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Prediction of antigenic epitopes for coat protein of *Potato virus Y*, Egypt

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Abstract

Isolated *Potato virus Y* Egypt (PVY- EG) obtained from naturally infected potato plants in El-Sharkia, Egypt, was identified by biological, molecular and serological assays. The aims of this study were to predict the antigenic determinants (epitopes) of the isolated PVY using immunoinformatics; to prepare antibodies then apply them for PVY screening in potato plants in the field, as well as comparison with antibodies against coat protein. The amino acid sequence of PVY isolate was expected by DNAStar protean system; in which four parameters for epitope prediction including antigenicity, surface probability, hydrophobicity and secondary structure were used. These models were applied to PVY as a case study to predict immunogenic peptides for antibodies production. The most hydrophilic regions of PVY-coat protein (PVY- CP) were; 36-40, 46-47, 67-74, 96-98, 130-133, 164-166 and 198-200, which showed high hydrophobicity of this protein. This indicated that this protein was one of the best candidates to be immunogenic, and capable of producing antibodies that cross react with PVY. The peptide was chemically synthesized and injected into a rabbit. Obtained antibodies were evaluated using Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), and Immune Dot-Blot assay. These antibodies positively reacted against PVY infected potato tissues.

Keywords: *Potyvirus*, Potato, Immunoinformatics, ELISA, PAbs

1. Introduction

Potato virus Y (PVY) is the type-species of genus *Potyvirus*, which is a member of Potyviridae family (Pringle, 1999; Abdel-Shafi *et al*., 2017; Dupuis; 2017; Gibbs *et al*., 2017). This genus of *Potyvirus* forms one of the largest and most economically important groups of plant viruses. PVY is a flexuous; helical, rod-shaped virus of approximately 700 nm length, 12 nm in diameter, and 3.4 nm helical pitches. It has a single stranded positive sense RNA genome of approximately 10 kb length; encapsidated by about 2000 copies of a single coat protein of approximately 30 KD size. During the infection process; this RNA is translated into a large precursor polyprotein; that is cleaved co- and post-translationally into 10 mature proteins (Riechmann *et al*., 1992; Han *et al*., 2017).

Accordingly; more efforts are urgently needed to introduce epitope prediction tools with a better predictive performance, and hence more reliability. Machine learning is a scientific discipline that deals with the construction and study of algorithms that we can learn from data. Such algorithms operate by building a model based on inputs; and using that to make predictions or decisions, rather than following only explicitly programmed instructions. Machine learning can be considered a subfield of computer science and statistics. It has strong ties to artificial intelligence and optimization; which deliver methods, theories and application domains to the field. In addition; it offers one of the most cost-effective and hence widely used approaches to develop epitope prediction tools. In the last few years; several advances in the machine learning research have emerged (El-Manzalawy *et al*., 2008a).

However, several studies have pointed the limitation of existing methods in reliably identifying potential epitopes (Blythe and Flower, 2005; EL-Manzalawy *et al*., 2008a, c; Gowthaman and Agrewala, 2008). B-cell epitopes can be classified into two types; linear (continuous) epitopes, and conformational (discontinuous) epitopes. Langeveld *et al*., (2001); identified linear epitopes as short peptides; corresponding to a contiguous amino acid sequence fragment of a protein In contrast; conformational epitopes are composed of amino acids that are not contiguous in primary sequence, but are brought into close proximity within the folded protein structure.

Immunoinformatics; a newly emergent branch of bioinformatics has already become a familiar and useful tool for selecting epitopes from immunologically relevant proteins, as well as for further development of information about different epitopes (Zheng *et al*., 2011). According to Groot *et al*., (2002); Liu *et al*., (2012); Fleri *et al*., (2017); immunoinformatics combines molecular biology and related clinical data to collate, contrast and develop on the basis of homology modeling. It effectively reduces the antigen epitope screening range and experimental workload; improves efficiency, allows the discovery of new epitopes between 10 and 20 times more efficiently and thus saves research funds, in addition to accelerating the process of new epitope discovery.

2. Material and methods

2.1. Isolation of PVY

The infected potato plants gave positive results with PVY specific antibodies used for PVY isolation and identification. A differential host *Datura metel* was mechanically inoculated with infectious sap of infected potato for PVY isolation. Indicator plant; *Chenopodiumam amaranticolor* L., was inoculated with the virus isolate for single lesion isolation and then grown under greenhouse conditions. The five morphologically identical local lesions were separated and crushed in 0.1 M phosphate buffer (pH 7) between two slide glasses. This crude extract was mechanically inoculated into *Nicotiana tabacum* cv. white burly for virus propagation. Inoculated plants were kept under greenhouse conditions for symptoms development at 26± 2°C.

