Pathotypic diversity of *Hyaloperonospora brassicae* collected from *Brassica oleracea*

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Abstract Downy mildew caused by *Hyaloperonospora brassicae* is an economically destructive disease of brassica crops in many growing regions throughout the world. Specialised pathogenicity of downy mildews from different *Brassica* species and closely related ornamental or wild relatives has been described from host range studies. Pathotypic variation amongst *Hyaloperonospora brassicae* isolates from *Brassica oleracea* has also been described; however, a standard set of *B. oleracea* lines that could enable reproducible classification of *H. brassicae* pathotypes was poorly developed. For this purpose, we examined the use of eight genetically refined host lines derived from our previous collaborative work on downy mildew resistance as a differential set to characterise pathotypes in the European population of *H. brassicae*. Interaction phenotypes for each combination of isolate and host line were assessed following drop inoculation of cotyledons and a spectrum of seven phenotypes was observed based on the level of sporulation on cotyledons and visible host responses. Two host lines were resistant or moderately resistant to the entire collection of isolates, and another was universally susceptible. Five lines showed differential responses to the *H. brassicae* isolates. A minimum of six pathotypes and five major effect resistance genes are proposed to explain all of the observed interaction phenotypes. The *B. oleracea* lines from this study can be useful for monitoring pathotype frequencies in *H. brassicae* populations in the same or other vegetable growing regions, and to assess the potential durability of disease control from different combinations of the predicted downy mildew resistance genes.

Introduction

Brassica downy mildew is a foliar disease caused by an oomycete recently re-classified as *Hyaloperonospora brassicae* (Gäumann) Göker, Voglmayr, Riethmüller, Wei and Oberwinkler (syn. *Hyaloperonospora parasitica* (Pers. ex Fr.) Constant. and *Peronospora parasitica*) (Göker et al. 2009). The disease can cause economically significant damage in Brassica crops, with a worldwide distribution wherever vegetable and oilseed brassicas are grown. It can be especially severe in climates with warm days (20 and 24°C) and cool nights (8 and 16°C) with high humidity (RH>80%) (Channon 1981). Young seedlings are most vulnerable to the pathogen, especially in protected environments for transplant production. Adult plants of highly susceptible cultivars can also be damaged, and infection may extend into the inflorescence causing discoloration within cauliflower curds, broccoli, and cabbage heads and reducing the postharvest quality of the crop.

Several *Hyaloperonospora* species with relatively narrow host ranges have been recently re-defined based on molecular analysis of nuclear ribosomal ITS and LSU sequences. For example, *H. brassicae* includes mainly pathogens of the genus *Brassica* and possibly pathogens of closely related genera like *Raphanus*, *Sinapis* and *Armoracia* (Göker et al. 2009). Pathogenic variants (referred to as physiologic races or pathotypes) of downy mildew have been described from several host range studies, which exhibit specialised pathogenicity in different *Brassica* species and closely related ornamental or wild relatives (Dickinson and Greenhalgh 1977; Sherriff and Lucas 1990; Nashaat and Awasthi 1995; Silué et al. 1996). Genotype specific variation within downy mildew isolates pathogenic on *B. oleracea* was first described by Natti et al. (1967); in this

study a broccoli and a cabbage plant introduction showed differential resistance to downy mildew isolates considered to be different pathotypes or races. Another pathotype has been identified subsequently, late in the season, on senescing broccoli leaves (Dickson and Petzoldt 1993). Other studies have also shown genotype/cultivar specificity in the interaction *H. brassicae-B. oleracea* (Greenhalgh and Dickinson 1975; Dickinson and Greenhalgh 1977; Sherriff and Lucas 1990; Moss et al. 1991b; Thomas and Jourdain 1992; Silué et al. 1996; Leckie et al. 1998, Jensen et al. 1999; Agnola et al. 2003; Monot and Silué 2009). The methods and host lines vary amongst these studies, so it has been difficult to compare results and determine whether distinct pathotypes are maintained in populations of *H. brassicae* within growing regions or over wider geographic distances (Wang et al. 2000).

