An - Najah Univ. J. Res. (N. Sc.) Vol. 25, 2011

# Incidence and Genetic Variability of *Erysiphe cichoracearum*, the Causal Agent of Powdery Mildew on Squash and Cucumber in Tubas and Jenin

# دراسة مدى الانتشار والاختلافات الوراثية لمسبب مرض البياض الدقيقي على محصولي الخيار والكوسا في طوباس وجنين

**Hazem Sawalha**

**حازم صوالحة**

Department of Biology and Biotechnology, Faculty of Arts and Sciences, Arab American University, Jenin, Palestine

E-mail: hsawalha@aauj.edu

Received: (2/12/2010), Accepted: (14/8/2011)

## Abstract

An experiment was carried out in 2008 to study the incidence of *Erysiphe cichoracearum,* the causal agent of powdery mildew on squash and cucumber in different growing seasons of the Tobas and Jenin districts. The incidence was studied in both open fields and plastic houses. Also, the research studied the genetic variability between the fungal isolates using RAPD. The maximum fungal incidence was recorded on squash, compared with cucumber in all growing seasons. The maximum disease incidence of 70% was recorded on squash during both spring and summer growing seasons in Tobas district. Autumn was considered the second most serious growing season of both crops as the fungal incidence ranged from 4 to 53% and 3 to 42% on squash and cucumber, respectively. In late winter growing season, the fungal incidence was much lower on both crops planted in Tobas district compared with the other growing seasons (4 to 20% on squash and 2 to 18% on cucumber) . In addition, studying the genetic variability between the fungal isolates showed that these isolates were genetically different between Tobas and Jenin districts. Furthermore, polymorphism was in squash and cucumber from obtained isolates the between observed Tobas district while no polymorphism was recorded in Jenin district.

**ملخص**

اجريت دراسة لمعرفة مدى انتشارمرض البياض الدقيقي على نباتي الكوسا والخيار في محافظتي طوباس وجنين خلال موسم 2008. كذلك تم في هذا البحث ايضا دراسة الاختلاف الجيني بين العزلات الفطرية في منطقتي الدراسة باستخدام RAPD. وبشكل عام كانت الاصابة بهذا المرض عالية على الكوسا مقارنة بالخيار في المنطقتين وفي جميع المواسم الزراعية، كذلك كان انتشار المرض الاعلى خلال موسمي الزراعة الربيعية والصيفية مقارنة بمواسم الزراعة الاخرى، حيث سجلت اعلى اصابة في هذين الموسمين على محصول الكوسا في محافظة طوباس، اذ وصلت 70%. احتل موسم الزراعة الخريفية المرتبة الثانية من حيث مدى انتشار المرض فيها حيث تراوحت الاصابة من 4-53% ومن 3-42% على محصولي الكوسا والخيار على التوالي. اما بالنسبة للزراعة الشتوية فقد كانت اكثر امنا لدى المزارع، كون الاصابة فيها كانت قليلة مقارنة بالمواسم الاخرى، اذ تراوحت الاصابة من 4-20% على الكوسا ومن 2-18% على الخيار. ومن ناحية اخرى بينت الدراسة ان العزلات الفطرية تختلف وراثيا بين محافظتي طوباس وجنين. كذلك تبين ايضا وجود اختلافات وراثية للعزلات الفطرية التي عزلت من محصولي الكوسا والخيار في محافظة طوباس في حين اثبتت الدراسة عدم وجود هذه الاختلافات بين العزلات من كلا المحصولين في محافظة جنين.

## Introduction

Cucumber and squash are one the major vegetable crops in Palestine depending on the availability of irrigation. The total area in Palestine planted with these crops is about 5600 hectares with an annual production of 21000 metric tons (Palestinian Central Bureau of Statistics (PCBS), 2008). The crops offer excellent versatility in the kitchen and provide colorful additions to any meal. However, they are associated with a number of health and nutrition benefits (Daoud 2008; Ali-Shtayeh and Jamous 2008; Wardlaw *et. al.* 2004).

Powdery mildew is one of the most devastating diseases of cucurbit crops in the world (Agrios 1997). The disease attacks cucumbers, muskmelons, squash, gourds, and pumpkins grown both in field and greenhouse conditions. The infection with a powdery mildew causes retardation in plant growth , foliage loss, as well as reduction in yield (Oetting and Yunis 2004, Agrios 1997). Furthermore, the disease can reduce marketable yields of the crop by 100% during certain years (Gent *et. al.* 2006).

