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Coat Protein Gene Sequence Analysis of *Potato virus X* and *Potato virus Y*: Conserved Regions to Design Gene Silencing Cassette

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Abstract: *Potato virus X* (PVX) and *Potato virus Y* (PVY) are two of the three most prevalent viruses that cause significant yield declines in potato. Twenty-seven PVX and thirty-seven PVY accessions were analyzed for nucleotide sequence variation of the coat protein gene. The average and variance of genetic distance for PVX were estimated at 0.118 and 0.004 and 0.118 and 0.005 for PVY using the neighbour joining method. Results of phylogenetic trees and their certification via stepwise discriminant analysis led us to classify of PVX sequences in four groups and PVY sequences in three groups. One purpose of this project was to determine suitable conserved regions to make of gene silencing constructs. Length of identified conserved regions were enough to silence of the virus coat protein genes on infected plants, many of which were located consequently with short gap spacers. In this term, some of groups were divided into subgroups to obtain conserved regions under minimum length of 25 nt, enough length to design specific diagnostic-primers.

Key words: Coat protein, conserved region, genetic distance, neighbour joining, silencing constructs

INTRODUCTION

Potato (*Solanum tuberosum*) originated in the highlands of South America, where it has been consumed for more than 8000 years. Potato is very low in fat and it has more protein than maize. At present, potato is the fourth most important food crop in the world, in both total production and area cultivated. Also, potato is a good bioreactor for recombinant protein production (Artsaenko *et al.*, 1998) and is a one of the selected plants for production of the edible vaccine for oral virus immunization (Richter *et al.*, 2000). An estimated 22% of potato yield is lost per year due to diseases and pests (Ross, 1986). Therefore, the need for genetic improvements of potato was recognized as a primary target for plant genetic engineering. As immediate needs, virus and insect resistance were recognized as important and attainable goals. Development of resistance to the potato viruses were selected as priority goals, because these are the most economically important pest and diseases of potato in the around of world (Kaniewski and Thomas, 2004). PVX and PVY are two of the three the most widespread potato viruses with significant yield loss. PVX and PVY are positive sense ssRNA viruses. They belong to family-genus of *Flexiviridae-potexvirus* and *Flexiviridae-potyvirus*, respectively (Mayo and Brunt, 2005).

Gene silencing results in no expression or very low expression of a gene or RNA sequence that was formerly expressed, or likely would be expressed in the absence of the gene-silencing phenomenon (Atkinson *et al.*, 1998). There is great consensus that gene silencing is an adaptive defense mechanism against viruses and transposable elements. Therefore, application of gene silencing is a useful method for production of plant virus resistance cultivars (Waterhouse *et al.*, 2001). The development of genetically engineered resistance depends on exploiting genes from the pathogen, known as Pathogen-Derived Resistance (PDR) (Hamilton, 1980; Sanford and Johnston, 1985). It has been shown that the PVX Coat Protein (CP) is transported through the phloem of potato, unloads into the vascular tissue and is subsequently transported between cells during the course of infection (Cruz *et al.*, 1998). Two distinct mechanisms for viral cell-to-cell movement have been described (Maule, 1991; Carrington *et al.*, 1996). One strategy is typified by TMV. It is independent of the CP (Siegel *et al.*, 1962; Takamatsu *et al.*, 1987) and requires a single Movement Protein (MP) that can traffic between cells (Waigmann *et al.*, 1994). A second well-described movement strategy used by the various viruses, e.g., comoviruses and nepoviruses is CP-dependent and involves the transport of virions through virus-induced tubules that span the cell walls of adjacent

cells (Wellink and Van Kammen, 1989; Van Lent *et al.*, 1990; Suzuki *et al.*, 1991; Dolja *et al.*, 1994, 1995; Forster *et al.*, 1992; Chapman *et al.*, 1992; Sit and AbouHaidir, 1993; Cruz *et al.*, 1998). Therefore, CP of viruses have important role at virulence and applicable to produce transgenic PDR cultivars. Virus-resistant transgenics have been developed in many crops by introducing either viral CP or replicase gene encoding sequences. Resistance obtained by using CP is conventionally called Coat Protein Mediated Resistance (CPMR). Coat protein genes have been shown high efficiency in preventing or reducing infection and disease caused by homologous and closely related viruses (Gonsalves and Slightom, 1993). Coat protein-mediated protection has been reported for *Tobacco mosaic virus*, (TMV) (Nelson *et al.*, 1988), *Tomato mosaic virus*, (ToMV) (Sanders *et al.*, 1992), *Cucumber mosaic virus*, (CMV) (Namba *et al.*, 1991; Quemada *et al.*, 1991), *Alfalfa mosaic virus* (AIMV) (Loesch-Fries *et al.*, 1987; Tumer *et al.*, 1987), PVX, (Hemenway *et al.*, 1988), PVY, (Perlak *et al.*, 1994) and potato leaf roll virus, PLRV, (Kamiewski *et al.*, 1993). Transgenic potato with the coat protein genes of PVX and PVY that expressed both CP genes were resistant to infection by PVX and PVY by aphid transmission and mechanical inoculation (Lawson *et al.*, 1990). Since CP plays a major role in vector transmission, CPMR confers additional advantage of resistance to vector inoculation in a majority of cases. For example, potato, which expresses PVX and PVY CP and tobacco, tomato and cucumber expressing CMV CP were seen to be highly resistant to aphid transmissions (Lawson *et al.*, 1990; Guo *et al.*, 1999). In potato, expression of the antisense RNA prevented virus infection even after grafting with scions from infected plants and therefore this transformant might be regarded as immune to the virus (Paucha *et al.*, 1998). Here we analyzed the PVX and PVY coat protein gene sequences using a phylogenetic approach in order to determine applicable conserved sequences that could be used to design specific diagnostic-primers, in gene-silencing studies to produce virus or co-virus resistance cultivars and correlation between coat protein gene sequences and geographical distribution.

MATERIALS AND METHODS

This study was conducted in Agriculture Biotechnology Research Institute for Northwest and West of Tabriz, Iran.

Sequence alignment and phylogenetic tree construction: In this study, 27 CP PVX and 37 PVY gene sequences

from GenBank were submitted to multiple sequence alignment performed by ClustalX (ver 1.8) to find relationships among sequences for gap opening, gap extension, presence of divergent sequences and DNA transition weight of 10.00, 0.20, 30 and 0.50, respectively. Phylogenetic tree construction via the Neighbour Joining (NJ) method of Saitou and Nei (1987) was performed between all pairs of sequence from a multiple alignment. Bootstrapping of sequences was performed 1000 iterations and 500 iterations for random number generator seeds. Genetic pair distances and distances from root of trees obtained by PhyloDraw (ver 0.8). Stepwise discriminant analysis, based on distances from root of trees, (Jennrich and Sampson, 1985; Pimentel, 1979; Jennrich, 1977; Lanchenbruch and Goldstein, 1979) was carried out using SPSS (ver 9.0) to verify the cluster analysis.

