



Plant Pathology Journal

ISSN 1812-5387

science
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Revolutionize in Protein Patterns of Different *Vicia faba* L. Cultivars Infected with *Broad bean true mosaic virus* under Salicylic Acid Treatments

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Abstract: A variety of faba bean cultivars infected with BBTMV showed symptoms as severe mosaic, mottling, crinkling, size decline and malformation. Salicylic Acid (SA) application before inoculation specially 100 μ M concentrate was reduced the virus symptoms. In Infected plants under all SA treatments were decreased the viral concentration, percentage of infection and severity of diseases compared with decreasing under BBTMV infection without SA treatments. Increase the level of SA play role important for resistance against BBTMV infection in all faba bean cultivars. Possessions of viral disease with SA application were tested after 21 days from inoculation measured up to healthy control on some protein characters include the quantity of protein bands, molecular weight of band and percentage of the protein band in all diverse faba bean cultivars within this investigation. The results showed that all SA treatments increased all properties of protein patterns. The highest increase was observed when seedling treated by 100 μ M (SA and BBTMV) compared with uninfected control. The highest increase of protein contents was observed in infected cv. Sakhal and lower increase was noticed in cv. Tribe White. In the largest parts of SA applications, the all protein characters were mentioned above an increased includes protein amounts and numbers. Total soluble proteins were accumulated when healthy plants were treated by SA. Stimulation of broad bean cultivars to BBTMV infection under SA application were observed in found many modifications in protein patterns. Probably, the polypeptides accumulation is depending on the SA level. The main purpose of this research was to invistegate the effects of BBTMV and SA treatments on symptoms development, virus concentrations, percentage of infections, disease severity, protein patterns in different broad bean leave cultivars, protein bands numbers, molecular weight bands and percentage of amounts in susceptible and tolerance broad bean cultivars.

Key words: Salicylic acid, *Vicia faba*, *Broad bean true mosaic virus*

INTRODUCTION

Faba bean, *Vicia faba* L. (Fabaceae), is considered as one of the most important food legumes in Egypt. The faba bean is a major staple food crop in Africa. Farmers plant around four million hectares of beans which correspond to 20% of total crop area planted (Bond, 1987). Broad beans can get better soil productiveness and decline the incident of infections, under certain environmental conditions (Mwanamwenge *et al.*, 1998). A number of viruses may cause severe diseases in faba bean crops. There are 15 different viruses in the genus *Comovirus* of the family Comoviridae, such as BBTMV. *Vicia faba* L. one of the most important host plant to BBTMV, including other legume plants like peas, (Quantz, 1953). Symptoms of BBTMV infection can be

severe in cool includes mild mosaic, malformation and necrosis (Paul and Quantz, 1959). Salicylic Acid (SA) is a natural signaling molecule involved in plant defense response against pathogen infection. Its function in plant disease resistance is well recognized for plants, where it is required for basal resistance against pathogens as well as for the inducible defense mechanism, Systemic Acquired Resistance (SAR) which confers resistance against a broad-spectrum of pathogens. (Chaturvedi and Shah, 2007). Our study appraised the special effects of exogenous treatment SA on the disease severity, BBTMV infection percentage, protein patterns in cultivars, protein bands numerals, molecular weight for each band and percentage of bands in susceptible and tolerance broad bean cultivars.

MATERIAL AND METHODS

The DAS-ELISA technique was applied as described by Clark and Adams (1977) to measure BBTMV concentrations in the infected and (SA+V)-treated leaves using three antibodies BBTMV, BBSMV and BYMV were obtained from International center for Agricultural Research in the Dry Areas (ICARDA), Plant virology, P.O. Box 5466, Aleppo, Syria.

Four cultivars of faba bean, cv. Tribe White, Giza 3, Giza 717 and Sakha 1) were planting in natural and favorable conditions suitable for broad bean growth. BBTMV used in these experiments was prepared from fresh severely infected leaves of faba bean cv. Giza 3. After 21 days of growth, plants with similar size were selected and divided into five groups. Each group consists of four replicates (a replicate is one pot containing three healthy plants). The namely of the groups was as follows, group 1: Control (sprayed with water), group 2: Inoculated with virus at the same time with the other groups, group 3: 50 µM (SA and BBTMV), group 4: 100 µM (SA and BBTMV), sprayed by SA and inoculated with virus 3 days later (SA and BBTMV) and group 5: 100 µM SA, sprayed without followed virus inoculation.

Every part of plant leaves were sprayed by SA treated. All leaves were mechanically inoculated by BBTMV inoculation after three days from SA spraying (Radwan *et al.*, 2008). Disease severity and percentage of infection were calculated after 21 days from seedling inoculation and were recorded according to the subsequent balance (0) No external symptoms, (1) Light mottling and crinkling, (2) Mottling and crinkling, (3) Strict mosaic, size diminution and (4) Deformation. Disease Severity (DS) values were calculated using the following formula according to Yang *et al.* (1996):

$$DS (\%) = \frac{\text{Disease grade} \times \text{No. of plants in each grade}}{\text{Total No. of plants} \times \text{Highest disease grade}} \times 100$$

The youngest developed leaves from both control and treated plants from four cultivars broad bean were collected after three weeks from inoculation for analysis of changes, 50 mg dry tissues of each cultivar from four broad bean leave cultivars infected and uninfected by BBTMV were treated with different salicylic acid concentrations and compared with control. the leaves cultivars were crocheted and extracted with SDS reducing buffer to determine the total soluble protein contents.

SDS-PAGE procedure was portrayed by Laemmli (1970). Gels were photographed and scanned by Gel Doc Bio-Rad System (Gel-Pro analyzer V3) t determine the

percentage of similarity of four broad bean leaves cultivars of each treatment group and electrophoretic profile of total soluble protein extracted from treated and untreated four broad bean leaved cultivars.

ANOVA type one-way were used to calculated the noteworthy diversity in the averages of the experimental treatments. A probability at level of 0.05 or less was measured considerable.

