

Full Length Research Paper

Evaluation of a core collection of *Brassica rapa* vegetables for resistance to *Xanthomonas campestris* pv. *campestris*

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Accepted 27 September, 2010

The identification of juvenile resistance to isolates Xcc512 (race 1) and Xcc524 (race 4) of *Xanthomonas campestris* pv. *campestris* (Xcc) was performed in a screening of *Brassica rapa* vegetable core collection with 210 accessions representing the genetic and geographic diversity of the specie. Twenty-four plants per accession were screened against the isolates Xcc524 (race 4) and Xcc512 (race 1) at the third true-leaf. The conventional rating criteria of the mean Disease Index (DI) and the percentage of resistant seedlings (%R) were compared and adopted as the criteria to rank the accessions for their interest as sources of resistance. A great variety of reactions was found between and within accessions of the *B. rapa* core collection, ranging from complete resistance to full susceptibility. Sources of resistance to isolate Xcc524 were found among the broccoleto, choy-sum, and Chinese cabbage gene pools. The turnip cultivar type group was the only one where the number of resistant accessions was significantly lower than expected. One hundred and twenty one accessions presented at least 20% of resistant plants to this isolate. Thirty-six accessions presented more than 70% of resistant plants to this isolate and so, they should be considered as potential and useful sources for direct use in breeding programs of Xcc resistance. In contrast, from the 210 accessions tested with isolate Xcc512 (race 1), it was found that 195 (92%) were completely susceptible with all the plants rated in the 7 to 9 classes and only one accession, the Chinese cabbage “Chang Puh Early” (B-31), presented 25% of resistant plants. This resistance is rare. Besides, other accessions that were screened such as Chinese cabbage “Chang Puh Medium Early” (B-32), and broccoletos “Cima di rapa tardivo di Marzo” (K-9011) and “Tardivo” (HRI-5213), are presented respectively 19, 16, and 16%. All these four accessions are new sources of resistance and they can be exploited in breeding programmes for blackrot resistance with race 1 isolates. Since they exhibit resistance to the two major worldwide Xcc races (1 and 4), they can be used to transfer this resistance through interspecific hybridization to other *B. rapa* morphotypes and to *Brassica oleracea*, where there are no cultivars with complete resistance to these Xcc races.

Key words: *Brassica rapa*, turnip, broccoleto, oriental vegetables, disease resistance, diversity, host–pathogen interaction, black rot.

INTRODUCTION

Brassica rapa is an important vegetable crop and to a minor extent, also an oil seed crop. *B. rapa* vegetables are consumed worldwide and provide a large proportion of the daily food intake in many regions of the world. There is a large variation in the plant organs that are consumed (leaves, roots, and inflorescences), which has resulted in the selection of different morphotypes. Because *B. rapa* has been

cultivated for many centuries in different parts of the world, the variability within the specie has increased as a result of ongoing breeding. Based primarily on the organs used and secondly on their morphological appearance, a number of major cultivar type groups, which have been given subspecies names, can be distinguished (Diederichsen, 2001). The recent studies suggest that cultivated subspecies of *B. rapa* originated independently in two different centers: Europe with turnip and broccoleto types and Asia with the oriental leafy and flowering vegetable types such as Chinese cabbage,

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pak-choi, choy-sum, mizuna and tsa-tsai (Song et al., 1988; Chen et al., 2000; Guo et al., 2002). Oriental leaf vegetable types are among the ten most important consumed vegetables in the world and have been introduced with success in Europe, America and Australia contributing to the diversification of traditional *Brassica* vegetable production systems.

Black rot of crucifers caused by *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson (Xcc) is considered to be one of the most important diseases of crucifers worldwide (Williams, 1980). It is usually the major disease of *Brassica* crops in tropical regions and warm and humid areas. Xcc is seed-borne and also can overwinter on plant debris, crucifer weeds and wild relatives of cultivated brassicas (Cook et al., 1952; Schaad and Dianese, 1981). The bacteria develop a systemic infection in susceptible hosts, penetrating the plant through the hydathodes or wounds and it spreads easily under favourable conditions to nearby plants by rain splash (Williams, 1980). The pathogen invades the xylem and colonizes the mesophyll. The symptoms of black rot include marginal leaf chlorosis, necrosis and darkening of leaf veins and vascular tissue within the stem. Wilting and necrosis also occur as the disease progresses. Control of the disease is difficult and it is usually attempted through the use of healthy planting material (seeds or transplants) and the elimination of other potential inoculum sources such as infected crop debris and cruciferous weeds (Taylor et al., 2002). The disease has a wide geographical distribution and has been identified in all the continents where cruciferous are cultivated. It is particularly destructive to *Brassica oleracea* vegetables causing reduction in yield and quality (Williams, 1980), but it can also attack all other *Brassica* spp. and other cruciferous crops, cruciferous weeds and ornamentals. In *B. rapa*, the disease can be serious in turnip and turnip greens (Vicente 2004) and it has also been reported in Chinese cabbage and other oriental *B. rapa* vegetable crops (Schaad and Thaveeschai, 1983; Ignatov et al., 2000a).

