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USE OF PATHOGENICITY AND ELECTROPHORETIC PROTEIN PATTERNS TO DISTINGUISH ISOLATES OF *Fusarium oxysporum* f.sp. *vasinfectum* PATHOGENIC ON COTTON

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ABSTRACT

Eight isolates of *Fusarium oxysporum* f.sp. *vasinfectum* (FOV) were tested for levels of pathogenicity on 45-day-old greenhouse-grown seedlings of six cotton genotypes. Isolate x genotype interaction was a very highly significant ($p = 0.0094$) source of variation in wilt incidence suggesting that isolates responded differently to different genotypes. Due to the significance of isolate x genotype interaction, a least significant difference (LSD) was used to compare between the individual isolate means within genotypes, based on these comparisons, it was easy to differentiate between some of FOV isolates by their differential pathogenicity on some of the genotypes. On the other hand, some isolates was indistinguishable because they showed nonsignificant differences on any of the genotypes. Proteins of the isolates were compared by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Gels were stained with Coomassie Brilliant Blue R-250 (CBB) or silver nitrate (SN). Cluster analysis of the protein banding patterns by the unweighted pair-group method based on arithmetic means (UPGMA) placed the isolates in several groups. Similarity levels among the isolates on staining with SN were less than those of CBB, which indicates that SN was more sensitive than CBB to detect the differences among the isolates in protein banding patterns. There were isolates specific bands by which isolates, particularly those belonged to different groups, could be identified. Some isolates, which were indistinguishable by pathogenicity test, were easily distinguished by their specific bands after staining with SN. The results of the present study indicate that isolates of FOV could be identified by their differential pathogenicity on a set of cotton genotypes, combined with their specific parotein bands separated by electrophoresis and stained with SN.

INTRODUCTION

Fusarium wilt (*Fusarium oxysporum* Schlecht f.sp. *vasinfectum*, (Atk.) Snyd. and Hans.) of cotton (*Gossypium* spp.) was first described by Atkinson (1892) in the USA. The earlist report of the disease outside the USA came from Egypt (Fahmy, 1927), where it spread rapidly with the release of the Sakal cultivar during the 1920s. Fusarium wilt now occurs in all the main cotton-growing areas of the world (Watkins, 1981). *Fusarium oxysporum* f.sp. *vasinfectum* (FOV) casued serious losses in the commercial Egyptian cottons (*G. barbadense* L.) in the late fifties. Since than, an extensive cotton-breeding program was initiated to develop cultivars resistant to the disease.

The Egyptian race of FOV (race 3) has long been known in the Nile Valley, where it remains one of the most damaging pathogens on *G. barbadense*

cultivars (Watkins, 1981). This race also attacks *G. barbadense* in the former Soviet Union (Watkins, 1981) and Israel (Netzer *et al.*, 1985).

Fusarium wilt remains a potential threat to cotton production in Egypt because it seems that FOV is still well established in the Egyptian soil (A.A. Aly, *personal observations*), which increases the proability the new isolates of race 3 will arise to confound cotton breeders.

Therefore, differentiation of FOV isolates involved in cotton wilt disease is important for improving our understanding of the ecology of these isolates and the epidemiology of the disease.

The conventional method of differentiating among isolates belonging to different formae speciales of *F. oxysporum* is the observation of the differences in virulence when the pathogen isolates interact with a set of genotypes (Armstrong and Armstrong, 1981). However, this method is expensive, time consuming, and may be influenced by variability inherent in the experimental system (Aly, 1988; Bhatti and Kraft, 1992). Furthermore, the differential disease reactions do not provide information about the genetic relationship among the pathogen isolates (Perez-Artes *et al.*, 1995).

