# Analysis of the serological variability of Lettuce mosaic virus using monoclonal antibodies and surface plasmon resonance technology

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# Short Communication

# Analysis of the serological variability of *Lettuce mosaic virus* using monoclonal antibodies and surface plasmon resonance technology

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A panel of 19 monoclonal antibodies (mAbs) was used to study the immunological variability of *Lettuce mosaic virus* (LMV), a member of the genus *Potyvirus*, and to perform a first epitope characterization of this virus. Based on their specificity of recognition against a panel of 15 LMV isolates, the mAbs could be clustered in seven reactivity groups. Surface plasmon resonance analysis indicated the presence, on the LMV particles, of at least five independent recognition/binding regions, correlating with the seven mAbs reactivity groups. The results demonstrate that LMV shows significant serological variability and shed light on the LMV epitope structure. The various mAbs should prove a new and efficient tool for LMV diagnostic and field epidemiology studies.

*Lettuce mosaic virus* (LMV), a member of the genus *Potyvirus*, is probably the most detrimental virus on lettuce crops worldwide (for reviews on LMV see Dinant & Lot, 1992; Le Gall, 2003). Symptoms are quite variable and depend on the particular isolate–host combination but frequently include dwarfing, failure to form proper heads, leaf distortion, leaf mosaic or mottling, vein clearing and sometimes necrotic reactions. As with other potyviruses, LMV is transmitted efficiently by aphids in a non-persistent manner (Tomlinson, 1970) and rapid epidemics can develop in susceptible cultivars, leading to losses of up to 80–100 % (Dinant & Lot, 1992). In addition, a number of LMV isolates can also be transmitted through infected seeds (Grogan *et al.*, 1952; Tomlinson, 1970).

LMV is currently controlled by a mixture of genetic (deployment of resistance genes) and epidemiological (lettuce seed certification, enforcement of lettuce-free periods) approaches. However, none of these approaches is completely satisfactory, since there are now LMV isolates that are able to overcome the *mo1$^1$* and *mo1$^2$* resistance genes used worldwide by the lettuce breeders (Pink *et al.*, 1992a, b; Dinant & Lot, 1992; Bos *et al.*, 1994) and which

encode alleles of the cap-binding protein, eIF4E (Nicaise *et al.*, 2003), and, since weeds and ornamental plants can sometimes serve as reservoirs of LMV (Costa & Duffus, 1958; McLean & Kinsey, 1963; Zerbini *et al.*, 1995, 1997), such resistance-breaking isolates may be able to survive in the environment even in the absence of lettuce crops. Detection of LMV in infected plants or in seed lots is routinely carried out using immunological techniques such as ELISA (Clark & Adams, 1977; Jafarpour *et al.*, 1979; Falk & Purcifull, 1983) or radioimmunosorbent assay (Ghabrial & Sheperd, 1982). More recently, efforts have been made to develop more sensitive techniques for the detection of LMV based on the PCR (Van der Vlugt *et al.*, 1997; Revers *et al.*, 1999; Peypelut *et al.*, 2004).

Despite a large body of knowledge on the biological (Dinant & Lot, 1992; Bos *et al.*, 1994; Revers *et al.*, 1997a; Krause-Sakate *et al.*, 2002, 2005) and molecular (Zerbini *et al.*, 1995; Revers *et al.*, 1997a, b, 1999; Krause-Sakate *et al.*, 2002) variability of LMV, there is to date no information on the serological variability of this virus or on the correlations between these different levels of variability. There is also very little information on the immunological structure of LMV and, more generally, on that of poty-viruses. However, it has been shown in a few cases such as *Johnsongrass mosaic virus* (Shukla *et al.*, 1988, 1994) or

Details of the mAbs used in this study are available as supplementary material with the online version of this paper

*Plum pox virus* (Candresse *et al.*, 1998) that most of the strain- or virus-specific epitopes are located in the N-terminal hypervariable region of the capsid protein (CP) that is exposed on the surface of the virions (Allison *et al.*, 1985; Shukla *et al.*, 1988), while the group- or genus-specific epitopes are believed to be located in internal, more conserved parts of the CP (Jordan & Hammond, 1991).

