Identification of Sources of Resistance to *Xanthomonas campestris* pv. *campestris* in *Brassica napus* Crops

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Abstract

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Black rot, caused by *Xanthomonas campestris* pv. *campestris*, is one of the most important diseases affecting *Brassica* crops worldwide. Nine races have been differentiated in *X. campestris* pv. *campestris*, with races 1 and 4 being the most virulent and widespread. The objective of this work was to identify sources of resistance to races 1 and 4 of *X. campestris* pv. *campestris* in different *Brassica napus* crops, mainly in the underexplored *pabularia* group. Seventy-six accessions belonging to four *B. napus* groups were screened for resistance to two *X. campestris* pv. *campestris* races (1 and 4). The strain of race 1 used in this study was more virulent on the tested materials than the strain of race 4. No race-specific resistance was found to race 1. Most cultivars were susceptible except Russian kale, from the *pabularia* group, which showed some resistant plants and some other accessions with some partially resistant plants. High levels of race-specific resistance to race 4 were found in the *pabularia* group, and great variability within accessions was identified. Three improved cultivars (Ragged Jack kale, Friese Gele, and Valle del Oro) and four landraces (Russian kale, MBG-BRS0037, MBG-BRS0041, and MBG-BRS0131) showed plants with some degree of resistance to both races, which may indicate that race-nonspecific resistance is involved. These accessions could be directly used in breeding programs, either as improved cultivars or as donors of race-specific resistance to other *Brassica* cultivars.

Black rot is one of the most devastating diseases in *Brassica* crops and has worldwide distribution. This bacterial disease is caused by *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson. The seedborne pathogen can survive in crop debris or crucifer weeds and is especially damaging in vegetable *Brassica* crops, mainly in warm and humid climates. In coastal temperate areas of Europe, the disease is not able to kill the plant (35) but it makes V-shaped necrotic lesions on leaf margins, which decrease the quality on the fresh market. Moreover, this disease weakens plant tissues, potentially leading to infection from other pathogens. Infected seed are the primary source of inoculum. When optimal conditions for bacterial development are not present, no symptoms are expressed; however, the pathogen can remain in the vascular system of these asymptomatic plants. Symptoms may be expressed on a susceptible cultivar when conditions favor disease development.

In Spain, which is an important *Brassica* spp. producer and consumer, the presence of *X. campestris* pv. *campestris* was described in the 1970s by Urquijo et al. (29) and later by Ortega and López (18). Urquijo et al. (29) determined the presence of *X. campestris* pv. *campestris* in *Brassica napus*, *B. oleracea*, and *B. rapa* crops from northwest, central, and eastern Spain based on the symptoms of the disease in infected plants. Ortega and López (18) identified *X. campestris* pv. *campestris* from different *B. oleracea* crops (broccoli, Brussels sprout, cabbage, and cauliflower) from the Mediterranean coast of Spain based on morphological and biochemical characteristics of the pathogen and in a pathogenicity test (isolation, growth of the bacterium in pure culture, and reinoculation of susceptible host plants). In northwestern Spain, black rot has been recently identified in several *Brassica* crops (14) and a study referred to the incidence of *X. campestris* pv. *campestris* races is being carried out (*unpublished data*). According to our pre-liminary results, race 4 is the most prevalent in this area, followed by races 1 and 6, although it is possible that other races are also present (races 2, 7, and 9). In this region, the production is mainly by small growers who do not use healthy plant material or disease-free seed; consequently, the pathogen can spread rapidly. No studies involving either the pathogen or screens for resistance in this area have been conducted.

Williams (34) illustrated a number of control measures against this disease; specifically, the use of resistant cultivars and the exploration of new sources of resistance. Currently, disease management is still complicated and limited sources of resistance are available. Recently, the search for new sources of resistance has focused on race-specific resistance since the existence of six races of the pathogen was described by Vicente et al. (31). The authors analyzed 164 *X. campestris* pv. *campestris* isolates from various origins worldwide and established a *Brassica* differential series, thus modifying previous classifications by Kamoun et al. (13) and Ignatov et al. (11). The interaction between differential cultivars and *X. campestris* pv. *campestris* races is explained by using a gene-for-gene model. In addition to monogenic race-specific resistance (4,8,31,32), a quantitative race-nonspecific resistance has been described (3,7,23,25,32). Recently, Fargier and Manceau (5) analyzed 47 representative strains of *X. campestris* and added three new races (races 7, 8, and 9) to the six races described by Vicente et al. (31). It is recognized that races 1 and 4 are the most virulent and widespread, accounting for most black rot cases around the world (6,31).

