Funded Plan of Work:

Development of soybean varieties or germplasm resistant to Sclerotinia stem rot

Dechun Wang

MICHIGAN STATE UNIVERSITY

MSU research related to development of resistant soybean varieties or germplasm

- Incorporate partial resistance from old or new sources into elite germplasm
- Identify DNA markers linked with partial resistance in the new resistance sources
- Develop new greenhouse methods for evaluation of partial resistance to the disease

MICHIGAN STATE UNIVERSITY

Incorporate new or old sources of resistance into elite germplasm

- NKS 19-90
- Asgrow A2506
- Colfax
- Corsoy 79
- Vinton 81

- Plant introductions (PIs)
  - PI 391589B
  - FC 030233
  - PI 089001
  - PI 153259
  - PI 437764
  - PI 548404
  - PI 548312

General progeny evaluation procedures

- Multiple stage evaluations in Michigan
  - 1 location 2 replications, single row plots
  - 2 locations 2 reps at each loc, 6-row plots
  - 4 locations 2 reps at each loc, 6-row plots
- Regional trials
  - Collaborative research
    - North Central states
    - Soybean Uniform Tests, Northern states
- Greenhouse evaluations
  - with the cut-petiole method in the past
  - with the spray-mycelium and the drop-mycelium methods now

New resistant variety release

- Skylla
  - High yielding
    - The highest and 2nd highest yielding line in 2001 and 2003 Soybean Uniform Test II, respectivley.
    - It has been one of the highest yield varieties in Michigan South Conventional Soybean Performance Trials since it was entered in the trials in 2001.
  - With partial resistance to Sclerotinia stem rot
    - It is a progeny of S19-90, a widely used resistant check

Disease severity index values of lines for the four most infected disease nurseries in 2004 NC Uniform White Mold Trials

Line	Mean	Wiscons	Iowa	Ohio	ND
NKS 19-90	44.1	31.8	49.2	38.9	61.3
Skylla	48.5	57.0	53.0	38.0	44.0
Dwight	69.0	79.0	71.0	49.4	77.0

MICHIGAN STATE UNIVERSITY

New resistant germplasm release

- A x N -1-55 (Jointly released with UIUC)
  - A progeny from a cross between two partially resistant parents S19-90 and A2506
  - With better resistance than either parent

Disease severity index values of lines for the four most infected disease nurseries in 2004 NC Uniform White Mold Trials

Line	Mean	Wiscons	Iowa	Ohio	ND
A X N-1-55	37.0	32.0	25.6	36.3	54.0
A2506	55.6	61.0	54.5	43.5	63.2
NKS 19-90	44.1	31.8	49.2	38.9	61.3
Skylla	48.5	57.0	53.0	38.0	44.0
Dwight	69.0	79.0	71.0	49.4	77.0

MICHIGAN STATE UNIVERSITY

Genetic mapping of genes underlying partial resistance in PI 391589B

MICHIGAN STATE UNIVERSITY

Materials and Methods

- Mapping population
  - 106 F_2 derived lines from a cross PI 391589B x IA2053

- Resistance evaluation
  - Field evaluations with inoculations
    - In 2003, cut-petiole inoculation, 3 reps
    - In 2004, drop-mycelium inoculation, 6 reps
  - Greenhouse evaluations
    - Drop-mycelium inoculation

Genotyping

- The parents were first tested with 1132 SSR markers to identify polymorphic markers
- Then the populations were tested with the polymorphic markers (109 total)