RESULTS AND DISUSSION

The leaf cultivars of broad bean (Tribe White, Giza 3, Giza 717 and Sakha 1) were exhibited systemic rigorous symptom under BBTMV inoculation compared with non-inoculated. Novel symptoms appeared including mosaic and crinkling, size diminution and malformation in susceptible cultivars cv. Giza 3 and Tribe White (Fig. 1), other than, mild mosaic, size reduction and light mottling in tolerant cultivars CV. Giza 717 and Sakha 1 (Fig. 2). On the other hand, SA treatments diminished the exterior of damaging symptoms reasoned by virus progress, principally when the leaves were treated with 100 µM SA under BBTMV infection while 100 µM (SA+V) treated leaves had no celebrate symptoms. On the other hand, mild BBTMV symptoms were observed by 50 µM (SA+V) application (Radwan *et al.*, 2007; Radwan *et al.*, 2008). This type of the systemic resistance might be initiated by SA is spraying in broad bean plants against BYMV Elbadry *et al.* (2006).

The products of several disobedience genes were triggered appearance and connected with plant tolerance, possibly will playing significant responsibility in the constraint of pathogen development and dispersal. Typical characteristic indicator genes of Systemic Acquired Resistance (SAR) are for instance encoding pathogenesis-related protein PR1 (Van Loon and Van Kammen, 1970; Fraser, 1982; Van Loon *et al.*, 1994; Van Loon and Van Strien, 1999).

Results showing in Fig. 3a-c, revealed that BBTMV infection inoculated plants have higher percentage of infection, severity of disease and concentration of virus while lower in case of SA treated plants and control. This regular lessen of infection percentage, severity of disease and concentration of virus with an increase level of SA play role important for resistance against BBTMV infection in all cultivars of faba bean compared with untreated one. Radwan *et al.* (2008) also recorded that SA treatments reduced the concentration of BYMV, infection percentage and severity of disease.

The results tabulated in (Table 1 and 2) and illustrated in (Fig. 4) showed that the susceptible cultivars cv. Giza 3 and Tribe White were recorded a low number of



Fig. 1(a-b): Stimulation of susceptible faba bean leaf cultivars cv. Giza 3 and Tribe White to BBTMV infection showing mosaic and crinkling, size diminution and malformation compared with control (a) Susceptible cultivars cv. Giza 3 and (b) Tribe white

protein bands in all treated groups (6, 4; 6, 6; 5, 6; 7, 10 and 6, 8 PBs), respectively, when inoculated by BBTMV under SA treatments compared with control. Their Molecular Weights (MW) were arranged between 84 to 28 KDa in cv. Giza 3 and arranged between 162 to 29 KDa in cv. Tribe white. While, The results tabulated in (Table 3 and 4) and illustrated in (Fig. 5) showed that the tolerance cultivars cv. Giza 717 and Sakha 1 were recorded high number of protein bands (7, 11; 8, 12; 9, 12; 10, 14 and 8, 11 PBs) respectively, in all treated groups when inoculated by BBTMV under SA treatments compared with control and (MW) were arranged between 265 to 28 KDa in cv. Giza 717 and arranged between 157 to 33 KDa in cv. Sakha1 and these results are in agreement with (Radwan *et al.*, 2007).

Stimulation of infected broad bean under SA application on percentage of protein band amount (% of Amo.) in difference faba bean leave cultivars were presented in (Table 1-4) and illustrated in (Fig. 4 and 5). In susceptible Giza 3 and Tribe white cultivars (% of Amo.) arranged between 1.758-18.257 and 3.245-16.349% in treated group1; 6.032 to 17.825 and 1.996-14.289% in treated group 2; 6.658-15.024 and 1.987-17.289% in treated group 3; 7.008-21.929 and 1.720 to 21.389% in treated group 4 plus 6.203-18.937 and 1.542-19.242% in treated group 5, respectively while, in tolerance Sakha1 and Giza 717 cultivars (% of Amo.) arranged between 2.417-21.133 and 5.853-16.239%; 3.634-23.695 and 5.397-21.341%; 3.663-26.038 and 5.702-23.750%; 3.484-35.996 and 5.990 to 35.451% also 3.581-30.193 and



Fig. 2(a-b): Stimulation of tolerance cultivars cv. Giza 717 and Sakha 1 to BYMV infection showing mild mosaic, size reduction and light mottling compared with control (a) Tolerance cultivars cv. Giza 717 and (b) cv. Sakha 1

3.512-32.747%, respectively. Therefore, the percentage of protein amount bands in infected lanes were less than that of the control in cv. Tribe white and Giza 3 but, more than in cv. Sakha 1 and Giza 717.

On the other hand, it can be distinguishable that sole bands include a high percentage of the protein band amount were presented in infected cultivars under all treatments by salicylic acid and absented in control healthy such as band number 9 (MW. 52 and RF. 0.699) of cv. Giza 3 absent in treated group 1 (control healthy) and was recorded 12.321, 13.538, 15.822 and 13.367% in treated groups (2-5), correspondingly. Alternatively, band number 7 (MW. 55 and RF. 0.659) present in healthy control group 1 was recorded 12.862% and absent in treated group (2-5).

The bands number 16 (MW. 50 and RF. 0.737) and 19 (MW. 42 and RF. 0.803) of cv. Tribe white were absent in treated group 1 and were recorded (14.591, 13.137, 17.439 and 13.367%) and (13.755, 17.289, 21.389 and 19.242%) in treated group (2-5), in that order. Conversely, bands number 17 (MW. 47 and RF. 0.757) and 20 (MW. 41 and RF. 0.817) present in healthy control group 1 were recorded 19.296% and 16.349, respectively and absent in treated group (2-5).

In addition to, the bands number 24 (MW. 115 and RF. 0.366), 39 (MW. 54 and RF. 0.685) and 44 (MW. 33 and RF. 0.953) of cv. Sakha 1 were absent in treating group 1 and were recorded (17.987, 26.038, 26.049 and 24.095%), (23.695, 24.461, 26.921 and 25.947%) and (16.779, 19.631, 29.321 and 19.956%) in treated group (2-5), correspondingly. On the other hand, bands number 27 (MW. 102 and RF. 0.413) and 32 (MW. 76 and RF. 0.525) present in healthy control group 1 were recorded 16.071% and 21.133, respectively and absent in treated group (2-5).

Finally, the bands number 10 (MW. 83 and RF. 0.548), 14 (MW. 65 and RF. 0.651) and 15 (MW. 58 and RF. 0.698) of cv. Giza 717 were absent in treated group 1 and were recorded (21.341, 21.680, 28.538 and 27.998%), (13.234, 19.896, 33.047 and 21.063%) and (17.974, 17.721, 30.570 and 26.080%) in treated group (2-5), respectively. Then again, bands number 9 (MW. 85 and RF. 0.536) and 20 (MW. 49 and RF. 0.772) present in healthy control group 1 were recorded 14.265% and 15.065, respectively and absent in treated group (2-5).

