Study on the viruses of *Petunia* in Hungary

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Summary: Virus susceptibility of 11 varieties of vegetatively propagated petunia against 2 virus strains (TMV-C/U1 and PVY^{NTN}) were examined. Neither resistant nor tolerant varieties of petunia were found. The virus infection of the *Petunia* genus was examined in Hungary. The most common pathogens were the *Tobacco mosaic tobamovirus* (TMV) and the *Tomato mosaic tobamovirus* (ToMV), but the samples also contained *Alfalfa mosaic alfamovirus* (AMV), *Cucumber mosaic cucumovirus* (CMV) and *Potato Y potyvirus* (PVY). *Potato X potexvirus* (PVX) was isolated in the varieties of trailing petunia for the first time.

Key words: *Petunia* varieties, virus susceptibility, natural virus infection, DAS-ELISA

Introduction

Cultivated species of the genus of *Petunia* Juss. have important role in the market of annual ornamental plants. Their popularity is due to the relatively simple cultivation technology and their excellent ornamental quality. In Hungary an estimated number of 60 million annual ornamental plants are set out every year. From this quantity 25 million a year belong to the species of *Petunia* genus, which are propagated by seed and 0.5–1 million which propagated by vegetative way. Earlier in cultivation we could only meet the species of *P.* x *hybrida* propagated by seed, the improvement of which has been examined since 1830.

The improvement of the species propagated in vegetative way started only at the end of the 1980s. The cross-fertilization of the annual *P.* x *hybrida* and the South American perennial *P. pendula* resulted varieties whose cultivation values exceed the ones propagated by seed. The varieties were selected on the basis of the growing characteristics of the feral parent and the aesthetic qualities of the ancestor. Groups of varieties (*Cascadias, Dream, Microtunia, Million Bells, Surfinia, Surprise* and *Solana*) can be found in the flower-market propagated by authorised firms of foreign countries. The characteristic feature of these varieties was that they produce very few viable seeds, therefore vegetative propagation is inevitable. The soft, velvety leaf of petunia, which is densely covered with hairs, promotes the mechanical transmission of viruses, which can cause serious problems in growing. In virus research, varieties of petunia propagated by seed, which proved to be good host plants, have been used as test plants for a long time.

The virological problems of the varieties of petunia propagated vegetatively, got into the centre of research in Europe as well as Hungary only in the 1990 (*Feldhof*, 1994; *Verhoeven*, 1994; *Lesemann & Dalchow*, 1995; *Roenhorst & Verhoeven* 1995; *Bellardi* et al., 1996; *Kaminska & Rudzinska-Langwald*, 1996; *Lesemann*, 1996; *Mavric* et al., 1996; *Salamon* 1996; *Feldhoff* et al., 1998; *Boonham* et al., 1999; *Cohen* et al., 1999). In studies concerning the virus diseases of the genus *Petunia* virologists used seven species of the genus (*Petunia axillaris* Lam., *P. compacta* Juss., *P.* x *hybrida* Vilm., *P. inflata* Juss., *P. parodii* Juss., *P. parviflora* Juss., *P. violacea* Lindl.) several varieties of *P.* x *hybrida* and its vegetatively propagated varieties, which have become well-known in the last decade, in different virological tests against 88 viruses altogether (*Baracsi & Horváth*, 1998). Knowing the virus susceptibility of the species, during vegetative propagation, care must be taken of the health condition of the parent plant, which should be free from viruses. It is also important to take care of fertilisation because high pH level, the lack of iron and nitrogen, under or over irrigation can influence growing, can cause chlorosis or symptoms, which are similar to virus symptoms. Mistakes in plant protection can also cause symptoms similar to virus infections. Therefore it is absolutely necessary to eliminate symptoms caused by a mistake in the production or virus infections (*Lesemann & Dalchow*, 1995).

Our purpose was to determinate the virus susceptibility of the varieties of vegetatively propagated petunia, which can be found on the market in Hungary. By examining with diagnostic methods the collected plants showing virus symptoms, our aim was to estimate the level of virus infection of the *Petunia* genus in Hungary.

Material and method

Study of resistance

11 trailing petunia varieties: *'Bright Dream', 'Cascadias Pink', 'Choice', 'Microtunia Blue', 'Microtunia Purple Vein', 'Million Bells Cherry', 'Million Bells Trailing Blue, 'Solana Royal Karl Gustav', 'Surfinia Purple', 'Surprise Blue Sky', 'Surprise White Carrousels'* grown in Hungary were tested. Virus free plants with 4–6 leaves were inoculated with tissue sap of virus infected host plants (*Nicotiana tabacum* cv. Samsun, *Nicotiana tabacum* cv. Xanthi-nc) in suitable conditions of the Departement of Plant Pathology and virology (DPPV) using phosphate buffer (pH 7.2) and carborundum (400 mesh) as an abrasive.