Statistical Analysis

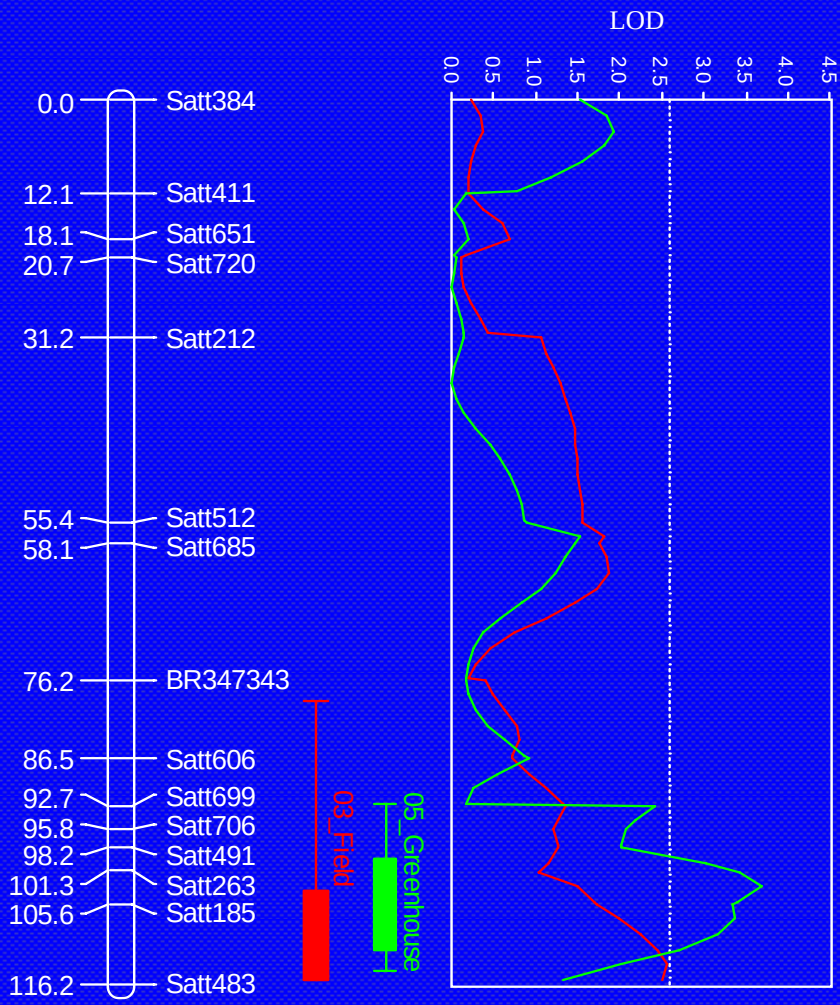
- JoinMap was used to construct genetic linkage maps
- The computer program QTL-Cartographer was used to locate the quantitative trait loci (QTL) underlying white mold resistance
  - Composite interval mapping method used

SSR markers associated with partial resistance to Sclerotinia sem rot

Marker	Linkage Group	Position	P-value			Resistance Source
			2003 field evaluation	2004 field evaluation	2005 greenhouse evaluation	
Satt411	E	12.92	0.014 *	0.011 *	0.277	PI 391589B
Satt720	E	20.8	0.095	0.019 *	0.314	PI 391589B
Satt651	E	32.1	0.005 **	0.000 ****	0.931	PI 391589B
Satt212	E	32.27	0.019 *	0.006 **	0.769	PI 391589B
Satt606	E	39.77	0.276	0.594	0.022 *	IA 2053
Satt699	E	41.24	0.239	0.432	0.001 ***	IA 2053
Satt706	E	43.36	0.243	0.381	0.002 **	IA 2053
Satt491	E	43.64	0.305	0.693	0.003 **	IA 2053
Satt185	E	44.76	0.066	0.262	0.000 ***	IA 2053
Satt483	E	44.98	0.894	0.185	0.011 *	IA 2053
Satt263	E	45.4	0.333	0.838	0.001 ***	IA 2053
AW186493	F	?	0.034 *	0.015 *	0.147	PI 391589B
Satt149	F	18.13	0.025 *	0.035 *	0.859	PI 391589B
Satt419	I	21.9	0.037 *	0.035 *	0.477	PI 391589B

