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Autonomous cell death, temperature sensitivity and the genetic control associated with resistance to cucumber mosaic virus (CMV) in diploid potatoes (*Solanum* spp.)

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Abstract Cucumber mosaic virus (CMV) is a commonly occurring plant virus that causes severe damage in many crops, including the diploid crop species tomato and pepper (*Lycopersicon* spp. and *Capsicum* spp., respectively) of the family Solanaceae, but it is neither common nor economically important in cultivated potatoes (*Solanum tuberosum;* Solanaceae). Resistance to CMV was examined in two diploid (2n=2x=24), highly heterozygous potato populations (*Solanum* spp.; Solanaceae) consisting of 76 and 126 progeny. Resistance to long-distance transport of CMV controlled by one locus with a major effect and functional at a low temperature (18°C) but overcome at a high temperature (28°C) was identified in one population. In the other population, resistance was controlled by two loci with major effects. In both populations, additional genes with minor effects were probably also involved. Induced resistance to CMV, associated with autonomously developing cell death lesions (Anl) previously not known in potato, was expressed in one parental line. The mechanisms of resistance to CMV may be associated with an inherent or developmental lack of host factors required for compatible CMV-host interactions in viral long distance transport and/or inability of CMV to efficiently suppress the host gene silencing mechanism in potatoes. Polyploidy (gene dose) and high heterozygosity (multiple homologous genes) of potato cultivars may be significant in conferring the durable resistance to CMV. These data provide explanations why CMV is not common and economically important in cultivated potatoes, even though CMV commonly occurs in other crops, weeds and wild plants in potato production areas.

Key words Cucumber mosaic virus (CMV) Solanaceae · Autonomous cell death · Temperature sensitivity

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Introduction

Cucumber mosaic cucumovirus (CMV) has the largest number of host species of all known plant viruses and is transmitted by over 80 species of aphids in a non-persistent manner (reviewed by Palukaitis et al. 1992). It causes great losses in many crop species of different families, including tomato (*Lycopersicon esculentum* Mill.) and pepper (*Capsicum* spp.) of the family Solanaceae (Watterson 1993). It is therefore surprising that CMV has little economic importance in cultivated potatoes (*Solanum* spp.) that belong to the same family (Hooker 1981; De Bokx and van der Want 1987). Indeed, potatoes are resistant to CMV; many potato cultivars and wild potato species remain apparently non-infected following inoculation or develop detectable amounts of CMV only in inoculated leaves (Valkonen et al. 1995; Valkonen 1997; Celebi et al. 1998). Furthermore, in the systemically infected potato plants, transmission of CMV to the next crop via tubers occurs at a low frequency (Anon 1957; Celebi et al. 1998).

Lycopersicon spp. and *Capsicum* spp. have little resistance to CMV. Only partial resistance and tolerance are known in some accessions and cultivars, which may show complicated inheritance and be difficult to transfer by crosses (Watterson 1993; Caranta et al. 1997). Therefore, potatoes might be an alternative source of resistance genes to be used in the related solanaceous crop species. However, before this may be proposed, a better understanding of the mechanisms and genetic control of resistance to CMV in potatoes under various environmental and physiological conditions is required.

To date nothing has been reported on the genetic basis of resistance to CMV in potatoes. The long-distance

transport of CMV is blocked in many resistant potato genotypes (Celebi et al. 1998), and environmental conditions might affect the expression of resistance (Valkonen et al. 1995). The study presented here was carried out to examine the mechanisms and genetic basis of resistance to CMV in potato. Diploid potato genotypes and crosses were used to enable the formulation of conclusive genetic models based on F_1 populations (Boniarbale et al. 1988; Gebhardt et al. 1989; Watanabe 1994). Selection of the parental clones was based on independent genetic backgrounds in order to include several potentially different resistance genes and mechanisms. The same potato lines and crosses have been extensively examined for resistance to several common potato viruses, such as potato Y potyvirus (PVY), potato A potyvirus (PVA) and potato X potexvirus (PVX) (Valkonen et al. 1994a; Hämäläinen et al. 1998; Tommiska et al. 1998). Thus, direct comparison between resistance to CMV and the well-described models of single gene-mediated resistance to PVY, PVA and PVX (Cockerham 1970; Ross 1986) were possible. This study also reports that induced resistance to CMV can be associated with autonomous cell death (i.e. development of 'disease mimic lesions') previously not described in potato.

Materials and methods

Plant material

The diploid (2n=2x=24) potato genotypes selected for this study were *S. phureja* line IvP35 (Hermsen and Verdenius 1973), line 2x(v–2)7 derived from tetraploid (2n=4x=48) *S. tuberosum* through haploid induction (Watanabe et al. 1994) and interspecific hybrid lines 87HW13.7 (Watanabe et al. 1995) and 84.194.30 (Watanabe et al. 1994) produced by crossing diploid lines derived from *S. tuberosum* with diploid interspecific hybrid lines that have several wild and cultivated potato species in their pedigree. The cross 87HW13.7×IvP35, including 126 progeny, segregates for the dominant gene Nx_{phu} that controls resistance to PVX derived from line IvP35. Nx_{phu} has been mapped to chromosome IX (Tommiska et al. 1998). The cross 84.194.30×2x(v–2)7, including 76 progeny, segregates for resistance to PVA, PVY and potato V potyvirus (PVV), each resistance being controlled by a different dominant gene derived from line 2x(v–2)7. Ra_{adg} and Ry_{adg}, which control resistance to PVA and PVY, respectively, have been mapped to the same region of chromosome XI (Hämäläinen et al. 1998).

