Growth stage-dependent resistance to the potyviruses lettuce Italian necrotic virus and Lettuce mosaic virus displayed by Lactuca sativa introgression lines carrying the Mo3 locus from L. virosa

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Abstract

The single dominant gene *Mo3* from accession PIVT1398 of *Lactuca virosa* was shown previously to confer resistance to all tested isolates of *Lettuce mosaic virus* (LMV; genus *Potyvirus*), including *mo1*-resistance-breaking isolates. Because accession PIVT1398 is also resistant to the recently described potyvirus lettuce Italian necrotic virus (LINV), this study checked whether the *Mo3* resistance locus could be involved in resistance to LINV. Introgression lines of *Mo3* into two different *L. sativa* genetic backgrounds (Girelle and Mantilia) were resistant to isolates LMV-9, LMV-13 and LINV-Fr (a French isolate of LINV), whereas Girelle and Mantilia were susceptible to these virus isolates. The resistance reaction in inoculated leaves of the introgression lines varied from absence of symptoms and absence of virus detection for LMV-9, to hypersensitive-like necrotic lesions and low virus titres for LINV-Fr and large necrotic lesions and relatively abundant virus detection for LMV-13. At the systemic level, the resistance phenotype was growth stage-dependent for LINV-Fr and LMV-13. Inoculation of ≥28-days-old plants led to absence of symptoms and no virus with the three tested potyvirus isolates in almost all plants. Inoculation of younger plants (≤22-days-old) led to chlorotic/necrotic lesions on some apical leaves with low virus titre in a varying proportion of plants with LINV-Fr and on all plants with efficient virus detection with LMV-13. These results show that the *Mo3* gene, or a tightly linked locus, in *L. virosa* PIVT1398 controls resistance to LINV in addition to LMV and could be used to breed *L. sativa* cultivars with broad-spectrum potyvirus resistance.

Keywords: lettuce, linkage, *Mo3* locus, pleiotropy, *Potyvirus*, resistance

Introduction

Lettuce (*Lactuca sativa*, family Asteraceae) crops are affected by several potyviruses (genus *Potyvirus*; family *Potyviridae*), including *Lettuce mosaic virus* (LMV), *Turnip mosaic virus* (TuMV), *Bidens mottle virus* (BiMoV), *Endive necrotic mosaic virus* (ENMV) and lettuce Italian necrotic virus (LINV) (Christie *et al*., 1968; Zink *et al.*, 1973; Zitter & Guzman, 1974; Provvidenti & Hampton, 1992; Blancard *et al.*, 2006; Ciuffo *et al*., 2016; Desbiez *et al.*, 2017). LINV was recently described in lettuce crops in Italy and southeast France and induces severe necrosis and plant wilting, leaf distortion and/or vein clearing. Its natural host range seems to be limited to *L. sativa* cultivars. However, experimental inoculations showed that it can infect plants of other species in the family Asteraceae (*Calendula* spp., *Cichorium* spp., *Helianthus annuus, Tragopogon pratensis* and *Zinnia elegans*) and *Nicotiana benthamiana* (Desbiez *et al.*, 2017). Transmission by *Myzus persicae* (Homoptera: Aphididae) was demonstrated experimentally (Ciuffo *et al*., 2016; Desbiez *et al.*, 2017). LINV is phylogenetically related to LMV in the genus *Potyvirus*. No source of resistance to LINV was found in *L. sativa* accessions carrying known potyvirus resistance genes such as the *mo1*^{1} or *mo1*^{2} alleles conferring LMV resistance, or the *Tu* gene conferring TuMV resistance (Desbiez *et al*., 2017). However, a few accessions of the wild species *L. virosa* and *L. perennis* were found to be resistant to a French isolate of LINV (LINV-Fr). The *L. virosa* accession PIVT1398 appears particularly interesting because it has a broad resistance spectrum including to LMV and ENMV-Fr isolates (Desbiez *et al.*, 2017) and because of the possibility of obtaining fertile intercrosses between *L. virosa* and *L. sativa* (Maisonneuve *et al*., 1995). The LMV resistance of PIVT1398 was shown to be conferred by the single gene *Mo3* in F_1, F_2 and F_3 progenies derived from crosses with the LMV-susceptible accession *L. virosa* PIVT280 (Maisonneuve *et al*., 1999). *Mo3* was inherited as a dominant gene after inoculation with LMV-0 but as an incompletely dominant gene after inoculation with LMV-13, the heterozygous plants

