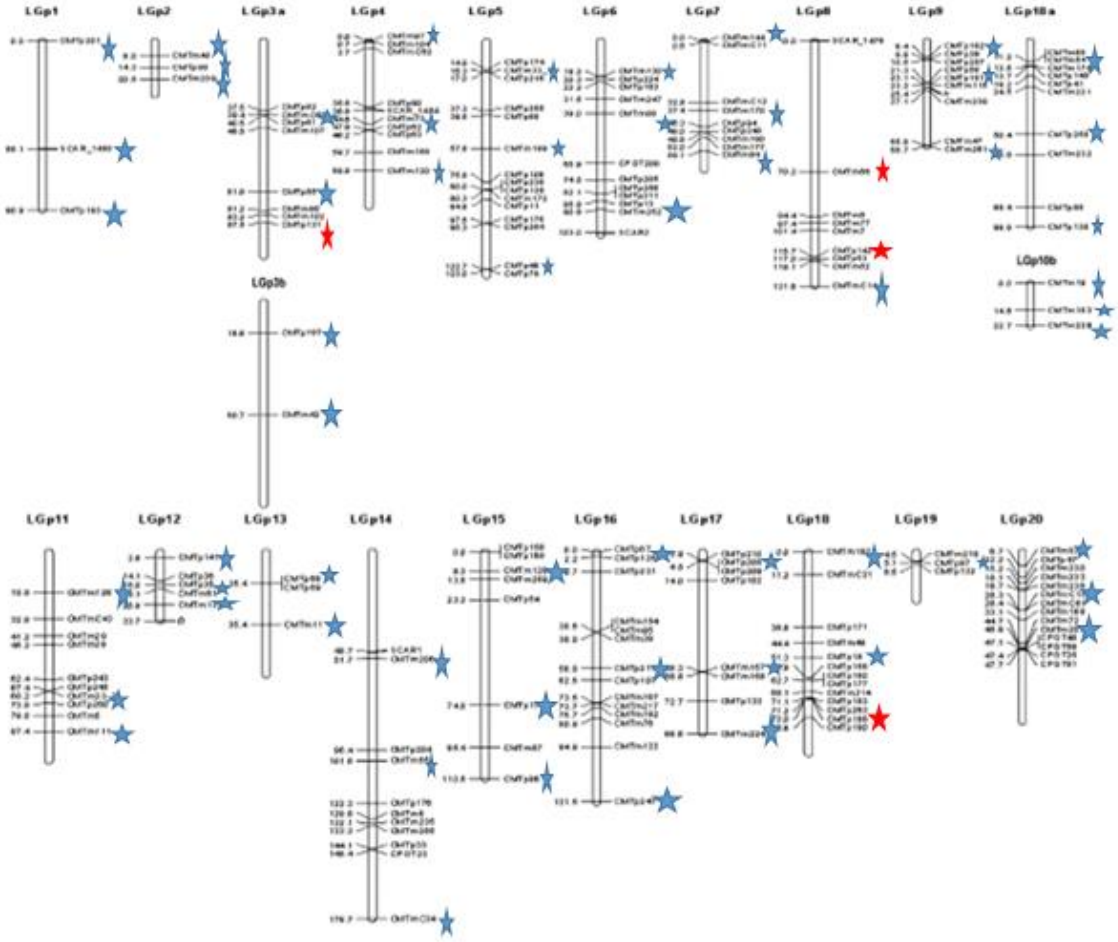


Figure 12. This map shows the genetic linkage of SSR markers in *Cucurbita pepo* L. (Gong et al., 2008)

3.5. Polymerase Chain Reaction (PCR)

The specified conditions were used for PCR analysis. These processes denaturating DNA at 94°C for 3 min, 30 cycles were determined for the amplification of PCR products. For this purpose 94°C for 1 min, following 40-60°C for 1 min according to chosen SSRs marker, and then 72°C for 1 min were used. Finally for extention 72°C for 10 min was used. The annealing temperature was determined by using Gradient PCR.

Table 4

Components of the PCR reaction

PCR Components	Concentration	Volume (µl)
Buffer	10 X	3,5
dNTP	2.5 mM	2
SSR Primer (Forward)	10 µM	1
SSR Primer (Reverse)	10 µM	1
(Taq) DNA Polymerase	5 U/µl	1,2
Genomic DNA	50 ng/µl	2
dH_2O		14,3

3.6. Agarose Gel Electrophoresis

200 ml TBE buffer (0.5X) was measured by a graduated cylinder and add 4 g agarose in a 250 ml flask. This flask was put in the microwave until the solution was completely clear. 5 µl of RedSafeTM Nucleic Acid Staining Solution (20,000x) was added to the flask after cooling the agarose solution. Prepared agarose solution poured into the gel tray. Finally, samples were loaded on the gel and carried out electrophoresis. The bands of PCR products were investigated under UV illumination.

Table 5

Components of Agarose gel electrophoresis

Chemical Agent	Ingredients
0.5X TBE Buffer	Tris-HCl (pH:8.3) EDTA Borate
Loading dye	RedSafeTM Nucleic Acid Staining Solution

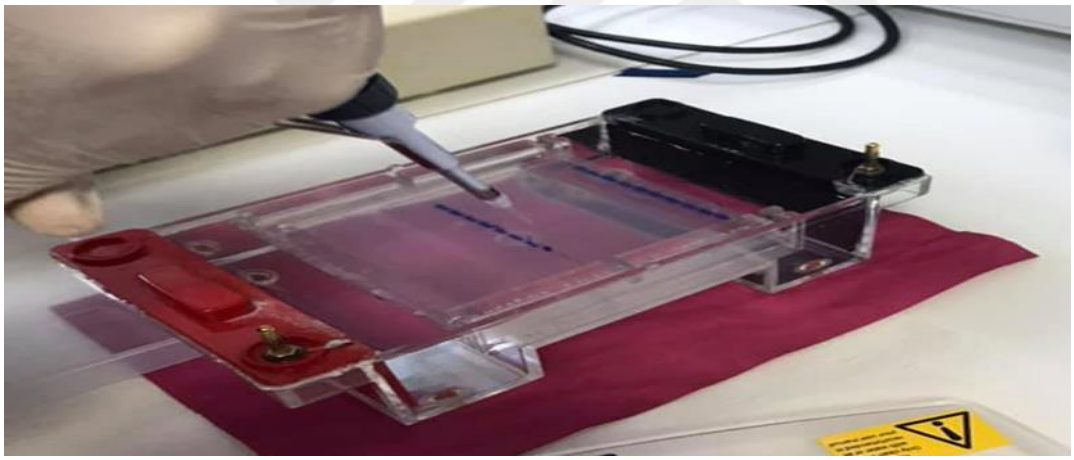


Figure 13. Scanning PCR samples by gel electrophoresis.

CHAPTER 4

RESEARCH FINDING

4.1. Screening PCR Product Results In Agarose Gel

In this study, the polymorphic band profiles were shown in Figure 14. Angelina and Regina parental genotypes have two PCR bands. Arrow indicates resistant genotypes and the circle indicates susceptible genotypes. One of the bands indicated with an arrow distinguishes resistant genotypes and the circle indicated band can distinguish susceptible genotypes. Polymorphic band sizes were given in Table 6.

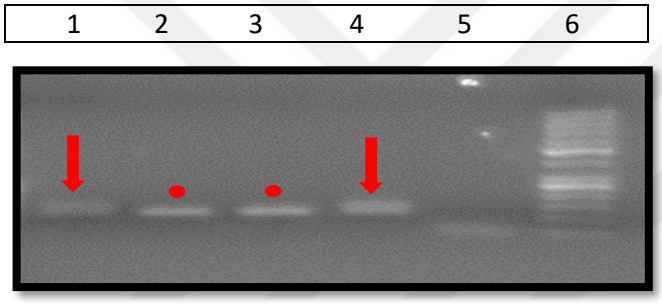


Figure 14. This image shows PCR products of *C.pepo* that obtain with SSR markers in 2% agarose gel 1. Algelina (CMTp131 marker) 2.Kabuklu K10 (CMTp131 marker) 3.Sulu Saray (CMTp131 marker) 4.Regina (CMTp131 marker) 5. Negative control 6. Thermo scientific 50bp DNA ladder.

