Virus susceptibility and resistance of Hungarian pepper varieties

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Summary: The aim of our study was to examine susceptibility or resistance of 18 pepper varieties to four viruses [tobacco mosaic *tobamovirus* (TMV), sowbane mosaic *sobemovirus* (SoMV), NTN strain of potato Y *potyvirus* (PVY^{NTN}) and cucumber mosaic cucumovirus (CMV). Out of the 18 varieties, 13 were resistant to CMV infection. Thirteen varieties were susceptible to TMV, while five ones (Dabora F1, Brill F1, Fehérözön Synthetic, Ciklon F1, Cecil F1) showed only local hypersensitive reaction. All of the tested pepper varieties showed resistance to SoMV. Eight varieties (Tuba, Fehérözön Synthetic, Boni, Alba Regia, Korona, Édesalma, Cecil F1, Star) were found to be resistant to PVY^{NTN}. Out of the examined varieties five (Boni, Alba Regia, Korona, Édesalma, Star) were resistant to three viruses (SoMV, CMV and PVY^{NTN}). Only one (Cecil F1) displayed complex, extreme resistance to SoMV, PVY^{NTN}, CMV and hypersensitive reaction to TMV, therefore this hybrid is very important in pepper breeding and growing for virus resistance.

Introduction

Peppers (*Capsicum* spp.) originating in Mexico, Southern Peru and Bolivia are now grown world-wide under various environmental and climatic conditions *(Pickersgill* et al. 1979). Last decades vegetable growing under glass- and foil-house conditions achieved considerable results in Hungary. Forced pepper play the most important role in this respect. The consumption of our traditionally national product – sweet pepper – increases not only in Hungary but abroad as well; therefore changing of eating habit creates good export possibilities for Hungarian primeur pepper *(Budai* et al. 1996). Besides this, red pepper is an important national product of Hungary.

Among pathogens, viruses cause the most important diseases of paprika. Virus diseases are one of the major limiting factors in successful pepper cultivation *(Martelli & Quacquarelli,* 1983, *Horváth,* 1986b, *Duriat,* 1996). The extent of infection varies between 20 and 60%, and 5–40% yield losses may occur due to virus infection. The species of *Capsicum* genus are known as natural and artificial hosts of 55 and 44 plant viruses, respectively *(Horváth,* 1981, *Horváth,* 1986a, *Green & Kalloo,* 1994, *Edwardson & Christie,* 1997). Response of different pepper varieties, breeding lines, the different *Capsicum* species and accessions to virus infections have been extensively studied *(Horváth,* 1986c, *Mijatovi,* 1997, *Castagnoli* et al., 1997, *Sudarsono* et al., 1998, *Wang* et al., 1998). Out of them new sources of resistance have been found, which could be used for pepper breeding programs *(Sowell,* 1982, *Lane* et al., 1997). Viruses and other pathogens often occur in complex infection, increasing injuries *(Gáborjányi* et al., 1997, 1998a, *Kazinczi* et al., 1998a,b,c). In Hungary 13 viruses have been isolated from infected pepper plants so far. The extremely stable, mechanically transmitted *tobamoviruses* are found to be the major problems under cover conditions, while the dominance of the aphid transmitted *cucumo-, poty-* and *alfamoviruses* were demonstrated in the open fields *(Salamon,* 1996). Recently new viral diseases of pepper, named: pepper yellow vein mosaic and pepper yellow line and ring pattern have been occurred under cover in Hungary and abroad. In spite the fact, that the etiology of these diseases is intensively studied, viruses have not been exactly identified so far *(Fletcher* et al., 1987, *Rast,* 1988, Salamon *& Szürke,* 1990, *Salamon & Némethy,* 1995, *Burgyán & Szittya,* 1996, *Szittya & Burgyán,* 1996). Due to a regular survey has been made since 1970's in South-Hungary, it was confirmed that tobacco mosaic *tobamovirus* (TMV), tomato mosaic *tobamovirus* (ToMV), cucumber mosaic *cucumovirus* (CMV), alfalfa mosaic *alfamovirus* (AMV) and potato Y *potyvirus* (PVY) are the most widespread under covers causing severe yield losses, particularly in complex infections *(Kiss,* 1996). First descriptions of pepper pathogen viruses in Hungary are listed in *Table 1*.

