

THE RELATIONSHIP BETWEEN COTYLEDON AND ADULT PLANT RESISTANCE TO DOWNY MILDEW (*Peronospora parasitica*) IN *BRASSICA OLERACEA*

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Abstract

410 accessions of a "core collection" of *Brassica oleracea* were screened at the cotyledon stage for resistance to downy mildew with isolates P501 (Portugal), P005 and P006 (both UK). Seedlings were assigned to one of six previously described interaction phenotypes. Seed of 20 accessions that showed a high proportion of seedlings in non-sporulating classes and of four accessions with an intermediate and a high proportion of seedlings in sporulating classes was used to produce plants which were assessed for response to downy mildew as adult plants in a field trial in Central Portugal. Field evaluation employed a five class scale of increasing susceptibility based on the number of infected leaves per plant and on the size of sporulating lesions. The incidence of plants exhibiting field resistance was higher than cotyledon resistance with some accessions showing more than 95% of plants with no sporulating lesions. The accessions with more than 20% of the seedlings in non-sporulating classes following inoculation with any of the isolates all expressed moderate to high resistance in the field. There was no accession with higher resistance at the cotyledon than at adult plant stage. In contrast, accession HRI 9846 (ornamental kale) was very susceptible at the cotyledon stage and yet was resistant in the field.

1. Introduction

The fungus *Peronospora parasitica* (Pers. ex. Fr.) Fr.) is the causal agent of crucifer downy mildew, an important disease of cole crops. Damage is economically greatest at the seedling stage when the disease may kill plants or retard development. The fungus may also infect adult plants reducing yield and crop quality. In the field, the disease is first observed on the lower leaves from which it progresses to the upper leaves; flower parts may also be affected and the fungus can develop internally in heads of cauliflower and broccoli causing a characteristic necrosis. Fungicide resistant forms of *P. parasitica* are common and there is an interest in identifying and characterising genetically determined resistance for use in integrated disease management programs. Most studies on resistance to downy mildew have concentrated on cotyledons or young leaves. Cotyledon resistance is easy to assay on large number of plants and observed interaction phenotype (IP) are stable since tests are usually conducted under controlled environment conditions. Field resistance of adult plants is commercially important but has received

less attention than cotyledon resistance. IPs for adult plants in the field are variable due to the fluctuating and uncontrolled environment as well as being difficult to assess. In addition, space availability and cost limit the number of accessions that can be readily tested in the field.

The objective of this study was to compare cotyledon with adult plant response to downy mildew in a selected group of accessions of *Brassica oleracea* to determine if cotyledon resistance is an indicator of adult plant field response.

2. Materials and methods

2.1. Cotyledon evaluation

A core collection of 410 accessions of *B. oleracea* was screened for resistance to downy mildew (*P. parasitica*) at the cotyledon stage using isolates P501 from Batalha, Portugal and P005 and P006 both from UK (Leckie, *et al.*, 1996). Fifty seedlings per accession were tested with the Portuguese isolate and ninety with the UK isolates. The tests were carried out under controlled conditions. Evaluation consisted on independent observation of pathogen reproduction and host response, the plants being classified according to six interaction phenotypic classes (Table 1). Classes NN, HN and FN exhibited no sporulation and either no macroscopically host response or visible necrotic flecking. Classes SS, CS and HS exhibited increasing amounts of sporulation with associated necrotic host response (Leckie, *et al.*, 1996).

Table 1 - Host-pathogen IP classes for cotyledon evaluation in the laboratory.

IP classes	Host	Pathogen
NN	No host response.	No sporulation.
HN	Light necrotic flecking.	
FN	Heavy necrotic flecking.	
SS	Any host response.	Sparse sporulation confined to point of infection.
CS		Sparse dispersed sporulation or moderate sporulation confined to point of infection.
HS		Moderate to heavy sporulation dispersed over whole cotyledon.

From: Leckie, *et al.* (1996). *Acta Horticulturae* 407: 95-101.

2.2. Adult-plant evaluation

Twenty-four accessions were chosen following evaluation of response at the cotyledon stage for field evaluation of resistance to downy mildew. These included 20 accessions that exhibited a high proportion of seedlings in non-sporulating classes and four accessions with an intermediate or a high proportion of seedlings in sporulating classes (Tables 2 and 3). The selection was non-random since the choice was biased towards accessions showing a high proportion of plants in cotyledon non-sporulating IP classes.

