J. Agric. Sci. Mansoura Univ., 31 (4): 2027 - 2039, 2006

USE OF PEROXIDASE ISOZYMES TO QUANTIFY VIRULENCE AND AGGRESSIVENESS OF ISOLATES OF *Fusarium oxysporum* f.sp. *vasinfectum* PATHOGENIC ON COTTON

Aly,A. A.*;E. M. Hussein*; M. T. M. Mansour* and M. A. Tag El- Din**

*** Plant Pathology Research Institute, Agric. Res. Center, Giza, Egypt.**

****Dept. of Biochemistry,Faculty of Agric.,Ain Shams Univ.,Cairo, Egypt.**

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. Isolates differed significantly (p=0.0000) in their pathogenicity on the genotypes. Similarly, differences among genotypes were very highly significant (p=0.0000) when they were tested against the isolates. Isolate x genotype interaction was a very highly significant (p=0.0094) source of variation in wilt incidence suggesting that isolates responded differently to the different genotypes. These results imply that the pathogenicity of the tested isolates is a mixture of both aggressiveness and virulence and there are significant differences among isolates in both types of pathogenicity. Similarly, resistance of the tested genotypes is also a mixture of both horizontal and vertical resistance, and the genotypes significantly differ in both types of resistance. Assessment of the relative contribution of each of these factors in the explained (model) variation in wilt incidence revealed that isolate aggressiveness accounted for 27.79% of the explained variation, horizontal resistance of the genotypes accounted for 34.79%, and virulence of the isolates or vertical resistance of the genotypes accounted for 35.12%. Peroxidase isozymes from mycelium and conidia of the isolates were separated by polyacrylamide gel electrophoresis (PAGE), and the obtained banding patterns were visualized by using a specific staining system. Data for virulence or aggressiveness (dependant variables) and amounts of isozymes (independent variables or predictors) were entered into a computerized stepwise multiple regression analysis. Using the predictors supplied by stepwise regression, five regression models were constructed to predict virulence of isolates on genotypes. Three of the generated models proved effective in predicting isolate virulence on the genotypes 417/98, 476/98, and 545/98. Coefficient of determination (R^2) values of the models were 88.19, 83.01, and 67.76%, respectively. It is noteworthy that the one-variable model of aggressiveness showed the lowest R^2 value (44.25%), which may indicate that peroxidase isozymes are of limited value as biochemical markers to predict aggressiveness of FOV isolates. The results of the present study suggest that peroxidase isozymes of isolates may provide a supplementary assay to greenhouse tests to quantify virulence of FOV isolates.

INTRODUCTION

Fusarium wilt (*Fusarium oxysporum.* Schelecht f.sp. *vasinfectum*, (Atk.) Snyd. and Hans.) of cotton (*Gossypium* spp.) was first described by Atkinson (1892) in the USA. The earliest 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) caused serious losses in the commercial Egyptian cottons (*G. barbadense* L.) in the late fifties. Since

then, 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 probability that new races other than race 3 or new biotypes of race 3 will arise to confound cotton breeders.

Quantification of virulence and aggressiveness of FOV isolates is important for improving our understanding of the ecology of these isolates and the epidemiology of the disease. The conventional method for quantification of virulence and aggressiveness of isolates belonging to different formae speciales of *F. oxysporum* (FO) is the observation of the differences in virulence or aggressiveness when the pathogen isolates interact with a set of host 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).

Therefore, another reliable method either alternative or complementary to that based on the differential interaction between FOV isolates and cotton genotypes, is required for quantification of virulence and aggressiveness of FOV isolates.

Isozymes (Isoenzymes) are defined as genetically determined multiple molecular forms of an enzyme. There are three main causes of formation of multiple molecular forms of enzymes. These are (1) the presence of more than one gene locus coding for the enzyme. (2) the presence of more than one allele at a single gene locus coding for the enzyme, and (3) the post translation modifications of the formed enzymatic polypeptide resulting in formation of nongenetic or so-called "secondary" isozymes. The term isozymes is usually used to denote multiple molecular forms deriving from different genetic loci, where the term "allozymes" is used to denote multiple molecular forms deriving from different alleles of the sane genetic locus. The term "allelic isozymes" is also used by isozymologists (Manchenko, 1994).

