RECIPROCAL COMPLEMENTATION OF MOVEMENT FUNCTION OF POTATO VIRUSES X AND A

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Summary. – During isolation of strains of potato viruses X (PVX) and A (PVA) from indigenously collected potato germplasm, an inseparable association between these viruses was discovered. As a result, all the hosts of PVX, used to free PVX from PVA, also showed infection of PVA along with PVX. Furthermore, *Nicandra physaloides*, which is a host of PVA but not PVX, also did not free PVA from PVX. These results suggested a reciprocal complementation of movement function of these viruses due to which they together infected various hosts sensitive to PVX or PVA. Relative concentration of PVX, in all the hosts tested, was much higher than that of PVA.

Key words: potato virus A; potato virus X; movement function; complementation

Introduction

PVX is an ubiquitous potato virus causing serious potato diseases upon combination with other potato viruses (MacLachlan *et al.*, 1953; Hooker, 1981). PVA has been reported to be recently prevalent (Browning *et al.*, 1995). Both these viruses cause heavy yield losses particularly upon their combination and even with other viruses (Hooker, 1981). A synergistic interaction between PVX and potato virus Y (PVY) upon coinfection leads to increased concentration of PVX and a more severe disease (Rochow and Ross, 1955; Khurana and Raychaudhari, 1988).

Superinfection of a PVX-infected host with PVY enables the former virus to spread systemically even at temperatures which otherwise restrict it to inoculated leaves (Close, 1964). In other words, superinfection complements the movement function of PVX at restrictive temperatures.

Such a complementation of movement function of PVX by as diverse viruses as tobacco mosaic, cauliflower mosaic, henbane mosaic, brome mosaic and barley stripe mosaic viruses is also known (Close, 1964; Zachos, 1957; Malyshenko *et al.*, 1989).

Though the synergistic interaction between PVX and PVA with regard to disease symptoms has been reported long back (Hooker, 1981; MacLachlan *et al.*, 1953), no study with respect to complementation of movement function of PVX and PVA and *vice versa* has appeared. The present paper reports about reciprocal complementation of movement function of PVX and PVA. Complementation of movement function of a potyvirus and a potexvirus, as described here, appears reported for the first time.

Materials and Methods

Ten out of many indigenously collected germplasm clones, maintained at Central Potato Research Institute, Shimla, showing different symptoms, were used to isolate PVX and PVA. All these clones showed virus disease symptoms and carried mixtures of 5 to 7 potato viruses, namely PVX, potato virus S (PVS), PVA, potato virus M (PVM), PVY, potato leaf roll virus (PLRV) and potato aucuba mosaic virus (PAMV). Isolation of PVX was undertaken by passaging the viruses through *Datura stramonium* and *Capsicum pendulum*, specific hosts of PVX and non-hosts to many other potato viruses, namely PVS, PVA, PVY, and PVM (de Bokx, 1987). Isolation of PVA was attempted by passaging the viruses through

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Abbreviations: IEM = immune electron microscopy; PAMV = potato aucuba mosaic virus; PLRV = potato leaf roll virus; p.i. = post inoculation; PVA = potato virus A; PVM = potato virus M; PVS = potato virus S; PVX = potato virus X; PVY = potato virus Y

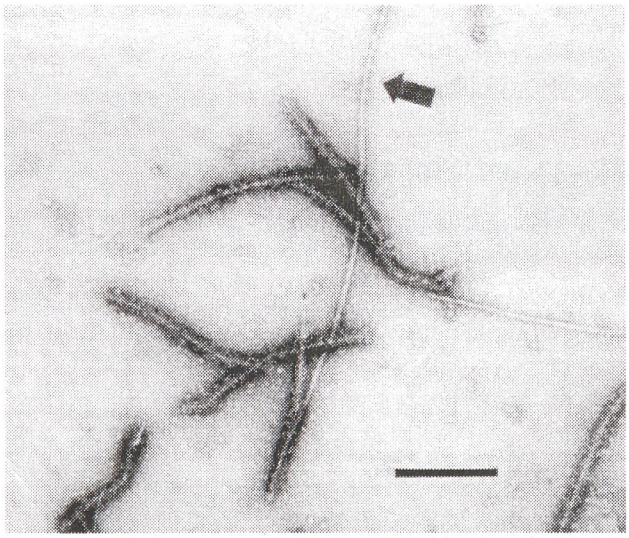

Fig. 1
Immune electron microscopy of PVX and PVA
Decorated virions of PVX and undecorated ones of PVA (arrow) upon trapping and decoration with antiserum to PVX. Bar = 250 nm.

