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ORIGINAL ARTICLE

Management of *Potato virus Y* (PVY^{-NTN}) causing potato tuber necrotic ringspot disease (PTNRD) in potato by prior treatment with a mild PVY strain

Osama A. Abdalla*, Amal I. Eraky, Safynaz A. Mohamed, Fikry G. Fahmy

Department of Plant Pathology, Faculty of Agriculture, Assiut University, Assiut Governorate 71515, Egypt

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*Corresponding address:
osama-mohammad@utulsa.edu

Abstract

Potato virus Y (PVY) is one of the most destructive viruses infecting potato in Egypt and worldwide. Recent research has shown that a necrotic PVY^{-NTN} strain is infecting potato in Upper Egypt. Chemical control is not effective to control this viral pathogen. An alternative to control PVY infecting potato is using a mild PVY strain to elicit systemic cross protection in potato plants against infection with a severe necrotic strain of PVY. Results of this study showed that a PVY necrotic strain produced a significant lesser number of local lesions on diagnostic plants (*Robinia pseudoacacia* L.) when these plants were treated first with a mild PVY strain. Data obtained from greenhouse and field experiments indicated that treatment of potato plants (variety Burna) with a mild PVY strain significantly protected potato from infection with a severe necrotic PVY strain, and resulted in a significant increase in tuber yield compared with infected plants without prior treatment with a mild PVY strain. The highest increase in potato tuber yield was obtained when potato plants were inoculated with a mild PVY strain 3 days before challenging with the severe necrotic PVY strain. This study proved that using a mild strain of PVY can significantly protect potato plants from infection with a severe strain of this virus under both greenhouse and field conditions and can present a potential method to reduce losses due to infection of this virus in Assiut governorate and Upper Egypt.

Key words: cross protection, mild strain, potato, *Potato virus Y* (PVY), severe strain

Introduction

Potato (*Solanum tuberosum* L.) is being infected with more than 40 viruses (Abdalla *et al*. 2015). Among these viruses *Potato virus Y* is infecting potato in most production regions worldwide (Nolte *et al*. 2004). *Potato virus Y* (PVY) is a member of the genus Potyvirus in the family Potyviridae (Maki-Valkama *et al.* 2001). PVY is transmitted by aphids in the non-persistent manner with optimal acquisition and transmission probe durations of about a minute (Harrington *et al.* 1986).

PVY causes severe losses in many crops especially potato, tobacco, tomato and pepper (Cuevas *et al*. 2012; Quenouille *et al.* 2013). In Egypt, PVY is considered to be one of the most limiting factors of potato production (El-Mohsen *et al.* 2003; El-Helay *et al.* 2012), especially necrotic strains PVY^{-NTN} which cause potato tuber necrotic ringspot disease (PTNRD) (Abdalla *et al.* 2016b). Traditional control strategies are not useful to control plant viruses (Boquel *et al*. 2014) since aphids can transmit PVY before being killed by insecticides (Perring *et al*. 1999). An alternative method to control plant viruses is cross protection or using a mild strain to confer resistance against infection with the severe strain (Hamilton 1980). Cross protection was first reported by McKinney (1929), who found tobacco plants systemically infected with a "light green strain" of *Tobacco mosaic*

virus (TMV), became resistant to infection with the severe TMV strains. Cross protection could be practiced to protect plants from viral infection (Yeh and Gonsalves 1984). It was applied successfully against different viruses including *Sugarcane mosaic virus* (Fahmy *et al.* 1986), and against *Potato virus X* (Lawson *et al.* 1990), *Potato virus A* (Valkonen 2002), and *Pepino mosaic virus* (Hasiów-Jaroszewska *et al.* 2014).

The aim of this present study was to evaluate the possibility of using a mild PVY strain to protect potato from infection with the severe necrotic PVY^{-NTN} strain under field and greenhouse conditions.

Materials and Methods

Source and identification of severe *Potato virus Y*

Potato virus Y was isolated from the Burna potato variety, which showed typical, naturally curly mosaic symptom of leaves, and was identified using biological, serological, and molecular tests as previously described by Abdalla *et al.* (2016a). This PVY strain was characterized as PVY^{-NTN} according to its biological properties and nucleotide sequences of coat protein gene as described by Abdalla *et al.* (2016b).

