Genetic Control and Host Range of Avrulence Toward *Brassica napus* Cultivars Quinta and Jet Neuf in *Leptosphaeria maculans*

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ABSTRACT

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Leptosphaeria maculans causes blackleg of oilseed rape. Gene-for-gene interactions between race PG3 and *Brassica napus* cv. Quinta were related to interaction between the fungal avirulence (*Avr*) gene *AvrLm1* and the corresponding resistance gene *Rlm1*. *AvrLm1* isolates were avirulent on cvs. Doublol, Vivol, Columbus, and Capitol, and no recombinant phenotypes were observed in the progeny of two *AvrLm1* × *avrLm1* crosses, suggesting that all of these cultivars may possess *Rlm1* or genes

displaying the same recognition spectrum, or that a cluster of *Avr* genes is present at the *AvrLm1* locus. In one cross, segregation distortion was observed at the *AvrLm1* locus that could be explained by interaction between *AvrLm1* and one unlinked deleterious gene, termed *Dell*. Incompatibility toward cvs. Jet Neuf and Darmor.bzh was governed by a single gene, unlinked to *AvrLm1* or *Dell*. This avirulence gene was termed *AvrLm4*. Preliminary plant genetic analysis suggested the occurrence of a corresponding dominant resistance gene, termed *Rlm4*, present in the Quinta line analyzed and linked to *Rlm1*.

Additional keywords: *Phoma lingam*.

The filamentous Ascomycete *Leptosphaeria maculans* (anamorph *Phoma lingam*) is the causal agent of blackleg of oilseed rape (*Brassica napus* L.). Blackleg is one of the most economically important disease of oilseed Brassicas worldwide (16,30). Control of the pathogen can be achieved with fungicide treatments (9). However, their efficacy remains at a low level due to the lack of clear-cut forecasting schemes to optimize the use of fungicides (12). In consequence, genetic resistance remains the most environment friendly and reliable method of control. Genetic resistance to *L. maculans* is usually divided into adult-stage resistance and seedling resistance (27,30). The former is the sum of many still uncharacterized general resistance mechanisms strongly interacting with the environment and growing conditions. Adult plant resistance is generally evaluated in the field by scoring symptoms at the end of the growing season. In contrast, seedling resistance is usually ascribed to single-gene race-specific resistance (2). Seedling resistance is easily scored following inoculations in controlled environment conditions, either on cotyledons or leaves (1,2). Race-specific resistance aims at preventing the fungus from entering the plant, which is a prerequisite for biotrophic systemic growth within the plant that eventually leads to stem canker (17). In consequence, race-specific resistance is efficient to protect plants under field conditions whenever corresponding avirulent races are prevalent in local populations (3).

At present, two *B. napus* differential sets have been described (5,18,26). They comprise either the three cvs. Westar, Quinta, and Glacier, which discriminates three pathogenicity groups (PG), termed PG2 to PG4, within the *Tox*⁺ group of the *L. maculans*

species complex (14), or the four cvs. Lirabon, Quinta, Glacier, and Jet Neuf, discriminating six PGs termed A1 to A6 (Table 1). Even though genetic characterization of race-specific resistance in *B. napus* was described as early as 1980 (10), it is only recently that genetic control of a few specific interactions between *B. napus* and *L. maculans* was established on both the pathogen and the host plant; the avirulence (*Avr*) genes *AvrLm1* and *AvrLm2* in *L. maculans* are responsible for a specific resistance response in cvs. Quinta and Glacier, respectively (1,2). The corresponding dominant resistance genes *Rlm1* and *Rlm2* were identified in cvs. Quinta and Glacier, respectively, providing the first evidence for a gene-for-gene relationship in the *L. maculans*-*B. napus* interaction (2). Another gene-for-gene interaction between the *LEM1* resistance locus in cv. Major and the *Avr* gene *alm1* in isolate PHW1245 was reported (11,28). To date, only a few additional sources for specific resistance have been identified in *B. napus*, and no other *Avr* locus has been described. Moreover, among the few major genetic loci operating at the seedling stage described in *B. napus*, no data are available regarding the possible allelism or redundancy of some of these genes, because genetic or quantitative trait loci studies were performed on seemingly unrelated cultivars, i.e., Major (11), Shiralee and Maluka (24), or Quinta and Glacier (2).

