Lactuca virosa, a source of disease resistance genes for lettuce breeding: results and difficulties for gene introgression

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Abstract: Four *L. virosa* accessions have been used as sources of disease resistance. Resistance to *Bremia lactucae* and two viruses, BWYV and LMV, were identified. To transfer these resistances to lettuce, *in vitro* embryo rescue must be used to obtain the first BC with *L. sativa.* Two resistances to *Bremia* were studied in fertile lines of lettuce issued from interspecific crosses followed by several BCs and selfing. The resistance introgressed from each *L. virosa* was efficient against all tested isolates. The hypothesis of two dominant genes will be discussed in relation with segregation obtained in segregating populations (F_2 and F_3). The study of inheritance of virus resistance was conducted in a segregating population between *L. virosa* genotypes. The hypotheses of one dominant gene (*Bw*) for resistance against BWYV and one partly dominant gene (*Mo3*) for resistance to LMV were proposed. Many heterozygous plants for *Mo3* were necrotic after LMV inoculation. The introgression into *L. sativa* of the virus resistance genes from *L. virosa* was very laborious due to physiological problems of plants growth especially with *Mo3*. After several BCs with different varieties and self-pollinations, the plants were still necrotic; many plants died at the vegetative stage due to very weak roots, especially the heterozygous one, or at the bolting due to stem necrosis. The BWYV resistant lines, several self-pollinations after only one BC, had small heads or had not yet headed. Nevertheless, after several BCs, some fertile resistant lettuce plants were obtained.

Keywords: *Lactuca sativa, L. virosa*, lettuce, interspecific cross, disease resistance, inheritance, *Bremia lactucae*, Lettuce Mosaic Virus, Beet Western Yellow Virus

Introduction

Several diseases can be particularly damaging for lettuce production; some are responsible of loss of quality with symptoms on leaves or loss of the plants in early infection. Among these diseases, that caused by the fungus *Bremia lactucae* is present all year round and that caused by viruses such as Lettuce Mosaic Virus (LMV) and Beet Western Yellow Virus (BWYV) are observed in the field. Because it is necessary to have leaves without symptoms and without residues of pesticides, genetic resistance is the best solution for protection of lettuce.

Genetic resistance found in *Lactuca sativa* or in *L. serriola* is easy to use in breeding programs (e.g. R18 against *B. lactucae* or *mo1* against LMV). But sometimes there is not resistance in these species and it is necessary to look at other species more distantly related to lettuce (Koopman et al., 1998). *L. virosa* is one of these wild species only partly compatible with cultivated lettuce.

In *L. virosa*, several resistances have been found to *B. lactucae* (Maxon Smith and Langton, 1989), aphids (Eenink and Dieleman, 1981) and BWYV (Maisonneuve et al., 1991). This species is diploid with n=x=9 chromosomes as for *L. sativa*; but the interspecific F_1 hybrids with lettuce are completely sterile. With some accessions, it is possible to obtain back-crosses using lettuce as the male plant. Then the plants are somewhat fertile and it is possible to harvest few seeds. With some accessions, the use of *in vitro* rescue of immature embryos can produce more hybrids (Maisonneuve, 1987).

In this paper, the use of four genotypes of *L. virosa* for fungus and virus resistance are presented with the introgression of the resistance in lettuce.

Material and methods

Plant material

Four *L. virosa* accessions were used in this study: 2 genotypes from CPRO (Wageningen, NL) supplied by Eenink, PIVT 280 (= CGN04683) and PIVT 1398 (= CGN09365), and 2 genotypes collected by INRA (LS 238 and LS 241) in France. Some F_1 interspecific hybrids and all BC_1 of these hybrids were made by *in vitro* rescue of immature embryos (Maisonneuve, 1987; Maisonneuve et al., 1995).

