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Effects of Potato X Potexvirus on the Development of Late Blight in Selected Potato Genotypes

M. Hossain, O.S. Opina[1], U. Jayasinghe[2] and N.B. Bajet[1]

Tuber Crops Research Centre
Bangladesh Agricultural Research Institute
Joydebpur, Bangladesh

ABSTRACT. *Effects of potato X potexvirus (PXV) on the development of late blight in potato and, purification and antiserum production of the virus were studied in Philippines during two seasons. Inoculation of plants with PXV delayed the onset and reduced the severity of late blight infection caused by Phytophthora infestans inseveral potato genotypes. Inoculation with PXV also reduced the zoospore penetration and capacity of sporulation of P. infestans and size of the lesions developed. A higher peroxidase activity was found in the genotypes infected with the virus than those with P. infestans alone. Potato genotypes inoculated with PXV + P. infestans gave a significantly higher tuber yield when compared to those infected with the fungus alone.*

The virus was purified through sucrose gradient centrifugation. The yield of the purified PXV varied with the location of infection. The PXV antiserum produced in rabbits showed a titer between 10^{-6} to 10^{-7} in ELISA and was able to detect the presence of PXV in field samples of potato.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is an important staple food crop that is grown in many countries. However, this crop suffers from many viral, fungal, bacterial and mycoplasmal diseases during growth and development. Among the viral diseases, potato X potexvirus (PXV) is the third most important after potato leaf roll luteovirus (PLRV) and potato Y potyvirus (PYV). A total of seven viruses of potato namely, PLRV, PYV, PXV, potato S carlavirus (PSV), potato M carlavirus (PMV), potato A potyvirus (PAV), and tobacco mosaic tobamovirus (TMV) have so far been reported in the

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1 Department of Plant Pathology, University of the Philippines Los Baños, College, Laguna 4031, Philippines.

2 International Potato Centre, Baguio, Philippines.

Philippines (Talens, 1979; Balaoing *et al.*, 1979; Balaoing and Verzola, 1980; Luis, 1981).

Gupta *et al.* (1985) reported that the common viruses such as PYV and PLRV reduced the tuber yield of potato up to 60–75% while the mild ones such as PXV, PSV and PMV reduced yield by 10–30%. In the Philippines, single infection by PXV and PSV reduced the tuber yield of potato by 3.6–13% and 8%, respectively (Luis, 1981). There has been no concerted and sustained efforts to increase productivity of potato in the country by managing the viral diseases. One drawback for this is the unavailability of more reliable and sensitive techniques to detect and identify these viruses in the field or to ascertain the presence of these viruses in planting materials.

Among the fungal diseases, late blight caused by *Phytophthora infestans* (Mont.) de Bary is the most important in potato, and has been recorded worldwide in places where the crop grows. This pathogen affects the leaves, stems, petioles, and tubers and causes serious yield losses (Alexopoulos and Mims, 1979).

Under field conditions, double infection by both fungal and viral diseases are common in potato plant. As multiple diseases are common in field conditions, it is necessary to develop a variety tolerant or resistant to combined effects of the pathogens. Resistance of a host against a pathogen may be changed by the presence or interaction with another pathogen. Several reports are available on the interaction between viral and late blight of potato (Darsow and Wulfert, 1989; Kalra *et al.*, 1989a; 1989b). Mukhopadhyay and Sengupta (1967) reported that pre–infection with viruses that cause mild mosaics may have synergistic effect on the natural susceptibility of potato to the late blight in the field. On the other hand, Kalra *et al.* (1990) reported that the effect of PYV infection inhibited the liberation and germination of zoospores of *P. infestans*.

Thus, the objectives of this study were: (i) to quantify the effect of PXV on the development of late blight in relation to yield of some potato genotypes, (ii) to determine the peroxidase activity of potato as influenced by PXV and late blight and, (iii) to purify and produce antiserum of PXV.