Seed of the same batches remaining from cotyledon tests was used to produce seedlings for two field experiments, at Batalha, Portugal, in 1995 and 1996. Different accessions were tested in each of two experiments using a randomised block design with three replicates of 36 plants per replicate. Four accessions were tested in both. The seedlings were transplanted in September, spaced 0.5 x 0.5 m and sprinkle irrigated when necessary. No fungicides were applied. Natural infection occurred in October and disease was evaluated in November and December after epidemic development when the plants had more than 12 leaves. Disease assessment used five classes taking into account the

number of infected leaves per plant and the size of sporulating lesions (Table 4). Data is presented as percentage of plants per IP class.

Table 2 - *B. oleracea* accessions tested in the field in 1995.

Accession	Sub species	Crop type	Cultivar	Origin
CGN 15123	<i>acephala medullosa</i>	marrow stem kale	Hoge Groene	NLD
CGN 15149	<i>acephala sabellica</i>	borecole	Halvhoj Kruset-Konserva	DNK
HRI 3598 ^(a)	<i>acephala sabellica</i>	borecole kale	Westland Winter Verdura	GBR
CGN 14038	<i>alboglabra</i>	Chinese kale	Kailan	THA
HRI 6254 ^(a)	<i>botrytis botrytis</i>	autumn cauliflower	Tasman	AUS
HRI 8571	<i>botrytis botrytis</i>	romanesco cauliflower	Cavolo Broccolo Romanesco	ITA
HRI 4771 ^(a)	<i>capitata</i>	summer cabbage	Cavolo Cappuccio Medio Nap	ITA
HRI 5652 ^(a)	<i>capitata</i>	cabbage	Shetland Cabbage	GBR
HRI 10590	<i>capitata</i>	spring cabbage	Spring Glory	JPN
CGN 11160	<i>capitata alba</i>	white cabbage	Delikatesse	DNK
HRI 8362	<i>capitata sabauda</i>	savoy cabbage	Ferrus HKz Gs	DEU
HRI 9579	<i>tranchuda</i>	tranchuda cabbage	Penca	PRT

^(a) Accessions repeated in 1996.

Table 3 - *B. oleracea* accessions tested in the field in 1996.

Accession	Sub species	Crop type	Cultivar	Origin
CGN 11125	<i>acephala</i>	fodder kale	Westfälische Furchenkohl	BRD
CGN 14111	<i>acephala</i>	vegetable kale	Butzo	ESP
HRI 7547	<i>acephala</i>	kale	Furchenkohl	DEU
HRI 9846	<i>acephala</i>	ornamental kale	Red on Green	JPN
HRI 3598 ^(a)	<i>acephala sabellica</i>	borecole kale	Westland Winter Verdura	GBR
HRI 5555	<i>acephala sabellica</i>	hybrid kale/borecole	Arsis Rs (F1)	NLD
HRI 2838	<i>botrytis botrytis</i>	cauliflower	Algromajo No 2	NLD
HRI 4856	<i>botrytis botrytis</i>	romanesco cauliflower	Romanesco Tardivo	ITA
HRI 6254 ^(a)	<i>botrytis botrytis</i>	autumn cauliflower	Tasman	AUS
HRI 5416	<i>botrytis italica</i>	sprouting broccoli	Cavolo Cavolina Rizza	ITA
OL 87098	<i>botrytis italica</i>	broccoli	Broccoli	USA
HRI 4771 ^(a)	<i>capitata</i>	summer cabbage	Cavolo Cappuccio Medio Nap	ITA
HRI 5652 ^(a)	<i>capitata</i>	cabbage	Shetland Cabbage	GBR
CGN 7121	<i>capitata sabauda</i>	savoy cabbage	Schelk-sel. Willems	NLD
HRI 5443	<i>gongylodes</i>	purple kohlrabi	Cavolo Forte	ITA
ISA 85	<i>tranchuda</i>	tranchuda cabbage	Couve Tranchuda de Aveiro	PRT

^(a) Accessions repeated in 1996.

Table 4 - Host-pathogen IP classes for adult plant evaluation in the field.

IP classes	Host	Pathogen
RR	No host response.	No sporulation.
R	Any host response.	One/two leaves with small (<1cm) sporulated lesions or only one leaf with a large (>1cm) sporulated lesions.
RS		One/two leaves with large or small sporulated lesions or three leaves with small sporulated lesions.
S		Two to four leaves with large or small sporulated lesions.
SS		Five or more leaves with large sporulated lesions.

