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Summary

New European isolates of *B. lactucae* and of LMV are able to overcome the disease resistance genes used in commercial varieties. Wild *Lactuca* spp. constitute a large pool for new genes. Several new resistance genes against European *B. lactucae* isolates were identified in wild *Lactuca* species and introduced in *L. sativa* lines. The resistance from two *L. virosa* accessions was efficient against every isolate. The results suggested at least 2 dominant genes in each accession controlled the resistance (2 independent in one accession, two tightly linked in the other one). A complete stable resistance was observed in artificial tests with absence of spores. Therefore, this resistance was assumed to be comparable to *Dm* gene. The spectra of resistance of lines derived from two *L. saligna* were similar with resistance from cv. Ninja. This mechanism seems more complex than *Dm* gene with apparition of late and sparse sporulation on many plants in some segregating populations. This late sporulation was also observed in some commercial varieties and other INRA resistant lines derived from *L. serriola.*

In a large collection of different *Lactuca* spp., one *L. virosa* (PIVT1398), was identified as resistant to different European LMV isolates overcoming *mo1[1]* or *mo1[2]* genes. A study of *L. virosa* segregating F_2 population suggested one major dominant gene for this resistance. *Mo3* was proposed to designate this gene. Its expression in heterozygous plant was variable with different isolates. Its expression was consistent with a dominant gene against LMV-0, but incomplete dominant gene against LMV-13 (necrotic phenotype). The introgression of this gene in *L. sativa* is in progress despite several difficulties.

An accumulation in one genotype of several resistance genes against each disease could be a strategy to increase the durability of these new genes.

Lactuca serriola, L. saligna, L. virosa, LMV, *Bremia lactucae*, resistance genes, genetics of resistance, accumulation of genes

Introduction

Lettuce is an important vegetable crop for fresh market. For consumer, the leaves must be exempt of disease symptoms and of pesticide residues. Among the most important diseases, downy mildew (*Bremia lactucae* Regel) and lettuce mosaic potyvirus (LMV) are constant risks for this crop. For both diseases, resistance genes have been introduced in cultivars since 30 to 40 years (1, 2, 11). Nevertheless, several isolates identified in Europe can overcome the *Dm* and *mo* resistance genes used in commercial varieties.

For *B. lactucae,* several genes efficient against NL16 isolate were overcome in the last few years, e.g. resistance from Samourai (RZ), Ninja (S&G) or Bacarès (Vilmorin) (presented in a communication at this meeting). Likewise, European virus isolates can

overcome the LMV tolerance conferred by *mo1*^{1} and *mo1*^{2} (9). Therefore, we are looking in wild *Lactuca* spp. for new sources of resistance to both diseases.

Several genes from *L. serriola* have been introduced into some cultivars (e.g. several *Dm* genes; (2)) because the two species can be easily crossed. The use of *L. saligna* and *L. virosa* has been more restricted; however, some resistance genes from *L. saligna* have been introduced into *L. sativa* (8, 10). A possible method for overcoming the crossability barrier with *L. saligna* and *L. virosa* was achieved by *in vitro* rescue of immature hybrid embryos (5).

Investigations of new sources of resistance to *B. lactucae* in *L. serriola*, *L. saligna* and *L. virosa* have been in progress at INRA for several years. Some resistance efficient to NL16 have been introgressed into *L. sativa* and we are controlling their efficiency against new European isolates.

For LMV, we have recently screened a large collection of *Lactuca* with virulent isolates. One accession of *L. virosa* appeared interesting, consequently a program to study this resistance and introgress it into *L. sativa* has been investigated.

Material and methods

Plant material

Two morphologically different accessions of *L. saligna* (CR16 and CR17) received from I. Crute (NVRS, Wellesbourne) and two accessions of *L. virosa* collected by INRA (LS238 and LS241) were used for *B. lactucae* resistance studies. The interspecific crosses with *L. saligna* and the BC_1 [(*L. sativa* x *L. virosa*) x *L. sativa*] were made by *in vitro* culture of immature embryo (5). The progenies obtained at INRA from 3 *L. serriola* resistant to NL16 were also tested with new European isolates. Lines derived from these interspecific crosses by backcrosses with several *L. sativa* (e.g. cv. Girelle, cv. Ravel) or crosses between interspecific progenies; and then selfpollination were generated. Evaluation of this resistance and its inheritance study were made on this material issued from four to seven cross generations before selfing; the genetic background could be considered as *L. sativa* one.

