Journal

J Biol Chem Environ Sci, 2006, Vol. 1(3): 575-594 www.AcepsAg.org

APPLICATION OF PROTEIN RELATED INDEX AND ISOZYMES IN DEFINING ZICCINI YELLOW MOSAIC POTYVIRUS IN INFECTED SQUASH PLANTS

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ABSTRACT

Squash plants naturally infected by Ziccini yellow mosaic potyvirus (ZYMV) were classified into three groups according to the degree of symptoms i.e. mosaic, curly and crinkling, and malformation leaves. The disease was transferred from each group to various cucurbaceae plants by sap mechanical technique then diagnosed by biological and serological (DAS-ELISA) tests, and change in chlorophyll contents to ensure that all these symptoms resulted from ZYMV. The use of protein related index (PR-index) and isozyme separation to detect ZYMV infection in squash plants and to distinguish each degree of symptom was evaluated. SDS-PAGE of protein separation showed a distinct protein pattern and protein contents of control and infected plants. DISC-PAGE isozyme separation of control and the three infected groups showed 7, 6, 6 and 8 esterase isozymes, and 4, 6, 2 and 7 peroxidase isozymes respectively. Similarity analysis confirmed the difference in protein and isozyme patterns between control and each of infected plant groups, which suggests using these techniques in ZYMV characterization.

Key words: Soluble proteins, Peroxidase and esterase isozymes, ZYMV, Biological and serological assays, Photopegment, Squash plants.

INTRODUCTION

Ziccini yellow mosaic disease is widely distributed in Egypt and has been reported as a main problem for squash crop production in open fields and protected agriculture, besides causing severe losses in cucurbit crops (Lisa *et al.*, 1981 and Farag 1999). The disease showed several types of symptoms including severe mosaic, leaf distortion, crinkle, malformation, blisters, green vein banding and chloritic local lesion followed by vein netting, yellow often necrosis and plant death (Ibrahim 1986; Abo El-Nasr *et al.*, 2004 and Othman *et al.*, 1985). Therefore, it was important to define the etiology of this disease by symptomatology, biology, serology and protein related index tests.

Leal and Lastra (1984) revealed that the reduction in chlorophylls, soluble proteins and nitrogen contents was evident in leaves infected by *tomato yellow mosaic virus* that causes severe symptoms of yellowing eduction of the leaf lamina and stunting. The subcellular distriction of virus stimulates the synthesis of soluble proteins which were detected by SDS-PAGE. The most prominent alteration detected in protein pattern of virus infected tomato leaves, as judged by the intensity of their staining, is the enhancement of 14.3, 20, 39, 58 and 97 KDa proteins (Gianinazzi *et al.*, 1980; Hadidi 1988; El-Dougdoug 1996 and Sherif and El-Dougdoug 2000). Certain host proteins were increased dramatically as part of a general physiological response to infection by viruses and other pathogens (Camacho-Henriquez and Sanger 1982).

The relationship between isozyme composition of host plant and plant resistance or susceptibility to diseases has been studied in some pathosystems. Isozyme spectra of malate dehydrogenase, peroxidase and esterase of 10 flax cultivars were separated by PAGE. Data indicated that PAGE of isozymes may provide a supplementary assay to greenhouse and field tests to distinguish quantitatively between powder mildew resistant or susceptible cultivars (Ali *et al.*, 2005). Peroxidase and polyphenol oxidase activities were found to be considerably higher in infected tomato leaves than in healthy ones. Viral infection with yellow leaf curl and leaf roll of tomato exhibited higher activity of peroxidase and polyphenoloxidase (Sherif and El-Habbaa 2000).

The present study aims to the use of some biochemical tests e.g. virus related proteins, isozymes and photopegment contents, in

addition to biological and serological assays in the detection of virus infection.

MATERIAL AND METHODS

Naturally infected squash plants (*Cucurbita oepo* var. Eskandarani) were collected from experimental farm of Fac. of Agric., Ain Shams Univ., Shoubra El-Kheima in a winter season. The examined plants revealed the following three characteristic symptoms: mosaic, curl and crinkle and malformation leaves These plants and some healthy plant, used as control, were subjected to the following tests and determinations.

1. Biological tests:

a) Leaf samples of naturally infected plants were used to infect healthy squash seedlings by sap mechanical inoculation technique by using phosphate buffer (0.1 M, pH 7.2) under greenhouse condition to ensure that these symptoms were due to a virus infection.

b) Different host test: Healthy plants were divided into 3 groups each group contains 8 different species (Table 1). Each group was infected with one of each symptom to confirm that the three symptoms were due to ZYMV.

2. Serological assay: DAS-ELISA technique (Clark and Adams 1977) was used for ZYMV detection in naturally infected plants and differential hosts. The virus kit were obtained from Agric. Gen. Eng. Res. Institute (Giza).

3. Photopigment contents: Chlorophyll a, b and carotenoid contents were determined using the method described by Broughan (1960). Statistical analysis was preformed by using SPSS program (V. 11.5).

4. Protein extraction: Samples of squash leaves were taken from ZYMV infected and healthy plants. Water soluble proteins were extracted by grounding 0.2 g of each sample in 1.0 ml extraction buffer (6 ml, 1.0 M Tris, pH 8.8, 800 µl, 0.25 M EDTA and 93.2 ml H_2O), with shaking throughly. The extracts were transferred to Eppendorof tubes and centrifuged for 15 min. at 10.000 rpm under cooling. Supernatants (water soluble proteins) were transferred to fresh tubes and used for SDS-PAGE analysis and extraction of isozymes was used as described by Jonathan *et al.* (1990).

