Differential Responses of *Brassica oleracea* and *B. rapa* Accessions to Seven Isolates of *Peronospora parasitica* at the Cotyledon Stage

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ABSTRACT

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Nineteen accessions of *Brassica oleracea* var. *italica* (broccoli), 31 of *B. oleracea* var. *botrytis* (cauliflower), two of *B. oleracea* var. *capitata* (cabbage), three of *B. rapa* subsp. *rapifera* (turnip), one of *B. rapa* subsp. *pekinensis* (Chinese cabbage), and three of *Raphanus sativus* (radish) were tested for their response to isolates of *Peronospora parasitica* (downy mildew) at the cotyledon stage. Of the seven isolates tested, four were from crops of cauliflower in France, two from oilseed rape (*B. napus* subsp. *oleifera*) in the UK, and one was from mustard (*B. juncea*) in India. Twenty-one differential responses to *P. parasitica* isolates from *B. oleracea* and two from *B. rapa* were identified. 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. The differential resistance to *P. parasitica* identified in *B. oleracea* and *B. rapa* can be used for future studies of the genetics of the host-pathogen interaction and for breeding for disease resistance.

Downy mildew (*Peronospora parasitica* (Pers.:Fr.)Fr.) is the most frequently recorded disease on horticultural and agricultural members of the genus *Brassica*. The disease mainly affects young plants that may, in severe cases, be stunted or killed. Infection at later stages results in the debilitation and reduction in performance and quality of the host plant. Infection may extend to the curds of cauliflower, broccoli, and cabbage heads both in the field and post harvest (2). Predictions of climate warming (8) and the possibility of milder, wetter winters in Europe may favor downy mildew and thus aggravate the problem of the disease. Chemical control of the disease may not always be reliable as resistance has developed in *P. parasitica* to metalaxyl, which at one stage proved outstandingly effective in the control of downy mildew (1,3). Thus, there is clearly a need to breed sources of host resistance that would counter pathogenic variation. It is also possible that differential sources of host resistance could be useful in programs of integrated control if they were deployed together with fungicides; this would potentially prolong the effectiveness of both control procedures. Evidence has been reported for differential responses in *B. oleracea* and *B. rapa* capable of discriminating four and two pathotypes of the fungus, respectively, derived from the same host species (4,5). Differential resistance to *P. parasitica* expressed at the cotyledon stage has also been reported in other *Brassica* spp. (6,7). Characterization of further resistance to pathogenic variation in *Brassica* spp. is necessary for efficient breeding and may also provide markers for fungal genetic studies. Isolates of *P. parasitica* from different *Brassica* spp. were found to be most virulent in their species of origin, but were nevertheless able to grow, although generally not as well, on other *Brassica* species (10). Therefore, *P. parasitica* derived from a *Brassica* sp. may be used to characterize resistance in other related species. The purpose of the present study was to identify differential sources of resistance to downy mildew in 52 accessions of *B. oleracea* using pathogen isolates derived from *B. oleracea*, *B. napus,* and *B. juncea.* Four accessions of *B. rapa* and three accessions of *R. sativus* were also included for comparison and to identify differential responses in these species to the seven pathogen isolates of *P. parasitica* used in this study.

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MATERIALS AND METHODS

Source and maintenance of *P. parasitica* isolates. Seven single-spore isolates of *P. parasitica* were used in this study: FP03 to FP06 were collected from crops of cauliflower (*B. oleracea* L. var. *botrytis*) in France (FP03 and FP04 were collected from Brittany, FP05 from Manche, and FP06 from Yonne). Isolates P003 and R1 were collected from crops of winter oilseed rape (*B. napus* L. subsp. *oleifera*) in the UK (P003, obtained from the University of Nottingham, was a sexual progeny isolate recovered from a homothallic field isolate collected from Leicestershire; isolate R1 was collected from Hertfordshire). Isolate IP02 was collected from mustard (*B. juncea* (L.) Czernj. & Coss.) at Pantnagar, North India (brought to the UK under MAFF Import Licence No. PHF 1307B/14/102). All isolates were maintained separately on 6-day-old cotyledons; isolates FP03-FP06 were maintained on cauliflower cv. Billabong, isolates P003 and R1 were maintained on winter oilseed rape cv. Ariana, and isolate IP02 was maintained on mustard line PPBJ1 (6).

