Investigation on the transmission of some Tobamoviruses by pollen and seed in pepper *(Capsicum annuum L.)*

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Key words: Pepper, Tobamovirus, transmission by pollen and seeds.

Summary: Five pepper cultivars were mechanically inoculated with isolates of three Tobamovirus species, viz. the "Gelb" strain of tobacco mosaic virus (TMV-G), the XM-isolate of dulcamara yellow fleck virus (DYFV-XM) and the Nov/H isolate of pepper mild mottle virus (PMMV-Nov/H), respectively. Symptoms caused by the viruses were characterised. The viruses were sucessfully re-isolated from different organs (roots, leaves, fruit parts) of susceptible peppers to test plants. It was estabilished, that the pollen of diseased peppers carried infective virions at least on their surface. Washes of seeds were highly infective, but no infectivity was found after treatment of the seeds with 2% NaOH or 10% Na3PO4. No infectivity of inocula prepared from seed-coats of alkaline treated seeds was established. Infection of young seedlings grown from untreated seeds was demonstrated, while the seedlings came from alkaline treated seeds remained free of infective virus. The possible role of pollen and seed in the epidemiology of Tobamoviruses pathogenic to pepper as well as the importance of seed treatment is discussed.

Introduction

Plant viruses are obligate intracellular pathogens parasiting the transcriptional and translational machinery of the host cells. The great majority of them cause systemic infection in plants, i.e. they spread from the initially infected cells to healthy cells and invade the host plant. With the exception of some viruses, which are transmitted in soil and infect the root systems (e.g. *tobacco necrosis Necrovirus, beet necrotic yellow vein Furovirus*), they invade all organs of the host plant including the reproductive ones. A great number of viruses are known not only present in, but also transmitted by pollen and seeds (see Ref. *Mink*, 1993; *Johansen* et al., 1994). This way of natural transmission helps the viruses to perpetuate in nature and to distribute vertically and horizontally in the plant ecosystems. Pollen and/or seed transmission are genetically determined in the virus-host systems, i.e. both the viral and the host genes are collectively involved in them. Transmission of viruses by pollen, especially of the viruses belonging to the genera *Ilarvirus, Hordeivirus & Nepovirus* has a great importance in the viral epidemics in agricultural crops (*Hamilton* et al., 1977, *Michon,* 1982, *Stace-Smith & Hamilton,* 1988, *Mink,* 1993).

The virus species classified to the *Tobamovirus* genus (*Brunt* et al., 1996) have no specific animal or fungal vectors. They transmit from plant-to-plant mainly by the way of mechanical transmission. They have extremely stable rod shaped particles which remain infective for a long time in the soil, in water systems and in contaminated surface of agricultural mashines and instruments all of which may serve as a source of infection. *Tobamoviruses* are also known transmissible by pollen and/or seeds of infected plants (see for Ref. *Hamilton & Valentine,* 1984, *Mink,* 1993, *Johansen* et al., 1994).

Seven *Tobamovirus* species have been described infecting pepper (*Capsicum spp.*) produced either in field or under protected conditions *(Table 1)*. Transmission of

Table 1 *Tobamovirus* species naturally infecting pepper (*Capsicum* spp.)*

Tobamovirus species	Pathotypes
Tobacco mosaic virus (TMV)	P_0
Tomato mosaic virus (ToMV)	P_0
Bell pepper mottle virus (BcPMV)	P_0
Tobacco mild green mosaic virus (TMGMV)	P_0, P_1
Pepper mild mottle virus (PMMV)	$P_{1,2}$; $P_{1,2,3}$
Paprika mild mottle virus (PaMMV)**	P_1
Dulcamara yellow fleck virus (DYFV)***	P_1

* According to Green & Kim (1991) with some modification.

** PaMMV is identical with the pepper tobamovirus strain P11 isolated by Rast (1982, see. Garcia-Luque et al., 1993)

*** In contrast to some authors (e.g. Tóbiás et al., 1982; Gáborjányi et.al., 1999) our investigations have showed that DYFV (syn.: Tobamovirus-Ob) infected *C. frutescens* cv. Tabasco that carry the resistance gene L2 only locally (Salamon, 1993). Therefore we classified it as P1 pathotype. *Dulcamara yellow fleck virus* which was first showed as a new species of the *Tobamovirus* genus in Hungary (Salamon et al., 1987) and have several strains (including the Ob strain) was renamed to *Solanum dulcamara* yelow fleck-Ob by Salfacon et al. (1993). We originally used and propose to use the name dulcamara yellow fleck virus by analogy of the virus name *dulcamara mottle virus* approved by the ICTV (Brunt et al., 1996).

