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Introgression and Characterization of Black Rot Resistance Derived from *Brassica carinata* in Cole Crops

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Abstract: Black rot (*Xanthomonas campestris* pv. *campestris*) is one of the most serious diseases of cole crops in NY State, particularly during warm damp seasons. Resistance to black rot from Ethiopian mustard has been introgressed into broccoli lines using protoplast fusion and hybrid crosses with cabbage and cauliflower have been made. This resistance source has been studied by comparing molecular polymorphisms with disease severity segregations following greenhouse inoculations of plants. Disease severity ratings at the juvenile and mature plants stages indicated that complete resistance to black rot was being recovered in F_2 populations derived from the broccoli lines 11 and 11B crossed with cauliflower. Plants exhibiting intermediate resistance to black rot were also observed in the juvenile and mature plant inoculation trials. The segregation results suggested that more than one gene may control the resistance. However, closer studies with molecular markers suggested that the source of resistance may indeed be a single dominant gene. The resistance may not be fully stabilized resulting in low recovery of resistant plants in the F_2 populations derived from 11 and 11B. Markers may be important in pyramiding resistance genes to other pests simultaneously even if seedling disease screening is possible. Other black rot resistant material was evaluated, in total 124 lines were field screened and inoculated with black rot during summer 2000, and significant screening of black rot material derived from *B. carinata* was made in greenhouse trials. Selections of resistant material have been made, and these selections are being crossed to the major vegetable cole crops.

Introduction:

Black rot (*Xanthomonas campestris* pv. *campestris*) is one of the most serious diseases of cole crops, especially during warm, damp seasons. It is easily spread from contaminated seed in nurseries and through mechanical transmission in the fields. Symptoms of the disease include V-shaped lesions originating from the margin of the leaf. There are no effective chemicals for the control of black rot, although copper bactericides are applied and have a limited effect. The most effective approaches to controlling black rot are through good farm management practices, hot water treatment of seeds and the use of cultivars with resistance to the disease. Hot water treatment may reduce seed viability and does not fully eradicate the disease. Host plant resistance while partially effective, is not complete and can still result in spread of the disease throughout plantings. A new source of black rot resistance reported to be controlled by a single dominant gene was identified in a *Brassica napus* accession PI 199947 (later identified as *B. carinata*) and was used to transfer resistance to broccoli by protoplast fusion (Guo *et al.*, 1991; Hansen and Earle, 1995). Following identification of a somatic hybrid between PI 199947 and rapid cycling *Brassica,* additional crosses to the broccoli cultivar 'Green Comet' were made to stabilize the resistance. Lower than expected ratio's of resistant plants were observed in F_2 populations generated from resistant broccoli lines, and the resistance source was further studied to better clarify the genetic control. This study examined the genetic control of black rot resistance derived from *B. carinata*, examining segregations of molecular markers associated with black rot resistance. Evaluations and breeding of germplasm was also undertaken to introgress this resistance source into the major vegetable cole crops (cabbage, cauliflower, broccoli)

Materials and Methods:

Black rot resistant broccoli lines (11B-1-12 and 11-1-2) developed from *B. carinata* were crossed to the black rot susceptible cauliflower cultivar 'Snowball', and the hybrids were self-fertilized to form F_2 populations segregating black rot resistance. The objective of this study was to examine the relationship between RAPD polymorphisms (RAPDs) segregating within the generated F_2 population, and form associations with disease severity ratings of plants at juvenile and mature stages.

One-hundred F_2 seedlings generated from an 11B-1-12 x cauliflower cross were sown in $(3.8\ cm)^3$ 'Speedling' flats for inoculation and re-planted into six-inch pots at the 6-week stage. Four isolates of black rot were grown on plates of YDCP at 28ºC for 48 hours before inoculation. Plants were wound inoculated with each isolate by puncturing the leaves with two black rot infected needles either side of the mid-rib. Plants were placed in a mist chamber for 48 hours at 28ºC with a 14-hour photoperiod following inoculation to encourage infection. The inoculation was performed on the 4-week old seedlings from the generated F_2 population and 32 control plants of broccoli and cauliflower varieties 'Marathon' and 'Delira' were simultaneously inoculated to ascertain infection rate. The plants were re-inoculated at the 10-week stage to test for mature plant resistance. Plants were rated on a scale of 0 – 5, where 0 = completely resistant, and 5 = completely susceptible.