For the present study we assembled a set of genetically uniform lines of *B. oleracea* (doubled haploid or self-pollinated inbred lines) derived from our previous efforts to identify sources of downy mildew resistance from European diversity of the species (Coelho et al. 1998; Monteiro et al. 2005; Vicente et al. 2012). Our objective was to use this host differential set to characterize pathotypic variation of *H. brassicae* within a European sample of the pathogen collected from various crop types of *B. oleracea*. At least six pathotypes were distinguished, indicating a potential use of the host differential for monitoring pathotype frequencies in *H. brassicae* populations, and to assess the potential durability of downy mildew control from a broad spectrum resistance (*R*) gene described in previous work (Vicente et al. 2012) either singly or in combination with other genes.

Materials and methods

This study was carried out simultaneously in two laboratories at the Instituto Superior de Agronomia, Technical University of Lisbon, Portugal, and at Warwick HRI (now School of Life Sciences, Wellesbourne campus), University of Warwick, UK. Standard material and methods were used in the two laboratories wherever possible, unless otherwise noted below.

Plant materials

Four *B. oleracea* lines developed in the UK were selected based on results obtained in previous work to define different sources of seedling resistance (Vicente et al. 2012), and three other lines were selected based on results of field trials performed in Portugal (Coelho et al. 1998; Monteiro et al. 2005) (Table 1). A rapid-cycling brassica line cv. Senna (GK97362) was included in all the tests as a universally susceptible control. All host lines were either doubled haploid or self-pollinated inbreds (Table 1), and exhibited good germination rates, good size of cotyledons at 7-9 days after sowing, and good self-fertility for relatively easy seed multiplication.

For the tests performed in Portugal, the seeds were sown in trays (3 x 3 x 5-cm cells) filled with a peat-based substrate (Levington F2, Fisons, UK), covered with a layer of vermiculite, watered by capillary matting and maintained in a growth room (20±1°C, 70±10% RH with 20 h photoperiod under cool-white fluorescent light at 250 $\mu mol\ m^{-2}\ s^{-1}$), until the cotyledons were fully expanded. For the tests performed in the UK, the seeds were sown in plug trays (2 x 2 x 3-cm cells), also filled with Levington F2 compost and covered with a layer of vermiculite, and placed in a covered propagator

in a growth room at 20±2ºC with 10 h photoperiod. All host lines were sown in the same tray and only one isolate was tested per tray. Six plants per line were evaluated, and three replications were done at different times. A total of 18 plants of each host line were tested with each isolate.

In Portugal, all isolates were maintained in rapid-cycling cv. Senna, the seeds were sown in small pots (6-cm diameter) and grown inside propagators. In the UK, the isolates were maintained in kale cv. Maris Kestrel. Approximately 10 seeds were sown per 4-cm square module filled with compost (Levington F2) soaked in water and covered with a layer of vermiculite.

Pathogen isolates

The *H. brassicae* isolates used in this study came from UK and Portuguese collections (Table 2). The six isolates tested in the UK were collected from various crop types and growing regions in the UK, as previously described by Vicente et al. (2012). Three isolates tested in Portugal (Hp530, FP06 and Hp006) were obtained from European collections (Italy, France and UK respectively) and the four Portuguese isolates were collected in three different locations from leaves that were naturally infected in untreated field plots. At the laboratory, leaves with sporulated lesions were agitated in distilled water to dislodge the spores and conidial suspensions were drop inoculated on eight-day-old detached cotyledons from seedlings of the maintenance host cv. Senna. Detached cotyledons were placed on moist filter paper in Petri dishes (9 x 1.5-cm), sealed with adhesive tape to avoid cross-contamination of isolates, and incubated to stimulate germination of spores in the dark (at 16ºC, high RH for 24 h). The Petri dishes

were transferred to the growth room for disease development, and six days after inoculation, were again incubated to promote asexual sporulation of the pathogen in the dark. Non-contaminated field isolates were obtained after some transfers into fresh plant materials. The isolates were subcultured four times, each based on single-spore culture adapted from the method described by Moss et al. (1994), and the emerging populations were assumed to be pure cultures. The Hp541 was a field population isolate that was subcultured several times.

