National Sclerotinia Initiative

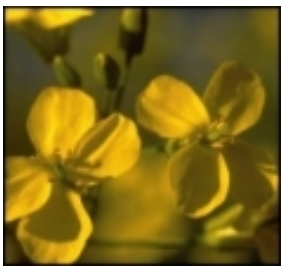
USDA-ARS

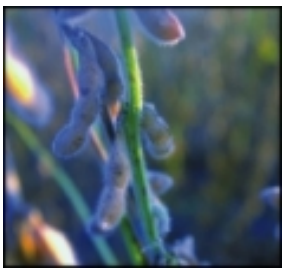
National Sclerotinia Initiative

2019 Annual Meeting

Fargo, ND

March 14-15, 2019



2019 National Sclerotinia Initiative Meeting

March 14-15, 2019

Radisson Hotel Fargo
201 North 5th Street, Fargo, ND

AGENDA

2019 National Sclerotinia Initiative Meeting
March 14-15, 2019

Thursday – March 14, 2019

11 am-1 pm	Registration & Poster Set-Up (lunch on your own) *(posters are displayed for the entire meeting)*	**City A**
1:00 pm	Welcome & Introductions – **Mike Grusak, USDA-ARS, Fargo, ND**	**City B**
1:10 pm	Welcome & Update from the Plains Area – **Bryan Kaphammer, USDA-ARS, Fort Collins, CO**	
1:20 pm	Welcome & Update from Office of National Programs – **Roy Scott, USDA-ARS, Beltsville, MD**	
	Sclerotinia Research Activities – Session 1 **Moderator – Phil Miklas, USDA-ARS, Prosser, WA**	**City B**
1:30 pm	Targeting essential genes in Sclerotinia sclerotiorum to achieve Sclerotinia stem rot resistance in soybean (Abstract p. 11, Poster #19) – **Mehdi Kabbage, University of Wisconsin, Madison, WI**	
1:50 pm	Characterizing pathogenicity effectors of Sclerotinia sclerotiorum preferentially expressed under acidic conditions and during plant infection (Abstract p. 26; Poster #9) – **Weidong Chen, USDA-ARS, Pullman, WA**	
2:10 pm	Biological Control of White Mold Using the Mycovirus SsHADV-1-Infected Hypovirulent Strain DT-8 of Sclerotinia sclerotiorum – **Weidong Chen and Zheng Qu, USDA-ARS, Pullman, WA**	
2:30 pm	Break	**City A**
3:00 pm	Identification of Sclerotinia sclerotiorum virulence determinants relevant to infection of multiple host plants by association mapping (Abstract p. 9) – **William Underwood, USDA-ARS, Fargo, ND**	
3:20 pm	Developing environmental friendly fungicides for managing white mold (Abstract p. 12, 21; Poster #2, #7) – **Shin-Yi Marzano, South Dakota State University, Brookings, SD**	
3:40 pm	Improved white mold resistance in dry and snap beans through multi-site screening and pathogen characterization throughout major production areas (Abstract P. 17, 22, 24; Poster #1, #3, #6, #15) – **Sydney Everhart, University of Nebraska, Lincoln, NE**	

4:30-5:30 pm	Break and Poster Session	**City A**
5:30-7:30 pm	Dinner & Posters	**City A**

Friday – March 15, 2019

7:00-8:00 am	Steering Committee Breakfast Meeting	**Metropolitan**
7:00 am	Continental Breakfast	**City A**

Sclerotinia Research Activities – Session 2 **City B**
Moderator – Shin-Yi Marzano, South Dakota State University, Brookings, SD

8:30 am	Enhancing soybean for resistance to Sclerotinia stem rot (Abstract p. 14; Poster #11) – **Zixiang Wen, Michigan State University, East Lansing, MI**
8:50 am	Fine mapping of loci for resistance to Sclerotinia stem rot in Glycine latifolia (Abstract p. 16; Poster #5) – **Leslie Domier, USDA-ARS, Urbana, IL**
9:10 am	Characterizing resistance and pathogenicity genes associated with infection of B. napus by S. sclerotiorum (Abstract p. 10; Poster #4, #13) – **Kishore Chittem, North Dakota State University, Fargo, ND**
9:30 am	Improving resistance to Sclerotinia sclerotiorum in spring canola (Abstract p. 13, 18; Poster #8, #10) – **Luis del Rio, Fereshteh Shahoveisi, and Jayanta Roy, North Dakota State University, Fargo, ND**
9:50 am	Enhancing basal resistance to Sclerotinia sclerotiorum in Brassica (Abstract p. 28, Poster #20) – **Chenggang Wang, University of Florida, Gainesville, FL**
10:10 am	Leveraging Arabidopsis genomic resources to identify genes governing quantitative resistance to Sclerotinia (Abstract p. 20) – **William Underwood, USDA-ARS, Fargo, ND**
10:30 am	Break

Sclerotinia Research Activities – Session 3 **City B**
Moderators – Mike Grusak, USDA-ARS, Fargo, ND; Bryan Kaphammer, USDA-ARS, Fort Collins, CO

11:00 am	Discussion Topic: *The Future of the National Sclerotinia Initiative*

Noon	Lunch	**City A**
Noon	Lunch and Poster Viewing	

	Sclerotinia Research Activities – Session 4 **City B** **Moderators – Sydney Everhart, University of Nebraska, Lincoln, NE**
1:20 pm	Refining genomic tools for *Sclerotinia* resistance and agronomic breeding of sunflower – towards dissection of the resistance phenotype (Abstract p. 23; Poster #16) – **Brent Hulke, USDA-ARS, Fargo, ND**
1:40 pm	QTL mapping of Sclerotinia basal stalk rot resistance derived from sunflower wild species (Abstract p. 25; Poster #17) – **Zahirul Talukder, USDA-ARS, Fargo, ND**
2:00 pm	Improving stalk rot phenotyping by evaluation of environment, pathogen, and host factors for S. sclerotiorum infection in sunflower disease nurseries (Abstract p. 19) – **William Underwood, USDA-ARS, Fargo, ND**
2:20 pm	Screening for resistance sources to Sclerotinia white mold in recently acquired germplasm of cool season grain legumes (Abstract p. 27, Poster #18) – **Weidong Chen, USDA-ARS, Pullman, WA**
2:40 pm	Coffee Break
3:10 pm	White mold resistance-QTL: identification, interactions, and fine mapping in common bean (Abstract p. 15, 29; Poster #12, #14) – **Phil Miklas, USDA-ARS, Prosser, WA; Jim Myers, Oregon State University, Corvallis, OR; Phil McClean, North Dakota State University, Fargo, ND**
4:10 pm	Meeting Wrap-Up & Future Plans **– Mike Grusak, USDA-ARS, Fargo, ND**
4:45 pm	Safe Travels Home or Networking at Local Restaurants