Sources of resistance to *X. campestris* pv. *campestris* in *Brassica* genomes have been examined by Bain (2), Hunter et al. (10), Guo et al. (8), Nogueira and Dias (17), Ribeiro and Dias (19), Westman et al. (33), Taylor et al. (25), Massomo et al. (16), Tonguç and Griffiths (28), Jensen et al. (12), and Griffiths et al. (6). To the authors' knowledge, the use of resistant cultivars has only had limited success, and available sources with useful levels of resistance are scarce. Moreover, most of this work focused in cabbage (*B. oleracea* var. *capitata*) due to its economical value, while the search for resistance in *B. napus* has been more restricted. Guo et al. (8) identified two highly resistant accessions of *B. napus* (plant introduction [PI] 199947 and PI 199949) but they were reidentified later as *B. carinata* (25). In this study, several crops from the *Brassica*-

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ceae family were evaluated, including 38 accessions of *B. napus*, and resistance to race 1 was not found. Recently, Griffiths et al. (6) evaluated 558 *B. napus* accessions, and incomplete resistance to *X. campestris* pv. *campestris* was found in two accessions from South Korea (PI 469733 and PI 469828).

From the taxonomic point of view, *B. napus* can be divided into four groups according to the edible part of the plant (seed, roots, and leaves) and their main end use (seed oil production and fresh consumption of vegetable parts; Table 1). Four classification groups are as follows: *B. napus* L. *napobrassica* (L.) Reichb. group (synonymous with *B. napus* L. *rapifera* (Metzg) group), which includes rutabaga or swede; *B. napus* L. *napus* group, which includes forage and fodder rape; *B. napus* L. *oleifera* (DC.) group, which includes oilseed rape; and, finally, *B. napus pabularia* (DC.) Reichb. group, which includes leaf rape, rape kale, Siberian kale, and Hanover salad (Table 1). Crops belonging to this last group are popular for human consumption in northwestern Spain and northern Portugal, where they are known as "nabicol" and "couve-nabiça", respectively (20,22). Black rot can be a serious problem in leafy-green vegetable crops belonging to this last group, where leaves must be completely free of any lesion.

Specific resistance to races 1 or 4 has been reported in *B. carinata*, *B. juncea*, *B. nigra*, *B. rapa*, and *B. napus* (6,8,25,27,28). Within *B. napus* species, resistance has been only found in rapeseed (8), in one couve-nabiça cultivar (25), and in two accessions with unknown use collected from South Korea (6). However, an evaluation including the different groups from *B. napus* species has not been performed thus far, and breeding for *X. campestris* pv. *campestris* resistance has not been undertaken in vegetable crops of *B. napus*. Therefore, the objective of this work was to identify sources of resistance to races 1 and 4 of *X. campestris* pv. *campestris* in different *B. napus* crops, mainly in the underexplored *pabularia* group. The relationship between *X. campestris* pv. *campestris* resistance and the different end-use of *B. napus* crops is also discussed.

Materials and Methods

Plant accessions. In total, 76 *B. napus* accessions (47 landraces and 29 commercial and improved cultivars) from different geographic origins were evaluated (Table 2). Accessions were divided based on the end-use of the material following the European *Brassica* database nomenclature (http://documents.plant.wur.nl/cgn/pgr/brasedb/) into four sets: (i) the *napus* group included 10 accessions used as forage crops, (ii) the *oleifera* group included 9 accessions of spring and winter rapeseed used as oilseed crops, (iii) the *napobrassica* group included 9 accessions used as root crops, and (iv) the *pabularia* group included 48 accessions, mostly from Spain, used as leafy-green vegetable crops.

A differential resistance series established by Vicente (31), including cv. Wirosa F_1 (*B. oleracea*, susceptible to both races), doubled-haploid line S×D1 (*B. oleracea*, susceptible to both races), doubled-haploid line Cob60 (*B. napus*, derived from line 14R of cv. Cobra, resistant to race 4), cv. Seven Top Turnip (*B. rapa*, resistant to race 4 although variable), inbred line PIC1 (*B. carinata*, a selection of PI 199947, resistant to races 1 and 4), and doubled-haploid line FBLM2 (*B. juncea*, derived from Florida Broad Leaf Mustard, resistant to races 1 and 4), was used (Table 2).

Four pots containing four plants were sown by accession. Pots were watered by subirrigation using a flood-bench system. Water was delivered from below and was allowed to flood the bench for 30 min, after which excess water was drained. This process was repeated twice per week.

