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BOLU ABANT İZZET BAYSAL UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
Department of Biology

RECOVERY OF *Verticillium dahliae* FROM CERTIFIED SEED POTATOES AND CHARACTERIZATION OF STRAINS BY VEGETATIVE COMPATIBILITY AND AGGRESSIVENESS

MASTER OF SCIENCE

ELİF YAŞAR

ACADEMIC SUPERVISOR
Prof. Dr. Nusret ZENCİRCİ

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APPROVAL OF THE THESIS

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Examining Committee Members	**Signature**
Supervisor Prof. Dr. Nusret ZENCİRCİ Bolu Abant Izzet Baysal University	.....
Member Prof. Dr. Hakan ULUKAN Ankara University	.....
Member Assoc. Prof. Dr. Songül GÜREL Bolu Abant Izzet Baysal University	.....

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ABSTRACT

RECOVERY OF *Verticillium dahliae* FROM CERTIFIED SEED POTATOES AND CHARACTERIZATION OF STRAINS BY VEGETATIVE COMPATIBILITY AND AGGRESSIVENESS
MSC THESIS
ELİF YAŞAR
BOLU ABANT IZZET BAYSAL UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF BIOLOGY
(SUPERVISOR: PROF. DR. NUSRET ZENCİRCİ)

BOLU, JANUARY 2022
(xiii + 35)

Potato (*Solanum tuberosum* L.) is a very important agricultural crop in Turkey and throughout the world. It is one of the four major vegetable sources such as wheat (*Triticum* spp), corn (*Zea mays* L.), rice (*Oryza* spp.) and is especially used for the food industry and many different fields. There are lots of factors that affect the production of potato crops; bacteria, fungi, viruses, mycoplasmas, viroids, and nematodes. *Verticillium* wilt is a very important disease of the potato (*Solanum tuberosum* L.) that is caused by the potato early dying by the soilborne fungus *Verticillium dahliae*. In this thesis, we worked on 110 *Verticillium dahliae* isolates, 63 of these VCGs have belonged to VCG4A, 24 are VCG4B, 19 are VCG2A and one isolate belonges to VCG2B that was applied pathogenicity test. In this study, we performed to the aggressiveness of *V. dahliae*, and to test the resistance of widely cultivated potato varieties against vegetative compatibility groups of *V. dahliae*.

KEYWORDS: Potato (*Solanum tuberosum* L.), *Verticillium* wilt, *Verticillium dahliae,* Vegetative Compatibility Group

ÖZET

Verticillium dahliae'NIN SERTİFİKALI TOHUMLUK PATATESLERDEN ELDE EDİLEREK PATOJEN IRKLARININ VEJETATİF UYUM VE SALDIRGANLIK AÇISINDAN KARAKTERİZE EDİLMESİ
YÜKSEK LİSANS TEZİ
ELİF YAŞAR
BOLU ABANT İZZET BAYSAL ÜNİVERSİTESİ
LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ
BİYOLOJİ ANABİLİM DALI
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Patates (*Solanum tuberosum* L.), Türkiye'de ve dünyada çok önemli bir tarım ürünüdür. Buğday, mısır, pirinç gibi dört ana bitkisel üründen biridir ve özellikle gıda sanayi ile birçok farklı alanda kullanılmaktadır. Patates'in üretimini etkileyen birçok faktör vardır. Bunlar bakteriler, mantarlar, virüsler, mikoplazmalar, viroidler ve nematodlardır. *Verticillium* solgunluğu; toprak kaynaklı mantar olan *Verticillium dahliae* tarafından erkenden ölüme neden olan çok önemli bir patates hastalığıdır. Bu tezde, patojenite testi ile 110 *Verticillium dahliae* izolatı üzerinde çalışılmış olup, bu VCG'lerin 63'ü VCG4A'ya, 24'ü VCG4B'ye, 19'u VCG2A'ya ve bir izolat ise VCG2B'ye aittir ve *V. dahliae*'nın saldırganlığı ile yaygın olarak yetiştirilen patates çeşitlerinin *V. dahliae*'nin vejetatif uyumluluk gruplarına karşı direnci de test edilmiştir.

ANAHTAR KELİMELER: Patates (*Solanum tuberosum* L.), *Verticillium* Solgunluğu, *Verticillium dahliae,* Vejetatif Uyum Grupları

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LIST OF ABBREVIATIONS AND SYMBOLS

AUDPC **:** Area under the disease progress curve

FAO **:** Food and agriculture organization

G **:** Gram

Kg **:** Kilogram

LSD **:** Least Significant Difference

ml **:** Milliliter

PDA **:** Potato dextrose agar

PED **:** Potato early dying

VCG **:** Vegetative Compatibility Group

°C **:** Degrees celsius

% **:** Percentage

Ø : Diameter

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First of all, I wish to express my deepest gratitude to my supervisor Prof. Dr. Nusret Zencirci for his guidance, advice, criticism, encouragement and insight throughout the research.

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Finally, I would like to thank Prof. Dr. Mehmet Erhan Göre, I benefited from his knowledge, experience and for this project presented to us.

1. INTRODUCTION

1.1 Why Potatoes?

Potato (*Solanum tuberosum* L.) is one of the most important vegetable food sources in the world. In fact, it is a “foreigner” to our country and continent, and even to the world outside of the American continent; so, potatoes went everywhere except the continent of America (1). But it has been "indigenous" everywhere. Why?

First, it is rich in starch, which is great extent facilitates the feeding. It is a good source of nutrients (1). Second, it is easy to digest. Third, it is easy to cook and easy to consume. Fourth, it is easy to grow and easy to produce. Fifth, because it grows under the ground, it is little affected by the attack and looting of the animals. Sixth, it grows in almost any climate (it seeks mainly is mild and cool climate). The seventh is also used as animal feed, and eighth is convenient for harvesting and storage.

Because of these situations, it has made countries, societies and people dependent on them.

1.2 The history of Potatoes

Potatoes appeared as a wild plant species in the Andes mountains 8,000 years ago. The homeland of potato is South America. It came from here to Colombia, Venezuela, Chile and Argentina. Indians were planted it first time in Peru. While the Spaniards invaded Peru, they encountered potatoes with the treasure. Pedro Cieza de Leon was a Spanish conquistador and chronicler of Peru and Popayán who brought the potato from Spain to Europe for the first time. Later, it passed to England at the end of the 1500s. It spread all over Europe in the 16th century. But potatoes did not create interest in Europe (1). It was rediscovered 50 years after its discovery by an Englishman (1). By the time, potato cultivation started firstly in Italy and then in Germany, Russia, and France. Although it attracted attention in England, the potato was used here as pork and horse feed. The Irish understood the true value of potatoes firstly. At the beginning of the 17th century, potatoes started to be grown in this country for the first time in this country. The potato entered from Italy to Germany and Austria in 1745. Initially, potatoes were not very popular in Europe. Moreover, it was found to be a source of disease and was shown as the cause of the leprosy (Hansen’s disease-HD-*Mycobacterium*

leprae) disease, which was an epidemic at that time. Antoine Augustin Parmentier, an officer and chemist in the French army who has researched the benefits of potatoes due to the risk of famine in Europe, proved that the potatoes are not a poisonous plant and has become a national hero, saving people from starving.

