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Patente de Invención	■	Patente de Modelo de Utilidad		☐
Vía Nacional				
Vía PCT (fase nacional)	■	Cap. I	■	Cap. II ☐

No. Solicitud Internacional PCT/NL2008/050232
Fecha de Presentación Internacional 22/Abril/2008

Como resultado del Examen de Fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Folios 4 a 21	03/Noviembre/2009
Reivindicaciones:	Folios 22 a 25	03/Noviembre/2009
Prioridad:	HL 2000622	15/Marzo/2001

2. Análisis de la Prioridad
Se acepta la reivindicación de Prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486), y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención
Título de la solicitud: "Plantas *Brassica oleracea* resistentes al *Albugo candida*"
El problema planteado en esta solicitud consiste en proporcionar métodos y plantas de *Brassica oleracea* resistentes a *Albugo candida*
Para resolver este problema técnico la solicitud en estudio proporciona plantas *Brassica oleracea* que contienen un gen resistente a *Albugo Candida*, causante de la roya blanca de las cruciferas. El descubrimiento esta asimismo relacionado con un metodo para la obtención de una planta *Brassica oleracea* con resistencia al *A. Candida*.El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A01H 5/00; C12Q 1/58

4. Excepción a la Patentabilidad (Art 20 D 486 literales (c))

El objeto de las reivindicaciones 1 a 9 y 18 no es patentable de acuerdo con el Art citado, ya que se refiere a organismos completos (plantas). El método de la reivindicación 10 y de las reivindicaciones dependientes 11 a 17, no es patentable bajo el artículo citado ya que se limita al cruzamiento de plantas, un proceso se considera esencialmente biológico. Se aclara al solicitante que según lo divulgado en la descripción (folio 8 líneas 7 a 28), el gen reclamado como característico en las reivindicaciones de método de obtención de plantas, corresponde a un gen que hace parte de un variedad de *B. oleracea*, y el solicitante simplemente lo identificó y aisló, pero no proporciono modificaciones sobre el mismo. Se recuerda al solicitante que este tipo de aislamientos no son patentables bajo el Art. 15 literal b D486. Además, las secuencias de la tabla 1 y 2 corresponden a aislamientos de secuencias de *Brassica oleracea*, dicha materia no es patentable bajo el Art. 15 literal b D486.

5. Reivindicaciones relativas a un uso o a un segundo uso (Art 14 y 21 D 486)
El uso de la reivindicación 19 no es patentable de acuerdo con el Art 14.

A pesar de que la solicitud contiene materia no patentable, se realizó una búsqueda y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación*
D1	Genetic Resources and Crop Evolution 51: 713–722	Evaluation of a core collection of Brassica oleracea accessions for resistance to white rust of crucifers (Albugo candida) at the cotyledon stage	2004

D1 divulga plantas de *Brassica oleracea* que contienen un gen resistente a *Albugo Candida* (roya blanca).

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.

JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (C)

El anterior requerimiento fue notificado por fijación en lista, de fecha 25 SET. 2012

Elaborado por: Sandra Carolina Crespo Cárdenas
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Expediente 09-123698 Primer Artículo 45

Genetic Resources and Crop Evolution 51: 713–722, 2004.

Evaluation of a core collection of *Brassica oleracea* accessions for resistance to white rust of crucifers (*Albugo candida*) at the cotyledon stage

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Abstract

The identification of seedling resistance to white rust of crucifers was performed in a screening of a *B. oleracea* core collection with 400 accessions representing the genetic and geographic diversity of the species. Fifty seedlings per accession were tested against the Portuguese isolate *Ac502* using the methodology and evaluation procedures developed by Williams (1985) and Leckie et al. (1996). The percentage of resistant seedlings (%R) and the conventional rating criteria of the mean Disease Index (DI) based on the two different evaluation procedures of disease expression used, were compared and adopted as the criteria to rank the accessions for their interest as sources of resistance. A great variability of reactions was found between and within accessions of the core collection, ranging from complete resistance to full susceptibility. Sources of resistance were found namely among the cauliflowers, broccoli and tronchuda cabbages gene pools. Forty-seven accessions presented at least 20% of resistant seedlings. Nine accessions (the kales INRA18 and INRA62, the cauliflowers HRI4856, HRI4866 and HRI5424, the loose-head cabbage HRI11555, the savoy cabbage BRA848, the black broccoli HRI6318 and the Portuguese tronchuda cabbage ISA207) presented 50–78% of resistant seedlings and so they should be considered as potential and useful sources for direct use in breeding programs for white rust resistance. Fourteen inbred lines, representing the full range of disease expression, derived from resistant accessions of the core collection were also tested for resistance to other two Portuguese isolates (*Ac503* and *Ac504*) and to a UK isolate. The results provided no evidence of differential reaction to the *A. candida* isolates tested.