Pathogen isolates. Bacterial isolates of race 1 type strain HRI3811, synonymous with PHW1205 (collected on *B. oleracea* in the United States) and race 4 type strain HRI1279A (collected from *B. oleracea* var. *capitata* in the United Kingdom), typed by Vicente et al. (31), and provided by Warwick HRI, Wellesbourne, UK, were used. Bacterial cultures were grown in bacterial screening media 523 at 30°C during 48 h prior to inoculation. Bacterial culture was diluted in sterile tap water until suspension reached a density of 5×10^8 cfu ml^{-1}. This concentration corresponded to a spectrophotometer reading of 0.5 at a wavelength of 600 nm.

Inoculations and disease resistance assays. Sixteen plants per accession were inoculated 4 to 5 weeks after planting. Two different leaves of the same plant were used for inoculations. The third leaf was inoculated with race 1 and the fourth leaf with race 4. After inoculation, greenhouse conditions were maintained at 14 h of light, mean temperature 24°C at night and 28°C during the day and relative humidity between 80 and 90%. Multiple needles were used for inoculation according to Lema-Márquez et al. (15). Plants were rated for disease reaction twice, at 14 and 21 days after inoculation. Disease evaluations were made on a visual 1-to-9 rating scale based on the relative lesion size, where 1 = no visible symptoms; 3 = an isolated, diffuse chlorotic zone around necrotic lesions with blackened veins of <25% of the inoculated area; 5 = chlorotic zones around necrotic lesions with blackened veins occupying <50% of the inoculated area; 7 = complete chlorosis, with black veins and usually necrotic centers of the inoculated area and chlorotic or necrotic lesions extending beyond the inoculated area; and 9 = severely diseased, typical leaf edge damages, with large V-shaped chlorotic lesions presenting areas with blackened veins. For each inoculated leaf, two disease scores were taken (14 and 21 days post inoculation). Accessions with mean disease scores from 1 to 3 were considered resistant, 4 to 6 were partially or moderately resistant, and 7 to 9 were considered susceptible.

Statistical analyses. Analyses of variance were performed for disease score and were combined across races by using the general linear model procedure of SAS (21). Accessions and races were considered as fixed effects whereas replications (plants within accessions) were considered as a random factor. Comparisons of means were performed for each trait by using Fisher's protected least significant difference at the 0.05 level of probability (24). The sums of squares for accessions were orthogonally divided into groups according to their final use.

Results

The combined analysis of variance across races showed that accessions and races were significantly different ($P \le 0.05$) for disease scores (*data not shown*). The race–accession interaction was also significant, indicating that accessions performed distinctly against two races involved in race-specific resistance. Because this interaction was significant, individual analyses of variance for each race were conducted (*data not shown*). Therefore, results will be discussed separately for each race. The ranges of resistance responses and mean disease score of 76 accessions of *B. napus* inoculated with isolates of two *X. campestris* pv. *campestris* races (races 1 and 4) shown by individual accessions are in Table 2. The results of the resistance tests by taxonomic group are shown in Table 3.

Race 1. In the analyses of variance, significant differences were found for disease score among the 76 *B. napus* accessions evaluated, among the four taxonomic groups defined a priori (*napobrassica*, *napus*, *oleifera*, and *pabularia*) and among accessions within each taxonomic group. No race-specific resistance to race 1 was

Table 1. Description of the main taxonomical groups included in *Brassica napus*

Group	Crops, use	Part used	End use	Consumption
napobrassica or *rapifera*	Rutabaga or swede	Root	Fresh	Animal and human
napus	Forage or fodder rape	Leaf	Fresh	Animal
oleifera	Oilseed rape or rape	Seed	Oil	Human
pabularia	Leaf rape, rape kale, Siberian kale, and Hanover salad	Leaf	Fresh	Animal and human

found in the genotypes evaluated. Accessions from the *napus* group were slightly different from other groups, although all of them were susceptible based on their values for mean disease score (Table 3). Russian kale, a rape kale cultivar, included into the *pabularia* group, showed some degree of resistance to this race, having 25% resistant plants. This cultivar differed significantly from all other accessions evaluated and had the lowest mean disease score (5.3), ranging from 2 to 9 (Table 2). Three cultivars—Friese Gele (a commercial rutabaga), Ragged Jack kale (an improved forage rape), and Valle del Oro (a commercial winter oilseed rape)—along with three nabicol landraces from north-

western Spain (MBG-BRS0037, MBG-BRS0041, and MBG-BRS0131, included into the *pabularia* group) presented some plants with moderate resistance with disease scores ranging from 5 to 6 (Table 2).

