Chapter 5
Physiologic Specialization (Pathogenic Variability)

5.1 Introduction

Specificity in the downy mildew fungus on crucifers is very complex since it occurs on a wide range of wild hosts as well as agricultural and horticultural species. However, there has been little sustained effort to introduce resistance to the disease, and hence there has been less selection pressure exerted on the pathogen population than is the case with many other obligate parasites. Further impetus has been added by the exponential growth in research on the wild crucifer *Arabidopsis thaliana*, as a host for *Hyaloperonospora parasitica* and serving as a model system for genetic and molecular analysis (Uknes et al. 1992). Discontinuities in the host range of isolates from different host genera and species suggest that the fungus may exist as a series of pathotypes adapted to each host of origin, although some cross-infections may occur. There is also growing evidence that within host species, specificity may be determined by genotype-specific interactions consistent with a gene for gene recognition system (Lucas et al. 1988, 1994; Nashaat and Awasthi 1995). Specificity might therefore be expressed at several levels including family, genus, species, and cultivar or accession. In view of the close cytogenetic relationship between the major *Brassica* species, coupled with the strongly outbreeding nature of several of these, some overlap in the host range of species-adapted isolates is perhaps predictable.

At the generic level, pathogenic specialization has been observed by several workers all around the world. Gardner (1920)) and Kobel (1921) suggested that *H. parasitica* is highly specialized and seldom occurs in the same biological form on more than one crucifer. An isolate of *H. parasitica* obtained from turnip is able to infect seedlings of turnip but not rutabaga or radish (Gardner 1920). In Holland disease on cabbages is classified in two groups, both representing distinct biological forms of the fungus. The first is characterized by short, ellipsoid conidia and the second by larger, elongated conidia with protuberant apices. The average dimen-

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sions of the later group are 32.51 × 25.66 μm and that of the former 26.67 × 23.13 μm, the corresponding ratios of length to breadth being 1.26 and 1.11, respectively (Thung 1926a). However, Gaumann (1926) subdivided *H. parasitica* (= *H. brassicae*) into three biological strains, namely, 1. f. sp. *brassicae*, the chief hosts of which are *B. oleracea*, *B. napus*, *B. rapa*, *B. nigra*, *B. juncea*, *B. tournefortii,* and *B. fruticulosa*, but can also cause some infection on *Sinapis arvensis*, *S. alba*, *Raphanus raphanistrum*, *R. sativus*, and *Eruca sativa*; 2. f. sp. *sinapidis*, the principal hosts of which are *S. arvensis* and *S. alba*, but are also able to produce sub-infections on all the above-mentioned species of *Brassica* (except *B. rapa* and *B. juncea*) and *Raphanus*, with occasional conidiophore formation on *B. oleracea*; and 3. f. sp. *raphani*, the chief hosts of which are *R. raphanistrum* and *R. sativus*, but can also produce sub-infections on all the above-mentioned species of *Brassica* (except *B. fruticulosa*), as well as on *S. arvensis* and *S. alba*, with occasional conidiophore formation on *B. oleracea* and *B. napus*. The downy mildew on radish does not attack cabbage (*B. oleracea* var. *bullata* and *capitata*) and is slightly pathogenic on Chinese cabbage (*B. pekinensis*, *B. chinensis*), rape (*B. campestris*), and mustard (*B. juncea*) (Hiura and Kanegae 1934). Conversely, the form derived from *B. pekinensis* does not infect radish but is allied to one on *B. chinensis* and rape. The forms derived from *B. pekinensis*, *B. chinensis,* and rape are mutually pathogenic on one another (Hiura and Kanegae 1934).

5.2 Pathogenic Variability

The identification of pathogenic variability seems to be initiated in 1944 with the observation of Wang (1944) who classified the reaction of the hosts into four categories: 1. susceptible with normal symptoms; 2. resistant, showing large necrotic spots; 3. para-immune, showing slightly visible necrotic dots; and 4. immune, with no visible symptoms. Three pathotypes of *H. parasitica* were differentiated*: H. parasitica Brassicae* on *Brassica*, *H. parasitica Raphani* on *Raphanus,* and *H. parasitica Capsellae* on *Capsella*. The three pathotypes were not mutually compatible with each other's host. Six forms of *H. parasitica Brassicae* were differentiated by their reaction to *B. chinensis*, *B. oleracea*, *B. juncea*, and *B. napobrassica*. Wang (1944) prepared a dichotomous key to physiological forms from China as follows:

A. *Capsella bursa-pastoris*, immune
B. *Raphanus sativus*, immune or para-immune variety Brassicae
C. *B. oleracea*, resistant or para-immune
D. *B. chinensis*, susceptible
E. *B. juncea* (Meitan Dav Yu Tsai), susceptible Ph.fm. 1
EE. *B. juncea* (Meitan Dav Yu Tsai,) resistant
F. *B. napobrassica*, immune Ph. fm. 2
FF. *B. napobrassica*, resistant Ph. fm. 3
DD. *B. chinensis*, resistant
E. *B. juncea* (Dav Ching Tsai), susceptible Ph. fm. 4

