MASTER THESIS

NİĞDE ÖMER HALİSDEMİR UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

T.R.
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GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF AGRICULTURAL GENETIC ENGINEERING

DETERMINATION OF RESISTANCE LEVELS OF SOME ONION CULTIVARS OR INBREED LINES WITH FUSARIUM TESTING AT SEEDLING STAGE

EBRAR KARABULUT

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Supervisor

Assist. Prof. Dr. Ali Fuat GÖKÇE

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Ebrar KARABULUT tarafından **Dr. Öğr. Üyesi Ali Fuat GÖKÇE** danışmanlığında hazırlanan "**Determination of resistance levels of some onion cultivars or inbreed lines with Fusarium testing at seedling stage**" adlı bu çalışma jürimiz tarafından Niğde Ömer Halisdemir Üniversitesi Fen Bilimleri Enstitüsü **Tarımsal Genetik Mühendisliği Anabilim Dalı**'nda Yüksek Lisans (İngilizce) tezi olarak kabul edilmiştir.

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Prof. Dr. Murat BARUT
MÜDÜR

THESIS CERTIFICATION

I announce that all the information in the thesis has been obtained and presented accordance with scientific and academic rules. Furthermore, this study has been prepared inside of the rules of writing the thesis, and that all sources of expression and information that do not belong to me are fully cited.

Ebrar KARABULUT

SUMMARY

DETERMINATION OF RESISTANCE LEVELS OF SOME ONION CULTIVARS OR INBREED LINES WITH FUSARIUM TESTING AT SEEDLING STAGE

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This master thesis study was carried out to determine the resistance levels of some onion genotypes in Yalova gene pool by Fusarium testing during the seedling stage. The isolate used in the study was *Fusarium oxysporum* f. sp. *cepae*, which causes damping off during the seedling stage and basal rot in onion bulb. The pathogenic isolate concentration which is pathogenic was adjusted as $1x10^6$ ml^{-1} by hemocytometer. The number of germinated seeds and the number of survival and dead seedlings were taken at regular intervals 10 days after sowing. The variance analysis for the onion seedling test and the mean differences against control were analyzed by using General Linear Model of the Tukey test. The germination rate of control seeds varied between 72% to 98%, while the germination rate of inoculated seeds varied between 39% to 93%. 'Texas Early Grano 502' showed the highest level of resistance with a survival rate of 83.87%, and resistance levels of '19Y07' and '19Y142' genotypes were higher than other genotypes. Resistance levels of '19Y51', '19Y15' and '19Y73' genotypes were lower than other genotypes. ‘Akgün 12’ showed moderate resistance with a survival rate of 59.64%. Determining the resistance levels of these onion genotypes to this pathogen during the seedling stage may be a preliminary step towards further studies.

Key words: Onion, *Fusarium oxysporum* f. sp. *cepae,* damping off, Fusarium seedling testing

ÖZET

BAZI SOĞAN ÇEŞİTLERİ VEYA ISLAH HATLARINDA FİDE DÖNEMİ FUSARIUM TESTLEMESİ İLE DAYANIKLILIK SEVİYELERİNİN BELİRLENMESİ

KARABULUT, Ebrar
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Bu yüksek lisans tez çalışması Yalova gen havuzundaki bazı soğan genotiplerinin fide döneminde Fusarium testlemesiyle dayanıklılık seviyelerinin belirlenmesi amacıyla yürütülmüştür. Çalışmada kullanılan izolat soğanda basal çürüklüğe ve fide döneminde çökertene neden olan *Fusarium oxysporum* f. sp. *cepae*'dır. Patojenik olan izolat konsantrasyonu $1x10^6$ ml^{-1} olarak hemositometre ile ayarlanmıştır. Ekimden 10 gün sonra düzenli aralıklarla tohumların çimlenen tohum sayıları, hayatta kalan ve ölü fide sayıları alınmıştır. Soğan fide testi için varyans analizi ve kontrole karşı ortalamaların farkları Tukey testinin Genel Doğrusal Modeli kullanılarak analiz edilmiştir. Kontrol tohumlarının çimlenme oranı %72 ila %98 arasında değişirken, inokule edilmiş tohumlarının çimlenme oranı %39 ila %93 arasında değişmiştir. 'Texas Early Grano 502' %83.87 sağkalım oranı ile en yüksek seviyede dayanıklılık göstermiştir ve '19Y07' ve '19Y142' genotiplerin direnç seviyeleri diğer genotiplerden daha yüksektir. '19Y51', '19Y15' ve '19Y73' genotiplerin direnç seviyeleri diğer genotiplerden daha düşüktür. 'Akgün 12' %59.64 sağkalım oranıyla orta derecede dayanıklılık göstermiştir. Fide aşamasında bu soğan genotiplerinin bu patojene karşı direnç seviyelerinin belirlenmesi, daha ileri çalışmalar için bir ön adım olabilir.

Anahtar kelimeler: Soğan, *Fusarium oxysporum* f. sp. *cepae*, çökerten, Fusarium fide testleme

PREFACE

In this master's study, it was carried out to determine the resistance levels of some onion genotypes against *Fusarium oxysporum* f. sp. *cepae* at seedling stage. The numbers of germinated seeds, alive and dead seedlings of onion genotypes at regular intervals 10 days after sowing were taken by comparing with control. At the end of the onion seedling test, significant difference was not observed between control and inoculated seeds in some genotypes. In other genotypes, it also was observed that resistance levels were low.

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SYMBOLS AND ABBREVIATIONS

Symbols	Description
%	Percentage
µl	Micro liter
^{o}C	Celsius degree
>	Greater than
<	Less than

Abbreviations	Description
A. niger	Aspergillus Niger
Adj MS	Adjusted Mean Squares
Adj SS	Adjusted Sum of Squares
ANOVA	Analysis of Variance
DF	Degrees of Freedom
DM	Dry Matter
Exp	Experiment
FAO	Food and Agriculture Organization
FBR	Fusarium Basal Rot
FOC	Fusarium Oxysporum f. sp. Cepae
f. sp.	Formae Specialis
FW	Fresh Weight
G	Grouping
GLM	General Linear Model
IGS	Intergenic Spacer
min.	Minute
ml	Milliliter
mm	Millimeter
N	Number of Replication
PDA	Potato Dextrose Agar
Pf	Pseudomonas Fluorescents

RBR	Root and Basal Rot
RFLP	Restriction Fragment Length Polymorphism
R-Sq (adj)	Adjusted R-Squared
SE	Standard Error
Seq SS	Sequential Sums of Squares
Std Dev	Standard Deviation
TEG	Texas Early Grano
TH	Trichoderma Harzianum
TSS	Total Soluble Solids
TUIK	Turkey Statistical Institute
UK	United Kingdom
USA	United States of America

CHAPTER I

INTRODUCTION

Onion (*Allium cepa* L.) has been cultivated for thousands of years and it has been found in Turkey, Iran, Afghanistan, Kazakhstan and Western Pakistan that showed a wide spread in time as summurized by Gökçe et al. (2012). Onion belonging to the Alliaceae family is a significant vegetable crop worldwide. Onion is a herbaceous biennial crop and it has wide range of landraces and cultivars with edible bulbs (Singh et al., 2018; Nasr-Esfahani, 2018).

Onion is one of the oldest and most widely consumed and used crops in Turkey, it is a vegetable that is used for cooking on a daily basis throughout the year (Gökçe et al., 2012). Onion is preferred as both dry and green, has rich nutritional values, vitamins and minerals. An average of 100 grams of edible onion contains approximately 85% water, 0.9% fiber and 2% protein. The nutrient content of onions changes according to the variety of onion (Rodrigues et al., 2003).

Onion is consumed raw or cooked in the daily diet or made into different onion products. Onion is an important food source and have many known beneficial health effects. Thanks to onion bioactive compounds, onion has significant benefits on cardiovascular diseases, bone metabolism and prevention of colon cancer (Corzo-Martinez and Villamiel, 2012).

Edible onion has an important place in the world economy. In FAO resources, the first order is China, followed by India, USA, Egypt, Iran and Turkey (FAO, 2018). More than half of the world's total production is owned by China, India and USA. However, in recent years, onion production in Egypt has shown a significant increase and has reached the level of USA onion production (FAO, 2018).

Onion cultivation area in Turkey in 2019 was increased by 16.4% compared to 2018 and took place in 61.3 thousand ha areas. Turkey produced approximately 2.2 million tons of onion in 2019. Ankara is the first city with 510 thousand tons of onion production. Ankara was followed by Amasya as second city with 249 thousand tons of

production, Çorum with 166 thousand tons, Adana with 152 thousand tons, Tokat with 112 thousand tons and Bursa with 110 thousand tons (TUIK, 2019). Also, the average amount of green onion production in Turkey was 140 thousand tons in the last four years (TUIK, 2015, 2016, 2017, 2018, 2019).

As with other agricultural products, there are disease factors, pests and weeds, which cause serious damage during the production of onion. One of the factors that causes onion diseases is fungal pathogens. The onion plant can be infected by soil- and seedborne fungal pathogens, resulting in significant yield and quality losses. *Fusarium oxysporum* Schlechtend.: Fr. f. sp. *cepae* (Hans) Synder and Hans (FOC) is caused seedborne and soilborne diseases such as damping off and basal root rot (Özer et al., 2003; Köycü and Özer, 1997; Özer, 1998). This pathogen may be seen and noticed in different growth stages of onion and can cause serious loss in the field and during storage (Özer et al., 2003; Fantino and Schiavi, 1987).

Symptoms of Fusarium basal rot (FBR) are appeared on leaves, roots, basal plates, on the bulb scales of small seedlings, on the mature and dormant plants (Cramer, 2000; Özer et al., 2003). Infected onion plants show symptoms both in the underground and aboveground (Behrani et al., 2015). Symptoms are pre- and post emergence damping off of seedling in the field, root rot in older plants, discoloration in onion stem plate, basal rot in bulbs during storage (Cramer, 2000; Abawi and Lorbeer, 1972).

Fusarium oxysporum f. sp. *cepae* (FOC) exist in many countries such as the United States, Brazil, South Africa, the Netherlands, India, England, Iran, Sweden, Japan and Uruguay, where onions are grown around the world (Cramer, 2000; Swift et al., 2002; Galvan et al., 2008; Dissanayake et al., 2009; Southwood, 2010; Lager, 2011; Ghanbarzadeh et al., 2014; Ünsal et al., 2019) and also FOC exist in Turkey in onion production areas (Türkkan and Karaca, 2006; Bayraktar and Dolar, 2011).

Fusarium oxysporum f. sp. *cepae* pathogen can lead to losses reaching up to 50% in the field and 75% in the greenhouse (Brayford, 1996; Stadnik and Dhingra, 1996; Ünsal et al., 2019). For this reason, development and application of the most effective control methods of this destructive pathogen is vital. This pathogen can be controlled with some control methods such as resistance of host plant, crop rotation, solarization, various

biological applications and fungicide applications (Cramer, 2000; Ünsal et al., 2019). However, the use of resistant varieties is economic, applicable in large scale and stated as the best opinion (Özer, 1998; Cramer, 2000; Özer et al., 2003; Özer et al., 2004; Nasr-Esfahani et al., 2012).

