Inheritance of True Leaf Stage Downy Mildew Resistance in Broccoli

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ABSTRACT. Downy mildew, incited by the biotrophic fungal parasite, *Peronospora parasitica* (Pers. Fr.) Fr., is one of the most destructive diseases of broccoli (*Brassica oleracea* L., Italica Group) and other related crop species throughout the world. Cultivation of resistant cultivars is the most desirable control method because it provides a practical, long-term, and environmentally benign means of limiting damage from this disease. The commercial hybrid cultivar, Everest, has been shown previously to contain a high level of downy mildew resistance. Doubled-haploid (DH) lines developed from that hybrid were also shown to exhibit a similar, high level of resistance at the three- to four-leaf stage. To determine the mode of inheritance of this true leaf resistance, the resistant DH line was crossed to a susceptible line (derived from 'Marathon') to produce an F₁ hybrid. Subsequently, F₂ and backcross (BC) populations were developed from the hybrid. In addition, a DH population of ≈100 lines was developed from the same F₁ used to create the F₂ and BC. All populations were evaluated for response to artificial inoculation with *P. parasitica* at the three- to four-leaf stage. F₁ plants were resistant like the resistant parent and F₂ populations segregated approximately nine resistant to seven susceptible. Using the resistant parent as recurrent parent, BC populations contained all resistant plants, while the BC to the susceptible parent fit a 1 resistant : 3 susceptible segregation ratio. These results can be explained by a model with two complementary dominant genes. This model was confirmed by the DH population that segregated ≈1:3, resistant to susceptible. Due to the dominant nature of this resistance, controlling genes should be easily incorporated into F₁ hybrids and used commercially to prevent downy mildew.

Downy mildew, incited by the biotrophic fungal parasite, *Peronospora parasitica*, is one of the most destructive diseases of cruciferous crops (Channon, 1981). The disease has worldwide distribution and has been reported on many economically important species in the Brassicaceae, including *Brassica oleracea* L. (broccoli, cabbage and other cole crops), *B. napus* L. (canola), *Raphanus sativus* L. (radish), and *B. Rapa* L., Chinensis Group (e.g., Chinese cabbage, pak-choi). This pathogen infects most all plant organs including leaves, stems, and flower parts (Channon, 1981; Sherf and MacNab, 1986).

Broccoli (*Brassica oleracea*, Italica Group) is susceptible to infection by *P. parasitica* at all growth stages. Infection at the seedling stage can be particularly destructive because cotyledons and young leaf tissues are highly vulnerable to attack, and when infected, can result in stunted or killed seedlings. Fungicide applications provide an effective means of controlling downy mildew (Brophy and Laing, 1992). However, registered fungicides may be lost in the future due to concerns about their possible detrimental environmental effects (Flint et al., 1992), concerns about food safety, and development of fungicide resistance by the pathogen (Crute et al., 1985, 1994). The potential loss of effective fungicides has created strong incentives to develop effective alternative methods for downy mildew control. Use of resistant cultivars is one such alternative that would provide a practical, long-term solution to effective disease control.

Natti et al. (1967) studied two sources of downy mildew resistance in *B. oleracea* expressed at the cotyledon stage and found that

each was controlled by single dominant genes. More recently, Hoser-Krause et al. (1987) identified a single recessive gene responsible for downy mildew resistance at the four- to five-leaf stage in broccoli. There are no reports that any of the above-described resistance genes have been incorporated into cultivars. Based on gradations observed in disease reaction phenotype, Dickson and Petzoldt (1993) hypothesized that modifying genes probably act in concert with major genes to confer variable levels of downy mildew resistance. This is supported by observations of Jensen et al. (1999) who characterized moderate resistance in breeding lines of broccoli.