TMV-C/U1 and the Maradona isolate of PVY^{NTN} virus strains were used (Virus gene bank of the Laboratory of DPPV).

The symptoms were evaluated 1–4 weeks after the inoculation and DAS-ELISA (*Clark & Adams*, 1977) was carried out. After the incubation optical density was measured at 405 nm, with Metertech N°6 Miniphotometer or Labsystems Multiscan RC ELISA Reader.

Back inoculations of the viruses were carried out with the samples which appeared to be negative in the serological examinations. In the case of PVY^{NTN} virus the *Nicotiana tabacum* cv. Xanthi-nc test plant, in the case of TMV C/U1 virus *Nicotiana tabacum* cv. Samsun test plants were used.

Tests of commercial plant samples

In different parts of Hungary vegetatively propagated petunia plants showing disease symptoms were collected. The samples were tested on 15 viruses by ELISA according to *Clark & Adams* (1977). There were viruses among them, which can often be isolated from species of the *Solanaceae* family (*Alfalfa mosaic alfamovirus*, AMV; *Cucumber mosaic cucumovirus*, CMV; *Tobacco mosaic tobamovirus*, TMV; *Tomato mosaic tobamovirus*, ToMV; *Potato X potexvirus*, PVX; *Potato Y potyvirus*, PVY; *Tomato spotted wilt tospovirus*, TSWV). During the serological examination our samples were tested for viruses which were described on other annual ornamental plants too (*Arabis mosaic nepovirus*, ArMV; *Bean yellow mosaic potyvirus*, BYMV; *Carnation mottle carmovirus*, CarMV; *Chrysanthemum B carlavirus*, CBV; *Petunia asteroid mosaic tombusvirus*, PeAMV; *Sowbane mosaic sobemovirus*, SoMV; *Tomato aspermy cucumovirus*, TAV; *Tobacco necrosis necrovirus*, TNV). Many of the above mentioned ornamentals are often planted together with trailing petunias therefore infection risk was possible.

The following varieties were tested: *'Cascadias Pink', 'Champagne', 'Charme', 'Chateau', 'Improved Charme', 'Surfinia Blue var. Sunblue', 'Surfinia Blue Vein Sunsolos', 'Surfinia Hot Pink var. Marrose', 'Surfinia Revolution Purple', 'Surfinia Revolution White', 'Surprise Big Spark', 'Surprise Blue Spark' 'Surprise Blue Vein', 'Surprise Pink Spark', 'Surprise Pink Vein'*.

Results and discussion

Study of resistance

One week after the inoculation local necrotic or chlorotic spots on the leaves were observed except of 3 varieties (*'Microtunia Blue', 'Million Bells Cherry' and 'Million Bells Trailing Blue'*). Later the symptoms were systemized on ten varieties. Leaf deformation, blistering and mosaic and colour breaking on the flowers were noticed *(Fig. 1 and 2)*. In the case of the varieties *'Bright Dream'* systemic symptoms did not appeared. The serological examination gave a negative result only in three cases but after the back inoculation on the test plants these plants proved to be infected as well.

Figure 1 Systemic colour breaking on flower, on *'Surprise White Carrousels'* inoculated with TMV-C/U1

Figure 2 Systemic leaf blistering on *'Surfinia Purple'* inoculated with TMV-C/U1

The result of the experiment is summarized in *Table 1*.

Results of the infection test with the TMV-C/U1 strain indicate that the 11 petunia varieties tested are susceptible to the virus. In accordance with *Cohen* et al. (1999) we could not find either a tolerant or a resistant variety.

Systemic symptoms on the plants inoculated with the PVY^{NTN} strain appeared mainly in the form of chlorosis, mosaic, leaf blistering, leaf and flower deformation and colour breaking on the flowers *(Fig. 3 and 4)*, but local symptoms did not appear. During the serological test and back isolation we obtained a negative result in some cases, which was probably caused by the circumstances of inoculation.