Introduction

The Oomycete *Albugo candida* (Pers.) Kuntze, is the causal agent of white rust or white blister of crucifers. This pathogen occurs on numerous members of the *Cruciferae* or *Brassicaceae* family, including the cultivated vegetable and oilseed *Brassica* species where it can produce two types of infection: local and systemic. Local infection is characterised by the formation of conspicuous white pustules on leaves and shoots surrounded by chlorotic areas. Systemic infection affects mostly the apical buds and the inflorescences, causing hypertrophy and hyperplasia. The floral organs become severely distorted and sterile (stagheads) and eventually turn necrotic and die. Commonly *A. candida* occurs in close association with downy mildew, incited by *Peronospora parasitica* (Pers.) Fr., which is often found parasitizing colonies of *A. candida* (Bains and Joothy 1985; Chaurasia et al. 1982). Although traditionally regarded as a minor disease on cole crops (*Brassica oleracea* L.), the white rust has gained some importance in Europe following some severe outbreaks, after the 1980s in the UK, Netherlands, France, Spain and Portugal, on different cole crops like Brussels sprouts, cauliflowers and cabbages. Reasons for this increase in disease incidence are not fully understood, but it is likely that a combination of the use of seed contaminated by

oospores, the employ of systemic fungicides exerting a selection pressure against less virulent pathotypes, the resistance of some pathotypes to metalaxil fungicides, and the cultivation of modern F_1 hybrids cultivars with a narrow genetic base which lack of resistance genes has contributed to this situation. At present, there are no viable alternatives to chemical control of the disease and in certain cases the control is achieved only with limited success. The development of resistant cultivars would constitute a more environmentally and friendly alternative of control that could be used in integrated crop protection strategies to reduce pesticide inputs. The use of resistant cultivars has been successively employed in the control of white rust of other *Brassica* species, like oilseed *B. rapa* L., and *B. juncea* (L.) Czernj. and Coss.

For the development of a breeding programme to incorporate resistance to white rust in vegetable brassicas it is essential to know the variability of response to this pathogen present in the *B. oleracea* germplasm. Despite the large *B. oleracea* gene pool that offers a great variability for disease resistance, this remains largely underexploited. Reports in the literature about the variation of response of *B. oleracea* accessions to white rust are scarce, apart of some works with portuguese coles (Monteiro and Williams 1989; Santos et al. 1996).

This paper reports the results obtained in the first evaluation at the cotyledon stage against a Portuguese *A. candida* isolate, of a representative and structured core collection of 400 *B. oleracea* accessions and a further evaluation of 14 cole inbred lines with a full range of disease expression to three other isolates, two from Portugal and another from the UK.

Materials and methods

Plant material and isolates

The plant material used for the screening consisted of a core collection of 400 *B. oleracea* accessions (Table 1), selected from six European genebank collections and representing the available range of crop types and ecogeographical location within the species with a minimum of duplication. A complete discrimination of all the accessions used was presented by Santos (2000).

One Portuguese *A. candida* isolate, *Ac502*, collected on cauliflower leaves from a grower's field at Lourinhã (middle west Portugal) was used.

Table 1. Composition of the *B. oleracea* L. core collection originated from six European genebanks and 39 countries.

Botanical name *B. oleracea* L.	Common name	Number of assessions
var. *acephala* DC.	Kales	76
var. *alboglabra* Bailey	Chinese kales	10
var. *botrytis* L.	Cauliflowers	67
var. *capitata* L.	Cabbages	99
var. *costata* DC.	Tronchuda cabbages	37
var. *gemmifera* DC.	Brussel sprouts	45
var. *gongylodes* L.	Kohlrabis	19
var. *italica* Plenck	Broccolis	46
var. *oleracea*	Wild cole	1

