Investigation on the response of Iranian oriental and semi oriental tobacco genotypes to Potato Virus Y under controlled conditions

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ABSTRACT

Potato virus Y (PVY) as one of the serious diseases on tobacco (*Nicotiana tabacum* L.), reduces leaf yield and its quality. Use of resistant genotypes is being considered as the most effective, economical and a safe way to reduce damages of the virus. In the present study, the response of 90 oriental and semi-oriental tobacco genotypes was evaluated against PVY using randomized complete block design with three replications under controlled conditions. Each replicate consisted of 6 seedlings. The leaves of each genotype were inoculated mechanically via rubbing method at 4-6 leaf stage with PVY. Four weeks following inoculation, the studied genotypes showed different severity symptoms associated with PVY. ELISA assay were applied on the inoculated plants by the indirect method using the polyc lonal antibody specific to PVY.

Optical density value of each infected sample was read at 405 nm using an ELISA reader. Analysis of variance on ELISA data showed significant differences among genotypes for concentration of PVY. Results revealed that 'Erzeogovina' genotype with $OD_{=}0.68881$ contained the highest virus concentration whereas the 'KB101' genotype with $OD_{=}0.0005$ contained the lowest virus concentration. The differed reactions of genotypes to PVY virus encourage the use of resistance genetic resource for the reduction of disease damages.

Key words: ELISA, *Nicotiana tabacum* L., PVY

INTRODUCTION

Potato virus Y (PVY) is a member of the Potyvirus genus in the family Potyvirideae, the largest and highly destructive family in the world of plant viruses (Shukla *et al.,* 1994). The viral genome consists of a single stranded positive-sense RNA molecule of 10 kb in length, with a VPg protein covalently attached to its 5' end and a poly-A tail at its 3' end (Riechmann *et al.,* 1992). PVY was first reported by Smith in 1931 on potato (De Box and Huttinga, 1981). PVY is transmitted by more than 50 aphid species in a non-circulative and non-persistent manner, among which *Myzus persi-*

cae is the most effective vector (Sigvald, 1984). PVY infects different crop species from *Solanaceae* family including potato (*Solanum tuberosum* L.), tobacco (*Nicotiana tabacum* L.), tomato (*Lycopersicon esculentum* Mill.), and pepper (*Capsicum* spp.) as well as Solanaceous and non-Solanaceous weeds, and even ornamental plants (Valkonen, 2007). Approximately 400 experimental hosts for PVY have been reported (Edwardson and Christie, 1997). PVY symptoms are classified into two classes (Crosslin, 2013): 1) typical symptoms consist of severe vein necrosis, vein clearing and vein banding and mottling, 2) questionable symptoms consist of mild mottling, chlorotic spots and slight deforming. Veinal necrosis is a serious symptom with high economic importance.

Tobacco is unique among agricultural crops in the world because about 90% of the commercially important part of the plant, i. e. leaves, is consumed in the form of smoke (Chaplin, 1975). It is also used as a model plant in biotechnology researches and has a great potential to be used in molecular farming for producing commercially important substances such as medical drugs and vaccines (Gadani *et al.,* 1995). It is cultivated in more than 100 countries on approximately 4.2 million hectares of crop land. Tobacco is an important commercial non-food crop in Iran. Northwest Iran, due to its geographical situation, is one of the most favorable regions for oriental tobacco cultivation. Oriental tobacco is well known for its desirable odor and thus is a major constituent of blend cigarette stocks. Because of the suitability of the climate conditions for disease spread, this region is also a hotspot for PVY epidemy. PVY causes serious damage in potato (10-100%) and other *Solanaceae* crops worldwide (Warren *et al.,* 2005).

Controlling PVY is performed by destroying infected source, planting healthy seeds, application of chemical agents, and using resistant cultivars (Franc, 2001). Due to economic impact, environmental problems and health risk of chemical agents to farmers and consumers, the use of resistant cultivars is preferred. Susceptibility of different *N. tabacum* cultivars was investigated against PVY and three levels of resistance were described according to the amount of induced necrotic symptoms (Blancard *et al.,* 1995). Partial resistance to PVY in *N. tabacum* has been reported to be controlled by recessive alleles (*va*, *va*1, *va*2) (Gupton and Burk, 1973; Miller, 1987; Reddick *et al.,* 1991). The *va* gene reduces cell to cell movement of viral particles and development of vein necrosis symptoms during PVY infection, but it does not prevent plants from viral infection (Acosta-Leal and Xiong, 2008). The '*va*' gene has been mainly used in tobacco breeding programs in order to control other potyviruses such as tobacco vein mottling virus (TVMV) and tobacco etch virus (TEV) (Miller, 1987; Reddick *et al.,* 1991).