Other sources of resistance to downy mildew in *B. oleracea* have been identified (Coelho et al., 1998; Laemmlen and Mayberry, 1984; Silue et al., 1995; Sousa et al., 1997). To date, genes controlling these resistances have not been characterized or are currently under study (Agnola et al., 2000; Farinho et al., 2000). Dickson and Petzholt (1993) showed that the F₁ hybrid broccoli cultivar, Everest, exhibits a relatively high level of resistance at several leaf stages, and the observations of Wang et al. (2000) at the three- to four-leaf stage confirmed this. Because the parentage of 'Everest' is protected as a trade secret, inheritance of its three- to four-leaf stage resistance has never been described. Accordingly, we developed doubled-haploid (DH) lines of broccoli using 'Everest' as a parent to identify homozygous lines with high levels of resistance similar to 'Everest' for use in inheritance studies (Wang et al., 2000). Among our developed lines, we identified some lines that were susceptible at the cotyledon stage, but highly resistant at a three- to four-leaf stage (Wang et al., 2000). The primary objective of this study was to determine inheritance of this resistance originally derived from 'Everest' and expressed in a DH line developed from the cultivar.

Materials and Methods

PARENTAL MATERIALS AND GENETIC POPULATIONS. Two homozygous broccoli lines (USVL012 and USVL047) were evaluated in a previous study (Wang et al., 2000). USVL012 exhibits three- to four-leaf stage downy mildew resistance, and USVL047 is suscep-

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tible to downy mildew at all stages. USVL012 was derived from the commercial hybrid cultivar, Everest (Syngenta Seed, Gilroy, Calif.), and served as the resistant (R) parent in studies described herein. USVL047 was derived from the commercial hybrid cultivar Marathon (Sakata Seed America, Inc., Salinas, Calif.) and served as the susceptible (S) parent. Both lines were developed as DH lines at the U.S. Vegetable Laboratory (USVL), Charleston, S. C. (Farnham, 1998). The R and S inbreds were crossed in both directions to create two reciprocal F₁ hybrids (F_{1(RS)} and F_{1(SR)}). Both F₁ hybrids were used to 1) generate four backcross (BC) populations (BC_{1(RS-R)}, BC_{1(RS-S)}, BC_{1(SR-R)}, and BC_{1(SR-S)}) by backcrossing to the respective R or S parental inbred; and 2) make two F₂ populations [F_{2(RS)} and F_{2(SR)}] by selfing the respective hybrids.

The same F_{1(RS)} plants used to generate the conventional segregating populations described above also produced microspore-derived DH populations using techniques described previously (Wang et al., 1999). The ploidy of all regenerated plantlets (R₀) was determined at the four- to five-leaf stage using DNA flow cytometry procedures (Farnham, 1998). Only diploid regenerants with normal anthers and viable pollen were self-pollinated to produce seed. Harvested seed lots from all individual diploid, R₀ plantlets represent a population of DH lines (R1 generation) segregating for three- to four-leaf stage downy mildew resistance.

DOWNY MILDEW EVALUATIONS. Two downy mildew resistance evaluations were performed at the three- to four-leaf stage. The first was carried out in Fall 1998 and consisted of 14 to 15 seedlings of each parental line and each F₁, 50 to 55 seedlings of each BC₁, and ≈90 seedlings of each F₂. The second evaluation was conducted in Fall 1999 and consisted of 98 DH lines derived from F_{1(RS)}. Except during periods when plants were incubated in dew chambers, both seedling evaluation studies were conducted in a walk-in growth room set at 22 °C with a 14-h photoperiod of ≈240 μmol·m⁻²·s⁻² [measured using an integrating quantum photometer (LI-188B; LICOR, Lincoln, Nebr.) immediately above plants] provided by sodium vapor lamps. In preparation for both rounds of evaluation, individual seeds were planted in 145-cm³ plastic pots filled with Metro Mix 360 (Grace Sierra, Milpitas, Calif.). Seeds were germinated and seedlings were grown to the three- to four-leaf stage at which point they were inoculated with *P. parasitica*.

