

T.C.
AKDENİZ UNIVERSITY



**MARKER ASSISTED BACKCROSS BREEDING FOR FUSARIUM WILT
(*FUSARIUM OXYSPORUM* SCHLECHT.
F. SP. *MELONGENAE*) IN EGGPLANT**

Derya SAMUR

**INSTITUTE OF NATURAL AND APPLIED SCIENCES
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MASTER DEGREE THESIS**

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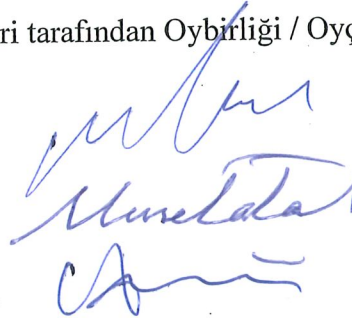
MASTER DEGREE THESIS

Bu tez 19/06/2019 tarihinde jüri tarafından Oybirliği / Oyçokluğu ile kabul edilmiştir.

Prof. Dr. Nedim MUTLU

Doç. Dr. Mürsel ÇATAL

Yrd. Doç. Dr. Hasan PINAR



ÖZET

MARKIR YARDIMLI GERİYE MELEZLEME İLE FUSARIUM SOLGUNLUĞUNA (*FUSARIUM OXYSPORUM* SCHLECHT. F. SP. *MELONGENAE*) KARŞI DAYANIKLI PATLICAN GELİŞTİRİLMESİ

Derya SAMUR

Yüksek Lisans Tezi, Tarımsal Biyoteknoloji Anabilim Dalı

Danışman: Prof. Dr. Nedim MUTLU

Haziran 2019; 42 sayfa

Patlıcan hem açık tarlada hem serada yetiştirilmekte ve hastalıklar önemli verim kaybına sebep olmaktadır. *Fusarium oxysporum* Schlecht. f. sp. *melongenae*, FOM) toprak kökenli bir patojen olup, fusarium solgunluğu hastalığına sebep olur. Markır yardımcı seleksiyonda kullanılmak üzere tek dominant genle kontrol edilen *Fusarium* solgunluğuna dayanıklılık veren gene bağlı moleküler markır daha önce geliştirilmiştir. Bu çalışmanın amacı markır yardımcı geriye melezleme yöntemi ile *Fusarium* solgunluğuna dayanıklı patlıcan hatlarının geliştirilmesidir. Donör ebeveynler “LS2436” (*Solanum melongenae*) hattından geliştirilen ve ilgili moleküler markır taşıyan *Fusarium* solgunluğuna karşı dayanıklı olduğu belirtilen ileri seviye patlıcan hatlarıdır. Patlıcan ıslah materyalleri ilk önce ilgili moleküler markırlar ile taranmış ve ardından FOM izolatu ile klasik testlemeye tabi tutulmuştur. 533 adet genç fide kök daldırma yöntemi ile BATEM (Antalya/Türkiye)’den temin edilen FOM izolatu ile test edilmiştir. Moleküler markırlar ile dayanıklı olarak belirlenen tüm fidelerin inokülasyonda da dayanıklı oldukları görülmüştür. Markır yardımcı seleksiyon ve geri melezleme BC1F1 generasyonuna kadar devam ettirilmiş olup BC1F1 fideleri moleküler markırla taranmış ve ardından klasik testleme yapılarak markırın işaret ettiği dayanıklı fidelerin klasik testlemede de dayanıklı olduğu sonucuna ulaşılmıştır. Dayanıklı: hassas oranı beklendiği gibi 1:1 olarak gözlenmiştir. Elde edilen sonuçlar markırın güvenilir olduğunu ve geliştirilecek olan *Fusarium oxysporum* f.sp. *melongeae*’ya dayanıklı patlıcan hatlarıyla ülkemizde sorun olan ve giderek yayılması beklenen FOM’a dayanıklı çeşitler geliştirilerek üreticilerin ekonomik anlamda zarar görmesi önlenebilecektir.

ANAHTAR KELİMELER: : Dayanıklılık, Fusarium Solgunluğu, Geriye Melezleme, Hastalık, Markır Yardımlı Seleksiyon, Patlıcan

JÜRİ: Prof. Dr. Nedim MUTLU

Doç. Dr. Mürsel ÇATAL

Dr. Öğr. Üyesi Hasan PINAR

ABSTRACT

MARKER ASSISTED BACKCROSS BREEDING FOR FUSARIUM WILT (*FUSARIUM OXYSPORUM* SCHLECHT.

F. SP. *MELONGENAE*) IN EGGPLANT

Derya SAMUR

MSc Thesis in Agricultural Biotechnology

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Eggplants are produced in both greenhouse and open field, and fungal diseases cause significant yield loss. *Fusarium oxysporum* Schlecht. f. sp. *melongenae*, FOM) is a major soil-borne pathogen, causing vascular wilt disease in eggplant. A molecular marker tightly linked to single dominant gene (FOM) was previously available for use in marker assisted selection (MAS). The aim of the study was to develop eggplant lines resistant to *Fusarium* wilt by using a marker assisted backcross breeding approach. Donor parents were advanced eggplant lines known to have fusarium wilt resistance originating from “LS2436” (*Solanum melongenae*) lines. The eggplant breeding materials was first screened with the molecular markers linked to the FOM gene. Then, the 533 young seedlings representing various populations claimed to be resistant to the pathogen were both root-dip inoculated with FOM isolate obtained from BATEM institute (Antalya, Turkey), and screened with the molecular marker. All the seedlings identified as resistant using the markers survived the infection. Marker assisted selection and backcross programme was continued to BC1F1. The seedlings of BC1F1 population along with the parents and checks were again screened with molecular marker and then classical test. Results showed that all the plants selected by MAS showed resistance response to FOM in classical test. Resistance vs susceptible ratio was 1:1 as expected in BC1F1 generation. In conclusion, the marker is reliable for selection against FOM and developing new eggplant cultivars resistant to FOM via marker assisted backcross selection is feasible.

KEYWORDS: Backcross, Disease, Eggplant, Fusarium Wilt, Marker Assisted Selection, Resistance

COMMITTEE: Prof. Dr. Nedim MUTLU

Assoc. Prof. Dr. Mürsel ÇATAL

Assist. Prof. Dr. Hasan PINAR

ACKNOWLEDGEMENT

The aim of the study is to develop eggplant (*Solanum Melongenae*) lines resistant to Fusarium wilt using a marker assisted backcross breeding approach. This makes available economic damage to producers can be prevented and also breeding with marker assisted selection will save time for breeders.

First I would like to thank to my supervisor, Prof. Dr. Nedim MUTLU, for his help, guidance, encouragement and scientific advice throughout my time as his student.

I would like to thank my manager (Dr. Duran Şimşek) and all colleagues from AXIA Tohum Co. for sharing their experiences with me.

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AKADEMİK BEYAN

Yüksek Lisans Tezi olarak sunduđum “Marker Assisted Backcross Breeding for Fusarium Wilt (*Fusarium Oxysporum* Schlecht. F. Sp. *Melongenae*) in Eggplant” adlı bu çalışmanın, akademik kurallar ve etik deđerlere uygun olarak yazıldığını belirtir, bu tez çalışmasında bana ait olmayan tüm bilgilerin kaynađını gösterdiğimi beyan ederim.

19/06/2019

Derya Samur



SYMBOLS and ABBREVIATIONS

Symbols

cM	: Centimorgan
mm	: Millimeter
μL	: Micro liter
Kb	: Kilobase
PDA	: Potato Dextrose Agar
g	: Gram
hr	: Hour
Mm	: Millimeter
°C	: Celsius temperature
%	: Percent
mg/kg	: Milligram per kilogram
∞	: Infinity

Abbreviations

AU	: Akdeniz University
BATEM	: Batı Akdeniz Tarımsal Araştırma Enstitüsü Müdürlüğü
CC-NBSLRR	: Contained Coil-Nucleotide Binding Site-Leucin Rich Repeat
CTAB	: Cetyl Trimethyl Ammonium Bromide
DNA	: Deoksiribo Nükleik Asit
FAO	: Food and Agriculture Organization
FOM	: <i>Fusarium oxysporum</i> Schlecht. f. sp. <i>Melongenae</i>
MAS	: Marker Assisted Selection

PCR : Polymerase Chain Reaction

QTL :Quantitative Trait Loci

TÜİK : Türkiye İstatistik Kurumu

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1. INTRODUCTION

Eggplant (*Solanum melongena* L.) is the third most economically important Solanaceous crop after potato and tomato (Rotino et al. 2005). Eggplant is one of the most cultivated fruit plants worldwide with an 1.858.253 ha harvested area, and more than 50 million tones production in the world. (FAOS 2019)

Production areas are mainly within subtropical zone for both greenhouse and openfield. World production area and total yield is condensed in Asia, Africa, Mediterranean Basin and South America (Mutlu et al. 2008).

In European countries, eggplant is an outlandish vegetable but in Asia and the Mediterranean it is an important and valuable nutrient ingredient, it is called king of vegetables (Şekara et al. 2007).

Eggplant is susceptible to various diseases especially fusarium, verticillum and bacterial wilt (Kalloo 1993). Soil-borne diseases (e.g. bacterial and fungal wilts), are the most serious diseases reducing the yield and quality of eggplants both in greenhouse and in open field cultivations (Sihachakr et al. 1994). Fungal wilts caused by *Verticillium dahliae* (Vd) Kleb. (Fradin et al. 2006) and *Fusarium oxysporum* f. sp. *melongenae* (FOM) (Cappelli et al. 1995) are two main fungal diseases in eggplant.

Fusarium wilt, is one of the most devastating and widespread disease of eggplant. Matsuo and Ishigami was published first study for Fusarium Wilt then, fundemental researches have printed with the aim of identifying resistant eggplant allies.

