Resistance to *Peronospora parasitica* in Chinese Cabbage

J. E. YUEN, Former Associate Plant Pathologist, The Asian Vegetable Research and Development Center, Shanhua, Tainan 741, Taiwan

ABSTRACT

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Some Chinese cabbage (*Brassica campestris* subsp. *pekinensis*) lines developed at The Asian Vegetable Research and Development Center, both open-pollinated and F_1 hybrids, are moderately resistant to downy mildew caused by *Peronospora parasitica*. In field trials, these lines had a reduced rate of disease increase when disease was measured as either percentage of leaf area infected or number of diseased leaves. Resistant lines had fewer and smaller lesions than the susceptible check 7–9 days after inoculation. In vitro tests with detached cotyledons showed a reduced rate of sporulation in resistant lines, with no evidence of maternal effects in hybrids. Comparisons of F_1 hybrids and their parents tested in vitro suggest that resistance may be an additive characteristic.

Chinese cabbage (*Brassica campestris* L. subsp. *pekinensis* (Lour.) Olsson) is a popular vegetable in Asia and has received much attention from The Asian Vegetable Research and Development Center (AVRDC). Breeding programs at AVRDC have made significant progress in improving resistance in Chinese cabbage to downy mildew, caused by *Peronospora parasitica* (Pers.:Fr.) Fr. Although AVRDC material has been reported as "resistant" (2–4), this resistance has not been fully described.

Past studies of resistance to *P. parasitica* have emphasized reactions in other crops (10) or host-pathogen interactions (9,12). In addition, much past work has dealt with what can be interpreted as qualitative resistance (10,12). Quantitatively expressed resistance, such as that described by Crute for lettuce downy mildew (6), is characterized by a slower rate of disease development. Quantitatively expressed resistance may have several components, but these have not been described in Chinese cabbage lines resistant to *P. parasitica*.

This study was initiated to characterize quantitatively expressed resistance to *P. parasitica* (herein referred to as partial resistance) in Chinese cabbage and to explore methods for quantifying resistance under both field and laboratory conditions. A preliminary report of part of this work has been published (5).

Present address of the author: Department of Plant Pathology, University of Hawaii at Manoa, Honolulu, HI 96822.

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MATERIALS AND METHODS

Field trials. Two field trials were conducted to examine downy mildew resistance under natural growing conditions. In 1983, two resistant open-pollinated Chinese cabbage lines, 77M(3)-27 and 77M(3)-35, and a susceptible line, B-14, were tested in a field trial with three replicates transplanted 10 May. Each plot consisted of 10 plants in a double-row bed (30 × 30 cm within beds, 1 m between beds), and replicate blocks were separated by a strip of maize 3 m wide. In 1985, six F_1 hybrids (58, 59, 62, 82-46, 82-156, and 82-157), reciprocal crosses of three hybrids (58R, 62R, and 82-46R), the two resistant lines from 1983, and a susceptible check were tested in a similar trial with four replicates transplanted on 12 March.

On 17 May 1983 and 27 March 1985, plants were sprayed with a suspension of conidia (10^4/ml) gathered from naturally infected plants. Plants were furrow-irrigated immediately after inoculation to provide high relative humidity to encourage infection.

Nine days after inoculation in 1983 and 7 days after inoculation in 1985, the number of visible lesions per plant and a visual estimate of the mean lesion size for each plant were recorded. Fourteen, 21, 28, and 35 days after inoculation, the percentage of the area of each plant that was infected was estimated using a key based on lettuce downy mildew (7), and the number of infected leaves on each plant was counted. For all data, single-plant values were used to calculate plot averages, which were then used in the data analysis.

Data on number and size of lesions were analyzed with an ordinary analysis of variance (ANOVA). Regression analysis was used to analyze the weekly data on disease progress: the percentage of plant area infected or the number of infected leaves was considered the dependent variable, with days after inoculation as the independent variable. The error variances from analysis of data from the 1985 trial were heterogeneous (11), and the data were transformed (the arcsine–square-root transformation for the percentage of leaf area infected, and the square-root transformation for the number of infected leaves) to stabilize the variance. Various submodels were considered in the regression analysis to examine differences among regression lines. ANOVA tables were calculated to compare, via *F* tests, the sources and the amount of variation (1).