EE. *B. juncea* (Dav Ching Tsai), resistant Ph. fm. 5
CC. *B. oleracea*, susceptible Ph. fm. 6
BB. *R. sativus*, susceptible variety *Raphani*
AA. *C. bursa-pastoris*, susceptible variety *capsellae*

Felton and Walker (1946) and Natti (1958) differentiated the races of *H. parasitica* found on *R. sativus* and *B. oleracea* on the basis of their host specificity. Morris and Knox-Davies (1980) also indicated distinct races of *H. parasitica* on *B. oleracea* and *R. raphanistrum* based on host specificity. *H. parasitica* f. *brassicae* on cabbage, f. rapae on turnip, f. rapifera on *B. rapa*, f. *rapifera*, f. *napi* on *rape*, f. *raphani* on radish, and f. *sinapsidis* on *Sinapis alba* have been distinguished as special forms of *H. parasitica* from Leningrad though all are similar morphologically (Dzhanuzakov 1963).

Three vars. of *H. parasitica* have been differentiated in 35 samples of downy mildew from *B. pekinensis* and other crucifers, namely, f. sp. *brassicae* on *Brassica*, f. sp. *raphani* on *Raphanus*, and f. sp. *capsellae* on *Capsella* (Chang et al. 1964). *Hyaloperonospora parasitica* f. sp. *brassicae* exists in at least three different subforms (*pekinensis*, *oleracea*, and *juncea*). Isolates from *B. pekinensis*, *B. chinensis*, and turnip were classified in the same group and can attack all three hosts but did not infect *Capsella bursa-pastoris*, radish, cabbage, Chinese mustard, and *B. juncea* var. *multiceps*. *B. juncea* var. *megarrhiza* expressed various reactions to these isolates. Isolates from Chinese mustard, *B. juncea* var. *megarrhiza* and *B. juncea* var. *multiceps,* were limited to these hosts, except that those from Chinese mustard did not infect some vars. of *B. pekinensis*, *B. chinensis*, and turnip. Radish isolates are of two types, one infects only radish, the other vars. of *B. pekinensis*, cabbage, and turnips as well as radish. Isolates from cabbage and *C. bursa-pastoris* are host specific. In Norway, cross-inoculation experiments with downy mildew from cabbage, turnip rape, and radish indicated the occurrence of different races on cabbage and radish (Semb 1969).

According to Natti et al. (1967), the predominant physiologic race of *H. parasitica* pathogenic to broccoli and other types of *B. oleracea* grown commercially in New York were race 1 and race 2. The later race was pathogenic to plants resistant to race 1. Dickinson and Greenhalgh (1977) observed a wide variation in the reaction of seedlings of different crucifers' species to isolates of *Hyaloperonospora* derived from *Brassica* and *Raphanus* species (Table 2.3). Masheva et al. (1996a) found that the pathogenicity of two isolates of *H. parasitica* (one from Plovdiv and one from Goma Oriahovitsa, Bulgaria) towards ten hybrids of cabbage, broccoli, and cauliflower differed in pathogenicity and host specificity.

In India, *H. parasitica* isolates from different hosts vary in host range. Isolates from *Brassica*, *Raphanus*, *Eruca*, and *Sisymbrium* are not cross-infective (Bains and Jhooty 1983). Mehta and Saharan (1994) tested the host range of 9 isolates of *H. parasitica* collected from the leaves and stag heads of 6 host species on 17 host differentials (Tables 5.1 and 5.2). Isolates from *Brassica* oilseeds infected all species, except *B. alba*, whereas isolates from cauliflower leaves did not infect *B. carinata*, *B. alba*, *B. nigra*, *B. chinensis*, *B. pekinensis*, and *B. napus* (Table 5.2). There was no significant difference among the conidial size of the isolates collected from

Table 5.1 Host differentials of *Hyaloperonospora parasitica* (Mehta and Saharan 1994)

Common name	Botanical name	Cultivar
Indian mustard (raya)	*Brassica juncea*	RH-30
Toria	*Brassica campestris* var. toria	TH-68
Yellow sarson	*B. campestris* var. yellow sarson	YSPb-24
Brown sarson	*B. campestris* var. brown sarson	BSH-1
Ethiopian mustard	*B. carinata*	HC-1
White mustard	*B. alba*	Local
Black mustard	*B. nigra*	Local
Chinese mustard	*B. chinensis*	Local
Chinese mustard	*B. pekinensis*	Local
Rapeseed	*B. napus*	GSL-1
Wild turnip	*B. tournefortii*	Local
Cabbage	*B. oleracea* var. *capitata*	Pride of India
Cauliflower	*B. oleracea* var. *botrytis*	Snowball-16
Turnip	*B. rapa*	White Purple Top
Knol khol	*B. caulorapa*	Early White Vienna
Taramira	*Eruca sativa*	Local
Radish	*Raphanus sativus*	HR-1

leaves and stag heads, but significant differences were observed among these groups (Table 5.3). The isolates were classified into two distinct pathotypes, one from cauliflower and the other from oilseeds *Brassica*. There was no significant difference between the isolates in percentages of spore germination (Table 5.4). Specific populations of *H. parasitica* differing in pathogenesis and host specificity were reported from Bulgaria (Masheva et al. 1996a, b). The populations formed at a lower temperature were more aggressive on cabbage heads.