Orientation of lofty quantity of proteins was attended to cooperate a significant position in resistance reply in opposition to viruses. Van Loon (1985) confirmed that in more than a few plants groups infected by many viruses

Table 1: Effect of salicylic acid treatments on protein bands in infected faba bean Giza 3 cultivar after 21 days from inoculation by BBTMV

		Properties of leaf protein bands in Giza3 cultivar														
Bands No.	M.W (kDa)	Group 1			Group 2			Group 3			Group 4			Group 5		
		RF	Bands	Protein band (%)	Bands	Protein band (%)	Bands	Protein band (%)	Bands	Protein band (%)	Bands	Protein band (%)	Bands	Protein band (%)		
1	84	0.504	-	-	-	6.451	-	-	-	-	-	-	-	-	-	-
2	83	0.576	-	-	-	-	-	-	-	-	-	-	-	-	-	6.203
3	70	0.578	+	1.758	-	-	-	-	-	-	-	-	-	-	-	-
4	66	0.605	-	-	-	-	-	-	-	-	-	-	-	-	-	15.337
5	63	0.612	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	62	0.643	+	3.874	-	-	-	-	-	-	-	-	-	-	-	-
7	55	0.659	+	12.862	-	-	-	-	-	-	-	-	-	-	-	-
8	53	0.672	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	52	0.699	-	-	-	12.321	-	-	-	-	-	-	-	-	-	-
10	47	0.714	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	46	0.727	+	16.689	-	-	-	-	-	-	-	-	-	-	-	-
12	39	0.808	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	38	0.819	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	32	0.909	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	31	0.938	+	5.744	-	-	-	-	-	-	-	-	-	-	-	-
16	30	0.946	-	-	-	6.032	-	-	-	-	-	-	-	-	-	-
17	28	0.996	+	18.257	-	-	-	-	-	-	-	-	-	-	-	-
						17.825	+	10.825	+	11.536	+	10.825	+	11.536	+	-
						6.813	-	6.658	+	19.934	-	6.658	+	19.934	-	-
						9.187	-	9.187	+	21.929	-	14.61	+	21.929	-	-
						9.187	-	9.187	+	14.61	-	14.61	+	14.61	-	-
						12.321	-	12.321	+	15.822	-	15.822	+	15.822	-	-
						13.538	-	13.538	+	10.079	-	10.079	+	10.079	-	-
						15.024	-	15.024	+	10.037	-	10.037	+	10.037	-	-
						15.024	-	15.024	+	7.008	-	7.008	+	7.008	-	-

Table 3: Effect of salicylic acid treatments on protein bands in infected faba bean Sakhal cultivar after 21 days from inoculation by BBTMV
Properties of leaf protein bands in Sakhal cultivar

Bands No.	M.W (kDa)	RF	Group 1		Group 2		Group 3		Group 4		Group 5	
			Bands	Protein band (%)	Bands	Protein band (%)	Bands	Protein band (%)	Bands	Protein band (%)	Bands	Protein band (%)
1	265	0.076	-	-	-	-	-	-	+	-	-	-
2	229	0.125	-	-	-	-	-	-	-	-	+	9.358
3	212	0.151	-	-	-	-	-	-	-	-	-	-
4	205	0.162	+	2.417	+	3.634	+	11.279	-	-	-	-
5	197	0.177	+	18.336	+	13.678	-	-	-	-	-	-
6	192	0.186	-	-	-	-	+	8.481	-	-	-	-
7	189	0.191	-	-	-	-	-	-	+	8.240	-	-
8	187	194	-	-	-	-	-	-	-	-	+	3.613
9	183	0.210	-	-	-	-	+	3.663	-	-	-	-
10	182	0.204	-	-	-	-	-	-	+	18.192	+	3.581
11	157	0.255	+	6.047	-	-	-	-	-	-	-	-
12	156	0.257	-	-	+	11.870	-	-	-	-	-	-
13	152	0.267	+	10.638	-	-	-	-	-	-	-	-
14	151	0.268	-	-	+	5.678	-	-	-	-	-	-
15	150	0.272	-	-	-	-	-	-	+	28.721	-	-
16	149	0.273	-	-	-	-	-	5.898	-	-	-	-
17	146	0.281	-	-	-	-	-	-	+	35.996	+	27.964
18	143	0.288	-	-	-	-	+	11.896	-	-	-	-
19	142	0.292	-	-	-	-	-	-	-	-	+	11.218
20	123	0.342	+	5.970	-	-	-	-	-	-	-	-
21	120	0.351	-	-	+	6.023	-	-	-	-	-	-
22	119	0.356	+	8.164	-	-	-	-	-	-	-	-
23	117	0.362	-	-	-	-	+	16.057	-	-	-	-
24	115	0.366	-	-	+	17.987	+	26.038	+	26.049	+	24.095
25	114	0.371	-	-	-	-	-	-	-	-	-	-
26	112	0.378	-	-	-	-	-	-	-	-	+	8.097
27	102	0.413	+	16.071	-	-	-	-	-	-	-	-
28	100	0.418	-	-	+	16.811	-	-	-	-	-	-
29	99	0.423	-	-	-	-	-	-	+	21.014	-	-
30	97	0.431	-	-	-	-	+	9.086	+	20.969	+	30.193
31	85	0.483	-	-	-	-	-	-	-	-	-	-
32	76	0.525	+	21.133	-	-	-	-	-	-	-	-
33	75	0.531	-	-	-	-	-	-	-	-	-	-
34	72	0.548	-	-	-	-	-	-	-	-	-	-
35	71	0.554	-	-	-	-	-	-	+	3.484	-	-
36	69	0.625	-	-	-	-	-	-	+	10.631	-	-
37	68	0.575	-	-	-	-	-	-	-	-	+	8.686
38	65	0.651	-	-	-	-	-	-	+	7.952	-	-
39	54	0.685	-	-	+	23.695	+	24.461	+	26.921	+	25.947
40	48	0.730	-	-	+	7.642	-	-	-	-	-	-
41	45	0.758	-	-	+	9.572	-	-	-	-	-	-
42	44	0.827	+	9.314	+	-	+	9.536	-	-	+	10.325
43	34	0.945	+	10.173	-	-	-	-	-	-	-	-
44	33	0.953	-	-	+	16.779	+	19.631	+	29.321	+	19.956
45	29	0.966	-	-	+	6.827	-	-	+	13.638	+	9.571
46	28	0.996	+	16.622	-	-	+	17.416	-	-	-	-