Laboratory studies. Resistance in both open-pollinated lines [77M(3)-27 and 77M(3)-35] and F_1 hybrids (58, 59, 62, 82-46, 82-156, and 82-157) was further investigated in laboratory tests that measured the rate of spore production on detached cotyledons. Because incubator space was limited, these tests were done in five separate batches. Each batch also included the susceptible check, B-14. Parental lines were also included when hybrid material was tested. The reciprocal crosses of three F_1 hybrids (58R, 62R, and 82-46R) were also included.

P. parasitica was isolated from the field and maintained through weekly transfers of conidial suspensions (10^4–10^5 conidia/ml) to detached cotyledons (10–12 days old) of Chinese cabbage line B-14 grown in a greenhouse. The inoculated cotyledons were then kept in an illuminated (12-hr photoperiod) incubator at 14 C. The fungus generally sporulated 1 wk after inoculation.

Seeds of each line were sown, and the cotyledons were removed when plants were 12 days old. The cotyledons were placed on moist filter paper in 5-cm petri dishes with the abaxial surface up. Five dishes (each containing five cotyledons) were prepared for each line. Each cotyledon was inoculated on the abaxial surface with 100 μl of a suspension of conidia. Spore concentration varied by batch but ranged from 1.0 to 3.0 × 10^4 conidia per milliliter.

Starting 4 or 5 days after inoculation, all five cotyledons were removed from

one dish of each line. Each cotyledon was placed in a test tube containing 5 ml of water. Tubes were then shaken with a vortex mixer to remove the conidia. The concentration of the resulting spore suspension was determined with a hemacytometer. This procedure was repeated daily until all five petri dishes of each inbred line were tested. The spore suspension in each tube was considered a statistical unit, thus yielding five replicates for spore production from each of the 5 days.

These data were analyzed by multiple regression and a preplanned model sequence to determine whether the slopes of the lines differed. Spore concentration (after square-root transformation) was the dependent variable, and days after inoculation was the independent variable. The preplanned model sequences used indicator variables to selectively group data points from different treatments during regression (1). In this manner, F tests could be used to compare lines. Analyses were also performed using untransformed data, but these showed nonrandom error distributions, and the results are not presented here.

RESULTS

Field trials. In 1983, the two open-pollinated lines 77M(3)-27 and 77M(3)-35 showed clear resistance to downy mildew. These lines had fewer and smaller lesions than the susceptible line B-14 9 days after inoculation (Table 1). In addition, disease developed more slowly in the resistant lines (Table 1). When disease progress was measured in terms of the percentage of infected area per plant, the two resistant lines together were significantly ($P < 0.01$) different from B-14, with an F value of 275 with 2 and 32 degrees of freedom ($F_{2,32} = 275$), but were not significantly ($P = 0.05$) different from each other ($F_{2,30} = 1.28$). When disease progress was measured in terms of the number of infected leaves per plant, the same pattern emerged ($F_{2,32} = 268$; $F_{2,30} = 2.81$).

Seven days after inoculation in 1985, differences among lines in both lesion size and number were clearly visible (Table 2). All lines tested had smaller and fewer lesions than the susceptible check B-14, although not all differences were statistically significant.

Regression models indicated that the lines tested in the 1985 field trial varied significantly ($P < 0.01$) in the rate of disease increase measured either by transformed area infected per plant ($F_{22,157} = 23$) or by transformed number of infected leaves per plant ($F_{22,117} = 33$). A statistic similar to the least significant difference was calculated to compare the slopes (8) by multiplying the estimated standard deviation of the difference between two means by the appropriate t statistic. When the percentage of infected area was used as the disease measurement, the susceptible check had a significantly higher rate of disease increase than any of the other entries. When the number of infected leaves was used as the disease measurement, however, the regression lines for several entries were essentially the same as that for the susceptible check (Table 2). These data were also analyzed without transformation but showed nonrandom error distributions, so the results are not presented here.