Conserved regions: Conserved regions detected by BioEdit (ver 5.0.6) were defined following parameters of maximum average entropy and maximum entropy per position each being were 0.2 with no gaps tolerated and a minimum segment length defined as 25.

RESULTS

Alignment: Multiple alignment of the 27 PVX coat protein gene sequences indicated a gap from position of 85 to 120 for all of sequences exceptions X88781, X88782 and X88785. Total average and variation of genetic distances of pair sequences were 0.118131 and 0.004304, respectively for these sequences. Maximum distance and minimum similarity between sequences were 0.225 with similarity of 74% (between Fujian isolate and N14 strain) and 55% with distance of 0.165 (between HB and DY strains), respectively. Minimum distance was zero with similarity of 100% between NC001455 and M72416 accessions (Table 1).

Multiple alignment of the 37 PVY coat protein gene sequences indicated an N-terminal gap from position 1 to 180, positions of 236 and 274 to 280 for all of sequences exceptions AY459605, AY459607 and AY459609. Total average and variation of genetic distances were 0.118368 and 0.004776, respectively. Maximum distance and minimum similarity between sequences were 0.448 with similarity of 49% (between Fengyang-8-1 isolate-China and VTSBTschilombo isolate-South Africa) and 48% with distance of 0.405 (between VTSBTschilombo isolate-South Africa and XCH43 isolate- China). Minimum distance was 0.12 with similarity of 99% between DQ157179 and AY745492 (Table 2).

Table 1: Similarity and genetic distance (10^{-2}) of PVX coat protein genes

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1		0.00	0.27	0.27	2.77	3.47	3.01	2.97	3.54	3.53	4.30	4.03	4.91	3.66
2	100		0.28	0.28	2.78	3.48	3.01	2.97	3.54	3.53	4.30	4.03	4.91	3.66
3	99	99.00		0.55	3.10	3.76	3.30	3.25	3.82	3.81	4.58	4.31	5.18	3.91
4	99	99.00	99.00		3.05	3.75	3.28	3.25	3.81	3.81	4.58	4.31	5.18	3.93
5	96	96.00	96.00	96.00		1.27	2.13	2.10	2.95	2.95	3.72	3.45	4.32	3.07
6	96	96.00	96.00	96.00	98.00		2.83	2.80	3.66	3.65	4.43	4.15	5.03	3.78
7	96	96.00	96.00	96.00	97.00	96.00		0.28	3.19	3.18	3.96	3.68	4.56	3.31
8	96	96.00	96.00	96.00	97.00	96.00	99.00		3.15	3.15	3.92	3.65	4.52	3.27
9	96	96.00	96.00	96.00	96.00	96.00	96.00	96.00		0.30	3.98	3.70	4.58	3.33
10	96	96.00	96.00	96.00	96.00	96.00	96.00	96.00	99.00		3.97	3.70	4.57	3.32
11	95	95.00	95.00	95.00	95.00	95.00	95.00	95.00	96.00	96.00		0.27	4.78	3.91
12	95	95.00	95.00	95.00	95.00	95.00	95.00	95.00	96.00	96.00	99.00		4.50	3.64
13	94	94.00	94.00	94.00	96.00	94.00	95.00	95.00	94.00	94.00	94.00	95.00		4.51
14	96	96.00	96.00	96.00	96.00	95.00	96.00	96.00	96.00	96.00	96.00	96.00	95.00	
15	95	95.00	95.00	95.00	95.00	95.00	95.00	95.00	96.00	96.00	95.00	95.00	94.00	98.00
16	95	95.00	94.00	94.00	94.00	94.00	94.00	94.00	95.00	95.00	95.00	95.00	93.00	97.00
17	95	95.00	95.00	95.00	96.00	95.00	96.00	96.00	96.00	96.00	95.00	95.00	95.00	97.00
18	93	93.00	93.00	93.00	94.00	93.00	94.00	94.00	93.00	93.00	93.00	93.00	93.00	94.00
19	95	95.00	95.00	95.00	95.00	95.00	96.00	96.00	94.00	94.00	94.00	94.00	93.00	95.00
20	79	79.00	79.00	79.00	78.00	79.00	78.00	78.00	79.00	79.00	79.00	79.00	78.00	79.00
21	58	58.00	57.00	58.00	57.00	57.00	57.00	57.00	58.00	58.00	57.00	57.00	57.00	57.00
22	79	79.00	79.00	79.00	79.00	79.00	78.00	78.00	79.00	79.00	79.00	79.00	78.00	79.00
23	80	80.00	80.00	80.00	79.00	80.00	79.00	79.00	79.00	79.00	79.00	79.00	78.00	79.00
24	79	79.00	79.00	79.00	80.00	79.00	80.00	80.00	79.00	79.00	78.00	79.00	79.00	79.00
25	75	75.00	75.00	75.00	75.00	75.00	75.00	75.00	74.00	74.00	75.00	75.00	74.00	74.00
26	76	76.00	76.00	76.00	76.00	75.00	75.00	75.00	75.00	75.00	75.00	75.00	75.00	74.00
27	76	76.00	75.00	75.00	75.00	75.00	75.00	75.00	75.00	75.00	75.00	74.00	74.00	74.00