MATERIALS AND METHODS

The experiments were conducted at the Northern Philippines Root Crops Research and Training Center (NPRCRTC), La Trinidad, Benguet for

two seasons (dry : January–April, 1996 and wet : May–August, 1996) both in the field and greenhouse conditions. Laboratory experiments were conducted at the Department of Plant Pathology, University of the Philippines Los Baños (UPLB), Laguna, the Virology laboratory of the Entomology and Plant Pathology Division of the International Rice Research Institute (IRRI), Los Baños, Laguna and, NPRCRTC, La Trinidad, Benguet from October, 1995 to May, 1997.

Propagation of the potato genotypes

Potato (*Solanum tuberosum* L.) genotypes were received from the International Potato Centre (CIP), Lima, Peru as *in vitro* plantlets. The genotypes were multiplied through successive apical stem cuttings (5–6) to achieve around 500 seedlings per genotype and allowed to grow roots for 21 days in paper cups in the greenhouse and used in the study.

Isolation and propagation of P×V isolate

Isolates of PXV were collected from the field at Benguet and the Institute of Plant Breeding, UPLB, Laguna and were identified through host range, ELISA test and EM studies in addition to physical properties (Hossain, 1997). The ringspot strain of PXV was propagated in *Nicotiana glutinosa* and *N. tabacum var.* White Burley and were used in the study.

Preparation of inoculum of *P. infestans*

Potato leaves with typical symptoms of late blight were collected from the field at La Trinidad, Benguet, Philippines, washed with distilled water and inserted to a sliced potato tuber which was previously surface sterilized with burning alcohol. The tubers were then kept in a cool chamber at 18°C in plastic bags with sufficient moisture for 24 h to enhance sporulation. The sporangiospores were scraped and suspended in distilled water and placed in the refrigerator (about 6°C) for 2 h for zoospore release. The amount of zoospore in the suspension was standardized to about 3000 motile zoospore ml^{-1} by counting a 10 ml aliquot from the suspension under microscope (Diccion, 1994).

Artificial inoculation of *P. infestans*

The test plants in the green house were inoculated with a zoospore suspension of *P. infestans* (3000 zoospore ml^{-1}) using a plastic hand sprayer following the procedure of Chien (1989) with some modifications. The inoculated plants, both in wet and dry seasons, were covered with polyethylene sheets for 15 h. In both field and green house experiments, the inoculated plants were sprayed with water for 7 days after inoculation to maintain high relative humidity. Nine and six potato genotypes with known level of reactions to PXV and *P. infestans*, respectively, were used in the experiments.

Recommended doses of fertilizer at the rate of 120–120–120 kg ha^{-1} of N, P_2O_5 and K_2O, respectively, and 50% of the dosage of complete fertilizer and chicken manure (at the rate of 4 tons ha^{-1}) were basally applied to the soil. Fifty percent of the requirement of complete fertilizer mixture and, urea at the rate of 50 kg ha^{-1} were side dressed one month after planting during ridging. The fungicides Dithane M–45 and Ridomil MZ–58 were sprayed on to plants in the plots alone with PXV and control plots to control late blight infection. All the plots were sprayed with the insecticide Tamaron to control vectors of the viruses.

The unit plot size was 2.1 m × 2.1 m and each plot comprised of 21 plants with 70 cm×30 cm spacing. The treatments were arranged in a split plot design with three replicates. The main plot treatments were; (1) potato plants inoculated with PXV at 30 days after planting, (2) potato plants inoculated with *P. infestans* at 45 days after planting, (3) potato plants inoculated with PXV at 30 days after planting and followed by *P. infestans* at 15 days after inoculation of PXV, and (4) plants without inoculation (control).

Data collection

Late blight development was determined using standard rating scale (Henfling, 1982) and zoospore penetration, incubation period, latent period, sporulation, peroxidase activity (as below), tuber yield per plant, were determined/conducted. Data were analyzed statistically.

