National Sclerotinia Initiative

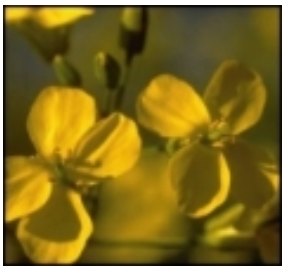
USDA-ARS

National Sclerotinia Initiative

2013 Annual Meeting

Bloomington, MN

January 23-25, 2013






National Sclerotinia Initiative
2013 Annual Meeting

January 23-25, 2013

Crowne Plaza Hotel & Suites
Three Appletree Square, Bloomington, MN

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AGENDA

National Sclerotinia Initiative – 2013 Annual Meeting

January 23-25, 2013

Wednesday – January 23, 2013

6-8 pm	Poster Session/Reception *(posters are displayed for the entire meeting)*	**McIntosh/Jonathan**

Thursday – January 24, 2013

7:15 am	Registration/Continental Breakfast	**McIntosh/Jonathan**
8:15 am	Welcome, Introductions & Meeting Charge – **Bill Kemp, USDA-ARS, Fargo, ND**	
8:25 am	Welcome and Update from the Northern Plains Area – **Mickey McGuire, USDA-ARS, Fort Collins, CO**	
8:35 am	ARS Office of National Programs Update – **Roy Scott, USDA-ARS,Beltsville, MD**	
8:45 am	***Featured Speaker*** Dissecting the quantitative resistance to *Sclerotinia sclerotiorum* in Asian *Brassica napus* germplasm – **Lone Buchwaldt, Agriculture and Agri-Food, Canada, Saskatoon, Saskatchewan**	
10:00 am	Discussion Break	**Ballroom Foyer**

Sclerotinia Research Activities – Session 1 **Fireside**
Moderator – Michael Wunsch, North Dakota State University, Carrington, ND

10:30 am	(1) Development and evaluation of canola breeding populations for resistance to *Sclerotinia sclerotiorum*; (2) Managing *Sclerotinia* stem rot of canola with fungicides (Abstract p. 13) – **Luis del Rio, North Dakota State University, Fargo, ND**
10:45 am	Identification of resistance and pathogenicity genes associated with *Sclerotinia sclerotiorum* infection in canola (Abstract p. 24) – **Rubella Goswami, DuPont Crop Protection, Newark, DE; Luis del Rio, North Dakota State University, Fargo, ND**
11:00 am	Genetic variation and virulence of *Sclerotinia sclerotiorum* in the United States (Abstracts p. 18, 20) – **Berlin Nelson, North Dakota State University, Fargo, ND**
11:15 am	Characterization of growth, pathogenicity, and apothecial development of *Sclerotinia sclerotiorum* isolates from different geographic regions in contrasting temperature regimes (Abstract p. 12) – **Glen Hartman, USDA-ARS, Urbana, IL**

11:30 am Improved white mold resistance in dry and snap beans through multi-site screening and pathogen characterization throughout major production areas (Abstract p. 30) – **James Steadman, University of Nebraska, Lincoln, NE**

11:45 am Working Lunch **McIntosh/Jonathan**

Sclerotinia Research Activities – Session 2 **Fireside**
Moderator – Weidong Chen, USDA-ARS, Pullman, WA

12:45 pm Discovery of novel sources of resistance to head rot and stalk rot in cultivated sunflower and wild *Helianthus* (Abstract p. 31)**– Thomas Gulya, USDA-ARS, Fargo, ND**

1:00 pm Facilitating management of *Sclerotinia* head rot of sunflowers through screening hybrids for resistance and evaluating fungicides for efficacy (Abstract p. 29) – **Michael Wunsch, North Dakota State University, Carrington, ND**

1:15pm Evaluation of wild *Helianthus* species for resistance to *Sclerotinia* stalk rot (Abstract p. 16) – **Charles Block, USDA-ARS, Ames, IA**

1:30 pm Advances towards a Marker Assisted Selection (MAS) breeding program in sunflower for *Sclerotinia* disease resistance (Abstract p. 9) – **Zahirul Talukder, North Dakota State University, Fargo, ND; Brent Hulke, USDA-ARS, Fargo, ND**

1:45 pm Deployment of novel sources of *Sclerotinia* resistance in sunflower – 2012 progress (Abstract p. 15) – **Lili Qi& Brent Hulke, USDA-ARS, Fargo, ND**

2:00 pm Use of a transformation system in sunflower for *Sclerotinia* resistance studies (Abstract p. 33) – **John Finer, The Ohio State University, Wooster, OH**

2:15 pm Transferring *Sclerotinia* resistance genes from wild *Helianthus* species into cultivated sunflower (Abstract p. 32) – **Chao-Chien Jan, USDA-ARS, Fargo, ND**

2:30 pm Break & Poster Session **McIntosh/Jonathan**

Sclerotinia Research Activities – Session 3 **Fireside**
Moderator – Brent Hulke, USDA-ARS, Fargo, ND

3:00pm White mold resistance-QTL: Identification, interactions, and fine mapping in common bean (Abstracts p. 11, 28, 34) – **Phil Miklas, USDA-ARS, Prosser, WA; James Myers, Oregon State University, Corvallis, OR; Phil McClean, North Dakota State University, Fargo, ND**

3:30 pm Pyramiding QTL for white mold resistance in Mesoamerican beans (Abstract p. 10) – **James Kelly, Michigan State University, East Lansing, MI**

3:45 pm Inheritance studies of new potential sources of resistance to white mold in dry bean (Abstract p. 25) – **Juan Osorno, North Dakota State University, Fargo, ND**

4:00 pm	Characterization of the genetic basis for partial resistance to *Sclerotinia sclerotiorum* in pea (Abstract p. 14) – **Kevin McPhee, North Dakota State University, Fargo, ND; Lyndon Porter USDA-ARS, Prosser, WA**	
4:15 pm	Expression profiling of the pea-*Sclerotinia sclerotiorum* interaction for genomics-assisted breeding (Abstract p. 17) – **Martin Chilvers, Michigan State University, East Lansing, MI)**	
4:30 pm	Wrap-up & Adjourn (Dinner on your own)	

Friday – January 25, 2013

7:00 am	Steering Committee Breakfast Meeting	**Beacon**
7:15 am	Continental Breakfast	**McIntosh/Jonathan**
	Sclerotinia Research Activities – Session 4 **Moderator – Rubella Goswami, DuPont Crop Protection, Newark, DE**	**Fireside**
8:30 am	Functional analyses of copper-zinc superoxide dismutase gene, a virulence factor, of *Sclerotinia sclerotiorum* (Abstracts p. 26, 27) – **Weidong Chen, USDA-ARS, Pullman, WA**	
8:45 am	Identification of novel loci for resistance to *Sclerotinia* stem rot in perennial soybean accessions (Abstract p. 23)**– Leslie Domier, USDA-ARS, Urbana, IL**	
9:00 am	Enhancing soybean for resistance to *Sclerotinia* stem rot (Abstract p. 19)**– Dechun Wang, Michigan State University, East Lansing, MI**	
9:15 am	Functional verification of candidate defense-related genes to *Sclerotiniasclerotiorum* in soybean and *Arabidopsis* (Abstract p. 21) – **Steven Clough, USDA-ARS, Urbana, IL**	
9:30 am	Break	**Ballroom Foyer**
	Sclerotinia Initiative Research: The next steps **Moderator – Bill Kemp, USDA-ARS, Fargo, ND**	**Fireside**
10:00 am	***Guest Speaker*** Strategic Planning & Reporting Progress – **Rich Wilson, USDA-ARS, Office of National Programs–Retired, Raleigh, NC**	
10:30 am	Strategic Plan Discussion – Writing Team Input/Revisions	
11:15 am	Agreements Update – Marcie Currie-Gross, USDA-ARS, Fort Collins, CO	
11:45 am	Working Lunch	**McIntosh/Jonathan**
1:00 pm	Assignment of Additional Tasks & Wrap-up of Initiative Business	
2:00 pm	Adjourn (Travel Safely!)	