The aim of the present study was to evaluate the reaction of oriental and semi-oriental tobacco genotypes in Urmia Tobacco Research Center to PVY under controlled conditions. The information presented here could assist tobacco breeders to choose parents of crosses for PVY resistance breeding programs.

MATERIAL AND METHODS

Plant material

The susceptibility of 90 oriental and semi-oriental tobacco genotypes was evaluated against PVY under greenhouse conditions. Some agronomic characteristics of studied lines are presented in Table 1 (Data: Urmia Tobacco Research Center). The "SPT" lines known as "Chopogh" tobacco are inbred lines selected from local landraces by single-plant or pure-line selection method at Urmia Tobacco Research Centre (Table 1). The "PD" lines are recombinant inbred lines (RILs) derived from the cross between Basma S. 31 and Dubec 566 (Table 1). The "Jahrom" is an Iranian water pipe tobacco line selected from the local landraces by the single-plant or pure-line selection method (Table 1). Other genotypes are inbred lines from CORESTA (Cooperation Center for Scientific Research Relative to Tobacco, Paris, France) collection or pure lines kindly provided by Tirtash Tobacco Research Centre (Iran).

Methodology of experiment

Tobacco genotypes were planted in plastic pots with 8 cm diameter filled with post grond H in a greenhouse with 24/18°C light/dark temperatures and natural light. The experiment was arranged in a randomized complete block design with three replications. Each replicate consisted of two pots each containing 3 seedlings. The leaves of each genotype were inoculated by the sap extract of

PVY in 4 ml 0.1 M sodium phosphate buffer (0.2% sodium diethyldithiocarbamate (DIECA), 1.4 gr K_2HPO, 1.2 gr KH_2PO_4 and 75 mg ml^{-1} of carborundum 400 mesh, pH 7.4), mechanically at 4-6 leaf stage. Leaf samples were taken from virus-inoculated plants 4 weeks post inoculation. The samples were investigated for the presence of PVY individually via indirect-enzyme-linked immunosorbent assay (I-ELISA). One gr of infected leaves were ground in 10 ml of the coating buffer (50 mM sodium carbonate, pH 9.6 and 0.01% sodium azide). Crude extracts were added to wells of an ELISA plate as the coating antigen and were kept at 4°C overnight. In each plate, 1 well was considered as a positive control (sap extract of PVY), 2 wells as the negative control (healthy sample) and 1 well as a blank (coating buffer).

Table 1. Mean and standard deviation values of some agro-morphological characteristics in 90 tobacco genotypes (Data: Urmia Tobacco Research Center).

