INTEGRATED APPROACHES IN WHITE MOLD MANAGEMENT

Megan McCaghey[1], Jaime Willbur[1], Ashish Ranjan[2], Scott Chapman[3], Carol Groves[4], Jake Kurcezewski[5], Mehdi Kabbage[6], and Damon L. Smith[7]

Introduction

Sclerotinia sclerotiorum, the causal organism of white mold, can cause significant yield losses to growers when environmental conditions are favorable for disease. Management of white mold includes a multi-prong approach of rotation with non-susceptible hosts, chemical control, and deploying tolerant varieties. However, commercial varieties lack a high level of white mold resistance. This work provides a useful and novel breeding method for selecting lines that have physiological resistance to white mold while maintaining agronomic qualities. The germplasm identified will serve as a valuable sources of physiological resistance to white mold that can be improved through further breeding. The full assessment of lines can be found in the open access article entitled, "Development and Evaluation of Glycine max Germplasm Lines with Quantitative Resistance to *Sclerotinia sclerotiorum*" (McCaghey and Willbur et al., 2017). Subsequent crosses with promising lines analyzed in this study were performed in 2016, and new lines were identified for novel crosses that will be performed in the field and greenhouse in 2018.

As an additional approach to enhance resistance to white mold, we are using the biotechnological method of RNA-interference (RNAi). RNAi reduces the expression of proteins that are essential to pathogenic success, and it has demonstrated effectiveness against various pests including *Meloidogyne* (Huang et al., 2006), *Fusarium* (Koch et al., 2013), and *S. sclerotiorum* (Andrade et al., 2016). *S. sclerotiorum* requires the secretion of oxalic acid (OA), a key virulence factor, to avoid host recognition and facilitate infection. Virus-induced gene silencing (VIGS) using *Bean pod mottle virus* (BPMV) was used to target OA biogenesis in *S. sclerotiorum*. Results from VIGS studies indicate promise for durable resistance through the generation of transgenic RNAi soybean plants that can effectively reduce the pathogenicity of *S. sclerotiorum.*

[1] Graduate Research Assistant, Dept. of Plant Pathology, 1630 Linden Dr. Univ. of Wisconsin-Madison, Madison, WI, 53706.
[2] Post-doctoral Researcher, Dept. of Plant Pathology, 1630 Linden Dr., Univ. of Wisconsin-Madison, Madison, WI, 53706.
[3] Researcher, Dept. of Entomology and Dept. of Plant Pathology, 1630 Linden Dr., Univ. of Wisconsin-Madison, Madison, WI, 53706.
[4] Researcher, Dept. of Plant Pathology, 1630 Linden Dr. Univ. of Wisconsin-Madison, Madison, WI, 53706.
[5] Undergraduate Research Assistant, Dept. of Plant Pathology, 1630 Linden Dr., Univ. of Wisconsin-Madison, Madison, WI, 53706.
[6] Assistant Professor, Dept. of Plant Pathology, 1630 Linden Dr., Univ. of Wisconsin-Madison, Madison, WI, 53706.
[7] Assistant Professor and Extension Specialist, Dept. of Plant Pathology, 1630 Linden Dr., Univ. of Wisconsin-Madison, Madison, WI, 53706.

Enhanced resistance in soybean germplasm and RNAi transgenic plants will provide another set of tools to add to our "toolset" of using cultural practices, predictive models, and fungicide application to manage white mold.

Objectives

1. Improve white mold resistance and agronomic properties of soybean through breeding in multiple environments and through multiple generations.
2. Improve white mold resistance through RNA-interference and transgenic plants.

Materials and Methods

Soybean germplasm was developed by crossing two sources of white mold resistance, W04-1002 and AxN-1-55, with lines exhibiting resistance to *Heterodera glycines* and *Cadophora gregata* in addition to favorable agronomic traits. Following greenhouse evaluations of 1,076 inbred lines derived from these crosses, 31 lines were evaluated for resistance in field tests during the 2014 field season. Subsequently, 11 white mold resistant breeding lines were moved forward for field evaluation in 2015, and seven elite breeding lines were selected and evaluated in the 2016 field season. The seven elite germplasm lines were also re-evaluated within a greenhouse using a cut petiole technique with multiple *S. sclerotiorum* isolates to test the durability of physiological resistance of the lines in a controlled environment. Following these evaluations, crosses were conducted with the assistance of Dr. Asheesh Singh's lab at Iowa State University between 51-23 and 52-82B and SSR 51-70 and 51-23 to further improve disease resistance and yield, and seed was increased in the greenhouse. During the spring and summer of 2017, F2 plants were selected in the greenhouse at West Madison Agricultural Research Station (WMARS), at Arlington Agricultural Research Station (ARS) in a field with a prior history of soybean cyst nematode (SCN) and sudden death syndrome (SDS), and at Hancock Agricultural Research Station (HARS) in a white mold nursery.