Electrophoresis of isozymes has been widely used for studying phylogenetic relationships of FO isolates (Aly, 1988; Katan *et al.*; 1991,Granada *et al.*, 1997; Skovgaard and Rosendahl, 1998; Paavanen-Huhtala *et al.*, 1999; and Abd El-Salam, 2004). However, as far as we know, no attempts have been made to quantify virulence and aggressiveness of FO isolates by using electrophoresis of isozymes. In the present study, peroxidase isozymes, separated by polyacrylamide gel electrophoresis (PAGE), were employed for quantification of virulence and aggressiveness of FOV isolates.

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 50g of sorghum grains and 40ml 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 isolate at a rate of 0.5g/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. Percentage of infected seedlings, which showed external or internal symptoms (Aly *et al.*, 2000) were recorded 45 days after planting.

Extraction of fungal proteins:

Protein extracted 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 mycylium was harvested by filtration through cheesecloth, 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, 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 clarification supernatant by adding ammonium sulfate at 70% of saturation (60g/100ml) then kept in the refrigerator for 30hr. 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.

PAGE of native protein:

Thawed protein-extract supernatant was mixed with an equal volume of a solution containing 20% glycerol (vol/vol) and 0.1% bromophenol blue (vol/vol) in 0.15 M tris-HCL, pH 6.8. Twenty microliters of the resulting suspension (40 to 60 µg of protein) was subjected to electrophoresis in 25 mM Tris buffer containing 192 mM glycin at pH 8.3. Electrophoresis was conducted at room temperature (approximately 20 to 25°C) for 9 hr on a 15% polyacrylamide gel with a 6% stacking gel, at 20 and 10 mA, respectively, until the dye reached the bottom of the separating gel (Laemmli, 1970 and Latorre *et al.*, 1995). Electrophoresis was preformed in

a vertical slab mold (16.5 x 14.5 x 0.1 cm). Gels were stained according to Manchenko (1994) for the detection peroxidase (EC 1.11.1.7) isozymes.

Statistical analysis:

The experimental design of the pathogenicity test was a randomized complete block with five replicates (blocks). Analysis of variance (ANOVA) of the data was performed with 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 compare isolate means.

Gels were scanned for band R_F (position) and amount (%) by the documentation system AAB (Advanced American Biotechnology 1166). Linear correlation coefficient was calculated to evaluated the degree of association between virulence and aggressiveness and the amount of each isozyme. Stepwise regression technique with the greatest increase in R^2 as the decision criterion was used to describe the effects of isozymes on virulence and aggressiveness. Correlation and regression analyses were preformed with a computerized program.

RESULTS AND DISCUSSION

External symptoms of Fusarium wilt were evident in the susceptible seedlings of the tested genotypes 20 days after planting. These seedlings were usually killed within 25 to 30 days after planting or they might survive showing external wilt symptoms on cotyledons. The symptoms were discrete areas of vein discoloration in the cotyledonary leaves, usually began at the margin, turn yellow or brown, eventually, the entire leaf wilted.

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 positive correlation between vascular discoloration of cotton seedlings (cultivar Giza 74) and each of wilt incidence ($r = 0.93$, $p < 0.01$) and wilt severity ($r = 0.98$, $p < 0.01$). In the present study, we used rigorous criteria for disease rating. According these criteria, the seedlings were considered healthy only if they were completely free of any internal and external symptoms. Thus, the seedlings were considered susceptible if they showed internal discoloration even though they were free of any external symptoms. Isolates of FOV differed significantly ($p = 0.0000$) in their pathogenicity on cotton genotypes. Similarity, differences among cotton genotypes were very highly significant ($p = 0.0000$) when they were tested against FOV isolates. Isolate x genotype interaction was a very highly significant ($p = 0.0094$) suggesting that isolates responded differently to the 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 rando.n, while each of isolate and genotype is fixed.

According to Vanderplank (1984) these results imply that the pathogenicity of the tested isolates is a mixture of both aggressiveness and virulence and there are significant differences among isolates in both types of pathogenicity. Similarly, resistance of the tested genotypes is also a mixture of both horizontal and vertical resistance, and the genotypes significantly differ in both types of resistance.

