

Evaluation of *Brassica carinata* Accessions for Resistance to Black Rot (*Xanthomonas campestris* pv. *campestris*)

Muhammet Tonguç and Phillip D. Griffiths¹

Department of Horticultural Sciences, Cornell University N.Y. State Agricultural Experiment Station, Geneva, NY 14456

Additional index words. ethiopian mustard, wound inoculation, *Brassica oleracea*

Abstract. Black rot, caused by *Xanthomonas campestris* pv. *campestris* (Pam.) Dawson (Xcc), is a major bacterial disease of *Brassica oleracea* L. vegetables. In this study the related species *Brassica carinata* Braun (ethiopian mustard), which can be used to generate interspecific crosses with *B. oleracea* was evaluated for resistance to Xcc. Fifty-four accessions and susceptible control plants were wound inoculated with four isolates of Xcc race 4 at the juvenile stage. Of the 54 accessions tested, A 19182 and A 19183 exhibited no symptoms when inoculated with Xcc for all plants tested, and the accessions including PI 199947, PI 199949 and PI 194256 segregated for resistance to Xcc.

Black rot is a bacterial disease of *Brassica* species caused by *Xanthomonas campestris* pv. *campestris* (Xcc). The disease has a wide geographic distribution and is particularly destructive to the *Brassica* vegetables (Williams, 1980). Xcc is a seedborne pathogen (Cook et al., 1952) that can overwinter on cruciferous weeds and wild relatives of cultivated *Brassica* crops (Schaad and Dianese, 1981). The principal methods for disease control are hot water treatment of seeds, good horticultural practices, and use of copper bactericides. However, the disease continues to be problematic, particularly during hot, humid seasons. Symptoms of black rot may vary between host species, with most plants expressing V-shaped chlorotic regions originating from leaf margins following infection through the hydathodes. Under ideal conditions disease symptoms appear 10 to 14 d after infection (Williams, 1980).

Earlier studies focused on black rot resistance derived from *B. oleracea*, including the cabbage cultivar 'Early Fuji' (Bain, 1952), the inheritance of which was reported to be controlled by a single recessive gene with two modifiers (Williams et al., 1972). Resistance has also been documented in *B. oleracea* plant introductions (PIs) including the cabbage accession PI 436606 from China. The resistance from this source was reported to be controlled by a single recessive gene with one or two modifiers (Dickson and Hunter, 1987; Hunter et al., 1987). These sources have been used in the development of black rot resistant germplasm, however; the resistance is typically incomplete and difficult to incorporate into hybrid cultivars (Camargo et al., 1995). Other sources of resistance have been reported in *B. nigra*, *B. juncea* and other *Brassica* species (Taylor et al., 2002; Westman et al., 1999) including *B. carinata* accessions PI 199947 and PI 199949 both formerly classified as *B. napus* (Guo et al., 1991).

Vicente et al. (2001) classified Xcc into six distinct races. The most important of these races are races 1 and 4, which account for over 90% of black rot disease worldwide (Vicente et al., 2001). In addition, an association between occurrence of races 1 and 4 with *B. oleracea* crops or weedy *Brassica* species has been observed (Vicente et al., 2001). Evaluation of *B. carinata* material has indicated that resistance is controlled by a single dominant gene that controls resistance to races 1, 3 and 4 of black rot (Guo et al., 1991; Vicente et al., 2001).

The major *Brassica* crop species are closely related forming the *Brassica* triangle (Prakash et al., 1999). These species have three genome types AA (*B. rapa*), BB (*B. nigra*) and CC (*B. oleracea*). The three genomes have naturally combined and formed amphidiploid species *B. juncea* (AB), *B. carinata* (BC), and *B. napus* (AC). When resistance to specific races was evaluated, race 4 resistance appeared to be common in A genome *Brassica* species (*B. rapa*, *B. juncea* and *B. napus*). Resistance to races 1, 3, and 4 was common in B genome *Brassica* species (*B. nigra*, *B. juncea* and *B. carinata*) (Taylor et al., 2002). *Brassica oleracea* accessions have not been identified that exhibit complete resistance to races 1 and 4, but several accessions exhibit incomplete resistance to these races (Taylor et al., 2002), which has been attributed to the presence of quantitative trait loci (QTL) (Camargo et al., 1995).