Screening procedures

Fresh spores for inoculation were obtained from mature pustules on cotyledons of maintenance plants grown under controlled conditions (20 °C; 16 h daylength). Screening procedures used were similar to those developed by Williams (1985). Seeds were sown 0.5 cm deep in propagation trays with 16 × 10 modules/tray and module size of 30 × 30 × 50 mm, filled with Levington F2 compost and covered superficially with a layer of vermiculite. Trays were placed in a growth room with 20 ± 1 °C, 80–90% RH and 24 h daylength on capillary mating. Seedlings were inoculated 7 days after sowing. The inoculum was prepared by collecting the fresh sporangial dust with a cyclone spore collector and suspending it in a flask with an appropriate volume of double distilled water. The flask was placed for 2–3 h in an incubator at 12 °C in the dark for the release of zoospores. The zoospore concentration was measured and adjusted to 10^5 swimming zoospores per mL using pre-chilled double distilled water. A calibrated Eppendorf micropipette was used to deliver two 10 µL droplets of inoculum to the adaxial surface of each cotyledon. Both cotyledons on each plant were inoculated with the isolate. After inoculation seed trays were placed in a dew chamber at 20 °C for 18 h in the dark and then returned to the growth room. The rapid-cycling *B. oleracea* CrGC3.4 and a commercial 'Thousand Head' kale were used as susceptible controls.

Disease assessment

Ten days after inoculation, the plant response defined by the presence or absence and intensity of chlorosis and necrotic flecking, and the pathogen sporulation defined by the amount and size of pustules, on each surface of the cotyledons, were visually assessed. This innovating recording system allows an accurate registration of inoculation results and it is independent of whatever scale is used for classification. Based on these observations the host-parasite interaction phenotype (IP) was recorded according to one of the six classes of resistance/susceptibility (Leckie et al. 1996): NN = no symptoms macroscopically; HN = chlorosis and/or slight necrotic flecking restricted to the inoculation zone on the upper surface of the cotyledon; FN = Heavy necrotic flecking extending to the lower surface of the cotyledon; S1 = Sparse sporulation on the upper surface of the cotyledon, no sporulation on the lower surface of the cotyledon; S2 = Sparse sporulation on both surfaces of the cotyledon; S3 = Heavy sporulation with large to coalescent pustules on the lower surface of the cotyledon. In parallel each seedling was also rated according to the numerical 0–9 rating scale proposed by Williams (1985). A complete randomised block design with five replications of 20 plants per accession was used to evaluate the level of resistance of the 400 accessions of the core collection.

Isolate specificity

The efficacy of the protection action of the resistance genes detected in the screening will depend on their effectiveness against a wide range of pathogen biotypes. The test of the entire core collection against several isolates was not feasible considering the great number of accessions and their genetic heterogeneity. To contour this situation it was also performed a screening with a set of 14 *B. oleracea* inbred lines (Table 2), which represented the range of interaction phenotypes observed in the screening of the core collection, and three other *A. candida* isolates, *Ac503* (Costa de Caparica, Portugal), *Ac504* (Santa Cruz, Portugal) and *Ac001* (Maidstone, UK) from important cole growing regions of Portugal and the UK. Screening procedures were similar to those described for the screening of the core collection, except that in this test 40 seedling cotyledons of each cole line were inoculated simultaneously with the four isolates (including again *Ac502* as control), representing a total of 560 interactions for each isolate.

Table 2. Identification of the 14 inbred lines of *B. oleracea* L. used in the evaluation of resistance to four isolates (*Ac502*, *Ac503*, *Ac504* and *Ac001*), their interaction phenotype (IP) and resistant parent accession.

Line No.	IP[a]	Resistant parent accession	Core number
1	HN	Algarvia	ISA 207
2	HN	Romanesco Verde Medio Precoce	HRI 4866
3	HN	Romanesco Precoce	HRI 4857
4	FN	Algarvia	ISA 207
5	FN	Algarvia	ISA 207
6	FN	Romanesco Verde Medio Precoce	HRI 4866
7	FN	Algarvia	ISA 207
8	S1	Algarvia	ISA 207
9	S1	Romanesco Verde Medio Precoce	HRI 4866
10	S1	Marzolo	HRI 5424
11	S1	Marzolo	HRI 5424
12	S1	Marzolo	HRI 5424
13	S2	Algarvia	ISA 207
14	S3	Algarvia	ISA 207

[a] HN = resistant: chlorosis and/or slight necrotic flecking restricted to the inoculation zone on the upper surface of the cotyledon; FN = resistant: Heavy necrotic flecking extending to the lower surface of the cotyledon; S1 = Moderately susceptible: Sparse sporulation on the upper surface of the cotyledon, no sporulation on the lower surface of the cotyledon; S2 = Moderately susceptible; Sparse sporulation on both surfaces of the cotyledon; S3 = Susceptible; Heavy sporulation with large to coalescent pustules on the lower surface of the cotyledon.

Data analysis

Data obtained from the screening of the core collection was expressed as the frequency of seedlings in each IP class and seedlings were considered resistant when rated as NN, HN and FN, moderately susceptible when rated as S1 and S2 and susceptible when included in the S3 class. 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 Disease Index (DI), calculated according to Williams (1985), 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 characterisation of the accessions.

