Journal of Agricultural Chemistry and Biotechnology

Journal homepage: www.jacb.mans.edu.eg
Available online at: www. jacb.journals.ekb.eg

Characterization of some Cotton Genotypes Highly Resistant to Fusarium Wilt Disease by Electrophoresis of Peroxidase Isozymes and Seed Proteins

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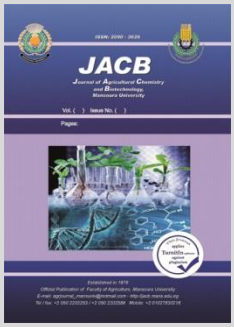

ABSTRACT

Cluster analysis was used to evaluate the genetic diversity among 12 cotton genotypes highly resistant to Fusarium wilt disease. Cluster analysis was carried out based on electrophoretic banding patterns of peroxidase isozymes and seed proteins. Cluster analysis of peroxidase isozymes divided the genotypes into three distinct remotely related groups. Peroxidase isozymes were reliable to differentiate among genotypes included in the different groups; however, the isozymes were of limited reliability to differentiate among genotypes within groups. The genotypes were separated into a larg number of remotely related subclusters based on cluster analysis of their protein banding patterns. Therefore, it was easy to differentiate among the genotypes of the different subclusters. The present study demonstrated that cotton genotypes could be identified by their electrophoretic banding patterns of peroxidase isozymes and seed proteins. These results could be of practical value for cultivar identification and for seed purity tests.

Keywords : Cotton genotypes ; Fusarium wilt disease ; peroxidase isozymes ; seed protein ; electrophoresis.

INTRODUCTION

Fusarium wilt of cotton is a serious fungal disease responsible for significant losses in yield and quality in the major cotton–producing areas in the world. *Fusarium oxysporum* Schlecht. f. sp. *vasinfectum* (ATK) Snyd and Hans (FOV) is the causal pathogen of this disease. FOV penetrates taproots of cotton behind the root tip. Wilt symptoms that appear on the infected plants are due to the occlusion of the xylem vessels. This occlusion is the consequence of the combined effects of fungal metabolites and the production of lipodial compounds by host in response to infection (Hillocks, 1984). FOV has a very good survival ability in soil and hence it is very difficult to eradicate it from infested fields. (Watkins, 1981).

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 and Konarev, 1988).

Sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE) is the most widely used analytical technique in protein studies. It is considered a low–cost, reproducible and rapid method for quantifying, comparing, and characterizing proteins (Abd El-Salam *et al*., 2005).

The basis of this method is that a charged molecule migrates in an electric field in a rate that is determined by its molecular weight (Bohinski, 1983).

Some attempts were made to differentiate among cotton genotypes by using protein electrophoresis. For example, when Abd El-salam *et al*. (2005) used electrophoresis of water soluble proteins to evaluate genetic diversity among eight cotton genotypes of *G. barbadense*, they found that some specific bands could be used to distinguish the genotypes

Protein electrophoresis showed that interspecific hybrids between *G. barbadense* and *G. hirsutum* had higher number of protein bands than their parents (Ali, 2006)

Abd El-Fattah (2010) used protein electrophoresis to detect the genetic variability and relationships among nine cotton varieties (*G. barbadense*). Protein analysis revealed qualitative differences among the nine varieties. Thus, a protein fraction with 70 KD was detected only in Giza 83, Giza 85, and Giza 91, while another protein fraction of 46 KD was only expressed in Dandera, Giza 75, Giza 80, Giza 89, and Giza 91.

Proteins extracted from seeds of three highly resistant genotypes used as male parents, three highly susceptible varieties used as female parents, and their F_1's hybrids were electrophoretically analysed by SDS-PAGE. One protein band with molecular weight of 32.83 KD was absent from all female parents, which were highly susceptible to damping-off disease disease while it was present in all male parents, which were highly resistant to the disease. This band was also detected in some crosses. This band was considered as a positive marker for resistance and can be used as biochemical marker on selecting for such an important triat (Darweesh, 2014)).

Isozyme (isoenzymes) are defined as genetically determined multiple molecular forms of an enzyme (Manchenko, 1994).

Genetic diversity among plant genotypes can be evaluated with seed isozyme markers. For instance. Abd El-Tawab *et al.* (1990) electrophoretically analyze esterase isozymes of four cotton cultivars. Esterase zymograms were quit similar for the two cultivars of *G.barbadense* (Dandera and Giza 80), while they were different from those of *G. hirsutum* (McNair 235 and Tamcot sp. 3741).