Germ plasm screening and evaluation. Host accessions and their sources are listed in Table 1. Seedlings were grown in 5 cm "Jiffy-pots" containing soil-less compost, in plant propagators (46 × 22 × 15 cm) in a glasshouse at 18 ± 2°C. Supplementary light was given to maintain a 12-h photoperiod. Eight adjacent Jiffy-pots were used for each accession in each propagator that contained up to 10 accessions, including a susceptible control. To ensure uniform water supply, the seedlings were watered via holes through the base of the propagator trays by immersing the propagators for 7 days prior to the inoculation in approximately 1 cm of dip water. Ten seedlings per accession were grown in each propagator. Up to 30 seedlings per accession distributed between three propagators were tested.

Conidial suspensions of *P. parasitica* isolates were prepared separately by washing off the conidia from the cotyledons on which isolates had been maintained (approximately 2×10^4 conidia/ml). Seedlings were inoculated using a micropipette by placing 20 µl of the conidial suspension on each side of the cotyledon. The propagators were sealed after inoculation to allow the relative humidity to increase to approximately 100%, and then placed in growth cabinets at 16°C with 12 h of darkness followed by a 16-h photoperiod under 120 µE s^{-1} m^{-2} of irradiance. Interaction phenotypes (IP) were observed 7 days after inoculation using the following 0 to 9 scale (7):

0 = No symptoms or signs of *P. parasitica.*

1 = Minute scattered necrotic flecks under inoculum drop, no sporulation.

2 = Larger necrotic flecks under inoculum drop, no sporulation.

3 = Very sparse sporulation, one to a few conidiophores, necrotic flecking. often present, tissue necrosis present.

5 = Sparse sporulation, tissue necrosis.

7 = Abundant sporulation, tissue necrosis and chlorosis may be present.

9 = Heavy sporulation, cotyledon collapsed.

A disease index (DI) was calculated using the following formula (12): DI = $(1/n)\Sigma$ for $i = 0$ to 9 $(i \times j)$, in which n = total plants, i = IP class and j = number of plants per class.

Host material was classified into three categories: susceptible (DI = 6 to 9), partially resistant (4 to 5) and resistant (0 to 3).

RESULTS AND DISCUSSION

Accessions from all host species tested were grouped according to their response to seven isolates of *Peronospora parasitica*.

All accessions of *Raphanus sativus* L. (radish) were classified in a single category "A." Accessions of *B. rapa* L. subsp. *rapifera* (turnip) and *B. rapa* subsp. *pekinensis* (Chinese cabbage) were classified in two categories, "A" and "B," respectively. The largest group was *B. oleracea*. This group which was classified into 21 categories, "C" to "W," included 21 accessions of *Brassica oleracea* var. *italica* Plenck. (broccoli), 39 of *B. oleracea* var. *botrytis* L. (cauliflower), and two of *B. oleracea* var. *capitata* L. (cabbage) (Table 2). Accessions for which seedling populations exhibited a heterogeneous reaction to some isolates, were classified in a separate category "X" (Table 1). This group included accessions of *B. oleracea* var. *capitata, italica,* and *botrytis*.

The heterogeneity for IP of accessions in category "X" may have occurred because no consideration was given to resistance to downy mildew during their breeding. During seed multiplication, a combination of self-pollination and cross-pollination would cause the observed variation (11).

