HortScience 26(12):1545-1547. 1991.

Brassica napus Sources of Resistance to Black Rot in Crucifers and Inheritance of Resistance

H. Guo[1], M.H. Dickson[2], and J.E. Hunter[3]
New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456-0462

Additional index words. Xanthomonas campestris pv. *campestris,* bridge line, *Brassica oleracea*

Abstract. **Resistance to black rot caused by the bacterium *Xanthomonas campestris* pv. *campestris* was studied in *Brassica oleracea, B. campestris,* and *B. napus. Two* accessions of *B. napus,* PI 199947 and PI 199949, exhibited the highest resistance so far found in cultivated *Brassica* spp. In *B. napus,* the high level of resistance was conferred by one dominant gene. In *B. campestris, two* Chinese cabbage accessions showed quantitative inheritance for moderate levels of resistance. Resistance was transferred to *B. campestris* from *B. napus,* but a unilateral incongruity was observed for black rot and morphology, but not for stem color or bolting. The bridge line 15 was used to transfer resistance to *B. oleracea.***

Black rot (BR), incited by the bacterium *Xanthomonas campestris* pv. *campestris* (Shaw and Kado, 1988), is a serious disease of crucifer crops, particularly when growing conditions are warm and wet. The pathogen may be seedborne and can survive in crop debris in soil and on host weeds. In nature, the bacterium invades the foliar vascular system of the host mainly through hydathodes. Staub and Williams (1972) studied the factors involved in disease development and developed artificial inoculation methods known as hydathode inoculation and vein inoculation. Shaw and Kado (1988) compared various inoculation procedures and concluded that pressure infiltration inoculation was not suitable for consistent induction of symptoms on turnip and cauliflower, whereas hydathode and wound inoculation procedures proved successful on *Brassica oleracea* and *B. campestris.*

Resistance to BR was discovered by Bain (1952) in the Japanese cabbage cultivars Early Fuji and Hugenot, as well as other species. Williams et al. (1972) demonstrated that resistance in 'Early Fuji' was due to one dominant major gene *f,* modified by one dominant and one recessive gene when *f* is in the heterozygous condition. Breeders in India (Sharma et al., 1972) reported that resistance to BR in cauliflower was governed by dominant polygenes. Dickson and Hunter (1987) found the cabbage accession PI 436606 to have juvenile and mature plant resistance. The objective of this study was to elucidate the inheritance of resistance in some crucifer species.

Plant materials were selected as follows. Resistant sources PI 199947, 199949, and 273640 *(B. napus)* were selected as a result of tests on the plant introduction collection of cabbage that identified PI 436606 cabbage (Dickson and Hunter, 1987) as a new source of resistance. BR resistant B162 and B171 *B. campestris* were obtained from the Asian Vegetable Research and Development Center, Taiwan. CC55 and PI 357374 *B. napus* and PI 418984 and 498988 ***B. campestris*** were identified as susceptible by earlier test

Received for publication 26 Nov. 1990. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[1]Visiting Fellow, Inst. of Vegetable and Floricultural Crops, Chinese Academy of Agricultural Sciences, 30 Baishiquiao Ave., Beijing, China.
[2]Professor, Dept. of Horticultural Sciences.
[3]Professor, Dept. of Plant Pathology.

Table 1. Segregation for resistance and susceptibility to black rot derived from crosses between lines of *B. napus* with high resistance (HR) and susceptibility (S) using the wound-colony inoculation method.[z]

Pedigree	No. plants in disease class 0	1	2	3	4	5	HR	S	χ^2	*P*
P_1 (PI 199947)	24						24	0		
P_2 (CC55)		1	11	6	4	2	1	23		
$(P_1 \times P_2)$ F_1	8		3				8	3		
$(P_2 \times P_1)$ F_1	9	3					12	0		
BC_1P_1	63	4	4	1			67	5		
BC_1P_2	21	10	7	5	22	1	31	35	0.242	>0.5
$(P_1 \times P_2)$ F_2	123	23	16	19	15	5	146	55	0.135	>0.8

[z]Disease classes are 0 = disease free, 1 = trace infection, 2 = 0.5- to 0.9-cm^2 lesions, 3 = l- to 1.4-cm^2 lesions, 4 = 1.5- to 1.9-cm^2 lesions, 5 = more than 2-cm^2 lesions. HR = classes 0 to 1. S = classes 2 to 5.

