Molecular characterization of *mo1*, a recessive gene associated with *Lettuce mosaic potyvirus* resistance in lettuce

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Abstract: Lettuce mosaic, caused by the potyvirus LMV, is a serious viral disease of lettuce crops world-wide. Beside seed control, one of the leading strategies to control LMV is the use of the recessive resistance genes *mo1*¹ and *mo1*². In an attempt to characterize the functional role of the translation initiation factor eIF4E in lettuce susceptibility to LMV, we cloned *Ls-eIF4E* cDNAs from a collection of lettuce cultivars. Data including sequence co-variation among nineteen lettuce genotypes, genetic co-segregation and functional complementation using two different bio-assays indicated that *Ls-eIF4E* is *mo1*.

Keywords: *Lactuca sativa*, cap-binding protein, eIF4E, recessive resistance, host resistance, virus movement

Introduction

Genetic resistance is a major approach to control plant diseases in the field. Cloning plant genes associated with resistance to viruses or other pathogens gives insight into the mechanisms underlying the resistance phenotype, and will also inform the design of strategies to provide more durable resistance strategies. Several genes for Leucine-rich repeat (LRR) proteins belonging to one of several classes and controlling hypersensitivity-based resistance to various types of plant pathogens in a dominant manner, have been cloned in recent years (Pontier et al., 1998).

In the case of viruses and especially potyviruses, a number of resistance phenotypes used by breeders have been associated with recessive alleles of single genes (Provvidenti and Hampton, 1992). Resistance is therefore probably associated with other mechanism(s) than hypersensitivity. A working hypothesis is that recessive resistance genes encode host factors recruited by the virus, and that their debilitation by mutation or inactivation reduces the virus ability to complete its cycle. In several plant-potyvirus models, sequence variations in the viral protein called VPg have been associated with the success of certain virus isolates to infect and produce symptoms in plants normally protected by a recessive gene (Keller et al., 1998, Nicolas et al., 1997, Schaad et al., 1997). VP has been shown to interact *in vitro* with isoforms of the cap-binding eukaryotic translation initiation factor eIF4E (Léonard et al., 2000, Schaad et al., 2000, Wittmann et al., 1997).

We have investigated the role of eIF4E in the compatibility between a potyvirus, *Lettuce mosaic virus* (LMV) and its host plant, lettuce (*Lactuca sativa*), in relation with the presence of the apparently allelic recessive genes *mo1*¹ and *mo1*² (Dinant and Lot, 1992, Ryder, 1970).

Material and methods

Plant material and viral constructs

The LMV susceptible lettuce genotypes Fiona, Girelle, Jessy, Mariska, Salinas, Vanguard, Trocadéro and 87-20M, the *mo1*¹ genotypes Alizé, Classic, Floribibb, Malika, Mantilia, Oriana and Presidio and the *mo1*² genotypes Autumn Gold, Desert Storm, Vanguard 75 and Salinas 88 (Candresse et al., 2002) were used. F1 hybrids between Mantilia on the one hand and Mariska or Girelle on the other hand were produced and self-pollinated to obtain F2 progenies. Plants were routinely grown under greenhouse conditions.

LMV-0-4E^{o} and LMV-0-4E^{1} were constructed to express the Ls-eIF4E^{o} or Ls-eIF4E^{1} cDNAs as *in vivo* processed translational fusions with the polyprotein of LMV-0, a common isolate of LMV. Symptoms were recorded 10 to 15 days after inoculation. Detection of the viral progeny was made by RT-PCR or DAS-ELISA. DAS-ELISA was performed after ten-times dilution of the plant extracts, so that the relationship between OD_{405} and antigen concentration was linear in our concentration range.

RT-PCR amplification, cloning and sequencing of cDNAs

Total cDNA was synthesized from total lettuce RNA using AMV Reverse Transcriptase, and PCR-amplified using *Taq* DNA Polymerase and synthetic oligonucleotide primers. The cDNA 5' and 3' ends were amplified using commercial RACE PCR kits. All amplified cDNAs were cloned in pGEM®-T Easy and sequenced using automated DNA sequencing.

Sequence analysis

The eIF4E and eIF(iso)4E sequences from a variety of plant and animal species were retrieved from GenBank. Multiple sequence alignments were generated using ClustalW (Thompson et al., 1994).

The 3D structures of cap-bound human and murine eIF4E were retrieved from PDB. Comparative protein modeling was elaborated online using Swiss-Model and Swiss-PdbViewer (Guex and Peitsch, 1997) and 3D-Jigsaw (Bates et al., 2001). The fit between 3D structure models was evaluated in Swiss-PdbViewer by calculating the root mean square (RMS) deviation (Chothia and Lesk, 1986) after iterative fitting.