As a prerequisite to identify nonredundant resistance sources in *B. napus*, our objective is to genetically characterize races of the pathogen that will be used to (i) discriminate or identify resistance sources in different plant genetic backgrounds, and (ii) develop race-specific molecular markers for large-scale dynamic survey of the pathogen whenever novel resistance are used. In the present paper, we describe (i) the genetic characterization of *AvrLm4* responsible for avirulent phenotype toward cv. Jet Neuf, (ii) linkage analysis of *AvrLm4* with *AvrLm1*, and (iii) preliminary data on genetic control of resistance to *AvrLm4* isolates.

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MATERIALS AND METHODS

Fungal strains and culture maintenance. The a.2 (Mat⁺, *AvrLm1*) and H5 (Mat⁻, *avrLm1*) isolates of *L. maculans* were obtained from single ascospores isolated from diseased *B. napus* stubble (1,15). Isolate 4.4.07 (Mat⁺, *avrLm1*) was obtained following in vitro crosses between one single-ascospore field isolate and one progeny of the cross a.2 × H5 (22). Isolate IBCN5 is a single-ascospore isolate from the International Blackleg of Crucifers Network collection (29), formerly named T11aC25 by the originator, H. H. Hoppe (18,31). IBCN5 is a Mat⁻, A5 isolate, i.e., avirulent on *B. napus* cv. Jet Neuf and virulent on the other genotypes of the differential set (Table 1) (18,31).

All fungal cultures were maintained on V8-juice agar medium. Long-term storage was ensured by storing agar plugs collected from actively growing cultures in sterile mineral oil at 4°C. For pathogenicity tests, 12-day-old sporulating cultures were obtained and conidia were collected as previously described (1).

In vitro crossing. In vitro crosses and tetrad or random ascospore recovery were performed according to Gall et al. (15) and Balesdent et al. (8). Progeny from in vitro crosses were named as follows: (i) in the case of tetrads, isolates are designated “i.j.kl” where “i” is the cross number, “j” refers to the pseudothecium from which spores are recovered, “k” refers to the ascus number, and “l” is a randomly attributed number from one to eight; and (ii) for random ascospores, isolates are termed “vi.j.k”, where “i” and “j” refer to the cross number and pseudothecium number, respectively, and “k” is a randomly attributed number to distinguish isolates recovered from the same pseudothecium. Following tetrad recovery, the twin isolates within each tetrad were identified on the basis of the similarity of their electrokaryotypes, as previously established (22). Tetrads were considered complete when at least one isolate of each of the four genotypes of the tetrad was recovered. However, in most cases up to seven or eight isolates were recovered, allowing us to perform independent replicates of the inoculation tests by inoculating the twin isolates.

Ten tetrads and 88 random ascospores isolated from the a.2 × H5 cross (cross 11) (1,2) were analyzed in the present study. In addition, seven complete tetrads were recovered from the cross IBCN5 × 4.4.07 (cross 21), and 89 random ascospores were recovered from the cross 21.3.01 × v11.3.6 (cross 23) (Table 2).

Plant material. Seeds of cvs. Westar, Glacier, and Quinta were provided by G. Séguin-Swartz (AAFC-SRC, Saskatoon, Canada), H. K. Love (Svalof Weibull Seed LTD, Lindsay, Canada), and H. H. Hoppe (Georg-August-Universität, Göttingen, Germany), respectively.

One plant of cv. Quinta displaying leaf resistance under field conditions (2,3) was selected for selfing. For further multiplication, open pollination between a few selected S1 plants took place in the greenhouse. The resulting line was termed Quinta S2-V95. Line Quinta S1-3636 was obtained by selfing selected plants of the original seed lot cultivar. Additional selfings of individual plants produced five S2 lines: Quinta S2-V1 to S2-V4 and Quinta S2-4999. F2 and testcross populations from a cross between Quinta S1-3636 and plants of cv. Score were also used (2). The line Darmor.bzh was obtained as described earlier (13). Seeds of the winter oilseed rape cvs. Vivol, Capitol, Doublol, and Columbus were obtained from Semences Cargill (Boissay, France).

