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USE OF MORPHOLOGICAL TRAITS AND ELECTRO-PHORESIS OF PROTEINS TO IDENTIFY THE TELEOMORPH OF *Oidium lini* INVOLVED IN POWDERY MILDEW OF FLAX

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ABSTRACT

Powdery mildew (PM) of flax is caused by the obligate parasite *Odium lini*. One obvious gap in our current knowledge of *O. lini* is the lack of consensus regarding the name of its teleomorph, which has been referred to as *Erysiphe polygoni* or *E. cichoracearum*. As an attempt to reslove this problem, this study used biostatistical analysis (BSA) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of proteins to evaluate the degree of relatedness between *O. lini* and some anamorphs of PM fungi, whose teleomorphic taxonomy is well established. In BSA, measurements were made for some quantitative morphological traits of the anamorphs of various fungi belong to *E. cichoracearum* and *E. polygoni*. The obtained measurements were subjected to cluster analysis by the unweighted pair-group method based on arithmetic means (UPGMA) and a phenogram was constructed. This phenogram showed that morphotypes 1 and 2 of *O. lini* belong to *E. polygoni*, while morphotype 3 belongs to *E. cichoracearum*. In SDS-PAGE, protein profiles of *O. lini* were compared with those of *E. polygoni* from peas and *E. cichoracearum* from sunflower. On the basis of electrophoretic dissimilarities among protein banding patterns, fungi were grouped by cluster analysis (UPGMA) and the results were expressed as a phenogram. This phenogram confirmed that morphotypes 1 and 2 of *O. lini* belong to *E. polygoni*.

Keywords: *Oidium lini*, Morphology, SDS-PAGE.

INTRODUCTION

Powdery mildew (PM) of flax (*Linum usitatissimum* L.) is caused by the obligate parasite *Odium lini* Škoric. This fungus is found on flax in Egypt only in its imperfect (conidial) stage. The pathogen infects all the aboveground flax organs including stems, leaves, flowers, and capsules. PM occurs annually in all flax production areas in Egypt. Physiological races of the pathogen have not been identified because no differential lines are available to date (A.A. Aly, *personal observations*). One obvious gap in our current knowledge of *O. lini* is the lack of consensus regarding the identification of its teleomorph, which has been referred to as *Erysiphe polygoni* (McKay, 1947; Dickson, 1956, and Nyval, 1981) or *E. cichoracearum* (McKay, 1947; Dickson, 1956, and Agrios, 1988).

Morhpological traits have been used to study the taxonomy of genus *Erysiphe*. For example, Zeller (1995) reported that the genus *Erysiphe* had been suggested as an ancestral form within Erysiphaceae characterized by a

set shared of relatively primitive morphological characters and might infact be polyphyletic. However, several groups within *Erysiphe* were delimited by other sets of important morphological differences, resulting in its subdivision into at least three evolutionary lines (sections) and other allied genera. He concluded that morphology does show some usefulness in phylogentic comparisons between sections of *Erysiphe*, but appears to have much limited usefulness for distinguishing among species within a section. Khan and Sharma (1995) examined characteristics of *Sphaerotheca fuliginea* and *E. cichoracearum* anamorphs from a large number of cucurbits collected from 9 states in India, to evaluate their use in identifying the species. Conidial shapes, presence of fibrosin bodies in conidia, conidial dimensions and length/breadth index, morphology of germ tubes and point of their origin, and development of appressoria were consistent characters, irrespective of cucurbit host and locality. It was suggested that these characteristics were of great taxonomic importance and could be reliably used to identify the species.