Genotype	NL		Yield (Kg/26.25 m^2)		D50F (Day)		Genotype	NL		Yield (Kg/26.25 m^2)		D50F (Day)	
	Mean	SD	Mean	SD	Mean	SD		Mean	SD	Mean	SD	Mean	SD
Erzeogovina	24.83	1.65	2.28	0.25	64.00	2.83	[4]Mutant N.3	24.33	1.89	1.90	0.87	62.00	7.07
FK 40-1	30.50	2.59	1.23	0.01	57.00	4.24	L17	27.33	1.41	2.54	0.76	61.00	5.66
Pobeda 2	38.50	0.24	1.44	0.13	55.50	4.95	Ch.T. 209-12e	37.50	3.54	2.27	0.85	117.00	1.41
Alborz 23	37.17	1.65	2.43	0.28	70.00	5.66	PD325	32.17	4.95	1.69	0.42	50.50	0.71
Nevrokop261	31.17	0.71	2.20	0.55	59.50	3.54	SPT408	13.00	2.83	1.50	0.22	32.00	0.00
Krumovgrad42	40.33	8.01	1.90	0.16	61.00	1.41	Basma16-10	30.33	0.47	1.91	0.33	54.50	3.54
Nevrokop	32.67	1.41	2.43	0.01	58.50	2.12	TK FK40-1(Mutant GH)	30.17	0.71	1.77	0.06	57.00	0.00
Harmanli 11	36.33	0.47	2.25	0.16	61.00	5.66	Basma 104-1	35.50	0.71	1.90	0.06	59.00	2.83
[1]SPT436	16.17	0.24	1.65	0.14	44.50	2.12	Samsoun Dere	42.33	0.94	1.78	0.35	66.50	0.71
[2]Jahrom 14	23.83	0.24	1.60	0.39	58.00	9.90	SS289-2	37.33	3.30	1.75	0.04	66.00	0.00
TR1	22.67	0.94	1.74	0.14	57.00	4.24	Ch.T.273.38	33.17	0.24	1.89	0.11	66.00	0.00
KP14/a	28.67	0.47	1.11	0.09	61.00	4.24	Kharmanli163	35.50	4.01	1.89	0.25	54.50	3.54
TR21	22.83	1.65	1.67	0.33	57.00	0.00	Melnik 261	31.33	6.13	2.03	0.72	60.00	0.00
Samsoun959	34.33	4.71	1.20	0.27	55.00	2.83	PD381	30.83	0.71	1.51	0.07	62.50	4.95
Ohdaruma	23.17	0.71	4.41	0.72	74.50	0.71	Ch.T.269-12 × FK401	36.83	2.12	1.86	0.01	66.00	0.00
[3]PD371	28.17	0.71	1.77	0.39	51.00	0.00	SPT432	14.17	0.71	1.58	0.24	40.50	2.12
PD328	29.67	2.83	1.28	0.06	54.00	4.24	Samson Katerini	25.83	0.24	0.98	0.71	61.00	5.66
PD329	30.33	4.24	1.98	0.53	61.00	2.83	Xanthi	27.33	1.89	0.95	0.14	40.50	9.19
TS8	34.17	2.59	2.49	0.28	66.50	10.61	SPT409	14.33	1.89	1.51	0.45	35.50	4.95
TK28	35.33	3.30	1.32	0.02	57.00	0.00	Basma Mahalades	23.67	0.00	0.85	0.21	51.00	0.00
Trabzon	36.17	3.54	2.16	0.63	56.00	1.41	SPT430	16.67	0.94	1.48	0.04	39.50	0.71
Basma 181-8	35.33	1.89	1.74	0.17	62.50	3.54	PZ17	28.33	0.94	1.90	0.11	58.00	2.83
SPT 414 × Pobeda	29.67	0.47	1.95	0.08	57.00	0.00	Ch.Trabzon 269-12B	46.33		3.45	0.74	122.50	16.26
Trabzon H.T.1	30.00	2.83	2.04	0.57	57.50	3.54	SPT410	16.00	1.41	1.68	0.37	40.00	7.07
PBD 6 × Mut 4 (F_1)	28.00	0.00	2.76	0.25	66.00	0.00	SPT413	11.83	0.71	0.57	0.25	25.00	0.00
SPT434	18.17	0.24	1.74	0.05	44.50	9.19	Kuklen 6	38.33	2.36	3.62	0.49	70.50	3.54
L16	26.67	1.41	2.42	0.02	62.00	2.83	PD336	31.67	0.47	1.53	0.03	51.50	0.71
Basma 12-2	29.67	1.89	1.89	0.09	54.50	4.95	Ch.T 269-12	41.00	3.77	3.25	0.58	108.00	4.24
Pobeda 1	40.50	0.24	1.56	0.06	59.50	3.54	SPT406	15.67	3.30	1.34	0.24	31.00	1.41
PD345	30.00	3.77	1.99	0.40	58.00	2.83	PD324	27.17	2.59	1.58	0.10	58.50	2.12

