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Molecular mapping of the viral determinants of systemic wilting induced by a *Lettuce mosaic virus* (LMV) isolate in some lettuce cultivars

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Abstract

The isolate AF199 of *Lettuce mosaic virus* (LMV, genus *Potyvirus*) causes local lesions followed by systemic wilting and plant death in the lettuce cultivars Ithaca and Vanguard 75. Analysis of the phenotype of virus chimeras revealed that a region within the P1 protein coding region (nucleotides 112–386 in the viral genome) and/or another one within the CI protein coding region (nucleotides 5496–5855) are sufficient together to cause the lethal wilting in Ithaca, but not in Vanguard 75. This indicates that the determinants of this particular symptom are different in these two lettuce cultivars. The wilting phenotype was not directly correlated with differences in the deduced amino acid sequence of these two regions. Furthermore, transient expression of the LMV-AF199 proteins, separately or in combination, did not induce local necrosis or any other visible reaction in the plants. Together, these results suggest that the systemic wilting reaction might be due to RNA rather than protein sequences.

Keywords: *Lactuca sativa*; Agroinfiltration; Infectious cDNA; Necrosis; *Potyvirus*

Lettuce mosaic virus (LMV, genus *Potyvirus*) is potentially the most destructive virus of lettuce (*Lactuca sativa)* field crops. Transmitted by seeds and aphids, LMV is distributed worldwide, probably due to the exchange of contaminated seed lots (Dinant and Lot, 1992; Le Gall, 2003). Virus particles are long, flexuous rods of approximately 750 nm × 13 nm. The LMV genome is composed of one molecule of a single-stranded positive-sense RNA 10,080 nucleotides long (Revers et al., 1997b) with a 5′-bound VPg and a 3′ poly-A, and translated into a polyprotein of 3255 amino acids. Three LMV resistance genes have been described in lettuce: two alleles (*mo1*^{1} and *mo1*^{2}) of the recessive gene *mo1*, which encodes the cap-binding protein eIF4E (Nicaise et al., 2003), and the dominant gene *Mo2* (Bos et al., 1994; Pink et al., 1992a, 1992b). Two main sub-groups of seed-borne LMV isolates are the major agents of field epidemics, LMV-Common and LMV-Most (Krause-Sakate et al., 2002). Unlike LMV-Common, LMV-Most is not controlled by *mo1*^{1} and *mo1*^{2}.

As with most viral diseases, the severity of symptoms induced by LMV isolates varies considerably depending on the host genotype, the stage of infection and the environmental conditions. Typical LMV symptoms on mature butterhead lettuce include dwarfing, defective heading, mottling, leaf distortion, yellowing, necrotic spots and, more rarely, systemic wilting followed by plant death (Dinant and Lot, 1992; Le Gall, 2003). Several examples of necrotic LMV isolates have been described, but necrosis was usually more or less dependent on environmental conditions (Dinant and

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Lot, 1992; Pink et al., 1992a; Zerbini et al., 1995). Lethal isolates of LMV have been described only three times in the literature. LMV-L (Zink et al., 1973) causes severe necrosis in older leaves and death of some lettuce cultivars, including Gallega de Invierno (*mo1*^{1}). LMV-lethal, a Greek isolate (Kyriakopoulou, 1985), induces necrotic local lesions in Cos-type lettuce, which gradually expand to the midribs and to the whole plant, eventually causing wilting and plant death, even when inoculated late in the development. Finally, two isolates from Brazil, LMV-AF198 and LMV-AF199, were lethal in some lettuce cultivars such as Ithaca (*Mo2*) and LMV-AF199 also in Vanguard 75 (*Mo2*, *mo1*^{2}) (Krause-Sakate et al., 1999). In addition, in independent experiments, another LMV isolate, LMV-1 (Dinant and Lot, 1992) caused systemic wilting in the cultivar Sigla (unpublished results). Systemic lethal necrosis or systemic wilting has also been observed for other potyviruses in other hosts (Chu et al., 1997; Collmer et al., 2000; Dogimont et al., 1996; Kyle and Provvidenti, 1993; Risser et al., 1981).