The fungus, *Erysiphe cichoracearum*, is the major causal agent of powdery mildew on cucurbits (Cook and Braun 2009). The disease symptoms appear on leaves, petioles, and young stems as white powdery colonies composed of mycelium and countless numbers of spores (Agrios 1997). Under favorable environmental conditions, the colonies coalesce and the entire top surface of the leaf may be covered with the powdery fungus within a few days. In addition, the host tissue becomes chlorotic and usually senesces early (Pérez-García *et. al.* 2009). In severe cases both the size and number of fruit may be reduced (Hansen 2009).

The pathogen has a widespread occurrence in Mediterranean countries (Gomez-Guillamon & Tores 1992; Vakalounakis *et al.* 1994; Bardin 1996; Oetting and Yunis 2004). In Arab countries including Jordan, Syria and Libya, the pathogen was reported to cause serious diseases of cucurbits (Abu-Blan *et. al.* 2002; Al-Maghribi and Tobbache 2000; Fadeil and El-Ammari 2000).

Squash and cucumber production in Palestine has not approached their full potential and farmers still take the risk and continue to cultivate these crops (PCBS) 2008). The reasons for this dramatic situation may be attributed to the climatic conditions and poor husbandry and, above all, the high level of diseases. As powdery mildew is expected to be the most significant fungal disease of squash and cucumber responsible for the continuous crop failure, the current work aimed at studying the incidence and genetic variability of this disease on these crops in North West bank particularly Jenin and Tobas districts (PCBS) 2008). These districts were selected for this study because they are considered the major cucurbit growing sites in the country. The annual cucurbit production in these regions comprises 33% of the total cucurbit production in the West Bank (PCBS) 2008). Since precise knowledge is required, polymerase chain reaction (PCR)-based methods were employed in this research.

## Materials and Methods

### Regions of study

Several fields of squash and cucumber were selected in Al-Far'a and Sanoor regions to represent the production of the crops in Tobas and Jenin districts respectively.

Squash and cucumber were planted in the study regions as follows:

Open field culturing was practiced in late winter, spring, summer and autumn growing seasons. Both late winter and autumn growing seasons are mainly practiced in Tobas district.

Plastic house culturing was practiced by a few cucumber farmers in Tobas district.

### Field visits and sample collection

The selected fields were visited twice a month for sample collection from February until December, 2008. Leaf samples showing symptoms of powdery mildew were collected and prepared for lab testing.

### Morphological testing

The fungal pathogens were identified in the laboratory according to morphology of conidia and conidiophores. The conidia are ovoid to ellipsoid whereas the conidiophores have elongated cells with a curved foot one (Alexopolus *et. al*. 1996).

### PCR testing

DNA was extracted from spores following the CTAB procedure detailed in McDermott *et. al*. (1994). The internal transcribed spacer (ITS) and the 5.8S regions of ribosomal genes in *E. cichoracearum* were amplified using primers PN23 (5'-CACCGCCCGTCGCTACTACCG-3') and PN34 (5'-TTGCCG CTTCACTCGCCGTT-3') (Mouyna & Brygoo 1993). The PCR reactions were set up in a 25 µl volume containing 1 µl of DNA sample, 1.5 mM MgCl2, 1.25 U of proofreading DNA polymerase. 1 µM of each primer, 200 µM of dNTP mixture and 2.5 µl of 10x reaction buffer. The cycling conditions was as follows: 94°C for 3 min 30 s, followed by 40 cycles of 94°C for 30 s, 55°C for 1 min and 72°C for 1 min 30 s and a final extension of 72°C for 8 min 30 s. PCR amplicons were separated electrophoretically on 2% agarose gels and visualized by staining with ethidium bromide (Bardin *et. al.* 1997).

### Disease incidence

The disease incidence was studies by collecting leaf samples randomly from plant rows of the studied fields. The disease incidence was determined by dividing the number of infected samples over the total number of the collected ones. The sample was considered infected if it has lesions with 2.0-8.0 mm in diameter according to Moret *et. al.* (2009).