Inoculum production

Fresh inoculum was regularly produced in each laboratory. A conidial suspension of each isolate was prepared following the method described by Leckie et al. (1996) with minor modifications. In Portugal, frozen isolates (-18ºC) were inoculated in detached cotyledons of the maintenance plant cv. Senna. One week later, freshly sporulating cotyledons were dipped in distilled water and gently agitated to dislodge conidia. Spore suspensions were centrifuged at 370 *g* for 3 min twice, and spore concentration was adjusted to 2.5 x 10^4 spores ml^{-1} for cotyledon inoculation. In the UK, the isolates were recovered from infected cotyledons preserved in a -76°C freezer. The suspensions of conidia were inoculated in cotyledons of approximately 40 nine-day-old seedlings of the maintenance plant stock cv. Maris Kestrel. Plants were incubated for seven days in a growth room at 15±2ºC with a day length of 12 h and fresh spore suspensions were prepared from cotyledons that showed sporulation. The spore concentration was measured using a haemocytometer and adjusted to 4 x 10^4 spores ml^{-1}.

Experimental inoculation

In Portugal, eight-day-old seedlings were drop inoculated by placing two 10-µl droplets of the isolate spore suspension on opposite lobes of each cotyledon. The plants in experimental trays were enclosed in sealed propagators and incubated for 24 h in the previously referred conditions, and returned to growth room for disease development. Six days later the propagators were incubated again during 24 h and the seedlings were evaluated at the 7th day with the aid of an illuminating magnifier (2x). In the UK, nine-day-old seedlings were drop-inoculated as performed in Portugal and the trays were covered with a propagator lid with the vents closed and transferred to a growth room at 15±2ºC with a day length of 12 h. The plants were also evaluated at the 7th day after inoculation.

Disease phenotypic evaluation and data analysis

Plants were individually evaluated using seven interaction-phenotype (IP) scores that take into account the degree of visible host plant response and the relative amount of asexual sporulation (Table 3). Compatible interactions resulted in asexual sporulation recorded 7 days after inoculation. The experiment for assessing host reactions was repeated in three separate occasions, in order to assess reproducibility of the tests. A mean IP score and standard error (IP±S.E.) were calculated for each combination of host line and pathogen isolate, and summarised into three phenotypic categories: incompatible IP<2.5; intermediate 2.5≤IP<4.5; and compatible IP≥4.5.

Results

The Hp717 isolate was tested in the UK and Portugal to confirm that experimental conditions between the two laboratories were suitable and comparable for development of downy mildew. Although there were some differences in the conditions in the two laboratories, the results confirmed that reactions to the inoculation of the various hosts with Hp717 were comparable (Table 4). Information was regularly exchanged and the researchers visited each other's laboratories to ensure that the experiments worked consistently in both countries. The susceptible line Senna was used in all tests as a universally susceptible control and showed high levels of sporulation consistently.

The eight *B. oleracea* lines were evaluated for their phenotypic reaction to the thirteen European isolates of *H. brassicae*. The host lines exhibiting a similar phenotypic pattern to different isolates were clustered into six classes (A to F) according to their response to the isolates (Table 4). The control line cv. Senna, as expected, was highly susceptible with no visible host response to all of the pathogen isolates tested (designated class A) (mean IP≥5.9); showing a consistent susceptible response throughout the experiments in both laboratories. The two class F lines (PCA20.14 and EBH527) were resistant or moderately resistant to all thirteen isolates (mean IP≤3.0). The remaining five lines exhibited resistance to at least one, but not all of the isolates including: a single line in class B (EBH508) which exhibited resistance to three isolates (Hp006, Hp539 and Hp704) and was highly susceptible to the remaining isolates; a class C line (EBH502) which was resistant at least to some degree to seven isolates; a class D line (EBH525) which was fully resistant to eleven isolates; and two class E lines

(PC10 and PC11) which were resistant to all of the isolates except for high susceptibility to the single FP06 isolate (from France).