Peroxidase assay

Peroxidase activity (PA) was assayed according to the procedure described by Kar *et al.* (1985). Discs (12 mm dia.) from each leaf blade (3rd leaf from tip), which were collected from 5 different plants from each treatment, were pooled together and kept at 4°C for 30 min. The leaf discs were then homogenized with cold 0.1 M phosphate buffer (pH 6.0;100 mg leaf in 2 ml buffer) using a pre–chilled mortar and pestle. The homogenate was centrifuged at 4,250 rpm for 20 min and the supernatant was used as the enzyme preparation for the assay.

An aliquot of 3.5 ml of the assay mixture consisting of 2.5 ml of buffer–substrate solution (1:1:1 v/v/v/ of 0.03% hydrogen peroxide: 1% pyrogallol in 50% ethanol: 0.1 M phosphate buffer, pH 6.0) and 1 ml enzyme preparation (0.5 ml enzyme extract and 0.5 ml of 0.1 M phosphate buffer, pH 6.0) was allowed to react for 3 min at 27°C. The reaction was stopped by adding 0.5 ml of 2N H_2SO_4. The amount of purpurogallin formed was determined by the absorbance at 420 nm. Enzyme activity was expressed in units where one unit is the amount of purpurogallin formed which raised the absorbance by 0.1/min under assay conditions. The PA was tested before inoculation, 7 days after inoculation with PXV, and 7 and 15 days after inoculation with *P. infestans*.

Purification of PXV

Virus purification was done according to the CIP protocol with some modification (CIP, 1993). Leaves of *Nicotiana glutinosa* and *N. tabacum var.* White Burley with typical symptoms of the viral infection were harvested 20–22 days after inoculation. The infected leaves were homogenized in a pre–cooled blender with 2 volumes of cold 0.1 M phosphate buffer (pH 8.0) containing 0.2% 2–mercaptoethanol and 10% ethanol. The sap was extracted through 3 layers of gauze cloth and centrifuged at about 8,000×g in a Sorvall RC–2B Centrifuge for 20 min at 4°C. After adding 1% Triton X–100, the supernatant was stirred for 1 h at 4°C and clarified again by centrifugation at 6,000×g for 20 min at 4°C. The virus purification was done through PEG precipitation followed by sucrose gradient centrifugation (20–50%). The virus band was collected manually and diluted with 0.01 M phosphate buffer (pH 7.2) and the virus was sedimented through centrifugation at 125,000×g (Beckman Ti45 rotor) for 90 min. The viral pellet was then re-suspended with

0.01 M phosphate buffer (pH 7.2). The concentration of the virus was determined in a UV spectrophotometer using a PXV extinction coefficient of 2.97 (Noordam, 1973) and the yield per kg plant sample was calculated.

Antiserum production

A rabbit was immunized intramuscularly at 7 and 14 day intervals. For each injection 0.2 mg purified virus was used with 500 µl of phosphate buffer (0.01 M, pH 7.2) and 500 µl of adjuvant. After the 4th injection, the rabbit was killed and the serum collected was clarified through low speed centrifugation and kept at -20^{0}C. The antiserum titer was determined by comparing reaction with the pre–immune serum with infected leaf samples and purified virus samples in ELISA. The antiserum was tested against its homologous antigen (purified virus) in ELISA. Its applicability to detect the virus in field samples of potato, tomato and pepper was compared with the antiserum obtained from International Potato Centre (CIP).

RESULTS AND DISCUSSION

Incubation and latent periods of *P. infestans*

In the field experiments, the mean incubation period of *P. infestans* was higher in genotypes inoculated with both the virus and fungus when compared to those inoculated with the fungus alone (Table 1), in both dry and wet seasons. In the greenhouse experiment conducted during dry season, the mean incubation period of *P. infestans* in the genotypes inoculated together with the virus was about 3.67 days which was significantly higher than that of the genotypes inoculated only with the fungus. Significantly higher latent period was noted in virus inoculated plants compared to those inoculated with *P. infestans* alone.