Assessment of the relative contribution of each of these factors in the explained (model) variation in wilt incidence revealed that isolate aggressiveness accounted for 27.79% of the explained variation, horizontal resistance of genotypes accounted for 34.79%, and virulence of isolates or vertical resistance of the genotypes accounted for 35.12% (Table 2).

Taken together, these results indicate that isolate aggressiveness is the least important contributor in determining the variation in wilt incidence. Aggressiveness of isolates is less important than virulence, while horizontal resistance of genotypes is almost as important as their vertical resistance in determining the variation in wilt incidence.

Table 2. Relative contribution of *Fusarium oxysporum* f.sp. *vasinfectum* isolates, cotton genotypes, and their interaction to variation in wilt incidence.

Source of variation	Relative contribution[a] to variation in wilt incidence
Isolate (S)	27.79
Genotype (G)	34.79
S x G	35.12

[a] Calculated as percentage of sum squares of the explained (model) variation.

Due to the significant interaction between isolates and genotypes, LSD was used to compare between the individuals isolate means within genotypes, these comparisons showed that the interaction between isolates and genotypes was due to change in the magnitude of the differences between isolates. For example, the difference between S_1 and S_9 was nonsignificant on genotype 545/98; however, on genotype 45/99 the difference was highly significant. S_4 and S_5 showed the same virulence on genotype 545/98; however S_5 was significantly more virulent than S_4 on genotype 19/99 (Table 3).

Amino acid sequence of polypeptides (components of isozymes) are dependent on nucleotide sequence of their coding genes; therefore, an analysis of isozymic variation among FOV isolates by PAGE, approximates an analysis their genetic variation (Markert and Faulhaber, 1965).

Table 3. Wilt incidence[a] on cotton genotypes inoculated with isolates of *Fusarium oxysporum* f.sp. *vasinfectum* under greenhouse conditions.

Genotype	Isolate									Genotype
	S1	S 3	S 4	S 5	S 8	S 9	*S 11*	*S13*	*Control* [b]	mean
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 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 = 25.40 ($p < 0.05$) or 33.53 ($p < 0.01$).

Electrophoresis patterns of isoenzymes can be obtained rapidly and the growing conditions have no influence on these patterns (Koberhel and Gautier, 1974).

In the present study, a total of 23 peroxidase isozymes were identified among the 8 isolates that were analyzed (Figs 1, 2, and Table 4). No single isolate was stained for all the 23 isozymes. Similarly no single isozyme was common to all the isolates.

Fig. 1. Peroxidase isozyme patterns obtained by PAGE from 8 isolates of *Fusarium oxysporum* f.sp. *vasinfectum*.

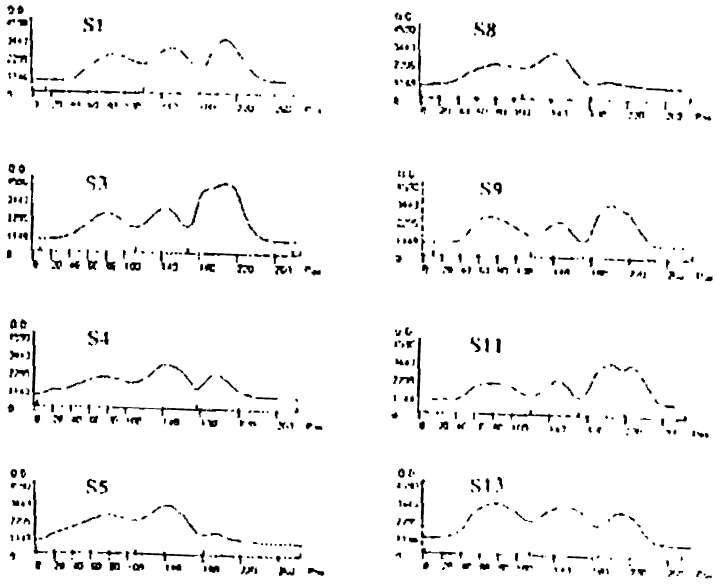

Fig. 2. Densitometer scanning of peroxidase isozyme patterns obtained by PAGE from 8 isolates of *Fusarium oxysporum* f.sp. *vasinfectum*.