The fungus penetrates into the roots and proliferates in the vascular tissue. Wilting progresses from lower to upper leaves, followed by collapse of the plant. When the stem and roots are cut diagonally, reddish-brown streaks are visible in the vascular tissues. (Altinok 2005).The pathogen can live for many years in the soil (Nelson et al. 1983; Katan et al. 1999; Altinok et al. 2006).

Fungicides can not control Fusarium wilt effectively, other solutions, such as soil fumigation or grafting might work well but they are either additional cost or hazardous to the environment and humanity. (Lee et al. 1994; Fradin et al. 2006; King et al. 2010).

For economic and safety reasons, resistant crop breeding is the most efficient way to avoid this disease (Kaur et al. 2014).

Fusarim wilt resistance source has been identified in *Solanum aethiopicum* Gilo Group and *S. aethiopicum* Aculeatum Group which are *Solanum melongena*'s relatives. (Daunay et al. 2001; Mutlu et al. 2008).These genotypes are LS1934, LS174, and LS2436 which have been defined to be completely resistance source (Mochizoki et al. 1997; Monma et al.1997; Sakata et al. 1996). Rfosa1 is a single dominant gene identified as a resistance locus with cleaved amplified polymorphic sequances, CAPS. tightly linked to gene of interest. (Toppino et al. 2008). SCAR markers linked to a Fusarium Resistance locus in eggplant line, LS2436 with bulked segragant analyses published by Mutlu in 2018.

Conventional breeding and molecular marker analysis can be used to increase disease resistance and improve yield traits for cultivated eggplant.

A molecular marker tightly linked to single dominant gene (FOM) was developed for use in marker assisted selection (MAS).

The aim of this study is to develop eggplant lines resistant to Fusarium wilt using a marker assisted backcross breeding approach. This makes available economic damage to producers can be prevented. and also breeding with molecular marker assisted will save time for breeders.

2. LITERATURE REVIEW

2.1. Definition, Origin, Cultivation and Domestication of Eggplant

Eggplant (*Solanum melongena* L.) belongs to the wide Solanaceae (nightshade family), contains ~3,000 species distributed in 90 genera (Vorontsova et al. 2012). The *Solanum* genus divided into 13 clades, eggplant is under the *Leptostemonum* clade which is known as the “spiny *Solanum*” group due to the presence of sharp epidermal prickles on stems and leaves (Vorontsova et al. 2013).

Solanum L. is one of the enormous genera have about 2300 species (Sekara et al. 2007). The number of species in the *Solanum* genus is reported differently according to different sources (Sakata et al. 1994, Isshiki et al. 1994c, Lester 1997, Daunay et al. 1998) Important crops such as potato (*Solanum tuberosum* L.) and tomato (*Solanum lycopersicum* L.), as well as many other minor crops. Eggplant ranks third economically important crop in Solanaceae family after potato and tomato. All eggplant production data were taken from FAO (Food and Agriculture Organization of United Nations) (FAOS 2018)

Subgenus *Leptostemonum* contains more than 400 species distributed worldwide (Knapp et al. 2013), many of them originated in the New World (Vorontsova et al.2012).

Eggplant (*Solanum melongena*) is probably originated in India (Daunayet al. 2008).

It has spread to all Asian countries and to Europe through Anatolia. In Turkey, eggplant has been cultivated since the beginning of the 17th century.

According to scients; the origin of eggplant is *S. melongena* may have been indirectly derived from the wild *S. incanum*, domesticated in India and Southeast of China. *S. aethiopicum* and *S. macrocarpon* were domesticated in Africa from their wild relatives *S. anguivi* and *S. dasyphyllum* (Lester et al. 1998)

Eggplant has three close relatives, endemic to the Old World, belonging to the genus *Solanum* L. subgenus *Leptostemonum*

- *S.melongena*
- *S. macrocarpon*
- *S. aethiopicum*

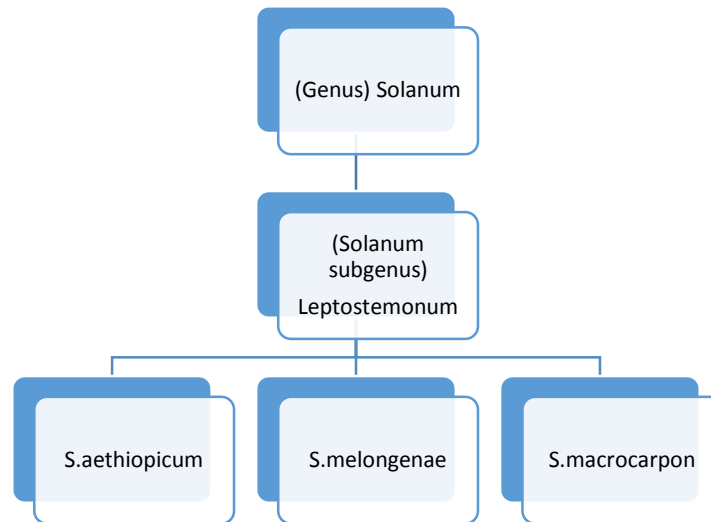


Figure 2.1. *Solanum melongena*'s cultivated relatives in the genus *Solanum* L., subgenus *Leptostemonum*

Eggplant has a basic chromosome number of $n = 12$ (Chiarini et al. 2010) and is an autogamous diploid with $2n=24$ (Şekara et al. 2007) a genome size of approximately 956 Mbp (Bennett et al. 2004).

Eggplant takes its name from its shape and (Lester et al. 1998) egg-like form varieties in USA and Canada, Europe calls it Aubergine and Asia and Africa knows as Brinjal. There are also other known names are melongen, garden egg, and guinea squash (Nothmann et al.1986, Choudhury et al. 1995, Lawande et al.1998, Daunay et al. 1999 and Kashyap et al. 2003).

2.2. Economic Value

Eggplant is a very popular native vegetable in Asia and the Mediterranean basin. China (17 mln tons per year) and India (8 mln tons), are the two countries which are the primary cultivation centers and have the highest production (Lawande et al.1998, Lester et al.1998, Daunay et al. 2001, Doganlar et al. 2002a, Doganlar et al. 2002b, FAOS 2018). After India and China cultivation spread to Japan and today Japan is the important producer (Frery et al. 2007). Entrance to the West was primarily around the Mediterranean region which is the secondary “domestication region” and covers Turkey (0.8 mln ton), Syria, and Persia (Nothmann et al.1986, Daunay et al. 2001, Kashyap et al. 2003).

The average yield is extremely variable, depending on climate, cultural system, crop duration and grower technology. The Netherlands is the number one country with yields of 390 tones per hectare (Doganlar et al. 2002a). Later on; other Mediterranean countries such as Italy, Spain, France, and Greece became eggplant producers (Lawande et al. 1998, Daunay et al. 2001, Frery et al. 2007). Today, Turkey ranks the first in Europe

in terms of total eggplant production (Economic Research Service, USDA 2017). There is a wide difference in the yield of eggplant production which is due to the growth environment, technology and varieties.

Overall, eggplant is now a globally cultivated plant species (Daunay et al. 2001, Doganlar et al. 2002a). The world production was approximately about 52 million tons in 2017, China with about 32 million tons and India with 12 million tons are the greatest producers (FAOS 2017). Eggplant is a winter-spring vegetable for greenhouse and summer vegetable for open field production and consumption in Turkey.

Depends on FAOS, Turkey ranks fourth in top ten eggplant producers in the world, other countries production quantities and harvested area information are given in the below.

Table 2.1. Area and production of eggplant in the top ten producer countries in the world

	Area Harvested (ha)	Production (tonnes)
China	786.266	32.908.763
India	733.000	12.510.000
Egypt	48.253	1.307.793
Turkey	25.592	883.917
Iran	21.255	654.149
Indonesia	43.905	535.436
Japan	9160	307.800
Italy	9449	286.473
Philipinnes	21.446	241.901
Spain	3580	225.912

The global eggplant production reaches nearly 50 million tones in 2 million hectar in 2016.

Table 2.2. Total production and yield quantities of eggplants in the world

Production / Yield Quantities of Eggplants in the World (Total)	
World area harvested eggplants (ha)	1.858.253
World production eggplants (tones)	52.309.119

Table 2.3. List of global harvested area for important solenaceaces group vegetables (FAOS 2017)

Area harvested (Global, 2017)	
Potatoes	19.302.642
Tomatoes	4.848.384
Eggplants	1.858.253
Pepper	568.299

2.3. Types of Eggplant

Eggplant has three main botanical varieties under the species *melongena*. *S. melongena* is characterized by morphological diversity. Usually consumed and sold types of eggplants are the produced from these varieties. (Nothmann et al. 1986; Lawande et al. 1998 and Kashyap et al. 2003).

These varieties are;

- 1) Var. *esculentum* - The round, oval or egg-shaped cultivars (common eggplant)
- 2) Var. *serpentinum* - The long, slender types (snake eggplant)
- 3) Var. *depressum* - The tiny fruits and messy plants (dwarf eggplant)

2.4. Fruit Diversity in Eggplant

Eggplants species have several morphological qualities; color of fruit, shape, flavour are the important traits that show differences among individuals (Collonnier et al. 2001; Kashyap et al. 2003; Nothmann et al. 1986, Daunay et al. 2001; Frary et al. 2007).

Eggplant fruit sizes may vary considerably in weight and length (Nothmann et al. 1986; Daunay et al. 2001). They range from nearly round through an elongated tear drop shape to long and cylindrical. Two color pigments, chlorophyll a and b and anthocyanins which are in different amounts and in combination controls the color of the fruit (Nothmann et al. 1986; Daunay et al. 2001; Frary et al. 2007). These color pigments effects for appearance for fruit controlled by more than one gene (Nothmann et al. 1986; Frary et al. 2007).

Fruit colors range varies from shiny black, light, dark purple, green, yellowish, white. Skin color uniformity may vary striped or spotted color.