Table 2. Segregation for resistance and susceptibility to black rot derived from crosses between lines of *B. napus* with high resistance (HR) and susceptibility (S) using wound-colony inoculation.[z]

Pedigree	No. plants in disease class 0	1	2	3	4	5	HR	S	χ^2	*P*
P_3 (PI 199949)	24						24	0		
P_4 (PI 357374)			3	2	19		3	21		
$(P_3 \times P_4)$ F_1		7		4	1			7	5	
$(P_4 \times P_3)$ F_1		18						18	0	
BC_1P_3	67	1	4				68	4		
BC_1P_4	35		25	7	1		32	36	0.042	>0.5
$(P_3 \times P_4)$ F_2	159		40	8	2		159	50	0.129	>0.5

[z]Disease classes are 0 = disease free, 1 = trace infection, 2 = 0.5- to 0.9-cm^2 lesions, 3 = l- to 1.4-cm^2 lesions, 4 = 1.5- to 1.9-cm^2 lesions, 5 = more than 2-cm^2 lesions. HR = classes 0 to 1, S = classes 2 to 5.

Table 3. Segregation for resistance and susceptibility to black rot derived from crosses between lines *B. napus* with moderate resistance (MR) and susceptibility (S) using wound-colony inoculation.[z]

Pedigree	No. plants in disease class 0	1	2	3	4	5	HR	S	χ^2	*P*
P_5(PI 273640)	4	5	1	2			10	1		
P_6(CC55)				4	5	3	0	12		
$(P_5 \times P_6)$ F_1		2		5		4		7	4	
BC_1P_5		3	3	18	27	8	6	53	9.76	>0.001
BC_1P_6	0	1	14	31	14	8	15	53	0.033	>0.05
$(P_5 \times P_6)$ F_2	4	24	27	65	61	19	55	145	0.158	>0.5

[z]Disease classes are 0 = disease free, 1 = trace infection, 2 = 0.5- to 0.9-cm^2 lesions, 3 = 1- to 1.4-cm^2 lesions, 4 = 1.5- to 1.9-cm^2 lesions, 5 = more than 2-cm^2 lesions. MR = classes 0 to 2, S = classes 3 to 5.

Table 4. Segregation for resistance and susceptibility derived from a cross between medium-resistant and susceptible *B. campestris* plants inoculated by the wound-suspension procedure.[z]

Pedigree	No. plants tested	No. plants in disease class 0	1	2	3	4	5	$\bar{X}$
P_7 (B 171)	23	0	3	9	7	4	0	2.52
P_8 (PI 498988)	24	1	2	1	10	6	4	3.25
$(P_7 \times P_8)$ F_1	16	0	3	6	4	3	0	2.44
$(P_8 \times P_7)$ F_1	16	0	3	5	4	4	0	2.56
BC_1P_7	70	0	10	22	24	14	0	2.40
BC_1P_8	68	0	3	9	15	20	21	3.69
$(P_7 \times P_8)$ F_2	136	2	5	40	68	21	0	2.74

[z]Disease classes are 0 = disease free, 1 = trace infection, 2 = 0.5- to 0.9-cm^2 lesions, 3 = 1- to 1.4-cm^2 lesions, 4 = 1.5- to 1.9-cm^2 lesions, 5 = more than 2-cm^2 lesions.

results. Line 15 (Quazi, 1988) was used as a bridge to transfer the resistance from *B. napus* to *B. oleracea.*

The hydathode inoculation has been most widely used for breeding for resistance in *B. oleracea.* However, preliminary comparison of hydathode and wound inoculation procedures showed that hydathode inoculation was not very effective on *B. campestris* due to lack of guttation. Only wound colony inoculation distinguished between moderate and high levels of resistance in *B. napus.* Wound suspension inoculation separated the more moderate levels of resistance from susceptibles in *B. campestris.*

Inoculation. Strain PHW-117 from P.H. Williams was used for the screening and genetic studies. The bacterium was grown on YDCP medium (yeast, dextrose, calcium phosphate) at 28C for 48 h before inoculation.