The results demonstrate that the virulence frequencies of *P. parasitica* isolates derived from *B. napus* on *B. oleracea* accessions are not much different and in some cases are the same as those derived from *B. oleracea* accessions (Table 3). The virulence frequency of the isolate derived from *B. juncea* (IP02) on *B. oleracea* accessions seems to be much less than those of isolates derived from *B. napus* (P003 and R1) and *B. oleracea* (FP03, FP04, FP05, and FP06). These results may be partly interpreted in terms of the genetic relationships between *Brassica* species (9). *B. oleracea* is diploid with the genomic designation *CC*. *Brassica juncea* and *B. napus* are amphidiploid derivatives with the genomic designations *AABB* and *AACC* indicating their cytogenetic origins.

Table 1. Host accessions, sources of seed, and other characteristics of *Raphanus sativus* (radish); *Brassica rapa* subsp. *rapifera* (turnip) and *B. rapa* subsp. *pekinensis* (Chinese cabbage); *B. oleracea* var. *capitata* (cabbage), *botrytis* (cauliflower), and *italica* (broccoli) arranged in groups according to their responses at the cotyledon stage to seven isolates of *Peronospora parasitica* (see Table 2)

Host species / accessions	Seed source[a]	Groups[b]	Other characteristics
Raphanus sativus			
Bamba, Noir Long d'Horloge, Rave à Forcer	CSP	A	Cultivars
Brassica rapa subsp. *rapifera*			
Long Blanc de Croissy, Stanis, Jaune Boule d'Or	CSP	A	Cultivars
B. rapa subsp. *pekinensis*			
Cadix	CSP	B	F_1 hybrid
B. oleracea var. *botrytis*			
OBS 1	OBS, INRA	C	Selfed Line
B. oleracea var. *capitata*			
Sel Isa	CSP	D	Cultivar
B. oleracea var. *botrytis*			
BR 18	OBS	E	Selfed line
B. oleracea var. *botrytis*			
BR 14, LT 02, BR 27	OBS	F	Selfed lines
B. oleracea var. *botrytis*			
BR 21	OBS	G	Selfed line
B. oleracea var. *botrytis*			
BR 08, BR 15, BR 16, BR 26	OBS	H	Selfed lines
B. oleracea var. *botrytis*			
BR 17	OBS	I	Selfed line
B. oleracea var. *italica*			
Hmart $33A_1A_1$, Hmart 52B, Hmart $46A_6B_1$	CSP	J	Double haploid
B. oleracea var. *italica*			
SAM I	CSP	J	Breeding line
B. oleracea var. *italica*			
Mart I	CSP	K	Breeding line
B. oleracea var. *botrytis*			
BR 03	OBS	K	Selfed line
B. oleracea var. *italica*			
Hmart 43B, Hmart 29B, Hmart 16B, Hmart 27B, Hmart 53B	CSP	L	Double haploid
B. oleracea var. *botrytis*			
BR 11	OBS	L	Selfed line
B. oleracea var. *italica*			
Hmart $35A_1$	CSP	M	Double haploid
B. oleracea var. *italica*			
Hmart 21A	CSP	N	Double haploid
B. oleracea var. *italica*			
Emp 1, Ski 1	CSP	O	Breeding lines
B. oleracea var. *botrytis*			
Cl 142	CSP	O	F_1 hybrid
B. oleracea var. *italica*			
Siria	CSP	P	F_1 hybrid
B. oleracea var. *italica*			
SOF 2	CSP	Q	Breeding line
Hmart 54B	CSP	Q	Double haploid
B. oleracea var. *botrytis*			
Nancy	CSP	R	F_1 hybrid
B. oleracea var. *italica*			
Empereur	CSP	S	Breeding line
B. oleracea var. *botrytis*			
Cl 89	CSP	T	F_1 hybrid
Fanch	OBS	T	F_1 hybrid
B. oleracea var. *botrytis*			
C 13	OBS	U	F_1 hybrid
B. oleracea var. *botrytis*			
BR 10, BR 12	OBS	V	Breeding lines
Hypolyte	CSP	V	F_1 hybrid
B. oleracea var. *botrytis*			
BR 02, BR 05, BR 06, BR 07, BR 28	CSP	W	Breeding lines
Billabong, CL 965	CSP	W	F_1 hybrid
B. oleracea var. *capitata*			
Sel Eco	CSP	X	Cultivar
B. oleracea var. *italica*			
GOP 1, Vet 1	CSP	X	Breeding lines
B. oleracea var. *botrytis*			
Nautilus	CSP	X	F_1 hybrid
BR 19, BR 24	CSP	X	Breeding lines