tobacco mosaic virus (TMV) and of other *Tobamovirus* species by pepper seeds has been demonstrated for a long time (e.g *Szirmai,* 1948, 1950, *McKinney,* 1952, *Tosic* et al., 1980). It was reported by several authors that the *Tobamoviruses* contaminate the outer seed coat of pepper and infect the seedlings through small wounds (cf. *Szirmai,* 1950, *Glaser,* 1976, *Molnár & Tóbiás,* 1978, *Mink,* 1988, *Pares & Gunn,* 1989, *Black* et al., 1991; *Beczner* et al., 1997). Others have reported that *Tobamoviruses* could be located inside the seeds as well (*McKinney,* 1952, *Demski,* 1981). Sites of the presence of virions on the seeds are important because of control strategies. The outer contaminations could be eliminated by seed treatment using solvents of virus-inactivators (i.e. NaOH, Na_3PO4, *Szirmai,* 1950, *Kapeller,* 1967, *Rast & Stijger,* 1982), but in the case of inner contamination we have no good measures of elimination.

Pollen grains of differrent hosts have also been demonstrated to carry *Tobamoviruses* on their surface, and rarely in the cytoplasm, too (*Michon,* 1982, *Hamilton & Valentine,* 1984). The role of pollen in the distribution or in the estabilishment of seed contamination of *Tobamoviruses* in general and particularly in the case of pepper is poorly understood. This article deals with our preliminary investigations on the role of pollen and seed in the epidemiology of pepper *Tobamoviruses*. The results were shortly presented earlier (*Salamon & Kaszta,* 1998).

Materials and methods

Viruses and plants

Isolates of three *Tobamovirus* species, namely *dulcamara yellow fleck virus* (DYFV), *pepper mild mottle virus* (PMMV) and *tobacco mosaic virus* (TMV) were maintained in the virus collection of the seniour author. Their origin, isolate marks as well as propagative and indicator host plants are presented in *Table 2*. Seeds of five pepper cultivars treated by 2% NaOH were sown in steril soil. Seedlings were transplanted in 18 cm pots. Experiments were done in greenhouse conditions at the Agricultural Biotechnology Center, Gödöllő.

Inoculation of pepper plants and re-isolation to test plants

Five individuals of each pepper cultivars at the age of four to six leaf stage were mechanically inoculated with the viruses, respectively. For inoculation carborundum was used as an abrasive. Symptoms on the plants were observed continously during the experiments. For re-isolation of viruses to test plants, root pieces, leaves, and friut parts of diseased peppers were ground in sterile mortar by adding sterile distilled water (1: 5–10 w/v). Test plants reacted with local lesions (*Nicotiana tabacum* cv. Xanthi-nc for TMV, and PMMV, and *N. megalosiphon* for DYFV) were inoculated to check the infectivity of the inocula. For rubbing the leaves of test plants sterile cotton pads were used for each inocula, respectively.

Table 2 Tobamovirus species, isolates and their propagative (donor) and indicator hosts used for the experiments

Virus names	Isolates	Donor plants	Indicator plants
Tobacco mosaic virus (TMV)	G*	Nicotiana tabacum cv. Samsun	Nicotiana tabacum cv. Xanthi-nc.
Dulcamara yellow fleck virus (DYFV)	XM*	Nicotiana tabacum cv. Xanthi-nc	Nicotiana megalosiphon
Pepper mild mottle virus (PMMV)	Nov/H*	Capsicum frutescens cv. Tabasco	Nicotiana tabacum cv. Xanthi-nc.

* The G (=Gelb) isolate of TMV was kindly donated by Dr. J. Richter (Aschersleben, Germany). This strain has unusual electrophoretic mobility (Salamon, not reported). DYFV-XM was isolated by the senior author from a complex of pathological strains of Tobamovirus-Ob, kindly supplied by Dr. G. Csilléry in 1979. PMMV was isolated from C. annuum cv. Novator in 1982 in Hungary and classified as a pathotype P1.2.3 (Salamon, 1993).

Infectivity tests for the detection of viruses on the pollen and seeds

Pollen grains of diseased pepper plants were carefully collected from the pollinating flowers and dropped on leaves of test plants. Then the leaves were rubbed with wet steril cotton pads. Pollen of healthy pepper plants were used as controls.

Seeds were collected from mature fruits and dried for a week on filter papers. To demonstrate their surface contamination by viruses, groups of 10 seeds were soaked in 2 ml steril water in Eppendorf tubes for 30 minutes, 50 ul drops of water were pipetted on leaves of indicator plants and rubbed with sterile cotton pads.