In this paper, we have analysed the molecular variability and epitope structure of LMV using panels of 19 mAbs and 15 isolates of LMV. The mAbs used were obtained in three independent fusion experiments, following immunization of mice with purified viral particles corresponding to three different LMV isolates: LMV-A231 (fusion performed in Indooroopilly by J. T.), LMV-0 and LMV-E (fusions performed in Bordeaux by T. D.). LMV-A231 and LMV-0 belong to the LMV-Common group of isolates, while LMV-E belongs to another phylogenetic group (Krause-Sakate *et al.*, 2002). The names of the mAbs, the LMV isolates they were prepared against and, when available, their type are given in Supplementary Table S1 available in JGV Online. All mAbs were used as tissue-culture supernatants. Working dilutions were optimized in ELISAs and are also given in Supplementary Table S1. The 15 LMV isolates used have been described in detail before (Revers *et al.*, 1997a; Krause-Sakate *et al.*, 2002) and were selected as representing the geographical, genetic and biological variability of the virus. Their origins and characteristics are summarized in Table 1.

The reactivity of the 19 mAbs against the panel of LMV isolates was evaluated by triple-antibody-sandwich (TAS) ELISA, with all reagents adjusted to a standard volume of 100 µl. For coating the plates, immunoglobulins from a polyclonal antiserum (El IV, obtained by H. L.) showing broad reactivity against LMV isolates were used at 0.5 µg ml$^{-1}$ (3 h at 37 °C in carbonate buffer). Plant extracts were prepared by grinding infected lettuce leaves (1 : 5, w/v) in PBS-Tween containing 2 % (w/v) polyvinylpyrrolidone and 20 mM diethyldithiocarbamate and were incubated overnight at 4 °C in the coated plates. Following washes, mAbs were applied (2 h, 37 °C), using the dilution of tissue-culture supernatant indicated in Supplementary Table S1. Finally, an alkaline phosphatase-labelled goat anti-mouse conjugate (Sigma) was used at a 1 : 8000 dilution. As a control, a double-antibody-sandwich (DAS) ELISA, using coating immunoglobulins and a polyclonal conjugate derived from the El IV antiserum, was also performed in parallel.

The results are presented in Table 2. All isolates were propagated in susceptible lettuce plants and reacted, although to varying extents, against the broad-reactivity polyclonal reagents derived from the rabbit El IV antiserum. The 19 mAbs could be divided in seven reactivity groups. Although two groups (group I containing LM-3, LM-4, LM-5, MB-1 and MB-2 and group II containing only MB-4) showed very broad reactivity, none of the 19 mAbs reacted against all 15 tested isolates. At the other end of the spectrum, MB-6, the only mAb in this study that was derived from the immunization with LMV-E, showed a very narrow reactivity. Four other reactivity groups could be defined, showing variable degrees of specificity against subsets of the LMV panel. In two groups (I and VI), mAbs derived

**Table 1.** Origins and characteristics of the various LMV isolates used in this study

Hosts refer to the original isolation host. The major LMV phylogenetic group to which each isolate belongs is listed (see Krause-Sakate *et al.*, 2002).

| Isolate | Host | Origin | *mo1¹/mo1²* breaking | Phylogenetic group |
|---|---|---|---|---|
| LMV-0 | Lettuce | France | No | LMV-Common |
| LMV-1 | Lettuce | France | Yes* | LMV-RoW |
| LMV-9 | Lettuce | France | Yes* | LMV-RoW |
| LMV-E | Lettuce | Spain | Yes | LMV-RoW |
| LMV-13 | Lettuce | France | Yes | LMV-Most |
| LMV-Aud | Lettuce | France | Yes | LMV-Most |
| LMV-CSB0 | Lettuce | Chile | Yes | LMV-Most |
| LMV-Dg | Osteospermum | Denmark | No† | LMV-Common |
| LMV-Br6 | Lettuce | Brazil | No | LMV-RoW |
| LMV-Br21 | Lettuce | Brazil | Yes | LMV-Most |
| LMV-Gr4 | *Helminthia* (=*Picris*) *echioides* | Greece | Yes | LMV-Gr |
| LMV-Gr5 | *H. echioides* | Greece | Yes | LMV-Gr |
| LMV-GrB | *H. echioides* | Greece | Yes | LMV-Gr |
| LMV-Yar | Lettuce | Yemen Arab Republic | Yes | LMV-Yar |
| LMV-A435 | Lettuce | Australia | No | LMV-RoW |

*Overcomes *mo1¹* but not *mo1²*.
†Induces extremely mild symptoms only.