Many management strategies have been developed in the world for the detection and control of *Fusarium oxysporum* f. sp. *cepae* pathogen. But over time, control methods have become limited and inadequate. Therefore, it has become very important to identify and develop varieties that are tolerant or resistant to this pathogen.

The goal of this study is in order to develop onion genotypes for commercial onion production. Classical disease resistance test is effective and economic. Developing of resistance cultivars will prevent the loss of crops in the field and storehouse and will contribute to the national economy. This thesis aims to determine tolerance / resistance level of some onion genotypes at seedling stage. In this study, resistance levels of some onion genotypes to this pathogen were determined.

CHAPTER II

LITERATURE REVIEW

2.1 Onion

Onion is grown in all areas between the 50th in the southern and the 65th parallels in the northern hemisphere. However, onions adapted to different climates are grown in ideal conditions for their climate requirements. Onions adapted to different climatic requirements of a day length below 12 hours for bulbing are called short day onions; the ones that require a day length of 12 to 14 hours are called intermediate day onions; and lastly those that need a length of more than 14 hours are called long day onions (late, winter or storage) (Gökçe, 2015).

Short day (early) onions are planted in the fall in the mediterranean coastline, which is not cold in the winter; intermediate day onions are planted in the early spring months in cold weather places; long day onions are cultivated in march and april in the inner and northern regions of Turkey. In addition, sufficient moisture and temperature of between 10 to 15 °C are required in order to onion seed germinate. During the development and growth periods, between 10 to 25 °C temperature for maturing of the heads and between 25 to 30 °C temperature for the drying of the leaves is thought ideal. It is known that there is sunburn at high temperature (Gökçe, 2015; Pike, 1986; Şalk et al., 2008).

Onion requires two growth seasons or years to form seed from seed. In the first year, bulb onion is formed by seed and the other year, leaves and flower stalk develop. Based on the strength of the onion, 1 to 3 flower stalks develop from each growth point. In some regions, depending on the conditions of the cultivated climate, sets (small onions) instead of seed is also used (Gökçe, 2015). Seed production process with sets cultivation includes three growth seasons. In addition, in breeding lines, onion production is made with seedlings because of the seeds are valuable (Gökçe, 2010; Gökçe 2011).

2.2 Fungal Diseases of Onion

Onions are susceptible to a great number of fungal pathogens that are foliar, bulb and root fungal pathogens (Brayford, 1996; Cramer, 2000; Javaid and Rauf, 2015; Javaid et al., 2017) that reduces its yield and quality (Javaid and Rauf, 2015; Javaid et al., 2017). Purple blotch (*Alternaria porri*), brown stain (*Botrytis cinerea*), leaf blight (*Botrytis squamosa*), downy mildew (*Peronospora destructor*), powdery mildew (*Leveillula taurica*), smut (*Urocystis cepulae*) are the major and most common fungal foliar diseases of onion in the world. White rot (*Sclerotium cepivorum*), pink root (*Pyrenochaeta terrestris*), basal rot (*Fusarium oxysporum* f. sp. *cepae*) and damping off (*Pythium* spp., *Phytophthora* spp., *Rhizoctonia solani* and *Fusarium* spp.) are the widespread fungal diseases infecting onion from the soil. Also, neck rot (*Botrytis* spp.), black mold (*Aspergillus niger*), blue mold rot (*Penicillium* spp.) are the main fungal diseases of stored bulbs worldwide (Brewster, 2008).

The degree of loss caused by Fusarium species differs not only by their type, but also by the type of isolate (Dissanayake et al., 2009; Kintega et al., 2020). *Fusarium* spp. has been many formae speciales that one of them is *Fusarium oxysporum* f. sp. *cepae* (Burgess et al., 1994; Cramer, 2000). *F. oxysporum* f. sp. *cepae* an significant pathogen, leading to disease symptoms like wilting, basal plate rot, root rot and damping off. As other *Fusarium* spp., *F. solani, F. acuminatum, F. equiseti, F. culmorum, F. proliferatum, F. subglutinans, F. redolens*, and *F. tricinctum* also have been notified to be observed with onion growing areas (Abawi and Lorbeer, 1972; Bayraktar and Dolar, 2011).

2.2.1 *Fusarium oxysporum* f. sp. *cepae*

Fusarium oxysporum f. sp. *cepae* pathogen is able to form chlamydospores, macroconidia and microconidia. Chlamydospores are thick walled, round and formed exceedingly in soil. Contaminated seeds, bulbs and soil have been defined as the main source of inoculum (Köycü and Özer, 1997; Nasr-Esfahani et al., 2013) that chlamydospores are the major source of inoculum under the field conditions (Burgess et al., 1994; Cramer, 2000; Taylor et al., 2013). The pathogen produces chlamydospores that have long term survival structures. Thus, pathogen can resist in the soil for several

years due to chlamydospores (Brayford, 1996; Cramer, 2000). Macroconidia are falcate, thin walled and generally 3-septate. Microconidia are generally non-septate and formed abundantly in culture (Burgess et al., 1994; Cramer, 2000).

While disease factor prefers between 28 to 32 °C as optimum temperature range, this disease factor may also occur in low temperatures such as 15 °C (Abawi and Lorbeer, 1972; Türkkan and Karaca, 2006; Bayraktar and Dolar, 2011; Taylor et al., 2013). Soil temperatures of 12 °C or less were ended up light disease or no disease development (Abawi and Lorbeer, 1972).

Fusarium oxysporum f. sp. *cepae* is one of the most destructive pathogens for onion (Özer and Köycü, 2004; Nasr-Esfahani et al., 2013). Root and basal rot disease of onion caused by FOC is one of the most important diseases causing enormous losses in onion growing areas in the world (Özer and Köycü, 2004; Nasr-Esfahani, 2018). *Fusarium oxysporum* is a complicated fungal species composed of omnipresent soil-borne plant pathogens (Nasr-Esfahani, 2018; Debbi et al., 2018; Nasr-Esfahani et al., 2013; Nirmaladevi et al., 2016; Burgess et al., 1994; Shinumura et al., 1998). This fungus is a significant soilborne disease that causes a significant loss of yield in the areas, where the onion grows and is also common during onion bulbs storage (Nasr-Esfahani, 2018; Nasr-Esfahani and Koupaee, 2015; Özer et al., 2004). The losses for Fusarium basal rot (FBR) during storage are greater than the losses seen in the field. Moreover, small FBR infections in the basal plate of onion can not be detected at harvest (Brayford, 1996).

Fusarium oxysporum f. sp. *cepae*, which leads to significant losses in both field and greenhouse conditions has been reported to exist in many countries such as the United States, Brazil, South Africa, the Netherlands, India, England, Iran, Sweden, Japan and Uruguay, where onions are grown around the world (Cramer, 2000; Swift et al., 2002; Galvan et al., 2008; Dissanayake et al., 2009; Southwood, 2010; Lager, 2011; Ghanbarzadeh et al., 2014; Ünsal et al., 2019). In some countries in which onion cultivation is intensive, the disease can lead to losses up to 50% in the field and 75% in the storehouse (Brayford, 1996; Stadnik and Dhingra, 1996; Ünsal et al., 2019). FOC is the most common pathogen species in Turkey, in the onion production areas (Türkkan and Karaca, 2006; Bayraktar and Dolar, 2011).

Fusarium basal rot also known as Fusarium wilt, has damage rate to onion more than 50% (Ghanbarzadeh et al., 2014; Cramer, 2000). This pathogen can be transported with seed and soil, and is able to continue to exist in the soil as chlamydospore for years. Accordingly, it is very challenging to produce Fusarium-free onions in the contaminated field soil (Brayford, 1996; Köycü and Özer, 1997).

This pathogen may create a delay in the growth of onion seedlings and may also lead to damping off before and after seedling emergence (Abawi and Lorbeer, 1972; Galvan et al., 2008; Dissanayake et al., 2009; Taylor et al., 2013). Damping off is occurred by *Fusarium* spp*., Pythium* spp. and *Rhizoctonia solani*, and damping off causes death of seedling roots (Schwartz, 2011).

2.2.1.1 Symptoms

Symptoms of Fusarium basal rot (FBR) can be observed on onion leaves, roots and basal stem plate of onion, on bulb scales of small seedlings, on mature onion and dormant bulbs (Cramer, 2000; Özer et al., 2003). Diseased onion plants show signs of disease in both aboveground and underground parts. Symptoms such as general wilting, stunted growth of onion with yellowing, browning of onion leaves are observed in the affected plants (Behrani et al., 2015).

Symptom of first aboveground of FBR is that chlorosis would be seen on all leaves of mature plants. Chlorosis leads to tip necrosis. Tip necrosis appears on the entire leaf and the plant eventually dies (Brayford, 1996; Cramer, 2000). *F. oxysporum* f. sp. *cepae* infects the basal parts of the bulb scales in the severe cases and white mycelium can be seemed on the basal parts of external bulb scales (De Visser et al., 2006; Cramer, 2000). FBR provides an entry way for secondary pathogens to infect the bulb (Cramer, 2000).

Other organisms and mechanical damage injure plant tissue. Thus, this plant become clarified to infection due to the tissue injury (Brayford, 1996). The death and abscission of roots are come true due to the infection within the basal plate. The separation of stem plate from roots easily during uprooting is a remarkable symptom of FBR. *Fusarium oxysporum* f. sp. *cepae* within the basal plate leads to browning of the basal plate tissue. When the all basal plate is ruined, it can be observed that the stem plate can be

immediately removed from the rest of the bulb (Cramer, 2000). Certain symptom of disease for onion seedling is delayed emergence and pre- and post emergence damping off (Ghanbarzadeh et al., 2014; Lager, 2011).

2.2.1.2 Control

Fusarium oxysporum f. sp. *cepae* can be controlled with some control methods such as resistance of host plant, crop rotation, solarization, biological applications and fungicide applications (Cramer, 2000; Ünsal et al., 2019). Also it can be controlled with farm hygiene, destruction of infected plants and avoidance of fields with basal rot incidence (De Visser et al., 2006). Optimizing of disease control strategies is essential to control of Fusarium basal rot. Control of Fusarium basal rot is possible when a reliable and cost effective detection procedure is applied for soil samples and plant samples (De Visser et al., 2006).

Crop hygiene is very necessary for disease control. Potential plant ruins and volunteer plants should be defined and removed. The spread of disease residues to worker boots and farm equipment should be avoided (Brewster, 2008).

Crop rotation is a desirable practice to prevent diseases (Brewster, 2008). 4 or 5 years as crop rotation is applied in order to decrease incidence and severity of Fusarium basal rot (Özer and Köycü, 2004). When susceptible crops are not planted successive recently, they are protected from the potential disease residues of previous crops. However, the rotation of susceptible plants is not economical every time, especially in the intense production areas (Brewster, 2008).

Fungicides are divided into two as protectors and systemics. While preservatives are used before infection, systemic fungicides are used to destroy the fungus after infection. Fungicide resistance occurs in plants after a while, so they are either used as a mixture or in consecutive fungicide treatments by changing their active ingredients (Brewster, 2008).