	15	16	17	18	19	20	21	22	23	24	25	26	27
1	4.24	4.85	3.68	5.83	5.04	20.20	19.40	20.00	19.80	19.90	21.60	201.00	21.10
2	4.24	4.85	3.68	5.85	5.04	20.20	19.40	20.00	19.80	19.90	21.60	21.00	21.10
3	4.52	5.13	3.96	6.13	5.30	20.50	19.70	20.30	20.10	20.20	20.20	21.30	21.40
4	4.52	5.30	3.96	6.13	5.32	20.50	19.70	20.30	20.10	20.20	21.90	21.30	21.40
5	3.66	4.27	3.10	5.27	4.46	19.60	18.90	19.50	19.30	19.30	21.00	20.40	20.50
6	4.36	4.98	3.81	5.67	5.17	20.30	19.60	20.20	20.00	20.00	21.70	21.10	21.20
7	3.89	4.51	3.34	5.51	4.70	19.80	19.10	19.70	19.50	19.50	21.30	20.60	20.80
8	3.86	4.47	3.30	5.47	4.66	19.80	19.10	19.70	19.50	19.50	21.20	20.60	20.70
9	3.91	4.53	3.36	5.53	4.72	19.80	19.10	19.70	19.50	19.60	21.30	20.70	20.80
10	3.91	4.52	3.35	5.52	4.71	19.80	19.10	19.70	19.50	19.60	21.30	20.60	20.80
11	4.49	5.12	3.94	6.11	5.30	20.40	19.70	20.30	20.10	20.10	21.80	21.20	21.40
12	4.22	4.83	3.66	5.83	5.02	20.20	19.40	20.00	19.80	19.80	21.60	21.00	21.10
13	5.10	5.71	4.54	6.71	5.90	21.10	20.30	20.90	20.70	20.70	22.50	21.80	22.00
14	1.96	2.63	3.93	5.22	4.41	19.50	18.80	19.10	19.20	19.30	21.00	20.40	20.50
15		3.51	3.21	5.81	4.99	20.10	19.40	20.00	19.80	19.80	21.60	20.90	21.10
16	96.00		3.83	6.42	5.61	20.70	20.00	20.60	20.40	20.50	22.20	21.60	21.20
17	96.00	95.00		5.25	4.44	19.60	18.80	19.40	19.30	19.30	21.00	20.40	20.50
18	94.00	92.00	95.00		5.96	21.10	20.40	20.10	20.80	20.80	22.50	21.90	22.00
19	95.00	94.00	94.00	92.00		19.50	18.70	19.30	19.10	19.20	20.90	20.30	20.40
20	78.00	79.00	79.00	78.00	80.00		0.78	13.90	13.70	13.70	17.70	17.10	17.30
21	57.00	57.00	57.00	56.00	57.00	71.00		13.10	12.90	13.00	17.00	16.40	16.50
22	78.00	78.00	79.00	78.00	80.00	88.00	62.00		5.91	12.10	17.60	17.00	17.10
23	79.00	79.00	80.00	79.00	81.00	86.00	60.00	94.00		11.90	17.40	16.80	17.00
24	78.00	79.00	80.00	79.00	79.00	86.00	61.00	88.00	86.00		17.40	16.80	17.00
25	73.00	74.00	75.00	74.00	75.00	79.00	56.00	78.00	76.00	78.00		2.02	3.67
26	74.00	75.00	75.00	75.00	76.00	79.00	56.00	79.00	77.00	78.00	97.00		3.05
27	74.00	74.00	75.00	75.00	75.00	79.00	55.00	80.00	77.00	79.00	96.00	96.00	

NC001455(1), M72416(2), E01310(3), M38655(4), X88783(5), U19790(6), X88787(7), Z34261(8), AF528555(9), M95516(10), AF260641(11), AF260640(12), X88788(13), AF272736(14), AB056718(15), AB056719(16), X88784(17), AF485891(18), AY763582(19), Z23256(20), X72214(21), AF172259(22), M63141(23), X88786(24), X88785(25), X88782(26), X88781(27)

It is mentionable that similarity could not be alone as a reliable parameter corresponds to the weak correlation between reported genetic distances and similarities. Therefore, conserved regions and genetic distances are reliable than similarity to analyze sequence based functions or Phylogenetic relationship.

Phylogenetic relationships: Cluster analysis based on 27 and 37 sequences of PVX and PVY coat protein gene achieved by the neighbour joining method (Fig. 1). These sequences segregated into four groups. Stepwise discriminant analysis on groups from the cluster analysis using genetic distances of sequences from root of trees