Six different pathotypes of *H. brassicae* were identified by classifying the mean IP as either being compatible (mean IP≥4.5), incompatible (mean IP<2.5) or intermediate (Table 4). Isolates showing the same virulence pattern on the differential set of host lines were grouped and considered to belong to the same pathotype. Pathotypes 1, 4 and 6 were represented by a single isolate, Hp006, Hp806 and FP06 respectively. Other pathotypes were represented by more than one isolate including two examples of pathotype 2 (Hp539 and Hp704), five of pathotype 3 (Hp530, Hp533, Hp535, Hp717, and Hp710), and three of pathotype 5 (Hp541, Hp702, and Hp801). The three referred cases with multiple isolates included examples from different countries. For example, pathotype 3 includes isolates from Italy, two diverse locations in Portugal (Odemira and Azores Island) and the UK.

Discussion

Lettuce downy mildew (caused by *Bremia lactucae*) provided the seminal gene-for-gene model more than three decades ago for plant-oomycete interactions, which has since been validated by extensive genetic proof of the predicted pairs of corresponding pathogen avirulence (*Avr*) and host resistance (*R*) genes (Crute and Johnson 1976; Norwood and Crute 1984; Hulbert and Michelmore 1985; Hott et al. 1987, 1989). Analogous models have been proposed for other plant-oomycete pathosystems including spinach downy mildew (caused by *Peronospora effusa* syn. *P. farinosa* f. sp. *spinaciae*, Irish et al. 2003; Correll et al. 2007), potato late blight (*Phytophthora*

infestans, Armstrong et al. 2005), soybean root rot (*Phytophthora sojae*, MacGregor et al. 2002; Shan et al. 2004), sunflower downy mildew (*Plasmopora halstedii*, Delmotte et al. 2008) and Arabidopsis downy mildew (*H. arabidopsidis*, Holub 2008).

A minimum of five pairs of host *R* and pathogen *Avr* genes are required to explain the IP observed in this current investigation of brassica downy mildew. Several hypotheses can be considered for the allocation of *R* and *Avr* genes. The model presented in Table 5 is the simplest hypothesis involving a small number of genes. Five lines (EBH508, EBH502, EBH525, PC10 and PC11) showed isolate-specific resistance with a uniform susceptible response to FP06 isolate and distinct responses to other isolates. These lines contain at least one *R* gene. A rapid, localised host cell response was observed in many interactions. The resistance in some of these lines may be conferred by more than one gene. For example, we have hypothesised that the resistance in two class E lines (PC10 and PC11) is conferred by two genes (R4 and possibly R1); if only a single *R* gene (R4) confers resistance to pathotypes 1 to 5 of *H. brassicae*, the five pathotypes will have to contain an *Avr* gene (A4). The resistance of line EBH525 could be also controlled by one gene (R3) or a combination of two genes (R1 and R3). The cotyledon resistance in the Portuguese cabbage 'Algarvia' is suggested to be dominant and controlled by two non-independent loci (Bahcevandziev 2003).