Genotype	NL		Yield (Kg/26.25 m^2)		D50F (Day)	
	Mean	SD	Mean	SD	Mean	SD
Ch.T.283-8	33.67	0.00	2.91	0.19	63.00	4.24
SPT441	16.67	0.47	1.82	0.17	43.00	1.41
[4]Mutant N. 4	29.83	2.12	2.60	0.07	61.50	6.36
Ch.T.266-6	30.50	2.12	2.41	0.25	61.50	6.36
Izmir	24.33	2.36	0.99	0.11	38.50	4.95
Ploudiv	36.17	0.24	2.95	1.30	71.50	3.54
PD364	33.83	1.65	1.71	0.02	58.00	1.41
SPT405	18.83	0.71	1.80	0.08	35.00	1.41
PL7	28.67	0.47	2.09	0.29	59.50	3.54
SPT439	10.00	1.89	1.48	0.83	29.50	0.71
PD371	32.50	0.24	1.66	0.65	56.00	5.66
Dubec 566	26.83	1.18	1.63	0.23	58.00	1.41
Matianus	-	-	2.23	0.13	-	-
SPT433	11.67	0.47	0.78	0.13	26.50	4.95
Line 20	46.00	2.36	2.07	0.11	61.50	3.54
TK23	38.00	0.47	1.57	0.05	56.50	0.71
Orumia 379 (PD 379)	33.33	0.47	1.99	0.40	58.50	0.71
Basma S. 31	31.50	0.24	1.95	0.05	50.50	0.71
SPT403	18.83	0.71	1.73	0.07	41.50	0.71
Krumograd	33.17	0.24	1.38	0.32	66.00	0.00
N.H.H.659	26.00	1.41	1.37	0.10	50.50	0.71
GD165	27.33	4.24	1.69	0.34	55.00	7.07
SPT412	16.67	0.94	1.60	0.27	38.50	2.12
Trabzon No 23	35.17	0.24	2.68	0.00	67.00	0.00
TB22	36.67	0.47	1.31	0.21	57.00	0.00
SPT420	15.67	0.47	1.12	0.35	31.50	2.12
Trimph (Virginia Type)	26.33	0.47	4.46	0.78	73.00	8.49
Krumovgrad Kanti						
Orumia 205 (PD 205)	30.50	2.59	2.41	0.02	58.00	0.00
KB101	26.33	4.71	3.39	0.35	89.00	1.41

[1]The "SPT" lines known as "Chopogh" tobacco are inbred lines selected from local landraces by single-plant or pure-line selection method in Urmia Tobacco Research Centre. [2]The "Jahrom" line is Iranian water pipe's tobacco lines selected from local landraces by single-plant or pure-line selection method in Urmia Tobacco Research Centre. [3]The "PD" lines are recombinant inbred lines (RILs) derived from the cross between Basma S. 31 and Dubec 566 in Urmia Tobacco Research Centre. [4]Early-maturing mutants selected in Urmia Tobacco research center. SD: standard deviation, NL: number of leaf, D50F: days to 50% flowering. Yield: dry leaf weight per 26.25 m^2.

Table 2 Analysis of variance for PVY concentration in 90 oriental and semi-oriental genotypes inoculated with Potato Virus Y under controlled conditions.

Source of variation	df	SS	MS	F	P-value
Genotype	89	14.4062	0.1618	1.89**	0.0002
Replication	2	2.2037	1.1018	12.84**	<.0001
Error	177	15.1976	0.0858		
Total	268	31.8557			

df, degree of freedom. SS, sum of square. MS, mean of square. ** Significant at P = 0.01 probability level.

Table 3. Genetic variability for PVY concentration in oriental and semi-oriental tobacco genotypes.