Therefore, another reliable method, either alternative or complementary to that based on the differential interaction between FOV isolates and cotton genotypes, is required for identification of FOV isolates. Electrophoresis of proteins seems to be a suitable method to achieve this goal. The suitability of this method to detect the genetic differences among isolates of FOV is due to the fact that amino acids sequences of polypeptides (components of proteins) are dependent on nucleotide sequences of their coding genes; therefore, an analysis of protein variation among isolates of FOV by electrophoresis, approximates an analysis of their genetic variation (Markert and Faulhaber, 1965).

Electrophoresis of proteins has been widely used for studying variation in *F. oxysporum*. For exmple, Lo and Sun (1986) found that protein pattern in vertical slab electrophoresis of *F. oxysporum* from radish different from that of *F. oxysporum* from mustard and *F. oxysporum* from kale. Scala *et al.* (1989) distinguished pathotype 2 of *F. oxysporum* f.sp. *dianthi* by electrophoresis of native protein due to a unique band with MMc 30 kDa. Lodwig *et al.* (1999) used electrophoretic profiles of total proteins to distinguish isolates 27 and 30 of *F. oxysporum* f.sp. *cubense* and *F. oxysporum* from *Triticum* sp. Aly *et al.* (2000) reported that electrophoretic banding patterns of dissociated proteins provided a reliable method for grouping the four Egyptian races of *F. oxysporum* f.sp. *ciceris*.

The present investigation was initiated to determine whether the isolates of FOV can be distinguished by their differential pathogenicity on a set of cotton genotypes, in conjunction with their electrophoretic protein patterns.

MATERIALS AND METHODS

Isolates of FOV:

Isolation, purification, and identification to species level of the isolates used in the present study were carried out at Cotton Pathology Lab., Plant Path. Res. Inst., Agric. Res. Cent., Giza.

Pathogenicity test of FOV isolates on cotton genotypes:

Substrate for growth of each isolate was prepared in 500-ml glass bottles, each bottle contained 50 g of sorghum grains and 40 ml of tap water. Contents of bottles were autoclaved for 30 minutes. Isolate inoculum, taken from one-week old culture on PDA, was aseptically introduced into the bottle and allowed to colonize sorghum for 3 weeks. The present test was carried out by using autoclaved clay loam soil. Batches of soil were infested separately with inoculum of each isoalte at a rate of 0.5 g/kg of soil. Infested soil was dispensed in 10-cm-diameter clay pots and these were planted with seeds of the tested genotypes (10 seeds/pot). In the control treatments, the genotypes were grown in autoclaved soil. Pots were randomly distributed on a greenhouse bench under a temperature regime ranged from 23±3°C to 33 ±2.5°C. Percentgae of infected seedlings, which showed external or internal symptoms (Aly *et al.*, 2000) were recorded 45 days after planting. The experimental design of pathogenicity test was a completely randomized with five replications. Analysis of variance (ANOVA) of the data was performed with the MSTAT-C Statistical Package (A Microcomputer Program for the Design, Management and Analysis of Agronomic Research Experiments, Michigan State Univ., USA). Least significant difference (LSD) was used to compare isolates means.

Extraction of fungal proteins:

Protein extracts from FOV isolates were prepared according to Hussein (1992), in the following way. Fungal isolates were grown for 22 days at 25-30°C on liquid Czapec medium. The mycelium was harvested by filteration through cheescloth, washed with distilled water several times, and freezed-dried. This frozen mycelium was suspended in phosphate buffer pH 8.3 (1-3 ml/g mycelium), mixed thoroughly with glass beads, and ground in liquid nitrogen to a fine powder. The ground mycelium was centrifuged at 19,000 rpm for 30 minutes at 0°C. The protein content in the supernatant was estimated according to Bradford (1976) by using bovine serum albumin 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 the refrigerator for 30 hr. Pellets, collected by centrifugation at 11,000 rpm for 30 minutes, were resuspended in phosphate buffer pH 8.3 and subjected to dialysis for 24 hr against the buffer and centrifugation at 11,000 rpm for 30 minutes. Protein was estimated in the obtained supernatant.