Table 2: Similarity and genetic distance (10^{-2}) of PVY coat protein genes

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
1		3.61	4.71	7.17	7.25	7.21	7.21	7.18	7.30	7.26	7.62	7.47	7.23	9.24	9.24	9.04	9.16	9.30	9.19
2	96.0		5.35	7.80	7.87	7.85	7.84	7.80	7.92	7.88	8.25	8.09	7.85	9.86	9.86	9.78	9.66	9.93	9.81
3	96.0	93.00		7.49	7.56	7.54	7.53	7.50	7.61	7.58	7.94	7.78	7.55	9.55	9.55	9.35	9.48	9.62	9.51
4	93.0	92.00	92.00		0.12	0.15	0.30	0.71	0.83	0.79	1.35	1.66	2.24	8.60	8.60	8.40	8.52	8.67	8.55
5	92.0	92.00	92.00	99.00		0.23	0.37	0.79	0.90	0.87	1.42	2.32	1.74	8.67	8.67	8.59	8.47	8.74	8.63
6	92.0	92.00	92.00	99.00	99.00		0.35	0.76	0.88	0.84	1.40	1.71	2.29	8.65	8.65	8.45	8.57	8.72	8.60
7	93.0	92.00	92.00	99.00	99.00	99.00		0.75	0.87	0.83	1.39	1.70	2.28	8.64	8.64	8.44	8.56	8.71	8.59
8	93.0	92.00	92.00	99.00	99.00	99.00	99.00		0.37	0.50	1.35	1.67	2.25	8.61	8.61	8.41	8.53	8.67	8.56
9	93.0	92.00	92.00	99.00	99.00	99.00	99.00	99.00		0.62	1.47	1.78	2.36	8.72	8.72	8.52	8.64	8.79	8.68
10	93.0	92.00	92.00	99.00	99.00	99.00	99.00	99.00	99.00		1.43	1.75	2.33	8.68	8.68	8.48	8.61	8.75	8.64
11	93.0	92.00	92.00	98.00	98.00	98.00	98.00	98.00	98.00	98.00		2.11	2.70	9.05	9.05	8.85	8.97	9.12	9.00
12	93.0	92.00	92.00	98.00	98.00	98.00	98.00	98.00	98.00	98.00	98.00		2.53	8.89	8.89	8.69	8.81	8.96	8.85
13	93.0	92.00	93.00	98.00	97.00	97.00	98.00	97.00	97.00	97.00	97.00	97.00		8.66	8.66	8.46	8.58	8.72	8.61
14	90.0	89.00	89.00	91.00	91.00	91.00	91.00	91.00	90.00	91.00	90.00	90.00	90.00		0.75	0.75	0.87	1.08	0.97
15	90.0	89.00	89.00	91.00	91.00	91.00	91.00	91.00	91.00	91.00	90.00	90.00	91.00	99.00		0.75	0.87	1.08	0.97
16	90.0	89.00	89.00	91.00	90.00	90.00	91.00	90.00	90.00	90.00	90.00	90.00	90.00	98.00	98.00		0.62	0.88	0.77
17	90.0	89.00	89.00	91.00	91.00	91.00	91.00	91.00	91.00	91.00	90.00	91.00	91.00	99.00	99.00	99.00		1.00	0.89
18	90.0	89.00	89.00	91.00	91.00	91.00	91.00	91.00	91.00	91.00	90.00	90.00	90.00	98.00	98.00	98.00	99.00		0.62
19	84.0	83.00	83.00	84.00	84.00	84.00	84.00	84.00	84.00	84.00	84.00	84.00	84.00	92.00	92.00	92.00	92.00	92.00	
20	90.0	89.00	90.00	91.00	91.00	91.00	91.00	91.00	91.00	91.00	90.00	91.00	91.00	99.00	99.00	99.00	99.00	99.00	92.00
21	89.0	89.00	89.00	89.00	89.00	89.00	89.00	89.00	89.00	89.00	89.00	89.00	89.00	96.00	96.00	96.00	96.00	96.00	89.00
22	88.0	88.00	88.00	89.00	89.00	89.00	89.00	89.00	89.00	89.00	88.00	88.00	89.00	96.00	96.00	96.00	96.00	96.00	89.00
23	89.0	88.00	89.00	89.00	89.00	89.00	89.00	89.00	89.00	89.00	89.00	89.00	89.00	96.00	96.00	96.00	96.00	96.00	89.00
24	89.0	88.00	88.00	89.00	89.00	89.00	89.00	89.00	89.00	89.00	88.00	88.00	89.00	96.00	96.00	96.00	96.00	96.00	90.00
25	89.0	88.00	89.00	89.00	89.00	89.00	89.00	89.00	89.00	89.00	89.00	88.00	89.00	96.00	96.00	96.00	96.00	96.00	89.00
26	89.0	89.00	89.00	89.00	89.00	89.00	89.00	89.00	89.00	89.00	89.00	89.00	89.00	97.00	97.00	97.00	97.00	97.00	90.00
27	89.0	89.00	88.00	89.00	89.00	89.00	89.00	89.00	89.00	89.00	89.00	89.00	89.00	96.00	96.00	96.00	96.00	96.00	89.00
28	88.0	88.00	88.00	88.00	88.00	88.00	88.00	88.00	88.00	88.00	88.00	88.00	88.00	93.00	93.00	92.00	92.00	92.00	86.00
29	88.0	88.00	88.00	88.00	87.00	88.00	88.00	88.00	88.00	88.00	88.00	88.00	88.00	92.00	92.00	92.00	92.00	92.00	86.00
30	90.0	89.00	90.00	92.00	92.00	92.00	92.00	92.00	92.00	92.00	91.00	91.00	91.00	97.00	97.00	97.00	97.00	97.00	97.00
31	91.0	90.00	91.00	93.00	92.00	92.00	93.00	93.00	93.00	93.00	92.00	93.00	92.00	96.00	96.00	96.00	96.00	96.00	96.00
32	91.0	91.00	91.00	94.00	94.00	94.00	94.00	94.00	93.00	94.00	93.00	94.00	93.00	95.00	95.00	95.00	95.00	95.00	95.00
33	91.0	91.00	91.00	93.00	93.00	93.00	93.00	93.00	93.00	93.00	93.00	93.00	93.00	93.00	93.00	93.00	93.00	93.00	93.00
34	94.0	92.00	94.00	92.00	92.00	92.00	92.00	93.00	93.00	93.00	92.00	92.00	92.00	90.00	91.00	90.00	91.00	91.00	91.00
35	50.0	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	49.00	50.00	49.00	50.00	50.00	50.00	50.00	50.00	50.00
36	49.0	50.00	49.00	49.00	49.00	49.00	49.00	49.00	49.00	49.00	49.00	49.00	49.00	49.00	50.00	50.00	50.00	50.00	50.00
37	49.0	49.00	49.00	49.00	49.00	49.00	49.00	49.00	49.00	49.00	49.00	49.00	48.00	49.00	49.00	49.00	49.00	49.00	49.00
20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37		
1	8.86	10.20	10.50	10.20	10.20	10.40	10.30	9.62	13.80	13.90	8.73	7.98	8.06	8.69	5.75	39.5	39.9	41.0	
2	9.48	10.80	11.10	10.80	10.80	10.90	10.90	10.20	14.40	14.50	9.35	8.60	8.68	9.31	6.37	40.2	40.5	41.7	
3	9.17	14.20	10.80	10.50	10.50	10.70	9.94	10.70	14.10	10.50	9.04	8.30	8.37	9.00	6.07	39.8	40.2	41.3	
4	8.22	9.57	9.82	9.56	9.55	9.71	8.99	9.70	13.10	13.30	8.09	7.34	7.42	8.05	6.51	38.9	39.2	40.4	
5	8.29	9.64	9.89	9.63	9.62	9.78	9.06	9.78	13.20	13.40	8.17	7.42	7.49	8.13	6.56	38.9	40.5	39.3	
6	8.27	9.62	9.87	9.61	9.60	9.76	9.04	9.75	13.20	13.30	8.14	7.39	7.47	8.10	6.56	38.9	39.3	40.4	
7	8.26	9.61	9.87	9.60	9.59	9.75	9.03	9.74	13.20	13.30	8.13	7.38	7.46	8.09	6.55	38.9	39.3	40.4	
8	8.23	9.58	9.83	9.57	9.56	9.72	8.99	9.71	13.20	13.10	8.10	7.35	7.43	8.06	6.52	38.9	39.2	40.4	
9	8.34	9.70	9.94	9.68	9.67	9.83	9.11	9.83	13.30	13.40	8.21	7.50	7.54	8.17	6.64	39.0	39.4	40.5	
10	8.30	9.66	9.91	9.65	9.80	9.63	9.07	9.79	13.20	13.40	8.18	7.43	7.51	8.14	6.60	39.0	39.3	40.5	
11	8.67	10.20	10.30	10.00	10.20	10.00	9.44	10.20	13.60	13.70	8.54	7.79	7.87	8.50	6.96	39.3	39.7	40.8	
12	8.51	9.86	10.10	9.85	9.84	10.00	9.28	10.00	13.40	13.60	8.38	7.63	7.71	8.34	6.81	39.2	39.5	40.7	
13	8.28	9.63	9.88	9.62	9.61	9.77	9.04	9.76	13.20	13.30	8.15	7.40	7.48	8.11	6.57	38.9	39.3	40.5	
14	0.73	3.47	3.72	3.46	3.45	3.61	2.88	3.60	7.05	7.19	2.73	4.14	4.85	6.30	8.58	38.6	39.0	40.1	
15	0.73	3.47	3.72	3.46	3.45	3.61	2.88	3.60	7.05	7.19	2.73	4.14	4.85	6.30	8.58	38.6	39.0	40.1	
16	0.53	3.27	3.52	3.26	3.25	3.41	2.68	3.40	6.85	6.99	2.53	3.94	4.65	6.08	8.38	38.4	38.8	39.9	
17	0.66	3.39	3.64	3.38	3.37	3.53	2.81	3.53	6.97	7.12	2.65	4.06	4.77	6.20	8.50	38.6	38.9	40.1	
18	0.80	3.54	3.78	3.52	3.51	3.68	2.95	3.67	7.11	7.26	2.80	4.20	4.92	6.35	8.64	38.7	39.1	40.2	
19	0.69	3.42	3.67	3.41	3.40	3.56	2.84	3.56	7.00	7.15	2.83	4.09	4.80	6.23	8.53	38.6	38.9	40.1	
20		3.09	3.34	3.08	3.07	3.23	2.50	3.22	6.67	6.81	2.35	4.76	4.47	5.90	8.20	38.3	38.6	39.8	
21	96.00		0.75	0.49	0.50	0.88	1.38	2.10	5.95	6.09	3.70	5.11	5.82	7.25	9.55	39.6	40.0	41.1	
22	89.00	99.00		0.74	0.75	1.13	1.63	2.35	6.19	6.34	3.95	5.36	6.07	7.50	9.80	39.9	40.2	41.4	
23	89.00	96.00	99.00		0.49	0.87	1.37	2.09	5.94	6.08	3.69	5.10	5.81	7.24	9.54	39.6	40.0	41.1	
24	90.00	96.00	99.00	99.00		0.86	1.36	2.08	5.92	6.07	3.68	5.09	5.80	7.23	9.53	39.6	40.0	41.1	
25	89.00	96.00	99.00	98.00	99.00		1.52	2.24	6.09	6.23	3.84	5.25	5.96	7.39	9.69	39.8	40.1	41.3	
26	90.00	97.00	98.00	98.00	98.00	98.00		1.24	5.36	5.51	3.12	4.53	5.24	6.67	8.96	39.0	39.4	40.5	
27	89.00	96.00	98.00	97.00	98.00	97.00	97.00		6.08	6.22	3.83	5.24	5.95						