To inactivate the viruses on the seed surface, seeds were treated with 2% NaOH, or 10% Na_3PO_4 for 10 minutes, washed in water intensively, then dried overnight on filterpapers. The presence of infective viruses on them was investigated as above.

For demonstration of viruses in the inner seed-coat, washed seeds treated by alkalines were germinated on wetted filter papers placed in the bottom of closed boxes. After germination, the seed-coats were carefully collected from the top of cotyledons with sterile tweezers, ground in water, of which the infectivity was checked by inoculation of test plants.

Groups of 25 young seedlings that were germinated from treated and untreated seeds of infected pepper plants were ground in 5 ml destilled water. The infection of seedlings were demonstrated by rubbing of test plants with inocula, respectively. Seeds of healthy pepper plants were used as controls for all of the experiments.

Results and discussion

Symptoms of Tobamoviruses on pepper and results of re-inoculation

All of the three *Tobamoviruses* used for inoculation were highly infective to pepper cultivars, but induced different

Table 3 Symptoms caused by *Tobamoviruses* on pepper (*Capsicum annuum* L. cvs.)

Pepper cultivars	Virus isolates and symptoms					
	TMV-G		DYFV-XM		PMMV-Nov/H	
	L*	S	L	S	L	S
Golden California Wonder	cl	vc ymo	cl	ymo yr fd	sl	mmo fdc fd
Szintetikus Cecei	nl dl	sl	ys	yr ymo fd	sl	mmo d bnf
Újmajori	es	vc gmo ys	ys	ymo yr fd	sl	mmo fd bnf
Tizenegyes	nl dl	sl	ys	ys ld fd	sl	mmo fd bnf
Almapaprika	sl	w, tn smo	sl	ymo ygy yf fd	sl	mmo bnf
Evita	nl dl	sl	sl	ymo fd	sl	mmo fd bnf

* Abbreviations: L = local symptoms, S = systemic symptoms, bnf = brown necrotic spots of fruits, fdc=fruit discoloration, mmo = mild mosaic, vc = vein clearing, h = wilt, cs = chlorotic spots, cl = chlorotic lesions, ld = leaf deformation, dl = drop of leaves, sbd = súlyos bogyódeformáció, ys = yellow spots, yr = yellow rings, ymo = yellow mosaic, sl = symptomless, tn = total necrosis, gmo = green mosaic, nl = necrotic lesions

reactions and caused different symptoms *(Table 3)*. TMV-G induced hypersensitive reaction s.a. necrotic lesions followed by leaf abscision, on plants of three cultivars (cvs. Tizenegyes, Evita, Szintetikus cecei). As the inoculated leaves fell down, these plants remained virus-free, indicating that the resistance gene L1, incorporated into these cultivars, provided a good control to this unusual strain of TMV.

The isolates DYFV-XM and PMMV-Nov/H, however, infected all of the cultivars systemically causing severe symptoms. The symptoms caused by DYFV on peppers carrying the L1 gene was extremely severe, often leading to the death of plants. Usually, DYFV and TMV-G caused brilliant yellow mosaic spots and rings on the leaves of systemically infected plants, while PMMV induced mild

Table 4 Infectivity of different parts of pepper plants (cv. Golden California Wonder) inoculated by *Tobamoviruses*

Plant parts	Control	Viruses		
		TMV-G	DYFV-XM	PMMV-Nov/H
Fruit flesh	–	+++ *	++	+++
Diaphragm	–	+++	+++	+++
Seed column	–	+++	+++	+++
Leaf	–	+++	+++	+++
Pollen	–	++	++	++
Root	–	+++	+++	+++

*Number of local lesions on a single leaf of test plants: – = no lesions, + =1–5 lesions, ++ = 6–30 lesions, +++ = > 30 lesions

green mottle. The fruits of susceptible pepper plants became distorted, discoloured, and necrotic spots or streaks were often appeared on the fruit flesh. Re-isolation tests have revealed, that all of the parts of infected plants contained highly infective virus *(Table 4)*.

Our pathological tests cearly showed that pollen grains collected from diseased plants carry infective virions at least on their surface. As a consequence, the pollen could be an important source of *Tobamoviruses* in pepper. *Tobamoviruses* could be easily distributed via contaminated pollen grains by the wind and/or by flower visiting insects. It could be assumed, that initial infections of pepper plants in greenhouses come from outside via pollen grains of infected wild plants or crops. Pollinating infected plants may also dangerous sources of virus contaminations even in the research greenhouses. The presence and infectivity of *Tobamoviruses* inside the pepper pollen grains remained, however, unknown.