National Sclerotinia Initiative Poster Session

Epidemiology & Disease Management		
Poster No.	Title	Author(s)
1	Characterization of metconazole sensitivity of Sclerotinia sclerotiorum isolates from North Central US	G. Ameen, L.E. del Rio

Genomics		
Poster No.	Title	Author(s)
2	Expression profiling of the pea-*Sclerotinia sclerotiorum* interaction for genomics-assisted breeding	X. Zhuang, K. McPhee, T. Coram, M. Chilvers
3	Fungal gene expression patterns during infection of Brassica napus lines by Sclerotinia sclerotiorum	K. Chittem, W. Yakima, R. Goswami, L. del Rio
4	Genome-wide association study identifies multiple loci associated with soybean resistance to *Sclerotinia sclerotiorum*	Z. Wen, R. Tan, M. Chilvers, D. Wang
5	High density genotyping of Sclerotinia sclerotiorum	R. Brueggeman, C. Qiu, B.D. Nelson
6	Identification and functional analysis of soybean candidate defense-related genes against *Sclerotinia sclerotiorum*	D.H. Simmonds, L. Blahut-Beatty, L. Buchwaldt, Y. Zhang, B. Calla, D.J. Neece, S.J. Clough
7	Identification of novel loci for partial resistance Sclerotinia stem rot in perennial soybean accessions	S. Chang, L.L. Domier, G.L. Hartman
8	Identification of resistance and pathogenicity genes associated with *Sclerotinia sclerotiorum* infection on canola	R.S. Goswami, K. Chittem, W. Yajima, L. del Rio Mendoza

Pathogen Biology &Development		
Poster No.	Title	Author(s)
9	Characterization of growth, pathogenicity, and apothecial development of *Sclerotinia sclerotiorum* isolates from different geographic regions in contrasting temperature regimes	G.L. Hartman, L.J. Koga, C.B. Hill, C.V. Godoy
10	Genetic variation and aggressiveness of *Sclerotinia sclerotiorum* in the United States	C. Qiu, B.D. Nelson
11	Improved white mold resistance in dry and snap beans through multi-site screening and pathogen characterization throughout major production areas	J.R. Steadman
12	Oxalate-deficient mutants of *Sclerotinia sclerotiorum* accumulate fumaric acid and remain pathogenic	L. Xu, M. Xiang, D. White, W. Chen
13	Susceptibility of sunflowers to Sclerotinia head rot after bloom and implications for screening sunflowers for resistance	M.J. Wunsch, S. Halley, A. Arens, L. Besemann, K. Rashid, J. Bergman, M. Schaefer, B. Schatz

National Sclerotinia Initiative Poster Session

Variety Development/Germplasm Enhancement		
Poster No.	Title	Author(s)
14	Advances towards a Marker Assisted Selection (MAS) breeding program in sunflower for Sclerotinia disease resistance	Z.I. Talukder, B.S. Hulke, L. Qi, T.J. Gulya
15	Analysis of defense genes response in different common bean P. vulgaris genotypes	W. Mkwaila, F. Carneiro, J.D. Kelly
16	Can the negative association between yield and white mold resistance for NY6020 bean resistance be broken?	J. Myers, C. Will, J. Davis
17	Characterization of the genetic basis for partial resistance to Sclerotinia sclerotiorum in pea	K. McPhee, B. Tashtemirov, L. Porter
18	Deployment of novel sources of Sclerotinia resistance in sunflower	Y. Long, T.J. Gulya, B.S. Hulke, K. McPhee, L. Qi
19	Evaluation of wild sunflower species for Sclerotinia stalk rot resistance	C.C. Block, L.F. Marek, T.J. Gulya
20	Head rot and stalk rot resistance sources confirmed in USDA sunflower germplasm and status of ascospore production experiments	T. Gulya, B. Hulke, Z. Talukder, B. Harveson, M. Wunsch, M. Boosalis, R. Schafer
21	Identification and validation of QTL for white mold in two pinto bean RIL populations	W. Mkwaila, E. Wright, J.D. Kelly
22	Intrachromosomal recombination suggests outcrossing in Sclerotinia sclerotiorum populations	R.N. Attanayake, W. Chen
23	Preliminary evaluation of the Bean CAP snap bean panel for white mold resistance	J. Myers, J. Davis, P. Miklas, P. McClean
24	Update on transferring Sclerotinia resistance genes from wild perennial Helianthus species into cultivated sunflower	Z. Liu, X. Cai, G.J. Seiler, T.A. Gulya, K.Y. Rashid, C.C. Jan
25	Use of a transformation system in sunflower for Sclerotinia resistance studies	Z. Zhang, J. Finer

Advances towards a Marker Assisted Selection (MAS) breeding program in sunflower for Sclerotinia disease resistance

Zahirul I. Talukder[1], Brent S. Hulke[2], Lili Qi[2] and Thomas J. Gulya[2]
[1]North Dakota State University, Fargo, ND 58102
[2]USDA-ARS, Northern Crop Science Laboratory, Fargo, ND

Funded Plan of Work: Pyramiding Sclerotinia head rot and stalk rot resistances into elite sunflower breeding lines with the aid of DNA markers

ABSTRACT:
Sclerotinia sclerotiorum (Lib.) de Bary is the most destructive pathogen in sunflower (*Helianthus annuus* L.), causing two important diseases, the stalk rot and the head rot. Both the mode of infection and the genetics of resistance are completely different for the two diseases, effectively doubling the effort required to control the diseases. Resistance to *S. sclerotiorum* is under polygenic controland no major resistance gene is known against this pathogen in cultivated sunflower. We have identified molecular markers associated with Sclerotinia disease resistance in order to establish an efficient marker-assisted selection (MAS) breeding program in sunflower. Both the candidate gene association mapping (CGAM) and the genome-wide association mapping (GWAM) approaches have been employed to accomplish the goal of the project. The stalk-rot AM population comprised of 260 sunflower lines of which 11 were USDA-ARS released inbred lines and 249 were plant introductions (PIs) collected from all over the world. Among the different AM models tested, a general linear model (GLM) with modification to account for population structure was found to be the best fitting model for this population and used for association analysis. CGAM revealed that the sequence polymorphisms in both paralogs of *HaCOI1* were strongly associated with Sclerotinia stalk-rot resistance and explained 7.4% of phenotypic variation in the association mapping (AM) population. The head-rot AM population is comprised of 230 sunflower lines of which 61 were USDA-ARS released inbred lines and 196 were PIs. A mixed linear model (MLM) with modification to account for kinship was found to be the best fitting model for this population and used for association analysis. Preliminary analysis showed that the genes *HaDET3* and one paralog of *HaEIN2* are strongly associated with head rot resistance and explained a total of 13% of the phenotypic variation in the population. GWAM was conducted with the genotypic data generated using ~8700 single nucleotide polymorphism (SNP) markers developed by National Sunflower Association (NSA). We are currently fitting models to these data and hope to provide preliminary results at the meeting. The SNP markers associated with Sclerotinia disease resistance can together form a selection index model, which will simplify selection based on markers, thus improving the efficiency of MAS during cultivar development. The fact that a wide diversity of lines was surveyed in this population suggests that the SNP loci should be robust across breeding programs, which we will test starting in 2013.

