Report to the NY IPM Program 2000

Breeding and Characterization of Thrips Resistance in Cabbage

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Abstract: Onion thrips (*Thrips tabaci*) are a major pest of the cabbage industry in New York State and the northeast, largely due to the ineffectiveness of chemical control. Host plant resistance is the most effective defense against thrips damage, but it is difficult to evaluate even with labor-intensive screening. Recent screens for thrips damage have identified varieties with high levels of thrips resistance, this resistance needs to be characterized in order to maximize the benefit of host plant resistance in new varieties and breed this resistance into varieties of different maturities. In 1999 a total of 800 primers were used to amplify molecular regions in Fresco (a thrips resistant variety) and Bartolo (a thrips susceptible variety). When compared to three other Bejo varieties Bronco (a partially resistant half-sib of Bartolo), Brutus (partially resistant) and its susceptible half-sib Gideon, A total of 15 polymorphisms were exclusive to Fresco and were not found in the other four Bejo lines, 15 polymorphisms were observed in all other Bejo lines, but not in Fresco, other polmorphisms were present in one or more resistant varieties, but absent in the susceptible varieties. Thirty-three primers were used to amplify polymorphisms in commercial varieties based on these results. A total of 20 cabbage varieties were chosen that had been previously evaluated for thrips damage, and varied in maturity and type. The two varieties exhibiting the highest levels of resistance to thrips 'Fresco' (Bejo seeds) and 'Bobcat' (Reeds seeds) were vernalized and self-fertilizations and crosses to susceptible types are being made to generate populations for future screening. Self-fertilized 'Fresco' seed has been harvested and some plants screened for thrips damage during the 2000 season, although the results are somewhat erroneous due to variable type and poor weather conditions leading to low damage levels. Polymorphisms were identified that were exclusive to both 'Fresco' and 'Bobcat', polymorphisms were also identified that were present in both and types and at least one other varietal type. These polymorphisms indicate some potential similarity between thrips resistant types although no clear similarities were identified in this study.

Introduction:

Cabbage is the most important vegetable crucifer in NY State with an estimated annual value in excess of $50 million (USDA, National Agricultural Statistics 1998). Onion thrips are a major pest of cabbage causing bronzing of the leaves and damage up to 22 leaves inside the cabbage head. Substantial damage has been caused in northeast and midwest cabbage production since the early 1980s and thrips are currently the most problematic insect pest in NY State. The thrips inside the cabbage head escape contact

with chemicals and are largely unaffected by traditional control strategies. Evaluation of insecticide trials has indicated that only two of the seven trials conducted resulted in reduced thrips damage. Varietal differences in thrips populations occur on commercial cultivars of cabbage, with many cultivars showing limited resistance to thrips. Work by Curtis and Shelton at NYSAES (*personal communication*) funded by the USDA pest management alternatives program has helped identify some of the most resistant and susceptible varieties, and has resulted in an accurate and effective screening method for rating thrips resistance.

Host plant resistance is currently the most important component in the management of thrips on cabbage. The high levels of resistance found in the fresh-market varieties 'Fresco' and 'Bobcat' need to be identified, so that the resistance can be moved into other varieties of varying maturity effectively. One of the major limiting factors in breeding for thrips resistance is the labor-intensive screening process. This could be alleviated, by breeding for host-plant resistance based on marker-assisted selection. Molecular markers linked to thrips resistance could be used to move resistance into new cultivars quickly and effectively, while selecting for other desirable traits or genes. The purpose of this research is to identify polymorphisms associated with thrips resistant varieties, and make self-fertilizations/crosses to susceptible types generating segregating populations for marker analysis. The polymorphisms identified in all studies are PCR markers, as they are most practical for screening large numbers of plants within a breeding program.

Materials and Methods:

A total of twenty cabbage varieties were identified for screening with polymorphisms identified in the variety 'Fresco'. These varieties including their maturity, type and resistance are listed in Table 1. Twenty-six primers generating thirty-three molecular polymorphisms were used to screen these cabbage varieties, all primers were 10-mer oligonucleotides obtained from the University of British Colombia.

VARIETY	COMPANY	D/M.	TYPE	THRIPS RES.
Fresco	Bejo	75	FM/P green	VT
Bobcat	Reed's	76	FM green	VT
Transam	Bejo	105	P	VT
Vitaro	Bejo	105	S red	T
Rona	Petoseed	115	FM/S/P red	T
Primax		60	heirloom	?
Atlantis	Petoseed	68-72	FM green	?
Huron	Petoseed	115	S green	T
Ramada	Bejo	83	FM green	VS
Gideon	Bejo	83	FM green	S
Geronimo	Bejo	78	FM green	S
Hinova	Bejo	100	P	S
Upton	Bejo	105	P	S
Marvellon	Reed's	82	P	VS
Rinda	Petoseed	75	P	VS
Cecile	Bejo	80	FM/P green	S
Genesee	Petoseed	98	P	VS
Bartolo	Bejo	115	S green	VS
Storage Hybrid #4	Reed's	85-90	FM/S green	VS
Bronco	Bejo	78	FM green	VS

Table 1: Varieties evaluated to observe presence/absence of molecular polymorphisms previously identified between a thrips resistant cabbage (Fresco) and thrips susceptible cabbage (Bartolo). FM = Fresh Market, S = Storage, P = Processing, VS = Very Susceptible, S = Susceptible, T = Tolerant, VT = Very Tolerant.