Race 4. The analyses of variance indicated differences in disease score according to accessions, groups, and accessions within each corresponding group. Differences among groups were greater than those found for race 1. A high level of race-specific resistance to race 4 was found in the *pabularia* group. This group had the highest number of accessions, including more than one resistant plant (42 of 48), and showed the lowest mean disease score (3.4), being

Table 2. Description and percentage of resistant plants and mean and range of disease score for *Xanthomonas campestris* pv. *campestris* races 1 and 4 for 76 *Brassica napus* accessions for resistance to *Xanthomonas campestris* pv. *campestris* conducted in Pontevedra (Spain) in 2008

| | | | | | | | | | Disease score (1–9)[s] | | |
| | | | | | Resistant (%) | | Race 1 | | Race 4 | |
Accession no.[t]	Status[u]	Accession name	Origin	Source[v]	Race 1	Race 4	Range	Mean	Range	Mean
Differential accessions										
BE0116	AC	S×D$_1$ (*B. oleracea*)	United Kingdom	WHRI	0	0	9	9.0	7–9	8.3
BE0117	C	Seven Top Turnip (*B. rapa*)	United Kingdom	WHRI	0	82	9	9.0	1–9	2.6
BE0118	AC	Cob60 (*B. napus*)	United Kingdom	WHRI	0	100	9	9.0	1–3	1.8
BE0119	AC	FBLM2 (*B. juncea*)	United Kingdom	WHRI	100	100	1–2	1.1	1–2	1.2
BE0120	AC	PIC 1 (*B. carinata*)	United Kingdom	WHRI	100	100	1	1.0	1–3	1.1
BE0121	C	Wirosa F$_1$ (*B. oleracea*)	Spain	Germisem	0	0	9	9.0	9	9.0
Forage crops[w]										
BE0051	C	Colza Rocalba	Spain	MBG	0	70	7–8	7.2	1–7	3.2
BE0130	AC	Lair	United Kingdom	CGN	0	73	7–9	8.6	1–7	3.2
BE0132	AC	SjG (6)	The Netherlands	CGN	0	56	7–9	8.5	1–8	3.6
BE0128	AC	Zls (4)	The Netherlands	CGN	0	23	8–9	8.9	2–7	4.7
BE0131	AC	O.W. (1)	The Netherlands	CGN	0	39	7–9	8.6	2–9	4.8
BE0055	AC	Mara	The Netherlands	CGN	0	26	7–9	8.5	2–8	4.9
BE0054	AC	Vysokopol'skij 12	Russia	CGN	0	13	7–9	8.3	2–9	6.3
BE0053	AC	Ragged Jack kale	New Zealand	CGN	0	12	6–9	7.9	2–9	7.2
BE0057	AC	Potem	France	CGN	0	8	7–9	8.7	2–9	7.8
BE0129	AC	Ramon	The Netherlands	CGN	0	0	9–9	9.0	7–9	8.9
Mean	…	…	…	…	…	…	…	8.4	…	5.4
CV	…	…	…	…	…	…	…	8.6	…	36.1
LSD (0.05)	…	…	…	…	…	…	…	0.5	…	1.3
Leafy vegetable crops[x]										
MBG-BRS0092	L	Nabicol	Spain	MBG	0	100	7–9	8.7	1–2	1.2
MBG-BRS0085	L	Nabicol	Spain	MBG	0	100	7–9	8.7	1–3	1.3
MBG-BRS0087	L	Nabicol	Spain	MBG	0	100	7–9	8.9	1–2	1.3
MBG-BRS0088	L	Nabicol	Spain	MBG	0	100	7–9	8.8	1–2	1.4
MBG-BRS0105	L	Nabicol	Spain	MBG	0	100	9	9.0	1–3	1.4
MBG-BRS0107	L	Nabicol	Spain	MBG	0	100	7–9	8.8	1–3	1.4
MBG-BRS0073	L	Nabicol	Spain	MBG	0	100	7–9	8.0	1–3	1.5
MBG-BRS0543	L	Nabicol	Spain	MBG	0	100	7–9	8.4	1–3	1.5
MBG-BRS0028	L	Nabicol	Spain	MBG	0	100	9	9.0	1–2	1.6
MBG-BRS0090	L	Nabicol	Spain	MBG	0	100	9	9.0	1–3	1.7
MBG-BRS0044	L	Nabicol	Spain	MBG	0	100	9	9.0	1–3	1.7
MBG-BRS0034	L	Nabicol	Spain	MBG	0	93	7–9	8.4	1–4	1.8
MBG-BRS0041	L	Nabicol	Spain	MBG	0	100	5–9	8.6	1–3	1.8
MBG-BRS0110	L	Nabicol	Spain	MBG	0	100	7–9	8.2	1–3	1.8
MBG-BRS0113	L	Nabicol	Spain	MBG	0	100	7–9	8.9	1–3	1.8
MBG-BRS0356	L	Nabicol	Spain	MBG	0	100	9	9.0	1–3	1.8
MBG-BRS0378	L	Nabicol	Spain	MBG	0	100	9	9.0	1–3	1.9
MBG-BRS0054	L	Nabicol	Spain	MBG	0	100	9	9.0	1–3	2.1
MBG-BRS0056	L	Nabicol	Spain	MBG	0	93	7–9	8.2	1–5	2.1
MBG-BRS0048	L	Nabicol	Spain	MBG	0	88	9	9.0	1–5	2.2
MBG-BRS0065	L	Nabicol	Spain	MBG	0	100	8–9	8.9	1–3	2.2
MBG-BRS0039	L	Nabicol	Spain	MBG	0	94	9	9.0	1–4	2.3
MBG-BRS0014	L	Nabicol	Spain	MBG	0	100	7–9	8.9	1–3	2.4