Table 2: Continued

	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
34	84.00	91.00	90.00	90.00	90.00	90.00	90.00	90.00	90.00	89.00	89.00	91.00	91.00	91.00		38.90	39.20	40.40
35	52.00	50.00	51.00	50.00	50.00	51.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00		0.90	6.51
36	52.00	50.00	50.00	50.00	50.00	51.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00			6.84
37	51.00	49.00	50.00	50.00	50.00	50.00	50.00	50.00	49.00	49.00	49.00	50.00	49.00	49.00	49.00		50.00	93.00

AJ439544(1), AJ390307(2), AJ439545(3), DQ157179(4), AY745492(5), DQ157178(6), AY884985(7), DQ008213(8), AJ889868(9), AJ890350(10), AJ890349(11), AY841265(12), AY841266(13), AJ890345(14), AJ390288(15), AJ890347(16), AJ890344(17), AJ890343(18), AJ890342(19), AY884982(20), AJ390285(21), E07484(22), AY884984(23), AJ390286(24), E03317(25), AY884983(26), AJ890346(27), AY841267(28), AY742719(29), S74813(30), AY841257(31), AY841260(32), AY840082(33), AJ890348(34), AY459607(35), AY459605(36), AY459609(37)

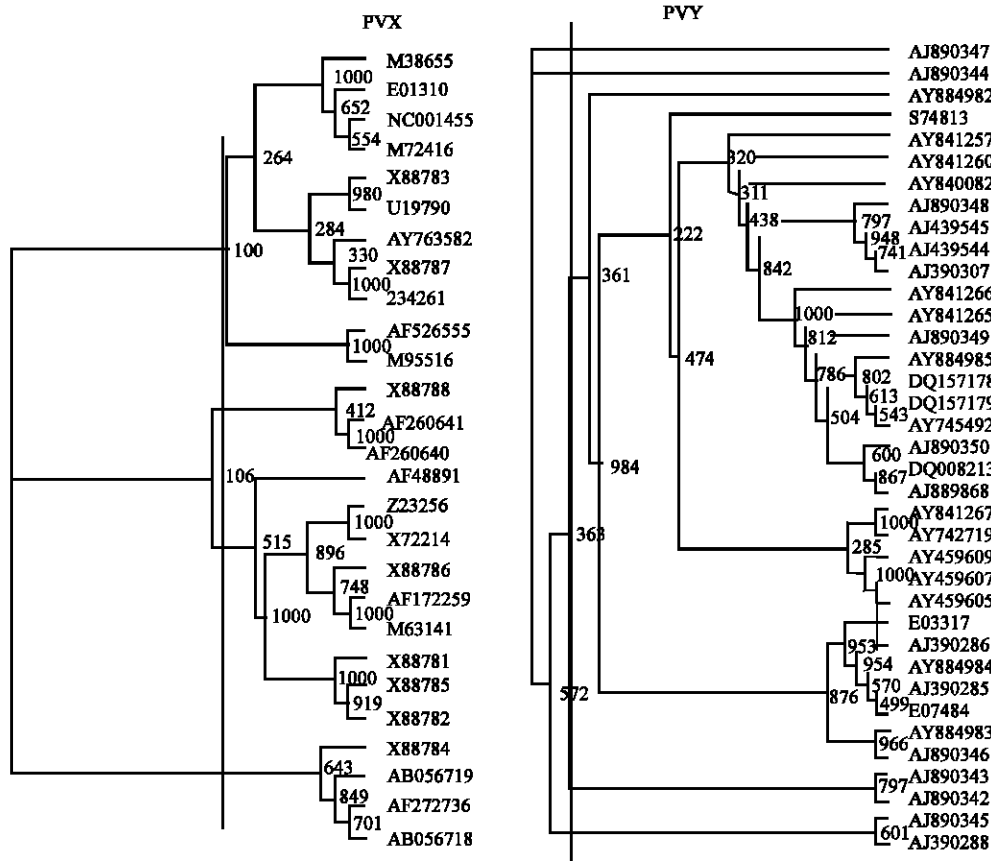


Fig. 1: Phylogenetic trees of PVX and of PVY coat protein genes. Nucleotide sequences were determined with accession number (Vertical lines are as cutting positions)

verified the groups of PVX sequences but not for PVY sequences groups. For PVX sequences, difference between groups was determined with Wilks lambda of 0.131 ($F_{3,23} = 50.742, p < 0.000$). Also, Chi-squared test was significant ($F_{3,23} = 4782.816, p < 0.000$). When we changed the tree of PVY sequences, discriminant analysis on new made groups verified the new groups with significant Wilks lambda of 0.661 ($F_{2,34} = 8.738, p < 0.001$) and Chi-squared test ($F_{2,34} = 1242.105, p < 0.000$). The new groups are as follow: group 1 included AJ439544, AJ390307, AJ439545, AJ890348, DQ157179, AY745492, DQ157178, AY884985, DQ008213, AJ889868, AJ890350, AJ890349,

AY841265, AY841266, AY459607, AY459605, AY459609, AY840082, AY841260, AY841257, AY742719 and AY841267, group 2 included AJ390285, E07484, AJ890346, AJ390286, E03317, AY884983 and AY884984 and group 3 included AJ890343, AJ890342, AJ890345, AJ390288, S74813, AY884982, AJ890347 and AJ890344.