The structure of the core collection, which is divided into crop type groups of accessions sharing

common morphological and geographical characteristics, allows the analysis of results to be made at various levels of characterisation of the *B. oleracea* germplasm. On the assumption that this core collection is a balanced sample of the variation found in the *B. oleracea* genepool, chi squared (χ^2) tests were used to compare expected and observed number of resistant accessions in each group of coles.

Inoculation data obtained from the isolates test was subject to non-parametric analysis of variance. The interaction phenotypes rated by the scale of Leckie et al. (1996) obtained for each of the 14 cole inbred line seedlings against the four isolates were compared using the Friedman's analysis of variance by ranks (Zar 1996) applied after numerical transformation according to the equivalence established with the Williams 0–9 scale. The statistic value of the Friedman's test traduced by χ^2_r was utilised for the calculation of F_F (Zar 1996).

Results

Screening of the core collection

A complete discrimination of data obtained was described by Santos (2000). As expected a great variability of reactions was found between and within accessions of the core collection, ranging from complete resistance to full susceptibility. From the 400 accessions tested it was found that 242 (61%) were completely susceptible with all the seedlings rated in S3 class and only 47 (12%) presented at least 20% of resistant seedlings. A list of these 47 accessions considered resistant, with at least 20% of resistant seedlings, and their corresponding percentage of Interaction Phenotype (IP) classes and Disease Index (DI) is presented in Table 3. The nine most resistant accessions with 50–78% of resistant seedlings were: (i) the 'kales' of French origin INRA18 (66%) and INRA62 (58%); (ii) the Italian cauliflowers, HRI4866 (60%), HRI5424 (71%) and HRI4856 (71%); (iii) a loosehead cabbage from Portugal, HRI11555 (78%); (iv) a savoy cabbage from Switzerland, BRA848 (54%); (v) a Italian black broccoli, HRI6318 (54%); and (vi) a Portuguese tronchuda cabbage, ISA207 (54%).

Table 4 presents a summary of the results of the evaluation of the *B. oleracea* core collection obtained for each cultivated crop type. The accession of the wild *B. oleracea* is not listed since it was completely susceptible. The coles crop types with more resistant reactions observed were the broccolis, the cauliflowers and the tronchuda cabbages. In these crop types a high frequency of intermediate reactions was also observed (see Table 4). Kale and cabbage crop types presented also some resistant reactions.

Broccoli crop type accessions (see Table 4) had the highest frequency of resistant (14%) and moderately susceptible reactions (36%). Seven accessions (HRI2405 to HRI6318) presenting 20–54% of resistant seedlings (Table 3) were considered resistant. This resistant material is all originated from Italy with exception of 'Late Purple Sprouting' broccoli (HRI11064), which is from the UK but originally was also from Italy.

Cauliflower crop type group (see Table 4) was characterised by high frequencies of resistant and moderately resistant reactions. Twelve accessions (CGN15135 to HRI5424) presenting 20–71% of resistant seedlings (Table 3) were considered resistant. Most of this resistant germplasm was originated from Italy and constituted by 'romanesco' cauliflowers.

Tronchuda cabbages crop type group (see Table 4) exhibited a continuous range of resistant seedlings between 0 and 49%. Classes of intermediate response (S1 and S2) represented 25% of all the seedlings evaluated. Even the most resistant accessions, like 'Couve Algarvia' (ISA 207) had a very uniform distribution in all IP classes. Considering the distribution of seedlings within the range of IP classes, 76% of this germplasm group had more than 20% of the seedlings in non-sporulating and low sporulating IP classes (NN to S2) and 29% presented more than 50% resistant or moderately resistant seedlings. Eight accessions (HRI9574 to ISA207) presenting 20–54% of resistant seedlings (Table 3) were considered resistant.

In the kales crop type group variation for response to white rust isolate *Ac502* was great (see Table 4). Eight accessions (ISA108 to INRA18) presenting 20–66% of resistant seedlings (Table 3) were considered resistant. From these the most resistant were two breeding lines of fodder kales from France (INRA62 and INRA18) but one half of the resistant accessions were Portuguese kales. Expression of reactions in all the IP classes and particularly in moderately susceptible IP classes S1 and S2 was a distinctive characteristic of this crop type germplasm, which influenced the DI values. For example, 'Couve de Horto' (ISA 248) with a DI = 3.4, presented 48% of resistant seedlings and 80% of seedlings were resistant or moderately susceptible, and the two most resistant accessions of this group, the fodder kales, INRA62 and

Table 3. Percentage of Interaction Phenotype (IP) classes and Disease Index (DI) of core collection accessions with at least 20% of resistant seedlings to Portuguese *A. candida* (Pers.) Kuntze isolate *Ac502*.

