DIFFERENTIAL RESISTANCE TO PERONOSPORA PARASITICA AND *ALBUGO CANDIDA* IN *BRASSICA OLERACEA*

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Abstract

A sample of 22 accessions of *Brassica oleracea* were screened for their responses at the seedling stage to twelve isolates of the fungal pathogen *Peronospora parasitica* (downy mildew), and 14 accessions were screened for their responses at the seedling stage to nine isolates of *Albugo candida* (white blister). The results suggested that classic gene-for-gene interactions operate in the *P. parasitica* pathosystem whereas in the *A. candida* pathosystem the results could be explained either by a gene-for-gene model or an additive gene model. Interpretation of the results for both pathogens was complicated by the heterogeneous nature of the host plant accessions and differential lines are therefore being developed in a homogeneous genetic background to allow further investigation of these pathosystems to be carried out.

1. Introduction

The oomycete fungus *Peronospora parasitica* is the causal agent of downy mildew in crucifer species. Infection by the pathogen generally occurs in mild and damp conditions and, in compatible hosts, asexual sporulation is evident after about 7 days. In commercially grown *Brassica oleracea* types infection at the seedling stage can cause uneven growth and lack of uniformity in the crop at maturity resulting in economic losses. In addition, systemic infection of crops such as cauliflower and broccoli can lead to internal necrosis and a reduction in marketable yield. The closely related oomycete fungus *Albugo candida* causes white blister in crucifer species. Damp conditions are necessary for infection, and asexual sporulation in the form of white pustules, generally on the underside of leaves, occurs 1-2 weeks after infection. The major economic effects in commercial Brassica crops are due to infection of the buttons of Brussels Sprouts, the foliage of leafy Brassica such as Spring greens and the production of "staghead symptoms" on broccoli florets.

A collaborative CEC funded project has evaluated a structured sample of the *B. oleracea* genepool for response to the two pathogens with the aim of identifying and genetically characterising potentially useful sources of resistance (Leckie, *et al.*, 1996). The core collection, comprising 410 accessions representing a range of crop types and ecogeographical origins, was screened with three isolates of *P. parasitica* and two isolates of *A. candida*. Forty-five accessions were identified as having some resistance to *P. parasitica*, fifty-two had some resistance to *A. candida* and fifteen accessions were found to possess resistance to both pathogens. Analysis of some of these sources of resistance suggests that the responses are under simple genetic control involving alleles at one or two loci. The utility of these resistance genes in providing protection for Brassica crops will depend on their effectiveness against a range of pathotypes, therefore two samples of *B. oleracea* accessions from the original core collection were tested with a number of isolates of the pathogens. In addition, it is necessary to have diagnostic pathogen isolates that allow unambiguous gene identification in order to select and map such genes as a prelude to their isolation. A breeding programme has therefore been

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established to produce for each pathogen a set of host differential lines in a common genetic background.

2. Materials and methods

The 22 *B. oleracea* accessions tested with *P. parasitica* isolates and the 14 accessions tested with *A. candida* isolates are shown in Tables 1 and 2, respectively. Forty-five seedlings of each accession were screened with each isolate. Twelve isolates of *P. parasitica* and nine isolates of *A. candida* were used. Seedlings of the test accessions were produced in a glasshouse, inoculated with two isolates of one of the pathogens and then incubated in a growth room, as described in Leckie, *et al.* (1996). Scoring took place at 7 days after inoculation for response to *P. parasitica* and 12 days after inoculation for response to *A. candida*. For each pathogen six interaction phenotype classes were used to describe the host plant response and the growth of

Figure 1 (a) Scoring system for response to *P. parasitica*

Interaction Phenotype	**NN**	**HN**	**FN**	**SS**	**CS**	**HS**
Host Response	No host response	Light necrotic flecking	Heavy necrotic flecking	Any host response		
Pathogen Growth	No sporulation			Sparse sporulation	Sporulation confined to point of inoculation	Medium to heavy sporulation dispersed over whole cotyledon

Figure 1 (b) Scoring system for response to *A. candida*

Interaction Phenotype	**NN**	**HN**	**FN**	**SS**	**CS**	**HS**
Host Response	No host response	Light necrotic flecking	Heavy necrotic flecking	Any host response		
Pathogen Growth	No sporulation			Small pustules on upper surface of cotyledon	Small pustules on lower surface of cotyledon	Large pustules

the pathogen. These classes are shown in Figure 1(a) and (b). Any seedling scored as NN (no host response, no pathogen growth) was assumed to be an "escape" i.e. had not been inoculated, and was therefore discounted from the analysis of the results.

3. Results and discussion

The results of screening 22 accessions of *B. oleracea* with 12 isolates of *P. parasitica* are given in Table 1. The data shown are the total percentage of seedlings scored in the two interaction phenotype classes HN and FN as described in Figure 1(a). It can be seen that there were no accession x isolate combinations for which 100% of the seedlings were in these non-sporulating classes indicating the high level of heterogeneity within the accessions. It should also be noted that these accessions were selected on the basis of their high %(HN+FN) in the original screening of the core collection with at least one of the isolates P005, P006 and P501, whereas in this experiment a number of accessions have very low %(HN+FN) with all the isolates used, including the three used in the original screen. This again suggests that there is a high level of heterogeneity within the accessions leading to problems with reproducibility of data due to sampling variation. An example of this is accession HRI5555 for which we have evidence that segregation at a single locus determines resistance to isolate P005 but which in this experiment would be considered susceptible to this isolate. Nevertheless the results clearly show that there are reciprocal interactions between host and isolate pairs (for example, CGN11125 and HRI5652 with P218 and P501) indicating the possibility of a gene-for-gene relationship in which individual resistance loci in the host are matched by specific avirulence loci in the pathogen. Four lines (CGN07104, BRA963, HRI4771 and CGN11140) appear to have similar responses to all the isolates used although they are of diverse origins (two cabbages and two borecole kales) and it will be interesting to determine whether or not they carry the same gene(s).