Disease resistance could potentially provide low cost and sustainable means of controlling black rot in *B. rapa* vegetable. However, breeding black rot resistant cultivars is complicated by the existence of at least six races (1 to 6) of the pathogen (Vicente et al., 2001). Worldwide, races 2, 3, 5 and 6 are rare, and races 1 and 4 appear to be the most important (Vicente et al., 2001). Therefore, resistance to both of these two races is a minimum requirement to be of value in controlling black rot. In order for the development of a breeding program to incorporate resistance to Xcc in *B. rapa* and other vegetable brassicas, it is essential to know the variability of response of this pathogen present in the *B. rapa* gene pool. Despite the fact that a large gene pool offers a great variability for disease resistance, it still remains largely under exploitation. There is a dearth of written

information regarding the variability of resistance of *B. rapa* vegetables to the bacterial disease caused by Xcc. Recently, Griffiths et al. (2009) in a screening of collection of different crucifers, identified 5 resistant accessions of *B. rapa* with oilseed plant growth type (PI633154, A9285, PI340208, PI597831, and PI173847) as resistant to Xcc races 1 and 4. Ignatov et al. (1998, 2000b) described a high level of resistance to race 4 and rare resistance to race 1 in Indian oilseed and Japanese turnips, but found that Chinese cabbage, pak-choy and European turnips were more susceptible. The *B. rapa* Chinese cabbage accession B162 was also previously reported to pose quantitative resistance to *X. campestris* pv. *campestris* (Guo et al., 1991). This was confirmed by Taylor et al. (2002) who found that this Chinese cabbage accession had potential broad spectrum resistance to type isolates of Xcc races of the pathogen. Soengas et al. (2007) study the genetics of broad spectrum resistance in this Chinese cabbage B162, using QTL analysis of resistance to races 1 and 4 of the pathogen. Resistance to both races was correlated and a cluster of highly significant QTL, that explained 24 to 64% of the phenotypic variance, was located on A06. Two additional QTLs for resistance to race 4 were found on A02 and A09. Markers closely linked to these QTL could assist in the transfer of the resistance into different *B. rapa* cultivars or into *B. oleracea*.

The large *B. rapa* gene pool remains largely unexploited regarding the variability against Xcc. Therefore, the identification of sources of resistance is the necessary step for the development of resistant cultivars, which will contribute to a more sustainable production of these *Brassica* vegetables by reducing the levels of agrochemicals application. Also, these *B. rapa* accessions can become important resistance sources, since they present less barriers to the transfer of Xcc to *B. oleracea*, than those encountered in transferring resistance from *B. juncea* and *B. carinata* accessions (Tonguc et al., 2003; Tonguc and Griffiths, 2004). If interspecific hybrids with *B. oleracea* can be successfully generated, we will contribute to the reduction of the world limits on the production of vegetable brassicas by Xcc.

The aim of the present study is to evaluate a core collection of *B. rapa* at the juvenile stage in order to quantify the presence, the frequency and the potential use of resistant accessions for introgression of Xcc resistance. To achieve this, a *B. rapa* core collection from five seed banks was inoculated and subsequently evaluated for resistance, with two isolates belonging to race 4 and race 1 of Xcc.

MATERIALS AND METHODS

Screening of the germplasm collection

A selection of 210 accessions from a *B. rapa* germplasm collection

Table 1. Composition of the *B. rapa* core collection originated from five seed banks.

Subspecies of <i>B. rapa</i>	Cultivar group name	Nr. of accessions
<i>Chinensis</i>	Pak-choy	26
<i>Parachinensis</i>	Choy-sum, flowering pak-choy	15
<i>Pekinensis</i>	Chinese cabbage	56
<i>Rapifera</i>	Turnip	72
<i>Utilis</i>	Broccoleto	36
<i>Narinosa</i>	Tsa-tsai	2
<i>Nipposinica</i>	Mizuna	3

Table 2. *Xanthomonas campestris* pv. *campestris* isolates used in this study.

Isolate ¹		Host ²	Race ³
Code	Origin		
Xcc524	Patacão, Faro, PRT	Cauliflower (Bo)	4
Xcc512	Geraldes, Peniche, PRT	Portuguese kale (Bo)	1
Xcc501	Santa Cruz, Torres Vedras, PRT	Portuguese kale (Bo)	1
PHW117	Louisiana, USA	Cabbage (Bo)	1

¹Isolates Xcc501 and PHW117 were only used for screening of Chinese cabbage "Hsia Sheng" (B-162).

²Bo=*Brassica oleracea*

³The determination of the races was based on the differential cultivar screenings using Vicente et al. (2001) classification. The isolates belong to the same races using the classification of Kamoun et al. (1992).

