

Sclerotinia resistance enhanced by accumulation of QTL and transgenic approaches

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Abstract

This project has two goals involving research on germplasm enhancement and variety development, including biotechnology. The first goal is to increase the level of resistance to *Sclerotinia sclerotiorum* in soybean. Objective 1 is to combine quantitative trait loci (QTL) that were previously mapped and identified with the resistance phenotype into single breeding lines. Three single-cross populations were developed to combine independent QTL into single soybean lines using SSR primers to mark the QTL regions. During 2003 we screened F2 plants and F3 plants from selected F2:3 progeny rows from the crosses to identify individual F3 plants that had the desired genotypes for resistance at important QTL based on microsatellite (SSR) markers. The goal is to obtain F4-derived lines that are homozygous for up to 8 of the identified resistance QTL identified on 7 different linkage groups in soybean. Objective 2 is to determine if a novel antifungal synthetic peptide expressed in soybean will confer resistance to *S. sclerotiorum*. To date, 18 primary transformant lines have been established in the greenhouse. T1 seeds were harvested from the lines and progeny analysis was begun in the greenhouse during October 2003. We will identify plants expressing the inserted gene construct by screening with glyphosate herbicide. During the winter, we will conduct a detached leaf assay and petiole test on the tolerant lines, and wild-type controls including the parental line Thorne to evaluate resistance to *S. sclerotiorum*. The second goal is to improve the use of calcium cyanamide as a control option for *S. sclerotiorum*. Objective 1 is to field-test Ca-cyanamide tolerant transgenic plants. Development of Ca-cyanamide tolerant plants would allow post-planting application for more effective inhibition of apothecial development and ascospore release. A preliminary yield evaluation of the transgenic cah-gene lines was conducted. Average yield of the transgenic lines was lower than the parental line 'Thorne' and the check cultivar NE3001. Objective 2 is to determine the lowest levels of Perka™ (granular Ca-cyanamide) needed to control ascospore release and effectively reduce white mold severity in soybean. A greenhouse experiment is in progress, and a field evaluation during 2004 is planned.

Goals and Objectives

Goal 1: Increase the level of resistance to *Sclerotinia sclerotiorum* in soybean

- Combine QTL that were previously mapped and identified with the resistance phenotype into single breeding lines
- Determine if a novel antifungal synthetic peptide expressed in soybean will confer resistance to *S. sclerotiorum*

Goal 2: Improve the use of calcium cyanamide as a control option for *S. sclerotiorum*

- Field test Ca-cyanamide tolerant transgenic plants
- Determine the lowest levels of Perka™ (granular Ca-cyanamide) needed to control ascospore release and effectively reduce white mold severity in soybean

QTL Progress

- Developed three populations from resistant cultivars and RILs (Table 1)
- Evaluated over 1,000 F2 plants and 2,000 F3 plants from selected F2:3 lines for SSR marker genotypes to identify F3 plants with the desired marker locus genotypes (Table 2)
- 2,000 F4 plants from selected F3 plants are growing in the greenhouse. Genotype analysis with SSR markers will occur to identify F4 plants homozygous for SSR alleles that are linked to up to 8 QTL on 7 different Linkage Groups (Figure 1).
- Homozygous F4:5 lines will be grown in the field during 2004 for phenotypic evaluation of resistance to *S. sclerotiorum*
- F4:5 lines from F4 plants that are still heterozygous for some of the marker loci will be grown to obtain homozygous F5 plants. Subsequent phenotypic evaluation of F5-derived lines will occur.

Table 1. Mean lesion size (cm²) and QTL marker genotypes for soybean recombinant inbred lines and cultivars based on 8 replications of the detached leaf assay during 2000 and 2001.

Line [†]	Lesion Size (cm ²)	Std Err.	Allele associated with smaller lesion size [‡]														
			A	B	A	B	A	A	A	A	C	B	B	B	B		
			Marker														
			Linkage Group														
			O	O	A2	D1a	D1a	D1b	G	G	F	L	O	O	O	O	O
Dassel	7.01	0.64	B	B	B	B	A	B	B	B	A	B	B	B	B	B	B
NKS 1990	7.55	0.67	B	B	B	B	A	B	B	B	B	B	C	B	B	B	B
UX989-78	7.49	0.64	B	B	A	A	A	A	A	A	A	B	B	B	B	B	A
UX991-06	7.38	0.64	A	B	B	B	B	A	A	A	A	A	A	A	A	A	A
UX991-31	7.74	0.62	B	B	B	B	B	A	A	A	A	C	B	A	A	A	A
UX991-35	6.63	0.62	B	B	A	B	A	B	A	A	C	A	A	A	A	A	A
Vinton 81	7.61	0.72	A	B	A	B	A	B	B	A	B	D	B	A	A	A	A
Williams 82	9.17	0.62	C	A	A	A	B	A	A	A	B	A	A	A	A	A	A

[†]Allele designations were modified from Arachana et al. (2001) from A=Williams 82 allele and B="not Williams 82" to designations based on size of the amplicon: A=largest, B=next smaller, C=next smaller, etc. for each primer. Shading and bold indicate marker allele associated with smaller lesion size based on Arachana et al. (2001).

[‡]UX lines are F4-derived recombinant inbred lines; UX989=Dassel x Williams 82, UX991=NKS 1990 x Williams 82.

Table 2. Soybean crosses made and resulting genotypes[†] at QTL marker loci.

Cross [‡]	Allele associated with smaller lesion size													
	A	B	A	B	A	A	A	A	C	B	B	B	B	B
	Marker													
	Linkage Group													
	O	O	A2	D1a	D1a	D1b	G	G	F	L	O	O	O	O
Dassel x UX991-35	B	B	A	B	A	B	A	A	C	B	B	B	B	B
UX989-78 x UX991-31	B	B	A	B	A	A	A	A	C	B	B	B	B	A
Vinton 81 x UX991-06	A	B	B	A	A	A	A	A	BD	B	A	A	A	A

[†]The bold underline indicates alleles that are heterozygous in the cross. Shading indicates loci with no favorable allele.

