Al-Azhar J. Agric. Sci. Sector Res.,
Vol.3 : pp 83-96 (December)2007

REACTION OF CUCUMBER CULTIVARS TO POWDERY MILDEW PATHOGEN AND ITS RELATED WITH HOST PROTEIN

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ABSTRACT: *Sphaerotheca Fuliginea* isolate from El-Ayat (Giza) was more virulent compared with the other tested isolates where it infected 17 out of 18 cucumber cultivars, while the other isolates infected only 14 to 15 cucumber ones.

Differences between the tested cucumber cultivars were highly significant. Some cucumber cultivars possess superior levels of resistance to *Sphaerotheca fuliginea* (Delight Green, Marmar, Medina, Premo, Sweet crunch, Thamin and Yasmina), some exhibited partial resistance reaction (Joy and Sure Green). Others showed susceptible reaction (Afdal, Beit Alpha, Betostar, Chinease Long, Delta star, Green Bowl, Hesham, Maram, Nil, Pasandra, Rawa, Sahara and Shrouqe.

The results revealed that the susceptible cucumber cultivars, Beit Alfa, Afdal and Rawa had an increase in protein bands compared with the partial resistant ones, Joy and Sure Green. Meantime, the resistant group consists of two hybrids, Sweet Crunch and Thamin. Also, this study aimed to develop their protein fingerprinting patterns in relation to their levels of resistance to powdery mildew disease.

New types of protein bands were found in the susceptible cultivars by using this protein fingerprint technique. Data revealed that gel electrophoresis is a powerful technique to differentiate between resistant and susceptible cultivars toward plant pathogens. The electrophoretic technique, divided cucumber cultivars into two subcluster (SL = 82.09%).

The higher SL, included the susceptible hybrids (Afdal, Beit Alpha and Rawa) which formed a subclusters (SL = 96.57%) . The other treatments which included the partial resistant hybrids (Joy and Sure Green), and the resistant ones (Sweet Crunch and Thamin) which formed the another subcluster (SL= 88.52%).

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the most important vegetable crops in Egypt. Cucumber powdery mildew (*Sphaerotheca fuliginea*) which reduce the quality and quantity of fruit yield. Cultivation of resistant cultivars is considered the best method for disease control. Resistant phenomenon of the cultivars may be attributed to certain morphological and physiological host characters. The virulence pathogen variations may also play an important role in the mechanism of resistance.

Imam *et al* (1975) stated that cucumber, cvs. Poinsett and Yomaki were resistant to powdery mildew disease. Helal *et al* (1978) pointed out that cucumber, cv. Beit-Alpha was highly susceptible to the disease, while cv. Poinsett was highly resistant. Abd El-Sattar *et al.* (1985) reported that cucumber, cv. Polmeto was highly resistant whil cv. Wanda was moderately resistant. El_Desouky (1989) found that cucumber cv. Dema was highly susceptible to powdery mildew, while cvs. Medina and Conquere F., were highly resistant.

On the contrary, cvs. Poinsett 76 and TMG1 were found to be immune to the infection. Ahmed (1995) found that cucumber, cvs. Aschel was highly susceptible to the disease, while hybrids Amira, Katia and Medina were moderately susceptible, mean while hybrids conquere F1, Sweet Crunch and Seliperti were resistant ones. Bardin *et al.* (1997) demonstrated the virulence variations of *Sphaerotheca fuliginea.* They observed heterothallism for all tested isolates. Most cucumber hybrids grown in Egypt are susceptible to powdery mildew disease. El-Desouky *et al.* (2003) evaluated nineteen cucumber genotypes and five cultivars under green house conditions for resistance to powdery mildew disease. Genotypes 14 x 15 F1, 14 Munger F1, Joy hybrid and Delight Green hybrid exhibited resistance reaction, while 765 F1, 757 F1 and Sure Green hybrid showed partial resistance. Genotypes 728 F1, 723 F1, Green Bowl hybrid and Beit-Alpha hybrid were susceptible.

Abd El-Sayed (2000) and El-Desouky and El-Deweny (2001) mentioned that cucumber powdery mildew intensity in Egypt was highly decreased in Autumn season than in spring one. At the same time, during the growing season, the earliest planting resulted in lowest percentage of disease intensity.

Markert and Faulhaber (1965) reported that an analysis of protein in variation among plant cultivar by electrophoresis is approximate an

analysis for their genetic variation. Sweilm *et al.* (2002) used sodium dodocyle sulphate-polyacrylamide gel electrophoresis in fingerprint of both resistant and susceptible cucumber cultivars.