Source and identification of the mild strain

Mild mosaic symptoms were observed on naturally infected Cherry tomato (*Solanum lycopersicum*) growing in Assiut governorate. A purified particle of infected leaves was examined under an electron microscope as described in Abdalla *et al.* (2016a). The RNA was extracted from infected leaves and tested in RT-PCR using specific PVY primers as previously described by Abdalla *et al.* (2016a). Plant sap from the infected leaf of this virus was crushed using phosphate buffer 0.1 M pH 7.2 and mechanically inoculated into 5-day-old potato plants after the appearance of the first leaves by rubbing carborundum dust leaves as described by Abdalla *et al.* (2016a). Potato plants were maintained in an insect proof greenhouse at 22 ± 2°C for further experiments.

Evaluation of the pathogenicity of the PVY mild strain

To biologically assess the symptoms induced by this strain on these diagnostic plants, infected tomato leaves showing the mild mosaic symptoms were crushed in 0.1 K M H_2KPO_4 buffer and mechanically inoculated into 14 different diagnostic plants (Table 1) which are known to be susceptible against PVY infection.

Table 1. Diagnostic plants used in the bioassay of the PVY mild strain

No.	Common name	Scientific name
1	Broad bean	*Vicia fabae* L.
2	Common rue	*Ruta graveolens* L.
3	Common sage	*Salvia officinalis* L.
4	Datura	*Datura stramonium* L.
5	False acacia	*Robinia pseudoacacia* L.
6	Garden petunia	*Petunia hybrid* L.
7	Grape tomato	*Solanum lycopersicum* L.
8	Kidney beans	*Phaseolus vulgaris* L.
9	Nabak, cidir	*Ziziphus spina-christi* L.
10	Neem tree	*Azadirachta indica* L.
11	Potato	*Solanum tuberosum* L.
12	Sweet basil	*Ocimum basilicum* L.
13	Tobacco	*Nicotiana tabacum* L.
14	White lupin	*Lupinus termis* L.

Effect of prior inoculation of a mild PVY strain on disease development caused by the severe PVY strain

Leaves from cherry tomato plants (naturally infected with a mild PVY strain) were crushed thoroughly in a sterilized mortar with 0.1 M phosphate buffer pH 7, then filtered through clean muslin and mixed with carborundum. *Robinia pseudoacacia* L. plants (30-days old) were inoculated with the aforementioned solution.

The inoculum of the severe necrotic PVY strain was prepared as described in the mild PVY strain, pre-treated plants were challenged with the severe PVY strain 0, 1, 2 and 3 days after inoculation with the mild strain. The number of developed local lesions was calculated as the average number of 12 plants in each treatment. The experiment was repeated twice, under both greenhouse and field conditions during the 2013/2014 and 2014/2015 growing seasons.

Effect of dual inoculation with mild and severe PVY strains on tuber yield of potato plants

Under greenhouse conditions

Potato tubers free from viruses [as confirmed by serological tests as described by Abdalla (2016a)] were planted in 6 inch sterilized pots filled with 700 g of sterilized clay soil. Twelve potato plants, 5 days after the appearance of the first leaves, were inoculated with the aforementioned solution using a spray gun under an air pressure of 4.1 bar as described by Mandal *et al.* (2007). Subsequently, inoculation with the severe strain of PVY was carried out at different times:

a) at the same time as inoculation with the mild strain; b) 1 day after inoculation with the mild strain; c) 2 days after inoculation with the mild strain and d) 3 days after inoculation with a mild strain. Yield per gram was recorded after harvest for each plant. An infected control with the severe isolate alone was used and also a non-infected control was used. The experiments were repeated in two successive seasons (2013/2014 and 2014/2015).

Field experiments

Potato tubers (Burna variety) free from viral infection were planted in rows, and the width between cultivated rows was 60 cm. The inoculums of both mild and severe PVY strains were prepared in 0.1 m phosphate buffer pH 7.0 and carborundum 320 grit, Potatoes were inoculated first with chilled inoculum of mild PVY strain using an air pressure of 4.1 bar as described by Mandal *et al*. (2007). Later potato plants were inoculated with the severe strain 0, 1, 2, 3 days after inoculation with the mild strain (12 plants for each application time). The experiment was designed as a randomized block design, in two consecutive growing seasons (2013/2014 and 2014/2015). Reduction in disease severity was measured as an increase in potato tuber weight. The weight of tubers from each plant was recorded after harvest.