The average latent periods for different genotypes under field conditions during dry season were 5.67 and 6.67 days when inoculated with *P. infestans* alone and PXV + *P. infestans*, respectively (Table 1). However, in the greenhouse experiments the latent period in the genotypes inoculated with *P. infestans* alone was from 5 to 7 days and it varied from 5 to 8 days in those inoculated with both virus and fungus. In the wet season, under green house conditions, the average latent periods of *P. infestans* were 5.44 and 5.93

Table 1. Effect of potato X potexvirus (PXV) infection on incubation and latent periods of *P. infestans* on selected potato genotypes at La Trinidad, Benguet.

Treatment/Genotype	Incubation period (days)				Latent period (days)			
	Dry season		Wet season		Dry season		Wet season	
	Field	GH	Field	GH	Field	GH	Field	GH
Pi alone								
CIP 375335.1	3	3	3	3	6	5	5	5
CIP 384112.8	3	3	4	4	6	5	7	7
CIP 384112.14	3	3	3	3	5	5	5	4
CIP 384724.13	3	4	3	4	6	6	5	6
CIP 390353.3	3	4	-	4	5	6	-	6
CIP 676025	3	3	-	3	5	5	-	5
CIP 720090	3	3	-	3	5	5	-	4
CIP 800953	3	3	3	3	5	5	5	5
CIP 800983	5	4	5	4	8	7	7	7
Mean	3.22	3.33	3.5	3.44	5.67	5.44	5.67	5.44
PXV+Pi								
CIP 375335.1	3	3	4	4	6	5	6	6
CIP 384112.8	4	4	4	4	7	7	7	7.3
CIP 384112.14	4	4	3	4	6	6	5	6
CIP 384724.13	4	4	4	4	8	7	6	6
CIP 390353.3	4	4	-	4	6	6	-	6
CIP 676025	4	3	-	3	6	5	-	5
CIP 720090	3	3	-	3	5	5	-	4
CIP 800953	3	3	4	3	6	5	6	5
CIP 800983	6.3	5	6	5	10	8	8	8
Mean	3.92	3.67	4.17	3.78	6.67	6.0	6.33	5.93
CV (%)(Main Plot)	10.1	6.7	19.9	6.5	7.6	4.1	4.8	2.4
LSD (5%)(Main Plot)	0.42	0.27	NS	0.27	0.55	0.27	0.41	0.16

Pi - *P. infestans* GH - Greenhouse
\- - Not included in the study
PXV+Pi - Inoculated with PXV prior to *P. infestans*

days in genotypes inoculated with the fungus alone and PXV + *P. infestans*, respectively. Similar variations in incubation and latent periods of *P. infestans* have also been reported by Kalra *et al.* (1989a).

Efficiency of infection

The mean infection efficiency (zoospore penetration) of *P. infestans* was significantly higher (77.50%) in potato genotypes inoculated only with the fungus than that observed in genotypes pre-inoculated with PXV (Table 2). Among the genotypes pre-inoculated with PXV, efficiency of infestation of *P. infestans* was significantly reduced in CIP 384112.8, CIP 384724.13 and CIP 800983, where the latter recorded the least infection efficiency.

The increased resistance to the penetration of *P. infestans* in PXV infected plants which observed in the present studies is similar to the findings of Pietkiewicz (1974). Increase of resistance to penetration of fungal zoospores after virus infection may have resulted from the destruction of mycelium or even of the infection pegs.

Sporulation period

Significant variations of mean sporulation periods *P. infestans* were observed between those inoculated with the fungus alone and those with PXV + *P. infestans*. The average sporulation periods of different genotypes inoculated with *P. infestans* alone and those with PXV + *P. infestans* were 8.57 and 7.27 days, respectively. Among the different genotypes infected with the fungus alone, the highest mean sporulation period of 10.80 days was observed in CIP 800953 and the lowest (7.2 days) in CIP 375335.1 (Table 2). The reduction of sporulation period of *P. infestans* with prior PXV infection observed in the present study may be due to the change in the host nutritional status by virus infection.

