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Resistance of Faba Bean Accessions to *Bean Yellow Mosaic Virus* and *Broad Bean Stain Virus*

Sherin Mahfouze[1*] • Eman Khattab[2] • Nour Gadalla[2,3]

[1] Genetics and Cytology Department, Genetic Engineering and Biotechnology Division, National Research Center, Dokki, 12622, Egypt
[2] Virus and Phytoplasma Research Department, Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt
[3] Biological sciences Department, Faculty of Science, King Abdul Aziz University (KAU). Jeddah, Kingdom of Saudi Arabia

Corresponding author: * **Sherinmahfouze@yahoo.com**

ABSTRACT

The primary objective of this study was to find new faba bean (*Vicia faba*) accessions resistant of *Bean yellow mosaic virus* (BYMV) and *Broad bean stain virus* (BBSV). In addition, since little genetic information is available on the resistance of faba bean to these viruses, this study aimed to investigate the changes in peroxidase activity and protein composition in faba bean leaves resistant to both BYMV and BBSV. The 15 faba bean accessions had different resistance to BYMV and BBSV based on disease index. Accessions No. 3, 4, 8 and 12 were immune while accessions No. 1, 5, 7, 9, 10, 11, 13 and 14 were highly resistant; accessions No. 6 and 15 were moderately resistant and accession No. 2 was tolerant. SDS-PAGE and POD-isozymes patterns were used to studying genetic variability among the immune, tolerant, or susceptible 15 faba bean accessions under mixed infection with both BYMV and BBSV. 12 protein markers with molecular sizes ranging from 145.2 to 6 KDa were observed in immune, tolerant and resistant plants but which were not present in the control. The highest number of markers appeared in accession No. 3 (six markers). Most pathogen-related proteins were observed in diseased plants with both BYMV and BBSV, e.g., accession No. 14 in which four bands appeared (92, 86.5, 21 and 14.8 KDa). Peroxidase activity increased in all faba bean accessions except for accessions No. 2, 11 and 15. Increasing peroxidase activity was related with host resistance to both viruses.

Keywords: DAS-ELISA, pathogen-related proteins, Peroxidase isozymes, SDS-PAGE

INTRODUCTION

Plants in nature are constantly challenged by a diverse array of pathogenic microorganisms. Often, their protective mechanisms involve an inducible defense system. The ability of plants to invoke such a defense reaction is assumed to be mediated by an initial recognition process that involves the detection of certain unique signal molecules of incompatible pathogens by receptor-like molecules in plants, resulting in a cascade of biochemical events that leads to the expression of resistance and susceptibility to a disease (Rylas *et al.* 1994). Host-pathogen interactions are assumed to generate signals that activate nuclear genes involved in plant defense responses leading to the induction of stress-related enzymes and differential expression of proteins (Kauss 1987; Keen 1990; Hammond and Jones 1996; Alfano *et al.* 1997).

Faba beans (*Vicia faba*) are widely grown as vegetable plants in Egypt. The species is considered a major staple food crop that is important for human and animal nutrition (Bond 1987; Hemida 2005). Under certain environmental conditions, beans can improve soil fertility and reduce the incidence of weeds, diseases and pests when grown in rotation with other crops (Mwanamwenge *et al.* 1998). Many viral diseases can affect faba bean plants, and this is considered a serious problem worldwide. Infection by certain viruses causes significant yield reduction and economic losses (El-Bramaw and El-Beshehy 2011). Viral diseases have an important status because they not only cause direct damage to the host but also predispose the plant to secondary invaders (Beute 1970). Among faba bean viruses, *Bean yellow mosaic virus* (BYMV) (family *Potyviridae*; genus *Potyvirus*) causes mosaics and necrosis in legumes, depending on the host genotype and virus strain (Derks *et al.* 1980) and *Broad bean stain virus* (BBSV) (family *Secoviridae*; genus *Comovirus*) (Kumari *et al.* 2010) which causes staining of the seeds. BYMV and BBSV are the most widespread and frequent viruses (Morales and Castaño 1987).

Pathogenesis-related proteins (PRPs) are known to be induced in plant tissues in response to pathogen infections, especially during the hypersensitive and systemic response (Van Loon and Van Strien 1999). However, the biological and biochemical functions of these PRPs during the defense reactions and developmental processes are unclear.

Peroxidase (POD; E.C. 1.11.1.7) catalyses the oxidation of various hydrogen donors in the presence of hydrogen peroxide (H_2O_2) and oxidizes phenolic substances. Their reaction products are highly reactive and toxic to pathogens. They play important role in the host-parasite interaction, disease development and defense reaction of infected plants. Enhanced phenol synthesis and POD activity in various host parasite combination was correlated with disease resistance (Ghosal *et al.* 2004).

