Studies on the Tobamovirus resistance of the pepper (*Capsicum annuum* L.) cultivar *Greygo*

INTERNATIONAL JOURNAL OF HORTICULTURAL SCIENCE

AGROINFORM Publishing House, Hungary

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Key words: *Capsicum/Tobamovirus*/Resistance

Summary: Resistance of the Hungarian pepper (*Capsicum annuum* L.) cultivar *"Greygo"* to *Tobamoviruses* has been investigated. All plants of the population of *Greygo* proved to be resistant to tobacco mosaic and tomato mosaic viruses (TMV, ToMV), both represent the pepper pathotypes P_0 of *Tobamoviruses*. Individuals of *Greygo*, however, were found to be susceptible to pathotypes $P_{1.2}$ and $P_{1.2.3}$ of pepper mild mottle virus (PMMV). When inoculated with the XM isolate of dulcamara yellow fleck virus (DYFV, pathotype P_1) the population of *Greygo* segregated in resistant and susceptible plants. These results as well as inoculations of the progenies of three TMV resistant plants clearly showed, that besides the resistance allele L^1 the cultivar *Greygo* possesses also an another allele. This allele, provisionally marked by L^{2g} behaves like to the allele L^2 characteristic to *Capsicum frutescens* cv. *Tabasco*. Determination of the identity of the allele L^{2g} to the allele L^2 needs further genetic and pathological informations. Relations between the *Tobamoviruses* pathogenic to pepper and the alleles of the resistance gene L are outlined for the discussion.

Introduction

Viruses belonging to the *Tobamovirus* genus cause serious problems in the production of pepper (*Capsicum annuum L.*) all over the world (*Green & Kim*, 1991; *Christie & Edwardson*, 1997). The biological properties of *Tobamoviruses* (e.g. extreme stability and infectivity of particles, transmission by plant extracts, seeds and pollen) as well as the common growing practices of vegetable pepper make the conventional control measures often insufficient to prevent the appearance and spreading of diseases caused by this group of viruses. The production of resistant cultivars is the only real possibility to control them.

Resistance to *Tobamoviruses* in pepper is based on the hypersensitive reaction (HR) of the plants (*Holmes*, 1937). On hypersensitive plants the virus causes rapid cell and tissue necrosis in and around the initial sites of infection and then being localized. The susceptible plants, however, react to the infection with chlorotic local spots and become infected systemically expressing differrent kinds of symptoms (e.g. necrosis, green or yellow mosaic of leaves, fruit deformations).

The resistance to *Tobamoviruses* in pepper is known to be controlled by a single locus called L (*Daskalov & Poulos*, 1994). The susceptible genotypes are marked by L^+L^+. Five resistance alleles marked by the symbols of L^1, L^{1C}, L^2, L^3 and L^4, respectively, are known responsible for HR (*Boukema*, et.al. 1980; *Daskalov & Poulos*, loc.cit). Each of them inherites a single dominant trait in order of the dominance of $L^4>L^3>L^2>L^{1C}>L^1$.

The alleles of the L gene except the L^{1C} can be distinguished phenotypically from one another by inoculation of plants with a series of *Tobamovirus* "pepper pathotypes" classified as P_0, P_1, $P_{1.2}$ and $P_{1.2.3.}$, respectively. The alleles L^1 and L^{1C} are differentiated by growing inoculated plants at elevated temperature (*Daubeze* et al., 1990). Classification of *Tobamoviruses* as "pepper pathotypes" does not correspond with the taxonomic status of the viruses (viz. *Green & Kim*, 1991; *Brunt* et al., 1996; *Salamon & Kaszta*, 2000). Relations between the resistance alleles and the pepper *Tobamovirus* pathotypes is presented in *Table 1*.