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

Thawed protein-extract supernatant was mixed with an equal volume of a solution containing 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 minutes. Twenty-microliter samples (40 µg of proteins) were subjected to electrophoresis in 15% polyacrylamide prepared in 0.1% SDS. Electrophoresis was conducted at room temperature (approximately 20 to 25°C), for 9 hrs on an 15% polyacrylamide gel with a 6% stacking gel, at 20 and 10 mA, respectively, until the dye reached the bottom of the separating gel. Electrophoresis was performed in a vertical slab mold (16.5 x 14.5 x 0 1 cm). Gels were stained with Coomassie Brilliant Blue R-250 (Laemmli, 1970 and Latorre *et al.*, 1995) or with silver nitrate (Sammons *et al.*, 1981).

Gel analysis:

A gel documentation system (Advanced American Biotechnology 1166 E. Valencia Dr. Unit 6C, Fullerton CA 92631) was used to document the results, to calculate the protein band masses by interpolation from a known standard, and to cluster the protein patterns by the unweighted pair-group method based on arithmetic mean (UPGMA).

RESULTS and DISCUSSION

External symptoms of Fusarium wilt were evident in the moderately susceptible seedlings and in the susceptible seedlings of the tested genotypes 20 days after planting. The susceptible seedlings were usually killed within 25 to 30 days after planting, while the moderately susceptible ones survived showing external wilt symptoms on cotyledons.

A distinctive characteristic of Fusarium wilt is dark brown discoloration of the root and stem xylem. However, there is no consensus of opinions regarding the diagnostic importance of this vascular discoloration for judging susceptibility to Fusarium wilt in a seedling test. For example, Armstrong and Armstrong (1978) stated that vascular discoloration was a questionable standard for judging susceptibility to wilt in a seedling test. Zink *et al.* (1983) found no clear relationship between the severity of external symptoms in surviving muskmelon seedlings and the extent and degree of internal vascular discoloration. On the other hand, Salgado *et al.* (1994) used vascular discoloration as a criterion for judging susceptibility of tepary bean seedlings to Fusarium wilt. Osman (1996) found highly significant correlation between vascular discoloration of cotton seedlings (cultivar Giza 74) and each of wilt incidence ($r = 0.93$, $P < 0.01$) and wilt severity ($r = 0.98$, $P \leq 0.01$) In the present study, we used rigorous criteria for disease rating. According to these criteria, the seedlings were considered moderately susceptible if they showed internal discoloration even though they were free of any external symptoms. Thus, the seedlings were considered healthy only if they were completely free of any internal and external symptoms.

Eight isolates of FOV were tested for levels of pathogenicity on 45-day-old greenhouse-grown seedlings of six cotton genotypes.

Isolates of FOV differed significantly (P = 0.0000) in their pathogenicity on cotton genotypes. Similarly, differences among cotton genotypes were very highly significant (P = 0.0000) when they were very tested against FOV isolates. Isolate x genotype interaction was a very highly significant (P = 0.0094) suggesting that isolates responded differently to different genotypes (Table 1).

Table 1: Analysis of variance of the interaction between isolates of *Fusarium oxysporum* f.sp. *vasinfectum* and cotton genotypes under greenhouse conditions.

Source of variation[a]	D.F.	M.S.	F. value	P > F
Block	3	500.978	1.5145	0.2129
Isolate (S)	8	2265.113	6.8474	0.0000
Genotype (G)	5	4537.592	13.7171	0.0000
S x G	40	572.503	1.7307	0.0094
Error	159	330.797		

[a] Replication is random, while each of isolate and genotype is fixed.

Due to the significance of isolate x genotype interaction, a least significant difference (LSD) was used to compare between the individual isolate means within genotypes, based on these comparisons, it was easy to differentiate between some of FOV isolates by their differential pathogenicity on some of the genotypes. For example, S1 was easily distinguished from S3 on the genotype 19-99. On this genotype S1 was significantly more pathogenic than S3. Isolates S9 and S13 were differentiated from each other on the genotype 417/98. Susceptibility of this genotype to S9 was significantly higher than its susceptibility to S13. Isolates S8 and S13 were easily distinguishable on the genotype 545/98. Isolate S1 was pathogenic on the genotype 45-99, while S9 was nonpathogenic on this genotype. On the other hand, some isolates like S3 and S5 were indistinguishable because they showed non-significant differences on any of the genotypes. Similarly S11 and S13 were indistinguishable on nay genotype (Table 2).