Core No.	Variety	Common name	Country	% IP Classes						DI
				NN	HN	FN	S1	S2	S3	
	B. oleracea var. *acephala* DC. (Kales):									
ISA108		Couve de Desfolhar	PRT	0	0	20	0	10	70	6.7
ISA34		Couve Ratinha	PRT	0	12	19	12	12	45	5.2
ISA245		Couve de Horto	PRT	0	4	26	10	20	40	5.0
HRI4302		Covo Kale	ZIMB	7	14	10	2	10	57	5.7
HRI6226		Giant Jersey Kale	GBR	12	23	7	2	0	56	5.2
ISA248		Couve de Horto	PRT	0	0	48	16	16	20	3.4
INRA 62		Fodder kale	FRA	2	16	40	10	10	22	3.3
INRA18		Fodder kale	FRA	4	10	52	4	6	24	3.1
	B. oleracea var. *alboglabra* Bailey (Chinese kales):									
CGN14044		Golden Kailan	TAIW	2	10	8	2	4	74	6.8
	B. oleracea var. *botrytis* L. (Cauliflowers):									
CGN15135		Cauliflower Alpha Record	NLD	0	2	18	14	24	42	5.4
HRI7520		Romanesco	ITA	7	14	7	11	0	61	6.6
CGN15136		Cauliflower Danova	DNK	0	2	20	24	24	30	4.7
HRI5294		Cavolfiore Farnese Tardivo	ITA	8	12	3	12	5	60	5.9
HRI8567		Cavolo Broccolo Romanesco	ITA	0	26	0	5	5	64	6.2
CGN11103		Cauliflower Lecerf Lero	NLD	0	0	28	12	28	32	4.8
HRI8571		Cavolo Broccolo Romanesco	ITA	5	35	3	10	7	40	4.5
HRI4857		Cavolo Broc. Romanesco Precoce	ITA	13	31	4	8	4	40	4.2
HRI10757		Cauliflower Gwenael no. 20	FRA	0	18	30	16	30	6	3.0
HRI4866		Cavolo Broc. Rom. Medio Precoce	ITA	5	25	30	5	5	30	3.5
HRI4856		Cavolo Broc. Romanesco Tardivo	ITA	10	44	17	13	10	6	2.0
HRI5424		Marzolo Cavolfiore	ITA	0	65	6	19	0	10	2.2
	B. oleracea var. *capitata* L. (Cabbages):									
HRI11490		Couve repolho Bacalã	PRT	0	20	14	8	6	51	5.3
HRI7824		Jersey cabbage	GBR	8	36	4	16	10	26	3.6
HRI11555		Couve repolho Bacalã	PRT	10	58	10	0	6	16	2.4
HRI11615		Lombarda	PRT	0	10	10	0	16	64	6.5

Table 3. Continued.

Core No.	Variety	Common name	Country	% IP Classes						DI
				NN	HN	FN	S1	S2	S3	
ISA88		Couve Lombarda de Lisboa	PRT	0	6	14	0	9	71	6.8
CGN7123		Savoy cabbage (Selection Silvertand)	NLD	2	18	2	0	18	60	6.3
CGN14082		Savoy cab. Bewaargele (Sel. V. Hout.)	NLD	0	28	2	14	14	42	5.0
CGN7121		Savoy cabbage Shelk. (Sel. V. Willems)	NLD	2	7	30	5	9	47	5.0
BRA254		Savoy cabbage Fruehkopf	GER	4	44	0	0	16	36	4.3
BRA848		Savoy cabbage Haba 200	CHE	0	20	34	10	24	−12	3.1
	B. oleracea var. *costata* DC. (Tronchuda cabbages):									
HRI9574		Couve Penca de Gondomar	PRT	0	4	16	10	20	50	5.8
ISA7		Couve Troncha	PRT	0	20	0	0	0	80	7.1
ISA307		Couve Troncha	PRT	0	0	20	30	20	30	4.7
ISA405		Couve Murciana	PRT	4	16	0	0	10	70	6.7
HRI9489		Couve da Horta	PRT	4	4	14	16	31	31	4.8
ISA368		Coivão	PRT	0	0	36	20	4	40	4.6
HRI9467		Coivão	PRT	0	4	45	4	21	26	3.9
ISA207		Couve Algarvia	PRT	18	18	18	16	22	8	3.6
	B. oleracea var. *gongylodes* L. (Kohlrabis):									
HRI5443		Cavolo Forte	ITA	0	8	12	10	20	50	5.8
	B. oleracea var. *italica* Plenck (Broccolis):									
HRI2405		Broccolo Apriloto	ITA	0	12	8	36	8	36	4.8
HRI5425		Cavolo Broccolo Natalino	ITA	0	4	16	16	14	50	5.7
HRI8631		Sicilian Purple Lake	ITA	20	0	0	0	30	50	5.8
HRI10654		Late Purple Sprouting Broccoli	GBR	9	4	11	15	15	45	5.4
HRI5419		Broccolo di Mojo	ITA	0	19	15	11	36	19	4.1
HRI2398		Cavolo Broccoli Piccolini di Palermo	ITA	4	6	30	44	16	0	2.5
HRI6318		Cavolo Broccoli di Lavagna	ITA	4	44	6	18	4	24	3.3