inoculated with LMV-13 being often necrotic (Maisonneuve *et al.*, 1999). LMV-0 is avirulent towards the *mo1*^{1} or *mo1*^{2} resistance alleles that are present in many cultivars of *L. sativa*, whereas LMV-13 is virulent towards both alleles (Dinant & Lot, 1992). Later on, *L. sativa* introgression lines (ILs) carrying the *Mo3* locus were developed in several genetic backgrounds via a backcross programme (Maisonneuve, 2003). The results of inoculation with either LMV-0 or LMV-9 (an *mo1*^{1} virulent isolate; Dinant & Lot, 1992), depending on the recurrent *L. sativa* cultivar (carrying the *mo1*$^{+}$ or *mo1*^{1} gene, respectively), fit to the hypothesis of one gene and strong necrosis associated with heterozygous plants. Some F_3 and F_4 populations tested with LMV-0 or LMV-9 displayed uniform resistance reactions with neither symptoms nor virus detection in apical, uninoculated leaves. The objectives of the present study were to analyse the phenotypic reaction of *L. virosa* PIVT1398 to LINV and LMV inoculation and to check if the *Mo3* locus could also be involved in the LINV resistance using data obtained from the reaction of ILs carrying this genetic factor(s).

Materials and methods

Plant material

The *Mo3* gene from *L. virosa* PIVT1398 (= CGN09365) was independently introduced by backcrosses into two butterhead lettuce cultivars, Girelle and Mantilia, producing ILs Girelle-Mo3 and Mantilia-Mo3, respectively (Tables S1 & S2). Two other *L. sativa* cultivars, Columbus and Mariska, were used for the backcrosses (Tables S1 & S2). Mantilia carries the *mo1*^{1} gene, that confers resistance to LMV-0. The three other *L. sativa* cultivars (Columbus, Girelle and Mariska) are susceptible to LMV (*mo1*$^{+}$). Girelle-Mo3 was developed from the F_1 interspecific cross Columbus × PIVT1398, followed by seven backcrosses to *L. sativa* and three generations of selfing (= BC_7S_3). Mantilia-Mo3 was developed from the interspecific

cross PIVT1398 × Columbus followed by nine backcrosses to *L. sativa* cultivars and three generations of selfing (= BC_9S_3). Eight and four generations of selfing were also inserted between the backcrosses in the genealogies of Girelle-Mo3 and Mantilia-Mo3, respectively (Tables S1 & S2). For both ILs, selection for LMV resistance was made in different generations, with LMV-13 in the first generations and with LMV-0 or LMV-9 from BC2. The phenotypes of Girelle-Mo3 and Mantilia-Mo3 were similar to those of Girelle and Mantilia, respectively, in terms of plant appearance, growth and development. LMV-susceptible accession *L. virosa* LS238 was also used as control.

Virus isolates and inoculation procedure

The origins of the French isolate LINV-Fr and of isolates LMV-9 and LMV-13 are described in Desbiez *et al.* (2017) and Dinant & Lot (1992), respectively. LMV-9 overcomes the $mo1^1$ resistance allele, whereas LMV-13 overcomes both $mo1^1$ and $mo1^2$ resistance alleles. The inoculation procedure was as described in Desbiez *et al.* (2017), with virus being propagated in Mantilia. After sowing on paper in Petri dishes (2 days at 6 °C without light, then 5–7 days in a climate chamber at 24/18 °C and 16 h light), the germinated seeds were transplanted in soil in 0.5 L pots in an insect-proof greenhouse for growing and tests. The seedlings were inoculated 16–34 days after sowing to compare virus infection and symptoms in different growth stages: from young plants with two to three leaves to older plants with up to six to seven leaves. Whatever the stage at inoculation, the two oldest leaves were rub-inoculated. A comparison of four growth stages at inoculation (performed from 18 to 34 days post-sowing) was made on Girelle-Mo3 in December/January; then comparisons of two inoculation stages (16 vs 28 or 20 vs 28 days post-sowing) were made in April/May and August, respectively.

Virus detection

Symptoms were recorded regularly and the infection status of the plants was confirmed by enzyme-linked immunosorbent assay (ELISA), at 7–10 days post-inoculation (inoculated leaves), or at 2–6 weeks post-inoculation (apical, uninoculated leaves). LINV-Fr was detected by antigen-coated plate (ACP)-ELISA with a generic potyvirus-group kit (Agdia). LMV was detected by double antibody sandwich (DAS)-ELISA with a specific polyclonal antiserum as described in Maisonneuve *et al*. (1999). Virus detection was arbitrarily considered positive when the absorbance at 405 nm (A_{405}) was more than three times that of the healthy controls. LINV was also detected by reverse transcription-polymerase chain reaction (RT-PCR). Total RNAs were purified from 200 mL of the leaf extracts used for ELISA using the Tri-Reagent kit (Merck) according to the manufacturer's instructions. Two-step RT-PCRs were performed. Primer 7091CP-R (5′-GCTAGCTGATTCTTGGTGGC-3′) and *Avian myeloblastosis virus* reverse transcriptase (Promega) were used for RT. Primers 7091CP-R and 7091Nib-F (5′-TGGTTCATGTCTCATAGGGG-3′) and *Taq* DNA polymerase (Promega) were used for PCR. The primers were designed based on the sequence of isolate LINV-Fr (accession number KU941945) to amplify a region of *c*. 650 nucleotides (nt) at the junction of the NIb and coat protein cistrons. Mock-inoculated plants of the same genotype(s) were used as negative controls and plants with symptoms, of the susceptible genotypes Mantilia or Girelle, as positive controls. The PCR programme was 5 min at 95 °C; 40 cycles at 95 °C for 30 s, 57 °C for 30 s and 72 °C for 45 s; and 72 °C for 10 min.