(continued on next page)

[s] Disease score based on a rating scale from 1 to 9 (1 = plants without lesions and 9 = severely diseased plants), where 1 to 3 = resistant, 4 to 6 = moderately or partially resistant, and 7 to 9 = susceptible. Range indicates minimum and maximum mean disease score.

[t] CV = coefficient of variation, LSD = least significant difference among accessions within groups, and LSD all = LSD among all accessions.

[u] AC = advanced or improved cultivar, C = commercial cultivar, and L = traditional or local cultivar or landrace.

[v] Seed sources: CGN = Center for Genetic Resources, Wageningen, The Netherlands; COMAV = Centro de Conservación y Mejora de la Agrodiversidad Valenciana, Valencia, Spain; WHRI = Warwick - Horticulture Research International, Wellesbourne, UK; MBG = Misión Biológica de Galicia (CSIC), Pontevedra, Spain.

[w] Fodder or forage rape = *napus* group.

[x] Couve-nabiça, Hanover kale, leaf-rape, nabicol, rape kale, or Siberian kale = *pabularia* group.

[y] Oilseed rape or rapeseed = *oleifera* group.

[z] Rutabaga or swede = *napobrassica* or *rapifera* group.

significantly lower than those observed in *napus* (5.4), *oleifera* (6.2), and *napobrassica* (8.0) groups (Table 3).

Based on the mean disease score of each accession, leafy vegetable crops showed the highest percentage of resistant accessions with respect to the total accessions evaluated within each group (73%), followed by forage (20%), oilseed (11%), and root vegetable (0%) crops (Table 2). Approximately 50% of total accessions were resistant to race 4, having a mean disease score less than 4 on a 1-to-9 rating scale (Table 2). Of these, 19 accessions, all belonging to *pabularia* group, were uniformly resistant. In the four groups, some accessions classified as susceptible or as moderately resistant were highly variable. In fact, a high proportion of accessions evaluated (40 of 76) produced variable resistance reactions, including both resistant and susceptible plants (Table 2).

In the *pabularia* group, nabicol accessions named MBG-BRS0028, MBG-BRS0087, MBG-BRS0088, and MBG-BRS0092 were more resistant to race 4, with all plants having disease scores between 1 and 2 (Table 2). Within the same group, the rape kale cv. Russian kale, found to be partially resistant to race 1, can also be classified as resistant to race 4 based on its disease score, although levels of resistance were highly variable. Three commercial cultivars included in this group were also resistant or moderately resistant to race 4, although resistance was highly variable. Apart from the *pabularia* group, two *napus* cultivars (colza Rocalba and Lair)

Table 2. (*continued from preceding page*)

