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USE OF PROTEIN ELECTROPHORESIS TO QUANTIFY RESISTANCE OF COTTON TO FUSARIUM WILT DISEASE

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ABSTRACT

Six experimental crosses and 6 commercial cultivars of Egyptian cottons (*Gossypium barbadense* L.) were evaluated for Fusarium-wilt resistance under greenhouse conditions in 2007 growing season. The experimental crosses showed varying levels of susceptibility to Fusarium wilt disease, while the commercial cultivars were highly resistant. Seed proteins of the genotypes (crosses and cultivars) were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and the obtained banding patterns were visualized by using the Coomassie Brilliant Blue staining system. Data for Fusarium wilt ratings and amounts of protein fractions were entered into a computerized stepwise multiple regression. Using the predictors supplied by stepwise regression, a five-factor model was constructed to predict wilt incidence. This model showed that wilt incidence differences were due to the protein fractions no_s. 4, 30, 32, 7 and 55, which accounted for 99.98% of the total variation in wilt incidence ratings. This result indicates that SDS-PAGE of seed proteins may provide a supplementary assay to greenhouse tests to distinguish quantitatively between Fusarium-wilt-resistant or susceptible genotypes.

INTRODUCTION

Fusarium wilt (*Fusarium oxysporum*. Schelecht f.sp. *vasinfectum*, (Atk.) Snyd. and Hans.) of cotton (*Gossypium* spp.) has long been known in the Nile Valley, where it caused serious losses in the commercial Egyptian cottons (*G. barbadense* L.) in the late fifties (Bakry *et al.*, 1958). Since then, an extensive cotton-breeding program was initiated to develop cultivars resistant to the disease. In this program, cotton genotypes are screened under greenhouse conditions, in soil infested with the wilt fungus.

Currently, screening of breeding materials under greenhouse conditions is the only reliable method to distinguish the Fusarium-wilt highly resistant genotypes. The test is time consuming, and may be influenced by variability inherent in the experimental system (A.A. Aly, *personal observations*). The first symptoms of the disease appear on susceptible genotypes after 20 days from planting date under very favorable environmental conditions and may require a longer period of time under less favorable conditions.

Therefore, another reliable method, either alternative or complementary to the greenhouse tests, is required for identification of the Fusarium-wilt highly resistant genotypes.

The use of gel electrophoresis to analyze plant protein and hence distinguish between and identify cultivars of crop species is a firmly established technique (Cook, 1988). Proteins are primary products of gene expression and reflect gene system specificity in the best manner. Therefore,

they are used as very effective markers for genotype identification and evaluation of the species and cultivar constitution (Konarev, 1988).

Some attempts were made to differentiate among flax cultivars by using protein electrophoresis. For instance, Kasymova *et al.* (1988) determined the optimum conditions for polyacrylamide gel electrophoresis of seed storage proteins in cotton. They also presented data on the component composition of the albumins, globulins, and glutelins for 3 *G. hirsutum* and 5 *G. barbadense* varieties as well as *G. arboreum* and *G. herbaceum*. Differences in the number and intensity of albumin and globulin bands were sufficient to differentiate species, while differences in glutelin bands were sufficient for variety classifications.

Electrophoretic analysis of the total protein and the globulin fraction in a collection of *G. hirsutum* varieties revealed that some banding patterns were variety specific, while others were common to several varieties (Shadmanov *et al.*, 1989).

Wang *et al.* (1990) evaluated the range of genetic variation in 73 accessions of *G. herbaceum* by electrophoretic analysis of water-soluble seed proteins. The accessions were divided into 18 groups according to the pattern of their electrophoretic bands. Cultivars or lines of the same group were generally distributed in the same geographical region or in neighbouring countries. It was suggested that groups including fewer cultivars or lines were more primitive in evolution. Results also suggested that electrophoresis of soluble seed proteins can be used in identifying inheritance and variation of a plant species and in classification.

On the basis of an electrophoretic analysis of the proteins in the seeds of 74 *G. hirsutum* and 30 *G. barbadense* varieties, Yunuskhanov *et al.* (1992) found 4 protein markers (A, B, C, and/or D, with mobilities of 0.44, 0.50, 0.55, and 0.60, respectively, or in different experimental conditions 0.35, 0.40, 0.45, and 0.52). *G. barbadense* varieties did not differ in this respect and all carried markers A and C. The *G. hirsutum* ones fell into several groups, the largest group having B, C, and D, with A, B, C, and D being somewhat rarer.