Results and discussion

Cloning and sequence analysis of the lettuce eIF4E and eIF(iso)4E cDNAs

The central region of the *eIF4E* cDNA from the susceptible lettuce genotype Salinas was PCR-amplified using degenerate oligonucleotides derived from seven plant *eIF4E* sequences, including three from *A. thaliana*. The 169-bp sequence obtained was used to design new oligonucleotides for 3'RACE and 5'RACE amplification of the cDNA ends. The terminal sequences determined from the corresponding cDNAs served to design a pair of oligonucleotides allowing PCR-amplification of the nearly full-length *eIF4E* cDNA, including the entire coding region. The assembled full-length sequence encoded a 26 kD protein. The identity between the predicted amino-acid sequence and eleven eIF4E sequences from plants, vertebrates and insects ranged between 40 and 70% (data not shown). Similarly, a nearly full-length Salinas *eIF(iso)4E* cDNA encoded a 22 kD protein homologous to other plant *eIF(iso)4E*.

Correlation between mutations in the eIF4E cDNA and the presence of mo1¹ or mo1²

The coding region of the *eIF4E* cDNA from seven additional lettuce genotypes (Floribibb, Mantilia, Malika, Salinas 88, Vanguard, Vanguard 75 and 87-20M) was PCR-amplified,

cloned and sequenced as described above. On the basis of the presence of few variations, these sequences could be classified into three types, Ls-eIF4E^{o}, Ls-eIF4E^{1} and Ls-eIF4E^{2} (Table 1). There was a strict correlation between *Ls-eIF4E*^{1} and the presence of *mo1*^{1}, and between *Ls-eIF4E*^{2} and the presence of *mo1*^{2}, while the susceptible genotypes all had *Ls-eIF4E*^{o}. This correlation was maintained even in the two independent pairs of genotypes nearly isogenic for *mo1*^{2}, Salinas / Salinas 88 and Vanguard / Vanguard 75. It was also confirmed and extended when the central domain of the *Ls-eIF4E* cDNA from eleven additional genotypes was sequenced: Fiona, Girelle, Jessy and Mariska (*Ls-eIF4E*^{o}, susceptible), Alizé, Classic, Oriana, and Presidio (*Ls-eIF4E*^{1}, *mo1*^{1}), and Autumn Gold and Desert Storm (*Ls-eIF4E*^{2}, *mo1*^{2}) (not shown).

A three dimensional (3D) model of the Ls-eIF4E protein was predicted based on the known 3D structures of human and murine cap-bound eIF4E molecules (Marcotrigiano et al., 1999, Tomoo et al., 2002). The amino-acids that differ between the thre e Ls-eIF4E types were predicted to be at or near the surface of the protein, in two different loops near the cap-recognition pocket (not shown).

In contrast, no sequence difference was detected between the *eIF(iso)4E* sequences from Salinas and Vanguard and from their related *mo1*^{2} genotypes Salinas 88 and Vanguard 75. An identical sequence was also found in Mantilia (*mo1*^{1}). This suggests that eIF(iso)4E sequence variations are not directly linked with the *mo1*-related phenotypes.

Table 1. Three types of eIF4E sequences in lettuce. The nucleotide sequence differences found in seven genotypes compared to Salinas are shown, with their corresponding amino-acid differences. Position 730 is in the 3' non-coding region and therefore translation does not apply (n.a.).

		Nucleotide (Amino-Acid) positions									
		228 (70)		299 (93)		344-349 (108-110)		576 (186)		730 (n.a.)	
		nt	aa	nt	aa	nt	aa	nt	aa	nt	aa
eIF4E^{o}	Salinas Vanguard 87-20M	G	Ala	C	Phe	AGG- -AGC	QGA	G	Ala	C T	n.a.
eIF4E^{1}	Floribibb Malika Mantilia			T	Phe	(del)	His	T	Ser		
eIF4E^{2}	Salinas 88 Vanguard 75	C	Pro							T	n.a.

Genetic co-segregation between mo1^{1} and Ls-eIF4E^{1}

The 6-nucleotide deletion found at positions 344-349 in *Ls-eIF4E*^{1} is associated with the presence of a *Pag*I restriction site, a variation that could easily be scored after PCR amplification of the central region of *Ls-eIF4E* and *Pag*I digestion. The segregations of this CAPS marker and of LMV resistance were analysed in parallel in two [susceptible x *mo1*^{1}] F2 progenies, [Mariska x Mantilia] and [Girelle x Mantilia]. Three distinct CAPS pattern were observed (Figure 1). All resistant plants were homozygous for the presence of *Pag*I. The susceptible plants were either homozygous for the absence of *Pag*I for about one third of them, or showed a partial digestion, and were thus identified as heterozygous for *Pag*I for the remaining two-thirds. Thus, a complete map co-segregation was observed between *mo1*^{1}-associated LMV resistance and the CAPS marker characteristic of *Ls-eIF4E*^{1}.

