Evaluation of Broccoli and Cauliflower Germplasm for Resistance to Race 2 of *Peronospora parasitica*

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Abstract. Evaluations for resistance against race 2 of downy mildew, incited by *Peronospora parasitica*, were conducted on 240 U.S. Plant Introductions (PI) classified as *Brassica oleracea* var. *botrytis* (consists of both broccoli and cauliflower types). Plants were inoculated at the two-expanded leaf stage with 5.0×10^3 sporangia per ml. Inoculated plants were incubated in a dark 16C dew chamber for 24 hr and were then placed in a 22C growth chamber with a 12-hr photoperiod. On the 7th day after inoculation, plants were returned to the dew chamber for 30 hr and ratings for downy mildew reaction phenotypes were made at 9 days postinoculation on a 0-9 scale of increasing disease severity. A disease index (DI) was calculated for each entry. Based on the DI, no PI entries were highly resistant. PI entries 181860, 188562, 204765, 204768, 204772, 204773, 204775, 204779, 241612, 264656, 291567, 373906, and 462225 were moderately resistant. (DI of 3.1-5).

Downy mildew of broccoli (*Brassica oleracea* var. *italica* L.), cauliflower (*B. oleracea* var. *botrytis* L.), and other crucifers is incited by *Peronospora parasitica* Pers. ex Fr. The disease is serious in the cooler parts of Europe, the United States, India, Russia, South America, and Australia (Sherf and Macnab, 1986). The high humidity, warm days, and cool nights of the southeastern United States result in severe damage from this disease (Walker and Williams, 1977). Biological races of *P. parasitica* occur on the Cruciferae and three races have been recognized on *B. oleracea* (Natti et al., 1967; M.H. Dickson, personal communication).

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The use of resistant cultivars is potentially the most economical and reliable method of

managing this disease. Sources of resistance must, however, be identified for use in plant breeding programs. One possible source of resistance is the U.S. Plant Introduction collection.

Natti (1958) tested four broccoli and 39 cauliflower plant introductions (PIs) against an isolate of *P. parasitica* that was subsequently designated as race 1. Natti et al. (1967) tested 230 crucifer PIs for downy mildew resistance. They reported that 16 accessions contained one or more plants resistant to race 2 and, in some cases, race 1 of the pathogen. The former report specified the broccoli and cauliflower PIs that were tested, but the latter report did not specify which crucifer accessions were tested. The purpose of the work reported here was to test all available broccoli and cauliflower accessions in the U.S. Plant Introduction collection for resistance to a local isolate of *P. parasitica*.

The isolate of *P. parasitica* that was used in these tests was obtained in 1987 from field-grown plants of *B. oleracea* cv. 'Calabrese' at Charleston, S.C., and was maintained on cotyledons of the broccoli cultivars Waltham 29 and Green Duke. The techniques described by Williams (1985) were used. The race designation of this isolate was deter-

Table 1. Reaction of *Brassica oleracea* differential genotypes and control cultivars to a Charleston, S.C., isolate of *Peronospora parasitica*. Plants inoculated at the two-expanded leaf stage.

Differential genotype	Resistant to race(s)	Reaction phenotype ^z						Disease index ^y (resistance class)
		0	1	3	5	7	9	
B-O-23*	1	0	0	0	6	26	0	6.6 (S)
MDR-1 ^w	1	0	0	0	3	13	0	6.6 (S)
Broccoli ^w	1	0	0	0	1	20	0	6.9 (S)
M7-2 ^w	2	0	16	1	10	0	0	2.6 (VR)
M11-5 ^w	2	0	30	3	1	0	0	1.3 (VR)
M15 × M24 ^w	1 and 2	0	0	10	11	2	0	4.3 (MR)
MDR-3 ^w	3	0	0	0	7	27	0	6.6 (S)
8775-1 ^x	3	0	0	5	13	24	0	5.9 (LR)
Waltham 29	None	0	0	0	7	26	1	6.6 (S)
Green Duke	None	0	0	0	2	36	0	6.9 (S)

^zReaction classes: 0 = no symptoms; 1 = small necrotic flecks, no sporulation; 3 = necrotic flecks, one to few sporangia present; 5 = necrotic lesions, sparsely scattered sporulation usually confined to necrotic areas; 7 = necrotic lesions, sometimes with accompanying chlorosis, scattered, heavy to abundant sporulation in both chlorotic and necrotic areas; 9 = necrosis and some chlorosis may be evident, uniformly heavy sporulation over abaxial surface of leaf.

