**An-Najah National University Faculty of Graduate Studies** 

# Characterization of novel sources of Fusarium resistance in Faqous (*Cucumis melo* subsp. *melo* var. *flexuosus*) by phytopathological approach

By

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### **Dedication**

To my mother "God bless her soul", my father whom I carry his name, my wife Muna for her endless support, my daughters Tasneem and Leen, my sons Rebhi, Abd-Alrahman and Omer, my dear brothers and sisters for their support and encouragement, with love and respect.

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∨ الإقرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

Characterization of novel sources of Fusarium resistance in Faqous (Cucumis melo subsp. melo var. flexuosus), by phytopathgological approach

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#### **Declaration**

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's name:	اسم الطالب:
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Characterization of novel sources of Fusarium resistance in Faqous (*Cucumis melo* subsp. *melo* var. *flexuosus*), by phytopathgological approach

 $\mathbf{B}\mathbf{y}$ 

# Imad Subhi Ibrahim Eid Supervisor

#### Dr. Munqez Shtaya

#### **Abstract**

**Background:** Palestinian snake melon (Faqous) is grown in open fields on a significant scale in Palestine, where it exhibits good climatic adaptation, and some stress and disease tolerance traits.

**Objectives:** The aim of this study was to search for novel genetic resources for breeding *Fusarium* resistant melons, concentrating on the locally adapted landraces grown by small farmers.

**Methods:** Faqous accessions from 47 Fields were screened for resistance to wilt caused by *Fusarium oxysporum* f. sp. *melonis* (Race 0,1, and 2) 39 of which screened for race 1.2 using inoculum concentration at 1x10<sup>6</sup> spores/ml, according to the Guidelines for the Identification of Races of *Fusarium oxysporum* f. sp. *melonis* using Differential Melon Lines as controls.

**Results**: All tested accessions were resistant to FOM 0 and FOM 2, while all the accessions were susceptible to FOM1.2 and FOM1. The lowest mean 0.52 of area under the disease progress curve (rAUDPC) for FOM1.2 was for accession RB38 from Batonia. Most of the rAUDPC values for

FOM1 were close to each other, with the highest rAUDPC value of 0.81 for accession AB59 from Bardala while the lowest was 0.41 for SD30 accession from Dear Baloot. There were no significant differences in the rAUDPC values for FOM 1.2 between districts from which Faqous accessions were collected, whereas, the accessions collected from Salfit showed significantly lower rAUDPC than most of the accessions for FOM1.

**Conclusions:** All Palestinian snake melons tested were resistant to FOM race 0 and race 2, and were susceptible to partially resistant to FOM1 and FOM 1.2.

**Keywords:** Spores concentration, Differential lines, *Fusarium oxysporum* f. sp. *Melonis*, rAUDPC, Resistance.

#### **Chapter One**

#### **General Introduction**

The *Cucurbitaceae* family includes 118 genera and 825 species. The most economically important crop species are melon (*Cucumis melo* L.), cucumber (*Cucumis sativus* L.), watermelon (*Citrullus lanatus* (Thunb.) Mat. & Nak.), and members of the genus *Cucurbita* L., including summer and winter squash, pumpkins, and gourds (Bisognin 2002). Munger & Robinson (1991) proposed a further-simplified division of *C. melo* into a single wild variety, C. *melo* var. *agrestis*, and six cultivated ones: Included *flexuosus* 

In Palestine, cucurbit crops are among the most widely grown vegetables, total cultivated area with cucumber, squash, and snake melon were 30,355, 2,484, and 4,573 dunums, respectively (Table 1) (Palestinian Ministry of Agriculture, MoA, 2015-2016).

Table 1: Cucurbit crops area (dunum) and production (ton/year) in Palestine (2015/2016)\*

Стор	Total Area (Dunum)	Total Production (Ton)
Cucumber	25999	155,392
Squash	20453	40,680
Muskmelon	1515	5,634
Snake cucumber (Faqous)	4608	2,813
Pumpkin	1084	2,568
Gourd	1369	2,350
Watermelon	1184	6,589
*Palestinian Ministry of Agriculture, MoA, 2015-2016. One dunum=1000m <sup>2</sup>		

Landraces of cucumber-looking melons of ancient domestication, called Faqous (snake melon), are grown in the open field on significant scale in Palestine, where they exhibit good climatic adaptation, and some stress and disease tolerance traits. Farmers have preserved the diversity of snake melon in the form of landraces, as a primary gene pool.

Soilborne diseases cause important economic loses in plant production. They influence seedlings in nurseries until harvest. *Fusarium* wilts are known to be one of the limiting factors in the production of cucurbits in the West Bank. *Fusarium* wilt of melon is a soilborne disease caused by *Fusarium oxysporum* f .sp. *melonis* (FOM). No fungicide treatment is effective against FOM, while effective soil fumigation has been banned due to its heavy environmental impact. Planting resistant cultivars and grafting on resistant rootstocks are very effective in controlling the disease.

#### **Problem hypothesis**

Fusarium wilt, caused by races of *Fusarium oxysporum* f.sp. *melonis*, have a devastating impact on melon in Palestine and the Mediterranean region in general. Identifying sources of genetic resistance and incorporating them in cultivars and rootstocks would have been the best solution, but it was never applied to locally important landraces such as Faqous melons that are not in the focus of commercial breeding. This research seeks to collect *Cucumis melo* landraces, and develop genetic resources for breeding locally adapted melons, focusing on Fusarium resistance as our first goal.

By studying various resistance sources (i.e., resistant or tolerant melon genotypes) one can elucidate different ways by which plants cope with FOM.

#### **Objectives**

Fusarium wilt is difficult to control, and has a devastating impact on melon in Palestine. This research seeks to search for novel genetic resources for breeding *Fusarium* resistant melons, concentrating on the locally adapted landraces grown by small farmers.

#### **Specific Objective**

Screen the collection for *Fusarium* wilt resistance and search for novel genetic resources for breeding *Fusarium* resistant melons, concentrating on the locally adapted landraces grown by small farmers.

### **Chapter Tow**

#### **Literature Review**

#### 2.1 Importance of melon and other cucurbit crops in Palestine

The *Cucurbitaceae* family includes 118 genera and 825 species. The most economically important crop species are melon (*Cucumis melo* L.), cucumber (*Cucumis sativus* L.), watermelon (*Citrullus lanatus* (Thunb.) Mat. & Nak.), and members of the genus *Cucurbita* L., including summer and winter squash, pumpkins, and gourds (Bisognin 2002).

Cucurbit crops are grown over a large area of the world and are consumed in large quantities in the traditional diet. Annual production of cucurbits in the world amounted to 31948349 tons melons (include cantaloupes), 118413465 tons watermelon and 83753861 tons cucumber (FAOSTAT 2017). In Palestine, cucurbit crops are among the most widely grown vegetables, total cultivated area with cucumber, squash, and snake melon were 30,355, 2,484, and 4,573 dunums, respectively (Palestinian Ministry of Agriculture, MoA, 2015-2016).

#### 2.2 The Cucumis melo germplasm: taxonomy and genetic variation

Cucumis melo is considered as the most diverse species of the genus Cucumis. Great morphological variation exists in fruit characteristics such as size, shape, color, texture, taste and composition (Whitaker & Davis 1962; Jeffrey 1980; Kirkbride 1993). The species comprises, wild and cultivated varieties; the latter includes sweet "dessert" melons, as well as

non-sweet forms that are consumed raw, pickled or cooked. *Cucumis melo* has 2n=24 chromosomes. It was long assumed that Africa is likely the origin and its closest relatives are a clade of 24-chromosome African species, while *Cucumis sativus*, the cucumber (2n=14), arose in India (Ghebretinsae et al. 2007). Recently, Australia was suggested as melon's likely origin, following a detailed molecular phylogeny of related species and genera that challenged previous views on *Cucumis* evolution and domestication (Sebastian et al. 2010).

The extensive variation found in *C. melo* led botanists to propose intraspecific classification schemes. *C. melo* was parted by Grebenscikov (1953) and Jeffrey (1980) in two subspecies, *ssp. melo* and *ssp. Agrestis*. Naudin (1859) developed a classification scheme based on a live collection of 2000 melon specimens, divided melons into 10 varieties. Munger & Robinson (1991) proposed a further-simplified division of *C. melo* into a single wild variety, *C. melo* var. *agrestis*, and six cultivated ones: *cantalupensis, inodorus, conomon, dudaim, flexuosus* and *momordica*.