Amino acid sequence of polypeptides (components of proteins) are dependent on nucleotide sequences of their coding genes; therefore, an analysis of protein variation among fungi by electrophoresis, approximates an analysis of their genetic variation (Markert and Faulhabor, 1965). Very few attempts; however, have been made to utilize this technique to study the taxonomy of genus *Erysiphe*. For example, Koch and Koehler (1990) used unspecific stained proteins as biochemical-genetic markers to study genetic variation within and between *E. graminis* f.sp. *hordi*, *avenae*, *secalis* and *tritici*. The genetic relationships between powdery mildew formae speciales were computed by cluster analysis from similarity (F) and dissimilarity (D) coefficients and illustrated by phylogentic trees. Marked correspondence was found between *E. graminis* f.sp. *secalis* and *tritici* (F: 82-90%). Lower homologies were obtained from the comparison of these formae speciales, respectively, with *E. graminis* f.sp. *hordi* (F: 28-34%) and *avenae* (F : 24-32%).

The objectives of the present study was to identify the teleomorph of *O. lini* by comparing its morphological traits and electrophoretic protein patterns with those of *E. polygoni* and *E. cichoracearum*.

MATERIALS AND METHODS

Characterization of morphological traits of *O. lini* compared with those of *E. cichoracearum* and *E. polygoni*:

Leaves of flax, okra, tobacco, peas, and alfalfa infected with powdery mildew were cleared for 10-60 min-according to the age of the leaves-at 70°C or overnight at room temperature in an ethanol-chloroform (75: 25 v/v) mixture containing 0.15% trichloroacetic acid, with frequent changes of the solution. Five cleared leaves were placed on microscope slides (one leaf/slide) and then stained in 0.2% trypan blue in lactophenol. The trypan blue stain was prepared by dissolving 600 mg trypan blue in 200 ml lactic acid then 400 ml of glycerol were added and the volume was made up to one liter by deionized water (Hammett, 1977). The following traits were examined under light microscope:

1. Shape of the conidium on the top of the conidiophore.
2. Length of the conidiophore (u).
3. Number of the conidiophore cells.
4. Dimensions of the conidiophore basal cell (u).
5. Dimensions of the first cell above the basal cell (u).
6. Dimensions of the cell in the middle of the conidiophore (u).
7. Dimensions of the conidium on the top of the conidiophore (u).

Fifteen conidiophores were examined for estimation of the previously mentioned traits. Means of the traits were subjected to cluster analysis.

Electrophoretic protein patterns of *O. lini* compared with those of *E. cichoracearum* and *E. polygoni*:

A. Extraction of fungal protein:

Fungal growth (conidia, conidiophores, and mycelium) of *O. lini* from flax, *E. cichoracearum* from sunflower, and *E. polygoni* from peas were collected by a fine drawing brush, suspended in 5 ml sterile water, and freezed-dried. The frozen growth was suspended in phosphate buffer pH 8.3 (1-3 ml/g fungal growth), mixed thoroughly with glass beads, and ground in liquid nitrogen to a fine powder. The ground growth was centrifuged at 19, 000 rpm for 30 min at 0°C. The protein content in supernatant was estimated according to Bradford (1976) by using bovine serum albumine as a standard protein. If protein concentration was low, protein would be precipitated from the clarified supernatant by adding ammonium sulfate at 70% of saturation (60 g/100 ml), then kept in a refrigerator for 30 hr. Pellets, collected by centrifugation at 11, 000 rpm for 30 min, were resuspended in phosphate buffer pH 8.3 and subjected to dialysis for 24 hr against the buffer and centrifugated at 11,000 rpm for 30 min. Protein was estimated in the obtained supernatant. The supernatant containing the soluble protein was stored at -20°C until usage.

B. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE):

Thawed protein-extract supernatant was mixed with an equal volume of a solution consisting of (by volume) 64% buffer (0.15M Tris-HCl), pH 6.8); 20% glycerol; 6% sodium dodecyl sulfate (SDS); 10% 2-6-mercaptoethanol; and 0.1% bromophenol blue, before boiling in a water bath for 3 min. Twenty-microliter samples (40 ug of proteins) were subjected to electrophoresis in 5-20% gradient polyacrylamide prepared in 0.1% SDS (Laemmli, 1970) with a 3.5% polyacrylamide stacking gel. Electrophoresis was conducted at 10°C, for 4 hr at 30 and 15 mA for the separating gel and the stacking gel, respectively, until the dye reached the bottom of the separating gel (Laemmli, 1970). Electrophoresis was performed in a vertical slab mold (16 x 18 x 0.15 cm). Gels were stained with silver nitrate for the detection of protein bands (Sammons *et al.*, 1981).