Results

Resistance to LINV-Fr

Following inoculation with LINV-Fr at 20 days after sowing, all plants of Girelle-Mo3 and Mantilia-Mo3 (278 in total) showed necrotic lesions on inoculated leaves, similar to hypersensitive reactions (HR), at 5–7 days post-inoculation (dpi). The donor of the *Mo3* resistance gene, *L. virosa* accession PIVT1398, showed rare and tiny necrotic lesions on inoculated leaves at the same time. In contrast, the *L. sativa* parental lines Girelle and Mantilia as well as the *L. virosa* susceptible control accession LS238 did not show any symptoms on inoculated leaves. At 6–12 dpi, Girelle and Mantilia showed systemic vein clearing and leaf curling, progressively evolving into a general wilting and the death of the plants one month after inoculation (Table 1; Fig. S1). *Lactuca virosa* LS238 showed milder mosaic symptoms on apical, uninoculated leaves, which did not induce death of plants. No symptoms were observed at the systemic level in *L. virosa* PIVT1398 or in a large majority (81%) of plants belonging to Girelle-Mo3 and Mantilia-Mo3. However, chlorotic/necrotic lesions (CNL) developed on the first two apical leaves of 22% and 14% of the plants belonging to Girelle-Mo3 and Mantilia-Mo3, respectively, from 6 to 14 dpi (Table 1; Fig. S2). Most of the plants showing these CNL symptoms at the systemic level continued to grow and newly developed leaves did not show any symptoms. However, 7.2% of the total Girelle-Mo3 plants stayed stunted with limited growth, and new leaves also showed CNLs. Overall, 95% of IL plants did not show symptoms on apical leaves at 38 dpi (Table 1). ACP-ELISA was performed using samples from the apical leaves with the aim of detecting virus infection. All Girelle, Mantilia and *L. virosa* LS238 plants reacted positively, whereas none of the plants of either *L. virosa* PIVT1398 or *L. sativa Mo3* ILs were positive, regardless of the presence of CNLs. A few plants

with CNLs showed absorbance values (A_{405}) close to twice those of negative controls, which may indicate very low virus accumulation or replication.

Resistance to LINV-Fr and plant growth stage

The resistance status of Girelle-Mo3 plants inoculated at different growth stages was compared in three independent experiments (Table 2) in order to gain insight into the peculiar range of LINV-Fr resistance phenotypes observed in the ILs (showing either complete resistance to systemic infection, limited systemic invasion followed by a recovery, or systemic invasion leading to plant stunting). For all experiments, plants had two expanded leaves at the youngest growth stage. This stage corresponded to 16–20 days after sowing, depending on the season in which the experiment was conducted. In all experiments, 100% of the Girelle susceptible controls showed infection, and 100% of the *L. virosa* PIVT1398 controls showed resistance at the systemic level, based on symptom occurrence and ELISA. All plants belonging to the ILs showed necrotic HR-like lesions on inoculated leaves. The systemic infections in IL plants were stage-dependent in the three experiments. Overall, CNLs were observed at the systemic level only on plants inoculated with LINV-Fr at young stages, from 16 to 22 days after sowing. No plant showed these kind of symptoms when inoculated 28 days after sowing or later (Table 2). The percentage of plants showing CNLs at the systemic level varied greatly among different experiments (2.4% for Girelle-Mo3 plants inoculated 22 days after sowing in experiment 1 to 96% for plants inoculated 20 days after sowing in experiment 3), which could be related to the climatic conditions (lowest percentage in winter, intermediate in spring and highest in summer) or to the inoculum concentration. As previously, no evidence of virus detection in leaves showing CNLs was obtained by ELISA.

