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Movement and Distribution of Potato Leafroll Virus Antigen in Resistant Potato Genotypes

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Abstract

Twelve potato clones with variable degrees of resistance to potato leaf roll virus (PLRV) were co-infected with potato virus X (PVX) or potato virus Y (PVY) before or after inoculations with PLRV. In some PLRV resistant clones, PVX and PVY facilitated the movement and also increased the concentration of PLRV antigen. PLRV could not be rub-transmitted in mixed infections with the sap-transmissible viruses, PVX and PVY. PLRV did not observably affect the movement and concentration of PVX and PVY in resistant potato clones. PLRV also did not affect the movement of PVX into non-inoculated leaf tissue of cultivars Saco and USDA seedling 41956, nor did it cause systemic movement of PVX in hypersensitive *Gomphrena globosa*. PLRV was detected in infected plants by enzyme-linked immunosorbent assay (ELISA) and tissue blotting on nitrocellulose membranes, using polyclonal antibodies. Tissue blotting proved to be a more sensitive method, especially for detection of [ow titer antigens from plant tissue. These studies indicate that an essential function of PLRV inhibited in resistant plants may be at least partially complemented by active function of PVX or PVY.

Introduction

Potato is one of the most important dicotyledonous agronomic crops worldwide and is a major food source in most countries, especially those with temperate climates (Rich, 1983). Potatoes are usually vegetatively propagated by planting seed pieces or whole tubers, or by using lateral buds. This method of propagation perpetuates many diseases, especially viruses. Among the most important of these are PLRV, PVX and PVY viruses.

PLRV is aphid transmitted in a circulative, persistent, but non-propagative manner and is a one of the most serious and economically important viruses of potato (Jones *et al*., 1982; Rich, 1983; Rochow and Duffus, 1981). Particularly in chronic infections, PLRV decreases tuber yields. Current season infection with PLRV often causes necrosis of phloem tissue of tubers (called net necrosis) of some cultivars, a defect that renders tubers unmarketable. Net necrosis is particularly severe in the popular Russet. Burbank cultivar. PVX, a mechanically transmitted virus, occurs wherever potatoes are grown. Yields have been reduced 15 percent or more by PVX compared to virus free plants (Anonymous, 1986; Hooker, 1981). PVY has long been recognized as infecting cultivated potatoes. It can be divided into different strain groups (De Bokx and Huttinga, 1981; Jones, 1981, 1990) and can be transmitted mechanically as well as by aphids in the non-circulative and non-persistent manner.

Barker and Harrison (1985) reported that the PLRV occurs in lower concentrations in leaves of the several potato cultivars with higher resistance ratings. The lower virus concentration found in infected plants of resistant genotypes may reduce the chances of aphid transmission from infected plants (Barker and Harrison, 1986).

With respect to resistance, a number of different interactions between various viruses in the same host have been reported. Potato clones resistant to PLRV commonly do not degenerate rapidly, but after a period, they may become susceptible when infected with another virus. In Peru, a case was reported (Jayasinghe *et al*., 1989) in which the cultivar Mariva, which has a high level of resistance under greenhouse conditions, is susceptible in the field. The loss of resistance in these plants has been associated with the presence of other viruses.

A number of cases are known in which one virus complements the movement of another unrelated virus in resistant hosts (Carr and Kim, 1983; Jayasinghe *et al*., 1989; Ross, 1954). Also, in the presence of one virus, the concentration of other viruses may be increased many times (Barker, 1989). The restriction of beet curly top virus to the phloem was broken in beans and its concentration was increased ten fold by the presence of localized, necrotic tobacco mosaic virus (TMV) infection on primary leaves. Similarly, the restriction of TMV to local lesions on N gene containing Nicotiana glutinosa leaves was overcome by the co-infection with PLRV (reported in previdus sections of this dissertation).

This work was undertaken to determine whether co-infection with sap-transmissible viruses would affect resistance of potato clones to PLRV.