Table 5. Values of the percentage of resistant seedlings (%R) and mean Disease Index (DI) for ten accessions of the core collection.

Core number	Accession name	Crop type	%R	DI
ISA383	'Penca da Póvoa'	Tronchuda cabbage	0	8.6
BRA1419	'Brimo'	Broccoli	0	3.0
HRI9842	'Green Valiant' (F_1)	Broccoli	0	3.5
ISA255	'Couve de Desfolhar'	Galega kale	4	5.5
HRI4813	'Marzatico Napoletano'	Cauliflower	11	3.5
HRI4302	'Covo'	Kale	31	5.7
HRI2398	'Piccolini di Palermo'	Broccoli	40	2.5
HRI6226	'Giant Jersey'	Kale	42	5.2
BRA848	'Haba 200'	Savoy cabbage	54	3.1
HRI11555	'Bacalã'	Cabbage	78	2.4

Table 6. Results of the screening of 14 *B. oleracea* L. inbred lines against four *A.candida* (Pers.) Kuntze isolates.

Interaction phenotype classes	*A. candida* isolates*			
	Ac503	*Ac504*	*Ac502*	*Ac001*
NN+HN+FN (Resistant)	291	284	271	265
S1+S2 (Moderately susceptible)	177	184	198	198
S3 (Susceptible)	90	90	89	95

* *Ac502* (Lourinhã, Portugal), *Ac503* (Costa da Caparica, Portugal), *Ac504* (Santa Cruz, Portugal), and *Ac001* (Maidstone, UK).

values were low a small variation in DI could represent a large variation of %R of the accessions as observed in broccoli 'Piccolini di Palermo' (HRI2398) and cabbage 'Bacalã' (HRI11555; Table 3). This also happened in accessions like kales 'Couve de Desfolhar' (ISA108) and 'Giant Jersey' (HRI6226; Table 3) which had similar and intermediate DI values and a 38% difference in the %R. The ranking of F_1 hybrids like 'Green Valiant' or other accessions with very uniform response, like 'Brimo' (see Table 5) which presented only seedlings in the moderately susceptible S1 class, using the number of resistant seedlings as primary criteria could be very misleading of the real interest of these accessions for selection, comparing for example to the fully susceptible 'Penca da Póvoa'. In opposition, if the DI is considered, 'Brimo' and 'Green Valiant' were supposed to be similar to savoy cabbage 'Haba 200' (BRA848; Table 3) with an equal DI but with 54% of resistant seedlings.

Isolate specificity

Table 6 presents the inoculation results of the 14 inbred lines tested against the four *A. candida* isolates. No significant differences ($F_{(a = 4;b = 558)} = 2.70$; $F_{(3;1677)} = 3.12$, $P = 0.05$) were observed between the reactions of these lines to the four isolates. The distribution of interaction phenotypes for each line was consistent to the previous evaluation with *Ac502* and the frequency peaks observed corresponded to the interaction phenotype proposed. The maximum variation in the number of incompatible interactions occurred between isolates *Ac503* and *Ac001*, corresponding to 4.6% of the total number of incompatible interactions observed. This was accompanied by a variation of 3.8% in the number of partial compatible interactions S1 and S2 and a 0.8% variation in the number of S3 compatible interactions. These small differences were due to the variability of reactions observed in some of the lines exhibiting FN or S1 interaction phenotypes, and probably related to differences in the shape and size of the cotyledons that could have influenced the evaluation rating.