Table 4. Peroxidase isozyme patterns for eight isolates of *Fusarium oxysporum* f.sp. *vasinfectum* (FOV) obtained by polyacrylamide gel electrophoresis.

Isozyme		FOV isolate							
No.	Position	*S 1*	S 3	S 4	S 5	S 8	S 9	*S 11*	*S 13*
1	**72**	**0.00**[a]	0.00	0.00	0.00	0.00	36.69	0.00	0.00
2	**77**	0.00	0.00	34.24	0.00	0.00	0.00	32.83	40.76
3	**79**	0.00	0.00	0.00	41.58	0.00	0.00	0.00	0.00
4	**80**	0.00	28.74	0.00	0.00	0.00	0.00	0.00	0.00
5	**83**	0.00	0.00	0.00	0.00	39.40	0.00	0.00	0.00
6	88	33.90	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	**143**	0.00	0.00	0.00	37.27	0.00	0.00	0.00	0.00
8	**145**	0.00	21.49	0.00	0.00	37.63	0.00	0.00	0.00
9	**146**	0.00	0.00	34.76	0.00	0.00	0.00	0.00	0.00
10	**147**	0.00	0.00	0.00	0.00	0.00	20.08	0.00	0.00
11	150	0.00	0.00	0.00	0.00	0.00	0.00	17.64	0.00
12	152	28.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00
13	154	0.00	0.00	0.00	0.00	0.00	0.00	0.00	32.70
14	195	0.00	0.00	0.00	21.14	0.00	0.00	0.00	0.00
15	**197**	0.00	0.00	31.00	0.00	0.00	0.00	0.00	0.00
16	198	0.00	0.00	0.00	0.00	22.97	0.00	0.00	0.00
17	201	0.00	0.00	0.00	0.00	0.00	43.24	0.00	0.00
18	204	0.00	0.00	0.00	0.00	0.00	0.00	26.17	0.00
19	206	0.00	0.00	0.00	0.00	0.00	0.00	0.00	26.54
20	210	37.54	0.00	0.00	0.00	0.00	0.00	0.00	0.00
21	211	0.00	48.63	0.00	0.00	0.00	0.00	0.00	0.00
22	225	0.00	0.00	0.00	0.00	0.00	0.00	23.35	0.00
23	281	0.00	1.13	0.00	0.00	0.00	0.00	0.00	0.00

[a] **Amount (%) of the designated isozyme.**

Since the present study included a limited number of isolates. It is unlikely that these isolates represent the full range of variation within FOV. Despite this limitation, 23 peroxidase isozymes were detected. To allow interpretation of this considerable level of isozymic variation, it is assumed that FOV exhibits a typical. *F. oxysporum* nuclear structure (Puhalla, 1981). This means it is a haploid fungus, and diploids, if they exist, are extremely unstable and transitory. Accordingly, if a given locus, say peroxidase locus mutates frequently, every new mutation is expressed, in contrast to the way in which it can be hidden in a diploid fungus. Each isolate was characterized by a unique set of isozymes. For instance, isozymes no_s. 6, 12, and 20 were unique to S_1. S_3 was characterized by the unique isozymes 4, 21, and 23. Each of S_3 and S_{11} showed a total of 4 isozymes, while each of the other isolates showed a total of 3 isozymes.

Pearson correlation coefficient was calculated to measure the degree of association between virulence of isolates on each genotype and the amount (%) of each separated isozyme (Table 5). Isozymes no_s. 3, 7, and 14 were positively correlated ($p<0.05$) with virulence of isolates on Giza 74, while isozymes no_s. 1, 10, and 17 were positively correlated with virulence of isolates on genotype 417/98. None of the other isozymes was significantly correlated with virulence of isolates on any genotypes.

Table 5: Correlation between virulence (Y_s) of *Fusarium oxysporum* f.sp. *vasinfectum* isolates on six cotton genotypes and content of peroxidase isozymes of these isolates.

