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Evaluations of Tobacco Cultivars Resistance to *Tobacco mosaic virus* and *Potato virus Y*

[1]Wei Chen, [1]Ting Huang, [1]Jin Dai, [1]Wenting Liu, [2]Julong Cheng and [1]Yunfeng Wu
[1]State Key Laboratory of Crop Stress Biology for Arid Areas and Key Laboratory of Crop Pest Integrated Pest Management on the Loess Plateau of Ministry of Agriculture,
College of Plant Protection, Northwest A and F University, Yangling, 712100, Shaanxi, China
[2]Shaanxi Tobacco Research Institute, 710061, Xi'an, China

Abstract: *Tobacco mosaic virus* (TMV) and *Potato* virus Y (PVY) are two destructive plant viruses which cause significant economic losses to tobacco. Breeding resistant cultivars is one of the most important strategies to control these viruses. In this study, the responses to TMV and PVY of 22 tobacco cultivars were evaluated based on resistance index and virus titre obtained from ELISA in both greenhouses and fields during five years (2007-2011). *Nicotiana glutinosa* resistant to TMV and Virgin A Mutant resistant to PVY were used as resistant checks; NC89 susceptible to both TMV and PVY was used as the susceptible check. The results showed that there were significant differences in disease index and ELISA value among the 22 tobacco cultivars after inoculation. Specifically, 2 cultivars (Coker86 and Jiyan5) were identified to be resistant or moderate resistant to TMV and 3 cultivars (Liaoyan8, Jinxing6007 and Qinyan98) to PVY, while the other cultivars were susceptible or moderate susceptible to TMV or PVY. The resistant cultivars may be used as resistant resources to TMV and PVY in future breeding projects.

Key words: *Tobacco mosaic virus*, *Potato* virus *Y*, resistant cultivars, resistance resources

INTRODUCTION

Tobacco mosaic virus (TMV), typical species of the *Tobamovirus* genus, belongs to the positive-sense single stranded RNA virus. TMV with a very wide host range is known to infect at least 125 individual plant species, which causes huge production losses (Lian *et al*., 2011). Since it was first reported in 1886 (Zaitlin, 1998), TMV has mutated into many stains. Some stains such as TMV-O, TMV-C and TMV-N can infect most members of the *Solanaceae* (Holmes, 1946), which have been the destructive pathogens of tobacco, pepper and tomato.

Potato virus Y (PVY), the member of the family *Potyviridae*, can infect tobacco, tomato and cucumber, especially the members in the Solanaceae family (Ramirez-Rodriguez *et al*., 2009). Based on primary hosts, symptoms and serological reaction, PVY has been classified into three main strains: PVY-N, PVY-O and PVY-C (Ellis *et al*., 1997). The PVY-N strain induces severe vein necrosis on *Nicotiana tabacum* leaves and restricts the yield and quality of tobacco seriously (Ramirez-Rodriguez *et al*., 2009).

TMV and PVY are the members of the most serious plant diseases on tobacco. In China, TMV and PVY have been found in 16 provinces, such as Liaoning, Heilongjiang, Shandong, Henan, Shaanxi, Anhui and Guangdong (Chen *et al*., 1997). The two tobacco viruses can con-infect with other viruses such as Potato virus X (PVX), Cucumber mosaic virus (CMV) and Tobacco vein banding mosaic virus (TVBMV), causing more serious decrease in yield quality and quantity of tobacco (Dai *et al*., 2012; Wu *et al*., 2005). It is more difficult to control plant virus diseases than fungal diseases, and the chemical treatments seem to be ineffective in controlling viruses when they occurred (Bagley, 2002). To control these virus diseases, the integration of cultural practices and resistant cultivars has been developed primarily (Lian *et al*., 2011). The purpose of Cultural practices is to reduce sources of the virus and limit the spread of virus (Bagley, 2002; Holmes, 1946; Koike *et al*., 2000). And the efforts to identify resistant cultivars have also made some achievements. For example, three genes (Tm-1, Tm-2 and Tm-2a) in tomato, four genes (L1-L4) in pepper and N gene in