Virus purification was carried out according to Rupar *et al*., (2013) with some modification as follows: Five hundred grams of freshly infected *N. tabacum* cv. *samsun* leaves were homogenized in a cold warning blender in ice-cold grinding buffer (0.1 M Tris HCl, pH 8.2) containing 0.1 M NaCl, 0.1% sodium sulfite and 1% mercaptoethanol (1.5 ml/g tissues). This homogenized extract was stained through two layers of cheesecloth; clarified by adding 14% cold n-butanol, and then stirred for 30 min. at 5°C. The mixture was centrifuged at 9000 rpm for 15 min. The aqueous phase was centrifuged at 28000 rpm for 90 min. The pellets were re-suspended in 0.01 M Tris-HCl buffer (pH 7.2) containing 0.1% mercaptoethanol, and stirred overnight at 4°C. The supernatant was collected by centrifugation at 8000 rpm at 4°C for 15 min.; and then the virus was concentrated by two cycles of differential centrifugation at 32000 rpm for 2 h, and at 8000 rpm for 10 min. The pellets were re-suspended in 1.2 ml of 0.01 M Tris-HCl buffer (pH 7.2) without additives.

Finally, virus concentration was estimated spectrophotometrically using an extinction coefficient of 2.8 for 1mg\ ml\ cm.

2.2. Epitope prediction

2.2.1. Bioinformatics analysis and criteria for epitope selection

Antigenic epitope analysis was performed using DNAStar protean software in reference to Shi-hui *et al*., (2011).

2.2.1.1. Protein sequence

The coat protein sequence of PVY was made; and then analyzed with a view to recognize the immunologically relevant regions carried out by studying; antigenicity, surface probability, hydrophobicity and secondary structure. This allowed identification of active sites to find out potential targets of PVY. The potential hydrophilic regions of the protein were essential to find out the antigenic determinants with the location of continuous epitopes. Hydrophobicity and surface probability analysis of the nucleoprotein was performed according to Kyte and Doolittle, (1982); Emini *et al*., (1985), respectively.

2.2.1.2. Secondary structure prediction

Secondary structure prediction of a protein aimed to predict alpha regions, Beta-values and coil regions. The antigenic index of the nucleoprotein was determined using the Jameson-Wolf method (Jameson and Wolf, 1988). In addition, secondary structure of the nucleoprotein was predicted using the Chou-Fasman method (Chou and Fasman, 1978), and Garnier-Robson method (Garnier *et al*., 1978).

2.2.1.3. Peptide synthesis

Peptides corresponding to predicted regions as potential epitopic sites are shown in Table (1). One of them was synthesized using standard Fmoc. (9-fluorenylmethoxy carbonyl); whereas, solid phase peptide was synthesized by SBS Genetech. Co., Ltd. (Shanghai, China), http://www.sbsbio.com. The purity of the peptides was greater than 95% with (50 mg), as assessed by high performance liquid chromatography (HPLC). The molecular mass of the synthesized peptides was confirmed by Electrospray ionization-mass spectrometry, and then the peptides were stored at -20°C until use.

2.3. Serological studies

2.3.1. Preparation of PVY antigens

2.3.1.1. Intact virus antigen

The purified virus particles were broken into small fragments using sonication method described by Walkey, (1985) before immunization processes.

2.3.1.2. Predicted epitope antigen

Antigenic determinants of amino acid sequence coat protein were predicted using DNAStar Protean system, and chemically synthesized by SBS Genetech. The four properties of the amino acids sequences chosen as parameters for epitope prediction were; antigenicity, surface probability, hydrophobicity, and secondary structure. The predicted polypeptide (50 mg) was diluted in 10 ml of 0.1M phosphate buffer (pH 7.0), to obtain 1ml peptide suspension (5mg/ml).

2.3.2. Immunogenicity of PVY isolate

The prepared virus antigen and predicted epitope coat protein of PVY isolate were used for injection into rabbits. The immunogens were emulsified with Freund's complete adjuvant using the adapted system until a stiff white emulsion was formed. Rabbits were injected with 25 and 30 mg of each virus particles and predicted epitope (peptide), respectively. 10 ml of blood samples were collected from a cut in one of the ears of the immunized rabbits for separation of antibodies after four weeks from injection according to Abdel Salam, (1989). Produced antisera (virus particles and epitope) should be stored at 28°C, and remain stable for 5 months only. The titer of the specific immunoglobulin in sera was assessed by tube precipitin, and indirect ELISA.

Table 1: Linear epitopes predicted by combined immunoinformatic methods

Number	Source (position)	Sequence	Length of sequence	M. wt (Dalton)
Peptide 1	36-40	QGLGK	5	573
Peptide 2	46-47	RP	2	289
Peptide 3	67-74	HKRRTQRG	8	1163
Peptide 4	96-98	ISP	3	351
Peptide 5	130-133	KRWQ	4	670
Peptide 6	164-166	DEP	3	395
Peptide 7	198-200	TSS	3	329

Where; Q: Glutamine G: Glycine L: Leucine
K: Lysine R: Arginine P: Proline
H: Histidine T: Threonine I: Isoleucine
S: Serine W: Tryptophan D: Aspartic acid
E: Glutamic acid

2.3.3. Evaluation of the produced antisera

The produced antisera against the virus particles and predicted peptide were evaluated using the following assays:

2.3.3.1. Tube precipitin

The precipitation test was used for measuring antiserum titre according to Ali, (2014).