No.[a]	Y_1[b]	Y_2	Y_3	Y_4	Y_5	Y_6
1	- 0.234[c]	0.939**	- 0.139	- 0.339	0.198	- 0.444
2	- 0.056	- 0.423	- 0.457	- 0.426	- 0.510	- 0.414
3	0.708*	- 0.205	0.568	0.335	0.375	0.067
4	- 0.010	0.193	- 0.085	0.031	0.045	0.486
5	- 0.100	- 0.205	- 0.356	0.542	- 0.560	- 0.229
6	- 0.369	- 0.102	0.624	- 0.032	0.672	0.699
7	0.708*	- 0.205	0.568	0.335	0.375	0.067
8	- 0.098	- 0.088	- 0.375	0.519	- 0.495	0 045
9	- 0.154	- 0.187	- 0.007	0.333	- 0.161	- 0 041
10	- 0.234	0.939**	- 0.139	- 0.339	0.198	- 0.444
11	0.529	- 0.205	- 0.085	- 0.179	- 0.216	- 0.175
12	- 0.369	- 0.102	0.624	- 0.032	0.672	0.699
13	- 0.369	- 0.229	- 0.520	- 0.690	- 0.353	- 0.363
14	0.708*	- 0.205	0.568	0.335	0.375	0.067
15	- 0.154	- 0.187	- 0.007	0.333	- 0.161	- 0.041
16	- 0.100	- 0.205	- 0.356	0.542	- 0.560	- 0.229
17	- 0.234	0.939**	- 0.139	- 0.339	0.198	- 0.444
18	0.529	- 0.205	- 0.085	- 0.179	- 0.216	- 0.175
19	- 0.369	- 0.229	- 0.520	- 0.690	- 0.353	- 0.363
20	- 0.369	- 0.102	0.624	- 0.032	0.672	0.699
21	- 0.010	0.193	- 0.085	0.031	0.045	0.486
22	0.529	- 0.205	- 0.085	- 0.179	- 0.216	- 0.175
23	- 0.010	0.193	- 0.085	0.031	0.045	0.486

[a] Number of isozyme.

[b] Y_1, Y_2, Y_3, Y_4, Y_5, and Y_6 are virulences of the tested isolates on genotypes Giza 74, 417, 476, 545, 19, and 45, respectively.

[c] Pearson correlation coefficient (r), which measures the degree of association between virulence of the tested isolates and amount of the designated isozyme. Value of r is significant at $p < 0.01$ (**) or $p < 0.05$ (*).

Data for virulence of isolates on each of the tested genotypes and amounts of isozymes were entered into a computerized stepwise multiple regression analysis. The analysis constructed predictive models by adding predictors, in this case, amounts of isozymes, to the models in order of their contribution to R^2. The analysis was effective in eliminating those isozymes with little or no predictive value by incorporating into the model only those isoenzymes that made a satisfactory significant contribution to the R^2 value of the models (Podleckis *et al.*, 1984).

Using the predictors supplied by stepwise regression, 5 models were constructed to predict virulence (Table 6). R^2 values of the models ranged from 45.11 to 88.19%. It is noteworthy that no regression model could be constructed to quantify virulence on genotype 45/99.

None of the isozymes was satisfactory correlated with aggressiveness (Table 7). Amount of isozymes no. 13 accounted for 44.25% of the tota' variation in aggressiveness (Table 8).

Table 6. Stepwise regression models that describe the relationship between virulence (Y_s[a]) of *Fusarium oxysporum* f.sp. *vasinfectum* isolates on six cotton genotypes and content (X_s[b]) of peroxidase isozymes of these isolates.

Genotype	Stepwise linear regression model	Coefficient of determination (R^2)	F. value [c]
Giza 74	$Y_1 = 6.22 + 0.89\ X_{14}$	50.19%	6.04 *
417	$Y_2 = 14.24 + 1.32\ X_{10}$	88.19%	44.80 ***
476	$Y_3 = 6.15 + 0.46\ X_6 + 0.35\ X_3$	83.01% [d]	12.22 *
545	$Y_4 = 20.37 - 0.62\ X_{13} + 0.37\ X_5$	67.76% [e]	5.25 x
19	$Y_5 = 38.32 + 1.06\ X_{20}$	45.11%	4.93 x
45	f	...	...