All parental lines and progeny lines were maintained as in vitro shoot cultures. For virus tests, in vitro plantlets were transferred to and grown in soil. Experiments in the greenhouse were carried out at the University of Helsinki, Viikki (latitude 60°N), Finland, under natural daylight supplemented with illumination from sodium halide lamps (photoperiod 18 h; 150 µmol s^{-1} m^{-2}, measured at crop height) from 1995 to 1997. Minimum and maximum temperatures were recorded daily. In November–February, the temperature and light intensity in the greenhouse could be reliably adjusted using heating and artificial illumination, respectively, because the outdoor temperatures (<0°C) and daylight intensity were low. During September–October and March–April, light intensity and temperature were temporarily higher. During the summer (May–September), the greenhouse was shaded with curtains and no tests for resistance to CMV were carried out; only infected and healthy plants of line 87HW13.7 were kept growing by cutting the shoots periodically. Experiments in growth chambers (Fi-totron 600H, FISONS Environmental Equipment, Loughborough, UK) were carried out at the Department of Plant Biology, SLU, Uppsala, Sweden. In the growth chambers, temperature was constant (18°C or 28°C) and the photoperiod 18 h (250 µmol s^{-1} m^{-2}). Plants were watered daily and fertilized weekly with 0.5% N:P:K=5:7:6 ferilizer (Kukkien Y-lannos, Kemira OY, Vaasa, Finland).

Inoculation and detection of CMV

The isolate Fny-CMV was obtained from Prof. Peter Palukaitis, SCRI, Scotland. It was selected for study because it has been previously used in all studies carried out on potatoes (Valkonen et al. 1995; Valkonen 1997; Celebri et al. 1998; Valkonen and Rokka 1998) and because it is well-characterized (Palukaitis et al. 1992; Kaplan et al. 1997; Blackman et al. 1998).

Two plants of each progeny and 3–5 plants of the parental genotypes were inoculated in each experiment. For mechanical inoculation, inoculum was prepared by grinding CMV-infected leaves of tobacco (*N. tabacum* 'Samsum') in sterile distilled water at 1 g/5 ml). The sap was rubbed on two Carborundum-duster lower leaves of each plant. Inoculated leaves were marked by a puncture through the leaf tip. Subsequent to this, three tobacco plants were inoculated with the same inoculum to verify that the inoculum was still highly infectious. CMV-infected plants of line 87HW13.7 were used as sources of infected shoots for graft-inoculation. Plants were cut down, and the new axillary shoots with mosaic symptoms were used as scions for grafting. Graft-inoculation was carried out as previously described (Valkonen et al. 1994b) by side-grafting one apical shoot of CMV-infected potato onto each test plant.

Polyclonal antibodies and alkaline phosphatase-conjugated antibodies to CMV were obtained from Dr. Frank Rabenstein, Federal Centre for Breeding Research on Cultivated Plants, Aschersleben, Germany, and used for detection of CMV by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) as previously described (Valkonen et al. 1995). The uppermost fully-expanded leaves, or the mechanically inoculated leaves, were sampled, weighed and ground in ELISA sample buffer at 1 g/3 ml. Two aliquots (100 µl) were transferred from each sample to two wells of a microtitre plate (Greiner Labortechnik, Frickenhausen, Germany).

Results

Autonomous necrotic lesions (Anl) in line 87HW13.7

In the experiments to test the progeny of the cross 87HW13.7×IvP35 for resistance to CMV in the greenhouse during September–October, both the inoculated and non-inoculated plants of line 87HW13.7 and 23 progeny developed chlorotic spots, vein chlorosis and brown vein necrosis symptoms in the upper leaves. Necrotic lesions also developed on stems and petioles. These symptoms were designated as '*a*utonomous *n*ecrotic *l*esions' (Anl). Autonomously developing cell death lesions have been previously detected in other plants species and named 'disease lesion mimics' (les or Les) in maize (Johal et al. 1995; Hu et al. 1998) and 'lesions simulating disease' (lsd) or 'accelerated cell death' (acd) lesions in *Arabidopsis* (Dietrich et al. 1994). They were considered further in this study because it was assumed that they could be associated with induced pathogen resistance, as previously shown in other plant species (Dangl et al. 1996).