The presence of LINV-Fr was also checked by RT-PCR in plant samples corresponding to experiment 2 (Fig. 1). A strong PCR signal was obtained for the Girelle susceptible control

on inoculated leaves, and no evidence of virus presence was obtained by RT-PCR on the inoculated leaves of *L. virosa* PIVT1398 showing tiny necrotic lesions. On Girelle-Mo3 inoculated leaves showing larger necrotic lesions, a large number of samples reacted positively in RT-PCR (10/13 tested plants inoculated 16 days after sowing; 1/5 tested plants inoculated 28 days after sowing), although only faint DNA bands were visible, indicative of low virus titres (Fig. 1). For Girelle-Mo3 plants, positive PCR signals were also observed for apical leaves in six of 10 tested plants showing CNLs at the systemic level (four of these six plants are shown in Figure 1). In contrast, no signal was observed in apical leaves of the other, symptomless plants (0/10 tested plants). In symptomless apical leaves collected on plants that showed the CNLs, LINV-Fr was only rarely detected by PCR (1/6 tested plants with a faint DNA band; Fig. 1).

Leaves of the plants belonging to Girelle-Mo3 that showed CNLs were also used to inoculate plants of *Lactuca* spp. genotypes, either LINV-Fr susceptible or resistant. For this, a pool of leaves of the four plants that showed CNLs in experiment 1 were used. Plants of the susceptible genotypes developed symptoms at a rate varying from 44% (*L. virosa* LS238; 11/25 plants) to 56% (*L. sativa* 'Girelle'; 28/50 plants). In addition to the absence of virus detection in ELISA and the absence of, or weak signals in RT-PCR, this confirmed the low virus titre in leaves showing CNLs. In contrast, no infection was obtained in inoculated plants of the resistant genotypes *L. virosa* PIVT1398 and Girelle-Mo3 (0/25 each), suggesting the absence of resistance-breaking LINV-Fr mutant in leaves showing CNLs. As a control, back-inoculations from Girelle plants with symptoms to Girelle led to 100% (10/10) of plants with symptoms.

Resistance phenotypes of *L. sativa Mo3* introgression lines against LINV-Fr and LMV

In the third experiment, the resistance to LINV-Fr was compared to that expressed against two LMV isolates (Table 2). The resistance phenotypes observed upon inoculation with LMV-13 were similar to those observed with LINV-Fr except that symptoms were more severe with LMV-13 (Fig. S2). In Girelle-Mo3 plants inoculated at the youngest stage (20 days after sowing), necrotic lesions were observed on inoculated and apical leaves for all plants. They were larger than those observed with LINV-Fr and the plants remained stunted afterwards with no recovery. Strong positive reactions were observed in ELISA in these plants. At the oldest stage (28 days after sowing), only one plant of 22 was infected at the systemic level with necrotic lesions and evidence of virus infection in ELISA (Table 2). LMV-13 infected all inoculated Girelle plants (Fig. S1) but none of the *L. virosa* PIVT1398 plants at the systemic level. In PIVT1398, necrotic lesions in inoculated leaves were more visible than with LINV-Fr. In relation to LMV-9, there was no evidence of infection in Girelle-Mo3 (Fig. S2) or in *L. virosa* PIVT1398 in inoculated leaves or at the systemic level, and based either on symptom recording or ELISA, whatever the inoculation stage. However, LMV-9 infected all Girelle plants with mosaic symptoms (Fig. S1) and strong ELISA reactions at the systemic level.

Discussion

All results are in agreement that the region encompassing the *Mo3* locus (or the *Mo3* gene itself) determines resistance against several LMV isolates in *L. virosa* PIVT1398 (Maisonneuve *et al.*, 1999 and this study) and is also involved in resistance to LINV-Fr. Indeed, all plants belonging to Girelle-Mo3 and Mantilia-Mo3 showed a very high resistance level to LINV-Fr compared to the Girelle and Mantilia recurrent parents: (i) no dead plants were observed in *L. sativa Mo3* ILs at 38 dpi, while 100% Girelle and Mantilia plants were dead at

this evaluation time; (ii) most of the inoculated IL plants did not show symptoms on apical leaves; (iii) none of them showed clear evidence of virus infection at the systemic level based on ELISA, even in the presence of CNL; and (iv) a frequent recovery was observed from 14 to 38 dpi in plants showing early CNL symptoms. Moreover, IL plants resulting from two independent interspecific crosses and with two different genetic backgrounds showed a similar high proportion of symptomless plants at the systemic level at 14 dpi.