Breeding peppers for resistance to *Tobamoviruses* has been started some 30 years ago in Hungary by incorporation of the allele L^1 into the vegetable pepper *D-Cecei SH* (*Zatykó*, 1974). The L^1 allele proved to be effective to protect the plants to the infection of *Tobamovirus* pathotypes P_0 until the appearance of dulcamara yellow fleck virus

Table 1 *Tobamovirus* species infecting pepper *(Capsicum* spp.), their pathotypes and the reactions of pepper genotypes*

Tobamovirus species	Pathotype	Reactions of pepper genotypes carrying different alleles of the L gene				
		L^+	L^{1**}	L^2	L^3	L^4
Tobacco mosaic virus (TMV)	P_0	S	R	R	R	R
Tomato mosaic vírus ToMV)	P_0	S	R	R	R	R
Bell pepper mottle virus (BePMV)	P_0	S	R	R	R	R
Tobacco mild green mosaic virus (TMGMV)	P_0/ P_1	S	R/S	R	R	R
Paprika mild mosaic virus (PaMMV)	P_1	S	S	R	R	R
Dulcamara yellow fleck virus (DYFV)	P_1	S	S	R	R	R
Pepper mild mottle virus (PMMV)	$P_{1.2}$	S	S	S	R	R
	$P_{1.2.3}$	S	S	S	S	R

* Compiled according to *Green& Kim* (1991), *Salamon & Kaszta* (2000) with some modification. S= susceptible, R=resistant

** In respect of differentiation of Tobamovirus pathotypes the alleles L^{1C} and L^1 could not be distinguished

(DYFV, formerly suspected to be a strain of ToMV) and pepper mild mottle virus strains (PMMV, pathotypes $P_{1.2}$ and $P_{1.2.3}$) in Hungary (*Csilléry* et al.,1983; *Tóbiás* et al.,1982; Salamon et al., 1987; *Salamon*, 1993;). The new Hungarian vegetable pepper hybrids (e.g. *Novares F1, Ciklon F1, Himes F1*) already carry the L^3 or the L^4 alleles derived from *Capsicum chinense* and *C. chacoense*, respectively (*Ságí* et al. 1999).

The cultivar *Greygo* belongs to the tomato (or squash) type of vegetable peppers. It was bred by selection from a population of pepper originated from Canada (*Zatykó*, 1990) and registered in 1992 as a cultivar having the *Tobamovirus* resistance allele L^1 (*Fehér*, 2000). However, *Zatykó* (loc.cit.) noticed, that the *Tobamovirus* resistance of *Greygo* covered a broader range of *Tobamovirus* pathotypes than that of TMV (P_0). By contrast, the only resistance allele L^1 was reported by *Gáborjányi* et al. (1999) to be present in cv. *Greygo*.

In 1998, the senior author of this work collected seeds of *Greygo* from plants produced in polyethylene tunnel at Mindszent (South-East Hungary). Seedlings were investigated for resistance to some *Tobamoviruses* and individuals resistant to DYFV-XM (pathotype P_1) have been found among the susceptible ones.

Based on these data we presumed that, besides the allele L^1 the population of *Greygo* carries also an another allele of resistance to *Tobamoviruses* which was not characterised earlier. This paper presents the results of investigations directed to characterise this unidentified allele in more detail.

Material ad methods

In this work isolates of the following *Tobamovirus* species were used: tobacco mosaic virus (TMV-U1), tomato mosaic virus (ToMV-D/H), dulcamara yellow fleck virus (DYFV-XM) and pepper mild mottle virus (PMMV-P8 and P14). TMV and ToMV were propagated on *N. sylvestris* and *Lycopersicon esculentum cv. Rutgers*, respectively. DYFV-XM was maintained on *N. tabacum cv. Xanthi*-nc. PMMV isolates, kindly supplied by I. Tóbiás were propagated in *Capsicum frutescens* cv. *Tabasco*. Each of the virus isolates were tested for "pathotype purity" by inoculation of pepper cultivars having different alleles of the gene L .