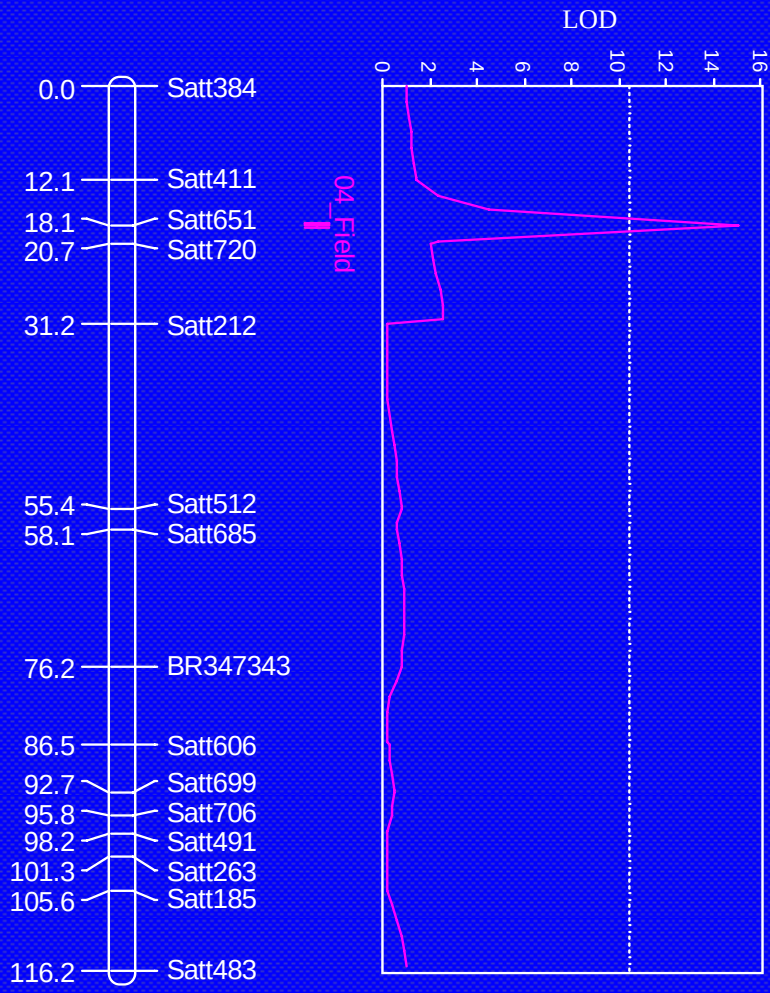
MICHIGAN STATE UNIVERSITY

LG E



MICHIGAN STATE UNIVERSITY

LG E



MICHIGAN STATE UNIVERSITY

Development of new inoculation methods

MICHIGAN STATE
UNIVERSITY

The need for a reliable evaluation method

- Both breeding and QTL mapping studies need a reliable method to evaluate resistance to white mold
- Field evaluation through natural infection often yields no results (no or not enough disease pressure)
  - Too dependent on natural environments
- Several greenhouse-laboratory evaluation methods have been used by different research groups
  - Results were often inconsistent among different tests.
  - Not suitable for large-scale evaluations

Objectives

- Develop new methods
  - High throughput, large-scale evaluation
  - Low cost
  - Consistent results

Inoculum preparation

- Culture the fungus in potato dextrose broth (PDB, 24g PDB/L) at room temperature (22 ºC) for 5 days with shaking to produce liquid inoculum
- Before inoculation the mycelia were homogenized in the culture media by blending for 10 seconds with Hamilton Beach® blender

Spray-mycelium method

Spray homogenized mycelium suspension

Even coverage with the inoculum

Incubate in a humid chamber

Determine plant mortality 2 weeks after inoculation

Drop-mycelium method

- Place 0.5 - 1 ml of homogenized mycelium suspension onto the apical meristem of soybean plants
- Incubate the inoculated plants in a humid chamber
- Determine plant mortality 2 weeks after inoculation

Both methods were published in 2005 in Plant Disease 89:1268-1272.

Advantage of the two new methods

- No wounding to the plants during the inoculation
  - Resistance due to preformed structural barriers can be evaluated with these two methods
- Easy inoculum preparation
- Quick and easy inoculation
  - Can be used for large-scale inoculation
- Low material cost
  - Drop-mycelium cost <10% of cut-petiole or cut-stem
  - Spray-mycelium cost ~30% of cut-petiole or cut-stem
- Easy data collection
  - Just count dead plants and determine plant mortality two weeks after inoculation

Compare the results obtained with the two methods with the results obtained with the cut-petiole method

- Use 18 genotypes with different level of resistance to *S. Sclerotiorum*
- 3 replications with 24 plants per method in each replication
- Randomized complete block design