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Analysis of defense genes in response to white mold infection in different *P. vulgaris* genotypes

Wezi Mkwaila, Flavia Carneiro & James D. Kelly, Michigan State University, East Lansing, MI

Funded Plan of Work: Pyramiding QTL for white mold resistance into Mesoamerican beans

ABSTRACT:
Several QTL studies have been conducted with different marker systems to identify white mold resistance in common bean populations derived from different genetic backgrounds (Soule et al., 2011). The focus of QTL analysis however is shifting from the discovery of QTL associated with quantitative disease resistance to determining the biological function underlying the QTL. The interpretation of QTL results is usually limited due to lack of information on the genetics, biochemistry and physiology underlying trait expression. Understanding the function of quantitative resistance genes and their mode of expression is necessary to enable breeders to select which QTL to deploy in marker assisted selection. We investigated the role of PGIP, Glucanase, and phenylanine ammonia lyase (PAL) genes in the defense response of different genotypes (AN-37, P02630, Beryl and G122) following infection with *Sclerotinia sclerotiorum.* In general there was no glucanase activity detected in the AN-37 and P02630 genotypes at 0hpi while low levels of transcription took place in the other two genotypes. Activity peaked at both 24 and 48 hpi in all the genotypes except in Beryl which had loss of expression at 48hrs. At 7 days all the genotypes had some level of glucanase activity with P02630 having the strongest signal relative to rest of the other genotypes. Overall PAL had low expression levels in response to *Sclerotinia sclerotiorum.* There was no transcription in response to wounding in all the genotypes, however, Beryl and G122 peaked at 24 hrs while the most abundant transcripts were at 48 hrs in the two pinto lines. All the genotypes retained some enzyme activity at 7 days. There was also variable activity of PGIP1 among the genotypes. In contrast to the other genes PGIP was induced with different signals in response to wounding at 0 hpi. The resistant line G122 showed the strongest signal at 0 and 48 hpi while the line AN-37 expressed the gene only at 48 hpi. At 7 dpi there was no signal except in P02630. These results suggest that the resistance reported in different bean genotypes to white mold is due to different defense pathways. The induction of defense genes at wounding may have a confounding role in the interpretation of results from the greenhouse straw test which usually correlates poorly with field tests. There is need to investigate the role of other genes from the genomic regions containing these defense genes to gain a better understanding of the variation in reaction to white mold in different genotypes of common bean.

Contact Information – James D. Kelly, Michigan State University, 370 Plant and Soil Sciences, Dept. Plant, Soil and Microbial Sciences, East Lansing, MI, 48824; (517) 355-0271 Ext. 1181; kellyj@msu.edu

Can the negative association between yield and white mold resistance for NY6020 bean resistance be broken?

Jim Myers, Ceely Will, and Joel Davis, Oregon State University, Corvallis OR

Funded Plant of Work: White mold resistance-QTL: Identification, interactions, and fine mapping in common bean

ABSTRACT:
NY6020-4 (and a sister line NY6020-5; henceforth referred to as NY6020) are snap bean breeding lines released from Cornell University in 1996 with partial resistance to white mold conditioned by the WM 8.3 QTL. They are low yielding in Oregon green bean processing trials (about 7 T/A compared to 10-12 T/A for bush blue lake [BBL] cultivars). Advanced lines derived from crosses between BBL lines and NY6020 also tend to be low yielding and are not acceptable to processors. Empirical observations of the NY6020 partially resistant breeding lines suggested that they had few flowers per inflorescence and/or inflorescences. This may account for the lower yields and it suggests that resistance may be in part a function of fewer flowers providing fewer infection points for Sclerotinia ascospores. NY6020 shows partial resistance in the straw test indicating that the line does have physiological resistance, but avoidance traits may work in tandem to reduce disease levels in the field. We examined several phenological traits associated with white mold avoidance, measured yield, and rated NY6020 derived advanced breeding lines for white mold response in the field in two seasons, and in the greenhouse using the straw test. We found that overall there is a weak but statistically significant negative association between disease score and yield supporting the idea that the NY6020 white mold resistant type is associated with lower yield. On the other hand, there were a number of lines with economically acceptable yields and high levels of disease resistance. Of the phenological traits measured, leaf size showed a significant negative correlation with disease score. It appears that high yielding lines with partial white mold resistance can be developed, but whether resistance levels as high as NY6020 can be achieved in BBL breeding lines remains an open question.

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Characterization of growth, pathogenicity, and apothecial development of *Sclerotinia sclerotiorum* isolates from different geographic regions in contrasting temperature regimes

Glen L. Hartman, USDA-ARS, University of Illinois, Urbana, IL; Lucimara J. Koga and Curtis B. Hill, University of Illinois, Urbana, IL;
and Cláudia V. Godoy, Embrapa Soja, Londrina, PR, Brazil

Funded Plan of Work: Characterization of growth, pathogenicity, and apothecial development of *Sclerotinia sclerotiorum* isolates from different geographic regions in contrasting temperature regimes

ABSTRACT:
The fungus *Sclerotinia sclerotiorum* is an important pathogen of multiple crops worldwide. There is little knowledge on the possible existence of ecotypes, sub-groups of the pathogen that have different pathogenic capacities in different environments. Among the experiments to determine if ecotypes exist, we have preliminary results from *S. sclerotiorum* mycelial-growth in potato dextroxeagar cultures. Two experiments with different sets of isolates from Brazil and the U.S were evaluated. In the first experiment, nine isolates from one site in Brazil and nine from different regions of the U.S were tested at 22°C and 28°C. In the second experiment, 10 isolates from different regions of Brazil and six from different regions of the U.S were tested at 20°C and 30°C. Colony diameter measurements were recorded, growth rates and area under colony growth curves were calculated, and data subjected to ANOVA. Results showed that independent of the country or geographic origin of *S. sclerotiorum* isolates, overall growth rates and area under colony growth curves were higher at 20°C and 22°C compared to 28°C and 30°C. In addition, within each temperature tested, growth rates and area under colony growth curves differed among isolates in both experiments. A test of carpogenic germination of multiple isolates in autoclaved sand at 20°C and 30°C with vernalized and un-vernalized sclerotia of each isolateis in progress. Inoculation of several crop species at different temperatures is planned for the next reporting period.

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Characterization of metconazole sensitivity of *Sclerotinia sclerotiorum* isolates from North Central US

G. Ameen, and L. E. del Río, Department of Plant Pathology, North Dakota State University, Fargo, ND

Funded Plan of Work: Evaluation of fungicide alternatives for control of *Sclerotinia* stem rot of canola

ABSTRACT:
Sclerotinia sclerotiorum (Lib.) de Bary is a necrotrophic plant pathogenic fungus that affects many broadleaf crops of economic importance to states in North Central US. Farmers in the region depend largely on fungicide applications to control this pathogen. Fungicides registered for use against *S. sclerotiorum* include pyraclostrobin, thiophanate methyl, boscalid, and metconazole. Of these, the most recently introduced fungicide is metconazole. To establish baseline sensitivity to metconazole, 89 *S. sclerotiorum* isolates collected from 13 states in North Central US were cultured in potato dextrose medium amended with technical grade metconazole at different concentrations. EC_{50} values ranged from 0.05 to 1.64 µg/ml. Two isolates had EC_{50}> 1 µg/ml; researchers studying the sensitivity of other organisms to metconazole have suggested this value as a threshold to separate resistant from susceptible strains. Greenhouse studies in which these isolates were inoculated on sunflower plants indicated that these "resistant" isolates are still controlled by commercial formulations of metconazole applied at the recommended doses.

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Characterization of the genetic basis for partial resistance to *Sclerotinia sclerotiorum* in pea

Kevin McPhee and Behzod Tashtemirov, North Dakota State University, Fargo, ND and Lyndon Porter, USDA-ARS, Prosser WA

Funded Plan of Work: Characterization of the genetic basis for partial resistance to *Sclerotinia sclerotiorum* in pea

ABSTRACT:
Sclerotinia sclerotiorum (Lib.) de Bary, the causal agent of white mold, can cause severe yield losses in pea. Partially resistant pea accessions have been previously identified. Utilization of these accessions in breeding program is challenging due to poor knowledge of genes involved in resistance. Previous research established a skeletal genetic map of the pea genome using a population of 190 F_2 plants developed from the cross 'Lifter'/PI240515. While phenoptyping the population it became clear that stem morphology was potentially introducing some bias into disease development. The recent research efforts were aimed at clarifying this impact and establishing a method to overcome this bias. A detached stem assay was established to assess the rate of lesion growth and the interference of the node on lesion transmission.