Pathogenicity tests. Isolates were inoculated on cotyledons of *B. napus* as previously described (15). Four different isolates, i.e., one on each half-cotyledon were inoculated on each plant. At least 10 plants were inoculated with each isolate, and most experiments were repeated twice. Plants were incubated in a growth chamber at 16°C (night) and -24°C (day) with a 12-h photoperiod. Symptoms were scored 14 to 21 days after inoculation (dpi) with a modified Williams and Delwiche rating scale (34), where symptom scorings attributable to the Tox⁰ members of the *L. maculans* species complex (7,14), i.e., infection classes (IC) IC2 and IC5 are

omitted. This new scale, termed IMAScore scale and described by Volke (31), comprises six IC, where IC1 is the typical hypersensitive response (HR), IC2 represents a larger (1.5 to 3 mm) dark necrotic lesion, IC3 is a nonsporulating lesion that may or may not show tissue collapse as in IC4 to IC6, but that is always sharply delimited by a dark necrotic margin that may extend within the lesion. IC4 to IC6 are characterized by gray-green tissue collapse without a darkened margin, and showing no sporulation (IC4), a few pycnidia (IC5), or profuse sporulation (IC6). IC1 to IC3 are considered to be resistance responses, whereas IC4 to IC6 are considered to be susceptibility (1,7).

RESULTS

***AvrLm1* confers avirulence toward cvs. Quinta, Doublol, Vivol, Capitol, and Columbus.** In cross 11, the *AvrLm1* parental isolate a.2, which was described as avirulent on cv. Quinta and virulent on cvs. Westar and Glacier (1), was also avirulent on cvs. Doublol, Vivol, Capitol, and Columbus, whereas H5 was virulent on all of these cultivars (Table 3). Previous results showed that avirulence toward cv. Quinta segregates as a single trait in the 10 tetrads and 88 random ascospores of cross 11 (1,2). Random progeny of cross 11 was further assessed for pathogenicity on Doublol, Vivol, Capitol, and Columbus. No recombinant phenotypes were recovered in the progeny, as all isolates that displayed the *AvrLm1* phenotype (avirulence on cv. Quinta) were also avirulent on cvs. Doublol, Vivol, Capitol, and Columbus (Table 3). These data suggest that *AvrLm1*, or genes tightly linked with *AvrLm1*, is responsible for inducing resistance in cvs. Doublol, Vivol, Capitol, and Columbus. Because resistance to *AvrLm1* isolates in cv. Quinta is due to the presence of the corresponding resistance gene *Rlm1* (2), cvs. Doublol, Vivol, Capitol, and Columbus will be referred to as “*Rlm1*-related cultivars” in the following text.

In the case of incompatible interactions on *Rlm1*-related cultivars, the most frequently observed IC was IC2 (Fig. 1). Quinta S2-V95 and cv. Capitol could be distinguished from the other *Rlm1*-related cultivars by the high frequency of IC3 (29.1 and 24.6%, respectively, versus less than 10% for the other cultivars) (Fig. 1). In the case of the *avrLm1*-*Rlm1* (compatible) interactions, the most frequent class was IC4 or IC5, depending on the cultivar (Fig. 1). For instance, cv. Vivol was characterized by a slow development of susceptibility symptoms, with only 13.6% of sporulating lesions at 15 dpi (IC5 and IC6), compared with more than 35% for the other *Rlm1*-related cultivars (Fig. 1). Even in the case of compatible interactions, a significant percentage of resistance responses was observed at 15 dpi, because 6% of the ≈2,900 inoculation sites observed belonged to IC1, IC2, or IC3 (Fig. 1). This was mainly the case for cvs. Columbus (9.7%) and Vivol (19.7%).

TABLE 1. Differential interactions expressed at the cotyledon stage between genotypes of *Brassica napus* and pathogenicity groups (PGs) of *Leptosphaeria maculans*

<i>L. maculans</i> ^a		<i>B. napus</i> genotypes ^b			
Interaction 1	Interaction 2	Westar and Lirabon	Glacier	Quinta	Jet Neuf
PG4	A1	C	C	C	C
	A5	C	C	C	I
PG3	A2	C	C	I	C
	A6	C	C	I	I
PG2	A4	C	I	I	C
	A3	C	I	I	I

^a Interaction 1 described by Mengistu et al. (26) and interaction 2 described by Hoppe et al. (18). Cvs. Westar, Glacier, and Quinta are described as differential according to Mengistu et al. (26), whereas Hoppe et al. (18) uses cv. Lirabon as susceptible control and cv. Jet Neuf as an additional differential.