In this investigation, pathogenic variations of cucumber powdery mildew isolates were detected to determine the most virulent isolate, evaluation of the local and imported cucumber cvs. to word the virulent isolate and study of some components of resistance is also of extreme importance. Furthermore, electrophoresis studies were carried out on protein banding patterns of resistant and susceptible plant tissues of infected and healthy hybrids.

MATERIALS AND METHODS

The course of the study was started by testing the pathogenicity using different isolates from *S. fuliginea* to determine the variability in their virulences. Fresh cucumber leaves showing typical powdery mildew symptoms were collected from a chose fields. Each sample is represented one Egyptian governorate. Disease-free leaves of the susceptible cultivar Beit Alpha were inoculated with these samples individually by dusting conidia onto detached leaves in Petri dishes as described by Warkenitin *et al.* (1996). After 3 to 4 days from inoculation powdery mildew colonies were examined under dissecting microscope. The individual colonies from these leaves were isolated and multiplied on seedlings of the susceptible c.v. Beit Alpha which were grown in isolated was cages under greenhouse conditions (20±4°C). Each isolate was multiplied and maintained in an isolated cage separated from others.

Inoculum of *S. fuliginea* was obtained from freshly sporulating colonies (lesion) 9-12 days after inoculation. Conidia were gently brushed into a small quantity of distilled water and counted with the aid of a heamocytometer and adjusted to give a suspension of 2-3 x 10^4 condia/ml (Reuveni *et al.* 1995).

Artificial inoculation was done by spraying the 2nd and 3rd true leaf stages of the plants with a suspension of *S. fuliginea* conidia of (Floris and Alvarez, 1996).

Seeds of seven local cucumber cultivar i.e. Afdal, Hesham, Medina, Nile, Sahara, Shrouq and Yasmina and nine imported cucumber ones, i.e. Beit Alpha, Betostar, Chineses Long, Deltastar, Premo, Pasandra, Rawa, Sweet Crunch and Thamin were planted in 25 cm diameter plastic pots

filled with Sandy clay soil (1 : 1 w/w), (2 seeds/pot x 10 replicates) and placed inside isolated cages under greenhouse conditions at Giza during the Autumn season 2005.

The plants were left to appearance of the first symptoms of powdery mildew after 7 days from inoculation. Disease infection was recorded as the number of infected leaves per plant and expressed as mean percent leaves infected (Raju and Anilkumar, 1990). Disease severity was monitored weekly starting from the appearance of first symptoms using 0-9 rating scale based on the percentage of leaf area affected (Warkentin *et al.* 1996), where 0 = no infection, 1 = < 1% , 2 = 1-5% , 3 = 5-10% , 4 = 10-20% , 5 = 20-40% , 6 = 40-60% , 7 = 60-80% , 8 = 80-90% , 9 = > 90% of leaf area affected.

Types of infection were assessed and scored using the above mentioned scale where scores from 0.0 to 1.0 were classified as resistant (R) and 2-3 were partial resistance (PR) while others from 4 to 9 are susceptible (S).

Maximum severities of mildew cover %, (Peak severity PS%) was estimated. Also the area under disease progress curve (AUDPC) was calculated using the procedure of Shaner and Finney (1977) as follows :

$$\text{AUDPC} = \sum_{i}^{n} \left[\left((Y_{i+n1} + Y_i) / 2 \right) \right] \left[X_{i+1} - X_i \right] \quad \text{, in which}$$

Y_i = mildew severity (per unit) at the i *th* observation.

Xi = Time (days) at the i *th* observation.

n = Total number of observations.

Susceptibility among cucumber genotypes was determined by performing a one way anlaysis of variance of the data using MSTAT statistical software. Mean comparisons were made among genotypes with Fishers LSD (0.05). Pearson's correlation coefficients (r) between various parameters of mildew assessment also calculated.

Evaluation of host resistance:

Seeds of each of 22 cucumber hybrids were planted in 25 cm diameter plastic pots in an isolated greenhouse (20±4°C). Plants were inoculated with condial suspension as previously mentioned at 2nd to 3rd true leaf stage.Plants were left under observation for the first signs of

disease symptoms. This experiment was repeated twice under the same conditions. After 7 days the disease assessed weekly and the different parameters of the disease, i.e. disease incidence (DI%) disease severity (DS%), peak severity (PS%) and area under disease progressive curve (AUDPC) were recorded.