2.3.3.2. Indirect Enzyme linked immunosorbent assay (ELISA)

ELISA was used for measurement of PVY activity according to Engvail, (1980). A microtitre plate reader with a band width of 10 mm or less; and O.D. range of 0.0 to 3.0 at wavelength of 405 nm, was accepted for use in absorption measurement. Results can be evaluated semi-quantitatively by calculating the ratio of the positive control extinction value over the extinction value of sample. The following formula was used to calculate the ratio:

Ratio of absorption=

$$\frac{\text{Extinction value of positive control}}{\text{Extinction value of sample}}$$

The results were interpreted as following; Ratio < 0.8 negative, Ratio ≥ 0.8 to 1.1, Ratio ≥ 1.1 positive.

2.3.3.3. Ouchterlony double diffusion assay

The gel double diffusion technique (Ouchterlony, 1970) was carried out to determine the relatedness of antigens.

2.3.3.4. Dot blot immunoassay (DBIA) and Tissue printing immunoassays (TPIA)

Dot blot (DBIA) and Tissue printing (TPIA) immunoassays were performed as described by Lin *et al*., (1990). Ten leaf samples were cut from 10 plant species mainly; *Solanum tuberosum* cv. Diamond, *S. tuberosum* cv. Spounta, *S. tuberosum* cv. Nikola, *S. tuberosum* cv. loady rosette, *Datura metel*,

Lycopersicon esculentum, *Ocimum basilicum*, *Capsicum annuum*, *Syzygium aromaticum*; and *Chrysanthemum* sp.; collected from different fields of Zagazig governorate, Egypt, showing viral symptoms, in addition to PVY isolate and predicted epitope. The leaf samples were crushed in PBS by blender. The infectious sap was spotted; the petioles were printed on nitrocellulose membrane, and then treated with antisera specific for the PVY isolate and the predicted epitope.

2.3.4. Sensitivity of the performed serological assays

Detection limit is defined as a value of three times the standard deviation of an analyte free sample, and is the smallest detectable antibody titer. The detection limits were determined for PVY particle and epitope IgG using specific antisera by indirect ELISA.

2.3.5. Specificity of the performed serological assays

To define the specificity of ELISA assay; several PVY isolates were tested for cross reactivity, however there was no cross activity observed. In addition, quality of the antigen and its source ensured high specificity of ELISA.

Cross reactivity (%) =

$$\frac{PVY \text{ isolates (serotype) concentration}}{PVY \text{ isolate (used) or epitope}} X\ 100$$

3. Results

3.1. Epitope prediction

Antigenic epitope analysis of PVY-CP was performed using DNAStar protean software. The hydrophobicity scores which actually give the index for hydrophilic regions were carried out using the hydrophobicity scale of Kyte and Doolittle, (1982). This scale puts a positive score for the non-polar residues, and negative score for polar residues of a given protein. From these data; we assayed the hydrophilic regions of PVY-CP as they were supposed to be antigenic in nature, and more exposed to the surface of the protein. The regions with maximum hydrophilic scores were analyzed as antigenic sites; because these regions were unstructured and solvent accessible, thus become easier for the antibodies to recognize the native proteins. For PVY-CP; the minimal value in Kyte and Doolittle, (1982) hydrophobicity scale was - 0.54444, whereas, maximal value was 2.87778 (Table 2). This scale puts positive values for hydropathic residues in protein. According to Kyte and Doolittle, (1982) hydrophobicity scale, the most hydrophilic regions of PVY-CP were 36-40, 46-47, 67-74, 96-98, 130-133, 164-166, and 198-200 (Table 2). These hydrophilic regions occupied a high proportion of the sequence indicating the high hydrophobicity of the protein. The frequent appearance of surface and flexibility regions indicated that the sequence was flexible; stretched, and easily exposed outside. This stretched structure was confirmed by flexibility and surface accessibility analysis.

The local hydrophilic region of the protein which was typically more exposed to the surface was detected as the antigenic site; whereas, the corresponding amino acids of these sites were detected as the antigenic peptides. The high antigenic index demonstrated the high capacity of the protein sequence to form epitopes. The same results were obtained according to Jameson-Wolf, (1988) antigenicity scale, where amino acids possessing antigenicity index values above 2 were classified as highly antigenic for binding with the corresponding antibodies. These amino acids were found at the same positions of the hydrophobicity scale (Table 2). The DNAStar provided prediction of the secondary structure of PVY-CP. The turn region of PVY-CP had a frequent appearance in the whole sequences that indicated its high possibility for being an epitope. These turn and coil regions had an exposed outer edge, thus might easily bind to antibodies. On the other hand; the alpha and beta sheet regions were non-deforming and hard to

Table 2: Predicted epitope site location of *Potato virus Y*- coat protein (PVY-CP) by numerical value for DNAStar protean system analysis