^yResistance classes based on disease indices (DI) calculated by Williams' formula: VR (very resistant) = DI of 0-3, MR (moderately resistant) = DI of 3.1-5; LR (lowly resistant) = DI of 5.1-6; S (susceptible) = DI of 6.1-7; VS (very susceptible) = DI of 7.1-9.

^xSupplied by M.H. Dickson.

^wSupplied by Petoseed, Woodland, Calif.

mined by inoculating it to the *B. oleracea* differentials developed by M.H. Dickson, New York Agricultural Experiment Station, Geneva (Natti et al., 1967; M.H. Dickson, personal communication). The isolate was also used to inoculate broccoli and cauliflower PIs and commercial cultivars to evaluate these accessions for resistance to downy mildew.

Both sets of inoculations and subsequent evaluations of interaction phenotypes were conducted using the following protocol. All work was conducted in glasshouses and growth rooms. A randomized complete-block design with four replicates was used. The number of plants per replicate varied depending on availability and viability of seed of each entry. Eight differential genotypes, 240 broccoli and cauliflower PIs, and nine commercial cultivars were tested. Because the growth chamber could not accommodate all of the entries that were tested, the data represent the compilation of four tests. The commercial cultivars Green Duke and Waltham 29 were included in each of these four tests as controls. Data were tested by analysis of variance to determine least significant difference. Based on the reactions of the controls, the tests were homogeneous.

Seed of each entry were sown to Jiffy Mix (Jiffy Products of America, West Chicago) in No. 812 Com-Packs trays (T.O. Plastics, Minneapolis, Minn.). Seedlings were grown in the glasshouse until the cotyledons had fully expanded and were then transferred to a 22C growth room with a 12-hr photoperiod from sodium vapor lamps. Plants were inoculated when they reached the two-expanded leaf stage. Just before inoculation, the 12 cells of each of the Com-Packs trays were separated to produce four sets of three cells each. One three-cell set of each of the resultant four sets was randomized and placed in larger plant trays to provide the four replicates.

Inoculum was prepared from the culture of *P. parasitica* maintained on broccoli cotyledons. Cotyledons that supported abundant sporulation by the pathogen were collected and placed in 50 ml of distilled water in a 250-ml flask. The flask was capped and shaken vigorously to dislodge the sporangia. The resulting sporangial suspension was filtered through one layer of cheesecloth and the concentration was determined with the aid of a hemacytometer and adjusted to 5×10^5 sporangia per ml. The adaxial surfaces of leaves one and two of all test plants at the three-leaf stage were sprayed with this inoculum to incipient run-off with a Paasche Type-H airbrush (Paasche Airbrush Co., Chicago) at 138 kPa. Inoculated plants were placed in a dark&w chamber at 16C for 24 hr. Plants were then removed from the dew chamber and returned to the 22C growth room. On the 7th day after inoculation, plants were returned to the 16C dew chamber for 30 hr and ratings of reaction phenotypes were made 9 days postinoculation.

Leaves one and two were observed with the aid of a stereo microscope at magnifications up to $\times 65$ and assigned to the fol-

Table 2. Reaction of selected U.S. Plant Introductions (PI) and commercial cultivars of broccoli and cauliflower to inoculation with *Peronospora parasitica* race 2.^a PI accessions listed are those in which plants with a resistant reaction phenotype ≤ 3 were identified.