This study shows a high level of pathotypic variation amongst *H. brassicae* isolates from *B. oleracea*. Some pathotypes (1, 4 and 6) were represented by single isolates whilst others (2, 3 and 5) were represented by more than one isolate from different locations including examples from the UK and Portugal. This indicates that a wide longitudinal distribution of similar pathotypic variation in the population of *H. brassicae* might occur in west-European crops of *B. oleracea*. Additional pathotypic

variation may still exist in the current sample of *H. brassicae.* For instance, the range of resistance phenotypes exhibited by the class C line EBH502 to isolates of pathotype 3 may indicate allelic variation or multiple *R* genes, and/or additional separation of pathotypes in *H. brassicae.* It is also very likely that additional pathotypes will be identified when isolates from other collections are tested. Clearly, molecular genetic studies in both the host and the pathogen will be needed to validate or modify the gene-for-gene predictions for the current model of the brassica downy mildew pathosystem.

The French FP06 isolate was the most virulent example in the current study (Table 4). Interestingly the same isolate was used in a previous study (Monot and Silué 2009) and only exhibited compatibility tested at the cotyledon stage with two genotypes (cauliflower cv. Billabong and broccoli cv. Marathon) out of nine brassica lines. In this previous study performed in France, a Portuguese isolate Hp501 (collected in 1994 in the Batalha region) was the most virulent exhibiting compatibility in the same nine host lines.

The existence of isolate-specific resistance to downy mildew in *Brassica* spp. lines, at the cotyledon stage, has been reported in a number of previous studies (Lucas et al. 1988; Moss et al. 1991a; Nashaat and Rawlinson 1994; Nashaat and Awasthi 1995). In this study, we have confirmed the resistance in lines used in our previous research in Portugal (Coelho et al. 1998; Monteiro et al. 2005) and the UK (Vicente et al. 2012). As all tests were performed at the cotyledon stage, it will be important to test the lines at a later stage of development with a range of *H. brassicae* isolates, as resistance in adult leaf plants can be independent controlled from seedling resistance (Monteiro et al. 2005).

A major constraint in previous studies of pathogenicity in *H. brassicae* was genetic heterogeneity within the host lines that were used to characterise isolates, leading to problems with reproducibility of phenotype assessment. *B. oleracea* is often difficult to work with because of the long generation time of many crop types, as a consequence of required vernalization treatment and/or biennial habit. Several lines used in our study were generated from crosses with rapid-cycling lines and do not require vernalization (Vicente et al. 2012). The development of monogenic differential hosts is valuable for future studies of the variability in the pathogen populations (Tapsoba and Wilson 1996).

The wide spectrum of resistance in the two class F lines (PCA20.14 and EBH527) to all thirteen *H. brassicae* isolates (Table 4) are potentially examples of broad spectrum, race-nonspecific resistance. Weak resistance, with sparse sporulation, was observed with some isolates in either host line. This may indicate allelic variation or multiple genes in the host and or pathogen genotypes. However, these host lines can be most useful for breeding programmes and are good targets for future molecular genetic investigation. Combination of resistance from classes D, E and F lines would appear to provide substantial downy mildew resistance in European crops of *B. oleracea*.

The use of resistant cultivars, including strong and durable resistance to diseases, has clear advantages for the consumers, the producers and the environment, but the existence of several pathotypes of *H. brassicae* presents a difficulty for the development and deployment of resistance cultivars. New cultivars and germplasm collections should be tested with different pathotypes to determine if useful resistance is present, and most

importantly whether it provides the broadest spectrum of resistance for a given growing region.

A standard nomenclature for designating pathotypes of downy mildew is essential for seed companies to release and promote cultivars with accurate information of the disease resistance they possess (Irish et al. 2003). Thus, a standard differential set of host lines will be invaluable to identify the pathotypes present within a region or across countries in order to select the most adequate cultivars to be introduced to control the disease. This testing is particularly important if genetic material from different countries is introduced into breeding programs. The durability of *R* genes will then depend on changes in population pathogen virulence.

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Table 1. Experimental lines of *Brassica oleracea* used in this study as a standard host differential to classify pathotypes of *Hyaloperonospora brassicae*.