Zhang *et al.* (1998) reported that electrophoretic bands of water-soluble proteins were plentiful, clear, polymorphic, and could be used for cultivar identification, and the study of cotton germplasm resources.

In the present study, sodium dodycyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of proteins was employed to develop a regression model to quantify Fusarium-wilt resistance in selected cotton genotypes. To the best of our knowledge, this approach has not been employed previously in the quantification of cotton resistance to Fusarium wilt.

MATERIALS AND METHODS

Evaluation of cotton genotypes for Fusarium-wilt resistance or susceptibility:

The genotypes evaluated in this test included 6 experimental crosses and 6 commercial cultivars (Table 1). All the genotypes were submitted by Cotton Breeding Section, Cotton Research Institute. The inoculum used in

this test was a mixture of equal parts (w/w) of 50 isolates of *F. oxysporum* f.sp. *vasinfectum* (FOV) race 3. These isolates were obtained from the fungal collection of Cotton Pathology Lab., Plant Path. Res. Inst., Agric. Res. Cent., Giza. Autoclaved clay loam soil was infested with the mixture of the isolates at a rate of 10 g/kg of soil. 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 the bottle 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. Infested soil was dispended in 10-cm-diameter clay pots and these were planted with 10 seeds per pot. There were 5 replications (pots) for each genotype.

Pots were distributed on a greenhouse bench in a randomized complete block design of 5 replications. The greenhouse was equipped with a heating system assuring that the minimum temperature in the greenhouse was maintained at 28°C; however, due to the lack of a cooling system, the maximum temperature was out of control fluctuating from 30 to 35°C depending on the prevailing temperature during the day (the test was conducted on January and February, 2007). Percentage of wilted seedlings, were recorded 40 days from planting date. The wilted seedlings included the dead seedlings and the surviving seedlings, which showed external symptoms. These symptoms usually began at the margin of cotyledon as yellowing along the veins (vein-clearing), eventually, the entire cotyledon turned yellow and dropped from the seedlings.

Extraction of proteins from cootonseeds:

Protein extract was prepared according to Hussein (1992) in the following way: Seeds of the genotypes were slightly ground and defatted by diethyl ether or chloroform for 4 to 5 days. After drying at room temperature, ground seeds were suspended in a solution (1-3 ml/g seeds) consisting of 12.5 g glucose and 1 g ascorbic acid dissolved in 100 ml phosphate buffer pH 8.3 and ground in a liquid nitrogen to a fine powder. After thawing, the powder suspended in the buffer, was centrifuged at 19,000 rpm for 30 minutes at 0°C. The protein content in the supernatant was adjusted to a concentration of 3 to 4 mg/ml according to Bradford (1976) spectrophotometric method by using bovine serum albumin as a standard protein.

Electrophoresis of dissociated protein (SDS-PAGE):

For electrophoresis of dissociated protein, each supernatant was mixed with an equal volume of a solution containing of (by volume) 64% buffer (0.15 M tris-HCL, pH 6.8); 20% glycerol; 6% 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 15% polyacrylamide gel with 6% stacking gel, at 20 and 10 mA, respectively, until the dye band reached the bottom of the separating gel. Electrophoresis was preformed 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).

Table 1. Reaction of 12 cotton genotypes to artificial infection by *Fusarium oxysporum* f.sp. *vasinfectum* (race 3) under greenhouse conditions in 2007.

No.	Genotype	Family	Wilt incidence [a] (%) [b]	
1	Cross Giza 80 x Australian genotype	27/99	90.0 [c]	A
2	Cross Giza 83 x Australian genotype	51/99	84.8	AB
3	Cross Giza 80 x Australian genotype	19/99	32.6	C
4	Cross Giza 83 x Australian genotype	47/99	75.3	B
5	Cross Giza 85 x Australian genotype	501/99	24.4	C
6	Cross Dendera x Australian genotype	64/99	81.4	AB
7	Giza 80	-------	0.0	D
8	Giza 83	-------	0.0	D
9	Giza 85	-------	0.0	D
10	Giza 86	-------	0.0	D
11	Giza 88	-------	0.0	D
12	Giza 89	-------	0.0	D

[a] Wilt incidence was calculated according to the following formula:
[infected seedlings/emerging seedlings] x 100. Infected seedlings included the dead seedlings and the surviving seedlings, which showed external symptoms.
[b] Percentage data were transformed into arc sine angles before carrying out the analysis of variance to produce approximately constant variance.
[c] Means followed by the same letter(s) are not significantly different ($p<0.05$) according to Duncan's multiple range test. Each value is the mean of five replicates (pots).