2.3. Dual cotyledon-adult plant evaluation

In 1995 a special procedure was followed to compare the IP of the same individual plant at both cotyledon and adult plant stages. One hundred and eight seedlings of Tronchuda cabbages accessions: 'Couve Algarvia' (CT 16.4), 'Couve Murciana' (CT 54/93) and 'Couve de Corte' (CT 06/93); and two broccoli accessions: OL 87125 and OL 87098 were tested at the cotyledon stage using the method described in 2.1. The *P. parasitica* isolate for cotyledon inoculation (P512) had been collected the previous year in the field where the adult plants were tested. After cotyledon evaluation the diseased cotyledons were removed and the seedlings grown on until they reached a size appropriate for transplanting. Each plant was tagged and planted out in a replicated field experiment, at Batalha, to be evaluated for adult plant response to downy mildew using the procedure described in 2.2. Data is presented as percentage of plants per IP class.

3. Results and discussion

The distribution of plants per cotyledon IP class was quite variable between accessions and isolates (Tables 5 and 6). Some accessions e.g. HRI 5443, HRI 5652 and HRI 6254 exhibited more than 50% of individuals in classes HN and FN, while all seedlings of other accessions e.g. OL 87098 and HRI 9846 were in class HS (Tables 5 and 6). There was also a very clear differential interaction with the three isolates, which was reported in more detail elsewhere (Leckie, *et al.*, 1997). For instance, about 50% of seedlings of accessions HRI 5555, HRI 5652 and HRI 6254 exhibited non-sporulating IPs after inoculation with one or two of the isolates but full susceptible IPs with the third one. The differential accession x isolate interaction made it impossible to establish a meaningful overall susceptibility grade for accessions at cotyledon stage.

Adult plant response to downy mildew in the field was quite variable between accessions. The accessions could be clustered in three groups taking into account the number of infected plants and the disease intensity. The first group comprised seven accessions (HRI 3598, CGN 15149, CGN 15123, HRI 5555, CGN 7121, CGN 11125, and HRI 7547) having more than 90% of plants in class RR and less than 5% in classes RS, S or SS. These accessions were almost disease free and therefore appeared to exhibit a high degree of field resistance to downy mildew. The second group included 10 accessions (HRI 4771, HRI 5652, HRI 6254, HRI 8362, HRI 9579, HRI 8571, HRI 9846, HRI 5443, CGN 14111, and HRI 4856) with more than 49% of plants in class RR or R. These accessions showed some field resistance, with a moderate to high proportion of infected plants, but with a slow rate of disease progress. The last group of seven accessions (HRI 10590, CGN 11160, CGN 14038, HRI 2838, ISA 85, HRI 5416, and OL 87098) had more than 50% of plants in classes RS, S or SS. In this group the sporulating

Table 5 - Percentage^(*) of plants per IP class at the cotyledon and adult plant stage in 1995.

Accession	Isolate	Cotyledon						Adult plant				
		NN	HN	FN	SS	CS	HS	RR	R	RS	S	SS
Borecole	P501	-	-	-	-	14	86	99	1	-	-	-
HRI 3598 ^(a)	P005	-	4	17	1	14	64					
	P006	1	-	5	2	23	69					
Borecole	P501	-	-	-	2	44	54	96	3	1	-	-
CGN 15149	P005	4	-	23	9	6	58					
	P006	3	-	16	1	12	68					
Kale	P501	-	-	-	-	70	30	91	4	3	2	-
CGN 15123	P005	4	-	27	7	9	53					
	P006	1	-	13	3	14	69					
Cabbage	P501	-	-	-	2	88	10	87	11	-	1	1
HRI 5652 ^(a)	P005	-	-	51	-	-	49					
	P006	-	-	51	-	-	49					
Summer cabbage	P501	-	-	2	3	65	30	87	5	5	3	-
HRI 4771 ^(a)	P005	-	-	19	4	19	58					
	P006	-	-	19	4	19	58					
Cauliflower	P501	-	-	-	-	26	74	81	9	7	1	2
HRI 6254 ^(a)	P005	-	-	-	-	2	98					
	P006	1	-	55	6	12	26					
Savoy cabbage	P501	-	-	-	-	6	94	67	14	8	10	1
HRI 8362	P005	-	-	40	5	6	49					
	P006	1	-	38	3	6	52					
C. Tronchuda	P501	-	-	8	26	58	8	61	13	16	10	-
HRI 9579	P005	-	-	6	3	17	74					
	P006	-	-	17	-	13	70					
Romanesco	P501	-	-	2	4	38	56	38	11	26	18	7
HRI 8571	P005	1	2	26	3	21	47					
	P006	-	-	2	3	26	69					
Spring cabbage	P501	-	-	-	-	96	4	17	25	39	18	1
HRI 10590	P005	-	-	-	2	74	24					
	P006	-	-	-	2	73	25					
White cabbage	P501	-	-	-	-	-	100	9	21	43	23	4
CGN 11160	P005	3	-	22	12	8	55					
	P006	5	-	10	10	9	66					
Kale	P501	-	-	-	2	82	16	2	7	41	32	18
CGN 14038	P005	-	-	12	17	25	46					
	P006	1	-	-	5	11	83					