Various fungicides such as benomyl, benomyl + mancozeb, carbendazim, carboxin, maneb, methoxymethyl mercury chloride, prochloraz, penncozeb, tebuconazole, thiram

and vinclozolin have been used in onion seed and shallot against the disease and these fungicides have been reported to minimize rot damage of root and bulb in onions (Köycü and Özer, 1997; Özer and Köycü, 1998; Cramer, 2000; Sintayehu et al., 2011; Ünsal et al., 2019). Chemical application to seed greatly reduces disease incidence, but this disease control is not economic every time. However, chemicals reach groundwater and cause groundwater contamination (Özer and Köycü, 1998). In addition some of the fungicides are currently banned in the world and in Turkey due to their negative effects on human and environmental health (Ünsal et al., 2019).

In the opinion of Valdez et al. (2007), the correct crop rotation and use of fungicide is used for disease control. Genetic resistance is a good alternative for disease control. It is also important that breeders and producers analyze the behavior of local varieties against different isolates.

Although solarisation, fumigation and rotation in order to shrink the initial inoculum can control some soilborne pathogen (Özer and Köycü 1998; Cramer 2000; Özer et al., 2003; Özer et al., 2004; Nasr-Esfahani et al., 2013), the use of naturally resistant cultivars has been considered as the best way worldwide (De Visser et al., 2006) and searching for resistant or tolerant varieties against to this disease is necessary, economic and applicable in large scale (Özer, 1998; Cramer, 2000; Özer et al., 2003; Özer et al., 2004; Nasr-Esfahani et al., 2012; Nasr-Esfahani et al., 2013). While the resistance mechanisms about this disease remain unknown, resistant cultivars have been improved successfully (Cramer, 2000; Özer et al., 2003).

The most effective and economic strategy for treatment is the using of resistant varieties in the method, owing the fact that the chemical treatment on the disease is low effect and disease factor stands in the form of chlamydospore for long year in the soil (De Visser et al., 2006; Caligiore Gei et al., 2014). The best method to treat this disease is to develop a resistant / tolerant variety.

According to a study of Cramer (2000), many intermediate day and long day onion hybrids have moderate levels of resistance against to FBR. Despite this resistance is not certain, losses caused by FBR can be remarkably decreased via the using of FBR

resistant cultivars. Definitely, whereas there are resistant cultivars of intermediate day and long day onions, there are not resistant cultivars of short day onions.

Researchers in Turkey and around the world have reported that onion genotypes react differently to FOC and that tolerant and resistant lines and varieties are determined (Lopez and Cramer, 2004; Özer, 1998; Özer et al., 2004; Saxena and Cramer, 2009; Dissanayake et al., 2009; Galván et al., 2008; Nasr-Esfahani et al., 2012; Nasr-Esfahani et al., 2013; De Visser et al., 2006).

Onion is a very important agricultural product in our life and it can be damaged by the Fusarium pathogen in many stages of cultivation. Especially, *Fusarium oxysporum* f. sp. *cepae* pathogen, which leads basal rot and damping off before and after emergence and induces serious damage to onion production. Many studies have been carried out about the identification, detection and control of the FOC pathogen in the world and Turkey.

2.2.1.3 Studies on *F. oxysporum* f. sp. *cepae* in onion in the world

Kehr et al. (1962) conducted a study to specify the pathogenicity of four FOC isolates in the temperature range of 20 and 38 °C. According to their study, they were proved that all *F. oxysporum* f. sp. *cepae* isolates was the major invaders of onion seedlings at the temperatures provided. In addition, they identified that the most resistant lines against to *F. oxysporum* f. sp. *cepae* were Zuckerman '59 Yellow Sweet Spanish, B 2264 and TEG 951.

Abawi and Lorbeer (1972) conducted a study to determine the inoculum density and temperature effect on disease development under controlled environmental conditions, to observe the germination, growth and establishment of the pathogen in organic soils, and to determine the form of propagule containing the pathogen in organic soil. According to the results of their studies, they noticed that the optimum temperature range for the growing of *Fusarium oxysporum* f. sp. *cepae* in culture is between 24 to 27 °C. There was a close correlation between the temperature and the damping off of onion seedlings, and a close correlation occurred between FOC inoculum density and onion seedling infection in organic soils. In their studies, in artificially infested, steam-treated soil, stored for several years in laboratory conditions pathogen existed as

chlamidospores (73.8%), macroconidia (6.6%), microconidia (9.8%) and hyphal fragments (9.8%) Also, it was proven that this pathogen existed in the form of chlamidospores in naturally infected soils.

Fantino and Schiavi (1987) performed mass selection in naturally infected soils and recurrent selection with three applications in naturally or artificially infected soils in the selected onion populations in the years of 1984 and 1985. These three applications are: soil naturally infested, greenhouse spore suspension and Fusarium colonized wheat seeds. The purpose of this breeding program is not only to develop a population tolerant to Fusarium; it is also to create populations with specific bulb features. Selected 'ERSO-1' and 'ERSO-2' onion populations determined their tolerance levels after 4 cycles of mass selection in naturally infected soil and multiplicated the selected populations.

Fusarium oxysporum f. sp. *cepae* damage not only onion but also other *Allium* species such as garlic. Rengwalska and Simon (1986) conducted a laboratory study to screen Pink root (*Pyrenochaeta terrestris)* and Fusarium basal rot resistance in garlic and onion. Garlic clones were collected from 5 different countries, while onion seeds were collected from United States of America. According to the results of their studies, PI 493118 (from Poland) garlic clone was pink root-resistance but was Fusarium-susceptible. PI 493112 (from Poland) was most resistant to Fusarium. They thought that the phenotypic variation observed between and within garlic clones could be genetically variable for the disease reaction.

Rajendran and Ranganathan (1996) conducted a research on the biological control method of onion basal rot by united practice with fungal and bacterial antagonists in Coimbatore, India. In this study, *Trichoderma viride, T. harzianum, T. hamatum, T. koningii, T. pseudokoningii* were used as fungal antagonists, while bacterial antagonists were used as *Pseudomonas jluorescens* and *Bacillus subtilis*, and they were found to be efficient against to FOC. The combined seed treatment of *T. viride* + *P jluorescens* has also found that it can lower the incidence of onion basal rot both in pots and in the field.

Stadnik and Dhingra (1997) investigated the root infection and the relationship of stored 'Baia Periforme' onions with bulb rot at different stages of growth in the field and in the

greenhouse in Brazil. According to the study, they noted that in the early stages of plant growth, inoculation of *Fusarium oxysporum* f. sp. *cepae* has a more effective colonization of the roots and the root plate. The percentage of rotten bulbs and the disease index, evaluated at 90 days of storage, were declared to be higher in early stage inoculated plants.

Ko et al. (2002) enlarged and stored 12 onion varieties with different storage degrees between 1995 and 1997 for 3 months. At the end of 3 months of storage, percentage of diseased bulbs were calculated according to effects caused by bacterial soft rot, black mold and Fusarium basal rot. They recorded knowledge on the bulb characteristics such as bulb fresh weight (FW), dry matter (DM) content, total soluble solids (TSS) and pyruvic acid content during harvest. The aim of this study was to determine the variability in short-term onion storability, to identify the responsible factors for onion storage losses over the years, and to determine the relationship between storage losses and bulb characteristics. According to the results of this study, they indicated that 'Texas Early Grano' was the most susceptible variety against Fusarium basal rot. 'Red Pinoy' and 'Serrana' showed good storability. As stated by the study, they pointed out that varieties with high TSS and DM had better storability and were less susceptible to storage diseases. In addition, it has been observed that varieties with good storability tend to have high pyruvic acid levels.

Taylor et al. (2013) collected *Fusarium oxysporum* isolates from onion fields in the UK between 2008 and 2010, and also taken from institutes from the Netherlands and Ireland in order to use onion seedling assay. They developed rapid, simple and repeatable seedling test to show numerous onion cultivars for resistance against to FOC in the UK. In this study, seedling assay was applied to commercial onion cultivars and determined the aggressiveness level of different FOC isolates taken. It is reported that the best method is to use onion cultivars with increased FOC resistance and the second option is in order to use non-pathogenic Fusarium isolates as biological control.

Saxena and Cramer (2009) screened FBR-resistant and FBR-susceptible onion seedlings based on the death of seedlings caused by Fusarium basal rot (FBR) in the New Mexico onion fields using *Fusarium oxysporum* f. sp. *cepae* (FOC) isolates picked up from New Mexico onion fields. The combinations of 17 FOC isolates and 8 onion entries were

evaluated at 25 °C for 20 days and disease incidence was calculated. A variation analysis proved that there is a significant interaction between FOC isolate and onion entry for FBR incidence. In this study, 'NuMex Chaco' and 'NuMex Crispy' onion entries had the widest distribution and FOC isolate ‘CSC 515’ has been observed to be the best FOC isolate for future screenings due to its high average and variation of FBR incidence.

Ghanbarzadeh et al. (2014) collected eighty Fusarium isolates from red onion bulbs on infected onion fields in the East Azarbaijan. They determined that 17 out of 38 selective isolates were pathogenic in onion. In their morphological and molecular characteristic study, one of the these isolates was found as *Fusarium oxysporum* and they specified that *Fusarium oxysporum* causes severe rot and damping-off on onion bulb and seedlings. Also they reported that *Fusarium oxysporum* causes 96% damping off rate on the onion cultivation according to the isolation damping percentage.

Nasr-Esfahani et al. (2012) conducted a study to test various onion genotypes against to FOC incidence in the greenhouse and field conditions at different growth stages in Iran. In this study, Texas Early Grano was one of the most susceptible onion varieties at three stages of early, flowering and seed setting. Under field conditions, disease severity of TEG was 21.8%, 32.8% and 43.6%, respectively. In greenhouse conditions, disease severity of TEG was 62.5%, 75% and 100% for 3 growth stages, respectively.

Nasr-Esfahani et al. (2013) tested various onion genotypes against to FOC incidence in greenhouse and field conditions at different growth stages. They randomly examined infected plant samples in the field and greenhouse conditions and watched fewer onion root and basal rot disease (RBR) symptoms during the early planting stage of the plants. They established RBR incidence the severity of the disease in three stages like early, flowering and seed placement. ‘Texas Early Grano’ was one of the most susceptible onion varieties in all three stages. The most resistant varieties were ‘Kashan-White’ and ‘Ghom-White’ in pot conditions, and ‘Kashan-White’ in field conditions.

Cramer et al. (2000) initiated to evaluate the adaptation and performance of onion varieties and improved breeding lines in the south New Mexico, USA in 2000. Each onion variety was considered to be short, medium and long day onion varieties and was

applied by taking into account the sowing and harvesting times. Two varieties (fall-seeded and spring-transplanted) were grown in onion fields. They observed and calculated the pink root and Fusarium basal rot (FBR) of these onion entries. The basal plates of 25 onion bulbs randomly selected were cut transversely and subjectively graded by giving them 1 (no disease tissue) and 9 (70% or more disease tissue). Based on the result of this study, the FBR incidence for fall planted cultivars ranged from 0.6% to 40.3% whereas for spring planted cultivars ranged from 2.9% to 29.2%.

