Final Project Report to the NYS IPM Program, Agricultural IPM 2002-2003

Title: Breeding Cabbage for Resistance to Black Rot (*Xanthomonas campestris*).

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Type of grant: Pest-resistant crops; allelopaths

Project location(s): All of NY

Abstract:

Fresh-market cabbage is the highest value vegetable crop in NY State. Black rot is a serious disease of cabbage especially during warm, damp seasons in the northeast. Breeding for improved resistance to black rot will be important for the future protection of this crop in NY State, through development of new varieties with enhanced resistance to this disease.

Background and justification:

Black rot is a serious disease of cole crops (including cabbage, broccoli and cauliflower) and is easily spread from contaminated seeds 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, especially as it has limited effect on this disease.

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 approxumately 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 in greenhouse trials, and has been been introgressed to broccoli by Lisa Earle. These lines have been used to derive plants which have complete resistance to NY isolates at juvenility and maturity in greenhouse trials, however, F_2 populations derived from this material do not segregate with expected frequencies in the populations tested. These segregating populations have been used to identify molecular markers that are linked to a major dominant gene controlling resistance.

Crosses of this material were been made to cabbage, cauliflower, broccoli and Brussels sprouts in 2000/2001 and the germplasm was advanced and field tested in summer 2001 following a greenhouse inoculation of seedling plugs. 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. Considerable greenhouse and field screening of breeding lines will enable introgression of this resistance to commercial types, and evaluation of the material relative to resistance derived from *B. oleracea*. Molecular markers have also been identified with linkage to this gene, thanks to support from NY IPM in 2000 and 2001. 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 evaluate black rot breeding lines for resistance derived from *B. carinata* and *B. oleracea*.

[2] To screen *B. carinata* accessions to identify additional resistant material for utilization in breeding for black rot resistance.

[3] To self-fertilize all selections from the 2002 trial, and make crosses of the most resistant material to cabbage, broccoli and cauliflower.

Procedures:

[1] Breeding lines will be evaluated with controls in replicated plots during 2002. The lines will represent populations advance to the F_3 generation and beyond, and new crosses made with resistant material. The seed will be planted in 128 cell speedling trays, grown to the 3-week stage and hardened in cold-frames for planting. Four replicated plots with 10 plants per plot will be transplanted to the field with 18" spacing between plants and 3' spacing between rows. Plants will be spray inoculated at 6-weeks with a slurry of four NY isolates of black rot grown for 4-days on YDCP. An overhead irrigation sprinkler plot will be set up to maintain high humidity and moisture content in the field that is conducive to pathogen spread. Plants will be inoculated a second time at the 8 weeks, and the irrigation will be used daily to

encourage pathogen spread. Plants will be evaluated for disease severity at 10 and 12-weeks after planting, relative to susceptible and tolerant resistant control varieties. Field plots will be grown at Geneva with isolation to prevent any risk of spread to neighboring trials or farms. Plants will be evaluated for black rot damage using a scale of 0-5, where 0= completely resistant, and 5= completely susceptible. Comparisons between resistant lines derived from *B. carinata* will be contrasted to those with *B. oleracea* derived resistance. Selections from the most resistant lines will be removed from the field, placed in 10" pots and transferred to vernalization chambers for 3-months to induce flowering.

[2] Sixty-two *B. oleracea* accessions obtained from USDA (Ames, Iowa) were previously tested with greenhouse inoculations, and two lines were identified with complete resistance to black rot, and several others with segregating resistance. These lines will be field evaluated in replicated plots during 2002 to compare the level of field resistance to black rot with accessions previously used for introgression (PI 199947 and PI 199949 and PI 436606). Resistance in the greenhouse can break down under heavy field pressure, and this will allow the identification of more resistant material to introgress resistance to black rot from.

[3] Standard breeding methodologies will be employed to advance germplasm and make crosses to commercial material for further testing. Plants will be removed from vernalization in December, cut back and encouraged to flower at 60°C. The flowers are largely self-incompatible, this will be overcome by self-pollination of unopened flowers with mature pollen from mature flowers of the same plant. Crosses will also be made by using unfertilized flowers. Seed will be collected and cleaned for further field and greenhouse testing in 2003.

Results and discussion:

[1] A total of 71 breeding lines and twelve control varieties/accessions were seeded to screen for black rot resistance in field plots during 2002. Breeding lines were transplanted to the field in replicated plots in June, and were inoculated twice at 4 and 8 weeks using a backpack mist blower. Field plots were irrigated prior to inoculation late afternoon to create high turgor pressure in plants facilitating inoculation. Plants were irrigated daily for seven days after each inoculation using an overhead sprinkler system. Control varieties were used (cabbage, cauliflower and broccoli) representing susceptible and tolerant varietal controls. The most resistant lines were based on crosses from Ethiopian mustard derived material (PI 199947). A total of 82 selections were made (32 cabbage, 35 broccoli and 15 cauliflower) for self-pollination and crossing.