Figure 3 Systemic chlorotic spots and veinal necrosis on *'Surfinia Purple'* inoculated with PVY^{NTN}

Figure 4 Systemic flower distortion and colour breaking on flower on *'Surprise White Carrousels'* inoculated with PVY^{NTN}

Table 1 Summarized data of the petunia varieties infected with TMV C/U1 strain

Varieties	Number of plant	Symtoms[1]		ELISA[2] ($OD_{405\ nm}$)	Bioteszt[3]
		Local	Systemic		
Bright Dream	1	Nl	–	0.537	0
	2	Nl	–	0.591	0
	3	Nl	–	0.611	0
	4	Nl	–	0.620	0
	5	Nl	–	0.718	0
Cascadias Pink	1	Nl	Ch, M	0.708	0
	2	Nl	M	0.631	0
	3	Nl	Ch	0.670	0
	4	Nl	Ch, M	0.619	0
	5	Nl	M	0.815	0
Choice	1	Nl	Bli, M	0.704	0
	2	–	Bli, Fdef	0.549	0
	3	–	Ch, M,	0.597	0
	4	–	Fdef, M	0.751	0
	5	–	Bli	0.477	0
Microtunia Blue	1	–	Bli, Cb, M	0.466	0
	2	–	Ch, M,	0.447	0
	3	–	Bli, M	0.482	0
	4	–	Cb, M	0.460	0
	5	–	Bli	0.361	0
Microtunia Purple Vein	1	–	–	**0.218**	+
	2	Nl	M	0.642	0
	3	Nl	Bli	0.619	0
	4	Nl	Bli, M	0.712	0
	5	Nl	–	**0.186**	+
Million Bells Cherry	1	–	M	0.395	0
	2	–	Ch	0.622	0
	3	–	Ch	0.567	0
	4	–	Ch, M	0.654	0
	5	–	D	0	0
Million Bells Trailing Blue	1	–	M	0.504	0
	2	–	Ch, M	0.512	0
	3	–	Ch, M	0.532	0
	4	–	Ch	0.553	0
	5	–	Ch, M	0.593	0
Solana Royal Karl Gustav	1	Chl	Ldef, M	0.797	0
	2	Chl	M	**0.213**	+
	3	Chl	Ldef, M	0.620	0
	4	Chl	M	0.549	0
	5	Chl	M	0.477	0
Surfinia Purple	1	Chl	Cb, M	0.708	0
	2	Chl	Ldef, M,	0.536	0
	3	Chl	Cb, M	0.678	0
	4	Chl	Cb, Ldef	0.485	0
	5	Chl	Ldef, M	0.642	0
Surprise Blue Sky	1	Chl	Bli	0.563	0
	2	Chl	Bli	0.497	0
	3	Chl	Bli, Ldef	0.792	0
	4	Chl	Bli, Ldef	0.812	0
	5	Chl	Ldef	0.736	0
Surprise White Carrousels	1	Nl	Bli, Cb, Gr	0.530	0
	2	Nl	D	0	0
	3	Nl	Gr, Ldef	0.443	0
	4	Nl	Bli, Cb, Gr	0.437	0
	5	Nl	Bli, Cb	0.429	0

[1] Bli: blistering, Cb: colour breaking, Ch: chlorosis, Chl: chlorotic lesions, D: plant death, Fdef: flower deformation, Gr: growth reduction, Ldef: leaf deformation, M: mosaic, Nl: necrotic lesions.

[2] The negative plants are printed in bold. 0: death of the plant

[3] +: Symptoms appeared on the test plant, 0: not tested

Table 2 Summarized data of the petunia varieties infected with PVY^{NTN} strain

Varieties	Number of plant	Symtoms[1]		ELISA[2] ($OD_{405\ nm}$)	Bioteszt[3]
		Local	Systemic		
Bright Dream	1	–	M	0.705	0
	2	–	Ch, M	1.313	0
	3	–	Ch, M	1.917	0
	4	–	M	0.465	0
	5	–	Ch, M	2<	0
Cascadias Pink	1	–	Bli, Ldef, M	2<	0
	2	–	Ldef, M	2<	0
	3	–	Ldef, M	2<	0
	4	–	–	**0.116**	–
	5	–	Bli, Ldef, M	2<	0
Choice	1	–	Ch, Fdef, M	2<	0
	2	–	Ch, M	1.816	0
	3	–	M	1.609	0
	4	–	Ch, Fdef	1.962	0
	5	–	Ch, Fdef, M,	2<	0
Microtunia Blue	1	–	Cb, Ch, M	1.254	0
	2	–	Ldef, M	1.056	0
	3	–	Cb, M	1.368	0
	4	–	Cb, Ldef	1.579	0
	5	–	Cb, Ch, Ldef	2<	0
Microtunia Purple Vein	1	–	Ch, Ldef	1.609	0
	2	–	Ch, Ldef, M	1.917	0
	3	–	Ch, M	1.313	0
	4	–	Ch	0.824	0
	5	–	Ch, M	1.112	0
Million Bells Cherry	1	–	–	**0.030**	–
	2	–	–	**0.049**	+
	3	–	Ch, Gr	0.412	0
	4	–	–	**0.034**	–
	5	–	Ch, Gr	0.514	0
Million Bells Trailing Blue	1	–	Ch	**0.086**	+
	2	–	Ch, M	0.353	0
	3	–	Ch, M	0.836	0
	4	–	–	**0. 045**	–
	5	–	–	**0. 051**	–
Solana Royal Karl Gustav	1	–	–	**0. 058**	–
	2	–	–	**0. 094**	–
	3	–	Ch, M	1.413	0
	4	–	Ch, M	1.072	0
	5	–	–	**0.029**	–
Surfinia Purple	1	–	Bli, Cb, Ldef	1.058	0
	2	–	Cb, Ldef	1.625	0
	3	–	Bli, Vn	1.086	0
	4	–	Bli	0.849	0
	5	–	Cb, Ldef	1.576	0
Surprise Blue Sky	1	–	Ch, M	1.415	0
	2	–	Ch, M	1.234	0
	3	–	Ch	0.842	0
	4	–	M	0.689	0
	5	–	Ch, M	0.936	0
Surprise White Carrousels	1	–	Cb, Ch, M	2<	0
	2	–	Cb, Ch, M	2<	0
	3	–	Ch	**0.026**	+
	4	–	Ch, M	**0.209**	+
	5	–	Cb, Ch	0.801	0