Rajamohan et al. (2019) evaluated for the potential of various biocontrol agents to conduct the basal rot of onion in vitro. The tested *Trichoderma viride* isolates gave the largest (82.86%) inhibition, and *Pseudomonas* fluorescents (Pf2) provided the greatest (80.82%) reduction in *F. oxysporum* mycelium growth.

Valdez et al. (2007) conducted a study to identify a credible method for the pathogenicity test isolates of *Fusarium* spp. They isolated 56 monosporic *Fusarium* spp. isolates from different onion growing fields in Argentina and conducted pathogenicity tests on paper discs and sand. They realized that the pathogenicity test on the sand showed more reliable results than the pathogenicity tests on the filter paper discs, since the sand germination gave results in the tolerance range. According to the study, there are differences in aggressiveness on *Fusarium* spp. isolated from onion in onion production areas in Argentina. Pathogenicity test on sand was the best method for isolate differentiation. There was no clear resistance to the disease noticed on the tested Argentinean varieties. Also, as claimed by Valdez et al., (2007), low germination percentage indicates that the inoculated isolate is aggressive; higher germination percentage may indicate that the plant has resistance to the inoculated isolate.

Galvan et al. (2008) conducted a study to examine the levels of Fusarium basal rot resistance using genetically different Fusarium isolates in onion varieties and related Allium species. According to the resistance levels against Fusarium basal rot, *A. pskemense*, *A. roylei* and *A. galanthum* showed moderate resistance, whereas *Allium fistulosum* and *A. schoenoprasum* entries showed high resistance level to each isolate. Of the five *Allium cepa* varieties, 'Rossa Savonese' was moderately resistant, while ‘Texas Grano Early 502’ was less resistant.

Akhtar and Javaid (2016) conducted a study in order to control basal rot of onions caused by *Fusarium oxysporum* f. sp. *cepae*. In this study, they concluded that united treatment of *Withania somnifera* dry leaf material and biological control agent *Trichoderma harzianum* could be used for control of basal rot of onion.

Behrani et al. (2015) used seedling infestation and soil infestation methods to confirm the pathogenicity of *Fusarium oxysporum* f. sp. *cepae* isolates. They recorded the highest plant mortality rate and pathogen infection in the soil infestation method. In addition, they used antracol, carbendazim, copper oxychloride and kingmil MZ fungicides against *Fusarium oxysporum* in different concentrations. According to their study, they remarked that every concentration of carbendazim fungicide was more effective than other fungicides. In fact they mentioned that the high concentration of fungicides was more effective than the medium and low concentrations. Moreover, they reported that as the fungicide concentration was increased, height and weight of plant also increased.

Malathi (2015) conducted a study by isolating biocontrol agents from soils in various places of Tamil Nadu, India. Among the isolated biocontrol agents, *Trichoderma harzianum* (TH3) provided 83% inhibition and *Pseudomonas* sp. (Pf12) decreased mycelial growth of FOC with 75%. As a matter of fact, bacterial and fungal biocontrol agents (Pf12 + Pf27 + TH3) have been shown to diminish disease by 85%.

Kintega et al. (2020) managed a study in order to test pathogenicity of 33 isolate obtained from *F. oxysporum, F. thapsinum, F. proliferatum, F. solani and F. fujikuroi* fungus species in Burkina Faso. As maintained by the study, in the evaluation of isolates on onion seedlings, they reported that *F. proliferum, F. thapsinum and F. solani* isolates caused seedling damping off with 58.33% - 70.83% rate. They declared that the most pathogenic isolates in onion bulbs were *F. proliferum and F. oxysporum*, which created the decay of 21.67 to 25 mm in the bulbs.

Caligiore Gei et al. (2014) conducted a study that tested onion varieties reported as tolerants in previous studies with 5 *Fusarium* spp. isolates using 4 inoculum concentrations in Mendoza, Argentina. These onion varieties were ‘Antártica- INTA’, ‘Grano de Oro-Seminis’, ‘Valcatorce-INTA’ and ‘TW-2007’. They observed that high

concentrations of inoculum enhance the ability of the isolates to generate disease. In study, they realized that ‘TW-2007’ cultivar was tolerant, while other varieties were susceptible.

Lopez and Cramer (2004) applied a seedling screening procedure to short-day onion entries from the National Plant Germplasm System. They grew onion seeds in the growth cabinet and calculated their percentage of seedling survival. They also planted surviving seedlings in the field to measure the incidence of FBR in the bulb stage. According to the results of the study, ‘Serrana’ was the only onion variety that was moderately resistant to FBR. Other short-day onion varieties showed low resistance or no resistance. While there was a powerful and positive correlation between the incidence and severity of FBR, both have reported a weak correlation with seedling life.

Gutierrez et al. (2006) conducted a study in order to demonstrate for winter sown onion germplasm for FBR resistance by using a mature bulb screening at harvest and after 4 weeks in storage. From the tested entries, ‘NMSU 99-30’, 'NuMex Arthur' and 'NuMex Jose Fernandez' were observed to show the least disease severity and incidence every two years at harvest and after 4 weeks of storage. They also thought that it would be logical to use these onion entries in the improvement of FBR resistant varieties.

2.2.1.4 Studies on *F. oxysporum* f. sp. *cepae* in onion in the Turkey

Köycü and Özer (1997) managed a study in order to determine seedborne fungi in onion and their infection to onion sets. In this study, they collected seeds from 7 different regions in the Turkey and coat, endosperm and embryo of onion seed were cultured. Thus, seedborne fungi were identified and their contamination to onion sets was analyzed in both sterile and field soils. On the authority of the results of the study, all of the detected fungi have turned out to be a natural contagious of seeds. *F. acuminatum, F. culmorum, F. equiseti, F. graminearum* and *F. sambucinum* were probably contaminated by seeds from sterile soil to onion sets, but were restrained by other organisms in the field soil, according to observations. *A. niger* and *F. oxysporum* were transferred from onion and soil to onion sets.

Özer (1998) have carried out a study to examine the response of local and imported some onion varieties used in Turkey to infections caused by *F. oxysporum* and *A. niger* in 1996-1997. Seed and soil invasion methods were used for both pathogens. According to the study, 'TEG 502' was the most susceptible cultivar to infections occurred before emergence by seed and soil infestations of *A. niger* and *F. oxysporum*. 'Banko' was also a susceptible onion variety to *A. niger* and *F. oxysporum*. 'Kantartopu' had a greater tendency to damping off after emergence with both invasion methods of *A. niger*. 'Akgün 12' and 'Alex' showed more tolerance for infection caused by two types of pathogens than other varieties.

Özer et al. (2003) analyzed the ability of two FOC isolates (FOC6 and FOC8) in order to colonize onion seedlings, polygalacturonase activity and pectin lyase production. In this study, 'Akgün 12' and 'Rossa Savonese' appeared resistant against to FOC6 and FOC8 isolates, while 'Kantartopu' and 'Texas Early Grano 502' appeared excessively susceptible, especially when FOC8 was inoculated. As specified by the study, FOC8 has more aggressive FOC6 and FOC8 has higher amount of pectolytic enzymes. Enzyme activity was not found in healthy onion tissues. Besides, different antifungal compounds have been shown in germinated onion seedlings.

Özer et al. (2004) performed greenhouse and field evaluation of onion for resistance to FBR caused by *Fusarium oxysporum* f. sp. *cepae*. In this study, 'Akgün 12' and 'Rossa Savonese' cultivars in advance determined as resistant during seedling stage were used and it is determined that these cultivars were resistant to disease at the bulb stage. Also, extraction of onion sets were applied by using thin layer chromatography to explain their content of antifungal compounds.

Özer and Köycü (2004) evaluated the activities of benomyl, thiram, prochloraz and tebuconazole against *F. oxysporum* on the seed. They found that this fungicide treatment increased the emergence of seedlings and reduced the percentage of damping off after emergence.

Bayraktar et al. (2010) conducted a study by collecting 75 isolates of FOC from 7 cities of Turkey to identify the genetic variability of pathogen populations. They indicated that this study was the first study of genetic variability among isolates of *F. oxysporum*

f. sp. *cepae* in Turkey. According to the results achieved in the seedling test were observed a high level of variability in the aggressiveness level of *F. oxysporum* f. sp. *cepae* isolates. They stated that most of the isolates were extremely lethal and there was no relationship between virulent and the geographical origin of the isolates.

Bayraktar and Dolar (2011) conducted a study to determine Fusarium species, resulting root and basal rot in onions in Turkey, in 2007. Isolates were defined accordance with their morphological and cultural characteristics, which are *F. oxysporum, F. solani, F. acuminatum, F. equiseti, F. proliferatum, F. redolens*, and *F. culmorum*. *Fusarium oxysporum* was the most common Fusarium species, making up 66.57% of the total Fusarium species. *F. oxysporum, F. solani, F. acuminatum, F. proliferatum,* and *F. redolens* were excessively pathogenic and these Fusarium species were reported to cause severe precipitate on Texas Early Grano onion plants.

Ünsal et al. (2019) used calcium salts to prevent or lessen mycelial growth of fungus in order to control Fusarium basal rot. While the concentration of 1% calcium hydroxide and oxide has been indicated to completely obstruct fungal mycelial growth, calcium propionate has been observed to reduce mycelial growth by 72.72% even at a concentration of 2%. They showed that calcium propionate can be used to control Fusarium basal rot in onions, since calcium propionate has been found to significantly reduce the severity of FBR.

Türkkan and Karaca (2006) conducted a study to determine root rot disease factors and their incidence and severity in the 110 onion growing field in the years 2000-2001 in Amasya, Turkey. They have obtained some isolates from onion plants with root rot disease. The disease severity of these isolates was followed respectively that *Fusarium* spp. with 91.6%, *Rhizoctonia* spp. 4.2%, *Sclerotium cepivorum* with 1.9%, *Pythium oligandrum* with 1.5% and *Trichoderma harzianum* with 0.8%. As a result of pathogenicity tests, it was determined that virulent of *F. oxysporum* and *R. solani* were high and virulent of *P. oligandrum* was very low.

Türkkan and Erper (2014) evaluated twelve sodium salts as probable choice to synthetic fungicides for the control of onion basal rot caused by FOC. As a result of in vitro tests, they found that there were significant differences between the inhibitory impacts of

sodium salts on mycelium growth. They also reported that sodium metabisulfite and sodium fluoride sodium salts with a 2% concentration completely inhibit the mycelium growth of the fungus.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant materials

The material of the study were formed from 13 onion genotypes and 2 onion varieties. The onion genotypes were 19Y01, 19Y06, 19Y07, 19Y15, 19Y16, 19Y17, 19Y18, 19Y19, 19Y34, 19Y46, 19Y51, 19Y73 and 19Y142. 2 onion varieties were Akgün 12 and Texas Early Grano 502. 13 genotypes and Akgün 12 were provided from Yalova gene pool and Texas Early Grano 502 was provided by Bayram Seed Company.