No. of amino acid	Antigenicity index	Hydrophobicity	Surface probability
36	2.570000	1.87778	0.819664
37	2.510000	1.56667	0.927001
38	3.400000	1.21111	1.046614
39	2.760000	1.25556	1.657139
40	2.320000	1.25556	4.018563
46	2.060000	2.38889	2.673267
47	2.400000	2.63333	3.363142
67	2.520000	2.37778	5.981237
68	2.460000	2.33333	8.609357
69	3.400000	2.87778	4.2603
70	3.060000	2.7	3.139169
71	2.880000	2.44444	2.478291
72	2.400000	2.44444	2.655312
73	2.120000	1.73333	3.003031
74	2.140000	1.34445	1.517321
96	2.540000	-0.54444	1.550813
97	3.100000	-0.15556	0.650959
98	2.640000	0.033333	1.187042
130	2.020000	0.85556	3.361104
131	2.760000	1.74444	2.068372
132	3.400000	1.93333	1.727197
133	2.560000	1.07778	1.170037
164	3.060000	0.11111	1.88622
165	3.400000	0.3	1.443773
166	2.610000	0.48889	0.446882
198	2.400000	1	2.277854
199	3.000000	1	2.975906
200	2.550000	1.42222	2.635802

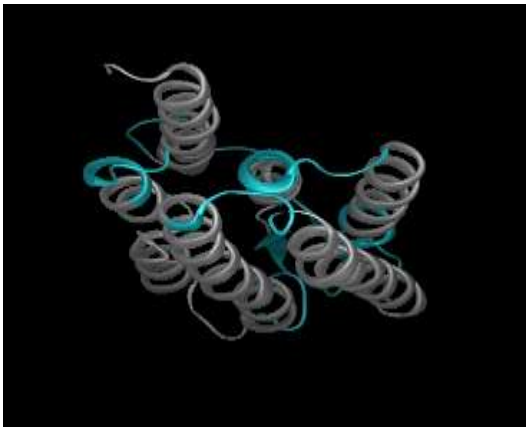


Fig. 1: Three dimensional structure of *Potato virus Y* coat protein predicted by DNAStar protean

integrate with antibodies, suggesting that these regions were not epitopes. The three dimensional secondary structure of the protein predicted by DNAStar protean was shown in Fig. (1). According to the antigenicity analysis; all amino acids possessing antigenicity index values above 2 were classified as highly antigenic, thus binding with corresponding antibodies was mostly probable.

3.2. Antisera production and titration

3.2.1. Tube precipitin

The highest titre of antisera of virus particles and predicted epitopes were determined using tube precipitation method. The titre orders were (1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024, 1:2048 and 1:4096). The titres of antisera were 1:512 and 1:128 against virus particles and epitope of PVY isolate, respectively (Fig. 2).

3.2.2. Indirect enzyme-linked immunosorbent assay

Each of the virus particles and epitopes gave serological reactions with antisera produced against them with different levels (Table 3). While blank ELISA (no antigen PVY isolates) gave no serological reaction (Table 3).

Results recorded in Table (3) apparently indicated that the antigens of virus particles and epitopes of PVY isolates (dilution 1:10) reacted positively with different levels of antisera. It was found that; the epitope of PVY coat protein produced highest amount of PVY antigen (30 mg/ml), and gave high absorption values of optical density (O.D) (1:128), compared with virus particles (25 mg/ml) which presented absorption values of (1:512). Comparison between antiserum of virus particles and those of predicted epitope of PVY/CP are demonstrated in Table (4). The strong serological precipitation reaction varied with the specific antiserum of each of the virus particles, and the peptide of predicted epitopes as clear in Table (4) and Fig. (3). These serological results were interpreted to know the exact status of PVY infection (Table 5).

3.2.3. Quchterlony double diffusion assay

This test was carried out for studying the serological relationship between peptide of (predicted epitope) coat protein, and 6 PVY isolates [Bean (1), Tomato (2), PVY isolate (3), *D. metel* (4), Potato (5), and Pepper (6)]. A sharp precipitin band was detected near the well containing the epitope of coat protein (antiserum against it). Clear precipitin bands were formed near the wells of potato, PVY isolate, and *D. metel* isolate; however, no clear bands were formed near the wells of bean and tomato isolates (Fig. 4). The serological relationship between the six PVY isolates was clear, observed through the formation of precipitin bands on using polyclonal antibodies specific for the virus particles. Sharp precipitin bands were detected near the wells of four PVY isolates (tomato, pepper, potato and *D. metel*), whereas a moderate precipitin band was formed near the well of the bean isolate (Fig. 4).

3.2.4. Dot blot immunoassay (DBIA) and tissue print immunoassay (TPIA)

Results of DBIA and TPIA assays presented in Table (6), Fig. (5) revealed that the ten plant species showed different PVY symptoms; gave positive reactions with different degrees on using PVY polyclonal antibody, PVY isolate and the predicted epitope. DBIA assay showed strong to very strong serological reactions with all the plant species. On the other hand, TPIA assay presented moderate to strong serological reactions with the plant species as well as with the epitopes. The plant species; *Solanum tuberosum* cv. (diamond, spounta, nikola and loady rosette), *D. metel*, *Lycopersicon esculentum* and *Capsicum annuum* serologically reacted with different degrees with the epitopes antiserum, while *Ocimum basilicum*, *Syzygium aromaticum* and *Chrysanthemum* sp. did not give serological reactions with the epitopes antiserum.