Genotype	$\bar{x}_{OD\ value}$	Genotype	$\bar{x}_{OD\ value}$	Genotype	$\bar{x}_{OD\ value}$
Erzeogovina	0.68881 [a]	[4]Mutant N.3	0.31572 [a-i]	Ch.T.283-8	0.2055 [c-i]
FK 40-1	0.67489 [ab]	L17	0.31567 [a-i]	SPT441	0.20394 [c-i]
Pobeda 2	0.595 [abc]	Ch.T. 209-12e	0.30994 [a-i]	[4]Mutant N. 4	0.20088 [c-i]
Alborz 23	0.57211 [a-d]	PD325	0.30733 [a-i]	Ch.T.266-6	0.20009 [c-i]
Nevrokop261	0.54202 [a-e]	SPT408	0.29972 [a-i]	Izmir	0.19889 [c-i]
Krumovgrad42	0.5415 [a-e]	Basma16-10	0.2945 [a-i]	Ploudiv	0.19428 [c-i]
Nevrokop	0.49189 [a-f]	TK FK40-1(Mutant GH)	0.29131 [a-i]	PD364	0.19111 [c-i]
Harmanli 11	0.49072 [a-f]	Basma 104-1	0.29037 [a-i]	SPT405	0.18362 [d-i]
[1]SPT436	0.48756 [a-g]	Samsoun Dere	0.28289 [a-i]	PL7	0.18057 [d-i]
[2]Jahrom 14	0.48394 [a-g]	SS289-2	0.28143 [b-i]	SPT439	0.17772 [d-i]
TR1	0.48078 [a-g]	Ch.T.273.38	0.26844 [c-i]	PD371	0.17278 [d-i]
KP14/a	0.45936 [a-h]	Kharmanli163	0.26318 [c-i]	Dubec 566	0.1705 [d-i]
TR21	0.43817 [a-h]	Melnik 261	0.25983 [c-i]	Matianus	0.16961 [d-i]
Samsoun959	0.432 [a-h]	PD381	0.25211 [c-i]	SPT433	0.1666 [d-i]
Ohdaruma	0.43028 [a-h]	Ch.T.269-12 × FK401	0.25056 [c-i]	Line 20	0.16429 [e-i]
[3]PD371	0.428 [a-h]	SPT432	0.24862 [c-i]	TK23	0.16289 [e-i]
PD328	0.41739 [a-h]	Samson Katerini	0.24778 [c-i]	Orumia 379 (PD 379)	0.16194 [e-i]
PD329	0.40306 [a-h]	Xanthi	0.24496 [c-i]	Basma S. 31	0.158 [e-i]
TS8	0.39679 [a-i]	SPT409	0.23967 [c-i]	SPT403	0.13968 [e-i]
TK28	0.38306 [a-i]	Basma Mahalades	0.23883 [c-i]	Krumograd	0.13275 [f-i]
Trabzon	0.36317 [a-i]	SPT430	0.23713 [c-i]	N.H.H.659	0.13222 [f-i]
Basma 181-8	0.35967 [a-i]	PZ17	0.22807 [c-i]	GD165	0.12695 [f-i]
SPT 414 × Pobeda	0.35154 [a-i]	Ch.Trabzon 269-12B	0.227 [c-i]	SPT412	0.12522 [f-i]
Trabzon H.T.1	0.34248 [a-i]	SPT410	0.22639 [c-i]	Trabzon No 23	0.11863 [f-i]
PBD 6 × Mut 4 (F_1)	0.33806 [a-i]	SPT413	0.2235 [c-i]	TB22	0.10422 [f-i]
SPT434	0.333 [a-i]	Kuklen 6	0.21667 [c-i]	SPT420	0.10019 [f-i]
L16	0.32728 [a-i]	PD336	0.21528 [c-i]	Trimph (Virginia Type)	0.08689 [f-i]
Basma 12-2	0.32683 [a-i]	Ch.T 269-12	0.21528 [c-i]	Krumovgrad Kanti	0.08322 [g-i]
Pobeda 1	0.32409 [a-i]	SPT406	0.21494 [c-i]	Orumia 205 (PD 205)	0.07361 [hi]
PD345	0.3164 [a-i]	PD324	0.20817 [c-i]	KB101	0.0005 [i]

$\bar{X}_{OD\ value}$: Average level of PVY concentration in infected tissue of tobacco genotypes compared with Tukey test P=0.05 ($r_=3$). [1]The "SPT" lines known as "Chopogh" tobacco are inbred lines selected from local landraces by single-plant or pure-line selection method in Urmia Tobacco Research Centre. [2]The "Jahrom" line is Iranian water pipe's tobacco lines selected from local landraces by single-plant or pure-line selection method in Urmia Tobacco Research Centre. [3]The "PD" lines are recombinant inbred lines (RILs) derived from the cross between Basma S. 31 and Dubec 566 in Urmia Tobacco Research Centre. [4]Early-maturing mutants selected in Urmia Tobacco research center.

Each well was then thoroughly washed 3 times with phosphate-buffered saline (PBS) plus 0.05% Tween-20 (PBST), for 3 minutes each. Then, 50 µl of polyclonal antibody, specific to PVY, in conjugate buffer (PBS plus 0.05% Tween 20, 2% polyvinylpyrrolidone-40 and 0.2% egg albumin, diluted 1:1000) were added to each well and were incubated at 37°C for 3 hours. The plates were then washed with PBST and blocked with blocking buffer (PBST plus 2% egg albumin) at 37°C for 1 hour. The plates were again washed with PBST. Then 50 µl of secondary antibody (antirabbit) in the conjugate buffer (diluted 1:3000) were added to each well and incubated at 4°C overnight. The plate was washed 3 times with PBST for 3 minutes and then 50 µl ρ-nitrophenyl phosphate dissolved in the substrate buffer (9.7% diethanolamine, pH 9.8) were added to each well and incubated at room temperature for 120 min in dark. Optical density (OD) value of each well was read at 405 nm using an Anthos ELISA reader model 2020.