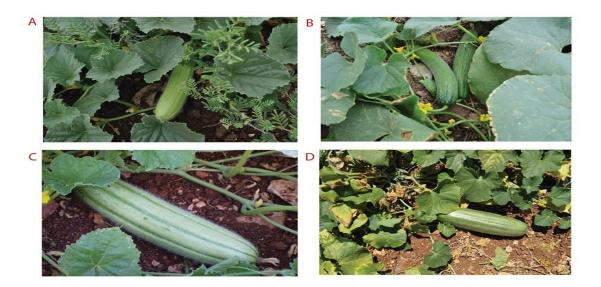
#### 2.3 Faqous (snake melon) landraces in Palestine

A landrace is a variety with a high capacity to tolerate biotic and abiotic stresses, resulting in high yield stability, and intermediate yield with a low level of inputs (Zeven 1998). Landraces of cucumber-looking melons of ancient domestication, called Faqous (snake melon, Figure 1), are grown in the open field on significant scale in Palestine, where they exhibit good climatic adaptation, and some stress and disease tolerance traits. Farmers

have preserved the diversity of snake melon in the form of landraces, as a primary gene pool.

Faqous, a rain-fed crop, is thought to be resistant to soil-borne diseases, the plant has heat resistance of the summer season, and the ability of Palestinian Faqous to thrive without irrigation could represent another trait of great interest as a rootstock for more delicate melon varieties.

Faqous fruits are picked 1-2 weeks after anthesis (flowering period), eaten immature like cucumbers, and their downy fruits are appreciated for their rich taste. The fruit color changes to yellow when ripe with a sharp muskmelon aroma. The seeds shape is more like muskmelon than cucumber, but they are rather slender like cucumber seeds. One week immature fruits have a cucumber-like taste and are consumed as alternative to cucumber (C. sativus L.) in many parts of the world (Hammer et al. 1986; Grebebshcikov 1986; Munger and Robinson 1991; Rohlf 1993).



**Figure 1**: Palestinian Faqous landraces, A: White Baladi, B: Green Baladi, C: White Sahouri and D: Green Sahouri.

#### 2.4 Fagous Properties and medical Value

C. melo var. flexuosus fruits raw or cooked have a flavor rather like cucumber and are very refreshing when eaten raw in hot weather. They can also be added to curries, cooked, preserved, or pickled. Seaweed is rich in oil with a nutty flavor, but very fiddly to use because the seed is small and covered with a fibrous coat. The seed contains between 12.5% and 39.1% oil. An edible oil is obtained from the seed (Facciola, 1998). The fruits can be used as a cooling, light cleanser, or moisturizer for the skin. They are also used as a first aid treatment for burns and abrasions. The flowers are expectorant and emetic. The fruit is stomachic. The seed is antitussive, digestive, febrifuge, and vermifuge. When used as a vermifuge, the whole seed complete with the seed coat is ground into a fine flour, then made into an emulsion with water and eaten. It is then necessary to take a purge in order to expel the tapeworms or other parasites from the body. The root is diuretic and emetic (Duke and Ayensu 1985).

#### 2.5 Melon Breeding

#### 2.5.1 Improvement of Muskmelon using Conventional Breeding

Conventional selection breeding method in muskmelon has led to a considerable varietal improvement. High sexual incompatibility restrictions at the interspecific and intergeneric levels have restricted the utlze of that genetic potential to find new and enhanced muskmelon cultivars (Robinson and Decker-Walters 1999). Choi et al. (1994) indicated that Muskmelon improvement by traditional hybridization is slow and limited to a restricted

gene pool. In contrast Dane (1991) demonstrated the possibility to produce viable intraspecific muskmelon hybrids between wild type and commercial varieties to transfer some particular muskmelon genetic characters, like disease resistance to fungi, bacteria, virus and insects, or tolerance to environmental factors, such as salinity, flooding, drought, and high or low temperature, to commercial muskmelon varieties. As indicated by Karchi (2000) 'Galia' muskmelon was the leader hybrid muskmelon produced by Research Center of the Agricultural Research Organization (ARO), Israel. Goldman (2002) showed that 'Galia' has green-fleshed qualities of 'Ha' Ogen' type, which is a smooth-skinned and sutured muskmelon, is used as the female parental line. 'Galia' has also a golden-yellow netted rind from 'Krymka', which was used as the male parental line. 'Ha' Ogen' type muskmelon is considered as individuals from *cantaloupensis* variety, whereas 'Krymka' cultivar belongs to the *reticulatus* variety.

#### 2.5.2 Improvement of Muskmelon using Recombinant Technology

Numerous considerations have given to tissue culture of the muskmelon than the intently related cucumber. Buds and shoots have been gotted in vitro directly from muskmelon cotyledons (Shetty et al. 1992) and indirectly from callus derived from cotyledons (Molina et al. 1995), root Kathal et al. 1994), hypocotyls (Molina et al. 1995) and leaves (Yadav et al. 1996). Embryogenesis in muskmelon directly from cotyledonary explants (Gray et al. 1993) has also been reported. Shoot multiplication from apical (Adelberg et al. 1993) or lateral buds (Ohki et al.1991) of

muskmelon was reported as forms of axillary multiplication. The tissue culture techniques developed, which are efficient and reproducible, are important to plant propagation and improvement of muskmelon production by genetic engineering.

Two main genetic transformation technique have been utilized to produce muskmelon transgenic plants; co-cultivation with Agrobacterium tumefaciens and particle gun bombardment (Sultana et al. 2014). Akasaka-Kennedy et al. (2004) showed that transformation success by Agrobacterium or particle gun bombardement is genotype-explant sourceand in vitro culture conditions-dependent. Zitter et al. (1998) reported that Muskmelon is attacked by many viral, bacterial, mycoplasmal and fungal diseases, Zitter also indicated that definitive disease can be managed by utilizing genetically resistance muskmelon cultivars. Transgenic 'EG360' and 'Sunday Aki' Yoshioka et al. (1993) reported that muskmelon plants which over-expressed the CMV-CP gene, cultivated in greenhouse revealed resistant to infection after inoculation with a low-dose of CMV.

Appearance, shuch as color, texture, and look of any sign of damage or disease are the top criteria for consumers to buy muskmelon. Customers are looking for nutritional and ripening information in store displays. Expanded shelf life in muskmelon fruit is an important quality attribute because increase the opportunity to commercialize muskmelon products. The first transgenic muskmelon plants carrying genes involved in fruit ripening process were founded by (guis et al. 1997).

# 2.5.3 Improvement of Muskmelon using by Polyploidization and Somaclonal Variation

Debeaujon and Branchard (1992) and Ezura et al. (1995) reported that when plant tissue culture was utilized to Cucumis melo inorder to found reliable regeneration protocols, somaclonal variation was a common observable fact, so tetraploid, octaploid, mixoploid, and aneuploid plants were easily recovered from in vitro cultures. Ezura et al. (1995) indicated the longer muskmelon cells are held under in vitro conditions, the more possibility to increase the ploidy levels in those cells. The frequency of chromosomal variation leading to aneuploid (hyperploid and hypoploid) plants at diploid, tetraploid and octaploid levels also increases. The tetraploid line of muskmelon production using somaclonal variation as well as colchicine treatment is important to production of a triploid muskmelon by hybridization a tetraploid and diploid although nowadays the triploid is not good hybrid cultivar for commercial production.

#### 2.6 Melon diseases

**Damping off:** Damping off is one of the first diseases to appear after seedlings are susceptible. Three fungi are commonly associated with damping off: *Pythium* spp., *Rhizoctonia solani* and *Thielaviopsis basicola*.

**Angular leaf spot**: Angular leaf spot is a bacterial disease that can be economically important on cucumbers, honeydew melon and zucchini. The bacteria, *Pseudomonas syringae* pv. *Lachrymans*, infect leaves, stems and fruit.

Alternaria leaf blight: Alternaria leaf blight is a fungal disease caused by the pathogen *Alternaria cucumerina*. Infected plants eventually lose their leaves, reducing fruit size and quality. It can be particularly severe on muskmelon but also affects squash, cucumbers and watermelon. Infection is most likely to occur on vine crops weakened by poor growing conditions or aging.

**Powdery mildew**: Powdery mildew caused by *Erysiphe cichoracearum* and *Sphaerotheca fuliginea* is a foliar fungal disease that occurs late in the season on muskmelons. It is less common on watermelon. On susceptible crops, this disease is often severe enough to significantly reduce yields.

**Bacterial wilt**: Bacterial wilt is a common and severe disease of vine crops caused by the bacterium, *Erwinia tracheiphila*. Muskmelons are the most severely infected but watermelons are also susceptible.

**Fusarium wilt**: A second wilt disease of cucurbits is Fusarium wilt. Each host crop is susceptible only to its own particular strain of the fungus. *Fusarium oxysporum* f.sp. *melonis* affects muskmelon. Within each strain of the fungus, different races attack various cultivars.

**Fusarium fruit rot**: Fusarium fruit rot of muskmelon is caused by the soilborne fungus *Fusarium roseum*. Usually ripe fruit is affected.