Table 3: Titration of PVY isolate antisera produced from immunized rabbits with purified virus particles and predicted epitope by indirect ELISA

Antigens / Antisera dilutions	PVY isolate antigens			
	Virus particles		Predicted epitope of coat protein	
	Non-treated buffer	Intact virus particle	Non-treated buffer	Treated epitope
1/1	+	+	+	+
1/2	+	++	-	++
1/4	-	++	-	+++
1/8	-	++	-	+++
1/16	-	+++	-	++++
1/32	-	++++	-	+++
1/64	-	+++	-	+++
1/128	-	+++	-	+++
1/256	-	+++	-	-
1/512	-	++	-	-
1/1024	-	-	-	-
1/2048	-	-	-	-
1/4096	-	-	-	-

Where; Serological reaction was measured at 405 nm by indirect ELISA. Where; - no reaction, + weak reaction, ++ moderate reaction, +++strong reaction, ++++ very strong reaction

Table 4: Comparison of serological reaction of PVY antisera at O.D. of (405 nm) by indirect ELISA

Antigen / Antisera	PVY immunogens						Blank
	Virus particles			Epitope coat protein			
	Serological reaction	ELISA value	Concentration (mg/ml)	Serological reaction	ELISA value	Concentration (mg/ml)	
Virus particles	++++	1.475	30	++	0.720	21	-
Epitope coat protein	++	0.975	24	++++	1.275	28	-
+ Control	+++	0.525	10	+++	0.425	15	-
- Control	-	0.110	-	-	0.120	-	-

Where; Concentration of antigen (mg/ml); Blank = Phosphate buffer saline; - no serological reaction, + weak reaction, ++ moderate reaction, +++ strong reaction, ++++ very strong reaction

Table 5: Interpretation criteria of PVY particle and epitope serological profile

Infection*	PVY particle IgG	Epitope IgG
No infection	-	-
Latent (past)	+	±
Recent	+	+
Reactivation	+	±

*Potato plants infected with PVY isolate

Table 6: Results of PVY isolate hosts with specific produced antisera against PVY isolate and produced epitope by DBIA and TPIA

Antisera / PVY hosts	PVY isolate		Epitope	
	DBIA	**TPIA**	**DBIA**	**TPIA**
1- *Solanum tuberosum* cv. diamond	+++	++	++	+++
2- *Solanum tuberosum* cv. spounta	+++	++	+++	+++
3- *Solanum tuberosum* cv. nikola	+++	+++	++	+
4- *Solanum tuberosum* cv. loady rosette	+++	++	+	++
5- *Datura metel*	+++	+++	++	+
6- *Lycopersicon esculentum*	++	++	+	+
7- *Ocimum basilicum*	+++	+++	-	-
8- *Capsicum annuum*	++++	++	++	++
9- *Syzygium aromaticum*	+++	++	-	-
10- *Chrysanthemum* sp.	+++	++	-	-
11- PVY isolate +	++++	+++	++	++
12- Predicted epitope +	++++	+++	+++	+++

Where; - Negative results, + weak reaction, ++ moderate reaction, +++ strong reaction, ++++ very strong reaction. DBIA: Dot blot immunoassay, TPIA: Tissue print immunoassay.

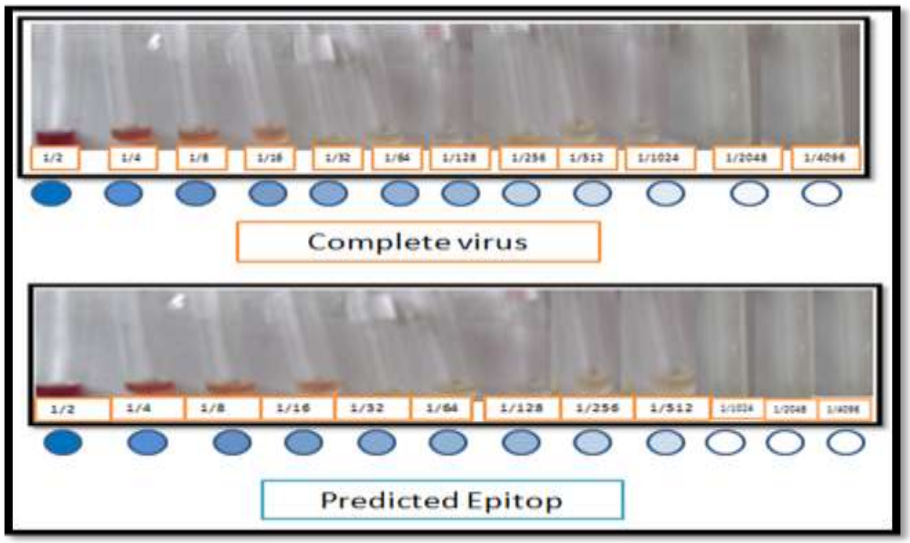


Fig. 2: Tube precipitin test for determination of antisera titers of: a-Intact virus particles; b-Predicted epitope of PVY-CP