Statistical analysis of the data:

The experimental design of the greenhouse experiment was a randomized complete block with five replications. Analysis of variance (ANOVA) of the data was performed with the MSTAT-C statistical package. Duncan's multiple range test was used to compare genotype means. Percentage data were transformed into arc sine angles before carrying out the ANOVA to produce approximately constant variance.

Gels were scanned for band R_F (position) and amount (%) by the gel documentation system AAB (Advanced American Biotechnology 1166). Stepwise regression technique with the greatest increase in R^2 as the decision criterion was used to describe the effects of proteins (predictors or independent variables) on wilt incidence (dependent variable). Correlation and regression analyses were preformed with a computerized program.

RESULTS AND DISCUSSION

External symptoms of Fusarium wilt were evident in the susceptible seedlings 20 days after planting. The highly 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 an olive brown discoloration of the root and stem xylem. However, this symptom was a questionable standard for judging susceptibility to wilt in a seedling test (Armstrong and Armstrong, 1978), and there was no clear relationship between the severity of external symptoms in surviving muskmelon seedlings and the extent and degree of internal vascular discoloration (Zink *et al.*,

1983). Therefore, in the present test, vascular discoloration was not used as a criterion for judging susceptibility of cotton seedlings to wilt.

Resistance or susceptibility of the genotypes to Fusarium wilt is shown in Table 1. The genotypes were classified into two distinct groups. The susceptible group included all the experimental crosses, while the resistant group included all the commercial cultivars. Of the susceptible genotypes, family 27/99 showed the highest level of susceptibility, while the family 501/99 showed the lowest level of susceptibility. The other genotypes showed intermediate levels of susceptibility between these two extremes.

In this test, the genotypes were screened in the greenhouse under conditions very favorable for unrestricted development of the wilt fungus. The soil was autoclaved, the temperature was optimal most of the time, and the inoculum density was relatively high. However, FOV was unable to induce visible symptoms on any of the commercial cultivars.

Amino acid sequences of polypeptides (components of proteins) are dependent on nucleotide sequence of their coding genes; therefore, an analysis of protein variation among cotton genotypes by SDS-PAGE approximates an analysis of their genetic variation (Markert and Faulhaber, 1965). Electrophoretic patterns can be obtained rapidly and with small amount of tissues. Therefore, large number of single plant selections can be tested without sacrificing the plants (Wheeler *et al.*, 1971).

In the present study, a total of 60 protein bands were identified among the 12 genotypes that were analyzed (Figs 1 and 2 and Table 2)..

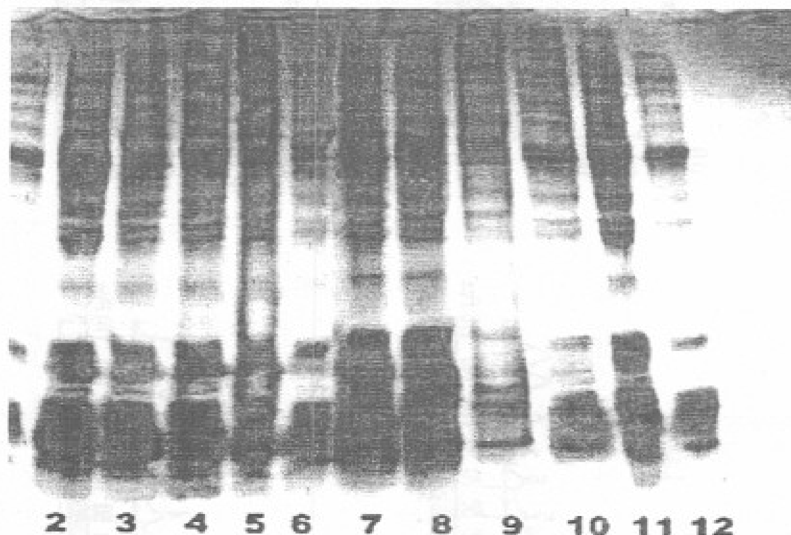


Fig.1. Protein patterns obtained by SDS-PAGE from seeds of 12 cotton genotypes. Lane (M) is a protein marker, while lanes from 1 through 12 are cotton genotypes. Identification of the genotypes is shown in Table 1.