### Random Amplified Polymorphic DNA (RAPD)

Twelve fungal isolates collected from squash and cucumber in both Al-Far'a and Sanoor regions were cultured on cucumber planted under glasshouse conditions to be a fungal source for genetic analysis (Table 1). Seven Operon primers that yielded consistent and reproducible banding patterns were selected for analyzing the isolates (Table 2). The primers were purchased from Operon Technologies Inc., Alameda, USA. Each primer was tested twice to ensure the consistency of RAPD patterns. RAPD polymorphism among isolates was scored from fragments of 100-2000 bp. RAPD fragments of the same size were considered identical.

**Table (1):** Fungal isolates, their hosts and collection regions.

| **Isolate** | **Region** | **Host** |
|---|---|---|
| FC1 | Al-Far'a | Cucumber |
| FC2 | Al-Far'a | Cucumber |
| FC2 | Al-Far'a | Cucumber |
| FC4 | Al-Far'a | Cucumber |
| FS1 | Al-Far'a | Squash |
| FS2 | Al-Far'a | Squash |
| FS3 | Al-Far'a | Squash |
| FS4 | Al-Far'a | Squash |
| SC1 | Sanoor | Cucumber |
| SC2 | Sanoor | Cucumber |
| SS1 | Sanoor | Squash |
| SS2 | Sanoor | Squash |

**Table ( 2):** Operon primers used to study the RAPD.

| **Primer** | **Sequence** |
|---|---|
| OPC12 | TGTCATCCCC |
| OPE03 | CCAGATGCAC |
| OPF01 | ACGGATCCTG |
| OPH02 | TCGGACGTGA |
| OPH07 | CTGCATCGTG |
| OPF03 | CCTGATCACC |
| OPG03 | GAGCCCTCCA |

### Statistical analysis

Statistical analysis of the data was done using the Two-Sample Tests of Proportions ( TSTP ) to compare disease incidence on both crops in the study regions. The results were analysed using a level of significance when ά = 0.05 (Lind *et. al* . 2005).

## Results

### Morphological testing

Based on the morphology of conidia and conidiophores, the causal agent was identified as *E. cichoracearum.* Conidia are ovoid to ellipsoid with a smooth surface. Conidiophores were significantly long and conidia were formed in chains of 3 to 5 bulbous cells, representing conidia at different stages of differentiation. The conidiophore stalks consisted of three elongated cells including a curved foot cell (Plate 1).

.

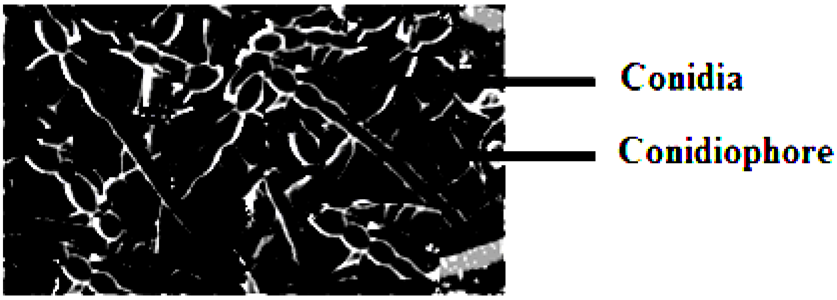



**Plate (1):** Microscopic image (150X) showing the conidia and conidiophores of *E. cichoracearum.*

### PCR testing

The PCR was able to detect *E. cichoracearum* from infected squash and cucumber by developing obvious bands of nucleic acid when electrophoresed in agarose gel . Primers PN23 and PN34 allowed the PCR amplification of a fragment of similar size (about 800 bp) corresponding to the ITS and 5.8S regions of the ribosomal DNA (Plate 2).

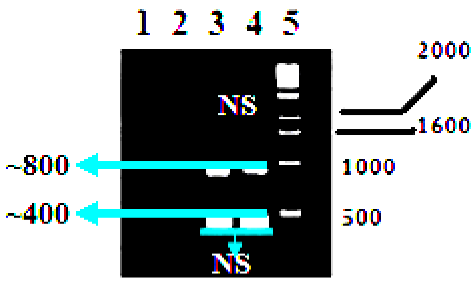



**Plate (2):** Agar gel electrophoreses of amplified PCR products of *E. cichoracearum* DNA from infected squash and cucumber plants.