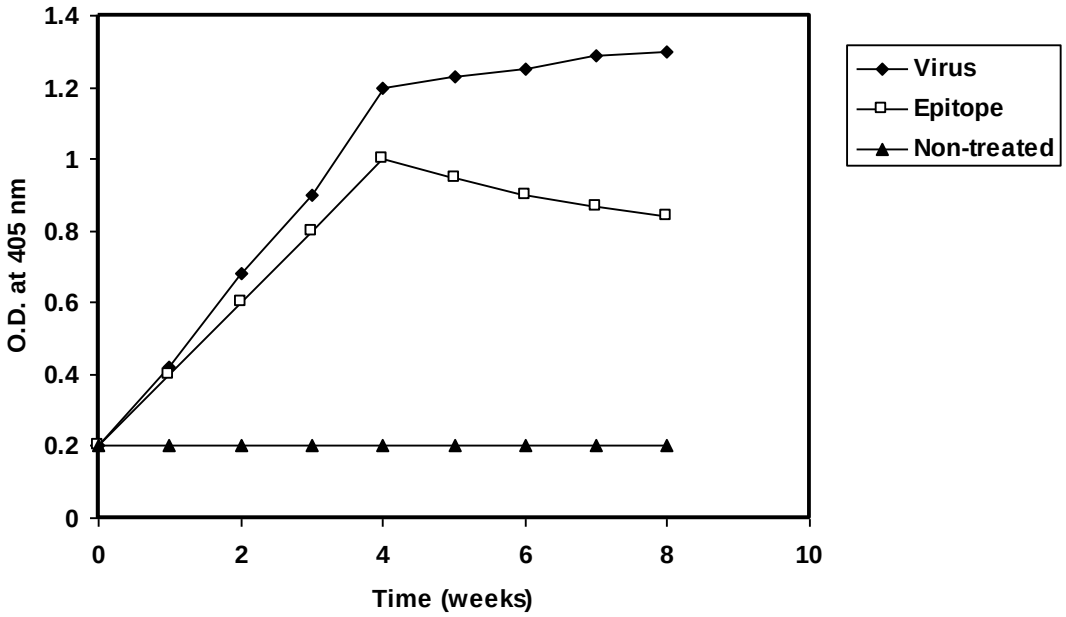
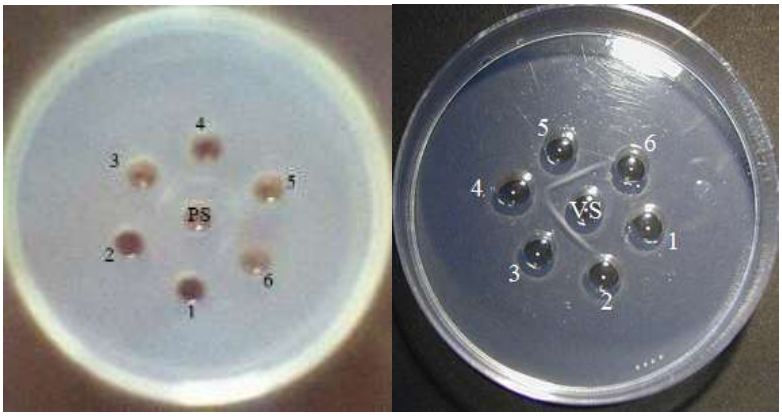
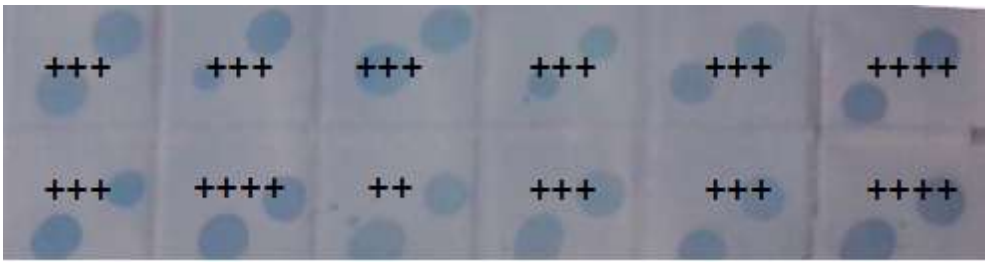


Fig. 3: Antisera titer of intact virus, epitope and none treated samples of immunized rabbits