Statistical analysis

All data were subjected to statistical analysis and means were compared using the LSD test Gomez and Gomez (1984).

Results

Evaluation of the pathogenicity of the PVY mild strain

Symptoms induced by the mild PVY strain were evaluated on different diagnostic plants. The results of this assay are presented in Table 2, and show that most of the tested diagnostic plants were symptomless 30 days after inoculation, including Datura, Common rue, Cidir, Neem tree, Sweet basil, False acacia, while in the other diagnostic plants including petunia, tomato and tobacco only mild mosaics were observed on these plants. However, the most severe symptoms were observed on kidney bean and broad bean as local lesions and mottling appeared on these plants. This mild PVY did not produce any symptoms on potato plants. All diagnostic plants were tested in ELISA to confirm the presence of PVY.

Table 2. Evaluation of the pathogenicity of the PVY mild strain

No.	Diagnostic plants	Reaction (symptoms)
1	Broad bean (*Vicia fabae*)	L.L
2	Common rue (*Ruta graveolens*)	N.S.
3	Common sage (*Salvia officinalis*)	N.S.
4	Datura (*Datura stramonium*)	N.S.
5	False acacia (*Robinia pseudoacacia*)	M.Y
6	Garden petunia (*Petunia hybrid*)	M.Ms
7	Grape tomato (*Solanum lycopersicum*)	M.Ms
8	Kidney beans *Phaseolus vulgaris*)	L.L
9	Nabak, cidir (*Ziziphus spina-christi*)	M.Y
10	Neem tree (*Azadirachta indica*)	M.M
11	Potato (*Solanum tuberosum*)	N.S.
12	Sweet basil (*Ocimum basilicum*)	M.Y
13	Tobacco (*Nicotiana tabacum*)	M.ML
14	White lupin (*Lupinus termis*)	N.S

L.L = local lesions; M.ML = mild mottling 30 days after inoculation; M.Ms = mild mosaic; M.Y = mild yellowing; N.S = No significant symptoms

Effect of prior treatment with a PVY mild strain on disease development in *Robinia pseudoacacia* infected with a severe PVY strain

Under greenhouse conditions

Results presented in Table 3 showed that a prior inoculation of *R. pseudoacacia* with a mild PVY strain significantly reduced the number of local lesions produced on plants challenged later with a severe PVY strain. These data revealed that the efficiency of mild PVY to reduce local lesions depended on the interval period between treatment with the mild and severe PVY strains, and this efficiency increased as the interval period increased. The highest reduction in the number of local lesions was observed when *R. pseudoacacia* was inoculated with the mild PVY strain 3 days before challenging with the severe PVY strain in both successive trials under greenhouse conditions.

Under field conditions

Similar results were obtained under field conditions, as treatment with the mild PVY strain significantly reduced the number of local lesions on *R. pseudoacacia* subsequently challenged with the severe PVY strain in both consecutive growing seasons (2013/2014 and 2014/2015). Data presented in Table 4 displays that the highest reduction was observed when *R. pseudoacacia* were treated with the mild PVY strain 3 days before challenging with the severe PVY strain. There were no significant differences between these plants and healthy plants.

Table 3. Effect of prior inoculation with a mild PVY strain on the number of local lesions on *Robinia pseudoacacia* caused by a severe PVY strain (under greenhouse conditions)

Trials / Treatments	Number of local lesions on *R. pseudoacacia*		
	trial one 2013/2014	trial two 2014/2015	mean
Severe PVY only	49.50*	46.00*	47.75
Inoculation of mild and severe PVY strains at the same time	13.5	13.00	13.25
Inoculation of mild PVY strain one day before severe strain	10.75	9.75	10.25
Inoculation of mild PVY strain two days before severe strain	6.5	4.5	5.50
Inoculation of mild PVY strain three days before severe strain	2.0	1.0	1.50
Healthy control	0.0	0.0	0.00
Mean	13.70	12.37	13.04
*average number of local lesions on 12 plants			
LSD at 5%:	3.91	2.50	

Table 4. Effect of prior inoculation with a mild PVY strain on the number of local lesions on *Robinia pseudoacacia* caused by a severe PVY strain (under field conditions)