Table 6

The polymorphic band sizes of parental genotypes were shown with CMTp131 marker via Logger Pro 3.16.2

Name of Genotype	Angelina	Kabuklu K10	Sulu Saray	Regina
Band Length	154 bp	-	-	165 bp
	124 bp	122 bp	125 bp	129 bp

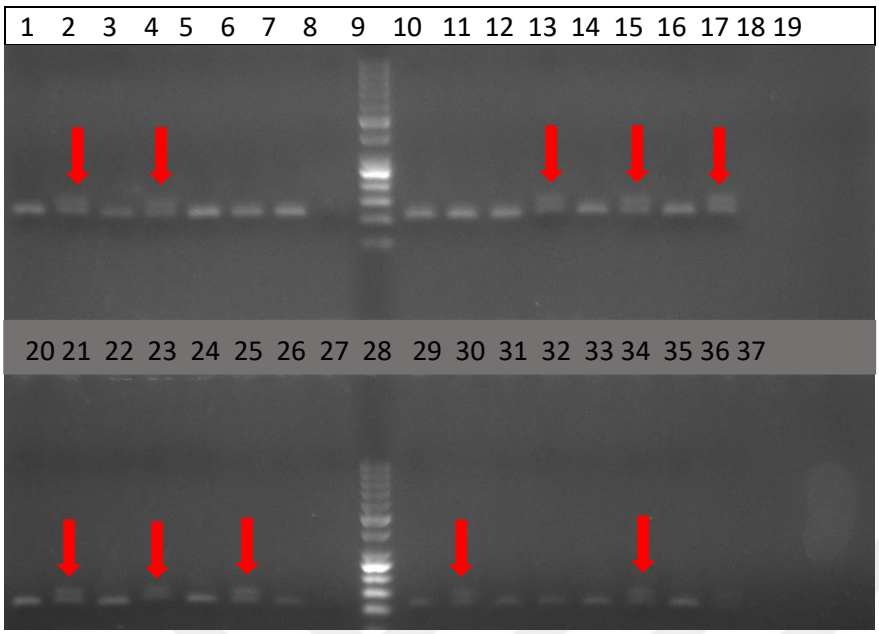


Figure 15. This image shows PCR products of parental *C.pepo* that obtain with CMTp131 SSR markers in 2% agarose gel 1.Kabuklu K10-1 2. Angelina-1 3. Sulu Saray-1 4. Regina-1 5. Kabuklu K10-2 6. Angelina-2 7. Sulu Saray-2 8. Regina-2 9. Invitrogen 50 bp DNA ladder 10.Kabuklu K10-3 11. Angelina-3 12. Sulu Saray-3 13. Regina-3 14.Kabuklu K10-4 15. Angelina-4 16. Sulu Saray-4 17. Regina-4 18. Negative Control 20. Kabuklu K10-5 21. Angelina-5 22. Sulu Saray-5 23. Regina-5 24. .Kabuklu K10-6 25. Angelina-6 26. Sulu Saray-6 27. Regina-6 28. Invitrogen 50 bp DNA ladder 29. Kabuklu K10-7 30. Angelina-7 31. Sulu Saray-7 32. Regina-7 33.Kabuklu K10-8 34. Angelina-8 35. Sulu Saray-8 36.Regina-8 37. Negative Control.

CMTp131 primer was used for the determination of polymorphisms between susceptible and resistant genotypes. Figure 15 shows 10 individuals of each parental genotype and arrows indicating resistant individuals.

Figure 16 shows 56 F_2 individuals of hybrid genotypes formed by crossing parental genotypes, Regina x Sulu Saray and Angelina x Sulu Saray with CMTp131, and the arrow indicates resistant individuals.

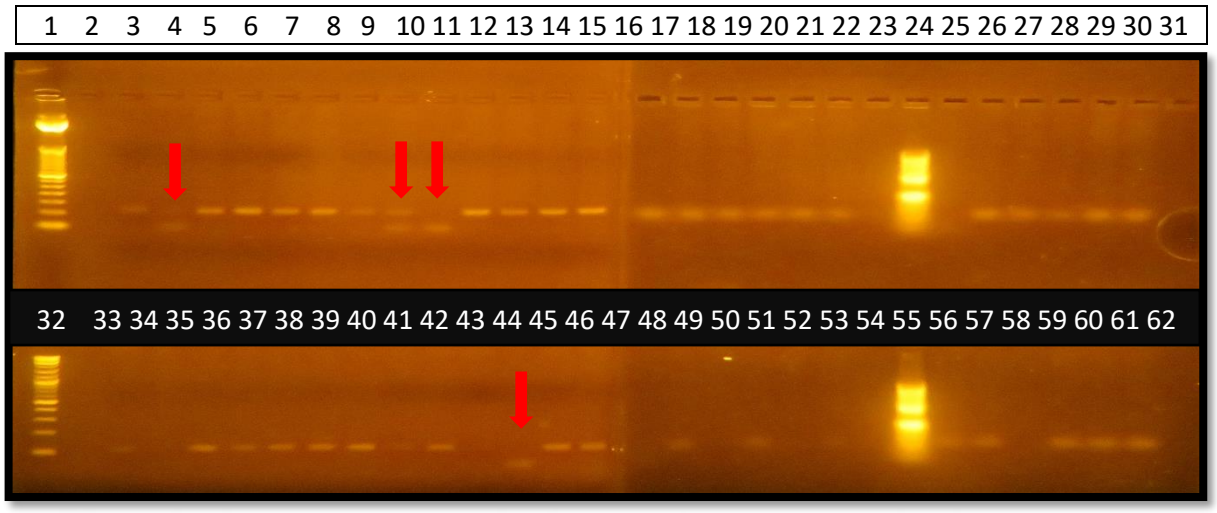


Figure 16. This image shows PCR products of *C.pepo* that obtain with SSR markers in 2% agarose gel 1, 24, 32 and 55: Invitrogen 50bp DNA ladder 3-23 and 25-31: Regina- Sulu Saray- F_2 individuals (CMTp131 marker) 34-54 and 56-62: Angelina- Sulu Saray- F_2 individuals (CMTp131 marker) 2 and 33: Negative Control.

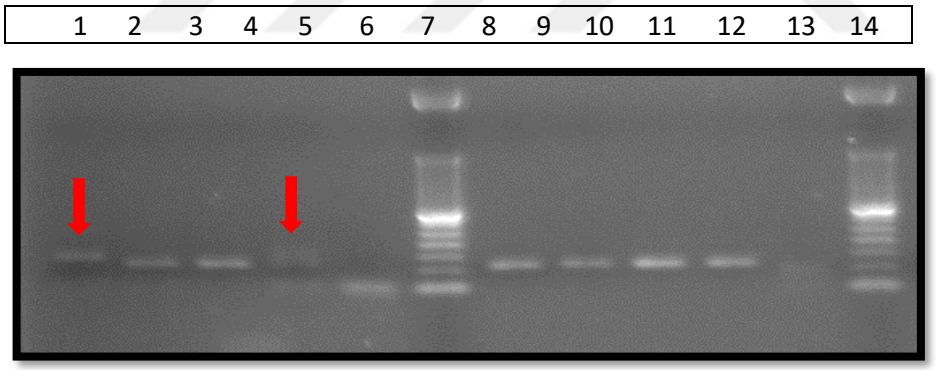


Figure 17. This image shows PCR products of *C.pepo* that obtain with CMTm66 SSR marker in 2% agarose gel 1. Angelina (CMTp131 marker) 2.Kabuklu K10 (CMTm66 marker) 3.Sulu Saray (CMTm66 marker) 4.Regina (CMTm66 marker)6. Invitrogen 50bp DNA ladder.