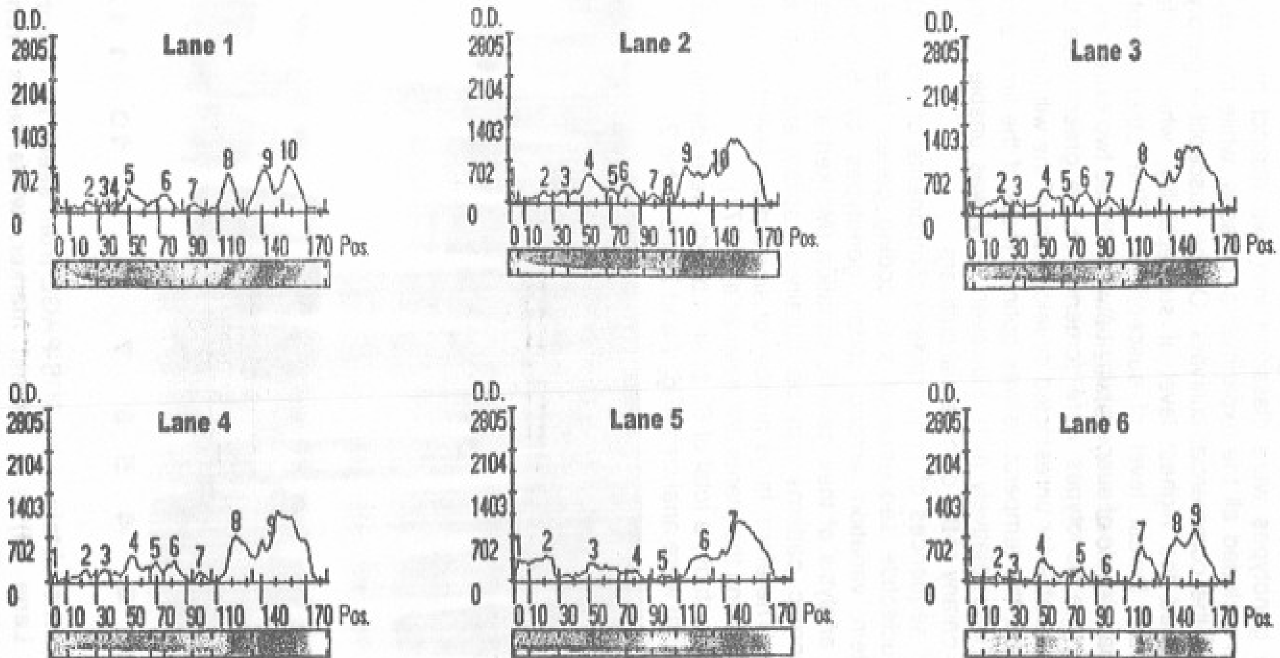
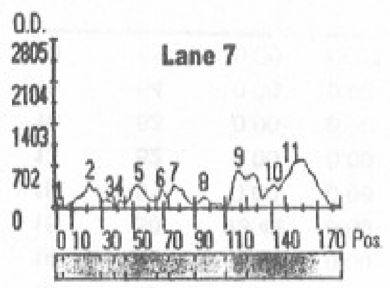
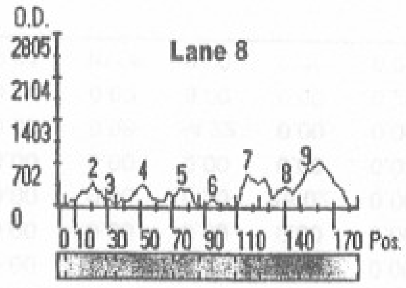
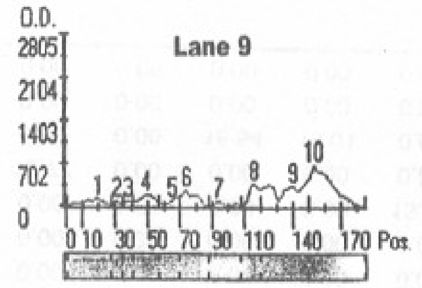
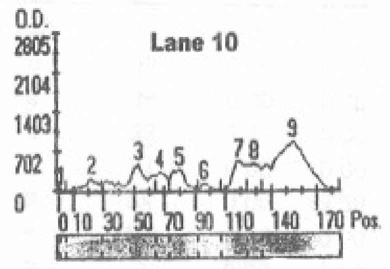