Commercial varieties were also evaluated for black rot resistance to identify the most promising cultivars currently on the market for reducing black rot damage. These varieties included a Wisconsin inbred ('Badger') developed from *B. oleracea* and used by seed companies in the development of current varieties. The varieties 'Matsumo' and 'Silver Dynasty' also evaluated as potential cabbage cultivars with high levels of black rot tolerance.

Variety	Source	Type	Seedling Rating	Field Rating
Badger	Wisconsin inbred	C	2.75	1.26
Scorpion	Seminis	C	3.34	1.67
Silver Dynasty	Seminis	C	3.32	1.78
Matsumo	Bejo	C	4.06	1.87
Platinum Dynasty	Seminis	C	3.58	2.03
Tenacity	Enza Zaden	C	3.39	2.08
Montecristo	Takii	B	4.06	2.53
Blue Dynasty	Seminis	C	3.77	2.76
Conquistador	Enza Zaden	C	3.45	2.78
Chieftain	Seminis	B	4.53	3.16
Royal Dynasty	Seminis	C	4.03	3.42
Hinova	Bejo	C	4.19	3.49
Miracle	Bejo	CA	4.31	3.59
Snow Mystique	Takii	CA	4.56	4.03
Atlantis	Seminis	C	3.88	4.16
Superdane	Reed's	C	4.00	4.19
PIA19182	USDA	EM	1.00	x
Wiroso	Bejo	C	-	2.29
Captain	Seminis	B	-	2.50
Fresco	Bejo	C	-	3.80
Transam	Bejo	C	-	4.84
PI 436606	USDA	C	3.25	-
Graffiti	Daehnfeldt	CA	4.17	-
Marathon	Sakata	B	4.33	-
Titleist	Takii	B	4.43	-

Table 1: Evaluation of commercial cabbage and Brassica vegetable varieties for black rot resistance in field (mature, mist inoculation) and greenhouse (juvenile, wound inoculation) trials (C= Cabbage, B=Broccoli, Ca=Cauliflower, EM=Ethiopian Mustard).

No commercial varieties for completely resistant, although low disease severity was associated with 'Silver Dynasty', 'Scorpion' and 'Matsumo'. While this resistance may be adequate if the disease pressure is low, the genetic control of resistance derived from *B. oleracea* is complicated and prevents efficient utilization in cultivar development.

[2] Previous evaluation of *B. carinata* accessions detected new sources of resistance to black rot, of which, the most promising were only added to the US collection (USDA, Ames, Iowa) in 1990 from the Vavilov Institute in St. Petersburg, Russia (A-19182 and A-19183). The USDA Ethiopian mustard (*B. carinata*) collection contains 62 accessions that are available for screening. All accessions were inoculated at juvenility and maturity for black rot resistance including accessions previously used for protoplast fusion hybrids (PI 199947 and PI 199949). Resistance was identified in several accessions (including PI 199947 and PI 199949) but was not found in all plants tested (other resistant accessions were PI 194251, PI 194252, PI 194904, PI 195921, PI 231046, PI 272637, PI 280230, PI 331378, PI 360884). Complete resistance was observed in all plants of two new accessions (A-19182 and A-19183) when wound-inoculated at the juvenile stage. Evaluation at maturity was not practical due to early leaf abscission in the early

maturity crops. These temporary accessions (A-19182 and A19183) were completely resistant to all NY black rot isolates tested (isolates representing races 1 and 4). The accessions did not cross to several *B. carinata* accessions, however, suggesting that they may be incorrectly classified as Ethiopian mustard. Interspecific hybrids were created during 2002 between A-19182 and *B. oleracea* sub-species (cabbage and broccoli) that are male sterile (one plant was male fertile) - but suitable as female parents. Interspecific hybrids were created with difficulty by rescuing hybrid siliques prior to their premature abscission, followed by embryo rescue from tissue culture. No interspecific hybrids were developed from the crosses with A-19183. The interspecific hybrids represent a new source of black rot resistance hybridized to *B. oleracea* – potentially eliminating many of the problems associated with protoplast fusion.

Approximately 30 interspecific crosses between A-19182 and *B. oleracea* were generated by removing the fertilized silique 5-10 days after the initial crosses were made, culturing the silique for a further 14 days and then performing embryo rescue techniques. These are being backcrossed to *B. oleracea* using an identical approach in order to move **B. carinata* (* possibly a related mustard species) chromosomes into potentially fertile plants with a high similarity to *B. oleracea*. To date two backcross plants have been recovered, and selfed flowers of one of the 30 interspecific crosses are being rescued to develop an F_2 population.

Prior work on black rot resistance has resulted in the development of breeding lines from *B. oleracea* and *B. carinata* derived resistance sources. The new interspecific hybrids created using A-19182 will provide additional germplasm for characterization, and could be the most effective approach to counter-acting the commercial losses from black rot.

[3] All selections of black rot resistant material were removed from the field and transplanted to 10" pots. Cabbage selections were moved to cold storage for 3 months to overcome the biennial life cycle of cabbage through vernalization. Plants were placed in greenhouses, and are being self-pollinated and crossed to advance breeding lines and create new populations segregating black rot resistance that can be used for future varietal production.