^(*)Number of plants evaluated: P501 = 50, P005 and P006 = 90, at the field = 108.

(a) Accessions repeated in 1996.

Table 6 - Percentage^(*) of plants per IP class at the cotyledon and adult plant stage in 1996.

Accession	Isolate	Cotyledon						Adult plant				
		NN	HN	FN	SS	CS	HS	RR	R	RS	S	SS
Borecole	P501	-	-	-	-	52	48	97	3	-	-	-
HRI 5555	P005	-	2	46	6	12	34					
	P006	-	2	45	6	18	29					
Borecole	P501	-	-	-	-	14	86	96	4	-	-	-
HRI 3598 ^(a)	P005	-	4	17	1	14	64					
	P006	1	-	5	2	23	69					
Savoy cabbage	P501	-	-	14	20	58	8	96	4	-	-	-
CGN 7121	P005	-	-	11	-	31	58					
	P006	2	-	20	-	25	53					
Fodder kale	P501	-	-	20	38	34	8	92	7	1	-	-
CGN 11125	P005	2	-	26	6	13	53					
	P006	2	-	9	1	25	63					
Kale	P501	-	-	8	68	24	-	90	7	1	2	-
HRI 7547	P005	1	-	15	7	19	58					
	P006	-	-	22	2	37	39					
Purple kohlrabi	P501	-	-	56	40	4	-	88	8	4	-	-
HRI 5443	P005	-	-	26	15	27	32					
	P006	-	-	9	20	40	31					
Summer cabbage	P501	-	-	2	3	65	30	71	18	10	1	-
HRI 4771 ^(a)	P005	-	-	19	4	19	58					
	P006	-	-	19	4	19	58					
Ornamental kale	P501	-	-	-	-	-	100	66	32	2	-	-
HRI 9846	P005	1	-	-	-	-	99					
	P006	-	-	-	-	-	100					
Cabbage	P501	-	-	-	2	88	10	66	30	4	-	-
HRI 5652 ^(a)	P005	-	-	51	-	-	49					
	P006	-	-	51	-	-	49					
Kale	P501	-	-	2	-	56	42	66	21	12	1	-
CGN 14111	P005	2	1	6	8	13	70					
	P006	2	-	5	2	12	79					
Romanesco	P501	-	-	25	12	55	8	57	30	11	2	-
HRI 4856	P005	-	-	25	3	33	39					
	P006	1	-	12	3	40	44					
Cauliflower	P501	-	-	-	-	26	74	39	22	31	8	-
HRI 6254 ^(a)	P005	-	-	-	-	2	98					
	P006	1	-	55	6	12	26					
Cauliflower	P501	-	-	-	-	4	96	6	17	55	22	-
HRI 2838	P005	1	-	-	2	32	65					
	P006	1	-	-	1	33	65					
C. Tronchuda	P501	-	-	10	22	58	10	4	10	63	23	-
ISA 85	P005	-	-	2	6	26	66					
	P006	-	-	8	1	25	66					
Broccoli	P501	-	-	-	8	66	26	-	41	57	2	-
HRI 5416	P005	-	-	17	7	3	73					
	P006	-	-	28	7	3	62					
Broccoli	P501	-	-	-	-	-	100	-	6	30	64	-
OL 87098	P005	-	-	-	-	-	100					
	P006	-	-	-	-	-	100					

^(*)Number of plants evaluated: P501 = 50, P005 and P006 = 90, at the field = 108.

(a) Accessions repeated in 1996.

lesions were large and the disease progressed rapidly from the lower to the upper leaves. Accession HRI 3598 (in group 1) and accessions HRI 4771, HRI 5652, and HRI 6254 (in group 2) were present in both the 1995 and 1996 experiments.