While Akgün 12 onion variety was used as tolerant, Texas Early Grano 502 variety was used as susceptible against *F. oxysporum* f. sp. *cepae* as reported according to previous researches. The susceptibility levels of other onion genotypes were not known. Moreover, all of the seeds had been not treated by any chemical application such as fungicide.

3.1.2 Fungal material

One pathogenic and virulence isolate (S22) to be used in the thesis study was provided by Prof. Dr. Harun Bayraktar from Ankara University, Agriculture Faculty, Plant Protection Department. The isolate was cultured by incubating in Potato Dextrose Agar (PDA) medium at 20 °C.

3.2 Methods

3.2.1 Onion seedling test

Onion seedling test was carried out by the method of inoculation of onion seeds. The symptoms and changes observed on the plant over time were noted. All the steps were described in detail below.

3.2.1.1 Sterilization of onion seeds

Surface sterilization of onion seeds from each genotype was performed in the flow cabinet in the mycology laboratory, Plant Protection Department, Atatürk Horticultural Crops Central Research Institute. The seeds belonging to genotypes taken from Yalova gene pool were kept in 1000 µl 1% sodium hypochlorite for 3 minutes in the eppendorf tubes to disinfect surface, then seeds were rinsed in the sterile distilled water 3 times and left to get dry on sterile filter papers.

3.2.1.2 Preparation of spore suspension

Fusarium oxysporum f. sp. *cepae* pathogen was cultured on PDA medium at 20 °C for 10 days. Sterile water was added into the petri dish to allow the conidia to pass into the water onto the developing culture and filtered through the sterile cheesecloth by gentle mixing and the intensity of the spore was adjusted to a density of $1x10^6$ ml^{-1} by the hemocytometer. Each onion genotypes was inoculated by standing in 1 ml of spore suspension for 1 hour and the seeds used for control was kept in 1 ml sterile purified water for 1 hour. After 1 hour, seeds were rinsed and put back into petri dishes.

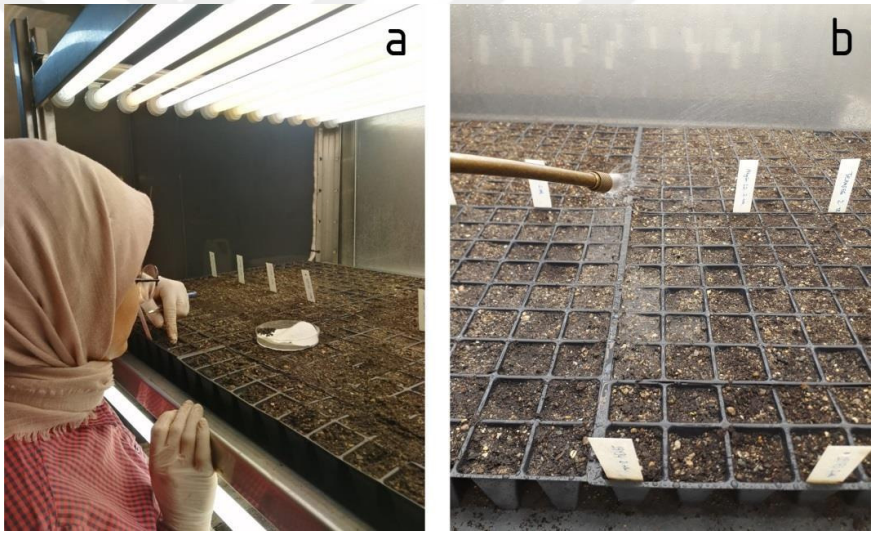
All processes in the laboratory were handled in the laminar flow cabinet under the sterile conditions (Picture 3.1). During the calculation of the spore concentration in the fungal spore suspension, shape of microconidia were observed (Picture 3.2).

Picture 3.1. Preparation stages of spore suspension

Picture 3.2. View of microconidia of *Fusarium oxysporum* f. sp. *cepae* on the microscope

3.2.1.3 Sowing of onion seeds

Before sowing the seeds, the 1/3 garden soil + 1/3 farm manure + 1/3 stream sand mixture was filled in fireproof bags and placed in the autoclave machine (60 min., 121 °C) to be sterile soil mixture. For each treatment, 100 seeds were planted for each genotype with independent 4 replications in each replication with 25 seeds that the random blocks were made (Picture 3.3a). The seeds planted in the viols were placed in the climate chamber and the climate chamber was adjusted to be 25 °C day / 18 °C night and 16 hour light / 8 hour dark and 60% relative humidity. Irrigation was done carefully to avoid contamination between inoculated seeds and non-inoculated (control) seeds (Picture 3.3b).



Picture 3.3. Sowing of onion seeds (a) and irrigation of viols (b)

3.2.1.4 Counting of germinated seed, alive and dead seedling

The counting of survival seedling was performed twice a week after 10 days of planting and continued for 3 weeks (Özer et al., 2004; Taylor et al., 2013). The survival percentage of the genotypes was calculated according to control genotypes in order to ensure a variation of natural in seed germination (Taylor et al., 2013). The count was calculated in %, in comparison with the control as damping off before and after the seedling emergence (Picture 3.4).

Picture 3.4. Counting of seedling before and after emergence in the climate chamber

Counting of dead and alive seeds and seedlings were taken after two weeks and four weeks and the percentage of emergence and survival were calculated (Saxena and Cramer, 2009).

$$\% \text{ Emergence} = \frac{\text{Number of emerged seedlings}}{\text{Total number of planted seeds}} \text{ X } 100 \quad (3.1)$$

$$\% \text{ Survival} = \frac{\text{Number of survival seedlings}}{\text{Total number of emerged seedlings}} \text{ X } 100 \quad (3.2)$$

3.2.1.5 Statistical analysis

At the end of 4 weeks, the data obtained before and after the seedling emergence were compared with the control. Paired Sample T-Test for comparison of control and inoculated alive seedlings was analyzed by using Microsoft Excel. The variance analysis for the onion seedling test were analyzed by using General Linear Model (GLM) of the Tukey test. Variance analysis were performed with Minitab 16 software.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Onion seedling test

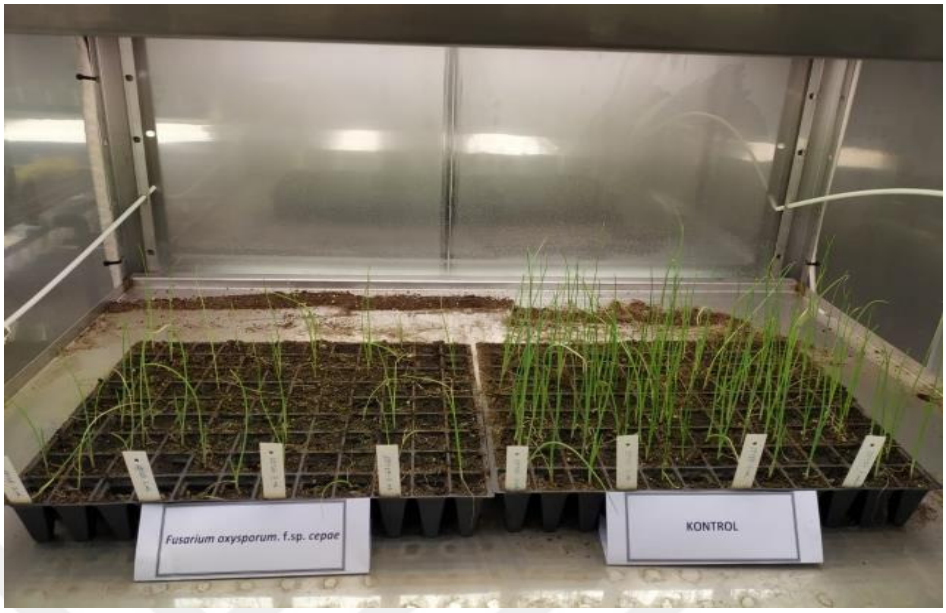
A pathogenic and virulent pathogen was inoculated to the seeds of some onion genotypes and seeds were planted in the viols. Symptoms caused by this pathogen were observed. Percentages of germinated seeds, alive seedlings and dead seedlings were shown in the tables (Table 4.1, 4.2, 4.3, 4.4). Resistance levels of onion genotypes were determined as a result of onion seedling test.

4.1.1.1 Observation of disease symptoms

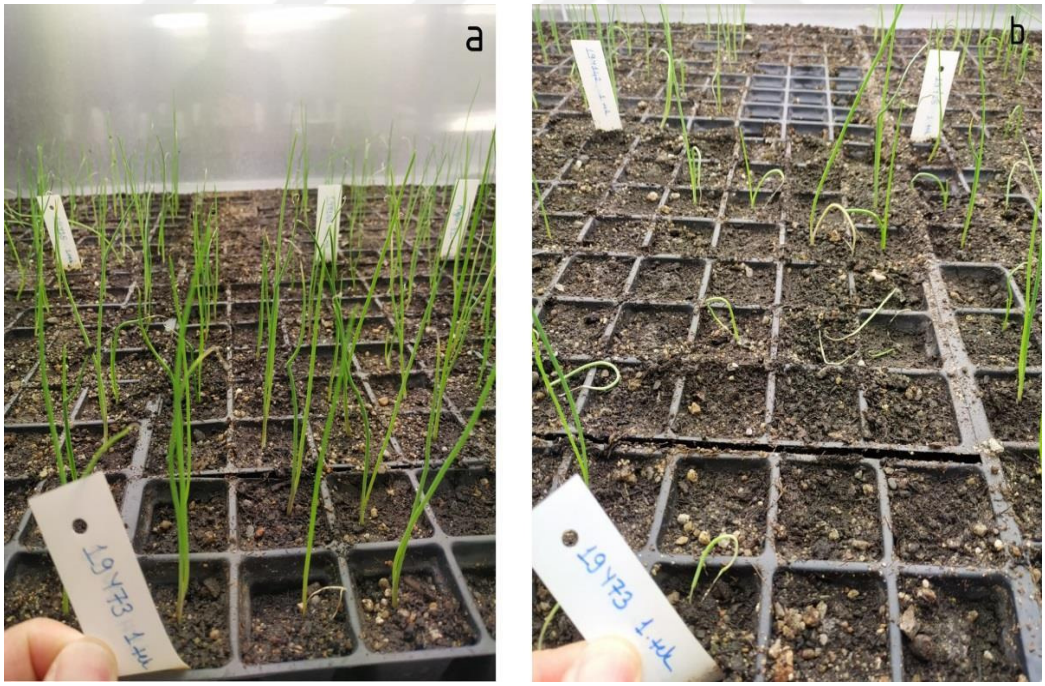
Development differences and symptoms of disease were observed inoculated plants by comparing with control plants. Each of the control genotypes and inoculated genotypes were taken photo to compare with each other, and the symptoms between them were significant difference (Picture 4.1 and Picture 4.2).

Ten days after inoculation, germination almost completed for inoculated and non-inoculated (control) seeds. Twelve days after inoculation, the initial disease symptoms began to seem such as the appearance of white mycelium on the soil and the death of several small seedlings.