**Gummy stem blight:** Gummy stem blight, caused by the fungus *Mycosphaerella melonis*, is a common disease of muskmelon, watermelon and cucumber

**Anthracnose:** Anthracnose, caused by the fungus *Colletotrichum lagenarium*, can be a destructive disease of muskmelons during warm, wet growing seasons. The disease also attacks watermelon, cucumber and gourds.

**Downy mildew:** Downy mildew, caused by the fungus *Pseudoperonospora cubensis*, affects muskmelon and cucumber. The disease can reduce yield and fruit quality. If plants are infected early in the season, downy mildew can kill off plants. The fungus causes irregularly shaped, yellowish to brown spots on the upper leaf surface (Production guideline for muskmelons).

#### 2.7 Fusarium wilt

#### 2.7.1 Causal Agent

Fusarium wilts are known to be one of the limiting factors in the production of cucurbits in the West Bank. Fusarium wilt of melon is a soilborne disease caused by Fusarium oxysporum f .sp. melonis (FOM). Four physiological races (0, 1, 2, and 1.2) of FOM have been identified, according to their reaction with differential melon genotypes (Risser et al. 1976).

As a soil fungus, it has the ability to survive through various means, one of which is its high capacity for change, morphologically as well as physiologically, to adapt environments changes. Wind-blown soil, irrigation water and contaminated seed are capable to disseminate the

fungus (Muskett and Colhoun 1947). Highly interest, chlamydospores have a high germination rate and cause severe disease even at low spore concentrations compared to micro conidia. (Houston and Knowles 1949).

#### 2.7.2 Mode of infection, Symptoms and Genetics of resistance

The symptoms of soilborne diseases brought about by various pathogens are very similar showing root rot, root darkening, wilt, yellowing, stunting or seedling damping-off, bark cracking and twig or branch dieback.

F. oxysporum found in farming soils as a saprotroph and overwinters as thick-walled chlamydospores. The development of host establishes in their nearness induce spore germination. The hyphae attack the root either directly or through injuries and grow intercellularly through the cortex. The hyphae go inside the xylem vessels through the pits, and at that point the fungus persists exclusively in the plant vasculature. Microconidia might be created inside the xylem and moved by the transpiration stream. (Michielse and Rep 2009).

Durining the infection procedure, the xylem components turn out to be progressively impeded with mycelia, spores, parasitic exudates and gels, and gums result from oxidation of cell materials. Fusaric acid and other toxins created by the fungus inspire the quick division of parenchyma cells neighboring the xylem elements, making them to narrow. The parts of the plant above the plugedvessels wilt, leaves become discolored, and finally plant decease. After plant death, the pathogene spreads throughout all tissues and delivers spores at or close to the epidermis. This cycle is

exclusively asexual. The teleomorph either does not exist or has not been founded. The genetics of resistance to F. oxysporum is extremely variable among the species of plants it attacks and includes mono-, oligo-, and polygenic resistance patterns. Major (gene-for-gene) resistance (R) genes to *F. oxysporum* have been founded in tomato, bean, pea, melon, and cucumber (Desjardins 2006). In other host-pathogen combinations, resistance is inherited quantitatively and molded by multiple QTL.

#### 2.7.3 Controls of Melon Fusarium wilt

Like many other crops, melon is vulnerable to various foliar and root fungal pathogens that cause diseases and lessen yield and fruit quality. Among these, Fusarium wilt is caused by a soil-borne pathogen, Fusarium oxysporum Schlechtend: Fr. f. sp. Melonis (H.N. Hansen) W.C. Snyder & H.N. Hans (Fom). This fungus survives in the soil as chlamydospores, and has the potential of colonizing crop residues and roots of most crops grown in rotation with melon (Gordon et al. 1989). Subsequently, crop rotation provided limited protection against melon *Fusarium* wilt (MFW) disease (*Crino et al. 2007*).

Soil sanitization utilizing different chemicals mainly methyl bromide (Cebolla et al. 2000) was a conventional practice to control Fom in greenhouses. In view of natural and human wellbeing concerns (Brimner and Boland 2003), this fumigant was restricted in industrialized nations.

Soil solarization is another effective procedure to decrease soil inoculum and control wilt disease (Tamietti and Valentino 2006), however isn't

confortable for intensive vegetable cultivation system, where time required to solarize the soil is extremely constrained. In addition, soil solarization is frequently restricted by local climate constrains, for example, temperature and relative humidity (Shlevin et al. 2004).

Melons grafting onto resistance rootstocks is promising technique to control soil-borne diseases in vegetables, especially for MFW (Cohen et al. 2002; King et al. 2008). In contrast, the additional cost still constrains its attainability just to melon varieties with high financial value.

Utilization resistant cultivars probably the best effective and practical methods of controlling MFW. The achievement of breeding programs for MFW resistance is influenced by many elements, like: the nature of the pathogen and variation of virulence in the population; availability, diversity and type of genetic resistance; or the effectiveness of procedure and apparatuses, such as molecular markers, used for assessing plant resistance (Oumouloud et al. 2013).

#### 2.8 Resistance to Fusarium oxysporum f. sp. Melonis

Four physiological races (0, 1, 2, and 1.2) of FOM have been identified, according to their reaction with differential melon genotypes (Risser et al. 1976). Resistance to race 1 and race 2 is conferred by single dominant genes, *Fom-2* and *Fom-1*, respectively. Both genes also confer resistance to race 0. These loci have been genetically mapped and DNA markers for breeding were developed (Oumouloud et al. 2008; Tezuka et al. 2009). The *Fom-2* gene has been cloned by a map-based approach and shown to

encode an NB-LRR protein (Joobeur et al. 2004). Among genotypes that lack either the *Fom-1* or *Fom-2* alleles, variable levels of quantitative tolerance to FOM exist (Burger et al. 2003).

Resistance to race 1.2 is more difficult to obtain. This FOM race is rapidly expanding and becoming more virulent in melon growing areas, while fully resistant cultivars are not widely available. In a field survey of FOM isolates done in the southeastern Anatolia, 58.8 % of the isolates belonged to race 1.2 (Kurt et al. 2002). Villeneuve and Maignien (2008) reported aggravating phytosanitary problems in the major melon growing area of Southern France, where the FOM1.2 strains isolated from the fields have become more aggressive compared to the standard laboratory strains. This has led farmers to increasingly rely on grafting melons on Cucurbita rootstocks, despite the added cost (Cohen et al. 2007). Partial resistance to race 1.2 in melon cultivar 'Isabelle' was shown to be polygenic (Perchepied&Pitrat 2004; Perchepied et al. 2005). In Italy, Ficcadenti et al. (2002) generated doubled-haploid lines from a cross with Isabelle, and obtained acceptable FOM1.2 resistance. The need for novel sources for FOM1.2 resistance has motivated more screening of the melon germplasm for this trait (e.g., Chikh-Rouhou et al. 2010). Zvirin et al. (2010) have described novel sources of FOM 1.2 resistance and two recessive genes conferred full resistance (Herman & Perl-Treves 2007). The line served as parent of the commercial hybrid melon 'Adir'. In the heterozygous state, it displays good field resistance, but under severe inoculation in the lab the genes appear recessive and a homozygous state at two loci is required to

attain full resistance (Herman and Perl-Treves 2007; Oumouloud et al. 2013).

Jeong et al. (2015) studied the resistance degrees to the fusarium oxysporum f. sp. Melonis race 1 (GR isolate) of 22 commercial melon cultivars and 6 rootstocks for melon plants. All tested rootstocks indicated no symptoms of Fusarium wilt. Among the tested melon cultivars, only three cultivars were susceptible and the other cultivars displayed moderate to high resistance to the GR isolate.

Patel et. al. (2016) showed that fifty melon accessions of muskmelon and its close relatives (snap melon and wild melon) were screened for Fusarium oxysporum f. sp. melonis (Fom) under artificial conditions in a replicated experiment. Seedlings were exposed for five minutes to Fusarium inoculum with spore suspension (1x10<sup>6</sup> spores mL<sup>-1</sup>) at expanded cotyledonary stage. Out of 50 accessions, nine accessions have high level of resistance and three accessions have moderate level of resistance to local *Fusarium oxysporum* f. sp. *melonis* isolate.

Sixty five melon germplasm was utilized by Park et al. (2013) to screen for resistance to *Fusarium oxysporum* f. sp. *melonis* (Fom). The screening test revealed 35 accessions that are highly resistant to Fom race 1.

## Chapter Three Materials and Methods

#### 3.1 Plant material

Faqous accessions from 47 Fields were screened for resistance to wilt caused by *Fusarium oxysporum* f. sp. *melonis* (Race 0, 2 and 1.2) (Table 2) 39 of which screened for race 1 (Table 3) using inoculum concentration at  $1 \times 10^6$  spores/ml, according to the Guidelines for the Identification of Races of *Fusarium oxysporum* f. sp. *melonis* using Differential Melon Lines, Charantai-Fom2, CharantaiT, Isabella, Margot, Vedrantai as controls. The susceptibility or the resistances of the differential lines for FOM's were previously Known, (Table 4) (Sandlin & Webb, 2016).