[‡]UX991=NKS 1990 x Williams 82, UX989=Dassel x Williams 82.

Antifungal peptide

- Synthetic antifungal peptide D4E1
- 19 amino acids long
- A lytic peptide shown to reduce infection by a number of pathogens, including fungi (Demegen, Inc., pers. comm. to T. Clemente)
- Goal - express the peptide in soybean and characterize the derived transformants for resistance against *S. sclerotiorum*
- 18 primary transformants were grown in the greenhouse. T1 seeds were harvested.
- T1 plants are being grown. We will identify plants expressing the inserted gene construct will by screening with glyphosate herbicide.
- Phenotypic evaluation of Sclerotinia resistance will occur on identified plants.

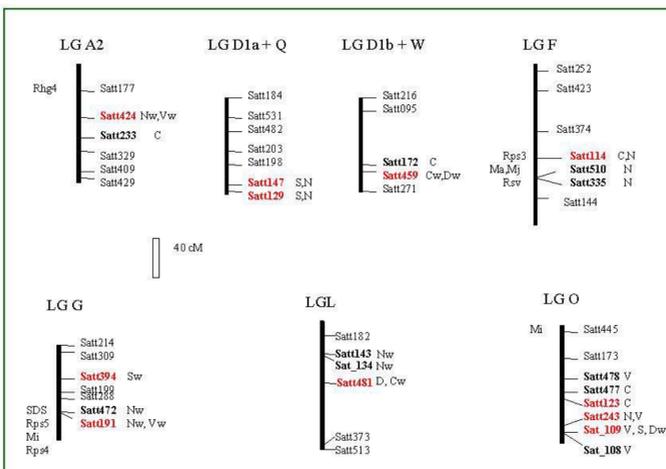


Figure 1. Linkage Group map for SSR alleles at QTL (shown in red).

Table 3. Soybean strain means for Perka Yield Test 2003 at Mead, NE.

Strain	Plot Wt g/plot	Maturity days ^{††}	Plant Height in	Lodging score	Seed Weight g/100 seed
341-1-1	297.0	51.0	30.5	1.0	17.4
341-1-2	346.6	58.0	36.0	2.0	14.8
341-1-3	477.9	51.0	32.0	2.0	17.0
341-1-4	439.6	58.0	31.5	1.0	16.8
341-1-5	358.5	58.0	36.5	1.0	17.7
341-8-1	339.0	58.0	36.0	2.0	17.4
341-8-2	376.8	58.0	38.0	2.0	16.9
341-8-3	303.2	58.0	32.0	1.0	16.9
341-8-4	390.9	58.0	32.5	2.0	17.2
341-8-6	385.1	58.0	34.0	2.0	16.7
NE3001	598.7	58.0	32.5	1.0	17.9
Thorne	523.9	62.0	37.0	1.0	15.8

LSD (0.05) 113.5 0 1.8 0 2.1
^{††}Plot weight and seed weight are on a 13% moisture basis.
^{†††}Number of days after 31 August.

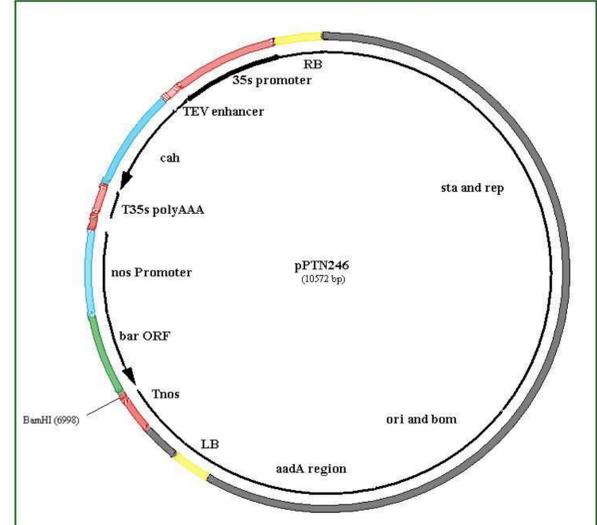
Table 4. Soybean family means for Perka Yield Test 2003 at Mead, NE.

Family	Plot Wt g/plot	Maturity days ^{††}	Plant Height in	Lodging score	Seed Weight g/100 seed
341-1	376.8	55.4	33.1	1.3	16.9
341-8	356.1	58.0	34.6	1.8	17.1
NE3001	598.7	58.0	32.5	1.0	17.9
Thorne	523.9	62.0	37.0	1.0	15.8

LSD (0.05) 79.8 0.0 1.3 0.0 1.5
^{††}Plot weight and seed weight are on a 13% moisture basis.
^{†††}Number of days after 31 August.

Cah gene Progress

- 2 events, 5 T2-derived lines per event
- An ascospore inhibition experiment with Perka™ is in progress in the greenhouse.
- A preliminary yield test of Perka resistant lines (*cah* gene) was conducted during 2003 (Table 3, Table 4). Data are from 2 replicates at one location



Perka® Tolerance Screen



Control soybeans with Perka® application rates of: 1.5 g, 3.0 g, 4.5 g, 6.0 g and 7.5 g (left to right). Photo taken two weeks post application

Perka® Tolerance of Event 341-1



T₁ individuals derived from event 341-1 two weeks post application with 7.5 g of Perka®. Plant on far right glyphosate sensitive T₁ derived from 341-1. Plant at left and in the center glyphosate tolerant T₁ individuals

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