Necrotic lesions on inoculated leaves and CNLs on some apical leaves of plants of the ILs combined with low virus titres in these leaves suggest that the resistance is linked to HR mechanisms, consistent with its dominant inheritance for LMV (Maisonneuve *et al.*, 1999). Pleiotropic effect of the *Mo3* gene conferring resistance to both LMV and LINV-Fr is supported by the fact that the Girelle-Mo3 and Mantilia-Mo3 ILs have undergone 17 potential rounds of meiosis, which could have separated two potential resistance genes by recombination. There are other examples of large spectrum resistance genes or loci conferring resistance to several potyvirus species, like the *Pvr4* locus in *Capsicum annuum* conferring resistance to at least six potyviruses (Janzac *et al*., 2009) and the *I* locus in bean (*Phaseolus vulgaris*) conferring resistance to at least 10 potyviruses (Meziadi *et al*., 2017). However, the analysed ILs result from interspecific crosses between *L. virosa* and *L. sativa*, for which recombination could have been suppressed in parts of the genome as observed in other plant interspecific crosses (Frary *et al*., 1996; Wenzl *et al*., 2006; Ganal *et al*., 2011; Sim *et al*., 2012). Hence, it is concluded that the single gene *Mo3* could have a pleiotropic effect, conferring resistance to both LMV and LINV-Fr. However, it is not possible to exclude categorically the hypothesis of two distinct loci, one (*Mo3*) conferring resistance to LMV and the other conferring resistance to LINV-Fr. Under this alternative hypothesis, the two loci would be located in the same genome region and recombination between them could be impaired because of the incompatibility between the *L. virosa* and *L. sativa* genomes. The *L.*

virosa PIVT1398 accession is also resistant to a third potyvirus, ENMV-Fr, but the link with *Mo3* using the *L. sativa* ILs could not be tested because the recipient parents, Girelle and Mantilia, are also resistant to ENMV-Fr (Desbiez *et al.,* 2017).

The phenotype of the resistance was found to be dependent on the virus species, on the virus isolate (for LMV) and on the plant growth stage at inoculation. With the LMV-9 isolate, the phenotype resembles an extreme resistance, with no manifestation of an HR in inoculated leaves of *L. virosa* PIVT1398 or of the tested *Mo3* ILs, and no virus detected in inoculated or apical leaves. Growth stage-dependent resistance was observed with LINV-Fr and LMV-13. At the youngest stage for inoculation, the resistance was not sufficient to block systemic infection. A proportion of plants showed systemic infection, as attested by ELISA, RT-PCR and inoculation tests. For these two viruses, resistance was associated with expression of necrotic lesions in inoculated leaves and, in some cases, in apical leaves. Hence, the resistance phenotype resembles an HR, but resistance does not completely hamper the virus movement at the systemic level when plants are young and/or when the virus is highly aggressive, like LMV-13. The CNLs observed at the systemic level following inoculation with LINV-Fr may therefore correspond to a secondary induction of plant resistance. As the plant grows, these successive inductions of plant defences at different leaf levels may ultimately confine the virus, which would explain the recovery phenotype observed in most of the plants. These differences of resistance level of *Mo3* have been observed previously in the *L. virosa* genetic background between LMV-0 and LMV-13 (Maisonneuve *et al.*, 1999). Furthermore, a recovery phenotype was observed for LINV-Fr plants inoculated at a young stage and expressing symptoms at the systemic level. Later on, in most of the plants, newly emerged leaves were devoid of symptoms and no virus was detected.

The comparison between the resistance phenotypes of *L. virosa* PIVT1398 and the *L. sativa Mo3* ILs also suggests an effect of the genetic background of the *Mo3* resistance locus

(or its genome region). Indeed, the *L. virosa* PIVT1398 resistance to LINV-Fr and LMV-13 is almost complete, with a lack of infection at the systemic level and very small necrotic lesions on inoculated leaves, whereas frequent systemic infections were observed for the ILs in the two *L. sativa* backgrounds when inoculated at a young stage and local lesions were much larger. That could be due to a loss of some complementary genes efficient against LINV-Fr or instability of the expression of *Mo3* in the *L. sativa* background. Additional studies analysing the resistance reaction of the F_2 progeny of PIVT1398 × LS238 would be necessary for more conclusive evidence.

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Table 1 Symptoms observed on apical uninoculated leaves in *Lactuca* spp. genotypes inoculated with LINV-Fr, 20 days after sowing.

Plant genotype	Symptoms 14 dpi[a]	Symptoms 38 dpi
L. virosa PIVT1398	0/14	0/14
L. virosa LS238	14/14 Mo[b], vein clearing	14/14 Mo
L. sativa 'Girelle'	14/14 Mo, stunting	14/14 dead
Girelle-Mo3	39/180**x**[c] CNL[d] (21.7%)	13/180**y** stunting (7.2%)
L. sativa 'Mantilia'	14/14 Mo, stunting	14/14 dead
Mantilia-Mo3	14/98**x** CNL (14.3%)	0/98**x**

[a]days post inoculation.

[b]mosaic.

[c]**x** and **y** indicate significant differences between the introgression lines Girelle-Mo3 and Mantilia-Mo3 for a given date (Fisher exact test; p=0.005).

[d]chloro-necrotic lesions.