Table 4: Effect of salicylic acid treatments on protein bands in infected faba bean Giza717 cultivar after 21 days from inoculation by BBTMV Properties of leaf protein bands in Giaz 717 cultivar

Bands No.	M.W (kDa)	RF	Group 1		Group 2		Group 3		Group 4		Group 5	
			Bands	Protein band (%)	Bands	Protein band (%)	Bands	Protein band (%)	Bands	Protein band (%)	Bands	Protein band (%)
1	157	0.287	-	-	-	-	+	5.702	-	-	-	-
2	145	0.317	+	6.542	+	9.343	+	10.164	-	-	-	-
3	139	0.335	-	-	-	-	-	-	+	18.286	+	17.474
4	111	0.428	-	-	-	-	-	-	-	-	+	3.512
5	104	0.454	-	-	-	-	+	9.077	-	-	-	-
6	102	0.462	-	-	-	-	-	-	+	5.990	-	-
7	100	0.471	-	-	-	-	-	-	-	-	+	4.794
8	98	0.477	-	-	+	14.065	-	-	+	6.935	-	-
9	85	0.536	+	14.265	-	-	-	-	-	-	-	-
10	83	0.548	-	-	+	21.341	+	21.680	+	28.538	+	27.998
11	81	0.557	-	-	-	-	-	-	-	-	-	-
12	69	0.625	-	-	-	-	-	-	-	-	-	-
13	66	0.645	+	5.853	-	-	-	-	-	-	-	-
14	65	0.651	-	-	+	13.234	+	19.896	+	33.047	+	21.063
15	58	0.698	-	-	+	17.974	+	17.721	+	30.570	+	26.080
16	57	0.710	+	6.671	-	-	-	-	-	-	-	-
17	53	0.739	-	-	-	-	-	-	-	-	-	-
18	51	0.761	-	-	-	-	-	-	+	25.626	-	-
19	50	0.766	-	-	+	10.846	-	-	-	-	-	-
20	49	0.772	+	15.065	-	-	-	-	-	-	-	-
21	44	0.827	-	-	-	-	+	17.596	-	-	-	-
22	43	0.832	+	6.562	+	5.397	-	-	+	35.451	+	32.747
23	34	0.942	-	-	-	-	-	-	-	7.883	-	-
24	33	0.953	+	16.239	+	16.107	+	11.043	+	16.999	+	16.683

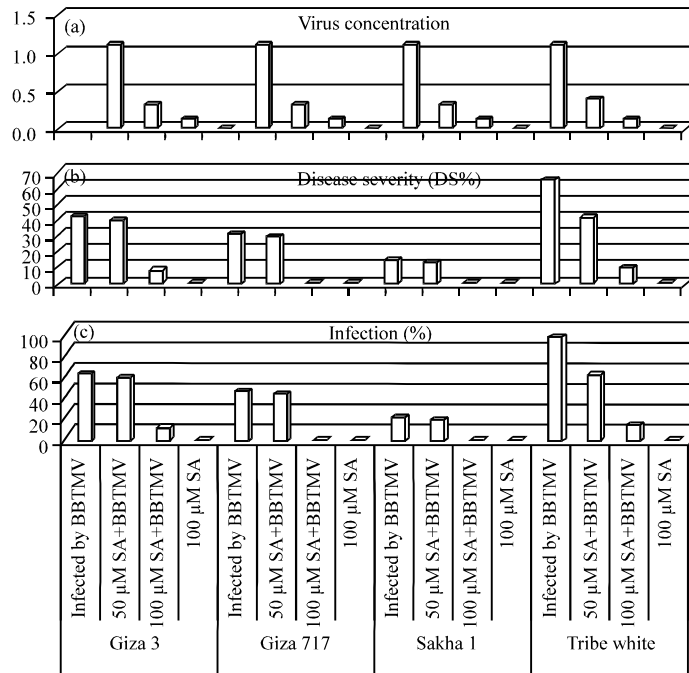


Fig. 3(a-c): Virus concentration, percentage of infection and diseases severity between faba bean cultivars, BBTMV and SA treatments calculated of (a) Virus concentration between faba bean cultivars, BBTMV and SA treatments, (b) Diseases severity between faba bean cultivars, BBTMV and SA treatments and (c) Percentage of infection between faba bean cultivars BBTMV and SA treatments