Protein electrophoresis :

Polyacrylamide gel electrophoresis (SDS-PAGE) was used according to Broglie *et al.*, 1986 to determine the quantitative changes that occur in the soluble proteins of the previously inoculated cucumber cultivars, grown under greenhouse conditions at Giza, in relation to their reaction of the *S. fuliginea* virulent isolate. Leaves at similar stage of the tested cucumber plants of two local cultivars (Afdal and Marmar) and sex imported hybrids (Beit – Alpha, Dehigt Green, Chinease Long,Thamin, Sweet Crunch and Rawa) showing different reaction to powdery mildew disease were taken. Two grams of each sample were ground in 0.05 M Sodium acetate buffer + sea sand in a mortar in liquid nitrogen at 4 °C . After that, 50 mg of the extract plus 0.7 ml of ES (4% SDS, 5 % sucrose, 50% mercaptoethanol) were shaken for 10 min at room temperature with gentle steering. The extract was centrifuged at 18.000 rpm for 30 min and the clear supernatant was heated at 100°C for 2-5 min and then cooled to room temperature. Proteins were precipitated by adding cold (20 °C) acetone (8 volume of the supernatant). Protein content of the supernatant was determined using the method described by Ekhramoddoullah and Davidsan (1995) in the same time bovine serum albumin (BSA) was used as a standard.

Silver staining method for protein described by Hochstrasser *et al.*, 1988 was used, where the membrane was stained with 0.1% coomassie blue R-250 in 50% methanol for 48 min and then destined in 50% methanol for 8 min at room temperature. The stained membrane was then raised with water for 10 min and scanned by a laser scanner. Proteins separated by SD-PAGE were electrophorotically transferred to Immobilon-P membrane as described by Matsudaria, (1987).

RESULTS AND DISCUSSION

Pathogenic isolate variations:

Eighteen cucumber cultivars with different phenotypes and genotypes were tested against sever Egyptian isolates of *S. fuliginea* to

define the variability in their virulences. Data in Table (1) indicated that *S. fuliginea* isolate from El-Ayat (Giza) was more virulent (score 4,5, 8 and 9 for most of the tested cvs.) as the infected 17 out of 18 cucumber cultivars compared with the other tested isolates where it infected 14 to 15 cucumber ones. Generally, many known resistant hybrids remained resistant and known susceptible hybrids remained susceptible to all the isolates. Some hybrids exhibited slight resistance reaction to all isolates. Other hybrids were completely susceptible to all isolates.

Table(1): Reaction of cucumber cultivars to artificial inoculation with *S. fuliginea* during Autumn season 2005 under greenhouse conditions 20 ± 4 °C at Giza.

Cucumber cultivar	Isolate No.* / Disease score **						
	1	2	3	4	5	6	7
Local cucumber cultivar							
Afdal	7	9	6	7	7	7	6
Hesham	3	5	3	3	3	3	3
Medina	2	4	2	2	3	2	3
Ni'e	0	1	0	0	0	0	0
Sahara	6	8	7	7	7	7	7
Shrouq	7	8	6	6	6	7	6
Yasmina	0	0	0	0	0	0	0
Imported cucumber cultivar							
Biet-Alpha	9	9	9	9	9	9	9
Betostar	7	8	7	6	6	6	6
Chinease Long	3	5	3	2	4	3	3
Deltastar	3	4	2	2	2	2	2
Premo	0	1	0	0	0	0	0
Pasandra	0	1	0	0	0	1	0
Joy	2	2	3	2	3	3	2
Rawa	3	5	3	3	3	3	3
Sure Green	3	2	2	3	2	2	3
Sweet Crunch	3	5	3	2	3	2	2
Thamin	2	4	2	3	3	3	3

* Isolate number : 1 = Baltim (Kafr El-Sheik), 2 = El Ayat (Giza), 3 = Kassassin (Ismailia), 4 = Gamassa (Demiatta), 5 = Nubaria (Behira), 6 = Qaha (Qalubiya), 7 = Shibin El_Kom (Minufia).