Table 2 Symptoms observed on apical uninoculated leaves in the introgression line Girelle-Mo3 inoculated with LINV-Fr, LMV-9 or LMV-13 at different growth stages. In each experiment, *L. sativa* 'Girelle' susceptible controls showed 100% infection, either with mosaic and wilting (with LINV-Fr) or mosaic only (with LMV). In contrast, *L. virosa* PIVT1398 plants did not show any infection at the systemic level.

Experiment	Virus	Plant stage at inoculation[a]	Plants showing symptoms on apical leaves / total	A_{405} values[b]
1	LINV-Fr	18	3 / 36 CNL[c]	1.6
1	LINV-Fr	22	1 / 41 CNL	1.1
1	LINV-Fr	28	0 / 38	
1	LINV-Fr	34	0 / 38	
2	LINV-Fr	16	13 / 30 CNL[d]	1.5
2	LINV-Fr	28	0 / 30	
3	LINV-Fr	20	24 / 25 CNL	Not tested
3	LINV-Fr	28	0 / 22	
3	LMV-9	20	0 / 25	
3	LMV-9	28	0 / 22	
3	LMV-13	20	25 / 25 Nec[e]	32.0
3	LMV-13	28	1 / 22 Nec	6.9

[a]in days after sowing.

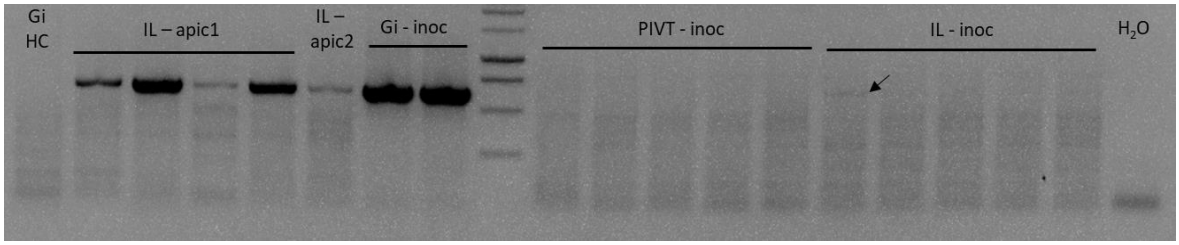
[b]mean absorbance values at 405 nm in ELISA of symptomatic leaves relative to mock-inoculated plants (mean of at least five replicas).

[c]chloro-necrotic lesions.

[d]RNA was purified from 8 of these 13 plants to check virus presence by RT-PCR.

[e]necrosis.

Figure 1 Detection of LINV-Fr by RT-PCR. Each lane corresponds to leaf RNA extracts from one individual plant. The arrow indicates a faint band corresponding to LINV-Fr. Gi: Girelle; HC: healthy control; IL: introgression line Girelle-Mo3; apic1: apical leaf showing chlorotic/necrotic lesions; apic2: apical leaf with no chlorotic/necrotic lesions; inoc: inoculated leaf; PIVT: *Lactuca virosa* PIVT1398.



Supporting Information

Figure S1 Symptoms observed on cv. Girelle plants inoculated with either LINV-Fr or LMV. (a), (b) Plants inoculated with LINV-Fr showing vein clearing (VC) on apical leaves at 7 days post-inoculation (dpi) or wilted at 22 dpi, respectively. (c) Plant inoculated with LMV-9 showing VC on apical leaves at 7 dpi. (d) Plant inoculated with LMV-13 showing VC on apical leaves at 7 dpi. (e) Mock-inoculated control plant. inoc: inoculated leaves.