Table 1 First descriptions of pepper pathogen viruses in Hungary

Viruses	References
Cucumber mosaic *cucumovirus* (CMV)	Szirmai (1941)
Alfalfa mosaic *alfamovirus* (AMV)	Szirmai (1944)
Tobacco mosaic *tobamovirus* (TMV)	Szirmai (1950)
Potato X *potexvirus* (PVX)	Szirmai (1950)
Potato Y *potyvirus* (PVY)	Horváth (1967)
Tomato aspermy *cucumovirus* (TAV)	Beczner et al. (1979)
Broadbean wilt *fabavirus* (BBWV)	Salamon et al. (1980)
Dulcamara yellow fleck *tobamovirus* (DYFV)	Salamon et al. (1987)
Tomato mosaic *tobamovirus* (ToMV)	Csilléry et al. (1983)
Tomato spotted wilt *tospovirus* (TSWV)	Gáborjányi et al. (1995)
Henbane mosaic *potyvirus* (HeMV)*	Gáborjányi et al. (1997)
Sowbane mosaic *sobemovirus* (SoMV)*	Gáborjányi et al. (1997)
Pepper mild mottle *tobamovirus* (PMMV)	Kálmán & Gáborjányi (2000)

*serologically detected viruses

First of all 'újhitűség' disease of pepper was described by *Szirmai* (1944), due to CMV infection. At the moment, CMV is the most important pepper pathogen virus in Hungary. The degree of the infection varies from year to year, depending on ecological factors *(Tóbiás* et al. 1978, *Tóbiás & Molnár,* 1983). The majority of the tested pepper varieties proved to be susceptible or at least tolerant *(Zatykó,* 1982). Six varieties (*Édesalma, Suptol, Szintetikus Cecei, Táltos Synthetic, Boni, Korona*) were tolerant to CMV infection *(Fehér & Kristóf,* 1995, *Fehér,* 1996). *Grube* et al. (1996) developed CMV resistant genotypes and tools for marker-assisted selection (MAS) to facilitate further transfer of CMV resistance.

Among economically important viruses TMV was mentioned at first time by *Szirmai* (1950). The breeding program against *tobamoviruses* started with the incorporation of L genes into commercial pepper varieties. Today many of the pepper varieties contain L^1, L^2 and L^3 genes *(Gáborjányi* et al., 1998b). At present eleven varieties contain the L^1 and seven ones (*Novares F1, Rapires F1, Dabora F1, Savó F1, Brill F1, Cecil F1, Bölény*) the L^2 gene *(Gáborjányi* et al., 1998b). Recently a new variety, *Ciklon F1* was reported to have the L^3 gene (Sági & Salamon, 1998). First hybrid (*H 19-6 F1*) containing the L^4 gene was created first by Csilléry in 1983 using *Capsicum chacoense* as a source of resistance and a green hot pepper variety (*Hímes F1*), which was registered in 1997 *(Salamon,* 1997, *Sági & Salamon,* 1998).

Before 1968 all the tested varieties were susceptible to TMV, CMV, potato X *potexvirus* (PVX) and PVY infections *(Horváth,* 1967, *Beczner & Horváth,* 1969, *Horváth,* 1969). After this time a breeding program was started to built the resistance genes into different pepper varieties *(Zatykó,* 1982). Resistance of varieties and sources of resistance to viruses was first summarized by *Horváth* (1983). In 1985 only 10%, but in 1995 31% of the registered Hungarian pepper varieties had some degree of virus resistance *(Gáborjányi* et al. 1998b).

To know the susceptibility or resistance of the different pepper varieties is of great importance. Therefore the aim of our study was to examine virus susceptibility of 18 varieties to four viruses.