^b C = compatible interaction; I = incompatible interaction (resistance).

Genetic control of avirulence toward cv. Jet Neuf. Incompatibility of IBCN5 toward cv. Jet Neuf, described by Hoppe et al. (18) was confirmed (Table 4, Fig. 2). In contrast to the *AvrLm1*–Quinta incompatibility, the IBCN5–Jet Neuf interaction phenotype was a typical HR response, with only IC1 and IC2 (data not shown). IBCN5 also induced HR on Darmor.bzh, although it caused disease on the related cv. Darmor (Table 4). It was avirulent on Quinta lines S2-V95, S2-V1 to S2-V4, S1-3636, and S2-4999, whereas it was virulent on cvs. Doublol, Vivol, Capitol, Columbus, and the original Quinta seed lot (Table 4) (data not shown).

Incompatibility of IBCN5 toward cv. Jet Neuf segregated as a single trait in the progeny of cross 21 (Table 2), because half the genotypes within each tetrad behaved as IBCN5 (Table 4, Fig. 2). It is thus postulated that one *Avr* gene, present in IBCN5, confers avirulence toward cv. Jet Neuf. All progeny of cross 21 that were avirulent on cv. Jet Neuf were also avirulent on Quinta S2-V95 and Darmor.bzh, but were virulent on the other cultivars (Table 4). The interaction phenotype of these isolates on Quinta S2-V95 was a typical HR (Fig. 3), clearly distinct from the late resistance response characteristic of the *AvrLm1*–*Rlm1* interaction (Fig. 1) (3).

To further analyze the genetic linkage between *AvrLm1* and the locus conferring avirulence toward cv. Jet Neuf, a cross was performed between isolate 21.3.01 (virulent on *Rlm1*-related cultivars

and avirulent on cv. Jet Neuf) and v11.3.6 (*AvrLm1* and virulent on cv. Jet Neuf) (Table 5). The 89 random ascospore isolates recovered were inoculated on cvs. Westar, Glacier, Vivol, Doublol, Capitol, Columbus, and line Quinta S2-V95. All isolates were virulent on cvs. Glacier and Westar (data not shown). Based on a 55:45 avirulent/virulent ratio in the progeny, a single-gene control of avirulence toward cv. Jet Neuf was confirmed (chi-squared = 0.91, df = 1, *P* > 0.05) (Table 5). Recombinant phenotypes, i.e., avirulent on both cv. Jet Neuf and *Rlm1*-related cultivars or virulent on all cultivars, were obtained with a 20.22 and 28.10% frequency, respectively, which is compatible with the hypothesis that the locus conferring avirulence toward cv. Jet Neuf is unlinked to *AvrLm1* (chi-squared = 0.1012, df = 1, *P* > 0.05) (Table 5). The locus responsible for avirulence toward cv. Jet Neuf was termed *AvrLm4*, and it is suggested that the genotypes of the parental isolates of cross 23 are *avrLm1*–*AvrLm4*, for isolate 21.3.01, and *AvrLm1*–*avrLm4*, for isolate v11.3.6 (Table 5).

However, as compared with cross 11, the segregation of *AvrLm1* in cross 23 cannot be explained by a single gene, because a 63:37 virulent/avirulent segregation ratio is obtained at the *AvrLm1* locus (chi-squared for a 50:50 expected ratio = 5.94, df = 1, *P* < 0.05). One hypothesis to explain this segregation distortion could be the occurrence of a deleterious gene involved in cross 23, but not in cross 11, termed *Dell*, so that *AvrLm1*–*Dell* genotypes are not viable. Based on this hypothesis, the genotypes of parental isolates in cross 23 would be *avrLm1*–*AvLm4*–*Dell* (21.3.01) × *AvrLm1*–*avrLm4*–*dell* (v11.3.6) (Table 5). The observed *avrLm1*/*AvrLm1* ratio in cross 23 fits with the expected two-thirds/one-third ratio (chi-squared = 0.562, df = 1, *P* > 0.05). In addition, the four phenotypic classes in the progeny of cross 23 should be recovered with the expected ratios of two-sixths (*avrLm1*–*AvrLm4*)/one-sixth (*AvrLm1*–*avrLm4*)/one-sixth (*AvrLm1*–*AvrLm4*)/