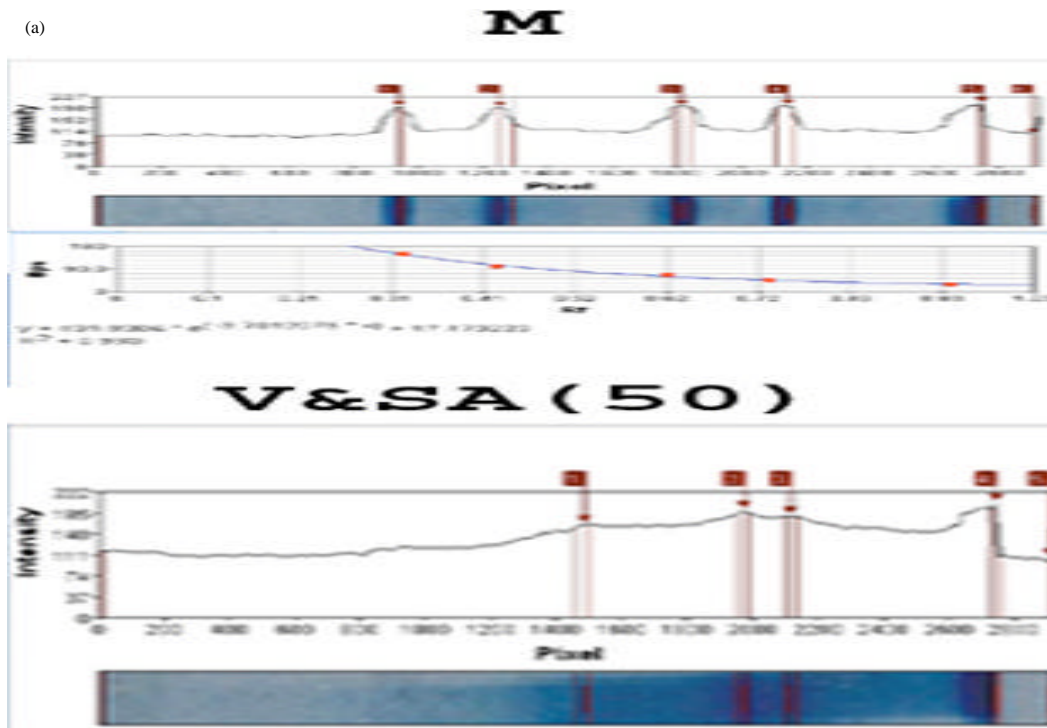


Fig. 4(a-f): Continue

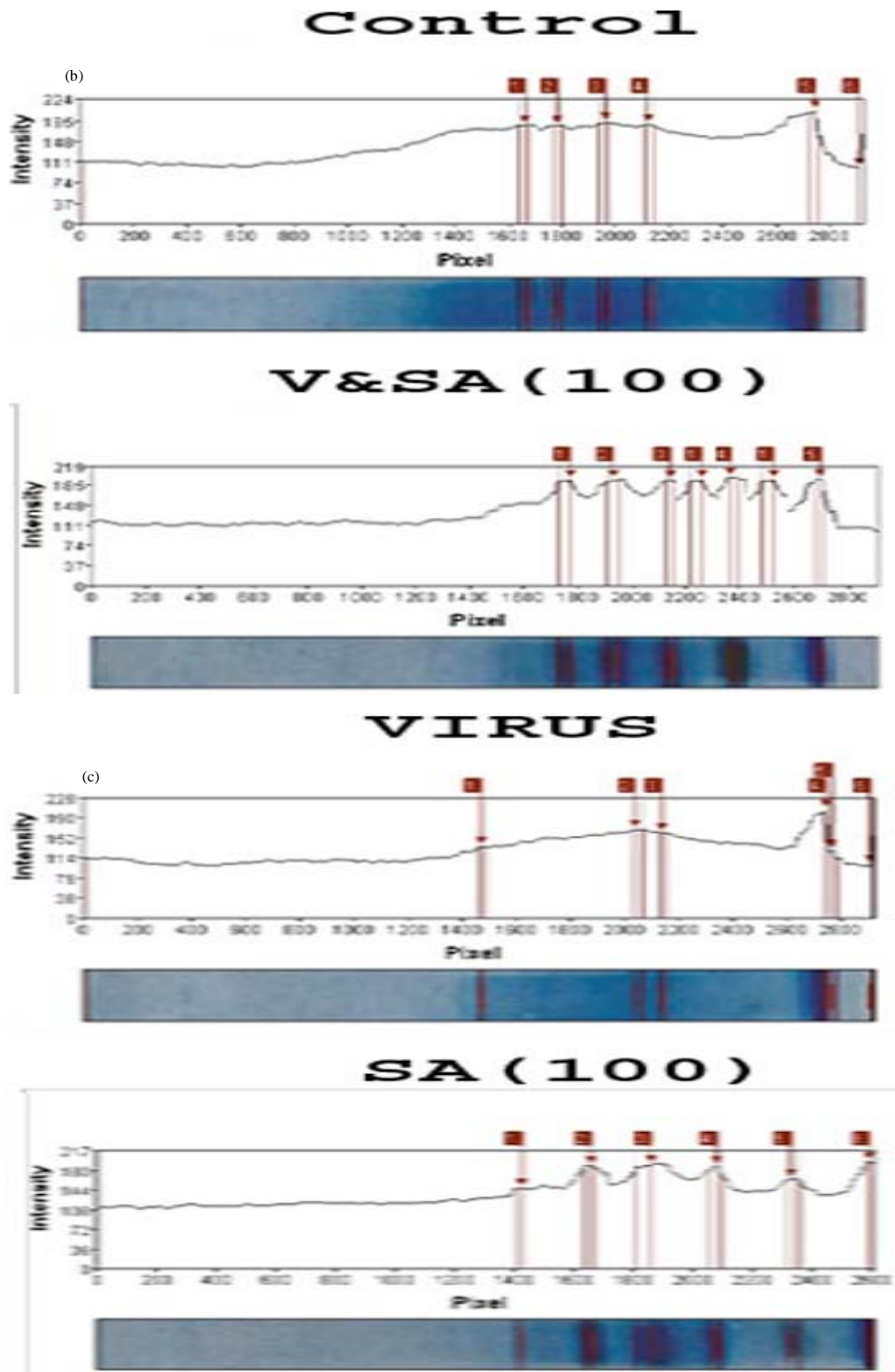


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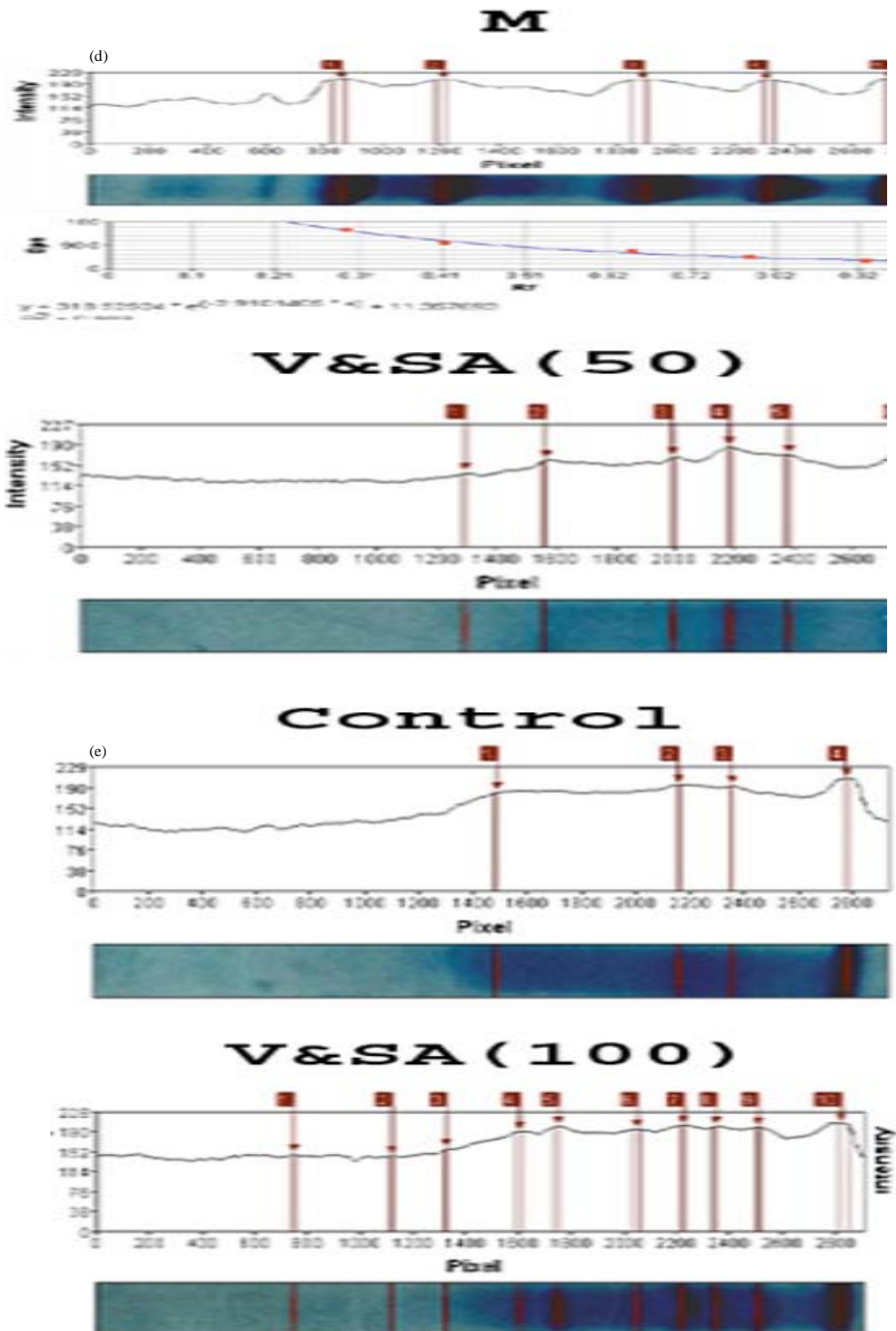


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