Sporulation capacity

The mean sporulation capacity of *P. infestans* varied significantly between the genotypes inoculated with the fungus alone (697.59 sporangia per 125 mm^2) and PXV + fungus (423.11 sporangia per 125 mm^2) (Table 2). Among the genotypes, the fungus sporulation was the highest in CIP 720090. Less fungal sporulation was observed in plants infected with *P. infestans* +

PXV than those with the fungus alone. Similar results have been reported by Kalra *et al.* (1989a).

Table 2. Effect of potato X potexvirus (PXV) on infection efficiency (%), sporulation period (days) and sporulation capacity (Sporangia per 125 mm²) of *P. infestans* in different potato genotypes at La Trinidad, Benguet.

Genotypes	Infection efficiency (%)*		Sporulation period (days)		Sporulation capacity (Sporangia/125 mm²)	
	Pi alone	PXV+Pi	Pi alone	PXV+Pi	Pi alone	PXV+Pi
CIP 375335.1	89.33 a	67.67 a	7.20 e	6.00 d	258.33 e	217.33 e
CIP 384112.8	69.67 b	50.00 c	8.20 c	7.00 c	887.00 d	564.00 c
CIP 384112.14	86.33 a	68.67 a	7.80 d	7.60 b	1133.33 c	351.67 d
CIP 384724.13	74.67 b	60.67 b	7.40 e	6.73 c	319.00 e	194.67 ef
CIP 800953	83.67 a	65.63 ab	10.80 a	7.07 c	1308.67 b	864.00 b
CIP 800983	61.33 c	43.33 b	10.00 b	9.20 a	57.00 f	38.67 g
CIP 390353.3	-	-	-	-	55.67 f	64.00 g
CIP 676025	-	-	-	-	133.00 f	109.67 fg
CIP 720090	-	-	-	-	2126.33 a	1404.00 b
Mean	77.50	59.33	8.57	7.27	697.59	423.11
CV (%) (main plot)	3.5		1.3		4.1	
LSD (1%) (main plot)	6.51		0.33		61.63	

Within each column, means followed by the same letter are not significantly different at p = 0.05 by the Duncan's New Multiple Range Test.

Pi - *P. infestans*
PXV+Pi - inoculated with PXV prior to *P. infestans*
* - Data were analysed after transformation using arc sine
\- - Not included in the study

Degree of infection of *P. infestans*

The PXV infection prior to inoculation of plants with *P. infestans* significantly reduced the severity of infection of the fungus both in the field and greenhouse experiments (Table 3). Among the genotypes inoculated with PXV and *P. infestans*, the level of the fungal infestation ranged from 1.00 (CIP 800983) to 8.67 (CIP 720090) and in those with *P. infestans* alone from 1.67 (CIP 800983) to 9.0 (CIP 720090) at 82 days after planting (DAP). In the greenhouse study during the dry season, the level of infestation of *P. infestans* in the presence PXV was lower than when the genotypes were infected with the fungus alone (Table 3).

The level of infection of *P. infestans* at different time intervals after planting were lower in plants infected with PXV + fungus than in those with the fungus alone. These results agree with the findings of Muller and Munro (1951), Nagaich and Prasad (1971), Pietkiewicz (1974,1975), Kalra *et al.* (1989a). In contrary, Mukhopadhyay and Sengupta (1967) reported that the virus infected plants with mild mosaic symptoms were severely infected with *P. infestans*, although the identity of the viruses was not confirmed. On the other hand, Nagaich and Prasad (1971) reported that the susceptibility of potato varieties to *Alternaria solani* increased due to PYV and, decreased due to PXV infection.

Peroxidase activity

Higher levels of peroxidase activity were observed in potato plants with host-resistance to late blight (Table 4). These results are agreement with the findings of Umaerus (1959) and Groza and Olteanu (1982).