A single South Carolina isolate of *P. parasitica* from field-grown plants of the broccoli cultivar, Calabrese (Thomas and Jourdain, 1990) was used in all studies. Conidial suspensions of this isolate were used as inoculum. Inoculum suspensions were prepared by washing off with deionized water conidia from cotyledons of 'Green Duke' broccoli having an abundant sporulation of *P.*

parasitica. Concentration was adjusted to 10⁴ conidia/ml. The entire adaxial and abaxial surfaces of all leaves of test plants at the three- to four-leaf stage were sprayed uniformly with the inoculum suspension using a Paasche Type-H airbrush (Paasche Airbrush Co., Chicago) at 138 kPa. Immediately after inoculation, seedlings were placed in a dark dew chamber at 16 °C and 100% relative humidity for 24 h to facilitate infection, after which plants were returned to the growth room at conditions described above. Seven days after inoculation, seedlings were again placed in the dew chamber for 24 h to promote sporulation. The following day, plants were evaluated for downy mildew reaction phenotype under a stereomicroscope at 65× magnification.

Ratings of reaction phenotype (RP) were assigned using the following 0 to 9 scale: 0 = no symptoms; 1 = small necrotic flecks, no sporulation; 3 = necrotic flecks, one to few sporangioophores present; 5 = necrotic lesions, sparse scattered sporulation usually confined to necrotic areas; 7 = necrotic lesions, sometimes with accompanying chlorosis, with heavy to abundant sporulation in both chlorotic and necrotic areas on the adaxial surface and scattered sporulation on the abaxial surface; and 9 = necrosis and some chlorosis usually evident, uniformly heavy sporulation over adaxial and abaxial surfaces. This rating scale is very similar to that described originally by Williams (1986). Seedlings with a RP <3, i.e., lacking any sporulation, were designated as resistant. Seedlings with a RP rating ≥3 were considered susceptible. All data were collected on an individual seedling basis.

Chi-square tests were used to determine goodness of fit to hypothesized models based on observed and expected numbers of resistant and susceptible individuals in F₂, BC, and DH populations.

Results

All plants of the susceptible USVL047 parent were uniformly and highly susceptible to infection by *P. parasitica* at the three- to four-leaf stage (mean RP = 7.9). Conversely, all plants from the resistant USVL012 parent were uniformly and highly resistant (mean RP = 1.3). All F₁ plants from reciprocal crosses were resistant. There was no significant difference in RP rating between F_{1(RS)} and F_{1(SR)} (mean RP = 2.0 for both).

The F_{2(RS)} population segregated 53 resistant to 37 susceptible (Table 1). The reciprocal F_{2(SR)} population gave similar results segregating 52 resistant to 39 susceptible (Table 1). The reciprocal BC populations to the susceptible parent were similar to each other with BC_{1(RS-S)} segregating 15 resistant to 37 susceptible and BC_{1(SR-S)} segregating 17 resistant to 34 susceptible. The reciprocal BC

Table 1. Segregation of downy mildew resistant and susceptible plants in reciprocal F₁, F₂, and BC₁ populations derived from the cross of USVL012 (R) and USVL047 (S) parents. Expected ratios for resistant (R) versus susceptible (S) are based on the model with two complementary dominant genes conditioning resistance.

Population	Observed plants		Expected ratio	χ ²	P
	R	S			
USVL012 (R)	14	0			
USVL047 (S)	0	14			
F _{1 (RS)}	15	0			
F _{1 (SR)}	15	0			
F _{2 (RS)}	53	37	9:7 (51:39)	0.18	0.5–0.7
BC _{1(RS-R)}	55	0	1:0 (55:0)	0.00	1.0
BC _{1(RS-S)}	17	34	1:3 (13:38)	1.65	0.1–0.2
F _{2 (SR)}	52	39	9:7 (51:40)	0.05	0.8–0.9
BC _{1(SR-R)}	50	1	1:0 (51:0)	0.02	0.8–0.9
BC _{1(SR-S)}	15	37	1:3 (13:39)	0.38	0.5–0.8