Lane 1: Healthy squash

Lane 2: Healthy cucumber

Lane 3: Infected squash

Lane 4: Infected cucumber

Lane 5: DNA size marker

NS: Area of non-specific reaction

## Disease incidence

Infection of both squash and cucumber plants with powdery mildew in late winter season began to develop about three months after crop transplanting in Al-Far'a region. The disease incidence in this region started in the beginning of April and grew to reach 18% and 20% infection within four months for cucumber and squash plants respectively (Fig 1).

In spring growing season, the first record of the disease infection of the crops appeared in April in both regions. The maximum fungal infections of squash were 48% and 70% in Sanoor and Al-Far'a respectively. In case of cucumber planted in these regions, the maximum disease incidences were 65% and 50% respectively (Fig. 2).

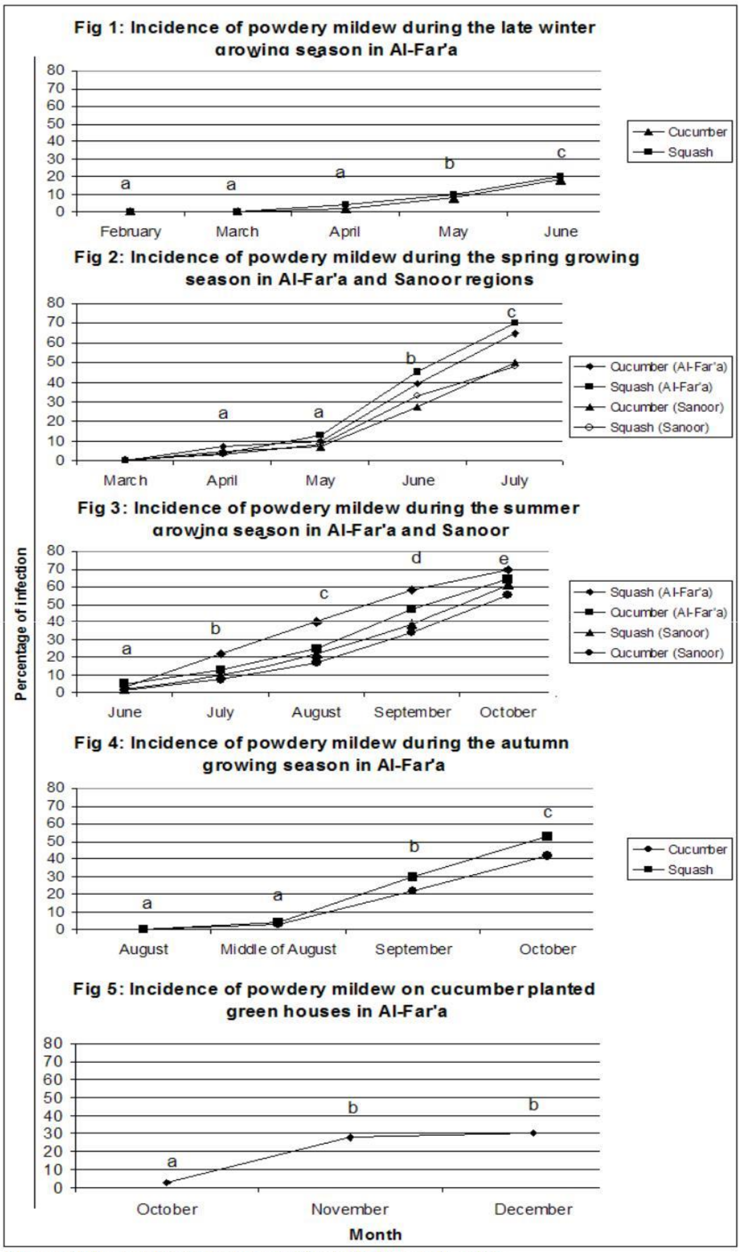
In summer growing season, the fungal infection in both regions appeared a month after transplanting. Moreover, the disease incidence grew rapidly to reach the maximum value in October. In this month, the maximum infections, recorded in Al-Far'a, were 70% and 64% on squash and cucumber respectively. On the same plants, the maximum fungal incidences recorded in Sanoor were 61% and 56% respectively (Fig 3).

In autumn growing season of Al-Far'a, the first disease infection was recorded in the middle of August. In this season, the disease infection started from 3% in the middle of August and continued until the maximum infection of 53% and 42% in October for both squash and cucumber plants respectively (Fig 4).

For cucumber planted under greenhouse conditions in Tobas district, the fungal infection appeared in October and grew slowly until reached 30% in December (Fig 5).