In order to generate RNAi silencing constructs, a sequence of 366 bp, corresponding to a *S. sclerotiorum* gene essential for OA biogenesis, was inserted into a BPMV vector in an antisense orientation. BPMV constructs were introduced into soybean using particle bombardment, and viral symptoms paired with RT-PCR were used to confirm viral replication prior to rub inoculations. Leaves confirmed to possess the silencing vector were then used to rub inoculate unifoliate seedlings. At the V4 growth stage, the plants were petiole-inoculated at the third trifoliate with pipette tips containing agar plugs of *S. sclerotiorum.* Disease progress was monitored by measuring lesions with digital calipers over five days and calculating the area under the disease progress curve (AUDPC), and the experiment was repeated. To assess expression of the silenced target, 6 cm of stem tissue surrounding the petiole was collected from plants 0-5 days post inoculation (DPI) for RNA extractions and RT-qPCR. Three biological replicates will be collected from each time point and expressions levels will be compared.

Results and Discussion

This work demonstrates that genetic gain can be made for white mold resistance in soybean while maintaining agronomic qualities, protein and oil content, and resistance to other pathogens. Breeding efforts using a novel source of white mold resistance followed by greenhouse and field screening, resulted in the development of several promising soybean lines for release as cultivars or use as parents in breeding programs. These candidate lines include 91-38, 52-82B, SSR51-70, and 51-23. Line 91-38 achieved an average yield of 2,802.5 kg ha^{-1} (44.8 bu ac^{-1}), which is 360.2 kg ha^{-1} (5.8 bu ac^{-1}) higher than W04-1002, the white mold resistant parent, and a mean DSI value of 11.4 across all field years evaluated. Line 91-38, which possessed the novel resistance-associated marker region on chromosome 16, also had one of the lowest disease severity rankings in both field and greenhouse trials compared to the susceptible check, Dwight, and other commercial lines in 2016. Additionally, line 52-82B had one of the best yields, a three-year mean of 3,547.1 kg ha^{-1} (56.8 bu ac^{-1}), and a low DSI mean of 27.5. Line SSR51-70 consistently exhibited among the lowest disease scores for all years in both field (mean DSI of 10.7) and greenhouse studies. With a three-year mean yield of 2,972.5 kg ha^{-1} (47.6 bu ac^{-1}) and DSI of 26.2, line 51-23 also exhibits promising yield potential and a high level of white mold resistance. All lines yielded on average between 2,700 (43.2) and 3,600 kg ha^{-1} (57.6 bu ac^{-1}) and were consistently near or above the yearly state averages for 2014 (2,953.03 kg ha^{-1}), 2015 (3,322.15 kg ha^{-1}), and 2016 (3,691.27 kg ha^{-1}) (National Agricultural Statistics Service et al., 2014-2016). Overall, the yield performance, elevated disease resistance, and high protein and oil contents of these four lines provides strong evidence for their candidacy in future white mold resistance breeding programs. 2016 field and greenhouse results are available in Figure 1, and complete evaluations are available in McCaghey and Willbur et al. (2017). F3 seed from all six populations originating from 2016 crosses was sent to Chile for increase this winter in order to enhance the efficiency of the inbreeding and selection process. Selection will continue with increased seed in the summer of 2018. Additional lines for novel crosses continue to be identified with the objectives of improving white mold resistance and agronomic properties.

Concurrent work to enhance soybean resistance through RNAi has produced encouraging results. Plants containing BPMV vectors targeting OA biogenesis showed enhanced resistance to *S. sclerotiorum* compared to empty-vector control plants, in replicated experiments (Figure 2) and expression of the OA target sequence trends to reduction based on preliminary results. We propose that RNAi strategies targeting OA biogenesis, and perhaps other pathogenicity factors, will provide new tools for resistance to *S. sclerotiorum* in soybean. These promising results provide confidence to move forward with the generation and screening of resistant, transgenic plants targeting OA biogenesis.

Summary

In this study, we identified four germplasm lines; 91-38, 51-23, SSR51-70, and 52-82B exhibiting a high level of white mold resistance combined with desirable agronomic traits, including high protein and oil contents. We have validated a proof of concept that genetic gain for physiological white mold resistance can be achieved, independent of escape mechanisms such as flowering date or plant architecture that confer field tolerance, through selection in a controlled greenhouse environment. We were able to identify several soybean lines that have excellent potential as parents in a breeding program or as varieties themselves, as evidenced by the planned release of 91-38. In addition, crosses have been performed using lines 51-23, SSR51-70, and 52-82B to identify new germplasm lines with enhanced resistance through combining sources of resistance while maintaining yield potential. Improved germplasm and RNAi strategies will provide new tools for resistance to *S. sclerotiorum* in soybean.

References

Andrade, C.M., Tinoco, M.L.P., Rieth, A.F., Maia, F.C.O., & Aragão, F.J.L. (2016). Hostinduced gene silencing in the necrotrophic fungal pathogen *Sclerotinia sclerotiorum*. *Plant Pathology*, *65*(4), 626-632.

Huang, G., Allen, R., Davis, E.L., Baum, T.J., & Hussey, R.S. (2006). Engineering broad root-knot resistance in transgenic plants by RNAi silencing of a conserved and essential root-knot nematode parasitism gene. *Proceedings of the National Academy of Sciences*, 103(39), 14302-14306.