In contrast, the research on LMV resistance was achieved in wild species. In preliminary experiments, 116 *L. sativa* genotypes were screened for resistance to virulent LMV-E isolates. Because no interesting resistance was found, then 115 wild *Lactuca* accessions were screened. The most interesting accession, *L. virosa* (PIVT1398) received from CPRO (Wageningen, NL), was extensively studied for its LMV resistance. The study was made on *L. virosa* material: F_1 between susceptible *L. virosa* (PIVT280) and resistant PIVT1398 and derived F_2 and F_3 families.

Tests for resistance to *B. lactucae*

European isolates has been used in this study, NL16 and isolates overcoming NL16 resistant varieties. The new isolates SAR1, SAR3 and I3 attacking respectively Ninja, Samourai and Bacarès, were supplied by Novartis Seeds (France); 49/83, overcoming previously Mariska resistance (6), was supplied by A. Lebeda. Lettuces were tested at seedling stage in closed plastic boxes with soil substratum. After sowing, the lettuce seeds were incubated at 6 °C for 24 h to 48 h to overcome seed dormancy; and then the boxes were transferred to a growth chamber for 3 weeks (16/12 °C day/night tempera-

tures, 16 h per day). Isolates were multiplied on susceptible cultivar seedlings cultivated on filter paper moistened with a nutrient solution. Seven day-old seedlings were inoculated by spraying a spore suspension (2 x 10^5 spores/ml). Seven and 14 days after inoculation, presence or absence of asexual sporulation were scored.

Tests for LMV resistance

European isolates were used in this study, the common isolate (LMV-0) and some isolates identified on some tolerant varieties: LMV-E, LMV-1, LMV-13 (3). The inoculum was an extract from 2-3 week-old infected lettuce plants (cv. Trocadero for LMV-0 or cv. Mantilia for other isolates). One gram of infected leaf tissue was grinded in 4 ml buffer containing 100 mg/ml of activated charcoal and carborundum. Young plants were mechanically inoculated once or twice (day 0 and 2). Plants were maintained in a growth chamber (22 ˚C /12 to16 ˚C day/night temperatures, 16 h per day) for LMV-0 and LMV-E. Experiments using the other strains (LMV-1 and LMV-13) were conducted at Montfavet with plants in an insect-proof greenhouse. Symptom observation as well as DAS-ELISA (Double Antibody Sandwich-ELISA) was used to evaluate the plant resistance. One gram of leaf tissue was collected from different young leaves, grinded with a roller press in 4 ml of PBS-Tween-PVP buffer and tested by DAS-ELISA using polyclonal antibodies.

Results and discussion

New sources of resistance to *B. lactucae*

Seven sources of resistance to *B. lactucae*, previously identified against NL16 and 49/83, were tested against new European isolates in comparison with new resistance of commercial varieties (Table 1). Four out of these 7 lines were resistant to every isolate (two *L. serriola* and the both *L. virosa*). As expected, TTE, genitor of one of the resistance used by Vilmorin, was overcome by I3. Isolate SAR3, identified on Ninja, attacked the both *L. saligna*.

Observation of plants with sparse and late sporulation was made in several lines and varieties in our test conditions; this late sporulation was scored especially in interaction I3/R18, but also with different isolates on R38 and R36 resistance factors as well as on our lines derived from *L. saligna*, and sometimes on TTE and FHD.

In segregating population with resistance from CR16 or CR17, this phenotype with late and weak sporulation was screened 14 days after inoculation (dpi), and then increased the percentage of susceptible plants. For example, in F_2 (SaBV x Girelle) the ratio resistant to susceptible plants at 7 dpi (120 to 33 with NL16 and 110 to 42 with 49/83) was consistent with one dominant gene ($\chi^2 = 0.96$ and 0.56 respectively). But at 14 dpi, some sporulation was scored on more than 50% of plants (51 res to 92 susc with NL16 and 66 res to 83 susc with 49/83). In experiment with weaker inoculation (2 x 10^3 sp/ml), the ratio at 14 dpi fitted with 3 : 1 in some segregating populations. These results suggested that the mechanism of resistance in these both *L. saligna* CR16 and CR17 as observed previously in other *L. saligna* (4) was different from the *Dm* gene mechanism. A difference of susceptibility between SaBV and SaAB observed with SAR2, isolate supplied by Novartis (results not shown), suggested that the both resistant factors must be different.