Discussion

The present study identified 47 new sources of resistance to white rust of crucifers with at least 20% of resistant seedlings. From those nine accessions presented 50–78% of resistant seedlings, and so, they could be immediately exploited in breeding programmes for white rust resistance. Besides some of these accessions could also be explored as sources of

multiple disease resistance to various pathogens at the cotyledon stage which was already attempted on *B. rapa* by Mitchell-Olds et al. (1995) against *A. candida*, *P. parasitica* and *Leptosphaeria maculans* (Desm.) Ces. and de Not. For example, tronchuda cabbage 'Algarvia' (ISA207) and kale 'Galega' (ISA243) were also found to be resistant to seven *P. parasitica* isolates from Portugal (Sousa et al. 1997) and to two *P. parasitica* isolates from the USA (PHW630) and the UK (PHW828) (Dias et al. 1993). Besides 'Galega' kale (ISA 243) was also reported by Ferreira et al. (1993) to be moderately resistant, namely to the highly pathogenic isolate PHW1223 of *L. maculans* from Australia. Several other accessions of tronchuda cabbage 'Coivão' were reported to be resistant to downy mildew (Sousa et al. 1997) and black rot caused by *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson (Ferreira et al. 1993).

Sources of resistance were identified among all the gene pools where diversity is greatest. The origin of the resistant accessions appears to be geographically related to the three regions of domestication of *B. oleracea* in Europe proposed by Dias (1992, 1995b): the first in Italy with broccoli and cauliflower, the second in Portugal with the tronchuda cabbages and galega kales and the third in Central and Northern Europe where various forms of cabbages and kales were developed. A more detailed analysis of each crop type group revealed that the degree of resistance could be related to certain cole morphotypes. In the cauliflower crop type group a high degree of resistance was consistently found in 'romanesco' cauliflowers, which share a common origin (Rome, Italy). In broccoli such morphotypes relation was not so evident, but there was some evidence that purple and dark green broccoli forms originated from Sicily were more resistant than other broccolis. The resistant accessions of Portuguese origin were represented in several crop types, like kales, tronchuda and common cabbages, which confirms the interest of these coles as sources of resistance as previously reported by Monteiro and Williams (1989) and Santos et al. (1996). In tronchuda cabbages a certain affinity was also observed between the degree of resistance and certain landraces according to Dias (1992, 1995a) classification. For example 'Coivão' and 'Algarvia' morphotypes presented a high degree of resistance; 'Couve de Corte' morphotypes had a great number of intermediate S1 and S2 reactions; and the majority of 'penca' morphotypes like 'Penca de Mirandela', 'Penca de Chaves', and 'Penca da Póvoa' that presented great susceptibility.

Surprisingly, no resistance was found in the wild *B. oleracea* and in the Chinese kales, which are close relatives to primitive *B. oleracea* forms in terms of crop evolution (Song et al. 1990; Dias 1995b). This finding may indicate that resistance was developed as a later event, probably as a result of strong selection pressures exerted in each center of domestication, along with mutational events that frequently occur in *B. oleracea*.

A great variability in reactions was found within the accessions studied, ranging from complete resistance with almost absence of symptoms to full susceptibility with abundant sporulation of the pathogen on both sides and lack of host response. Complete resistance was expressed by characteristic symptoms of hypersensitive response (HR) and traduced by two distinct interaction phenotypes HN and FN. Expression of these degrees of incompatibility could be under different gene control in analogy to what was suggested for *Arabidopsis thaliana* (L.) Heynh. by Holub et al. (1995). Besides complete resistance, a considerable number of accessions of broccoli, cauliflower, tronchuda and kale crop type groups expressed partial resistance traduced by restricted sporulation sometimes accompanied by chlorosis or necrosis. This kind of response was also observed in *B. rapa* (Edwards and Williams 1987; Kole et al. 1996), in *R. sativus* L. (Williams and Pound 1963), and in *B. juncea* (Bansal et al. 1999). In *B. juncea* the partial resistance phenotype at the cotyledon stage was shown to be under control of a single dominant gene with variable expression and significantly correlated with adult or field plant resistance (Bansal et al. 1999), as adult plants originated from partial resistant seedlings did not develop hypertrophic growth or stagheads under greenhouse and field conditions. This situation should also be explored in *B. oleracea* with important implications in breeding for resistance, especially of crop types like cauliflowers and broccolis where floral parts are consumed.