Materials and Methods

Plant Material: All potato clones used in this study were supplied by Dr. Charles Brown, Potato Breeder, USDA-ARS, Route 2, Box 2953-A, Prosser, WA 99350, USA. Clone 88A is the Fl of *Solanum chacoense*, a species resistant to PLRV and *Solanum phureja*, a susceptible species. Crosses

were made to get potato genetic material used in the breeding strategy to obtain suitable clones having variable degrees of resistance against potato leafroll virus. Crossing procedure is described as under:

BC-160	=.	*Solarium chacoense*
IVPB-2	=	*Solarium phureja*
BC 160 x IVPB-2	=	FI (88A)11 (88A) x
IVPB-2	=	88 R

Clones designated 88R are 88A backcrossed with *S. phureja*. A number of these progenies were selected based on various characteristics such as height, bushy, slender. Some of these clones showed resistance to PLRV, some were susceptible and others were intermediate in response. Clones from all 3 categories were included to study responses to mixed infections.

Tubers of clones used in this study were grown in 15-cm clay pots containing a mixture of sand, loam and peat moss. These pots were maintained in an insect-free glass greenhouse with supplementary light provided as needed to maintain 16-hour day length. The lights were rotary, low pressures, 1000 Watt, sodium lamps (70,000 lux). The temperature was maintained between 20-24°C. When plants were 20-25 cm tall, 4-5 cm long cuttings with at least one lateral bud, were made and were planted in vermiculite and kept in the same glass greenhouse. Liquid fertilizer consisting of 6 per cent nitrogen, 6 percent phosphorus and 6 per cent potassium diluted 1:200 was continuously added to the irrigation water throughout the experiment. When cuttings were 8-10 cm tall with 4-5 fully extended leaves (about 4-5 weeks); they were transferred to 12.5-cm diameter clay pots with the same potting mixture and were inoculated.

Virus Isolates: PLRV isolate No.7 was maintained on *Datura tatula* kept in an isolated insectory at 22-25°C with aphids (*Myzus persicae*). The PVX and PVY were local strains isolated from infected potatoes and maintained on *Nicotiana tabacum* cultivar Xanthi nc. grown at 20-24°C.

Virus Transmission: Plants were inoculated with PLRV using viruliferous aphids (*M. persicae*) reared on infected *D. tatula* plants. Aphids, 10-15 per plant, were allowed to feed for 48 hours, then killed using nicotine sulfate fumigation at the end of the transmission-period. PVX and PVY were rub-inoculated on Carborundum-dusted (600 mesh) lower leaves of each plants with a cheesecloth pad moisten with the inoculum. Inoculum for both viruses was prepared by grinding fresh diseased leaf tissue diluted 1:10 w/v in 50 mM potassium phosphate buffer, pH 7.2, using a mortar and pestle. Two of the lower expanded leaves were lightly dusted with 600 mesh Carborundum powder and rubbed with the virus suspension. Plants were divided into 2 sets with each receiving a different inoculation regime. In one set, PLRV was inoculated first. After initial establishments of virus infection, about 7-10 days, PVX or PVY inoculations were first and after 4-6 days PLRV inoculations were made. Inoculated plants were kept in an insect-produce greenhouse at 18-24°C with supplementatary illumination during the winter months. Non inoculated plants served as controls.

Virus Detection and Assay: PLRV, PVX and PVY were detected from systemic leaf tissue by using a two stage (Kaniewski and Thomas, 1988) modification of the double antibody sandwich ELISA (SAS-ELISA) described by (Clark and Adams, 1977). Samples were diluted 1:50 for all these viruses. The antibodies used in this study were product previously in our laboratory. ELISA readings were read as optical density at 405 nm using a Gifford model EIA reader. The presence of PLRV in plant tissue was also detected using a tissue blot method developed to determine the specific locations of PLRV antigen in infected plants. The method is described in detail in section 3 of the dissertation. Briefly, freshly cut free hand cross sections of infected to healthy plants were pressed lightly on a nitrocellulose membrane for 30 seconds. These membranes were processed to produce an insoluble reaction product at sited where antigen was bound. The results were observed and photomicrographed with a binocular stereoscope.

Results

Effects of PVX on resistance to PLRV: Of the 12 potato clones inoculated with PLRV alone, 5 (88R 13.27, 88R 15.19, 88R 15.37, 88R 16.18, 88R 16.6) were infect and 7 (88R 14.45, 88R 17.20, 88R 17.28, 88R 14.2 88R 14.41, 88R 14.9, 88R 17.22) were not infected according to ELISA (Table 1). Among the 7 that were not infected by PLRV alone, plants of 3 clones (88R 14.45, 88R 17.20, 88R 17.28) could be infected with PLRV if th were first infected with PVX. However, the concentration of the PLRV antigen in plants of these 3 clones was mud lower than in the 5 clones susceptible or in Russet Burbank control. Concentration of virus in the 5 clones infectable PLRV alone was comparable to that in the Russet Burbank control. infection with PVX after inoculations with PLRV not affect the initial virus infection or the concentration PLRV antigen in any of the 12 clones.