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Deployment of novel sources of Sclerotinia resistance in sunflower-2012 progress

Yunming Long[1],Thomas J. Gulya[2], Brent S. Hulke[2], Kevin McPhee[1], Lili Qi[2]
[1]Department of Plant Sciences, North Dakota State University, Fargo, ND
[2]USDA-ARS, Northern Crop Science Laboratory, Fargo, ND

Funded Plan of Work: Deployment of novel sources ofSclerotinia resistance and tools for breeding resistance in sunflower

ABSTRACT:
Stalk rot, caused by *Sclerotinia Sclerotiorum*, is a devastating disease of sunflower worldwide. Identification of wild resistant sources, introgression, and mapping of resistance genes could provide new insight into the genetic basis underlying the resistance and resistance breeding. The specific objectives of this study were to 1) pre-breed novel Sclerotinia resistance from wild annual species of *H. argophyllus*, *H. debilis*, *H. praecox*, and *H. petiolaris* into cultivated sunflower, and develop an advanced backcross population for QTL mapping, 2) investigate inheritance of Sclerotinia resistance in introgressed lines. During the winter of 2011 and the spring of 2012, we screened a total of 4,288 BC_2F_2 plants derived from eight original resistant accessions of *H. argophyllus*, *H. debilis*, *H. praecox*, and *H. petiolaris*, and selected 303 resistant plants to advance to the BC_2F_3 generation. Seventy-one BC_2F_3 families from one accession each of the four wild species were evaluated for their resistance to stalk rot in field trials at Carrington, ND, and Crookston, MN, in 2012. Overall, seven BC_2F_3 families had disease incidence of 0% and 10 had disease incidence lower than 10% at both locations, which were superior to the resistant check, Croplan 305 (12.36%). Out of 71 BC_2F_3 families tested in the field, 23 derived from *H. petiolaris* PI 435843 and *H. argophyllus* PI 494573 were also evaluated for their resistance to stalk rot in the growth chamber in the summer of 2012. Combining field and greenhouse data, the two most resistant lines, 11-256-053 from *H. petiolaris* and 11-275-037 from *H. argophyllus*, were identified. Whole genome scans of the resistant line 11-275-037 with sunflower SSR markers revealed the presence of introgressed chromosome segments located on linkage groups 9, 10, and 11, indicating these chromosomes may associate with stalk rot resistance. We continued to develop an advanced backcross (AB) population to facilitate genetic characterization of novel QTL for stalk rot resistance derived from *H. argophyllus* PI 494573. A total of 250 plants derived from 14 BC_2F_1 plants were advanced to the BC_2F_3 generation by single-seed descent. Our goal is to produce an AB population of $BC_2F_{2:6}$ lines by an additional three cycles of single-seed descent.

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Evaluation of wild *Helianthus* species for resistance to Sclerotinia stalk rot

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Funded Plan of Work: Evaluation of Wild *Helianthus* Species for Resistance to Sclerotinia Stalk Rot

ABSTRACT:
The objective of this project is to identify new sources of resistance in wild sunflower germplasm to Sclerotinia stalk and root rot, caused by *Sclerotinia sclerotiorum.* The USDA-ARS sunflower collection at Ames, IA contains wild annual species (1,358 accessions) and perennial species (824 accessions). Accessions are tested under high disease pressure in the greenhouse, with the goal of identifying accessions showing significantly better survival percentages than the most resistant hybrid check, Croplan 305.

In 2012, six perennial species were evaluated including *Helianthus decapetalus, H. giganteus, H. maximiliani, H. nuttallii, H. simulans,* and *H. verticillatus.* All of the perennial species showed remarkable resistance with every accession performing better Croplan 305. Good disease pressure usually kills 95-100% of the susceptible check, Cargill 270, and 60-80% of the resistant check, Croplan 305. Sixty percent plant survival is viewed as an indicator of a high level of resistance in an accession. For *H. decapetalus*, 13 of 14 acc. had ≥90% plant survival and 10 accessions had 100% survival (60 plants per accession). For *H. giganteus,* 14 of 17 accessions had ≥ 90% plant survival and five had 100% survival. For *H. maximiliani*, 42 of 45 acc. had ≥90% plant survival and ten had 100% plant survival. *Helianthus nuttallii* also showed superior resistance. Of the 39 accessions, 34 had ≥90% plant survival and 11 accessions had 100% plant survival. The last two perennial species evaluated were *Helianthus simulans* (4 acc.), and *H. verticillatus* (2 acc.). For *H. simulans*, 3 of 4 accessions had >80 plant survival and the fourth had 100%. *Helianthus verticillatus* had one accession at 77% and the other accession at 95% at the end of the test.

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Expression profiling of the pea-Sclerotinia sclerotiorum interaction for genomics-assisted breeding

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Funded Plan of Work: Expression profiling of the pea-Sclerotinia sclerotiorum interaction for genomics assisted breeding

ABSTRACT:
The overall project goal is to utilize genomic resources and techniques to study the host-pathogen interaction of *Pisumsativum* and *Sclerotinia sclerotiorum*, at the genetic level to ultimately improve white control. We have been conducting gene expression profiling via RNA seq. Around 300 million pairs of reads were produced by the Illumina GAIIx sequencing platform with a 75bp paired end sequencing protocol of a susceptible (Lifter) and a partially resistant (PI240515)line over three time points with corresponding mock inoculated samples. De novo assembly of the paired end reads was conducted to generate EST libraries and our published tBlastx method was utilized to parse the *Sclerotinia* and *P. sativum* reads (BMC Genomics 13:668). The resulting 44,998 ESTs (average length: 1,081 bp) from Lifter and 48,174 ESTs (average sequence length: 1,118 bp) from PI240515 were annotated by queries against the proteomes of Arabidopsis, Medicago and Soybean. To enable differential gene expression between the susceptible (Lifter) and resistant (PI240515), we utilized transcripts with high similarity to annotated Arabidopsis genes. From this we generated a list of 20,526 genes that we could perform differential gene expression profiling which was conducted for each time point. We have also compared our gene expression profiling data set against 145 Arabidopsis microarray data sets. From this preliminary analysis it appears as though ABA mediated transcriptional regulation might be involved in the resistance response to *S. sclerotiorum* in the early stages of infection, and salicylic acid (SA) mediated signal pathways might play important defense roles in the mid and late time points of our study. We are completing these analysis as well as conformational real-time PCR assays to identify potential resistance genes for which markers can be developed for the screening and mapping of these genes in progeny from the white mold resistance mapping populations.

In addition, 541 EST-derived SSR markers were identified by comparing the sequences from the RNA seq analysis of the two pea lines. These markers may also be incorporated into genetic linkage maps. The work described above will lead to a greater understanding of the genetics of host resistance and *Sclerotinia* pathogenicity and will be utilized to improve resistance to white mold in pea cultivars.

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Genetic variation and aggressiveness of *Sclerotinia sclerotiorum* in the United States

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Funded Plan of Work: Genetic variation and virulence of *S. sclerotiorum* in the United States

ABSTRACT:
The genetic variation and aggressiveness of S. *sclerotiorum* from crops in the United States was examined. Sixty nine Isolates of the pathogen were obtained from 14 states outside of the north central region. A previous study examined isolates within the north central region. These were collected from 17 different crops. Isolates were evaluated for mycelial compatibility group and microsatellite haplotype at eleven loci. There were 54 mycelial compatibility groups (MCG's) identified; 44 MCG's contained one isolate, 8 contained 2 isolates, one had three isolates and one contained five isolates. These isolates were paired with 33 previously identified MCG's from the north central region and six of the previously identified MCG's were identified within the isolates. The isolates were genotyped with 11microsatellite markers and74 polymorphic loci were detected. Polymorphic loci varied depending on the marker; the greatest polymorphism was with microsatellite 106-4 which had 22polymorphic loci and the lowest was5-2 with three polymorphic loci. Sixty six haplotypes were identified and specific haplotypes were associated with MCG's. Although most MCG's consisted of one haplotype, seven MCG's had multiple haplotypes varying from two to five. Aggressiveness of 65 isolates was tested on four crops, canola, dry bean, sunflower and soybean. A cut stem inoculation technique was used on five week old plants growing in the greenhouse. Canola and soybean were in one set of experiments and dry bean and sunflower were in another set of experiments. The experimental design was a split plot with crop as the main plot and isolate as the subplot with four replications and the experiments were repeated. Following inoculation, plants were maintained in a mist chamber for 72 hours and then the length of stem lesions were measured using electronic calipers. All data were analyzed with analysis of variance. All isolates were pathogenic on the four crops. In the canola-soybean experiments there were significant differences (P=0.05) among isolates when averaged over crops. Crop was not a significant factor (P=0.05) and the interactions of experiment by crop and crop by isolate were not significant. In the dry bean-sunflower experiments there were significant differences among isolates when averaged over crops and there was a significant difference between the crops, with longer lesions on the sunflower compared to the dry bean. The experiment by crop interaction was also significant, but not the crop by isolate interaction. The isolates used in this study are part of a collection of approximately 170 isolates from the United States that is being used to study various aspects of the biology of *S. sclerotiorum*.