In the symptoms of the disease, before germination, it either germinated later than controls or death occurred. After germination, inoculated seedlings developed later than control. Yellowing occurred on the onion leaves. White mycelium were seen on the soil of some seedlings (Picture 4.3). There was browning and softening in the root parts. It was noteworthy that when the root part of the seedlings that looked healthy in the soil was removed from the soil, it was already brown and broken (Picture 4.4). If the study had continued, seedlings would continue to die.



Picture 4.1. Final comparison between inoculated and control seedlings



Picture 4.2. Comparison of control (a) and inoculated seedlings (b) of 19Y73

Picture 4.3. View of dead seed and seedling and the formation of white mycelium (a, b, c and d)

Picture 4.4. Comparing of the alive and dead seedling in the same inoculated genotype

4.1.1.2 Evaluation of onion genotypes for resistance to *F. oxysporum* f. sp. *cepae*

A total of 15 onion genotypes inoculated by the seed inoculation method were evaluated about resistance against FOC. There were highly significant differences among onion genotypes due to infection caused by *F. oxysporum* f. sp. *cepae*. The data obtained in this study were given in detail in the following tables (Table 4.1, 4.2, 4.3, 4.4, 4.5).

Mean percentages of the germinated seed, alive and dead seedling of control and inoculated seeds were evaluated in Table 4.1.

Table 4.1. Treatment, mean percentages of the germinated seed, alive and dead seedlings

		Mean (%)		
Treatment	N	Germinated seed	Alive seedling	Dead seedling
Control seed	60	79.73a	79.73a	20.26a
Inoculated seed	60	56.80b	34.27b	22.53a

Different letters following the mean in the same column signify that the mean are statistically significant difference (ANOVA p = 0.05, Tukey test). N represents number of replication.

According to Table 4.1, germinated seed mean of control was significantly different than germinated seed mean of inoculated seeds. Alive seedling mean of control was significantly different than alive seedling mean of inoculated seeds. The mean of germinated seed and alive seedling in control seeds were the same because there was no adverse condition affecting seedlings in control seeds. Therefore, germinated all control seeds survived throughout the study.

In addition, dead seedling mean of control was not significantly different than dead seedlings mean of inoculated seeds in Table 4.1. However, these data are mean data of control and inoculated seeds. In Table 4.2, the mean percentages of germinated seed, alive seedling and dead seedling of each genotype were given separately. In this way, the difference between them could be observed better.

Table 4.2. Genotypes, mean percentages of the germinated seeds, alive and dead seedlings

		Germinated seeds		Alive seedlings		Dead seedlings	
Genotypes	N	Mean	G	Mean	G	Mean	G
TEG 502	8	95.50	a	88.00	a	8.50	b
19Y142	8	78.00	a	67.50	b	18.00	ab
19Y18	8	76.00	b	63.00	bc	19.00	ab
19Y06	8	75.50	bc	57.50	bcd	28.50	a
19Y17	8	73.00	bcd	60.50	bcd	20.50	ab
19Y19	8	71.50	bcd	57.00	bcd	23.00	ab
19Y16	8	70.00	bcd	60.00	bcd	20.00	ab
Akgün 12	8	65.50	bcd	54.00	bcd	24.50	a
19Y34	8	64.00	bcd	52.00	bcd	25.00	a
19Y07	8	61.00	bcd	55.50	bcd	16.00	ab
19Y73	8	61.00	bcd	49.00	cd	23.50	a
19Y01	8	60.50	bcd	52.50	bcd	19.50	ab
19Y46	8	60.50	bcd	50.00	cd	23.50	a
19Y51	8	56.50	cd	44.00	d	26.50	a
19Y15	8	55.50	d	44.50	d	25.00	a
General Mean		68.26		57.00		18.53	
General SE Mean		3.86		3.46		3.01	

Different letters following the mean in the same column signify that the mean are statistically significant difference (ANOVA p = 0.05, Tukey test). N represents number of replication. G represents grouping.

As it can be seen in Table 4.2, the effect of *Fusarium oxysporum* f. sp. *cepae* on germinated seeds, alive and dead seedlings of onion genotypes were evaluated. The mean percentages of control and inoculated seeds were shown together in Table 4.2.

From the 15 onion genotypes, Texas Early Grano 502 ranked first with 95.5% germination rate and 19Y142 ranked second with 78% germination rate. 19Y18 followed them with 76% germination rate. Onion genotypes with the rate of 19Y15 with 55.5%, 19Y51 with 56.5% and 19Y46 with 60.5% had the lowest germination percentages in Table 4.2.

In Table 4.2, when mean percentages of alive seedling were examined, onion genotypes with the highest percentage of alive seedling were TEG 502 with 88%, 19Y142 with 67.5% and 19Y18 with 63%. Onion genotypes with the lowest percentage of alive seedling were 19Y51 with 44%, 19Y15 with 44.5% and 19Y73 with 49% with effect of the pathogen.

In addition, according to percentages of dead seedling of onion genotypes, highest percentages of dead seedling was observed in the onion genotypes of 19Y06, 19Y51 and 19Y15, whereas lowest percentages of dead seedling was observed in Texas Early Grano 502, 19Y07 and 19Y142 in Table 4.2.

Moreover, in Table 4.3, the mean percentages of germinated seed, alive and dead seedlings of onion genotypes were given comparatively for both control and FOC. In addition, general mean and SE mean of the values of onion genotypes were also shown.

Table 4.3. Genotypes, experiment, mean percentages of the germinated seeds, alive and dead seedlings of control and inoculated seeds

			Germinated seeds		Alive seedlings		Dead seedlings	
Genotypes	Experiment	N	Mean	G	Mean	G	Mean	G
TEG 502	Control	4	98.00	a	98.00	a	2.00	c
TEG 502	FOC	4	93.00	ab	78.00	a	15.00	abc
19Y18	Control	4	88.00	abc	88.00	a	12.00	bc
19Y142	Control	4	85.00	abcd	85.00	a	15.00	abc
19Y17	Control	4	84.00	abcd	84.00	a	16.00	abc
19Y19	Control	4	83.00	abcde	83.00	a	17.00	abc
19Y16	Control	4	80.00	abcde	80.00	a	20.00	abc
19Y07	Control	4	79.00	abcde	79.00	a	21.00	abc
19Y06	Control	4	79.00	abcde	79.00	a	21.00	abc
19Y01	Control	4	77.00	abcde	77.00	a	23.00	abc
19Y73	Control	4	77.00	abcde	77.00	a	23.00	abc

Different letters following the mean in the same column signify that the mean are statistically significant difference (ANOVA p = 0.05, Tukey test). N represents number of replication. G represents grouping.

Table 4.3. (Continue) Genotypes, experiment, mean percentages of the germinated seeds, alive and dead seedlings of control and inoculated seeds

			Germinated seeds		Alive seedlings		Dead seedlings	
Genotypes	Experiment	N	Mean	G	Mean	G	Mean	G
19Y34	Control	4	74.00	abcdef	74.00	ab	26.00	ab
19Y46	Control	4	74.00	abcdef	74.00	ab	26.00	ab
Akgün 12	Control	4	74.00	abcdef	74.00	ab	26.00	ab
19Y15	Control	4	72.00	abcdefg	72.00	ab	28.00	ab
19Y06	FOC	4	72.00	abcdefg	36.00	cd	36.00	a
19Y51	Control	4	72.00	abcdefg	72.00	ab	28.00	ab
19Y142	FOC	4	71.00	abcdefg	50.00	bc	21.00	abc
19Y18	FOC	4	64.00	bcdefgh	38.00	cd	26.00	ab
19Y17	FOC	4	62.00	cdefgh	37.00	cd	25.00	abc
19Y19	FOC	4	60.00	cdefgh	31.00	cd	29.00	ab
19Y16	FOC	4	60.00	cdefgh	40.00	cd	20.00	abc
Akgün 12	FOC	4	57.00	defgh	34.00	cd	23.00	abc
19Y34	FOC	4	54.00	efgh	30.00	cd	24.00	abc
19Y46	FOC	4	47.00	fgh	26.00	cd	21.00	abc
19Y73	FOC	4	45.00	fgh	21.00	d	24.00	abc
19Y01	FOC	4	44.00	gh	28.00	cd	16.00	abc
19Y07	FOC	4	43.00	gh	32.00	cd	11.00	bc
19Y51	FOC	4	41.00	h	16.00	d	25.00	abc
19Y15	FOC	4	39.00	h	17.00	d	22.00	abc
General Mean			70.26		57.00		21.40	
General SE Mean			5.45		4.89		4.26	

Different letters following the mean in the same column signify that the mean are statistically significant difference (ANOVA p = 0.05, Tukey test). N represents number of replication. G represents grouping.

According to Table 4.3, dead seeds shown in the control seeds were non-germinated seeds. In other words, these dead seeds were percentage of seeds that were not germinated by their nature. In the case of inoculated seeds, it was showed the percentage of seedlings that died after germination.

It was observed that germinated seeds and alive seedlings of onion genotypes had similar values, while their dead seedlings had significant differences. While the mean percentage of germinated seeds was found to be 70.26, the mean percentage of alive seedlings was found to be 57.00. The mean percentage of dead seedlings was found to be 21.40. In addition, when looking at dead seedlings, significant differences were observed between genotypes both according to control genotypes and among themselves in Table 4.3.

Then, mean of control and inoculated alive seedlings at the end of the experiment were given comparatively in Table 4.4.

Table 4.4. Genotypes, comparison of control and inoculated alive seedlings

	Control Alive Seedling		Inoculated Alive Seedling		
Genotypes	Mean	Std Dev	Mean	Std Dev	p-value
19Y01	0.77	0.09	0.44	0.12	0.005
19Y06	0.79	0.14	0.72	0.09	0.427
19Y07	0.79	0.02	0.43	0.23	0.021
19Y15	0.72	0.03	0.39	0.07	0.000
19Y16	0.80	0.09	0.60	0.05	0.012
19Y17	0.84	0.09	0.62	0.10	0.016
19Y18	0.88	0.09	0.64	0.09	0.008
19Y19	0.83	0.11	0.60	0.16	0.063
19Y34	0.74	0.11	0.54	0.07	0.043
19Y46	0.74	0.07	0.47	0.07	0.002
19Y51	0.72	0.09	0.41	0.13	0.009
19Y73	0.77	0.04	0.45	0.12	0.003
19Y142	0.85	0.10	0.71	0.10	0.223
TEG 502	0.98	0.04	0.93	0.04	0.253
Akgün 12	0.74	0.16	0.57	0.04	0.110

(Paired Sample T-Test, p = 0.05)

When Table 4.4 was examined, significant difference was not observed between control and inoculated live seedlings of some genotypes. Examples of these genotypes were 19Y06, 19Y19, 19Y142, Akgün 12 and TEG 502 ($p > 0.05$).

2 onion varieties and 13 onion genotypes were inoculated by a FOC isolate (S22). Then, the survival rates of these onion genotypes at 2nd and 4th weeks were compared with each other in the Table 4.5. This evaluation was done with using the formula given by Saxena and Cramer, (2009).