In the study, PCR products were obtained from SSR analysis of each individual by giving a single polymorphic band. In Figure 17 both Angelina and Regina are visualized polymorphic bands profile according to Sulu Saray and Kabuklu K10. Also, differences

between resistant and susceptible varieties of PCR products, and the formation of different band sizes can easily determine polymorphism in Table 7.

Table 7

The polymorphic band sizes of parental genotypes were shown with CMTm66 marker via Logger Pro 3.16.2

Name of Genotype	Angelina	Kabuklu K10	Sulu Saray	Regina
Band Length	160 bp	-	-	160 bp
	-	133 bp	139 bp	131 bp

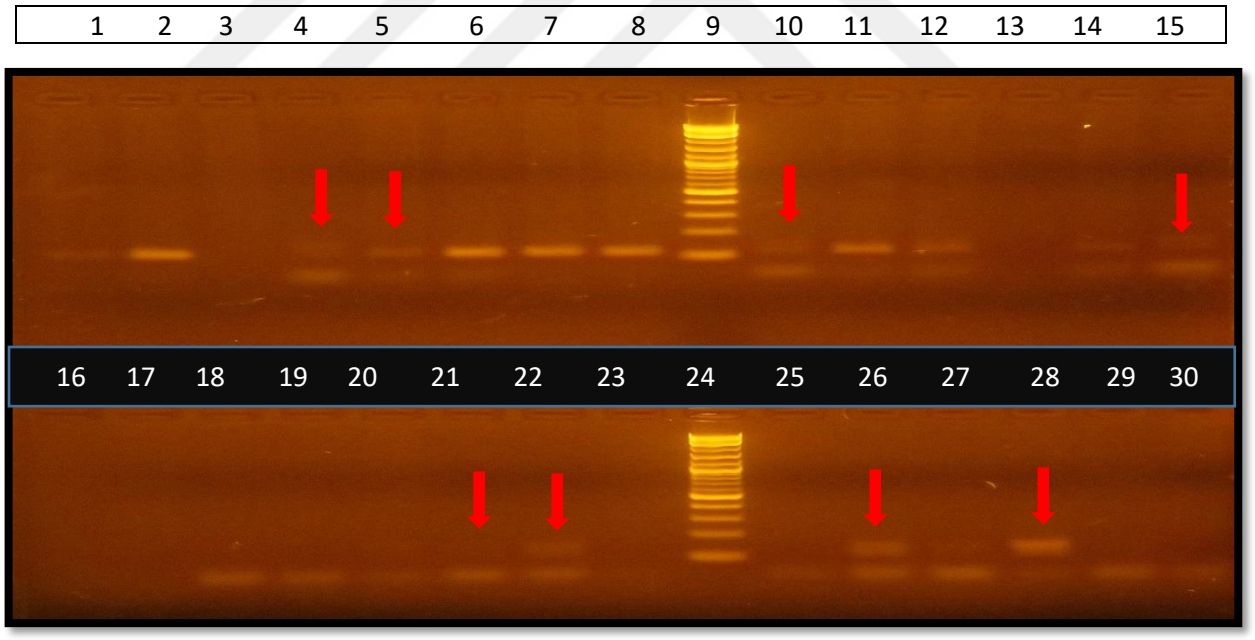


Figure 18. PCR products of *C.pepo* that obtain with CMTm66 SSR markers in 2% agarose gel. 1 and 16: Negative Control 2-8 and 10-15: Regina- Sulu Saray- F_2 individuals 9 and 24: Invitrogen 50bp DNA ladder 17-23 and 25-30: Angelina- Sulu Saray- F_2 individuals (CMTm66 marker).

PCR products of F_2 individuals of Angelina X Sulu Saray and Regina X Sulu Saray were shown in Figure 18.

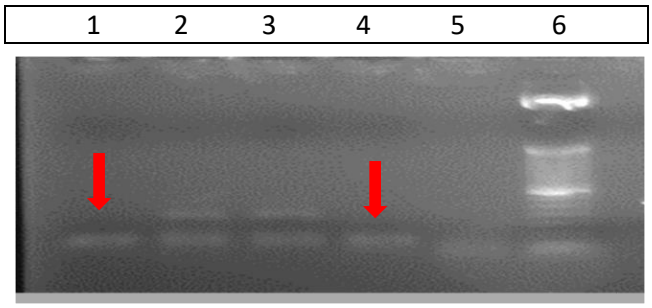


Figure 19. This image shows PCR products of *C.pepo* that obtain with SSR markers in 2% agarose gel 1.Angelina (CMTp190 marker) 2.Kabuklu K10 (CMTp190 marker) 3.Sulu Saray (CMTp190 marker) 4.Regina (CMTp190 marker) 6. Invitrogen 50bp DNA ladder.

PCR products were evaluated by visualizing the agarose gel under UV light. Susceptible individuals (Sulu Saray and Kabuklu K-10) showed two band formations but resistant individuals (Angelina and Regina) had only one band. Arrow indicates resistant parental genotypes.

Table 8

The polymorphic band sizes of parental genotypes were shown with CMTp190 marker via Logger Pro 3.16.2

Name of Genotype	Angelina	Kabuklu K10	Sulu Saray	Regina
Band Length	-	196 bp	196 bp	-
	99 bp	99 bp	97 bp	97 bp

This table showed the correlation between genotypes and band size. Both Angelina and Kabuklu K10 have a 99 bp band and both Sulu Saray and Regina have a 97 bp band. Nevertheless, Kabuklu K10 and Sulu Saray have 196 bp band. This 196 bp band links to increasing susceptibility to ZYMV. Resistant individuals had no 196 bp band formation.

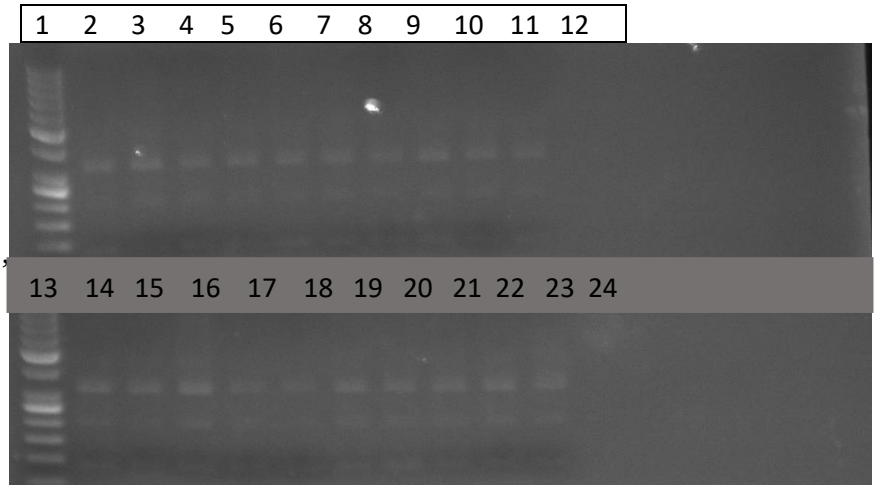


Figure 20. This image shows PCR products of parental *C.pepo* samples that obtain with CMTp190 marker SSR markers in 2% agarose gel 1. Invitrogen 50bp DNA ladder 2.Kabuklu K10- 3. Kabuklu K10- 4. Kabuklu K10- 5. Kabuklu K10- 6. Kabuklu K10- 7. Kabuklu K10- 8. Kabuklu K10- 9. Kabuklu K10- 10. Kabuklu K10- 11. Kabuklu K10- 12. Negative Control 13. Invitrogen 50bp DNA ladder 14. Sulu Saray- 15. Sulu Saray- 16. Sulu Saray- 17. Sulu Saray- 18. Sulu Saray- 19. Sulu Saray- 20. Sulu Saray- 21. Sulu Saray- 22. Sulu Saray- 23. Sulu Saray- 24. Negative Control.