Tuber yield of potato

The mean yields of potato genotypes under the various treatments were significantly different (Table 5). The highest mean yield (343.52 g per plant) was observed in the uninoculated genotypes and was not significantly different from the yield obtained with the genotypes infected with PXV alone (336.48 g per plant) in the dry season. However, the yield of genotypes inoculated with *P. infestans* + PXV (289.15 g per plant) and *P. infestans* alone (264.15 g per plant) were significantly lower than the non-inoculated and those inoculated with PXV alone. In addition, the average yields were significantly different between the genotypes inoculated with

Table 3. Level of infection of *P. infestans* (1–9 scale) at different times after planting in some potato genotypes as influenced by potato X potexvirus (PXV) infection during dry season (January–April, 1996) at La Trinidad, Benguet.

Treatment/Genotype	Level of infestation of *P. infestans* (scale 1-9)									
	Field					Greenhouse				
	Days after planting					Days after planting				
	54	61	69	76	82	56	63	70	77	84
Pi alone										
CIP 720090	3.00	8.33	8.33	9.00	9.00	3.33	4.33	5.67	6.33	7.33
CIP 384112.14	2.33	4.67	5.00	5.00	6.67	2.00	2.33	3.00	4.00	4.67
CIP 800953	2.00	3.67	3.67	4.00	6.33	1.67	3.00	3.33	4.00	4.67
CIP 375335.1	2.67	5.33	5.33	5.33	5.33	2.33	3.33	4.00	5.00	5.00
CIP 676025	1.00	2.00	2.33	3.00	4.00	1.00	2.00	2.33	3.00	3.33
CIP 384724.13	1.00	2.33	2.67	3.00	3.33	1.00	2.00	3.00	3.33	3.67
CIP 384112.8	1.00	2.67	3.00	3.00	3.00	1.00	1.67	2.00	2.33	2.67
CIP 390353.3	1.00	1.00	1.67	1.67	2.00	1.00	2.33	2.67	3.33	3.67
CIP 800983	1.00	1.00	1.00	1.33	1.67	1.00	1.00	1.33	1.33	1.67
Mean	1.67	3.44	3.67	3.93	4.59	1.59	2.44	3.04	3.63	4.07
PXV + Pi										
CIP 720090	2.67	7.67	7.67	8.33	8.67	2.33	4.00	5.00	5.67	6.67
CIP 384112.14	1.67	4.00	4.00	4.00	6.00	1.33	2.00	2.33	2.67	3.67
CIP 800953	1.67	3.00	3.00	3.33	5.33	1.33	2.33	3.00	3.33	3.33
CIP 375335.1	2.33	4.33	4.33	4.33	4.33	1.67	2.33	2.67	3.00	3.67
CIP 676025	1.00	1.00	1.67	2.00	3.00	1.00	1.67	2.00	2.33	2.33
CIP 384724.13	1.00	2.00	2.67	3.00	3.00	1.00	1.67	2.00	3.00	3.00
CIP 384112.8	1.00	1.33	2.33	2.67	2.67	1.00	1.33	1.33	1.67	2.00
CIP 390353.3	1.00	1.00	1.00	1.33	1.67	1.00	1.67	1.67	2.00	2.33
CIP 800983	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mean	1.48	2.81	3.07	3.33	3.96	1.30	2.00	2.33	2.74	3.11
CV (%)	10.7	9.3	11.1	14.1	10.4	9.4	16.1	6.7	7.1	8.4
LSD (5%)	NS	0.09	0.12	0.15	0.12	0.08	0.06	0.06	0.07	0.07
LSD (1%)	-	0.14	0.17	0.22	0.17	0.11	0.15	0.10	0.11	0.10

The scale was adopted from Henfling (1982), and ranges from 1 (no infection) to 9 (highest degree infection).