populations to the resistant parent were also similar to each other. The BC_{1(RS-R)} population did not segregate (exhibiting only resistant plants), and BC_{1(SR-R)} contained all resistant individuals except one. Chi-square tests revealed a close fit to a 9:7 ratio for the reciprocal F₂ populations. BC populations using the susceptible USVL047 as the recurrent parent fit a 1 resistant : 3 susceptible segregation ratio; however, the goodness of fit was not as high for these populations as with the F₂ and other BC populations.

Twenty-one of ninety-eight individual DH lines from the population derived from F_{1(RS)} exhibited resistance at the three- to four-leaf stage similar to the resistant parent. Chi-square analysis indicated a good fit ($0.4 < P < 0.5$) to a 1:3 ratio of resistant to susceptible.

Discussion

The two F₁ populations formed by crossing USVL012 and USVL047 and the two respective F₂ populations exhibited the same response or segregation ratios indicating no maternal or cytoplasmic effect for the three- to four-leaf stage downy mildew resistance expressed by USVL012. In addition, the highly resistant phenotype expressed by the reciprocal F₁ hybrids indicates dominance for this resistance. Based on the segregation ratio in the F₂ and BC populations, and goodness of fit for Chi-square tests, it is proposed that two unlinked, complementary dominant genes (designated as DM_{TL1} and DM_{TL2}) interact to confer the resistance in USVL012.

Strong evidence supporting the two-gene model comes in the observed 1:3 segregation ratio for resistance and susceptibility in the DH population. Although 16 genotypes are possible with a two-gene model in the F₂, in a DH population only four homozygous genotypes (DM_{TL1}DM_{TL1}DM_{TL2}DM_{TL2}, DM_{TL1}DM_{TL1}dm_{TL2}dm_{TL2}, dm_{TL1}dm_{TL1}DM_{TL2}DM_{TL2}, and dm_{TL1}dm_{TL1}dm_{TL2}dm_{TL2}) can result from the F₁ genotype DM_{TL1}dm_{TL1}DM_{TL2}dm_{TL2}. Only the first (homozygous dominant) of the possible genotypes is predicted to be resistant. Thus, the observed segregation in the DH population matches very closely what would be expected if two dominant genes conferred the downy mildew resistance. These results present the first use of a DH population to determine inheritance of downy mildew resistance in *B. oleracea*.

The two-gene control for the high level of three- to four-leaf stage resistance in USVL012 is unique when compared to other downy mildew resistances reported in *B. oleracea*. Natti et al. (1967) cited two different single dominant genes identified from different *B. oleracea* sources (one cabbage and one broccoli plant introduction) and concluded that the two genes were effective against different races of *P. parasitica* at the cotyledon stage. Examining a different *B. oleracea* source (in broccoli), Hoser-Krause et al. (1987) described a high level of downy mildew resistance expressed at the four- to five-leaf stage conditioned by a single recessive gene. These variable observations described by different investigators are likely the result of different resistance genes present in different *B. oleracea* germplasm sources.

Because three- to four-leaf stage resistance to downy mildew in USVL012 is controlled by only two genes, this resistance should prove easy to transfer to new breeding lines when USVL012 is used as a parent. In addition, the dominant expression of resistance in USVL012 will allow it, and other lines that contain the same resistance (Wang et al., 2000), to be used as inbred parents for developing resistant F₁ hybrids. The dominant resistance genes of USVL012 are especially useful because they need be incorporated into only one of the parental inbreds to be totally effective. Also, lack of maternal control for expression of this dominant resistance makes it possible for any potential resistant parents to be used as either a

male or female in hybrid combinations. Thus, the true leaf stage resistance described herein, could be deployed readily in new commercial broccoli cultivars.

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