Electrophoretic banding patterns of Coomassie Brilliant Blue R-250 (CBB) are shown in Fig 1 and Table 3, while those stained with silver nitrate (SN) are shown in Fig 2 and Table 4. Thirty three bands were detected with CBB. Of these bands, only one (3.03%) was common to all the isolates. The number of detected bands decreased to thirty one on staining with SN. None of these bands was common to all the isolates. The isolates showed variable decreases or increases in the number of bands when SN was used to visualize the protein patterns. Thus, protein profiles of S1, S8 and S9 showed negligible increases in the number of bands by 8.3, 10, and 9.1%, respectively. On the other hand, the bands of S3, S4, S5 and S13 decreased by 16.7, 10, 16.7 and 45.5%, respectively. Isolate S11 was the only isolate, which maintained the same number of bands whether the gel was stained with CBB or SN.

Table 2: Wilt incidence[a] on cotton genotypes inoculated with isolates of *Fusarium oxysporum* f.sp. *vasinfectum* under greenhouse conditions.

Genotype	Isolate									Genotype mean
	S1	S3	S4	S5	S8	S9	S11	S13	Control[b]	
Giza 74	0.00[c]	8.33	5.00	25.00	6.25	3.13	20.83	0.00	0.00	7.62
Giza 75 x Sea Island – 417/98	15.03	22.32	12.95	12.50	12.50	40.71	12.50	11.91	0.00	15.60
Giza 75 x Sea Island – 476/98	21.91	8.33	9.82	20.83	3.13	7.29	8.33	0.00	0.00	8.85
Giza 67 x Pima 7 – 545/98	18.75	20.54	29.17	29.20	35.12	10.00	14.58	0.00	0.00	17.48
Giza 80 x Australian genotype – 19/99	78.13	45.64	34.92	64.73	14.29	53.57	32.07	25.00	0.00	38.71
Giza 83 x Australian genotype – 45/99	44.35	36.11	15.63	19.84	8.33	0.00	10.42	3.13	0.00	15.31
Isolate mean	29.70	23.55	17.92	28.68	13.27	19.12	16.46	6.67	0.00	

[a] Wilt incidence is the percentage of the infected seedlings, which showed external and internal symptoms or only internal symptoms.
[b] The designated genotype was grown in autoclaved soil.
[c] Mean of four replicates.
LSD for isolate x genotype interaction = 26.40 ($P < 0.05$) or 33.53 ($P \leq 0.01$).

Any isolate was distinguished from the other isolates by isolate specific bands. The number of these specific bands ranged from 1-3 on staining with CBB or from 1-4 on staining with SN (Table 5). SN is a highly sensitive visualization technique for detection of the small amounts of proteins, which cannot be seen with CBB. Generally, SN is 100-fold more sensitive than CBB (Andrews, 1986). At this point, the question that may arise is why SN did not show the expected staining efficiency in case of S3, S4, S5 and S13. Ionic amino acid side chains may play an important role in the silver staining of proteins, so it seems likely that proteins of S3, S4, S5 and S13 had a low content of these and were therefore poorly stained. CBB was more efficient in staining such isolates by virtue of the sulphonic acid groupings on the dye molecules (Andrews, 1986).