Material and methods

Seeds of 18 varieties (*Cecil F1, Ciklon F1, Star, Synthetic Cecei, Tuba, Tizenegyes, Fehérözön Synthetic, Boni, Korona, Brill F1, Táltos Synthetic, Alba Regia, Gigant F1, Greygo, Édes Alma, Hímes F1, Dabora F1, Rapires F1*) of pepper were sown in sterilized boxes in the virological glasshouse free of vectors. The seedlings were planted in plastic pots (12 cm in diameter) containing a soil mixture of sand (pH 6.96, humus% 0.27): peat (pH 6.78, humus% 9.98) 1:3. Seven plants at 6–8 leaves stages of each varieties/hybrids were mechanically inoculated with four viruses: TMV (isolated from tomato), CMV-U/246 *(Schmidt & Horváth,* 1982), Maradona isolate of PVY^{NTN} *(Beczner* et al., 1984), H isolate of sowbane mosaic *sobemovirus* (SoMV). Sörensen phosphate buffer (pH 7.2) in the ratio 1:1 was used for inoculation. The inoculated plants were symptomatologically checked for infection. Five weeks after inoculation the varieties were tested using direct double-antibody sandwich ELISA (DAS ELISA) method, after *Clark & Adams* (1977). Substrate absorbance was measured twenty minutes after adding the substrate at 405 nm wavelength on Labsystems Multiskan RC ELISA Reader. Out of the seven plants of each variety the highest absorbency values were recorded. Test samples were considered positive if their absorbance values exceeded twice those of the healthy control samples. To confirm the results of serological tests and in latent host-virus relations back inoculation was also carried out to *Nicotiana tabacum* cv. Xanthi, *N. tabacum* cv. Samsun, *N. glutinosa* and *Chenopodium quinoa*, as indicator plants.

Results and discussion

Out of the studied varieties five (*Tuba, Greygo, Brill F1, Fehér özön Synthetic, Rapires F1*) were susceptible to CMV infection. Among them *Rapires F1* was symptomless, while others showed systemic symptoms (leaf deformation, mosaic, vein necrosis, chlorotic rings). 13 varieties were resistant to CMV infection, similar to results of *Fehér & Kristóf* (1995), *Moór & Zatykó* (1995) and *Fehér* (1996) *(Table 2)*. Wang et al. (1998) showed that the CMV resistance of pepper varieties at the seedling stage cannot always represent their resistance at the maturation stage. Generally speaking, resistance to CMV was enhanced from the seedling stage to the maturation stage more in pepper or chilli varieties than in sweet pepper varieties.

In spite of the fact that more Hungarian pepper varieties have some degree of resistance to TMV (Gáborjányi et al.,

Table 2 Reaction of pepper varieties to CMV

Varieties	Symptoms*	Absorbance	Biotest
Tuba	-/Mo, Led, Chl, Ri	0.225	+
Greygo	-/Vn, Led, Mo	0.332	+
Dabora F1	-/-	0.191	–
Hímes F1	-/-	0.183	–
Syn. Cecei	-/-	0.116	–
Táltos Synthetic	-/-	0.179	–
Brill F1	-/Led	0.341	+
Fehérözön Synthetic	-/Led, Mo, Vn	0.638	+
Boni	-/-	0.126	–
Alba Regia	-/-	0.106	–
Gigant F1	-/-	0.158	–
Korona	-/-	0.159	–
Édesalma	-/-	0.190	–
Ciklon F1	-/-	0.109	–
Tizenegyes	-/-	0.123	–
Cecil F1	-/-	0.126	–
Rapires F1	-/-	0.249	+
Star	-/-	0.134	–
Positive control		0.440	+
Negative control		0.110	–

* local/systemic symptoms; Mo, mosaic; Led, leaf deformation; Chl, chlorotic lesions; Ri, ringspot; Vn, vein necrosis; -, symptomless

Table 3 Reaction of pepper varieties to TMV

Varieties	Symptoms*	Absorbance	Biotest
Tuba	-/Mo	0.479	+
Greygo	-/Mo	0.468	+
Dabora F1	HR/-	0.228	-
Hímes F1	HR/-	0.635	+
Syn. Cecei	-/Mo	0.353	+
Táltos Synthetic	-/Mo, Led	0.748	+
Brill F1	HR/-	0.146	-
Fehérözön Synthetic	HR/-	0.215	-
Boni	-/Mo, Led	0.476	+
Alba Regia	-/Mo, Led	0.341	+
Gigant F1	HR/-	0.340	+
Korona	-/Mo, Led	0.352	+
Édesalma	HR/-	0.328	+
Ciklon F1	HR/-	0.136	-
Tizenegyes	-/Vn, Tn	0.408	+
Cecil F1	HR/-	0.129	-
Rapires F1	-/-	0.173	+
Star	-/Mo, Led	0.414	+
Positive control		0.507	+
Negative control		0.134	-

* local/systemic symptoms; Mo, mosaic; Led, leaf deformation; Vn, vein necrosis; Tn, top necrosis; HR, hypersensitive reaction; -, symptomless