The present investigation was initiated to determine whether cotton genotypes can be distinguished by their electrophoretic banding patterns of seed proteins and peroxidase isozymes.

MATERIALS AND METHODS

Cotton genotypes:

The genotypes used in the present study were randomly selected from the collection of germplasm

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DOI:10.21608/jacb.2019.67367

available at the Department of Cotton Breeding, Cytology and Genetics Unit, Cotton Research Institute (Table 1).

Table 1. Cotton genotypes used in the present Study.

Genotype number	Pedigree
1	Giza84 x (Giza74 x Giza 68)/187
2	Pima Type 1 (high Yield)/64
3	Giza 71 / 172
4	(Giza 74 x Giza 77)/182
5	(Giza 87 x Giza 81)/180
6	(Giza 86) /98
7	(Giza 87)/146
8	(Giza 45)radiated/192
9	Australian (okra leef)/41
10	Sea Island 2
11	(Giza 68 x Giza 45) x (Giza 45 x Sea Island)/190
12	Giza 90/35

Evaluation of cotton genotypes against Fusarium wilt race 3 under greenhouse conditions :

Evaluation of the tested genotypes was carried out under greenhouse conditions (Aly *et al*., 2007 and Abd-El salam *et al*, 2009)

Assessment of Fusarium wilt incidence:

Incidence of Fusarium wilt was assessed on the tested genotypes according to Aly *et al*. (2007) and Abd-El salam *et al*. (2009).

Extraction and estimation of seed proteins:

Seed proteins of the tested genotypes were extracted and estimated according to Hussein (1992) and Bradford (1976).

Electrophoresis of native protein (PAGE) for the detection of peroxidase isozymes:

Electrophoresis was carried out according to Laemmli, (1970) and Manchenko (1994) for the detection of peroxidase (EC.1.11.1.7) isozymes.

Electrophoresis of dissociated protein (SDS-PAGE):

Electrophoresis was carried out as previously mentioned in PAGE and gels were stained with silver nitrate for the detection of protein bands (Sammons *et al*., 1981).

Gel analysis :

Peroxidase and protein patterns obtained by PAGE and SDS-PAGE, respectively were clustered by a gel documentation system (Uvitec, Cambridge, UK) by the unweighted pair group method based on arithmetic means (UPGMA) according to Sneath and Sokal, (1973). Similarity coefficient matrix among peroxidase and protein banding patterns was calculated based on the number of shared bands between pairs of genotypes (Nei and Li, 1979).

RESULTS AND DISCUSSION

Data shown in Table 2 indicated that 75 % of the tested genotypes were valuable sources of resistance to Fusarium wilt disease as they were very highly resistant. This reaction class was the maximum level of resistance to the disease. It is well known that development of resistant cultivars is the most reliable method for controlling Fusarium wilt of cotton (Watkins, 1981).Thus, the healthy seedlings in this class ranged from 93.44 to 100 %. Therefore, it is important to maintain the purity of these genotypes and to develop reliable methods to test this purity.

Peroxidase isozyme banding patterns show in Fig. 1 were used to calculate SCM shown in Table 3. A phenogram based on similarity level (SL) generated from cluster analysis of SCM is shown in Fig. 2. The greater the SL, the more closely the genotypes were related in their banding patterns (zymograms). At the similarity level of 30%, the genotypes were divided into 3 distinct remotely related groups. The first group included genotypes 1, 8, 10, and 2. The second group included genotypes 9, 11, and 12. Genotypes 3, 4, 5, 6, and 7 were included in the third group. The genotypes showed 9 banding patterns (zymograms) for peroxidase. The phenogram shown in Fig. 3 indicated that peroxidase isozymes were reliable to differentiate among genotypes included in the different groups; however, the isozymes were of limited reliability to differentiate among genotypes within groups.Abd El-Tawab *et al*. (1990) reported almost similar results when they used esterase isozymes to compare between two groups of cotton cultivars.

Table 2. Reactions of 12 cotton genotypes to Fusarium wilt disease under greenhouse conditions.

Genotype[a] number	Heathy Seedlings[b] (%)	Reaction class[c]
1	96.67	VHR
2	56.68	R
3	86.17	HR
4	93.44	VHR
5	96.77	VHR
6	100.00	VHR
7	100.00	VHR
8	96.67	VHR
9	80.10	HR
10	96.67	VHR
11	96.67	VHR
12	100.00	VHR

[a]Pedigrees of the tested genotypes are shown in Table 1.
[b]Seedlings, which are completely free from any external internal symptoms.
[c]Reaction class was determined based on the percentage of healthy seedlings according to the following Scale: Very highly Suscoptible (VHS0) = 0-10, highly Susceptible (HS) = 11-30, Susceptible (S) = 31-50, resistant (R) = 51-70, highly resistant (HR) = 71-90, and very highly resistant (VHR) = 91-100.