Table 4.5. Genotypes, percentages of emergence and survival rate of onion seedlings

	Inoculated Seeds		
Genotypes	% Emergence*	% Survival* (2nd week)	% Survival* (4th week)
19Y51	41	90.24	39.02
19Y15	39	89.70	43.58
19Y73	45	93.33	46.66
19Y06	72	93.05	50.00
19Y19	60	90.00	51.66
19Y46	47	97.87	55.31
19Y34	54	96.29	55.55
19Y18	64	93.75	59.37
Akgün 12	57	91.22	59.64
19Y17	62	90.32	59.67
19Y01	44	95.45	63.63
19Y16	60	98.33	66.66
19Y142	71	98.59	70.42
19Y07	43	97.67	74.41
TEG 502	93	100.00	83.87
Mean	56.80	94.38	58.63

*Emergence rate and survival rate of onion seedlings at 2nd and 4th week was calculated using the formula given by Saxena and Cramer, 2009.

In the context of the Table 4.5, the survival seedling rates among onion genotypes ranged from 89.7% to 100% in the 2nd week, while survival seedling rates ranged from

39.02% to 83.87% in the 4th week. If the study had continued, it would have estimated that the survival seedling rates, would continue to decrease in the following weeks.

In Figure 4.1, percentages of the germinated seed, alive and dead seedling of were given in order to compare between the control and inoculated seeds.

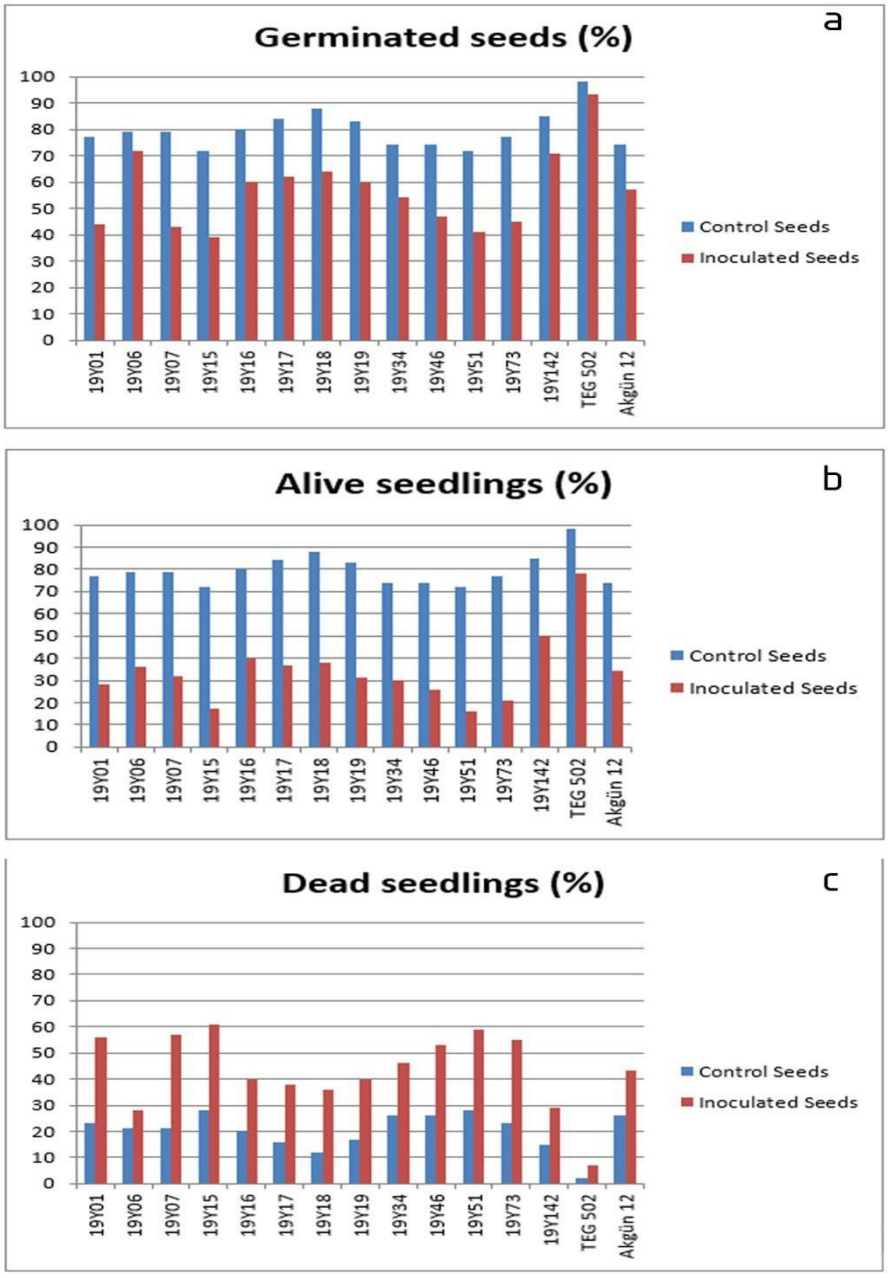


Figure 4.1. Comparing of control and inoculated seeds with percentages of germinated seed (a), alive (b) and dead seedling (c)

The counting of germinated seed (a) was completed at the end of the 2nd week, while the counting of alive seedling (b) and dead seedling (c) were completed at the end of the 4th week (Figure 4.1).

Lastly, in Figure 4.2, dead seeds (pre damping off) before germination and dead seedlings (post damping off) after germination were given comparatively.

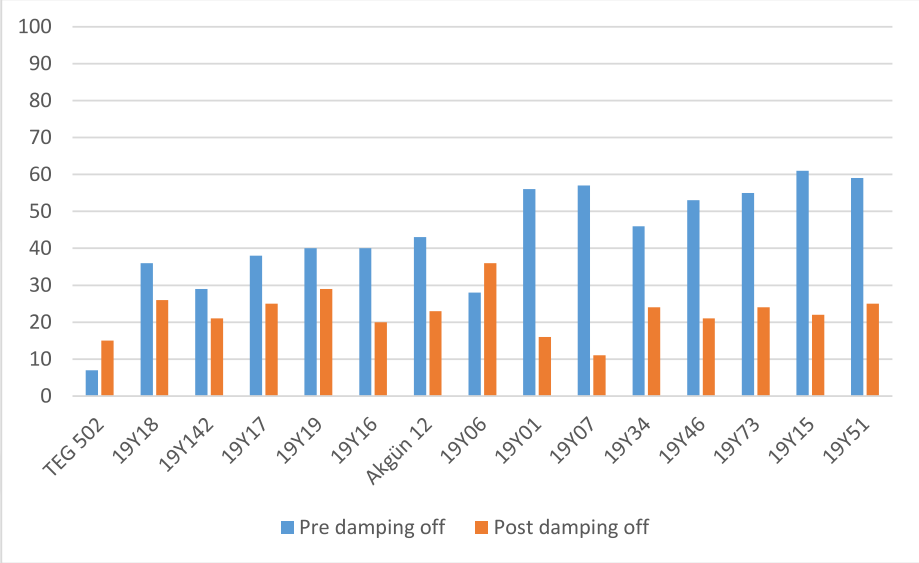


Figure 4.2. Comparing of dead seeds (pre damping off) and dead seedlings (post damping off) of onion genotypes

According to Figure 4.2, pre damping off refers to the percentage of seeds that die before germination, while post damping off refers to the percentage of seedlings that die after germination.

It was showed that rate of dead seeds before germination were significantly higher than the rate of dead seedlings after germination in Figure 4.2.

4.2 Discussion

F. oxyporum f. sp. *cepae* causes basal root rot and damping-off in edible onions and also creates great damage in the field, in the post-harvest storage and in the pre emergence and post emergence (Cramer, 2000; Ko et al., 2002; Galvan et al., 2008; Dissanayake et al., 2009; Lager, 2011; Ghanbarzadeh et al., 2014; Ünsal et al., 2019). Chemical control has been enounced for the control of diseases caused by FOC (Köycü and Özer, 1997; Özer and Köycü, 1998; Cramer, 2000; Ünsal et al., 2019). But the chemical control has a low and insufficient effect since disease factor lives in soil in the form of chlamidospore for many years (De Visser et al., 2006). In addition, many fungicides are prohibited due to the negative effects of chemical control on human and environmental health (Ünsal et al., 2019). Therefore, the most effective control method against the disease is resistance of host plant (Özer, 1998; Özer ve Köycü, 1998; Cramer, 2000; Özer et al., 2003; De Visser et al., 2006).

When FBR resistant cultivars were used, losses of caused by Fusarium basal rot of onion (FBR) could be significantly reduced (Cramer, 2000). Many resistance studies have been carried out in both the seedling stage and the bulb stage in edible onions (Fantino and Schiavi, 1987; Stadnik and Dhingra, 1997; Cramer, 2000; Ko et al., 2002; Özer, 1998; Özer et al., 2003; Özer et al., 2004; Galvan et al., 2008; Saxena and Cramer, 2009; Nasr-Esfahani et al., 2012; Nasr-Esfahani et al., 2013; Taylor et al., 2013; Lopez and Cramer, 2004; Caligiore Gei et al., 2014; Gutierrez et al., 2006).

Results obtained from disease studies may vary from region to region. Environmental states vary year by year and the performance of tested onion varieties can also vary every year (Cramer et al., 2000). Therefore, the obtained data may show differences in the resistance levels of the onion entries or the percentage of disease severity.

This study was conducted to determine the resistance levels of onions by Fusarium testing during the seedling stage. When FOC, which is a pathogenic fungus species in onion, was applied to onion seeds at conidia concentration of 10^6 ml^{-1}, it caused serious damage such as a decrease in germination of onion seeds, late ripening of onion seedlings and death of onion seedlings.

F. oxysporum f. sp. *cepae* caused germination, plant death and adverse effects on the length and weight of the root and stem in plant disease studies (Behrani et al., 2015). On that account, significant differences were noted between plant growth of inoculated and control plants. In addition, Cramer (2000) noticed that white mycelium may be on the basal plate of external bulb scales. Similar to the result of Cramer (2000) study, white mycelium was noticed on the soil of the onion seedling during seedling stage in this study (Picture 4.4).

Onion genotypes can be contrasted with standard commercial varieties. Because standard commercial varieties are widely grown and generally are resistant to root and basal rot (RBR). Applied experiments involving both breeding lines and standard commercial varieties realistically evaluate whether the new germplasm can have commercial potential (Nasr-Esfahani et al., 2013). In this study, onion genotypes were compared not only with their controls, but also with standard commercial varieties (Texas Early Grano 502 and Akgün 12).

In this study, the percentage of germinated seeds, the percentage of alive seedlings and the percentage of dead seedlings in the inoculated onion seeds were calculated by comparing them with the control seedlings. At the end of the 4-week counts, the obtained data were evaluated, analyzed and the resistance levels against damping off were determined among the onion genotypes (Table 4.1, 4.2, 4.3).

In accordance with the Table 4.2, TEG 502 has the highest germination rate with 95.5% and the highest rate of alive seedling with 88% among other onion genotypes. Thus, the resistance level of the TEG 502 appears to be highest against FOC. In other words, resistance level of 19Y15 and 19Y51 onion genotypes appear to be lowest against FOC due to having low germination rate and low alive seedling rate.