Sulu Saray and Kabuklu K-10 parental genotypes are susceptible genotypes to ZYMV and PCR products showed two band formation with CMTp190 marker in Figure 20.

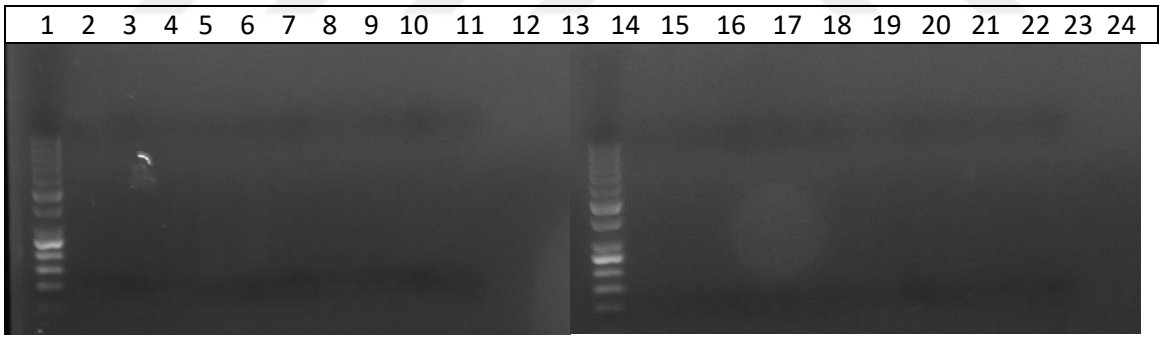


Figure 21. This image shows PCR products of parental *C.pepo* samples obtain with CMTp190 marker SSR markers in 2% agarose gel 1. Invitrogen 50bp DNA ladder 2.Regina-1 3. Regina-2 4. Regina-3 5. Regina-4 6. Regina-5 7.Regina-6 8.Regina-7 9.Regina-8 10.Regina-9 11.Regina-10 12.Negative Control 13. Invitrogen 50bp DNA ladder 14.Angelina-1 15.Angelina-2 16.Angelina-3 17. Angelina-4 18.Angelina-5 19.Angelina-6 20.Angelina-7 21.Angelina-8 22.Angelina-9 23.Angelina-10 24. Negative Control.

Regina and Angelina parental genotypes are resistant to ZYMV and there is no band formation when CMTp190 marker is used for screening in Figure 21.

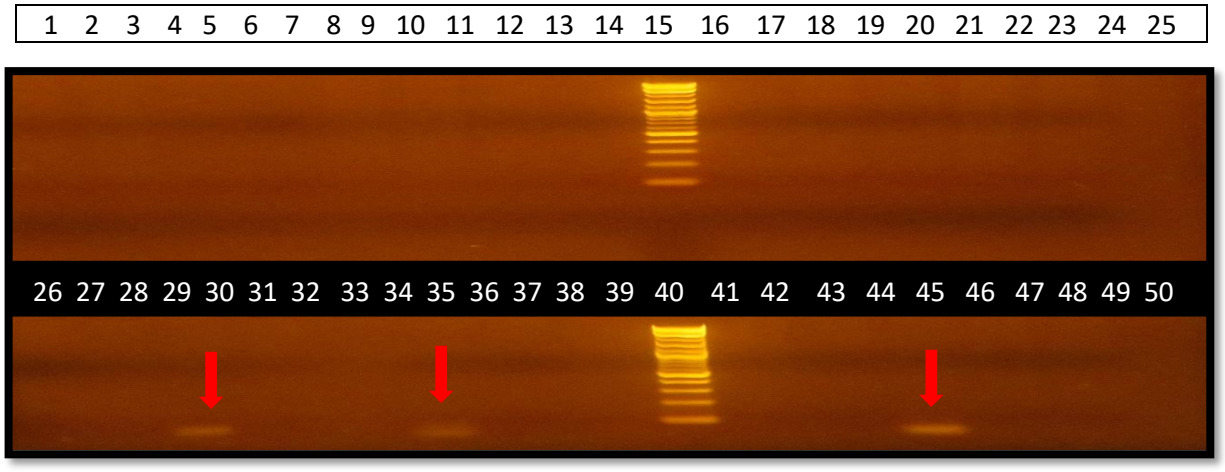


Figure 22. This image shows PCR products of *C.pepo* samples that obtain with CMTp190 marker SSR markers in 2% agarose gel 1-14 and 16-24 : Regina- Sulu Saray F_2 individuals 15 and 40 : Invitrogen 50bp DNA ladder 26-39 and 41-49: Angelina and Sulu Saray F_2 25 and 50: Negative Control

PCR products of Regina X Sulu Saray F_2 individuals and Angelina XSulu Saray F_2 individuals with CMTp190 marker were shown in Figure 22. Regina Sulu Saray F_2 individuals had no band formation also some of the Angelina –Sulu Saray F_2 individuals contain only one band. An arrow was indicating these band formations.

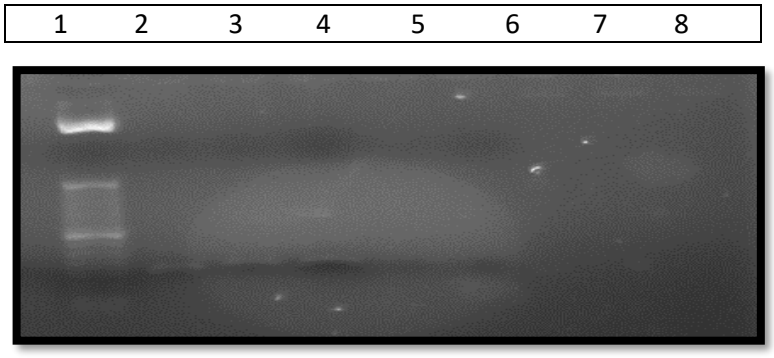


Figure 23. This image shows PCR products of *C.pepo* that obtain with SSR markers in 2% agarose gel 1. Invitrogen 50bp DNA ladder 2. Algelina (CMTp142 marker) 3.Kabuklu K10 (CMTp142 marker) 4.Sulu Saray (CMTp142 marker) 5.Regina (CMTp142 marker).

Table 9

The polymorphic band sizes of parental genotypes were shown with CMTp142 primer via Logger Pro 3.16.2

Name of Genotype	Angelina	Kabuklu K10	Sulu Saray	Regina
Band Length	165 bp	179 bp	174 bp	-

Table 9 showed that Kabuklu K10 and Sulu Saray PCR products have larger sizes than Angelina. Figure 23 shows the differences between resistant and susceptible genotypes.