TABLE 2. In vitro *Leptosphaeria maculans* crosses analyzed in this study

Cross	Parental isolates		Progeny	
	1	2	Recovered	Selected
11	a.2	H5	10 tetrads, 88 random ascospores	v11.3.06
21	IBCN5	4.4.07	7 tetrads	21.3.01
23	21.3.01	v11.3.6	89 random ascospores	

TABLE 3. Pathogenicity of the 88 random ascospore progeny of the *Leptosphaeria maculans* cross a.2 (*AvrLm1*) × H5 (*avrLm1*)

Isolates	<i>Brassica napus</i> cultivar ^a							
	Westar	Glacier	Quinta S2-V95	Doublol	Vivol	Capitol	Columbus	Jet Neuf
Parental isolates								
a.2	C	C	I	I	I	I	I	C
H5	C	C	C	C	C	C	C	C
Interaction phenotypes in progeny								
1 (43 isolates)	C	C	I	I	I	I	I	nd
2 (45 isolates)	C	C	C	C	C	C	C	nd

^a C = compatible interaction; I = incompatible interaction; nd = not determined.

Fig. 1. Interaction phenotypes observed when inoculating Quinta S2-V95 and *Rlm1*-related cultivars with **A**, *AvrLm1* or **B**, *avrLm1* isolates from cross 11. Plants were inoculated at the cotyledon stage. Symptoms were rated 15 days after inoculation using the IMAScore scale, where infection class (IC) IC1 to IC6 are representative of hypersensitive resistance, IC3 is late resistance, and IC4 to IC6 are susceptibility ratings. **A**, The number of inoculation sites observed was 992 for cv. Quinta, 470 for cv. Doublol, 523 for cv. Columbus, 364 for cv. Capitol, and 354 for cv. Vivol. **B**, The number of inoculation sites observed was 1,277 for cv. Quinta, 503 for cv. Doublol, 546 for cv. Columbus, 293 for cv. Capitol, and 264 for cv. Vivol.

two-sixths (*avrLm1-avrLm4*). The observed ratio for these four classes (Table 5) support the hypothesis (chi-squared = 1.4735, df = 3, $P > 0.05$). Finally, as in cross 11, no recombinant phenotypes were observed when considering the set of *Rlm1*-related cultivars (Table 5).

Preliminary assessment of genetic control of resistance to *AvrLm4*. The occurrence of the dominant resistance gene *Rlm1*, interacting with *AvrLm1*, was previously demonstrated following genetic analyses of F1, F2, and testcross populations of Quinta × Score crosses (2). The Quinta line used in these crosses was resistant toward *AvrLm4* isolates, whereas cv. Score was susceptible to *AvrLm4* isolates (Table 6). Consequently, the F2 and testcross progeny of Quinta × Score crosses were used to analyze the genetic control of the resistance of cv. Quinta to isolates harboring *AvrLm4*. F2 and testcross plants were inoculated with isolates v11.1.2 (*AvrLm1-avrLm4*) and v23.2.1 (*avrLm1-AvrLm4*). Gene-for-gene interaction between *AvrLm1* and *Rlm1* in cv. Quinta (2) was confirmed in both F2 and testcross populations, because the segregation ratio of the resistance to v11.1.2 was highly significant of a 1:1 and 3:1 resistant to susceptible ratio in testcross and F2 populations, respectively (chi-squared = 0.277 and 0.891, respectively, df = 1, $P > 0.05$) (Table 6). Similar results were obtained when considering the segregation of resistance of Quinta S1-3636 to v23.2.1, because a 98:79 resistant to susceptible ratio in testcross, and a 127:38 resistant to susceptible ratio in the F2 (chi-squared = 2.04 and 0.341, respectively, for a 1:1 and 3:1 ratio, df = 1, $P > 0.05$) were obtained. It is thus suggested that a

Fig. 2. Example of the segregation of interaction phenotypes on cvs. Jet Neuf (black) and Quinta S2-V95 (gray). Symptoms were rated 15 days after inoculation using the IMAScore scale. 4.4.07 and IBCN5, parental isolates of cross 21; other isolates are members of one tetrad resulting from this cross. Twin genotypes within the tetrad share the same letter between parentheses.