Presence of infective viruses in pepper seeds and seedlings

Washes of seeds collected from infected plants were always highly infective, showing the contamination of the seed surface *(Table 5)*. Treatments with NaOH and Na_3PO_4 proved to be effective in destroying the viruses on the seeds, even in the cases of DYFV and PMMV. However, inocula prepared from seed coats, that were collected from seedlings

Table 5 Infectivity of inocula prepared by soaking of treated and untreated pepper seeds in sterile water

Pepper cultivar	Viruses and treatments									
	Control	TMV-G			DYFV-XM			PM M V–Nov/H		
		Ø*	NaOH	Na_3PO_4	Ø	NaOH	Na_3PO_4	Ø	NaOH	Na_3PO_4
Golden California Wonder	0	+++	0	0	++	0	0	++	0	0
Szintetikus cecei	0	0	.	.	+	0	0	++	0	0
Újmajori	0	+++	0	0	+	0	0	++	0	0
Tizenegyes	0	0	.	.	+++	0	0	+++	0	0
Almapaprika	0	+++	0	0	++	0	0	+++	0	0
Evita	0	0	.	.	++	0	0	+++	0	0

* Notes: Ø = untrated seeds; NaOH = seeds soaked in 2% NaOH; Na_3PO_4 = seeds soaked in 10 % Na_3PO_4; 0 = no infectivity; + = 1–5 lesions; ++ = 6–30 lesions; +++ => 30 lesions; . = not investigated

Table 6 Infectivity of inocula prepared from seed coats and young seedlings of pepper plants artificially infected by Tobamoviruses

Pepper cultivar	Sources of inocula	Viruses and treatments								
		TMV-G			DYFV-XM			PM M V–Nov/H		
		∅*	NaOH	Na_3PO_4	∅	NaOH	Na_3PO_4	∅	NaOH	Na_3PO_4
Golden California	seedcoat	.	.	.	+++/++	.	.	.	.	0/0
Wonder	seedling	.	.	.	0/0	.	.	.	.	0/0
Szintetikus ceeei	seedcoat	.	.	.	.	0/0	.	.	0/0	0/0
	seedling	.	.	.	.	0/0	.	.	0/0	0/0
Újmajori	seedcoat	+++/++++	0/0	.	.	0/0	0/0	+++/+	.	0/0
	seedling		0/0	.	.	0/0	0/0	+/+	.	0/0
Tizenegyes	seedcoat	.	.	.	++/++	.	.	.	.	.
	seedling	.	.	.	+/+	.	.	.	.	.
Almapaprika	seedcoat	+++/+++	.	.	.	0/0	0/0	+++/+	0/0	0/0
	seedling	+++/+	.	.	.	0/0	0/0	++/+	0/0	0/0
Evita	seedcoat	.	0/0	.	.	.	.	.	0/0	0/0
	seedling	.	0/0	.	.	.	.	.	0/0	0/0

* Notes: ∅ =non treated seeds; NaOH = seeds treated with 2% NaOH; Na_3PO_4 = seeds treated with 10 % Na_3PO^4; 0 =no infectivity; + = 1–5 lesions; ++ = 6–30 lesions; +++ = > 30 lesions, · = not investigated; / = the results of two experiments in which 25 seeds or seedlings were ground to make an inoculum are separated by /.

grown from treated seeds proved to be non infective *(Table 6)*. It showed, that viruses were either not present or not present in infective form on the inside surface of seed-coat.

Infectivity was demonstrated in some groups of young seedlings germinated from untreated seeds, while it was never detected in seedlings came from the treated ones *(Table 6)*. This indicate, that the treatment of seeds of *Tobamovirus* infected pepper plants with NaOH or Na_3PO_4 is sufficient for the prevention of contamination of seedlings. In other words, the *Tobamoviruses* used in our experiments could not be transmitted through the endospermium or in the embryos of pepper seeds. If *Tobamoviruses* were capable to reach these parts of seeds either maternally or paternally (through germinating pollen), they should be eliminated or lose their infectivity during the seed maturation and/or germination.

To our opinion, pollen and seed contamination played and play an important role in the distribution of *Tobamoviruses* throughout the world. PMMV, for example, which was first detected as an unusual strain of TMV (*McKinney*, 1952), probably was imported by seeds to Europe, where it was distributed suddenly in the past 20 years. *Beczner* et al., (1997) mentioned, that PMMV was transported to Canada via seeds of pepper originated from Hungary some 15 years ago. To our opinion, these seeds, were probably non treated with NaOH, or the treatment was not suited for the official requerements. Breeders, botanical gardens, hobby-collectors and companies who exchange or transport seeds (and sometimes pollen as well) have great responsibility for the prevention of distribution of a range of plant viruses, especially of *Tobamoviruses* and other stable viruses *e.g Potexviruses, Sobemoviruses* and *Tymoviruses.*

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