**Table 2.** Reactivity of the 19 mAbs tested towards 15 isolates representing the geographical, genetic and biological diversity of LMV

All 15 isolates were obtained from fresh infected lettuce leaves; 'Healthy' refers to healthy lettuce leaves used as a control. ELISA reactivities are expressed as follows: $+++$, $A>1$; $++$, $1>A>0.5$; $+$, $0.5>A>0.1$; $+/-$, $0.1>A>$blank control; $-$, $A<$blank control. pAb refers to polyclonal antibody reagents derived from the El IV antiserum.

| Isolate | Group I | | | | | Group II | Group III | | Group IV | | | | Group V | Group VI | | | | | Group VII | pAb |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LM-3 | LM-4 | LM-5 | MB-1 | MB-2 | MB-4 | LM-2 | LM-9 | LM-7 | LM-11 | LM-12 | LM-16 | LM-14 | LM-8 | LM-10 | LM-13 | LM15 | MB-5 | MB-6 | |
| LMV-0 | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | + | ++ | + | ++ | ++ | ++ | ++ | ++ | + | − | +++ |
| LMV-1 | +++ | ++ | ++ | +++ | +++ | +++ | ++ | +++ | ++ | + | ++ | + | − | − | − | − | − | − | + | + |
| LMV-9 | +++ | + | + | +++ | +++ | +++ | +++ | +++ | − | − | − | − | − | − | − | − | − | +/− | − | + |
| LMV-E | +++ | +++ | ++ | +++ | +++ | +++ | +++ | +++ | ++ | + | ++ | + | − | − | − | − | − | +/− | + | ++ |
| LMV-13 | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | ++ | ++ | ++ | ++ | +++ | ++ | +++ | +++ | ++ | | +++ |
| LMV-Aud | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | ++ | ++ | ++ | ++ | +++ | ++ | +++ | +++ | ++ | − | +++ |
| LMV-CSB0 | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | + | ++ | + | + | ++ | ++ | ++ | ++ | + | − | +++ |
| LMV-Dg | +++ | +++ | ++ | +++ | +++ | +++ | +++ | +++ | ++ | + | + | + | + | ++ | + | ++ | ++ | + | − | +++ |
| LMV-Br6 | − | − | − | − | − | +++ | ++ | +++ | ++ | + | + | + | + | − | − | − | − | − | − | + |
| LMV-Br21 | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | + | ++ | + | ++ | ++ | ++ | ++ | ++ | + | − | +++ |
| LMV-Gr4 | ++ | + | + | ++ | ++ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | + |
| LMV-Gr5 | +++ | ++ | ++ | +++ | +++ | +++ | − | − | − | − | − | − | − | − | − | − | − | − | +/− | + |
| LMV-GrB | +++ | ++ | ++ | +++ | +++ | +++ | − | − | − | − | − | − | − | − | − | − | − | − | +/− | + |
| LMV-Yar | ++ | + | + | ++ | ++ | ++ | + | − | − | − | − | − | − | − | − | − | − | − | − | + |
| LMV-A435 | ++ | − | + | + | + | +++ | +++ | +++ | +++ | + | ++ | + | ++ | − | − | − | − | − | | ++ |
| Healthy | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |

from two immunization procedures using different LMV isolates showed similar reactivity profiles (Table 2). Within group I, some level of variation was observed between the mAbs when their spectra were analysed quantitatively: LM-4 and LM-5 gave weaker signals with LMV-9 and LMV-Gr isolates than the three other group I mAbs. Interestingly, mAbs of reactivity group III appeared to be specific for LMV isolates belonging to the LMV-RoW major phylogenetic group (Krause-Sakate *et al.*, 2002), and failed to react with isolates representative of the two other major phylogenetic groups of LMV isolates, LMV-Gr (isolates Gr4, Gr5 and GrB) and LMV-Yar.