Seeds of pepper cultivars from the pepper collection of the Vegetable Crops Research Institute were sown in compost. The seedlings were grown in light boxes at 24–28 C° with a daylenght of 16 h. Inocula were prepared by grinding the leaves of virus donor plants in sterile mortars by adding water (1/10 w/v) and carborundum (ca. 0.1 g/ ml). The plants were inoculated by rubbing fully expanded cotyledons or the first or second true leaves with sterile glass spatula dipped into inocula. The reactions of pepper plants were evaluated weekly. Some TMV resistant seedlings of Greygo were transplanted in 5L black polyethylene bags and were grown in greehouse. Resistance of their self-pollinated progenies were investigated as described above.

Results and discussion

Inoculation experiments have showed that all of the plants of *Greygo* reacted with local lesions and were resistant to the pathotype P_0 of Tobamoviruses (TMV ad ToMV). All of the seedlings inoculated by the pathotypes $P_{1.2}$ and $P_{1.2.3}$ of PMMV, however, proved to be susceptible systemically *(Table 2)*.

Table 2 Resistant and susceptible plants of some pepper cultivars inoculated by different *Tobamovirus* "pepper pathotypes"

Pepper cultivars	Viruses			
	ToMV D/H*	DYFV XM	PMMV P8	PMMV P14
Albaregia (L^+L^+)	0/15**	0/15	0/10	0/10
D-Cecei-SH (L^1L^1)	11/0	0/14	0/15	0/15
Greygo (?)	6/0	7/13	0/12	0/13
Tabasco (L^2L^2)	13/0	16/0	0/17	0/14
Ciklon (L^3L^+)	13/0	16/0	10/0***	0/10
Himes (L^4L^+)	13/0	13/0	12/0***	12/0

* ToMV-D/H and TMV-U1 caused similar reactions on the pepper sortiment.

** number of resistant/susceptible individuals

*** besides local lesions systemic necrosis appeared on several individuals

DYFV-XM caused brilliant yellow mosaic on both the cvs. *Albaregia* (L^+L^+) and *D-Cecei-SH* (L^1L^1) */Fig.1/*, but induced necrotic local lesions on *C. frutescens* cv. *Tabasco* (L^1L^1). As a consequence, DYFV-XM could be classified to pathotype P_1.

The population of *Greygo* inoculated with DYFV-XM segregated in susceptible and resistant induviduals. On the susceptible plants local yellow spots followed by yellow systemic mosaic appeared, while necrotic lesions developed on the cotyledons of the resistant individuals. As the cotyledons showing necrotic lesions felt down, the plants

Figure 1 Symptoms caused by DYFV-XM on the seedling populations of the cvs. *D-Cecei-SH* (D) and *Greygo* (G). Note the segregation of *Greygo* in susceptible and resistant plants.