To develop *B. oleracea* varieties with host plant resistance it will be necessary to identify and incorporate resistance genes that protect the plants against races 1 and 4. It will also be necessary to use sources that have more manageable genetic control of resistance than current *B. oleracea*-derived breeding lines. *Brassica carinata* accessions provide an important germplasm resource for the development of hybrid *Brassica* vegetables because the resistance has been documented as complete and single gene dominant when incorporated into cabbage and cauliflower germplasm. The purpose of this study was to evaluate available

B. carinata accessions from the USDA collection for resistance to race 4 of Xcc using a wound inoculation technique.

Materials and Methods

Plant material. The USDA *B. carinata* collection (North Central Regional Plant Introduction Station, Ames, Iowa) consists of 65 accessions primarily from Ethiopia. Fifty-four of these were available for evaluation. In total, 36 seeds of each of the 54 accessions were sown in 32-cell (125-cm³) Styrofoam trays in 'Cornell Mix' (Boodley and Sheldrake, 1982) with one seed per cell (Speedling, Sun City, Fla.) on 10 Apr. 2001. Cultivars of broccoli ('Marathon', 'Titleist'), cauliflower ('Snow Ball', 'Delira'), and cabbage ('Atlantis', 'Bartolo') were planted as susceptible controls.

Inoculation. The accessions were grown for 3 weeks to the two-leaf stage in a greenhouse at 23/20 °C day/night with a 14-h photoperiod under 1000 W metal halide lamps (300 μmol·m⁻²·s⁻¹) in preparation for the inoculation. Four isolates of Xcc isolated from infected cabbages in New York were obtained from D. Reed (Reed's Seeds, Cortland, N.Y.). These were screened with differential cultivars and characterized as race 4. These 4 Xcc isolates were grown on YDCP medium (Shelton and Hunter, 1985) for 3 d and used to needle-inoculate the 54 accessions and control plants. The wound inoculation involved piercing the true leaf either side of the midrib with 2 Xcc infected needles for each of the four isolates (Shaw and Kado, 1988). Two inoculations were undertaken, the first when seedlings were at the two- to three-true-leaf stage, and the second when the seedlings were at the four- to six-true-leaf stage. All plants were moved to a 100% humidity chamber for 48 h following the inoculation.

Plants were evaluated 10 to 14 d after the inoculation using a rating scale of 1 to 5 (1 = completely resistant, 5 = completely susceptible) (Tonguç et al., 2003). Diseased leaves were clipped carefully following the first rating and plants were allowed to develop new leaves over a 2-week period for the second inoculation. Disease severity ratings were analyzed for mean separations using Duncan's multiple range test (SAS, 1997).

Results

All control plants exhibited black rot symptoms when wound inoculated. Broccoli controls exhibited slower symptom development than cabbage and cauliflower controls. The mean disease severity rating of 54 *B. carinata* accessions tested was 4.2 for rating 1 and 4.35 for rating 2 (Table 1). Only two accessions (A 19182 and A 19183) exhibited complete resistance in all plants tested for both ratings. All plants of seven accessions were completely susceptible for rating 2, four of these (PI 195923, PI 197402, PI 360883, and PI 209023) were also completely susceptible at rating 1. Fifteen accessions contained five or more uninfected plants at rating 1. Rating 2 identified only five accessions that had five

Received for publication 11 Feb. 2003. Accepted for publication 26 Aug. 2003

¹To whom reprint requests should be addressed; e-mail pdg8@cornell.edu.

Table 1. Mean disease severity ratings of 54 *Brassica carinata* plant introductions (PIs) following inoculation with four isolates of Xcc race 4.