(Table 1), representing a range of morphotypes, its uses and their ecogeographical origins obtained from five seed banks, was screened with two isolates of *X. campestris* pv. *campestris* belonging to races 4 (Xcc524) and 1(Xcc512) (Table 2). Twenty four plants of each accession were screened per isolate in a complete randomised block design with 3 replications of 8 plants per accession. A susceptible turnip "Globo" was used as control. Seeds were sown 0.5 cm deep in seedling trays, with 8 × 11 modules/tray and module size of 40 × 40 × 60 mm, filled with Levington F2® (Scotts Company) compost and covered superficially with a thin layer of vermiculite. In each tray, only six accessions were sown to allow the growth of plants and to "facilitate" the inoculation procedure. Trays were placed in a growth room Fitoclima 16.000 E® at a temperature of 22 ± 1°C, 80 to 90% relative humidity, 24 h day length and a light intensity of 180 mmol.m⁻².s⁻¹ on capillary mating.

Plants inoculation

Plants were inoculated two weeks after sowing in the third true-leaf, by inserting an hypodermic syringe with a needle in the wounded area of the main vein and also two secondary veins and a 20 µl droplet of a bacterial suspension containing 10⁸ CFU/ml (21.14% absorbance at 540 nm). Isolates were grown on "Nutrient Broth" medium at 25 ± 1°C for 48 h before inoculation. After inoculation, the plants were placed in plastic propagators for 24 h in the dark in order to maintain relative humidity of about 100% level. It was then kept in the growth room Fitoclima 16.000 E® at a raised temperature of 24 and 20°C (day and night), 80 to 90% relative humidity, and 16/8 h photoperiod and a light intensity of 90 mmol.m⁻².s⁻¹

to enhance disease responses.

Disease assessment

After one week of inoculation and every two or three days during each week, the number of the three inoculation points showing symptoms and the severity of the symptoms were recorded. The last and final evaluation was made 15 days after inoculation. Based on these observations, the host-parasite interaction phenotype (IP) was recorded according to a modified 0 to 9 scale adopted from Williams (1985), where: 0 = no of symptoms surrounding the inoculation points; 1 = minute chlorosis/necrosis surrounding the inoculation points; 3 = small V-shaped lesion of 0.5 to 1.5 cm, sometimes with necrosis near the inoculation points; 5 = small to medium V-shaped lesion, with more than 1.5 cm, reaching half way to mid rib in the inoculations of the secondary veins; 7 = medium to big V-shaped lesions, reaching mid-rib in the inoculations of the secondary veins; 9=great lesion reaching the base of the leaf, sometimes with death of the leaf and/or system invasion of plant. Generally, plants without lesions or with a few small lesions (IP= 0, 1 or 3) were considered resistant and plants with small system lesions and only one reduced part infected (IP=5) were considered moderately susceptible or partially resistant. All the other plants, with more extensive systemic lesions (IP=7 or 9) were considered susceptible. All the resistant plants were reevaluated four to five weeks after sowing in the young leaves.

Data analysis

The results were presented as a Disease Index (DI) (Williams,

1985) based on the mean score of 24 plants interaction phenotype of each accession. One accession was considered resistant, when the percentage of resistant plants corresponding to the sum of incompatible interactions was at least 20%. The percentage of resistant seedlings (%R) and the mean DI were adopted as the criteria to rank the accessions for their interest as sources of resistance. Both approaches were compared in the range of the core collection results to determine which measure would be the most reliable for the characterization of the accessions.

The structure of the core collection, which is divided into cultivar type groups of accessions, sharing the same morphological characteristics, allows the analysis of results to be made at various levels of characterisation of the *B. rapa* germplasm. On the assumption that this core collection is a balanced sample of the variation found in the *B. rapa* gene pool, chi-squared (χ^2) tests were used to compare expected and observed number of resistant accessions in each group of *B. rapa* vegetables.

RESULT

Screening of the vegetable core collection

As expected, a great variety of reactions were found between and within accessions of the *B. rapa* collection with the two isolates ranging from complete resistance to full susceptibility. From the 210 accessions tested with isolate Xcc524 (race4), it was found that only 27 (13%) were completely susceptible with all the plants rated in the 7 to 9 classes and 121 (58%) presented at least 20% of resistant plants. A list of these 121 accessions considered resistant, with at least 20% of resistant plants and their corresponding Disease Index (DI), is presented in Table 3. The thirty-six most resistant accessions with more than 70% of resistant plants to this isolate were: (i) the pak-choi B-87 (71%); (ii) the choy-sums: CGN-15166 (96%), CGN-15164 (100%), CGN-7211 (82%), B-82 (79%), and K-8478 (83%); (iii) the Chinese cabbages: B-40 (96%), B-148 (92%), BRA-231 (92%), B-71 (88%), B-32 (88%), BRA-1314 (96%), B-138 (82%), BRA-1303 (88%), BRA-1300 (83%), B-446 (82%), B-552 (83%), B-74 (83%), B-314 (88%), BRA-1312 (83%), B-35 (75%), B-31 (70%), BRA-1133 (75%), B-30 (71%), B-162 (75%), and BRA-235 (75%); (iv) the turnips: HRI-4052 (88%), CGN-15220 (88%), HRI-3341 (88%), HRI-5306 (88%), HRI-3119 (88%) and CGN-6673 (75%); (v) the broccoletos: K-6457 (86%), BRA-1230 (71%), BRA-1225 (73%), and K-9011 (71%).