| | | | | | Resistant (%) | | Disease score (1–9)[s] | | | |
| | | | | | | | Race 1 | | Race 4 | |
Accession no.[t]	Status[u]	Accession name	Origin	Source[v]	Race 1	Race 4	Range	Mean	Range	Mean
MBG-BRS0068	L	Nabicol	Spain	MBG	0	92	9	9.0	1–9	2.5
MBG-BRS0434	L	Nabicol	Spain	MBG	0	93	7–9	8.3	2–7	2.8
MBG-BRS0035	L	Nabicol	Spain	MBG	0	83	8–9	8.9	1–8	3.1
MBG-BRS0346	L	Nabicol	Spain	MBG	0	82	9	9.0	1–7	3.1
MBG-BRS0388	C	Couve-nabiça	Portugal	MBG	0	94	9	9.0	2–9	3.1
MBG-BRS0423	L	Nabicol	Spain	MBG	0	75	7–9	7.5	2–4	3.2
MBG-BRS0542	L	Nabicol	Spain	MBG	0	71	7–9	8.2	2–7	3.2
MBG-BRS0582	L	Nabicol	Spain	MBG	0	74	7–9	8.8	1–9	3.2
MBG-BRS0373	C	Couve-nabiça	Portugal	MBG	0	81	9	9.0	2–8	3.3
MBG-BRS0029	L	Nabicol	Spain	MBG	0	75	7–9	8.7	1–9	3.4
BE0002	C	Nabicol Rocalba	Spain	MBG	0	43	7–9	8.6	2–7	3.5
BE0085	L	Russian kale	United Kingdom	WHRI	25	50	2–9	5.3	2–7	3.5
MBG-BRS0541	L	Nabicol	Spain	MBG	0	75	9	9.0	2–7	3.9
MBG-BRS0374	L	Nabicol	Spain	MBG	0	60	9	9.0	1–9	4.1
MBG-BRS0134	L	Nabicol	Spain	MBG	0	47	9	9.0	2–7	4.5
MBG-BRS0061	L	Nabicol	Spain	MBG	0	38	7–9	8.2	1–8	5.2
MBG-BRS0037	L	Nabicol	Spain	MBG	0	33	6–9	8.1	2–9	5.9
MBG-BRS0131	L	Nabicol	Spain	MBG	0	33	5–9	7.8	2–9	5.9
MBG-BRS0337	L	Nabicol	Spain	MBG	0	13	9	9.0	2–9	6.6
MBG-BRS0465	L	Nabicol	Spain	MBG	0	7	7–9	7.9	2–9	6.9
MBG-BRS0333	L	Nabicol	Spain	MBG	0	0	9	9.0	7–9	7.5
MBG-BRS0329	L	Nabicol	Spain	MBG	0	7	9	9.0	3–9	8.1
MBG-BRS0079	L	Nabicol	Spain	MBG	0	0	7–9	8.9	7–9	8.2
MBG-BRS0583	L	Nabicol	Spain	MBG	0	0	9	9.0	7–9	8.5
MBG-BRS0063	L	Nabicol	Spain	MBG	0	0	9	9.0	7–9	8.8
Mean	…	…	…	…	…	…	…	8.7	…	3.4
CV	…	…	…	…	…	…	…	7.8	…	44.8
LSD (0.05)	…	…	…	…	…	…	…	0.5	…	1.1
Oilseed crops[y]										
BE0114	C	Saphir	Spain	MBG	0	95	7–9	8.8	1–7	2.5
BE0052	C	Valle del Oro	Spain	MBG	0	47	6–9	8.6	2–8	3.9
BE0059	AC	Petranova	Germany	CGN	0	56	7–9	8.6	1–9	4.1
MBG-BRS0392	L	Kapusta zepak	Poland	MBG	0	50	9	9.0	2–9	4.8
BE0115	C	Jura	Spain	MBG	0	9	7–9	8.7	3–9	6.8
BE0068	AC	Belinda	The Netherlands	CGN	0	20	7–9	8.2	2–9	7.1
BE0049	C	Express	Germany	MBG	0	13	7–9	8.4	2–9	7.6
BE0050	C	Quest	Canada	MBG	0	0	8–9	8.9	8–9	8.9
BE0136	C	Topas	Sweden	MBG	0	0	9	9.0	9	9.0
Mean	…	…	…	…	…	…	…	8.7	…	6.1
CV	…	…	…	…	…	…	…	7.6	…	29.0
LSD (0.05)	…	…	…	…	…	…	…	0.5	…	1.3
Root vegetable crops[z]										
BE0125	AC	York	Germany	CGN	0	41	7–9	8.3	1–9	4.8
BE0064	AC	Friese Gele	The Netherlands	CGN	0	33	5–9	7.1	2–9	5.3
BE0001	C	Colinabo Rocalba	Spain	MBG	0	6	7–9	8.6	2–9	7.4
BE0124	AC	York/Wilhelms-burger Hybrid	Germany	CGN	0	0	7–9	8.3	7–9	8.4
BE0058	AC	Lollo	Germany	CGN	0	0	7–9	8.7	7–9	8.5
BE0126	AC	–	New Zealand	CGN	0	0	7–9	8.8	7–9	8.8
MBG-BRS0023	C	Colinabo amarillo cuello verde	Spain	MBG	0	0	9	9.0	7–9	8.8
BE0127	AC	Hollandse Gele Roodkop	The Netherlands	CGN	0	0	7–9	8.8	7–9	8.9
BE0123	L	Nabo Murcia	Spain	COMAV	0	0	9	9.0	9	9.0
Mean	…	…	…	…	…	…	…	8.6	…	8.0
CV	…	…	…	…	…	…	…	7.7	…	17.9
LSD (0.05)	…	…	…	…	…	…	…	0.5	…	1.0
LSD all (0.05)	…	…	…	…	…	…	…	0.5	…	1.1

were resistant to race 4, showing disease scores of 3.2. Again, the resistance test in these genotypes showed a variable reaction, including both resistant and susceptible plants. In the *oleifera* group, only the commercial rapeseed cv. Saphir was highly resistant to race 4 (95% of resistant plants showing a disease score of 2 or 3). No resistance to race 4 was found in the *napobrassica* group, even though most accessions evaluated were improved cultivars. The only exceptions were Friese Gele and York, which exhibited moderate resistance, with a mean disease score around 5 in the 1-to-9 rating scale (Table 2).