The primary objective of this study was to find new faba bean accessions resistant to BYMV and BBSV. In addition, we wanted to investigate the changes in POD and protein composition in leaves of faba bean plants resistant to BYMV and BBSV when compared to susceptible and control plants since little genetic information is available on the resistance of faba bean to BYMV and BBSV.

MATERIALS AND METHODS

Plant materials

Fifteen accessions of faba bean (**Table 1**) (Centre for Genetic Resources, The Netherlands; origin = Egypt) were tested against faba bean viruses (BYMV and BBSV) using the double antibody sandwich-enzyme linked immune sorbent assay (DAS-ELISA). 15 accessions of faba bean were planted in the greenhouse over three seasons (2009-2011).

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Original Research Paper

Table 1 Faba bean (*Vicia faba*) accessions used in this study.

No.	Accessions	No.	Accessions
1	CGN 7866	9	CGN 13510
2	CGN 07740 Rebaya 40	10	CGN 07737 Nr. 49
3	CGN 15619 Nr. 50	11	CGN 10391
4	CGN 7790	12	CGN 10314
5	CGN 15626 Giza 3	13	CGN 15572
6	CGN 13460 Giza 2	14	CGN 7865
7	CGN 13515 Nr. 56	15	CGN 10346
8	CGN 13497 Nr. 46		

Response of faba bean to mixed infection (BYMV and BBSV)

100 seedlings from each accession were planted in sand and clay (1: 2, v/v) in pots (14 cm Ø). After 21 days of growth, plants with similar sizes were mechanically inoculated in 0.1 M phosphate buffer pH 7.4 (1:2, w/v) containing BYMV and BBSV isolates (BYMV and BBSV isolates supplied from Dr. Eman A. Khattab, Serology Laboratory, Virus and Phytoplasma Research Department, Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt).

The inoculated plants and control were maintained in an insect-proof greenhouse at 17-22°C supplemented with a 12-h photoperiod at 400–700 µmol m^{-2} s^{-1}. The plants were fertilized with a 19: 19: 19 N-P-K solution (Wadielnil, Egypt) and insecticides such as 0.2% Malathion (Wadielnil) were applied to ensure vigorous growth and plants free of insects. Plants were observed daily every two months for visible symptoms. The susceptibility of cultivars was confirmed by DAS-ELISA. The percentage of infected plants and Disease index (DI) values were calculated using the following formula (Tian *et al.* 1985): (0) no symptoms; (1) mild mosaic, (2) mottle, (3) mild mosaic + yellowing, (4) severe mosaic + yellowing, (5) yellowing + vein banding, (6) severe mosaic + stunting, (7) severe mosaic + stunting + yellowing, and (8) yellowing + severe mosaic + crinkle.

$$DI\ (\%) = \Sigma \frac{(\text{No. of plants of each grade} \times \text{Disease grade})}{(\text{Total number of plants} \times \text{the highest grade})} \times 100$$

Detection of BYMV and BBSV

All the samples were tested for the presence of BYMV and BBSV by technique the Double Antibody Sandwich-Enzyme-Linked Immuno Sorbent Assay (DAS-ELISA) as described by (Clark and Adams 1977), BYMV and BBSV ELISA. Polystyrene plates were coated with IgGs (BYMV and BBSV IgGs were supplied from Dr. Eman A. Khattab) diluted in coating buffer (pH: 9.6) and incubated at 37°C for 4 h. The plate then washed three times with washing buffer for 3 min intervals. 100 µl samples were loaded duplicate wells of polystyrene microtitre plate. After loading the diluted extracts of 100 µl the plates were incubated overnight at 4°C. Following washing, 100 µl of conjugated antibodies were added to each well and the plate was incubated at 37°C for 4 h. After 3 additional washes, freshly prepared *p*-nitrophenylphosphate in substrate buffer (1 mg/ml) were loaded to each well. The plate was incubated at room temperature and photometric measurement was done at 405 nm after 2 h. Samples were considered as positive if their absorbance values were more than 2.5 times the negative control. ELISA test was carried out with four repetitions including positive and negative controls.

Electrophoretic analysis of protein by SDS-PAGE

SDS-PAGE was used to detect genetic variability among immune, resistant, tolerant and susceptible faba bean accessions via quantitative and qualitative determination of the total proteins. This method was done according to Laemmli (1970) as modified by Studier (1973). In this protocol, electrophoresis is in a vertical slab gel between glass plates. Two gels consisted of two parts, the upper stacking gel (5%) and the lower resolving gel (15%). 80 µl of samples containing 1 mg/ml protein were denatured at 100°C for 3 min in 1% SDS containing 100 mM β-mercaptoethanol and Tris-glycine buffer at pH 6.8. Electrophoresis was conducted at 25 mA for 6-7 h. The two gels were stained with solution (10% v/v methanol, 10% v/v acetic acid, 0.0125% w/v Coomassie Brilliant blue R-250) and shaken gently for 24 h. The staining solution was replaced with destaining solution (5% v/v methanol, 7% v/v acetic acid) and shaken gently for 24 h. The gels were viewed by trans-illuminator (Uvitec, UK) and the position of the bands was recorded by photographing the gel.