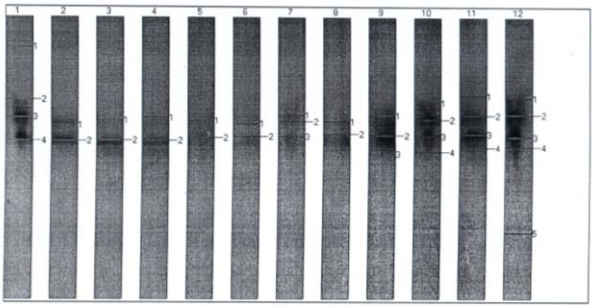

Fig. 1. Peroxidase isozyme banding patterns obtained by PAGE from 12 cotton genotypes.

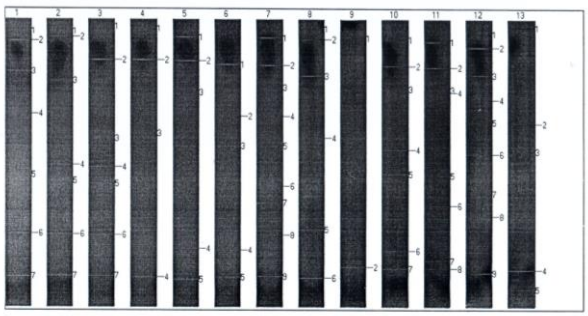

Fig. 2. Phenogram based on average linkage cluster analysis of electrophoretic peroxidase isozyme banding patterns obtained by PAGE from 12 cotton genotypes.

Table 3. Similarity coefficient matrix (SCM)[a] among peroxidase isozyme patterns separated by PAGE for 12 cotton genotypes

	Genotype[b]											
Genotype	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	1.00											
2	0.33	1.00										
3	0.67	0.50	1.00									
4	0.67	0.50	1.00	1.00								
5	0.67	0.50	1.00	1.00	1.00							
6	0.00	0.50	1.00	0.50	1.00	1.00						
7	0.29	0.00	0.80	0.80	0.80	0.80	1.00					
8	0.33	0.00	0.50	0.50	1.00	1.00	0.80	1.00				
9	0.29	0.00	0.00	0.40	0.40	0.40	0.67	0.40	1.00			
10	0.50	0.00	0.33	0.33	0.67	0.33	0.57	0.67	0.57	1.00		
11	0.25	0.00	0.00	0.33	0.33	0.33	0.57	0.33	0.57	0.50	1.00	
12	0.44	0.00	0.29	0.57	0.29	0.29	0.50	0.29	0.50	0.44	0.89	1.00

[a] **SCM was calculated based on the number of shared isozymes between pairs of genotypes (Nei and Li, 1979).**
[b] **Pedigrees of the tested genotypes are shown in Table 1.**

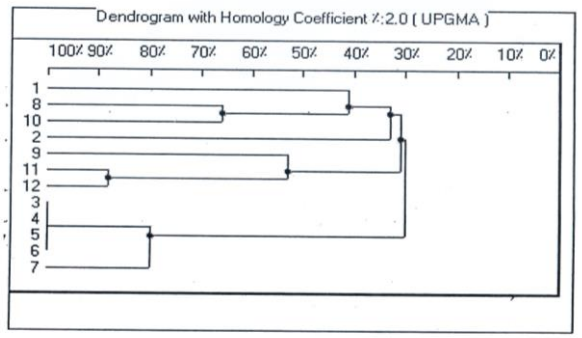

Fig.3. Protein banding patterns obtained by SDS-PAGE from 12 cotton genotypes. Column 13 is a protein marker.

The phenogram shown in Fig. 4 was constructed based on the protein banding patterns shown in Fig. 3 and SCM shown in Table 4. It is apparent that the genotypes separated into a large number of subclusters based on their protein banding patterns (Manicom *et al*, 1990) Thus, a low overall similarity level of about 10 % was found among the subclusters. In other words, the subclusters showed 90 % dissimilarity, which indicated a very high level of variability in the protein banding patterns of the tested genotypes. Therefore, it was easy to differentiate among the genotypes of the different subclusters. It is worthy of mention that genotype 12 showed an unique banding pattern compared with the other genotypes.