All studies on *Bremia* resistance were made on the progenies of the interspecific BC_1 [(PXD x LS) x cv. Ravel] where PXD is an INRA line close to cv. Girelle. Several crosses with winter butterhead cultivars and self-pollinations were made to obtain the material used in this study: ViBK and ViAE are progenies from *L. virosa* LS 241, ViCP and ViCQ are progenies from *L. virosa* LS 238. The inheritances were studied in F_2 and F_3 populations between a susceptible variety Cobham Green and some homogeneous resistant lines, ViAE and ViCQ.

Virus resistance was first studied in the *intra-virosa* crosses between both of the interesting PIVT numbers, PIVT 1398 and PIVT 280. Then the progenies of the interspecific BC_1 were used. For LMV resistance, 2 resistant lines were the genitors of the material: DC10 = F_5 [(cv. Columbus x PIVT 1398) x cv. Columbus]2-c-16-3-10 and JA7 = F_2 {cv. Mariska x F_3 [(PIVT1398 x cv. Columbus) x cv. Girelle]2-A-119}7. Back-crosses with several lettuce varieties were made to produce several quasi-isogenic lines. For BWYV, the self-pollinations (F_2 to F_6) of several BC_1 [(PIVT280 x cv.) x. butterhead] were studied.

***Tests for resistance to* B. lactucae**

European isolates were used in this study, Tv from England, NL16 from Netherlands and isolates overcoming NL16 resistant varieties. The new isolates Bl: 20 and Bl: 21 attacking respectively cv. Samourai and cv. Ninja (van Ettekoven and van der Arend, 1999) were supplied by Novartis Seeds (France); the Czech isolate 49/83, overcoming previously cv. Mariska resistance (Maisonneuve et al., 1994), was supplied by Lebeda. FR20/00 is a French isolate identified on cv. Argelès in South-East (Perpignan area) and supplied by Dubois in 2000.

Lettuces were tested at the seedling stage in closed plastic boxes with soil substrate at 16°C/12°C day/night temperatures, 16h per day, as described previously (Maisonneuve et al., 1999). The inoculum concentration was 1 - 2 x 10^5 spores/ml. The presence or absence of sporulation was scored at 7 and 14 days after inoculation (dpi). The score of the resistance was easy because the resistant plants were green with no spores on cotyledons at 14 dpi and the susceptible plants showed a profuse sporulation at 7 dpi.

Tests for LMV resistance

European isolates were used in this study, the common isolate (LMV-0) and some isolates identified on tolerant varieties : LMV-9, LMV-E, LMV-13 (Dinant and Lot, 1992). Mechanical inoculations and ELISA tests were made as described previously (Maisonneuve et al., 1999). Plants were maintained in a growth chamber (22°C/12 to 16°C day/night temperatures, 16 h per day) or in an insect-proof greenhouse. Symptom observation as well as DAS-ELISA (Double Antibody Sandwich-ELISA) were used to evaluate the plant resistance.

Tests for BWYV resistance

The French isolate BWYV-FL1, isolated in 1982 in southeastern of France, maintained by replication of *Myzus persicae* on *Physalis floridana* for several years in laboratory, was used.

Inoculations were made using small pieces of infected leaves of *P. floridana* carrying viruliferous aphids as described previously (Maisonneuve et al., 1991). Symptom observation as well as DAS-ELISA were used to evaluate the plant resistance. The DAS-ELISA used with the interspecific populations was identical to the method used for evaluation of the *L. virosa* population (Maisonneuve et al., 1991).

Table 1. *B. lactucae* resistance in F_2 (Cobham Green x Vi) and segregating F_4 ViBK or ViCQ population. Segregation and testing of some hypotheses of inheritance.