Among the investigated PVX sequences, those isolates from Korea, Japan and Taiwan sequence diversity was related to geographic origins. Korean isolates were classified in group number 4 and Japanese and Taiwanese isolates were classified in group number 3 (Table 3). This relation was achieved only for six, three and two derived

isolates from China, South Africa and French, respectively among 37 subjected PVY sequences (Table 4).

Conserved regions: Conserved regions for PVX and PVY sequences groups were determined separately (Table 5 and 6). Group number 2 for PVX and group number 1 for PVY were divided into 2 and 3 subgroups, respectively, to find at least one conserved region under working conditions. There were not any conserved regions under working conditions for all of the sequences when based on virus type. Therefore, to determine conserved regions

for all of the sequences, minimum segment length parameter was decreased to 10. Under this condition two conserved regions for PVX sequences and one conserved region for PVY sequences were found (Table 5 and 6).

Coat protein of plant viruses affect virulence efficiency via different mechanisms (Cruz *et al.*, 1998;

Table 3: PVX strains-isolates/cluster comparison

Group	Accession No. in NCBI	Strain-isolate-location
1	NC-001455	-----
	M72416	-----
	E01310	-----
	M38655	-----
	X88783	KP
	U19790	China
	X88787	XA
	Z34261	Ms- Argentina
	AF528555	Shandong isolate (China)
	M95516	-----
	AY763582	EG2 isolate (Egypt)
2	Z23256	HB- Harpenden isolate
	X72214	HB- Bolivia
	AF172259	CP4
	M63141	South American isolate
	X88786	WS2
	X88785	N14
	X88782	EX
	X88781	DY
	AF485891	Fujian isolate (Chinas)
	AF272736	Taiwan-Taiwan isolate
	AB056718	OS- Japanese isolate
3	X88784	N11
	AB056719	bs- Japanese isolate
	AF260641	KO2-Korea
4	AF260640	KO1-Korea
	X88788	XS

Table 4: PVY strains-isolates/cluster comparison

Group	Accession No. in NCBI	Strain-isolate-location
1	AJ439544	SON41 isolate-France
	AJ390307	O- Tom isolate-Portugal
	AJ439545	Spain: Canary Islands
	AJ890348	C- France
	DQ157179	N:O- OR-1 isolate
	AY745492	N:O-L56 isolate-Canada
	DQ157178	N:O- ID-1 isolate
	AY884985	N:O- Alt isolate-western USA
	DQ008213	PN10A isolate
	AJ889868	Wilga 156var- 156var isolate- Germany
	AJ890350	Wilga- 5 isolate-Germany
	AJ890349	O- Poland
	AY841265	XCH39 isolate-China
	AY841266	XCH43 isolate-China
	AY459607	PD12 isolate- South Africa
	AY459605	M521 isolate- South Africa
	AY459609	VTSBTschilombo isolate-South Africa
2	AY840082	NTN- Brazil
	AY841260	XCH30 isolate-China
	AY841257	AFY1 isolate-China
	AY742719	Fengyang-8-1 isolate-China
	AY841267	XCH47 isolate- China
	AJ390285	N-PVY-N- RB isolate- United Kingdom
	E07484	PVY-T
	AJ890346	Nicola isolate- Germany
	AJ390286	N-United Kingdom
	E03317	PVY-T
	AY884983	N- Mont isolate-western USA
3	AY884984	NA-N/NTN- RRA-1 isolate- western USA
	AJ890343	NTN- Gr99 isolate- Poland
	AJ890342	N- Poland
	AJ890345	NTN- Linda isolate- Germany
	AJ390288	NTN- United Kingdom
	S74813	-----
AY884982	NTN- western USA	
AJ890347	NTN- Satina isolate- Germany	
AJ890344	NTN- Ditta isolate- Poland	

Table 5: Conserved regions of grouped PVX coat protein genes sequences PVX

Sequence accession no.	Conserved region	Length	Positions with sequence	Average entropy
Group 1				
1	33	52	ACCACAAAAACTGCAGGCGCAACTCTGCCACA 84	0.000
2	29	229	TGGAAGGACATGAAGGTGCCACAGACAC 257	0.000
3	28	259	ATGGCACAGGCTGCTTGGGACTTAGTCA 286	0.000
4	29	454	AACAACAGTCCACCTGCTAACTGGCAAGC 482	0.000
5	26	493	AAGCCTGAGCACAAATTCGCTGCATT 518	0.000
6	50	520	GACTTCTCAATGGAGTCAACCAACCCAGCTGCATCATGCCCAAAGAGGG 569	0.000
7	38	595	GAAATGAATGCTGCCAAACTGCTGCCTTTGTGAAGAT 632	0.000
8	38	637	AAGGCCAGGCGACAATCCAACGACTTTGCCAGCCTAGA 674	0.000
Group 2				
Sub group 1				
1	76	10	CCAGCTAGCACCACACAAGCAGCCGGTCCACCACATCAACTAACACAGCTACTGCA GCGCAACTCCTGCCACTA 85	0.000
2	55	107	CTTCCGCCAGGCTTCAGGCTTATTCACAGTACCAGATGGGGACTTCTTTAGCAC 161	0.000
3	35	178	GCTAGCAACGCTGTTGCCACCAATGAGGACCTCA 212	0.000
4	59	226	ATCTGGAAGGACATGAAAAGTCCCTCTGACACTATGGCTCAGGCAGCTTGGGACCTTGT 284	0.000
5	38	322	GAAATGATAGGCACTGGCCCTTACTCCAATGGAGTTAG 359	0.000

Group 2 was divided to two subgroups (subgroup 1: X88781, X88782 and X88785, subgroup 2: Z23256, AF172259, M63141, X88786 and AF485891) as well as expelling of X72214 to obtain at least one conserved region