In contrast, from the 210 accessions tested with isolate Xcc512 (race 1), it was found that 195 (92%) were completely susceptible with all the plants rated in the 7 to 9 classes and only one accession, the Chinese cabbage “Chang Puh early” (B-31) presented at least 20% of resistant plants (25%; Table 3). Besides, with this isolate, only 15 accessions (Chinese cabbages: B-148, B-32, B-31, BRA-1133, and BRA-236; broccoletos: BRA-1225, K-9011, K-5552, BRA-1293, HRI-5213, BRA-1235, HRI-5273, HRI-5235, CGN-6829, and HRI4727) were found with resistant plants (Table 3).

Table 4 presents a summary of the results of the

evaluation of the *B. rapa* core collection obtained for each cultivar type group. The *B. rapa* cultivar types with more resistant reactions observed with isolate Xcc524 (race 4) were the broccoletos, choy-sums and Chinese cabbages. In these cultivar type groups, mainly with broccoletos, a relatively high frequency of intermediate reactions was also observed (Table 4). With isolate Xcc 512 (race 1), the variation for response for all the cultivar type groups was very small, with high susceptibility to this isolate being the general rule (mean DI, between 8.2 and 9.0; Table 4). Only broccoletos and Chinese cabbages presented resistant reactions (2 and 1%, respectively) and intermediate or medium susceptible reactions were only observed in broccoletos, Chinese cabbages and choy sums with 12, 4 and 2%, respectively (Table 4) Furthermore, with isolate Xcc 524, choy-sum cultivar type accessions (Table 4) had the highest frequency of resistant (56%) and 13% of moderately susceptible reactions. Twelve accessions (CGN-15166 to B-582) presenting 42 to 100% of resistant plants (Table 3) were considered resistant. These resistant materials originated from Indonesia, China, Malasia and Hong-Kong. One accession, which is from Netherlands, was originally from Indonesia as well. Chinese cabbage cultivar type accessions (Table 4) also presented a high frequency of resistant (51%) and 15% of moderately susceptible reactions. Forty-two accessions (B-40 to B-171) presenting 20 to 96% of resistant plants were considered resistant. These resistant materials are all from Asian countries. One accession, which is from the United States, was also originally from Asia. Broccoletto cultivar type group (Table 4) was characterised by high frequencies of resistant (39%) and 24% moderately susceptible reactions. Thirty-two accessions (K-6457 to HRI-4748) presenting 20 to 86% of resistant plants (Table 3) were considered resistant. All these resistant germplasms originated from Italy. Turnip cultivar type group (Table 4), exhibited only 18 and 10% of resistant and moderately susceptible reactions, respectively. Twenty-two accessions (HRI-4052 to HRI-3447), presenting 21 to 88% of resistant plants were considered resistant. The turnip cultivar type group was the only one in which the number of resistant accessions was significantly lower than expected (Table 4). In the pack-choy cultivar type group, variation in response to Xcc was great (see Table 4) and exhibited only 18 and 9% of resistant and moderately susceptible reactions, respectively. Eleven accessions (B-33 to BRA-1119) presenting 21 to 71% of resistant plants were considered resistant (Table 3). In turnip and pack-choy cultivar type groups with 72 and 74% respectively, the reactions were susceptible, which was reflected in the high mean DI values of 6.8 and 6.9, respectively (Table 4). In tsa-tsai and mizuna cultivar type groups, represented only by five accessions and included in the “others” cultivar type group, 8 and 5% of resistant and moderately susceptible reactions, respectively (Table 4), and two resistant

Table 3. List of the accessions of the *B. rapa* core collection considered resistant, with at least 20% of resistant plants, and their corresponding Disease Index (DI) screened with isolates Xcc524 (race 4) and Xcc512 (race 1).