Trials / Treatments	Number of local lesions on *R. pseudoacacia*		
	trial one 2013/2014	trial two 2014/2015	mean
Severe PVY only	44.25*	45.00*	44.62
Inoculation of mild and severe PVY strains at the same time	12.25	11.50	11.87
Inoculation of mild PVY strain one day before severe strain	10.00	10.75	10.37
Inoculation of mild PVY strain two days before severe strain	4.75	4.00	4.37
Inoculation of mild PVY strain three days before severe strain	1.00	1.00	1.00
Healthy control	0.00	0.00	0.00
Mean	12.04	12.0	12.020
*average number of local lesions on 12 plants			
LSD at 5%:	3.24	2.83	

Effect of prior inoculation with a mild PVY strain on tuber yield of potato plants infected with a severe PVY strain

Under greenhouse conditions

It was found that a prior inoculation of a PVY mild strain can significantly protect potato plants from infection with the severe strain of the same virus. This protection was measured on the basis of potato tuber yield, since infection with PVY usually resulted in a significant reduction in the tuber yield. Data presented in Table 5 show that a prior inoculation with a mild PVY strain significantly increased the potato tuber yield in plants (challenged later with a severe PVY strain), compared with plants challenged only with a severe PVY strain without prior treatment with a mild PVY strain. This significant increase in potato tuber yield occurred in all treatments, but the increase in tuber yield was correlated with the interval period between inoculation of a mild and a severe PVY strain. The highest increase in tuber yield was obtained when plants were inoculated with a mild PVY strain 3 days before challenging with the severe PVY strain in both successive trials (2013/2014 and 2014/2015).

Under field conditions

Similar results were obtained in field trials. Prior inoculation with a mild PVY protected potato from PVY infection and significantly increased the potato tuber yield in both 2013 and 2014 growing seasons. Data presented in Table 6 indicate that when the interval period between inoculation of mild and severe strains increased, the tuber yield increased. The highest production was achieved when potatoes were treated with a mild PVY strain 3 days before challenging with a severe PVY strain. Interestingly, there were no significant differences between the tuber yield of non-infected and infected potato plants in this last treatment in both (2013/2014 and 2014/2015) growing seasons.

Table 5. Effect of prior inoculation with a mild PVY strain on tuber yield of potato plants infected with a severe PVY strain (under greenhouse conditions)

Trials / Treatments	Potato tuber yield [g]*		
	trial one 2013/2014	trial two 2014/2015	mean
Severe PVY only	93.67*	297.15*	195.41
Inoculation of mild and severe PVY strains at the same time	166.98	160.00	163.49
Inoculation of mild PVY strain one day before severe strain	308.35	370.35	339.35
Inoculation of mild PVY strain two days before severe strain	713.93	838.93	776.43
Inoculation of mild PVY strain three days before severe strain	914.8	964. 8	914.80
Healthy control	863.23	908.78	886.00
Mean	510.16	515.04	512.60
*average gram per 12 plants			
LSD at 5%	52.448	78.98	

Table 6. Effect of prior inoculation with a mild PVY strain on tuber yield of potato plants infected with a severe PVY strain (under field conditions)

Trials / Treatments	Potato tuber yield [g]*		
	trial one 2013/2014	trial two 2014/2015	mean
Severe PVY only	503.07*	467.84*	485.45
Inoculation of mild and severe PVY strains at the same time	1139.73	1262.8	1201.26
Inoculation of mild PVY strain one day before severe strain	1390.58	2195.08	1792.83
Inoculation of mild PVY strain two days before severe strain	1365.63	1703.33	1534.48
Inoculation of mild PVY strain three days before severe strain	1812.23	3105.60	2508.915
Healthy control	1868.82	2854.00	2361.41
Mean	1346.67	1948.10	1647.39
*average gram per 12 plants			
LSD at 5%	204.2	309.6	

Discussion

Losses of potato yields due to viral pathogens are still considered to be some of the most restricting factors of potato production worldwide (De Bokx and Huttinga 1981). *Potato virus Y* is one of the most common viruses threatening potato production (Dupuis *et al.* 2017). It causes serious losses in the potato yield in Egypt (EL-Absawy *et al.* 2012; Aseel *et al.* 2015), especially, the PVYNTN strain which causes severe tuber necrotic ringspot disease (PTNRD) and was reported to cause serious disease in Egypt (Elwan *et al.* 2017). A recent study found that this strain is replacing the old and ordinary strain in the traditional potato production area in Upper Egypt (Abdalla *et al.* 2016b). This situation makes it urgent to find a method to control this destructive disease in Egypt. Using insecticides seems to have low impact on the spread of PVY (Boquel *et al.* 2014), as aphids often transmit PVY before being killed (Perring *et al.* 1999).