5. Protein related index (PR-index): Fractionation electrophoresis was performed under identical conditions on sodium dedocyl sulfate polyacrylamide gel (SDS-PAGE) (15.0% W/V) vertical slab using Sigma Aldrich Techware 1.5mm according to the method of Laemmli (1970) as modified by Studier (1973). The molecular weights of proteins were estimated relative to marker, a low molecular weight proteins (Pharmacia Motranl).

6. Iosozymes electrophoresis : Polyacrylamide gel electrophoresis (DISC-PAGE) was performed in 10% (W/V) slab gel (Davis 1964). The gel was stained after run according to Tanksley and Rick (1980) for esterase isozymes and Graham *et al.*, (1964) for peroxidase isozymes. The staining gel was incubated at 37°C in dark for complete staining after adding the appropriate substrates and staining solutions.

7. Gel documentation: For quantitative measurements and similarity analysis, a charge-coupled device camera imaging system and UVI soft V 99 analysis (Gel documentation and analysis system, Uvitec, Cambridge, UK) were used to capture the image and to calculate band intensities.

RESULTS

Leaf crinkling and malformation symptoms started as discoloration of leaves at interveinal areas of infected squash var. Eskandarani. This discoloration was more pronounced on the leaf edges with dark green mosaic bisters leaf distoration remaining along the thick veins vinal yellowing. The symptoms were then developed into three main features in the cultivated area and thus they were classified into the following three categories:

1. The leaves were mosaic and blisters (Fig. 1-B).
2. The leaves were curly, crinkling and decreased in size (Fig. 1-C).
3. Leaves showed reduction in size and filiform or shoestring shapes (Fig. 1-D).

By aging of all infected plants, the stem, branches and petioles became distorted, thick, rough and twisted. The rate of epical growth decreased and the plant became markedly stunted. The infected plants produced few flowers. Considerable flower shedding occurred in 45-50% of infected plants compared with 5-10% of healthy ones (Fig. 1-A).

The prevalence of the viral disease in squash plants was gradually increased. Observation showed a progressive increase in the numbers of aphids and whiteflies throughout the season. Some healthy squash plants (group 1) and the infected plants, classified into three groups (2, 3 and 4) according to the previously mentioned symptoms respectively, were subjected to the following examinations.

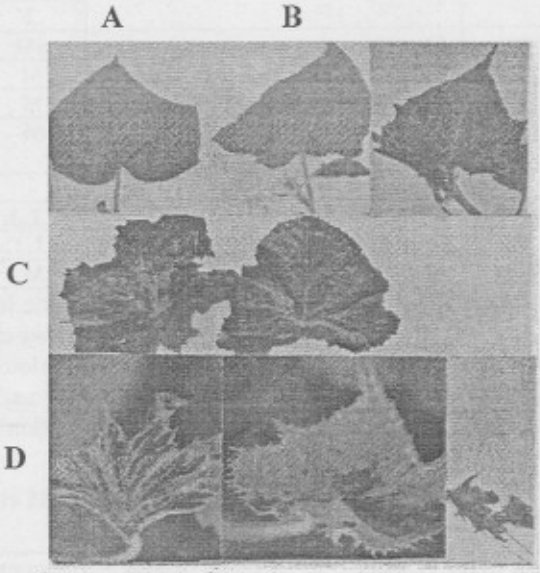


Fig. (1): Symptoms of naturally infected squash leaves by ZYMV disease.
A. Healthy leaves (group 1); B. Mosaic symptoms (group 2), C. Curl and crinkle symptoms (group 3); D. malformation leaves (group 4).

Biological detection: Each of the three different feature symptoms which were revealed in the open field were tested individually. These symptoms were transmitted by standard sap inoculation technique to healthy squash seedlings. These plants showed disease symptoms and contained a sap transmissible virus. Eight plant species belonged to 5 families were mechanically inoculated with the three different ZYMV symptoms to investigate their reactions and differentiate the three symptoms as indicated in Table 1.

Chlorophyll and carotenoid: Data presented in Table (2) showed that chlorophyll a and b were significantly decreased in infected plants

(group 2 and 4) as a result of ZYMV infection whereas group 3 didn't show significantly different from healthy group. On the other hand, carotenoids were not significantly changed.

Table (1): Differential host reaction of naturally ZYMV symptoms transmitted by sap inoculation.

Family	Scientific name	symptom groups		
		2	3	4
Cucurbitaceae	*Cucurbita pepo* var. Escandrani	SM	B,D	MF
	Cucumis sativus var. peta alpha	M	M,B	MF
	Cucumis melo var. cantalpensis	M	SM	MF
Solanaceae	*Lycopersicon esculentum* var. castle rock	M	LC	-
	Physalis floridana L.	-	M	SM
Chenopodiaceae	*Chenopodium amaranticolor* L.	C.L.L	Y.CLL	CLL.pin
Amaranthaceae	*Gomphrena globosa*	C.L.L.	N	NS
Serophulariaceae	*Antirrhinum majus*	M	Ch.	SM

SM = Severe mosaic
B = Blisters
D = Distortion
Ch = Chlorosis
LC = Leaf crinkle
C.L.L. = Chlorotic local lesion
Y.C.L.L. = Yellow chlorotic local lesion
C.L.L (pin) = Chlorotic local lesion (pin)
NS = No symptoms. M = Mosaic
MF = Malformation N = Necrosis

Table (2): Chlorophyll a,b and carotenoid contents in naturally ZYMV infected squash leaves.