Table 5: Continued

PVX				
Sequence accession No.	Conserved region	Length	Positions with sequence	Average entropy
	6	101	388GAGGTGTGCACACTCAGGCAGTTTTGCAAGAAGTACGCTCC CGTAG TCTGGAATTGGATGCTGACAAACAA CAGCCCACCGGCAAGTTGGCAGGCGCAGGG 488	0.000
	7	45	516 CTTTGACTTCTTTGATGGAGTCACCAACCCCGCAGCCATTATGCC 560	0.000
	8	62	604 GCCGCCCAAAGTGTCTTTTGTCAAGATCACAAAAGCGAGGGCGCA GTCCAGTGACTTTGC 665	0.000
	9	41	667 AGTCTGGATGCCGCTGTCACTAGAGGCCGCATCACAGGAAC 707	0.000
Group 3				
Sub group 2				
	1	20	61 ACTGCAGGCGCAACTCCTGC 80	0.000
	1	25	224 CTATTTGGAAGGCATGAAGGTGCC 248	0.000
	2	68	265 CAGGCTGCTGGGACTTAGTCAGACACTGTGCTGATGTGGGCTCA TCTGCTCAACAGAAATGATAGA 332	0.000
	3	26	349 AACGCATCAGCAGAGCTAGACTGGC 374	0.000
	4	41	394 TGACACTTAGGCAATTTTGCATGAAATGCCCCAGTGGT 434	0.000
	5	44	484 CAAGGTTTCAAGCCTGAGCACAATTCGCTGCATTTCGACTTCTT 527	0.000
	6	53	544 CCAGCTGCCATCATGCCCAAAGAGGGGCTCATCCGGCCACCGTCTGAAGCTGA 596	0.000
	7	56	607 GCCCAAAGTGTGCTTTGTGAAAGATTACGAAGGCCAGGGCACAATCCAACGACTT 662	0.000
	8	64	671 TAGATGCAGCTGTCACTCGAGGTGATCACTGGAACAACAACCGCTGAG GCTGTTTCACTCT 734	0.000
Group 4				
	1	26	7 GCACCAGCTAGCACAACACAGACCAC 32	0.000
	2	47	34 GGGTCAACTACCTCAACTACCACAAAAAGTGCAGGCGCAACTCCTGC 80	0.000
	3	26	256 ACTATGGCACAGGCTGCTTGGGACTT 281	0.000
	4	26	379 GCGATTAAAGAGGTGTGCACACTTAG 404	0.000
	5	29	412 TGCATGAAGTATGCCCCAGTGGTATGGAA 440	0.000
	6	62	442 TGGATGTTGACTAACAAACAGTCCACCTGCTAACTGGCAAGCACAA GGTTTCAAGCCTGAGCA 503	0.000
	7	26	505 AAATTCGCTGCATTTCGACTTCTTCAA 530	0.000
	8	41	595 GAAATGAATGCTGCCCAAAGTGTGCTGCTTTGTGAAAGATTAC 635	0.000
	9	59	637 AAGGCCAGGGCACAATCCAACGACTTTGCCAGCCTAGAT GCAGCTGTCACTCGAGGTGC 695	0.000
	10	48	697 ATCACTGGAACAACAACCGCTGAGGCTGTTGTCACTCTCCACCACCA 744	0.000
	1	10	530 ATGGAGTCAC 539	0.0158
All of Seq	2	11	610 CAAACTGCTGC 620	0.0144

Table 6: Conserved regions of grouped PVY coat protein gene sequences

PVY				
Sequence accession No.	Conserved region	Length	Positions with sequence	Average entropy
Group 1				
Sub group 1				
	1	35	671 ATGGCACATTTCTCAGATGTTGCAGAAGCGTATAT 705	0.000
Sub group 2				
	1	29	290 GATGTGAATGCTGGTACATCTGGGACACA 318	0.000
	2	47	326 CCGAGAATCAAGGCTATCAGTCCAAAATGAGAATGCCAAAAGCAA 372	0.000
	3	140	413 GCTCCACAACAAATGATATTTCAAATACTCGGGCA ACTCAATCACAGTTTGATACGTGGTATGAGGCAGT GCGGATGGCATAACGACATA GGAGAACTGAGATGCCAACTGTGATGAATGGGCTTATGGTTTGGTGCAT 552	0.000
	4	51	554 GAAAATGGAACCTCGCCAAATGTCAACGGAGTTTGGGT TATGATGGATGGG 604	0.000
	5	56	662 AGGCAAATCATGGCACATTTCTCAGATGTTGCAGAAGCGTATA TAGAAATGCGCAA 717	0.000
	6	29	719 AAAAAAGGAACCATATATGCCACGATATGG 747	0.000
	7	38	818 ACACCAAGTGGGGCTAGGGAAAGCGCACATTCAAATGAA 855	0.000
	8	35	866 TTGAAATCAGCCCAACCTCGACTTTTCGGGTTGGA 900	0.000
	9	59	926 AACACAGAGAGGCACACCACCGAGGATGTCTCTCCAAGTAT GCATACTCTACTTGGAGT 984	0.000
Sub group 3				
	1	26	166 CAAAAAGCATTGAGGGAAGCCCGTGG 191	0.000
	2	35	215 CATACTTATGGGCGAGACACAGGACCACGTAGC 249	0.000
	3	54	296 AATGCTGGAACAGTAGGGACGTTTACAGTTCCAAGACTTC AAATTACATCAAGC 349	0.000
	4	26	356 AGATTGCCAATAGTTGACGGACGTCC 381	0.000
	5	26	383 GTAATCAACCTGGATCACTTGGCAGT 408	0.000
	6	41	413 GATCCAGAGCAAACAATCTTGCAAATACCAGATCAACACA 453	0.000
	7	50	455 GAACAGTTTAAAGGCATGGTATGAAGGTGTGAAGGGT GATTATGGGGTATC 504	0.000