TABLE 4. Characterization of the progeny of a cross between two *Leptosphaeria maculans* isolates differing in their interaction phenotype on *Brassica napus* cv. Jet Neuf

		<i>B. napus</i> cultivar ^b						
Isolates	Genotype ^a	Jet Neuf	Darmor. <i>bzh</i>	Quinta S2-V95	Doublol	Vivol or Capitol	Darmor	Glacier
Parental isolates								
IBCN5		I	I	I	C	C	C	C
4.4.07		C	C	C	C	C	C	C
Progeny								
21.1.01 and 21.1.03	a	I	I	I	C	nd	C	C
21.1.02	b	I	I	I	C	nd	C	C
21.1.04 and 21.1.08	c	C	C	C	C	nd	C	C
21.1.06 and 21.1.07	d	C	C	C	C	nd	C	C
21.1.11 and 21.1.13	a	C	C	C	C	C	C	nd
21.1.12 and 21.1.14	b	I	I	I	C	C	C	nd
21.1.15 and 21.1.16	c	C	C	C	C	C	C	nd
21.1.17	d	I	I	I	C	C	C	nd
21.2.11 and 21.2.14	a	I	I	I	C	C	nd	C
21.2.15 and 21.2.16	b	I	I	I	C	C	nd	C
21.2.17 and 21.2.18	c	C	C	C	C	C	nd	C
21.2.12 and 21.2.13	d	C	C	C	C	C	nd	C
21.2.21 and 21.2.24	a	C	C	C	C	C	nd	C
21.2.23 and 21.2.25	b	I	I	I	C	C	nd	C
21.2.22 and 21.2.27	c	I	I	I	C	C	nd	C
21.2.26	d	C	C	C	C	C	nd	C
21.3.01 and 21.3.08	a	I	I	I	C	C	C	C
21.3.02 and 21.3.04	b	I	I	I	C	C	C	C
21.3.03 and 21.3.05	c	C	C	C	C	C	C	C
21.3.06 and 21.3.07	d	C	C	C	C	C	C	C
21.3.12 and 21.3.13	a	C	C	C	C	C	nd	C
21.3.11 and 21.3.14	b	C	C	C	C	C	nd	C
21.3.15 and 21.3.16	c	I	I	I	C	C	nd	C
21.3.17 and 21.3.18	d	I	I	I	C	C	nd	C
21.3.22 and 21.3.28	a	I	I	I	C	C	nd	C
21.3.21 and 21.3.24	b	C	C	C	C	C	nd	C
21.3.25 and 21.3.26	c	I	I	I	C	C	nd	C
21.3.23 and 21.3.27	d	C	C	C	C	C	nd	C

^a Genotypes (a to d) within each tetrad were identified following pulsed-field gel electrophoresis of whole chromosomes (22). All isolates of the progeny between each a to d belong to the same tetrad.

^b C = compatible interaction; I = incompatible interaction; nd = not determined.

dominant resistance gene conferring resistance to *AvrLm4* is present in the Quinta line used in the crosses. The occurrence of recombinant phenotypes in both F2 and testcross populations (Table 6) suggests that the locus responsible for resistance to *AvrLm4* is not *Rlm1*. The dominant resistance gene recognizing *AvrLm4* was designated *Rlm4*. The observed ratio of plants in each of the four phenotypic classes (Table 6) suggests a genetic linkage between *Rlm1* and *Rlm4* (chi-squared = 26.89 and 10.53 for testcross and F2, respectively, df = 1, *P* < 0.05).

DISCUSSION

A gene-for-gene relationship was established between *AvrLm1* and the corresponding resistance gene, *Rlm1*, in cv. Quinta, and between *AvrLm2* and the corresponding resistance gene, *Rlm2*, in cv. Glacier (2). In this paper, we report on the genetic identification of a novel avirulence gene, *AvrLm4*, specifying incompatibility on cv. Jet Neuf. The cross between one *AvrLm1* and one *AvrLm4* isolate generated a progeny comprising 89 isolates, among which the ratio of recombinant genotypes is highly indicative of genetic independence between *AvrLm1* and *AvrLm4*. This data, along with previously demonstrated genetic linkage between *AvrLm1* and *AvrLm2* (2), indicates that at least two independent genomic regions of *L. maculans* are involved in avirulence toward *B. napus*. The *AvrLm4* region specifies incompatibility toward cv. Jet Neuf, whereas the *AvrLm1-AvrLm2* region specifies incompatibility