The screening with the set of 14 cole inbred lines derived from some of the resistant accessions detected in the *B. oleracea* core collection with the four isolates representative of pathogen populations of important cole growing regions of Portugal and the UK did not reveal differences in virulence between the isolates. This observation allows with a convenient precaution the generalisation of the results of the *B. oleracea* core screening obtained with Portuguese

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

Crop type	Disease Index (DI)		Percentage of reactions[a]			Number of resistant accessions[b]		χ_r^{2c}
	Mean	Range	R	MS	S	Expected	Observed	
Brussels sprouts	8.5	7.1–8.6	0	3	97	5	0	5.63*
Chinese kales	8.0	6.8–8.5	4	9	87	1	1	–
Kohlrabis	8.2	5.8–8.6	2	6	92	2	1	0.56
Cabbages	7.9	2.4–8.6	6	7	93	11	10	1.00
Kales	7.3	3.1–8.6	7	13	80	9	9	–
Broccolis	6.9	2.5–8.6	14	36	50	5	7	0.90
Tronchuda cabbages	6.8	3.6–8.6	9	25	64	4	7	2.52
Cauliflowers	6.7	2.0–8.6	10	23	67	8	12	2.27
Total (Core Collect.)	7.5	2.0–8.6	7	17	76	45	47	0.09

[a] R = Resistant (NN + HN + FN); MS = Moderately susceptible (S1+S2).

[b] $E^R = T^R \times N^\wedge / T^\wedge$; where E^R = the expected number of resistant accessions in crop type group; T^R = the total number of resistant accessions found in the core collection; $N^\wedge$ = the number of accessions in a crop type group; $T^\wedge$ = the total number of accessions in the core collection.

[c] Susceptible (S3). * = Significant for $P = 0.05$; $\chi_r^2 = 3.51$.

INRA18 presented higher numbers of resistant seedlings (58 and 66%) but had DI values similar to ISA 248, respectively, 3.3 and 3.1 (Table 3).

Cabbage crop type group (see Table 4) presented a great variation in the percentage of resistant seedlings ($0 < \%R < 78$). Despite, it was observed that these coles were also very homogeneous in the response to inoculations. About 40% of the accessions were completely susceptible, which was reflected in the high mean DI value of 7.9. Ten accessions in this crop type group (HRI11490 to BRA848) had 20–78% of resistant seedlings (Table 3). From those the two loosehead cabbages 'Bacalã' (HRI11490 and HRI11555) are cultivated in Portugal for a long time (Dias 1992, 1995a), the Jersey cabbage (HRI7824) is from the UK, and the seven savoy cabbages included two accessions from Portugal (HRI11615 and ISA88), three commercial selections from the Netherlands (CGN7123, CGN14082 and CGN7121), and the others were from Germany (BR254) and Czech Republic (BRA848).

The Brussels sprouts crop type group was the only one where the number of resistant accessions was significantly lower than expected (Table 4). The response of Brussels sprouts accessions to white rust isolate *Ac502* was very consistent and uniform, showing high susceptibility (mean DI = 8.5; $7.1 < DI < 8.6$) and less than 2% of resistant seedlings. Only one accession, the 'Cavolo di Bruxelles' (HRI4783), presented a significant number (48%) of seedlings moderately susceptible of the S2 class.

In the Chinese kale and kohlrabi crop type group's the variation for response to white rust was also very small with high susceptibility to the isolate *Ac502* being the general rule (mean DI, respectively, of 8.0 and 8.2; see Table 4). Only one accession of each crop type presented 20% resistant seedlings (Table 3): the Chinese kale 'Golden' CGN14044 (DI = 6.8), and a purple kohlrabi (the only purple included in the core collection) 'Cavolo Forte' (DI = 5.8). Cultivars of white kohlrabi with good horticultural characteristics, like 'Knauf Ideal' and 'White Vienna', were completely susceptible to the isolate *Ac502*.

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. oleracea* core collection were negatively and significantly correlated ($r_s = -0.76$; $t = -23.49$, $P = 000.1$). In some cases important differences using the two criteria (%R and DI values) were observed in ranking accessions according to their interest as sources of resistance (Table 5). For accessions with a low percentage of resistant seedlings (0–15%) values of DI were very variable ranging from 3.0 to a maximum of 8.6. This is due to the differences between the number of seedlings in moderately susceptible and susceptible IP classes. Similarly, when DI

isolate *Ac502* and assures that the resistance genes detected will be geographically effective, depending however of the capability of the pathogen in creating new forms of virulence.

From the comparison of the two evaluation procedures used (Williams and Leckie et al. criteria) it was observed that the mean Disease Index (DI) and the percentage of resistant seedlings (%R) were both necessary, and complementary criteria to define the level of resistance presented in each accession. The mean Disease Index proposed by Williams (1985) can be used as a first indication of the accession ranking. Although this information should be complemented with a more detailed analysis of the distribution of interaction phenotypes using the discrete scale of Leckie et al. (1996) which is more adequate to characterise the type and range of interaction phenotypes, observed in the *A. candida–B. oleracea* interaction.

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