Table 1. Lesion number, lesion size, and downy mildew disease increase in Chinese cabbage lines tested under field conditions in 1983

Line	Lesion number[a]	Lesion size[b] (mm^2)	Percentage of diseased area[c]		Number of infected leaves[d]	
			B_0	B_1	B_0	B_1
77M(3)-35	2.26	0.369	2.76	0.08	0.23	0.05
77M(3)-27	0.87	0.245	1.55	0.15	0.07	0.06
B-14	19.11	1.753	2.15	0.55	1.27	0.10
95% LSD[e]	0.90	0.053				

[a] Mean number of lesions per plant recorded 9 days after inoculation.
[b] Mean lesion size per plant recorded 9 days after inoculation.
[c] Increase in percentage of diseased area = $B_0 + B_1x$, where B_0 is the y-intercept, B_1 is the slope, and x is days after inoculation.
[d] Increase in number of infected leaves per plant = $B_0 + B_1x$, where B_0 is the y-intercept, B_1 is the slope, and x is days after inoculation.
[e] Least significant difference.

Table 2. Lesion number, lesion size, and downy mildew disease increase in Chinese cabbage lines tested under field conditions in 1985

Line	Lesion number[a]	Lesion size[b] (mm^2)	Percentage of diseased area[c]		Number of infected leaves[d]	
			B_0	B_1	B_0	B_1
Hybrid 58	30.95	0.1700	0.0253	0.0123	1.1140	0.0942
Hybrid 58R	32.93	0.1300	0.0329	0.0118	1.0140	0.0985
Hybrid 59	22.98	0.1250	−0.0732	0.0137	0.7602	0.1017
Hybrid 62	4.10	0.1125	−0.1382	0.0154	0.4331	0.1115
Hybrid 62R	5.68	0.1250	−0.1408	0.0161	0.5930	0.1084
Hybrid 82-46	8.13	0.1400	−0.0269	0.0107	1.5340	0.0514
Hybrid 82-46R	10.80	0.1600	−0.0552	0.0115	1.3630	0.0564
Hybrid 156	23.60	0.1725	0.0024	0.0145	0.9393	0.0963
Hybrid 157	20.98	0.1900	−0.0846	0.0192	0.9727	0.0977
77M(3)-27	10.98	0.1175	−0.0442	0.0095	0.6940	0.0916
77M(3)-35	8.70	0.1125	−0.0500	0.0104	0.6345	0.0929
B-14	47.85	0.2050	−0.1040	0.0228	1.2970	0.0965
95% LSD[e]	25.05	0.0009		0.0044[f]		0.0161

[a] Mean number of lesions per plant recorded 7 days after inoculation.
[b] Mean lesion size per plant recorded 7 days after inoculation.
[c] Increase in percentage of diseased area (after arcsine-square-root transformation) = $B_0 + B_1x$, where B_0 is the y-intercept, B_1 is the slope, and x is days after inoculation.
[d] Increase in square-root-transformed number of infected leaves per plant = $B_0 + B_1x$, where B_0 is the y-intercept, B_1 is the slope, and x is days after inoculation.
[e] Least significant difference.
[f] Because of unequal replication, this LSD is for all lines except 82-46 and 82-46R. For comparisons of 82-46 and 82-46R with other entries, LSD = 0.0058. For comparison of 82-46 with 82-46R, LSD = 0.0070.