Statistical analysis

Normality of the OD values was assessed according to Shapiro-Wilk test (PROC UNIVARIATE of SAS 9.2 software) (SAS Institute, Cary, NC). Analysis of variance (ANOVA) was carried out using the general linear model (GLM) in the SAS 9.2 software. The mean values of studied genotypes were compared using Tukey test at 5% probability level.

RESULTS AND DISCUSSION

Analysis of variance revealed significant differences among tobacco genotypes for PVY concentration. This is indicating the genetic control of resistance to PVY in the tested genotypes (Table 2). The virus concentration in the studied genotypes ranged from OD=0.0005 in 'KB101' genotype to OD=0.6888 in 'Erzeogovina' genotype with an average of OD=0.2825 (Table 3). Almost 4.40% of genotypes showed PVY concentration of less than 0.1, and 24.40% showed the OD values in the range of 0.1 to 0.2 (Table 3).

PVY concentration in the RIL subpopulation ranged from OD=0.0736 in Orumia 205 (PD205) to OD=0.4280 in PD371 with an average of 0.2545. The Basma S. 31 and Dubec 566 as the maternal and paternal lines of RIL subpopulation, showed the OD values of 0.1580 and 0.1705, respectively. However, with some individuals of RILs such as PD205, the PVY concentration was less than it was with their parents, while with others it was more severe (Table 3). Once the alleles responsible for increasing as well as decreasing traits are dispersed in the parental lines, the higher levels of favorable alleles in the progeny may result in better traits than in the parental lines; because of a phenomenon known as transgressive segregation. In transgressive segregation, the alleles with positive or negative additive effects accumulate in the offspring (Zhang *et al.,* 2001). Transgressive segregation has been reported for resistance to powdery mildew in oriental tobacco by Darvishzadeh *et al.* (2010). It has also reported for resistance to Phoma black stem in sunflower by Rachid Al-Chaarani *et al.* (2002), Bert *et al.* (2004) and Darvishzadeh *et al.* (2007); for partial resistance to *S. sclerotiorum* in sunflower by Micic *et al.* (2005), Davar *et al.* (2010); for resistance to powdery mildew in oat by Jones (1983) and for resistance to powdery mildew in bread wheat by Lillemo and Skinnes (2006).

PVY concentration in "SPT" subpopulation known as "Chopogh" tobaccos selected from local landraces in Urmia Tobacco Research Centre, vary between OD=0.1002 in SPT420 and OD=0.4876 in SPT436 line with an average of OD=0.2329. The OD value in 35.29% of "SPT" lines was in the range of OD=0.1 to OD=0.2 (Table 3). This shows that the genetic variability for susceptibility to PVY existed among local landraces. In our previous investigation, the "SPT" lines presented a high variability for different characters, such as chloride accumulation rates in leaves (Darvishzadeh *et al.,* 2011), partial resistance to Orobanche (unpublished data) as well as resistance to powdery mildew (Darvishzadeh *et al.,* 2010).

A high consensus was observed between the severity of disease symptoms and virus concentration in the studied genotypes. Majority of genotypes with a high virus concentration showed mosaic symptoms and sever necrosis such as midribs necrosis or veins and stalk necrosis. Disease severity on some genotypes in some replicates results in seedling dying. A striking example was 'Erzeogovina' genotype. Three genotypes, 'KB101', 'Orumia 205' and 'Krumograd Kanti' with low virus concentration did not express any disease symptoms particularly vein necrosis although the existence of virus was confirmed by the serological assay (ELISA test). The results are in accordance with the reports of Doroszewska and Depta (2011) that declared differences in susceptibility of wild *Nicotiana* species against PVY; visually (disease symptoms) as well as serologically (DAS-ELISA test). In their study *N. raimondii*, diploid and tetraploid forms of *N. knightiana*, as well as tetraploid *N. glauca* were found completely resistant to PVY. *N. benavidesii*, *N. wigandioides* and *N. noctiflora* were highly resistant to PVY. The other species showed various degrees of infection.