Fig. 1 Symptoms of mixed infection both BYMV and BBSV on faba bean accession No., 6 showed severe mosaic and yellowing (right) compared with healthy (left).

POD extraction

1 g of young leaf extracts from faba bean accessions (immune, resistant, tolerant and susceptible) were analyzed for POD activity on non-denaturing gels. Fresh leaves were ground in a mortar in a 0.1 M tris-HCl buffer, pH 7.1. The leaf/buffer ratio was 1: 2 (w/v). The homogenate was centrifuged at 12,000 × *g* for 30 min at 4°C. The supernatant was used as a crude enzyme extract. Aliquots of enzyme extracts mixed with equal volumes of 40% sucrose were prepared. All samples were stored at -20°C until enzyme analysis.

POD visualization

POD-isozymes were separated by the method of Vallejos (1983). POD-isozymes were detected by incubating the gels for 5-20 min in a reaction mixture containing 0.5 mM benzidine hydrohloride and 10 mM H_2O_2 in 0.05 M acetate buffer, pH 4.9.

Gel analysis

Gels were analyzed by UVI Geltec version 12.4, 1999-2005 (Fort Lee, NJ, USA).

RESULTS

Fifteen faba bean accessions were mechanically inoculated with both BYMV and BBSV isolates. All faba bean accessions differed in response to BYMV and BBSV isolates and showed different degrees of infection (DI %) despite variations in symptoms (**Table 2**). Faba bean accessions No. 3, 4, 8, and 12 were immune (0% DI) to BYMV and BBSV. Accessions No. 1, 5, 7, 9, 10, 11, 13 and 14 were highly resistant (26.25, 25, 5, 37.5, 10, 25, 15 and 12.50% DI, respectively), accessions No. 6 (**Fig. 1**) and 15 were moderately resistant (60 and 40% DI), while accession No. 2 was tolerant with a 7.5% DI. These results were confirmed by DAS-ELISA (**Table 2**).

SDS-PAGE protein analyses of BYMV and BBSV mixed infected faba bean

The SDS-PAGE protein profiles of total soluble proteins from the leaves of the 15 faba bean accessions under control and mixed infection with BYMV and BBSV showed differences in banding patterns when compared with the healthy plants (**Fig. 2**). The results of SDS-PAGE revealed a total number of 47 bands at different molecular weights (MWs) ranged from about 205 to 5 KDa. The 28 bands

Table 2 Effectiveness of selection for resistance of BYMV and BBSV in 15 faba bean accessions evaluated in the greenhouse.

Faba bean accessions	Symptoms	Total number of tested plants	Disease index (%)	Confirmed index DAS-ELISA	Degree of resistance
1-CGN 7866	SM, St, Y	6/20	26.25	+	Highly Resistant
2-CGN 07740 Rebaya 40	MM, Y	4/20	7.50	+	Tolerant
3-CGN 15619 Nr. 50	O	0/20	0	-	Immune
4- CGN 7790	O	0/20	0	-	Immune
5-CGN 15626 Giza 3	SM, Y	10/20	25	+	Highly resistant
6-CGN 13460 Giza 2	SM, C, Y	12/20	60	+	Moderately resistant
7-CGN 13515 Nr. 56	MM	8/20	5	+	Highly resistant
8-CGN 13497 Nr. 46	O	0/20	0	-	Immune
9-CGN 13510	SM, St	10/20	37.5	+	Highly resistant
10-CGN 07737 Nr. 49	Mo	8/20	10	+	Highly resistant
11-CGN 10391	SM, Y	10/20	25	+	Highly resistant
12-CGN 10314	O	0/20	0	-	Immune
13- CGN 15572	SM, Y	6/20	15	+	Highly resistant
14-CGN 7865	Y, VB	4/20	12.5	+	Highly resistant
15-CGN 10346	Y, SM, C	8/20	40	+	Moderately resistant

C = crinkle, MM = mild mosaic, Mo = mottle, O = no symptoms, SM = severe mosaic, St = stunting, VB = vein banding, Y = yellowing

Table 3 Specific markers of total soluble proteins for BYMV and BBSV in immune, resistant and tolerant faba bean accessions.