In the UK, differential host resistance in relation to pathogenic variation of isolates derived from the same host species was identified in *B. rapa* (Moss et al. 1991; Silve et al. 1996), *B. napus* (Nashaat and Rawlinson 1994), *B. juncea* (Nashaat and Awasthi 1995), and *B. oleracea* (Silve et al. 1996). Isolates from different *Brassica* species found to be most virulent on their species of origin were nevertheless able to grow to less extent on other *Brassica* species (Sherriff and Lucas 1990). Nashaat and Awasthi (1995) identified five groups of *B. juncea* accessions with differential resistance to UK isolates Rl and P003 derived from oilseed rape (*B. napus* ssp. *oleifera*) and Indian isolates IP01 and IP02 derived from mustard (*B. juncea*) (Table 5.5). All *B. juncea* accessions were resistant to isolates from *B. napus*, but at the same time, *B. napus* cv. *Arian* was resistant to isolates from *B. juncea*. Twenty-one differential responses to *H. parasitica* isolates from *B. oleracea* and two from *B. rapa* were identified. Of the seven isolates tested, four were from crops of cauliflower in France, two from oilseed rape in the UK, and one was from mustard in India. All *Raphanus sativus* accessions were resistant to all seven isolates (Silve et al. 1996).

Table 5.2 Response of seventeen *Brassica* species to nine isolates of *Hyaloperonospora parasitica* (Mehta and Saharan 1994)

		Sources of *H. parasitica* isolates/reactions							
	Toria	Yellow sarson		Brown sarson		Raya		Cauliflower	*B. nigra*
Differential hosts	leaf inoculum	Leaf inoculum	Inflorescence inoculum	Leaf inoculum	Inflorescence inoculum	Leaf inoculum	Inflorescence inoculum	Leaf inoculum	Inflorescence Inoculum
Brassica campestris var. toria	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)
B. campestris var. yellow sarson	+ (6)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)
B. campestris var. brown sarson	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)
Brassica juncea	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)
B. carinata	+ (6)	+ (6)	+ (4)	+ (6)	+ (4)	+ (4)	+ (4)	–	+ (4)
B. alba	–	–	–	–	–	–	–	–	–
B. nigra	+ (6)	+ (6)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	–	+ (4)
B. chinensis	+ (6)	+ (6)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	–	+ (4)
B. pekinensis	+ (6)	+ (6)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	–	+ (4)
B. napus	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	–	+ (4)
B. tournefortii	+ (6)	+ (6)	+ (4)	+ (6)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)
Eruca sativa	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)
Raphanus sativus	+ (4)	+ (4)	+ (6)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)
B. oleracea var. *capitata*	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)
B. oleracea var. *botrytis*	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)
B. rapa	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)
B. caulorapa	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)

(), incubation period in days; +, infection; –, no infection; B, *Brassica*; 1, leaf inoculum; 2, inflorescence inoculum

Table 5.3 Conidial size of *Hyaloperonospora parasitica* isolates derived from eleven *Brassica* species (Mehta and Saharan 1994)

Sources of isolates	*H. parasitica* isolates[a]	Conidial dimensions (μ)			
		Range		Average	
		Length	Width	Length	Width
Brassica campestris var. toria	T_1	19.50–29.25	16.62–27.30	25.93	19.30
B. campestris var. yellow sarson	YS_1	21.45–29.25	19.50–24.37	25.35	21.74
B. campestris var. yellow sarson	YS_2	19.50–29.25	19.50–24.37	26.81	23.39
B. campestris var. brown sarson	BS_1	21.45–29.25	19.00–24.37	25.35	21.45
B. campestris var. brown sarson	BS_2	21.93–29.25	19.50–26.81	26.56	23.64
Brassica juncea	R_1	20.47–29.25	19.50–26.32	25.64	21.84
Brassica juncea	R_2	19.50–29.25	19.50–26.81	27.05	22.90
Brassica oleracea var. *botrytis*	C_1	19.50–29.25	17.55–24.37	23.30	20.76
B. tournefortii	BT_2	19.50–24.37	16.57–19.50	23.59	20.96
Raphanus sativus	RS_2	19.50–24.37	18.52–21.45	22.03	19.69
B. nigra	BN_2	21.93–29.25	19.50–26.81	26.81	22.90
LSD (P< 0.05)		–	–	2.43	1.78

[a]source of inoculum; 1, leaves inoculum; 2, hypertrophied inflorescence

Table 5.4 Percent conidial germination of *Hyaloperonospora parasitica* isolates at 18°C (Mehta and Saharan 1994)