[a] Dependent variables (virulences of the isolates on the designated genotype).
[b] Identification of the predictors (X_s) is shown in Table 4.
[c] F. value is significant at $p < 0.10$ (x), $p < 0.05$ (*), or $p < 0.005$ (***).
[d] Relative contribution of the predictors X_6 and X_3 to R^2 are 38.96 and 44.06%, respectively.
[e] Relative contribution of the predictors X_{13} and X_5 to R^2 are 47.66 and 20.09%, respectively.
[f] No regression model could be constructed.

Table 7. Correlation between aggressiveness of *Fusarium oxysporum* f.sp. *vasinfectum* isolates on six cotton genotypes and content of peroxidase isozymes of these isolates.

No.[a]	r[b]	No.	r
1	- 0.016	13	- 0.665
2	- 0.658	14	0.483
3	0.483	15	- 0.078
4	0.215	16	- 0.321
5	- 0.321	17	- 0.016
6	0.536	18	- 0.155
7	0.483	19	- 0.665
8	- 0.184	20	0.536
9	- 0.078	21	0.215
10	- 0.016	22	- 0.155
11	- 0.155	23	0.215
12	0.536		

[a] Number of isozym.
[b] Pearson correlation coefficient, which measures the degree of association between aggressiveness and amount (%) of the designated isozyme.

Table 8. Stepwise regression model that describes the relationship between aggressiveness (Y) of *Fusarium oxysporum* f.sp. *vasinfectum* isolates on six cotton genotypes and content (X) of peroxidase isozymes of these isolates.

Stepwise linear regression model	Coefficient of determination (R^2)	F. value [a]
$Y = 21.24 - 0.45\ X_{13}$	44.25%	4.76 x

[a] F. value is significant at $p < 0.10$ (x).

The utility of the electrophoretic data depends on the method of statistical analysis. Multiple regression was logical choice for construction of predictive models, but the complex nature of banding patterns warranted a method to eliminate bands with no predictive values. Stepwise regression is the best variable selection procedure because it eliminates from the model any variable whose contribution to predictive ability is satisfactory insignificant (Draper and Smith, 1981 and Podleckis *et al.*, 1984).

In the present study, satisfactory visualization of peroxidase isozymes was obtained by using a specific staining system, and the stepwise regression models they generated proved effective in predicting isolate virulence on three of the genotypes (417/98, 476/98, and 545/98). Therefore, PAGE of peroxidase isozymes, such as that described herein may provide a supplementary assay to greenhouse tests to quantify virulence of FOV isolates on cotton genotypes.

REFERENCES

Abd El-Salam, K.A. 2004. Biochemical and molecular identification of *Fusarium* spp. Isolated from roots of some Egyptian cottons. Ph.D. Thesis. Suez Canal Univ. Ismailiya. 101p.

Aly, A.A. 1988. Incidence of Fusarium wilt of flax as influenced by biotic and abiotic factors. Ph.D. Thesis. South Dakota State Univ. Brookings, 173p.

Aly, A.A., H.A. Eisa, M.T.M. Mansour, S.M.E. Zayed, and M.R. Omar. 2000 Resistance to Fusarium wilt disease in families of some commercial cotton cultivars. pp. 375-384. In: Proc. 9th Cong. Egypt. Phytopathol. Soc., Giza.

Armstrong, G.M. and Joanne K. Armstrong. 1978. Formae speciales and races of *Fusarium oxysporum* causing wilts of Cucurbitaceae. Phytopathology 68: 19-28.

Armstrong, G.M. and Joanne K. Armstrong. 1981. Formae speciales and races of *Fusarium oxysporum* causing wilt diseases, pp. 391-399. In: Fusarium: Diseases, Biology, and Taxonomy (P.E. Nelson, T.A. Toussoun, and R.J. Cook, eds). Pennsylvania University Press.

Atkinson, G.F. 1892. Some diseases of cotton. Ala. Agric. Exp. Stn. Bull. No. 41, pp. 19-29.

Bhatti, M.A. and J.M. Kraft. 1992. The effects of inoculum density and temperature on root rot and wilt of chickpea. Plant Disease 76: 50-54.

Bradford, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

Draper, N.R. and H. Smith. 1981. "Applied Regression Analysis", 2nd Ed. John Wiley, New York, 709p.