Faba bean accession No.	MW (KDa)	Accessions														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
		R	T	I	I	R	R	R	I	R	R	R	I	R	R	R
1	145.2									1						
2	42										1					1
3	31			1												
4	26.5			1												
5	23.5								1							
6	21										1					
7	19		1	1												
8	15.9		1	1			1	1					1	1	1	
9	14.8			1									1	1		1
10	13									1						
11	11.5			1												
12	6											1				
Total = 12		0	2	6	0	0	1	1	1	2	2	1	2	2	1	2

MW* = molecular weight, R = resistant, I = immune, T = tolerant; 1 = band presence, 0 = band absent

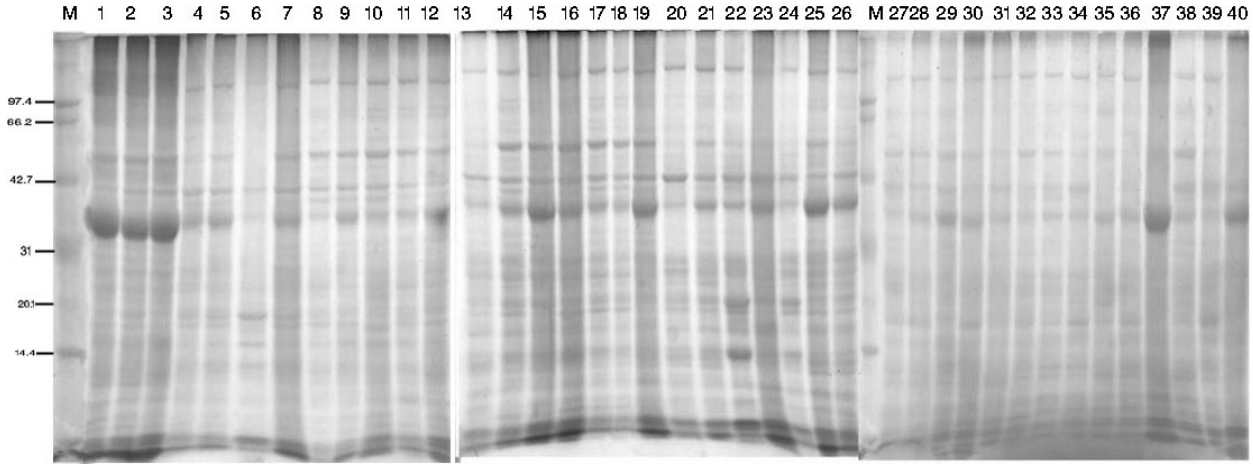

Fig. 2 SDS-PAGE profiles for total soluble proteins of faba bean accessions under mixed infection both BYMV and BBSV. M = marker (bp). Lanes 1, 2, 3 = control, immune and susceptible plants of accession No. 1, respectively. Lanes 4, 5 = control and tolerant plants of accession No. 2, respectively. Lanes 6, 7 = control and immune plants of accession No. 3, respectively. Lanes 8, 9 = control and immune plants of accession No. 4, respectively. Lanes 10, 11, 12 = control, resistant and susceptible plants of accession No. 5, respectively. Lanes 13, 14, 15 = control, resistant and susceptible plants of accession No. 6, respectively. Lanes 16, 17, 18 = control, resistant and susceptible plants of accession No. 7, respectively. Lanes 19, 20 = control and immune plants of accession No. 8, respectively. Lanes 21, 22, 23 = control, resistant and susceptible plants of accession No. 9, respectively. Lanes 24, 25, 26 = control, resistant and susceptible plants of accession No. 10, respectively. Lanes 27, 28, 29 = control, resistant and susceptible plants of accession No. 11, respectively. Lanes 30, 31 = control and immune plants of accession No. 12, respectively. Lanes 32, 33, 34 = control, resistant and susceptible plants of accession No. 13, respectively. Lanes 35, 36, 37 = control, resistant and susceptible plants of accession No. 14, respectively. Lanes 38, 39, 40 = control, resistant and susceptible plants of accession No. 15, respectively.

were varied in some distinctive faba bean accessions either in the immune, resistance, tolerant, or in susceptible plants and the remaining was monomorphic detected among all the accessions. On the other hand, 12 protein markers with molecular sizes ranged from 145.2 to 6 KDa were induced in immune, tolerant and resistant plants in faba bean accessions and disappeared in the healthy control. The highest number of markers scored in accession No. 3, (six markers)

Table 4 Specific markers of total soluble proteins for BYMV and BBSV in susceptible faba bean accessions.

Faba bean accession No.	MW (KDa)	Accessions						
		5	6	7	9	10	14	15
		S	S	S	S	S	S	S
1	92						1	
2	86.5						1	1
3	70				1			
4	23.5							1
5	22.8			1				
6	21		1				1	
7	16				1			
8	15.9	1				.		
9	15					1		
10	14.8	1					1	
Total = 10		2	1	1	2	1	4	2

