

Final Project Report to the NYS IPM Program, Agricultural IPM 2000 – 2001

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

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Type of grant: Pest-resistant crops; allelopaths

Project location(s): All of NY

Abstract:

Fresh-market cabbage is an important vegetable crop in NY State which had a farm-gate value of approximately \$80 million in 2000. Black rot (*Xanthomomas campestris* pv. *campestris*) is a major limiting factor to cabbage production in New York State especially during warm, damp seasons. Limited host plant resistance has been incorporated into several cabbage varieties, but is insufficient. Breeding a new source of resistance to black rot will be important for the future protection of this crop in NY State, through the development of new varieties with enhanced resistance to this disease. This research focuses on introgressing resistance from a related crop (Ethiopian mustard) into cabbage, and using molecular markers to aid the breeding work.

Background and justification:

Black rot is a serious disease of cole crops (cabbage, cauliflower, broccoli) and 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, and as the lesions enlarge the plant wilts and eventually rots. 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. Current sources of host plant resistance while partially effective, are not complete and still result in spread of the disease throughout plantings.

Incorporation of more effective resistance to black rot in cole crops will benefit growers economically, and environmentally, by reducing the need for chemical management.

Some cabbage varieties have limited resistance to black rot, but this is inadequate, particularly when there is high disease pressure. The major races to black rot are race 1 and race 4 which account for approximately 95% of the pathogen worldwide. Complete resistance to race 1 has not been found in *B. oleracea*, but is present in some of the mustard species (*B. carinata*, *B. nigra* and *B. juncea*). A source of resistance identified in Ethiopian mustard (*B. carinata* PI 199947) has been introgressed to broccoli using protoplast fusion and backcrossing. This source gives the plant complete resistance at the juvenile and mature stages, and has been introgressed to broccoli by Lisa Earle using PI 199947. Crosses of this material were made to cabbage, cauliflower, broccoli and brussels sprouts during spring 2000 and further crossing and backcrossing was undertaken in spring 2001. Evaluation of germplasm in the field, greenhouse and laboratory has suggested that the resistance may be controlled by a single dominant gene, but may need to be stabilized further. The resistance is superior to that available in any current germplasm and if successfully incorporated could fulfill a great need by the seed industry (where seed-borne contamination is a problem) and the NY growing community. Greenhouse and field screening of breeding lines will enable introgression to commercial types. Three molecular markers were identified with linkage to this gene in 2000, and more have since been isolated. These will help to accelerate introgression of this resistance into commercial types, and will allow simultaneous screening of germplasm for multiple traits important for the cabbage and cole crop industry.

Objectives:

- [1] To identify, polymorphic RAPD markers between black rot resistant and susceptible plants.
- [2] To screen populations segregating black rot resistance to identify polymorphic markers associated with the resistance.
- [3] To breed black rot resistance into cole crops as an alternative to copper bactericides

Procedures:

[1] Molecular markers can accelerate the incorporation of disease resistance into commercial types, selections showing high levels of resistance to black rot were compared to susceptible types to identify linked additional molecular markers from associations with molecular polymorphisms previously identified. RAPD markers were focused on due to the more efficient incorporation into breeding programs. SCAR markers can be created from these RAPD markers by cloning the bands and generating extended primer sequences from the clones. Markers will be used to identify resistant types at the seedling or mature plant stages in the future, and make the simultaneous incorporation of black rot resistance genes possible

[2] Several populations have been developed that segregate black rot resistance derived from Ethiopian mustard. These were further screened with identified molecular markers, and screened for disease severity to form associations with

molecular studies. A partial linkage map was constructed from associations of these markers with disease resistance.