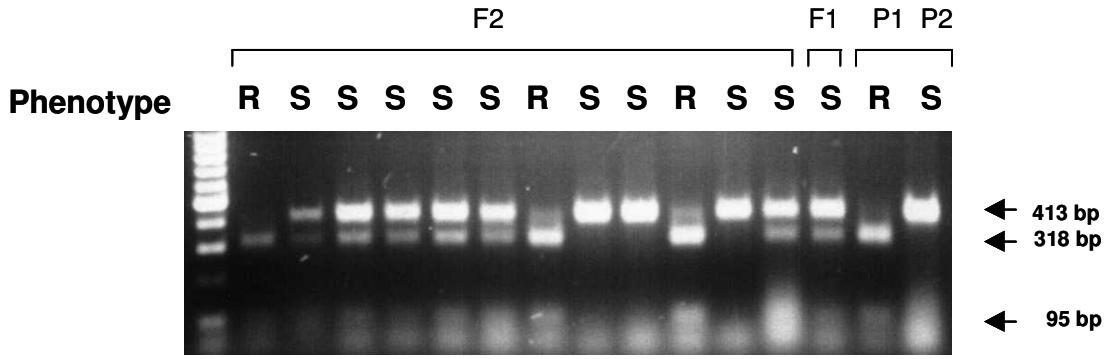

Figure 1. Co-segregation of *mo1*¹ and *Ls-eIF4E*¹. RT-PCR products from *eIF4E* from Mantilia (P1), Mariska (P2), their F1 progeny and a set of F2 progenies were generated and digested with *Pag*I. The original 448-bp product is digested into a 413-bp product due to the presence of a conserved *Pag*I site in all genotypes, used as an internal control. The 413-bp product was further processed in *Ls-eIF4E*¹.

Ls-eIF4E°, but not Ls-eIF4E¹, restores the infectivity of a recombinant LMV in* mo1 *plants. LMV-0-4E° and LMV-0-4E¹ were inoculated to Trocadéro, Salinas and Vanguard (susceptible), Floribibb, Malika and Mantilia (*mo1*¹), and Salinas 88 and Vanguard 75 (*mo1*²). As expected, LMV-0 caused symptoms only in the susceptible varieties (not shown). This was also the case of LMV-0-4E¹. However, typical LMV symptoms appeared in all plants inoculated with LMV-0-4E°.

No accumulation of non-recombinant LMV-0 was detected in the *mo1*¹ plants tested by ELISA, and accumulation was strongly reduced in *mo1*² plants compared to susceptible plants (Table 2). The same situation was observed for LMV-0-4E¹. On the other hand, LMV-0-4E° accumulated to similar levels in all three categories of genotypes.

Therefore, ectopic Ls-eIF4E expression can not only complement LMV accumulation in a normally resistant *mo1*¹ context, but also LMV symptom expression in a normally tolerant *mo1*² context, suggesting that resistance and tolerance, in this case, are functionnally related.

Agro-infiltration of Ls-eIF4E° in the upper, non-inoculated, leaves of Salinas 88 (*mo1*²) previously inoculated in their lower leaves with LMV-0-GFP restored the systemic accumulation of the virus in the infiltrated area (not shown). This was also observed for Ls-eIF4E¹ or Ls-eIF4E², but to a lesser extent (not shown), suggesting that quantitative expression could at least partly restore a qualitative defect of eIF4E.

Table 2. Virus accumulation was determined by ELISA 3 weeks after inoculation with LMV-0 containing the Ls-eIF4E° or Ls-eIF4E¹ coding sequence in two independent experiments. For each construct, the values were normalized to those obtained in the susceptible cultivar Salinas. LMV-0 and LMV-0-4E¹ were consistently not detectable in ELISA in Mantilia.

	Experiment 1		Experiment 2	
	LMV-0	LMV-0-4E°	LMV-0-4E°	LMV-0-4E¹
Mantilia (*mo1*¹)	n.a.	97.1 ± 11.5	111.7 ± 20.5	n.a.
Salinas 88 (*mo1*²)	8.7 ± 8.1	71.7 ± 26.0	105.7 ± 42.4	5.9 ± 11.0

Conclusions

Taken together, our results show not only a sequence co-variation and genetic co-segregation between *mo1* alleles and *eIF4E* in lettuce, but also functional complementation for LMV accumulation and symptom expression by ectopic expression. This strongly suggests that *Ls-eIF4E* is *mo1*. This is reminiscent of results recently obtained in pepper showing that the recessive PVY resistance gene *pvr2* is also *eIF4E* (Ruffel et al., in press), and that *eIF(iso)4E* disruption in *Arabidopsis thaliana* confers resistance to a variety of potyviruses including LMV (Duprat et al., in press, Lellis et al., 2002). This makes *eIF4E* the first natural recessive plant virus resistance gene cloned, and suggests that the large proportion of recessivity in resistance genes against potyviruses in crop plants (Provvidenti and Hampton, 1992) might reflect a conserved host-virus interaction mechanism still to be understood.

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