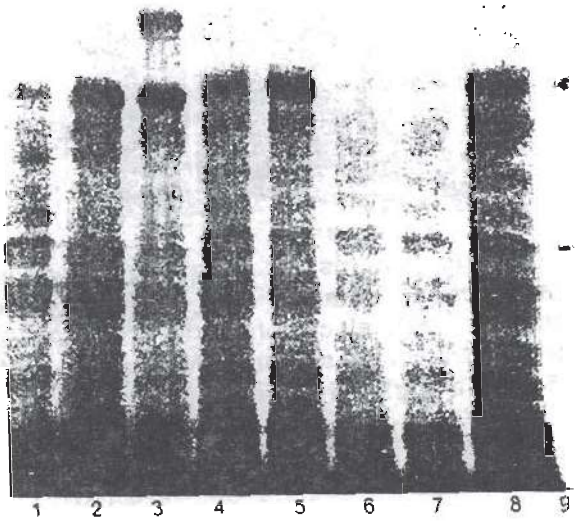

Fig. 1: **Protein banding patterns for isolates of *Fusarium oxysporum* f.sp. *vasinfectum* obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and stained with Coomassie Brilliant Blue R-250. Lanes 1 through 9 were S13, S11, S9, S8, S5, S4, S3, S1 and a marker, respectively.**

A problem with proteins as biochemical markers for typing or classification of fungi is the vast number, which can be generated. 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 other fungi have proven useful (Manicom *et al.*, 1990).

Fig. 3 showed the phenogram constructed based on the similarity levels (SLs) generated from the cluster analysis of electrophoretic banding patterns of CBB shown in Table 3. The greater the SL, the more closely the isolates were. Similarly, the phenogram of Fig. 4 was constructed based on the SLs generated from the cluster analysis of electrophoretic banding patterns of SN shown in Table 4.

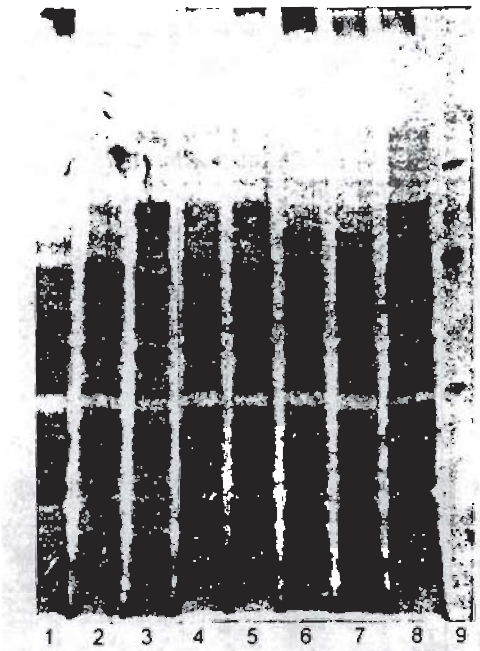

Fig. 2: Protein banding patterns for isolates of *Fusarium oxysporum* f.sp. *vasinfectum* obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and stained with silver nitrate. Lanes 1 through 9 were S13, S11, S9, S8, S5, S4, S3, S1 and a marker, respectively.

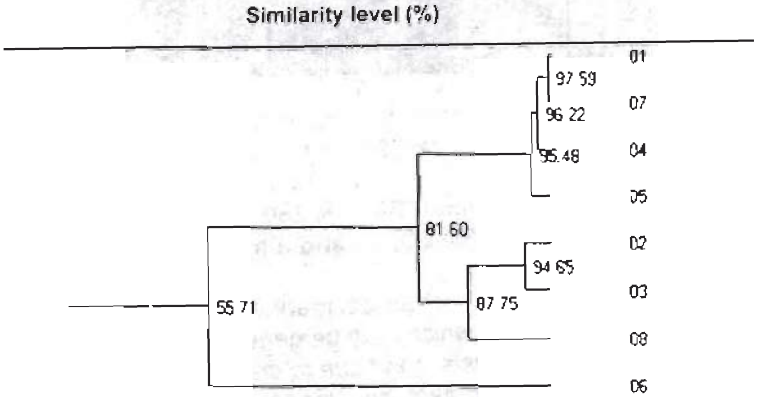

Fig. 3: Phenogram based on cluster analysis of electrophoretic banding patterns of isolates of *Fusarium oxysporum* f.sp. *vasinfectum* obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and stained with Coomassie Brillian Blue R-25. The isolates were S1 (01), S3 (02), S4 (03), S5 (04), S8 (05), s9 (06), s11 (07), AND s13 (08).