Table 2: Faqous accessions and differinial lines used for FOM 1.2 screening test.

No.	Accession Code	Collection site	Count
1	BERC-JB01	Jenin - Bear al-basha	30
2	BERC-JZ03	Jenin – Zababdeh	30
3	BERC-JZ04	Jenin – Zababdeh	30
4	BERC-JM05	Jenin – Meslyeh	30
5	BERC-JM06	Jenin - Meslyeh	30
6	BERC-JA07	Jenin – Mythaloon	28
7	BERC-JA09	Jenin – Mythaloon	30
8	BERC-JA10	Jenin – Mythaloon	27
9	BERC-UA11	Tubas – Aqqaba	30
10	BERC-TA12	Tulkarm – Anabta	30

NT.	A		G. A
No.	Accession Code BERC-TA13	Collection site  Tulkarm – Anabta	Count 30
11			
12	BERC-TA14	Tulkarm – Anabta	15
13	BERC-TB15	Tulkarm - Beat Lead	30
14	BERC-TB16	Tulkarm - Beat Lead	15
15	BERC-QH19	Qalqilia – Hajjah	9
16	BERC-QH20	Qalqilia – Hajjah	30
17	BERC-QJ21	Qalqilia – Jeat	21
18	BERC-QJ22	Qalqilia – Jeat	15
19	BERC-QG23	Qalqilia – Gensafoot	30
20	BERC-NT25	Nablus – Til	21
21	BERC-NT26	Nablus – Til	18
22	BERC-NF27	Nablus - Al-Fara'a	30
23	BERC-NS29	Nablus – Sabastiah	30
24	BERC-SD30	Salfit - Dear Baloot	30
25	BERC-SD31	Salfit - Dear Baloot	30
26	BERC-SD32	Salfit - Dear Baloot	30
27	BERC-RT33	Ramallah – Trmosayah	30
28	BERC-RT34	Ramallah – Trmosayah	30
29	BERC-RS35	Ramallah – Sinjil	30
30	BERC-RS36	Ramallah –Sinjil	30
31	BERC-RB37	Ramallah – Batonia	30
32	BERC-RB38	Ramallah – Batonia	30
33	BERC-RA39	Ramallah - Dear Ammar	30
34	BERC-RA40	Ramallah - Dear Ammar	30
35	BERC-RA41	Ramallah - Dear Ammar	30
36	BERC-HS43	Hebron – Suba	30

No.	Accession Code	Collection site	Count
37	BERC-HB47	Hebron - Beat Kahel	30
38	BERC-HO48	Hebron - Beat Ola	24
39	BERC-BB55	Bethlahem - Beit Sahour	9
40	Charantai-Fom2		33
41	CharantaiT		33
42	Isabella		3
43	Margot		27
44	Vedrantai		33

Table 3: Faqous accessions and differinial lines used for FOM 1 screening test.

No.	Accession Code	Collection site	Count
1	BERC-JB1	Jenin - Bear al-basha	30
2	BERC-JZ3	Jenin – Zababdeh	30
3	BERC-JZ4	Jenin – Zababdeh	30
4	BERC-JM5	Jenin – Meslyeh	30
5	BERC-JM6	Jenin - Meslyeh	30
6	BERC-JA7	Jenin – Mythaloon	28
7	BERC-JA9	Jenin – Mythaloon	30
8	BERC-JA10	Jenin – Mythaloon	27
9	BERC-UA11	Tubas – Aqqaba	30
10	BERC-TA12	Tulkarm – Anabta	30
11	BERC-TA13	Tulkarm – Anabta	30
12	BERC-TA14	Tulkarm – Anabta	15
13	BERC-TB15	Tulkarm - Beat Lead	30
14	BERC-TB16	Tulkarm - Beat Lead	15

No.	Accession Code	Collection site	Count
15	BERC-QH19	Qalqilia – Hajjah	9
16	BERC-QH20	Qalqilia – Hajjah	30
17	BERC-QJ21	Qalqilia – Jeat	21
18	BERC-QJ22	Qalqilia – Jeat	15
19	BERC-QG23	Qalqilia – Gensafoot	30
20	BERC-NT25	Nablus – Til	21
21	BERC-NT26	Nablus – Til	18
22	BERC-NF27	Nablus - Al-Fara'a	30
23	BERC-NS29	Nablus – Sabastiah	30
24	BERC-SD30	Salfit - Dear Baloot	30
25	BERC-SD31	Salfit - Dear Baloot	29
26	BERC-SD32	Salfit - Dear Baloot	30
27	BERC-RT33	Ramallah – Trmosayah	30
28	BERC-RT34	Ramallah – Trmosayah	30
29	BERC-RS35	Ramallah – Sinjil	30
30	BERC-RS36	Ramallah –Sinjil	30
31	BERC-RB37	Ramallah – Batonia	30
32	BERC-RB38	Ramallah – Batonia	30
33	BERC-RA39	Ramallah - Dear Ammar	30
34	BERC-RA40	Ramallah - Dear Ammar	30
35	BERC-RA41	Ramallah - Dear Ammar	30
36	BERC-HS43	Hebron – Suba	30
37	BERC-HB47	Hebron - Beat Kahel	30
38	BERC-HO48	Hebron - Beat Ola	24
39	BERC-BT53	Bethlahem - Taqoa'a	15
40	BERC-BT54	Bethlahem - Taqoa'a	15

No.	<b>Accession Code</b>	Collection site	Count
41	BERC-BB55	Bethlahem - Beit Sahour	24
42	BERC-BB56	Bethlahem - Beit Sahour	15
43	BERC-BB57	Bethlahem - Beit Sahour	15
44	BERC-UA58	Tubas – Aqqaba	3
45	BERC-AB59	Jericho – Bardala	3
46	BERC-AB60	Jericho – Bardala	3
47	BERC-AZ61	Jericho – Zbadat	3
48	CharantaiT		36
49	Vedrantai		36

#### 3.2 Screening Procedure

- 1. Seeds were sown in vermiculite, geminated and grown in a greenhouse at 25- 30°C with 16 hour photoperiod. Seedlings were not fertilized before inoculation (Figure 2).
- 2. Six days before inoculation of the plants, the inoculum suspension was prepared by inoculating V-8 broth (200 ml V-8 juice, 800 ml water, 3 g CaCO3) with by *Fusarium oxysporum* f. sp. *melonis* FOMs (FOM0, FOM1, FOM2, and FOM1.2) forming four difference inoculum suspensions (or cultures). The cultures were incubated for 1 week at 30°C on a rotary shaker set fast enough to keep the culture aerated.
- 3. Seedlings inoculations with FOMs were conducted when the cotyledons of the seedlings were fully expanded (10 to 14 days after sowing). The inoculum was prepared by filtrating the culture through sterile cheesecloth, and the spore suspension concentration in the culture was adjusted to 1 X

- 10<sup>6</sup> spores/ml (Figure 3). The seedlings were gently removed from the vermiculite, and the roots were washed in sterile water. The washed roots were cut using sterile scissors, and submerged in the spore suspension for 5 minutes. The seedlings were transplanted into sterile potting mix, and allowed to recuperate in a cool, dark, humid environment overnight.
- 4. The day after inoculation the plants were moved to a growth chamber, and maintained for three weeks at 24°C with 12 hours of light/day. The soil was kept moist, but not saturated. The plants were fertilized using 13-13-13 compound fertilizer 2gm/l of water, 10 days after inoculation.
- 5. The seedlings will typically regain turgor after inoculation, and then the susceptible plants started to wilt 5 to 7 days after inoculation. Three weeks after inoculation the results were clear, with resistant plants remained asymptomatic, while susceptible plants developed symptoms including wilt, stunting, vascular discoloration, and death. Sometimes it was necessary to cut the stems of stunted plants to look for vascular necrosis. The data was collected every two days according to the sore shown in Figure 4.



**Figure 2**: Inoculation procedures of Faqous seedlings with *Fusarium oxysporum* f. sp. *Melonis*. A: Seeds are sown in vermiculite, B: Melon seedlings grown in vermiculite, C: Removal of seedlings from vermiculite, D: Washing off the roots, E: Trimming the roots, F: seedlings soaking in *Fusarium oxysporum* f. sp. *melonis* inoculum, G: Melon seedlings transplanted into potting mix, H: inoculated seedlings in a growth chamber, I: Melon seedlings 2 wks after inoculation with Fom. Resistant seedlings are asymptomatic, but susceptible seedlings are dead or dying.