MW* = molecular weight, S = susceptible

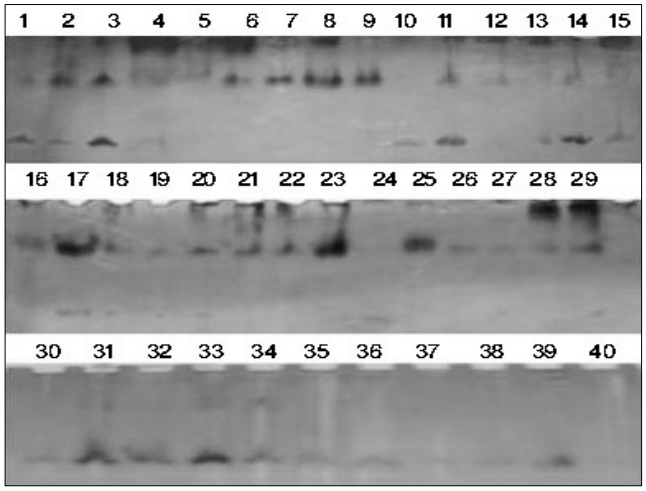

Fig. 3 POD-isozyme polymorphism profiles from healthy, immune, resistant, tolerant and diseased of faba bean accessions. Lanes 1, 2, 3 = control, immune and susceptible plants of accession No. 1, respectively. Lanes 4, 5 = control and tolerant plants of accession No. 2, respectively. Lanes 6, 7 = control and immune plants of accession No. 3, respectively. Lanes 8, 9 = control and immune plants of accession No. 4, respectively. Lanes 10, 11, 12 = control, resistant and susceptible plants of accession No. 5, respectively. Lanes 13, 14, 15 = control, resistant and susceptible plants of accession No. 6, respectively. Lanes 16, 17, 18 = control, resistant and susceptible plants of accession No. 7, respectively. Lanes 19, 20 = control and immune plants of accession No. 8, respectively. Lanes 21, 22, 23 = control, resistant and susceptible plants of accession No. 9, respectively. Lanes 24, 25, 26 = control, resistant and susceptible plants of accession No. 10, respectively. Lanes 27, 28, 29 = control, resistant and susceptible plants of accession No. 11, respectively. Lanes 30, 31 = control and immune plants of accession No. 12, respectively. Lanes 32, 33, 34 = control, resistant and susceptible plants of accession No. 13, respectively. Lanes 35, 36, 37 = control, resistant and susceptible plants of accession No. 14, respectively. Lanes 38, 39, 40 = control, resistant and susceptible plants of accession No. 15, respectively.

with MWs (31; 26.5; 19; 15.9; 14.8; and 11.5 KDa). Followed by, accessions No. 2, 9, 10, and 15 (two markers) with (19 and 15.19 KDa); (145.2 and 13 KDa); (42 and 21 KDa) and (42 and 14.8 KDa), respectively. Whenever, the accessions No. 12 and 13 were equally in number of markers (two markers) with the same MWs (15.9 and 14.8 KDa). The lowest number from markers induced in accessions No. 6, 7 and 14 with MWs 15.9 KDa and accession No. 11 with 6 KDa. On the other hand, the accessions No. 1, 4 and 5 have not appeared any markers. Additionally, three specific bands revealed in the immune plants of accession No., 3 with MWs 31, 26.5 and 11.5 KDa. The resistance plants of accession No., 9 with two markers at MWs (145.2 and 13 KDa). Moreover, the immune and resistance plants for accessions No., 8, 10 and 11 scored one specific band with MW 23.5, 21 and 6 KDa, respectively. Such newly induced bands are considered as protein markers for resistant to BYMV and BBSV infection and could be used as marker assistant selection (MAS) in faba bean breeding programs (**Table 3**).

BYMV and BBSV-related proteins

The BYMV and BBSV induce alterations in infected faba bean accessions proteins range from 92 to 14.8 KDa in total soluble protein (**Table 4**). The highest number of BYMV and BBSV-related proteins appeared in the susceptible plants of accession No. 14 at four proteins with MWs (92, 86.5, 21 and 14.8 KDa). However, the susceptible plants for accessions No. 5, 9 and 15 with two bands with MWs (15.9 and 14.8 KDa), (70 and 16 KDa), and (86.5 and 23.5 KDa), respectively. The lowest number from these bands showed in accessions No. 6, 7, and 10 with one band with MW 21, 22.8 and 15 KDa, respectively (**Table 4**).

POD electrophoresis

Analysis of isozyme profiles of POD in the 15 faba bean accessions under mixed infection with both BYMV and BBSV compared with the healthy control, as shown in (**Fig. 3**).The isozyme patterns of diseased and healthy faba bean plants produced similar types of banding patterns (**Fig. 3**). The bands were found to be hyperactive (Band intensity measured by O.D) in immune and resistant plants of all the accessions except the tolerant plants of accession No. 2 and the resistance plants of accessions No. 11 and 15. Additionally, the isozyme profiles of POD revealed the disappearance of some bands in the tolerant plants of accession No. 2, the immune plants of accessions No. 4 and 12 and the resistant plants of accessions No. 9, 13 and 14 which were present in their respective controls. In the case of the diseased plants isozyme profiling a clear extra band found in diseased plants such as accessions No. 1, 5, 6, 10 and 11 the other hyperactive bands observed in diseased plants compared with their respective healthy ones. The POD-isozyme profiles revealed the disappearance of some bands in diseased plants which were present in their respective controls such accessions No. 5, 7, 9, 12 and 14 (**Fig. 3**).