Fahmy, T. 1927. The Fusarium wilt disease of cotton and its control. Phytopathology 17: 749-767.

Granada, E.G., M. Amezquita, O. Arbelaez, G. Torres, E.G. De-Granada, and M.O. De-Amezquite. 1997. *Fusarium oxysporum* f.sp. *dianthi* race differentiation with the aryl esterase electrophoresis technique. Agronomia Colombiana 14: 66-71.

Hussein, E.M. 1992. Biochemical and serological studies for determining susceptibility of cotton cultivars to *Fusarium oxysporum* f.sp. *vasinfectum* (In Russian). Ph.D. Thesis. All-Union Institute of Plant Protection, Leningrad, USSR.

Katan, T., D. Zamir, M. Saraftii, and J. Katan. 1991. Vegetative compatibility groups and subgroups of *Fusarium oxysporum* f.sp. *radicis-lycopersici*. Phytopathology 81: 255-262.

Koberhel, A.S. and A.H. Gautier. 1974. Peroxidase isozymes from horseradish roots. J. Bio. Chem. 242: 2470-2473.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.

Latorre, B.A., G.F. Perez, W.F. Wilcox, and R. Torres. 1995. Comparative protein electrophoretic and isozymic patterns of *Phytophthora cryptogea* isolated from Chilean kiwifruit and North American deciduous fruits. Plant Dis. 79: 703-708.

Manchenko, G.P. 1994. "Handbook of Detection of Enzymes on Electrophoretic Gels". CRC Press, Inc., Boca Raton, Florida. 341p.

Markert, C.L. and I. Faulhaber. 1965. Lactate dehydrogenase isozyme patterns of fish. J. Exp. Zool. 159: 319-332.

Netzer, D., Y. Tai, A. Marani, and C. Weintall. 1985. Resistance of interspecific cotton hybrids (*Gossypium hirsutum* x *G. barbadense* containing *G. harknessii* cytoplasm) to Fusarium wilt. Plant Disease 69: 312-313.

Osman, Eman, A.M. 1996. Studies on the interrelationship among some Fusarium species with special reference to their pathogenicity on cotton. Ph.D. Thesis, Cairo Univ., Cairo. 125p.

Paavanen-Huhtala, S.J. Hyvonen, S.A. Bulat, and T.T. Yli-Mattila. 1999. RAPD-PCR, isozyme, rDNA RFLP, and rDNA sequence analysis in identification of Finnish *Fusarium oxysporum* isolates. Mycological Research 103: 625-634.

Podleckis, E.V., C.R. Crutis, and H.E. Heggestad. 1984. Peroxidase enzyme markers for ozone sensitivity in sweet corn. Phytopathology 74: 572-577.

Puhalla, J.E. 1981. Genetic considerations of the genus Fusarium, 291-305. In: Fusarium: Diseases, Biology, and Taxonomy (P.E. Nelson, T.A. Toussoun and R.J. Cook, eds.). Pennsylvania University Press.

Salgado, M.O., H.F. Schwartz, and M.A. Pastor Corrales. 1994. Resistance to *Fusarium oxysporum* f.sp. *phaseoli* in tepary beans (*Phaseolus acutifolius*). Plant Disease 78: 357-360.

Skovgaard, K. and S. Rosendahl. 1998. Comparison of intra- and extra cellular isozyme banding patterns of *Fusarium oxysporum*. Mycological Research 102: 1077-1084.

Vanderplank, J.E. 1984. "Disease Resistance in Plants", 2nd Ed. Academic Press, Orlando, Florida. 194p.

Watkins, G.M. Ed. 1981. Compendium of cotton diseases. The American Phytopathological Society. St Paul, Minnesota. 87p.

Zink, F.W., W.D. Guber, and R.G. Grogan. 1983. Reaction of muskmelon germplasm to inoculation with *Fusarium oxysporum* f.sp. *melonis* race 2. Plant Disease 67: 1251-1255.