The potatoes, entered into to Turkey in 1853 and began to be consumed by farmers in the 19th century, and farming of its beginning firstly in the Sakarya river valley of the Black Sea region and then ıt has spread all over the country from here (1). 15 years later, it started to be grown as field agriculture. With the introduction of robust and disease-free potato seeds from Marseille in 1908-1910 in France, significant increases were obtained in yield, and it was understood to be a lucrative and beneficial plant. Thereupon, potato production developed rapidly in our country and reached today's levels (1).

1.3 The importance of potatoes

Botanically, it is an annual plant that is a member of the Solanacae family which includes 2000 species, and 160-180 species of them has the ability to can form tubers (1). The potato plant is one of the basic nutrients consumed at the point of feeding the world population. It contains abundant carbohydrates, protein, vitamins and minerals(2).

On the other hand, raw potato consists of 70-80% water and 20% carbohydrates, mineral and vitamin content is at a very high level for a balanced diet of a person. 100 g potato tuber supply that minimum 7% of daily protein, 10% of iron, 20-50% of vitamin C, 10% of vitamin B1 and 3% of energy for needs a normal person (2). Similarly, it also contains plenty of vitamins A, C and B, as well as potassium, copper, tryptophan, manganese, diet pulp, magnesium and calcium (2). Potatoes with high nutritional value and are easy to digest are generally used in the production of chips and fried potatoes in the industry (3). Also, table potatoes are consumed by boiling, frying, or cooking. As with other foods, the processes applied to potatoes (cutting, cooking, frying) affect the components useful in the potato (3). The processing stages of the potato should be applied meticulously and accurately to preserve the availability of these components.

1.4 The Potato production and tuber yield in World

The potato is the most consumed nutrient after cereals in herbal nutrition. According to FAO 2017 data, 388.1 million tons of potatoes are produced in an area of 19.3 million hectares in the World (4). 29.9% of the world’s potato cultivation

areas are in China, 11.3% in India and 9.8% in Russia, these three countries realize 50.9% of world potato production. While the weight of the Central European countries is rapidly decreasing in world potato production, the shares of China and India are increasing day by day. Especially in China and India, where the population is high, potatoes have become an important product in terms of providing food security since more products are purchased from the unit area compared to other products. According to FAO 2017 data, 99.1 million tons of potatoes were produced in China and 48.6 million tons in India (4). The average potato yield in the world increased by 2.5% in 2017 compared to the previous year and reached 20.11 tons/ha. Potato trade in the world is mostly done among European Union countries. Germany ranks first place with 1.9 million tons of potato exports. France ranks second with 1.8 million tons, while Belgium ranks third with 898 thousand tons of exports. Belgium ranks first in world potato imports with 2.01 million tons, while the Netherlands ranks second with 1.6 million tons and Spain in third place with 715 thousand tons of imports. The Netherlands ranks first with 983 thousand tons in world seed potato exports and Egypt with 152 thousand tons in imports (4).

Table 1.1. Potato Data of World, FAO 2017 (thousand tons) (4)

	2013	2014	2015	2016	2017	Change (%)
Area (thousand ha)	19.293	18.879	18.914	19.077	19.303	1.2
Yield (ton/ha)	19.39	20.14	19.91	19.62	20.11	2.5
Production (ton)	374.070	380.265	376.577	374.252	388.191	3.7
Import (ton)	19.803	10.584	10.171	10.806	9.956	-7.9
Export (ton)	10.778	10.020	9.689	10.283	10.607	3.2
Export price ($/ton)	226	189	262	304	307	1.0

1.5 Production diversity and cultivation areas in Turkey

Turkey has a favorable geographical conditions for the cultivation of potatoes and 566560 hectares of potatoes are produced. Potato planting area is our largest province, Niğde with 23800 hectares (16.4%). Afyon is followed by Niğde with 13900 hectares, Konya with 10500 hectares and İzmir, Kayseri and Bolu respectively. As in the previous year, 4.7 million tons of potatoes were produced in 2017. With 892 thousand tons of potato production, Niğde ranks first in the production as in the cultivation area. Afyon with 477 thousand tons of production and İzmir, Kayseri and Nevşehir, respectively. 59.9% of the potato product are performed in these cities in Turkey.

Table 1.2. Potato Data of Turkey, FAO 2017 (thousand tons) (4)

	2012/2013	2013/2014	2014/2015	2015/2016	2016/2017	Change (%)
Area (thousand ha)	174	125	130	154	145	-6.0
Yield (kg/daa)	2.729	3.212	3.093	3.279	3.359	2.4
Production (ton)	4.821	3.955	4.175	4.763	4.750	-0.3
Import (ton)	4.490	3.843	4.212	4.643	4.316	-7.0
Export (ton)	9.43	88	129	86	75	-12.8
Export price ($/ton)	316	132	21	124	428	243.4

1.6 Potato Dıseases

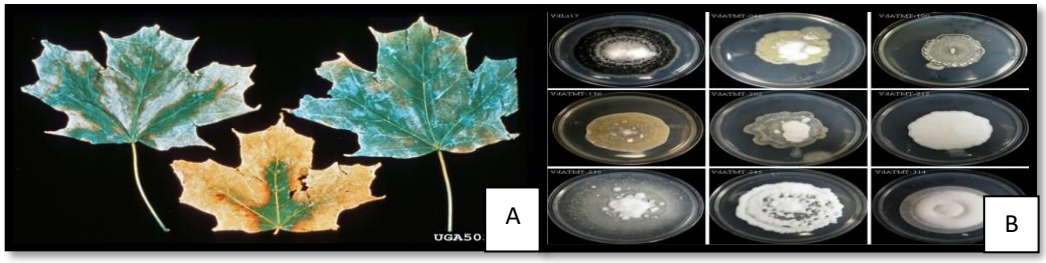
When a plant is disturbed for various reasons, the normal structure and functioning of the plant are disturbed and the plant is physiologically affected abnormally and becomes diseased (5). These stimuli, which affect the physiological and biochemical systems of the plant, enable the formation of the pathological conditions of the disease.

These diseases are divided into two classes as i) infectious and ii) noninfectious types according to the type of stimulus causing the disease. From them, i) infectious diseases caused by an agent of these diseases include bacteria, fungi, viruses, mycoplasmas, viroids, and nematodes (5). Another one is ii) noninfectious diseases, include those due to unfavorable environment, faulty nutrition, or other abiotic factors. Exposure to excessive heat, humidity and inconsistent oxygen, soil structure, mineral content and unsuitable growing conditions facilitate the process of noninfectious diseases. Because these factors are not organisms do not show the features of the host and have no reproduction capability.