The varieties were analyzed to identify molecular polymorphisms between the thrips resistant and thrips susceptible varieties, and to identify polymorphisms in 'Fresco' that may be common to other thrips resistant cabbage varieties. DNA was extracted from new leaves and stored at –20ºC. Random sequences of DNA were amplified using the primers in a modified PCR buffer (0.5M Tris pH 8.3, 10mM $MgCl_2$, 10mM tartrazine, 14% w/v Ficoll), and sequences were separated by gel electrophoresis in a 1.2% agarose gel. DNA was amplified using *Taq* polymerase and 0.2nM dNTPs for 45 cycles in Strategene thermocyclers. Each cycle consisted of 60 seconds at 94ºC, 60 seconds at 35ºC and 90 seconds at 72ºC. Gel banding patterns were visualized under UV light after staining with ethidium bromide (4ng/ml), and gel sequences were recorded on a BioRad gel documentation system.

Seed from self-fertilized 'Fresco' plants was sown in 128 cell s'Speedling' flats and moved to the field for evaluation of thrips damage. Damage was assessed on a 0-4 rating scale, and by the depth of the damage (the number of leaves inside the head that thrips damage was observed). The varieties 'Bobcat' and 'Fresco' were also planted in the field and greenhouse to obtain self-fertilized seed, and crosses to susceptible cabbage types. These self-pollinations and crosses will be advanced further to generate cabbage lines that differ in thrips resistance for more comprehensive analysis in subsequent seasons.

Results and Discussion:

The variety 'Fresco' was vernalized for crossing and self-pollinations in the winter of 1999/2000. Although self-pollinated seeds were obtained, the poor reproductive capacity of the variety prevented many crosses to susceptible types from being accomplished. The self-fertilized 'Fresco' seeds were field planted in early May 2000 to expose the plants to higher levels of thrips. The early season planting was disrupted by high levels of rainfall during the month of May that weakened the plants in flooded areas, and reduced the level of thrips that may typically be encountered. Thrips assessments were made (Table 2), although these were not sufficient for a comprehensive comparison with marker data, as high levels of damage were not observed. Ratings were also affected due to variations in heading type of the plants, as loose heads were not so amenable to damage ratings. As no significant damage was observed, no molecular data on these plants was undertaken. Several 'Bobcat' and 'Fresco' plants have been vernalized and are currently being self-pollinated and crossed for the development of breeding lines that can be used to study thrips resistance more effectively. It will be necessary to advance these at least 1 – 2 generations to select more uniform heading type lines, if accurate field assessments of resistance are to be made.

Backcross	Leaf layers damaged (0-10)	Damage severity (0-4)
T1	4	0.5
T2	1	0.25
T3	3	0.5
T4	3	0.25
T5	5	0.75
T6	2	0.25
T7	1	0.25
T10	2	0.5
T11	1	0.25
T12	1	0.5
T13	4	0.5
T17	1	0.25
T18	4	0.5
T19	7	1.0
T21	3	0.25
T22	1	0.25
T23	3	0.25
T24	6	0.75
T25	4	1.0
T28	1	0.25
T29	2	0.25
T31	3	0.75
T33	3	0.25
T34	2	0
T35	1	0.25
T36	1	0
T37	2	0.25
T40	1	0.25
T41	4	0.5
T42	1	0.25
T44	3	0.25
T45	1	0.25
T46	2	0.25
T47	5	0.5

Table 2: Assessment of thrips damage in 47 plants derived from selfs of ‘Fresco’

A total of 33 polymorphisms were scored to study their presence/absence in the 20 varieties (Table 3). No clear polymorphisms were identified that were present in the tolerant varieties and absent in all of the susceptible varieties. However, several of the polymorphisms may be useful if further segregation comparisons are undertaken. The primer p267 generated a polymorphism present in all varieties except for 'Bobcat' indicating that 'Bobcat' has a molecular region differing from all of the other varieties, a similar observation was made for primers p22, p586 in 'Fresco'. A marker present only in 'Bobcat' (p586b) was also observed. Six of the polymorphisms (p169, p267b, p351, p649, p203, p257) were observed in both 'Fresco' and 'Bobcat' and at least one other variety. These polymorphisms are all potential candidates for amplification of markers linked to genes controlling thrips resistance.