PI/cultivar	No. in reaction phenotype ^y						Total tested	Disease resistance ^x	
	0	1	3	5	7	9		Index ^w	Class
Brassica oleracea var. italica (broccoli)									
PI 188561	0	0	1	8	7	0	16	5.8	LR
PI 267721	0	0	1	10	6	0	17	5.6	LR
PI 462209	0	0	2	18	12	0	32	5.6	LR
Green Duke	0	0	0	15	104	12	131	7.0	S
Waltham 29	0	0	0	3	58	20	81	7.4	VS
Emperor	0	0	0	1	26	10	37	7.5	VS
Premium Crop	0	0	0	0	31	2	33	7.1	VS
Green Valiant	0	0	0	0	33	5	38	7.2	VS
Packman	0	0	0	9	26	1	36	6.6	S
Brassica oleracea var. botrytis (cauliflower)									
PI 163485	0	0	3	23	5	0	41	5.6	LR
PI 165488	0	0	1	14	12	0	27	5.8	LR
PI 173845	0	0	1	4	13	0	18	6.3	S
PI 181860	5	0	0	0	8	1	14	4.6	MR
PI 183213	1	0	6	25	12	0	44	5.2	LR
PI 183214	1	0	0	1	22	0	24	6.6	S
PI 188562	0	2	4	16	9	0	31	5.1	LR
PI 204758	0	0	2	3	15	1	21	6.4	S
PI 204760	0	0	1	8	13	0	22	6.1	S
PI 204763	0	0	1	17	13	0	31	5.8	LR
PI 204765	0	0	13	24	5	0	42	4.6	MR
PI 204768	0	0	6	9	7	0	42	5.0	MR
PI 204769	0	0	3	10	6	1	20	5.5	LR
PI 204771	0	0	2	32	4	0	38	5.1	LR
PI 204772	0	0	7	22	8	0	37	5.0	MR
PI 204773	0	2	8	16	7	0	33	4.7	MR
PI 204774	0	0	4	21	9	0	34	5.3	LR
PI 204775	0	0	4	14	3	0	21	4.9	MR
PI 204776	0	0	2	11	16	0	29	6.0	LR
PI 204779	0	0	7	22	4	0	33	4.8	MR
PI 204780	0	0	4	7	14	0	25	5.8	LR
PI 208477	0	0	1	5	21	6	33	6.9	S
PI 208478	0	0	1	5	19	1	26	6.5	S
PI 208482	0	0	1	26	14	0	41	5.6	LR
PI 208485	0	0	1	17	14	1	33	5.9	LR
PI 226504	1	0	1	28	19	3	52	5.8	LR
PI 231207	0	0	4	24	25	1	54	5.8	LR
PI 231209	3	0	2	21	26	6	58	6.0	LR
PI 241609	1	0	2	13	19	0	35	5.8	LR
PI 241612	1	0	0	4	0	1	6	4.8	MR
PI 261597	0	0	3	12	26	5	46	6.4	S
PI 264331	1	0	0	7	31	7	46	6.8	S
PI 264656	0	0	4	45	1	0	50	4.9	MR
PI 264658	0	0	1	12	18	4	35	6.4	S
PI 267725	0	0	1	18	11	0	30	5.7	LR
PI 291566	0	0	1	13	3	0	17	5.2	LR
PI 291567	0	0	1	29	1	0	31	5.0	MR
PI 343479	0	0	3	15	12	0	30	5.6	R
PI 345542	2	0	0	7	27	2	38	6.4	S
PI 373906	0	0	11	34	0	0	45	4.5	MR
PI 373908	0	0	5	21	10	0	36	5.3	LR
PI 373909	0	0	1	34	14	0	49	5.5	LR
PI 373921	0	0	4	36	37	0	77	5.9	LR
PI 385954	0	0	2	4	45	10	61	7.1	VS
PI 385955	0	0	1	7	12	1	21	6.2	S
PI 390968	0	0	1	12	40	1	54	6.5	S
PI 419064	0	0	1	20	14	0	35	5.7	LR
PI 462211	0	0	4	21	34	1	60	6.1	S
PI 462213	0	0	2	25	35	1	65	5.9	LR
PI 462214	0	0	4	5	26	0	75	5.6	LR
PI 462216	0	1	0	0	9	13	23	5.9	LR
PI 462218	0	0	1	27	24	0	52	5.9	LR
PI 462222	0	0	1	18	14	0	33	5.8	LR
PI 462223	0	0	1	27	55	0	83	6.3	S
PI 462224	0	0	1	9	21	0	31	6.3	S
PI 462225	0	0	19	31	5	0	55	4.5	MR
PI 478341	0	0	2	13	44	0	59	6.4	S
Snowball Y									
Improved	0	0	0	4	29	2	35	6.9	S
White Rock	0	0	2	28	2	0	32	5.0	MR
Snow Crown	0	0	10	28	3	0	41	4.7	MR