Table 1. Interaction of NL16 resistant genotypes with European isolates of *B. lactucae*

Variety/line	R factor [a] or source of resistance	Isolates of *B. lactucae* NL16	49/83	SAR1	SAR3	I3
Commercial varieties [b]						
Mariska	R18	R	+	+	R	R *
Samourai		R	+	+	R	*
Argelès	R38	R *	nd	R *	R	+
Bacarès		R	R	R *	R	+
Ninja	R36	R	R	R	+	R *
Angie	Nd	R	R	R	+	R
INRA lines						
Resistance from *L. serriola*						
F_7 YYD	LS102 (R17)	R	R	R	R	R
F_7 FHD	LS162	R	R	R	R	R
F_7 TTE	Nd	R	R	R	R	+
Resistance from *L. saligna*						
F_6 SaAB	CR16	73 R	73 R	27 R	20 +	20 R
F_4 SaBV	CR17	68 R	72 R	27 R	20 +	20 R
Resistance from *L. virosa*						
F_6 ViAE	LS241	197 R	340 R	349 R	410 R	143 R
F_4 ViCQ	LS238	250 R	357 R	383 R	413 R	219 R

[a] R factor proposed by IBEB (presented at this meeting);
[b] test on 10 to 50 plants per variety; interactions were scored as R for no sporulation, score R * for a sparse and late sporulation on only few plants of resistant variety; score * for mixed reaction (5R, 7*, 3+); score + for profuse sporulation on all plants.

Table 2. Segregation of resistance in populations derived from crosses between resistant *L. virosa* and susceptible cultivars inoculated with 4 isolates of *B. lactucae*

Isolates	ViBK6-2-2 (resistance from LS241)		ViCQ2-4-4 (resistance from LS238)		ViCP5-1-1	
	No of seedlings		No of seedlings		No of seedlings	
	Res : susc	χ^2 3 : 1	Res : susc	χ^2 15 : 1	Res : susc	χ^2 3 : 1
NL16	293 : 64	9.5 **	308 : 18	0.3	332 : 97	1.3
49/83	522 : 137	6.2 *	554 : 48	3.0	436 : 128	1.6
SAR1	524 : 118	15.0 **	296 : 25	1.3	345 : 93	3.3
SAR3	468 : 115	8.7 **	500 : 43	2.6	473 : 142	1.2

* Significantly different from hypothesis ($P<0.05$)
** Significantly different from hypothesis ($P<0.01$)

In contrast, the interaction between *B. lactucae* and our progenies from *L. virosa* was similar with reaction of *Dm* genes. At 7dpi, a strong sporulation was observed on susceptible plants and often the same segregation was scored at 7 and 14 dpi. Segregating populations with these resistance were scored after inoculation with 4 isolates (Table 2). The results suggested that two tightly linked dominant genes in ViBK and two independant genes in ViCQ and one dominant gene in ViCP controlled the resistance.

The complete resistance of ViBK (315 tested plants) and ViCQ (170 tested plants) after inoculation with I3, what is Avr7, was an indication of the transfer into these genotypes of *Dm*7 from the different cultivars used in this cross. The segregation for resistance to I3 scored in ViCP (ratio 350 res to 71 susc) suggested that the gene from LS238 present in ViCP could be linked to *Dm*7. Complementary tests on larger populations would be necessary to confirm these hypothesis. These loci could be designated R39 to R42 if there is no allelic gene between LS238 and LS241. Allelism tests are in progress.

New resistance to LMV

In a collection of 116 *L. sativa*, 76 *L. serriola*, 11 *L. saligna*, 22 *L. virosa* and 6 other *Lactuca* spp. tested with LMV-E only few accessions did not show severe symptoms. These resistant genotypes were tested with LMV-0, LMV-1 and LMV-13. Only one *L. virosa* and some plants from *L. perennis* showed interesting resistance to the four isolates.

The resistance of the *L. perennis* must be fixed before any program of protoplast fusion in order to transfer it into *L. sativa* that could be a very laborious work (7). Therefore, we initiated a study of the resistant *L. virosa* (PIVT1398). An immunity was observed with the different isolates (no virus detectable in ELISA in systemic but also in infected leaves). Because of the very low fertility of the interspecific crosses with *L. virosa* (hybrid sterile, very low fertility of the BC_1), the research on inheritance was initiated in segregating population derived of a cross between two *L. virosa* (PIVT280), a susceptible one (high virus multiplication) and PIVT1398. The F_1 and F_2 generation were inoculated with LMV-0 and LMV-13 (Table 3).