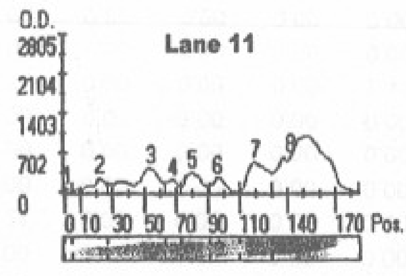
Fig. 2. Densitometer scanning of protein patterns obtained by SDS-PAGE from 12 cotton genotypes. Identification of the genotypes in lanes from 1 through 12 is shown in Table 1.











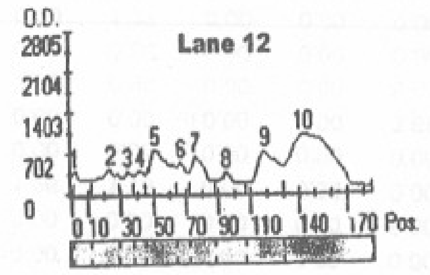


Fig. 2. Cont.

Table 2. Protein patterns for 12 cotton genotypes obtained by SDS-PAGE and stained with Coomassie Brilliant Blue R-250.

Band		Genotype [b]											
No.	Position	1	2	3	4	5	6	7	8	9	10	11	12
1	0	0.00[a]	0.00	0.00	0.00	0.00	0.00	0.00	0.21	0.00	0.00	0.00	0.00
2	2	1.72	0.97	0.90	0.00	0.00	0.00	0.22	0.00	0.00	0.25	0.41	4.01
3	3	0.00	0.00	0.00	1.15	5.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	23	4.70	0.00	0.00	3.79	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	24	0.00	3.26	7.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.60
6	31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.26	1.10	0.00	0.00	0.00
7	33	2.13	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00
8	34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.69
9	35	0.00	0.00	1.76	0.00	0.00	0.00	2.40	0.00	0.00	0.00	0.00	0.00
10	36	0.00	0.00	0.00	2.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11	38	0.00	2.84	0.00	0.00	0.00	0.00	0.00	0.00	1.33	0.00	0.00	0.00
12	40	1.50	0.00	0.00	0.00	0.00	0.00	1.31	0.00	0.00	0.00	0.00	0.00
13	42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.04
14	49	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.99	0.00	0.00	0.00
15	50	13.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16	51	0.00	0.00	0.00	0.00	0.00	15.02	0.00	0.00	0.00	0.00	0.00	13.02
17	52	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.77	0.00	0.00	0.00	0.00
18	53	0.00	0.00	0.00	0.00	14.22	0.00	0.00	0.00	0.00	15.54	15.01	0.00
19	54	0.00	0.00	0.00	0.00	0.00	0.00	9.20	0.00	0.00	0.00	0.00	0.00
20	55	0.00	13.07	9.81	10.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 2. Cont.

Band		Genotype											
No.	Position	1	2	3	4	5	6	7	8	9	10	11	12
21	65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.87	0.00	0.00	0.00
22	67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.29	0.00	4.52
23	68	0.00	0.00	0.00	0.00	0.00	0.00	3.46	0.00	0.00	0.00	3.43	0.00
24	69	0.00	0.00	3.72	3.71	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
25	71	0.00	2.86	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
26	74	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.86	0.00	0.00	0.00
27	75	7.85	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
28	76	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.69	0.00	0.00	0.00	8.30
29	77	0.00	0.00	0.00	0.00	0.00	0.00	7.98	0.00	0.00	0.00	0.00	0.00
30	79	0.00	6.25	0.00	0.00	0.00	4.93	0.00	0.00	0.00	0.00	0.00	0.00
31	80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.48	7.50	0.00
32	82	0.00	0.00	6.57	5.91	3.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00
33	93	2.55	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
34	94	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.23	0.00	0.00	0.00
35	95	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.41	0.00	0.00	0.00	5.47
36	96	0.00	0.00	0.00	0.00	0.00	0.00	3.26	0.00	0.00	1.86	4.56	0.00
37	97	0.00	0.00	0.00	0.00	0.00	1.13	0.00	0.00	0.00	0.00	0.00	0.00
38	99	0.00	2.22	3.57	1.91	1.37	0.00	0.00	0.00	0.00	0.00	0.00	0.00
39	109	0.00	0.36	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
40	116	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.96	0.00	0.00	0.00

Table 2. Cont.