Independence of the Anl development from CMV infection was verified, and its possible temperature-depen-

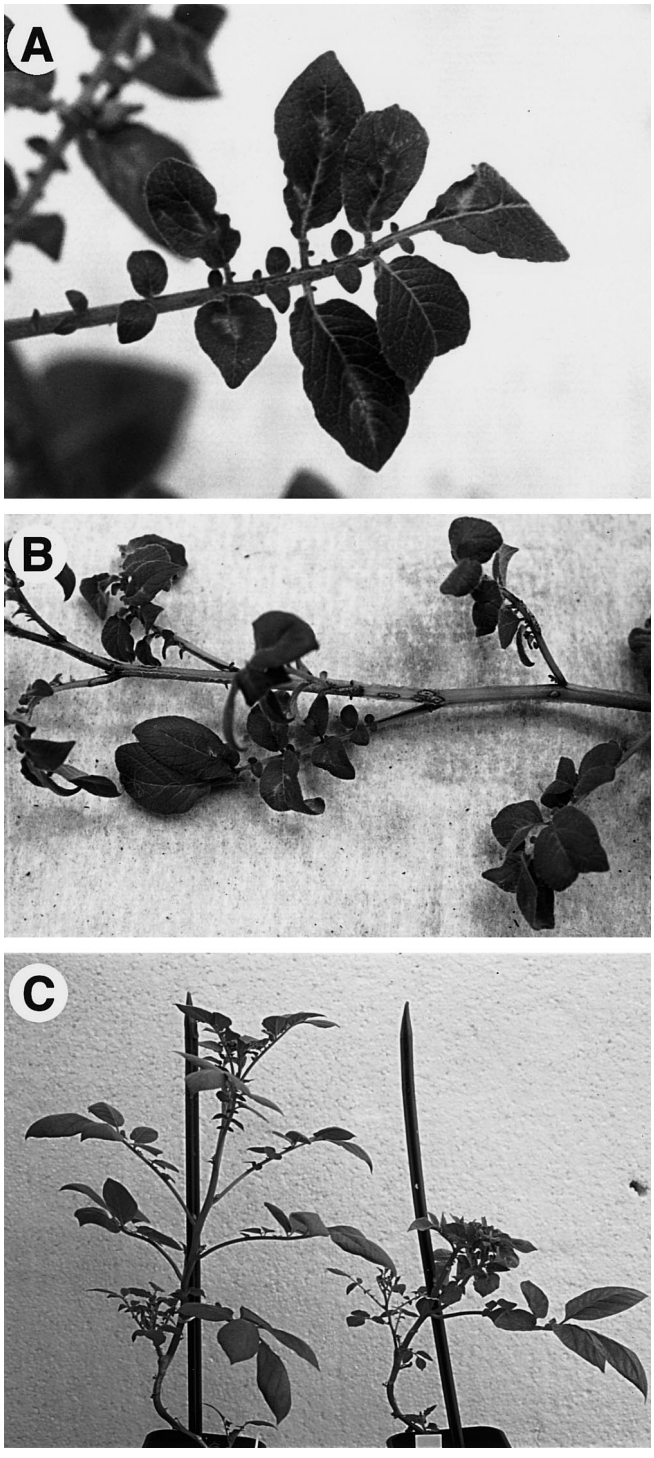

Fig. 1A, B Autonomously developing cell death lesions in line 87HW13.7: **A** vein chlorosis and necrosis, **B** necrotic lesions on stem and petioles. **C** *Right* stunting and yellowing symptoms at the plant top following systemic infection with Fny-CMV in a progeny derived from the cross 84.194.30×2x(v–2)7, *left* a resistant progeny not systemically infected and showing no symptoms following graft inoculation

dence tested in separate experiments. Healthy plants of line 87HW13.7, line IvP35, and 18 progeny (2–3 plants of each) were transferred from tissue culture (where Anl did not develop) to soil and grown at 18°C and 28°C in two growth chambers under the same light quality and intensity (250 µmol s^{-1} m^{-2}). Anl appeared in 2 progeny in 10 days, and in a total of 6 progeny and line 87HW13.7 (Fig. 1) during the 45 days of observation at both temperatures. These data indicated that the development of Anl was autonomously induced, thus not requiring infection with CMV, and similar at the two temperatures. Of the 6 progeny that developed Anl lesions in the growth chambers 5 also developed similar symptoms in the greenhouse in September–October. Thus, the conditions in the growth chambers were more conductive for Anl development than the conditions in the greenhouse and, consequently, the experiments in the greenhouse had, on average, revealed 5 out of 6 (83%) progeny lines with the ability to develop Anl.

Expression of many of the previously described autonomous cell death mutants is dependent on the light conditions (Dangl et al. 1996). In this study, a higher number of progeny lines developed Anl in the greenhouse in the early autumn (light intensity 250 µmolm^{-2} s^{-1}) than during the winter time (150 µmol m^{-2} s^{-1}) (Table 1). Line 87HW13.7 was severely affected by Anl development during the summer, while no Anl develeoped in new shoots in the middle of the winter. These data supported by the data from growth chamber experiments (see above) suggested that Anl development was positively correlated with increased light intensity. The factors involved in the induction of Anl were not investigated further in this study.