National Sclerotinia Initiative Poster Session

March 14-15, 2019
City A
Radisson Hotel Fargo

Epidemiology & Disease Management		
Poster No.	Title	Author(s)
1	Impact of spray droplet size on fungicide performance for management of white mold in soybeans and dry beans	M. Wunsch, J. Hafner, B. Kraft, K. Cooper, T. Miorini, S. Kallis
2	Metagenomic discovered soybean-leaf associated ssDNA virus infects and attenuates the virulence of white mold fungus	C. Feng, J. Feng, H. Saleem, S-Y Marzano
3	Optimizing fungicide applications through drop nozzles for management of white mold in soybeans and dry beans	M. Wunsch, J. Hanfer, B. Kraft, K. Cooper, T. Miorini, S. Kallis

Genomics		
Poster No.	Title	Author(s)
4	Characterization of two glyoxylate cycle pathway associated genes in pathogenicity of S. sclerotiorum	K. Chittem, S. Upadhaya, L. del Rio Mendoza
5	Fine mapping of loci for resistance to Sclerotinia stem rot in Glycine latifolia	L. Domier, N. McCoppin, T. Herman, Q. Liu, D. Chambers, G. Hartman

Pathogen Biology & Development		
Poster No.	Title	Author(s)
6	Fungicide sensitivity of populations of Sclerotinia sclerotiorum from soybean and dry bean in the U.S.	E. Nieto-Lopez, T. Miorini, S. Everhart
7	Heterologous expression of SlaGemV-1 mycovirus particle in planta to increase white mold resistance	Y. Fan, J. Feng, C. Feng, H. Saleem, S-Y Marzano
8	Phenotypic evaluation of the reaction of a Westar x PI436554 mapping population to Sclerotinia sclerotiorum	F. Shahoveisi, L. del Rio Mendoza
9	Sclerotinia sclerotiorum secretes an effector protein (SsE1) that specifically interacts with and mitigates the inhibitory effect of plant polygalacturonase-inhibiting proteins (PGIPs)	W. Wei. L. Xu., W. Chen

National Sclerotinia Initiative Poster Session

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City A
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Variety Development/Germplasm Enhancement		
Poster No.	Title	Author(s)
10	Effect of temperature on reaction of three Brassica napus plant introduction materials to Sclerotinia sclerotiorum	F. Shahoveisi, L. del Rio Mendoza
11	Enhancing soybean for resistance to Sclerotinia stem rot	Z. Wen, F. Lin, P. Collins, W. Li, C. Gu, M. Chilvers, D. Wang
12	Genetic improvement of dry bean for resistance to white mold using a MAGIC population	E. Escobar, P. Miklas, J. Osorno, P. McClean
13	Genome-wide associate study for Sclerotinia stem rot resistance in Brassica napus (L)	J. Roy, L. del Rio Mendoza, K. Chittem, M. Rahman
14	Field screening of recently identified sources of white mold resistance in common bean	J. Davis, J. Arkwazee, P. Miklas, P. McClean, J. Myers
15	New sources of white mold resistance derived from wide crosses in common bean and evaluated in the greenhouse and field using multi-site screening nurseries	R. Higgins, J. Steadman, S. Everhart
16	Refining genomic tools for Sclerotinia resistance and agronomic breeding of sunflower – towards dissection of the resistance phenotype	B. Hulke, N. Kane, J. Corwin
17	Sclerotinia basal stalk rot evaluation and QTL mapping in populations derived from wild annual sunflower species	Z. Talukder, W. Underwood, G. Seiler, X. Cai, L. Qi
18	Screening for and identification of resistance sources to Sclerotinia white mold in recently acquired germplasm lines of pea *(Pisum sativum)*	Y-C. Chen, C. Coyne, W. Chen
19	Targeting essential genes in Sclerotinia sclerotiorum to achieve Sclerotinia stem rot resistance in soybean	M. McCaghey, D. Shao, D. Smith, M. Kabbage
20	Understanding and improving basal resistance to Sclerotinia sclerotiorum	C. Wang, J. Rollins, Z. Mou

Association mapping of *Sclerotinia* genes contributing to aggressiveness on sunflower

Kassaye Belay[1], Roshan Sharma-Poudel[1], Berlin Nelson[1], Robert Brueggeman[1], and William Underwood[2]

[1]Department of Plant Pathology, North Dakota State University, Fargo, ND
[2]USDA-ARS, Northern Crop Science Laboratory, Fargo, ND

Funded Plan of Work: Identification of *Sclerotinia sclerotiorum* virulence determinants relevant to infection of multiple host plants by association mapping

ABSTRACT:

Sclerotinia sclerotiorum is one of the most economically important pathogens causing disease on sunflower in the primary US growing region in the Northern Great Plains. This pathogen causes three distinct diseases on sunflower. Head rot and mid-stalk rot are initiated by airborne ascospores, whereas basal stalk rot is initiated by mycelial invasion of root tissues. *S. sclerotiorum* exhibits an unusually broad host range of over 400 plant species. Though some progress has been made to identify virulence factors used by *S. sclerotiorum* to cause disease, it is not clear why this fungal pathogen is so successful in causing disease on such a large number of plant species. The goal of this project is to use a genome-wide association mapping approach to identify loci responsible for differences in aggressiveness among *S. sclerotiorum* isolates, with the assumption that the functions of the underlying genes will reveal novel insights into *S. sclerotiorum* pathogenicity. We assembled a collection of over 250 diverse isolates of *S. sclerotiorum* collected from numerous plant hosts and locations throughout the US. We have evaluated this isolate collection for aggressiveness of stem lesion formation, measured by lesion length, on two USDA sunflower inbred lines using a greenhouse assay. In parallel, we performed genotyping-by-sequencing on 227 of the isolates to discover ~6000 SNP markers which were used for genome-wide association mapping to identify candidate genes associated with aggressiveness on sunflower stem tissue. These efforts have identified a total of 15 makers significantly associated with aggressiveness representing 9 distinct loci. In the final year of this project, we are evaluating candidate genes at several loci for a role in pathogenicity by marker-replacement mutagenesis, focusing on genes that are differentially regulated during infection.

Contact Information – Dr. William Underwood, Sunflower and Plant Biology Research Unit, USDA-ARS Northern Crop Science Laboratory, 1616 Albrecht Blvd., Fargo, ND USA 58102-2765; (701) 239-1316, william.underwood@ars.usda.gov

Characterization of two glyoxylate cycle pathway associated genes in pathogenicity of *S. sclerotiorum*

Kishore Chittem, Sudha G.C. Upadhaya and Luis del Río Mendoza, North Dakota State University, Fargo, ND 58102.