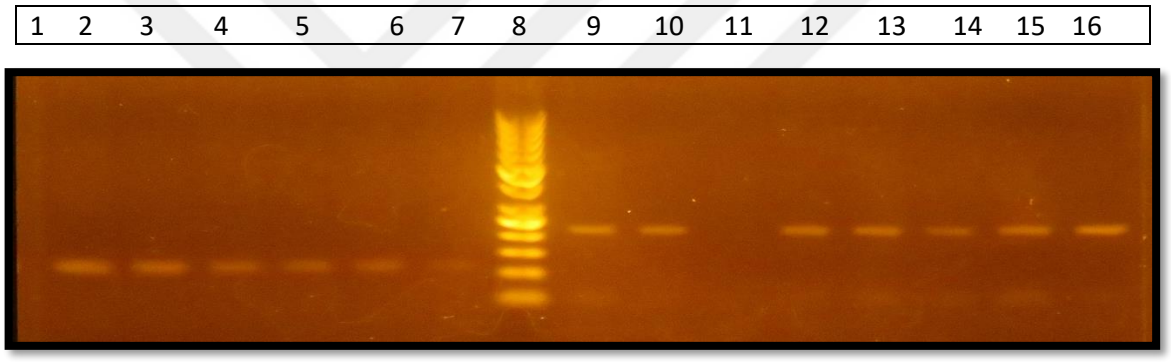


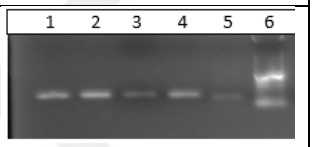
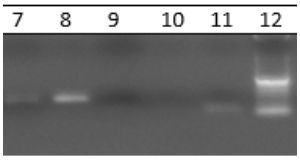
Figure 24. This image shows PCR products of *C.pepo* samples that obtain marker SSR markers in 2% agarose gel 1. Negative Control 2.Regina-Sulu Saray-1 (CMTp131) 3. Angelina-Sulu Saray-1(CMTp131) 4.Regina-Sulu Saray-2 (CMTp131) 5. Angelina- Sulu Saray-2 (CMTp131) 6.Regina-Sulu Saray-3 (CMTp131) 7. Angelina- Sulu Saray-4(CMTp131) 8. Invitrogen 50bp DNA ladder 9. Regina – Sulu Saray-1 (CMTp142) 10. Angelina- Sulu Saray-1 (CMTp142) 11. Regina – Sulu Saray-2 (CMTp142) 12.Angelina-Sulu Saray-2(CMTp142) 13. Regina – Sulu Saray-3 (CMTp142) 14.Angelina- Sulu Saray-3(CMTp142) 15. Regina – Sulu Saray-4 (CMTp142) 16.Angelina- Sulu Saray-4(CMTp142).

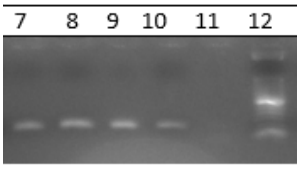
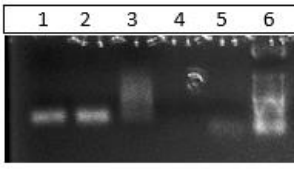
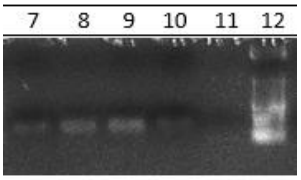
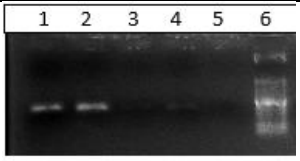
Table 10 showed the Linkage group of SSR primers that were chosen for PCR analysis and also the annealing temperatures of these SSR primers and Agarose gel images.

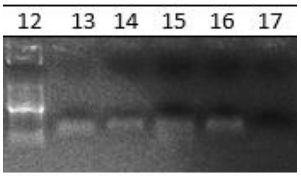
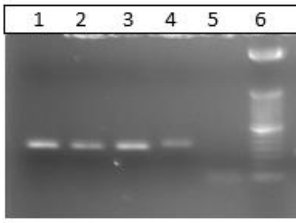
Resistant genotypes (Angelina- Regina) and susceptible genotypes (Sulu Saray-Kabuklu K-10) were analyzed with selected 60 SSR markers from Gong. Half of these markers showed PCR products. As a result of these analyses, 4 primers (CMTp190, CMTp142, CMTp131, CMTm66) had polymorphism between resistant and susceptible genotypes. 50℃ temperature is optimum for annealing. Also, polymorphic markers (CMTp190, CMTp142, CMTp131, CMTm66) are located on different chromosomes.

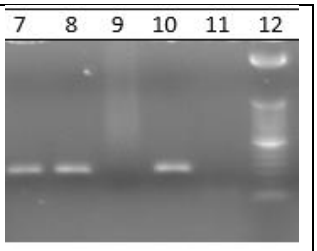
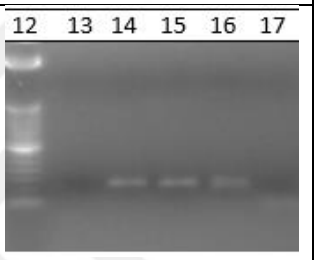
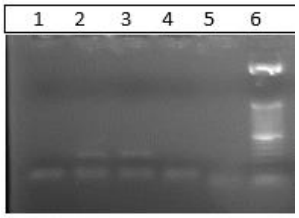
Table 10

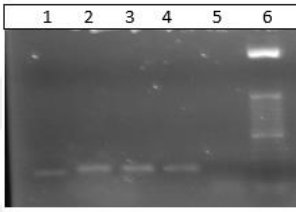
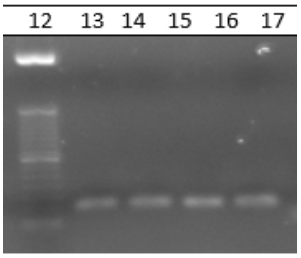
Linkage group and the annealing temperatures of SSR primers that were chosen for PCR analysis and agarose gel images

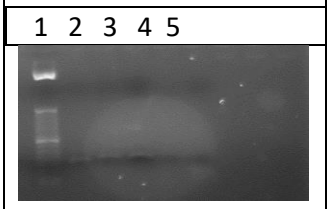
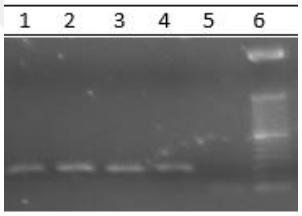
Linkage groups	SSR Primer	TEMPERATURE	PCR PRODUCTS ON AGAROSE GEL
LGp10b	CMTm19	50℃	 This image shows PCR products of C.*pepo* that obtain with SSR markers in 2% agarose gel 1.Angelina (CMTm19 marker) 2.Kabuklu K10 (CMTm19 marker) 3.Sulu Saray (CMTm19 marker) 4.Regina (CMTm19 marker) 6. Invitrogen 50bp DNA ladder.
LGp3a	CMTp55	50℃	 This image shows PCR products of *C.pepo* that obtain with SSR markers in 2% agarose gel 7.Angelina (CMTp55 marker) 8.Kabuklu K10 (CMTp55 marker) 9.Sulu Saray (CMTp55 marker) 10.Regina (CMTp55 marker) 12. Invitrogen 50bp DNA ladder.
LGp15	CMTp86	50℃	No band

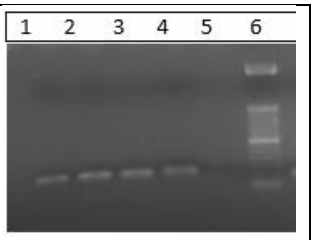
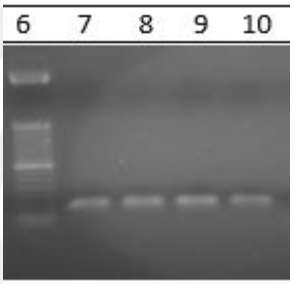
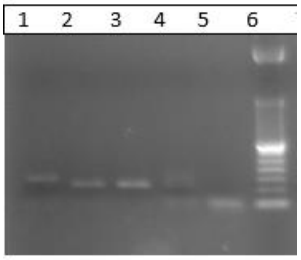
LGp9	CMTp151	50℃	 This image shows PCR products of *C.pepo* that obtain with SSR markers in 2% agarose gel 7.Angelina (CMTp151 marker) 8.Kabuklu K10 (CMTp151 marker) 9.Sulu Saray (CMTp151 marker) 10.Regina (CMTp151 marker) 12. Invitrogen 50bp DNA ladder.
LGp7	CMTm170	50℃	No band
LGp14	CMTmc34	50℃	 This image shows PCR products of *C.pepo* that obtain with SSR markers in 2% agarose gel 1.Angelina (CMTmc34 marker) 2.Kabuklu K10 (CMTmc34 marker) 3.Sulu Saray (CMTmc34 marker) 4.Regina (CMTmc34 marker) 6. Invitrogen 50bp DNA ladder.
LGp14	CMTm65	50℃	 This image shows PCR products of C.pepo that obtain with SSR markers in 2% agarose gel 7.Angelina (CMTm65 marker) 8.Kabuklu K10 (CMTm65 marker) 9.Sulu Saray (CMTm65 marker) 10.Regina (CMTm65 marker) 12. Invitrogen 50bp DNA ladder.
LGp2	CMTp98	50℃	