DISCUSSION

Fifteen faba bean accessions were mechanically inoculated with both BYMV and BBSV isolates. All *faba bean* accessions were revealed different responses to infection with two viruses depending on DI %. It was found that accessions No. 3, 4, 8 and 12 were immune, while accessions 1, 5, 7, 9, 10, 11, 13 and 14 were highly resistant, whenever accessions No. 6 and 15 were moderate resistant to infection with BYMV and BBSV. On the other hand, accession No. 2 was tolerant. These results were confirmed by DAS-ELISA. In spite of the importance of the diseases resistance in faba bean crop, the progress made in resistance breeding to BYMV and BBSV in faba bean is rarely and it should be taken into consideration in the future breeding programme. The spotlight has, therefore, shifted to host plant resistance. It is acknowledged that resistant faba bean varieties could potentially form the basis of sustainable management strategies for faba bean virus diseases. The selection of faba bean resistant varieties and continuous breeding programme for disease resistance appears to be the efficient means of controlling the disease considering faba bean viruses. (Jones and Mclean 1989; Asiedu 1998; El-Bramaw and El-Beshehy 2011). The selection of resistant accessions and continuous breeding programme for virus resistance appears to be the efficient means of controlling BYMV and BBSV. These results were in an agreement with (Gadh 1984) found that faba bean accessions varied in response to infection with BYMV from symptomless to immune.

The present investigator revealed enormous changes in biochemical components in faba bean accessions due to

mixed infection both BYMV and BBSV. The SDS-PAGE protein profiles of total soluble proteins from the leaves of the 15 faba bean accessions under the control and mixed infection with both BYMV and BBSV showed differences in band patterns when compared with their respective healthy plants (**Fig. 2**). These results were similar to those of Sela (1981), who mentioned that resistance-associated proteins are reported in several virus-host interactions. Plant pathogens such as viruses, bacteria, fungi and nematodes elicit the synthesis of plant proteins which help in restricting of the multiplication and spread of pathogens in the healthy tissues (Datta *et al.* 1999).

Analysis of pathogen-related proteins revealed that the content of such proteins was greater in the diseased plants with both BYMV and BBSV. Pathogenesis-related proteins (PRPs) are well known to be induced in plant tissues in response to pathogen infections, especially during the hypersensitive and systemic response. However, the biological and biochemical functions of these PRPs during the defense reactions and developmental processes are unclear (Kauss 1987; Keen 1990; Hammond and Jones 1996; Alfano *et al.* 1997; Van Loon and Van Strien 1999). Plants responded to pathogen attack by formation of new families of proteins called pathogenesis-related proteins or PR proteins. In this work, protein contents as well as protein profiles by SDS-PAGE were analyzed. Generally, induced amounts of soluble, insoluble and total proteins were detected in response to BYMV infection (Van Loon and Van Strien 1999). Radwan *et al.* (2010) found that BYMV infection leads to increase of about 15.28, 30.7 and 24.3% for soluble, insoluble and total proteins, respectively. Accumulation of proteins in virus-infected plants was reported previously by Fraser (1982) as pathogenesis-related proteins. Plants respond to various environmental challenges with diverse biochemical changes.