continued

Table 2. *continued*

PI/cultivar	No. in reaction phenotype ^y						Total tested	Disease resistance ^x	
	0	1	3	5	7	9		Index ^w	Class
Silver Star	0	0	0	24	5	0	29	5.3	LR
Snow March	0	0	0	21	7	0	28	5.5	LR

^yInoculation method is detailed in text.

^xReaction phenotypes: 0 = no symptoms; 1 = small necrotic flecks, no sporulation; 3 = necrotic flecks, one to few sporangiophores present; 5 = necrotic lesions, sparsely scattered sporulation usually confined to necrotic areas; 7 = necrotic lesions, sometimes with accompanying chlorosis, scattered, heavy to abundant sporulation in both chlorotic and necrotic areas; 9 = necrosis and some chlorosis may be evident, uniformly heavy sporulation over abaxial surface of leaf.

^wResistance classes based on disease indices (DI) calculated by Williams' formula: VR (very resistant) = DI of 0-3, MR (moderately resistant) = DI of 3.1-5, LR (lowly resistant) = DI of 5.1-6, S (susceptible) = DI of 6.1-7, VS (very susceptible) = DI of 7.1-9.

^zLSD ($P = 0.05$) of ± 0.9 .

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The results of the inoculation of the differential genotypes of *B. oleracea* indicated that the test isolate was race 2 of *P. parasitica* (Table 1). The reaction phenotypes and resultant DIs showed that the race 1- and race 3-resistant genotypes were susceptible to this isolate, while the race 2-resistant genotypes were resistant. The genotypes with both race 1 and race 2 resistance exhibited a moderately resistant reaction, which also supports the race 2 designation for the test isolate.

Among the broccoli accessions, PI 267721 and PI 462209, both with DIs of 5.6, were significantly more resistant than any of the commercial broccoli cultivars tested. None of the cauliflower PIs had DIs that indicated they were significantly more resistant than 'Snow Crown', the most resistant cultivar tested (DI = 4.7). Thirteen cauliflower accessions, PIs 181860, 188562, 204765, 204768, 204772, 204773, 204775, 204779, 241612, 264656, 291567, 373906, and 462225, had resistance ratings in the MR class (DI 3.1-5), as did the cultivars Snow Crown and White Rock. However, highly resistant plants that had a reaction phenotype ≤ 3 were identified from three broccoli and 57 cauliflower accessions (Table 2). Even though no accessions were identified that had a highly resistant overall DI, the identification of highly resistant plants within many of the tested PI accessions should be useful for the development of resistant cultivars. This list provides plant breeders with a wide

range of accessions having a great variety of horticultural characteristics from which to choose.

The resistances that have been used against *P. parasitica* thus far have been conditioned by highly specific major genes (Dixon, 1981), which has limited the usefulness of these resistances because of resistance-breaking races.

Additional testing against other races of downy mildew may indicate that some of the resistances identified in this study are more generalized in nature, which will make them more useful for the control of the disease.

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