Comparison of the disease incidence on cucumber and squash during the growing seasons

The results of statistical analyses revealed that the powdery mildew infections of squash and cucumber during spring and summer growing seasons were significant compared with the other growing seasons. Infection of both squash and cucumber revealed significant difference between Al-Far'a and Sanoor in spring growing season. No significant difference was recorded between disease incidences on these crops in other growing seasons in the study regions. In addition, comparing disease infection on both crops did not show any significant difference between the study regions in different growing seasons (Table 3).




Means with the same letter indicate no significant difference at $P = 0.05$

**Table (3 ):** Comparison of the disease incidence on cucumber and squash during the growing seasons using TSTP when Z-table (critical value) = 1.645.

| **The crop** | **Combinations of regions in regard to the disease incidence** | **Z-value of cucurbit growing seasons** | | | |
|---|---|---|---|---|---|
| | | **Late winter** | **Spring** | **Summer** | **Autumn** |
| Cucumber | Al-Far'a/Sanoor | --------- | 2.15(S) | 1.34(NS) | --------- |
| Squash | | --------- | 3.16(S) | 1.15(NS) | --------- |
| The region | Combinations of crops in regard to their infection with the disease | | | | |
| Al-Far'a | Cucumber/Squash | 0.35(NS) | 0.76(NS) | 0.90(NS) | 0.46(NS) |
| Sanoor | | --------- | 0.28(NS) | 0.72(NS) | --------- |

S:Significant
NS:Non significant
(--): Comparison was not done due to absence of crops in Sanoor in late winter and autumn growing seasons

**Random Amplified Polymorphic DNA (RAPD)**

One to five RAPD fragments from 100 to 2000 bp in size were observed per isolate after amplification with each Operon primer (Plate 3). Using OPC12 and OPH07 primers, several distinct RAPD fragments were recorded across the twelve isolates tested. The test showed that fungal isolates were genetically different between Al-Far'a and Sanoor regions. In addition, polymorphism was observed between the isolates obtained from squash and cucumber in Al-Far'a region. No polymorphism was recorded in Sanoor between isolates from both hosts (Plate 3).

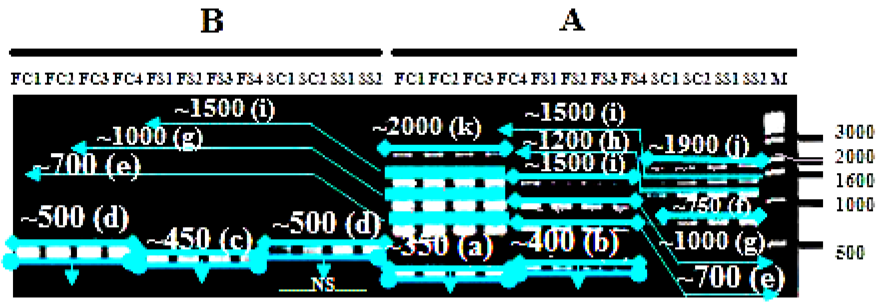



**Plate (3):** Random amplified polymorphic DNA of the *E. cichoracearum* isolates with primers (A) OPC12 and (B) OPH07 showing polymorphism between isolates. Lane M is a DNA marker. Similar letters represent similar band length. NS represents the area of non-specific reaction.

## Discussion

Testing the collected samples of leaves infected with powdery mildew in the north regions of West Bank showed that *E. cichoracearum* causes powdery mildew of squash and cucumber in this part of Palestine. These results are consistent with studies conducted in other Mediterranean regions including Spain (Gomez-Guillamon & Tores 1992), Crete (Vakalounakis *et. al.* 1994) and Italy (Bardin 1996). The differences in the fungus distribution and incidence during the growing seasons may be due to differences in the climatic conditions between regions. The warmer environment in Tobas district might have stimulated the growth and development compared with the cooler ones in Jenin which could have mildly obstructed the disease development in most growing seasons (Agrios 1997).

Furthermore, the results revealed that the powdery mildew infection was high in the spring and summer growing seasons in the study regions. This may explain why these seasons are considered the most critical seasons for cucurbit production in these regions because of the high rate of fungal infection. Similar results on the spread of the fungus were recorded by other researchers in other Mediterranean countries (Abu-Blan *et. al*. 2002).