Fruit length is between 4-45 cm, and thickness 2-35 cm, at different shapes and weight ranging between 15-1500 g. The fruits are set as single or in clusters, up to 5 fruits. Physiologically ripe fruits become brown, red or yellow (Swarup et al. 1995)

Bitterness might be occurred in taste of eggplant, different amount of chemicals and there are many types of eggplant are cultivated in Turkey round, semi-long and long and they used commonly in the Turkish kitchen. Although the morphological varieties of Turkish eggplant are distinctive.



(a)

(b)

(c)

Figure 2.2. Examples of various eggplant fruit types from the field

a) Eggplant in different colors and shapes; **b)** Topan type; **c)** Long cylindrical type

2.5. Growth Habit of Eggplant

Growing climate for eggplant spreads in wide temperate climate conditions to cover most of the world (Sihachakr et al. 1994). Eggplant (*Solanum melongena* L.) is a warm-loving plant that requires warm to hot conditions over a 5-6 months, between 22-30°C growing period for produce high efficiency and qualified fruit (Nothmann et al. 1986; Lawande et al.1998). During the growing period cool weather affect negatively plant growth and reduce yields. The plant is a biennial which is grown as an annual in general (Nothmann et al. 1986). Autogamy or self-pollination is the usual way of fertilization although cross-pollination is also possible by insect (Nothmann et al. 1986; Lawande et al. 1998; Daunay et al. 2001; Frary et al. 2007).

2.6. Nutritional and Medicinal Value

Eggplant has high content phenolic compounds such as chlorogenic acid in the fruit flesh and anthocyanins in the fruit skin (Mennella et al. 2012). Both phenolic acids and anthocyanins are beneficial for human health, helps slow the processes associated with aging and protects against many chronic diseases (Cao et al.1996; Plazas et al. 2013; Braga et al.2016).

Leaf extracts of eggplants have been used as a medicine to remedy for asthma, bronchitis, cholera and dysuria; fruits and the tissue are important for decreasing blood cholesterol (Kashyap et al. 2003).

Eggplant is a very low caloric healthiest vegetable has high content of vitamins, minerals and bioactive compounds for human health is also rich in bioflavonoids, which can provide protection from stomach cancer and atropine, nicotine and capsaicin are alkaloid derivatives that have impacts on the neural system and epithelium (Raigon et al. 2008; Plazas et al. 2014b; Docimo et al. 2016). Several chemicals that help lower cholesterol have been detected in eggplant, it has also potassium, magnesium, fiber help to maintain human health. It is known as ‘sodom apple’ and important for traditional medicine in East Africa it is used for chest pain, toothache, fever, stomachache and indigestion.

Eggplants also have negative effects on some people and cause an allergic reaction (Siddanakoppalu N. and Yeldur P. 2004).

2.7. Production Studies of Eggplant in Turkey

Turkey is an important country in producing Eggplant (*Solanum melongena L.*), which is according to FAOS, 2018 also, the production of eggplant in Turkey is varies between regions, and the largest region in eggplant production is Antalya. There are many different morphological types of the cultivated eggplant. Oblong or round fruited-types are used as stuffed or preserved, while the long cylindrical types used as grilled, fried or stuffed; and large or semi-long oblong types used as stewed or fried. Due to the huge demand on eggplant in Turkey, and other Middle Eastern and Asian countries, breeders are forced to improve many types. The most eggplant growing regions of Turkey are in western, southern and southeastern Anatolia, also there are some areas in the central Anatolia. Eggplant can be cultivated either in open fields or under cover. According to FAOS (2018), Turkey produce about 800,000 tons of eggplant every year, that cover 33,000 ha area.

2.8. Biotic and Abiotic Stresses

Biotic and abiotic stresses are significantly important for reducing productivity and severe affect of growth for eggplants.

These stresses should be control with urgent attention from horticulturalists either through breeding or by appropriate agronomic management (King et al. 2010; Schwarz et al. 2010).

Tomato (*Solanum lycopersicum*), eggplant (*Solanum melongena*) and chili/sweet pepper (*Capsicum spp.*) are three such vegetable crops of global importance threatened by common biotic and abiotic stresses to production.(Keatinge et al. 2014)

Eggplant has a comparatively long growth period and it is more exposed than other vegetable crops to a broad range of plant diseases, pests, nematodes, and weeds.(Keatinge et al. 2014)

Eggplant is under pressure for various insect pests including mites, whiteflies, aphids, eggplant fruit, and shoot borer, leafhopper, thrips, spotted beetles, leaf roller, stem borer, and blister beetle (Rotino et al. 1997; Kalloo et al. 1993; Sihachakr et al. 1994; Medakker et al. 2007).

Unpredictable weather with extreme temperatures, drought or flooding can sharply reduce yield and fruit quality.

Eggplant is susceptible to numerous diseases, these are bacterial wilt, verticillium wilt, fusarium wilt, anthracnose fruit rot, alternaria rot, damping off, phytophthora blight, phomopsis blight and fruit rot, leaf spot, little leaf of brinjal, and mosaic (Rotino et al., 1997).

Resistance to pest and diseases is really important due to general susceptibility to these agents in eggplant which results in serious effects on production and yield (Lawande and Chavan 1998; Daunay et al. 2001; Collonnier et al. 2001).

2.9. Biotic Stresses

Biotic stresses; diseases affecting eggplants are given in the table. (Source-www.seminis-us.com)

Table 2.4. List of biotic stresses and diseases for the eggplant production

Common Name in English	Common Name in Turkish	Factor
Late Blight	Domates Mildiyö Hastalığı	Phytophthora infestans
Early Blight	Patlıcan Erken Yanıklık Hastalığı	Alternaria solani
Cercospora Leaf Spot	Bakteriyel Benek Hastalığı	Cercospora spp.
Powdery Mildew Disease	Patlıcangillerde Külleme Hastalığı	Leveillula taurica
	Kök Çürüklüğü (Çökerten) Hastalığı	
Phythium Root Rot		Phythium spp.
Damping Off		Rhizoctonia spp
Fusarium Wilt		Fusarium spp
Leaf spot		Alternaria spp
Verticillium wilt		Verticillium dahliae
Bacterial Wilt	Bakteriyel Solgunluk	Ralstonia solanacearum
Sclerotinia Rot	Sebzelerde Beyaz Çürüklükler	Sclerotinia sclerotiorum
Grey Mould	Sebzelerde Kurşuni Küf Hastalığı	Botrytis cinerea
Torrado	Sebzelerde Beyaz Sinek Tütün beyazsineği	Bemisia tabaci
Whiteflies	Sera beyazsineği	Trialeurodes vaporariorum
Cutworms	Sebzelerde Bozkurt	Agrotis spp.
Crickets	Sebzelerde Dana Burnu	Gryllotalpa gryllotalpa
Mites	Kırmızı Örümcekler	
Two spotted mite	İki noktalı kırmızı örümcek	Tetranychus urtic
Mites	Pamuk kırmızı örümceği	Tetranychus cinnabarinus
Mites	Atlantik akarı	Tetranychus atlanticu
Green Vegetable Bug	Piskokulu Yeşil Böcek	Nezara viridula
Cotton Leafhopper	Sebzelerde Pamuk Yaprak Kurdu	Spodoptera littoralis
Cluster Caterpillar	Sebzelerde Tel Kurdu	Agriotes spp.
Aphids	Sebzelerde Yaprakbitleri	
Aphids	Bakla yaprakbiti	Aphis fabae
Aphids	Şeftali yaprakbiti	Myzus persicae
Aphids	Patates yaprakbiti	Macrosiphum euphorbiae
Aphids	Pamuk yaprakbiti	Aphis gossypii
Leafminer	Yaprak Galeri Sinekleri	
Broad mite	Sebzelerde Sarı Çay Akarı	Polyphagotarsonemus latus
Leafhoppers	Sebzelerde Yaprak Pireleri	Empoasca decipiens Paoli
Mites	Doömates Pas Akarı	Aculops lycopersici
Helioverpa	Sebzelerde Yeşilkurt [Heliothis armigera, Heliothis virescens]	Heliothis dipsacea
Western Flower Thrips	Sebzelerde Tripsler Tütün tripsi (Thrips tabaci) Çiçek tripsi	Frankliniella occidentalis
Tomato Spotted Wilt	Domates Lekeli Solgunluk Virüsü	Tospovirus
Root-knot nematodes	Kök-ur Nematodu	Meloidogyne javanica

2.10. Abiotic Stresses

- drought
- low or high temperatures
- salinity

2.11. *Fusarium* Wilt

Fusarium oxysporum Schlecht. f. sp. *melongenae*, FOM is a major soil-borne pathogen and one of the causal agents of vascular wilt disease threatening eggplant production in both greenhouses and open field. The disease causes economical loss and greatly reduces the yield and quality of eggplants (Kenneth et al. 1970; Kishi et al. 1974; Stravato et al. 1993; Urrutia et al. 2004; Altinok et al. 2005).

The fungus proliferates into vascular tissues from the roots to the upper leaves, first leaves colors turn slightly yellowish then followed by browning eventually plant fades completely. When the stem and roots are cut diagonally, brownish streaks can easily be visible in the vascular tissues (Mutlu et al.2008).

The fungus lives in the soil for several years and spreads by equipment, irrigation water and infected plant debris. According to researchs, FOM widely distributed in Turkey and it was also reported in other countries such as USA, Korea, Spain and China (Altinok et al. 2005).

Favorable conditions are warm soil temperatures (24-27° C) and high soil moisture for rapid disease development.