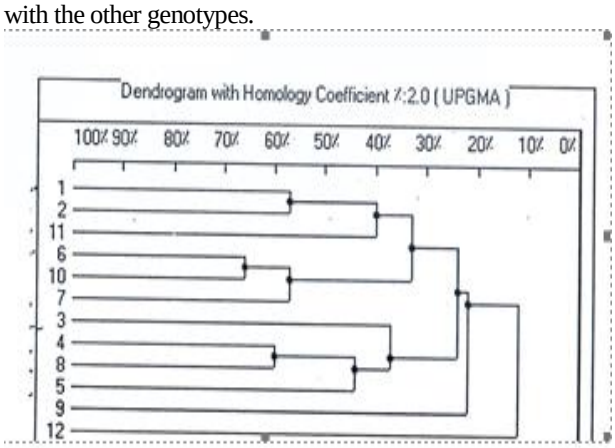

Fig. 4. Phenogram based on average linkage cluster analysis of electrophoretic protein banding patterns obtained by SDS-PAGE from 12 cotton genotypes.

The present study demonstrated that cotton genotypes could be identified by their electrophoretic banding patterns of peroxidase isozymes and seed proteins. These results could be of practical value for genotype identification and seed purity tests (Cook, 1988 and Konarev, 1988).

Table 4. Similarity coefficient matrix (SCM)[a] among protein banding patterns separated by SDS-PAGE for 12 cotton genotype

	Genotype[b]											
Genotype	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	1.00											
2	0.57	1.00										
3	0.29	0.57	1.00									
4	0.18	0.18	0.36	1.00								
5	0.17	0.33	0.33	0.44	1.00							
6	0.33	0.17	0.17	0.22	0.40	1.00						
7	0.38	0.38	0.38	0.15	0.14	0.57	1.00					
8	0.31	0.31	0.46	0.60	0.18	0.18	0.27	1.00				
9	0.22	0.22	0.22	0.00	0.00	0.00	0.36	0.00	1.00			
10	0.29	0.14	0.14	-.18	0.17	0.67	0.50	0.31	0.22	1.00		
11	0.40	0.27	0.13	0.17	0.15	0.15	0.35	0.14	0.20	0.53	1.00	
12	0.13	0.13	0.13	0.15	0.14	0.14	0.11	0.27	0.18	0.25	0.12	1.00

[a] **SCM was calculated based on the number of Shared bands between pairs of genotype(Nei and Li, 1979).**
[b] **Pedigrees of the tested genotypes are Shown in table1.**

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توصيف بعض التراكيب الوراثية للقطن عالية المقاومة لمرض ذبول الفيوزاريوم باستعمال التفريد الكهربى لمشابهات إنزيم البيرأوكسيديز و بروتينات البذرة

عـزت محمـد حسيــن ، ماريان منير حبيب ، أمل عبد المنجى عسران ،عبد الودود زكى عبد الله عاشور* و على عبـد الهـادى علـى

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

استعمل التحليل العنقودى لتقييم درجة التنوع الوراثى فى 12 تركيب وراثى للقطن عالية المقاومة لمرض ذبول الفيوزاريوم. أجرى التحليل العنقودى عن طريق الجمع بين استعمال أنماط مشابهات انزيم البيرأوكسيديز و أنماط البروتينات الناتجة من التفريد الكهربى. عند استعمال التحليل العنقودى لمشابهات إنزيم البيرأوكسيديز، أمكن تقسيم التراكيب الوراثية الى ثلاث مجموعات محددة توجد بينها درجة ضعيفة من التماثل فى أنماط مشابهات الإنزيم، و على ذلك يمكن القول بأن مشابهات إنزيم البيرأوكسيديز أظهرت قدرة مرتفعة على التفرقة بين التراكيب الوراثية التابعة للمجموعات المختلفة، فى حين كانت قدرتها محدودة فى التفرقة بين التراكيب الوراثية داخل المجموعة الواحدة. عند استعمال التحليل العنقودى لأنماط البروتينات، انقسمت التراكيب الوراثية الى العديد من المجموعات و التى أظهرت درجات ضعيفة من التماثل فى أنماط البروتين مما سهل من أمكانية التفرقة بين التراكيب الوراثية. تدل نتائج الدراسة الحالية على أنه من الممكن الجمع ما بين استعمال أنماط مشابهات إنزيم البيرأوكسيديز و بروتينات البذرة الناتجة من التفريد الكهربى للتفرقة بين التراكيب الوراثية للقطن . هذه النتائج لها أهمية تطبيقية فى التفرقة بين الأصناف وإجراء اختبارات النقاوة للتقاوى.