Accessions				Isolates			
Nr.	Subspecie/Cultivar group name	Code	Country	Xcc 524 (4)		Xcc 512 (1)	
				DI	%R	DI	%R
<i>B. rapa</i> ssp. <i>chinensis</i> (Pak-choy):							
1.	Pai Tsai 1	B-87	CHN	2.5	71	8.8	0
2.	Chang Puh Late	B-33	TWN	2.5	67	8.8	0
3.	Shirona Japanese greens	HRI-5166	JPN	3.1	63	8.8	0
4.	Unspecified landrace	HRI-5107	HKG	3.6	57	8.8	0
5.	Peng-Hop Pai-tsai	B-49	TWN	4.2	50	9.0	0
6.	Unspecified landrace	CGN-13924	CHN	5.0	33	9.0	0
7.	Unspecified landrace	BRA-446	CHN	5.3	36	8.9	0
8.	Pai Tsai 4	B-90	CHN	5.5	33	8.9	0
9.	Korean Gaesong Baechu	HRI-8829	PRK	6.0	38	9.0	0
10.	Hon Tsai Tai	HRI-8261	GBR	6.5	25	9.0	0
11.	Kirabu Santo	BRA-1119	JPN	7.0	21	9.0	0
<i>B. rapa</i> ssp. <i>parachinensis</i> (Choy-sum, flowering pak-choy):							
12.	Tsja Sin	CGN-15166	IDN	0.8	96	8.8	0
13.	Tsja Sin	CGN-15164	IDN	0.9	100	8.1	0
14.	Choy Sam	CGN-7211	NLD	1.8	82	8.6	0
15.	Choy sum Ex China 3	B-82	MAL	1.8	79	8.4	0
16.	Tsja Sin	K-8478	IDN	2.3	83	9.0	0
17.	Choy sum 80 days	B-95	CHN	3.0	67	9.0	0
18.	Tsja Sin	CGN-15168	IDN	3.0	63	8.8	0
19.	Choy sum 40 days	B-92	CHN	3.2	67	8.9	0
20.	Choy sum 60 days	B-94	CHN	4.1	58	8.9	0
21.	Choy sum 50 days	B-93	CHN	4.4	46	8.9	0
22.	Tsja Sin	K-8479	IDN	4.4	42	9.0	0
23.	Choy sum 60 days	B-582	HKG	5.0	46	8.8	0
<i>B. rapa</i> ssp. <i>pekinensis</i> (Chinese cabbage):							
24.	Yamato-Noen	B-40	JPN	0.8	96	8.8	0
25.	Hsiao Sheng Mao	B-148	TWN	1.0	92	8.3	4
26.	Tsinan Hsiao Pao Tou Pai Tsai	BRA-231	CHN	1.1	92	7.5	0
27.	Kyoto Nr. 3	B-71	JPN	1.1	88	9.0	0
28.	Chang Puh Medium Early	B-32	TWN	1.1	88	6.1	19
29.	Heian Chitosei	BRA-1314	MNG	1.2	96	8.8	0
30.	BB	B-318	KOR	1.4	82	8.8	0
31.	Unspecified landrace	BRA-1303	MNG	1.5	88	8.2	0
32.	Unspecified landrace	BRA-1300	MNG	1.5	83	7.8	0
33.	Heian Chitosei	B-446	JPN	1.6	82	8.8	0
34.	030001Wase Kanagawa	B-552	JPN	1.6	83	8.9	0
35.	Nozaki Early	B-74	JPN	1.7	83	8.8	0
36.	Improved KT	B-314	KOR	1.8	88	8.8	0
37.	Ta-Han-Tzu	BRA-1312	MNG	2.0	83	8.7	0
38.	Huang Gin	B-35	TWN	2.3	75	8.8	0
39.	Chang Puh Early	B-31	TWN	2.3	70	4.9	25
40.	Unspecified landrace	BRA-1133	CHN	2.5	75	7.8	4
41.	Dark Leaf Late	B-30	TWN	2.5	71	9.0	0

Table 3. Contd.