C. Densitometric scanning:

Protein banding patterns of fungi obtained by SDS-PAGE were scanned densitometrically (Scanning densitometer GS 300, Hoefer Scientific Instruments, San Francisco, CA, USA). The densitometer was connected

with a compterized program (Microsoft Windows GS 365 W. Version 301). The scanning included all major and minor bands of each sample. The position of each band was recorded as a rate of flwo (R_f) which ranged from 0.01 to 0.99. The presence of a band at a particular R_f was designated as + and its absence at the same R_f was designated as -. R_f values were calculated according to the following formula: Migration distance of a band (cm)/migration distance of the dye (cm). data were subjected to cluster analysis.

Cluster analysis:

Means of morphological traits and protein banding patterns were clustered by the average linkage technique (unweighted pair-group method based on arithmetic means). The results were expressed as phenograms (Joseph *et al.*, 1992). Cluster analysis was performed with a computerized program.

RESULTS and DISCUSSION

Microscopic examination of flax leaves infected with PM revealed that *O. lini* could be classified into three distinct morphotypes (Fig. 1) based on the morphology of the conidia, which were cylindrical (morphotype 1), bullet-shaped (morphotype 2), or barrel-shaped (morphotype 3). Morphotypes 1 and 2 were observed under field conditions during the growing season, while morphotype 3 was observed only in the greenhouse in September and October, i.e. befcre the beginning of flax growing season. The occurrence of this morphotype was restricted to cotyledons and never spread to true leaves. It caused severe stunting followed by quick death of the infected plants.

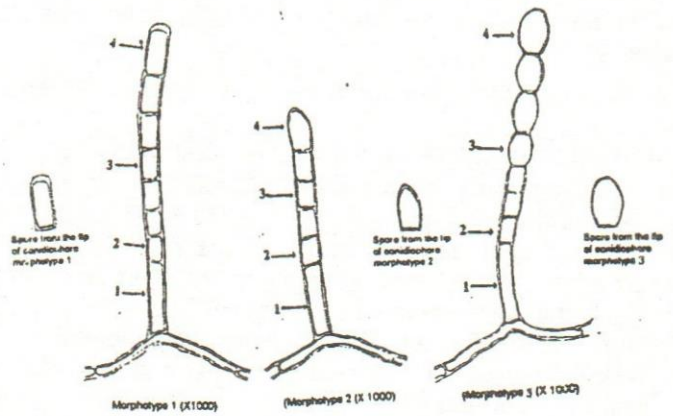

Fig. (1): Details of *O. lini* conidiophore on flax leaf. Morphotype 1 (left), morphotype 2 (middle), and morphotype 3 (right).

1. Conidiophore basal cell.
2. The first cell above the basal cell.
3. The cell in the middle of the conidiophore.
4. Conidium in the tip of conidiophore.

The phenogram of Fig. (2) was constructed based on the taxonomic distances (TDs) generated from cluster analysis of trait means showed in

Table (1). A taxonomic distance is the distance, which measures the degree of relatedness between organisms, the smaller the taxonomic distance, the more closely related the organisms are. The phenogram included three distinct clusters. The first one (TD = 10.6) included the morphotypes 1 and 2 of *O. lini* as well as *E. polygoni* from alfalfa. It should be noted that the morphological traits of morphotype 2 of *O. lini* and *E. polygoni* from alfalfa varied very little. Thus, the two fungi were included in a distinct subcluster showing the lowest dissimilarity level (TD = 0.6). The second cluster (TD = 10) included the two isolates of *E. cichoracearum* from okra and from tobacco in addition to the morphotype 3 of *O. lini*. This morphotype and *E. cichoracearum* from okra were included in a distinct subcluster showing a low dissimilarity level (TD = 2.4). The third cluster, which included only *E. cichoracearum* from okra were included in a distinct subcluster showing a low dissimilarity level (TD = 2.4). The third cluster, which included only *E. polygoni* from peas was unrelated to the other two clusters. In this phenogram, the isolates from different hosts but belonged to the same species were, as a general rule, clustered with one another. The only exception was the isolate of *E. polygoni* from peas, which was unrelated to the other isolate of *E. polygoni* from alfalfa. The phylogentic relationships as shown in Fig. 2 led us to draw a conclusion that morphotypes 1 and 2 of *O. lini* belong to *E. polygoni*, while morphotype 3 belongs to *E. cichoracearum*.