Certain details in Figs. 3 and 4 are worthy of mention. The overall SL among the isolates in case of staining with CBB was 55.71%; however, it was reduced to 50.09% when the gel was stained with SN. The SL among the tested isolates except S9 was 80.60% on staining with CBB and was reduced to 70.11% on staining with SN. The SL among S3, S4 and S13 was 87.75% and 75.98% when the gel was stained with CBB and SN, respectively. Taken together, these results indicate that SN was more sensitive than SBB in detecting the differences in protein profiles among isolates of FOV. Isolate S9 was placed in a separate subcluster whether the gel was stained with CBB or SN, which indicates that this isolate had a unique protein profile remotely related to those of the other isolates.

Table 3: Protein banding patterns for isolates of *Fusarium oxysporum* f.sp. *vasinfectum* obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and stained with Coomassie Brilliant Blue R-250.

Band		Isolate							
No.	MM[a]	S1	S3	S4	S5	S8	S9	S11	S13
1	19	+	+	+	+	+	+	+	+
2	21	-	+	+	+	+	+	+	-
3	23	-	+	-	-	-	-	-	-
4	26	+	-	-	+	+	-	+	-
5	27	-	+	+	-	-	+	-	+
6	30	+	+	-	+	+	+	+	-
7	31	-	-	+	-	-	-	-	+
8	32	+	-	-	-	-	-	-	-
9	33	-	+	-	+	-	-	+	+
10	34	-	-	+	-	-	-	-	-
11	35	-	-	-	-	-	+	-	-
12	36	+	+	-	-	-	-	-	+
13	37	-	-	-	-	+	-	-	-
14	38	-	-	-	+	-	-	-	-
15	40	-	-	+	-	-	-	-	-
16	42	+	+	-	-	-	+	-	+
17	44	-	-	-	+	+	-	-	+
18	46	+	-	-	-	-	+	-	-
19	47	-	+	+	+	+	-	+	+
20	53	-	-	-	-	-	-	-	+
21	54	+	+	+	-	-	-	+	-
22	55	-	-	-	+	+	-	-	-
23	58	-	-	-	-	-	+	-	-
24	59	+	+	-	-	+	-	+	-
25	60	-	-	-	+	-	-	-	-
26	64	+	-	-	-	-	+	+	-
27	65	-	-	-	+	-	-	-	+
28	66	-	-	+	-	-	-	-	-
29	68	+	-	-	-	-	+	-	-
30	69	-	+	-	-	+	-	-	-
31	70	-	-	-	+	-	-	-	-
32	71	-	-	-	-	-	-	-	+
33	73	+	-	+	-	-	+	+	-

[a] Molecular mass in kDa.
+ The designated band is present.
- The designated band is absent.

There were isolate specific bands by which isolates, particularly those belonging to different subclusters, could be identified. For example, there were only 7 differential bands (nos. 4, 8, 10, 11, 16, 44 and 45) between S3 and S4 which belonged to the same subcluster (Fig. 4); however, the differential bands between S1 and S3 increased to 13 (nos. 1, 2, 3, 4, 10, 11, 14, 15, 22, 23, 25, 26 and 29) because they belonged to different sub-clusters (Fig. 4). Some isolates, which were indistinguishable by pathogenicity test, were easily distinguished by their specific bands after staining with SN. For example, bands nos. 3, 4, 9, 11, 16, 22, 23 and 28 differentiated S3 from S5 (Fig. 4) although the two isolates were indistinguishable in the pathogenicity test (Table 2).