In addition, the results appeared low occurrence of the disease on the studied cucurbit plants during the late winter season. Such results may be attributed to the period of transplanting which is usually done in February, during which cold conditions prevail in the country (PCBS 2005). This case provides a chance for the cucurbit transplants to escape fungal infection during their early growth stages. In addition, the lower disease incidence in the autumn growing season of Tobas district may be due to the cold weather conditions starting from October especially during nights (PCBS 2005). These conditions might have negative effects on the disease as they can suppress the growth of the fungi for certain periods of time (Agrios 1997). The pathogen does not prefer regions with cold weathers as the low temperatures have drastic effects on the fungal growth and development (Agrios 1997; Oetting and Yunis 2004).

For cucumber planting in greenhouses in the Tobas district, the lower disease incidence compared with the open fields may be attributed to the well-developed protection systems and the intensive fungicide application practiced by the farmers in this region.

The genetic variability indicated within the Palestinian isolates of *E. cichoracearum* was related to the regional origin of the isolates as noticed between isolates of both Al-Far'a and Sanoor. Also, host specialization may be another reason for the genetic diversity between isolates. In this regard, the current results elucidated the genetic variability between isolates recovered from squash and cucumber in Al-Far'a regions. Similar results were recorded in France where the researchers observed genetic differences in the population of powdery mildews on cucurbits. The authors related such differences to host specialization (Bardin *et. al.* 1999) . In addition, Zeller and Levy (1995) characterized diversity among field collections of *E. cichoracearum* from a variety of hosts, and from other powdery mildew species, with RFLPs from a PCR amplified ribosomal DNA (rDNA) segment. The *E. cichoracearum* samples expressed six distinct RFLP haplotypes. Each haplotype was specific to either a single host or to a set of related host species. Similar researches were done on other fungi species. In this regard, Cunnington and Lawrie (2010) reported evolutionary lineages of the *Golovinomyces cichoracearum* complex on introduced plants in Australia. By comparing the rDNA ITS regions from 47 herbarium specimens using restriction fragment length polymorphism (RFLP) and sequencing, six RFLP groups were found, each corresponding to a previously reported evolutionary lineage in the complex.

On the other hand, Sheikholeslam *el. al.* (2005) successfully used RAPD-PCR experiment to investigate genetic diversity of 30 isolates of populations of *Erysiphe betae*, the causal agent of sugar beet powdery mildew in Iran. The author reported that 62% of DNA bands were polymorphic

The occurrence of genetic diversity in fungal isolates proposes that these isolates accumulated modifications which adapted them to their environments (Bardin *et. al.* 1999; Campbell and Reece 2004). The latter finding is a very competitive field for understanding the relationships among fungal genetics, host plants, and geographical isolation better. Further research is needed in this field.

**References**

- Abu-Blan, H. Al–Momany, A. & Al–Jaafreh, J. (2002). "Host Range and Survival of Powdery Mildew of Cucumber in Ghor El-Safi Area. Jordan". Dirasat (17). 23-33
- Agrios, G. (1997). Plant Pathology. Academic Press London. p 295.
- Alexopoulos, C. Mims, C. & Blackwell, M. (1996). Introductory Mycology. John Willey & Sons Inc. New York. p 462-487.
- Ali-Shtayeh, M. & Jamous, R. (2008). Traditional Arabic Palestinian Herbal Medicine. TAPHM. Biodiversity and Environmental Research Center (BERC). Til. Nablus. Palestine. p 221.
- Al-Maghribi, S. & Tabbache, S. (2000). "Survey of fungal diseases on some weeds in Syrian coast". Seventh Arab Congress of Plant Protection. 22-26 October. Amman. Jordan. 198.
- Bardin, M. (1996). "Diversite phenotypique et genetique des oidiums des cucurbitacees. *Sphaerotheca fuliginea* Et *Erysiphe*

*cichoracearum*." PhD Thesis. Villeurbanne. France. University of Lyon I.