(a)

(b)

Figure 2.3. Comparison of fusarium wilt symptoms on eggplant seedlings and leaves (a) Symptoms on eggplant seedlings; (b) Comparison of a leaf from an infected eggplant (left) with a leaf from a healthy plant (right)

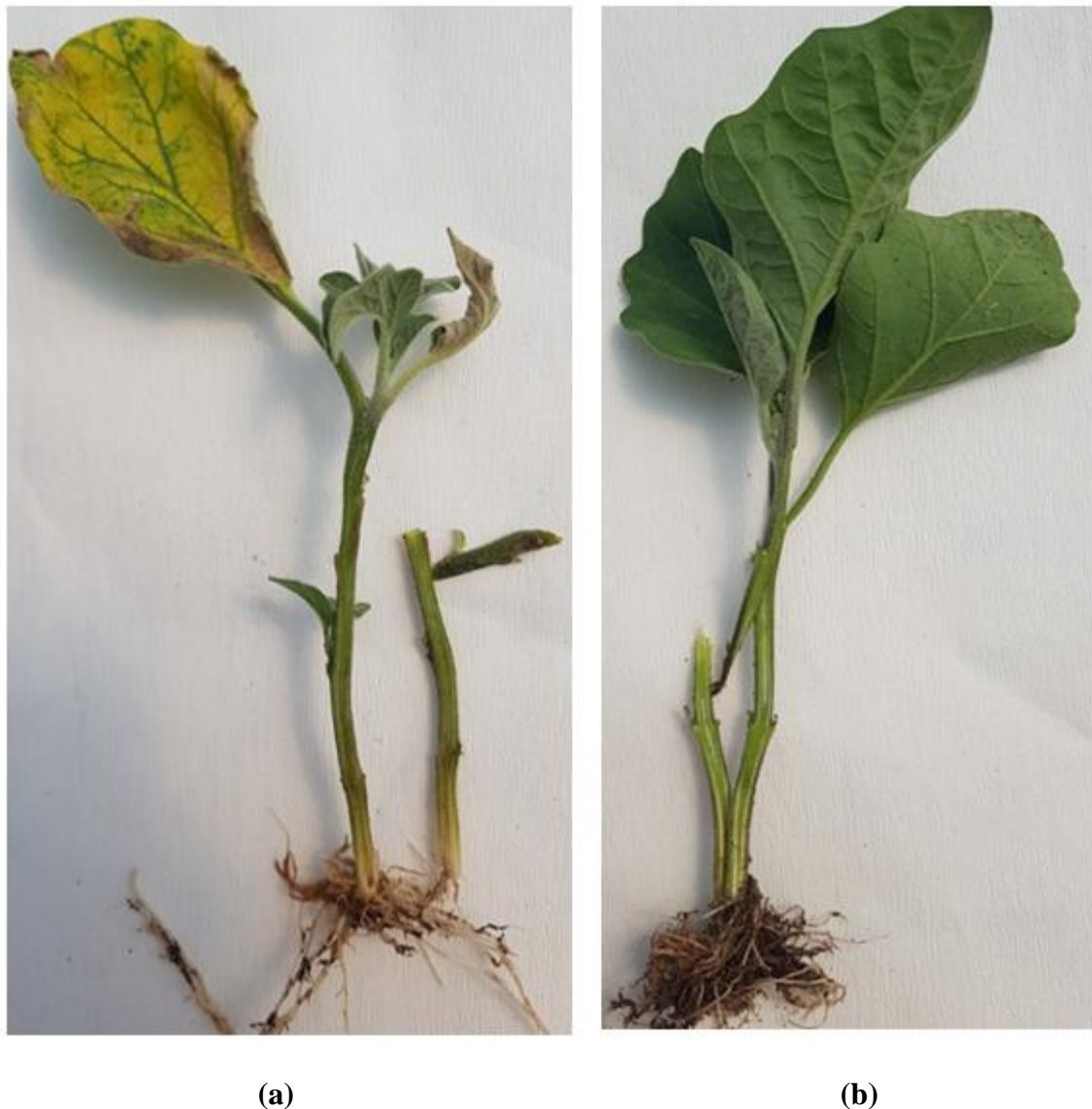


Figure 2.4. (a) Discoloured vascular tissue of the infected stem; (b) non infected

2.12. Molecular Studies for Resistance to Fusarium Wilt

First *F. oxysporum melongenae* (FOM) report was published in Japan in 1958 by Matsuo and Ishigami. Since this date, a significant number of studies have been carried out to identify resistant eggplant allies.

Resistance to *F. oxysporum f.sp. melongenae* has been identified in *S. melongenae* L. (Abdullaheva et al. 1988; Komochi et al. 1996; Mandhare et al. 1993) and *S. indicum*, *S. aethiopicum* L. aculeatum Group (*S. integrifolium*), *S. torvum*, *S. incanum*, *S. violaceum*, *S. sisymbriifolium* (Rizza et al. 2002, Gousset et al. 2005, Boyaci et al. 2010)

Three eggplant germplasms, LS1934, LS174, and LS2436, have been identified for resistance to the *Fusarium* wilt. (Mochizuki et al. 1997; Monma et al. 1997; Sakata et al. 1996). In 2014, the eggplant draft genome sequence was published (Hirakawa et al. 2014). *Fusarium* wilt resistance also studied in various economically important

horticultural crops such as tomato and melon. In tomato, four resistance genes, *I*, *II*, *I2*, and *I3*, derived from wild species (*Solanum pennellii* and *Solanum pimpinellifolium*) have been identified (Bournival et al. 1990; Hemming et al. 2004; Lim et al. 2008; Ori et al. 1997; Sarfatti et al. 1989; 1991; Segal et al. 1992; Simons et al. 1998). In melon, four resistance genes, *Fom-1*, *Fom-2*, *Fom-3*, and *Fom-4*, and a number of quantitative trait loci (QTLs) were reported (Brotman et al. 2005, 2012; Herman et al. 2008; Joobeur et al. 2004; Oumouloud et al. 2008, 2010; Perchepied et al. 2005; Tezuka et al. 2011; Wang et al. 2000; Zink et al. 1985). Tomato *I2* and melon *Fom-1* and *Fom-2* have been subjected to map-based cloning. All these three genes contained coil-nucleotide binding site-leucine rich repeat (CC-NBSLRR) class of plant resistance genes (Brotman et al. 2012; Joobeur et al. 2004; Simons et al. 1998).

There are 3 main studies carried –out for resistance locus for FOM

- 1) The resistance locus; *Rfo-sal*, which confers resistance to *Fusarium oxysporum* are LS1934, LS174, and LS2436 which have been defined to be completely resistance source were from an eggplant ally *Solanum aethiopicum* gr. *Gilo* on chromosome 2 (Barchi et al. 2010).
- 2) For the first time in cultivated eggplant line resistance locus from LS2436 based on bulked segregant analysis, was mapped on chromosome 4 (Mutlu et al. 2008). Sources of partial resistance to Fom and designated two markers were also detected in Asian landraces and introgressed in European eggplant genotypes (Mutlu et al., 2008).
- 3) Resistance locus with two alleles derived from LS1934, based on eggplant genome sequence by Fukuoka et al. 2012 and Hirakawa et al. 2014.

2.13. Control of Fusarium

Control of soilborne plant pathogen is difficult, because pathogen survives for many years in the soil.

Cultural practices include appropriate planting, use of clean materials, use of resistant crop cultivars, and use of clean tools, crop rotation and intercropping.

A long crop rotation (4-6 years) with cereals and grasses avoiding the use of any solanaceous crop is an alternative to reduce the fungal inoculum levels (Mishra et al. 1986).

Many of these methods can be used however, cultural control is not radical solution for the disease (Altinok et al. 2005; Daunay et al. 2008; Safikhani et al. 2013; Steekelenburg et al. 1976).

Physical methods such as solarization of the soil and heat treatment before planting can be combined with cultural methods for effective control but eggplant also produced in open field. Therefore solarization is not a good solution for open field cultivation

Strategies to control the disease by soil fumigation are either costly or only applied for greenhouse production (Gullino et al. 2002; Mandhare et al. 1993)

Use of chemical fungicides accumulates toxin in the environment and create residue problems, damage the nature and human health also extra cost for producer.

There have been several researches about Rhizospheric microorganisms to control soilborne plant pathogens such as *Trichoderma viride*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Aspergillus niger*.

Currently the preferred and efficient method for control of soilborne fungal diseases is to take advantage of resistant cultivars. Developing a resistant cultivar is a radical solution for controlling these disease, and the latest advances in molecular marker analyses can facilitate efficient breeding programmes.

Resistant commercial cultivars are yet to be developed, however, some eggplant cultivars susceptible to Fomg are grafted on resistant eggplant rootstocks. Such grafted plants are presenting a good level of resistance against Fomg so the best control method for environmental and financial reasons is planting resistant host plant (Gisbert et al. 2011; Altinok et al. 2014). Currently there are FOM resistant commercial eggplant rootstocks on which susceptible eggplant cultivars are grafted (Sato et al. 2004). Grafting productive scions onto resistant rootstocks has been a common practice to overcome such stresses in last years, especially in East Asia in both the Solanaceae and Cucurbitaceae (Lee 1994;Schwarz et al. 2010).

Grafting is a solution for producer but it is an extra cost. Producer pays for seedling of desired varity and also pays for resistant rootstock. Grafting vegetables on resistant rootstocks is a means of controlling root-knot nematodes and other soil-borne diseases in areas with intensive land use (Ioannou 2001; Kacjan Mars& Osvald 2004; Lopez-Perez et al. 2006; King et al. 2010; Rivard 2010).

The World Vegetable Center recommends rootstock accessions to grafting for Fusarium (Keatinge et al. 2014)



Eggplant rootstock accessions to grafting for Fusarium wilt recomended by World Vegetable Center:

- V1045276
- V1046104
- V1046101

3. MATERIAL AND METHOD

The study was carried out during February,2018 - May,2019 at Axia Tohum Co. Antalya, Turkey. Time table of the study is shown in the table below.