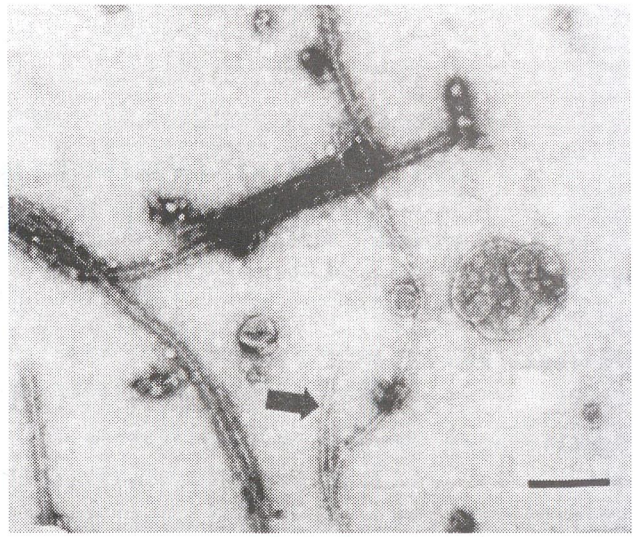

Fig. 2
Immune electron microscopy of PVX and PVA
Decorated virions of PVA and undecorated ones of PVX (arrow) upon trapping and decoration with antiserum to PVA. Bar = 250 nm.

N. physaloides, a specific host of PVA (de Bokx, 1987). Isolates of PVX obtained by mechanical passaging through *D. stramonium* and those of PVA obtained by mechanical passaging through *N. physaloides*, upon immune electron microscopy (IEM) checking, happened to carry both PVX and PVA besides PAMV and even PVY in some cases. Therefore, isolation of PVX from the PVX+PVA mixtures was tried by further mechanical passaging through *Chenopodium* spp., viz. *C. amaranticolor, C. quinoa*, and *C. murale*. PVX+PVA isolates obtained from *D. stramonium* were subjected to mechanical passaging through *N. physaloides* to free PVA from PVX. Similarly, PVX+PVA isolates obtained from *C. pendulum* were also used to inoculate *D. stramonium* and *N. physaloides* to obtain individual cultures of PVX and PVA, respectively.

All the test plants were raised in insect-proof glasshouse and 2–3-week-old seedlings were transplanted individually in earthen pots of 4 inch diameter, filled with a sterilized mixture of sandy loam soil and farm yard manure in the ratio of 2:1. Inoculations were carried out one week after transplantation. The virus inoculum was prepared by grinding infected leaves in 0.1 mol/l phosphate buffer pH 7.2 (2 ml of buffer per 1 g of leaf tissue). The slurry thus obtained was used to mechanically inoculate fully expanded leaves, predusted with carborundum (600 mesh), of the test plants. Test plants were raised during June when the day length was about

Table 1. IEM indexing of the potato germplasm clones

Potato clone	Relative concentration[a]						
	PVX	PVS	PVM	PVA	PVY	PAMV	PLRV
JG-11	++++	+++	–	++++	+	–	++
JG-30	++++	++	–	++++	–	–	+
JG18	+++	+++	++	+++	++	–	++
JG13	++++	++++	+	++	++++	–	–
JG-15	+++	++++	–	+	++++	–	–
BD-29	++++	++++	–	++	++++	–	++
JG-4	++++	++	–	++++	+	–	+
RKB-30	++++	++++	–	++	+++	–	–
PSK-107	+++	++++	–	++	+++	–	–
PCG-68	++++	++++	–	++	+++	+++	–

[a](–), (+), (++), (+++), and (++++) correspond to 0, up to 5, 10, and 20, and above 20 virions/20 cm^2 screen area, respectively, at magnification of 21,000 x.

Table 2. IEM indexing of *D. stramonium* (systemic host of PVX) leaves 10 days p.i. and *C. pendulum* (local lesion host of PVX) leaves 7 days p.i.