Accession no.	No. of plants with ratings 1 and 2	Uninfected plants with rating 1	Uninfected plants with rating 2	Rating 1 mean	Rating 2 mean
A 19182	30	30	30	1.0 o	1.0 o ^r
A 19183	30	30	30	1.0 o	1.0 o
PI 280230	30	24	14	1.7 n	2.6 n
PI 193760	30	10	0	2.5 m	3.4 lm
PI 194256	19	9	9	3.3 l	3.2 m
PI 194254	29	7	4	3.5 kl	3.6 l-k
PI 199947	30	8	7	3.6 j-l	3.6 l-k
PI 199949	30	5	5	3.6 j-l	3.8 j-k
PI 360884	30	3	0	3.7 j-l	4.2 f-i
PI 193460	30	7	1	3.8 h-k	4.1 g-j
PI 194251	30	6	1	3.8 h-k	4.4 a-h
A 19180	30	5	0	3.8 h-k	4.5 a-h
PI 360879	30	6	0	3.9 g-k	4.4 b-h
PI 360885	30	3	0	3.9 f-k	4.1 g-j
PI 194900	30	7	3	3.9 f-k	4.2 f-i
PI 131247	30	4	1	4.0 e-k	4.3 e-h
PI 194904	30	5	2	4.0 e-k	4.0 h-k
PI 331378	24	4	0	4.0 d-k	4.3 c-h
PI 226545	30	4	0	4.0 d-k	4.4 b-h
PI 193467	30	6	0	4.2 c-i	4.6 a-f
PI 231046	30	4	8	4.2 b-i	3.7 j-l
PI 273636	29	5	3	4.3 b-i	4.4 b-h
PI 195921	14	0	0	4.3 a-i	4.3 d-h
PI 194901	30	0	0	4.4 a-i	4.4 b-h
PI 193459	30	4	0	4.3 a-i	4.7 a-f
A 21217	30	0	0	4.3 a-i	4.5 a-h
PI 199950	30	7	3	4.4 a-h	4.5 a-h
PI 193759	30	2	0	4.5 a-h	4.5 a-h
PI 597822	30	0	0	4.5 a-h	4.5 a-h
PI 194252	30	3	0	4.5 a-h	4.7 a-f
PI 274283	30	1	0	4.5 a-g	4.6 a-f
PI 331377	30	1	0	4.6 a-g	4.6 a-g
PI 196836	30	1	0	4.6 a-g	4.6 a-g
PI 194253	30	3	0	4.6 a-g	4.8 a-d
PI 273640	30	3	0	4.6 a-g	4.8 a-d
PI 360882	30	2	0	4.6 a-f	4.8 a-e
PI 273637	30	0	0	4.6 a-e	4.6 a-g
PI 195552	30	0	0	4.7 a-d	4.7 a-f
A 19181	30	0	0	4.7 a-c	4.7 a-f
A 19184	30	0	1	4.7 a-c	4.7 a-f
PI 243913	30	1	0	4.8 a-c	4.8 a-e
PI 360886	30	1	0	4.7 a-c	4.8 a-d
PI 360881	30	1	0	4.8 a-c	4.8 a-d
PI 390133	30	1	0	4.8 a-c	4.8 a-d
PI 194903	30	0	0	4.9 ab	4.9 a-c
PI 197403	30	0	0	4.9 ab	4.9 a-c
PI 194255	30	1	0	4.9 ab	4.9 ab
PI 390134	30	0	0	5.0 a	4.8 a-e
PI 360887	30	0	0	5.0 a	4.9 ab
PI 360880	30	0	0	5.0 a	4.9 ab
PI 195923	20	0	0	5.0 a	5.0 a
PI 197402	30	0	0	5.0 a	5.0 a
PI 360883	30	0	0	5.0 a	5.0 a
PI 209023	30	0	0	5.0 a	5.0 a

^rMean separation within each column by Duncan's multiple range test, at $p \leq 0.05$. Means followed by the same letters are not significantly different from one another.

or more uninfected plants, including two accessions (PI 199947 and PI 199949) previously documented as black rot resistant (Guo et al., 1991).