Analysis of isozyme patterns (POD) in all faba bean accessions (the immune, resistant, tolerant and diseased) as shown in (**Fig. 3**) showed increased POD-activity except accessions No. 2, 11 and 15 was associated with resistance reaction which could be due to increased phenol concentration, where phenols were cofactor of POD and hence influenced resistance in the host. Increased POD-activity was associated with resistance reaction which could be due to increased phenol concentration, where phenols were cofactor of POD and hence influenced resistance in the host. These results were in an agreement with Solymosy *et al*. (1967) who compared the changes in isozyme spectrum in various host virus combinations and showed that the change was determined mainly by the host tissue and not by virus. Isozyme analysis is a powerful tool for estimating genetic variability identifying cultivars and germplasm accessions. The differences in the isozyme binding patterns are due to variation in the amino acid content of the molecule, which in turn is dependent on the sequence of nucleotides in DNA (Micales *et al.* 1986). Different bands obtained indicate different electrophoretic mobilities of the isozymes, which are coded by different alleles or separate genetic loci. Therefore, such studies are useful in identifying and characterizing resistance in faba bean caused by infection both BYMV and BBSV. Since enzymes control biochemical reactions, and their syntheses are under the control of specific gene, any change in the activity of an enzyme would reflect the pattern of gene expressions and corresponding metabolic events in the cell. Hence, enzymes can be used as tools to study the induced responses of plants showing disease symptoms at the biochemical level (Neog *et al.* 2004). In addition, phenol-oxidizing enzymes such as POD and PPO are associated with many diseases (Pegg 1985). Nadlong and Sequeira (1980) suggested that the increased POD-activity following virus infection was a reflection of physiological changes associated with, but not responsible for, induced resistance whereas up-regulated POD might be responsible for growth reductions and malformations in virus-infected plants. POD participates in a variety of plant defense mechanisms (Mareschbacher *et al.* 1986) in which H_2O_2 is often supplied by an oxidative burst, a common event in defense responses (Dixon and Lamb 1990). The cell wall of plants appears to be a major site for defense-related POD polymerization reactions such as lignification (Hammerschmidt and Kuc 1982), suberization (Espelie *et al*. 1986) and cross-linking of structural cell wall proteins (Fry 1986). PODs comprise one important class of PR proteins (PR-9) implicated in these "defense responses," in which an important role is to catalyze the formation of phenolic radicals at the expense of H_2O_2 (Gaspar *et al*. 1985). PODs may also oxidize phenolic monomers to form lignin (Grisebach 1981), function in H_2O_2 production (Mader *et al*. 1980), and metabolize indole acetic acid (Mato *et al.* 1988). Each plant species typically displays a unique pattern and number of soluble and wall-bound isozymes that may respond differentially to environmental stimuli. The factor has known to influence POD-isozyme expression is pathogen infection (Ye *et al*. 1990).

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REFERENCES

Alfano JR, Collmer A (1997) The type III (Hrp) secretion pathway of plant pathogenic bacteria: Trafficking harpins, Avr proteins, and death. *Journal of Bacteriology* **179**, 5655-5662

Asiedu R, Ngs YC, Bai KV, Ekanayake IJ, Wanyera NM (1998) Genetic Improvement. In: Orkwor GC, Asiedu R, Ekanayake IJ (Eds) *Food Yams*: *Advances in Research*, IITA and NRCRI Ibadan, Nigeria, pp 63-104

Beute MK (1970) Effect of virus infection on susceptibility to certain fungus diseases and yield of gladiolus. *Phytopathology* **60**, 1809-1813

Bond DA (1987) Recent developments in breeding field beans (*Vicia faba* L.). *Plant Breeding* **99**, 1-26

Clark ME, Adams AN (1977) Characteristics of microplate methods of enzyme linked immunosorbent assay for detection of plant viruses. *Journal of General Virology* **34**, 475-483

Datta K, Muthukrishnan S, Datta SK (1999) Expression and function of PR-Proteins genes in transgenic plants. In: Datta SK (Ed) *Pathogenesis-Related Proteins in Plants*, CRC, Boca Raton, pp 261-291

Derks AF, Vink-Van JL, Muller PJ (1980) *Bean yellow mosaic virus* in some iridaceous plants. *Acta Horticulturae* **110**, 31-37

Dixon RA, Lamb CJ (1990) Molecular communication in interactions between plants and microbial pathogens. *Annual Review of Plant Molecular Biology* **41**, 339-367

El-Bramaw MAE, El-Beshehy EKF (2011) The resistance of *bean yellow mosaic virus* (BYMV) in faba bean (*Vicia faba* L.) with diallel analysis. *Journal of Biology and Life Science* **2 (1)**, 1-15

Espelie KE, Franceshi VR, Kolattukudy PE (1986) Immunocytochemical localization and time course of appearance of an anionic peroxidase associated with suberization in wound healing potato tuber tissue. *Plant Physiology* **81**, 487-492

Fraser RSS (1982) Are pathogenesis-related proteins involved in acquired systemic resistance of tobacco plants to *Tobacco mosaic virus*? *Journal of General Virology* **58**, 305-313

Fry SC (1986) Cross-linking of matrix polymers in the growing cell walls of angiosperms. *Annual Review of Plant Physiology* **37**, 165-183

Gadh IPS, Bernier CC (1984) Resistance in faba bean (*Vicia faba*) to *bean yellow mosaic virus*. *Plant Disease* **68**, 109-111

Gaspar T, Penel C, Castillo FJ, Greppin H (1985) A two-step control of basic and acidic peroxidases and its significance for growth and development. *Physiology Plantarum* **64**, 418-423

Ghosal TK, Dutta S, Senapati SK, Deb DC (2004) Role of phenol contents in legume seeds and its effect on the biology of *Collosbrchus chinensis*. *Annual Plant Protection Science* **12**, 442-444

Grisebach H (1981) Lignins. In: Stumpf PK, Conn EE (Eds) *The Biochemistry of Plants* (Vol 7), Academic Press, New York, pp 457-478

Hammerschmidt R, Kuc J (1982) Lignification as a mechanism for induced systemic resistance in cucumber. *Physiology and Plant Pathology* **20**, 61-71