About 50 are caused by fungi, 30 by viruses, 10 by bacteria, and another 50 or so are either nonparasitic or due to unknown causes. Several others are due to nematodes or insects and one is caused by dodder (6).

1.6.1 What is the Potato Early Dying or *Verticillium* wilt?

The most popular disease is potato early dying which is known resulting of *Verticillium* wilt (7). It is caused by the soilborne fungus *Verticillium dahliae* (8). It is favored by warmer temperatures and can survive from 18 to 29 °C (9). The inhibition temperature is above 30°C. *Verticillium dahliae* survives in the soil during the seasons as microsclerotia which is a monocyclic disease. It lives in plant debris or like as *mycellium* in the plant vascular system. *Verticillium dahliae* likes the temperate climates and distribute a very large host range. Temperature is of major importance in disease development since it determines pathogen growth rate (10), kind of symptoms (11), and geographical distribution of the diseases. *Verticillium dahliae* includes over 200 dicotyledonous species and herbaceous annuals which are both herbaceous and woody perennials (Photograph 1.1 A) (12).



Photograph 1.1. A) *Verticillium* Wilt B) *Verticillum dahliae* (13).

Isolates of *V. dahliae*; (Photograph 1.1 B) can show growth capability at different temperatures. More aggressive isolates show more growth at very large temperature climates than less aggressive isolates. Nematodes; are synergistic interaction with *V. dahliae*. They complete the development in potato which is affected by *Pratylenchus* penetrans. The root-lesion nematodes have a mechanism of synergism. It is feeding sites providing entry for *Verticillium* which may increase root exudation by root-lesion nematodes (14). It stimulates germ with microsclerotia. Alternatively, nematode contact with microsclerotia as root-branching.

1.7 Pathogen

Several *verticillium* species, *Verticillium alboatrum* and *Verticillium dahliae* are the two most damaging species to crops. *Verticillium* is not common in all species. It has a short survival time and does not form microsclerotia. *Verticillium dahliae* can survive at higher temperatures and show more aggression than *alboatrum*. *V. alboatrum* lives in more normal temperatures not exceeding 21 °C, but Dahliae lives at exceeding 28°C (15). The reason why some of the *Verticillium dahliae* isolates show more aggressiveness is directly proportional to the ability of these isolates to adapt to even higher temperature changes (16).

1.7.1 The pathogen of *Verticillium dahliae*

One hundred ninety (190) species belonging to the genus of Verticillium. Species differ from each other by the size of the conidia, their structure, the formation of conidiospores, the yellow-pigmented hyphae and the number of phylids.

Verticillium dahliae is the most durable of the species, it can live for more than 10 years under the soil and causes *verticillium* wilt in more than 200 plant species. The disease increases pathogen development by inhibiting vascular transport under the soil.

Nematodes induce physiological changes to the host plant and have a disease-enhancing effect, which can be attributed to several causes (17). Nematodes cause wounds in the root part where the disease is easiest to transmit and allows fungal diffusion (18).

1.7.2 Pathogen morphology

The member of fungal class Deuteromycetes (Fungi Imperfecti) is *Verticillium* has not sexual stage and multinucleate form. The nuclei are haploid.

Conidia are single-celled and has ellipsoid shape. On the phialides, hyphae produce conidiophores (13).

1.7.3 Pathogen physiology

Verticillium dahliae; is caused by vascular wilt with disruption of the water transport system and xylem elements (12). Helping with xylem elements, conidia distribute and move through cell walls. Some symptoms are appeared as chlorosis, necrosis and wilting. The fungus lives around the nonvascular tissues as colonized. Leaves and stems are died by microsclerotia. (Photograph1.2)

Photograph 1.2 Chlorosis of potato leaves

The PED (potato early dying) syndrome is characterized by a general decline of plants 4 to 6 weeks earlier than normal maturity (13). Various degrees of chlorosis and necrosis appear in the foliage system sometimes showing dying on leaves and stems. A light brown color is appeared on around the leaves. It may not diagnostic color because it can be another stress factor that is not related to the PED. Developing of disease, plant system shows early symptoms. The plants will die after this severe case.

1.7.4 Host specificity

Verticillium dahliae has different morphology and pathogenicity. These are called Vegetative Compatibility Groups (19). *Verticillium* does not reproduce sexually and transfers information by anastomosis. In this way, differences between species occur. Heterokaryons formed by isolates by anastomosis contain genetically distinct nuclei (20).

The agent has different vegetative adaptation groups (VCG). Of these, VCG1 is the most destructive on cotton and causes sudden defoliation, shortening and death of diseased plants (21). VCG2 is divided into 2A and 2B in itself. Generally, VCG2B members are found to be more virulent than VCG2A.

1.7.5 Symptoms

Verticillium wilt has 2 types of symptoms: sudden and slow wilting.

Sudden wilting; occurs from late winter to early spring. Buds and branches dry out suddenly. These symptoms can be seen in one part of the plant or all over it. When longitudinal sections are taken from the plant, it is seen that the vascular bundles turn into a dark chestnut color. Diseased plant parts, buds and branches dry up and die. The leaves lose their greenish color and turn brown and curl backward along the midrib.

Slow wilting; begin to appear in the spring. Symptoms in flowers begin to appear before leaves. If the disease occurs at the beginning of the flowering period, the flowers fall. Flower buds turn brown and die. Leaves on diseased branches are dull green. The leaves, except the end leaves, are shed before they dry. Here, the vascular bundles of the diseased plant are brown. The disease causes low yield and death in the potatoes.

Photograph 1.3. *Verticillium* wilt taken by Elif YAŞAR

1.7.6 Life Cycle

There are two main stages in the life cycle of *Verticillium dahliae*. (Figure 1.1) (13).

Dormant phase: Many factors such as soil chemistry, temperature, hydration, micro-fauna and non-host products all have an impact on the viability of the resting structure (22). *Verticillium* spp. are very diverse, the basic life cycle of the pathogen is similar between species except for their survival structures. Survival structures vary by species. *V. dahliae* produces microsclerotia to survive (23). Microsclerotia have been reported to live for more than 10 years in fields planted with non-host crops (24). When the roots of a host crop approach the resting structure, root exudate promotes germination.

Parasitic phase: Microsclerotial germination tries to reach the vascular system in the interior of the plant to enter the plant. Natural root wounds are the easiest way to enter and these wounds occur naturally even on healthy plants due to soil erosion at the roots. Direct penetration of *Verticillium* into the roots has also been observed, but these infections rarely reach the vasculature, especially those that enter through the root hairs (25).