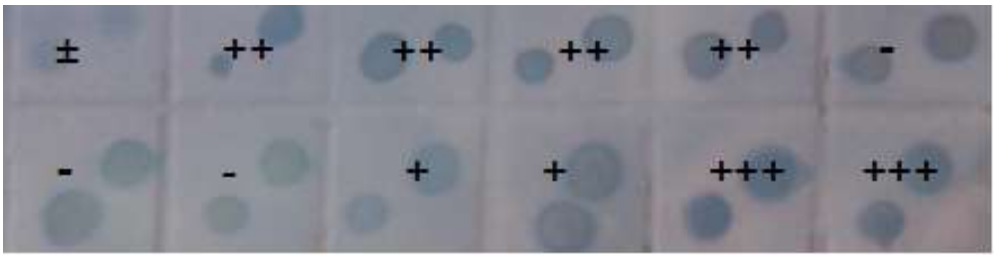


A: Intact virus B: Predicted epitope

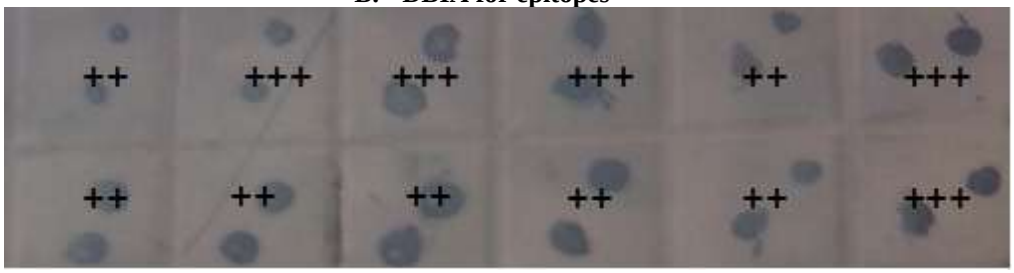
Fig. 4: Double diffusion assay on 8% agar showing the serological reactions between six PVY isolates (A); Predicted epitope coat protein (B), and the specific antisera. The six PVY isolates were from Bean (1), Tomato (2), PVY isolate (3), *D. metel* (4), Potato (5) and Pepper (6). Where; PS: Antiserum of virus particles, VS: Antiserum of predicted epitope.



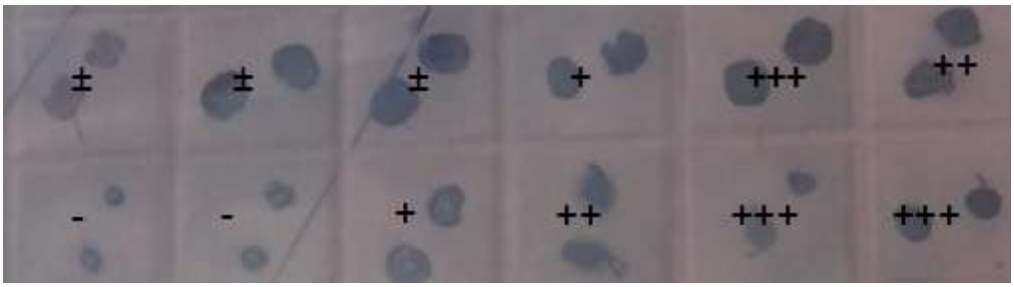
A. DBIA for PVY



B. DBIA for epitopes



C. TPIA for PVY



D. TPIA for epitopes

Fig. 5: Nitrocellulose membrane (A) and (B) spotted with PVY hosts (DBIA) and treated with produced antisera against PVY and epitopes; respectively. While; (C) and (D) represent petiole leaves spotted with PVY hosts (TPIA) and treated with produced antisera against PVY, and epitopes, respectively. Where; 1- *Solanum tuberosum* cv. (Diamond, 2- cv. Spounta, 3- cv. Nikola, 4- cv. loady rosette), 5-*D. metel*, 6-*Lycopersicon esculentum*, 7-*Ocimum basilicum*, 8-*Capsicum annuum*, 9-*Syzygium aromaticum*, 10- *Chrysanthemum* sp., 11-PVY isolate +, 12- Predicted epitope +

3.3. Performance characteristics

3.3.1. PVY detection

The minimum concentration of PVY was detected by indirect ELISA measured at O.D. of 0.525 and 0.425 nm, using antisera against virus particles and epitope, respectively. This means of zero standards was estimated to be 10 and 15 mg PVY isolate. The detection limits were determined using specific antisera by indirect ELISA as; 1:1024 and 1:512 for PVY isolate and produced epitope respectively (Table 3).

3.3.2. Specificity

Results of cross reactivity for PVY isolates obtained from different sources are summarized in Table (7). It was found that antiserum of virus particle gave high cross reactivity percentage than the epitope antiserum. This meant that the antiserum of the epitope coat protein had more specificity compared to the virus particle one.

Table 7: A cross reactivity of intact virus particles and epitope coat protein

PVY isolates	Antiserum specific (intact virus particle)	Antiserum specific epitope (coat protein)
Potato isolate	100	90
Tomato isolate	75	55
Pepper isolate	82	40
Bean isolate	75	25
D. metel isolate	95	45
Epitope	35	100

Where; This serological reaction was detected at O.D. of 405 nm

4. Discussion

According to Kyte and Doolittle, (1982), results of our hydrophobicity scale showed that the most hydrophilic regions of PVY-CP were; 36 to 40, 46-47, 67 to 74, 96 to 98, 130 to 133, 164 to 166 and 198 to 200. While Keller *et al*., (2005) previously found that the consensus sequence for the epitope of PVY strains was found at the amino acid positions 8-13, 33-36 and 45-50.This difference was related to the dissimilarity of isolates with different epitopes in positions and numbers. Based on the rules for locating the antigenic epitopes in the different regions including β turns and coil structures; with the high hydrophilicity and surface probability, the predicted epitopes were located in the region of amino acids positions; 8-22, 24-28, 45-55, 240-255, in reference to Shi-hui *et al*., (2011). The peptide analysis of PVY-CP contained 203 amino acids which pointed out 195 nanomers. These nanomers could be focused for designing a sero-diagnostic tool to detect PVY infection. By analyzing antigenicity; hydrophilicity, solvent accessibility and exposed surface area, we found the location of the potential epitopes at the sequences 41-50, 94-108 and 181-189 and might be sufficient for eliciting immune response targeted for virus detection as reported by Alam *et al*., (2013). Current results showed that the minimal value in Kyte and Doolittle, (1982) hydrophobicity scale was -2.655m, whereas maximal value was 3.577. Alam *et al*., (2013) reported that for PVY coat protein, maximal and minimal values were 0.030 and -10.074, respectively. The local hydrophilic region of the protein which was more exposed to the surface; was detected as the antigenic site, whereas the corresponding amino acids of these sites were detected as the antigenic peptides.