	Relative concentration[b] of potato virus(es) in													
	D. stramonium							*C. pendulum*						
Potato clone[a]	PVX	PVS	PVM	PVY	PVA	PLRV	PAMV	PVX	PVS	PVM	PVY	PVA	PLRV	PAMV
JG-11	+++	–	–	–	+	–	–	+++	–	–	–	+	–	–
JG-30	+++	–	–	–	+	–	–	+++	–	–	–	+	–	–
JG-18	+++	–	–	–	+	–	–	+++	–	–	–	+	–	–
JG-13	+++	–	–	–	+	–	–	+++	–	–	–	+	–	–
JG-15	+++	–	–	–	+	–	–	+++	–	–	–	+	–	–
BD-29	+++	–	–	–	+	–	–	+++	–	–	–	+	–	–
JG-4	+++	–	–	–	+	–	–	+++	–	–	–	+	–	–
RKB-30	+++	–	–	–	+	–	–	+++	–	–	–	+	–	–
PSK-107	+++	–	–	–	+	–	–	+++	–	–	–	+	–	–
PCG-68	+++[c]	–	–	–	+	–	+++	+++	–	–	–	+	–	+++[d]
Uninoculated (control)	–	–	–	–	–	–	–	–	–	–	–	–	–	–

[a]Indigenously collected germplasm clones showing different type of virus disease symptoms.
[b](–), (+), and (+++) correspond to 0, up to 5, and above 20 virions/20 cm^2 screen area, respectively, at magnification of 21,000 x.
[c]Infected plants showed only stunting.
[d]Infected plants showed local necrotic lesions on inoculated leaves and systemic necrosis.

15.5 hrs per day and a the maximum/minimum temperature was 30/20°C. Five plants were inoculated with each virus isolate. Both inoculated and uninoculated leaves were checked for the presence of potato viruses using IEM (Garg and Khurana,1992).

Polyclonal antisera to PVX, PVS, PVY, and PLRV were prepared at Central Potato Research Institute, Shimla (Khurana *et al.*, 1990; Singh *et al.*, 1990) while those to PVA and PVM were obtained from Dr. A. Kowalska (Institute Ziemniaka, Rozalin, Poland). The antiserum to PAMV was obtained from Dr. D.Z. Maat (Research Institute for Plant Protection, Wageningen, the Netherlands).

Results

Results of IEM checking of the germplasm clones are given in Table 1. All the clones were found to carry a mixture of 4 to 6 potato viruses. Table 2 and Figs. 1 and 2 show the results of inoculation of *D. stramonium* and *C. pendulum* with these virus mixtures. Inoculation of *D. stramonium* resulted in distinct mosaic in the case of nine potato clones and just in stunting in the case of the clone PCG-68 10 days post inoculation (p.i.). PVX+PVA were diagnosed in all cases with exception of PCG-68, where PAMV was also confirmed. Inoculation of *N. physaloides* with PVX+PVA taken from *D. stramonium* did not free PVA from PVX as both viruses continued occurring together. The symptoms induced in *N. physaloides* by all the clones comprised mosaic with necrotic spots about 12 days p.i.

Inoculation of *C. pendulum* resulted just in local necrotic lesions in the case of all the clones and also in systemic necrosis in the case of the clone PCG-68 within 7 days p.i. IEM checking revealed the presence of PVX+PVA in all the clones with exception of the PCG-68 clone where PAMV was also confirmed.

Inoculations from *C. pendulum* on *D. stramonium* or *N. physaloides* also did not separate PVX and PVA and both the viruses remained together. Table 3 presents the results of inoculation of *Chenopodium* species with PVX+PVA mixtures

Table 3. Reaction of PVX+PVA isolates on different species of *Chenopodium* after passaging through *D. stramonium* and *C. pendulum*

	Symptoms on *Chenopodium* spp.		
Potato clone	*C. amaranticolor*	*C. quinoa*	*C. murale*
JG-11	NS	CLL	NLL
JG-30	NS	CLL	NLL
JG-18	NS	CLL	NLL
JG-13	NS	CLL	NLL
JG-15	NS	CLL	NLL
BD-29	NS	CLL	NLL
JG-4	NS	CLL	NLL
RKB-30	NS	SS	NLL
PSK-107	NS	CLL	NLL
PCG-68	NS	NS	NS
Uninoculated (control)	NS	NS	NS

NS = no symptoms; CLL = local chlorotic lesions on inoculated leaves; NLL = local necrotic lesions on inoculated leaves; SS = symptomless systemic infection.