**Figure 3**: Preparation of inoculum suspension: Check the concentration of spores in the inoculum suspension under the microscope using a hemocytometer.

Each inoculation round included positive and negative control genotypes (Differential lines) in addition to non-inoculated control: The susceptible or resistance character to FOM 0, 1, 2 and 1.2 for each differential line was demonstrated, Charentais (T) has resistance to all races, Charentais (Fom2) has resistance to race 0, race 1 and susceptible to race 2 and 1.2, Vedrantais has resistance to race 0, race 2 and susceptible to race 1 and 1.2, Margot has resistance to race 0, race1, race 2 and susceptible to race 1.2, Isabella has resistance to race 0, race 1, race 2 and partial resistance to Race 1.2 (Table 4).

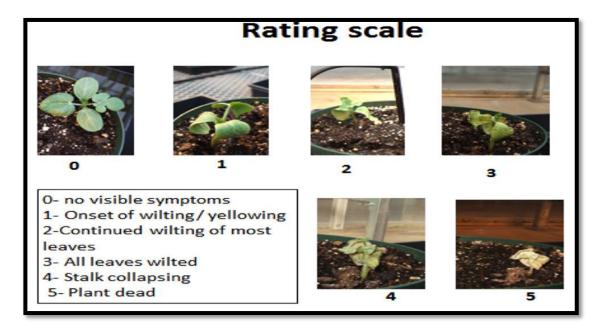
Table 4: Classification of Races of *Fusarium oxysporum* f. sp. *melonis* (Causing fusarium wilt) using differential host lines of Cucumis melo (Melon).

Differential hosts and genes for resistance									
Race	Charentais (T)	Charentais (Fom2)	Vedrantais	Margot	Isabella				
Race 0	S	R	R	R	R				
Race 1	S	R	S	R	R				
Race 2	S	S	R	R	R				
<b>Race 1.2</b>	S	S	S	S	PR				

 $\underline{S} = \underline{Susceptible}, R = \underline{Resistant reaction}, PR = \underline{Partial resistance}$ 

#### Scoring the symptom of fusarium wilting

Rating scale (0-5) was used to score the symptoms of *Fusarium* wilting (Figure 4).



**Figure 4**: Rating scale for Fusarium wilting symptoms (Courtesy: Department of Horticulture, University of Georgia; Athens, GA.).

#### 3.3 Data analysis

For statistical analyses, the values of the area under the disease progress curve (AUDPC) were used. The AUDPC integrates both the intensity of symptoms and the time taken between inoculation and symptoms expression.

The area under the disease progress curve (AUDPC) was calculated according to the formula proposed by Madden et al. (2007):

$$AUDPC = \sum_{i}^{n-1} \left[ \left( \frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i) \right]$$

Where:

(i): scoring period 0-5,

(yi): mean of the symptom scores for disease,

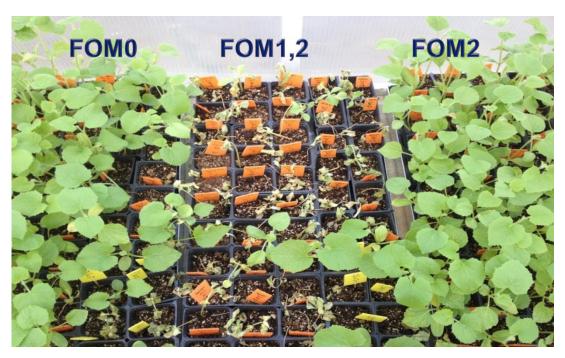
(ti+1 - ti): the numbers of days between scoring date i and scoring date i +1. AUDPC value not used across experiments, so relative AUDPC (rAUDPC) value was used.

The rAUDPC is calculated by dividing the AUDPC by the "maximum potential AUDPC."

### Chapter Four Results

#### 4.1 Fagous Screening for Fusarium oxysposrum melonis

All tested accessions were resistant to FOM 0 and FOM 2 (Figure 5), the relative area under the disease progress curve for the Faqous accessions inoculated with *Fusarium oxysporum melonis* (Race1& Race1.2) was calculated in terms of collection sites, districts and landraces.

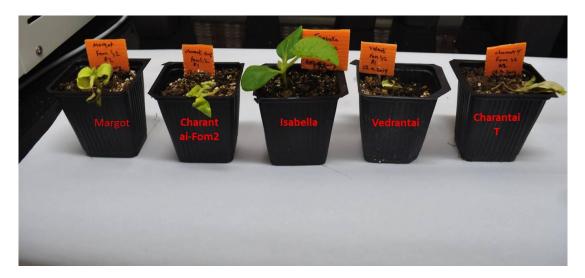


**Figure 5**: Example of snake melon (Faqous) response to Fusarium oxysporum f. sp. melonis (FOM) races

## 4.2 Relative area under the disease progress curve according to the site of collection.

For Fom1.2 the lowest relative area under the disease progress curve was found for the Isabella and the highest for BERC-RS35. The lowest mean 0.52 of rAUDPC for Palestinian accessions was found for BERC-RB38.

Significant differences in the rAUDPC were observed between Isabella and Margot and between Margot and BERC-NF27, BERC-NS29, BERC-NT26, BERC-RA41, BERC-TA13, BERC-RA39, BERC-JA10, BERC-UA11, BERC-NT25, BERC-SD31, BERC-TA12, BERC-BB55, Charantai-Fom2, CharantaiT, BERC-HO48, BERC-HS43, BERC-JA9, BERC-JM5, BERC-QH20, BERC-JM6, BERC-JZ3, BERC-QG23, BERC-HB47, BERC-JA7, BERC-QJ22, BERC-RS36, BERC-QJ21, BERC-JB1, BERC-QH19, BERC-RS35, BERC-RT34 were obtained. In addition, significant differences were found between BERC-RS35 and all accessions (Table 5). For FOM1 most of the rAUDPC values were comparable; the highest rAUDPC value 0.81 was for BERC-AB59 while the lowest 0.41 for BERC-SD30. However, there was significant differences between BERC-SD30 accession and BERC-NT26, BERC-JA7, BERC-QG23, BERC-JA9, BERC-NF27, BERC-RA39, BERC-BT54, BERC-RS36, BERC-BB55, BERC-QH19, BERC-JB1, BERC-BB57, BERC-HS34, BERC-RA41, BERC-HO48, BERC-TA12, BERC-QJ22, BERC-UA11, BERC-AB60, BERC-HB47, BERC-RT34, Vedrantai, CharantaiT, BERC-AZ61, BERC-JA10, BERC-BB56, BERC-RS35, BERC-UA58 and BERC-AB59 accessions. In addition, significant differences were observed between BERC-SD30, BERC-SD32, BERC-SD31, BERC-JM6, BERC-TB16, BERC-RB37, BERC-JZ4, BERC-JZ3, BERC-RB38, BERC-TA13, BERC-RA40, BERC-TB15, BERC-TA14, BERC-NT25, BERC-QJ21, BERC-NT26, BERC-JA7, BERC-QG23, BERC-JA9, BERC-NF27, BERC-NS29, BERC-RA39, BERC-BT54 and BERC-RS36.In contrast there was significant difference between Isabella and the others differential lines (Table 6 & Figure 6).



**Figure 6**: The response of the differinial lines inoculated with *Fusarium oxysporum* f .sp. *melonis* race 1.2.

Table 5: Relative area under the disease progress curve observed for different Faqous accessions from different locations and for the differential lines inoculated with Fom1.2.