1998b), the proportion of the TMV infected varieties was very high. No varieties showing extreme resistance (immunity) have been found. Only systemic symptoms (leaf deformation, mosaic, vein and top necrosis) have been noted in nine varieties (*Tuba, Greygo, Synthetic Cecei, Táltos Synthetic, Boni, Alba Regia, Korona, Tizenegyes, Star*) and the extinction values were high, similar to those of the positive control during serological tests. Three varieties (*Hímes F1, Gigant F1, Édesalma*) showed only local symptoms, but on the basis of serological and biological tests TMV was identified from the systemic leaves. *Rapires F1* showed no symptoms and TMV could not be detected by serological tests, but during back inoculation TMV was isolated from test plants (local necrotic lesions and systemic mosaic symptoms have been developed on *N. tabacum* cv. Xanthi and *N. tabacum* cv. Samsun, respectively, suggesting the presence of TMV) *(Table 3)*. It can be presumed that TMV occurs only at very low concentration in Rapires F1 hybrid.

Some varieties (*Dabora F1, Brill F1, Fehérözön Synthetic, Ciklon F1, Cecil F1*) showed only local hypersensitive reaction due to TMV infection. Necrotic lesions have been developed on the infected leaves 2–4 days after inoculation, and later the infected leaves dropped, preventing the spreading of the virus inside the whole plants *(Table 3)*. Plant resistance to virus infection expressed in the form of restricted systemic movement of the virus has been described for many other virus-host relations *(Nelson* et al., 1993, *Schaad & Carrington*, 1996, *Derrick & Barker*, 1997, *Guerini & Murphy*, 1999). However a temperature of 30 °C or more may overcome hypersensitive reaction (HR) in some host-virus systems. *Roggero* et al. (1996) reported that continuous high temperature can break the hypersensitivity of *Capsicum chinense* PI 152225 to tomato spotted wilt *tospovirus* (TSWV). The experiments reported are of practical interest because cultivated species of *Capsicum* are susceptible to TSWV and are generally grown where temperatures often exceed 30°C.

Because some varieties (*Tuba, Synthetic Cecei, Tizenegyes, Greygo*) -which formerly had been believed to be resistant to TMV- have been infected with TMV, it could be presumed that the TMV isolate used for infection has resistance breaking characteristics, similar to the Ob strain of ToMV (ToMV-Ob) *(Csilléry & Ruskó*, 1980, *Tóbiás* et al., 1982, *Csilléry* et al., 1983) and Italian isolates of TSWV which overcome the hypersensitive response of *Capsicum* varieties with resistance introgressed from *C. chinense* PI 152225 *(Roggero* et al., 1999).

All of the tested pepper varieties showed resistance to SoMV, in spite of the fact that this virus previously had been serologically detected from field pepper *(Gáborjányi* et al., 1997). Neither the inoculated nor the non-inoculated leaves showed symptoms and the virus could not be detected in them by serological and biological tests *(Table 4)*.

Tuber necrotic ringspot disease of potato, caused by the NTN strain of PVY was first described in Hungary by *Beczner* et al. (1984). PVY^{NTN} produces severe necrotic ring symptoms on the potato tubers and berries, too and has resistance breaking characteristics (Le *Romancer & Kerlan*, 1992, *Weidemann*, 1993, *Kus*, 1995). In spite of the fact, that this new strain cause severe injuries on potato, only mild mosaic symptoms could be seen on susceptible pepper varieties/hybrids due to PVY^{NTN}. Out of the investigated varieties ten were susceptible. Systemic latent infection have been occurred in case of *Hímes F1, Rapires F1* and *Gigant F1* hybrids. Eight varieties (*Tuba, Fehérözön Synthetic, Boni, Alba Regia, Korona, Édesalma, Cecil F1, Star*) were found to be resistant to PVY^{NTN} *(Table 5)*.