42.	Pe Tsai	BRA-79	CHN	2.5	67	8.8	0
43.	Hsia Sheng	B-162	TWN	2.6	75	7.7	0
44.	Heading	B-3	THA	2.6	63	8.8	0
45.	Sao-Baj-Kou	BRA-235	CHN	2.7	75	8.4	0
46.	Phoduran	BRA-989	PRK	2.8	67	8.9	0
47.	Taibyokashin	B-431	JPN	3.0	68	8.7	0
48.	Santung	B-300	TWN	3.0	67	8.7	0
49.	Tschifu	BRA-201	PRK	3.1	63	9.0	0
50.	Midget Nr.1	B-62	JPN	3.1	58	8.8	0
51.	Unspecified landrace	BRA-435	CHN	3.3	63	9.0	0
52.	Unspecified landrace	BRA-126	CHN	3.5	58	8.7	0
53.	Wase Kashin Santo	B-442	JPN	3.9	57	8.8	0
54.	Unspecified landrace	BRA-236	CHN	3.9	38	6.5	6
55.	Cantoner	ISA-801	CHN	4.0	54	8.1	0
56.	Jagerkohl	BRA-467	CHE	4.3	46	8.8	0
57.	Michihili	B-194	USA	4.5	33	8.0	0
58.	Unspecified landrace	BRA-1304	MNG	4.6	46	8.6	0
59.	One root Pai-tsai	B-302	TWN	4.7	46	8.9	0
60.	Tientsin	B-34	TWN	5.0	33	8.3	0
61.	Sandun	BRA-213	PRK	5.0	21	8.7	0
62.	Japro	ISA-802	CHN	5.1	38	9.0	0
63.	Unspecified landrace	BRA-1311	MNG	5.2	31	8.8	0
64.	Harumaki Santo	B-436	JPN	5.4	25	8.8	0
65.	Ho-Mei-Yung Ching	B-171	TWN	5.7	20	8.6	0
<i>B. rapa</i> ssp. <i>rapifera</i> (Turnip):							
66.	Long d'Alsace	HRI-4052	GBR	1.4	88	9.0	0
67.	Terauchi-kabu	CGN-15220	JPN	1.4	88	9.0	0
68.	Strubble Tyfon	HRI-3341	GBR	1.6	88	9.0	0
69.	Rapa 60 giorni	HRI-5306	ITA	1.9	88	9.0	0
70.	Unspecified landrace	HRI-3119	BTN	2.0	88	9.0	0
71.	Fodder Norfolk	CGN-6673	GBR	2.5	75	9.0	0
72.	Rapa Febuario	HRI-5308	ITA	2.6	63	9.0	0
73.	Petrovshaja	CGN-6861	SUN	4.1	50	9.0	0
74.	Milan Early White	HRI-3450	GBR	4.4	59	8.8	0
75.	Aberdeen Green Top Yellow	HRI-3272	GBR	4.6	38	9.0	0
76.	Rapa Aprile	HRI-5309	ITA	4.8	38	9.0	0
77.	Orange Jelly	HRI-3435	GBR	4.8	38	9.0	0
78.	Rapa natalino	HRI-5307	ITA	4.8	38	9.0	0
79.	York Globe	HRI-6155	NZL	4.9	38	9.0	0
80.	Oguni-kabu	CGN-15219	JPN	5.1	38	9.0	0
81.	Unspecified landrace	K-7833	CUB	5.3	31	9.0	0
82.	Unspecified landrace	K-7177	IRQ	5.3	31	9.0	0
83.	Winter turnip	CGN-7217	PAK	5.9	25	8.3	0
84.	Unspecified landrace	HRI-6584	EGY	6.4	25	9.0	0
85.	Salgam	K-8266	TJK	6.4	21	9.0	0
86.	Fodder Leielander Waasmunster	CGN-6811	BEL	6.5	21	9.0	0
87.	Early White Stone	HRI-3447	GBR	6.9	21	9.0	0
<i>B. rapa</i> ssp. <i>utilis</i> (Broccoletto):							
88.	Cima di rapa	K-6457	ITA	1.3	86	7.5	0
89.	Cima di rapa	BRA-1230	ITA	2.3	71	7.6	0

Table 3. Contd.

90.	Broccoletto	BRA-1225	ITA	2.4	73	6.9	10
91.	Cima di rapa tardivo di Marzo	K-9011	ITA	2.5	71	6.0	16
92.	Broccoletto	BRA-1233	ITA	2.5	69	6.8	0
93.	Cima di rapa	K-5552	ITA	3.3	61	7.3	8
94.	Broccoletto Natalino	CGN-6823	ITA	3.4	58	7.6	0
95.	Cima di rapa	BRA-1293	ITA	3.4	55	7.9	8
96.	Broccoletto tardivo	HRI-4733	ITA	3.6	48	7.5	0
97.	Broccoletto tardivo	HRI-5213	ITA	3.8	39	6.8	16
98.	Broccoletto	HRI-6818	ITA	4.2	32	8.3	0
99.	Cima di rapa cinquantina	BRA-1292	ITA	4.3	32	8.1	0
100.	Broccoletto	BRA-1235	ITA	4.5	56	8.0	4
101.	Cima di rapa novantina	HRI-5252	ITA	4.5	49	7.8	0
102.	Cima di rapa grande Natalina	HRI-5273	ITA	4.5	47	8.2	4
103.	Cima di rapa centoventina	HRI-11792	ITA	4.6	42	8.1	0
104.	Cima di rapa quarantina a cima grossa	HRI-5378	ITA	4.8	30	7.7	0
105.	Cima di rapa sessantina	HRI-4741	ITA	4.9	39	8.9	0
106.	Broccoletto Natalino	HRI-5235	ITA	5.1	31	8.1	4
107.	Rapa sponza	K-6464	ITA	5.2	29	8.2	0
108.	Broccoletto precoce quarantina	BRA-1228	ITA	5.2	26	7.9	0
109.	Broccoletto sessantina	CGN-6829	ITA	5.3	34	7.9	4
110.	Broccoletto	CGN-6824	ITA	5.3	33	8.2	0
111.	Rapa sponza	BRA-1234	ITA	5.4	33	8.4	0
112.	Cima di rapa novantina a cima grosso	HRI-4749	ITA	5.5	31	8.8	0
113.	Broccoletto novantino	HRI-4745	ITA	5.5	29	8.7	0
114.	Rapa da cima mezo tardivo	HRI-5366	ITA	5.6	33	8.5	0
115.	Broccoletto	BRA-1226	ITA	5.8	23	8.1	0
116.	Rapa da cima bradano novantino	HRI-4727	ITA	6.0	22	8.1	4
117.	Rapa da cima quarantina	HRI-3205	ITA	6.4	26	8.8	0
118.	Cima di rapa quarantina	HRI-4720	ITA	6.4	25	8.4	0
119.	Cima di rapa	HRI-4748	ITA	6.5	20	9.0	0
<i>B. rapa</i> ssp. <i>narinosa</i> (Tsa-tsai):							
120.	Senday Yukina	B-489	JPN	4.8	38	9.0	0
<i>B. rapa</i> ssp. <i>nipposinica</i> (syn. <i>japonica</i>) (Mizuna):							
121.	Isogo Kyo	B-500	JPN	5.2	33	9.0	0
Turnip Globo (<i>Susceptible control</i>)							
		ISA-800	PRT	8.5	0	9.0	0