Table 3.1. The steps and outputs of the MAS backcross program started in 2018

2018-1(February)	1) 192 Leaf samples taken from advance lines of eggplant breeding materials
	2) Molecular marker analyse for FOM and Classical Test
	3) Resistant plants are determined for backcross breeding
2018-1	Resistant (Donor) Parents X Susceptible (Recurrent) Parents  F1 FOM Resistance transferred by crossing to susceptible lines
2018-2	F1 X Susceptible (Recurrent) Parents  BC1F1 (Expected ratio: resistant:susceptible 1:1)
2019-1 (March)	Molecular marker analyse and classical test for BC1F1 plants

3.1. Plant Materials

Identification of Donor (Resistant) Parents

The resistance sources, P-R1, P-R2, P-R3 are cultivated eggplant advanced breeding lines developed from 'LS2436' which is a FOM resistant *S. melongena* genotype (Monma et al. 1996). The advanced breeding lines were thought to have Fusarium wilt resistance. The plant materials were first screened using molecular markers linked to FOM resistance (Mutlu et al, 2008). Then, both marker positive and negative plant materials were tested using Fusarium oxysporum f.sp. melongae isolate (BATEM institute, Antalya, Turkey) in a root dip inoculation method. The lines that were identified to be resistant using both the molecular marker and inoculation were used as donor parents to transfer FOM resistance into susceptible breeding material.

The susceptible advanced breeding lines P-S1, P-S2, P-S3, P-S4, P-S5, P-S6 were used as recurrent material in the experiment and hybridised with donor parents P-R1, P-R2, P-R3 to obtain F1 plants. Then, F1 plants were backcrossed to the recurrent parents in the following season, fall of 2018, to create segregating BC1F1 populations.

The seeds of BC1F1, the donor and recurrent parents were sown, and 6 weeks later seedlings were ready for classical and molecular test.

Table 3.2. List of the leaf samples taken from eggplant materials for first screening using molecular markers linked to FOM resistance

12.02.2018 Leaf Samples List							
Plate 1				Plate 2			
Plate no	Variety Name	Plate no	Variety Name	Plate no	Variety Name	Plate no	Variety Name
1-1	Amedeo F1	1-49	T 121 AÇ 5	2-1	P 217 AÇ 1	2-49	P 227 AÇ 4
1-2	A 117 F1	1-50	T 121 AÇ 6	2-2	P 217 AÇ 2	2-50	P 227 AÇ 5
1-3	Angela F1	1-51	P 202 AÇ 1	2-3	P 217 AÇ 3	2-51	P 230 AÇ 1
1-4	P 58AÇ	1-52	P 202 AÇ 2	2-4	P 217 AÇ 4	2-52	P 230 AÇ 2
1-5	P 59 AÇ 1	1-53	P 202 AÇ 3	2-5	P 217 AÇ 5	2-53	P 230 AÇ 3
1-6	P 61 AÇ 1	1-54	P 202 AÇ 4	2-6	P 218 AÇ 1	2-54	P 230 AÇ 4
1-7	P 63 AÇ 1	1-55	P 202 AÇ 5	2-7	P 218 AÇ 2	2-55	P 230 AÇ 5
1-8	P 91 AÇ 1	1-56	P 29 AÇ 1	2-8	P-R1	2-56	T 124 AÇ 1
1-9	P 91 AÇ 2	1-57	P 209 AÇ 2	2-9	P 218 AÇ 4	2-57	T 124 AÇ 2
1-10	P 91 AÇ 3	1-58	P 209 AÇ 3	2-10	P 218 AÇ 5	2-58	T 124 AÇ 3
1-11	P 91 AÇ 4	1-59	P 209 AÇ 4	2-11	P 219 AÇ 1	2-59	T 124 AÇ 4
1-12	P 91 AÇ 5	1-60	P 209 AÇ 5	2-12	P 219 AÇ 2	2-60	T 124 AÇ 5
1-13	P 201 AÇ 6	1-61	P 210 AÇ 1	2-13	P 219 AÇ 3	2-61	T 25 AÇ 1

Continuation of the Table 3.2.

1-14	P 201 AÇ 7	1-62	P 210 AÇ 2	2-14	P 219 AÇ 4	2-62	T 25 AÇ 2
1-15	P 201 AÇ 8	1-63	P- R2	2-15	P 219 AÇ 5	2-63	T 25 AÇ 3
1-16	P 201 AÇ 9	1-64	P 210 AÇ 4	2-16	P 220 AÇ 1	2-64	T 25 AÇ 4
1-17	P 201 AÇ 10	1-65	P-R3	2-17	P 220 AÇ 2	2-65	T 25 AÇ 5
1-18	P 201 AÇ 11	1-66	P 211 AÇ 1	2-18	P 220 AÇ 3	2-66	T 26 AÇ 1
1-19	P 201 AÇ 12	1-67	P 211 AÇ 2	2-19	P 220 AÇ 4	2-67	T 26 AÇ 2
1-20	P 201 AÇ 13	1-68	P 211 AÇ 3	2-20	P 220 AÇ 5	2-68	T 26 AÇ 3
1-21	P 201 AÇ 14	1-69	P 211 AÇ 4	2-21	P 221 AÇ 1	2-69	T 26 AÇ 4
1-22	P 201 AÇ 15	1-70	P 211 AÇ 5	2-22	P 221 AÇ 2	2-70	T 26 AÇ 5
1-23	P 201 AÇ 16	1-71	P 212 AÇ 1	2-23	P 221 AÇ 3	2-71	T 27 AÇ 1
1-24	P 201 AÇ 17	1-72	P 212 AÇ 2	2-24	P 221 AÇ 4	2-72	T 27 AÇ 2
1-25	T 122 AÇ 1	1-73	P 212 AÇ 3	2-25	P 221 AÇ 5	2-73	T 27 AÇ 3
1-26	T 122 AÇ 2	1-74	P 212 AÇ 4	2-26	P 222 AÇ 1	2-74	T 27 AÇ 4
1-27	T 122 AÇ 3	1-75	P 212 AÇ 5	2-27	P 222 AÇ 2	2-75	T 27 AÇ 5
1-28	T 122 AÇ 4	1-76	P 213 AÇ 1	2-28	P 222 AÇ 3	2-76	T 28 AÇ 1
1-29	T 122 AÇ 5	1-77	P 213 AÇ 2	2-29	P 222 AÇ 4	2-77	T 128 AÇ 2

Continuation of the Table 3.2.

1-30	T 122 AÇ 6	1-78	P 213 AÇ 3	2-30	P 222 AÇ 5	2-78	T 128 AÇ 3
1-31	T 122 AÇ 7	1-79	P 213 AÇ 4	2-31	P 223 AÇ 1	2-79	T 128 AÇ 4
1-32	T 122 AÇ 8	1-80	P 213 AÇ 5	2-32	P 223 AÇ 2	2-80	T 128 AÇ 5
1-33	T 122 AÇ 9	1-81	P 214 AÇ 1	2-33	P 223 AÇ 3	2-81	T 129 AÇ 1
1-34	T 122 AÇ 10	1-82	P 214 AÇ 2	2-34	P 223 AÇ 4	2-82	T 129 AÇ 2
1-35	T 119 AÇ 1	1-83	P 214 AÇ 3	2-35	P 223 AÇ 5	2-83	T 129 AÇ 3
1-36	T 119 AÇ 2	1-84	P 214 AÇ 4	2-36	P 224 AÇ 1	2-84	T 129 AÇ 4
1-37	T 119 AÇ 3	1-85	P 214 AÇ 5	2-37	P 224 AÇ 2	2-85	T 129 AÇ 5
1-38	T 119 AÇ 4	1-86	P 25 AÇ 1	2-38	P 224 AÇ 3	2-86	T 130 AÇ 1
1-39	T 120 AÇ 1	1-87	P 215 AÇ 2	2-39	P 224 AÇ 4	2-87	T 131 AÇ 1
1-40	T 120 AÇ 2	1-88	P 215 AÇ 3	2-40	P 224 AÇ 5	2-88	T 131 AÇ 2
1-41	T 120 AÇ 3	1-89	P 215 AÇ 4	2-41	P 225 AÇ 1	2-89	T 131 AÇ 3
1-42	T 120 AÇ 4	1-90	P 215 AÇ 5	2-42	P 225 AÇ 2	2-90	T 132 AÇ 1
1-43	T 120 AÇ 5	1-91	P 216 AÇ 1	2-43	P 225 AÇ 3	2-91	T 132 AÇ 2
1-44	T 120 AÇ 6	1-92	P 216 AÇ 2	2-44	P 225 AÇ 4	2-92	T 132 AÇ 3
1-45	T 121 AÇ 1	1-93	P 216 AÇ 3	2-45	P 225 AÇ 5	2-93	T 132 AÇ 4

Continuation of the Table 3.2.

1-46	T 121 AÇ 2	1-94	P 216 AÇ 4	2-46	P 227 AÇ 1	2-94	T 132 AÇ 5
1-47	T 121 AÇ 3	1-95	P 216 AÇ 5	2-47	P 227 AÇ 2	2-95	T 131 AÇ 4
1-48	T 121 AÇ 4	1-96	P 216 AÇ 6	2-48	P 227 AÇ 3	2-96	T 131 AÇ 5



(a)



(b)

Figure 3.1. Plants and fruits characteristics of both resistant and susceptible parents

(a) Resistant parents; (b) Susceptible parents

3.2. Classical Test for Fusarium Wilt Resistance Using *Fusarium Oxysporum f.sp. Melongae* Isolate

Classical test was carried out twice, first one was in April,2018 to verify the resistance to FOM, and the second one was in April 2019 to confirm resistance of segregating BC1F1 progenies. The *Fusarium oxysporum f.sp. melongae* isolate was supplied from BATEM institute, Antalya, Turkey.

The *Fusarium oxysporum f.sp. melongenae* isolate, was grown on the potato dextrose agar (PDA) at 24°C in dark for 10 days. Liquid medium were prepared from this culture. Liquid cultures were shaken at 50 rpm in a rotary shaker for 8 days at 24 to 25°C. The suspensions were filtered through cheesecloth. The spores were resuspended and spore density was adjusted to 1×10^6 conidia/ml.

The roots of seedlings were washed with clean top water to clear of soil. The 1/3 of roots were first trimmed with a sterile scissor to create scar tissue to promote infection. Wounded roots were submerged into the beaker that contain 10^6 concentration of FOM isolate for 5 minutes (Herman et al. 2007; Karimi et al. 2010). For control groups, 12 seedlings from each of parents submerged either into distilled water or into FOM isolate. The seedlings were planted into small pots and maintained in nursery.