J. Agric. Sci. Mansoura Univ., 31 (4), April, 2006

استعمال مشابهات إنزيم البيرأوكسيديز للتعبير الكمى عن القدرة المرضية المتخصصة والغير متخصصة لعزلات فطر فيوزاريوم أوكسيسبورم طراز متخصص فازينفكتم الممرض للقطن

على عبد الهادى على* ، عزت محمد حسين* ، محمود توفيق محمود منصور* و ممدوح أبو مسلم تاج الدين**

* معهد بحوث أمراض النباتات – مركز البحوث الزراعية – الجيزة – مصر.

**قسم الكمياء الحيوية – كلية الزراعة – جامعة عين شمس – القاهرة – مصر

قيمت ثمانى عزلات من فطر فيوزاريوم أوكسيسبورم من الطراز المتخصص فى اصابة القطن بالذبول، وذلك من حيث القدرة على إصابة ستة تراكيب وراثية من القطن- تتباين فيما بينها من حيث القابلية للإصابة بالمرض- تحت ظروف الصوبة. قيمت القدرة المرضية للعزلات على أساس تقدير النسبة المئوية للبادرات المصابة بالذبول عندما كان عمر البادرات ٤٥ يوما. أظهر تحليل التباين أن التراكيب الوراثية والعزلات وتفاعل التراكيب الوراثية X العزلات كانت كلها مصادر عالية المعنويه للتباين فى حدوث المرض. إن معنوية التراكيب الوراثية والعزلات وتفاعل التراكيب الوراثية X العزلات يدل على الآتى: (١) هناك قدر من التخصص الفسيولوجى بين عزلات الفطر عند إصابتها للتراكيب الوراثية. (٢) إن مقاومة التراكيب الوراثية هى خليط من المقاومة الأفقية والرأسية وأن التراكيب الوراثية تختلف فيما بينها فى نوعى المقاومة.(٣) إن القدرة المرضية لعزلات الفطر هى خليط من القدرة المتخصصة والغير متخصصة وأن عزلات الفطر تختلف فيما بينها فى نوعى القدرة المرضية. إن تقدير حجم التباين المفسر الراجع إلى كل مصدر من مصادر التباين أظهر أن القدرة المرضية الغير متخصصة للعزلات والمقاومة الأفقية للتراكيب الوراثية والقدرة المرضية المتخصصة للعزلات أو المقاومة الرأسية للتراكيب الوراثية كانت مسئولة عن ٢٧,٧٩و ٣٤,٧٩و ٣٥,١٢% من التباين المفسر، على الترتيب. إستعملت تقنية التفريد الكهربى لفصل مشابهات إنزيم البيرأوكسيديز من ميسيليوم وكونيديات العزلات، ثم إستعمل نظام صبغ متخصص لإظهار أنماط المشابهات الإنزيمية المتحصل عليها. أمكن-بستخدام أسلوب الانحدار المتعدد المرحلى- التوصل إلى خمسة نماذج إنحدار لوصف العلاقة بين القدرة المرضية المتخصصة للعزلات (متغير تابع) والنسب المئوية للمشابهات الإنزيمية المفصولة (متغير مستقل)-أظهرت ثلاثة من هذه النماذج كفاءة عالية فى التنبؤ بالقدرة المتخصصة للعزلات على إصابة التراكيب الوراثية ٩٨/٤١٧و ٩٨/٤٧٦ و ٩٨/٥٤٥، فقد كانت قيم معامل التحديد للنماذج الثلاثة هى ٨٨,١٩و ٨٣,٠١و ٦٧,٧٦%، على الترتيب. الجدير بالذكر أن نموذج الإنحدار الخاص بالقدرة المرضية الغير متخصصة أظهر أقل قيمة لمعامل التحديد (٤٤,٢٥%)، مما يدل على أن مشابهات البيرأوكسيديز ذات قيمة محدودة عند استعمالها كمعلمات بيوكيميائية للتنبؤ بهذه الصفة. تدل نتائج الدراسة الحالية على أنه من الممكن استخدام تقنية التفريد الكهربائى لمشابهات البيرأوكسيديز كوسيلة مكملة لإختبارات الصوبة للتعبير الكمى عن القدرة المتخصصة لعزلات فيوزاريوم أوكسيسبورم طراز متخصص فازينفكتم على إصابة التراكيب الوراثية للقطن.