Incubation period	Cauliflower	Toria	Brown sarson	Yellow sarson	Raya
	Leaf inoculum	Leaf inoculum	Leaf inoculum	Leaf inoculum	Leaf inoculum
0.0	–	–	–	–	–
0.5	0	0	0	0	0
1.0	0	0	0	0	10.57
1.5	12.20	13.73	15.72	11.87	32.46
2.0	36.50	33.25	45.87	39.31	52.68
3.0	66.30	63.25	69.12	61.06	69.12
4.0	84.25	83.86	80.99	81.92	88.85
5.0	86.02	84.91	82.61	84.16	88.85
6.0	86.51 (68.72)	87.36 (70.11)	82.90 (65.80)	85.42 (67.85)	89.19 (73.20)
LSD (P< 0.05)	NS				

Table 5.5 Sources of seed for accessions of *Brassica juncea*, arranged in five groups according to the response of their seedlings at the cotyledon stage to *Hyaloperonospora parasitica* and one accession of *B. napus* (Nashaat and Awasthi 1995)

Brassica juncea[a]	Seed source	*Brassica juncea*	Seed source
Group A		*Group C*	
RES-BJ01 (Kranti)	(India)	Ecotype/BGRC 34263	FAL
RES-BJ02 (Krishna)	(India)	BGRC 34283	FAL
RES-BJ03 (Varuna)	(India)	BGRC 34291	FAL
RES-BJ04 (BGRC 34253)	(FAL)	BGRC 34781	FAL
		BGRC 34789	FAL
		BGRC 46069	FAL
Group B			
Change Yang Huang Jie	HAU	BGRC 46071	FAL
BGRC 324294	FAL	PPBJ-1	India
		Skorosjelka-2	
Group C		BGRC 34275	FAL
Aurea/BGRC 28602	FAL	Stephniacka	
Blaze/BGRC 30288	FAL	BGRC 34274	FAL
Burgonde/BGRC 30289	FAL	Stoke/BGRC 51764	FAL
Commercial brown	Ag Cda	Yi Men Feng Wei Zi	HAU
Cutlass	Ag Cda	Zaria/BGRC 16254	FAL
Ecotype BGRC 34255	FAL		
Group D		*Group E*	
Hatano/BGRC 22527	FAL	BGRC 34282	FAL
BGRC 34239	FAL		
Landrace/BGRC 46323	FAL	*B. napus*	
Larja/BGRC 34273	FAL	Ariana	Semundo
Line/BGRC 34295	FAL		

[a]RES-BJ01 to RES-BJ04 lines selected from seedling population of accessions in parenthesis

Differential resistance of rapid cycling and commercial genotypes of *B. rapa* were also reported from the UK (Moss et al. 1988). A range of differential host responses were characterized by four homologous isolates (Table 5.6). Pathogen isolates were also characterized in relation to their sexual compatibility type and response to phenylamide fungicides (Moss et al. 1988).

Eleven isolates of *H. parasitica* tested on rapid cycling populations of *B. rapa* (aa, CrGc-1-1), *B. nigra* (bb, CrGc-2-1), *B. oleracea* (cc, CrGc-3-1), *B. juncea* (aabb CrGc-4-1), *B. napus* (aacc CrGc-5-1), *B. carinata* (bbccCrGc-6-1), and *R. sativus* (rr CrGc-7-1) indicated specificity towards particular genotypes within each rapid cycling population (Hill et al. 1988). Variation in the response of different host lines to *H. parasitica* has also been detected within wild crucifer species such as shepherd's purse (*Capsella bursa-pastoris*) and *Arabidopsis thaliana* (Lucas et al. 1994).

Moss et al. (1994) attempted to cross fungal isolates originating from different *Brassica* spp. by co-inoculating host lines previously identified as susceptible to these

Table 5.6 Differential virulence of *Hyaloperonospora parasitica* isolates from *B. campestris* on six host lines (Moss et al. 1988, 1991)

	Reaction of *H. parasitica* isolates			
Host lines	P007	P008	P013	P014
CA88014*[a]	+	+	+	+
JADE PAGODA	+	–	+	+
CA87063*	–	–	+	+
SNOWBALL	–	–	+	–
CA87068*	–	–	–	+
CA87065*	–	–	–	–

+, susceptible; –, resistant; a, universally susceptible; *, rapid cycling lines

isolates. A proportion of oospore progeny recovered from these crosses appeared to be hybrids and had reduced virulence on both hosts of origin. The differential resistance to *H. parasitica* identified in *Brassica* spp. can be used for future studies of the genetics of the host-pathogen interaction and for breeding for disease resistance.

The pathogenicity of four isolates of *H. parasitica* obtained from Japanese radish, rape, broccoli, and shepherd's purse (*Capsella bursa-pastoris*) was compared by observing infected cotyledons. These isolates eventually sporulated on host plant cotyledons (compatible combinations), but formed only small necrotic lesions on non-host cotyledons (incompatible combinations). Sheath encasement of haustoria and host cell death frequently occurred in the incompatible combinations. The *C. bursa-pastoris* isolate could only infect the host plant with haustorium formation in non-hosts inhibited mainly by papillae. Cytochemical observation showed that these papillae contained callose and polyphenol compounds but not lignin (Yoshida and Ohguchi 1998).