While a number of *potyviruses* infect pepper only three tobacco etch *potyvirus* (TEV), pepper mottle *potyvirus* (PepMoV) and PVY are dominant in Europe and North America. Out of them only PVY has economic importance

Table 4 Reaction of pepper varieties to SoMV

Varieties	Symptoms*	Absorbance	Biotest
Tuba	-/-	0.103	–
Greygo	-/-	0.116	–
Dabora F1	-/-	0.101	–
Hímes F1	-/-	0.126	–
Syn. Cecei	-/-	0.101	–
Táltos Synthetic	-/-	0.111	–
Brill F1	-/-	0.118	–
Fehérözön Synthetic	-/-	0.115	–
Boni	-/-	0.102	–
Alba Regia	-/-	0.115	–
Gigant F1	-/-	0.106	–
Korona	-/-	0.111	–
Édesalma	-/-	0.091	–
Ciklon F1	-/-	0.104	–
Tizenegyes	-/-	0.090	–
Cecil F1	-/-	0.104	–
Rapires F1	-/-	0.100	–
Star	-/-	0.196	–
Positive control		0.600	+
Negative control		0.105	–

*local/systemic symptoms; –, symptomless

Table 5 Reaction of pepper varieties to PVY^{NTN}

Varieties	Symptoms*	Absorbance	Biotest
Tuba	-/-	0.260	–
Greygo	-/Mo	0.620	+
Dabora F1	-/Mo, Led	0.462	+
Hímes F1	-/-	0.623	+
Syn. Cecei	-/Mo, Led	0.634	+
Táltos Synthetic	-/Mo, Led	0.599	+
Brill F1	-/Mo, Led	1.448	+
Fehérözön Synthetic	-/-	0.143	–
Boni	-/-	0.145	–
Alba Regia	-/-	0.163	–
Gigant F1	-/-	0.634	+
Korona	-/-	0.133	–
Édesalma	-/-	0.161	–
Ciklon F1	-/Mo	0.667	+
Tizenegyes	-/Mo	0.590	+
Cecil F1	-/-	0.179	–
Rapires F1	-/-	0.854	+
Star	-/-	0.134	–
Positive control		0.837	+
Negative control		0.134	–

* local/systemic symptoms; Mo, mosaic; Led, leaf deformation; –, symptomless

in Hungary. Several resistance genes for *potyviruses* are known in *Capsicum* genus. *Caranta* et al. (1997) reported the molecular mapping of *potyvirus* resistance factors from pepper line '*Perennial*' and an analysis of the genetic basis of multi*potyvirus* quantitative resistance, which allows us to make comparisons with the location of major genes and quantitative trait loci (QTLs) involved in virus resistance. *Dogimont* et al. (1996) studied the genetic basis of the broad spectrum resistance to *potyviruses* in a Mexican pepper line (CM 334) using doubled haploid lines. Segregation analyses indicated that resistance to pepper *potyviruses* in CM 334 is conferred by two genes. The first one, tentatively named *Pr4*, is dominant and confers the resistance to all known pathotypes P(0), P(1), P(1,2) of PVY and to PepMoV. The second one, named *Pr5*, is recessive; it confers only the resistance to common strain P(0) of PVY. Kyle & Palloix (1997) recently proposed the revision of the nomenclature for genetic loci in *Capsicum*, governing *potyvirus* resistance. At present there is clear evidence for four independent loci in *Capsicum*, each with alleles that confer resistance to viral isolates that belong to one or more of the viruses: PVY, PepMoV and TEV. Other studies determined the mechanism of resistance, e.g. the resistance of *C. annuum* cv. *Dempsey* to TEV is due to interference with virus RNA accumulation *(Deom* et al., 1997).

Out of the varieties examined five (*Boni, Alba Regia, Korona, Édesalma, Star*) were resistant to three viruses (SoMV, CMV and PVY^{NTN}). Only one (*Cecil F1*) displayed complex, extreme resistance to SoMV, PVY^{NTN}, CMV and hypersensitive reaction to TMV, therefore this hybrid is very important in pepper growing and breeding for virus resistance.

In our experiments each pepper variety was infected with only one virus. Mixed infections, i.e. infection of a plant by more than one type of virus, occur commonly in nature and may result in a range of effects on the host as well as on the levels of accumulation and degrees of movement of either of the viruses involved. Of particular interest of these works are those mixed infections in which the restricted ability of one virus to move is alleviated by co-infection with another virus *(Fuentes & Hamilton,* 1991, *Murphy & Kyle,* 1995). *Guerine & Murphy* (1999) showed that in *C. annuum* cv. *Avelar* plants co-infected with PepMoV and CMV, PepMoV is able to enter, accumulate in and move within internal phloem, thereby allowing the virus to invade young tissues systemically.

We plan mixed infections in future which can suppress or modify resistance or susceptibility of a given variety to viruses.

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