Funded Plan of Work: Characterizing resistance and pathogenicity genes associated with infection of *B. napus* by *S. sclerotiorum*

ABSTRACT:

The objective of this project has been to characterize putative resistance and pathogenicity genes associated with infection of *B. napus* by *S. sclerotiorum.* From differential gene expression data, genes involved in peroxisome associated pathways like fatty acid ß-oxidation and glyoxylate cycle were identified as novel potential pathogenicity candidates. The role of these pathways/genes has been shown to be essential for pathogenicity in several plant pathogens, including *Leptosphaeria maculans*, another important pathogen of canola. Based on these findings, two key glyoxylate pathway genes, isocitrate lyase (SS1G_04975) - *SsICL* and malate synthase (SS1G_05583) – *SsMLS1* were selected as candidate pathogenicity genes for functional characterization. Gene disruption mutants for *SsICL* and *SsMLS1* were developed by targeted gene replacement following split marker approach. PCR assays confirmed correct replacement of the genes of interest. The effect of deletion of these genes did not result in significant reduction in mycelial growth on potato dextrose medium compared to the wild-type. Results from the virulence/pathogenicity assays on canola using detached leaf assays will be presented.

Contact Information – Dr. Luis del Rio, Dept. of Plant Pathology, North Dakota State University, Dept. 7660 P.O. Box 6050, Fargo ND 58108-6050; (701) 231-7073; luis.delriomendoza@ndsu.edu

Controlling *Sclerotinia sclerotiorum* in *Glycine max* by Targeting Oxalic Acid Production Using Host-Induced Gene Silencing

Megan McCaghey, Dandan Shao, Damon Smith, Mehdi Kabbage. University of Wisconsin-Madison, Madison, WI

Funded Plan of Work: Targeting essential genes in *Sclerotinia sclerotiorum* to achieve sclerotinia stem rot resistance in soybean

ABSTRACT:

Sclerotinia sclerotiorum, the causal agent of Sclerotinia stem rot (SSR), is a yield-limiting, fungal pathogen of *Glycine max*. The pathogenic success of *S. sclerotiorum* requires the secretion of oxalic acid (OA), a virulence factor for this pathogen. Virus-induced gene silencing (VIGS) using *Bean pod mottle virus* (BPMV) was used to target OA biogenesis in *S. sclerotiorum*. A sequence of 366 bp, corresponding to the fungal oxaloacetate acetylhydrolase (*Ssoah1*), a key enzyme in OA biogenesis, was cloned into a BPMV vector in an antisense orientation. BPMV constructs were introduced into *G. max* using particle bombardment, and viral symptoms paired with RT-PCR were used to confirm viral replication prior to inoculation. Disease progress and target mRNA levels were monitored over a five-day period. Remarkably, plants containing BPMV vectors targeting Ss*oah*1 showed enhanced resistance to *S. sclerotiorum* compared to empty-vector control plants, in three replicated experiments. This phenotype was coupled by decreased expression of the target gene as determined by RT-PCR. These results provide evidence supporting host-induced gene silencing targeting virulence factors as a viable strategy to control SSR. Our study also evaluated the uptake of dsRNA and sRNA by *S. sclerotiorum*. Using fluorescence microscopy, we clearly show the uptake of labeled RNAs by the fungus, suggesting that the exogenous application of these molecules can also be used to target gene expression. Genetic resistance to SSR is inadequate in commercial soybean varieties, and fungicidal control can be inconsistent and expensive. Herein, we propose that RNAi strategies will provide new tools to control *S. sclerotiorum* in *G. max*.

Contact Information – Dr. Mehdi Kabbage, University of Wisconsin-Madison, 1630 Linden Drive, Madison, WI 53705; (608) 262 0506; kabbage@wisc.edu

Dissecting RNA Silencing Pathways in *Sclerotinia sclerotiorum*

Shin-Yi Lee Marzano

South Dakota State University, Brookings, SD

Funded Plan of Work: Developing Environmental Friendly Fungicides for Managing White Mold

ABSTRACT:

Recent studies have demonstrated that pathogenic fungi can use RNA silencing to silence host immunity genes through the delivery of small RNAs in addition to antiviral defense. This cross-kingdom RNA silencing is bidirectional with plant hosts capable of delivering small RNAs into fungal cells to silence fungal genes. These findings suggest the importance of RNA silencing mechanisms play in white mold growth and virulence. This study aimed to demonstrate the existence of antiviral RNA silencing mechanisms in *Sclerotinia sclerotiorum* by infecting wild-type and RNA-silencing-deficient strains of the fungus with an RNA virus and a DNA virus. Key silencing-related genes were disrupted to dissect the RNA silencing pathway. Specifically, dicer genes (*dcl*-1, *dcl*-2, and both *dcl*-1/*dcl*-2) were displaced by selective marker(s). Disruption mutants were then compared for changes in phenotype, virulence, and susceptibility to virus infections. Wild-type and mutant strains were transfected with a single-stranded RNA virus, SsHV2-L, and copies of a single-stranded DNA mycovirus, SsHADV-1, as a synthetic virus constructed in this study. Disruption of *dcl*-1 or *dcl*-2 resulted in no changes in phenotype compared to wild-type *S. sclerotiorum*; however, the double dicer mutant strain exhibited significantly slower growth. Furthermore, the Δ*dcl*-1/*dcl*-2 double mutant, which was slow growing without virus infection, exhibited much more severe debilitation following virus infections including phenotypic changes such as slower growth, reduced pigmentation, and delayed sclerotial formation. These phenotypic changes were absent in the single mutants, Δ*dcl*-1 and Δ*dcl*-2. Complementation of a single dicer in the double disruption mutant reversed viral susceptibility to the wild-type state. Furthermore, both the single Δ*agl*-2 and Δ*agl*-4 mutants had significantly slower growth and reduced virulence prior to and following virus infection. Additional studies indicated that the virus-infected wild-type strain accumulated virus-derived small RNAs (vsiRNAs) with distinct patterns of internal and terminal nucleotide mismatches, indicating RNA editing. The findings of these studies will broaden our understanding of RNA silencing and RNA-editing pathways in *S. sclerotiorum* and shed light on how these pathways can be exploited in the development of robust technologies to manage the spread and virulence of this fungal plant pathogen.

Contact Information – Shin-Yi Lee Marzano, 2140 North Campus Drive, SNP 252 Box 2140D, Brookings, SD 57007-2142; Shinyi.Marzano@sdstate.edu

Effect of temperature on reaction of three *B. napus* plant introduction materials to *Sclerotinia sclerotiorum*.