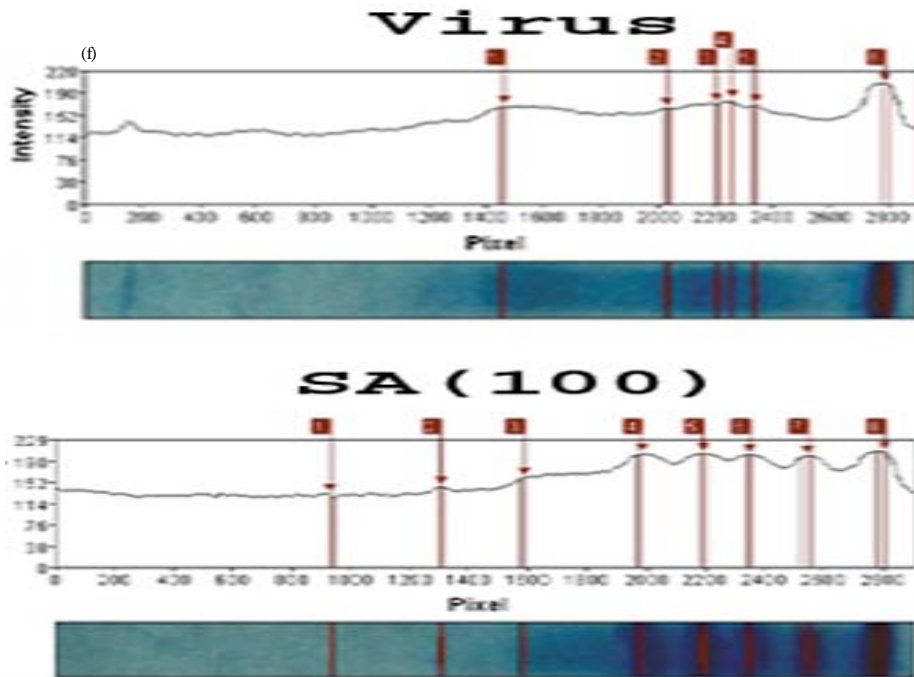


Fig. 4(a-f): Electrophoretic profile of total soluble protein extracted from treated and untreated four susceptible cultivars cv. Giza 3 and Tribe White under five treatment groups. (M) Protein marker