** Disease scores : 0 to 3 = Resistant (R) , 4 to 9 = Susceptible (S)

This may explain the dominance of such isolates under the Egyptian conditions. The variability in the virulence of *S. fuliginea* isolates was previously studied by many authors (Helal *et al.*, 1978, Abd El-Sattar, 1985, Cohen *et al.* 1995 and Abd El-Sayed, 2000) who found the causals of powdery mildew disease expressed clear differences in their ability to infect cucumber cvs. They attributed those variations to locality. Divergences of virulence pattern of *S. fuliginea* in Egypt could be caused

by wider geographical separation, different environmental conditions and / or the presence of different host genotypes biological differences associated year and time in the crop season which are greatly differed in Egypt according the agro-ecological zone. Similarly in Egypt, existence of *S. fuliginea* races 1 and 2 were reported in the Barragess location (El-Deweny *et al.*, 1989) and the variability among different geographical isolates was evident (El-Desouky and El-Deweny, 2001).

Reaction of cucumber cultivars resistance to *S. fuliginea* :

In this study, expression of four components of resistance to *S. fuliginea* i.e. disease incidence (DI), disease severity (DS), peak severity (PS) and area under the disease progressive curve (AUDPC) were used to discriminate among hybrids of cucumber for resistance to powdery mildew pathogen (Table 2). Generally, all components showed significant variation among hybrids and correlations between parameters were significants (Table 3).

The values of AUDPC came in a parallel line with the rest parameters and it were helpful in identifying resistant genotypes. AUDPC values have been employed more and more in identification of partial resistance (Shaner and Finney,1977, Norgaard Knudsen *et al.* 1986 and Raju and Anilkumar, 1990) Even the peak severity alon was able to give enough indication as the level of resistance. However, in such assessment based on peak severity ratings, it is likely that in many instances the useful genotypes may escape detection (Wilcoxson, 1986). Disese incidence is another useful parameter for identifying resistance source and in many cases, clear correlation between disease incidence and severity are known to exist (James, 1974).

In the other side, there was a clear correlation between AUDPC values and disease severity as well as disease incidence (r = 0.88 and 0.84 respectively). Data in Table (3) also showed a clear correlation between disease incidence and peak severity (r = 0.79). Raju and Anilkumar (1990), in powdery mildew of cowpea, reported such correlations between disease incidence with the rest parameters. Also, in powdery mildew of melon, phenotypic expression of quantitative and qualitative components of partial resistance to powdery mildew showed significant variations among genotypes and correlations between them were large and significant (Bioteux *et al.* 1995). Information about the contribution of each component and the correlation between them is a

Table(2): Evaluation of cucumber hybrids for some components (disese incidence (DI); disease severity (DS); peak severity (PS) and area under the disease progress curve (AUDPC) to inoculation with the virulent isolate of *S. fuliginea*, under greenhouse conditions (20 ± 4 C).

Hybrid	Components of resistance			
	DI(%)	DS(%)*	PS(%)*	AUDPC*
Afdal	75.6	44.25	28.62	545.15
Beit Alpha	86.8	52.00	39.77	572.80
Betostar	66.0	40.15	36.82	451.33
Chinease Long	12.0	11.19	12.35	126.05
Dlight Green	5.0	1.16	2.48	8.19
Dltastar	11.0	8.25	3.00	92.76
Green Bowl	21.0	11.55	42.54	249.98
Hesham	11.0	8.25	33.50	96.45
Joy	7.0	1.17	3.45	28.95
Maram	12.0	10.50	14.34	159.06
Marmar	6.0	1.17	2.34	8.19
Medina	4.0	2.34	2.34	52.20
Nile	13.0	1.17	2.34	26.85
Premo	20.0	5.46	16.39	57.10
Rawa	86.7	43.33	26.61	488.45
Pasandra	86.7	42.00	39.84	576.33
Sahara	21.0	3.51	7.02	43.83
Shrouqe	21.0	3.12	9.36	38.22
Sure Green	27.0	19.75	8.37	73.79
Sweet Crunch	4.0	1.17	1.17	6.88
Thamin	5.0	1.17	2.34	9.18
Yesmina	5.0	1.17	2.34	9.46
L.S.D at P = 0.05	3.22	3.16	1.43	75.06
L.S.D at P = 0.01	4.68	3.92	2.11	93.21

The different cucumber hybrids could be rentatively classified into three groups as follows :

a) Resistant group (R) hybrids such as Delight Green, Marmar, Medina, Premo, Sweet Crunch, Thamin and Yasmina.

b) Partial resistant group (PR) hybrids as in Joy and Sure Green.

c) Susceptible group (S) hybrids as found in Afdal, Beit Alpha, Betostar, Chinease Long, Deltastar, Green Bowl, Hesham, Maram, Nile, Pasandra, Rawa, Sahara and Shrouqe.