Corresponding Author: Yunfeng Wu, State Key Laboratory of Crop Stress Biology in Arid Areas and Key Laboratory of Crop Pest Integrated Pest Management on the Loess Plateau of Ministry of Agriculture, College of Plant Protection Northwest A and F University, 712100, Yangling, China Tel: +86-029-87092716 Fax: +86-029-87092716

tobacco (Whitham *et al*., 1996) have been found and applied in breeding to control TMV. Meanwhile, major dominant genes for resistantance to PVY have been identified in *Solanum* (Brigneti *et al*., 1997; Solomon-Blackburn and Barker, 2001). Extreme resistance to PVY has been identified in wild potato species (Cockerham, 1943), while hyper-sensitive resistance to PVY-O has also been reported (Cockerham, 1970; Celebi-Toprak *et al*., 2002; Valkonen, 1997). However, none of the above resistant genes are effective to control all the stains of TMV or PVY because plant virus mutants rapidly (Cai *et al*., 2011; Whitham *et al*., 1996), such as TMV-0b which has overcome *N* gene medicate resistance in tobacco (Csillery *et al*., 1983). Consequently, searching more resistant resources is in urgent need to control TMV and PVY.

In this study, the resistance of 22 tobacco cultivars to TMV and PVY were evaluated in both fields and greenhouses during five years (2007-2011). The objective was to determine whether the resources resistant to PVY and TMV exist in those tobacco cultivars.

MATERIALS AND METHODS

Plant materials and viruses: A total of 22 tobacco cultivars were evaluated under the field and greenhouse condition in this study, including Coker86, Coker176, CV87, C151, G28, G80, Honghuadajinyuan (Hhdjy), Jiyan5, Jingyehuang, Jinxing6007, K326, LJ981, Liaoyan8, Qinyan96, Qinyan98, RG11, Shuangkang70, Yunyan85, Yunyan87, Yunyan97, Zhangyan90 and Zhongyan103. *Nicotiana glutinosa* (*N. glutinosa*) resistant to TMV and Virgin A Mutant (VAM) resistant to PVY were used as resistant checks; NC89 susceptible to both TMV and PVY was used as the susceptible check. All the tobacco cultivars were provided by Shaanxi Tobacco Research Institute.

Virus isolates of TMV and PVY were stored and maintained in *Nicotiana tabacum* under the disease-free greenhouse condition at 25°C with a 16 h light period.

Disease index and resistant index: Grade and investigation methods of tobacco disease were carried out according to the tobacco industry standard of China (Zhu *et al*., 1996). Disease Index (DI) in the inoculated plants was assessed using the following 6 rating scales (Lian *et al*., 2011):

- 0 stands for no symptoms
- 1 stands for mild mosaic symptoms on leaves
- 2 stands for 50% of leaves showing obvious mosaic symptoms
- 3 stands for 100% of leaves showing severe mosaic symptoms
- 4 stands for severe mosaic and deformation of leaves
- 5 stands for severe mosaic and deformation of leaves with stunted growth

Table 1: Scales used to evaluate the resistant levels of tobacco cultivars

	Resistant level (RL)[b]			
Virus resistant Index (RI)[a]	Resistant (R)	Moderately Resistant (MR)	Moderately Susceptible (MS)	Susceptible (S)
TMV[c]	-1.0≤	-1-0	0-0.5	≥0.5
PVY[d]	-0.5≤	-0.5-0	0-1	≥1

[a]Resistant index (RI) = ln(DI/(100-DI)-ln Do/(100-Do)), DI: Disease index of experimental cultivars, Do: Disease index of susceptible check, RI standard for the reaction of the tobacco cultures to virus, [b]Resistant Level (RL) is divided into four kind of scale (R, MR, MS, S), each scale is determined by the value of RI, [c]TMV: Tobacco mosaic virus, [d]PVY: Potato virus Y

The Disease Index (DI) and Resistant Index (RI) were calculated using the following equation (Zhu *et al*., 1996):

$$\text{Disease index (DI)}=\sum \frac{\text{No. of infected tobacco}\times\text{Rating scale of the infected tobacco}}{\text{Total of the investigative tobacco}\times\text{Highest rating scale among the investigative rating scales}}\times 100$$

$$\text{Resistant index (RI)} = \ln(\text{DI}/(100-\text{DI})-\ln \text{Do}/(100-\text{Do}))$$

where, DI is disease index of experimental cultivars, Do is disease index of susceptible check.

Four Resistant Levels (RL) were used to assess the reactions to TMV and PVY (Zhu *et al*., 1996) according to the RI and the scales used to evaluate the resistant levels of tobacco cultivars were listed in Table 1.

Data from each test were statistically analyzed by the SPSS software.