Association of Anl development with resistance to CMV

Twelve progeny of the cross 87HW13.7×IvP35 and line 87HW13.7 were systemically infected and subsequently developed yellow mosaic symptoms in the upper non-inoculated leaves 20–25 days after mechanical inoculation in experiments carried out from September to November (temperature 16°/19°C). Movement of CMV from the inoculated leaves to the upper leaves took 20–25 days, which was much longer than the time needed (12–15 days) for the systemic infection with PVY, PVX and PVV in these same progeny lines (Tommiska et al. 1998; unpublished data). Some plants of line 87HW13.7 were infected with CMV and had yellow mosaic symptoms, while others developed Anl and were not infected (Table 1). Line IvP35 and 114 progeny contained no detectable amounts of CMV in the upper non-inoculated leaves 28 days after mechanical inoculation. Of these progeny, 23 lines developed Anl.

Results from graft-inoculation experiments carried out in December–January were similar but not identical to those obtained following mechanical inoculation. A total of 22 progeny and line 87HW13.7 were systemically infected with CMV following graft-inoculation,

Table 1 Phenotypic responses and CMV titres in plants of the cross 87HW13.7×IvP35 following inoculation with Fny-CMV in the greenhouse

Phenotype		Mechanical inoculation				Graft-inoculation	
		Exp. 1		Exp. 2		Exp. 3	
		n	Absorbance	*n*	Absorbance	*n*	Absorbance
Progeny:	ndi	91	0.07±0.02[b]	90	0.06±0.02	93	0.07±0.02
	YMo	12	1.51±0.34	12	1.78±0.23	22	1.23±0.44
	ndi+ANL	23	0.07±0.02	22	0.07±0.02	11	0.07±0.03
	YMo+ANL	0		0		0	
	Total:	126		124		126	
Parental:							
87HW13.7 ♀	ndi	0		0		0	
	YMo	3	1.85±0.08	2	2.02±0.10	2	1.01±0.17
	ndi+ANL	2	0.05±0.02	1	0.06±0.01	2	
	Total:	5		3		2	
IvP35 ♂	ndi	5	0.06±0.01	3	0.05±0.02	2	0.06±0.02
	YMo	0		0		0	
	ndi+ANL	0		0		0	
	Total:	5		3		2	
Controls:							
N. tabacum Samsun		5	3.44±0.12	4	3.51±0.09	4	3.39±0.10
Non-inoculated		4	0.07±0.02	4	0.06±0.02	4	0.06±0.01

[a] ndi, No symptoms and no CMV detected by DAS-ELISA; YMo, yellow mosaic symptoms; ANL, autonomous necrotic lesions, i.e. chlorotic spots, chlorotic and necrotic streaks in veins, and/or lesions on stems and petioles. ndi+ANL, and YMo+ANL: plants that show two phenotypic traits. *N. tabacum* plants had severe mosaic symptoms

[b] Mean ELISA absorbance values of two plants (two readings each)±SD in the uppermost fully-expanded leaves 28 days after inoculation

whereas other progeny lines and line IvP35 were not infected (Table 1). These data showed that in 10 of the progeny not infected following mechanical inoculation the resistance was overcome by graft-inoculation. These 10 progeny lines did not develop Anl under any conditions.

Only 11 progeny developed Anl during the graft-inoculation experiments in the middle of the winter (Table 1), whereas 23 progeny developed Anl in the autumn, as mentioned above. Of those 12 progeny that developed Anl only in the autumn and not during the graft-inoculation experiments, 11 were resistant to CMV following graft-inoculation. Line 87HW13.7 did not develop Anl and was infected with CMV following graft-inoculation.

Infected plants of line 87HW13.7 that were free of Anl symptoms in the winter were maintained in the greenhouse by cutting the shoots periodically. In May, Anl lesions reappeared in the new shoots, and at the same time the CMV titers were drastically reduced and were no longer detectable by ELISA (data not shown). Plants did not completely recover from CMV infection because later in the autumn the new shoots of these same plants developed yellow mosaic symptoms and contained high CMV titers. These data indicated that development of Anl coincided with induction of resistance to CMV in line 87HW13.7.

Genetic models for resistance to CMV and Anl development in cross 87HW13.7×IvP35

We refer below to the genetic models that were tested but explain in detail only those in which the hypothetical and observed segregation showed a high goodness of fit.

Data indicated that the parental line IvP35 was resistant to CMV under all the test conditions of this study. Thus, resistance in both the progeny class ‘ndi’ and ‘ndi+ANL’ could be due to resistance genes derived from line IvP35 or, alternatively, the 11 progeny of class ‘ndi+ANL’ could be resistant due to an induced mechanism associated with Anl development. The data from the graft-inoculation test were used for genetic modeling because this test was considered more stringent for the detection of susceptible genotypes.