Effects of PVY on resistance to PLRV: The affects of PLRV infection on susceptibility of the 12 clones to PLRV similar to those described for PVX. Among the 7 done that were not infected by PLRV alone, 4 (88R 14.45, 88R 17.20, 88R 17,22, 88R 17.28) could be infected was PLRV if they were first infected by PVY (Table 2). Pre-infection with PVY permitted infection of PLRV in additional clone (88R 17.22) that was not permitted PVX. Post infection with PVY permitted infection was PLRV in two clones (88R 14.45, 88R 17.20) that could not be infected by PLRV alone. Again, the concentration PLRV antigen in clones infected by PLRV, only in the presence of PVY was generally lower than in plants infection by PLRV alone.

Table 1: Effects of potato virus X on ELISA detectable potato leafroll virus antigen in potato plants

		Clones	ELISA readings_1/	
		PLRV alone	PLRV + PVX	PVX + PLRV
88R	13.27	1.999	1.999	1.999
88R	15.19	1.294	0.613	1.179
88R	15.37	0.610	1.028	1.671
88R	16.18	1.046	0.587	1.999
8811	16.60	1.131	1.999	1.320
88R	14.45	0.013	0.004	0.156
88R	17.20	0.014	0.012	0.418
88R	17.28	0.005	0.008	0.253
88R	14.20	0.017	0.007	0.013
88R	14.41	0.019	0.010	0.028
88R	14.90	0.003	0.017	0.023
88R	17.22	0.046	0.028	0.017
Russet Burbank (control)				

Table 2: Effects of potato virus Y on ELISA detectable potato leafroll virus antigen in potato plants

		Clones	ELISA readings_1/	
		PLRV alone	PLRV + PVX	PVX + PLRV
88R	13.27	1.999	1.999	1.999
88R	15.19	1.294	1.673	0.829
88R	15.37	0.610	0.803	0.571
88R	16.18	1.046	0.834	1.403
8811	16.60	1.131	1.999	1.320
88R	14.45	0.013	0.570	0.479
88R	17.20	0.014	0.159	0.104
88R	17.22	0.046	0.006	0.078
88R	17.28	0.005	0.003	0.073
88R	14.20	0.009	0.015	0.003
88R	14.41	0.019	0.019	0.023
88R	14.90	0.003	0.012	0.003
Russet Burbank (control)		1.036	1.017	0.961

_1/; Optical density at 405 nm

Table 3: Effects of potato leafroll virus on ELISA detectable potato virus X antigen in potato plants

		Clones	ELISA readings_1/	
		PLRV alone	PLRV + PVX	PVX + PLRV
88R	13.27	0.718	0.607	0.923
88R	14.20	1.649	1.999	1.861
88IR	14.41	0.493	0.494	0.647
88R	14.45	1.852	0.787	1.573
88R	14.90	1.348	1.600	1.613
88R	15.19	0.306	1.006	1.001
88R	15.37	1.999	0.698	0.717
88R	16.18	0.562	0.631	0.559
881R	16.6	1.510	1.893	1.000
88R	17.20	1.999	1.956	1.780
88R	17.22	0.426	0.693	0.981
88R	17.28	0.335	0.716	0.664
Russet Burbank (control)		0.924	0.717	1.063

Table 4: Effects of potato leafroll virus on ELISA detectable potato virus Y antigen in potato plants

		Clones	ELISA readings_1/	
		PLRV alone	PLRV + PVX	PVX + PLRV
88R	13.27	0.534	0.605	0.561
88R	14.45	0.903	1.127	0.745
88IR	16.18	0.512	0.481	0.609
88R	16.60	0.252	0.550	0.183
88R	14.20	0.002	0.009	0.006
88R	14.41	0.005	0.017	0.002
88R	14.90	0.012	0.004	0.006
88R	15.19	0.006	0.005	0.006
8811	15.37	0.014	0.009	0.006
88R	17.20	0.017	0.003	0.010
88R	17.22	0.001	0.007	0.057
88R	17.28	0.013	0.001	0.009
Russet Burbank (control)		0.585	0.714	0.722