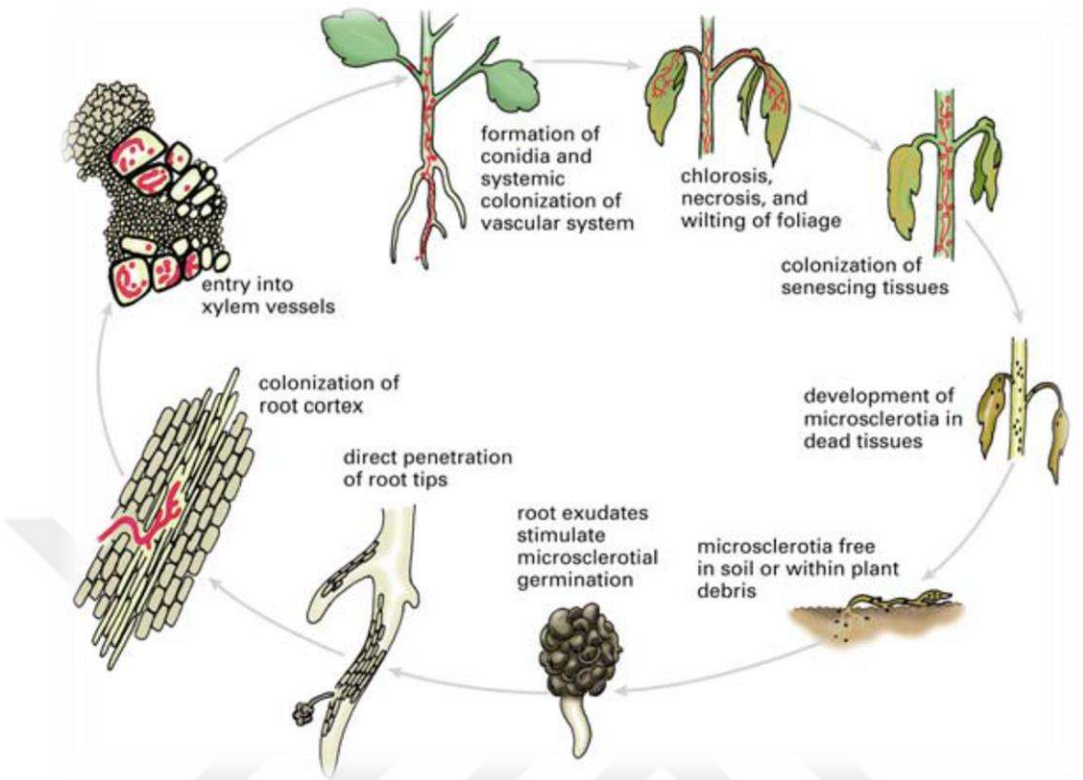


Figure 1.1. The life cycle of *Verticillium dahliae* (13)

After the pathogen enters the host, it makes its way to the vascular system and especially to the xylem. Fungi can spread in the plant as hyphae or as spores. *Verticillium*-producing conidia can spread through the xylem and rapidly colonize the plant.

A heavily infected plant may succumb to the disease and die. When this happens, *Verticillium* will form its survival structures, and when the plant dies, the survival structures will be where the plant fell, releasing the inoculations into the environment. Survival constructs will then wait for a host plant to grow nearby and restart the cycle. One study demonstrated that the formation of microsclerotia in senescent tissues of infected weeds failed to control rotationally *Verticillium dahliae* (26). Another study revealed that weeds can easily carry disease, helping *V. dahliae* survive (27).

2. AIM AND SCOPE OF THE STUDY

Verticillium wilt occurs in a wide host range but has similar devastating effects on most of these plants. In general, it causes tissue discoloration, stunting and premature defoliation and death, reducing the quality and quantity of the crop. Stocks from infected nurseries may be restricted. Once a plant is infected, there is no way to cure it. *Verticillium* wilt is a concern especially in temperate regions and irrigated areas. *Verticillium* spp. can occur naturally in forest soils and when these soils are cultivated, the pathogen will infect crops.

Potatoes grown on *Verticillium*-infested soils can have a 30-50% lower yield than potatoes grown on clean soils (13). *Verticillium* wilt begins as a mild, localized infection that will strengthen over several years as stronger strains of the fungus develop. If left unchecked, the disease will become so widespread that the crop will need to be replaced with resistant varieties or planted entirely new.

In this study, we aimed to learn *Verticillium dahliae* better, to show which subgenotype causes more damage to the potato plant, and how the disease progresses, which causes the most permanent damage to the potato among the vegetative compatibility groups and showing the production of potato affect some factors and preventing the disease effectively how is the more yield of potato.

3. MATERIALS AND METHODS

In this study, we were used 110 *Verticillium dahliae* isolates. The test pathogen isolate collection was obtained from the Republic of Turkey, Abant İzzet Baysal University, Faculty of Agriculture and Natural Sciences, Culture Laboratory Bank created by Prof. Dr. Erhan Göre.

All pathogenicity studies will be carried out on the Russet Burbank cultivar, which is known to be susceptible to the disease agent.

In the cultivar reaction tests section of the study, only the cultivars that have production permission in our country are envisaged to be tested. These species are Hermes, Gronala, Melody, Krone, Marabel, Lady Olympia, Lady Claine, Agria, Van Gogh, Bamba. Seed tubers of the potato were purchased from the Niğde Potato Research Instıtute.

Also, in this study, a temperature growth test was applied by looking at the development of the most virulent *Verticillium dahliae* isolates at respectively, 15, 20, 25, 30, 35 degrees.

3.1 MATERIALS

3.1.1 Test Plants

The Russet Burbank potato varieties were planted in the formalin disinfected soil after sprouting into tubers. 2-3 week old plants are then ready for testing.

Photograph 3.1. Potato tubers

Photograph 3.2. 2-3 weeks Potato plants

2-3 Week old healthy potato plants were grown in a climate room with 25±1°C temperature, 55-60% relative humidity and a 16 hour light period.

Roots of 2-3 week old plants were immersed in *Verticillium dahliae* spore suspension for 15 minutes (Photograph 3.3). The Russet Burbank potato variety was grown in the formalin disinfected soil with 3 plants in each pot (40 Ø) in the experiment to be established with 4 replications according to the randomized blocks trial design.

The reactions of some potato cultivars to the most virulent isolates of the identified VCGs were also tested in this way.

Photograph 3.3. Potato plants in *V. dahliae* spore suspensions and after inoculation in pots.

Photograph 3.4. 2-3 weeks potato plants

Photograph 3.5. inoculated plants

3.1.2 Test Pathogen

The isolates obtained from the culture laboratory were inoculated into petri dishes containing PDA and observed for 2 weeks of culture development. After 14 days of culture of *V. dahliae* isolates in PDA (potato dextrose agar), suspensions of 2-5.105 spores/ml were prepared and used in the pathogenicity test to be carried out in the climate chamber (Photograph 3.6,3.7).