The observed proportions of resistant (R) and susceptible (S) phenotypes (104:22 or 93:33, respectively) were clearly not consistent with any model assuming a single dominant or recessive gene controlling resistance to CMV at a heterozygous or homozygous locus. However, the model assuming two dominant genes (designated as *C1* and *C2*) at two independent heterozygous loci could not be rejected based on the data. According to this model, the assumed genotypes of line IvP35 and line 87HW13.7 would be *C1/c1:C2/c2* and *c1/c1:c2/c2*, respectively, and the expected progeny genotype ratio R:S=3:1 (χ^2=3.82 and 0.074, respectively; $\chi^2_{1,\ 0.05}$=3.84), which is supported by the data. Because

Table 2 Phenotypic responses and CMV titers in plants of the cross 84.194.30×2x(v–2)7 following graft-inoculation with Fny-CMV in the greenhouse (*nt* not tested)

Phenotype[a]		Exp.1		Exp. 2	
		n	Absorbance	*n*	Absorbance
Progeny:	ns	35	0.08±0.03[b]	29	0.06±0.02
	YMo, Stu	31	1.47±0.44	37	0.48±0.13
	Total:	76		76	
Parental:					
84.194.30 ♀	ns	nt		3	0.65±0.20
2x/v–2)7 ♂	ns	nt		3	0.15±0.04
Controls:					
87HW13.7	YMo	3	1.45±0.08	2	2.25±0.19
	ANL	0		1	0.07±0.01
Non-inoculated	ns	4	0.06±0.02	4	0.07±0.02

[a] ns, No symptoms; YMo, yellow mosaic symptoms; Stu, stunting, i.e. growth of the plant top ceased when symptoms appeared; ANL, autonomous necrotic lesions: chlorotic spots, chlorotic and necrotic streaks in veins, and/or lesions on stems and petioles. *N. tabacum* plants had severe mosaic symptoms

[b] Mean ELISA absorbance values of 2 plants (two readings each)±SD in the uppermost fully-expanded leaves

resistance to CMV was overcome by graft-inoculation in 10 progeny that resisted CMV following mechanical inoculation, additional genes may also be involved in resistance expression.

The observed proportions of R and S phenotypes of resistance to CMV were clearly different from the proportions of R and S phenotypes (54:55, respectively) of resistance to PVX controlled by the single dominant gene Nx_{phu} in the same cross (Tommiska et al. 1998). The genetic model of CMV resistance was not further tested on F_2 progeny because the progeny had low fertility (low pollen stainability and abnormal anther morphology in some lines), and all crosses that were attempted were unsuccessful.

Twenty-three progeny developed Anl in the greenhouse (Table 1), but a comparison of Anl development in the greenhouse and growth chambers suggested that, theoretically, a total of 28 progeny had the capacity to develop Anl (see above). Subsequently, the observed and theoretical proportions of progeny developing Anl and those not developing Anl were 23:103 and 28:98, respectively. Consistent with previous studies on autonomously developing cell death lesions in other plants species (Dangl et al. 1996), it was assumed that Anl development in line 87HW13.7 was due to a recessive mutation in a gene designated as *L*. Thus, line 87HW13.7 was assumed to be homozygous at this locus (*l/l*), whereas the corresponding locus in line IvP35 that did not develop Anl was assumed to be heterozygous (*L/l*) or homozygous (*L/L*). Because only the homozygous *l/l* genotypes were assumed to develop Anl, no model based on the assumption of one locus was consistent with the above-mentioned proportions of anl and non-Anl phenotypes. The genetic model described for the les mutant of maize (Hu et al. 1998) induced by the heterozygous condition of the *Les* locus also did not show a good probability of goodness of fit to the Aln data.

The only model which showed a good probability of goodness of fit was the one previously described for the 'autonecrosis lesion mutant' of tomato (Langford 1948; Dixon et al. 1996). It includes a recessive gene *ne* that induces necrosis in the presence of a dominant gene linked to the *Cf-2* resistance gene locus (Dixon et al. 1996). If it is assumed that Anl in line 87HW13.7 is induced by recessive gene *l* in the presence of a second gene, *Co* (a dominant co-factor of Anl development; genotype *llCoco*) and that line IvP35 lacks *Co* (*Llcoco*), the expected genotype ratio would be (*LlCoco*):(*llCoco*):(*llcoco*):(*llcoco*)=1:1:1:1 and the phenotype ratio would be Anl:anl=3:1. This model could not be rejected based on the observed proportion Anl:anl=23:103 (χ^2=3.058; $\chi^2_{1,\ 0,05}$=3.84) and the theoretical proportion Anl:anl=28:98 (χ^2=0.518; $\chi^2_{1,\ 0.05}$=3.84) of phenotypes.

The models presented for CMV resistance and Anl development fit the observed proportions of progeny with the combined traits, i.e. resistance to CMV and no development of Anl (91 progeny), resistance to CMV and development of Anl (23 progeny) and susceptibility to CMV and no development of Anl (12 progeny). The assumed genotypes of line 87HW13.7 (*c1c1c2c2llCoco*) and IvP35 (*C1c1C2c2Llcoco*) result in two and eight gametic genotypes, respectively, and the expected ratio of proportions of the three above-mentioned trait combinations would be 12:3:1 (χ^2=1.403; $\chi^2_{2,\ 0.05}$=5.99). Thus, the concomitant test of the two models does not allow us to reject them.