Silve et al. (1996) identified 21 differential responses to *H. parasitica* isolates from *B. oleracea* and 2 from *B. rapa*. All *Raphanus sativus* accessions were resistant to all seven isolates. Accessions for which seedling populations exhibited a heterogeneous reaction to some isolates were classified in a separate category. However, Mehta and Saharan (1994) classified 9 isolates of *H. parasitica* (collected from *Brassica* species) into 2 distinct pathotypes, 1 from cauliflower, and the other from oil-yielding *Brassica* species on the basis of their reaction to a set of 17 host differentials.

Variation has been found among interaction phenotypes when a range of *A. thaliana* (At) and *Brassica* accessions are inoculated with different isolates of *H. parasitica* (Hp). In *Brassica*, genetic characterization of this variation was rudimentary, and only one allele at a single locus associated with isolate-specific resistance to *H. parasitica* in *B. napus* has been defined. In contrast, and considering only those phenotypes that can be discriminated readily among segregating individuals, alleles controlling genotype-specific variation for response to these pathogens have been identified at 21 loci (RPP and RAC) in the At genome. Most of these loci now have been mapped with varying degrees of resolution and occur in several linkage groups in four of the five At chromosomes (Leckie et al. 1996).

Eight *B. oleracea* lines were evaluated for their phenotypic reaction to the thirteen European isolates of *H. brassicae* (Table 5.7) by Coelho et al. (2012). The host lines exhibiting a similar phenotypic pattern to different isolates were clustered into six classes (A–F) according to their response to the isolates (Table 5.8). The control lines cv. Senna were 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 (Tables 5.9 and 5.10). The two class F lines (PCA 20.14 and EBH 527) 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, Hp 539, and Hp 704), and were highly susceptible to the remaining isolates: a class C line (EBH 502) which was resistant at least to some degree to seven isolates, a class D line (EBH 525) 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 FP 06 isolates (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 5.8). 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 (Hp 530, 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 and two from diverse locations in Portugal (Odemira and Azores Island) and the UK (Coelho et al. 2012). Tham et al. (1994) used RAPD analysis to compare 16 isolates of *H. parasitica* from two different *Brassica* species, *B. napus* and *B. oleracea*. Two out of twenty random primers screened gave reproducible band patterns capable of discriminating between the different host-adopted isolates (Plate 5.1).

Table 5.7 *Brassica oleracea* standard host differentials to classify pathotypes of *Hyaloperonospora brassicae* (*H. parasitica*) (Coelho et al. 2012)

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

*Lines derived from crosses between rapid-cycling and the indicated ‘crop-type’ brassicas

Table 5.8 Mean interaction phenotypes in seedlings following cotyledon inoculations for different combinations of eight *Brassica oleracea* lines and thirteen European isolates of *Hyaloperonospora brassicae* (*H. parasitica*) (Coelho et al. 2012)

		IP[¥] ± S.E. for *Hyaloperonospora brassicae* isolates														
	Pathotype	1	2		3						4	5			6	
		006	539	704	530	533	535	717	717	710	806	541	702	801	FP06	
Class	Host line	PT[≠]	PT	UK	PT	PT	PT	PT	UK	UK	UK	PT	UK	UK	PT	Mean
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	PCA 20.14	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

[¥] Interaction phenotypes (IP) were assessed using seven scores (Table 5.10). Means and standard errors were calculated from a total of 18 plants (6 plants x 3 replicates) for each combination of host line and pathogen isolate. Combinations with IP<2.5 are incompatible; 2.5 ≤IP < 4.5 are moderately incompatible/ weakly compatible, and IP≥4.5 are compatible

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

Table 5.9 Origin of the *Hyaloperonospora brassicae* (*H. parasitica*) isolates collected from field samples on different crop types of *Brassica oleracea* (Coelho et al. 2012)

Isolates	Original host	Description[a]	Geographic origin country (region)	Year[b]	Country where it was tested
Hp702	Calabrese	SSI	UK (Cambridge shire)	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 (Acores)	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]Single-sporangiospore-derived isolate
[b]Date of entry in the UK or Portuguese collection
[c]Obtained from Dr. F. Branca (University di Catania, Italy)
[d]Obtained from Dr. D. Silve (Prince de Bretagne Biotechnie, France)

Table 5.10 Interaction-phenotype scores used to evaluate the response of *Brassica oleracea* cotyledons and relative amount of sporulation following inoculation with *Hyaloperonospora brassicae* (*H. parasitica*) (Coelho et al. 2012)

IP scores	Interaction phenotype
0	No host, 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