[3] Standard breeding methodologies were employed to advance germplasm and incorporate Ethiopian mustard derived black rot resistance traits into the vegetable cole crops. For the resistance screening, plants were inoculated at both the seedling and mature plant stages to evaluate for black rot resistance in trials performed in the field and greenhouse. Greenhouse inoculated plants were moved to mist chambers for 48 hours following wound inoculation of black rot using two needles punctured either side of the mid-rib. Field plant resistance was evaluated using a spray inoculation procedure at the 10-week stage. Field plots were grown at Geneva with isolation to prevent any risk of spread to neighboring trials or farms. Plants were evaluated for black rot damage using a scale of 0-5, where 0= completely resistant, and 5= completely susceptible. All selections are being self-fertilized and crosses of the best material will be made to cabbage, cauliflower, broccoli and brussels sprouts with good horticultural type for 2002 evaluation. A total of 273 lines were screened in 2001.

Results and discussion:

[1] The genetic control of the resistance based on PI 199947 was studied in greenhouse experiments, and PCR-based markers linked to an apparent major gene controlling resistance from this source were identified. For a single dominant gene, the expected ratio in the F₂ population of 100 plants would be 75 resistant plants. In the populations studied (based on 11B-1-12 and 11-1-2) there were only 8-18% of plants exhibiting complete resistance in greenhouse trials, and the cluster of RAPD markers in Table 1 associated with these plants. The recovery of resistant plants in F₂ populations was far lower than expected, indicating that the resistance is not being inherited in expected frequencies in these populations (Griffiths and Nickels, 2001).

Protoplast fusion involves the somatic hybridization of protoplasts containing different numbers of chromosomes. 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 before it can be used in commercial varieties. If stabilized, the apparent single dominant gene controlling resistance could be extremely valuable in the future breeding of commercial cabbage types. These results may be a consequence of incompatible chromosome pairing or aneuploidy associated with the initial fusion between *B. oleracea* and *B. carinata*. Further studies on advanced material will be undertaken to evaluate the utility and stability of this resistance source.

[2] A total of 100 F₂ plants were screened with RAPD markers. Linkage analysis showed that all linked markers were clustered, and formed one linkage group around the resistant locus. A partial linkage map was constructed and distances between markers and the locus were calculated. The closest marker (UBC320b) was located 4 cM away from the resistant locus. Primers UBC 72, UBC205, UBC320a, UBC 322, UBC327a

were clustered together since there was no recombination between them. UBC 221e was located 14 cM away on the other side of the resistance locus.

[3] A total of 273 lines were seeded to screen for black rot resistance. All lines were seedling inoculated using the needle wound procedure at the 3-week stage, and susceptible plants were removed. Of the plants remaining 177 lines were transplanted to evaluate resistance under field spray inoculation conditions. Plants were spray inoculated twice at 10 and 12 weeks, and infection was aided with overhead irrigation. All control varieties used (cabbage, cauliflower and broccoli) were completely susceptible, although cabbage varieties including 'Atlantis' that are listed as black rot tolerant, took a longer time to become fully infected. The most resistant lines were based on crosses from Ethiopian mustard derived material, although no line showed complete resistance in all plants. The susceptibility of plants within all lines further highlights some problems with stability of this resistance source. A total of 109 selections were made including 11 F₄ lines and 9 F₃ lines based on PI 199947 resistance. Selections were made from the most promising lines for evaluation of selfed progeny in 2002.

Primer Name	Primer sequence (5'-3')
UBC 66	GAGGGCGTGA
UBC 72	GAGCACGGGA
UBC 121	ATACAGGGAG
UBC 205	CGGTTTGGAA
UBC 221	CCCGTCAATA
UBC 320	CCGGCATAGA
UBC 322	GCCGCTACTA
UBC 327	ATACGGCGTC
UBC 426	TCTCCCGGTG
OPAB04	GGCACGCGTT

Table 1: RAPD markers associated with a major gene controlling black rot resistance in populations derived from PI 199947.

References:

Griffiths, P.D. and Nickels, J. L. (2001). Association of a molecular polymorphism with black rot resistance derived from Ethiopian mustard. *Cruciferae Newsletter* Nr. 23, p57-58.