Pi - Inoculated with *P. infestan* ; PXV+Pi - inoculated with PXV prior to *P. infestans*

* - Data were analyzed after square-root transformation

CV and LSD values for mean treatments

Table 4. **Peroxidase actvity (units per g fresh wt.) in different potato genotypes as influenced by potato X potexvirus (PXV) infection in the field during dry season (January–April, 1996).**

Genotypes	Peroxidase activity (units/g fresh weight)										
	Treatments										
	30 DAP	37 DAP		53 DAP				62 DAP			
	T4	T1	T4	T1	T2	T3	T4	T1	T2	T3	T4
CIP 375335.1	137	149	126	78	167	111	87	111	80	106	108
CIP 384112.8	140	119	119	132	161	115	184	114	101	112	106
CIP 384112.14	116	133	122	85	162	86	124	86	94	102	105
CIP 384724.13	178	163	148	154	182	125	168	154	140	175	126
CIP 390353.3	173	122	192	88	145	107	138	145	126	151	138
CIP 676025	161	133	200	119	127	108	127	127	111	114	121
CIP 720090	128	158	165	96	133	117	133	123	93	128	99
CIP 800953	154	168	210	107	167	120	167	120	127	133	147
CIP 800983	153	115	85	133	194	133	164	133	133	139	140
Mean	149	140	152	110	160	114	144	124	124	129	121

T1 - PXV alone
T2 - *P. infestans* alone
T3 - PXV+*P. infestans*
T4 - Control (without inoculation)
DAP - Days after planting

P. infestans alone and *P. infestans* + PXV. Among the genotypes in the control treatment, highest yield was obtained from CIP 384112.8 (701.33 g per plant) followed by CIP 384724.13 (422.67 g per plant) and thus recommended for variety development trials.

Potato genotypes with PXV infection reduced the disease severity of late blight. Thus, yield reduction was higher in plants inoculated with *P. infestans* alone than infection with PXV + *P. infestans*. The high disease severity resulted in less potato tuber yield. Large (1952) also reported that tuber production ceased when 75% of the foliage was affected by late blight. The low susceptibility to *P. infestans* in plants infected with PXV could also be due to reduced foliage as observed in this study. This reduced foliage may have affected the microclimate around the plant thus depressing the

development of the fungus. The low susceptibility of the virus-infected potato plants to the fungus may also be due to the production or depletion of certain metabolites (Blumer *et al.*, 1955) and change in the nutritional status of the host (Muller and Munro, 1951) resulting in inhibition of zoospore penetration.

Table 5. Tuber yield of different potato genotypes as influenced by potato X potexvirus (PXV), *P. infestans*, and PXV + *P. infestans* in the field during dry season (January–April, 1996).

Genotypes	Yield per plant (g per plant)			
	Treatments			
	PXV alone	Pi alone	PXV+Pi	Control
CIP 375335.1	384.00 cd	250.33 d	300.33 c	380.67 c
CIP 384112.8	670.67 a	562.00 a	601.67 a	701.33 a
CIP 384112.14	369.67 d	249.67 d	280.33 c	380.00 c
CIP 384724.13	422.00 b	350.33 c	373.67 b	422.67 b
CIP 390353.3	240.00 f	211.67 e	224.33 d	243.33 e
CIP 676025	141.67 g	116.00 f	125.33 e	149.67 f
CIP 720090	87.33 h	35.33 g	57.00 f	89.33 g
CIP 800953	310.00 e	212.67 e	239.00 d	306.33 d
CIP 800983	403.00 bc	389.33 b	400.67 b	416.33 b
Mean	336.48	264.15	289.15	343.52

Within each column, means followed by the same letter are not significantly different at p = 0.05 by the Duncan's New Multiple Range test.
Pi - *P. infestans*
PXV+Pi - inoculated with PXV prior to *P. infestans*

The inhibitory substances produced in or the surface of the leaf could destroy the infection pegs. Substances similar to phytoalexins have been suggested as responsible for increase resistance of virus-infected plants to late blight (Hecht and Bateman, 1964; Helton and Hubert, 1968). It has also been suggested that some physico–chemical changes brought about by the virus may reduce the susceptibility of plants to the fungus (Mahmood *et al.*, 1974). Moreover, viruses affect the protein metabolism of the host plants and

some of the proteins essential to the optimal development of *P. infestans* optimal could be eliminated or decreased by viral infections (Muller and Munro, 1951).