toward cvs. Glacier, Quinta (2), and *Rlm1*-related cultivars. These linkage data are consistent with the occurrence of races A2, A5, and A6 (Table 1) in natural populations (5), whereas field isolates avirulent toward cv. Glacier and virulent toward cv. Quinta have not been described (2,5,19,21,21,23,26). In addition, the lack of recombinant phenotypes in the 10 tetrads and 177 random progeny of two *AvrLm1* × *avrLm1* crosses, when inoculated on the set of cvs. Quinta, Doublol, Vivol, Capitol, and Columbus, suggested either that these cultivars all possess *Rlm1* or resistance genes displaying the same recognition spectrum, or that a cluster of tightly linked *Avr* genes is present in the *AvrLm1* region. Such *Avr* gene clusters have been described in two other plant–fungus interactions, i.e., *Mycosphaerella graminicola*–wheat and *Phytophthora sojae*–soybean (32,33). Finally, segregation distortion at the *AvrLm1* locus was observed in some, but not all, crosses, and could be explained by a lethal interaction between *AvrLm1* and one gene, termed *Dell*, unlinked to *AvrLm1* or to *AvrLm4*. This hypothesis, that still needs to be confirmed, implies that *Dell* could exert a counter-selection facilitating the dissemination of *avrLm1* isolates, therefore reducing the durability of *Rlm1* resistance.

The genetic evidence for a new avirulence gene, *AvrLm4*, suggested the occurrence of a corresponding resistance gene, that reacts to *AvrLm4* isolates, in cv. Jet Neuf and in some Quinta lines. Plant genetic studies indicate the occurrence of a single gene control of resistance to *AvrLm4* in the Quinta line used. This resistance locus, termed *Rlm4*, was found to be distinct from, but linked to, *Rlm1*. It can be hypothesized that *Rlm4* is also present in Quinta S2-V95 and cvs. Jet Neuf and Darmor.bzh. This result is in accordance with genetic studies performed by Delwiche (10) on breeding line R39, which later became cv. Jet Neuf.

Fig. 3. Interaction phenotypes observed when inoculating Quinta S2-V95 and cv. Jet Neuf with progeny of cross 21. White bar, isolates virulent on cv. Jet Neuf inoculated on cv. Jet Neuf; oblique stripes, isolates virulent on Quinta S2-V95 inoculated on Quinta S2-V95; black bars, isolates avirulent on cv. Jet Neuf inoculated on cv. Jet Neuf; horizontal bars, the same isolates, inoculated on Quinta S2-V95. Plants were inoculated at the cotyledon stage. Symptoms were rated 16 to 17 days after inoculation with the IMAScore scale. The data synthesized 170 ratings in the case of cv. Jet Neuf and 325 ratings for Quinta S2-V95.

TABLE 6. Segregation of resistance to *Leptosphaeria maculans* isolates harboring *AvrLm4* or *AvrLm1* in *Brassica napus* line Quinta S1-3636

Isolate	Plants analyzed	Plants per phenotypic class ^a			
		S-S	S-R	R-S	R-R
Parental lines					
Quinta	156	0	0	0	156
Score	36	36	0	0	0
Quinta × Score progeny					
Testcross	177	55	24	30	68
F2	165	16	22	20	107
Control cultivars					
Jet Neuf	25	0	0	25	0
Columbus	23	0	22	0	1

^a Each plant was inoculated with isolates v23.2.1 (*avrLm1-AvrLm4*) and v11.1.2 (*AvrLm1-avrLm4*). The interaction phenotypes were scored 14 days after inoculation. Each plant was classified in one of four interaction phenotype classes: S-S (susceptibility to both isolates), S-R (susceptibility to v23.2.1, resistance to v11.1.2), R-S (resistance to v23.2.1, susceptibility to v11.1.2), and R-R (resistance to both isolates).