To gain a preliminary understanding of the interactions between the epitopes and binding regions of the various mAbs, binding interference between the various mAbs was evaluated using surface plasmon resonance (Pollard-Knight *et al.*, 1990) implemented on a BIACORE 1000 apparatus (Biacore International AB). Polyclonal anti-LMV immunoglobulins were covalently immobilized on activated dextran-coated sensor chips CM5. Purified LMV-0 virions (30 µl, 50 µg ml$^{-1}$) were then trapped by the immunoglobulins [mean binding 93.6 relative units (RU)] and used as the target for the mAbs in the interference experiments. This isolate was selected as the binding target, as it is recognized by all mAbs with the exception of mAb MB-6 (group VII), which was therefore excluded from the analysis. A first mAb was applied as diluted cell-culture supernatant (30 µl, using the same dilution as for the ELISAs), followed by other mAbs applied in succession under similar conditions. The binding of as many as seven to ten mAbs could be evaluated and measured sequentially by recording modifications of the surface plasmon resonance, expressed in RU. The chip was then regenerated down to the covalently coupled anti-LMV immunoglobulins by an acid wash and the process was repeated to evaluate other binding interferences. The data collected were then processed to define groups of mAbs showing similar binding-interference patterns (i.e. binding interference within the group and absence of significant interference with members of other groups).

Although a few mAbs were not used in the analysis (LM-5, LM-16, LM-8), with the exception of group VII, all binding groups identified by TAS-ELISA (Table 2) were represented. Five binding-interference groups could be defined using the surface plasmon resonance analysis (Fig. 1). Two of these groups completely overlapped the TAS-ELISA reactivity groups II (MB-4) and IV (LM-7, LM-11, LM-12). Reactivity groups V and VI, which differed only in their reactivity towards LMV-Br6 and LMV-A435 (Table 2), showed complete binding interference, with the exception that mAb LM-15 showed binding interference with MB-5 only and not with other members of group VI. Surprisingly, although they belonged to the same reactivity group, no binding interference was observed between mAbs LM-3 and LM-4 on one hand and mAbs MB-1 and MB-2 on the other, indicating that, despite their similar reactivity patterns, these mAbs probably bind to different,
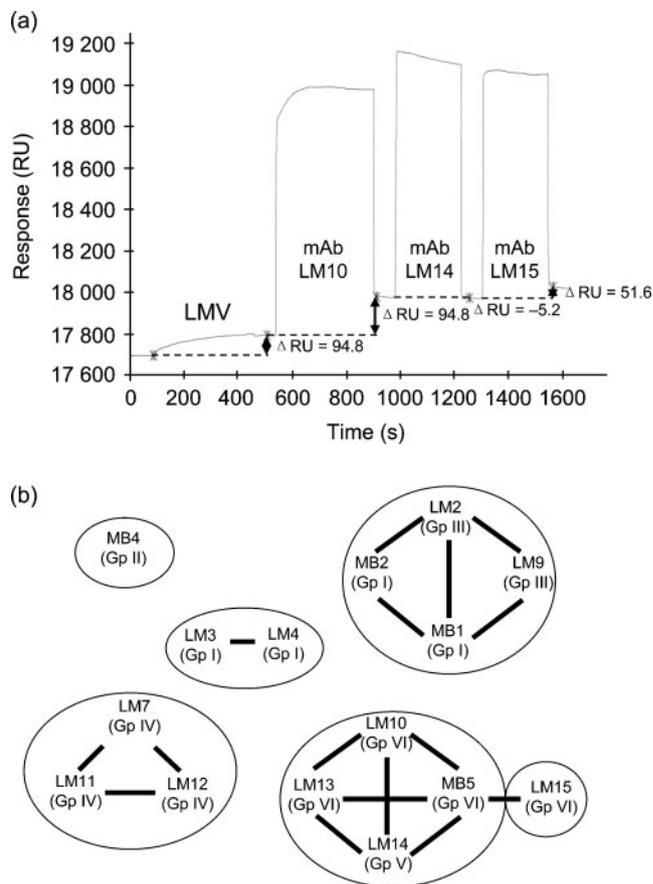