Segregating population	*Bremia* isolates	Observed segregation	Theoretical results: segregation R : S *		χ^2 (prob) ** for tested hypothesis *		
		R : S	3 : 1	15 : 1	13 : 3	9 : 1	12 : 1
			Progenies from LS 241				
F_2 (Cg x ViAE)	FR20/00	920 : 212	849 : 283	1061 : 71	0.00 (0.99)	95.8	>100
ViBK6-2-1	NL16	399 : 106	379 : 126	473 : 32	1.66 (0.20)	67.8	125.8
	49/83	507 : 94	451 : 150	563 : 38	3.81 (0.05)	21.3	53.5
	Bl: 20	511 : 98	457 : 152	571 : 38	2.82 (0.09)	25.1	60.5
	Bl: 21	629 : 125	566 : 189	707 : 47	2.33 (0.13)	36.3	83.9
Sums of 4 isolates		2046 : 423	1852 : 617	2315 : 154	4.24 (0.04)		
ViBK6-2-2	NL16	417 : 92	382 : 127	477 : 32	0.15 (0.70)	36.9	77.3
	49/83	522 : 137	494 : 165	618 : 41	1.80 (0.18)	85.23	159.2
	Bl: 20	524 : 118	482 : 161	602 : 40	0.06 (0.81)	50.1	103.3
	Bl: 21	468 : 115	437 : 146	547 : 36	0.36 (0.55)	61.3	118.9
Sums of 4 isolates		1931 : 462	17950 : 598	2243 : 150	0.49 (0.49)		
			Progenies from LS 238				
F_2 (Cg x ViCQ)	NL16	212 : 33	184 : 61	230 : 15	4.48 (0.03)	3.28 (0.07)	11.5
	FR20/00	1122 : 116	929 : 309	1161 : 77	71.5	0.55 (0.46)	4.91 (0.03)
Sums of 2 isolates		1334 : 149	1112 : 371	1390 : 93	73.7	0.00 (0.95)	11.6
ViCQ2-4-4	NL16	454 : 30	363 : 121	454 : 30	50.1	7.77 (0.01)	1.52 (0.22)
	49/83	554 : 48	452 : 150	564 : 38	45.9	2.75 (0.10)	0.07 (0.80)
	Bl: 20	296 : 25	241 : 80	301 : 20	25.3	1.74 (0.19)	0.00 (0.95)
	Bl: 21	500 : 43	407 : 136	509 : 34	41.8	2.61 (0.11)	0.04 (0.84)
Sums of 4 isolates		1804 : 146	1462 : 488	1828 : 122	162	13.7	0.12 (0.73)

* 3 R : 1 S = one gene dominant; 15 R : 1 S = two dominant independent genes; 13 R : 3 S = one dominant and one recessive independent genes; 9 R : 1 S = two dominant independent genes linked to incompatibility factors; 12 R : 1 S = two dominant independent genes with one of them linked to one incompatibility factor

** for $\chi^2 > 12$, prob <0.001

Results and discussion

Resistance to B. lactucae *from* L. virosa *LS 238 and LS 241*

The both homogeneous lines F_6 ViAE and F_4 ViCQ2-4-1 were completely resistant to every tested *Bremia* isolate (NL16, Tv, 49/83, Bl: 20, Bl: 21, FR20/00). A first study of inheritance was made in two types of segregating populations: two large F_2 and some segregating F_4 (ViBK for LS 241 and ViCQ2-4-4 for LS 238). The number of resistant (R) vs susceptible (S) plants is always between the number calculated for one dominant gene and for two

independent dominant genes (Table 1). These data are therefore compatible with the hypothesis of two dominant linked genes. But some other hypotheses could not be eliminated: one dominant and one recessive independent genes (segregation 13 R : 3 S) for LS 241 and two dominant genes with incompatibility factors linked to resistance genes (segregation 9 R : 1 S or 12 R : 1 S) for LS 238. The study of four F_5 issued from F_4 ViCQ2-4-4, tested with NL16 and FR20/00 (around 200 to 400 plants per F_5), confirms a likely segregation of 9 R : 1 S or 12 R : 1 S (data no presented). If the hypothesis of incompatibility factors linked to resistance is true, the plants with only one gene can not be produced; a pollen grain carrier of one resistance gene could fertilize only an ovule with the other resistant gene. To check these hypotheses a population of F_3 families has been analyzed for each source of resistance.