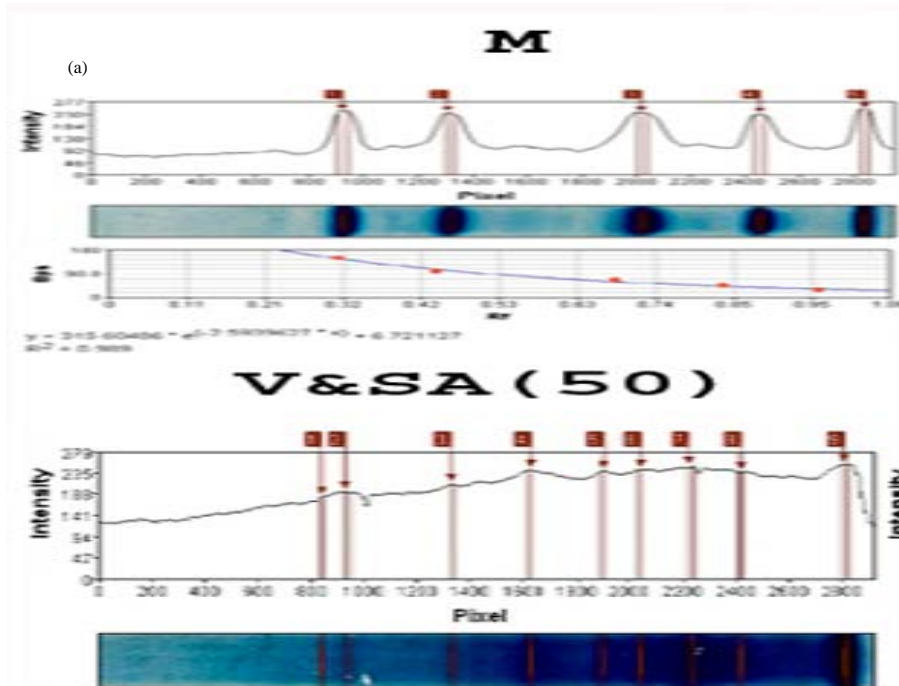


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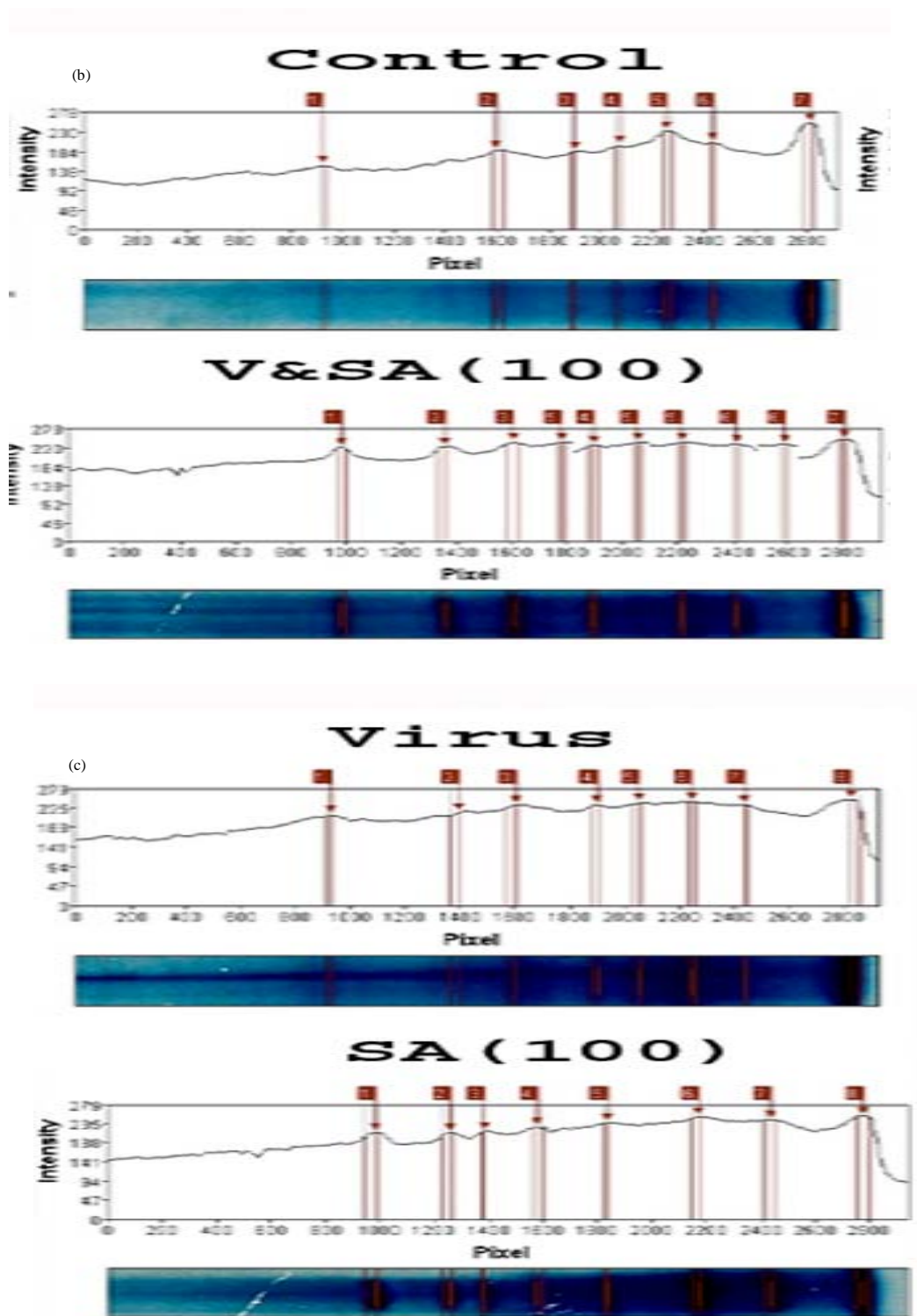


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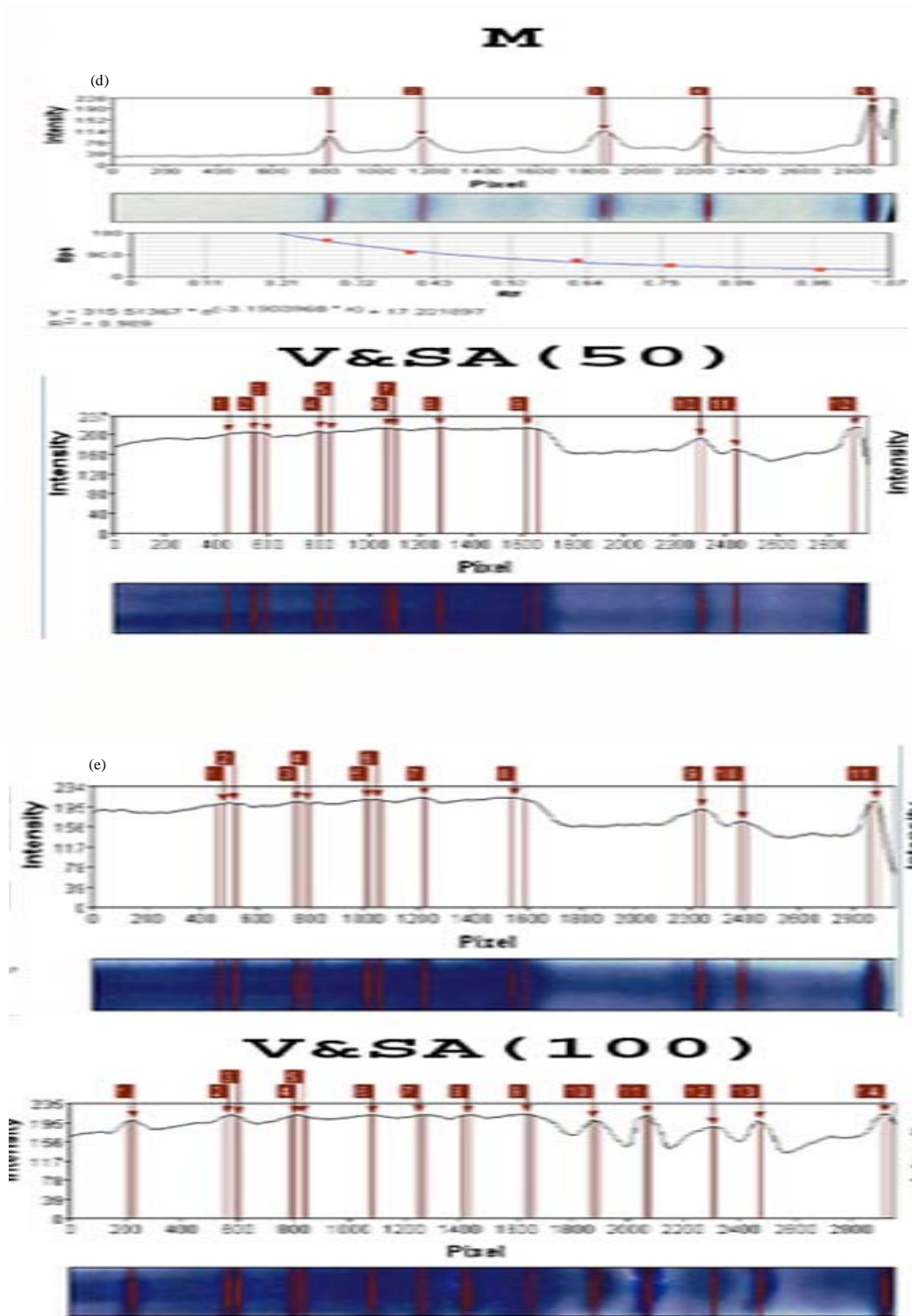