Inheritance of resistance to CMV in cross 84.194.30×2x(v–2)7

The 76 progeny and parental lines were graft-inoculated in the greenhouse in two experiments from February to

Table 3 Detection of Fny-CMV in mechanically inoculated plants of the cross 84.194.30×2x(v–2)7 grown at a constant temperature of 18°C or 28°C under similar light conditions in a growth chamber (*dpi* days post-inoculation)

Potato line	Phenotype in greenhouse[a]	Tested leaf[b]	Exp. 1[c] (18°C)			Exp. 2[c] (26 dpi)	
			10 dpi	22 dpi	30 dpi	18°	28°C
v2–7	YMo, Stu	IL	0.18	0.15	...	...	...
		SL	nt	0.05	...	...	...
v2–12	YMo, Stu	IL	0.38	nt	...	...	...
		SL	nt	0.06	...	...	...
v2–28	ns	IL	0.21	nt	0.23	...	...
		SL	nt	0.03	0.04	...	...
v2–29	YMo, Stu	IL	0.35	0.18	...	...	...
		SL	nt	0.06	...	...	...
v2–32	YMo, Stu	IL	...	...	...	nt	nt
		SL	...	...	...	0.03	2.05 (Stu)
v2–44	ns	IL	0.31	nt	nt	nt	nt
		SL	nt	0.03	0.05	0.04	2.85
v2–45	ns	IL	0.26	0.32	0.23	...	...
		SL	nt	0.03	0.04	...	...
v2–51	ns	IL	0.15	nt	0.13	...	...
		SL	nt	0.03	0.03	...	...
v2–107	ns	IL	0.13	nt	0.24	...	...
		SL	nt	0.03	0.05	...	...
v2–134	ns	IL	0.25	nt	nt	nt	nt
		SL	nt	0.04	0.06	0.04	1.65
2x(v–2)7	ns	IL	0.26	nt	0.24	nt	nt
		SL	nt	0.04	0.05	0.03	1.25
Controls							
Non-inoculated			0.02	0.02	0.03	0.04	0.03
Inoculated-1 h[d]			0.08	nt	nt	...	...
CMV (+) *N. tabacum*			3.54	1.01	1.70	1.42	
SE			0.04	0.03	0.07	0.02	0.16

[a] ns, No symptoms; YMo, yellow mosaic; Stu, stunting, i.e. growth of the plant top ceased when symptoms appeared
[b] IL, Inoculated leaves; SL, uppermost full-expanded non-inoculated leaves
[c] Mean of ELISA absorbance values of 2 plants (two readings each) measured 2 h after adding the substrate. nt, not tested; ..., not included in these experiments
[d] Four inoculated leaves of 2x(v–2)7 rinsed with tap water and tested by ELISA 1 h after inoculation

May (temperature 16°/19°C). Both parental lines were systemically infected 28 days after inoculation, but they showed no symptoms. The CMV titers in line 2x(v–2)7 were lower than in line 84.194.30 (Table 2), which indicated that line 2x(v–2)7 was moderately resistant to CMV. The phenotypic responses of progeny to CMV were different from both parents. Two distinct phenotypic classes of progeny were recognized: resistant (R), with no detectable systemic infection and symptoms in the upper leaves, and susceptible (S), with yellow mosaic symptoms, reduced growth (stunting) (Fig. 1C) and high CMV titers.

Properties of progeny expressing R and S phenotypes in the two experiments were 35:31 and 29:37, respectively (Table 2). Assuming a single recessive (*c3*) or dominant (*C3*) gene at a heterozygous locus in line 2x(v–2)7 and assuming line 84.194.30 to be homozygous for the allele of opposite dominance at the corresponding locus (*C3/C3* or *c3/c3*, respectively) would result in an expected genotype of *C3/C3:C3/c3:c3/c3*= 1:1:0 and 0:1:1, respectively. The expected phenotype ratio of R and S would be 1:1, irrespective of whether the gene was dominant or recessive. This model showed a good probability of goodness of fit with the observed phenotype proportions (χ^2=0.242 for 35:31; and χ^2=0.990 for 29:37; $\chi^2_{1,\ 0.05}$=3.84). Thus, the model predicting a single locus responsible for resistance could not be rejected. As with the other cross of this study, low fertility of the progeny prevented making further crosses and it could not be resolved whether this single locus-mediated resistance was recessive or dominant.

Resistance to CMV segregated independently (data not shown) from the resistance to PVY and PVA controlled by the genes Ry_{adg} and Ra_{adg}, respectively, on chromosome XI (Valkonen et al. 1994a; Hämäläinen et al. 1998). It also segregated independently from the genes Ny_{adg} and Nv_{adg} for resistance to PVY^0 and PVV, respectively (Valkonen et al. 1994a; unpublished data), which have not yet been localized on any chromosome in this cross.