After inoculation with LMV-0, the results were consistent with one dominant gene. This hypothesis was confirmed by the study of F_3 families derived from F_2 tested plants, because the progenies of all susceptible F_2 plants were homogeneous for susceptibility and the progenies of resistant F_2 plants were resistant or in segregation (data not shown). The results after inoculation with LMV-13 was different. Indeed, the expression of resistance in the heterozygous plants was sometimes irregular with often a necrotic phenotype. The same expression was observed after inoculation with LMV-E (data not shown). The results suggested an hypothesis of one incomplete dominant gene with these virulent isolates; we designated this locus *Mo3*.

In our strong test conditions (mechanical inoculation, often twice on young plant), some PIVT1398 susceptible plants were identified (2 out 30 plants inoculated with LMV-13 in greenhouse, 0 out of 114 plants inoculated with LMV-0 in growth chamber). A control of the virus multiplied in these plants revealed a LMV variant able to systemically infect PIVT1398. When these variants were inoculated to susceptible lettuce (cv. Trocadéro), they can lose their ability to overcome *Mo3*. According to the possibility of variant of LMV, for a good evaluation of *Mo3* in segregating population, the susceptibility of the plants must be checked by studying of the multiplied virus in back-inoculation on PIVT1398.

Table 3. Resistance to different LMV isolates in segregating population of *L. virosa*

Genotypes	LMV isolates	Number of plants susceptible	intermediate	resistant	χ^2 1:2:1 or 1:3
F_1 (PIVT1398 x PIVT280)					
	LMV-13	0	10	0	
F_1 (PIVT280 x PIVT1398)					
	LMV-13	3	8	7	
	LMV-0	0	0	16	
F_2 (PIVT280 x PIVT1398)					
	LMV-13	17	28	13	1.14
	LMV-0	26	4	56	1.25

Inoculation of plants cultivated in a growth chamber for LMV-0 or in a greenhouse for LMV-13 and then assay by DAS-ELISA. OD of susceptible plants were similar with inoculated PIVT280, the OD of resistant plant similar with inoculated PIVT1398 and not inoculated plants. The intermediate score corresponded to necrotic plants and/or plants with low virus multiplication

A test of F_3 lines were made to know whether the same gene confers the resistance to LMV-0 and to LMV-13. The 13 lines fixed susceptible to LMV-0 (11 to 74 tested plants) were susceptible to LMV-13 (5 to 28 plants tested) and the 11 lines fixed resistant to LMV-0 (34 to 88 tested plants) were resistant to LMV-13 (13 to 33 tested plants). However, in three resistant F_3 lines, one or two susceptible plants were identified; but a control of the virus revealed a variant of the isolate. These results suggested the same gene controlled the resistance to the both isolates. For this reason, it is possible to inoculate the plants with the common isolate (LMV-0) in breeding program in order to score *Mo3*; that is less hazardous for environment than to use a virulent isolate, especially LMV-13 that it could be seed born.

The introgression of *Mo3* into *L. sativa* were initiated (7) but it is a very laborious work due to the high mortality and to the low fertility of progeny maintained after several generations of BC with *L. sativa*.

Conclusions

Some new resistance genes were identified in *L. virosa*. Their introgression into *L. sativa* is more or less difficult with respect to the wild resistant accession. These resistance seem due to one or two dominant major genes in each accession that could be easy to use in breeding programs. For *B. lactucae* resistance, some other genes were identified in other species easier to cross with lettuce (*L. serriola* and *L. saligna*). But the expression of these genes with some partial resistance makes them not easy to screen and need very standardized conditions for the artificial test.

The main problem for disease resistance is the durability of the efficiency of the gene. For the both diseases, we were able in our test conditions to obtain variant of

isolate that overcome some resistance (isolates of *B. lactucae* overcoming *L. saligna* resistance and variant of LMV overcoming *Mo3*). The durability of these resistance in natural conditions is not known. In absence of other resistance efficient against the new European isolates of these both diseases, we will continue these programs with complementary genetic studies as in one hand allelism tests between *B. lactucae* resistance, in the other hand the introgression of *Mo3* in diversified cultivars. A strategy to increase the durability of the resistance could be the accumulation of the resistance genes in one genotype especially if mechanisms are different: for example, resistance against *B. lactucae* from *L. saligna* with genes from *L. virosa* or *Mo3* with one allele of *mo1* locus. Consequently, it will be important to identified some PCR based molecular markers of these genes; it is in progress for *Mo3*.

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