Regarding in the interaction between genotypes x experiment for germinated seeds in the study, no significant difference was observed ($p = 0.129$). However, regarding in the interaction between genotypes x experiment for alive seedlings ($p = 0.047$) and dead seedlings ($p = 0.028$), significant difference was observed (Appendix A). This situation proves that the infection has increased significantly over time and has caused more damage to genotypes.

In some genotypes, the mean of control dead seedling was observed to be higher than the mean of inoculated dead seedlings. These genotypes can be listed as follows: 19Y51, 19Y15, Akgün 12, 19Y46, 19Y34, 19Y01 and 19Y07. In the 19Y16 genotype, the mean of control and inoculated dead seedlings were equal. Other onion genotypes had higher average dead seedlings in inoculated genotypes as expected (Table 4.3). Another remarkable point was that TEG 502 onion variety have more mean of germinated seeds than other genotypes in both control and inoculated seeds (Table 4.3).

When mean of the control and inoculated alive seedlings were compared, there were no statistically significant differences in TEG 502 ($p = 0.253$), Akgün 12 ($p = 0.110$), 19Y142 ($p = 0.223$), 19Y19 ($p = 0.063$) and 19Y06 ($p = 0.427$) onion genotypes ($p > 0.05$). Significant differences were made an observation in other onion genotypes ($p < 0.05$) (Table 4.4).

In addition, seedling emergence rate and survival rate of onion seedlings at 2^{nd} and 4^{th} week were calculated using the formula given by Saxena and Cramer (2009) and the obtained data was given comparatively in the Table 4.5.

Each onion genotypes didn't differed significantly at 2^{nd} and 4^{th} weeks. For example, FOC isolate caused a little change of pre- and post emergence damping off, by a decrease from 100% survival rate after 2 weeks to 83.87% survival rate after 4 weeks in TEG 502 (Table 4.5). However, FOC isolate caused a significant amount effect of pre- and post-emergence damping off, with a decrease from 90.24% survival rate after 2 weeks to 39.02% survival rate after 4 weeks in 19Y51 (Table 4.5).

As another example, although 19Y07 onion genotype has more loss than 19Y142 in terms of damping off before germination, 19Y07 onion genotype has less loss in terms of damping off after germination than 19Y142 (Table 4.5). This example suggests that onion genotypes may differ between pre-germination resistance levels and post-germination resistance levels.

Moreover, in genotypes observed throughout the study, while the survival rate of genotypes 19Y15 and 19Y51, where the first symptoms were seen, was the lowest

compared to others, Texas Early Grano 502 onion variety showing the last symptom had the highest survival rate (Table 4.5).

On the report of a study of Özer et al. (2003) inoculated two different FOC isolates to onion varieties and measured disease severity 7 days after inoculation. The disease severity of Akgün 12, among the onion varieties, was 2.9% and 10%, respectively, while the disease severity of Texas Early Grano 502 was 48.5% and 72.5%, respectively. According to a study of Ko et al. (2002) remarked that TEG 502 was the most susceptible to FOC. Galvan et al. (2008) noted that the TEG 502 onion variety was less resistant in their study. Nasr-Esfahani et al. (2013) announced that Texas Early Grano was one of the susceptible onion genotypes in field and greenhouse conditions.

In contrast, according to the data obtained in this thesis study, at the end of the 4^{th} week, in the inoculated seeds, TEG 502 showed a 83.87% survival rate and 93% emergence rate (Table 4.5). Thus, unlike other studies, it was observed that the resistance level of Texas Early Grano 502 onion variety was highest against damping off before and after germination among other onion genotypes.

Breeding studies about the selection of genotypes against low aggressive races of *Fusarium* spp. can show onion genotypes as resistant or tolerant varieties. However, onion genotypes may show as susceptible varieties when more aggressive strains are used or when are grown in disease-prone environments (Caligiore Gei et al., 2014). Therefore, in previous studies, TEG 502 may be susceptible due to the use of a higher virulence pathogen. In other words, virulence of the pathogen used in this thesis study may be lower. Another option may show more obvious symptoms or losses in the later stages of the onion (after the 3-leaf seedling stage or during bulb stage).

According to a study of Özer et al. (2004), while Akgün 12 variety was found to be resistant in all bulb stages in their all experiments, in this thesis, the survival rate of Akgün 12 was showed 59.64% (Table 4.5). In this case, it was observed that Akgün 12 showed a moderate level of resistance against damping off during the seedling stage.

Akgün 12 may not appear more resistant compared to Texas Early Grano 502. However, regarding the germination of control seeds, it should be taken into

consideration that Akgün 12 has 74% germination rate and Texas Early Grano 502 has 98% germination rate (Figure 4.1a).

In general, it has been observed that FOC caused more damage to onion seeds before germination. In other words, it was observed that the pre damping off severity was higher than the post damping off severity in this study (Figure 4.2). The rate of dead seeds before germination were mostly observed in these genotypes: 19Y15, 19Y51, 19Y07, 19Y01 and 19Y73. The rate of dead seedlings after germination were mostly observed in these genotypes: 19Y06, 19Y19, 19Y18, 19Y51 and 19Y17 (Figure 4.2).

While the resistance levels of Texas Early Grano 502 and Akgün 12 onion varieties were known from the previous studies, the resistance levels of other onion genotypes used in this study were unknown. But this study can give an idea of the resistance levels of onion genotypes. According to the survival percentages at the end of the 4th week, the resistance levels of all onion genotypes at seedling stage were listed as follows. Texas Early Grano 502, 19Y07, 19Y142, 19Y16, 19Y01, 19Y17, Akgün 12, 19Y18, 19Y34, 19Y46 19Y19, 19Y06, 19Y73, 19Y15 and 19Y51 (Table 4.5). In the later stages, the reaction of onion genotypes to FOC may have a wider range of values.

It has been thought that differences in resistance levels changed over time and the result obtained in the 4^{th} week gave a more accurate result. In addition, the infection caused by the isolate have been thought to increase with time (Table 4.5).

Although alive seedling percentages gave similar results to germinated seeds percentages, there is a ranking difference between genotypes (Table 4.2). This proves that each genotype behave differently and show different results in pre-germination and post-germination stages. In order to determine the resistance levels between genotypes, observation and monitoring should be done carefully at each stage.

CHAPTER V

CONCLUSION

In this thesis, 13 onion genotypes and 2 onion varieties were used. While 13 genotypes and Akgün 12 were provided from the Yalova gene pool, Texas Early Grano 502 variety was provided from Bayram Seed Company. The *Fusarium oxysporum* f. sp. *cepae* isolate used was obtained from the Plant Protection Department, Ankara University. Symptoms of onion genotypes and their number before and after germination were followed.

While Akgün 12 variety was known to be tolerant from previous studies, Texas Early Grano 502 variety was known as susceptible. In this thesis study, Akgün 12 showed moderate resistance and Texas Early Grano 502 showed a high level of resistance. When the obtained results were analyzed, according to the survival percentages at the end of the 4th week, it is possible to specify the resistance levels of onion genotypes as follows: Texas Early Grano 502, 19Y07, 19Y142, 19Y16, 19Y01, 19Y17, Akgün 12, 19Y18, 19Y34, 19Y46, 19Y19, 19Y06, 19Y73, 19Y15 and 19Y51.

These 13 onion genotypes have never been used in a disease study. The resistance level of these genotypes was unknown. The results of Akgün 12 cultivar used in this study were similar to previous studies. Accordingly, one can have an idea about the resistance level of 13 other onion genotypes grown under the same conditions as Akgün 12. However, the result of Texas Early Grano 502 variety used in the study was not compatible with previous studies.

TEG 502 was used as susceptible control in this work. However, result showed that TEG 502 had highest tolerance rate among the evaluated genotypes. Unlike numerous other researchers' reports (cited elsewhere in this thesis), the result of the highest tolerance rate for TEG 502 might be speculated by two ways. One possibility is that FOC isolate was not pathogenic or virulent enough to cause severe damage to TEG 502. Hence, it is observed that only two genotype (19Y51 and 19Y15) showed significant differences between the germination rates of control and FOC application (Table 4.3). The other 13 genotypes had not significant germination rate between their control and

FOC applications. In the same table when genotypes were compared for their mean dead rates, almost none of them had a significant different value (Table 4.3). Hence experiment effect (control versus FOC application) on dead seedlings was not significant (Table 1.4 in Appendix - A). The other possibility is that TEG 502 somehow gained tolerance to used FOC isolate over the time.

In this thesis, since it was observed that the pre-damping off severity was higher than the post damping off severity, the production of onion at seed stage should be done more carefully and controlled. In addition, since the aggressiveness of pathogens changes from region to region and onion varieties found as resistant will not always show the same performance in time. Considering that plants and pathogens are living materials in the study, continuous repetition of disease studies will lead to more accurate and definite results. Therefore, disease studies should continue in a coordinated and sustainable manner in order to maintain crop productivity and quality.

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APPENDIX

A - Analysis of Variance

Table 1.1. Factor, type, levels and values used in variance analysis

Factor	Type	Levels	Values
Genotypes	fixed	15	19Y01, 19Y06, 19Y07, 19Y142, 19Y15, 19Y16, 19Y17,19Y18, 19Y19, 19Y34, 19Y46, 19Y51, 19Y73, Akgün 12, Texas Early Grano
Exp	fixed	2	Control, FOC

Table 1.2. Analysis of variance for germinated seeds, using adjusted SS test, testing the effects of genotypes, experiment and their interaction

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Genotypes	14	1.23035	1.23035	0.08788	7.37	0.000
Exp	1	1.57781	1.57781	1.57781	132.37	0.000
Genotypes*Exp	14	0.24939	0.24939	0.01781	1.49	0.129
Error	90	1.07280	1.07280	0.01192		
Total	119	4.13035				

S = 0.109179 R-Sq = 74.03% R-Sq (adj) = 65.66%

Table 1.3. Analysis of variance for alive seedlings, using adjusted SS test, testing the effects of genotypes, experiment and their interaction

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Genotypes	14	1.29880	1.29880	0.09277	9.67	0.000
Exp	1	6.20165	6.20165	6.20165	646.60	0.000
Genotypes*Exp	14	0.24515	0.24515	0.01751	1.83	0.047
Error	90	0.86320	0.86320	0.00959		
Total	119	8.60880				

S = 0.0979342 R-Sq = 89.97% R-Sq (adj) = 86.74

A - (Continue) Analysis of Variance

Table 1.4. Analysis of variance for dead seedlings , using adjusted SS test, testing the effects of genotypes, experiment and their interaction

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Genotypes	14	0.274080	0.274080	0.019577	2.70	0.002
Exp	1	0.015413	0.015413	0.015413	2.12	0.149
Genotypes*Exp	14	0.201387	0.201387	0.014385	1.98	0.028
Error	90	0.653600	0.653600	0.007262		
Total	119	1.144480				

S = 0.0852187 R-Sq = 42.89% R-Sq (adj) = 24.49%

CURRICULUME VITAE

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