Brandle *et al.* (1994) evaluated the reaction of fifty four tobacco cultivars (*N. tabacum* L.), seven species, and four somatic hybrids against PVY in Canada. They identified four cultivars: Virginia A Mutant (VAM), NC744, TN86 and PBD6 resistant to PVY. The resistance reaction of cultivars was confirmed by the ELISA test. In their study, resistant plants were similar to the healthy uninoculated controls in appearance. In their study, some cultivars such as Havana 307, Wanda, and Wisana were tolerant to PVY infection, exhibiting mild

mosaic symptoms with or without vein necrosis, and similar to the healthy controls in overall growth. Of the species tested, *N. kawakami* exhibited mild symptoms and reduced virus titer and *N. rustica* var. NRT presented a tolerance reaction to PVY. They concluded that genetic resistance to PVY was present both within the *N. tabacum* germ pool and among its wild relatives. Sievert (1978) evaluated the reaction of 11 burley tobacco (*N. tabacum* L.) cultivars and hybrids against PVY. Cultivars exhibited disease symptoms in the range of mild to severe rate. They identified 3 cultivars: Kentucky 10, Kentucky 12, and Kentucky 14 most tolerant to infection by PVY. Paunescu *et al.* (2002) developed some burley tobaccos (Burley 114, Burley 196, Burley 194 and Burley 190) resistance to PVY from local Romanian germplasm.

Gupton and Burk (1973) showed that resistance to PVY in *N. tabacum* is controlled by one locus: "va" localized on chromosome E (Gupton and Burk, 1973). They found that only homozygous "va va" plants show partial resistance to necrotic symptoms (vein necrosis) produced by PVY. Origin of 'va' locus goes back to Virgin A Mutant (VAM) line, which was selected from X-ray irradiated population (Koelle, 1961). 'va' gene has been utilized in commercial tobacco cultivars to provide resistance to three potyviruses; PVY, tobacco vein mottling virus and tobacco etch virus (Miller 1987; Reddick *et al.*, 1991). Resistance to PVY in Perevi, Bursana, Ensyu and Okinawa1 varieties have been shown to be due to recessive alleles of va, and these alleles were classified into three types (va, va1, and va2) according to the degree of resistance to PVY (Yamamoto, 1992). Acosta-Leal and Xiong (2008) found that genotypes without '*va*' gene produced vein necrosis on leaf but in contrast, its presence in tobacco cultivars associated with reduced symptoms of PVY after inoculation. Global results of the CORESTA PVY experiments suggest another putative resistance factors to PVY, different from "va". Witherspoon *et al.* (1991) showed that resistance in NC 602 (NCTG 52) line was controlled by a single gene with additive effect, independent from "va". NC 602 (NCTG 52) is a gameto clonal variant rescued from anther culture of PVY-susceptible MN 944 line. Resistant factors in NC95 and Habana 92 as a cigar wrapper tobacco are also reported different from "va" gene.

In the present study tobacco genotypes containing high virus concentration appeared sever vein necrosis in comparison with genotypes KB101, Orumia 205 and Krumograd Kanti. Probably the percentage of disease severity against PVY in KB101, Orumia 205 and Krumograd Kanti genotypes is influenced by *va* gene because any vein necrosis symptom was not observed on these genotypes. However, the existence of virus was confirmed with serological and biological test. To further elucidate the genetic control of PVY resistance in oriental tobacco, and investigate any allelic relations between factors controlling resistance in local lines with factors in internationally resistant lines (vava or VaVa lines) complementary studies is undertaken in our laboratory. As a consequence, new sources of resistance identified in present study to PVY could be used in resistance breeding programs against PVY, TVMV and TEV. An attempt to breed resistant cultivars allowed the development of breeding lines tolerant of all PVY isolates (Doroszewska, 2009).

CONCLUSION

A high genetic variation was observed among studied oriental and semi-oriental tobacco genotypes for susceptibility to PVY. In regard to the results of ELISA, Iranian local tobacco genotypes possessed resistant alleles which encourage tobacco breeders to improve PVY resistance through the selection method. The observed transgressive segregation makes it easier to find genomic regions controlling resistance to PVY in tobacco genome.

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