Host line (code)	Crop type	Description	Origin
EBH502 (JV06092)	Cabbage (subsp. *capitata*)	doubled haploid [a]	UK
EBH508 (JV06096)	Calabrese (subsp. *botrytis*)	doubled haploid [a]	UK
EBH525 (JV06085)	Borecole (subsp. *acephala*)	S_4 inbred [a]	UK
EBH527 (JV06103)	Romanesco cauliflower (subsp. *botrytis*)	doubled haploid [a]	UK
PC11 (OL87098)	Broccoli (subsp. *italica*)	S_5 inbred	Portugal
PC10 (OL87123)	Broccoli (subsp. *italica*)	S_4 inbred	Portugal
PCA20.14	Couve de Corte (subsp. *tronchuda*)	S_6 inbred	Portugal
Senna (GK97362)	Rapid-cycling brassica	doubled haploid	UK

[a] Lines derived from crosses between rapid-cycling and the indicated 'crop-type' brassicas.

Table 2. Origin of the *Hyaloperonospora brassicae* isolates collected from field samples on different crop types of *Brassica oleracea*.

Isolate	Original host	Description [a]	Geographic origin Country (region)	Year [b]	Country where it was tested
Hp702	Calabrese	SSI	UK (Cambridgeshire)	2001	UK
Hp704	unknown	SSI	UK (Lancashire)	2001	UK
Hp710	Cauliflower	SSI	UK (Lincolnshire)	2001	UK
Hp801	Cauliflower	Field isolate	UK (Somerset)	2007	UK
Hp806	unknown	Field isolate	UK (Lancashire)	2008	UK
Hp717	Calabrese	SSI	UK (Lincolnshire)	2001	UK and Portugal
Hp530	Mixed	SSI	Italy (Sicilia) [c]	1998	Portugal
Hp533	Portuguese cabbage	SSI	Portugal (Odemira)	2006	Portugal
Hp535	Portuguese cabbage	SSI	Portugal (Açores)	2006	Portugal
Hp539	Broccoli	SSI	Portugal (Batalha)	2007	Portugal
Hp541	Broccoli	Field isolate	Portugal (Batalha)	2008	Portugal
FP06	Cauliflower	SSI	France (Yonne) [d]	2002	Portugal
Hp006	*B. oleracea*	SSI	UK (Kirton)	1983	Portugal

[a] SSI - single-sporangiospore derived isolate.

[b] Date of entry in the United Kingdom or Portuguese collection.

[c] Obtained from Dr. F. Branca (Università di Catania, Italy).

[d] Obtained from Dr. D. Silué (Prince de Bretagne Biotechnologie, France).

Table 3. Interaction-phenotype scores used to evaluate the response of *Brassica oleracea* cotyledons and relative amount of sporulation following inoculation with *Hyaloperonospora brassicae*.

IP scores	Interaction phenotype
0	No host reaction, no sporulation.
1	Light host necrosis localized on the upper cotyledon surface, no sporulation.
2	Heavy host necrosis localized on the upper cotyledon surface, no sporulation.
3	Host necrosis localized on the upper cotyledon surface, weak sporulation (five conidiophores) localized on the lower cotyledon surface confined to the point of infection.
4	Host necrosis localized on the upper cotyledon surface, heavy sporulation localized on the lower cotyledon surface confined to the point of infection.
5	No necrosis on the upper surface, sparse to moderate sporulation dispersed over the whole cotyledon surface.
6	No necrosis on the upper surface, abundant and dense sporulation dispersed over the whole cotyledon surface.

Table 4. Mean interaction phenotypes in seedlings following cotyledon inoculations for different combinations of eight *Brassica oleracea* lines and thirteen European isolates of *Hyaloperonospora brassicae*.