Photograph 3.6. *V. dahliae* isolate

Photograph 3.7. *V. dahliae* suspensions of 2-5.105 spores/ml

The test pathogens used in this study were obtained from the study of Prof. Dr. Göre in 2012. VCG strains of *Verticillium dahliae* were isolated from potato cultivars cultivated in Turkey is shown that Table 3.1.

Table 3.1. Detection and vegetative compatibility characterization of *Verticillium dahliae* isolates from commercially available potato seed lots in Turkey (28).

Source		Seed lots infected/lots assayed	VCG distribution of isolates				
Cultivar	Company[z]		VCG4A	VCG4B	VCG2A	VCG2B	ND[y]
Adora	5	0/1					
Agata	8	1/1	11 isolates; Agt/92 - Agt/102				
Agria	3, 4, 6, 8, 11	2/6		6 isolates; Ag/25 - Ag/29 and Ag/45			
Alegria	4, 8	1/2	Al/50	4 isolates; Al/46 - Al/49			
Amora	8	0/1					
Anais	9	1/1	2 isolates; Ans/22 and Ans/23	Ans/21	Ans/24		
Anna	7	1/1			Ann/91		
Banba	8	1/1			Bn/107		Bn/106
Blondine	9	1/1	Bl/20				Bl/19
Borwina	4	1/1	2 isolates; Bo/58 and Bo/59		Bo/57		

Table 3.1. Detection and vegetative compatibility characterization of *Verticillium dahliae* isolates from commercially available potato seed lots in Turkey (28).

Continued…

Capri	8	1/1		6 isolates; Ca/84 - Ca/89			
Carrera	5	0/1					
Casablanca	4, 8	0/2					
Challenger	3, 5	0/3					
Chicago	4, 8	0/2					
Cripscall	5	1/1	4 isolates; Cr/37 - Cr/40				
Dione	5	0/1					
Electra	7	0/1					
Elfe	2	0/2					
Elodie	9	0/1					
Estrella	9	0/1					
Felsina	3, 5	1/3	2 isolates; Fe/43 and Fe/44	Fe/41	Fe/42		
Fosuc	8	0/1					
Galata	4, 8	2/2	6isolates ; Ga/51, Ga/53, Ga/55, Ga/56, Ga/103 and Ga/104		2 isolates; Ga/52 and Ga/54		
Granola	8, 10, 11	0/3					

Table 3.1. Detection and vegetative compatibility characterization of *Verticillium dahliae* isolates from commercially available potato seed lots in Turkey (28).
Continued…

Hanna	4, 8	1/2	2 isolates; Ha/80 and Ha/83		2 isolates; Ha/81 and Ha/82		
Hermes	11	0/1					
Innovator	3, 5	0/3					
Jaerla	8	0/1					
Krone	8	0/1					
Laderla	4	0/1					
Lady Anna	8	0/1					
Lady Blanca	3	0/2					
Lady Claire	8	0/1					
Lady Lenora	8	0/1					
Lady Olympia	8	1/1			Lo/90		
Lady Rosetta	11	0/1					
Lanorma	10	0/2					
Latona	5	0/1					
Laura	8	0/1					
Lindita	8	1/1		Li/108			
Madeleine	8	0/1					
Marabel	8, 11	1/2					Mr/109
Marfona	6, 11	2/2	2 isolates; Ma/33 and Ma/34	2 isolates; Ma/31 and Ma/32		Ma/30	

Table 3.1. Detection and vegetative compatibility characterization of *Verticillium dahliae* isolates from commercially available potato seed lots in Turkey (28).

Continued…

Melody	1	1/2		Me/2			
Musica	1, 4, 8	0/4					
Natascha	1	1/1			Na/110		
Nectar	7	0/1					
Orchestra	1, 8	1/2	Or/3				
Orlo	7	0/1					
Pomqueen	4, 8	1/2	18 isolates; Po/60 - Po/74, Po/76, Po/78 and Po/79		2 isolates; Po/75 and Po/77		
Russet Burbank	3, 4, 11						
Safrane	9	1/1	3 isolates; Sf/4 - Sf/6				
Sagitta	5	0/1					
Sante	8, 11	1/3		Sn/105			
Savanna	8	0/1					
Shepody	8	0/2					
Sifra	5	0/1					
Sissi	8	0/1					
Slaney	7	0/1					
Soleia	9	1/1	7 isolates; So/8, So/9, So/11, So/12, So/14, So/16 and So/17		5 isolates; So/7, So/10, So/13, So/15 and So/18		

Table 3.1. Detection and vegetative compatibility characterization of *Verticillium dahliae* isolates from commercially available potato seed lots in Turkey (28).

Continued…

Spunta	8	0/1					
Surya	8	0/1					
Sylvana	5, 8	0/2					
Taurus	5	1/1	Ta/36		Ta/35		
Toscana	1	1/1		To/1			
Triomphe	9	0/1					
Vangogh	5, 8	0/2					
Total		29/105	63	24	19	1	3

3.1.3 Potato Dextrose Agar (PDA) Medium

The preparation obtained by boiling 200 g potatoes in 1 liter distilled water is completed to 1 liter by filtering through cheesecloth. Twenty (20) g of agar-agar and 20 g of dextrose are added to the infusion, and autoclaved at 121°C at 15 pounds of pressure for 15 minutes poured into petri dishes in a sterile environment without cooling.

3.2 METHODS

Plants were grown in a climate chamber under 216-270 µEm-2s-1, 14h:10h L:D fluorescent lighting. The temperature and relative humidities were 22-24 °C and 50-70% in the light period, respectively, and 18-20 °C and 60-80% in the dark period. Disinfected seeds were planted in plastic pots filled with a sterilized potting mix (Sand: Clay Soil, Peat, 1: 1: 1, Volume: Volume). The plants were watered as needed in the climate room where a 16-hour light period was provided.

Three-week-old plants were gently removed from the potting mix, the roots were washed. Roots of control plants were similarly treated with sterile distilled water. Then, the plants were immersed for 10 minutes in the spore suspension (approximately 106 conidia per ml) prepared for each isolate with the pathogen cultures grown in petri dishes incubated in the dark at 24°C.

The seedlings which are 4 replications per pot were transplanted into 15 cm diameter plastic pots filled with sterilized potting mix.

Disease severity in individual plants was graded weekly from 1 to 6 according to the percentage of foliage affected by chlorotic, necrotic, and wilting symptoms in an acropetal progression.