Seedlings were planted into 48-well trays containing sterile torf. After inoculation, seedlings were kept at 27°C/18°C for 12-h photoperiod. Five weeks after inoculation disease symptoms were recorded; 1 (Resistant) no symptoms of disease and 0 (Susceptible) dead plant.



(a)

(b)

Figure 3.2. (a),(b) The roots of seedlings were washed with clean top water to cleared of soil



(a)



(b)

Figure 3.3. (a), (b) Wounded roots were submerged into the beaker that contain 10^6 concentration of FOM isolate for 5 minutes



(a)



(b)

Figure 3.4. (a), (b) The seedlings were planted into small pots and maintained in nursery

3.3. Molecular Marker Screening

Marker assisted selection were performed twice; first analysis was in February, 2018 to verify the resistance of donor parents against FOM, and second analysis was in April 2019 for selection of resistant BC₁F₁ plants among segregating progenies.

3.4. DNA Extraction

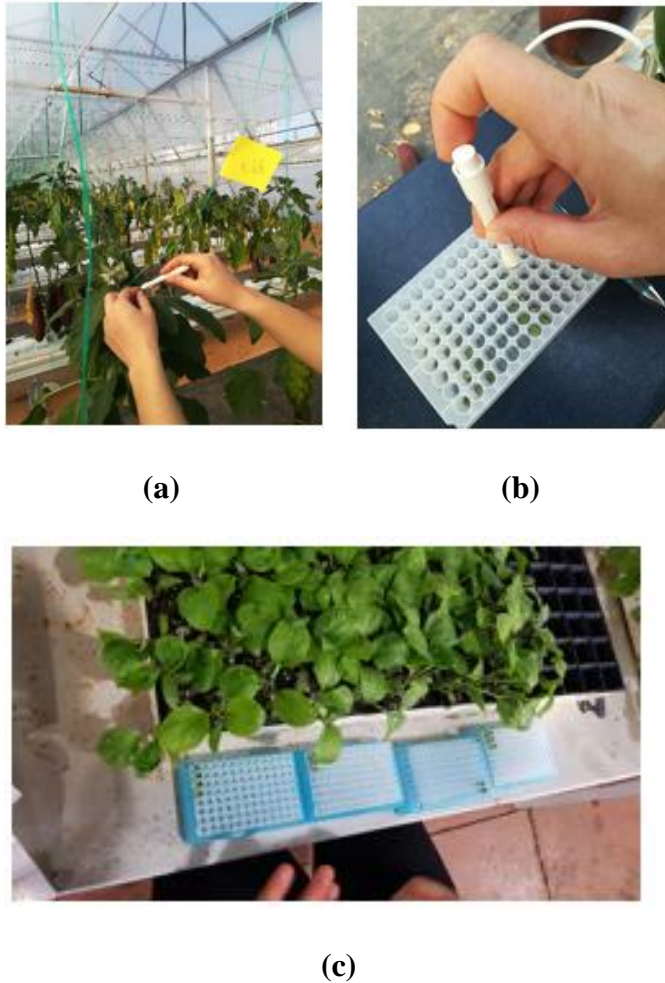


Figure 3.5. (a), (b) Taking leaf samples from young leaves of eggplants in 2018; (c) Taking leaf samples, in 2018 and 2019

DNA of parents, F1, and BC1F1 plants was extracted from young leaves using a modified CTAB extraction protocol (Doyle and Doyle 1990). Rapid and efficient disruption of leaf samples was carried out using Qiagen Tissulyser for DNA isolation. From fresh young leaves of eggplants, 200 mg of samples were taken and placed in 1.2ml microcentrifuge tubes containing 1 stainless steel bead. Then, 150 μ l CTAB (%2) and mercaptoethanol mix added in tubes. Lids of tubes closed carefully and placed in tissulyser, which was run for 15-30 minutes with 50 Hz. The samples were briefly centrifuged, 300 μ l CTAB and mercaptoethanol were added. The bottom cover of plates was removed and were incubated in water bath at 65 ° C for 2 hours. After incubation, 450 μ l chloroform (24 mikroliter chloroform, 1 mikroliter isoamyl alcohol, 24:1) was added and plates were turned upside down carefully. Then, the plates were centrifuged at 4000 rpm for 20 minutes. The DNA was pelleted by spinning with a centrifuge and the supernatant removed. The 300 μ l isopropanol was added onto the samples and stored at -20 ° C over night.

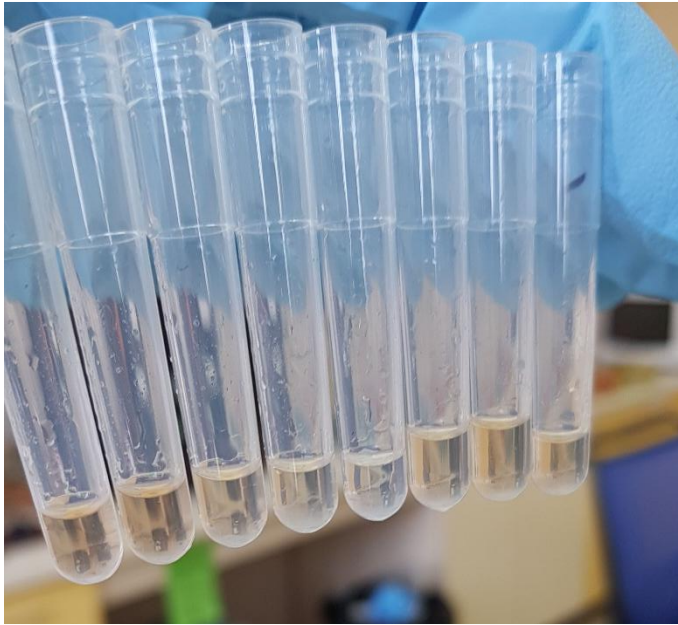


Figure 3.6. Extracted DNA Pellet

Next day samples were centrifuged again at 4000 rpm for 20 minutes. Pellet was seen at the bottom of tube and liquid was gently discarded without disturbing pellet. Then, 300 μ l of 70% ethanol was added on pellet and was centrifuged for 15 min at 4000 rpm. Ethanol was discarded, 300 μ l new 70% ethanol was added again and centrifuged for 10 min at 4000 rpm. Ethanol was discarded and pellet was left to dry in room temperature with open lid. Then, 150 μ l sterile distilled water was added to pellet and was kept 1 day at 4°C. The DNA was stored at -20°C until used.

3.5. PCR amplification

PCR reactions were performed in 15 μ L volumes in Akdeniz University, Agricultural Biotechnology Laboratory (MJ RESEARCH PTC-225 Peltier Thermal Cycler). All PCR products were separated on a 1.5% agarose gel (Thermoscientific Gel Tank), visualized with ethidium bromide staining under ultraviolet light, and photographed with Minilumi, DNR Bio-Imaging Systems.

SCAR426 primer was used in the study to determine the FOM Resistance (Mutlu et al. 2008).

Table 3.3. PCR reagents used in SCAR analyses

Components	Quantity
DNA	2 μ L
10x PCR Buffer ((NH ₄) ₂ SO ₄)	1.5 μ L
25 Mm MgCl ₂	1.5 μ L
5 U/ μ L Taq DNA polymerase	0.2 μ L
5 Mm dNTP	1.5 μ L
SCAR Forward Primer	1.8 μ L
SCAR Reverse Primer	1.8 μ L
H ₂ O	4.7 μ L
Total	15 μL

Table 3.4. The list of PCR cycle steps

Steps	Temperature	Time	Cycle
1	94 °C	3 min	
2	94 °C	30 sec	
3	57 °C	59 sec	
4	72 °C	59 sec	
5	GO TO Step 2		
6	72 °C	10:10 sec	
7	55 °C	30 sec	
8	45 °C	45 sec	
9	35 °C	45 sec	
10	25 °C	45 sec	
11	END		

Table 3.5. Forward and reverse primer sequences and melting temperature for SCAR 426 markers (Mutlu et.al. 2008)

Primer	Forward Sequences (5'-3')	Melting Temperature
SCAR 426	TGA GTC CAA ACC GGA CTA CAAG	62.1
Primer	Reverse Sequence (5'-3')	Melting Temperature
SCAR 426	GAC TGC GTA CGA ATT AAC TCT ACG	63.5

3.6. Gel Electrophoresis

To view PCR product under UV light 1,5% agarose gel was used. (400 ml TBE buffer solution, 6 g agarose, 6 μ L ethidium bromide)

TBE buffer solution, 108 g tris, 55 g boric acid and 7,5 g EDTA were solved in 1000 ml distilled water, to make 10X stock solution. This stock solution was diluted with 9 L distilled water to the 1X solution.

5 μ L loading dye (15 ml glycerol, 35 pure water, 0.05 g bromophenol blue) added to the PCR product and samples were loaded on gel. The 3 μ L of 1 kb DNA Ladder (Thermo, GeneRuller) was loaded in the first well of agarose gels. . Products were run at 110 V for 1 hour. (Thermo-Fisher Scientific Tank) Agarose gels were visualized by Minilumi, DNR Bio-Imaging Systems, and results were recorded.