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Genome-wide association study identifies multiple loci associated with soybean resistance to *Sclerotinia sclerotiorum*

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Funded Plan of Work: Enhancing soybean for resistance to Sclerotinia stem rot

ABSTRACT:
Genome-wide association analysis (GWAS) has become a popular method for dissecting the genetic basis of complex traits in plants. Here we show the results of GWAS based on genotyping 52,041 SNP variants across 720 diverse accessions of soybean elite cultivars that were evaluated for resistance to white mold (*Sclerotinia sclerotiorum*) in the greenhouse. The analysis revealed large differences in resistance to the disease in the population. The population was divided into two subpopulations based on the kinships among cultivars. The level of intra-chromosomal linkage disequilibrium was about 200 kb. We identified significant associations ($P < 6.56 \times 10^{-6}$) between SNP markers and the resistance to the disease at 4 loci, including 2 that were not reported in the literature. The SNPs at the identified loci explained 19% of the phenotypic variance. The identification of such associations may provide substantial insight into soybean resistance to white mold and may also facilitate marker-assisted selection in soybean.

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High density genotyping of *Sclerotinia sclerotiorum*

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Funded Plan of Work: Genetic variation and virulence of *S. sclerotiorum* in the United States

ABSTRACT:
Sclerotinia sclerotiorum is one of the most important broadleaf crop pathogens in the United States causing disease on diverse crops such as canola, dry bean, pea and soybean. Understanding the genetics of this necrotrophic pathogen is essential to broadening our knowledge of how this pathogen causes disease and how it interacts with the host. Genotyping of populations of *S. sclerotiorum* has relied on small numbers of markers, which does not permit accurate mapping of important phenotypictraits such as aggressiveness. In order to dissect the genetic mechanisms underlying aggressiveness, apothecia production, fungicide resistance and other important phenotypes of this pathogen, researchers must address the development of high-density genotyping of natural populations. The objective of this research is to genotype a diverse natural population of *S. sclerotiorum* from the United States. The overall goal is to develop the genetic tools that can be utilized by the research community to genetically characterize phenotypic traits through association mapping and aid in the identification of candidate genes underlying these identified loci utilizing the publicly available *S. sclerotiorum* genome sequence. The lack of genetically characterized populations due to the inability to cross isolates under controlled environments leaves the wealth of information in the genome sequence vastly underutilized. We have a diverse population of isolates of *S. sclerotiorum* from 24 states in the U.S. that were collected from different crops and environments. Most of these have been characterized for aggressiveness, mycelial compatibility groups, fungicide sensitivity, and other phenotypic characters. We are genotyping a select group of isolates using the Ion Torrent Personal genomics machine (PGM) that can be effectively utilized to achieve high-density genotyping of natural fungal populations. We have modified and adapted a restriction site associated DNA mapping method, a form of genotype by sequencing, originally developed for use with the Illumina sequencing platform and optimized it to run on the Ion Torrent PMG. We expect to identify ~10,000 single nucleotide polymorphism markers in the 38 Mb genome of *S. sclerotiorum*. This would place a molecular marker about every 4kb in each of the isolates. This density of genotyping would facilitate the high-resolution identification of genes underlying phenotypic variation with the accuracy of the phenotyping being the only constraint for association mapping. Generation of these genetic material and free access to them will greatly benefit the research community and provide a tool that is essential in order to utilize the publicly available *S. sclerotiorum* genome sequence.

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Identification and functional analysis of soybean candidate defense-related genes against *Sclerotinia sclerotiorum*

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Funded Plan of Work: Functional verification of candidate defense-related genes to *Sclerotinia sclerotiorum* in soybean and Arabidopsis

ABSTRACT:
In soybean, the wheat germingene (*gf-2.8*) confers a high degree of resistance to *Sclerotinia sclerotiorum*, a necrotrophic fungus. The transgene product, oxalate oxidase (OxO), catalyzes oxalic acid (OA) to produce H_2O_2. To identify genes with a role in defense, microarray studies have been used to examine the changes in soybean gene expression in response to infection of the transgenic (resistant) and parental (susceptible) lines. In addition, the effect of OA, a major virulence factor of *S. sclerotiorum*, was evaluated by leaf infiltration with OA. Thousands of genes were found to be significantly differentially expressed in each of the two studies. To identify genes related to defense, genes were classified functionally, based on the annotation of their best sequence match in public databases. Cluster analyses identified many defense-related genes that were induced across the studies, including genes annotated as GSTs, P450s, MMPs, PR proteins, WRKYs and genes of the phenylpropanoid pathway. Verification of a defense-related role of the candidate genes by over-expression or silencing is being assessed in soybean, Arabidopsis and in Nicotianabenthamiana. The goal of this study is to identify genes with the most relevant role in defense, either to aid molecular breeding or for transgenic modification to improve disease resistance.

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Identification and validation of QTL for white mold in two pinto bean RIL populations

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Funded Plan of Work: Identification and validation of QTL for white mold in pinto bean

ABSTRACT:
Pinto beans (*Phaseolus vulgaris*) are the most widely grown commercial class of dry beans in the U.S. and are among the most susceptible to white mold (*Sclerotinia sclerotiorum*). In order to enhance resistance the goal of this project was to identify quantitative trait loci (QTL) that were associated with resistance to white mold and to other agronomic traits associated with disease avoidance in two pinto bean recombinant inbred line (RIL) populations (AP630 and AP647). A genetic map of 727 cM was generated for the AP630 population using 107 markers spaced approximately every 8 cM. Eight traits, yield, white mold disease incidence, 100 seed weight, days to flowering, days to maturity, plant height, lodging, and greenhouse disease severity (straw test) were analyzed using the multiple QTL model. Twenty QTL were identified in different years for five traits (white mold disease incidence, seed yield, days to flowering, days to maturity, and the straw test) and these QTL were located across six linkage groups. LOD scores ranged from threshold values of 2.5 to 8.18. Significant QTL for seed yield were detected on bean chromosomes Pv02 and Pv05 accounting for 11 and 32% of observed variation based on 2007, 2008 and 2010 field data. The QTL on Pv02 is adjacent to the BM142 marker while the QTL on Pv05 is nearest to InDel marker NDSUind-Pt025. The favorable alleles in these QTL came from the P02630 parent. Significant QTL for field disease incidence were located on Pv01, Pv03 and Pv08 based on disease scores collected in 2007, 2008 and 2010. These QTL explained between 19 to 22% of total variation observed and the favorable alleles associated with these QTL originated from the AN37 parent. Significant QTL for flowering on Pv03 accounted for 23% of the total variation observed and this originated from the P02630 parent. QTL associated with the number of days to maturity explaining up to 31% of the variation were mapped on Pv05, and Pv08. Longer days to maturity were associated with alleles from the AN37 parent. There were also significant QTL for the straw test that measures physiological resistance in the greenhouse on Pv02, and Pv07 that explained up to 42% of total variation and most of the positive alleles came from the AN37 parent. In order to verify these QTL the same markers were screened on the second half-sib population (AP647). Screening revealed that 43 polymorphic markers associated with resistance from the first population (AP630) were also segregating in the AP647 population. Analysis of segregation patterns with significant markers in the AP647 population showed that the AN37 allele associated with BMd-34 marker, which was previously unlinked, now mapped near markers on Pv02 and increased field resistance significantly. This QTL on Pv02 is likely the same WM 2.2 QTL that was previously mapped by other authors. The favorable alleles from the AN37 parent associated with the marker BMd-1 in the QTL interval on Pv03 contributed an average of 10% increase in resistance in the greenhouse straw test in three separate tests. The QTL on Pv03 could be the same as WM3.1 that was mapped in the Aztec/ND population from which AN37 parent was originally selected. Confirmation of existing QTL is valuable as it provides bean breeders with more robust QTL markers to use in enhancing white mold resistance in future bean varieties.