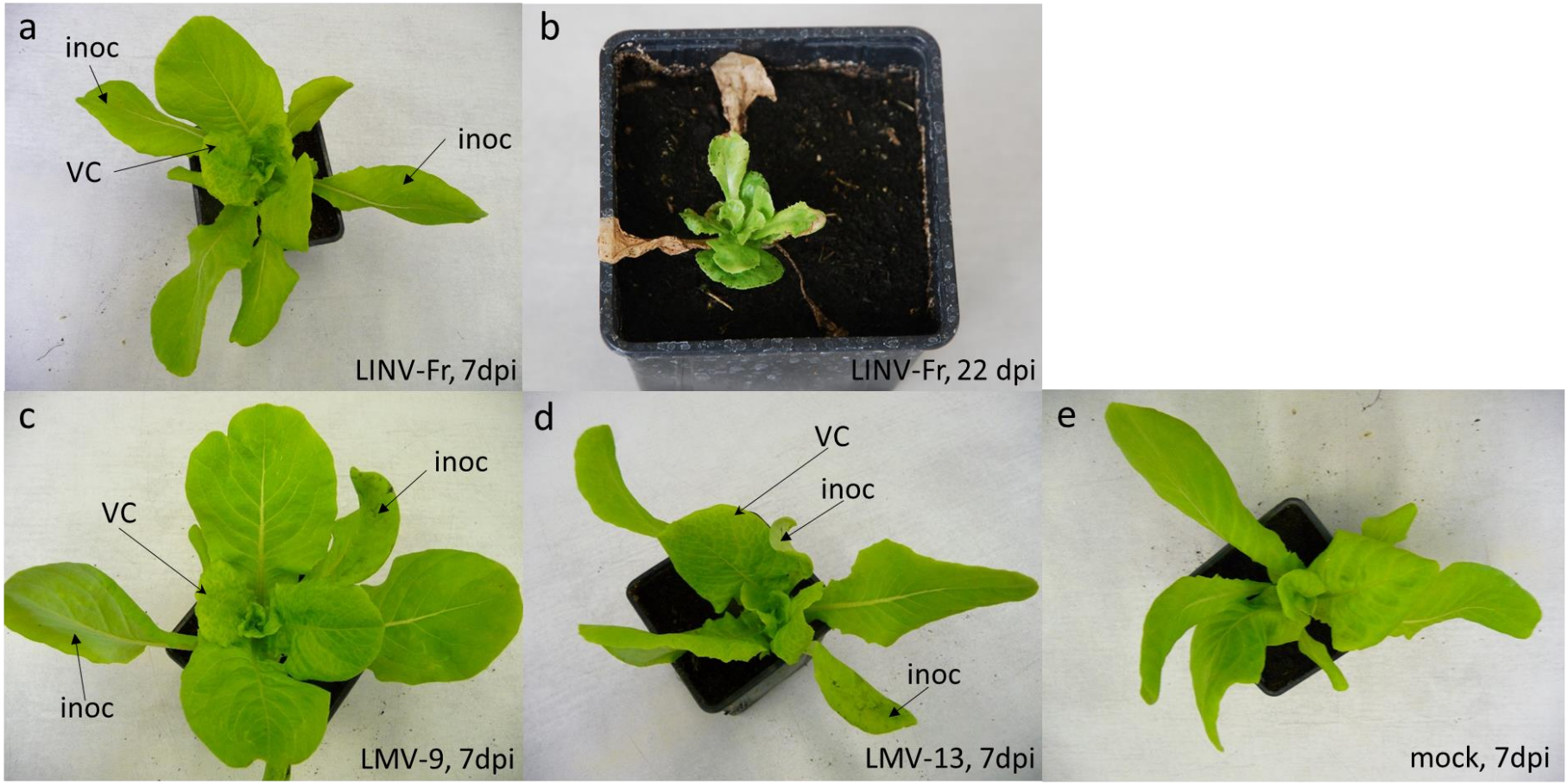


Figure S2 Symptoms observed on plants of the introgression line Girelle-Mo3 inoculated with either LINV-Fr or LMV. (a) Plant showing only necrotic lesions on leaves inoculated with LINV-Fr (inoc) at 7 days post-inoculation (dpi). (b) Plant inoculated with LINV-Fr and showing chlorotic lesions (CL) on an apical leaf at 7 dpi. (c) Plant inoculated with LINV-Fr and showing chlorotic/necrotic lesions (CNL) on apical leaves at 18 dpi. (d) Plant inoculated with LMV-9 and showing no symptoms in apical leaves at 7 dpi. (e) Plant inoculated with LMV-13 and showing necrotic lesions (NL) on inoculated and apical leaves at 7 dpi.