Squash plant groups	Chlorophyll (a)	Chlorophyll (b)	Carotenoids
Healthy leaf (group 1)	2.981	1.450	1.855
Mosaic leaf (group 2)	2.250	1.207	1.885
Curl and crinkle leaf (group 3)	2.752	1.425	1.925
Malformation leaf (group 4)	2.252	1.075	1.895
L.S.D 5%	0.250	0.180	0.095
1%	0.480	0.300	0.150

Serological assay of ZYMV: DAS-ELISA analysis revealed that all three naturally symptoms gave a positive reaction with IgG specific ZYMV. The percentage of ZYMV was 0.125; 0.575; 0.621 and 0.712 measured at 405 nm for healthy, mosaic, curl and crinkle, and malformation symptoms (groups 1-4) respectively.

Protein related index (PR-index): Infection by ZYMV disease was detected by protein related index. Proteins extracted from healthy (group 1) and infected squash leaves (groups 2-4) were subjected to SDS-PAGE analysis. The obtained data listed in Table (3) and Fig. (2

& 4) showed that the protein patterns of infected plants (groups 2-4) had more protein subunits (3, 6 and 7 respectively) than that of healthy plants (group 1). Moreover, a number of polypeptides disappeared in infected plants compared with the healthy plants. The molecular weight of each protein subunits was determined and listed in Table (3). The most prominent alteration in infected plants was the high intensity of protein bands 26.68, 20.10, and 19.35 KDa (group 2), 123.60, 37.65, 26.68, 23.26, 19.35 and 14.00 KDa (group 3) and 123.60, 37.65, 33.00, 26.68, 23.56, 20.10 and 14.00 KDa (group 4). Total protein contents increased in infected squash leaves with curl and crinkle (group 3) and malformation (group 4) symptoms while decreased in leaves with mosaic (group 2) symptom

Similarity relationship between protein contents of healthy and infected squash leaves is shown in Fig. (3). Results indicated a weak similarity between the protein contents of healthy leaves (group 1) and those of infected plants ($R \leq 0.13$). This result confirm the ability of using protein analysis as good indicator of ZYMV infection.

Esterase isozymes: Results of esterases separation are shown in Table (4) and Fig. (5). Each group of control and infected plants could be characterized by unique set of isozymes. The total number of esterase isozymes shown in all groups were 9 isozymes while those of each group (1-4) were 7, 6, 6, 8 respectively. Similarity correlation presented in Fig. (6) showed a low correlation between healthy (group 1) and infected (group 2-4) plants ($R \leq 0.40$). The characterized isozymes of each infected group were bands 1,3 (group 2), 3 (group 3), and 1, 3 (group 4).

Peroxidase isozymes: Results of peroxidase isozymes are shown in Table (5) and Fig. (8). Each group of control and infected plants could be characterized by unique set of isozymes. The total number of peroxidase isozymes shown in all groups were 7 isozmes while those of each group (1-4) were 4, 6, 2, 7 respectively. Similarity correlation presented in Fig. (7) showed a low correlation between healthy (group 1) and infected (groups 2, 4) plants ($R \leq 0.33$). The characterized isozymes of each infected group were bands 1, 2, 5 (group 2), 2 (group 3) and 1, 2, 5 (group 4).

Table (3): Protein related index of three different ZYMV symptoms

Marker protein (KDa)	Healthy		Symptom-1		Symptom-2		Symptom-3		PR-index
	Fraction %	M.W. (KDa)	Fraction %	M.W. (KDa)	Fraction %	M.W. (KDa)	Fraction %	M.W. (KDa)	
-	-	-	-	-	7.01	123.60	7.08	123.60	123.60
-	7.98	103.70	7.87	103.70	8.06	103.70	10.46	103.70	-
97.40	-	-	-	-	-	-	-	-	-
-	2.93	76.68	7.19	76.68	-	-	-	-	-
58.10	5.49	58.10	-	-	4.89	58.10	5.67	58.10	-
-	0.15	47.36	0.74	47.36	1.21	47.36	0.23	47.36	-
39.80	2.56	39.80	2.26	39.80	-	-	-	-	-
-	-	-	-	-	9.37	37.65	7.64	37.65	37.65
-	-	-	-	-	-	-	0.28	33.00	33.00
-	5.93	34.00	4.91	34.00	1.24	34.00	4.42	34.00	-
29.00	9.71	29.00	-	-	7.51	29.00	9.56	29.00	-
-	-	-	8.13	26.68	1.52	26.68	1.01	26.68	26.68
-	14.18	24.78	9.91	24.78	7.87	24.78	7.48	24.78	-
-	-	-	-	-	11.14	23.26	7.34	23.26	23.26
-	8.54	21.63	2.39	21.63	-	-	-	-	-
20.10	-	-	3.49	20.10	-	-	8.47	20.10	20.10
-	-	-	3.72	19.35	2.32	19.35	-	-	19.35
-	4.76	18.32	-	-	6.36	18.32	1.73	18.32	-
-	3.53	16.13	-	-	-	-	-	-	-
14.30	8.88	14.30	7.80	14.30	-	-	7.59	14.30	-
-	-	-	-	-	7.87	14.00	8.64	14.00	14.00
Protein content	74.64	-	58.4	-	76.37	-	87.6	-	-