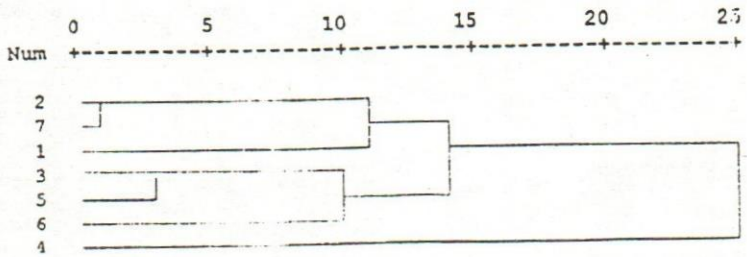

Fig. (2): Phenogram based on average linkage cluster analysis of morphological traits of isolates of *Oidium lini* and *Erysiphe* spp.

1. ***O. lini* morphotype 1,**
2. ***O. lini* morphotype 2,**
3. ***E. cichoracearum* from okra**
4. ***E. polygoni* from peas,**
5. ***O. lini* morphotype 3,**
6. ***E. cichoracearum* from tobacco,**
7. ***E. polygoni* from lalfalfa.**

A problem with proteins as biochemical markers to study fungal taxonomy is the vast number, which can be generated from a fungus. Faced with so much data, only sophisticated analysis can hope to draw meaningful

conclusions. The ready availability of computers has made numerical taxonomy more accessible and some later studies on fungi have proven useful (Manicom *et al.*, 1990). Therefore, in the present study, we used a computerized program for cluster analysis to differentiate among protein profiles of three PM fungi.

Table (1): Range and means of morphological traits used in cluster analysis of *Erysiphe* spp.

	Morphological trait									
	Length of conidio-phore (µ)	Conidiophore cells (No.)	Conidiophore basal cell		The first cell above the basal cell		Cell in the middle of the conidiophore		Conidium in the top of conidiophore	
			Length (µ)	Width (µ)	Length (µ)	Width (µ)	Length (µ)	Width (µ)	Length (µ)	Width (µ)
***O. lini* (1)** [a]										
Max. [b]	180.6	7	67.7	10.5	29.4	12.6	22.5	12.6	27.3	16.8
Min. [c]	121.8	5	40.0	8.4	12.6	8.4	16.8	8.4	21.0	10.5
Mean	143.4	5.7	54.5	9.1	19.6	9.6	19.6	10.4	23.8	11.5
***O. lini* (2)** [d]										
Max.	180.6	8	73.5	12.6	25.2	12.6	29.4	12.6	25.2	14.7
Min.	71.4	4	21.0	8.4	10.5	8.4	14.7	8.4	12.6	8.4
Mean	123.2	5.4	43.2	8.8	18.5	9.0	20.9	9.2	22.1	10.9
***E. cichoracearum* from okra**										
Max.	197.6	10	33.6	12.6	25.2	14.7	25.2	18.9	29.4	18.9
Min.	92.4	5	12.6	8.4	10.5	8.4	14.7	14.7	21.0	14.7
Mean	160.7	8.4	26.9	11.2	17.5	12.5	19.6	16.4	24.6	18.1
***E. polygoni* from peas**										
Max.	118.7	4	35.7	6.3	27.3	8.4	29.4	12.6	39.9	16.8
Min.	63.0	4	21.0	4.2	11.6	5.3	10.5	6.3	14.7	7.4
Mean	105.1	4	26.7	5.5	21.1	7.4	23.5	9.0	31.8	12.2
***O. lini* (3)** [e]										
Max.	214.2	9	63.0	12.6	21.0	12.6	21.0	14.7	14.7	16.8
Min.	126.0	6	25.2	6.3	10.5	8.4	14.7	10.5	10.5	10.5
Mean	164.5	8.1	47.2	9.1	14.9	9.0	18.0	12.4	12.4	14.3
***E. cichoracearum* from tobacco**										
Max.	193.2	8	117.6	14.7	12.6	12.6	21.0	16.8	27.3	16.8
Min.	113.4	6	35.7	6.3	8.4	8.4	10.5	10.5	14.7	10.5
Mean	154.0	6.7	72.8	10.1	9.5	9.5	15.4	11.2	22.4	14.6
***E. polygoni* from alfalfa**										
Max.	201.6	6	92.4	7.1	42.0	6.3	29.4	8.4	54.6	12.6
Min.	88.2	4	12.6	4.2	12.6	4.2	10.5	5.0	33.6	10.5
Mean	140.6	4.9	40.3	5.1	22.0	5.6	17.2	6.0	45.2	12.3