Table 4. Results of passaging of virus mixtures from different potato clones through *N. physaloides* (systemic host of PVA)

Potato clone	Symptoms (systemic)	Relative concentration[a] of virus(es)						
		PVX	PVS	PVM	PVY	PVA	PLRV	PAMV
JG-11	Mosaic with necrotic spots	+++	–	–	–	++	–	–
JG-30	-"-	+++	–	–	–	++	–	–
JG-18	-"-	+++	–	–	–	++	–	–
JG-13	-"-	+++	–	–	–	++	–	–
JG-15	-"-	+++	–	–	–	++	–	–
BD-29	-"-	+++	–	–	+	++	–	–
JG-4	-"-	+++	–	–	–	++	–	–
RKB-30	-"-	+++	–	–	–	++	–	–
PSK-107	-"-	+++	–	–	–	++	–	–
PCG-68	-"-	+++	–	–	–	++	–	++
Uninoculated (control)	Nil	–	–	–	–	–	–	–

[a](–), (+), (++) and (+++) correspond to 0, up to 5, 10 and more than 20 virions/20 cm^2 screen area, respectively, at magnification of 21,000 x.

obtained from *D. stramonium* and *C. pendulum*. There was no symptom induction in *C. amaranticolor* and PVX+PVA was observed only in the inoculated leaves of all the clones tested with exception of clone RKB-30 which caused a systemic infection. *C. quinoa* and *C. murale* showed local chlorotic or necrotic lesions except the PVX+PVA mixture from the RKB-30 clone which caused a symptomless yet systemic infection.

The results of mechanical passaging of the virus mixtures through *N. physaloides* are given in Table 4. There was systemic mosaic with necrotic spots with all the clones. IEM checking showed presence of PVX+PVA in all the clones while PVY and PAMV were also present in the clones BD-29 and PCG-68, respectively.

Discussion

Each plant virus has at least a few sepcific hosts under specific conditions. Thus, PVX infects some hosts not infected with PVA and *vice versa* when inoculated individually. Many a times this host specificity may be violated due to the presence of another virus. Under these conditions, a non-host may also serve as a host due to the phenomenon called complementation of virus movement function (Malyshenko *et al.*, 1989).

Our results clearly depict this phenomenon since many of the non-hosts of PVA, e.g. C. *amaranticolor, C. quinoa, C. murale, C. pendulum*, and *D. stramonium* (de Bokx, 1987) served as its host when both the viruses (PVX+PVA) coexisted. An interesting aspect of our results is the reciprocal nature of complementation since *N. physaloides*, which is the host of PVA and the non-host of PVX (de Bokx, 1987), turned out to be the host of PVX when coinfected with PVA.

Modus operandi of this phenomenon is complementation of the movement function of an incompatible virus and a compatible virus. Apparent non-infection of a host by a virus, many a times, is due to inability of the virus to move out of the initially invaded cell, even though the virus multiplies there. This phenomenon causes a subliminal infection (Sulzinski and Zaitlin, 1982). Such inability, however, is overcome, in some cases, in the presence of a virus able to move freely within the host plant. Recently, it has been reported that PVX infection modifies the plasmodesmata of infected cells and readily moves from cell to cell in the form of assembled virus particles through the modified plasmodesmata (Santa Cruz *et al.*, 1998). It is most likely that such modified plasmodesmata provide channel for free cell-to-cell movement of PVA. Complementation of virus movement function is not confined to any particular group(s) of viruses. It even takes place between unrelated groups of viruses (Fuentes and Hamilton, 1991; Malyshenko *et al.*, 1989)

Many instances have been reported when coinfection with two viruses, one of which is a potyvirus, results in severe disease and high titer of the non-potyvirus in the combination – a phenomenon called synergism (Rochow and Ross, 1955). The presented study also confirmed this as there was a much higher concentration of PVX than PVA even in *N. physaloides* (a non-host to PVX) when coinfected with PVA. A severe disease called potato crinkle caused by a combination of PVX+PVA is well documented (Hooker, 1981; Khurana and Raychaudhari, 1988; MacLachlan *et al.*, 1953).

Complementation of viruses for their systemic spread was demonstrated between PVX (helper) and red clover

mottle virus in *N. tabacum*, brome mosaic virus (helper) and PVX, and barley stripe mosaic virus and PVX in *Hordeum vulgare* (Malyshenko *et al.*, 1989). Similarly, complementation of the movement function of PVX by PVY, TMV, CMV (yellow strain) and henbane mosaic virus at 31°C has been also reported (Close, 1964).

To conclude, our results demonstrate reciprocal nature of complementation of transport function of PVX and PVA. They also suggest subliminal infection of *D. stramonium*, *C. pendulum* and *Chenopodium* spp. with PVA and of *N. physaloides* with PVX since complementation of the movement function of PVA by PVX and *vice versa* would not be otherwise easy as observed in this study.

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