Test under field condition: During five years (2007-2011), the field experiments for resistance evaluation were carried out in the experimental garden of Shaanxi Tobacco Research Institute (Xi'an, China). The field has a long history of continuous tobacco production and a very high incidence of tobacco virus disease such as TMV, CMV, TEV and PVY. In order to prevent the cross infection, the whole experiments were carried out in gray insect-proof net rooms. The experiments were conducted in a randomized complete block design with three replications. The tobacco cultivars were germinated in Shaanxi Tobacco Research Institute on 21 March 2007, 19 March 2008, 22 March 2009, 28 March 2010 and 22 March 2011. Transplant was carried out on 10 April 2007, 10 April 2008, 12 April 2009, 15 April 2010 and 12 April 2011. Thirty tobacco seedlings for each variety were planted in 30 m rows with 1m spacing between plans in each row and rows were 1.5 m apart. Irrigation, fertilizer (8-12 kg N per

thousand tobacco, and the radio of N: P_2O_5:K_2O was1:1:2.5) and so on were applied according to the local tobacco standard cultivation techniques. No pesticide was used during the whole experiments. In late May of every year, all tobacco cultivars were inoculated with the viral inoculums by mechanical inoculation. The viral inoculums used were prepared as follow: The infected leaves were ground and the sap was filtered through cheesecloth and diluted at the rate of 1 g of infected tissue 100 mL^{-1} of 0.01 mol L^{-1} phosphate buffer (PBS, pH = 7.4). The DI of all tobaccos was investigated in late July or early August.

Test under greenhouse condition: Ten tobacco plants with four or five leaves for each cultivar were chosen. And five plants were inoculated with the viral inoculums in greenhouse condition at 25°C with a 16 h light period. The inoculated method was the same as in the field tests. The rest five tobaccos per cultivar were inoculated with 0.01 mol L^{-1} PBS (pH = 7.4) as healthy control. Three weeks later, the non-inoculated new leaves were detected by DAS-ELISA. DAS-ELISA was carried out using a commercially available kit (Neogen Corporation, USA) according to the manufacturer's instructions. Optical density at 405 nm (OD_{405}) was measured by a micro-plate reader (Thermo Multiskan MK3, Shanghai, China) to estimate the virus load of the tested samples. Test samples were considered to be positive if their absorbance values exceeded twice that of negative control.

Detection of N gene: The sequence of N gene was obtained from NCBI (GeneBank: U15605.1) and aligned by the program DNAMAN. Based on the conserved sequence, a pair of primers (F5'-3': ATGGCATCTTCTTCTTC; R5'-3': AAGAACCCCACA CTTTGAG) was designed using the program Primer Premier 5.0.

Total nucleic acids were extracted using phenol/chloroform assay. Approximately 500 mg of leaf tissue was frozen in liquid nitrogen, ground in an RNase-and Dnase-free mortar and homogenized with 500 μL phenol/chloroform (1:1) and 500 μL extraction buffer in a 1.5 mL RNase-and DNase-free microfuge tube. Total nucleic acids were redissolved in 35 μL RNase-and DNase-free water. DNA isolated from tobacco samples was used as PCR template. Following optimization, 12.5 μL PCR mixture included 1 μL of template, 2.5 μL of 25 mM Mg^{2+} (Promega, MadisonWI), 2.5 μL of a dNTP mixture with each dNTP at 5 mM, 2.5 μL of 10×polymerase buffer (Promega, MadisonWI) and 1.5 μL of 5 U μL^{-1} Hot-start Taq polymerase (Promega, MadisonWI) and 1 μL of 10 μM respective upstream and downstream primers. The samples were amplified using the PTC-100 Peltier Thermal Cycler (Bio-Rad, Hercules, CA), with first denaturation at 94°C for 3 min, 35 cycles of denaturation at 94°C for 1 min, primer annealing at 53°C for 1min and primer extension at 72°C for 1min and the final extension at 72°C for 10 min. The size of PCR production was examined by 1.5% Ago-Gel under UV light.

RESULTS AND DISCUSSION

The resistance to both TMV and PVY of 22 tobacco cultivars from different tobacco productions in China was evaluated in the past five years. The present results showed that two cultivars (Coker86 and Jiyan5) were resistant or moderate-resistant to TMV. And three cultivars (Liaoyan8, Jinxing6007 and Qinyan98) were resistant or moderate resistant to PVY. No cultivars were proved to be resistant or moderate resistant to both TMV and PVY.