Symptom-1: Mosaic (group 2)
Symptom-2: Curling and crinkling (group 3)
Symptom-3: Malformation (group 4)
Fraction %: relative to the % of marker fractions

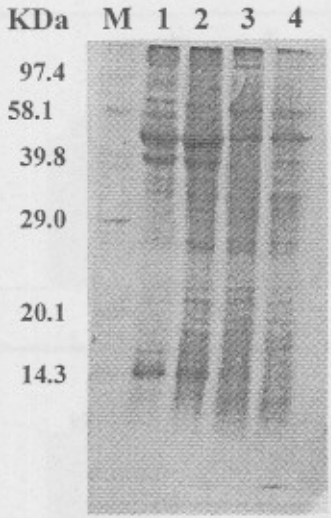
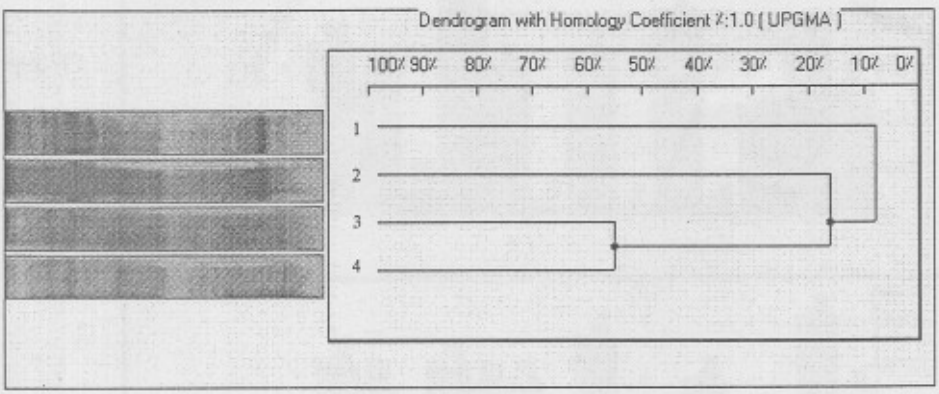


Fig. (2): SDS-PAGE (15%) of proteins extracted from squash leaves.
1. Healthy leaves, 2. Leaves showing naturally mosaic symptoms.
3. Leaves showing curl and crinkle naturally symptoms.
4. Leaves showing malformation symptoms (M = Marker protein).



Groups	1	2	3	4
1	1.00			
2	0.13	1.00		
3	0.00	0.12	1.00	
4	0.12	0.21	0.56	1.00

Fig. (3): Similarity analysis based on the protein content (group 1-4).