Fereshteh Shahoveisi and Luis E. del Río Mendoza, Department of Plant Pathology, North Dakota State University, Fargo, ND 58108

Funded Plan of Work: Improving resistance to *Sclerotinia sclerotiorum* in spring canola

ABSTRACT:

One of the objectives of this project is to characterize the resistance present in plant introduction materials 436554 and 458940 and transfer it into elite canola breeding lines. Characterization will be accomplished by identifying molecular markers associated with resistance and by evaluating the effect of incubation temperatures on the reaction of these lines. A doubled haploid mapping population derived from the cross of 436554 and Topas will be ready for phenotypic evaluation in the spring of 2019. A second mapping population, derived from the cross between 436554 and Westar, was advanced to the F_5 generation using single-seed descent. DNA was extracted from plants from this mapping population and will be sent for genotyping. Phenotypic data from this population will be collected in early 2019. To evaluate the effect of incubation temperature on disease development, a replicated trial was conducted using lines 436554, 458940, 633119 and the commercial hybrid 45H28. Lesion sizes were measured at two-day intervals 2-12 days after inoculation on plants incubated at 10, 25, and 30 ºC. Accessions 436554 and 458940 had significantly smaller lesions than the commercial hybrid when incubated at 10 and 25 ºC but lesions were similar at 30ºC. Interestingly, accession 633119 had statistically larger lesions than the other accessions and the hybrid when incubated at 30 ºC. We are conducting another set of experiments to confirm these results.

Contact Information – Dr. Luis del Rio, Dept. of Plant Pathology, North Dakota State University, Dept. 7660 P.O. Box 6050, Fargo ND 58108-6050; (701) 231-7073; luis.delriomendoza@ndsu.edu

Enhancing soybean for resistance to Sclerotinia stem rot

Zixiang Wen, Feng Lin, Paul J. Collins, Wenlong Li, Cuihua Gu, Martin Chilvers, and Dechun Wang, Department of Plant, Soil and Microbial Sciences, Michigan State University

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

ABSTRACT:

Sclerotinia stem rot of soybean is caused by *Sclerotinia sclerotiorum*, which is capable of infecting a wide range of plants. To enhance soybean for resistance to this disease, marker-assisted selections (MAS) were carried out for screening $F_{2:3}$ lines derived from 8 cross combinations between new resistance sources and elite cultivars. After MAS, a total of 518 $F_{3:4}$ lines enriched with resistance alleles were selected and undergone visual selection through single-row-plot trials in 2018. Moreover, to enlarge training population size for genomic selection, 80 advanced breeding lines were evaluated for white mold resistance in a disease nursery and were genotyped with SoySNP6K BeadChip. To date, genotypic and disease severity index (DSI) of 779 soybean lines have been acquired. Accuracies of genomic predictions value of DSI ranged from 0.55 to 0.60 among different non-parametric or parametric methods. However, no significant differences were observed in the prediction accuracy among these methods. The effect of marker number on accuracy began to plateau around 1600. Moreover, the diminishing return from increasing population size has been observed after the size surpass 400, but accuracy steadily climbed until the largest possible size (779) was used. Incorporating molecular data-driven decisions in soybean breeding holds good potential to expedite genetic gain of selection for resistance to Sclerotinia stem rot.

Contact Information – Dr. Dechun Wang, 1066 Bogue St., Rm. A384E, East Lansing, MI 48824-1325; 1-517-355-0271 Ext. 1188; wangdech@msu.edu

Field Screening of Recently Identified Sources of White Mold Resistance in Common Bean

Joel Davis[1], Haidar Arkwazee[2], Phil Miklas[3], Phil McClean[4], and James R. Myers[1]

[1]Department of Horticulture, Oregon State University, Corvallis, OR USA
[2] Department of Horticulture, University of Sulaimani, Iraq;
[3]USDA-ARS, Grain Legume Genetics and Physiology Research Unit, Prosser, WA USA
[4]North Dakota State University, Fargo, ND USA

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

ABSTRACT:

Snap bean shows a higher level of susceptibility to white mold compared to dry bean. This group is also a potential source of resistance QTL not found in dry bean. Our overall goal is to identify, evaluate and use resistance QTL from this germplasm pool. In 2018, a F5:7 recombinant inbred population (Unidor/OSU5630; n = 184) was screened in the field at the OSU Vegetable Research Farm near Corvallis, OR for resistance to white mold. The population exhibited a normally distributed reaction to white mold, with 19 lines showing a disease severity index (DSI) of 6 or less (DSI is the geometric mean of incidence and severity and ranges from 0 – 30 where 0 is highly resistant). A QTL analysis of the population is in process. This population was screened in 2017 for resistance using the straw test in the greenhouse. A single QTL on Pv03 was observed. This population has excellent architecture which may contribute to avoidance in the field. A nested association mapping (NAM) panel was also evaluated in the field for white mold resistance. The panel consists of four populations (Cornell 501/WMG904-20-3, n = 56; NY6020-4/WMG904-20-3, n = 69; M0070/WMG904-20-3, n = 60 and WMG904-20-3/A195, n = 62) totaling 247 lines with the common parent WMG904-20-3. Two populations in particular (Cornell 501/WMG904-20-3 and WMG904-20-3/A195 had greater than 50% of the lines with a DSI of nine or less. Genotyping of the NAM panel is underway.

Contact Information – Dr. Jim Myers, Department of Horticulture, Oregon State University, Corvallis, OR 97331; 541-737-3083 james.myers@oregonstate.edu

Fine mapping of loci for resistance to Sclerotinia stem rot in the wild perennial *Glycine latifolia*

Leslie L. Domier[1], Nancy K. McCoppin[1], Theresa K. Herman[2], Qiong Liu[2], Donald S. Chambers[2], and Glen L. Hartman[1], [1]USDA-ARS, [2]Department of Crop Sciences, University of Illinois, Urbana, IL 61801

Funded Plan of Work: Fine mapping of loci for resistance to Sclerotinia stem rot in *Glycine latifolia*

ABSTRACT:

Sclerotinia stem rot, caused by *Sclerotinia sclerotiorum*, is a damaging disease of soybean (*Glycine max*). Partial resistance to the disease is available in the soybean primary gene pool, but higher levels of resistance to Sclerotinia stem rot can be found among soybeans wild perennial relatives. Because it has been possible to recover fertile recombinant hybrid plants between soybean and only one perennial *Glycine* species, it has not been possible to utilize those genes for resistance to Sclerotinia stem rot or other pathogens and pests in soybean improvement. Advances in high-throughput genome sequencing and gene mapping along with gene editing technologies provide mechanisms to bypass barriers to standard genetic hybridization. Selected accessions of *Glycine latifolia*, one of soybean's 26 perennial relatives, show high levels of resistance to Sclerotinia stem rot. To map loci for resistance to Sclerotinia stem rot in *G. latifolia*, populations of 324 F6 recombinant inbred lines (RILs) and over 3000 individual F2 seed were produced from crosses between *G. latifolia* plant introductions resistant and susceptible to Sclerotinia stem rot. Four replications of the RIL population were evaluated for sensitivity to oxalic acid, a pathogenicity determinant for *S. sclerotiorum*, using stem cuttings of greenhouse-grown plants. Evaluations of the RIL population for sensitivity to inoculation with *S. sclerotiorum* are ongoing.