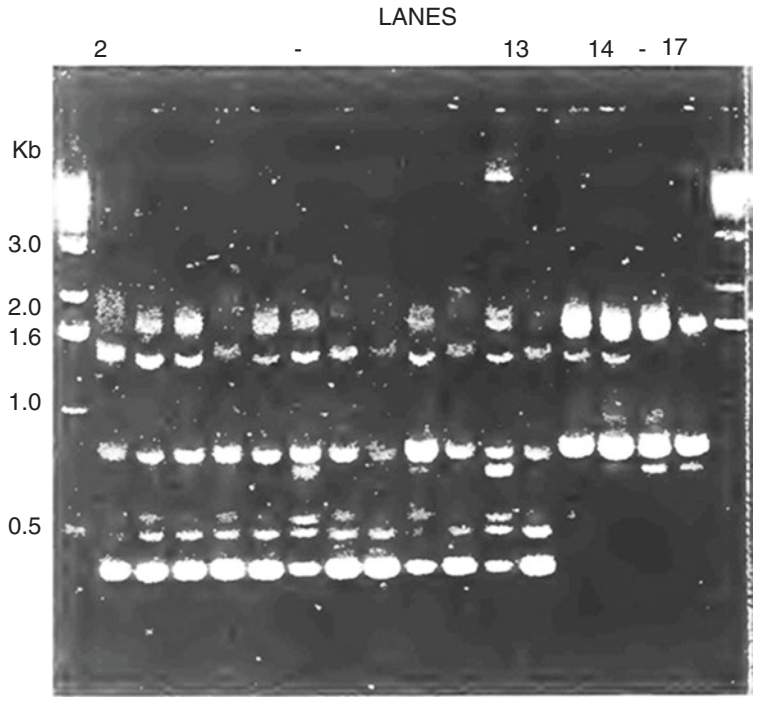

Plate 5.1 Random amplified polymorphic DNA (RAPD) from 16 isolates of crucifer downy mildew (*Hyaloperonospora parasitica*), lanes 2–13 are isolates from oilseed rape *Brassica napus*, and lanes 14–17 are isolates from cauliflower *B. oleracea* (Tham et al. 1994)

5.2.1 DNA Fingerprinting of H. parasitica

Different pathotypes can be distinguished on the basis of host specificity (Sherriff and Lucas 1990), but such tests are time-consuming and may not reveal the full extent of variations present. Other phenotypic markers such as sexual compatibility type (Sherriff and Lucas 1989a, b) or fungicide sensitivity (Crute et al. 1985) can be assessed, but they provide limited information for epidemiological studies or genetic analysis. There is need, therefore, for alternative molecular markers (Michelmore and Hulbert 1987) to further define variations in the pathogen.

Randomly amplified polymorphic DNAs (RAPDs) have been proposed as genetic markers that overcome many of the technical limitations of restriction fragment length polymorphism (RFLP) analysis (Williams et al. 1990; Welsh and McClelland 1990). RAPD scan can be used in the constructions of linkage maps (Williams et al. 1990; Reiter et al. 1992), in the identification of strains, and varieties by genomic fingerprinting (Welsh and McClelland 1990; Goodwin and Annis 1991; Hu and Quiros 1991; Schafer and Wostemeyer 1992; Kresovich et al. 1992; Klein-Lankhorst et al. 1991; Koller et al. 1993; Stiles et al. 1993) and following the cloning of amplified fragments may also serve as conventional RFLP probes. In the

study carried out by Tham et al. (1994), the potential use of RAPDs as a source of genetic markers in *H. parasitica* was evaluated. Several isolates from different host species were compared to determine whether reproducible banding patterns correlated with host specificity. As *H. parasitica* can only be cultured on living plant tissues, particular attention was paid to possible artefacts arising through contamination of sample DNA from plant or other microbial sources.

Random amplified polymorphic DNA (RAPD) fingerprints were generated from a range of *H. parasitica* isolates from different *Brassica* species (Table 5.11). Reaction conditions, in particular DNA template, primer, and Mg^{2+} concentrations, were optimized to ensure that amplifications were reproducible. Possible artefacts arising through host plant DNA were assessed by including such DNA in control reactions. Confirmation that diagnostic RAPD bands were generated from fungal DNA was also obtained by southern hybridization of a RAPD band to genomic fungal DNA. By screening 20 decamer primers, 2 were found to detect sufficient genetic variation to allow complete differentiation between pathotypes. These results illustrate the potential value of RAPDs for detecting polymorphisms between isolates of a non-culturable plant pathogenic fungus. Rehmany et al. (2000)

Table 5.11 Isolates used for RAPD analysis (Tham et al. 1994)

Isolate code	Source host	Year of isolation	Geographic origin	Mating type[a]	Response to metalaxyl[b]
P001	*B. napus*	1982	Leicestershire	Homothallic	S
P1072	*B. napus*	1992	Hertfordshire	Homothallic	nt
P1118	*B. napus*	1993	Leicestershire	Homothallic	nt
P1119	*B. napus*	1993	Leicestershire	Homothallic	nt
P1130	*B. napus*	1993	Nottinghamshire	Homothallic	nt
P1121	*B. napus*	1993	Essex	Homothallic	nt
P1122	*B. napus*	1993	Hertfordshire	Homothallic	nt
P003[c]	*B. napus*	1983	–	P1	S
P004[c]	*B. napus*	1983	–	P1	S
P033[c]	*B. napus*	1983	–	P2	S
P1100[c]	*B. napus*	1992	–	P2	nt
P1105[c]	*B. napus*	1992	–	P1	nt
P005	*B. oleracea*	1977	Tyne and wear	P1	S
P006	*B. oleracea*	1983	Lincolnshire	P2	R
P1091	*B. oleracea*	1991	Lincolnshire	nt	nt
P1092	*B. oleracea*	1991	Lincolnshire	nt	nt