4. RESULTS

4.1. Detection of the Fusarium Wilt Resistance Gene in Eggplant Breeding Materials using Molecular Markers

The SCAR426 marker was used to screen the breeding materials thought to be resistant to FOM. Of the 192 eggplant genotypes at F4 to F8 generation, 3 genotypes were identified as resistant and all the rest were susceptible (Table 4.1)

Table 4.1. Number of Resistant and susceptible plants obtained from the first screening with SCAR 426 marker. 3 donor parents with Fusarium Resistance determined

Resistant Plants	Susceptible Plants	Total plant
3 (P-R1, P-R2, P-R3)	189	192

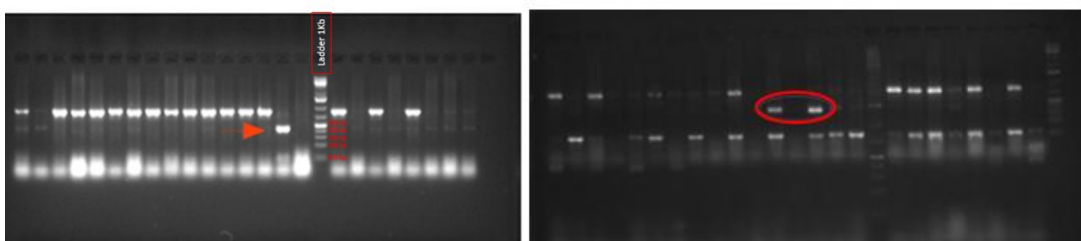


Figure 4.1. Molecular analyses with SCAR426 marker, in 2018 where resistant allele is marked with red arrow.(Ladder, L=1 Kb.) (a) One genotype is marked with red arrow indicates resistance gene of interest is between 400bp and 500 bp, expected size is 426 bp); (b)Two different genotype are marked with red circle, size of the allele; 426bp

4.2. Confirmation of Resistance with Classical Test

To confirm status of the three genotypes that was found to be resistant in molecular marker analysis, and to further verify response of the eggplant genotypes against FOM, inoculation test using FOM isolate was carried out for 533 seedlings, representing 20 lines at F1 to F6 generations

The seedlings were root-dip inoculated with FOM isolate obtained from BATEM institute, Antalya, Turkey.

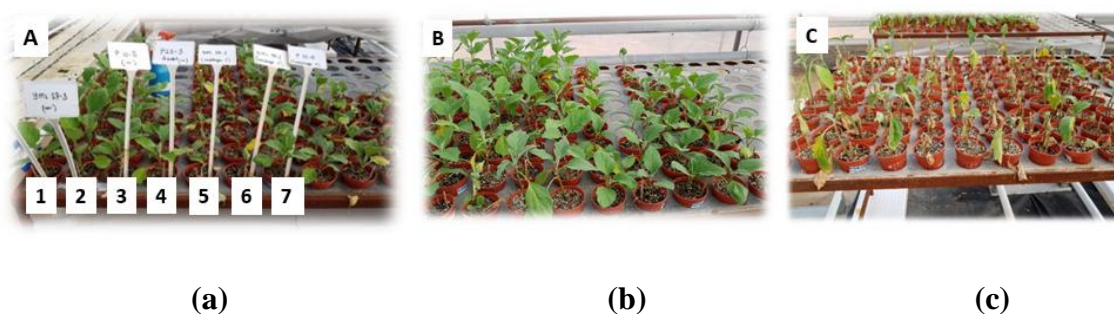


Figure 4.2. Root dip inoculation with *Fusarium oxysporum* f.sp. *melongea* isolate in 2018

a) Control group plants; first 4 rows are non-inoculated susceptible and resistant plants with no symptoms, 5th and 6th rows are susceptible plants showing symptoms after inoculation, 7th row is resistant plants with no symptoms after inoculation (b) Resistant plants with no symptoms after inoculation, 2018 (c) Susceptible plants showing symptoms of yellowing and wilting after inoculation

Table 4.2. Classical test results, 2018

	Variety	Inoculated Plant No	Results		Non- inoculated Plant No	Results	
			Dead	Healthy		Dead	Healthy
Susceptible	PY 09-1	10	8	2	10	0	10
Susceptible	PY 08-3	10	8	2	10	0	10
Resistant	P R-1	10	0	10	5	0	5
Resistant	P R-2	8	0	8	4	0	4
	PBF-1	5	2	3			
	PBF-2	5	0	5			
	PBF-3	5	0	5			
	PBF-4	5	4	1			
	PBF-5	5	0	5			
	PBF-6	5	0	5			
	PBF-7	5	0	5			
	PBF-8	5	0	5			
	P T22	82	0	82			
	P 101	91	25	66			
Resistant	P R-3	124	8	116			
	P 118	158	98	60			
TOTAL		533	153	380			

The three different genotypes marked as resistant using molecular marker were also observed as resistant with classical test. The PR-1 and PR-2 lines were fully resistant without any disease symptoms, however, 6.5% of the PR-3 line showed susceptible reaction, indicating segregation or heterogeneity within the line for FOM resistance.

After identifying the resistance material with molecular marker and confirming the resistance with inoculation, the three (3) resistant lines were used as donor parent in order transfer the resistance gene into advanced susceptible eggplant parents. Pollens were taken from donor parents and recurrent parents were emasculated, then, pollens were carefully placed on recurrent plants stigma. The flower was marked and observed for growth and F1 fruits were harvested.

Table 4.3. The hybridizations between donor and recurrent parents to obtain F1 hybrids

PS-1 (RECURRENT S) X PR-1(DONOR RR)	PS-1 (RECURRENT S) X PR-2(DONOR RR)	PS-1 (RECURRENT S) X PR-3(DONOR RR)
PS-2 (RECURRENT S) X PR-1(DONOR RR)	PS-2 (RECURRENT S) X PR-2(DONOR RR)	PS-2 (RECURRENT S) X PR-3(DONOR RR)
PS-3 (RECURRENT S) X PR-1(DONOR RR)	PS-3 (RECURRENT S) X PR-2(DONOR RR)	PS-3 (RECURRENT S) X PR-3(DONOR RR)
PS-4 (RECURRENT S) X PR-1(DONOR RR)	PS-4 (RECURRENT S) X PR-2(DONOR RR)	PS-4 (RECURRENT S) X PR-3(DONOR RR)
PS-5 (RECURRENT S) X PR-1(DONOR RR)	PS-5 (RECURRENT S) X PR-2(DONOR RR)	PS-5 (RECURRENT S) X PR-3(DONOR RR)
PS-6(RECURRENT S) X PR-1(DONOR RR)	PS-6(RECURRENT S) X PR-2(DONOR RR)	PS-6(RECURRENT S) X PR-3(DONOR RR)

The heterozygous resistant F1 seeds were harvested from recurrent parents, the seeds were sown, and seedlings were grown in a nursery at Axia Tohum Co., Antalya, Turkey.

The heterozygous F1 plants were then used as maternal lines where recurrent parents were also planted in greenhouse. Pollen from recurrent parents were transferred to the maternal individuals (F1) which were heterozygous resistant for FOM.

The pollinated flowers were labeled, and BC1F1 seeds were harvested at the end of season.

Table 4.4. The hybridizations between F1 and recurrent parents to obtain BC1F1 populations in Fall 2018.

			BC1F1
PS-1 (RECURRENT S)XPR-1(DONOR RR)	X	PS-1 (RECURRENT S)	PBC 1A-1
PS-1 (RECURRENT S)XPR-2(DONOR RR)	X	PS-1 (RECURRENT S)	PBC 1A-2
PS-1 (RECURRENT S)XPR-3(DONOR RR)	X	PS-1 (RECURRENT S)	PBC 1A-3
PS-2 (RECURRENT S)XPR-1(DONOR RR)	X	PS-2 (RECURRENT S)	PBC 2A-1
PS-2 (RECURRENT S)XPR-2(DONOR RR)	X	PS-2 (RECURRENT S)	PBC 2A-2
PS-2 (RECURRENT S)XPR-3(DONOR RR)	X	PS-2 (RECURRENT S)	PBC 2A-3
PS-3 (RECURRENT S)XPR-1(DONOR RR)	X	PS-3 (RECURRENT S)	PBC 3A-1
PS-3 (RECURRENT S)XPR-2(DONOR RR)	X	PS-3 (RECURRENT S)	PBC 3A-2
PS-3 (RECURRENT S)XPR-3(DONOR RR)	X	PS-3 (RECURRENT S)	PBC 3A-3
PS-4 (RECURRENT S)XPR-1(DONOR RR)	X	PS-4 (RECURRENT S)	PBC 4A-1
PS-4 (RECURRENT S)XPR-2(DONOR RR)	X	PS-4 (RECURRENT S)	PBC 4A-2
PS-4 (RECURRENT S)XPR-3(DONOR RR)	X	PS-4 (RECURRENT S)	PBC 4A-3
PS-5 (RECURRENT S)XPR-1(DONOR RR)	X	PS-5 (RECURRENT S)	PBC 5A-1
PS-5 (RECURRENT S)XPR-2(DONOR RR)	X	PS-5 (RECURRENT S)	PBC 5A-2
PS-5 (RECURRENT S)XPR-3(DONOR RR)	X	PS-5 (RECURRENT S)	PBC 5A-3
PS-6 (RECURRENT S)XPR-1(DONOR RR)	X	PS-6 (RECURRENT S)	PBC 6A-1
PS-6 (RECURRENT S)XPR-2(DONOR RR)	X	PS-6 (RECURRENT S)	PBC 6A-2
PS-6 (RECURRENT S)XPR-3(DONOR RR)	X	PS-6 (RECURRENT S)	PBC 6A-3

BC1F1 fruits were harvested, seeds were sown and seedling were grown in nursery at Axia in January, 2019. BC1F1 plants were screened with both molecular markers and root-dip inoculation method using FOM isolate on March, 2019.

Of the 18 BC1F1 populations derived from crosses involving the three resistant donor parent and six recurrent parent, 24 plants and 12 plants were tested either with inoculum or molecular markers, respectively.