MICHIGAN STATE UNIVERSITY

Plant mortality (%) with numbers in parentheses indicate the ranking with 1 being the most resistant

Genotypes	Cut-petiole	Spray-mycelium	Drop-mycelium
A2506	65 (7)	21 (1)	31 (2)
Chapman	52 (2)	36 (2)	36 (3)
Kenwood 94	60 (5)	36 (2)	67 (7)
NKS 19-90	44 (1)	39 (4)	39 (4)
Corsoy 79	58 (4)	40 (5)	75 (11)
BSR 101	63 (6)	46 (6)	28 (1)
Hardin 91	69 (8)	53 (7)	78 (12)
Vinton 81	77 (11)	54 (8)	83 (14)
Colfax	55 (3)	56 (9)	61 (5)
Conrad 94	81 (13)	58 (10)	72 (9)
Dunbar	73 (9)	61 (11)	64 (6)
Elgin 87	79 (12)	61 (11)	72 (9)
Jack	82 (14)	62 (13)	69 (8)
Olympus	83 (16)	62 (13)	83 (14)
Resnik	87 (17)	63 (15)	89 (17)
Ciba 3253	93 (18)	65 (16)	81 (13)
Felix	74 (10)	67 (17)	83 (14)
Fairbault	82 (14)	68 (18)	95 (18)
$LSD_{0.05}$	28	21	34

The rank correlation coefficients among the data obtained with the three inoculation methods

	Cut-petiole	Spray-mycelium	Drop-mycelium
Cut-petiole	1.00		
Spray-mycelium	0.81**	1.00	
Drop-mycelium	0.71**	0.77**	1.00

** Significant at the 0.01 probability level.

Compare the results obtained with the drop-mycelium method with the results obtained in field evaluations

- Use 35 genotypes with different level of resistance to *S. Sclerotiorum*
- 3 replications with 10 plants in each replication
- Field evaluations were conducted in Wisconsin, Ohio, and Iowa
- Randomized complete block design

MICHIGAN STATE UNIVERSITY

Plant mortality obtained with the drop-mycelium method and the DSIs obtained in field evaluations in Iowa, Ohio, and Wisconsin.

Genotype	Drop-mycelium	Iowa	Ohio	Wisconsin
	Mortality (%)	DSI	DSI	DSI
A X N-1-55	0	25.6	36.3	32
A X N-1-68	0	36.5	41.5	25
U409006	10	55.6	49.6	64
E99279	16.7	54.9	43	43
S19-90	25	49.2	38.9	27
U419020	41.7	38.3	37	55
E99250	44.4	49.9	34.5	52
U409014	44.4	66.3	38.2	51
U412014	50	34.8	54.1	36
LP02-222	54.5	72.3	50	76
OhioFG3	54.5	54.2	39.6	20
Skylla	58.3	53.3	38	57
A X N-2-55	60	43.7	41.1	43
LP02-221	66.7	62.2	47.8	77
LP02-250	75	66.4	45.9	84
Dwight	77.8	70.7	49.4	79
A2506	100	54.5	43.5	61
LP02-240	100	58.4	40	78
LP02-253	100	68.4	47.2	89

Correlation coefficients among the data obtained from greenhouse drop-mycelium method and field evaluations in Iowa, Ohio, and Wisconsin.

	Iowa	Ohio	Wisconsin	Drop-mycelium
Iowa	1.00			
Ohio	0.39*	1.00		
Wisconsin	0.76**	0.47*	1.00	
Drop-mycelium	0.45*	0.36*	0.68**	1.00

* : Significant at the 0.05 probability level.
**: Significant at the 0.01 probability level.

MICHIGAN STATE UNIVERSITY

Acknowledgement

- Yiwu Chen
- Xiaomei Guo
- John Boyse
- Yang Wang
- Brian Diers
- Craig Grau
- Anne Dorrance
- Jean Liu
- Berlin Nelson
- Ray Hammerschmidt

- Sclerotinia Initiative
- North Central Soybean Research Program
- Michigan Soybean Promotion Committee

MICHIGAN STATE UNIVERSITY