Effect of temperature on resistance to CMV in line 2x(v–2)7

Six progeny that expressed resistance to CMV in the first graft-inoculation experiment were found to be suscepti-

ble and developed yellow mosaic and stunting symptoms in the second experiment. This could be due to increased greenhouse temperatures during the second experiment. Effect of temperature on resistance expression was tested with line 2x(v–2)7 and 10 randomly chosen progeny which were mechanical inoculated in the grown chambers. The inoculated leaves were infected, but no plant was systemically infected with CMV at 18°C based on ELISA (Table 3). These data indicated that resistance was based on restricted long-distance movement following mechanical inoculation, as previously reported for line 2x(v–2)7 (Celebi et al. 1998). Subsequently, 3 progeny and line 2x(v–2)7 were mechanically inoculated with CMV at 18°C and 28°C in parallel experiments. No plant was systemically infected at 18°C, as previously, whereas all plants were systemically infected at 28°C and contained high CMV titers in the upper non-inoculated leves (Table 3), indicating that resistance was overcome at high temperature.

Discussion

This study has shown that several different mechanisms confer resistance to CMV in potatoes and that resistance may be expressed differently depending on the environmental conditions. The following main types of resistance to CMV were identified: (1) resistance to long-distance transport that was functional at a low temperature (18°C), controlled by a single locus and overcome at a high temperature (28°C) [line 2x(v–2)7]; (2) resistance controlled by duplicate loci and probably expressed as a function of the physiological and developmental stage of the plant (see below) (line IvP35); and (3) induced resistance associated with the induction of autonomous cell death (Anl) (line 87HW13.7).

The genetic basis and mechanisms of resistance to CMV in potatoes appeared to be partially similar to the resistance known to the common potato viruses PVA, PVY, PVX and potato leaf roll luteovirus (PLRV). In one of the two populations of this study, resistance to CMV was attributable to two genetic loci with major effects. There are some examples of resistance to the common potato viruses being controlled by the complementary action of multiple genetic loci in potato (Pehu et al. 1990; Barker et al. 1994; Valkonen et al. 1994b; Vallejo et al. 1995). Additional genes besides those with the major effects also affected expression of resistance to CMV, and novel resistance phenotypes different from the parents were discovered in progency. Expression of the dominant genes for resistance to, for example, PVY, can also be affected by other genes in potato, resulting in a differential expression of resistance depending on the genetic background to which the resistance gene is introduced (Ross 1986). Temperature-sensitive expression of resistance to CMV was found in this study, and resistance to PVY can also be lost at higher temperatures in some potato genotypes expressing the dominant gene *Ny* (Valkonen 1997; Valkonen et al. 1998). It could not be resolved in this study whether the temperature-sensitive resistance to CMV was dominant or recessive, but examples of recessive resistance to the common potato viruses in potato are few (Bagnall and Young 1972). Finally, resistance to CMV in line IvP35 may be physiologically and developmentally regulated and, thus, analogous to the so-called mature plant resistance to viruses in potato (Beemster 1987). This is because in the previous study line IvP35 was systemically infected with CMV following mechanical inoculation (Celebi et al. 1998), whereas in this study it was not systemically infected with the same isolate of CMV. The significant difference between the two studies is that in the previous study the plants of IvP35 were inoculated directly after introduction to the greenhouse from in vitro culture, whereas in this study plants were propagated for experiments by taking cuttings in the greenhouse.

It appears that no complete resistance to CMV infection capable of preventing CMV infection in inoculated leaves as well as inhibiting systemic infection has yet been found in potato. Systemic infection with CMV is blocked in many potato genotypes, but all potato genotypes tested by mechanical inoculation have shown a detectable accumulation of CMV in inoculated leaves (Celebi et al. 1998). This is different from the known extreme resistance to the common potato viruses. For example, the genes *Ry* and *Rx* prevent any detectable accumulation of PVY and PVX, respectively, in inoculated leaves of potato (Barker and Harrison 1984; Adams et al. 1986; Köhm et al. 1993; Gilbert et al. 1998). The development of necrotic symptoms is characteristic of the expression of virus-specific or virus strain-specific, single gene-mediated hypersensitive resistance (HR) controlled by the dominant *N* genes (Cockerham 1970; Ross 1986; Goodman and Novacky 1994; Barker 1997). Genes for HR to potato viruses are common in potato cultivars and wild species. In contrary, only few potato genotypes are known to react with necrotic symptoms to infection with CMV (Valkonen et al. 1995; Valkonen and Rokka 1998), and the genetic basis of these necrotic responses has not been studied. These features of resistance to CMV in potato are similar to the partial resistance to CMV in pepper (Caranta et al. 1997).