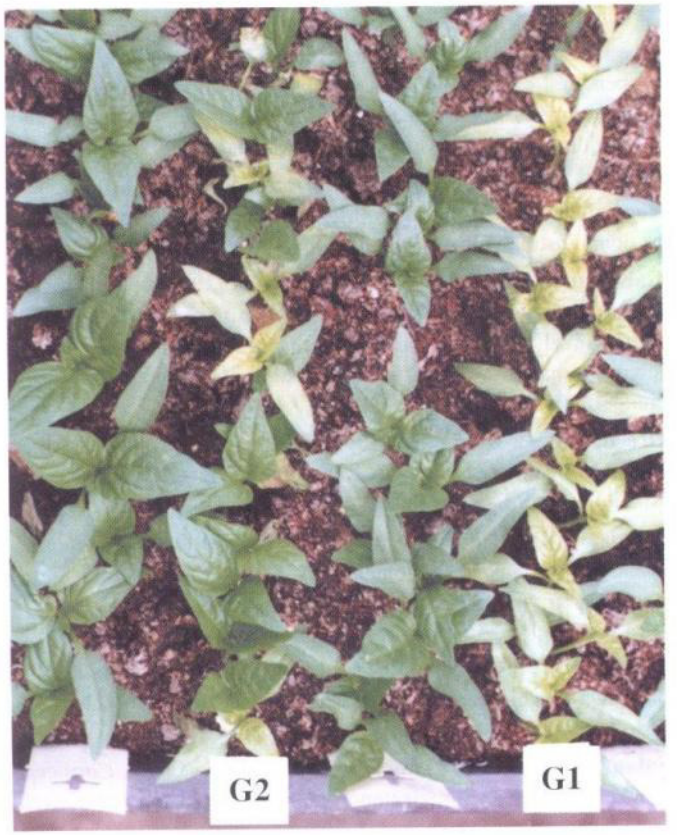

Figure 4 Progenies of the *Greygo* mother plants 2000/1 (G1) and 2000/2 (G2) inoculated by DYFV-XM.

Figure 2 Necrotic local lesions on true leaves of *Greygo* caused by DYFV-XM

Figure 3 Plants of Greygo for seed production

grew vigourously without any systemic symptoms *(Figure 1)*. Some of them were inoculated by DYFV-XM at the first and second true leaves again. On these leaves definite necrotic lesions were observed showing the hypersensitive response of the plants *(Figure 2)*.

Progenies of three TMV resistant individuals of *Greygo* (marked as mother plants of 2000/1-3, *Figure 3)* were inoculated by ToMV and DYFV, respectively. All of the progenies were found to be resistant to ToMV, but their reactions to DYFV varied in dependence on their parental plants *(Table 3)*. The progenies of the plant 2000/1 were susceptible to DYFV, while the seedlings of the plant 2000/3 proved to be resistant to this virus. *(Figure 4)* The first generation of the plant 2000/2 segregated in resistant and susceptible individuals *(Figure 5)*. These results indicated that in respect to the resistance to *Tobamoviruses* the selected mother plants represented differrent genotypes.

The DH line selected from the basic population of *Greygo* proved to be resistant to ToMV, but it was susceptible to DYFV *(Table 3)*.

Table 3 Susceptibility and resistance in some *Greygo* populations to Tobamovirus pathotypes P_0 (ToMV) and P_1 (DYFV)

Seed lots	Viruses	
	ToMV-D/H	DYFV-XM
Greygo I 98	16/0*	7/14
Greygo DH/I 96	14/0	0/15
Greygo 2000/1	20/0	0/20
Greygo 2000/2	23/0	14/5
Greygo 2000/3	16/0	20/0

* Number of resistant/susceptible plants

Figure 5 Seedlings of the *Greygo* mother plant 2000/3 (G3) inoculated by DYFV-XM

The results of investigations clearly showed, that in accordance with our hypothesis, the population of the pepper cultivar *Greygo* possesses at least two differrent Tobamovirus resistance genes or alleles of unknown frequency. One of them provides resistance exclusively to the Tobamovirus pathotype P_0 (TMV and ToMV). This allele can be for all probability identical to the allele L^1. It occurs in a number of plants of the basic *Greygo* population in homozygous form. The progenies of these plants (like to those of 2000/1) were found to be susceptible to DYFV-XM. Other plants of *Greygo* that showed resistance to DYFV-XM must be carrying a resistance allele either in homozygous or in heterozygous form that is apparently differrent from the alleles L^1, L^{1C}, L^3 and L^4, respectively. This allele behaves like the standard L^2 allele characteristic to *C. frutescens* cv. *Tabasco*. However, its identity to the allele L^2 could not have been established. Therefore we mark it provisionally by the symbol L^{2g} (g refers to the cv. *Geygo*). Determination of the identity of the allele L^{2g} to L^2 needs further genetic and pathological informations.

The origin of L^2 type of resistance in *Greygo* is unknown. It is worth to mention, however, that besides *C. frutescens* cv. *Tabasco* some cultivars and lines of *C. annuum*, e.g. *Chilifen* and "*P11*" carry the allele L^2, too. (*Boukema et al.* 1980 ; *Salamon*, 1983). More recently resistance to the *Tobamovirus* pathotypes P_0 and P_1 has also been discovered in a number of ornamental (small fruited) *C. annuum* lines (*Salamon*, 1999).

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