A comparison between cotyledon and adult plant IPs was not straightforward. The plants of accessions in Group 1, showing high field resistance, were either (i) resistant to P005 and P006 but susceptible to P501; (ii) resistant to P005 but susceptible to P006 and P501; or (iii) moderately resistant to P005, P006 and P501. The cotyledon reaction of plants in Group 2, having moderate to high adult plant resistance, ranged from very susceptible to P005, P006 and P501 (e.g. HRI 9846) to a very clear differential reaction (e.g. HRI 5652 and HRI 6254). The accessions in group 3, highly susceptible in the field, were either very susceptible to all isolates at the cotyledon stage (e.g. HRI 10590 and OL 87098) or exhibited a differential reaction (e.g. CGN 11160 and HRI 5416).

Evaluating the field resistance of adult plants is difficult. It is not enough just to estimate the relative susceptibility by measuring the size of sporulating lesions on a single leaf. It is also important to estimate the rate at which new leaves become infected, because slow disease progression is an important manifestation of resistance. Apparently, the plants of accessions in Group 1 are field resistant to infection. Those in Group 2 are susceptible to infection but the disease does not progress as fast as on plants in the third group, that included the most susceptible accessions. For an adult plant to develop extensive downy mildew lesions successive cycles of infection and sporulation are required. Under variable climatic conditions in the field some plants express moderate resistance because of slow progression of the disease. The low rate of infection of new leaves may also explain why in some years few plants are recorded as SS i.e. more than 5 fully infected leaves.

The comparison of IPs of plants dually inoculated at cotyledon and adult plant stages (Table 7) confirms the combinations of response observed in accessions of the core collection (Tables 5 and 6). 'Couve Algarvia' exhibited a high proportion of plants in non-sporulating classes both at the cotyledon and adult plant stages. Plants of 'Couve Murciana' and broccoli OL 87125 exhibited susceptibility at the cotyledon stage but were mostly assigned to non-sporulated classes as adult plants. Broccoli OL 87098 was susceptible both at the cotyledon and adult plant stages. 'Couve de Corte' exhibited slightly fewer plants in sporulating classes than broccoli OL 87098 at both cotyledon and adult plant stages.

The dual inoculation experiment yielded three combinations of cotyledon and adult plant IPs (data not shown): (i) non-sporulation for cotyledon and adult plant; (ii) sporulation for cotyledon and non-sporulation for adult plant; and (iii) sporulation for cotyledon and adult plant. There were no plants which exhibited non-sporulation at the cotyledon stage and sporulation in the field.

Table 7 - Percentage^(*) of plants per IP class when the same plants were evaluated at cotyledon and at adult plant stages.

Accession	Isolate	Cotyledon						Adult plant				
		NN	HN	FN	SS	CS	HS	RR	R	RS	S	SS
C. Algarvia - CT 16.4	P512	-	-	52	47	1	-	92	6	1	1	-
C. Murciana - CT 54/93	P512	-	-	1	8	72	19	79	17	3	1	-
C. Corte - CT 06/93	P512	-	-	5	37	50	8	8	13	34	44	1
Broccoli - OL 87125	P512	-	-	-	-	16	84	40	28	14	12	6
Broccoli - OL 87098	P512	-	-	-	-	20	80	-	-	8	40	52

^(*)Number of plants evaluated = 108.

There was no clear relationship between cotyledon IPs and adult plant IPs when plants of the core collection were tested at the cotyledon and plant adult stages and this was borne out by the several dual inoculation of Tronchuda and broccoli accessions. Therefore, adult plant response cannot be predicted by the cotyledon IP except in so far as non-sporulation at the cotyledon stage was never associated with high susceptibility at the adult plant stage. The situation is further complicated when there is differential interaction at the cotyledon stage to different *P. parasitica* isolates because there is no way of knowing the virulence characteristics and relative abundance of different *P. parasitica* variants in a field pathogen population.

It is possible that there are developmentally regulated pathogen recognition mechanisms operating which result in the difference between cotyledon and adult plant responses. Screening at the cotyledon stage is important to detect seedling resistance that is of high horticultural value during propagation. However, it may also serve to identify field resistant individuals since the probability of detecting individuals exhibiting a slow progression of the disease in the field was greater among plants exhibiting non-sporulating IPs at the cotyledon stage.

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