Reduced susceptibility to *Erysiphe graminis* was also recorded in barley plants infected with barley-dwarf virus than virus–free plants (Potter and Jones, 1981). Mahmood *et al.* (1974) reported that prior infection with bottle–gourd mosaic virus reduced the severity of powdery mildew fungus, *Sphaerotheca fuliginea* and that the symptoms of fungal infection appeared much later in plants which were inoculated with the virus at earlier stages.

Purification and production of antiserum of PXV

After sucrose density gradient (20-50%) centrifugation, virus containing zone was observed at about the middle part of the tube. The zone was broad and thick and approximately 2 cm wide. This band, when pelleted and examined spectrophotometrically, produced typical nucleoprotein spectrum with a maximum absorption at 260 nm and a minimum at 240 nm. The yield of purified virus varied with the location of infection (Table 6). In ELISA, a positive reaction with infected leaf samples was observed with the antiserum even when diluted at 1/10,000,000.

The ringspot strain of PXV was successfully multiplied in *N. glutinosa* and *N. tabacum* var. White Burley at La Trinidad, Benguet and Los Baños, Laguna. The purified virus had a spectrum typical of a nucleoprotein and a mean a 260/280 nm ratio of 1.68 (Table 6). Hyung *et al.* (1977) who observed a 260/280 nm ratio of 1.4. The preservation of infected samples at -20°C for 35 days did not reduce the yield of purified virus hence, was considered stable.

A higher virus yield was obtained from the samples propagated and multiplied in La Trinidad when compared to those in Los Baños. These observations further support the theory that this virus prefers or multiplies in hosts much more efficiently at lower temperatures such as in La Trinida in addition to the production of much more distinct/severe symptoms.

Reaction of antiserum with field samples

The PXV antiserum produced in rabbits was relatively of high titer and reacted to homologous antigen but not to PYV thus indicating specificity.

The locally produced PXV antiserum with a dilution of 5×10^{-6} performed as well as the antiserum obtained from CIP with a dilution of 2×10^{-3}. The PXV antiserum was able to detect the virus with field samples of potato, tomato and pepper in ELISA (Hossain, 1997).

Table 6. Yield of purified potato X potexvirus (PXV) obtained from samples maintained in La Trinidad and Los Banos.

Sample used	Yield	A 260/280
Freshly harvested leaves from La Trinidad, Benguet	49.84 mg kg^{-1}	1.93
Preserved leaves for 35 days at -20°C from La Trinidad	55.55 mg kg^{-1}	1.32
Freshly harvested leaves from Los Banos	12.88 mg kg^{-1}	1.80
Mean	39.42 mg kg^{-1}	1.68

CONCLUSIONS

The present study showed that prior PXV infection reduced the susceptibility of potato plants to *P. infestans*. Therefore, in the development of a potato variety, pathologists/ breeders should consider the interaction among pathogens and the reaction to individual pathogens under field conditions for better acceptance of the developed variety by the farmers. In the present study potato genotypes CIP 384112.8 and CIP 384724.13 produced a good tuber yield, immune to PXV and were resistant to late blight.

The present study revealed the occurrence of a PXV strain, its' successful propagation, purification and production of antiserum to this virus in Philippines. The availability of the antiserum to this virus and of PYV (to be reported in another paper) in the country opens up better prospects for a more efficient management of the respective viruses. In this regard, effective certification and production of clean planting materials by micro propagation,

and epidemiological studies are of major interest. In addition, experiments on the production of antibodies specific to a strain of the virus should be undertaken to have more efficient method of detection of the virus, including their strains.

Further studies are recommended to elucidate the physiological changes due to virus inoculation in plants, especially in hosts immune to the viruses. Emphasis should be given to study the interaction of *P. infestans* and PXV in order to explore the possibilities to control late blight disease in the presence of the virus.

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