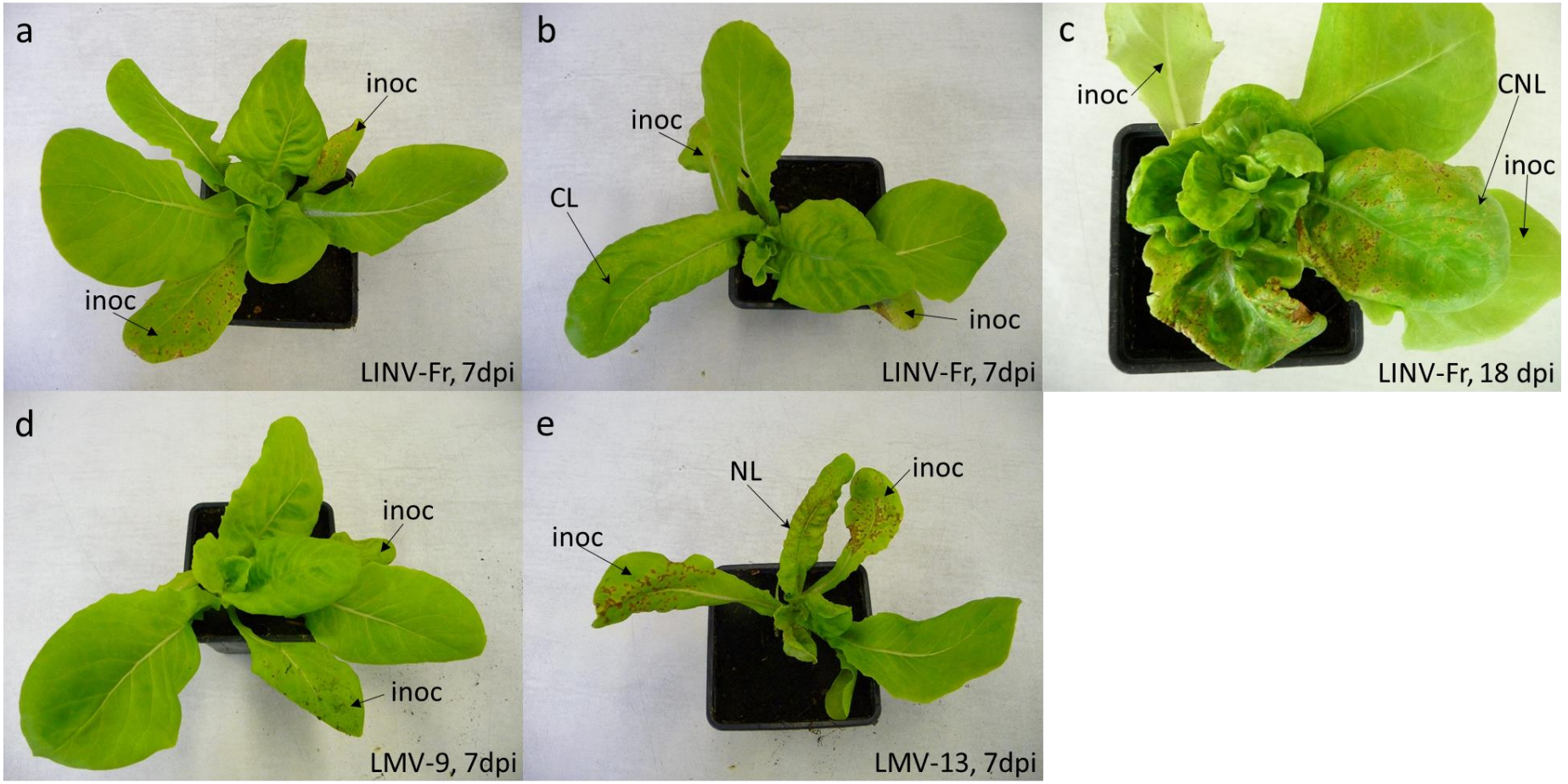


Table S1 Genealogy of introgression line Girelle-Mo3.

Generation	Interspecific F_1	BC_1	BC_2	BC_3	BC_4	BC_5	BC_6	BC_7
1	Columbus × PIVT1398							
2		Columbus (♂)						
3		Selfed						
4		Selfed*[a]						
5		Selfed*						
6		Selfed*						
7			Girelle Ms-7 (♀)*[b]					
8				Girelle (♂)				
9				Selfed				
10					Girelle Ms-7 (♀)*			
11					Selfed*			
12						Girelle Ms-7 (♀)*		
13							Girelle Ms-7 (♀)*	
14							Selfed*	
15							Selfed*	
16								Girelle Ms-7 (♀)*
17								Selfed
18								Selfed*
19								Selfed*

[a]*: a LMV-resistant plant was selfed or crossed with Girelle (or Girelle Ms-7).
[b]Girelle Ms-7 is a near-isogenic line of 'Girelle' with gene *Ms-7* for male sterility (Ryder E, 1971. Genetic studies in lettuce (*Lactuca sativa* L.). *Journal of the American Society for Horticultural Science* **96**, 826-828).

Table S2 Genealogy of introgression line Mantilia-Mo3.

Generation	Interspecific F_1	BC_1	BC_2	BC_3	BC_4	BC_5	BC_6	BC_7	BC_8	BC_9
1	PIVT1398 × Columbus									
2		Girelle (♂)								
3		Selfed								
4		Selfed*[a]								
5			Mariska Ms-7 (♀)*[b]							
6				Mariska Ms-7 (♀)*						
7					Mantilia (♀)					
8					Selfed					
9						Mantilia (♀)*				
10							Mantilia (♀)*			
11								Mantilia (♀)*		
12									Mantilia (♀)*	
13			,						Selfed	
14										Mantilia (♀)*
15										Selfed*
16										Selfed*
17										Selfed*

[a]*: a LMV-resistant plant was selfed or crossed with Mariska Ms-7 or Mantilia.

[b]Mariska Ms-7 is a near-isogenic line of 'Mariska' with gene *Ms-7* for male sterility (Ryder E, 1971. Genetic studies in lettuce (*Lactuca sativa* L.). *Journal of the American Society for Horticultural Science* **96**, 826-828).