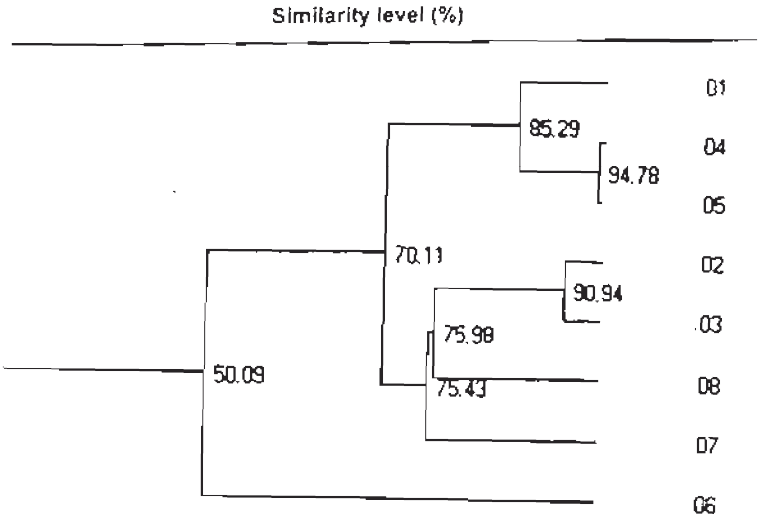

Fig. 4: Phenogram based on cluster analysis of electrophoretic banding patterns for isolates of *Fusarium oxysporum* f.sp. *vasinfectum* obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and stained with silver nitrate. The isolates were S1 (01), S3 (02), S4 (03), S5 (04), S8 (05), s9 (06), S11 (07), AND s13 (08).

Thus, it seems reasonable to conclude that the observed isolate specific bands may be useful as biochemical markers in ecological studies in soil where "marked" isolates are needed. The differential pathogenicity of FOV isolates, combined with their specific protein bands after staining with SN would facilitate their identification after reisolation from soil.

Table 4: Protein banding patterns for isolates of *Fusarium oxysporum* f.sp. *vasinfectum* obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and stained with silver nitrate.

Band		Isolate							
No.	MM[a]	S1	S3	S4	S5	S8	S9	S11	S13
1	12	-	+	+	+	-	-	-	-
2	13	+	-	-	-	-	-	-	-
3	14	-	+	+	-	-	+	+	+
4	15	+	-	+	+	+	-	-	-
5	16	-	-	-	-	-	+	-	+
6	17	+	+	+	+	+	-	+	-
7	18	-	-	-	-	-	-	+	-
8	19	+	+	-	+	+	+	-	-
9	20	-	-	-	+	+	+	-	-
10	22	+	-	+	-	-	-	+	+
11	23	-	+	-	-	-	+	-	-
12	24	-	-	-	-	-	+	-	-
13	25	+	+	+	+	+	-	+	+
14	29	-	+	+	+	-	+	+	-
15	30	+	-	-	-	+	-	-	-
16	33	+	+	-	-	-	+	-	-
17	36	-	-	-	-	-	-	+	+
18	37	+	+	+	+	+	+	-	-
19	40	-	-	-	-	-	-	-	+
20	41	-	-	-	-	-	-	+	-
21	42	-	-	-	-	-	+	-	-
22	43	+	-	-	+	+	-	-	-
23	44	-	+	-	-	-	-	-	-
24	45	-	-	+	-	-	-	-	-
25	48	+	-	-	-	+	-	-	-
26	54	+	-	-	-	-	+	-	-
27	55	-	-	-	-	-	-	+	-
28	56	-	-	-	+	+	-	-	-
29	66	+	-	-	-	-	+	-	-
30	67	-	-	-	-	+	-	-	-
31	69	-	-	-	-	-	-	+	-

[a] Molecular mass in kDa.
\+ The designated band is present.
\- The designated band is absent.

Table 5:Isolates specicif band of *Fusarium oxysporum* f.sp. *vasInfectum* detected after staining gels with Coomassie Brilliant Blue R-250 (CBB) or with Silver nitrate (SN).