		IP[y] ± S.E. for *H. brassicae* isolates													**Mean**
	Pathotype	**1**	**2**		**3**						**4**	**5**			**6**	
Class	**Host line**	**006 PT[z]**	**539 PT**	**704 UK**	**530 PT**	**533 PT**	**535 PT**	**717 PT**	**717 UK**	**710 UK**	**806 UK**	**541 PT**	**702 UK**	**801 UK**	**FP06 PT**	
A	Senna	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	5.9 ±0.1	6.0 ±0.0	6.0 ±0.0	6.0
B	EBH508	2.4 ±0.3	1.0 ±0.0	2.8 ±0.3	5.6 ±0.1	5.5 ±0.1	6.0 ±0.0	6.0 ±0.0	5.5 ±0.3	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	5.1
C	EBH502	1.2 ±0.1	4.9 ±0.2	4.9 ±0.2	1.6 ±0.2	1.1 ±0.1	3.3 ±0.2	3.0 ±0.3	2.8 ±0.4	2.4 ±0.2	3.8 ±0.1	5.0 ±0.2	5.9 ±0.1	5.9 ±0.1	5.8 ±0.1	3.7
D	EBH525	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.1 ±0.1	5.0 ±0.3	1.0 ±0.0	1.0 ±0.0	1.8 ±0.3	5.8 ±0.1	1.7
E	PC10	1.0 ±0.0	1.1 ±0.1	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	3.1 ±0.3	1.0 ±0.0	1.0 ±0.0	1.4 ±0.2	5.9 ±0.1	1.5
E	PC11	1.3 ±0.1	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.3 ±0.2	2.1 ±0.3	1.0 ±0.0	1.0 ±0.0	1.5 ±0.2	6.0 ±0.0	1.5
F	PCA20.14	1.8 ±0.2	2.4 ±0.3	2.1 ±0.3	1.4 ±0.1	1.9 ±0.3	1.4 ±0.1	1.1 ±0.1	1.2 ±0.2	2.3 ±0.3	2.8 ±0.5	2.9 ±0.3	1.0 ±0.0	2.0 ±1.0	1.5 ±0.2	1.8
F	EBH527	1.1 ±0.1	1.3 ±0.2	2.8 ±0.3	1.1 ±0.1	1.3 ±0.2	1.1 ±0.1	1.1 ±0.1	1.8 ±0.3	2.7 ±0.4	2.9 ±0.4	1.1 ±0.1	1.4 ±0.2	3.0 ±0.2	1.0 ±0.0	1.7

[y] Interaction phenotypes (IP) were assessed using a seven scores (Table 3). Means and standard errors were calculated from a total of 18 plants (six plants x 3 replicates) for each combination of host line and pathogen isolate. Combinations with IP<2.5 (black) are incompatible; 2.5≤IP<4.5 (grey) are moderately incompatible/weakly compatible; and IP≥4.5 are compatible.

[z] PT or UK indicates whether the data for each isolate was collected from an experiment either in Portugal or the UK, respectively.

Table 5. A postulated gene-for-gene model to explain the host-pathogen interactions following cotyledon inoculations between six pathotypes of *Hyaloperonospora brassicae* and eight *Brassica oleracea* lines.

						***H. brassicae* pathotype**					
						1	**2**	**3**	**4**	**5**	**6**
						A1	**A1**	**.**	**.**	**.**	**.**
						A2	**.**	**A2**	**A2**	**.**	**.**
						A3	**A3**	**A3**	**.**	**A3**	**.**
						.	**.**	**A4**	**A4**	**A4**	**.**
***B. oleracea* lines**						**A5**	**A5**	**A5**	**A5**	**A5**	**A5**
Senna	.	.	.	.	.	+	+	+	+	+	+
EBH508	R1	.	.	.	.	-	-	+	+	+	+
EBH502	.	R2	.	.	.	-	+	-	-	+	+
EBH525	.	.	R3	.	.	-	-	-	+	-	+
PC10 and PC11	R1	.	.	R4	.	-	-	-	-	-	+
EBH527 and PCA20.14	.	.	.	.	R5	-	-	-	-	-	-

'+' compatible interaction (host susceptibility to a virulent pathogen); '-' incompatible interaction (at least one matching gene pair of host resistance and pathogen avirulence).