It was reported that the incidence of TMV and PVY varied among years (Latorre *et al*., 1982; Li *et al*., 2009; Ramirez-Rodriguez *et al*., 2009). In present study, the incidence of TMV was the highest in 2009 followed by 2011, 2007, 2010 and the incidence was the lowest in 2008 (Table 2). For PVY, the highest was in 2009 followed by 2007, 2008, 2010 and the lowest was in 2011 (Table 3). The differences in the incidences of these two diseases could possible due to environmental factors since all the studies were carried out by the same ways.

In the field test, significant differences in DI were observed among 22 tobacco cultivars inoculated with TMV. Take 2009 for example, the DI ranged from 12.44 (*N. glutinosa*) to 44.13 (Jingyehuang). Concretely, Coker86 and Jiyan5 with low DI were found to be similar to resistant control *N. glutinosa* with the DI of 12.44 (Table 2). However, Qinyan98, Shuangkang70, C151, Qinyan96, G80, Jinxing6007, LJ981, K326, Zhongyan103, G28, Yunyan97, Jingyehuang, Hhdjy, RG11, Yunyan85 and Yunyan87 had become susceptible or moderate susceptible to TMV (Table 2). All the susceptible or moderate susceptible tobacco cultivars are grown widely in Shaanxi, Heilongjiang, Liaoning, Shandong and Yunnan, in all of which TMV cause huge economic loss to the tobacco (Chen *et al*., 1997). Therefore, those susceptible cultivars grown in the above regions should be either improved or replaced. The resistant or moderate resistant cultivars (Coker86 and Jiyan5) should be introduced to the above areas and applied into breeding to control TMV. In this study, 19 among the 22 tobacco cultivars had been identified to be susceptible or moderate susceptible to PVY (Table 3). The DI of the

Table 2: Reaction of tobacco cultivars to TMV under field and greenhouse condition

	2007		2008		2009		2010		2011		2012
Cultivars	DI[d]	RL[e]	DI	RL	DI	RL	DI	RL	DI	RL	EV
N. glutinosa	9.99^{ma}	R	6.75^{l}	R	12.44^{l}	R	8.28^{m}	R	10.03^{j}	R	0.216(-)[b]
Coker86	12.01^{l}	R	8.71^{k}	R	15.45^{k}	MR	9.38^{j}	MR	11.56^{i}	MR	0.863(+)
Jiyan5	12.92^{k}	R	8.75^{k}	R	15.46^{k}	MR	11.49^{k}	MR	11.65^{i}	MR	0.929(+)
Coker176	18.05^{j}	MR	22.20^{i}	MS	30.26^{h}	MS	21.67^{fg}	MS	26.01^{g}	MS	1.024(+)
CV87	18.09^{j}	MR	22.21^{i}	MS	30.15^{i}	MS	21.64^{g}	MS	26.17^{g}	MS	1.122(+)
Liaoyan8	18.11^{j}	MR	22.20^{i}	MS	30.14^{i}	MS	21.13^{i}	MS	26.67^{f}	MS	1.226(+)
Zhongyan90	18.15^{j}	MR	22.22^{hi}	MS	30.24^{hi}	MS	21.44^{gh}	MS	26.92^{e}	MS	1.265(+)
Qinyan98	30.79^{h}	MS	22.24^{fh}	MS	30.32^{h}	MS	21.49^{gh}	MS	26.99^{e}	MS	1.399(+)
Shuangkang70	31.12^{g}	MS	22.26^{f}	MS	30.32^{h}	MS	21.87^{f}	MS	26.94^{e}	MS	1.426(+)
C151	30.99^{g}	MS	22.87^{f}	MS	29.13^{j}	MS	21.26^{hi}	MS	27.44^{d}	MS	1.503(+)
NC89(CK)	29.01^{i}	MS	21.89^{j}	MS	29.16^{j}	MS	20.49^{j}	MS	23.69^{h}	MS	1.717(+)
Qinyan96	42.03^{f}	S	21.90^{j}	MS	44.28^{e}	S	21.40^{h}	MS	34.92^{c}	S	1.803(+)
G80	42.04^{f}	S	28.33^{e}	MS	44.32^{de}	S	26.6^{de}	MS	35.57^{b}	S	1.857(+)
Jinxing6007	43.07^{e}	S	28.32^{e}	MS	44.39^{d}	S	26.48^{e}	MS	35.73^{ab}	S	2.451(+)
Longjiang981	43.12^{de}	S	28.33^{e}	MS	44.43^{c}	S	26.74^{d}	MS	35.90^{a}	S	2.931(+)
K326	43.19^{de}	S	28.37^{d}	MS	44.92^{a}	S	26.99^{c}	MS	35.97^{a}	S	3.412(+)
Zhongyan103	43.32^{cd}	S	28.39^{d}	MS	44.93^{a}	S	32.17^{b}	S	-	-	3.428(+)
G28	43.42^{c}	S	28.39^{d}	MS	44.58^{b}	S	32.17^{b}	S	-	-	3.43(+)
Yunyan97	43.47^{c}	S	28.61^{c}	MS	44.84^{a}	S	32.39^{b}	S	-	-	3.432(+)
Jingyehuang	44.34^{a}	S	28.62^{c}	MS	44.13^{f}	S	33.99^{a}	S	-	-	3.433(+)
Hhdjy[f]	44.02^{b}	S	34.97^{b}	S	-	-	-	-	-	-	.442(+)
RG11	44.04^{b}	S	34.96^{b}	S	-	-	-	-	-	-	3.442(+)
Yunyan85	44.09^{b}	S	35.08^{a}	S	-	-	-	-	-	-	3.458(+)
Yunyan87	44.17^{ab}	S	35.09^{a}	S	-	-	-	-	-	-	3.459(+)
Negative control	-c	-	-	-	-	-	-	-	-	-	0.122
Positive control	-	-	-	-	-	-	-	-	-	-	3.454(+)
Healthy control	-	-	-	-	-	-	-	-	-	-	0.166(-)