accessions (B-489 and B-500) presenting 37.5 and 33.3% of resistant plants, respectively (Table 3) was observed.

Comparison between %R and DI criteria

The percentage of resistant plants (%R) and the mean Disease Index (DI) values observed in the screening of the *B. rapa* core collection were sometimes not

correlated. In the few resistant accessions to isolate Xcc512, important differences using the two criteria (%R and DI values) were observed in ranking accessions according to their interest as sources of resistance (Table 3). For example, Chinese cabbages B-31 and B-32 with %R of 25 and 19% have DIs of 4.9 and 6.1, respectively; and broccoletos HRI-5213 and K-9011, both with %R of 16% have DIs of 6.8 and 6.0, respectively. Besides there were other 11 accessions showing low percentages of resistant plants (4 to 10%) with DI values ranging from

Table 4. Mean and interaction interval of Disease Index (DI), percentage of reactions and expected and observed numbers of resistant accessions in each cultivar type of the *B. rapa* core collection.

Cultivar group	Disease Index		Isolate Xcc524 (4)						Isolate Xcc512 (1)				
			% of reactions ¹			Nr. Resist. Acces. ²			Disease Index		% of reactions		
	Mean	Range	R	MS	S	Exp.	Obs.	χ^2 ³	Mean	Range	R	MS	S
Pack-choy	6.9	2.5-9.0	18	9	74	15	11	1.58	8.9	8.6-9.0	0	0	100
Choy-sum	3.9	0.8-8.3	56	13	31	9	12	1.75	8.8	8.1-9.0	0	2	98
Chinese cabbage	4.0	0.8-8.8	51	15	34	32	42	2.63	8.2	4.9-9.0	1	4	95
Turnip	6.8	1.4-9.0	18	10	72	41	22	4.65*	9.0	8.5-9.0	0	0	100
Broccoleto	4.8	1.3-8.1	39	24	37	21	32	3.79	8.0	6.0-9.0	2	12	87
Others	7.9	4.8-8.8	8	5	87	3	2	0.79	9.0	9.0-9.0	0	0	100
Mean/Total	5.7	0.8-9.0	32	13	56	121	121	-	8.7	4.9-9.0	1	3	97

¹R=Resistant (IP=0,1,3); MS=Moderately susceptible (IP=5); S=Susceptible (IP=7,9)

² $E^R = T^R \times N^A / T^A$; where E^R =the expected number of resistant accessions in cultivar type group; T^R =the total number of resistant accessions found in the core collection; N^A =the number of accessions in a cultivar type group; T^A =the total number of accessions in the core collection (i.e. 210).

³*=Significant for $P=0.05$; $\chi^2_{r=3,84}$.

6.5 to a maximum of 8.3. This was due to the differences between the number of plants identified in moderately susceptible and susceptible IP classes. Similarly, when DI values were low, a small variation in DI could represent a large variation of %R of the accessions, as observed in Chinese cabbages B-442 and BRA-236, both with a mean DI of 3.9 for isolate Xcc524, but with 57 and 38% of %R, respectively. This also happened with accessions of pack-choy K-8478 and Chinese cabbage B-31, both with a mean DI of 2.3 for the same isolate, but with a %R of 83 and 70%, respectively. From another perspective and in screening with isolate Xcc 524, the pack-choy HRI-8829, the Chinese cabbages BRA-236 and ISA-802, the turnip CGN-15219 and the tsa-tsai B-489, all with a %R of 38%, had a DI of 6.0, 3.9, 5.1, 5.1 and 4.8, respectively (Table 3).