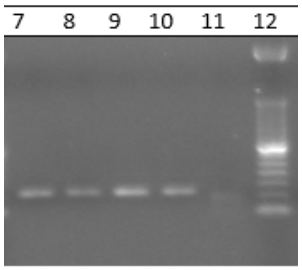
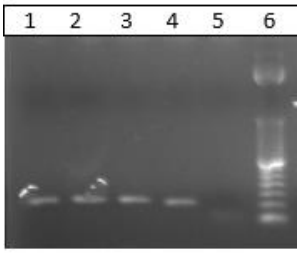
			This image shows PCR products of *C.pepo* that obtain with SSR markers in 2% agarose gel 1.Angelina (CMTp98 marker) 2.Kabuklu K10 (CMTp98 marker) 3.Sulu Saray (CMTp98 marker) 4.Regina (CMTp98 marker) 6. Invitrogen 50bp DNA ladder.
LGp1	CMTp201	50°C	 This image shows PCR products of C.pepo that obtain with SSR markers in 2% agarose gel 12. Invitrogen 50bp DNA ladder 13. Angelina (CMTp201 marker) 14.Kabuklu K10 (CMTp201 marker) 15.Sulu Saray (CMTp201 marker) 16.Regina (CMTp201 marker).
LGp1	CMTp193	50°C	No Band
LGp3b	CMTm42	50°C	 This image shows PCR products of *C.pepo* that obtain with SSR markers in 2% agarose gel 1.Angelina (CMTm42 marker) 2.Kabuklu K10 (CMTm42 marker) 3.Sulu Saray (CMTm42 marker) 4.Regina (CMTm42 marker) 6. Invitrogen 50bp DNA ladder.

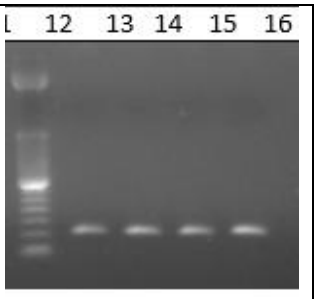
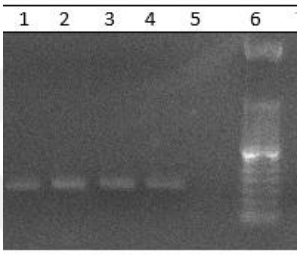
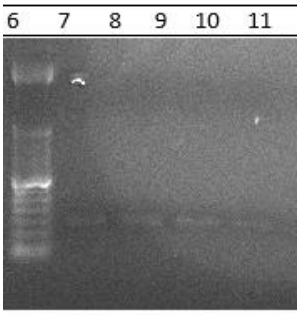
LGp4	CMTm97	50°C	 This image shows PCR products of *C.pepo* that obtain with SSR markers in 2% agarose gel 7.Angelina (CMTm97 marker) 8.Kabuklu K10 (CMTm97 marker) 9.Sulu Saray (CMTm97 marker) 10.Regina (CMTm97 marker) 12. Invitrogen 50bp DNA ladder.
LGp6	CMTm130	50°C	 This image shows PCR products of *C.pepo* that obtain with SSR markers in 2% agarose gel 12. Invitrogen 50bp DNA ladder 13. Angelina (CMTm130 marker) 14.Kabuklu K10 (CMTm130 marker) 15.Sulu Saray (CMTm130 marker) 16.Regina (CMTm130 marker).
LGp18	CMTp190	50°C	 This image shows PCR products of *C.pepo* that obtain with SSR markers in 2% agarose gel 1.Angelina (CMTp190 marker) 2.Kabuklu K10 (CMTp190 marker) 3.Sulu Saray (CMTp190 marker) 4.Regina (CMTp190 marker) 6. Invitrogen 50bp DNA ladder.

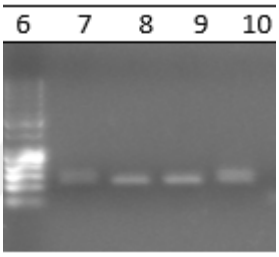
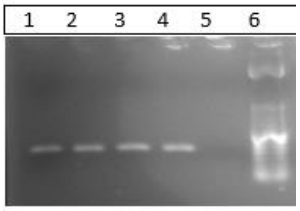
LGp14	CMTm206	50℃	No band
LGp16	CMTm247	50℃	No band
LGp13	CMTm11	48℃	 This image shows PCR products of *C.pepo* that obtain with SSR markers in 2% agarose gel 1.Angelina (CMTm11 marker) 2.Kabuklu K10 (CMTm11 marker) 3.Sulu Saray (CMTm11 marker) 4.Regina (CMTm11 marker) 6. Invitrogen 50bp DNA ladder.
LGp20	CMTm83	48℃	 This image shows PCR products of *C.pepo* that obtain with SSR markers in 2% agarose gel 12. Invitrogen 50bp DNA ladder13.Angelina (CMTm83 marker) 14.Kabuklu K10 (CMTm83 marker) 15.Sulu Saray (CMTm83 marker) 16.Regina (CMTm83 marker)
LGp19	CMTp97	48℃	No band

LGp15	CMTm129	48°C	No band
LGp12	CMTp142	48°C	 This image shows PCR products of *C.pepo* that obtain with SSR markers in 2% agarose gel 1. Invitrogen 50bp DNA ladder 2. Angelina (CMTp142 marker) 3.Kabuklu K10 (CMTp142 marker) 4.Sulu Saray (CMTp142 marker) 5.Regina (CMTp142 marker).
LGp8	CMTmc14	48°C	 This image shows PCR products of *C.pepo* that obtain with SSR markers in 2% agarose gel 1.Angelina (CMTmc14 marker) 2.Kabuklu K10 (CMTmc14 marker) 3.Sulu Saray (CMTmc14 marker) 4.Regina (CMTmc14 marker) 6. Invitrogen 50bp DNA ladder.
LGp3a	CMTmc67	48°C	No band
LGp13	CMTp68	48°C	No band

LGp16	CMTp217	48℃	 This image shows PCR products of *C.pepo* that obtain with SSR markers in 2% agarose gel 1.Angelina (CMTp217 marker) 2.Kabuklu K10 (CMTp217 marker) 3.Sulu Saray (CMTp217 marker) 4.Regina (CMTp217 marker) 6. Invitrogen 50bp DNA ladder.
LGp6	CMTm252	48℃	 This image shows PCR products of *C.pepo* that obtain with SSR markers in 2% agarose gel 6. Invitrogen 50bp DNA ladder 7.Angelina (CMTm252 marker) 8.Kabuklu K10 (CMTm252 marker) 9.Sulu Saray (CMTm252 marker) 10.Regina (CMTm252 marker)
LGp8	CMTm66	49℃	 This image shows PCR products of *C.pepo* that obtain with SSR markers in 2% agarose gel 1.Algelina (CMTm66 marker) 2.Kabuklu K10 (CMTm66 marker) 3.Sulu Saray (CMTpm66 marker) 4.Regina (CMTm66 marker) 6. Invitrogen 50bp DNA ladder.