Isolate No.	CBB		SN	
	No.[a]	MM[b]	No.	MM
S1	8	32	2	13
S3	3	23	23	44
S4	10	34	24	45
	15	40	...	..
	28	66	...	.
S5[c]	14	38	...	...
	25	60	...	...
	31	70	...	...
S8	13	37	30	67
S9	11	35	12	24
	23	58	21	42
S11[d]	...	...	7	18
	...	...	20	41
	...	...	27	55
	...	...	31	69
S13	20	53	19	40
	32	71	...	...

[a] No. of the specific band.
[b] Molecular mass of the specific band in kDa.
[c] No specific bands were detected after staining with SN.
[d] No specific bands were detected after staining with CBB.

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استعمال القدرة المرضية وأنماط البروتين الناتجة من التفريد الكهربى للتفرقة بين عزلات فطر فيوزاريوم أوكسيسبورم طراز متخصص فازينفكتم الممرضة للقطن

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قيمت ثماني عزلات من فطر فيوزاريوم أوكسيسبورم من الطراز المتخصص فى إصابة القطن بالذبول، وذلك من حيث القدرة على إصابة ستة تراكيب وراثية من القطن تتباين فيما بينها من حيث القابلية للإصابة بالمرض، وذلك تحت ظروف الصوبة. قيمت القدرة المرضية للعزلات على اساس تقدير النسبة المئوية للبادرات المصابة بالذبول عندما كان عمر البادرات ٤٥ يوما. تفاعل العزلات × التراكيب الوراثية كان مصدرا عالى المعنوية للتباين فى حدوث المرض، مما يدل على أن عزلات الفطر أظهرت استجابات مختلفة للتراكيب الوراثية. نظرا لمعنوية هذا التفاعل فإن أقل فرق معنوى استعمل للتفرقة بين متوسطات العزلات داخل التراكيب الوراثية. اظهرت هذه المقارنات أنه من السهولة بمكان التفرقة بين بعض العزلات نظرا للإختلافات المعنوية فيما بينها من حيث القدرة على إصابة التراكيب الوراثية. فى حين لم يمكن التفرقة بين بعض العزلات التى لم تظهر مثل هذه الفروق المعنوية. اجريت دراسة مقارنة لأنواع البروتينات المستخلصة من العزلات وذلك باستعمال تقنية التفريد الكهربى للبروتين المفكك باستعمال مادة صوديوم دوديسيل سلفيت. استعملت مادة كوماسى بريليانت بلو أو مادة نترات الفضة لإظهار أنماط البروتين المتحصل عليها. استعمل اسلوب التحليل العنقودى لتصنيف هذه العزلات إلى مجموعات بناء على ما بينها من تماثل فى أنماط البروتين، وتم التعبير عن النتائج فى فينوجرام لكل طريقة من طريقتى الصبغ. أظهرت الدراسة أن درجات التماثل بين أنماط البروتين للعزلات كانت اقل عند استعمال نترات الفضة لصبغ البروتينات، مقارنة بدرجات التماثل التى أمكن الحصول عليها عند استعمال الكوماسى بريليانت بلو، مما يدل على أن نترات الفضة هى الأكثر حساسية للكشف عن الفروق فى أنماط البروتين بين العزلات. أمكن التفرقة بين العزلات – خصوصا تلك التابعة لمجاميع مختلفة، باستعمال بروتينات مميزة للعزلات. كما أن هذه البروتينات نجحت فى التميز بين بعض العزلات التى لم يمكن تمييزها فى اختبار القدرة المرضية. تدل نتائج الدراسة الحالية على انه من الممكن تعريف عزلات فطر فيوزاريوم أوكسيسبورم المتخصص فى إصابة القطن بالذبول بتقييم الإختلافات فى القدرة المرضية بين العزلات مع استخدام تقنية التفريد الكهربى ثم الصبغ بنترات الفضة لإظهار البروتينات المميزة لهذه العزلات.