TABLE 5. Segregation analysis of interaction phenotypes in a cross between two *Leptosphaeria maculans* isolates differing in their interaction phenotypes on cv. Jet Neuf and *Rlm1*-related cultivars

Isolates	Brassica napus cultivars ^a			No. of isolates	Observed ratio	Expected ratio and putative genotype ^b	Expected ratio and putative genotype ^c
	Capitol, Vivol Columbus, Doublol	Jet Neuf	Quinta S2-V95				
Parental isolates							
21.3.01	C	I	I	...	...	<i>avrLm1 AvrLm4</i>	<i>avrLm1 AvrLm4 Dell</i>
v11.3.6	I	C	I	...	...	<i>AvrLm1 avrLm4</i>	<i>AvrLm1 avrLm4 dell</i>
Interaction phenotypes in progeny							
1	C	I	I	31	34.83%	1/4, <i>avrLm1 AvrLm4</i>	2/6, <i>avrLm1 AvrLm4 dell</i> or <i>Dell</i>
2	I	C	I	15	16.86%	1/4, <i>AvrLm1 avrLm4</i>	1/6, <i>AvrLm1 avrLm4 dell</i>
3	I	I	I	18	20.22%	1/4, <i>AvrLm1 AvrLm4</i>	1/6, <i>AvrLm1 AvrLm4 dell</i>
4	C	C	C	25	28.09%	1/4, <i>avrLm1 avrLm4</i>	2/6, <i>avrLm1 avrLm4 dell</i> or <i>Dell</i>

^a C = compatible interaction; I = incompatible interaction.

^b Expected ratio and putative genotypes for two unlinked *Avr* genes, *AvrLm1* and *AvrLm4*.

^c Expected ratio and putative genotypes for two unlinked *Avr* genes (*AvrLm1* and *AvrLm4*) and one deleterious (*Dell*) gene interacting with *AvrLm1*.

An increasing number of reports indicate that gene-for-gene incompatibility is common in the *L. maculans*-*B. napus* interaction (2,11,28). However, although very numerous specific interactions have been described (20), only few of them were submitted to genetic analysis. One prerequisite for developing genetic studies would be a standardized differential set using well-defined cultivars or DH lines, with characterized resistance genes (28). At present, as exemplified in the current study with cv. Quinta, major inconsistencies can be obtained when using different lines issued from the same cultivar. This is probably due to the heterogeneous nature of the *B. napus* cultivars grown in most parts of the world, as only a few countries require registration of pure lines. At least three different lines can be originated from cv. Quinta, harboring (i) *Rlm1*, (ii) *Rlm1* and *Rlm4* (this study), or (iii) none of these resistance genes (3,19). In consequence, depending on the Quinta line used in the differential set comprising cvs. Westar or Lirabon, Quinta, Glacier, and Jet Neuf, one will be able to discriminate six PGs, if the Quinta line does not harbor *Rlm4* (5,18) (Table 1), but only three PGs if the Quinta line harbors *Rlm1* and *Rlm4*. In the latter case, it is impossible to state whether PG3 isolates possess only *AvrLm1* (A2 isolates), *AvrLm4* (A5 isolates), or both *Avr* genes (A6 isolates) (Table 1). Even when using seed of the original Quinta cultivar, interpretation of the outcome of the interaction with *AvrLm1* isolates may be complicated by (i) the intermediate rating (IC3) usually resulting from this incompatibility reaction and (ii) the possibly high ratio of *rlm1* plants, susceptible to *AvrLm1* isolates (3). Both intermediate resistance and susceptibility ratings can result in an average rating indicative of susceptibility rather than incompatibility and of an erroneous interpretation of the data. In this case, the analysis should be focused on the ratio of resistant to susceptible plants rather than on the average disease index. In the present study, one *Rlm1*-related cultivar, Columbus displayed highly consistent responses either to *AvrLm1* or to *avrLm1* isolates, as compared with other *Rlm1*-related cultivars. Replacement of cv. Quinta by cv. Columbus could be a first step toward the standardization of the differential set.

The data presented here suggest that, due to the heterogeneity of some *B. napus* cultivars and the need for large-scale inoculation tests to determine unequivocally the outcome of one given interaction, molecular markers should be valuable for race identification at both the individual and population levels. For such a purpose, a genetic map of *L. maculans* is currently being constructed to identify markers linked to *AvrLm1* (6). However, as the ultimate marker of race in natural populations is the *Avr* gene itself, one progeny of cross 23, carrying both *AvrLm1* and *AvrLm4*, was chosen to construct a Bacterial Artificial Library and initiate map-based cloning of *Avr* genes (4).

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