				FOM 1.2 rAUDPC			
No.	Accession Code	Collection Site	Count	Minimum	Maximum	Mean	Mean+SD
1	BERC-JB01	Jenin - Bear al-basha	30	.45	.92	.76	0.76±0.12 <sup>ij</sup>
2	BERC-JZ03	Jenin - Zababdeh	30	.46	.82	.70	0.7±0.1 <sup>fghij</sup>
3	BERC-JZ04	Jenin - Zababdeh	30	.27	.84	.60	0.6±0.11 <sup>bcdefg</sup>
4	BERC-JM05	Jenin - Meslyeh	30	.57	.82	.68	0.68±0.04 <sup>defghij</sup>
5	BERC-JM06	Jenin - Meslyeh	30	.69	.69	.69	0.69±0 <sup>efghij</sup>
6	BERC-JA07	Jenin - Mythaloon	28	.43	.92	.71	0.71±0.1 <sup>ghij</sup>
7	BERC-JA09	Jenin - Mythaloon	30	.63	.69	.67	0.67±0.03 <sup>defghij</sup>
8	BERC-JA10	Jenin - Mythaloon	27	.63	.65	.64	0.64±0.01 <sup>cdefghij</sup>
9	BERC-UA11	Tubas - Aqqaba	30	.63	.65	.64	$0.64\pm0.01^{\text{cdefghi}}$
10	BERC-TA12	Tulkarm - Anabta	30	.61	.74	.64	$0.64\pm0.02^{\text{cdefghij}}$
11	BERC-TA13	Tulkarm – Anabta	30	.41	.71	.62	$0.62\pm0.09^{\text{cdefgh}}$
12	BERC-TA14	Tulkarm - Anabta	15	.50	.67	.58	0.58±0.08 <sup>bcdef</sup>
13	BERC-TB15	Tulkarm - Beat Lead	30	.50	.67	.57	0.57±0.06 <sup>bcdef</sup>
14	BERC-TB16	Tulkarm - Beat Lead	15	.53	.67	.60	$0.6\pm0.06^{\mathrm{bcdefg}}$
15	BERC-QH19	Qalqilia - Hajjah	9	.71	.82	.77	$0.77\pm0.04^{\rm j}$
16	BERC-QH20	Qalqilia - Hajjah	30	.60	.79	.68	0.68±0.05 <sup>defghij</sup>
17	BERC-QJ21	Qalqilia - Jeat	21	.68	.77	.73	$0.73\pm0.02^{\text{hij}}$
18	BERC-QJ22	Qalqilia - Jeat	15	.67	.77	.72	0.72±0.03 <sup>ghij</sup>
19	BERC-QG23	Qalqilia - Gensafoot	30	.53	.78	.69	0.69±0.08 <sup>fghij</sup>
20	BERC-NT25	Nablus - Til	21	.44	.79	.65	$0.65\pm0.14^{\text{cdefghij}}$
21	BERC-NT26	Nablus - Til	18	.37	.78	.62	0.62±0.17 <sup>cdefgh</sup>
22	BERC-NF27	Nablus - Al-Fara'a	30	.33	.78	.63	0.63±0.1 <sup>cdefgh</sup>
23	BERC-NS29	Nablus - Sabastiah	30	.45	.71	.63	0.63±0.07 <sup>cdefgh</sup>
24	BERC-SD30	Salfit - Dear Baloot	30	.55	.57	.56	0.56±0.01 <sup>bcd</sup>
25	BERC-SD31	Salfit - Dear Baloot	30	.55	.88	.65	0.65±0.13 <sup>cdefghij</sup>
26	BERC-SD32	Salfit - Dear Baloot	30	.55	.57	.56	0.56±0.01 <sup>bcde</sup>
27	BERC-RT33	Ramallah – Trmosayah	30	.45	.75	.58	$0.58\pm0.06^{\text{bcdef}}$

				FOM 1.2 r.	AUDPC		
No.	Accession Code	Collection Site	Count	Minimum	Maximum	Mean	Mean+SD
28	BERC-RT34	Ramallah - Trmosayah	30	.24	1.04	.89	$0.89\pm0.19^{k}$
29	BERC-RS35	Ramallah - Sinjil	30	.86	.99	.95	$0.95\pm0.05^{k}$
30	BERC-RS36	Ramallah -Sinjil	30	.24	.83	.72	$0.72\pm0.11^{ghij}$
31	BERC-RB37	Ramallah - Batonia	30	.24	.89	.60	$0.6\pm0.19^{\text{bcdefg}}$
32	BERC-RB38	Ramallah - Batonia	30	.32	.71	.52	0.52±0.1 <sup>bc</sup>
33	BERC-RA39	Ramallah - Dear Ammar	30	.39	.71	.64	$0.64\pm0.1^{\mathrm{cdefghi}}$
34	BERC-RA40	Ramallah - Dear Ammar	30	.29	.74	.56	0.56±0.13 <sup>bcde</sup>
35	BERC-RA41	Ramallah - Dear Ammar	30	.32	.75	.62	0.62±0.17 <sup>cdefgh</sup>
36	BERC-HS43	Hebron - Suba	30	.26	.75	.67	0.67±0.16 <sup>defghij</sup>
37	BERC-HB47	Hebron - Beat Kahel	30	.65	.75	.72	$0.72\pm0.04^{\text{ghij}}$
38	BERC-HO48	Hebron - Beat Ola	24	.23	.75	.69	0.69±0.14 <sup>defghij</sup>
39	BERC-BB55	Bethlahem - Beit Sahour	9	.50	.84	.68	0.68±0.13 <sup>defghij</sup>
40	Charantai-Fom2		33	0.00	.90	.68	0.68±0.17 <sup>defghij</sup>
41	CharantaiT		33	.39	.90	.65	0.65±0.15 <sup>defghij</sup>
42	Isabella		3	.17	.57	.37	0.37±0.2 <sup>a</sup>
43	Margot		27	0.00	.83	.49	0.49±0.2 <sup>b</sup>
44	Vedrantai		33	.25	.83	.60	0.6±0.15 <sup>bcdefgh</sup>

Tukey's b test was used to separate mean values

Table 6: Relative area under the disease progress curve observed for different Faqous accessions from different location and for the differential lines inoculated with Fom1.

		Fom 1 rAUDPC						
No.	Code C	ollection Site	Count	Minimum	Maximum	Mean	Mean±SD	
1	BERC-JB1	Jenin - Bear al-basha	30	0.54	0.87	0.65	0.65±0.11 <sup>defghijklmnopq</sup>	
2	BERC-JZ3	Jenin - Zababdeh	30	0.54	0.59	0.55	0.55±0.01 <sup>abcdefghi</sup>	
3	BERC-JZ4	Jenin - Zababdeh	30	0.51	0.56	0.54	$0.54\pm0.01^{\text{abcdefgh}}$	
4	BERC-JM5	Jenin - Meslyeh	30	0.43	0.56	0.5	0.5±0.04 <sup>abcde</sup>	
5	BERC-JM6	Jenin - Meslyeh	30	0.43	0.52	0.48	0.48±0.02 <sup>abcd</sup>	
6	BERC-JA7	Jenin - Mythaloon	28	0.47	0.92	0.59	0.59±0.16 <sup>cdefghijklmn</sup>	
7	BERC-JA9	Jenin - Mythaloon	30	0.47	0.83	0.61	0.61±0.11 <sup>cdefghijklmno</sup>	
8	BERC-JA10	Jenin - Mythaloon	27	0.63	0.86	0.76	$0.76\pm0.07^{\text{nopq}}$	
9	BERC-UA11	Tubas - Aqqaba	30	0.56	0.88	0.71	0.71±0.1 <sup>hijklmnopq</sup>	
10	BERC-TA12	Tulkarm - Anabta	30	0.42	0.89	0.71	0.71±0.15 <sup>hijklmnopq</sup>	
11	BERC-TA13	Tulkarm – Anabta	30	0.25	0.71	0.55	0.55±0.1 <sup>abcdefghi</sup>	
12	BERC-TA14	Tulkarm - Anabta	15	0.47	0.59	0.57	0.57±0.04 <sup>abcdefghijkl</sup>	
13	BERC-TB15	Tulkarm - Beat Lead	30	0.23	0.78	0.56	0.56±0.11 <sup>abcdefghijk</sup>	
14	BERC-TB16	Tulkarm - Beat Lead	15	0.4	0.63	0.51	$0.51 \pm 0.08^{abcdef}$	
15	BERC-QH19	Qalqilia - Hajjah	9	0.46	0.7	0.63	0.63±0.11 <sup>cdefghijklmnopq</sup>	
16	BERC-QH20	Qalqilia - Hajjah	30	0.31	0.63	0.48	0.48±0.07 <sup>abcd</sup>	
17	BERC-QJ21	Qalqilia - Jeat	21	0.44	0.65	0.58	0.58±0.06 <sup>abcdefghijklm</sup>	
18	BERC-QJ22	Qalqilia - Jeat	15	0.5	0.84	0.69	0.69±0.1 <sup>ghijklmnopq</sup>	
19	BERC-QG23	Qalqilia - Gensafoot	30	0.37	0.78	0.59	0.59±0.13 <sup>cdefghijklmn</sup>	
20	BERC-NT25	Nablus - Til	21	0.37	0.78	0.58	0.58±0.12 <sup>abcdefghijklm</sup>	
21	BERC-NT26	Nablus - Til	18	0.37	0.78	0.58	0.58±0.16 <sup>bcdefghijklm</sup>	
22	BERC-NF27	Nablus - Al-Fara'a	30	0.48	0.78	0.61	0.61±0.12 <sup>cdefghijklmno</sup>	
23	BERC-NS29	Nablus - Sabastiah	30	0.33	0.78	0.61	0.61±0.18 <sup>cdefghijklmno</sup>	
24	BERC-SD30	Salfit - Dear Baloot	30	0.39	0.45	0.41	$0.41\pm0.02^{a}$	
25	BERC-SD31	Salfit - Dear Baloot	29	0.25	0.84	0.47	$0.47\pm0.16^{abc}$	