Figure 4.3. Number of plants for selected plants for classical test and molecular analyse



Figure 4.4. Root dip inoculation with *Fusarium Oxysporum* symptoms on eggplant seedlings in 2019

Table 4.5. The result list of classical tests in 2019

Classical Test Results					
		Number of plants inoculated with FOM	Number of plants non-inoculated with FOM	Number of Dead Plants	Number of Live Plants
Resistant (Parent)	P R-1	12	3	0	15
Resistant (Parent)	P R-2	3	3	0	6
Resistant (Parent)	P R-3	3	3	0	6
Susceptible (control)	YML 9-1	8	8	8	8
Susceptible (Parent)	P S-1	3	3	3	3
Susceptible (Parent)	P S-2	3	3	3	3
Susceptible (Parent)	P S-3	3	3	3	3
Susceptible (Parent)	P S-4	3	3	3	3
Susceptible (Parent)	P S-5	3	3	3	3
Susceptible (Parent)	P S-6	not enough plant			
PBC 1A-1		24		11	13
PBC 1A-2		24		6	18
PBC 1A-4		24		19	5
PBC 2A-1		24		16	8
PBC 2A-2		24		12	12
PBC 2A-4		24		6	18
PBC 3A-1		24		20	4
PBC 3A-2		24		10	14
PBC 3A-4		24		12	12
PBC 4A-1		24		9	15
PBC 4A-2		24		15	9
PBC 4A-4		24		15	9
PBC 5A -1		24		11	13
PBC 5A -2		24		10	14
PBC 5A -4		24		15	9
PBC 6A-1		24		12	12
PBC 6A-2		24		4	20
PBC 6A-4		24		7	17
Total		432		210	222

Table 4.6. The result list of molecular tests in 2019

Molecular Analyse Results								
			Number of plants inoculated with FOM	Number of plants non-inoculated with FOM	Number of Dead Plants	Number of Live Plants	Number of plants analysed with SCAR Marker	Number of bands on gel
PBC 1A-2			24		6	18	12	5
PBC 2A-1			24		16	8	12	6
PBC 2A-2			24		12	12	12	5
PBC 3A-4			24		9	15	12	6
PBC 4A-4			24		11	13	12	5
PBC 5A -1			24		10	14	12	5
PBC 5A -4			24		4	20	12	8
TOTAL							84	40

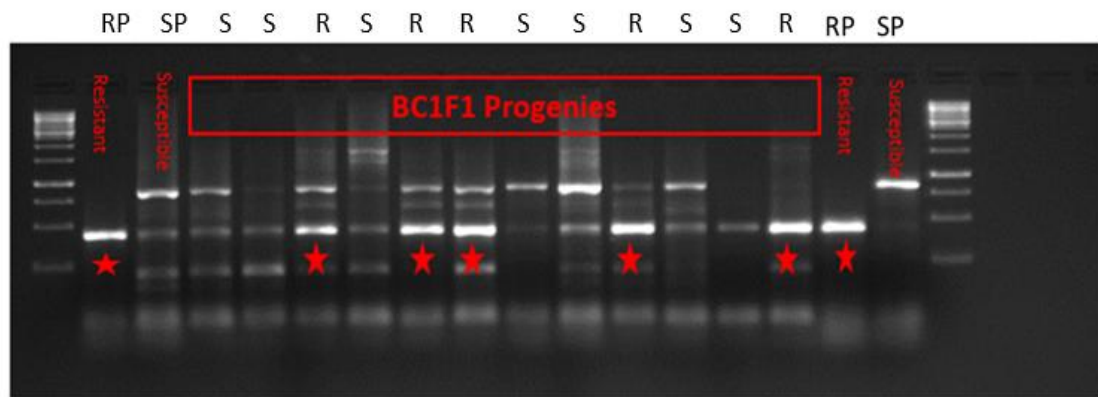


Figure 4.5. Molecular analyses with SCAR marker, in 2019 L=1kb ladder; (Resistant bands have been marked with red color and R=Resistant, S=Susceptible)

A chi-square goodness-of-fit test with 5 % significance level was used to test any deviation from single gene. The expected segregation ratio for BC1F1 progenies was 1:1 (resistant: susceptible). Because there two groups, resistant vs susceptible, the degrees of freedom (df) is $n-1=2-1=1$.

Degrees of freedom (df) = 2 classes (Resistant and Susceptible) - 1

df =n-1=2-1=1

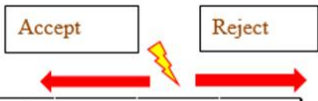
Pearson's Chi Squared test:

$$X^2 = \sum \frac{(O - E)^2}{E}$$

Null hypothesis

O = the number of observed, data collected

E = proportion of expected, data prediction

Table 4.7. Percentage points of the Chi-Square distribution


Accept Reject

←-----|-----→

Percentage Points of the Chi-Square Distribution									
Degrees of Freedom	Probability of a larger value x2								
	0.99	0.95	0.90	0.75	0.50	0.25	0.10	0.05	0.01
1	0.000	0.004	0.016	0.102	0.455	1.32	2.71	3.84	6.63
2	0.020	0.103	0.211	0.575	1.385	2.77	4.61	5.99	9.21
3	0.115	0.352	0.584	1.212	2.366	4.11	6.25	7.81	11.34
4	0.297	0.711	1.064	1.923	3.357	5.39	7.78	9.49	13.28
5	0.554	1.145	1.610	2.675	4.351	6.63	9.24	11.07	15.09

Table 4.8. The result of Chi-Square for all BC1F1 plants

Chi-Square for all BC1F1 plants					
	Obs	Exp	(Obs-Exp)	(Obs-Exp) ²	(obs-Exp) ² /Exp
Resistant	222	216	6	36	0,166666667
Susceptible	210	216	-6	36	0,166666667
Total Plants	432	432	Sum (Chi Squared Value)=		0,333333333

Table 4.9. Reaction of BC1F1 plants from resistant and susceptible eggplant cross to *Fusarium oxysporum* Schlecht. f.sp. *melongenae* isolate

Population	Resistant plants (no.)	Susceptible plants (no.)	Expected ratio	χ^2	Probability (P)
BC1F1	222	210	1:1	0.82	0.99

Table 4.10. Reaction of BC1F1 plants from resistant and susceptible eggplant cross to FOM marker SCAR 426

Population	Resistant plants (no.)	Susceptible plants (no.)	Expected ratio	χ^2	Probability (P)
BC1F1	40	44	1:1	0.76	0.96

In total, 105 heterozygous BC1F1 lines planted in greenhouse were evaluated for resistance against *Fusarium*. None of them show any symptoms after inoculation demonstrating that are resistant against *Fusarium*. Furthermore, the dominant SCAR marker, SCAR 426 was used to select the individual lines that possess gene of interest. 53 individual lines were heterozygous resistant against *Fusarium*.

5. DISCUSSION

Results confirmed the monogenic dominant inheritance of the fusarium wilt resistance derived from resistant eggplant breeding lines PR-1, PR-2, PR-3 and introgressed into susceptible advanced eggplant breeding materials.

Resistant materials derived from crosses involving LS2436. Previous studies also confirmed that LS2436 possessed single dominant gene for resistance against FOM. The SCAR426 marker was developed by Mutlu et al.2008 using LS2436 resistance source where the marker was 1.2 cM away from the gene. 1cM of genetic distance is approximately equal to 1% recombination. In another words, 1.2% probability of recombination during meiosis between the marker and the gene, and from every 100 plants selected based solely on marker results, 98 of them are expected to have the resistance gene while 2 plants may be susceptible have recombination.

The reliability of SCAR426 was validated in both breeding materials and segregating BC1F1 populations as confirmed with FOM inoculations. The marker offers many advantages, allowing seedling-stage selection of resistant genotypes without inoculation, waiting period and symptom detection.

In a similar study involving fusarium resistance in eggplant, a dominant single gene, *Rfo-sa1* for fusarium resistance in a somatic hybrid line introgressed from *Solanum aethiopicum* gr.Gilo was reported and it is mapped at the end of chromosome 2 (Barchi et al.2010, Portis et al.2014).

In recent years, the eggplant draft genome sequence was published by Hirakawa et al.2014 and followed by another study revealed that the responsible locus named as *FMI* mapped at the exact same position with *Rfo-sa1*. However *FMI* resistance derived from LS1934, the resistance loci originated from different species also geographically distant areas were mapped.

Linkage drag is the transfer of undesirable chromosome segments along with the gene of interest. Recombination can remove linkage drag. But, the removal of linkage drag depends upon the type of linkage between the genes. If it is complete linkage, it may be difficult to remove that drag. Therefore, introgression of resistance from closer species to recurrent material might help to prevent transfer of undesired genes. We have successfully transferred resistance gene against FOM from a cultivated-type donor parent into six different recurrent parents. Added resistance into advanced breeding lines is expected to protect the hybrids from FOM infection, nullifying the need for chemical application against the pathogen. The resistant hybrids would reduce the production cost, prevent yield loss due to FOM infection.

6. CONCLUSIONS

Eggplant is economically important crop from Solanaceae family. It has a good amount of nutrition for human health. Producing eggplant is ranked third after tomato and potato in world-wide

There are many diseases infect eggplant, fusarium wilt is one of the most destructive for producing eggplant in green house and open field. There is no efficient solution to protect the crop from this soil-borne disease. Pesticides and cultural solutions are extra cost and hazardous for environment. Preferred and efficient method for control of this soilborne fungal disease is to using resistant cultivars.

Fusarium wilt, caused by *Fusarium oxysporum* Schlecht. f. sp. *melongenae*, and Resistance for FOM identified in eggplant cultivars with significant studies.

In this study; in 2018 we screened our eggplant breeding materials with molecular analyses using SCAR 426 marker and then to confirm the markers reliability we inoculated the seedlings with *Fusarium Oxyporum* isolate, supplied from BATEM, Antalya.

Both classical and molecular analyses allowed to obtain resistant materials and these resistant materials have been marked as donor parents for backcross program.

With marker assisted backcross program we introgressed the resistancy to sensitive advanced eggplant lines. In 2019, BC1 plants obtained and tested with classical and molecular analyses.

Our results showed we successfully introgressed the gene of interest for resistance to FOM to the susceptible lines. This makes available to use marker for breeding programs to develop new resistant and eggplant lines.

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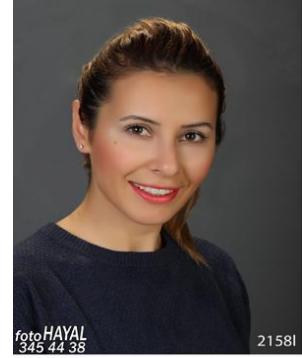
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