Results indicated that the immunodominant regions with the high surface probability and antigenic index were distributed along the entire protein. It has been reported that the antigenic epitope with the high hydrophilicity and surface probability was mainly distributed in the regions containing β-turn and coil structures. Prediction results of the computer program suggested that N-terminal amino acid; 36-40, 46-47, 67-74, 96-98, 130-133, 164-166 and 198-200, potentially participated in the formation of the epitope (Kyte and Doolittle, 1982; Keller *et al*., 2005).

In the current study, the potential antigenic epitopes were predicted using computer-assisted analysis, whereas, the antiserum of PVY was prepared based on the antigenic epitopes sequence. This was helpful for prediction of the epitope based on the tertiary structure of protein. The five different serological tests including; indirect ELISA, precipitation test, Ouchterlony, Dot blot and tissue printing immunoassay, were mainly used in this work for detection and evaluation of antibodies production against the epitope and PVY particle. In addition; comparison between availability, sensitivity, and efficiency of these assays were also studied for the virus and the predicted epitope.

The tube precipitin test was used mainly for determination of the antiserum titer, and virus end point (minimum concentration of the virus that can be detected). The titres of antisera were 1:512 and 1:128 against the virus particles and epitope of the PVY isolate, respectively. These results were in accordance with those of many investigators (Karasev *et al*., 2010; Nerway and Kassim, 2014; Tian *et al*., 2014).

For the indirect ELISA technique, minimum concentration of the PVY in the purified suspension which gave a positive reaction was 30 (mg/ml). Whereas for the predicted epitope, the minimum concentration of the virus detected was

21 (mg/ml). These results were in the range reported previously by Abdel Salam *et al*., (1989).

Ouchterlony double diffusion assay was carried out for studying the serological relationship between peptides of predicted epitope coat protein, and six PVY isolates (Bean, Tomato, PVY isolate, *D. metel*, Potato and Pepper). A sharp precipitin band was formed near the well containing the epitope coat protein (antiserum against it). In addition, clear bands were formed near wells of potato, PVY and *D. metel* isolates; however, no even weak bands were formed near wells of the bean and tomato isolates. These results were in the range with those obtained by Abdel Salam *et al*., (1989). It was concluded that the Ouchterlony double diffusion assay was less sensitive for determination of the antiserum titer. This was in agreement with Van Regenmortel *et al*., (1988); who concluded that the purified preparations as well as extracts from infected plants often contained variable amount of a small protein antigenically related to the virus, in addition to the intact virus particle. These soluble antigens represented subunits of the coat protein of the virus that were produced in excess in the infected cells; and were not encapsidated, or they might be produced from degradation of the virus particles.

The dot blot immunoassay (DBIA) and tissue print mmunoassay (TPIA) showed that plant species such as; potato (cv. Diamond, spounta, nikola and loady rosette), *D. metel*, tomato, pepper, and PVY isolate reacted serologically with different degrees with the epitope antiserum. *Ocimum basilicum*, *Syzygium aromaticum* and Chrysanthemum sp. did no show such serological reaction. These results were in accordance with findings of Bravo-Almonacid *et al*., (1992).

The five different serological assays used in this work were evaluated in many aspects. Outchterlony assay was less sensitive in virus diagnosis but required cheap chemicals and materials. Indirect ELISA techniques was highly sensitive for virus detection and diagnosis, even at very low concentration of virus in purified suspension and infectious sap. This was considered as a significant advantage of ELISA, but in general this assay suffered from high cost of chemicals, materials and equipment needed. The antibody of specific epitope predict gave positive precipitation reaction with epitope predicts, and no reaction with intact virus. On the contrary, antibody of intact virus antigen gave positive precipitation reaction with both intact virus and epitope predicted. These results confirmed that the serological relationship between predicted epitope and intact virus might be similar. Predicted epitope was similar with one or more epitopes of intact virus antigen. On the other hand; antibody specific predicted epitope reacted with some PVY infected hosts, whereas antibody of intact virus reacted with all of PVY infected hosts. According to the previous results, predicted epitope monoclonal antibodies were considered. Similar results were reported by Gomaa, (2003).

Conclusion

This study predicted the epitope (antigenic regions) on the PVY-CP by using immunoinformatics. Moreover, synthesis of antigenic determinants proteins and production of antibodies against the epitopes were carried out.

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