Races 1 and 4. Some accessions showed plants with either resistance or partial resistance to races 1 and 4. Among them, Russian kale had two plants with resistance to races 1 and 4 and another two plants partially resistant to both races. Other promising cultivars were Friese Gele (from *napobrassica* group), which showed three plants with partial resistance to race 1 and resistance to race 4; Ragged Jack kale (from *napus* group), which presented two plants with partial resistance to race 1 and resistance to race 4; and Valle del Oro (a winter oilseed rape from Spain, included in the *oleifera* group), which had one plant with partial resistance for both races. Nabicol accessions MBG-BRS0037, MBG-BRS0041, and MBG-BRS0131, belonging to the *pabularia* group, included some plants with partial resistance to race 1 and both resistance and partial resistance to race 4. In this case, race-nonspecific resistance could be involved and must be further studied.

Discussion

Black rot disease is particularly serious in leafy-green vegetable crops used for human consumption and belonging to the *pabularia* group. It would seem reasonable that this group showed the highest level of resistance because any lesion on the leaves is not acceptable. When the edible parts of the plant are seed or roots, as for accessions in the *oleifera* and *napobrassica* groups, this disease will probably not affect the marketable yield. However, it should be noted that black rot is an important constraint for these crops in early stages of the plant development. Infected young seedlings can show dwarfing, erratic growth, and one-sided biased, withered leaves (1). Tissue infected with *X. campestris* pv. *campestris* can serve as a point of entry for other fungal and bacterial organisms, further limiting seed and root yields.

No race-specific resistance to race 1 was found in the accessions evaluated. Even though strong and uniform resistance for race 1 was not found, the finding of variable reaction for resistance in some accessions is very relevant because resistance to race 1 is very unusual. As was already reported in several works (23,25), resistance to race 1 is very uncommon and has been found only in *Brassica* crops carrying the B genome (*B. carinata*, *B. juncea*, and *B. nigra*). These accessions must be further evaluated to find resistant plants that can be used in breeding programs when resistance could be fixed and available (i.e., through doubled-haploids techniques).

A high degree of variability for resistance was found within each accession, producing a high proportion of variable reactions. A similar result was found by Westman et al. (33), highlighting the variability for resistance to *X. campestris* pv. *campestris* found in *B. nigra* genotypes. Taylor et al. (25) also found a high intravarietal variability for resistance to *X. campestris* pv. *campestris* in different *B. oleracea* crops. As was discussed by Vicente et al. (31), extreme reactions for resistance to *X. campestris* pv. *campestris* within an accession could be explained when resistance is controlled by a single gene or if a mixture of genotypes within a cultivar occurs.

According to Vicente (30), who studied the identification and distribution of *X. campestris* pv. *campestris* races in Portugal, it could be possible to find race 4 in northwestern Spain, where most of the *pabularia* accessions included in this study were collected. Local vegetable crops such as nabicol have been sown for generations in the same location, favoring disease. The high level of resistance to *X. campestris* pv. *campestris* race 4 found in most of the nabicol landraces could be partly explained by the selection practiced by growers throughout the years, rejecting abnormal and diseased plants for both human consumption and seed production.

Most of the *B. napus* commercial and improved cultivars evaluated in this work belong to the *oleifera*, *napobrassica*, and *napus* groups. The low resistance level to both races found in *B. napus* commercial and improved cultivars from these three groups suggests that breeding for *X. campestris* pv. *campestris* resistance has not been a priority in those crops. Breeding in *B. napus* has been mainly focused on oilseed quality and fresh yield but not on disease resistance. Furthermore, breeding for *X. campestris* pv. *campestris* resistance in *Brassica* crops has had limited success, mainly due to the low number of available sources of resistance and the difficulties in transferring that resistance among *Brassica* spp. (26). However, resistance to race 4 found in commercial leafy vegetable *B. napus* crops from the *pabularia* group suggests that some type of indirect breeding for resistance to *X. campestris* pv. *campestris* could have happened, because damaged leaves are not allowed in the fresh market.