Contact Information – Dr. Leslie Domier, USDA-ARS, Department of Crop Sciences, University of Illinois, 1102 S. Goodwin Ave., Urbana, IL 61801; 217-333-0510; leslie.domier@ars.usda.gov

Fungicide sensitivity of populations of *Sclerotinia sclerotiorum* from soybean and dry bean in the U.S.

Edgar H. Nieto-Lopez[1], Thomas J. J. Miorini[2], and Sydney E. Everhart[1]
[1]University of Nebraska-Lincoln; [2]University of North Dakota State

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:

Management of white mold disease in soybean and dry beans, caused by *Sclerotinia sclerotiorum*, is based on the integration of several methods, including fungicide application as a cost-effective tool. Lack of sensitivity to fungicides dimethachlon and metconazole has been reported in *S. sclerotiorum* populations. Although *S. sclerotiorum* is predicted to have low risk of resistance development based on *expected* population genetic variability, intensive use of fungicides can select for fungicide resistance. Our recent work showed that a discriminatory concentration method (DC) was a more reliable method over use of a spiral gradient. Thus, our present study was conducted to develop a DC for *S. sclerotiorum* that could be used to predict the effective concentration of 50% inhibition (EC_{50}) for assessment of sensitivity from a large collection of *S. sclerotiorum* isolates. The first step was to determine the baseline sensitivity of *S. sclerotiorum* by evaluation and comparison fungicide sensitivity of isolates with and without fungicide field exposure. We selected 42 isolates for this step: 15 from soybean fields, 6 from soybean fields known to have fungicide exposure, and 21 from the dry bean white mold nurseries used for our multi-site screening. Sensitivity to thiophanate methyl (TM; FRAC-group 1), tetraconazole (T; FRAC-group 3), boscalid (B; FRAC-group 7), and picoxystrobin (P; FRAC-group 11), was determined using serial dilution of 5 concentrations. A dose-response curve was fit to estimate the EC_{50} for each fungicide. The second step was to identify which of the five concentrations was the best predictor of EC_{50} to serve as the DC. We used linear regression of percent mycelial growth vs. log-EC_{50} at each concentration. The DC's were 10 for TM, 2.0 for T, 0.2 for B, and 0. 01 for P (ppm). In the case of TM, concentrations below 10 ppm were unreliable from one experiment to the next, whereas growth at 10 ppm was similar and was thus selected as the DC. The third step is to use the DC for representative isolates from farmer fields, dry bean nurseries, and fungicide-exposed isolates from field trials. Preliminary results were generated using 154 isolates, which included: 86 from dry beans (from 9 states and 2 states from Brazil, with 1-17 isolates) and 66 from soybeans (6 NE, 18 IA, 13 WI & 18 MI and 4 from Mexico). Comparison of isolates by state showed no statistical difference, however, some isolates showed reduced sensitivity. Due to heterogeneity among isolates, further investigation is needed to obtain complete set of at least 10 isolates per field before conclusions can be drawn.

Contact Information – Dr. Sydney Everhart and Dr. James R. Steadman, Department of Plant Pathology, University of Nebraska, 406 Plant Science Hall, Lincoln, NE 68583-0722, 402-472-2879, everhart@unl.edu.

Genome-Wide Association Study for Sclerotinia Stem Rot Resistance in *Brassica napus* (L)

Jayanta Roy[1], Luis del Río Mendoza[2], Kishore Chittem[2], and Mukhlesur Rahman[1]

[1]Department of Plant Sciences, North Dakota State University, Fargo, ND
[2]Department of Plant Pathology, North Dakota State University, Fargo, ND

Funded Plan of Work: Improving resistance to *Sclerotinia sclerotiorum* in spring canola

ABSTRACT:

Sclerotinia stem rot (SSR) is one of the most destructive fungal diseases of canola caused by *Sclerotinia sclerotiorum* (Lib) de Bary, that significantly reduce seed yield as well as oil content and quality. In North Dakota, average yield losses have been estimated at 13-50%, and economic losses estimated up to 20.8 million dollars due to this disease. Since there is no completely resistant varieties available, identification of resistant genotypes and genes in diverged germplasm accessions is one of the best options to develop durable disease resistant cultivar for the growers. In the present study, a panel of 218 germplasm accessions originated from 29 countries were evaluated in a controlled environment using RCBD with three replications and five plants were grown in each replicates, to identify potential resistant germplasm. The germplasm was evaluated using single isolate (WM#031) by mycelial stem inoculation at flowering stage. For each accession, lesion length (cm) and width (%), along with a visual estimation of the percentage of the main stem that is girdled by the lesion, and the number of dead plants were recorded 3, 5, 7, 9, 11, 13 and 15 days after inoculation. Mean stem lesion length, width and mortality percentage were calculated to evaluate each accession. Seven promising lines have been identified as tolerant based on lower stem lesion length, the percentage of stem girdling and mortality. The germplasm accessions have been genotyped using Illumina genotyping-by-sequencing (GBS) platform at the Institute for Genomic Diversity at Cornell University, and 42,575 single nucleotide polymorphisms have been identified. Finally, genome-wide association study will be conducted using all three evaluated phenotypic values to identify the genomic region containing SSR resistant genes in *B. napus.*

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Improving sunflower phenotyping for resistance to basal stalk rot caused by *S. sclerotiorum*

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Funded Plan of Work: Improving stalk rot phenotyping by evaluation of environment, pathogen, and host factors for S. sclerotiorum infection in sunflower disease nurseries

ABSTRACT:

Basal stalk rot (BSR) of sunflower is relatively unusual among diseases of crop plants incited by *Sclerotinia sclerotiorum.* BSR is a root rot initiated upon invasion of sunflower roots by mycelium derived from myceliogenic germination of sclerotia in the soil. This mode of infection is not observed for other affected crop plants, with the exception of "lettuce drop" disease caused by both *S. sclerotiorum* and *S. minor.* As a consequence of the unique mode of infection, evaluation of sunflower germplasm and experimental materials for BSR resistance requires inoculation of the root zone with *S. sclerotiorum* mycelium. The ARS Sunflower & Plant Biology Research Unit has previously developed an inoculation method for field-scale evaluation of sunflower materials for BSR resistance using custom-build equipment to deposit *Sclerotinia*-infested millet seed into a furrow next to sunflower plants. This technique is capable of producing sufficient and even disease pressure in some years. However, the reliability of this method is relatively poor and frequent failures to produce adequate disease pressure are encountered. Additionally, plant vigor and time to flowering or maturity also appear to affect disease development in field nurseries. The initial goal of this project was to evaluate potential improvements to field BSR screening and we also initiated subsequent efforts to develop an alternative, greenhouse-based method for BSR evaluation. During the 2018 field season, we repeated several alterations and amendments to the field inoculation procedure. However, these efforts did not significantly impact disease pressure. In addition, we successfully developed a sensitive and quantitative greenhouse evaluation for BSR. Using a panel of 30 sunflower genotypes for which prior field screening data were available, we have demonstrated that the greenhouse procedure generally follows field results and allows for better statistical separation of highly and moderately resistant genotypes. The new greenhouse BSR methods should be useful for future germplasm evaluations and the approach is of sufficiently high throughput to facilitate evaluation of genetic mapping populations.