A total of 124 lines were field screened for black rot resistance based on selections and crosses from the 1999 season. These plants represented lines of cabbage,

broccoli, cauliflower and hybrids derived from both *B. carinata* sources, and lines based on surrently used resistance derived from *B. oleracea*. Seedlings were sown in $(3.8\ cm)^3$ 'Speedling' flats and transplanted to the field at the 5-week stage. All plants were individually inoculated at the ten week stage with four isolates of black rot, using the wound inoculation technique described. Selections were made at plant maturities based on their field resistance to black rot, all selections were removed from the field potted and placed self-fertilized after a vernalization period (3-weeks for cauliflower and broccoli, 3-months for cabbage).

Results and Discussion:

Disease severity ratings at the juvenile and mature plants stages indicated that complete resistance to black rot was being recovered in F_2 populations derived from 11 and 11B crosses with cauliflower (prior releases from Lisa Earle derived from *B. carinata*). Plants exhibiting intermediate resistance to black rot were also observed in the juvenile and mature plant inoculation trials. The proportion of completely resistant plants observed (approximately 10% in populations derived from 11 and 11B) suggested that more than two genes controlled the black rot resistance derived from PI 199947 which differed from two previous studies of this resistance (fig. 1). In one F_2 population of 100 plants derived from 11B-1-12 x 'Snowball', 10 plants rated 0 at the seedling stage, eight of which were still rated 0 at the mature stage suggesting that the resistance was consistent at the juvenile and mature plant stages.

A total of 16 RAPD polymorphisms were scored for one of the F_2 populations (derived from the 11B-1-12 x 'Snowball' cross) based on presence/absence of amplified bands. Mean disease severity rates were generated for band presence/absence and molecular polymorphisms exhibiting significant associations with resistance/ susceptibility were determined (Table 1). The most significant association with resistance was observed for the polymorphism OP AB04-2 (Operon primer OP AB04). All plants in which the primer was amplified were rated zero at the juvenile and mature stages except for one plant which rated as 1 in the juvenile inoculation test. However, only 9 plants out of the 100 scored amplified this polymorphism, far less than the 75 that would be expected using a dominant RAPD marker. It is possible that the resistance to black rot in this population was not fully stabilized chromosomally. The polymorphism (UBC 221-3) showed significant associations with black rot resistance, but also segregated in very low proportions suggesting similarities/linkage to OP AB04-2. The somatic fusion the B. carinata accession PI 199947 protoplast (n=17) with a *B. oleracea* protoplast (n=9) resulted in chromosomal instability for several generations of backcrossing. It is possible that the black rot resistance introgressed from PI 199947 is not fully stabilized in the plant used as a resistant parent (11B-1-12), and that further backcrossing to *B. oleracea* will be required to stabilize this resistance source for use in commercial varieties. These results suggest, that the source of resistance may indeed be a single dominant gene (due to the consistency of marker presence and juvenile/mature resistance) but may not be fully stabilized resulting in low recovery of resistant plants in F_2 populations derived from the plants used as parents. Once stabilized, the resistance source may not require molecular markers if it can be screened for efficiently at the seedling stage.

Selections of 111 plants were made, a total of fifty-eight field selections, a further fifty-three selections were made in the greenhouse, and twenty-three F_1 crosses with *B.*

carinata derived resistance (without sufficient numbers for field screening) were increased to the F_2 generation. Many of the greenhouse selections are being progeny tested, and the most resistant sources will be crossed to all major vegetable cole crops.