LGp6	CMTm88	49℃	 This image shows PCR products of *C.pepo* that obtain with SSR markers in 2% agarose gel 7.Angelina (CMTm88 marker) 8.Kabuklu K10 (CMTm88 marker) 9.Sulu Saray (CMTm88 marker) 10.Regina (CMTm88 marker)12. Invitrogen 50bp DNA ladder.
LGp11	CMTm111	49℃	No band
LGp16	CMTp125	49℃	 This image shows PCR products of *C.pepo* that obtain with SSR markers in 2% agarose gel 1.Angelina (CMTp125 marker) 2.Kabuklu K10 (CMTp125 marker) 3.Sulu Saray (CMTp125 marker) 4.Regina (CMTp125 marker) 6. Invitrogen 50bp DNA ladder.
LGp5	CMTm189	49℃	No band

LGp10a	CMTp258	49℃	 This image shows PCR products of *C.pepo* that obtain with SSR markers in 2% agarose gel 12. Invitrogen 50bp DNA ladder 13.Angelina (CMTp258 marker) 14.Kabuklu K10 (CMTp258 marker) 15.Sulu Saray (CMTp258 marker) 16.Regina (CMTp258 marker)
LGp18	CMTp18	51℃	 This image shows PCR products of *C.pepo* that obtain with SSR markers in 2% agarose gel 1.Angelina (CMTp18 marker) 2.Kabuklu K10 (CMTp18 marker) 3.Sulu Saray (CMTp18 marker) 4.Regina (CMTp18 marker) 6. Invitrogen 50bp DNA ladder.
LGp17	CMTm157	51℃	No band
LGp11	CMTp245	51℃	 This image shows PCR products of *C.pepo* that obtain with SSR

			markers in 2% agarose gel 6.Invitrogen 50bp DNA ladder 7..Angelina (CMTp245 marker) 8.Kabuklu K10 (CMTp245 marker) 9.Sulu Saray (CMTp245 marker) 10.Regina (CMTp245 marker)
LGp12	CMTp35	46℃	No band
LGp3a	CMTp131	46℃	 This image shows PCR products of *C.pepo* that obtain with SSR markers in 2% agarose gel 6. Invitrogen 50bp DNA ladder 7..Angelina (CMTp131 marker) 8.Kabuklu K10 (CMTp131 marker) 9.Sulu Saray (CMTp131 marker) 10.Regina (CMTp131 marker)
LGp10a	CMTp138	46℃	No band
LGp10b	CMTm183	46℃	No band
LGp17	CMTm224	46℃	No band
LGp18	CMTm187	47℃	 This image shows PCR products of *C.pepo* that obtain with SSR markers in 2% agarose gel 1.Angelina (CMTm187 marker)

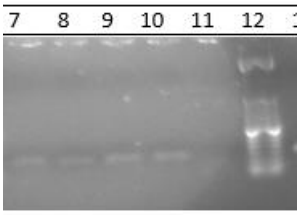
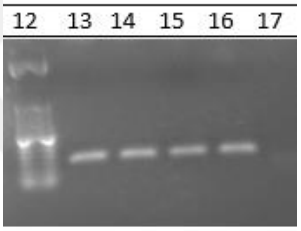
			2.Kabuklu K10 (CMTm187 marker) 3.Sulu Saray (CMTm187 marker) 4.Regina (CMTm187 marker) 6. Invitrogen 50bp DNA ladder.
LGp17	CMTp208	47℃	 This image shows PCR products of *C.pepo* that obtain with SSR markers in 2% agarose gel 7.Angelina (CMTp208 marker) 8.Kabuklu K10 (CMTp208 marker)9.Sulu Saray (CMTp208 marker) 10.Regina (CMTp208 marker) 12. Invitrogen 50bp DNA ladder.
LGp10b	CMTm229	47℃	 This image shows PCR products of *C.pepo* that obtain with SSR markers in 2% agarose gel 12. Invitrogen 50bp DNA ladder 13.Angelina (CMTm229 marker) 14.Kabuklu K10 (CMTm229 marker) 15.Sulu Saray (CMTm229 marker) 16.Regina (CMTm229 marker)
LGp11	CMTm126	43℃	No band
LGp12	CMTm131	43℃	No band

Table 11

Monomorphic and polymorphic band structures of SSR primers

Monomorphic Band Structures of SSR Primers	**Polymorphic Band Structures of SSR Primers**
CMTm19, CMTp55, CMTp151, CMTmc34, CMTm65, CMTp98, CMTp201, CMTm42, CMTm97, CMTm130, CMTm11, CMTm83, CMTmc14, CMTp217, CMTm252, CMTm88, CMTp125, CMTp258, CMTp18, CMTp245, CMTm187, CMTp208, CMTm229	CMTm66, CMTp190, CMTp142, CMTp131

Table 11 is showing results and a summary of all the SSR primers in table 10. Parental genotypes have two lines of the resistant and susceptible polymorphic band structure giving differences in band profiles. Polymorphic band profiles are CMTm66, CMTp190, CMTp142, and CMTp131.

CHAPTER 5
RESULTS AND RECOMMENDATIONS

5.1. Result and Recommendations

In the frame of this thesis, 60 SSR markers which were developed by Gong et al. (2008) were used. Gong determined 20 linkage groups. 60 SSR primers which cover 20 linkage groups used to investigate ZYMV resistance between resistant and susceptible parental genotypes. In this study, two ZYMV resistant parental genotypes (Angelina, Regina) and two ZYMV susceptible parental genotypes (Sulu Saray, K-10 Kabuklu) were used. Two hybrid genotypes formed by crossing parental genotypes were obtained from the Trakya Agricultural Research Institute, Edirne. These hybrid genotypes also were used to determine polymorphism between the hybrid of parental genotypes.

SSR PCR products of plant materials were identified by agarose gel electrophoresis. Alternatively, capillary electrophoresis can be used with florescent marked SSR primers. In this study, AGE was used for the identification of SSR markers because of low-cost and easy usage. Polymorphisms usually show containing addition, and deletion as different PCR product formations.

Firstly in this study, Gradient PCR was used to make optimization for proper annealing temperature. After the determination of suitable annealing temperature resistant parental genotypes (Angelina- Regina) and susceptible parental genotypes (Sulu Saray-Kabuklu K-10) were tested with selected 60 SSR markers from Gong et al. (2008). PCR products showed that most of the SSR primers can bind to template DNA at 50℃ and 49℃. Some of these 60 markers were eliminated because of low band producibility. Primers producing fragments were used for the polymorphism test. 10 parental genotypes were used for PCR analysis. Polymorphic band formation can be shown as a result of amplification with 45 primers between resistant and susceptible genotypes. However, not all of these polymorphic bands are markers. Polymorphic band profile to be a marker, it should be determined in susceptible parents and susceptible F_2 individuals but not found in resistant parents and resistant F2 “bulk”. The same goes for vice versa. The polymorphic band should be determined in resistant parents and resistant F_2 individuals but not found in susceptible

parents and resistant F2 “bulk”. The band structure seen in F_2 mixtures can also be observed in F_2 individuals forming these mixtures.

Parental genotypes were investigated by performing CMTp131 marker. Figure 15 showed that Regina and Angelina genotypes generated two bands (160 bp and 125 bp) and Sulu Saray and Kabuklu K10 generated a single band (125 bp). F_2 individuals were screen to see discrimination between two different band profiles. Out of 4 individuals, 56 individuals had two bands but the other individuals had only one band. This marker indicates polymorphism and this marker can be used for further analyses.

The determination of base pair length with the CMTp142 marker was close between resistant and susceptible F_2 individuals. This primer is not considered polymorphism for F_2 individuals. It can only distinguish parental genotypes.

CMTp190 is a significant primer because this primer includes multiple polymorphisms and easily distinguishes resistant and susceptible parental genotypes. Resistance individuals include one band or no band and susceptible individuals include two bands that are approximately 196 bp and 99 bp lengths. F_2 individuals (Regina- Sulu Saray and Angelina –Sulu Saray) with screening CMTp190 primer demonstrated polymorphism. Resistant individuals showed only one band or no band. Screening with CMTm66 primer in parental genotypes, two band profile formation was created. Single and two bands were created as 160 bp and 130 bp lengths. PCR analysis with F_2 individuals produced two types of band profiles in both genotypes. They are important primers that easily provide results. These primers were thought will be helpful for hybrid seed production studies of commercial zucchini genotypes in Türkiye.