Band		Genotype											
No.	Position	1	2	3	4	5	6	7	8	9	10	11	12
41	117	15.81	0.00	0.00	0.00	0.00	0.00	0.00	11.33	0.00	0.00	0.00	0.00
42	119	0.00	0.00	0.00	0.00	0.00	0.00	10.88	0.00	0.00	9.37	0.00	12.89
43	120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.81	0.00
44	121	0.00	0.00	0.00	0.00	0.00	17.69	0.00	0.00	0.00	0.00	0.00	0.00
45	122	0.00	14.13	16.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
46	123	0.00	0.00	0.00	17.86	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
47	127	0.00	0.00	0.00	0.00	13.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00
48	129	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.95	0.00	0.00
49	140	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	27.71	0.00	0.00	0.00
50	141	0.00	0.00	0.00	0.00	0.00	0.00	19.14	17.32	0.00	0.00	0.00	0.00
51	142	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	51.75	0.00
52	143	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	38.46
53	144	26.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
54	145	0.00	24.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
55	146	0.00	0.00	0.00	0.00	50.25	29.73	0.00	0.00	0.00	0.00	0.00	0.00
56	147	0.00	0.00	50.27	52.61	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
57	153	0.00	0.00	0.00	0.00	0.00	0.00	32.63	35.41	34.05	0.00	0.00	0.00
58	154	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	45.65	0.00	0.00
59	158	24.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
60	159	0.00	0.00	0.00	0.00	0.00	26.04	0.00	0.00	0.00	0.00	0.00	0.00

[a] Amount (%) of the designated protein fraction.

[b] Identification of the genotypes are shown in Table 1.

This large number of bands was due to the effect of SDS. which dissociated each oligomeric protein into its subunits (Bohinski, 1983). When protein preparations were treated with mercaptoethanol and SDS, the mercaptoethanol disrupted (reduced) all disulphide (-S-S-) bonds present in proteins, whereas the detergent SDS bound to all regions of protein associations. This resulted in total disruption of associated subunits organization and then yielded SDS-carrying, highly-anionic polypeptide chains (Clark and Switzer, 1977). No single genotype was stained for all the 60 bands. Similarly, no single band was common to al the genotypes. Family 501/99 showed the least number of bands (6 bands), while, Giza 80 showed the greatest number (11 bands). Each genotype was characterized by unique bands. For example, bands no_s. 15, 27, 33, 53, and 59 were unique to family 27/99.

Pearson correlation coefficient was calculated to measure the degree of association between wilt incidence and the amount of the separated protein fractions (Table 3). However, only two proteins (3.33%) were significantly ($p<0.05$) correlated with wilt incidence.

Data for wilt incidence and amount of protein fractions were entered into a computerized stepwise multiple regression analysis. The analysis constructed a predictive model by adding predictors, in this case, amounts of protein fractions to the model in order of their contribution to R^2. The analysis was effective in eliminating those variables with little or no predictive value by incorporating into the model only those variables that made a satisfactory contribution to the R^2 value of the model (Podleckis *et al.*, 1984). Using the predictors supplied by the stepwise regression, a five-factor model was constructed to predict wilt incidence (Table 4). This model shoed that wilt incidence differences were due to the protein fractions no_s 4, 30, 32, 7, and 55, which accounted for 99.98% of the total variation in wilt incidence ratings

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

In the present study, satisfactory visualization of banding patterns were obtained by using the Coomassie Brilliant Blue R-250 staining system for general protein, and the stepwise regression model they generated proved effective in predicting wilt incidence from banding patterns. Therefore, SDS-PAGE of proteins, such as that described herein, may provide a supplementary assay to greenhouse tests to distinguish between Fusarium-wilt resistant or susceptible genotypes quantitatively.