	Fom 1 rAUDPC						
No.	Code C	ollection Site	Count	Minimum	Maximum	Mean	Mean±SD
26	BERC-SD32	Salfit - Dear Baloot	30	0.39	0.45	0.42	0.42±0.02 <sup>ab</sup>
27	BERC-RT33	Ramallah – Trmosayah	30	0.39	0.63	0.47	$0.47\pm0.07^{abc}$
28	BERC-RT34	Ramallah - Trmosayah	30	0.2	0.84	0.73	$0.73\pm0.15^{\mathrm{jklmnopq}}$
29	BERC-RS35	Ramallah - Sinjil	30	0.71	0.81	0.78	$0.78\pm0.04^{\rm opq}$
30	BERC-RS36	Ramallah -Sinjil	30	0.24	0.71	0.63	0.63±0.09 <sup>cdefghijklmnop</sup>
31	BERC-RB37	Ramallah - Batonia	30	0.16	0.69	0.52	$0.52\pm0.16^{abcdefg}$
32	BERC-RB38	Ramallah - Batonia	30	0.42	0.67	0.56	0.56±0.08 <sup>abcdefghi</sup>
33	BERC-RA39	Ramallah - Dear Ammar	30	0.34	0.69	0.62	0.62±0.0 <sup>9cdefghijklmno</sup>
34	BERC-RA40	Ramallah - Dear Ammar	30	0.16	0.69	0.56	0.56±0.15 <sup>abcdefghij</sup>
35	BERC-RA41	Ramallah - Dear Ammar	30	0.39	0.75	0.67	0.67±0.11 <sup>efghijklmnopq</sup>
36	BERC-HS43	Hebron - Suba	30	0.26	0.75	0.67	0.67±0.16 <sup>efghijklmnopq</sup>
37	BERC-HB47	Hebron - Beat Kahel	30	0.65	0.75	0.72	$0.72\pm0.04^{ijklmnopq}$
38	BERC-HO48	Hebron - Beat Ola	24	0.23	0.75	0.69	0.69±0.14 <sup>fghijklmnopq</sup>
39	BERC-BT53	Bethlahem - Taqoa'a	15	0.33	0.78	0.64	0.64±0.14 <sup>cdefghijklmnopq</sup>
40	BERC-BT54	Bethlahem - Taqoa'a	15	0.41	0.78	0.63	0.63±0.13 <sup>cdefghijklmnop</sup>
41	BERC-BB55	Bethlahem - Beit Sahour	24	0.52	0.78	0.64	0.64±0.09 <sup>cdefghijklmnopq</sup>
42	BERC-BB56	Bethlahem - Beit Sahour	15	0.74	0.78	0.78	0.78±0.01 <sup>opq</sup>
43	BERC-BB57	Bethlahem - Beit Sahour	15	0.41	0.78	0.67	0.67±0.11 efghijklmnopq
44	BERC-UA58	Tubas - Aqqaba	3	0.74	0.82	0.79	$0.79\pm0.04^{pq}$
45	BERC-AB59	Jericho – Bardala	3	0.78	0.82	0.81	$0.81\pm0.02^{\rm q}$
46	BERC-AB60	Jericho – Bardala	3	0.61	0.82	0.72	0.72±0.11 <sup>ijklmnopq</sup>
47	BERC-AZ61	Jericho - Zbadat	3	0.71	0.78	0.74	$0.74\pm0.04^{\text{mnopq}}$
48	CharantaiT		36	0.33	1.29	0.74	0.74±0.29 <sup>lmnopq</sup>
49	Vedrantai		36	0.29	1.33	0.74	$0.74\pm0.3$ k <sup>lmnopq</sup>

Tukey's b test was used to separate mean values

4.3 The relative area under disease progress curve for Faqous accessions in terms of collection sites (Districts) and the differential lines inoculated with Fom1.2 and FOM1.

For FOM 1.2 Isabella and Margot were significantly lower than the accessions used 0.37 and 0.49 respectively, the highest rAUDPC was for the accessions collected from Qalqilia 0.71, the lowest rAUDPC was from the accessions collected from Salfit. There were no significant differences in the rAUDPC values between districts from which Faqous accessions were collected, whereas significant differences between Faqous accessions and the differential lines Margot and Isabella in rAUDPC were observed (Table 7).

For FOM1 the accessions collected from Salfit showed significantly lower rAUDPC than most of the accessions utilized in the rAUDPC, the highest rAUDPC was for the accessions collected from Jericho 0.76. There were no significant difference in the rAUDPC values between accessions collected from Qalqilia, Jenin, Tulkarm, Nablus and Ramallah. In addition there were also no significant differences between accessions collected from Bethlehem, Hebron, Tubas, Jericho and the differential lines Charantai T and Vedrantai. (Table 8).

Accessions collected from Salfit showed the lowest rAUDPC for both FOM1 and FOM1.2.

Table 7: The relative area under the disease progress curve observed for Faqous accessions collected from different districts and the differential lines inoculated with FOM1.2.

		rAUDPC				
		Count	Minimum	Maximum	Mean	Mean±SD
District	Bethlahem	9	0.5	0.84	0.68	0.68±0.13°
	Hebron	84	0.23	0.75	0.69	0.69±0.12°
	Jenin	235	0.27	0.92	0.68	$0.68\pm0.09^{c}$
	Nablus	99	0.33	0.79	0.63	$0.63\pm0.12^{c}$
	Qalqilia	105	0.53	0.82	0.71	0.71±0.06°
	Ramallah	270	0.24	1.04	0.68	0.68±0.19°
	Salfit	90	0.55	0.88	0.59	$0.59\pm0.09^{bc}$
	Tubas	30	0.63	0.65	0.64	0.64±0.01°
	Tulkarm	120	0.41	0.74	0.6	$0.6\pm0.07^{bc}$
Differential	Vedrantai	33	0.25	0.83	0.6	0.6±0.15 <sup>bc</sup>
lines	Charantai- FOM2	33	0	0.9	0.68	0.68±0.17°
	CharantaiT	33	0.39	0.9	0.65	0.65±0.15°
	Isabella	3	0.17	0.57	0.37	0.37±0.2 <sup>a</sup>
	Margot	27	0	0.83	0.49	$0.49\pm0.2^{b}$

Tukey's b test was used to separate mean values

Table 8: The relative area under the disease progress curve observed for Faqous accessions collected from different districts and the differential lines inoculated with FOM1.

			rAUDPC					
		Count	Minimum	Maximum	Mean	Mean±SD		
District	Salfit	89	0.25	0.84	0.43	0.43±0.09 <sup>a</sup>		
	Qalqilia	105	0.31	0.84	0.57	0.57±0.12 <sup>b</sup>		
	Jenin	235	0.43	0.92	0.58	0.58±0.12 <sup>bc</sup>		
	Tulkarm	120	0.23	0.89	0.59	0.59±0.13 <sup>bc</sup>		
	Nablus	99	0.33	0.78	0.6	0.6±0.15 <sup>bc</sup>		
	Ramallah	270	0.16	0.84	0.61	$0.61\pm0.14^{\text{bcd}}$		
	Bethlehem	84	0.33	0.78	0.67	$0.67\pm0.12^{cde}$		
	Hebron	84	0.23	0.75	0.69	$0.69\pm0.12^{de}$		
	Tubas	33	0.56	0.88	0.72	0.72±0.1 <sup>e</sup>		
	Jericho	9	0.61	0.82	0.76	$0.76\pm0.07^{e}$		
Differential	CharantaiT	36	0.33	1.29	0.74	0.74±0.29 <sup>e</sup>		
lines	Vedrantai	36	0.29	1.33	0.74	$0.74\pm0.3^{e}$		

Tukey's b test was used to separate mean values

# 4.4 The relative area under disease progress curve for Faqous accession in term of landrace names and the differential lines inoculated with FOM1.2 and FOM1.

The relative area under disease progress curve had no significant differences between the landraces inoculated with FOM1.2, in contrast significant differences were found between the four landraces (GB, GS, WB, and WS) and Isabella, and Margot (Table 9). The relative area under disease progress curve for FOM1 showed no significant differences between WB and GB and between WS and GS, in contrast significant differences were observed between WB and WS, and GS (Table 10).