Parental genotypes and F_2 individuals of elite *C. pepo* germplasm were investigated to obtain the relationship between ZYMV resistance and SSR primers. CMTm66, CMTp131, and CMTp190 have been distinguished as polymorphic markers. Three markers could be used to determine polymorphism and to select resistant and susceptible individuals. F_2 individuals were screened and evaluated for ZYMV resistance via these primers (CMTm66, CMTp190, CMTp131). These primers could be thought of as a molecular marker genetically linked with ZYMV resistance. This molecular marker can be used for screening resistant varieties and lines regarding ZYMV in plant breeding programs.

In this study, instead of acquiring new molecular markers tightly linked to the ZYMV resistance in zucchini, a screening study of zucchini genotypes that have commercial importance in Türkiye was performed.

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APPENDICES

APPENDIX 1. SSR markers used for assessing genetic diversity

Linkage groups	SSR Primer	Forward	Reverse
LGp1	CMTP201	AggAgTggTgggCTAATACg	TgAAATTgAgggAgggAgAg
LGp1	CMTP193	ggTgACggCAAgAAAAgCTA	gCTgACCCTCTCTCCCTCTC
LGp2	CMTM48	TTTgCAgTACgCTgCAgAAC	ggATCCTTCTCgTggTggTA
LGp2	CMTP98	gCAgAggAgAAgTgggTTTg	CTTTATCCgACCAAgCgTTC
LGp2	CMTM209	ATTAgAATCCCTTggAgAACg	gCAATCgTTTTTCTgTTggT
LGp3a	CMTMC67	ACATgTgggTgCAATgAgg	CACCATTgTggAAAATgAAACA
LGp3a	CMTP55	gTTgTTAATAATCggAACAAAgC	AAgACAgAAggTCAgAgATATATgAAT
LGp3a	CMTP131	gCACTTgAATCTTCgTCAAC	CgAgAAAgAATTAACgAgCA
LGp3b	CMTP187	AACAATCCTCgCCTCAAATC	ATgAAAATgggAAgCCAgAg
LGp3b	CMTM42	ATTggTgCCgAAgCTATCAC	CCCACgTTATggAgCAgAAT
LGp4	CMTM97	AgggggAACTgATAgTCATCg	ACCTCACCTTCCATCgTCAC
LGp4	CMTM73	ATgCTCAgACATCCATgCAC	gCgAAAgATTACCgATgCTC
LGp4	CMTM120	gCCAAAggTTCCAAATgACA	TgATTTgCgCACAaACAAAC
LGp5	CMTP174	gCCggAACCAgACTTCTC	CCCTCCCTTCCCATTAAAC
LGp5	CMTM189	AATTATATAgCTggCTggATCg	AACATACAACAgCAgggAATg
LGp5	CMTP46	TTCCCTTCTgCAgAgATgCT	CCATgCgCATAATTgTATCg
LGp6	CMTM130	CCCTCCACCACCTCCTTAg	CAgggggATCATAAAAgTgg
LGp6	CMTM88	CATCgACATTCgCCTCATC	AggCAgCTTCCAAATCAgC
LGp6	CMTM252	CCCATTCCCCATTTCTTTCT	CAAATTCgATCggCTTTACAC
LGp7	CMTM144	ACATgggCATACCTCgAATC	CACCTggCTgTTTTgTCTgA
LGp7	CMTM170	TCAgACCCACTCCCATgAAC	AgCgAACACgTgAAAACgTC
LGp7	CMTM84	TATCACCAAACgTgCAT	AggTTTTgAAgAACAAATTC
LGp8	CMTM66	ACgCgTTgTTgTTAgTgTgg	ggggATTTATgAACCCAACAT
LGp8	CMTM7	AACCAAACTCCggCAAgA	gTTCTCTCCgTTCAggATgg
LGp8	CMTMC14	AAgCCgTgATggACAACATT	CCACCACCCCTgATTTTAACT

LGp9	CMTP182	CACgAAgATTTgATggCCTTA	ggATTgggATggTgAAgATg
LGp9	CMTP151	CggAgAAACTCgACACTCC	CCCAgCACCATCAggATAC
LGp9	CMTM261	ggTggCCTCTgAACAATTTC	ACCTAACCAATgggCATgAg
LGp10a	CMTM175	gAgTgATgTTTTgAgTAAACAg	CTTgTTCATCATCATCTgTg
LGp10a	CMTP258	gggCTACTTCCCCTAAAgATg	ACgATgCgTATTCACACTCAA
LGp10a	CMTP138	AAAggTTTCCACATCCTTg	gAAAAggAAAAAgTgTTCAAAg
LGp10b	CMTM19	gCATgggAgATgAAggTTAg	ATTTCCTggTggTATgAgATTC
LGp10b	CMTM183	CTTTgCATTgCCACTCTTCT	TgAgggAAATTgAATgTggT
LGp10b	CMTM229	CTggAgCAgCTgAAgATgTg	gCCAAAAATTAACggAAACg
LGp11	CMTM126	TTTCATgTTATgATATTgTCAg	TCCAAAgCTTACgTTTACT
LGp11	CMTP245	AggTAACTgCACCCCAgCTA	CCCATCTCTCAAgCTCATCC
LGp11	CMTM111	CTCCATTCCCATggCTTC	CCATgAgCTTgAgAgAggTg
LGp12	CMTP142	TCAACCAAgTgCCAATCTCA	ACTgATCCACCgACTgATACg
LGp12	CMTP35	CCAgATTCTAAAgCAAAgAg	ACATCTAAgCCTCTCTCTCTC
LGp12	CMTM131	TAgAgTTCgATCAATgCAA	gTTgATACTTCAACCACgTT
LGp13	CMTP68	CACACCCATTTCATTTTgACC	ATTgATTgggACgTgAggAA
LGp13	CMTM11	TggAAggATTCTCCCACAgT	TACAATTTgACgTCCgCAAg
LGp14	CMTM206	AggAACggAgggATAgAAAAgA	CCTTgTCCCCAAgTTgCATA
LGp14	CMTM65	gAACTTCgTgTgTgCgTgTC	TTgCTggAACTTCCTCTCgT
LGp14	CMTMC34	CgACgAATCTTTTgCTCCTC	CTCAAATCCTCCCTCACTCg
LGp15	CMTM129	TgATCgAATTgTggCTggT	AATCAATCTgCTCCgTCgTC
LGp15	CMTP17	ACTgCTCAATAAggCAAggA	AAACAAgAgTgCACAAACAgg
LGp15	CMTP86	gCTCTTggACAAgAATggTCA	ATggCTTCTggTggTTTgTC
LGp16	CMTP125	CTTgTTCCgCAgCATCAg	AgTgAgAgggAgACgCAAAg
LGp16	CMTP217	CACgACTCCCAgAATCAATC	gATTggAAACTCgCTCCATA
LGp16	CMTM247	CCAAAATTCACTAgAAgTAACgATCA	ATgCAACgAAgTTggggTAg
LGp17	CMTP208	CgTgAACATTCgTTTgTTgg	TCATCCgTTTCCTTTTCAgC
LGp17	CMTM157	ggACAgAgTggCTAggTCTgAgT	CCAAACAAgAgATgggCAgA

LGp17	CMTM224	gTgggTCgCTCCTTAAA	ATAgCggATAAAgTggACTg
LGp18	CMTM187	ATCggTgAgTCCCAAAAATg	ATCACAAAgCgggAAAACAC
LGp18	CMTP18	TgACATCACTCCggCAACTC	ACACCTTCgCTTCCgACATC
LGp18	CMTP190	CggggAAgAgAggTTTAggT	CCCATACATTCCCATATAACACC
LGp19	CMTP97	CCACACACCAATCgTTgAAg	CgCAgAATCTCgAAACACAA
LGp20	CMTM83	ggCATTTCTgAgAACAgCTT	ACgTTAgTTATgCTATTTTgTAggC
LGp20	CMTMC61	TTATTgTCgTCgCTCTgCAC	TCCggAACTATTATAACAACgACA

APPENDIX 2. Invitrogen 50 bp DNA Ladder