DISCUSSION

The present study identified 121 new sources of resistance to isolate Xcc524 (race 4) with at least 20% of resistant plants. From the thirty-six accessions presented, more than 70% of resistant plants were immediately exploited in breeding programmes for Xcc resistance. Sources of resistance were identified among all the cultivar types/gene pools where diversity is the greatest. More than one half of the *B. rapa* accessions screened (from a total of 210) were resistant to this isolate Xcc524. This fact confirms the A genome origin of this resistance, as suggested by Ignatov et al. (2000a) and by Taylor et al. (2002).

The origin of the resistant accessions appears also to be geographically related to the two independent centers of origin of *B. rapa* in Asia (with the oriental leafy and

flowering *B. rapa* vegetables) and Europe with turnips and broccoletos as proposed by Song et al. (1988). A more detailed analysis of each cultivar type group revealed that the degree of resistance could be related to certain *B. rapa* morphotypes such as the flowering broccoletos and choy-sums, and the Chinese cabbages. Surprisingly, much less resistance was found in pack-choy and turnip cultivar groups compared with choy-sum (flowering pack-choy) and broccoletos (flowering turnips), respectively, which are close relatives in terms of crop evolution. This finding may indicate that resistance was lost or developed at a later stage, probably as a result of selective pressure exerted in each center of origin for certain morphotypes along with mutational events that occurred frequently in *B. rapa*.

The present study identified also, new sources of resistance to isolate Xcc512 (race 1). This resistance is rare or not so common. From these accessions, Chinese cabbages "Chang Puh Early" (B-31) and "Chang Puh Medium Early" (B-32), and broccoletos "Cima de rapa tardivo di Marzo" (K-9011) and "broccoleto tardivo" (HRI-5213), presented respectively 25, 19, 16 and 16% of resistant plants and so, they can be exploited in breeding programmes for blackrot resistance with race 1 isolates. These results show also that the mean Disease Index (DI) and the percentage of resistant seedlings (%R) are both necessary, and complementary criteria to define the level of resistance presented in each accession. The mean Disease Index (DI), proposed by Williams (1985), can be used as a first indication of the accession ranking; but as observed need to be complemented by the percentage of resistant seedlings (%R) or with a more detailed analysis of the distribution of the interaction phenotypes. Sources of resistance to isolate Xcc512 (race 1) were only identified among the Chinese cabbage

and broccoletos cultivar group types. The origin of the resistant accessions appears to be geographically related since the Chinese cabbages are from Taiwan and China and all the broccoletos are from Italy. Taylor et al. (2001) also found that a broccoletto, "broccoletto di rapa Natalino" (HRI-5235), appeared to be either resistant or partially resistant to isolates races 1 and 4. The Chinese cabbage, "Hsia Cheng" (B162), considered by Taylor et al. (2002) with potential broad-spectrum resistance and resistant to race 1 isolates of Xcc, was susceptible to isolate Xcc512. Dias (unpublished) have screened this accession with other two isolates of race 1 (Xcc501 and PHW117) and it was found that this accession was resistant to isolate PHW117 (ID=3.9), confirming the results of Guo et al. (1991), but not to Xcc501 (ID=6.3). This observation alerts us for the precaution of the generalisation of the results and agrees with the recognition made by Vicente et al. (2001) that there is a great variability within race 1 of Xcc in terms of aggressivity.

Those four accessions (B-31, B-32, K-9011 and HRI5213) screened in the present study, represent newly identified sources exhibiting resistance to the two major worldwide Xcc races 1 and 4. These accessions can be used to transfer this resistance through interspecific hybridization to other *B. rapa* morphotypes and to other *Brassica* vegetables, mainly *B. oleracea*, where there are no cultivars with complete resistance to these most common Xcc races 1 and 4. Previous work with interspecific sources of resistance has been undertaken, including the transfer of resistance from *B. carinata* PI199947 to *B. oleracea* (Hansen and Earle, 1995) through protoplast fusion, which could not be stabilized in a *B. oleracea* plant type (Tonguc et al., 2003). Complications arising from interspecific crosses with *B. oleracea* through sexual crosses include different chromosome number in the parents. These barriers can be overcome with techniques including embryo rescue and protoplast fusion, but complications such as aneuploidy in breeding lines can also occur (Tonguc and Griffith, 2004). These four identified *B. rapa* resistant accessions represent sources, which may have less barriers in the transfer of Xcc resistance to *B. oleracea* than those encountered in transferring resistance from resistant *B. carinata* or *B. juncea* accessions (Tonguc et al., 2003; Tonguc and Griffith, 2004). If interspecific hybrids with *B. oleracea* will be successfully generated, it will reduce the worldwide limitations on production caused by this pathogen in this species. The broad spectrum resistance from *B. rapa* on its own or in combination with strong race-specific resistance, can contribute to the long-term control of the disease.

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