In the present study, mild mosaic symptoms were observed on cherry tomato growing in Assiut governorate (Upper Egypt). Examination of a virus particle by electron microscopy revealed that the causal viral particles are long filamentous shaped rods, 650–750 nm in length. Serological and molecular identification tests determined that this virus is PVY. Bioassay of the virus on different diagnostic plants showed that this mild PVY strain produces no symptoms or very mild symptoms on these diagnostic plants, including potato which did not exhibit any symptoms and remained symptomless after inoculation with this mild strain, although these plants reacted positive against PVY in ELISA tests, indicating that the strain can infect potato without causing any symptoms. Identification of a mild PVY strain provided an indication about possibly using this mild strain to control severe ones.

The results obtained from this study indicate that prior treatment with a mild PVY strain can protect potato plants against infection with the severe PVY strain

and reduce losses in tuber potato yield. This protection against infection with PVY was assessed on the basis of reduced numbers of local lesions on diagnostic plants, or increased potato tuber yield as proposed by Latorre and Flores (1985).

This current study found that the degree of this protection depends on the interval time between treatment with the protecting virus (mild strain) and the challenging virus (severe strain). In general, when potato plants were treated with the protecting strain (mild PVY strain) a long time before challenging with the severe strain, the protection against PVY increased (potato tuber yield increased). The highest increase in tuber yield was achieved when potato plants were treated with a mild PVY strain 3 days before challenging with a severe PVY strain. These results agree with a previous conclusion proposed by Gal-On and Shiboleth (2006) namely, that a long time is required for a mild strain to protect plants from infection with a severe strain of the same virus.

The data from the present study showed that application of a mild protecting strain of PVY is an efficient method to reduce losses in potato tuber yield due to infection with the necrotic PVY^{-NTN} strains. Cross protection (pre-immunization) has already been reported against plant viruses including: *Tomato mosaic virus* (Ahoonmanesh *et al.* 1981), *Tobacco mosaic virus* (Cassells and Herrick 1977), *Citrus tristeza virus* (Costa and Muller 1980), *Potato virus Y* (Latorre and Flores 1985), and *Papaya ringspot virus* (Tennant *et al.* 1994; Chiang *et al.* 2007).

The mechanisms by which a mild strain can provide plants with protection against severe strains are not completely clear, but two main hypotheses were proposed to explain this phenomenon, either, the CP-mediated inhibition of virion disassembly, or the posttranscriptional gene silencing (PTGS) based on degradation of viral RNA (Valkonen 2002). CP-mediated resistance depends on treatment with a protecting mild strain which leads to prevention of uncoating (disassembly) of the challenge virus as it enters the plant, and thus thwarts the virus replication cycle (Culver 1996 and Lu *et al.* 1998). The other hypothesis proposes that a mild strain can induce RNA silencing (PTGS) in plants against a severe strain of the same virus. RNA silencing is a general term for host defense mechanisms that are targeted against invasive viruses, viroids (Xie *et al.* 2004) Plant viruses possess a counter-defense mechanism against RNA silencing and can suppress this gene silencing (Roth *et al.* 2004). However, it was suggested that treatment with a protecting mild strain can enhance plants to activate the RNA silencing against the severe strain and thus prevent the severe strain from suppression of plant defense mechanism RNA silencing and this agrees with the results of this study.

This study indicates that using a mild PVY strain can significantly protect potato plants from infection with the severe necrotic PVY strain and increase potato tuber production under field conditions.

Conclusions

Potato virus Y causes severe losses in potato production in Egypt, especially with the current spread of the PVY^{-NTN} necrotic strain causing PTNRD disease in Upper Egypt. The results of the present study revealed that using a mild PVY strain can significantly protect potato from infection with the severe necrotic PVY^{-NTN} strain under greenhouse and field conditions. The current study found that the greatest increase in potato tuber yield was achieved when potato plants were treated with a protecting mild PVY strain 3 days before inoculation with the challenging severe strain under field conditions

This current study suggests that using a mild PVY strain presents a possible solution to controlling the PVY^{-NTN} necrotic strain causing PTNRD disease in Upper Egypt under field conditions.

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