Uninfected plants from 13 accessions (PI 280230, PI 194256, PI 194254, PI 199947, PI 199949, PI 193460, PI 194900, PI 131247, PI 194904, PI 231046, PI 273636, PI 199950, and PI 194251) were transferred to plastic pots (10-cm-diameter) and were allowed to grow for 3 weeks before screening at maturity. Plants from PI 199947, PI 199949, and PI 194256 either remained free of disease symptoms or contained the spread of disease in leaves,

however; plants from the 10 other accessions developed disease symptoms when they were retested at maturity. Plants tested from PI 280230 had low disease severity ratings when they were grown in Styrofoam trays, but became infected when transferred to larger pots in part through leaf size

Discussion

Host plant resistance is the most practical way to control black rot, and resistance sources from *B. oleracea* have been evaluated extensively due to the economic importance

of vegetable *Brassicaceae* such as, cabbage, cauliflower, broccoli and brussels's sprouts (Bain, 1952; Hunter et al., 1987, Taylor et al., 2002). No *B. oleracea* varieties with complete resistance to the most common races, 1 and 4, have been developed. However, the cabbage breeding line Badger Inbred 16 (P.H. Williams, Univ. of Wisconsin) has been documented as incompletely resistant to race 1 (Taylor et al., 2002). The problems associated with *B. oleracea* derived sources highlight the need to use resistance genes from outside the species.

Black rot resistance can be expressed at the juvenile, mature, or both juvenile and mature plant stages (Hunter et al., 1987). Differences in disease reactions of some accessions in this study could be attributed to differences in expression of disease symptoms at different stages. Plants that showed no symptoms at both the juvenile and mature stages were considered to be the most useful sources as they would be able to prevent or reduce spread in seedling nurseries and field plantings.

Resistance from *B. carinata* accession PI 199947 has previously been transferred to *B. oleracea* (Hansen and Earle, 1995). However, *B. oleracea* breeding lines derived from the original *B. carinata* + *B. oleracea* protoplast fusion hybrids have not exhibited stable inheritance of the black rot resistance gene and this problem has continued to persist after many backcrosses (Griffiths and Nickels, 2001; Tonguç et al., 2003; Zhou et al., 1997). If a resistant gene is introgressed from a B genome crucifer, the accessions used may differ stability in interspecific crosses; therefore, the number of sources of resistance for interspecific crosses should be maximized.

It has been suggested that some *Brassicaceae* species contain resistance that is race nonspecific or provides resistance to at least races 1 and 4 (Taylor et al., 2002). This type of resistance will be the most practical and efficient for cultivar development if the interspecific crossing barriers can be overcome. Difficulties involved in interspecific crosses with *B. oleracea* through sexual crosses include different chromosome numbers in the parents. These barriers can be overcome with techniques including embryo rescue and protoplast fusion, but aneuploidy in breeding lines occurs. Successful introgression of black rot resistance using these approaches may be extended and more problematic if the resistance gene(s) is located on an aneuploid chromosomes in breeding lines.

PI 199947, PI 199949, PI 194256, A 19182, and A 19183 were considered to be the best sources of *B. carinata* accessions for introgression of Xcc race 4 resistance into *B. oleracea*. Although A 19182 and A 19183 were completely resistant to black rot all in plants tested at the seedling stage, it was not possible to field test these accessions at maturity due to early seed set and leaf abscission. It is also possible that the leaf morphology of PI 280230 might have compromised disease severity ratings as plants became infected when they were transferred to larger pots, although accurate readings could not be documented.

A 19182 and A 19183 are relatively new

additions to the *B. carinata* collection that were obtained from the Vavilov Institute, St. Petersburg, Russia, in 1972. They were morphologically very distinct from other *B. carinata* accessions evaluated. When these accessions were reciprocally crossed to highly susceptible *B. carinata* accessions (PI 360883, PI 209023, PI 195552, and PI 597822) and *B. oleracea* cultivars ('Captain', 'Titleist' and 'Marathon') they failed to produce seeds. However, A 19182 crossed to a *B. napus* genotype (PI 458949) with some success. These observations cast doubt on their identity as *B. carinata*.

Taylor et al. (2002) noted that accessions of *B. juncea* were uniformly resistant to Xcc races tested. Observations of some aborted seeds, unfilled siliques between the cross of A 19182 and PI 458949, cpDNA PCR-RFLP profiles (data not shown) and uniform resistance to race 4 suggest that these two accessions might belong to *B. juncea* and are currently mis-identified in the USDA collection as *B. carinata*. The accessions identified belong to a *Brassica* species and can be used to develop interspecific crosses with *B. oleracea*, to introgress resistance to Xcc race 4.