[a] **Morphotype (1) of *O. lini* from flax**
[b] **Maximum value.**
[c] **Minimum value.**
[d] **Morphotype (2) of *O. lini* from flax.**
[e] **Morphotype (3) of *O. lini* from flax.**

Electrophoretic banding patterns of SDS-PAGE are shown in Fig. 3 and Table 2. Thirty nine bands of dissociated proteins were detected (Table 2). Of these bands, 9 (23.08%) were common to all fungi. This large number of the observed bands was due to the effects of SDS, which dissociated each oligomeric protein into its subunits (Bohinski, 1983). Therefore, each band was a monomeric protein. Fig. 4 showed the densitometric scanning of proteins (DSP) obtained by SDS-PAGE from the three fungi. *E. polygoni* from peas and *O. lini* from flax showed almost identical DSP, which indicates that the common bands between the two fungi showed almost the same intensity. On the other hand, both fungi showed striking differences in DSP compared to *E. cichoracearum* from sunflower. It should be noted; however, that the

degree of staining is not necessarily directly proportional to the number of proteins in a given band. Staining intensity may be affected by the amino acid composition of the protein and its size as well as by multiple proteins at a given site (Partridge *et al.*, 1984). Accordingly, variations in intensity of bands were not used in cluster analysis, which was based entirely on presence or absence data. Fig. 4 showed the phenogram constructed based on TDs genersted from cluster analysis of electrophoretic banding patterns of SDS-dissociated proteins shown in Table 2. In this phenogram, *O. lini* and *E. polygoni* from peas belonged to a single cluster (TD = 0.6).

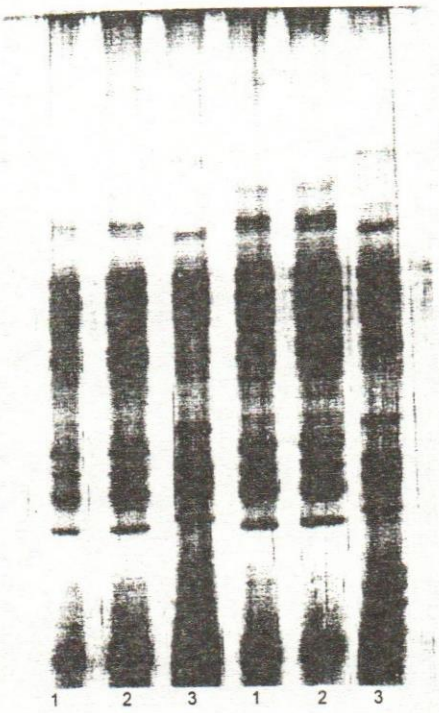

Fig. (3):Protein patterns obtained by SDS-PAGE for *E. polygoni* from peas (1), *O. lini* from flax (2), and *E. cichoracearum* from sunflower (3).