1-6 values according to plant symptom ratio (28,29). They are:

1 = no visible symptoms

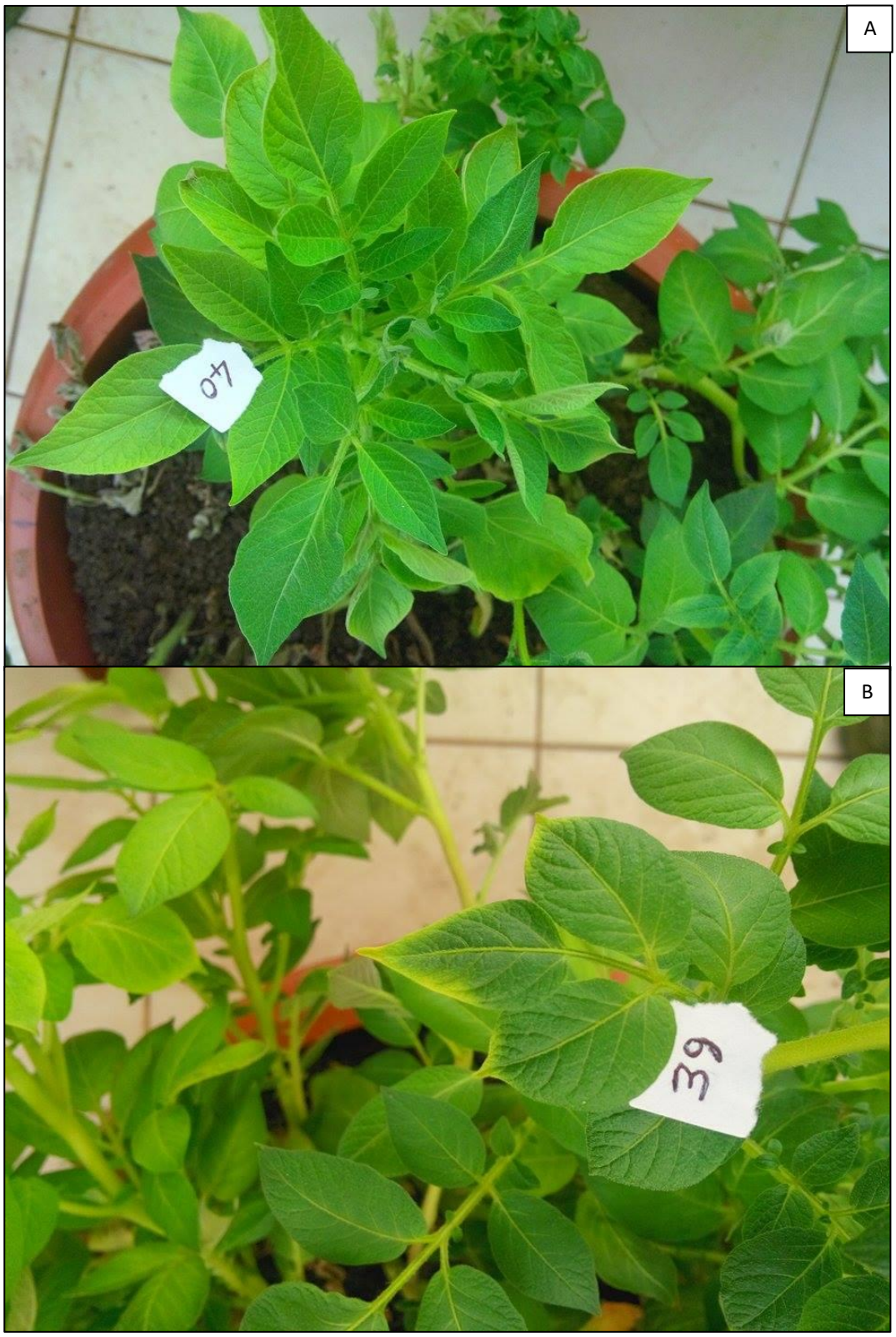
2 = slight chlorosis of lower leaves

3 = extensive chlorosis of lower leaves

4 = extensive chlorosis and some necrosis of lower and upper leaves

5 = severe suffocation with chlorosis and necrosis of the entire plant

6 = dead or nearly dead plants



Photograph 3.8. A) 1 = no visible symptoms, B) 2 = slight chlorosis of lower leaves

Photograph 3.9. A) 3 = extensive chlorosis of lower leaves, B) 4 = extensive chlorosis and some necrosis of lower and upper leaves

Photograph 3.10. A) 5 = severe suffocation with chlorosis and necrosis of the entire plant, B) 6 = dead or nearly dead plants

The plants, including the control group, were evaluated weekly and concluded with measurements made at the end of the 7th week.

For each isolate, the area under the disease progression curve (AUDPC) for each plant was calculated based on leaf symptom rates during the 1-7 week period after inoculation to determine aggressiveness (29).

Each experiment was run in a randomized whole block design with eight replicates each of a single potted plant.

Experiments were repeated once. Recorded values were averaged among plants within each experimental unit for further data analysis.

Disease severity data from all experiments were collected and the combined dataset was analyzed by one-way analysis of variance. The reactions of the determined VCGs against the most virulent isolates of potato cultivars allowed for production in our country were tested. The study will be carried out by the method specified in the “Pathogenicity test” section. Obtained AUDPC data were analyzed as one-way analysis of variance and LSD values will be evaluated at the P = 0.05 significance level.

Again in this study, the effect of the most virulent isolates on colony growth at different temperatures was examined. The development of isolates belonging to VCG 4A, 4B, 2A and 2B groups was observed in petri dishes 2 weeks after sowing, with 3 replications.

4. RESULTS AND DISCUSSIONS

4.1 Pathogenicity and Aggressiveness of Vegetative Compatibility Groups on the Russet Burbank

Disease severity, Median of disease severity ratios and areas under the disease development curve were calculated for the isolates of 110 sub-genotypes of *V. dahliae* on the Russet Burbank (Table 4.1).

In this study, there are differences between the aggressiveness and pathogenicity levels of *verticillium* isolates. VCG4A isolate showed more aggression than VCG4B and VCG2A based on AUDPC ratios. VCG2B isolates did not show any difference ($P < 0.05$) in AUDPC rates compared to VCG2A. However, it is possible to say that there are some exceptional differences. Some isolates of VCG4B and VCG2A were significantly found to be more virulent ($P < 0.05$) than some isolates in VCG4A. When this aggression rate in VCG4A is considered, it is seen that some isolates show more disease-causing effects (Table 4.1).

Considering the AUDPC rates, VCG4A (463.43) is clearly disease-causing on the species. VCG4B (384.10) ranks second in terms of disease severity, followed by VCG2A (356.96). It has also been demonstrated in previous studies that VCG4B isolates produce significantly higher disease severity than VCG2A (30). Considering the variability between species, the least difference was seen between VCG4A isolates. As a result, it was observed that VCG4A isolates had the highest lethal effect on potatoes in all cases previous studies on this subject have also shown that VCG4A is more virulent than other isolates (31). VCG2 and VCG4B (32). When the AUDPC values of the isolates in the VCG4A group were examined, thus showing how much the disease severity was observed that most of the isolates had a value above 300. Only 2 isolates found in VCG2A had AUDPC values above 300, but VCG2B isolates did not show a value above 300. VCG4A and VCG2A were the two most lethal and least lethal groups in this study. The first symptoms started to be seen in the inoculated plants from the first week. Growth retardation, chlorosis and necrosis were observed in diseased plants. In the study conducted with 110 isolates, death occurred in almost all of the plants. With mild chlorosis in VCG4B and VCG2, the plants completed the 7 weeks. There were no species that resulted in death in these plants.