[a]Within columns, means followed by the same letter are not significantly different at p = 0.05, [b]Within columns, (-) or (+) indicates virus negative or positive samples, respectively, [c]Within columns, means the test was not carried out, [d]Within columns, means disease index, [e]Within columns, means resistant level, [f]Within columns, means Honghuadajingyuan

Table 3: Reaction of tobacco cultivars to PVY under field and greenhouse condition

	2007		2008		2009		2010		2011		2012
Cultivars	DI[d]	RL[e]	DI	RL	DI	RL	DI	RL	DI	RL	EV
VAM(CK)	11.89^{ha}	R	11.73^{k}	R	12.44^{i}	R	9.20^{j}	R	7.86^{g}	R	0.109(-)[b]
Liaoyan8	11.92^{h}	R	11.75^{k}	R	12.46^{i}	R	9.26^{j}	R	7.89^{g}	R	0.111(-)
Jinxing6007	14.35^{f}	MR	17.20^{j}	MR	19.23^{h}	MR	9.28^{j}	MR	7.93^{g}	R	0.111(-)
Qinyan98	20.52^{g}	MS	17.21^{ij}	MR	19.24^{h}	MR	14.4^{i}	MR	9.92^{f}	MR	0.125(-)
Shuangkang70	20.52^{g}	MS	17.22^{ij}	MR	19.24^{h}	MR	14.44^{hi}	MR	9.93^{f}	MR	0.425(+)
Jiyan5	20.53^{g}	MS	17.25^{i}	MR	19.28^{h}	MR	14.5^{h}	MR	9.99^{f}	MR	0.764(+)
corker 86	28.03^{f}	MS	21.88^{h}	MS	29.17^{g}	MS	18.42^{g}	MS	13.64^{e}	MS	0.947(+)
Qinyan96	28.04^{f}	MS	21.89^{h}	MS	29.18^{fg}	MS	18.43^{g}	MS	13.67^{e}	MS	0.951(+)
Yunyan87	28.04^{f}	MS	21.91^{h}	MS	29.22^{fg}	MS	18.49^{f}	MS	13.70^{e}	MS	0.985(+)
zhangyan90	28.06^{f}	MS	28.33^{g}	MS	29.23^{f}	MS	18.49^{f}	MS	17.75^{d}	MS	1.043(+)
C151	28.59^{e}	MS	28.33^{g}	MS	29.40^{e}	MS	18.5^{f}	MS	17.77^{d}	MS	1.23(+)
LJ981	28.59^{e}	MS	28.34^{g}	MS	29.43^{e}	MS	18.98^{f}	MS	17.79^{d}	MS	1.565(+)
Jingyehuang	28.60^{e}	MS	28.38^{f}	MS	29.58^{d}	MS	18.99^{ef}	MS	17.8^{d}	MS	1.687(+)
G28	28.61^{e}	MS	28.39^{f}	MS	29.91^{c}	MS	19.04^{e}	MS	17.81^{d}	MS	1.826(+)
NC89(CK)	29.43^{d}	MS	28.4^{ef}	MS	29.93^{c}	MS	22.17^{d}	MS	19.13^{d}	MS	1.883(+)
Yunyan85	29.46^{d}	MS	28.43^{de}	MS	29.94^{c}	MS	28.94^{c}	MS	19.13^{c}	MS	2.405(+)
Hhdjy[f]	34.01^{b}	MS	28.45^{d}	MS	29.94^{c}	MS	28.99^{c}	MS	20.31^{b}	MS	2.509(+)
G80	34.02^{b}	S	34.97^{c}	S	33.31^{b}	S	31.85^{b}	S	32.12^{a}	-	2.656(+)
K326	34.03^{b}	S	34.98^{c}	S	33.31^{b}	S	31.89^{b}	S	32.14^{a}	-	2.766(+)
Coker176	34.29^{a}	S	35.09^{b}	S	33.33^{b}	-	31.89^{b}	-	32.21^{a}	-	2.976(+)
Yunyan97	34.29^{a}	S	35.09^{b}	S	33.35^{ab}	-	31.9^{b}	-	32.21^{a}	-	3.16(+)
RG11	34.29^{a}	S	35.10^{b}	S	33.38^{a}	-	32.29^{a}	-	32.24^{a}	-	3.22(+)
CV87	34.31^{a}	S	35.14^{a}	S	33.38^{a}	-	32.29^{a}	-	32.24^{a}	-	3.43(+)
Zhongyan103	34.32^{a}	S	35.16^{a}	S	33.39^{a}	-	32.3^{a}	-	32.27^{a}	-	3.431(+)
Negative control	-[c]	-	-	-	-	-	-	-	-		3.434(+)
Positive control	-	-	-	-	-	-	-	-	-		0.09
Healthy control	-	-	-	-	-	-	-	-	-		3.389(+)