Table 2 shows the results of screening 14 accessions of *B. oleracea* with 9 isolates of *A. candida* and it should be noted that the same problems due to the heterogeneity of the hosts were encountered. The results can be explained by an additive genetic model, in which an increasing number of resistance genes in the host lead to resistance to more isolates. An almost constant ranking of accessions and isolates is evident from examination of the data in Table 2. However, it is also possible that there is a gene-for-gene relationship operating but the isolates needed to detect clear reciprocal interactions have not yet been identified. Depending on the level of %(HN+FN) at which an accession is considered to be susceptible there are some reciprocal interaction evident within the data set, for example, accessions ISA91 and HRI4866 with isolates AcP20 and HRI-2.

The difficulties in interpretation of the data will be overcome with the development of host differential lines which are homozygous for particular identified resistance genes. At present five lines with differential resistance to *P. parasitica* (from accessions HRI5555, HRI8571, HRI4856, HRI4771, and ISA207) and four lines with differential resistance to *A. candida* (from accessions HRI4856, ISA207, HRI8571, and HRI6226) are being developed. These lines are selections from crosses between resistant plants of the original accessions and a rapid-cycling self compatible stock. The differential lines will be used to characterise pathogen isolates and to further investigate the host-pathogen interactions identified between *B. oleracea* and the two pathogens.

References

Leckie D, Astley D, Crute IR, Ellis PR, Pink DAC, Boukema I, Monteiro AA, Dias JS. (1996). The location and exploitation of genes for pest and disease resistance in European genebank collections of horticultural brassicas. Proc. Int. Sym. on Brassicas, Ninth Crucifer Genetics Workshop. Eds: JS Dias, IR Crute, AA Monteiro. Acta Hort. 407. ISHS 1996. pp. 95-101.

Table 1 - The responses of 22 accessions of *B. oleracea* to 12 isolates of *P. parasitica*. Figures are %(HN+FN) out of 45 seedlings tested for each isolate x accession combination.

	Isolate											
Accession	P005	P006	P202	P204	P211	P213	P214	P216	P217	P218	P501	P502
CGN07104	51	84	55	93	9	50	50	82	91	93	8	3
BRA963	34	43	38	55	0	49	49	41	58	56	0	0
HRI4771	38	70	49	67	15	33	33	60	35	35	6	6
CGN11140	13	27	29	55	13	46	46	39	23	22	0	0
HRI5389	47	69	56	71	7	45	45	49	51	62	49	49
ISA207	14	33	41	7	43	2	2	0	0	0	93	93
HRI7547	2	2	18	16	15	0	0	16	0	0	58	58
CGN11125	16	18	16	30	18	0	0	18	0	0	49	49
HRI5652	24	31	47	60	0	5	2	40	48	48	0	0
ISA62	5	20	31	31	0	32	30	11	36	38	0	0
CGN07121	42	31	14	22	12	0	0	5	0	0	12	14
HRI4856	12	7	12	5	10	0	0	4	0	0	25	28
HRI5555	11	13	16	20	2	18	13	0	22	22	0	0
HRI9549	2	24	7	22	7	2	0	18	9	9	9	9
HRI8362	12	17	5	29	7	9	9	5	15	15	0	0
HRI6254	5	33	0	0	0	0	0	2	0	0	0	0
HRI6226	0	15	0	2	0	0	0	2	0	7	2	2
HRI7520	0	11	0	4	0	0	0	4	0	0	0	0
HRI8571	7	2	0	0	0	0	0	0	9	11	11	13
HRI4773	9	9	0	0	0	0	0	0	2	2	7	7
CGN15152	2	2	0	7	0	0	0	0	0	0	0	0
HRI10590	2	2	0	2	0	0	0	0	2	5	0	0

Table 2 - The responses of 14 accessions of *B. oleracea* to 9 isolates of *A. candida*. Figures are %(HN+FN) out of 45 seedlings tested for each isolate x accession combination.

	Isolate								
Accession	A501	AcP15	AcP11	A001	HRI-1	AcP20	HRI-2	AcP-5	AcP-9
HRI4856	67	56	63	58	55	28	71	37	33
HRI6318	52	54	58	43	43	28	50	24	18
ISA207	43	41	35	24	35	28	47	13	9
ISA91		42	27	25	25	20	8	3	0
HRI4866	58	33	27	21	27	8	17	16	16
HRI7824	43	42	24	28	28	3	4	11	11
HRI8571	39	37	22	30	31	3	4	4	4
HRI4857	40	13	21	13	13	8	17	2	2
HRI6226	34	16	9	0	3	0	0	7	7
HRI10757	48	10	9	5	0	7	0	2	2
HRI4861	16	10	15	7	9	5	0	4	2
ISA82	0	2	7	8	8	0	2	0	0
HRI6628	0	2	2	2	0	4	3	0	0
CGN07102	0			0	0			0	0