The necrotic and chloritic lesions in line 87HW13.7 developed autonomously and independent from CMV infection, in contrast to the conclusions presented in the previous study where these lesions were observed in the greenhouse during a period of sunny and hot weather (Valkonen et al. 1995). The lesions (designated as AnI) and their development were quite similar to the previously described autonomously developing cell death lesions in *Arabidopsis* (Dietrich et al. 1994), maize (Johal et al. 1995) and barley (Büschges et al. 1997), that probably result from loss-of-function mutations in genes that suppress the genetically programmed cell death. Genetic modeling suggested that Anl development in line 87HW13.7 was analogous to the model described for the development of ‘autonecrosis’ in tomato (Langford 1948; Dixon et al. 1996). Data also suggested that the

Anl development coincided with induced resistance to CMV. In other plant species, the autonomous cell death lesions are commonly associated with induced defense responses that normally are triggered by pathogen infection (Dangl et al. 1996). For example, the *lsd1* and *acd2* mutant plants of *Arabidopsis* produce salisylic acid (SA), which is an important component in the signal transduction pathway leading to systemic acquired resistance (SAR) (Ryals et al. 1996). On the other hand, exogenous application of SA can inhibit the long-distance transport of CMV in tobacco plants (*Nicotiana tabacum* L.; Solanaceae) (Naylor et al. 1998). Thus, it will remain as an interesting subject for future studies to examine whether Anl development induces SA production and how the lesion development, resistance induction and the molecular pathways underlying these two responses are linked and regulated (Vidal et al. 1997; Naylor et al. 1998).

The most common type of resistance to CMV in potato seems to be that of restricted viral long-distance transport, expressed as a detectable infection in the inoculated leaves but a lack of systemic infection (this study; Celebi et al. 1998). Previous studies have found 8 of 12 (Anon 1957) and 21 of 25 (Celebi et al. 1998) tetraploid potato cultivars to be resistant to systemic infection with CMV. Also in many susceptible cultivars where CMV is transported systemically, CMV movement is slow compared to that of the common potato viruses (this study; Celebi et al. 1998), and the shoots grown from tubers of these CMV-infected plants are often free of CMV (Anon 1957; Celebi et al. 1998). Thus, the restricted systemic movement of CMV is a common trait, while full susceptibility to CMV is rare in tetraploid potatoes. This study showed that the block of systemic infection can be under simple genetic control. An inherent or developmental lack of compatible host factors required for CMV-host interactions during virus transport, and active host responses that inhibit virus movement but do not cause visible lesions characteristic of HR could explain the observed resistance. In CMV, the 3a protein and coat protein are involved in the long-distance transport of CMV (Kaplan et al. 1997; Blackman et al. 1998; Canto and Palukaitis 1998), but the plant genes and proteins that mediate virus transport are unknown. Because the tetraploid cultivars are usually highly heterozygous (Ross 1986), the lack of systemic movement of CMV is difficult to explain by a total absence of compatible host factors in the many independent, tetraploid cultivars in which systemic infection does not occur. This model would also include that the resistance is recessive (Nicolas et al. 1997; Schaad et al. 1997). Perhaps a gene-dose effect where the virus-compatible host factors are a minority among the putative host factors mediating cellular transport functions could partially explain the data. CMV might also be able to only partially suppress the host gene-silencing mechanism, which has recently been proposed to be the principal challenge to the virus in infecting any plant (Jones et al. 1998). In the heterozygous, tetraploid potato genotypes many homologous but non-identical genes might substitute for each other in mediating gene silencing. Much more severe symptoms than in either parent were expressed in the susceptible portion of the progeny from the cross between a tolerant (line 84.194.30) and moderately resistant [line 2x(v–2)7] potato genotype, which suggests that the locus controlling this resistance may in fact be involving in the host gene-silencing mechanism (Kasschau and Carrington 1998; Brigneti et al. 1998).

The multiple mechanisms controlled by several genes seem to make most potatoes ”poor hosts” to CMV. Potatoes are not severely affected by CMV in the field, although they are presumably frequently challenged by different strains of CMV transmitted to potatoes by aphids from other crops, weeds and wild plants commonly infected with CMV (Palukaitis et al. 1992). The highly heterozygous and tetraploid condition of potato cultivars (Ross 1986) probably facilitates maintenance of a broad variety of genes that can contribute to resistance. However, this may also mean that resistance to CMV can be difficult to transfer from potato to other solanaceous crops where novel sources of efficient resistance to CMV would be of great importance (Watterson 1993; Caranta et al. 1997). Gene transfer between solanaceous species has been achieved by overcoming the interspecific crossing barriers using somatic hybridization (Austin et al. 1985; Jacobsen et al. 1992; De Jong et al. 1993). For example, several somatic hybrids between potato cv ‘Pentland Dell’ (HR-type response to CMV) and a distantly related, non-tuber-bearing wild potato species (susceptible to CMV) express stronger resistance to CMV than either parental genotype (Valkonen and Rokka 1998). Nevertheless, the genes restricting CMV infection in potato could lose their effect in the foreign genetic background where homologous, but not necessarily allelic, genes may carry out the functions necessary for the virus. This may be why resistance to virus movement derived from the wild potato species *Solanum brevidens* Phil. has been lost while the gene dose from the cultivated, virus-susceptible potato has been increased in somatic hybrids (Pehu et al. 1990; Valkonen et al. 1994b; Rokka et al. 1995). Use of resistance to CMV from potatoes would seem more promising if complete, dominant, simply inherited resistance not affected by environmental conditions was found.

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