Table 4.1. Median (M) values, a mean area under the disease progress curve (AUDPC) values for Vegetative Compatibility Groups on the Russet Burbank

VCG	Isolate	M	AUDPC
4A	Sf/5	5.00	288.08
	So/8	5.25	323.56
	So/10	5.00	463.43
	So/12	6.00	442.55
	So/13	4.75	384.10
	Bl/20	4.00	263.03
	Cr/40	3.75	281.81
	Al/49	5.00	325.65
	Al/50	5.00	296.43
	Ga/56	4.00	254.68
	Po/63	4.50	308.95
	Po/65	5.50	384.10
	Po/67	5.75	361.14
	Po/68	5.50	404.98
	Po/71	6.00	338.18
	Po/72	6.00	342.35
	Lo/90	6.00	375.75
	Agt/92	6.00	367.40
	Agt/94	5.75	331.91
	Ga/103	5.50	296.43
4B	To/1	5.25	311.04
	Ag/29	5.00	384.10
	Ma/31	3.25	277.64
	Fe/41	3.75	290.16
	Ag/45	5.00	288.08
	Al/46	5.00	254.68
	Ha/81	3.75	198.31
	Ca/87	5.50	379.93
2A	So/17	3.00	125.25
	Ag/25	5.25	356.96
	Ta/35	3.00	192.05
	Fe/42	3.75	269.29
	Bo/57	4.00	317.30
	Ha/82	4.00	258.85
	Na/110	4.00	267.20
2B	Ma/30	3.75	302.69

In some studies, VCG4A isolates were found to be more virulent on the potatoes compared to other plants (33).

VCG2A is lethal only in tomato (*Lycopersicum esculentum* L.) plant, while VCG2B is lethal in both species with the potatoes (30) VCG4A isolates cause sudden plant death, while VCG4B and VCG2 cause slow plant chlorosis (34).

4.2 Pathogenicity and Aggressiveness of Vegetative Compatibility Groups on the Potato Varieties

In this part of the study, we calculated the disease severity ratios and AUDPC of VCG4A and VCG4B in the 10 potato species (Table 4.2). Here, we determined that VCG4B is more virulent than VCG4A in the potato species. Looking at the average disease severity, VCG4A showed sudden wilting in almost all species in the first weeks, after which recovery from shoots was observed. This proves once again that VCG4A is highly virulent on many species. It was observed that VCG4B showed chlorosis and growth retardation on the potato species from the first week. Here, looking at the mean and AUDPC ratios between species, it was seen that VCG4B showed a slow but more pronounced aggressiveness on the potato species.

Table 4.2. Median (M), a mean area under the disease progress curve (AUDPC), calculated for the Vegetative Compatibility Groups on the potato varieties ($^{abcdefghi}P<0.05$: For each isolate (horizontal line), letters refer to differences between host species.)

	VCG 4A		VCG 4B	
	M	**AUDPC**	**M**	**AUDPC**
HERMES (a)	4.3	92.17	4.0	91.00
GRANOLA (b)	4.0	114.34	6.0	121.34a
MELODY (c)	3.0	99.17	3.7	85.17b
KRONE (d)	4.7	130.67a	6.0	151.67abc
MARABEL (e)	3.7	127.17a	4.0	177.34abc
LADY OLYMPIA (f)	6.0	88.67de	6.0	126ace
LADY CLAINE (g)	6.0	137.67acf	6.0	154abcf
AGRIA (h)	6.0	114.34	6.0	109.67deg
VAN GOGH (i)	6.0	126f	6.0	149.34abceh
BAMBA (j)	6.0	126f	6.0	168abcfh

Looking at the AUDPC, we see that the most affected type is Marabel. We see a picture that results in wilting and stunting from the first week, and death at the end of the 7th week. Hermes (91.0), with the lowest AUDPC rate, was the least affected strain from both VCG4A and VCG4B isolates among other species. Almost all strains had a disease rate of over 100, while Hermes and Melody were the only two strains resistant to VCG4A and VCG4B isolates. When we look at the AUDPC rates for both VCG groups one by one, we see that *Verticillium* has a high lethal effect on the species. With this study, variety reaction testing became a guiding reference for potato production.

4.3 Development of *Verticillium dahliae* isolates at different temperatures

Based on the results in the first part of the study, it was determined that the most virulent isolates causing the disease were Ma30, Ca85, Ann91, Agt92. The development of isolates of VCG4A, VCG4B, VCG2A and VCG2B at 15, 20, 25, 30, 35 degrees was examined (Table 4.3).

Table 4.3. The development of the most virulent VCGs at different temperatures

	Diameter at 15 °C (cm)	**Diameter at 20 °C (cm)**	**Diameter at 25 °C (cm)**	**Diameter at 30°C (cm)**	**Diameter at 35°C (cm)**
Ma30 (VCG2B)	5.9	4.5	6.4	3.9	2.4
Ca85 (VCG 4B)	5.6	5.2	3.5	4.0	2.8
Ann91 (VCG 2A)	6.7	5.5	4.8	2.2	2.6
Agt92 (VCG 4A)	6.2	5.2	5.8	4.5	3.2

The growth diameters of *Verticillium dahliae* were measured in a PDA medium for 2 weeks at different temperatures of the incubator. Meanwhile, it is seen that *Verticillium dahliae* grows best between 15 degrees and 25 degrees. In

addition, while other isolates show little growth at 35 degrees, VCG4A seems to have grown more than the others. At 25 degrees with the greatest growth, VCG2B (6.4) grew almost twice as compared to the other isolates. This confirms that the suitable temperature for the development of *Verticillium* is between 20-25 degrees.

5. CONCLUSIONS AND RECOMMENDATIONS

V. dahliae, the cause of early death disease, has become one of the most important phytopathological problems in our country and around the world with the serious crop losses it has caused in recent years and has focused attention as one of the diseases that will be studied the most in the upcoming period.

At the same time with this study, it was ensured that the most virulent representatives of VCGs were determined by pathogenicity tests, and that these isolates were used as reference isolates both in this study and in future studies, and to determine the reactions to the detected VCGs of the potato varieties widely cultivated in our country.

After the outputs of this study, the existing knowledge about the pathogen has increased, and the studies that have focused on the fight against the agent until today will be for the first time to understand the difference and kinship between the agent and its individuals. For this reason, it can be expected that the outputs of the study will change the current fighting strategies against the agent.