Seed were planted in Cornell mix in styrofoam trays (5 × 5 × 5-cm cells) and allowed to grow at 25/20C (day/night) in the greenhouse for 2 weeks before inoculation. After inoculation, the seedlings were transferred to 30/27C (day/night) to accelerate disease development. The characteristic yellowing with vein blackening occurred in ≈5 days following inoculation. The inoculation procedures were: 1) wound suspension inoculation. A sterilized toothpick was inserted into a vein near the leaf margin and a 15-µl droplet of a bacterial suspension (1.5×10^9 cfu/ml, 10% transmittance at 600 nm) was placed on the wounded area. This system was used for inoculating *B. campestris.* 2) Wound colony inoculation: A sterilized toothpick was dipped in a bacterial colony growing on an agar medium and then inserted into a leaf vein near the leaf margin. This system was used for *B. napus* inoculation as it identified plants with the high level of resistance.

For both the wound inoculation procedures, the first two true leaves were inoculated, and reinoculation was done on the third and fourth true leaves. Two sites were inoculated on each shelf. For later tests, a florist's frog rather than a needle was used for inoculation.

Disease reactions were rated 8 to 10 days after wound inoculation procedures with a 0 to 5 scale: 0 = disease free, 1 = trace infection, 2 = 0.5 to 0.9, 3 = 1.4, 4 = 1.5 to 1.9, and 5 = more than 2 cm^2 of leaf diseased. For *B. campestris,* scores of 0, 1, and 2 were interpreted as resistant and higher scores as susceptible. For *B. napus,* 0 and 1 were interpreted as highly resistant, 2 as moderately resistant, and 3-5 as susceptible reactions. Different score systems were used because of the higher level of resistance available in *B. napus* and the more severe wound colony inoculation method used.

Inheritance of resistance. In the cross PI 199947 (HR) x CC55 (S) (Table 1), segregation ratios suggested a single dominant gene for the high level of resistance. The segregation ratios derived from the cross PI 199949 (HR) x PI 357374 (MR) were similar (Table 2), again indicating one single dominant gene for high resistance. Although the data on disease expression were recorded on a 0 to 5 scale, the results and subsequent observation (see Tables 5 and 6) fitted a single-gene model for resistance better than a quantitative inheritance pattern.

In the cross PI 273640 (MR) x CC55 (S) (Table 3), offspring from backcrosses to both parents were mostly susceptible. The F_2 population resulted in a segregation ratio of 1 resistant : 3 susceptible. We postulate that a homozygous recessive gene in PI 273640 was responsible for the moderate resistance. The scores of 0, 1, and 2 were classified as resistant or moderately resistant. The Chi-square test for the backcross to the MR parent did not fit any expected ratio. The moderate level of resistance in this cross, as opposed to the

Table 5. Reaction of interspecific F_1 plants derived from crosses between *B. campestris* × *B. napus* and *B. napus* × bridge line 15 to black rot 8 days after wound-colony inoculation.[z]

Cross	Resistance classification	Plants tested (no.)	Plants observed (no.)		
			Resistant	Susceptible	Reaction
B 162 x PI 199947	MR x HR	8	8	0	R
B 171 x PI 199947	MR x HR	13	13	0	R
PI 498933 x PI 199947	S x HR	5	5	0	R
B 171 x PI 357374	MR x S	12	0	12	S
PI 418984 x PI 357374	S x S	5	0	5	S
B 171 x PI 273640	MR x MR	13	0	13	S
B 171 x CC55	MR x S	5	0	5	S
B 162 x CC55	MR x S	14	0	14	S
PI 199949 x L15	HR x S	7	7	0	R
PI 199947 x L15	HR x S	6	6	0	R

[z]HR = highly resistant; MR = moderate resistance; R = resistant; S = susceptible.

Table 6. Segregation of backcross to Chinese cabbage of interspecific cross between B 162 *B. campestris* x PI 199947 *B. napus* after wound-colony inoculation.