- Bardin, M. Nicot, P. Normand, P. & Lemaire, J. (1997). "Virulence variation and DNA polymorphism in *Sphaerotheca fuliginea.* causal agent of powdery mildew of cucurbits". European Journal of Plant Pathology (103). 545-54.
- Bardin, M. Carlier, J. & Nicot, P. (1999). "Genetic differentiation in the French population of *Erysiphe cichoracearum*. a causal agent of powdery mildew of cucurbits". Plant Pathology (48). 531-540.
- Campbell, N. & Reece, J. (2004). Biology. Sixth Edition. Benjamin Cummings. 438-452.
- Cook, R. & Braun, U. (2009). "Conidial germination patterns in powdery mildews". Mycological Research (113). 616-636.
- Cunnington, J. & Lawrie, A. (2010). "Genetic characterization of the *Golovinomyces cichoracearum* complex in Australia". Plant Pathology (59). 158-164.
- Daoud, R. (2008). "Studies on Folkloric Medicinal Plants used by Palestinians in the Qalqilia District". Unpublished M.Sc. thesis. An-Najah National University. Palestine. 39.
- Fadeil, G. & El-Ammari, S. (2000). "The incidence and intensity of cucurbit powdery mildew in El-Jabal El-Akhter region". Seventh Arab Congress of Plant Protection. 22-26 October. Amman. Jordan. 185
- Gent, D. Mahaffee, W. & Turechek, W. (2006). "Spatial heterogeneity of the incidence of powdery mildew on hop cones". Plant Disease. (90).1433-1440.
- Gomez-Guillamon, M. & Tores, J. (1992). "Influence of temperature on the resistance to *Sphaerotheca fuliginea* in Spanish melon cultivars". In. Doruchowski RW. Kozik E. Niemirowicz-Szczytt K.

eds. Warsaw University of Agriculture. ed. Proceedings of the Fifth EUCARPIA Conference. Cucurbitaceae. Warsaw. Poland. 155-159.

- Hansen, M. (2009). Powdery Mildew of Cucurbits. Produced by Communications and Marketing. College of Agriculture and Life Sciences. Virginia Polytechnic Institute and State University. 1-2.
- Lind, D. Marchal, W. & Wathen, S. (2005). Statistical Techniques in Business & Economics. Twelfth Edition. McGraw-Hill Irwin. New York. 262-263.
- McDermott, J. BraÈndle, U. Dutly, F. Haemmerli, U. Keller, S. Mueller, K. & Wolfe, M. (1994). "Genetic variation in powdery mildew of barley. development of RAPD. SCAR. and VNTR markers". Phytopathology (84). 1316-1321.
- Moret, A. Muñoz, Z. & Garcés, S. (2009). "Control of powdery mildew on cucumber cotyledons by chitosan". Journal of Plant Pathology 91 (2). 375-380.
- Mouyna, I. & Brygoo, Y. (1993). Les champignons et la PCR. In. Larzul D. ed. La PCR. un Procede de Replication in Vitro. Paris. France. Collection Genie Genetique.Lavoisier. 335-341.
- Oetting, R. & Yunis, H. (2004). Field Guide to Common Insects. Mites. & Diseases of Greenhouse Grown Sweet Peppers. & Tomatoes. Hakohav Press. Kfar Qari. 12-13.
- Palestinian Central Bureau of Statistics (PCBS). (2005). Meteorological Conditions in the Palestinian Territories Annual Report. Ramallah– Palestine. 39-49.
- Palestinian Central Bureau of Statistics (PCBS). (2008). Agricultural Statistics. Ramallah– Palestine. 77-110.
- Pérez-García, A. Romero, R. Fernández-Ortuño, D. López-Ruiz, F. de Vicente, A y. & Torés, JA. (2009). "The powdery mildew fungus *Podosphaera fusca* (synonym *Podosphaera xanthii)*. a constant threat to cucurbits". Molecular Plant Pathology 10. 153-160.

- Sheikholeslami, M. Seidel, M. Okhovvat, S. Hedjaroude, G. & Javan-Nikkhah, M. (2005). "Study on DNA diversity of Iranian populations of *Erysiphe betae* causal agent of sugar beet powdery mildew". Commun Agric Appl Biol Sci. 70(3). 327-332.
- Vakalounakis, D. Klironomou, E. & Papadakis, A. (1994)."Species spectrum. host range and distribution of powdery mildews on Cucurbitaceae in Crete". Plant Pathology 43. 813-818.
- Wardlaw, G. Hampl, J. & Disvestro, R. (2004). The Perspectives of Nutrition. Sixth Edition. McGraw Hill. New York. 371-450.
- Zeller, K. & Levy, M. (1995). "Intraspecies differentiation in the powdery mildew *Erysiphe cichoracearum* determined with rDNA RFLPs". Molecular Ecology 4. 277-283.