Table 3. Regression coefficients for rates of production of conidia[a] of *Peronospora parasitica* on detached cotyledons of Chinese cabbage lines

Line	B_0	B_1
77M(3)-27	−1.5736	0.2647
77M(3)-35	−1.9472	0.2961
B-14	−2.7053	0.5387
Hybrid 58	−1.6177	0.7430
Hybrid 59	−2.6772	0.8129
E-7	−2.0879	0.5842
E-9	−2.9820	0.7018
N-4	−0.4671	0.7246
B-14	−2.7910	1.0703
Hybrid 62	−3.6245	0.9543
Hybrid 62R	−4.4749	1.1371
E-7	−2.4047	0.7739
B-18	−3.7201	0.9642
B-14	−3.2333	1.0983
Hybrid 82-46	−5.3969	1.0904
Hybrid 82-46R	−4.9987	1.0112
77M(3)-38-11-2-21	−0.2598	0.4808
B-162-3	−3.7425	0.8356
B-14	−4.1711	1.2403
Hybrid 82-156	−2.1217	0.4392
Hybrid 82-157	−1.4789	0.3429
PL-1	−1.1092	0.2328
N-4-2-41	−2.8056	0.6489
N-5-3-5-3-41	−3.8122	0.8332
B-14	−5.0718	1.0079

[a] Rate of conidial production (square root of number of conidia × 10^3/ml) = $B_0 + B_1x$, where B_0 is the y-intercept, B_1 is the slope, and x is days after inoculation.

In vitro tests. Detached cotyledons of resistant Chinese cabbage lines, both open-pollinated lines and F_1 hybrids, showed lower rates of spore production than those of the susceptible check B-14 (Table 3). Simplified models were used to determine whether regression lines were separate or whether a single line could explain the available data equally well (1).

Open-pollinated Chinese cabbage lines 77M(3)-27 and 77M(3)-35 showed a significantly ($P < 0.01$) lower rate of spore production than B-14 ($F_{2,56} = 74.48$). These two lines, however, did not differ significantly ($P = 0.05$) in the rate of spore production ($F_{2,59} = 0.68$). In the remaining tests, all hybrids and parental material grouped together showed a significantly ($P < 0.01$) lower rate of spore production than B-14 ($F_{2,116} = 16.24$ for hybrids 58 and 59, $F_{2,96} = 21.34$ for hybrid 62, $F_{2,91} = 48.22$ for hybrid 82-46, and $F_{2,116} = 18.48$ for hybrids 82-156 and 82-157).

Two of the tests with F_1 hybrids were designed to examine the possibility of maternal effects by including the reciprocal cross (62 and 62R, and 82-46 and 82-46R). In both cases, the hybrid and its reciprocal cross did not differ significantly ($P = 0.05$) in the rate of spore production ($F_{2,90} = 1.22$ for hybrid 62, and $F_{2,85} = 0.29$ for hybrid 82-46).

Tests with the F_1 hybrid material were arranged to examine both parental material and the hybrid simultaneously. For hybrids 58, 59, and 62, the rate of spore production of the combined parental lines was not significantly ($P = 0.05$) different from that of the hybrid itself ($F_{2,114} = 1.96$ for hybrids 58 and 59, and $F_{2,96} = 1.24$ for hybrid 62). For hybrids 82-46, 82-156, and 82-157, however, the sporulation rates of the F_1 lines were significantly ($P < 0.05$) different from the combined rates of the parental material ($F_{2,89} = 5.91$ for hybrid 82-46, and $F_{2,114} = 7.17$ for hybrids 82-156 and 82-157).

DISCUSSION

The open-pollinated lines 77M(3)-27 and 77M(3)-35 showed high levels of resistance relative to the susceptible check B-14 in these experiments. The F_1 hybrids also displayed varying levels of resistance, but none of them were immune to downy mildew. This resistance was expected because the inbred lines for these hybrids were developed, in part, from these open-pollinated lines.

When disease was evaluated 1 wk after a controlled inoculation, resistance was most apparent as a reduction in the number of lesions per plant. The variance with this parameter was so large, however, that not all differences were significant. Lesion size varied much less, and with this criterion all test lines appeared resistant compared to the susceptible check. A reduction in the number of lesions can be attributed to lower infection efficiency, and a reduction in lesion size can be attributed to slower growth of the pathogen within the plant.