Table(3): Correlation coefficient between various components of resistance to powdery mildew on 22 cucumber hybrids.

Components of resistance	PS	DI	DS	AUDPC
Peak severity	1.00	- 0.79**	- 0.88 **	- 0.84**
Disease incidence		1.00	0.84**	0.84**
Disease severity			1.00	0.98**
AUDPC				1.00

* Significant at P = 0.05 ; ** Significant at P = 0.01

great important to the development of resistance screening systems for cucumber powdery mildew. The cucumber genotypes with high level of resistance and partial resistance hybrids is an acceptable color type and hence could be used either directly or in breeding programs. Also, the identification of parameters which are mutually correlated may allow breeders to choose both the most appropriate and the simple component of resistance.

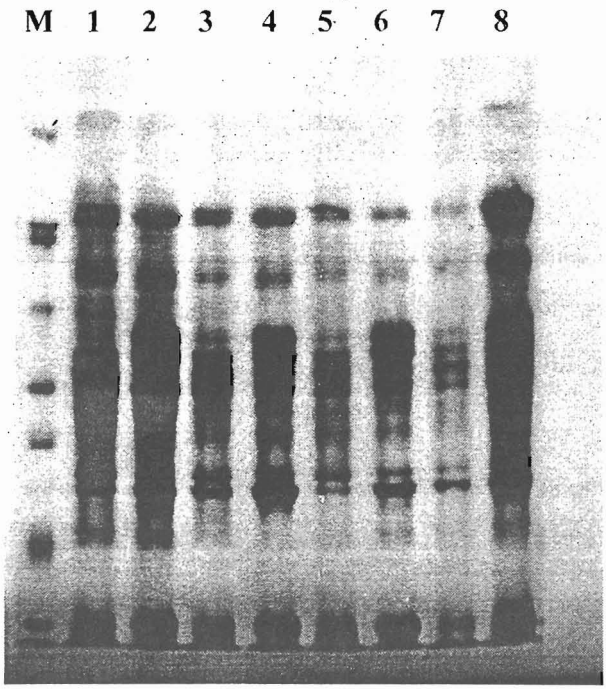
Protein electrophoresis:

The results presented in Fig.(1) reveal the possibility of using electrophoresis patterns to differentiate cucumber cultivars in relation to the levels of the disease resistance. A photograph of the Gel electrophoretic patterns of proteins obtained from the extracts are presented in Fig.(1) as well as illustrated in the cluster analysis as dendrogram of protein banding patterns (Fig. 2).

The results revealed that the susceptible cucumber cultivars, Afdal, Beit-Alpha and Rawa had an increase in protein bands compared with the partial resistant ones, Joy and Sure Green. Resistant cultivars, Sweet Crunch and Thamin showed low number of protein bands. New types of protein bands were found in the susceptible cultivars by using this protein fingerprint technique. Also, a computerized program for cluster analysis to differentiate the effect of host (hybrids) reaction to powdery mildew disease was used. This phenogram constructed is based on similarity levels (SLs) generated from cluster anaylsis of electrophoretic banding patterns of dissociated protein. The phenogram was divided into two subcluster (SL = 82.09%), the higher SL, included the susceptible hybrids (Afdal, Beit Alpha and Rawa) which formed a subcluster (SL = 96.57%). The other treatments which included the partial resistant hybrids (Chinease Long, Deligt Green and Maram) and the resistant ones (Sweet Crunch and Thamin) formed the another subcluster (SL = 88.52%).

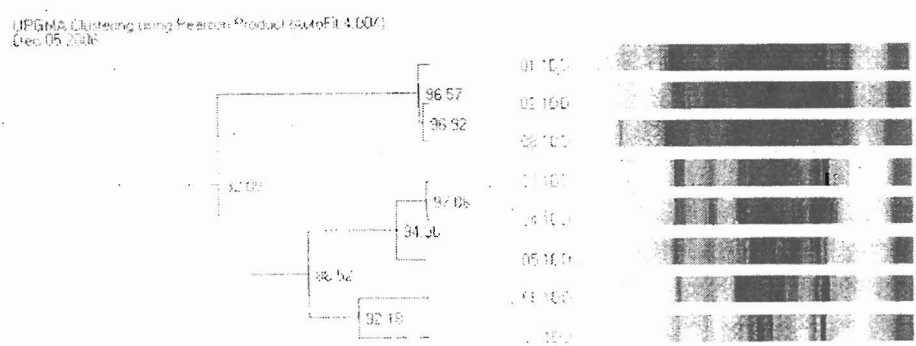
Results may be explained on the basis that susceptible varieties are more suitable for growth and reproduction of the pathogen causing some disappearance of proteins and formation of new proteins in these hybrids. Infection with powdery mildew resulted in the induction of new polypeptides. These results may indicate the formation of pathogen-related protein (PR – protein) as mentioned by Antoniwa *et al.* (1980). Generally, it could be concluded that (PR – Protein) reflected a particular type of stress protein which are induced during the infection. The present

results are in accordance with those reported by Sang and Joo (1992).