[1] Bli: blistering, Cb: colour breaking, Ch: chlorosis, Fdef: flower deformation, Gr: growth reduction, Ldef: leaf deformation, M: mosaic, Vn: veinal necrosis.

[2] The negative plants are printed in bold

[3] +: Symptoms on the test plants. –: Symptomless. 0: Not tested.

Results of the experiment are summarized in *Table 2*.

The resistance of petunia varieties propagated vegetatively was examined against potato Y virus. We established that all of the 11 varieties tested are susceptible to the PVY^{NTN} strain. The symptoms on the inoculated plants were the same as the ones published by *Mavric* et al. (1996) and *Boonham* et al. (1999).

In our study of resistance the pathological reactions of plants in case of virus infection was characterized. The knowledge of the visually perceptible symptoms helps the growers, who work in production, to recognize virus-infected plants and to prevent serious epidemics. As the development of garden culture results in the increase of the production of petunia propagated vegetatively, the significance of this work is obvious and it is important to realize a successful propagation and production technology.

Tests of commercial plant samples

Among the 160 petunia plants tested, 124 proved to be infected. 15 viruses were examined and the presence of 6 was justified (TMV, ToMV, AMV, PVY, CMV, PVX).

TMV could be found in most samples (69). This pathogen causes the most serious problems in the petunia stands propagated vegetatively in different countries of Europe as well (*Fedhof*, 1994; *Lesemann & Dalchow*, 1995; *Kaminska & Rudzinska-Langwald*, 1996; *Fedhoff* et al., 1998; *Cohen* et al.,1999; *Spence* et al., 2001).

ToMV, the presence of which was published by other researchers (*Lesemmann & Dalchow*, 1995; *Roenhorst & Verhoeven*, 1995; *Bellardi et al.*, 1996; *Lesemann*, 1996; *Cochen* et al., 1999) could be found in 28 samples.

As the appearance of AMV in Petunia is already known (*Verhoeven*, 1994; *Lesemann & Dalchow*, 1995; *Roenhorst & Verhoeven*, 1995; *Lesemann*, 1996; *Salamon*, 1996), we could also detect that virus in 15 plants out of 160 individuals.

PVY could be found in 7 cases. *Salamon* (1996) described the occurrence of the normal (PVY^0) and the vein necrosis (PVY^N) strain of the virus in petunia propagated vegetatively in Hungary. The PVY^{NTN} strain was identified in these Hungarian samples with monoclonal antibodies similarly to the method of *Boonham* et al. (1999).

PVX was found in three plant samples (two plants of '*Surprise Pink Vein*' varieties and one plant of '*Surprise Big Spark*' variety) the occurrence of which has not been mentioned in either the foreign or the Hungarian literature.

CMV was detected in only 2 samples. Regarding the risk of infection this virus must not be neglected because it can cause epidemy in Hungary just like in the big ornamental plant farms in Europe (*Feldhof*, 1994; *Lesemann & Dalchow*, 1995; *Bellardi* et al., 1996; *Lesemann*, 1996; *Mavric* et al., 1996).

In our experiments complex infections were detected. Four plants of the varieties '*Surfinia Revolution Purple*' were infected simultaneously by AMV and TMV. One plant of the '*Surfinia Revolution White*' was infected by both AMV and CMV. CMV and TMV were isolated from

'Surprise Pink Vein' variety. AMV, CMV and TMV complex virus infection was observed in the case of *'Surfinia Revolution White'*.

The presented results draw the attention of the growers and researchers to the constant risk of infection. It is also important to respect the plant health regulations in case of commercial plants and to check the import propagation material permanently.

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