**Fig. 1.** Surface plasmon resonance analysis of binding interference between the various anti-LMV mAbs. (a) Example of a Biacore sensorgram showing the successive binding on the sensor chip of LMV particles (LMV) and of three different mAbs (LM10, LM14, LM15) expressed as RU as a function of time in seconds. The change in RU (ΔRU) observed when the plateau is reached following each binding step is indicated. Previous binding of mAb LM10 inhibits binding of mAb LM14 (ΔRU of −5.2) but not of mAb LM15 (ΔRU of 51.6). (b) Diagrammatic representation of the binding interferences identified. The mAbs are named, together with their reactivity group (Gp), as defined in Table 2. mAbs showing binding interference are linked by thick lines, while groups of mAbs showing mutual interference are drawn within ellipses.

non-overlapping sites on the virus particle. However, interference was observed between mAbs MB-1/MB-2 and the mAbs of reactivity group III (LM-2 and LM-9), showing that the sites recognized by these two pairs of mAbs probably overlap.

The panel of 19 mouse mAbs directed against LMV virions was derived from three independent fusion experiments. Taken together, the TAS-ELISA and binding-interference data show that these 19 mAbs define a minimum of seven distinct reactivity specificities/binding sites defined on the LMV particle. Concerning the groups of mAbs defined by the binding-interference analysis, these groups cannot be regarded as defining epitopes for at least two reasons: (i)

some of the binding-interference groups contain mAbs belonging to different reactivity groups and (ii) binding interference cannot be taken as a proof that interfering mAbs bind to the same region of the CP, an alternative hypothesis being that binding of one mAb may promote conformational changes which in turn may inhibit binding of other mAbs (Saunal & van Regenmortel, 1995). Overall, however, a significant match was observed between the two analyses, indicating that, as might be expected, they provide complementary but somewhat redundant information.

The reactivities observed using a panel of 15 LMV isolates representative of all major phylogenetic lineages of LMV (Krause-Sakate et al., 2002) have some potentially interesting implications in practical terms. A prominent one is that LMV shows considerable serological variability and that, unless significant efforts are invested in the development of broad-reactivity mAbs, the best current chance to develop mAb-based polyvalent assays for the detection of LMV probably lies with the use of mixtures of several mAbs selected from different reactivity groups showing no interference in the surface plasmon resonance analysis reported here. For practical purposes, some of the mAb specificities observed may have applications, such as that of LM-9, which is uniquely able to recognize isolates of the LMV-RoW phylogenetic lineage, which contains the two major types of LMV isolates found in lettuce crops, LMV-Most and LMV-Common (Krause-Sakate et al., 2002). The same applies to the mAbs of reactivity group VI, which appear to be specific to these two types of LMV, which are of major epidemiological importance in lettuce crops worldwide (Krause-Sakate et al., 2002).

An LMV-0 mutant (LMV-0ΔDAG) in which the Asp–Ala–Gly triplet involved in aphid transmission has been deleted from the CP N-terminal region was constructed by fusion PCR from an infectious LMV-0 cDNA clone (Redondo et al., 2001). Due to the cloning procedure used, this mutant also contains an Ala to Thr point mutation of the third amino acid of the CP. The parental and recombinant LMV cDNA clones were inoculated by biolistics to susceptible lettuce (cultivar 'Trocadéro') as described previously (German-Retana et al., 2000). Progeny analysis was performed by direct sequencing after RT-PCR amplification of the cognate genomic region. The mutations introduced in the LMV-0ΔDAG recombinant isolate abolished reactivity towards mAb MB-5, which belongs to reactivity group VI (result not shown). Although long-range conformational effects of these mutations cannot be ruled out, the simplest explanation is that the site recognized by this mAb (and by the other mAbs belonging to reactivity group VI) lies in the CP N-terminal region and, more specifically, in close proximity to amino acids 3 and/or 6–8, which are the only mutations introduced in LMV-0ΔDAG. This hypothesis is in keeping with the complete sequence conservation observed in that region for the LMV-Common and LMV-Most isolates analysed to date (Krause-Sakate et al., 2002 and results not shown). The possibility exists that group VI mAbs could recognize a

conformational rather than a linear epitope, which might explain longer-range conservation. However, this hypothesis is not supported by observations showing that two of the group VI mAbs, LM-10 and MB-5, bind to denatured CP subunits in Western blot experiments (result not shown). Thus, the most likely hypothesis seems to be that group VI mAbs bind to the conserved extreme N terminus of the LMV CP.

## Acknowledgements

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