[a]Mating defined as in Sherriff and Lucas (1989b)
[b]S, sensitive; R, resistant; nt, not tested
[c]Single-spore oospores progeny from self of isolates P001, P003, and P033 segregated for virulence to the oilseed rape (*B. napus* var. *oleiferas*) cv. Cresor

compared downy mildew isolates from *A. thaliana* and *B. oleracea* using AFLP and ITS analysis dividing the isolates into five groups that correlated with taxonomic species and in most of the cases with host origin.

5.2.2 *Identification of Host Differentials and Nomenclature of Pathotypes*

Physiologic specialization/pathogenic variability have long been recorded in *H. parasitica* infecting cruciferous plants all over the world (Gardner 1920; Kobel 1921; Thung 1926b; Gaumann 1926; Hiura and Kanegae 1934; Wang 1944). However, nomenclature and designation of *H. parasitica* pathotypes/races were initiated by Natti et al. (1967) based on differential interaction of isolates on cruciferous hosts, viz. broccoli and *B. oleracea*. Natti et al. (1967) identified and designated race 1 and race 2 of *H. parasitica* showing differential reactions on these two hosts. Later, several reports have come from different countries on the host specificity of *H. parasitica* isolates collected from numerous cruciferous hosts (Felton and Walker 1946; Natti 1958; Dzhanuzakov 1963; Chang et al. 1964; Semb 1969; Dickinson and Greenhalgh 1977; Morris and Knox-Davies 1980; Hill et al. 1988; Sherriff and Lucas 1990; Moss et al. 1991; Mehta and Saharan 1994; Lucas et al. 1994; Tham et al. 1994; Silve et al. 1996; Masheva et al. 1996b; Yoshida and Ohguchi 1998). The methodology and host species/varieties/lines used in these studies were not comparable and reproducible in the absence of non-maintenance of isolates and the use of international standard set of host differential. It is difficult to compare the results and determine whether distinct pathotypes are the same or different. Mehta and Saharan (1994) designated distinct pathotypes, one from cauliflower and other from oilseed *Brassica* on the basis of differential interaction of 9 isolates on a set of 17 host cruciferous host differential set (Table 5.12). Nashaat and Awasthi (1995) identified five groups of *B. juncea* (Table 5.5) accessions with differential resistance to UK isolates R1 and P003 derived from oilseed rape (*B. napus* ssp. *oleifera*) and Indian isolates IP01 and IP02 derived from mustard (*B. juncea*). Coelho et al. (2012) assembled set of genetically uniform lines of *B. oleracea* (doubled haploid or self-pollinated inbred lines) to use as host differential set for the characterization of pathotypic variation of *H. parasitica* (*H. brassicae*) within a European sample of the pathogen collected from various crop types of *B. oleracea*. Six pathotypes (HP1–HP6) were distinguished indicating a potential use of the host differentials for monitoring frequencies in *H. parasitica* populations (Table 5.8). A naming system for the isolates of *H. arabidopsidis* was introduced by Dangl et al. (1992), Holub et al. (1994), and Slusarenko and Schlaich (2003) on the basis of geographical location and ecotypes infected (AHCO, ASWA, CALA, EDCO, EMWA, etc.). For detailed description, see chapter 1, section 1.11.6. However, still there is a need to standardize a set of host differentials (isogenic lines) and a system of nomenclature and designation of pathotypes of *H. parasitica* at international level. It is

Table 5.12 Identification of pathotypes of *Hyaloperonospora parasitica and H. arabidopsidis*

Designated pathotype	Country	International primary host	References
Isolate 1	USA	Turnip	Gardner (1920)
Biological forms 2	Holland	Cabbage	Thung (1926b)
Biological strains 3	Germany	Crucifers	Gaumann (1926)
Biological forms	Japan	Crucifers	Hiura and Kanegae (1934)
Pathotypes 3	China	*Brassica*, *Raphanus*, *Capsella*	Wang (1944)
Races	USA	*Raphanus*, *B. oleracea*	Felton and Walker (1946), and Natti (1958)
Races	South Africa	*B. oleracea*, Raphanus	Morris and Knox-Davies (1980)
Special forms	Leningrad	Crucifers	Dzhanuzakov (1963)
Three forms	China	Crucifers	Chang et al. (1964)
Races	Norway	Cabbage, radish	Semb (1969)
Races 1 and 2	USA (New York)	Broccoli, *B. oleracea*	Natti et al. (1967)
Isolates	India	Crucifers	Bains and Jhooty (1983)
Pathotypes 2 (9 isolates)	India	*Brassica*, cauliflower	Mehta and Saharan (1994)
Isolates P007, P008, P013, P014	UK	*B. rapa* (*B. campestris*)	Moss et al. (1991), and Silve et al. (1996)
Isolates	UK	*B. napus*	Nashaat and Rawlinson (1994)
IP01 and IP02	UK	*B. juncea*	Nashaat and Awasthi (1995)
R1 and P003	UK	*B. napus*	Nashaat and Awasthi (1995)
7 isolates	UK, France, India	Cauliflower (4) Rape (2), mustard (1)	Silve et al. (1996)
11 isolates	USA	Crucifer rapid cycling population	Hill et al. (1988)
2 isolates	Bulgaria	Plovdiv, Goma Oriahovitsa	Masheva et al. (1996a, b)
4 isolates	Japan	Japanese radish, rape, broccoli, shepherd's purse	Yoshida and Ohguchi (1998)
21 isolates	UK	*Brassica oleracea*	Silve et al. (1996)
2 isolates	UK	*B. rapa*	Silve et al. (1996)
6 pathotypes HP1–HP6	Portugal, UK	*B. oleracea*	Coelho et al. (2012)
5 groups of isolates through DNA fingerprinting (P001–P005)	UK	A. *thaliana*, *B. oleracea*	Tham et al. (1994)