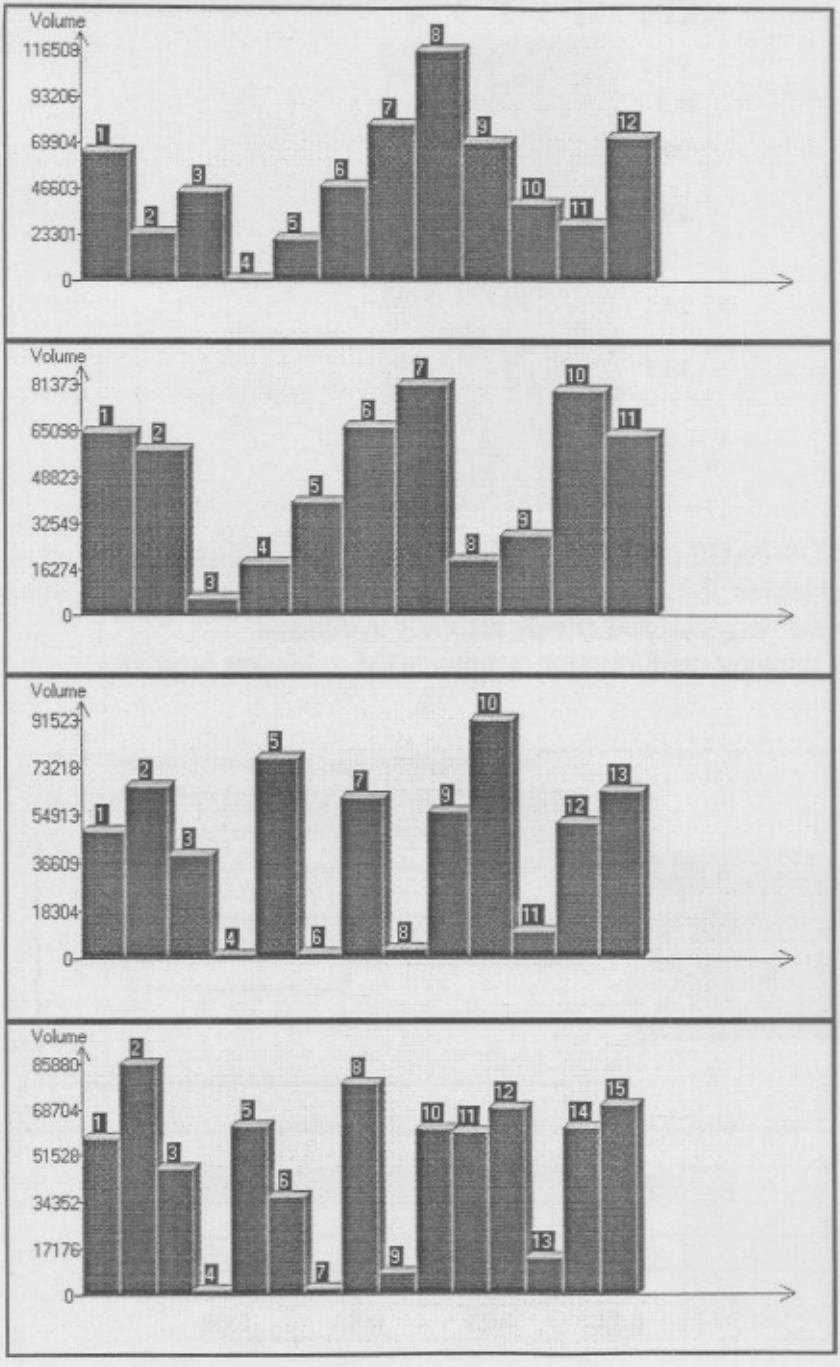


Fig. (4): Histogram illustrated protein patterns of (groups 1-4).