This cluster was unrelated to the other cluster, which included only *E. cichoracearum* from sunflower. It is worthy to mention that morphotypes 1 and 2 of *O. lini* occurred together on the infected flax plants; therefore, it was impossible to study them individually in electrophoresis; however, microscopic examination enabled us to do that in biostatistical analysis. Therefore, electrophoretic analysis led us to draw a conclusion that morphotypes 1 and 2 of *O. lini* belong to *E. polygoni*.

Table (2): Protein patterns obtained by SDS-PAGE for three powdery mildew fungi.

Band		Fungus		
No.	R_f	*E. polygoni* from peas	*O. lini* from flax	*E. cichoracearum* from sunflower
1	0.02	+	+	+
2	0.11	+	+	-
3	0.28	-	-	+
4	0.36	+	+	-
5	0.38	+	-	-
6	0.40	-	-	+
7	0.45	-	-	+
8	0.46	+	+	-
9	0.48	+	+	+
10	0.49	+	+	-
11	0.50	+	+	-
12	0.51	+	+	+
13	0.52	+	+	-
14	0.53	-	-	+
15	0.54	+	+	-
16	0.55	-	-	+
17	0.56	+	+	-
18	0.57	+	+	-
19	0.58	+	+	+
20	0.59	+	+	-
21	0.60	-	-	+
22	0.62	+	+	+
23	0.63	-	+	-
24	0.64	+	+	+
25	0.65	+	+	-
26	0.67	-	-	+
27	0.68	+	+	-
28	0.70	-	-	+
29	0.73	+	+	+
30	0.76	+	+	-
31	0.80	+	+	+
32	0.82	-	-	+
33	0.83	-	-	+
34	0.85	+	+	-
35	0.89	-	-	+
36	0.92	-	+	-
37	0.93	+	-	-
38	0.94	+	+	+
39	0.97	-	-	+

Rate of flow (R_f) was calculated according to the following formula:

$$R_f = \frac{\text{Migration distance of band (cm)}}{\text{Migration distance of marker (cm)}}$$

\+ The designated band is present.

\- The designated band is absent.