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Identification of novel loci for resistance to Sclerotinia stem rot in perennial soybean accessions

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Funded Plan of Work: Identification of novel loci for resistance to Sclerotinia stem rot in perennial soybean accession

ABSTRACT:
Work was continued to develop molecular resources for mapping of loci for resistance to Sclerotinia stem rot in Glycine latifolia, a perennial relative of cultivated soybean. During 2012,384 GoldenGate SNP markers and over 1,781 genotyping-by-sequencing (GBS) loci were evaluated in F_2 populations, which produced the first genetic maps for any perennial *Glycine* species and showed that most *G. max* and *G. latifolia* chromosomes were collinear. Non-destructive methods were compared for the phenotypic evaluation of *G. latifolia* for response to Sclerotinia stem rot, and showed that cut-stem assays using mycelial agar plugs or dilute solutions of oxalic acid produced the best discrimination between susceptible and resistant *G. latifolia* accessions. Preliminary mapping studies in an F_2 population identified a QTL (LOD=3.1; R^2=0.18) for resistance to Sclerotinia stem rot on the *G. latifolia*homeologue of soybean chromosome 17. RIL population development was continued. A draft genome sequence was assembled for *G. latifolia*. A manuscript describing the production and mapping of SNPs in *G. latifolia* was accepted for publication in *Theoretical and Applied Genetics.*

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Identification of resistance and pathogenicity genes associated with *Sclerotinia sclerotiorum* infection in canola

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Funded Plan of Work: Identification of resistance and pathogenicity genes associated with *Sclerotinia sclerotiorum* infection using next-generation sequencing.

ABSTRACT:
The goal of this study has been to identify white mold resistance genes in canola and pathogenicity genes in *Sclerotinia sclerotiorum* associated with infection of this host through transcriptome analysis. A double haploid homozygous progeny from a PI line of *Brassica napus* with significant resistance to white mold (NEP63) and one with almost no resistance (NEP32) to the disease have been used in this study. These lines were infected with an aggressive isolate of *S. sclerotiorum* using two different methods (petiole inoculation and leaf inoculation). cDNA libraries were created using RNA collected at different time-points after inoculation of each of these lines and the pathogen grown in culture. Approximately 95million, 76bp reads were obtained from these libraries using Illumina sequencing. The sequences were aligned to the *S. sclerotiorum* whole genome to filter out fungal genes and the remaining sequences were compared to the Brassica 95kEST assembly. Functional categorization of the differentially expressed ESTs of both plant and fungal origin was conducted and expression of significant number genes involved in defense response, signal transduction and immune response were detected among others. Interestingly, sequences that aligned to 400 Brassica ESTs representing unigenes in the 95K assembly were found to be only expressed during infection in the resistant and not on the susceptible line while using the petiole inoculation method, suggesting that these could potentially be involved in resistance to *S. sclerotiorum*. Fungal genes exclusively expressed *in-planta* during disease development that could possibly be associated with pathogenicity were also detected. Further analyses of sequences and characterization of genes is in progress.

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Inheritance studies of new potential sources of resistance to white mold in dry bean

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Funded Plan of Work: Inheritance studies of new potential sources of resistance to white mold in dry bean

ABSTRACT:
White mold is a devastating plant disease which is caused by the fungus *Sclerotinia sclerotiorum.* This fungusis causing severe yield losses in dry bean (*Phaseolus vulgaris* L.) especially in North Dakota and Minnesota, which are ranked as top producers of dry beans in the US. Discovery of resistant genotypes such as G112, Bunsi, PC- 50, I9365-25, A 195 and A55 has hampered white mold's pathogenicity. During summer 2009, breeding lines ND060514 (navy) and ND080547 (small red) from NDSU were found to have high levels of field resistance to white mold in addition to good agronomic performance.

The project focuses on the study the potential source of resistance to white mold in these lines. Screening of these lines at the molecular level was done with some known molecular markersand was compared with other resistant and susceptible genotypes. 17 RAPD (Random Amplification of polymorphism DNA) markers, 2 SRAP (Sequence-related amplified polymorphism) markers, 6 SCAR (Sequenced Characterized Amplified Region) markers and 7 SSR (Simple Sequence Repeats) markers linked to loci for resistance were used along with several resistant and susceptible genotypes. Out of these 10 markers (5 RAPD, 1 SRAP, 2 SCAR, and 2 SSR) showed polymorphism when screened. These lines showed similarities when compared with each other when scored with almost all of the polymorphic markers. Preliminary results showed that at least 10 QTL's could be positively associated with resistance in breeding lines ND060514 and ND080547. Loci WM8.4, WM5.4, WM6.1, WM7.1, WM 2.1, WM2.2, WM8.1, WM9.1, WM4.2, and WM5.3 were identified in theses lines. Pedigrees of these two lines also suggest that the resistance found in ND060514 may come from ICA-Bunsi while resistance in ND080547 is still not clear. Mapping populations are being developed to identify the resistance in line ND080547. The present study confirmed the presence of resistance in these breeding lines. Nonetheless, these lines and tagged QTL's will be useful in breeding programs and for understanding the source of resistance in the lines and selection of beneficial alleles at these loci for improvement of resistance.

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Intrachromosomal recombination suggests outcrossing in *Sclerotinia sclerotiorum* populations

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Funded Plan of Work: Variation in pathogenicity and fungicide sensitivity in relation to variation of neutral markers of *Sclerotinia sclerotiorum*

ABSTRACT:
Genetic structure and reproductive mode of the homothallic fungal pathogen *Sclerotinia sclerotiorum* have been widely studied using linkage disequilibrium (LD) tests with putatively unlinked molecular markers. Both clonal and recombining population structures have been reported from around the world. We previously observed random association between linked loci in *S. sclerotiorum* populations suggesting intrachromosomal recombination or high mutation rates at these loci. To detect linkage disequilibrium and recombination of linked loci, we used 12 microsatellite loci distributed over four chromosomes to genotype 230 isolates of *S. sclerotiorum* sampled from seven populations in the USA and China from a variety of crops. Each isolate carried a single allele for each of the 12 loci suggesting the isolates were haploid and homokaryotic. Pair-wise linkage disequilibrium (LD) tests (Hedrick's *D*, Fishers exact test and I_A) between physically linked loci showed relationships ranging from linked to random association with increasing distance between loci on three chromosomes. For the three loci on chromosome four, LD decay with increasing physical distance between loci was found in six of the seven populations. Likewise, LD decay was found for the three loci on chromosome six in four of the seven populations, and also for the loci on chromosome five in two of the populations. The reduced pair-wise linkage disequilibrium with increasing distance cannot be attributed to mutation alone, and thus the high intrachromosomal recombination is most likely due to meiotic recombination following outcross in these populations. Recombination hot spots and cold spots were detected.

Additionally, mating type loci in 59 isolates of two populations were genotyped using PCR with allele-specific primers. About 40% of the isolates showed both *MAT1-1* and *MAT1-2*idiomorphs, as expected for a homothallic species. However, the remaining 60% of the isolates had only the *MAT1-2*idiomorphdetected by the allele-specific PCR. Although the nature of the absence of the *MAT1-1*idiomorph remains to be determined, the results showed variations in mating type alleles in natural populations suggesting that some of the isolates may not be truly homothallic.