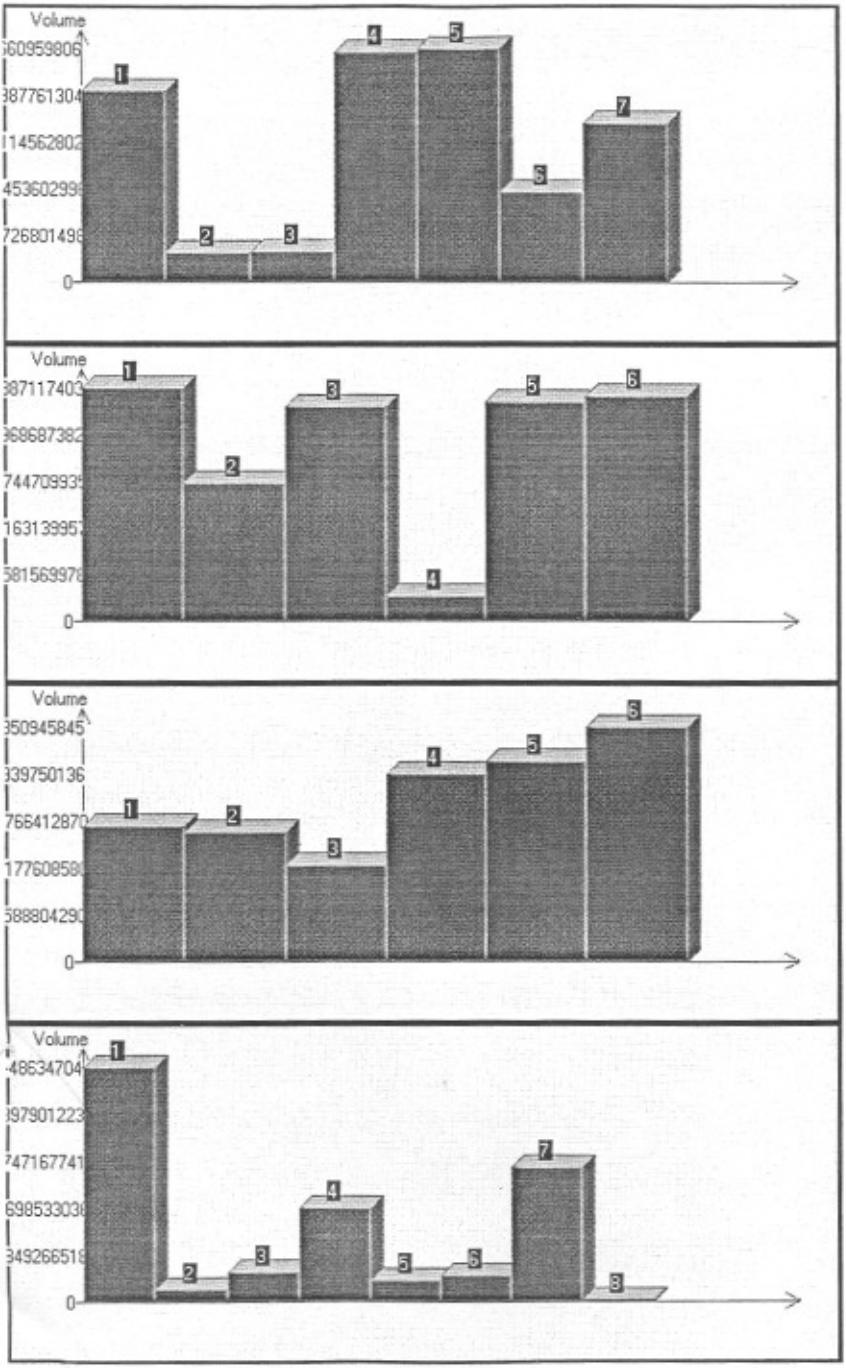
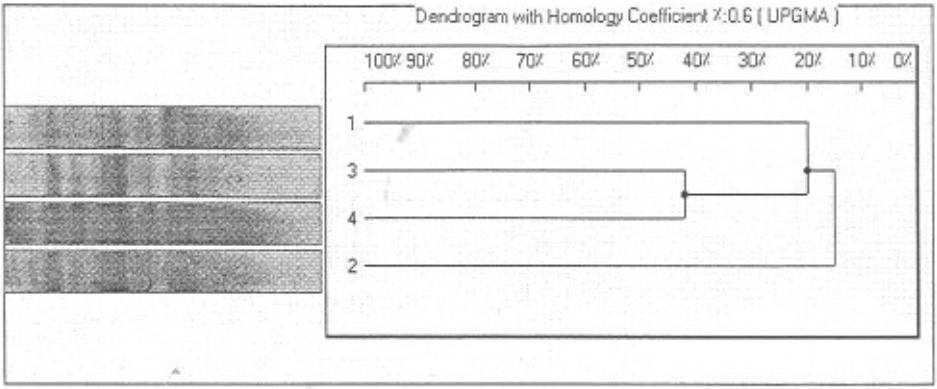
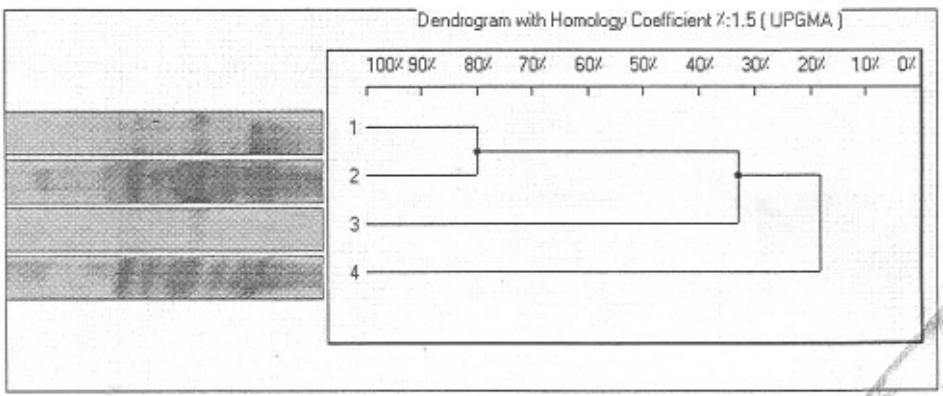