Hammond KKE, Jones JDG (1996) Resistance gene-dependent plant defense responses. *Plant Cell* **8**, 1773-1791

Hemida SK (2005) Effect of *bean yellow mosaic virus* on physiological parameters of *Vicia faba* and *Phaseolus vulgaris*. *International Journal of Agriculture and Biology* **7 (2)**, 154-157

Jones RAC, Mclean GD (1989) Virus diseases of lupins. *Annals of Applied Biology* **114**, 609-937

Keen NT (1990) Gene-for-gene complementarity in plant-pathogen interactions. *Annual. Review of Genetics* **24**, 447-463

Kauss H (1987) Some aspects of calcium-dependent regulation in plant metabolism. *Annual Review of Plant Physiology* **38**, 47-72

Kumari AN, Attar N, Loh MH, Vetten HJ (2010) First report of *Beet mosaic virus* infecting chickpea (*Cicer arietinum*) in Tunisia. *Plant Disease* **94 (8)**, 1068

Laemmli UK (1970) Cleavage of structural protein during the assembly of the head of bacteriophage T_4. *Nature* **227**, 680-689

Mader M, Ungemach J, Schlob P (1980) The role of peroxidase isoenzyme groups of *Nicotiana tabacum* in hydrogen peroxide formation. *Planta* **147**, 467-470

Mareschbacher BM, Kogel KH, Noll U, Reisener HJ (1986) An elicitor of the hypersensitivity lignification response in wheat leaves isolated from the rust fungus *Puccinia graminis f.sp. tritici*. I. Partial purification and characterization. *Zeitschrift für Naturforschung* **41**, 830-838

Mato MC, Rúa ML, Ferro E (1988) Changes in levels of peroxidases and phenolics during root formation in *Vitis* cultured *in vitro*. *Physiology Plantarum* **72**, 84-88

Micales JA, Bonde MR, Peterson GL (1986) The use of isozyme analysis in fungal taxonomy and genetics. *Mycotaxon* **27**, 407-449

Morales FJ, Castaño M (1987) Seed transmission characteristics of selected *bean common mosaic virus* strains in differential bean cultivars. *Plant Disease* **71**, 51-53

Mwanamwenge J, Loss SP, Siddique KHM, Cocks PS (1998) Growth. Seed yield and water use of faba bean (*Vicia faba* L.) in a short-season Mediterranean-type environment. *Australian Journal of Experimental Agriculture* **38**, 171-180

Nadlong L, Sequeira L (1980) Increases in peroxidase activities are not directly involved in induced resistance to *Tobacco mosaic virus*. *Physiological Plant Pathology* **16**, 1-8

Neog B, Yadav RNS, Singh ID (2004) Peroxidase, polyphenol oxidase and acid phosphatase activities in the stigma-style tissue of *Camellia sinensis* (L) O Kuntze following compatible and incompatible pollination. *Journal of the Indian Institute of Science* **84**, 47-52

Pegg GF (1985) Life in a black hole: The micro-environment of the vascular pathogen. *Transactions of the British Mycological Society* **85**, 1-20

Radwan DEM, Fayez KA, Mahmoud SY, Lu G L (2010) Modifications of antioxidant activity and protein composition of bean leaf due to *Bean yellow mosaic virus* infection and salicylic acid treatments. *Acta Physiologiae Plantarum* **32**, 891-904

Ryals J, Uknes S, Ward E (1994) Systemic acquired resistance. *Plant Physiology* **104**, 1109-1112

Sela I (1981) Plants-virus interaction related to resistance and localization of viral infections. *Advances in Virus Research* **26**, 201-237

Solymosy F, Szirmai J, Beezner L, Farkas GL (1967) Changes in peroxidase enzyme patterns induced by virus infection. *Journal of Phytopathology* **57**, 825

Studier FW (1973) Analysis of bacteriophage T, early RNAs and proteins of slab gel. *Journal of Molecular Biology* **79**, 237-248

Tian WH, Cao SX, Sun Q, Chang SQ, Chang XH, Tien P (1985) Biological properties of CMV-S51 containing satellite RNA. *Acta Phytopathologica Sinica* **15**, 145-149

Vallejos CE (1983) Enzyme activity staining. In: Tonskley SD, Orton TJ (Eds) *Isozymes in Plant Genetics and Breeding Part A*, Elsevier Science Publishers BV, Amsterdam. pp 469-516

Van Loon LC, Van Strien EA (1999) The family of pathogenesis related proteins, their activities and comparative analysis of PR-1 type proteins. *Physiological and Molecular Plant Pathology* **55**, 85-97

Ye XS, Pan SQ, Kuc J (1990) Activity, isozyme pattern, and cellular localization of peroxidase as related to systemic resistance of tobacco to blue mold (*Peronospora tabacina*) and to *tobacco mosaic virus*. *Phytopathology* **80**, 1295-1299