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Oxalate-deficient mutants of *Sclerotinia sclerotiorum* accumulate fumaric acid and remain pathogenic

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Funded Plan of Work: Identifying virulence factors of *Sclerotinia sclerotiorum* through transformation

ABSTRACT:
Sclerotinia sclerotiorum is a ubiquitous necrotrophic pathogen capable of infecting over 400 plant species including many economically important crops. Oxalic acid production has been shown in numerous studies to be a pathogenicity factor for *S. sclerotiorum* through several mechanisms. During our random mutagenesis study of *S. sclerotiorum* using *Agrobacterium*-mediated transformation, we identified three mutants that had lost oxalate production. Southern hybridization blots showed the mutation was due to a single T-DNA insertion, and plasmid rescue and DNA sequencing confirmed that the T-DNA insertion site was located in the ORF of oxaloacetate acetylhydrolase (*Ssoah*, SS1G_08218) of *S. sclerotiorum*. The mutants did not change the color of a pH-indicating medium (PDA amended with 50 mg/L bromophenol blue). The pH values of 6-day PDB culture filtrates were 1.8-2.0 for the wild type and 2.8-3.1 for the mutants. No oxalic acid was detected using HPLC in culture filtrates or in the mycelium of the mutants, but another acid compound was accumulated in culture filtrates of the mutants and detected by HPLC, and the compound was identified as fumaric acid using LC-MS. The mutants showed reduced vegetative growth on PDA and produced sclerotia that are beige in color and soft in texture. Artificial acidic conditions (pH 3.4 and 4.2) enhanced vegetative growth and promoted normal (black and hard) sclerotial formation of the mutants. Furthermore, the oxalate-minus mutants remained pathogenic on pea, green bean and faba bean in detached leaf assays and on intact plants of *Arabidopsis thaliana*. Their virulence levels were similar to that of the wild type strain on certain host plants, but varied depending on the plant species tested. The mutant had increased expression levels of cell wall-degrading enzymes such as polygalacturonases compared to the wild type strain during the process of infecting pea leaves. The results showed that a low pH condition is very important for growth and virulence of *S. sclerotiorum* on its wide range of host.

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Preliminary evaluation of the Bean CAP snap bean panel for white mold resistance

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Funded Plan of Work: White mold resistance-QTL: Identification, interactions, and fine mapping in common bean

ABSTRACT:
The Bean Coordinated Agricultural Project (CAP) is a multidisciplinary, multi-institutional USDA-NIFA supported endeavor focused on genomics and nutritional traits of common bean. Two panels of bean cultivars have been assembled. The dry bean panel consists of about 300 accessions of Middle American origin while there are 150 in the snap bean panel (both Middle American and Andean origin). Each panel has been evaluated separately, because of differences in phenotypic traits of interest. The panels have been genotyped using an Illumina 10,280 SNP GeneChip and this allows the evaluation for phenotypic traits beyond those of specific interest to the Bean CAP. In 2012, a subset of the snap bean panel with non-pole bean habit (134 lines) was grown in a white mold screening nursery at Corvallis, Oregon and evaluated for white mold incidence and severity and several phenological traits associated with disease avoidance. The geometric mean of incidence and severity ranged from 0 to 75% with a number of accessions showing levels of disease similar to the partially resistant checks. Some probably had avoidance traits (determinate habit, open canopy, late maturity) but others had type III habit and would normally be expected to have high levels of disease. Both Andean and Mesoamerican snap beans were found in the low disease group. The panel needs to be tested again in the field and evaluated by the straw test. The snap bean panel may possess novel sources of white mold resistance, which can be evaluated though association mapping.

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Sclerotinia head rot of sunflower: Improving the methods used to screen sunflowers for resistance and prospects for management with fungicides

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Funded Plan of Work: Facilitating management of Sclerotinia head rot of sunflowers though screening hybrids for resistance and evaluating fungicides for efficacy

ABSTRACT:
Sclerotinia head rot, caused by *Sclerotinia sclerotiorum,* is one of the most economically important diseases of sunflowers. The use of partially resistant sunflower hybrids is an important management tool, and, because full resistance to this disease does not exist, the availability of effective fungicides would improve disease management.

Field experiments evaluating the susceptibility of sunflowers to Sclerotinia head rot during and after bloom provided insights on how to improve the methods used to screen sunflowers for resistance to this disease. In an inoculation timing trial conducted in Langdon, ND in 2012, inoculations conducted at bloom resulted in an increase in Sclerotinia head rot relative to the non-inoculated control ($P < 0.05$); inoculations conducted after bloom did not. In a parallel trial conducted in Carrington, ND in 2012, inoculations conducted at the R5 (bloom) and R6 (flowering complete, ray flowers wilted) growth stages resulted in significant increases in Sclerotinia head rot relative to the control ($P < 0.05$), but disease in the resistant hybrid was significantly higher when inoculations were conducted at the R5 growth stage than at the R6 growth stage. These results are similar to findings from 2011 and suggest that obtaining replicable results in disease screening nurseries requires that inoculations be conducted over multiple days such that all plants in all entries are inoculated at the same growth stage.

A set of 25 sunflowers and breeding lines were screened for resistance to Sclerotinia head rot at three locations in 2012, and results from Carrington and Oakes, ND, but not Langdon, were strongly correlated with each other. In Carrington and Oakes, inoculations were conducted over 2 to 3 weeks such that each plant was inoculated at the same growth stage; in Langdon, inoculations were conducted over a span of 5 days, and each plant was not inoculated at the same growth stage.

Fungicides showed efficacy against Sclerotinia head rot in a field trial conducted in Scotttsbluff, NE but not in Langdon, ND in 2012. In Scottsbluff, three fungicides significantly reduced Sclerotinia head rot incidence and severity and four significantly reduced Sclerotinia stalk rot. In Langdon, none of the eight fungicides tested resulted in a significant reduction in Sclerotinia head rot relative to the inoculated control. Disease levels in a similar trial conducted in Carrington were too low to permit a rigorous assessment of fungicide efficacy.

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Sources of white mold resistance derived from wide crosses in common bean and progress in characterization of relevant pathogen isolates

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Funded Plan of Work: Improved white mold resistance in dry and snap beans through multi-site screening and pathogen characterization throughout major production areas

ABSTRACT:
In 2012 weather conditions were not conducive for the development of white mold in three of the six field screening nurseries. However, in those fields where beans were infected by the white mold pathogen, all nine bean entries derived from NSI funded research were classified as resistant. This resistance ranged from avoidance to moderate resistance similar to the resistant check but in adapted bean backgrounds with a range of seed classes. Thus, the advantage of multi-site screening for making progress each year on confirming resistance is validated. The assessment of new lines with resistance derived from wide crosses and a variety of sources but with limited seed was conducted by multiple cooperators using a straw test. From data sent by three collaborators who completed greenhouse straw tests, five lines with resistance similar to the resistant check were identified. Results from five other locations should be available by the meeting date. The evidence supporting aggressiveness being similar between clones and where there are significant differences that they occur between isolates in different clones has been consistent. Variation in number of clones at a location and percent shared clones between locations ranged from 8 - 27 and 11 – 75%, respectively. There is preliminary evidence that isolates collected from screening nurseries differ from isolates collected from grower fields in the same area with regard to shared clones. The recent weather patterns have decreased grower field incidence of white mold and thus grower field isolates have not been collected. We hope to collect more isolates in specific grower field locations to expand that data base. The use of isolate genotyping with 16 polymorphic microsatellite markers will produce haplotypes that can be evaluated for variability within and between locations. Genotyping will be a less time consuming and less costly method of assessing genetic variability. Characterized isolates that are widespread or local with high or low aggressiveness will be available from our lab for use in screening for resistance.

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Sunflower head rot resistance found in Hungarian land races, and continued studies on ascospore production demonstrate isolate differences

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Funded Plan of Work: Discovery of novel sources of resistance to head rot and stalk rot in cultivated sunflower and wild *Helianthus*

ABSTRACT:
In 2012, we had two inoculated stalk rot nurseries (Carrington, ND and Crookston, MN) and two inoculated, misted head rot nurseries (Staples and Sabin, MN), in which we evaluated a total of 4,600 rows of sunflower germplasm. Phenotyping of 250 USDA cultivated sunflower Plant Introductions (PIs) for head rot was completed, yielding four datasets over two years, which complemented the six datasets of stalk rot data and four datasets of Phomopsis stem canker data gathered previously, all of which will be used in association mapping. From this multi-year head rot analysis, we identified 25 accesions with an average of < 16% nfection, comapred to the HA 89 check at 76% infection, and the mean across all entries of 61% head rot. Seven of the ten most resistant accessions (0 to 6% infection) were old Hungarian land races. In addition to testing of the USDA PIs, we evaluated USDA breeding lines from four USDA scientists for head rot and stalk rot that were in various stages of selection. Several germplasm releases with improved head rot resistance are planned for 2013. Other work completed during 2012 was a multi-lab study whose objective was to document and potentially modify the very successful ascospore production method developed by retired U. Nebraska pathologist Mike Boosalis. The method developed by M. Boosalis was tested on five genetically diverse isolates of *Sclerotinia sclerotiorum* in four laboratories (Lincoln and Scottsbluff, NE, and Carrington and Fargo, ND). Two of the four labs had problems producing apothecia and ascospores, illustrating how tricky large-scale ascospore production can be. In the remaining labs, the method developed by M. Boosalis was effective at generating ascospores from two of the five isolates, including the isolate that M. Boosalis used to develop his method. For the remaining isolates, few if any apothecia and ascospores were produced. Slight modifications to the method did not change this outcome. The results suggest that this method is very useful for generating ascospores from specific isolates of *S. sclerotiorum* but will not be useful for all isolates. Further work is planned for 2013 in which other media components will be examined and well as sterilization proceudres to minimize contamination, a minor but essential consideration.