_1/; Optical density at 405 nm

We tried to mechanically transmit PLRV to *N. tabacum* cultivar Xanthi nc. plants using inocula from PLRV infected plants that were also co-infected with either PVX or PVY. ELISA was used to detect PLRV from systemic leaf tissue of inoculated plants, 10, 20 and 30 days after the inoculations. PLRV could not be detected from the systemic tissue of plants inoculated with PLRV alone or in combination with PVX and PVY, but PVX and PVY were readily detected in all 3 ELISA performed. There were no observable effects of PVX and PVY on the rub transmission of PLRV in N. tabacum cultivar Xanthi nc.

Effects of PLRV on resistance to PVX: All 12 potato clones and Russet Burbank were susceptible to PVX (Table 3) when inoculated with PVX alone, therefore the influence of PLRV on the systemic movement of PVX in resistant plants could not be determined. It was observed that the concentration of PVX in plants infected with PVX only or co-infected with PLRV was the same.

Potato variety Saco which is immune to PVX USDA seedling 41956 which is extremely resistant to PVX (Ross, 1954, 1960) and Gomphrena globose, a local lesion host for PVX (Berck, 1970), were separately inoculated with PVX only and PVX with pre or post inoculations with PLRV. In all 3 hosts, co-infection with PLRV failed to affect the infection and movement of PVX. ELISA could not detect PVX in non-inoculated leaves of these plants. No different was observed in the shape, size, or color of local lesions that developed on plants inoculated with PVX only or in plants co-infected with PLRV. PVX remained confined

to the local lesions and it could not be detected from non inoculated tissue even after 12 weeks of incubation. Also, PVX was not detected from non-inoculated tissue of Saco and USDA seedling 41956, inoculated with PVX only or co-infected with PLRV.

Effects of PLRV on resistance to PVY: Among 12 potato clones inoculated with PVY only, 4 were susceptible while 8 were resistant. In plants co-infected with pre or post PLRV inoculations, no change in susceptibility pattern was observed (Table 4). Resistant clones remained resistant to PVY in mixed infections and PLV infection did not make these plants susceptible. There was no significant difference in the concentration of PVY between co-infected or singly infected plants.

Effects on the movement of PLRV: Results of tissue blotting showed that PLRV doubly infected plants remained confined to the phloem tissue. Pre or post inoculations with PVX and PVY did not provide the movement function that PLRV requires to move from phloem cells to parenchyma cells. This pattern was observed in both resistant as well as in plants that became susceptible by co-infections.

Discussion

The fact that PVX and PVY assisted infection of PLRV suggests the possibility that resistance to PLRV is based on the repression of an essential step in PLRV infection and that this step may be performed by similar functiong of PVX and PVY. The fact that PLRV antigen was much lower in plants that were infected only in the presence of PVX or PVY suggests that PVX and PVY did not provide the required function efficiently. The data may provide some information concerning the PLRV function repressed in the resistant potato clones. In their studies on resistance to PLRV in potato plants, Barker and Harrison (1985) found that virus accumulated to similar extent in individual cells of resistant and susceptible potato genotypes but infected few cells in the resistant plants. They concluded that resistance was caused by partial inhibition of cell-to-cell spread of PLRV in phloem tissue. Among the plants in this study in which PVX or PVY infection assisted PLRV infection, no cells containing PLRV could be found by tissue blotting analysis unless the plants were also inoculated with PVX or PVY. If PLRV moved systemically in plants inoculated with PLRV alone, it was not concentrated in a few cells and its accumulation was restricted to levels not detectable by ELISA.

The capacity of prior, but not post, PVX infection to assist infection by PLRV suggested that resistance to initial infection, i.e. PLRV infected into plants at the time of inoculation did not survive long in the absence of PVX infection. However, the capacity of both prior and post PVY inoculation to assist PLRV infection did not support this hypothesis.

Tissue blotting on nitrocellulose membrances is easily used for the detection of plant viruses (Lin *et al*., 1990). We used polyclonal antibodies for the detection of PLRV, PVX and PVY and found that these viruses can be detected from all plant tissues including sterns, roots and leaves. A advantage of the tissue blotting method is that it can be used to determine the exact location of antigens in pla Moreover, this method is easy to use and tissue membranes can be stored for a long period of time and be re-examined whenever needed.

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