Table 9: The relative area under the progress curve observed for Faqous landraces collected from different districts and the differential lines inoculated with FOM1.2.

		rAUDPC					
		Minimum	Maximum	Mean	Mean+SD		
Landrace	GB	0.37	0.82	0.65	0.65±0.09c		
	GS	0.5	0.84	0.68	0.68±0.13c		
	WB	0.27	0.92	0.65	0.65±0.1c		
	WS	0.23	1.04	0.68	0.68±0.17c		
Differential	Charantai-						
lines	FOM2	0	0.9	0.68	0.68±0.17c		
	CharantaiT	0.39	0.9	0.65	0.65±0.15c		
	Isabella	0.17	0.57	0.37	0.37±0.2a		
	Margot	0	0.83	0.49	0.49±0.2ab		
	Vedrantai	0.25	0.83	0.6	0.6±0.15c		

Tukey's b test was used to separate mean values

Table 10: The relative area under the progress curve observed for Faqous landraces collected from different districts and the differential lines inoculated with FOM1.

		rAUDPC					
		Count	Mean	Count	Minimum	Maximum	Mean±SD
Landrace	WB	486	0.56	486	0.23	0.92	0.56±0.13 <sup>a</sup>
	GB	174	0.58	174	0.31	0.84	$0.58\pm0.14^{ab}$
	WS	384	0.64	384	0.16	0.88	$0.64\pm0.14^{bc}$
	GS	84	0.67	84	0.33	0.78	$0.67\pm0.12^{c}$
Differential	CharantaiT	36	0.74	36	0.33	1.29	$0.74\pm0.29^{d}$
line	Vedrantai	36	0.74	36	0.29	1.33	$0.74\pm0.3^{d}$

Tukey's b test was used to separate mean values

## Chapter Five

#### **Discussion**

Our data indicated that all tested accessions were resistant to FOM 0 and FOM 2, this is in agreement with those of other researchers (Matsumoto et al. 2014), who reported that melon cultivars were resistant to FOM 0 and FOM 2. The resistance of Faqous accessions to FOM 0 and FOM 2 might be explained by the fact that that these accessions possess the gene FOM-1 which is responsible for FOM 0 and FOM 2 resistance and lack the gene Fom-2 which confers resistance to race 1. Matsumoto and Miyagi (2012) demonstrated that single dominant gene confer the resistance to FOM 1.

In the present study the rAUDPC for FOM 1 ranged from 0.41 to 0.81 and for FOM 1.2 from 0.52 to 0.95. Based on the rAUDPC there were several slightly resistant accessions for FOM1, most of the Faqous accessions were susceptible to FOM1.2. Sensoy et al. (2012) collected fifty melon genotypes from Lake Van Basin several of which had resistance to FOM 0, most of the collected melon genotypes were found susceptible to FOM race 1.2.

Oumouloud et al. (2009) found a certain degree of resistance for FOM 1.2 within some accessions belonging to subsp. *Melo* this conforms with our result which indicated that the rAUDPC for FOM 1.2 for all accessions ranged from mild to high value (partial resistance to susceptible level. The absence of resistant accessions for FOM 1.2 may be related to

heterozygous state for genes responsible for FOM1.2 resistance in the accessions.

Resistance to race 1.2 is complicated and seems to be managed by multiple recessive genes (Chikh-Rouhou et al. 2007, 2008; Herman & Perl-Treves 2007; Perchepied et al. 2005). More recently Chikh-Rouhou et al. (2010, 2011) detected resistance in Far Eastern and Iberian lines, and demonstrated that this polygenic resistance is either dominant or recessive and four to eight elements are embroiled in the resistance to race 1.2 in these lines.

Polygenic resistance is based on minor genes, which may confer a higher level of resistance when all the genes are present together in a homozygous state (Clerjeau et al. 1981). Utilizing the partial resistance accession such as RB 38 (rAUDPC 0.52) as breeding material for resistance improvement may be useful. High concentration of the inoculum (3x10<sup>6</sup> spores per ml) result in no appearance for low resistance for FOM1.2 in the screened accessions (Zink et al. 1992). Herman and Perl-Treves (2007) demonstrated that under different inoculation conditions, different levels of resistance are expressed. In breeding programs, choosing the correct conditions to screen for pathogen resistance was critical. Inoculum concentration has been found to influence the response of the plants to the pathogen and it has been demonstrated that, when trying to introgress BIZ alleles for FOM 1.2 resistance, a concentration of 1x10<sup>6</sup> spores per ml was the most sufficient (Burger et al. 2003). Lesser concentrations resulted in wilting and/or

yellowing symptoms only in some of the plants. Under such inoculation conditions, many susceptible plants could escape infection and mistakenly be assigned as resistant (Burger et al. 2003).

Chikh-Rouhou et al. (2008) indicated variation in accessions results when inoculated with race 0, 1, 2 and 1.2 compatible with the present study.

There were no significant difference in the susceptibility to for FOM 1.2, between Districts where Faqous accessions were collected, whereas there were significant differences in the susceptibility to FOM 1, between Districts from where Faqous accessions were collected. This may be due to the heterozygous state for genes responsible for FOM1.2 and FOM 1 resistance and the surrounding conditions. The occurrence of Fusarium wilt symptoms after artificial inoculation might be affected by the hereditary background of the plant (Mas et al., 1981) and by environmental elements (Cohen et al., 1996).

#### **Chapter Six**

#### **Conclusions and Recommendations**

#### **6.1 Conclusions**

All Palestinian snake melons tested showed resistance to FOM race 0 and race 2. Partial resistance for FOM1 and FOM 1.2 was detected in few studied accessions. Partial resistance to *Fusarium oxysporum* f. sp. *melonis* race 1 and race 1.2 was only detected in a few Palestinian snake melon accessions. The highest partial resistance for race 1 was found in SD30, SD32, RT33 and SD31. In contrast the highest partial resistance for FOM 1.2 was detected in RB38, SD30 and SD32. Finding resistant (for race 0 and race 2) or partial resistance (for race 1 and race 1.2) accessions may create an opportunity to study the genetics of resistance inheritance and to develop molecular markers that will facilitate breeding resistant melon cultivars.

#### **6.2 Recommendations**

Further genetic identification resistance for FOM race 1 and 1.2 is required prior to the integration of Faqous in melon breeding approaches. In general all tested accessions can be utilized in melon breeding programs as new sources of resistance for FOM race 0 and race 2 while SD30 and SD32 accessions can be used for new source of resistance for FOM race 1 and race 1.2.

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جامعة النجاح الوطنية كلية الدراسات العليا

# تحديد مصادر مقاومة جديدة لمرض الفيوزاريوم في الفقوس بواسطة الطرق المرضية والجينية

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قدمت هذه الأطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في الانتاج النباتي بكلية الدراسات العليا في جامعة النجاح الوطنية نابلس – فلسطين

تحديد مصادر مقاومة جديدة لمرض الفيوزاريوم في الفقوس بواسطة الطرق المرضية والجينية إعداد

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الخلفية: يزرع الفقوس على نطاق واسع في فلسطين ويظهر تكيف جيد مع المناخ، بالإضافة الى تحمل بعض الامراض والظروف القاسية.

الاهداف: الهدف من هذه الدراسة البحث عن اصول جينية يمكن استخدامها في تهجين نبات شمام مقاوم للفيوزاريوم من خلال التركيز على الاصول المحلية المتكيفة مع المناخ والتي تزرع من قبل المزارعين الذين يملكون حيازات صغيرة.

المنهجية: تم فحص 47 سلالة من الفقوس تمثل 47 حقل من الضفة الغربية لمقاومتها للذبول المنهجية: تم فحص 47 سلالة من الفطر 37(0, 1, 2, السلالات الفطر 47 باستخدام تركيز هذه السلالات (سلالات الفقوس) تم فحصها لمقاومتها لسلالة الفطر 1.2 باستخدام تركيز 1 x10<sup>6</sup> لما بطريقة غمس الجذور بمعلق الابواغ، بالاضافة الى سلالات الفقوس تم استخدام (as controls) .

النتائج: جميع السلالات التي فحصت كانت مقاومة للفيوزاريوم سلالة صفر (FOM0) و والسلالة (rAUDPC). لقد كان ادنى متوسط 0.52 للمساحة تحت منحنى تقدم المرض (FOM2) كانت قريبه للفيوزاريوم FOM1.2 للسلالة RB38 معظم قيم AUDPC الفيوزاريوم تورية بردلة بينما كانت اقل قيمة من بعضها حيث كانت اعلى قيمة 0.81 للسلالة 9 AB59 من قرية بردلة بينما كانت اقل قيمة 0.41 للسلالة 3 BD30 من قرية دير بلوت. بالاضافة الى ذلك لم يوجد اي فروقات معنوية في POM1 للفيوزاريوم 1.2 FOM1 للفيوزاريوم FOM1 للفيوزاريوم 1.2 FOM1 بينما وجد فرق معنوي في rAUDPC للفيوزاريوم 1.2 FOM1 بين السلالات التي جمعت من مناطق اخرى.

الإستنتاجات: جميع الفقوس الذي تم فحصة مقاوم للفيوزاريوم 6 FOM و FOM وحساس الى مقاوم جزئيا للفيوزاريوم FOM 1 و FOM 1.2

**Keywords:** Fusarium oxysporum f. sp. melonis, Differential Melon Lines, rAUDPC سلالة، تركيز الابواغ،