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Update ontransferring Sclerotinia resistance genes from wild perennial *Helianthus* species into cultivated sunflower

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Funded Plan of Work: Transferring Sclerotinia resistance genes from wild *Helianthus* species into cultivated sunflower

ABSTRACT:
Cultivated sunflower lacks sufficient tolerance to Sclerotinia, whereas wild perennial *Helianthus* species are highly resistant. The main objective of this project is to incorporate the resistance genes from wild perennial species into a cultivated background using traditional crossing and backcrossing method. A four-year evaluation (2009-2012) for stalk and head rot has been conducted for the backcross progenies derived from wild perennial species of different ploidy levels. For stalk rot evaluation in 2012, 26 of 62 families of hexaploid*H. californicus* (crossed with HA 410), nine of 37 progenies derived from five interspecific amphiploids (crossed with HA 410), 12 of 55 families derived from three diploid species (crossed with HA 410), and nine of 94 families derived from two diploid species (crossed with HA 441) showed 0% infection. In addition to the 56 immune families, a total of 85 families had scores lower than the resistant checks. For head rot, 21 of 95 backcross families derived from two diploid species (crossed with HA 441) and two of 87 families derive from other crosses showed a 0-1.0 disease rating, while another 31 families showed a 1.1-2.0 rating, compared to 2.83 for the most resistant check. Results from replicated field tests for head rot (2009 and 2011) and stalk rot (2009 and 2010) were less conclusive, but enabled us to eliminate the most susceptible families. Among the three years of field evaluations, 2012 was unquestionably the best year allowing us to make adequate comparisons and selections for further confirmation in 2013. The outstanding families will be prepared for germplasm release, cytogenetic and molecular studies, and the initiation of QTL mapping populations. Over 200 advanced backcross progeny families were grown in Fargo in 2012 to provide a continuous supply of materials for field evaluation. To further diversify the resistance genes and increase the probability of identifying useful major QTLs (genes), new crosses were made between perennials *H. hirsutus, H. salicifolius, H. occidentalis, H. divaricatus,* and *H. resinosus*with NMS HA 89, HA 410 and/or HA 451 and backcrossed to cultivated lines in 2012. Extensive effort was made to obtain BC_1F_1 individuals, using the F_1 plants as female or male parent during backcrossing, aided by embryo rescue. Over 400 BC_1F_2 families/rows will be planted in 2013 for seed increase, and the BC_1F_3 families will be evaluated for both stalk and head rot resistance in 2014. A genomic *in situ* hybridization technique (GISH) distinguishing chromosomes of the perennials and the cultivated sunflower has been developed. This technique will be used to study meiotic chromosome pairing between chromosomes of wild perennials and the cultivated line, and to identify the chromosomes or segments of chromosomes introgressed into the cultivated background. We plan to release resistant germplasms derived from the various perennial species in late 2013 if field testing in 2013 confirms the 2012 results showing the successful transfer of Sclerotinia resistance gene(s) from wild perennial *Helianthus* species into cultivated sunflower lines.

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Use of a transformation system in sunflower for Sclerotinia resistance studies

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Funded Plan of Work: Use of a transformation system in sunflower for Sclerotinia resistance studies

ABSTRACT:
A reliable transformation system in sunflower is needed to take advantage of the current biotechnological tools to study and fully characterize Sclerotinia resistance in sunflower. The main problem in sunflower transformation is the inability to efficiently integrate *Agrobacterium*-mediated transformation and *in vitro* organogenesis. Previously, line RHA280was identified as having remarkably high response to shoot induction using a shoot induction medium (SIM). High numbers of adventitious shoots (>100 on average) can now be consistently obtained by placing excised cotyledon explants from mature, dry seed on SIM for three weeks.

Efforts were made to increase shoot elongation after induction by evaluating the effects of gibberellin, an anti-cytokinin, alternative cytokinins, auxin and gelling agents in an elongation medium. Use of gibberellic acid alone led to the most efficient adventitious shoot elongation. On average, up to fifteen elongated shoots could obtained from each cotyledon explants but with a relatively large variation among explants. Given the inefficiency in root induction of elongated shoots, micro-grafting was evaluated to recover plants from elongated shoots with a success rate up to 38%. Using cotyledon explants for *Agrobacterium*-mediated transformation with the *gfp*gene as the reporter, GFP expression was rarely observed at the shoot-forming surface but was displayed at high levels at the cut areas of the explant. Sonication was initially considered to generate micro-wounds all over explants including shoot-forming surface, but sonication did not appear to effectively generate micro-wounds at the target area, perhaps due to the hardness of dry explant tissue. Pre-culture of cotyledons was evaluated to increase tissue softness and enhance the susceptibility to SAAT. Placement of intact cotyledons in liquid SIM overnight, followed by SAAT, was used to increase transformation at shoot-forming regions. Multiple GFP shoot primordia were obtained from 8-10% explants using Agrobacterium with SAAT. In a separate approach for sunflower transformation, Agro-infiltration was evaluated using organogenesis of primary leaves from seven-day-old seedlings germinated on cytokinin-contained medium. The number of adventitious shoots obtained from leaves was lower than that using cotyledon explants, and shoots were more difficult to elongate for micro-grafting. GFP-expressing shoots were occasionally obtained from primary leaves placed on MS medium containing 13 mg/L (60 µM) kinetin and 7.5 mg/L Hygromycin B after Agro-infiltration. Efforts on both transformation systems continue to generate elongated GFP-expressing shoots and increase the recovery rate of transgenic plants.

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White mold resistance-QTL: Identification, interactions, and fine mapping in common bean

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Funded Plan of Work: Resistance-QTL: Identification, interactions, and fine mapping in common bean

ABSTRACT:
The identification of major genes (QTL) conferring physiological resistance to white mold (WM) from diverse sources including the promising *P. coccineus* source, and linking them with molecular markers to facilitate marker-assisted breeding for development of cultivars with partial resistance to WM, is the long term goal of this project. For 2012, five QTL, some apparently unique from *P. vulgaris* QTL were identified in interspecific populations. Interestingly, the interspecific lines do not approach homozygosity at the same rate as intraspecific lines when inbred over generations. Also, interspecific derived lines show a higher rate of outcrossing than do *P. vulgaris* lines. This last observation in combination with interspecific incompatibilities may help explain why approach to homozgyosity with inbreeding is slower than expected. A new comparative map containing 79 QTL, 27 for partial resistance, 36 for disease avoidance traits, and 16 for root traits, was generated. In summary, 13 QTL conferring resistance to white mold co-located with QTL conferring disease avoidance traits, six with strong and seven with weak associations. Canopy porosity and resistance to lodging was extremely important for reducing disease severity in both dry and snap bean (r = 0.61) across 11 trials conducted in MI, OR, and WA, from 2000 to 2011. Given the complexity of disease resistance as evidenced by the comparative QTL map, marker-assisted breeding for disease avoidance is not recommended at this time. Instead, selecting for resistance to white mold in the field, in combination with high yield potential and acceptable maturity, is the recommended strategy. Using the recently released genome assembly of common bean, we discovered that the WM8.3 peak QTL consisting of 13 co-segrating markers spans ~40Mb. Within that interval, is a gene that encodes for a receptor-like protein that is similar to some previously described disease resistance genes.

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