1- Afdal (S), 2- Beit Alpha (S), 3- Joy (PR), 4- Chinease Long (R), 5- Thamin (R), 6- Sure Green (PR), 7- Sweet Crunch (R), 8- Rawa (S)

Fig. (1): Photograph of protein banding patterns obtained from leaves extracts representing 8 cucumber hybrids grown under greenhouse conditions and showing different reaction types to artificial inoculation.



1- Afdal (S), 2- Beit Alpha (S), 3- Deligt Green (R), 4- Chinease Long (R),

5-Thamin (R), 6- Sure Green (PR), 7- Sweet Crunch (R), 8- Rawa (S)

Fig. (2): Dendrogram of Protein banding Patterns of eight cucumber hybrids included different reaction types to powdery mildew.

Further studies are needed using more varieties with different degrees of resistance and susceptibility to evaluate fingerprinting technique for differentiation of various genotypes. This technique may provide a method to differentiate cultivars, aid in the identification of diverse germplasm and protein changes appear may be a key to understanding host resistance to diseases.

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الملخص العربى

استجابة أصناف الخيار لمرض البياض الدقيقى وعلاقته ببروتين العائل

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كانت عزلة العياط (محافظة الجيزة) للفطر سفيروثيكا فيولجينا مسبب مرض البياض الدقيقى على الخيار فى مصر أكثر العزلات شراسة مقارنة بغيرها من العزلات المختبرة حيث تمكنت من إصابة ١٧ صنفاً من بين ١٨ صنفاً مختبراً من أصناف الخيار، بينما كانت العزلات الأخرى قادرة على إصابة ما بين ١٤ إلى ١٥ صنفاً فقط.

كانت الاختلافات بين أصناف الخيار المختبرة كبيرة. فبعض الأصناف أظهرت مستوى عالى من المقاومة للفطر الأكثر شراسة من المسبب وهى أصناف (دليت جرين ، مرمر، مدينة، بريمو، سويت كرانش، ثامين وياسمينا) بينما أظهرت أصناف أخرى مقاومة جزئية للإصابة وهى أصناف (جوى وسور جرين) كما أظهرت أصناف ثالثة قابلية واضحة للإصابة وهى أصناف (أفضل، بيت ألفا، بيتوستار، شينزلونج، دلتا ستار ، جرين باول ، هشام ، ما رام ، نايل ، باسندرا ، راوا ، سهارا، وشروق .

باستخدام طريقة التفريد الكهربى للبروتين باستخدام مادة صوديوم دوديسيل سلفيت أمكن التفريق بين الثمانية هجن المختبرة من الخيار فى تفاعلها ضد مرض البياض الدقيقى حيث تم الكشف عن حزم البروتين الموجودة .

كما تم أيضاً تطبيق أسلوب التحليل العنقودى لتصنيف الهجن بناء على ما بينها من درجة قرابة فى أنماط البروتين وتم التعبير عن النتائج فى فينوجرام.

أظهرت الدراسة أن أنماط البروتين المفكك بواسطة مادة صوديوم دوديسيل سلفيت تصلح للتفرقة بين هجن الخيار بناء على علاقة القرابة بين هذه الأصناف وقد تم تقسيم الأصناف إلى مجموعتين ارتبطتا بدرجة رد فعلهم للإصابة بالمرض (بدرجة قرابة ٨٢,٠٩%) وكونت مجموعة الهجن الحساسة تحت عنقود (بدرجة قرابة ٩٦,٥٧%) أما مجموعة الهجن المقاومة والهجن المقاومة جزئياً فقد كونت تحت عنقود (بدرجة قرابة ٨٨,٥٢%) وقد ظهرت أنواع جديدة من البروتين فى الأصناف الحساسة فى شكل حزم ممثلة للبصمة الوراثية للهجن المختلفة.