[a]Within columns, means followed by the same letter are not significantly different at p = 0.05, [b]Within columns, (-) or (+) indicates virus negative or positive samples, respectively, [c]Within columns, means the test was not carried out, [d]Within columns, means disease index, [e]Within columns, means resistant level, [f]Within columns, means Honghuadajingyuan

19 tobacco cultivars were higher or showed no significant difference ($p \leq 0.05$) with that recorded for NC89 (Table 3). PVY also frequently occurs in combination with other viruses such as TMV, CMV and PVX (Dai *et al*., 2012). This co-infection leads to tremendous economic losses. So, those cultivars susceptible to PVY should be avoided growing in the areas where PVY had been one of the most common viruses such as Henan, Yunnan and Liaoning (Chen *et al*., 1997). Liaoyan8, Jinxing6007 and Qinyan98 were considered to be resistant or moderate resistant to PVY, which will be the important resistant resources for anti-virus disease breeding. Those resistant tobacco cultivars also should be introduced into the areas where PVY had been popular to control PVY.

In summary, several results were confirmed based on symptomatology, (1) Fourteen cultivars (Yunyan85, Hhdjy, G80, K326, Yunyan97, RG11, Zhongyan103, Shuangkang70, C151, LJ98, Qinyan96, G28, Jingyehuang and Yunyan87) were thought to be susceptible or moderate susceptible to both TMV and PVY, (2) Five cultivars (Coker176, CV87, Coker86, Zhongyan90 and Jiyan5) were resistant or moderate resistant to TMV but susceptible or moderate susceptible to PVY, (3) Three cultivars (Liaoyan8, Jinxing6007 and Qinyan98) were susceptible to TMV but resistant or moderate resistant to PVY. There were no cultivars resistant or moderate resistant to both TMV and PVY.

Under the greenhouse condition, significant differences in ELISA values were observed among tobacco cultivars after inoculation. For TMV, the OD_{405} value of resistant check was lower than all the tobacco cultivars (Table 2). However, the virus titre in Corker86 with OD_{405} value of 0.76 and Jiyan5 with 0.929 kept at a low level (Table 2). This result confirmed that Corker86 and Jiyan5 were tolerant to TMV (Table 2). The OD_{405} values of 19 cultivars ranging from 1.024 to 3.549 were twice higher than negative check with OD_{405} value of 0.122 (Table 2). It was showed that these 19 cultivars were susceptible to TMV, which was similar to the field tests. For PVY, the ELISA values ranged from 0.109 (VAM) to 3.431(Zhongyan90) (Table 3). Although no cultivars showed more effective resistance to PVY than VAM, two cultivars (Liaoyan8 and Jingxing6007) with low virus concentration were considered to be negative for PVY (Table 3). This confirmed that Liaoyan8 and Jingxing6007 were resistant to PVY.