[a] CSP, Clause Semences Professionelles, Paris, France. OBS, Organisation Bretonne des Semences, Plougoulm, France. INRA, Institut National de la Recherche Agronomique, Plougoulm, France.

[b] Group X, Accessions expressing heterogeneous responses to *P.parasitica*.

Table 2. Responses of groups "A" to "W" of *Raphanus sativus* (radish); *Brassica rapa* subsp. *rapifera* (turnip) and *pekinensis* (Chinese cabbage); *B. oleracea* var. *capitata* (cabbage), *botrytis* (cauliflower), and *italica* (broccoli) accessions at the cotyledon stage to infection with seven isolates of *Peronospora parasitica*

Host species	Host category[a]	Disease index for *P. parasitica* isolates[b]						
		P003	IP02	FP05	FP06	FP04	R1	FP03
Raphanus sativus	A (3)	–	–	–	–	–	–	–
Brassica.rapa subsp. *rapifera*	A (3)	–	–	–	–	–	–	–
B. rapa subsp. *pekinensis*	B (1)	–	–	–	–	–	–	+
B. oleracea var. *botrytis*	C (1)	–	–	–	–	–	+	+
B. oleracea var. *capitata*	D (1)	–	–	–	–	+	+	+
B. oleracea var. *botrytis*	E (1)	–	–	–	+	+	+	+
B. oleracea var. *botrytis*	F (4)	–	–	+	+	+	+	+
B. oleracea var. *botrytis*	G (1)	–	–	±	+	+	+	+
B. oleracea var. *botrytis*	H (4)	–	+	+	+	+	+	+
B. oleracea var. *botrytis*	I (1)	–	±	±	+	+	+	+
B. oleracea var. *italica*	J (4)	+	–	±	+	+	+	+
B. oleracea var. *italica*	K (1)	±	–	±	+	+	+	+
B. oleracea var. *botrytis*	K (1)	±	–	±	+	+	+	+
B. oleracea var. *italica*	L (5)	+	–	+	+	+	+	+
B. oleracea var. *botrytis*	L (1)	+	–	+	+	+	+	+
B. oleracea var. *italica*	M (1)	±	–	+	+	+	+	+
B. oleracea var. *italica*	N (1)	+	–	+	+	+	±	+
B. oleracea var. *italica*	O (2)	+	+	–	+	+	+	+
B. oleracea var. *botrytis*	O (1)	+	+	–	+	+	+	+
B. oleracea var. *botrytis*	P (1)	+	±	–	+	+	+	+
B. oleracea var. *italica*	Q (2)	+	–	–	+	+	+	+
B. oleracea var. *botrytis*	R (1)	–	+	+	+	+	+	–
B. oleracea var. *italica*	S (1)	+	–	+	±	+	+	–
B. oleracea var. *botrytis*	T (2)	+	+	–	–	+	+	+
B. oleracea var. *botrytis*	U (1)	+	–	–	–	+	+	+
B. oleracea var. *botrytis*	V (3)	±	±	+	+	+	+	+
B. oleracea var. *botrytis*	W (7)	+	+	+	+	+	+	+

[a] Number of accessions of each group in parenthesis.
[b] + Susceptible (disease index 6-9), ± partially resistant (4-5), - resistant (1-3).