Fig. (5): Histogram illustrate esterase isozymes pattern volumes of healthy and infected plants (groups 1-4).



Groups	1	2	3	4
1	1.00			
2	0.31	1.00		
3	0.15	0.33	1.00	
4	0.40	0.43	0.43	1.00

Fig. (6): Similarity analysis based on esterase isozymes (groups 1-4).



Groups	1	2	3	4
1	1.00			
2	0.80	1.00		
3	0.33	0.50	1.00	
4	0.18	0.15	0.00	1.00

Fig. (7): Similarity analysis based on peroxidase isozymes (groups 1-4).

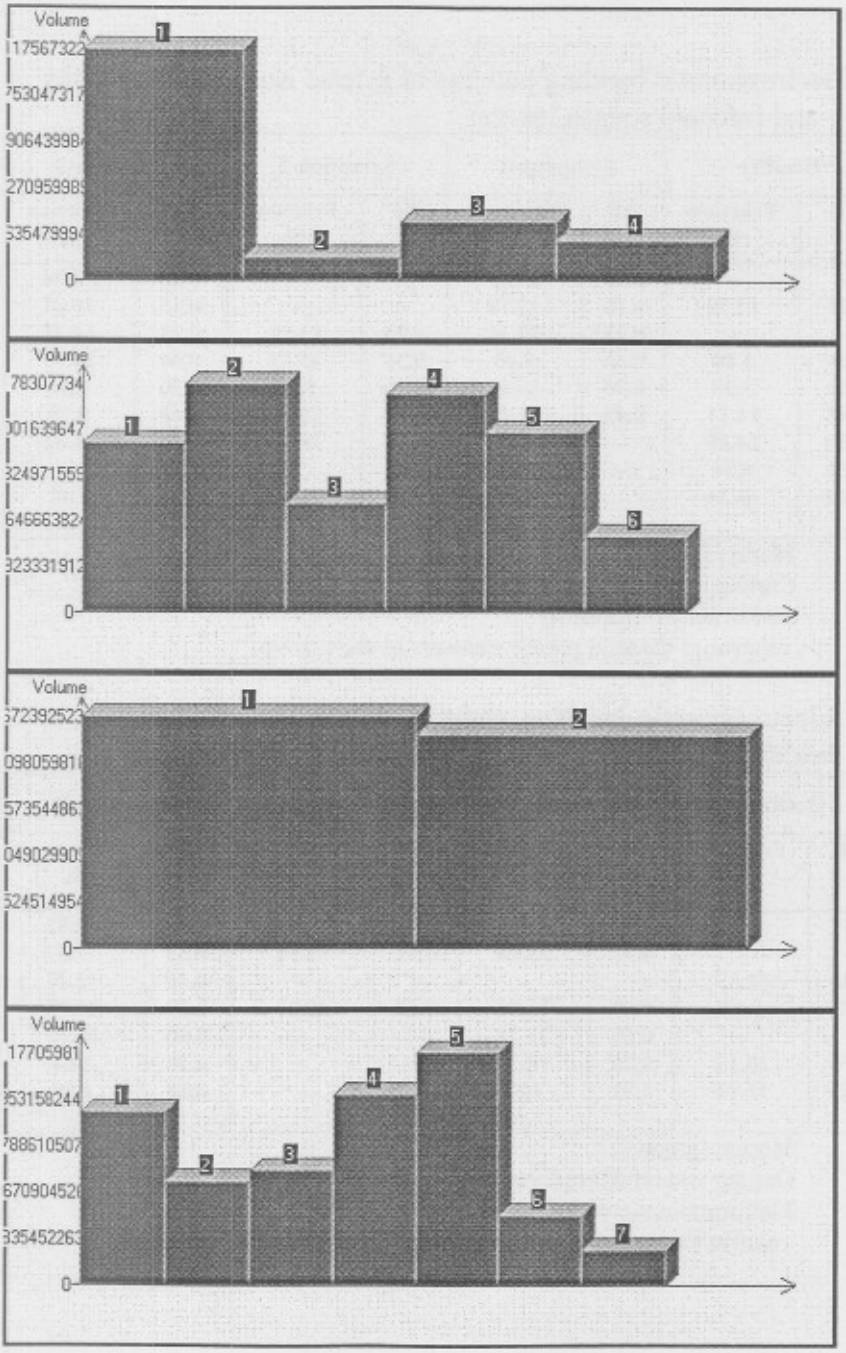


Fig. (8): Histogram illustrates peroxidase isozyme patterns and volumes of healthy and infected plants (groups 1-4).

Table (4): Electrophoretic banding patterns of esterse isozymes in healthy and infected squash leaves.

Band number	Healthy		Symptom-1		Symptom-2		Symptom-3	
	RF	Fraction %	RF	Fraction %	RF	Fraction %	RF	Fraction %
1	-	-	0.10	22.01	-	-	0.10	18.94
2	0.16	19.75	0.16	13.10	-	-	0.16	20.87
3	-	-	0.23	20.34	0.23	13.88	0.23	14.71
4	0.34	3.10	0.34	2.43	0.34	13.12	0.34	21.99
5	0.30	3.35	0.36	20.81	0.36	9.92	0.36	5.86
6	0.45	24.11	0.45	21.31	0.45	19.06	0.45	9.70
7	0.54	24.00	-	-	0.54	20.34	0.54	11.42
8	0.56	9.34	-	-	-	-	-	-
9	0.60	16.34	-	-	0.60	23.67	0.60	5.41

Symptom-1: Mosaic (group 2)
Symptom-2: Curling and crinkling (group 3)
Symptom-3: Malformation (group 4)
Fraction %: relative to the total protein contents in each group.

Table (5): Electrophoretic banding patterns of peroxidase isozymes in healthy and infected squash leaves.

Band number	Healthy		Symptom-1		Symptom-2		Symptom-3	
	RF	Fraction %	RF	Fraction %	RF	Fraction %	RF	Fraction %
1	-	-	0.12	17.26	-	-	0.12	18.82
2	-	-	0.31	23.24	0.31	51.91	0.31	11.37
3	0.40	62.33	-	-	-	-	0.40	12.38
4	0.57	6.52	0.57	11.06	0.57	48.09	0.57	20.75
5	-	-	0.60	22.31	-	-	0.60	25.08
6	0.76	20.15	0.76	18.30	-	-	0.76	7.61
7	0.82	11.00	0.82	7.82	-	-	0.82	3.99

Symptom-1: Mosaic (group 2)
Symptom-2: Curling and crinkling (group 3)
Symptom-3: Malformation (group 4)
Fraction %: relative to the total protein contents in each group.

DISCUSSION

Biological and serological detection of ZYMV are still being used (Ohtsu *et al.*, 1985 and Abo El-Nasr, 2004). However, biological tests require long period (30-45 day), experience and greenhouse conditions while serological methods are expensive. In addition, under certain condition, symptoms could be confusing and unclear. Therefore, new method should be developed to overcome obstacles. This method should meet two requirements. It should be independent of the pathogen, and should reflect the host genetics. Protein and isozyme analysis reasonably meet these requirements for several reasons. Amino acid sequence of polypeptides are dependent on nucleotide sequence of their coding genes; therefore, an electrophotetic analysis of proteins and isozymes of ZYMV infected leaves, showing different symptoms, approximates the analysis of their genetic variation. Electrophoretic patterns of protein and isozymes 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). The growing conditions have no influence on isozyme patterns (Kobrehel and Gautier, 1974).

The identification of the ZYMV based on symptomatology, host range mode of transmission and serology are still being used for ZYMV (Ohtsu *et al.*, 1985 and Abo El-Nasr, 2004). Symptoms of ZYMV disease on squash differed depending on the isolates as well as the environmental conditions (Makkouk *et al.*, 1979 and Aref and El-Dougdoug, 1996). By comparing the data presented in this study of the three viral symptoms mechanically transmitted with the results of Abo El-Nasr *et al.*, (2004), it was concluded that the three disease symptoms had ZYMV viral complex of one or more strains. Similar results were obtained for TYLCV-E (Aref and El-Dougdoug, 1996). Ohtsu *et al.*, 1985 and Abo El-Nasr *et al.*, 2004, reported that symptoms of ZYMV, mechanically transmitted, on *Antirrhinum majus, Chenopodium amaranticolor, Cucumis sativus, C. melo,* arer similar but different from Tomato yellow leaf curl virus, those appeared on *Physalis floridana*, tomato and *G. globosa*.