(continued)

Table 5.12 (continued)

Designated pathotype	Country	International primary host	References
AHCO, ASWA, CALA 2, CALA, CAND 3, EDCO, EMCO, EMOY, EMOY 2, EMWA 1, GOCO, GOWA, HIKS1, HIKS, HIND, HIND 2, HIND 4, MADI, MAKS, MAKS9, NOCO, NOCO 2, NOKS 1, WAND, WELA, WELA1, WELA 3	Germany, UK	*Arabidopsis accessions* Col-0, Ler-0, Oy-0, Nd-1, Ws-0, Wei-0	Dangl et al. (1992), Holub et al. (1994), Slusarenko and Schlaich (2003), and Schlaich and Slusarenko (2009)

essential to know whether pathotypes of *H. parasitica* reported so far from different countries are the same or different (Table 5.12). It will be very helpful to identify resistant genes and their combinations to breed resistant cvs. and their durability extent under different agroecological situations.

5.3 Heterothallism and Homothallism

Induction of the sexual process in several downy mildew species requires the presence of two strains of opposite mating type. Such heterothallic behaviour has been reported for *H. parasitica* (De Bruyn 1937; McMeekin 1960; Kluczewski and Lucas 1983; Sherriff and Lucas 1989b; Sequeira and Monteiro 1996). Homothallic forms of *H. parasitica* have also been observed (De Bruyn 1937; Sherriff and Lucas 1989b; Sequeira and Monteiro 1996). No relationship has been observed between geographic origin and mating types. Both heterothallic and homothallic isolates have been isolated from the same fields (Sequeira and Monteiro 1996). The two forms of sexual reproduction are very important for the maintenance and evolution of the fungal strains and epidemiology of the disease. Two mating type heterothallic isolates of *B. oleracea* and *B. campestris* designated as P1 and P2 have been identified. Isolates from oilseed rape *B. napus* have been found to be uniformly homothallic and remained self-fertile even after months of laboratory subculture (Sherriff and Lucas 1989b). In a cytogenetic study of heterothallic and homothallic isolates of *H. parasitica* at metaphase 1 of meiosis, a ring of four chromosomes is found (Sherriff and Lucas 1989a). This ring is interpreted as a reciprocal translocation complex between chromosomes carrying the mating-type alleles. In homothallic isolates a fifth chromosome is associated with the ring of four. The self-fertility of these isolates may therefore be due to the presence of a third mating-type allele on the fifth chromosome, a condition known as secondary homothallism. The determination of sexual compatibility type (SCT) of an unknown isolate can be achieved by mixing conidia in a 1:1 ratio with isolates of known SCT and inoculating to a common compatible host. In heterothallic isolates, oospores may form in combination with isolates of opposite SCT. Isoenzyme markers are particularly important in discriminating between self and true hybrid progeny (Moss et al. 1988).

5.4 Hybridization of *Hyaloperonospora* Isolates

Moss et al. (1994) produced viable oospore of *H. parasitica* under laboratory conditions and described recovered isolates (referred as sexual progeny) from these oospore populations. Oospores were produced when isolates of opposite sexual compatibility type, specialized to the same or different *Brassica* species, were grown together in seedling cotyledons of a host line capable of supporting growth of both isolates. Recovery of sexual progeny from oospore populations produced from two out of four pairing between isolates, is specialized in the same host species (homologous pairings) and proved to be relatively easy. On the basis of their characterization with respect to virulence, response to phenylamide fungicides, sexual compatibility type, and isoenzyme polymorphisms, there was evidence that the sexual progeny from these homologous pairing could be of hybrid origin. For the first time in a member of the Peronosporaceae, it provided possibility to recover and successfully characterize a new sexual progeny from pairing between isolates specialized to different host species (heterologous pairing). However, the majority of such isolates sporulated weakly and, as consequence, proved difficult to maintain and were lost. Nevertheless, it is concluded that some evidence for the hybrid nature of progeny from heterologous pairing was obtained.

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