Among the 67 F_3 progenies (Cobham Green x ViAE), 31 were homogeneous (23 resistant and 8 susceptible; study of 55 to 260 plants per F_3), 19 were segregating with over 75% of resistant plants per F_3 and 17 presented a segregation compatible with one dominant gene (study of 55 to 140 plants per F_3). Given that any F_3 population presented progeny with a majority of susceptible plants, the hypothesis concerning the presence of a recessive gene can be eliminated. These results were compared with different theoretical segregations for one or two genes (Table 2); the segregation with 7 homogeneous resistant F_3 : 4 F_3 in segregation 3 R to 1 S plant : 4 F_3 in segregation 15 R to 1 S plant : 1 homogeneous susceptible F_3 (two independent dominant genes) fits with our data ($\chi^2 = 0.16$). The both hypotheses with two independent genes linked to incompatibility factor give also a rather high χ^2 (0.04). These genes must be identical for resistance against NL16, Tv and FR20/00 because the reaction of the homogeneous F_3 families is the same with these 3 isolates.

Among the 68 F_3 progenies (Cobham Green x ViCQ2-4-1), 36 were homogeneous (30 resistant and 6 susceptible; study of 125 to 220 plants per F_3), 31 were segregating with over 75% of resistant plants per F_3 among which 27 fit with the hypothesis 9 R : 1 S (study of 60 to 210 plants per F_3, test with NL16 and FR20/00) and only one presented a segregation compatible with one dominant gene. The hypothesis of two dominant independent genes with linked incompatibility factors seems the likeliest for ViCQ2-4-1 and can explain that both genes were maintained after many crosses and self-pollinations (9 meioses). These genes must be identical for resistance against NL16, Tv, FR20/00 and Bl: 21 because the tests with these 4 isolates gave the same reaction for all homogeneous F_3 families.

Table 2. Segregation of F_3 (Cobham Green x Vi) families for resistance to *B. lactucae*.

Tested segregation		Population (Cg x ViAE) *		Population (Cg x ViCQ) *	
Hypothesis	Segregation	χ^2	probability	χ^2	probability
one dominant gene	1 : 2 : 1	7.1	0.03	17.2	<0.001
two independent dominant genes	7 : 4 : 4 : 1	5.1	0.16	27.3	<0.001
two independent dominant genes linked to incompatibility factors	5 : 4 : 1	6.7	0.04	1.4	0.49
two independent dominant genes, one linked to one incompatibility factor	6 : 4 : 2 : 1	8.1	0.04	13.6	0.004
one recessive and one dominant independent genes	7 : 4 : 2 : 2 : 1	22.4	<0.001	27.4	<0.001

* 67 F_3 (Cg x ViAE) tested with Tv and 68 F_3 (Cg x ViCQ) tested with FR20/00

Resistance to viruses from* L. virosa *PIVT 1398 and PIVT 280

The resistances of PIVT 1398 and PIVT 280 are complete resistances to LMV and to BWYV respectively. There were no symptoms and no virus detectable by ELISA tests in these *L.*

virosa accessions. The segregations for virus multiplication in F_2 (PIVT 280 x PIVT 1398) were compatible with the hypotheses of one dominant gene for each virus resistance, *Bw* and *Mo3* respectively (Maisonneuve et al., 1991, 1999). *Mo3* was efficient against all tested isolates, but *Bw* resistance was tested only with a laboratory isolate BWYV-FL1. The crosses between these two *L. virosa* accessions and 10 varieties of *L. sativa* were made for the two cytoplasms. Only few vigorous hybrid plants were obtained (Maisonneuve et al., 1995). After a large scale pollination of these F_1 and embryo rescues, few BC_1 plants were produced and selfed. The selection of the resistant plants was conducted in the progenies of these BC_1I_1 at each generation.