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Leveraging *Arabidopsis* genomic resources to identify genes governing quantitative resistance to *Sclerotinia*

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Funded Plan of Work: Not funded through competitive pool, Sclerotinia Initiative base

ABSTRACT:

Sclerotinia sclerotiorum is a necrotrophic fungal pathogen capable of causing disease on an exceptionally broad range of host plants, including many agriculturally important crop species. Resistance to *S. sclerotiorum* is quantitative and involves alleles of many genes contributing small effects, in contrast to the gene-for-gene type of resistance observed for many biotrophic and hemi-biotrophic phytopathogens. Consequently, mapping of loci contributing to resistance is challenging and the identities and functions of genes governing resistance to *Sclerotinia* are mostly unknown. The goal of this project is to utilize the genomic and functional genetic resources available for the model dicot *Arabidopsis thaliana* to identify and functionally characterize genes conferring *Sclerotinia* resistance. We have phenotyped an *Arabidopsis* diversity panel comprised of 325 accessions for resistance to two isolates of *S. sclerotiorum*. We evaluated all accessions for resistance at two time-points after leaf inoculation with the sequenced reference isolate, 1980, as well as a second, less aggressive isolate, BN325. As anticipated for the quantitative nature of resistance typical for this pathogen, we observed a continuous distribution of resistance/susceptibility, with a small number of accessions exhibiting relatively high levels of partial resistance. We have used our resistance phenotyping data in conjunction with publicly available SNP markers to conduct genome-wide association mapping of *Sclerotinia* resistance. In total, across both time-points and isolates, we identified 36 loci significantly associated with resistance. Unexpectedly, only a single locus was significantly associated with resistance to both isolates. Currently, we are attempting to functionally validate candidate genes at a subset of associated loci using available T-DNA insertional mutants. We anticipate that his dataset will be useful to identify plausible candidate genes within mapped intervals for affected crop species and to potentially develop new strategies for more efficient resistance breeding efforts.

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Metagenomic discovered soybean-leaf associated ssDNA virus infects and attenuates the virulence of white mold fungus

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Funded Plan of Work: N/A

ABSTRACT:

Soybean leaf-associated gemycircularvirus 1 (SlaGemV-1) is a single-stranded circular DNA virus belonging to a newly established Family *Genomoviridae* that has the potential of extracellular transmission and causes hypovirulence in fungi. In this study, we aimed to characterize SlaGemV-1 which was originally identified by metagenomics survey of soybean leaves without known fungal hosts. As SlaGemV-1 shares 55% of its coat protein sequence with *Sclerotinia sclerotiorum* hypovirulence associated DNA virus 1 (SsHADV-1), SlaGemV-1 was rescued and tested to determine if SlaGemV-1 is infectious in *Sclerotinia sclerotiorum*. Lacking the actual virion for further characterization, two infectious clones were constructed to rescue the virus. Both infectious clones were discovered to be replicating in *S. sclerotiorum.* Furthermore, SlaGemV-1 significantly reduced the virulence of *S. sclerotiorum* demonstrated by leaf assays. The study fulfilled Koch's postulates and establishes a rare reverse genetic system for studying ssDNA virus/fungal host interaction. Additionally, SlaGemV-1 is expected to be stable in the environment and capable of extracellular transmission, which is an important criterion for developing a biocontrol strategy. Because SsHADV-1 is notably transmitted by fungal-feeding insect vectors, we hypothesized that SlaGemV-1 also infects insects for effective transmission in nature. For the first time, we also report that mycoviruses SlaGemV-1 and SsHADV-1 replicate in bacteria, and that future efforts in realizing the potential of producing virocontrol biopesticides are warranted.

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New sources of white mold resistance derived from wide crosses in common bean and evaluated in the greenhouse and field using multi-site screening nurseries

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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:

The goal of our work over the past two years is to identify putative sources of resistance to white mold in adapted backgrounds at multiple sites located in most of the major bean-production areas of the northern states. This is the third year of our project funding. Our approach combines evaluations in greenhouses using a straw test method that is consistent in identification of sources of resistance in adapted and non-adapted bean germplasm and a multi-site field evaluation at white mold nurseries in several locations throughout the major dry bean production regions of the United States. A benefit of the straw test is that it requires only 24 bean seeds per site (~200 seeds) for evaluations to confirm resistance. A benefit of our multi-site field testing is that it mitigates variability in weather from site-to-site that can hinder effective evaluations. In 2018, breeders sent 11 lines for evaluations, which were pinto, navy, black, small red, and white kidney bean seed classes. Greenhouse trials were conducted using these 11 lines, plus the controls (G122, Beryl, and Bunsi). In preliminary analysis of greenhouse data from three sites, none of the tested lines had better straw test ratings than the resistant control, G122. However one pinto line, identified in the 2017 testing as having significantly reduced disease ratings, was again rated as having better performance than all other test lines outside the resistant control. Field trial preliminary data from six sites did not show a significant difference in lines (ANOVA; $p = 0.051$). Two lines (N14229, a navy, and NE5-16-101, a pinto) had lower disease ratings than Bunsi for the second year in a row, although were not rated lower than G122. Collectively, these preliminary results suggest these lines with disease resistance in the greenhouse show promise for increased disease resistance in the field. Over the past two years, we have evaluated a total of 15 bean lines in the greenhouse and/or multi-site fields, which is important for identification of new sources of resistance to white mold.

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Refining genomic tools for Sclerotinia resistance and agronomic breeding of sunflower – towards dissection of the resistance phenotype

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Funded Plan of Work: Refining genomic tools for Sclerotinia resistance and agronomic breeding of sunflower – towards dissection of the resistance phenotype.