References:

Guo, H., Dickson, M., Hunter, J. (1991). *Brassica napus* sources of resistance to black rot in crucifers and inheritance of resistance. Hortscience 26: p1545-1547.

Hansen, L. and Earle, E. (1995). Transfer of resistance to *Xanthomonas campestris* pv. *campestris* into *Brassica oleracea* L. by protoplast fusion. Theor. Appl. Gen. 91: p1293-1300.

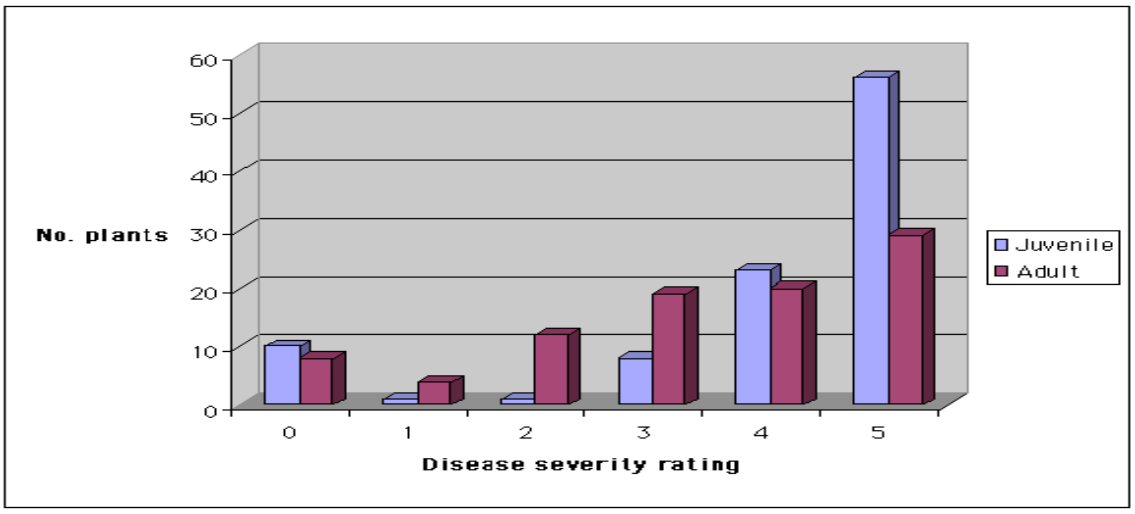

Fig. 1: Segregation of disease severity rates at juvenile and mature stages for F_2 progeny of 11B-1-12 x 'Snowball' cross.

Primer No.	Juvenile Marker Present	Juvenile Marker Absent	Mature Marker Present	Mature Marker Absent
UBC 218	4.39 a*	3.86 a	3.66 a	3.35 a
UBC 98	3.88 b	4.70 a	3.28 b	4.50 a
UBC 221-1	3.93 a	4.08 a	3.43 a	3.40 a
UBC 221-2	4.08 a	3.81 a	3.53 a	3.27 a
UBC 221-3	1.13 b	4.30 a	1.25 b	3.67 a
OP AB04-1	3.92 a	4.33 a	3.33 a	4.00 a
OP AB04-2	0.14 b	4.33 a	0 b	3.75 a
UBC 568	3.88 a	4.35 a	3.49 a	3.17 a
UBC 485-1	4.13 a	3.39 b	3.69 a	2.50 b
UBC 485-2	3.83 b	4.52 a	3.31 a	3.88 a
UBC 381	3.88 a	4.21 a	3.44 a	3.39 a
UBC 549	3.96 a	4.00 a	3.39 a	3.52 a
UBC 302-1	4.03 a	3.83 a	3.47 a	3.41 a
UBC 302-2	3.93 a	4.19 a	3.35 a	3.73 a
UBC 302-3	3.83 b	4.50 a	3.28 a	3.94 a
UBC 42	4.01 a	3.86 a	3.48 a	3.29 a

Table 1: Mean disease severity rates of plants showing presence/absence of RAPD polymorphisms.