All entries except 82-157 were more resistant than the susceptible check when the rate of increase in the percentage of area infected was used to measure resistance. Differences in resistance level were less obvious when the number of infected leaves was used to measure disease increase, and only 82-46 was resistant by this criterion. Although the number of infected leaves is a more objective measure of disease than the percentage of area infected, it is inherently less accurate because it cannot take into account differences in the size of the diseased area on each leaf. Thus, it is not surprising that differences between resistant and susceptible lines become less evident when the number of infected leaves is used to measure disease. The effects of interplot interference cannot be eliminated from this experiment and may also tend to obscure differences between resistant and susceptible material. In pure stands without interplot interference, the number of infected leaves may be a good way to evaluate disease severity. In this study, number of infected leaves per plant provided an objective disease measurement similar to disease incidence and maintained its usefulness even when disease incidence was high.

The hybrids varied in resistance, although some had levels of resistance approaching that of the open-pollinated lines 77M(3)-27 and 77M(3)-35. For the resistance described here, lesion size appeared to be the best single measure of disease, whereas the percentage of area infected was the best measure for epidemiological comparisons of resistance.

To investigate the possibility that the resistance described here is affected by the female parent (maternal effects), the reciprocal cross of three of the hybrids was examined in the 1985 field trial. A hybrid and its reciprocal cross contain the same nuclear chromosomes but have different cytoplasmic factors. The hybrids and their reciprocal crosses differed significantly ($P < 0.05$) only in lesion size 1 wk after inoculation. There was no evidence of maternal effects on lesion number 1 wk after inoculation or disease increase (measured either by percentage of diseased area or by number of infected leaves).

The exact nature of the resistance reported here is not clear. The interaction phenotypes that some plant breeders use to score downy mildew resistance (13) were not recorded during these studies because tissue necrosis was minimal or absent and all lines would have received ratings of seven to nine. Because these lines were not tested with different races of *P. parasitica,* whether the resistance described here is race-specific cannot be determined.

In vitro tests provided a more precise technique for examining partial resistance to downy mildew. Lines that were resistant under field conditions showed a reduced rate of spore production relative to the susceptible check in laboratory tests. This result agrees with results obtained in a study with broccoli (10), where sporulation was reduced on younger plants (seedlings with three to four leaves) of material known to be resistant under field conditions. Other plant breeders already use cotyledon reactions to screen for downy mildew resistance in Chinese cabbage (13).

The controlled measurements of partial resistance allowed several more detailed investigations. No maternal effects could be detected in in vitro tests with two F_1 hybrids (62 and 82-46) and their reciprocal crosses (62R and 82-46R); the rates of spore production in the hybrids and their reciprocal crosses were virtually identical. This result agrees with field observations when lesion number 1 wk after inoculation or disease increase was used to measure resistance.

Investigations aimed at determining the genetics of host resistance yielded mixed results. Comparisons of three F_1 hybrids with their parent lines confirmed that the resistance described here is an additive effect, since the rate of spore production for the hybrid did not differ from the rate for its combined parents. The rates of spore production for three other hybrids differed from the rates for their combined parents. In experiments with parental lines under field conditions (*unpublished*), the growth habit of the parental lines was so different from that of the F_1 hybrids that comparisons were not meaningful. If this partial resistance were additive, the resistance of a hybrid could be predicted from the levels of resistance of the parental lines. The in vitro tests reported here at present lack the sensitivity to unequivocally predict the performance of a hybrid from its parental lines.

Resistance to downy mildew exists in both open-pollinated and hybrid lines of Chinese cabbage developed at AVRDC. While not immunity, this resistance appears as a reduced rate of disease development and is the result of lower infection efficiency, reduced lesion development, and reduced rate of sporulation of the pathogen. This resistance can be detected in detached cotyledons as a reduced rate of spore production. Preliminary experiments with detached cotyledons from selected hybrid lines indicate the absence of maternal effects in two of the hybrids.

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