ABSTRACT:

Sunflower breeding has made huge gains in disease resistance and quality traits that are simply inherited, but lacks efficiency to adequately deal with Sclerotinia resistance, because of the complex genetic architecture. Many genes of small effect must work in concert to facilitate partial resistance. Lines exist with high levels of resistance in the field, as demonstrated by recent germplasm evaluations. They do not, however, bring the most favorable yield and agronomic characteristics to sunflower hybrids. The primary goal of this work is to better balance the intensity and efficiency of selection for Sclerotinia resistance and other agronomic traits, to make more breeding progress per generation on all traits proportional to their actual value to the producer. Genomic Selection (GS) is a new statistical technology we would like to investigate for this purpose. Up to this point in the project, we have used GBS or skim sequencing to genotype all of the lines we have evaluated for yield, agronomic traits, Phomopsis stalk canker, and Sclerotinia head and stalk rots since 2008. We have found that trio imputation with 5 to 10x whole genome shotgun data from parental lines can result in dense data sets for breeding populations, and that for single populations, can result in around 2000-3000 markers that show almost all of the recombination history in a biparental population with additional redundancy to account for genotyping error. We are adjusting our bioinformatics pipeline to better deal with large amounts of genotyping data, making it more efficient. We recently received new genomic data on our association mapping population and have increased coverage of our association analyses from 8,000 to 3 million markers, more than enough to cover regions which were not densely covered before. Our report will provide the most up-to-date knowledge from these investigations.

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Residual effect of fungicides applied for flower protection and control of Sclerotinia stem rot of soybean

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ABSTRACT:

Boscalid, picoxystrobin, tetraconazole, and thiophanate methyl are labeled in the U.S. to manage Sclerotinia stem rot (SSR), with application recommended at bloom to prevent flower infection. Since not all flowers are open at the time of application, the present study was carried out to characterize residual activity of four systemic fungicides over time. Soybean 'William 82' was grown under controlled conditions and fungicides were applied 30 days after emergence with 187 L ha^{-1} associated with nonionic surfactant (0.25% v/v), plus the control. Entire plants were collected at 1, 2, 4, 6, 8 and 10 days after application. Leaves of plants were removed and three mature (white) flowers per plant were inoculated with 10µl water with 5,000 ascospores of a single isolate of *S. sclerotiorum*. Plants were incubated at room temperature and disease severity evaluated daily at 6 to 15 days after inoculation and number of sclerotia counted. Area under the disease progress curve (AUDPC) of disease severity showed all fungicide applications reduced disease compared to the control at each day after application. To compare residual activity over time, AUDPC was converted to percent inhibition for each fungicide and linear regression performed. Boscalid and tebuconazole applications showed some evidence of systemicity, because of sustained disease suppression over time compared to fungicide thiophanate methyl. Sclerotia formation was reduced after all fungicide application, although results were inconsistent. Preliminary results suggest picoxystrobin may reduce formation of *S. sclerotiorum* sclerotia formation at 6 days after fungicide application ($p < 0.10$), which would be an important consideration for long-term disease management.

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Sclerotinia basal stalk rot evaluation and QTL mapping in populations derived from wild annual sunflower species

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Funded Plan of Work: QTL mapping of Sclerotinia basal stalk rot resistance derived from sunflower wild species

ABSTRACT:

In 2018, we completed the second year Sclerotinia basal stalk rot (BSR) screening trials of the three mapping populations derived from crosses of three wild annual sunflower species, *H. petiolaris* (Pop1, 174 BC_2F_4 families), *H. praecox* (Pop2, 174 BC_2F_4 families), and *H. argophyllus* (Pop3, 134 BC_1F_4 families) with cultivated sunflower lines. Field screening trials were conducted at Carrington, ND using randomized incomplete block design with three replications. Highly significant (p<0.001) genetic variations were observed for BSR disease incidence (DI) in all three populations in both individual and combined analysis. Spearman rank correlations were also highly significant (p<0.001) between DI scores of 2017 and 2018 (ρ= 0.34, 0.53, and 0.50 for Pop1, Pop2 and Pop3, respectively), suggesting a high degree of repeatability of the trials. The BSR DI data of Pop1 followed a normal distribution in both 2017 and 2018 seasons with mean DI of 51.9 and 47.5%, ranging from 4.8-88.6% and 2.2-83.9%, respectively. Combined analysis revealed no significant genotype × environment (G×E) interaction for the trait. However, the mean BSR DI scores were low for Pop2 in both 2017 (DI 12.4%) and 2018 (DI 21.8%) seasons, ranging from 0-51.5% and 0-71.0%, respectively. Combined analysis revealed significant (p= 0.013) G×E interaction for the trait. In 2018, the Pop2 was also screened in the greenhouse with three replications under high disease pressure. The repeatability of the greenhouse trials was also highly significant (p<0.001) with ρ = 0.38 and 0.40, respectively for disease rating and area under disease progress curve (AUDPC). The mean DI of Pop3 were 20.3% and 32.5%, ranging from 0-72.3% and 0-78.5%, respectively in 2017 and 2018 seasons with no significant G×E interaction observed in this population for the trait.

A genetic linkage map was developed for Pop2 using 1,328 SNP markers developed by GBS technology on 17 linkage groups (LGs) spanning 1,990.37 cM. Quantitative trait loci (QTL) analyses were performed using BSR DI data from the field trials and disease rating and AUDPC data from the greenhouse tests. Preliminary analyses identified QTL on twelve LGs associated with BSR resistance for the three data sets. QTL for BSR DI were detected in 16 genomic regions, seven of these regions had QTL detected in both years. Combined analysis also detected QTL in five of the seven genomic regions. QTL for greenhouse trials were detected in seven genomic regions of which QTL for both AUDPC and disease rating were detected in three genomic regions. Only two genomic regions on LGs 1 and 2 shared QTL from both field and greenhouse tests. Further refining of the QTL analysis will be performed when more phenotypic data become available.