To effectively study the use of these polymorphisms, it will be necessary to generate plant populations that can be adequately rated for thrips resistance, and improve the uniformity of the genetics within the lines. The current crosses that are being made this winter will be advanced in 2001 to help generate such lines. It is anticipated that germplasm may have to be advanced for at least another 1-2 seasons before the resistance can be adequately studied using these polymorphisms. It will also be necessary to generate a larger number of polymorphisms when better characterized near inbred lines have been evaluated for differences in thrips susceptibility.

As there is a need to advance the germplasm, funds will not be sought to support this project in 2001, even though the project will be ongoing. Advances in generations and identification of additional polymorphisms will be made during 2001. When these have been analyzed, the project will be modified to examine relationships in a more comprehensive manner.

The ability to accurately determine thrips resistance is a major limiting factor to breeding thrips resistant varieties. Ability to identify high levels of resistance to thrips using marker assisted selection could significantly improve the breeding effort, and allow introgression of resistance to thrips with other desirable traits for cabbage grown in the NY market.

Primer No.	Fresco	Bobcat	Transam	Vitaro	Rona	Primax	Atlantis	Huron	Ramada	Gideon	Geronimo	Hinova	Upton	Marvellon	Rinda	Cecile	Genesee	Bartolo	St. Hybrid #4	Bronco
22	2	1	1	1	1	.	1	1	1	1	1	1	1	1	1	1	1	1	1	1
152	1	2	1	2	2	2	2	1	2	2	1	2	1	2	2	1	2	2	2	2
166	2	1	1	1	2	1	1	1	1	2	1	1	2	1	1	1	1	1	1	1
203	1	1	2	2	1	2	2	2	2	2	2	2	2	2	1	2	2	2	2	2
212	1	2	1	2	2	2	2	1	2	1	1	2	1	2	2	1	2	2	1	2
257a	2	2	2	2	2	2	2	2	.	.	2	2	1	2	2	2	2	2	2	2
257b	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2	2	2	1
267a	2	1	1	2	2	2	2	1	1	2	1	1	1	2	2	1	1	1	1	1
267b	1	1	2	2	1	2	2	2	2	2	2	2	2	2	1	2	1	2	2	2
297	1	2	1	2	1	1	1	1	2	1	1	1	1	1	1	1	1	2	1	2
309	1	2	1	1	1	2	2	1	.	2	1	1	1	2	2	1	2	2	2	2
311	2	2	1	2	2	2	2	1	1	1	1	1	1	1	2	2	1	1	2	1
351	1	1	2	2	.	1	2	2	1	2	2	2	2	2	1	1	1	2	2	2
365a	2	1	1	1	1	1	1	2	2	2	2	2	2	1	1	2	1	1	2	2
365b	1	2	2	2	2	1	2	2	2	1	.	.	.	1	2	1	2	2	2	2
410	1	2	2	2	2	2	2	1	2	2	2	2	2	2	1	1	2	2	1	.
426a	2	1	2	2	1	2	1	2	1	2	2	2	2	1	2	1	2	1	2	2
426b	1	1	1	2	2	1	1	1	2	1	2	2	2	2	1	1	2	2	2	2
522a	1	2	1	1	2	2	2	2	2	1	2	2	1	2	2	2	2	2	1	2
522b	2	2	1	1	1	1	2	2	1	2	2	1	2	1	2	2	2	2	2	1
524	2	1	1	1	1	1	.	1	1	2	2	1	2	1	1	1	1	1	2	1
541	1	1	1	1	2	1	1	2	2	1	2	2	1	1	1	2	2	2	2	2
543	2	2	2	2	2	2	2	1	1	2	1	2	2	2	2	1	2	1	2	2
553	1	2	2	2	2	2	2	1	1	2	2	2	1	2	1	2	2	1	1	1
565	2	1	1	2	2	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1
586a	2	2	2	2	2	1	1	2	1	2	2	1	2	2	2	2	2	2	2	1
590	2	2	1	2	2	1	2	1	1	2	1	1	2	2	2	1	1	2	2	2
631a	1	2	2	2	1	2	2	2	2	2	1	1	1	2	1	2	2	2	1	2
631b	2	2	2	2	2	1	2	1	2	2	2	2	2	1	1	1	2	2	2	2
643	2	2	2	2	2	1	1	1	1	1	2	2	1	1	.	2	2	1	2	1
649a	2	1	1	1	1	1	1	2	1	1	1	1	1	1	1	2	.	1	2	2
649b	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2
661	2	1	1	2	2	2	2	1	1	1	1	1	1	1	2	2	1	1	2	1

Table 3: Analysis of 20 varieties of cabbage for presence of PCR polymorphisms indicating molecular differences between the thrips resistant variety ‘Fresco’ and susceptible varieties. 1 = polymorphism present, 2 = polymorphism absent.