In the gene-for-gene model established by Vicente et al. (31), resistance to race 1 is conferred by the R1 gene, possibly originating from the B genome of *B. nigra*, and resistance to race 4 is conferred by the R4 gene, possibly originating from the A genome of *B. rapa*. This is consistent with the hypothesis that, in our *B. napus* accessions (AC genome), the amphidiploid species derived from interspecific hybridization between *B. oleracea* (C genome) and *B. rapa* (A genome), resistance to race 4 is present and resistance to race 1 is practically absent (mean disease scores to races 4 and 1 were 5.8 and 8.6, respectively). In the model proposed by Vicente et al. (31) and Taylor et al. (25), in the AC genome of *B. napus*, the R1 gene conferring resistance to race 1 is not present. This supports the theory that the resistance found in some accessions could be due to a race-nonspecific resistance. However, inoculation with

Table 3. Frequency of resistant accessions and mean disease score for *Xanthomonas campestris* pv. *campestris* races 1 and 4 of 76 *Brassica napus* accessions included in this study and grouped according to their main end use

Use	Total no.[x]	No. of resistant accessions[v]		Disease score (1–9)[w]	
		Race 1	Race 4	Race 1	Race 4
Forage crops (*napus* group)	10	0	8	8.4 b	5.4 c
Leafy vegetable crops (*pabularia* group)	48	1	42	8.7 a	3.4 d
Oilseed crops (*oleifera* group)	9	0	6	8.7 a	6.1 b
Root vegetable crops (*napobrassica* group)	9	0	2	8.6 a	8.0 a
Mean	…	…	…	8.6	4.6
CV[y]	…	…	…	7.9	35.0
LSD (0.05)[z]	…	…	…	0.16	0.51

[v] Accessions including more than one resistant plant.

[w] Disease score based on a rating scale from 1 to 9, where 1 = plants without lesions and 9 = severely diseased plants. Means in columns followed by the same letter are not significantly different at $P \leq 0.05$.

[x] Total number of accessions.

[y] Coefficient of variation.

[z] Least significant difference.

other *X. campestris* pv. *campestris* races, particularly race 6, must be done to verify this finding. In a recent study performed by Soengas et al. (22), based on morphologic and agronomic traits of different *B. napus* crops, the Russian kale landrace was grouped apart from other accessions. This cultivar was completely different from other *B. napus* accessions evaluated, showcasing characteristic leaf morphology with divided and wavy leaf margins. A relationship between leaf morphological attributes and *X. campestris* pv. *campestris* resistance must be further investigated.

A comparison of black rot resistance among crops within *B. napus* species was achieved for the first time, emphasizing the search for sources of resistance in leafy vegetable crops. In this study, we suggest that different selection criteria applied on *B. napus* crops according to their use could have led to an indirect selection for resistance to *X. campestris* pv. *campestris*. Because resistance to race 1 seems to be absent in the *B. napus* genome, the moderate levels of resistance found in some cultivars turn them into promising sources of resistance. Because the inoculation method (juvenile wound inoculation by using a concentrated bacterial suspension) and conditions (favorable greenhouse environment for the *X. campestris* pv. *campestris* development) used in this study were severe, higher levels of resistance can be expected in the field in those moderately resistant cultivars (7). Resistance to race 4 was found in the *pabularia, napus*, and *oleifera* groups, mainly in local nabicol accessions belonging to the first group. Thus, the potential value of nabicol accessions to improve resistance to race 4 in *Brassica* crops is noteworthy, especially in those areas where vegetable *B. napus* crops are grown (i.e., the Atlantic coast of Europe) and black rot causes leaf damage, consequently decreasing crop quality and value. These accessions could be directly used in breeding programs to obtain resistant cultivars or used as donors of race-specific resistance. Related *Brassica* spp. have been used to introgress disease resistance and other agronomic and horticultural traits into *B. oleracea,* even though chromosome numbers differ, by using different approaches such as protoplast fusion (9) or embryo rescue (27). This is due to the high tolerance of aneuploidy and common ancestry in diploids. Resistant nabicol accessions could be used to transfer resistance to race 4 into other vegetable *Brassica* crops that share a common genome with *B. napus*, where resistance to races 1 and 4 of *X. campestris* pv. *campestris* is not present. Additionally, if new resistance genes are implicated in the response to black rot in our materials that differ from those detected by Griffiths et al. (6), then efforts can be made to complement and pyramid *Brassica* sources of black rot resistance to obtain durable resistance to *X. campestris* pv. *campestris*. Further theoretical and practical applications with these materials could focus on inheritance and mechanism of resistance studies and development of molecular markers for black rot resistance.

Acknowledgments

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