Fig. 5(a-f): Continue

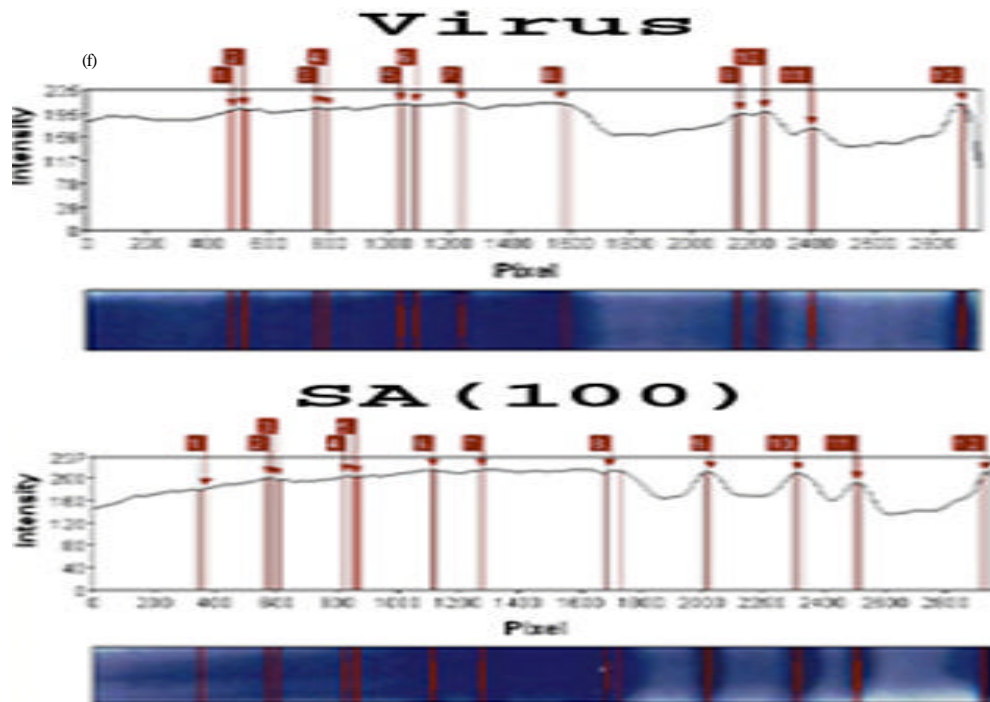


Fig. 5(a-f): Electrophoretic profile of total soluble protein extracted from treated and untreated four tolerance cultivars cv. Giza 717 and Sakha 1 under five treatment groups. (M) Protein marker

the expansion of symptoms is go together with manifestation of novel proteins, whose incidence from cooperation between specific pathogen and host origin.

Bean plants infected with BYMV were recorded higher percentage of protein contents compared with healthy plants. As a general rule of SA application were observed an increase in protein amounts (Radwan *et al.*, 2010). PVY infection was recorded higher whole comfortable of PR proteins compared with control (Sapotsky *et al.*, 2005).

Katoch (2007) observed that the PR proteins participate a significant responsibility in plant tolerance aligned with pathogens.

The bands presented in 100 μ M (SA+BBTMV) and 100 μ M SA treatments were greatly accumulated in treatments of tolerance cultivars. The protein subunits were less or more build up depending on SA application.

Due to BBTMV infection, level of 50 and 100 μ M (SA+BBTMV) and 100 μ M (SA) the protein outline was confirmed novel bands of weights compared to the control in all broad bean leave cultivars.

In the main, the concentration of protein bands of infected lanes was less than that of the control in susceptible cultivars but, more than resistance. SA treatments have a very important role in the accumulation of many PR-proteins responsible for induction of

resistance and the presence of a line of defense in plants against virus infection. Loake and Grant (2007) and Radwan *et al.* (2010) mentioned that the high increase from several novel protein subunit were detected. Infected \been leaves by BYMV observed novel manufactured polypeptide called PRPs in rejoinder to SA application under BYMV infection. In infected tobacco leaves by TMV PRPs were accumulated under SA treatments (White, 1979). Newly, pea leaves samples were collected after treatment with 5 mM salicylic acid (SA) resulted in the appearance of a new protein band of 96.7 kDa 48 h after treatment (Katoch, 2007).

In conclusion, the outcome of the their exploration proposed that the protective action of SA treatments may be associated with a reduction in concentration of virus, infection percentage and severity of BBTMV disease with virus infection. On the other hand, Overall, the BBTMV infection under SA applications caused accumulation of protein contents and founded novel bands of polypeptides in response to infection of BBTMV under SA application in *Vicia faba* L. plants leaves.

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