The first data to be obtained about the VCGs of the agent in our country, how they are distributed, their virulence and the reactions of the varieties to these VCGs will provide the basis and material for new phytopathological studies, thus contributing to the creation of new control strategies.

Project outputs will be used extensively by agronomists in breeding studies to be carried out to develop new varieties. In addition, it is expected that the project outputs will be used in the proposals of the existing varieties according to the regions.

6. REFERENCES

1. Cullen LM. Irish history without the potato. Past Present. 1968;40(1):72–83.
2. Prokop S, Albert J. Potatoes , nutrition and diet. Food Agric Organ Factsheet [Internet]. 2008;1–2. Available from: http://www.fao.org/potato-2008/en/potato/factsheets.html
3. Alexander J, Benford D, Cockburn A, Cravedi J, Dogliotti E, Domenico A Di, et al. Nitrate in vegetables - Scientific Opinion of the Panel on Contaminants in the Food chain. EFSA J. 2008;6(6):1–79.
4. Yegül Z. Tarım Ürünleri Piyasaları Patates. TC Tarım ve Orman Bakanlığı. 2019;(15).
5. Boyd AE V. Potato storage diseases. Plant Pathol. 1972;51(5):297–321.
6. Rich AE. Potato Disease. 1983.
7. Powelson ML, Rowe RC. Biology and management of early dying of potatoes. Phytopathology. 1993;31:111–26.
8. Pegg GF, Brady BL. Verticillium Wilts. Physiol Mol Plant Pathol. 2003;62(1):51–2.
9. Soesanto L, Termorshuizen AJ. Effect of temperature on the formation of microsclerotia of Verticillium dahliae. J Phytopathol. 2001;149(11–12):685–91.
10. Singh B, Lakra BS, Niwas R, Singh M. Influence of depth of planting on development of black scurf of potato (Rhizoctonia solani). Ann Biol. 2005;21(2):241–4.
11. Bouchek-Mechiche K, Pasco C, Andrivon D, Jouan B. Differences in host range, pathogenicity to potato cultivars and response to soil temperature among Streptomyces species causing common and netted scab in France. Plant Pathol. 2000;49(1):3–10.
12. Fradin EF, Thomma BPHJ. Physiology and molecular aspects of Verticillium wilt diseases caused by V. dahliae and V. albo-atrum. Mol Plant Pathol. 2006;7(2):71–86.
13. Rowe RC, Powelson ML. Potato early dying: Management challenges in a changing production environment. Plant Dis. 2002;86(11):1184–93.
14. Fan R, Cockerton HM, Armitage AD, Bates H, Cascant-Lopez E, Antanaviciute L, et al. Vegetative compatibility groups partition variation in the virulence of Verticillium dahliae on strawberry. PLoS One. 2018;13(2):1–21.
15. Zeise K, Von Tiedemann A. Host specialization among vegetative compatibility groups of Verticillium dahliae in relation to Verticillium longisporum. J Phytopathol. 2002;150(3):112–9.
16. Daayf F, Nicole M, Bélanger RR, Geiger JP. Hyaline mutants from Verticillium dahliae, an example of selection and characterization of strains for host-parasite interaction studies. Plant Pathol. 1998;47(4):523–9.
17. Back MA, Haydock PPJ, Jenkinson P. Disease complexes involving plant parasitic nematodes and soilborne pathogens. Plant Pathol. 2002;51(6):683–97.
18. Prot J-C. Biochemical and genetic basis of fungus-nematode interactions. Nematode Interact. 1993;288–301.
19. Ligoxigakis EK, Vakalounakis DJ, Thanassoulopoulos CC. Weed hosts of Verticillium dahliae in Crete: Susceptibility, symptomatology and significance. Phytoparasitica. 2002;30(5):511–8.
20. Puhalla JE. Vegetative Compatibility Groups within Verticillium dahliae . Phytopathology. 1983;73(9):1305.
21. Korolev N, Katan J, Katan T. Vegetative compatibility groups of Verticillium dahliae in Israel: Their distribution and association with pathogenicity. Phytopathology. 2000;90(5):529–36.
22. Butterfield EJ. Reassessment of Soil Assays for Verticillium dahliae. Vol. 77, Phytopathology. 1977. p. 1073.
23. Goud JKC, Termorshuizen AJ, Gams W. Morphology of Verticillium dahliae and V.

tricorpus on semi-selective media used for the detection of V. dahliae in soil. Mycol Res. 2003;107(7):822–30.

24. Chapman TA, Chambers GA, Kirkby K, Jiménez-Díaz RM. First report of the presence of Verticillium dahliae VCG1A in Australia. Australas Plant Dis Notes [Internet]. 2016;11(1):2014–7. Available from: http://dx.doi.org/10.1007/s13314-016-0197-2

25. Lysek H, Krajci D. Penetration of ovicidal fungus Verticillium chlamydosporium through the Ascaris lumbricoides egg-shells. 1987. p. 57–60.

26. Johnson WM, Johnson EK, Brinkerhoff LA. Symptomatology and formation of microsclerotia in weeds inoculated with Verticillium dahliae from cotton. Phytopathology. 1980;70(1):31–5.

27. Demirci E, Genc T. Vegetative compatibility groups of Verticillium Dahliae isolates from weeds in potato fields. J Plant Pathol. 2009;91(3):671–6.

28. Göre ME, Şenkal BC, Berk SK, Onaran H, Altin N, Ay E, et al. Recovery of Verticillium dahliae from commercially available potato seed lots planted in Turkey and characterization of isolates by vegetative compatibility and aggressiveness. Phytoparasitica. 2015;43(2):241–51.

29. Omer MA, Johnson DA, Rowe RC. Recovery of Verticillium dahliae from North American certified seed potatoes and characterization of strains by vegetative compatibility and aggressiveness. Am J Potato Res. 2000;77(5):325–31.

30. Tsror L, Hazanovsky M, Mordechi-Lebiush S, Sivan S. Aggressiveness of Verticillium dahliae isolates from different vegetative compatibility groups to potato and tomato. Plant Pathol. 2001;50(4):477–82.

31. Joaquim TR. Vegetative Compatibility and Virulence of Strains of Verticillium dahliae from Soil and Potato Plants . Vol. 81, Phytopathology. 1991. p. 552.

32. Strausbaugh CA. Assessment of vegetative compatibility and virulence of Verticillium dahliae isolates from Idaho potatoes and tester strains. Vol. 83, Phytopathology. 1993. p. 1253–8.

33. Bhat RG, Subbarao K V. Host range specificity in Verticillium dahliae. Phytopathology. 1999;89(12):1218–25.

34. Ashworth LJJ. Aggressiveness of random isolates of Verticillium dahliae from cotton and the quantitative relationship of internal inoculum to defoliation. Phytopathology. 1983;73:1292–5.