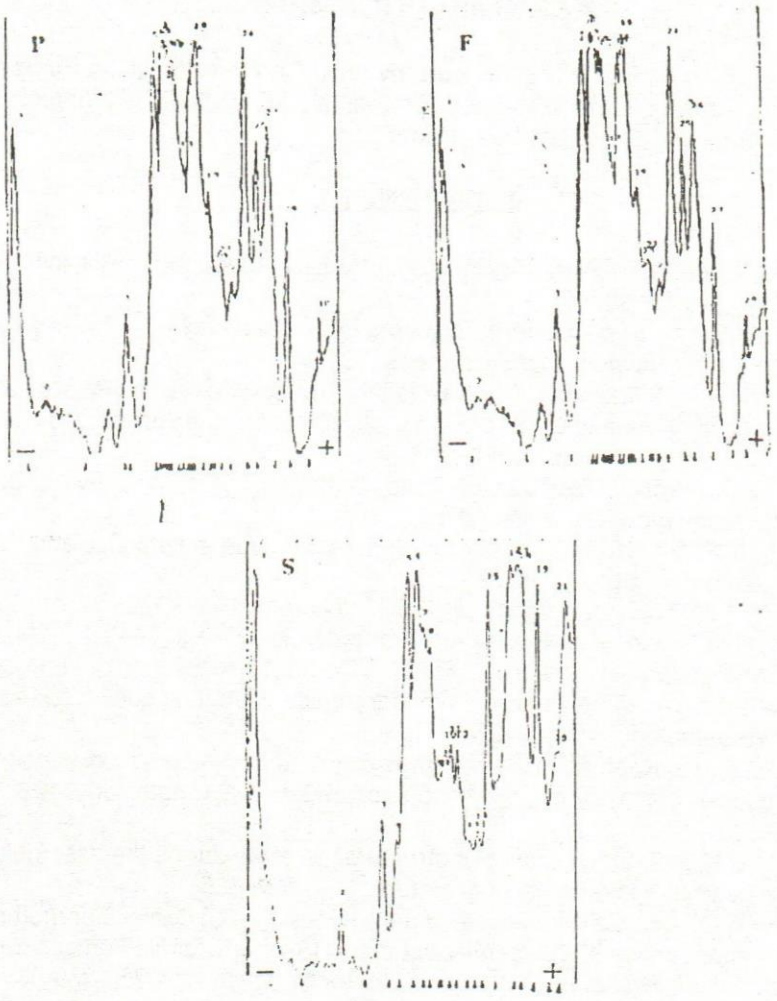

Fig. (4): Densitometric scanning of proteins obtained by SDS-PAGE for *E. polygoni* from peas (P), *O. lini* from flax (F), and *E. cichoracearum* from sunflower (S).

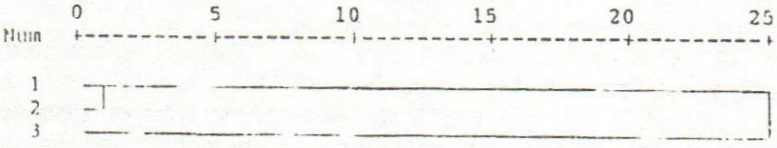

Fig. (5): Phenogram based on average linkage cluster analysis of electrophoretic protein patterns obtained by SDS-PAGE from *E. polygoni* of peas (1), *O. lini* of flax (2), and *E. cichoracearum* from sunflower (3).

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إستعمال الصفات المورفولوجية والتفريد الكهربى للبروتينات لتعريف الطور الكامل لفطر أويديم لينای المسبب لمرض البياض الدقيقى فى الكتان

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١- معهد بحوث امراض النباتات - مركز البحوث الزراعية - الجيزة - مصر.

٢- قسم النبات الزراعى - كلية الزراعة - جامعة الزقازيق - مشتهر - مصر

يتسبب مرض البياض الدقيقى فى الكتان عن الإصابة بالفطر الإجبارى التطفل أويديم لينای. هناك فجوة واضحة فى المعلومات المتوفرة عن هذا الفطر تتمثل فى الخلاف حول اسم طوره الكامل الذى يشار إليه أحيانا باسم إيريزيف بوليجونای وأحيانا أخرى باسم إيريزيف سيكوراسيرم. أمكن - فى الدراسة الحالية - تعريف الطور الكامل لفطر أويديم لينای عن طريق تحديد درجة القرابة بين هذا الفطر وبعض الأطوار الناقصة لفطريات البياض الدقيقى الأخرى. وقد روعى فى اختيار هذه الفطريات أن يكون الوضع التقسيمى لأطوارها الكاملة معروف بدقة. استخدمت طريقتين لتحديد درجة القرابة هما: التحليل الإحصائى لبعض الصفات المورفولوجية التى يمكن تقديرها كميا والفصل الكهربى للبروتينات بعد تفكيكها باستعمال مادة صوديوم دوديسيل سلفيت. أجرى التحليل العنقودى للقياسات التى أمكن الحصول عليها فى الطريقة الأولى وتم التعبير عن النتائج فى فينوجرام. اظهرت النتائج أن فطر إيريزيف بوليجونای هو الطور الكامل للطرازين ١ و ٢ لفطر أويديم لينای، فى حين كان فطر إيريزيف سيكوراسيرم هو الطور الكامل للطراز ٣ لفطر أويديم لينای فى الطريقة الثانية، أجرى التحليل العنقودى لأنماط البروتين المتحصل عليها من فطريات أويديم لينای من الكتان و إريزيف بوليجونای من البسلة وإيريزيف سيكوراسيرم من عباد الشمس. أكدت النتائج ما سبق التوصل إليه فى الطريقة الأولى من أن فطر إيريزيف بوليجونای هو الطور الكامل للطرازين ١ و ٢ لفطر أويديم لينای.