Biological assay was applied using local lesion on *Ch. Amaranticolor* (Mathews, 1991). Our results demonstrated that the naturally mosaic symptoms gave chlorotic local lesion, curl and

crinkle gave yellow chlorotic local lesion, and malformation symptom gave pin chlorotic local lesions (Table 2).

Serological assay of ZYMV using DAS-ELISA revealed that all the three naturally ZYMV symptoms gave positive reaction with IgG specific ZYMV by different absorbance at 405 nm. These results are in the range obtained by Salem (2004). Ghazalla (1998) concluded that visual inspection of plants was not an effective way for detecting the virus. He added that ELISA technique showed reliability for selecting virus-free stocks of plants as general.

Detection of ZYMV by protein related index (PR-index) was done *via* determination of polypeptides by using SDS-PAGE, and esterase and peroxidase using DISC-PAGE. Data presented in Table (3) indicated that the number of protein fractions increased in the infected plants (groups 2-4), on the other hand, protein contains increased in groups 3,4 while decreased in group 2. This result indicated that plant resistance could be due to increasing protein content or certain protein fractions or both together. For example group 2 showed decrease in protein content but increase in protein fractions 26.68, 20.10 and 19.35 KDa indicating that despite increasing the biodegradation of protein content, new protein types were detected due to virus infection. The new protein called b-protein or related protein. Groups 3 and 4 gave a new protein fraction at 14 KDa. The same observation was reported by (Hadidi, 1988 and El-Dougdoug, 1996). The synthesis of new proteins are due to the host-virus interaction. Previous results also indicated that protein contents could be increased dramatically (Camacho-Henriquez and Sanger, 1982) or decreased (Leal and Lastra, 1984).

Gene for tobacco b-protein has recently been identified (Parant and Asselin, 1984). Subsequently *do novo* synthesis of m-RNA of b-protein in infected tobacco by tobacco mosaic virus was reported. Furthermore probing of tobacco genomic DNA with cDNA clones specific to b-proteins identified that b-proteins are coded by family of genes and it has been suggested that these proteins play a role in induced resistance to viral infection (Parant and Asselin, 1984) and are interferon like (Gianinazi *et al.*, 1980).

The enzymatic pools and their metabolic pathways are the most important factors affecting pathogenicity especially with viruses. The results showed that the levels of peroxidase and esterase in infected leaves of groups 2-4 were higher than those in healthy leaves. These

high levels play an important role in the resistance in defence mechanism and detection of the ZYMV. Increase in these enzyme activity and isozymes has been detected after infection by pathogens in different host pathogen combinations (Hammeschmidt *et al.*, 1982; El-Dougdoug, 1996; Sherif and El-Dougdoug, 2000 and Sherif and El-Habbaa, 2000).

Related pathogen-protein index (PR-index) could also be used in identification, detection and presence of many plant pathogens e.g. virus like (viroid, phytoplasma), bacteria, and fungi in plant extracts using SDS-PAGE, Therefore, PR-index provides a rapid method for virus detection and suitable for large scale application.

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تطبيقات دلائل البروتينات والمشابهات الانزيمية فى التعرف على فيروس الموزيك الاصفر الزوكينى فى نباتات الكوسة المصابة

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مظاهر الاصابة فى الحقل لفيروس الموزيك الأصفر الزوكينى على نباتات الكوسة المصابة بالفيروس صنفت الى ثلاث مجموعات (الموزيك – التجعد – والتشوهات). إنتقلت هذة المظاهر بحقن العصير على نباتات كوسة سليمة تحت ظروف الصوبة. هذة الأعراض تم الكشف عنها وتمييزها بيولوجياً بالعوائل المفرقة وسيرولوجياً باستخدام إختبار الإليزا وكذلك التغير فى محتوى الكلوروفيل للتأكد أن كل من مظاهر الإصابة الثلاث ناتجة عن الأصابة لفيروس ZYMV .

تم تقييم استخدام البروتينات المصاحبة للاصابة بالفيروس ومشابهات الانزيمات كدليل للكشف عن الإصابة لفيروس الموزيك الأصفر الزوكينى فى نبات الكوسة والتمييز بين مظاهر الإصابة الثلاثة. وأظهرت البروتينات المفصولة بواسطة SDS-PAGE بروتينات مميزة ومحتوى بروتينى فى النباتات المصابة بالمظاهر (الموزيك ، التجعد ، والتشوهات) بالمقارنة بالنباتات السليمة.

كما أظهرت طريقة الفصل الكهربائية (DISC-PAGE) لمشابهات الانزيم من النباتات المصابة بالمظاهر الموزيك والتجعد والتشوة مقارنة بالنباتات السليمة عدد ٧، ٨،٦،٦ مشابهات لانزيم الإستيريز وعدد ٤، ٦، ٢، ٧ مشابهات لانزيم البيروكسيديز على الترتيب. وتحليل التشابهة الاحصائى يؤكد اختلاف التركيب البروتينى والمشابهات الانزيمية للثلاث مجموعات المصابة مقارنة بالنباتات السليمة مما يؤكد امكان استخدام الهجرة الكهربية للبروتينات المصاحبة ومشابهات الانزيمات لتمييز فيروس الموزيك الأصفر الزوكينى.

قام بتحكيم هذا البحث: أ.د/نجاح الشحات على و أ.د/حسين محمد جلال الدين على