Cross	Plants observed (no.)		P for expected 1:1	Stem color[z]	Bolting[y]
	Resistant	Susceptible			
PI 199947	6	0	---	pr	b
B162	0	6	---	gn	nb
F_2	120	0	---	pr	nb
BC_1 to B162 - 1	9	15	0.20	gn	nb
2	3	21	0.001	seg	seg
3	5	19	0.001	seg	seg
4	13	11	0.70	gn	nb
5	11	13	0.70	gn	nb
6	11	13	0.70	seg	nb
7	13	11	0.70	gn	seg

[z]pr = Purple, gr = green, seg = segregating.
[y]b = Bolting, nb = nonbolting.

high level, probably contributed to the lack of clear segregation for resistance.

The moderate resistance found in *B. campestris* appeared to be quantitative (Table 4). The results did not allow calculation of heritability because much variation was observed in both parents. By comparing the means of the backcross populations with that of the F_1 and the parents, the dominant effects of the polygenes for resistance were evident.

The highly resistant *B. napus* lines PI 199947 and PI 199949 exhibited strong dominance in the F_1 generation of intraspecific crosses (Tables 1 and 2) and interspecific crosses with *B. campestris* and line 15 (Tables 5 and 6); the moderate resistance (Table 3) was recessive. No differences in reaction to the disease were noted between reciprocal crosses.

In the interspecific crosses, progeny in the F_2 and backcrosses to the *B. campestris* parent showed resistance but little segregation for leaf morphological traits due to a unilateral incongruity frequently found following interspecific crosses. In the backcross F_2, the progeny segregated for resistance, stem color, and bolting (Table 6). The segregation of resistance and susceptibility did not give the expected ratio. These results may be explained by assortative recombination of chromosomes during gamete formation. When resistant backcross F_3 resistance selections were tested, some lines proved to be homozygous resistant with *B. campestris* morphology. Backcross F_3 plants showing resistance when young were tested again when 50 days old and were still found to be resistant.

The cross of resistant *B. napus* to L15 showed strong dominant resistance (Table 5).

PI 199949 showed resistance to 16 strains of X. *campestris.* pv. *campestris,* including strains causing symptoms intermediate between those of BR and leaf blight, and against *X. campestris* pv. *armoraciae* and *X. campestris* pv. *armoracea-like* organisms causing leaf spot and hydathode necrosis. A total of 16 strains was tested with the wound colony inoculation procedure that used a small florist's frog for inoculation. These strains were similar to the 16 strains used by Hunter et al. (1987) to test the resistance of PI 436606. The results indicate a dominant gene for high resistance in *B. napus* and, possibly, a second independent recessive gene for moderate resistance. Likewise, *B. campestris* lines with the high level of resistance from *B. napus* have been developed, rather than the moderate levels as in B171 and B162. More work might be done to clarify whether the resistance gene is the same as the *f* gene found in *B. oleracea* and if more modifiers are involved, as described by Williams et al. (1972). However, that appears to be unlikely since the level of resistance in our studies is higher and was expressed both at the juvenile and mature plant stage.

Literature Cited

Bain, D.C. 1952. Reaction of Brassica seedlings to black rot. Phytopathology 42:497–500.

Dickson, M.H. and J.E. Hunter. 1987. Inheritance of resistance in cabbage seedlings to black rot. HortScience 22:108–109.

Hunter, J. E., M.H. Dickson, and J. Ludwig. 1987. Source of resistance to black rot of cabbage expressed in seedlings and adult plants. Plant Dis. 71:263-266.

Sharma, B. R., V. Swarup, and S.S. Chatterjee. 1972. Inheritance of resistance to black rot in cauliflower. Can. J. Genet. Cytol. 14:363–370.

Shaw, J.J. and C.I. Kado. 1988. Whole plant wound inoculation for consistent reproduction of black rot of crucifers. Phytopathology 78:981–986.

Staub, T. and P.H. Williams. 1972. Factors influencing black rot lesion development in resistant and susceptible cabbage. Pbytopathology 62:722-728.

Williams, P. H., T. Staub, and J.C. Sutton. 1972. Inheritance of resistance in cabbage to black rot. Phytopathology 62:247–252.

Quazi, M.H. 1988. Interspecific hybrids between *Brassica napus* and *B. oleracea* L. developed by embryo culture. Theor. Appl. Genet. 75:309-318.