Table 3. Relationship between incidence of Fusarium wilt[a] on 12 cotton genotypes and protein content[b] of seeds from these genotypes.

No.[c]	r[d]	No.	r
1	- 0.262	31	- 0.388
2	0.151	32	0.216
3	0.012	33	0.466
4	0.609*	34	- 0.262
5	- 0.035	35	- 0.358
6	- 0.387	36	- 0.467
7	0.556	37	0.397
8	- 0.262	38	0.361
9	- 0.220	39	0.424
10	0.347	40	- 0.262
11	0.283	41	0.237
12	0.188	42	- 0.496
13	- 0.262	43	- 0.262
14	- 0.262	44	0.397
15	0.466	45	0.294
16	0.134	46	0.347
17	- 0.262	47	- 0.065
18	- 0.381	48	- 0.262
19	- 0.262	49	- 0.262
20	0.523	50	- 0.398
21	- 0.262	51	- 0.262
22	- 0.387	52	- 0.262
23	- 0.389	53	0.466
24	0.259	54	0.424
25	0.424	55	0.153
26	- 0.262	56	0.265
27	0.466	57	- 0.501
28	- 0.388	58	- 0.262
29	- 0.262	59	0.466
30	0.606*	60	0.397

[a] Wilt incidence was calculated according to the following formula: [infected seedlings/emerging seedlings] x 100. Infected seedlings included the dead seedlings and the surviving seedlings, which showed external symptoms.
[b] Amount of protein (%).
[c] No. of protein fraction.
[d] Pearson correlation coefficient, which measured the degree of association between wilt incidence and the designated protein fraction, was significant at $p<0.05$ (*).

Table 4. Regression equation that describes the effects of some protein fractions (X_s) on the incidence[a] of Fusarium wilt of cotton.

Stepwise regression model	R^2 [b]	F. value [c]
$Y = 0.04 + 12.07 X_4 + 13.68 X_{30} + 4.96 X_{32} + 15.73 X_7 + 0.18 X_{55}$	99.98%	13373.69 ***

[a] Wilt incidence was calculated according to the following formula: [infected seedlings/emerging seedlings] x 100. Infected seedlings included the dead seedlings and the surviving seedlings, which showed external symptoms.
[b] Coefficient of determination. Relative contribution of X_4, X_{30}, X_{32} X_7, and X_{55} to R^2 were 37.03, 54.82, 4.99, 2.70, and 0.44%, respectively.
[c] F. value is significant at $p = 0.0000$ (***).

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إستعمال التفريد الكهربى للبروتينات للتعبير الكمى عن مقاومة القطن لمرض ذبول الفيوزاريوم

علـــى عبـــد الهـــادى علـــى ، عـــزت محمـــد حـــسين ، معـــوض رجـــب عمـــر ,
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قيمت ستة هجن تجريبية وستة أصناف تجارية من الأقطان المصرية وذلك من حيث المقاومة لمرض ذبول الفيوزاريوم ، تحت ظروف الصوبة ، خلال موسم ٢٠٠٧. تباينت الهجن فيما بينها من حيث درجة القابلية للإصابة بالمرض ، أما الأصناف التجارية فكانت كلها على درجة عالية من المقاومة للمرض. إستعملت تقنية التفريد الكهربى لفصل بروتينات بذرة التراكيب الوراثية (الهجن والأصناف) ، وذلك بعد تفكيك هذه البروتينات باستعمال مادة صوديوم دوديسيل سلفيت. استعملت مادة الكوماسي بريليانت بلو لصبغ أنماط البروتينات المتحصل عليها. أمكن – باستخدام أسلوب الانحدار المتعدد المرحلى – التوصل الى نموذج رياضى لوصف العلاقة بين حدوث المرض (متغير تابع) والبروتينات المفصولة (متغير مستقل). أظهر هذا النموذج أن ٩٩,٩٨% من التباين الكلى فى حدوث المرض من الممكن أن يعزى إلى تأثير البروتينات أرقام ٤ و٣٠ و٣٢ و٧ و٥٥. تدل نتيجة الدراسة الحالية على أنه من الممكن إستخدام تقنية التفريد الكهربى لبروتينات البذرة – كوسيلة مكملة لاختبارات الصوبة – للتفرقة الكمية بين تراكيب القطن الوراثية المقاومة أو القابلة للإصابة بمرض ذبول الفيوزاريوم.