The tobacco cultivars with N gene are few in China. The N gene was found in *Nicotiana glutinosa* which confers a gene-to-gene resistance to TMV (Whitham *et al*., 1996). Because of its high level resistance, the N gene was initially introduced into tobacco breeding (Gerstel, 1945; Holmes, 1938). In this study, the N gene was found in 6 tobacco cultivars among 22 tobacco cultivars (Fig. 1). Therefore, it is an urgent task for Chinese breeders to fully use N gene.

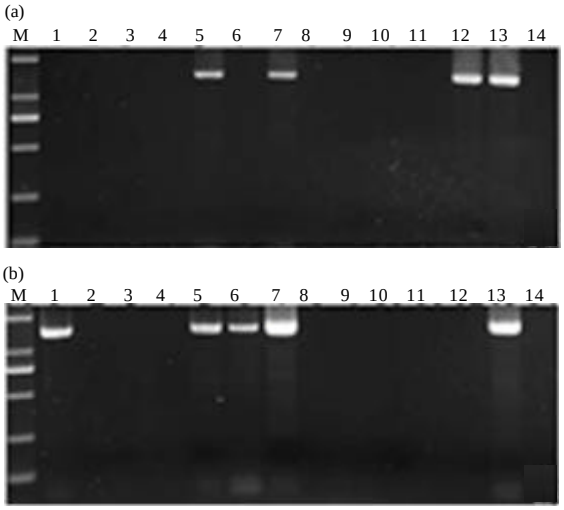

Fig. 1(a-b): Detect N gene in the tobacco cultivars by PCR, (a): M: DL2000, 1-12: Coker86, Coker176, CV87, C151, G28, G8, Honghuadajinyuan (Hhdjy), Jiyan5, Jingyehuang, Jinxing6007, K326, *N.glutinosa*; 13: positive control; 14: negative control and (b): M: DL2000, 1-12: LJ981, Liaoyan8, Qinyan96, Qinyan98, RG11, Shuangkang70, Yunyan85, Yunyan87, Yunyan97, Zhangyan90, Zhongyan103, NC89; 13: positive control; 14: negative control. The N gene was detected using the special primers (F5'-3': ATGGCATCTTCTTCTTC; R5'-3': AAGAACCCCACACTTTGAG) with the production of 1413bp among the 22 tobacco cultivars

The use of varieties resistant to TMV or PVY is considered to be one of the most effective and environment-friendly ways to protect tobacco (Koike *et al*., 2000). However, the loss-of-function of resistant genes has been the major limitation due to the development of new strains of virus that overcome the resistant genes. TMV-O overcoming the N gene is a typical phenomenon. In this study, although the N gene was found in 6 tobacco cultivars, 5 of 6 tobacco cultivars had been susceptible or moderate susceptible to TMV (Table 2). The reason for this phenomenon may be just as follow: (1) the function of N gene was affected by the environmental factors. N gene is sensitive to temperature. At temperature of 28°C and above, N gene mediated resistance is suppressed and TMV moves systemically (Whitham *et al*., 1996). (2) The mutation was in N gene.

The N gene is a member of the TIR-NBS-LRR class of plant resistance genes (Takabatake *et al*., 2006). The deletion and point's mutation of TIR can interfere with the wild-type N gene function and lead to loss-of-function (Takabatake *et al*., 2006). Thus, the basic production practices such as crop rotation and seed sterilization should also be taken into consideration to control virus diseases except for the selection and breeding resistant cultivars.

CONCLUSION

In conclusion, under both field and greenhouse conditions, resistance or moderate resistance to TMV was observed in 2 (Coker86 and Jiyan5) cultivars. And 3 (Liaoyan8, Jinxing6007 and Qinyan98) cultivars were resistant or moderate resistant to PVY. These resistant resources may be directly used in future breeding programs, genetic studies, identification of molecular markers. Meanwhile, the basic production practices such as crop rotation and seed sterilization should also be taken into consideration to control virus diseases.

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