Two homogeneous resistant interspecific lines, DC10 and JA7, were used as LMV resistant parents to introduce *Mo3* in six varieties by a back-cross program. In the initial BC generations, many plants were necrotic with weak root systems, corky stem bases and necrotic streak on stem. Among heterozygous plants, very strong necrosis of the incompatible reaction led to the death of the plant. Therefore the resistance tests were made on F_2 families alternated with the BCs. After 3 BCs, vigorous heading plants were produced with cv. Girelle, cv. Diana and cv. Mariska. But, the seedlings of the BC with cv. Vanguard75 and cv. Kordaat were so weak that it was very difficult to produce the BC_2. Some F_3 and F_4 populations, tested against LMV, were homogeneous resistant with no symptoms nor virus as estimated by ELISA (OD<3 OD of un-inoculated plants). A rather large F_3 of BC_3 with Diana were tested for resistance to LMV-E. The segregation obtained with an ELISA test at 20 dpi (11 vigorous plants with any symptom and any virus ; 11 vigorous plants with mosaic and virus (OD>2) ; 18 weak plants with stunted apex and strong necrosis that are dead at 20 dpi) fit with hypothesis of one resistance gene and strong necrosis in heterozygous plants (χ^2=0.40 and p=0.82). These data must be confirmed on larger F_3 populations with different genotypes.

From the interspecific crosses made with PIVT 280, only four BC_1 gave a progeny selected after several self-pollinations for BWYV-FL1 resistance (Table 3). The F_2s, and then every generation, have been tested for resistance in the summer. After three to four years, the F_3 to F_5 populations issued from only four F_2 plants were selected. Because the populations are issued from self-pollinations of only the first BC by lettuce, the material is not yet horticulturally interesting. However, some lines have lost different characters from *L. virosa* (reflexed involucre, spines on vein, lack of heading) and a large progress could be expected after several BCs by lettuce cultivars.

Table 3. Introgression of the resistance to BWYV-FL1 from PIVT 280 in lettuce.

Genotypes of F_1 hybrids	Number F_2 of BC_1 harvested	Selected F_2 after resistance tests of progenies	Resistance estimated by ELISA	
			Generation	Segregation R : S
(PIVT 280 x cv. Malika)	20 F_2 (BC with 16 cv).	(F_1 x Melina)1	F_4	12 : 0 10 : 2
(PIVT 280 x cv. Rossia)	20 F_2 (BC with 5 cv.)	(F_1 x VFd line)1	F_4	10 : 10 9 : 5 18 : 12
(PIVT 280 x cv. Capitan)	9 F_2 (BC with 3 cv.)	(F_1 x Saffier)1f	F_2	8 : 18
(PIVT 280 x cv. Cocarde)	5 F_2 (BC with 16 cv.)	(F_1 x Capitan)3	F_2	14 : 6

Conclusions

L. virosa presents a large reservoir of genes for lettuce breeding for resistance to *Bremia*, LMV, BWYV and aphids. It is possible to introgress resistance genes to lettuce as shown with the *Nr* gene in cv. Dynamite (van der Arend et al., 1999) and many other varieties with resistance to *N. ribisnigri*.

Different strategies can be chosen to use the resistance genes from *L. virosa* as described in this paper. They should be related to the method of testing the resistance, easy or laborious, and to the capacity of the wild accession to cross with lettuce. For *Bremia* resistance, the tests are very easy with a strong expression of resistance (no spores at 7 and 14 dpi). The resistances were introgressed quickly to lettuce without major problem with horticultural screening; at this stage of the program, the resistance tests were only conducted on small populations to keep the resistance. For this strategy, a resistant lettuce population was produced, then the genetic and efficiency of the resistance were studied. At present the material is fertile, like butterhead, but maybe some genes from the wild parent were lost during the back-crosses with *L. sativa*. For virus resistance, the tests are more laborious specially for BWYV. The distance with lettuce seems larger with PIVT 1398 than with other accessions. Therefore, inheritance was studied at first in *L. virosa* species and the introgression was made without horticultural evaluation. For LMV, the physiological problem of interspecific plants was resolved by crosses with several varieties. After producing resistance lettuce lines, a control of the introgressed genes would be necessary on large populations to know the expression of the gene in a lettuce background.

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