Literature Cited

- Bain, D.C. 1952. Reaction of brassica seedlings to black rot. *Phytopathology* 42:497–500.
- Boodley, J.W. and R. Sheldrake, Jr. 1982. Cornell peat-lite mixes for commercial plant growing. N.Y. Agr. Expt. Sta. Agr. Info. Bul. 43.
- Camargo, L.E.A., P.H. Williams, and T.C. Osborn. 1995. Mapping of quantitative loci controlling resistance to *Brassica oleracea* to *Xanthomonas campestris* pv. *campestris* in the field and greenhouse. *Phytopathology* 85:1296–1300.
- Cook, A.A., R.H. Larson, and J.C. Walker. 1952. Relation of the black rot pathogen to cabbage seed. *Phytopathology* 42:316–320.
- Dickson, M.D. and J.E. Hunter. 1987. Sources of resistance to black rot of cabbage expressed in seedlings and adult plants. *Plant Dis.* 71:263–266.
- Griffiths, P.D. and J.L. Nickels. 2001. Association of molecular polymorphism with black rot resistance derived from Ethiopian mustard. *Cruciferae Nwslt.* 23:57–58.
- Guo, H., M.H. Dickson, and J.E. Hunter. 1991. *Brassica napus* sources of resistance to black rot in crucifers and inheritance of resistance. *HortScience* 26:1545–1547.
- Hansen, L.N. and E.D. Earle. 1995. Transfer of resistance to *Xanthomonas campestris* pv. *campestris* into *Brassica oleracea* L. by protoplast fusion. *Theor. Appl. Genet.* 91:1293–1300.
- Hunter, J.E., M.H. Dickson, and J.W. Ludwig. 1987. Sources of resistance to black rot of cabbage expressed in seedlings and adult plants. *Plant Dis.* 71:263–266.
- Prakash, S., Y. Takahata, P.B. Kirti, and V.L. Chopra. 1999. Cytogenetics, p. 59–105. In: C. Gomez-Campo (ed.). *Biology of brassica coenospecies*. Elsevier, Amsterdam, Netherlands.
- SAS Institute. 1997. SAS user's guide. SAS Inst., Cary, N.C.
- Schaad, N.W. and J.C. Dianese. 1981. Cruciferous weeds as sources of inoculum of *Xanthomonas campestris* in black rot of crucifers. *Phytopathology* 71:1215–1220.
- Shaw, J.J. and C.I. Kado. 1988. Whole plant wound inoculation for consistent reproduction of black rot of crucifers. *Phytopathology* 78:981–986.
- Shelton, A.M. and J.E. Hunter. 1985. Evaluation of the potential of the flea beetle *Phyllotreta cruciferae* to transmit *Xanthomonas campestris* pv. *campestris*, casual agent of black rot of crucifers. *Can. J. Plant Pathol.* 7:308–310.
- Taylor, J.D., J. Conway, S.J. Roberts, D. Astley, and J.G. Vicente. 2002. Sources and origin of resistance to *Xanthomonas campestris* pv. *campestris* in *Brassica* genomes. *Phytopathology* 92:105–111.
- Tonguç, M., E.D. Earle, and P.D. Griffiths. 2003. Segregation distortion of *Brassica carinata* derived black rot resistance in *Brassica oleracea*. *Euphytica* 134:269–276.
- Vicente, J.G., J. Conway, S.J. Roberts, and J.D. Taylor. 2001. Identification and origin of *Xanthomonas campestris* pv. *campestris* races and related pathovars. *Phytopathology* 91:492–499.
- Westman, A.L., S. Kresovich, and M.H. Dickson. 1999. Regional variation in *Brassica nigra* and other weedy crucifers for disease reaction to *Alternaria brassicicola* and *Xanthomonas campestris* pv. *campestris*. *Euphytica* 106:253–259.
- Williams, P.H., T. Staub, and J.C. Sutton. 1972. Inheritance of resistance in cabbage to black rot. *Phytopathology* 62:247–252.
- Williams, P.H. 1980. Black rot: a continuing threat to world crucifers. *Plant Dis.* 64:736–742.
- Zhou, Z., N.F. Weeden, and M.H. Dickson. 1997. The expression of a resistant gene to black rot in progeny of the protoplast fusion broccoli (*B. oleracea*). *Cruciferae Nwslt.* 19:109–110.