Table 6: Continued
PVX

Sequence accession no.	Conserved region	Length	Positions with sequence	Average entropy
	8	65	506 GATGCTGAAATGGGCATACTCCTTAATGGCCTCATGGTTTGGTGTAT TGAGAAATGGTACATCACC 570	0.000
	9	120	583 AATGTGGGTGATGATGGACGGAGAAGAACAAGTAACT TATCCAATAAAACCTCTATTGGATCATGCTGTCC CCACATTTAGACAGATAATGACACACTTCAGCGACATAGCTGAAGCGTA 702	0.000
	10	112	704 ATTGAAAAGAGAAAACAGGATAAAGGCCTATATGCCA AGGTATGGCCTACAGAGGAATTTGACTGATATGA GTCTTGC GCGGTATGCATTTGATTTCTATGAACTCCACTCAA 815	0.000
	11	139	842 CATATGCAAATGAAAGCAGCAGCTTTAAAGAA TGCACAGAACC GCTGTTTGGTTGGATGAAAACGCTCCACGC AAGAAGAAGACACGGAGAGGCATACAACAACCTGATGTTACAAGAAA TATACATAACCTGTTAG 980	0.000
Group 2				
	1	25	294 TGAATGTGGAACATCTGGAECTCA 318	0.000
	2	38	326 CCACGAATTAAGCTATCACGTCCAAAATGAGAATGCC 363	0.000
	3	72	397 ACACTTACTCGAGTATGCTCCACAGCAAATGACATCTCAAATACT CGAGCAACTCAATCACAGTTTGTAT AC 468	0.000
	4	76	498 ACATAGGAGAAAACCTGAAATGCCAACTGTGATGAATGGGC TTATGGTTTGGTGCATTGA AAATGGAACCTCGCCAAA 573	0.000
	5	28	581 GGAGTTTGGGTTATGATGGATGGAGATG 608	0.000
	6	96	610 ACAAGTCGAATACCCACTGAAACCAATCGTTGAGAATGCAAAA CCAACACTTAGGCAAATCATGGCA CATTCTCAGATGTTGCAGAAGCGTATAT 705	0.000
	7	54	722 AAGGAACCATATATGCCACGATATGGTTTGTAGTTCTGTA TCTGCGGATGGAAGT 775	0.000
	8	133	777 TGGCTCGCTATGCTTTTGTACTTTTATGAAGTTACATCACGGACACCA GTGAGGGCTAGAGAGGCACACATT CAAATGAAGGCCCGCAGCTTTAAATCAGCTCAATCTCGACTTTTC GGATTGGATGGTGGCAT 909	0.000
	9	87	911 AGTACACAAGAGGAAAAACACAGAGAGGCACACCACCGAGGAT GTTTCTCAAAGTATGCA TACTCTACTTGGAGTGAAGAACATGTGA 997	0.000
Group 3				
	1	32	287 AAGGACGTGAATGTTGGAACATCTGGAECTCA 318	0.000
	2	44	329 CGAATTAAGCTATCACGTCCAAAATGAGAATGCCAAGAGTAA 372	0.000
	3	37	397 ACACTTACTCGAGTATGCTCCACAGCAAATGACATC 433	0.000
	4	34	435 CAAATACTCGAGCAACTCAATCACAGTTTGATAC 468	0.000
	5	62	499 CATAGGAGAAAACCTGAAATGCCAACTGTGATGAATGGGCTTATG GTTTGGTGCATTGAAAATG 560	0.000
	6	115	608 GAACAAGTCGAATACCCACTGAAACCAATCGTTGAGAATGC AAAACCAACACTTAGGCAAATCA TGGCACAATTTCTCAGATGTTGCAGAAGCGTATATAGAAATGCGCAACAAAA 722	0.000
	7	32	734 ATGCCACGATATGGTTTGTAGTTCTGTAATCTGCG 765	0.000
	8	90	791 TTTGACTTTTATGAGGTCACATCACGAACACCAGTGAGGGCTAGGGA AGCGCACATTCA AATGAAGGCCGACGATTGAAATCAGCCCAA 880	0.000
	9	44	911 AGTACACAAGAGGAGAACACAGAGAGGCACACCACCGAGGATGT 954	0.000
	10	40	956 TCTCAAAGTATGCATACTCTACTTGGAGTCAAGAACATGT 995	0.000
All of seq	1	11	539 ATGGTTTGGTG 549	0.1217

Group 1 was divided to three subgroups as well as elimination of the AJ390285 for obtaining of at least one conserved region. Sub group 1 includes the AY459605 to 607 and sub subgroup 2 includes the DQ157178, DQ157179, AY841492, AY841266, AY841265, AY884985 and AJ890349 and subgroup 3 includes the others

Maule, 1991; Carrington *et al.*, 1996; Siegel *et al.*, 1962; Takamatsu *et al.*, 1987; Waigmann *et al.*, 1994; Wellink and Van Kammen, 1989; Van Lent *et al.*, 1990; Suzuki *et al.*, 1991; Dolja *et al.*, 1994) and CP should be a suitable candidate gene to produce PDR cultivars via RNAi (Loesch-Fries *et al.*, 1987; Tumer *et al.*, 1987; Hemenway *et al.*, 1988; Perlak *et al.*, 1994; Kamiewski *et al.*, 1993; Kamiewski *et al.*, 1990; Van

dervlugt *et al.*, 1992; Hoekema *et al.*, 1989; Feher *et al.*, 1992; Kollar *et al.*, 1993; Sudarsono Young *et al.*, 1995). Some of conserved regions that should be useful to provide broad resistance for group 1 PVX might be specific to the endemic geographical regions for group 1. Sub group 2 of PVX group 2 have two candidate conserved regions at positions from 388 to 488 and from 604 to 707 to construct the gene silencing cassettes for

developing of virus resistance potato varieties. Also, the positions from 544 to 734 for PVX group 3 seem suitable as well. Positions from 379 to 530 and from 595 to 744 of PVX group 4 are applicable for use in silencing constructs. Applicable of conserved regions of PVY to use in gene silencing constructs are as follow: For subgroup 2 of group 1 are from 413 to 604, from 662 to 747 and from 818 to 984, for subgroup 3 of group 1 are from 296 to 815 and from 842 to 980, for group 2 are from 498 to 705 and from 722 to 997 and for group 3 are from 608 to 722, from 791 to 880 and from 911 to 995.

DISCUSSION

We have subjected 27 PVX and 37 PVY coat protein gene sequences in order to variation analysis. According to alignment results, it is apparent that diversity for PVX and for PVY sequences are approximately the same. Also, genetic distances are low for each virus genus among of coat protein sequences. Therefore, these virus isolates have seemingly relatively slow divergent evolution in the coat protein genes, which might be related to a conserved role in the virus life history.

Also, comparison of geological distribution with obtained cluster groups illustrate the possible artificial transportations of viruses isolates via plant materials or existence of the hot spot mutational positions on coat protein gene sequences which different source isolates could be placed into same cluster groups.

It might be possible to produce general PVX-PVY resistance potato by using dual conserved of both PVX and PVY conserved regions. Minimum length of transgene to silence of a gene is approximately 100 nucleotides (Wesley *et al.*, 2001) and therefore, there are numerous candidate sequences that could be efficient to reduce expression of the virus coat protein gene on infected plants. However, conserved sequences of groups or subgroups must be chosen correspond to geological distribution of viruses isolates to develop co-virus resistance potato at particular place.

Pathogen detection is the other application of conserved regions. The detection of viral pathogens is of critical importance in biology, medicine and agriculture. Presently, molecular detection of pathogens specially based on PCR has become an efficient, rapid and simple method in different area as well as plant viruses detection (Hadidi *et al.*, 1995; Haliloglu and Bostan, 2002; Elnifro *et al.*, 2000). Therefore, these detected conserve regions could be capable to use as candidate regions for specific primer designing in order to virus or isolates detection. According to the small length of conserved region based on all of the sequences for each virus,

detection of virus isolates must be via 3' specific based primer corresponds to the conserved region. Also, these conserved regions could be subjected for microarray based detection of viral families (Wang *et al.*, 2002), especially for detection of viral isolates or subgroups.

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