Table 3. Frequency of disease indices for the interaction phenotypes between *Peronospora parasitica* isolates and *Brassica oleracea* accessions

Disease index	*Peronospora parasitica* isolates and host origin						
	B. juncea	*B. napus*		*B. oleracea*			
	IP02	P003	R1	FP03	FP04	FP05	FP06
1 to 3	29	14	0	0	1	10	4
4 to 5	4	5	1	2	0	8	1
6 to 9	16	30	48	47	48	31	44

Brassica oleracea and *B. napus* isolates, adapted to the *C* genome that both of these host species share, are therefore likely to be more specialized than *B. juncea* isolates, adapted to both *A* and *B* genomes.

The differential resistance to *P. parasitica* isolates identified in this study is of a special value for breeding for disease resistance, particularly if resistance throughout the growth of the plant is confirmed. The resistance and matching virulence require further characterization using other pathogen isolates and a wider range of host genotypes with a view to future utilization in studies of host-pathogen genetics and breeding for disease resistance. The newly identified differential sources of resistance in this study are available from the first author.

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LITERATURE CITED

1. Brophy, T. F., and Laing, M. D. 1992. Screening of fungicides for the control of downy mildew on container-grown cabbage seedlings. Crop Prot. 11:160-164.
2. Channon, A. G. 1981. Downy mildew of Brassicas. Pages 321-339 in: The Downy Mildews. D. M. Spencer, ed. Academic Press, London.
3. Crute, I. R., Norwood, J. M., and Gordon, D. L. 1985. Resistance to phenylamide fungicides in lettuce and brassica downy mildew. Pages 311-314 in: Proc. Mixture Centenary Meeting, Bordeaux. British Crop Prot. Coun. Monog. No. 31.
4. Moss, N. A., Lucas, J. A., Crute, I. R., and Gordon, P. L. 1991. Sources of seedling resistance to *Peronospora parasitica* (downy mildew) in *Brassica oleracea*. Tests of Agrochemical and Cultivars 12. Ann. App. Biol. 118(supple.):94-95.
5. Moss, N. A., Lucas, J. A., and Crute, I. R. 1991. Evidence for differential response to isolates of *Peronospora parasitica* (downy mildew) in *Brassica rapa*. Tests of Agrochemical and Cultivars 12. Ann. App. Biol. 118(supple.):96-97.
6. Nashaat, N. I., and Awasthi, R. P. 1995. Evidence for differential resistance to *Peronospora parasitica* (downy mildew) in accessions of *Brassica juncea* (mustard) at the cotyledon stage. J. Phytopathol. 143:157-159.
7. Nashaat, N. I., and Rawlinson, C. J. 1994. The response of oilseed rape (*Brassica napus* subsp. *oleifera*) accessions with different glucosinolate and erucic acid contents to four isolates of *Peronospora parasitica* (downy mildew) and the identification of new sources of resistance. Plant Pathol. 43:278-283.
8. Parry, M. L., Carter, T. R. 1989. The greenhouse effect and the future of UK agriculture. J. R. Agric. Soc. Engl. 150:120-31.
9. Sherriff, C., and Lucas, J. 1987. Variation in host specificity in the *Brassica* population of *Peronospora parasitica*. Pages 333-335 in: Genetics and Plant Pathogenesis. P. R. Day and G. J. Jellis, eds. Blackwell Scientific Publications, Oxford.
10. Sherriff, C., and Lucas, J. 1990. The host range of isolates of downy mildew, *Peronospora parasitica*, from *Brassica* crop species. Plant Pathol. 39:77-91.
11. Thompson, K. F., and Hughes, W. G. 1986. Breeding and varieties. Pages 293-367 in: Oilseed Rape. D. H. Scarisbrick and R. W. Daniels, eds. William Collins Sons & Co. Ltd., London.
12. Williams, P. H. 1985. Downy mildew. Section D in: CrGr Resource Book. University of Wisconsin, Department of Plant Pathology, Madison.