When LMV-AF199 was inoculated onto lettuce cultivars such as Ithaca or Vanguard 75, necrotic local lesions were visible as early as 6–8 days post-inoculation (dpi) (Fig. 1A). The appearance of necrotic local lesions is a rather uncommon outcome of LMV inoculation in *Lactuca sativa*. This was followed (12–16 dpi) by necrosis in the vascular tissues (Fig. 1B) and generalized plant wilting (Fig. 1B and C). The inoculated plants collapsed and death finally occurred approximately 18–22 dpi (Fig. 1D). This course of events was apparently not affected by environmental conditions or by the age of the plants at the date of inoculation (data not shown). LMV-AF199 belongs to the Most type of LMV isolates (Krause-Sakate et al., 2002), but the ability to induce systemic wilting does not appear to be typical of LMV-Most since none of the other LMV-Most isolates tested did so (data not shown). On the other hand, another Brazilian isolate of the LMV-Common type, LMV-AF198, also induced the same reaction in Ithaca (Krause-Sakate et al., 1999). It could not be tested in Vanguard 75 because, like all Common type isolates, LMV-AF198 does not infect *mo1*^{2} cultivars. However, this was also not a general property of Brazilian LMV isolates since other isolates of the same origin did not induce this phenotype (data not shown).

To test whether this phenotype was specific to Ithaca and Vanguard 75, additional lettuce cultivars were inoculated with LMV-AF199. Cultivars Trocadéro, Gorga, Marisa, Salinas (susceptible), Brasil 330, Calona, Floribibb, Malika (*mo1*^{1}) and Salinas 88 (*mo1*^{2}) reacted to LMV-AF199 with mosaic symptoms. Unlike the two cultivars showing the wilting reaction, none of these cultivars carry the *Mo2* gene. These results therefore suggested that the lethal wilting phenotype could be associated with the presence of the *Mo2* gene (Krause-Sakate et al., 1999). However, this hypothesis was disproved by the observation that another *Mo2*-carrying accession, which we named IFR, showed a typical mosaic and failed to wilt upon LMV-AF199 inoculation. Mistakenly named Ithaca earlier (Revers et al., 1997a), IFR is a partially characterized Iceberg-type lettuce which does not have a *mo1* resistance allele, as assessed by its susceptibility to LMV-0, but which does carry *Mo2*, as assessed by its resistance to LMV-Yar and LMV-Gr4. Altogether, the results suggest that the lethal wilting induced by LMV-AF199 in Ithaca and Vanguard 75 is not dependent on the presence of *Mo2* or of any other previously characterised LMV resistance gene from lettuce. Alternatively, the lethal wilting could indeed be dependent on the presence of *Mo2*, but strictly dependent on a second factor, present in Ithaca and Vanguard 75 but absent in IFR. In any case, the genetic basis of this lethal reaction to LMV-AF199 remains to be determined.

Several conserved restriction sites in the cDNAs of LMV-E (Genbank accession no. X97705) and LMV-AF199 (Genbank accession no. AJ278854) allowed us to obtain infectious recombinant cDNAs by exchanging genomic regions between the full-length infectious cDNA clone of LMV-E (Yang et al., 1998) and partial cDNA clones of LMV-AF199 that had been used previously (Krause-Sakate et al., 2002) to determine the sequence of this isolate (Fig. 2). As previously described (Redondo et al., 2001), the recombinant cDNAs were inoculated using a portable biolistics device (Bio-Rad Gene Gun) onto the non-wilting lettuce Trocadéro, and these plants were used as inoculum for further mechanical inoculation. Dot-blot hybridization using an equimolar mixture of in vitro transcripts of LMV-E and LMV-AF199 cDNAs (positions 9035–9871 relative to the full-length genome) confirmed that the recombinant viruses accumulated to levels comparable to those reached by the parental isolates in Trocadéro (Fig. 3). Virus quantification from wilting lettuce cultivars infected with LMV-AF199 or recombinant viruses similarly inducing plant death was not reliable (data not shown).

None of the recombinant viruses were able to induce systemic wilting in Vanguard 75. Therefore, the LMV-AF199 region present in these recombinants (the largest one is in Rec1, from nucleotides 112 to 5855) is not sufficient to induce systemic wilting in Vanguard 75 when inserted in a LMV-E background. Thus, primary viral determinants of this phenotype must be located either in the 5′-NTR or in the 3′-half of the LMV-AF199 genome, and absent from LMV-E. The results obtained do not exclude the possibility that such determinants could be dependent of the region spanning nucleotides 112–5855 to induce wilting in Vanguard 75.

In Ithaca, three recombinants caused systemic wilting followed by plant death similar to the symptoms induced by LMV-AF199 (Figs. 1E and 2), while two other recombinants induced mosaic symptoms similar to those induced by LMV-E (Fig. 2). The identity of the progeny of each construct was verified by restriction analysis and, in the case of Rec5, by direct sequencing of the recombined regions (data not shown). Together with previous results, this clearly demonstrates that the basis for the wilting reaction is different in Ithaca and in Vanguard 75.

Of the three recombinants reproducing the LMV-AF199 lethal symptoms in Ithaca, the one with the smallest LMV-