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Sclerotinia sclerotiorum Secretes an Effector Protein (SsE1) that Specifically Interacts with and Mitigates the Inhibitory Effect of Plant Polygalacturonase-Inhibiting Proteins (PGIPs)

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Funded Plan of Work: Characterizing pathogenicity effectors of *Sclerotinia sclerotiorum* preferentially expressed under acidic conditions and during plant infection

ABSTRACT:

Sclerotinia sclerotiorum causes the necrotrophic disease white mold on more than 400 plant species including many economically important crops such as common bean, canola, soybean, sunflower and pea, chickpea and lentil. The most prominent symptom of white mold is maceration of host tissue, resulting in soft rot, due to degradation of cell wall component pectin. *S. sclerotiorum* produces a number of polygalacturonases (SsPGs) that degrade the major plant cell wall component pectin. In defending fungal infection, plants have evolved to produce polygalacturonase- inhibiting proteins (PGIPs) that bind to pathogen's PGs and inhibit PG activity in protecting plant cell wall from fungal PG degradation. The functions of PGs in fungal virulence and PGIPs in plant defense have been well documented. However, there have been no reports of pathogens' ability to counteract plant PGIPs. We have identified a small effector peptide (SsE1) of *S. sclerotiorum* that specifically interacts with *Arabidopsis thaliana* AtPGIP1, through a variety of techniques such as yeast two-hybrid (Y2H), co-immunoprecipitation (co-IP) and bimolecular fluorescence complementation (BiFC) assays. *SsE1* expression is significantly induced during early stages of infection. Targeted deletion of *SsE1,* as well as of *SsPG1,* resulted in significant reductions in virulence. In enzymatic assays using purified SsE1, SsPG1 and AtPGIP1 from heterologous expression in yeast cells, SsE1 reduced effectiveness of AtPGIP1 in inhibiting SsPG1 activity in degradation of polygalacturonic acid. SsE1 itself had no enzymatic activity of polygalacturonase. Over expression of *SsE1* in *Arabidopsis thaliana* plant increased susceptibility to fungal infection. Our results show that *S. sclerotiorum* secretes a special effector that interferes with plant PGIP, mitigating PGIP inhibitory effect and enhancing fungal PG activity in supporting maceration effect of *Sclerotinia* infection.

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Screening for and identification of resistance sources to Sclerotinia white mold in recently acquired germplasm lines of pea (*Pisum sativum*)

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Funded Plan of Work: Screening for resistance sources to Sclerotinia white mold in recently acquired germplasm of cool season grain legumes

ABSTRACT:

Sclerotinia white mold is an important disease of many economically important crops including dry pea. Management of the disease is through cultural practices, fungicide applications, and host resistance when available. Employing resistance is the only viable long-term approach to managing Sclerotinia white mold of pea as well as of other crops. However, resistance to Sclerotinia white mold is only at low to moderate levels in pea cultivars and in the early limited germplasm collections of pea. In order to identify new resistance sources to white mold in dry pea, attempts were made in screening the newly acquired germplasm accessions in the USDA Western Regional Plant Introduction Station in Pullman, Washington. The seeds of the newly acquired germplasm lines were increased in the USDA Central Ferry farm in 2017, which made it possible to have sufficient materials for screening. Ninety-five accessions of recently acquired germplasm lines from China along with two reference accessions (Shawnee and DSP) were evaluated for resistance to *Sclerotinia sclerotiorum*. Experiments were conducted with completely randomized designs with three replications, each replication consisted with three plants. Two-week old seedlings were inoculated at the stem 6 cm above the soil line by placing a 6-mm agar plug from the edge of actively growing *S. sclerotiorum* colonies. Disease progress was monitored by measuring the lesion length on the stem starting three days after inoculation at two-day intervals. Although no germplasm lines among the screened accessions were immune to white mold, ten accessions showed disease severity lower than the tolerant cultivar DPS used as a check. The most tolerant lines were W6 44561 and W6 44593 originally from Inner Mongolia and Qinghai provinces of China, respectively, which consistently showed lowest levels of disease. These lines could be potential new sources of resistance to white mold. Additional research is needed to screen the remaining accessions of the newly acquired germplasm lines and confirm their allelic differences among these tolerant lines.

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Understanding and Improving Basal Resistance to *Sclerotinia sclerotiorum*

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Funded Plan of Work: Enhancing basal resistance to *Sclerotinia sclerotiorum* in Brassica

ABSTRACT:

The goal of the project is to use the newly identified *Arabidopsis thaliana* gene hypersusceptible to *S. sclerotiorum* (*HSS1*) for engineering high levels of disease resistance in canola. Through map-based cloning, we have determined that *HSS1* encodes the Mediator complex subunit MED16, indicating that MED16 is a key component regulating basal resistance to *S. sclerotiorum*. Unfortunately, overexpression of the *Arabidopsis HSS1* gene did not provide an increase in resistance to *S. sclerotiorum*. To better understand these results, we analyzed the fate of the HSS1/MED16 protein during infection and discovered that *S. sclerotiorum* infection induces a modification followed by complete degradation of HSS1/MED16. We have screened other novel forms of HSS1/MED16 from fungi and non-host plants for their ability to confer resistance to *S. sclerotiorum*. We found that the rice *HSS1* (*OsHSS1*) homolog complemented the *A. thaliana hss1* mutant and the *S. sclerotiorum HSS1* homolog did not. Over-expression of *OsHSS1* in the *A. thaliana hss1* mutant background increased resistance to *S. sclerotiorum* above the wild-type level. In the past year we have developed a transient assay for testing proteolytic sensitivity of MED16 orthologs. Results indicate that the OsMED16 protein is sensitive to proteolysis resulting from plant infection with *S. sclerotiorum*. This indicates that the mechanism of enhanced resistance is likely not based on resistance to proteolysis. We are now focusing our efforts on engineering other components of the MED16 signaling cascade utilizing a rice ortholog of WRKY33 to determine if a further increase in OsMed16-mediated resistance is possible. Expression of interacting nonhost components of basal resistance pathways is expected to increase the functionality of the individual components and enhance the resistance response.

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

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

ABSTRACT:

Work continues toward validating the effect of meta-QTL for partial resistance to white mold in common bean populations. Eight meta-QTL were observed by GWAS across different dry bean diversity panels. The genomic intervals for existing meta-QTL were realigned with version 2.1 of the reference genome. We continued development, from F2 to F4 generation, for two recombinant inbred pinto bean populations: PT12-37/VCP-13 and PT9-5-6/USPT-WM-12 for validating the effects of meta-QTL WM2.2, WM3.1, and WM5.4. The great northern dry bean proposed germplasm release USGN-WM-3 with high levels of resistance to white mold in the straw test has been postponed because it exhibited extremely poor agronomic performance in advanced yield trials in 2018. Efforts instead have shifted toward examining potential release of pinto bean breeding line PS08-039A which outyield the previous pinto release USPT-WM-12 in 2018 trials. A red bean SR16-5 is also being considered for release because it exhibited intermediate disease reaction in the national Bean White Mold Nursery in 2019 and has good yield potential. A high level of partial resistance in the straw test was confirmed for three Andean genotypes: ADP-0014 (yellow landrace from Tanzania), ADP-0436 (red mottled cultivar 'JB-178' released by Univ. Puerto Rico), and ADP-0734 (red mottled breeding line from Malawi-CIAT). Disease reactions and linkage maps for genetic recombinant inbred populations (Raven/I9365-31; Montrose/I9365-25; and UI 537/I9265-25) segregating for WM5.4 were provided to McClean's lab to support fine mapping of this QTL.

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