Koch, A., Kumar, N., Weber, L., Keller, H., Imani, J., & Kogel, K. H. (2013). Host-induced gene silencing of cytochrome P450 lanosterol C14α-demethylase–encoding genes confers strong resistance to Fusarium species. *Proceedings of the National Academy of Sciences*, 110(48), 19324-19329.

McCaghey, M., Willbur, J., Ranjan, A., Grau, C.R., Chapman, S., Diers, B., & Smith, D.L. (2017). Development and Evaluation of Glycine max Germplasm Lines with Quantitative Resistance to *Sclerotinia sclerotiorum*. *Frontiers in Plant Science*, 8, 1495.

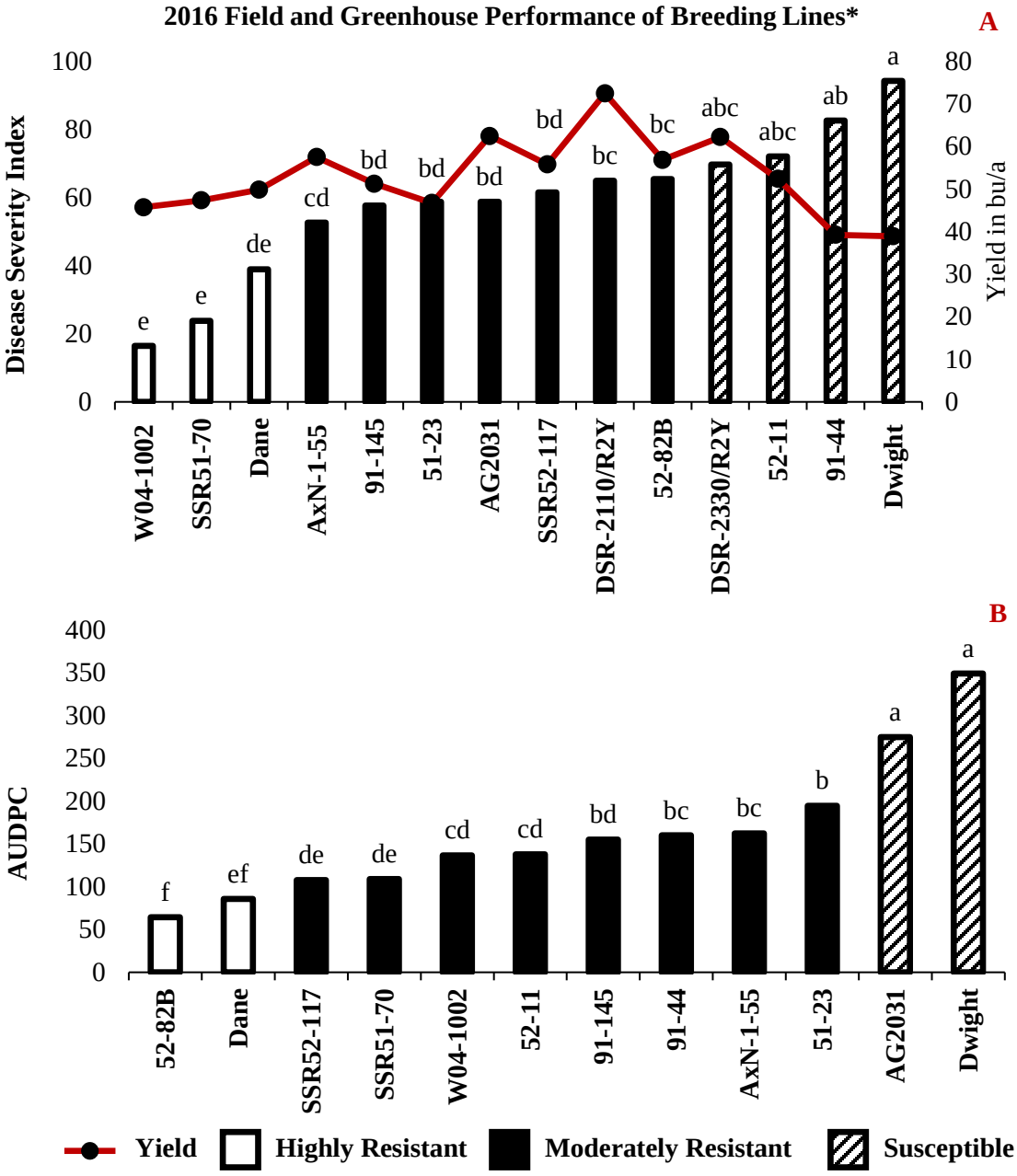

Figure 1. (A) White mold was measured using the disease severity index (DSI) which was generated by rating 30 arbitrarily selected plants in each plot and scoring plants on a 0-3 scale: 0 = no infection; 1 = infection on branches; 2 = infection on main stem with little effect on pod fill; 3 = infection on main stem resulting in death or poor pod fill. The scores of the 30 plants were totaled for each class and divided by 0.9. (B) Area under the disease progress curve (AUDPC) results from 2016 greenhouse evaluations.
*Means followed by the same letter are not significantly different based on Fisher's Least Significant Difference (LSD; α=0.05).

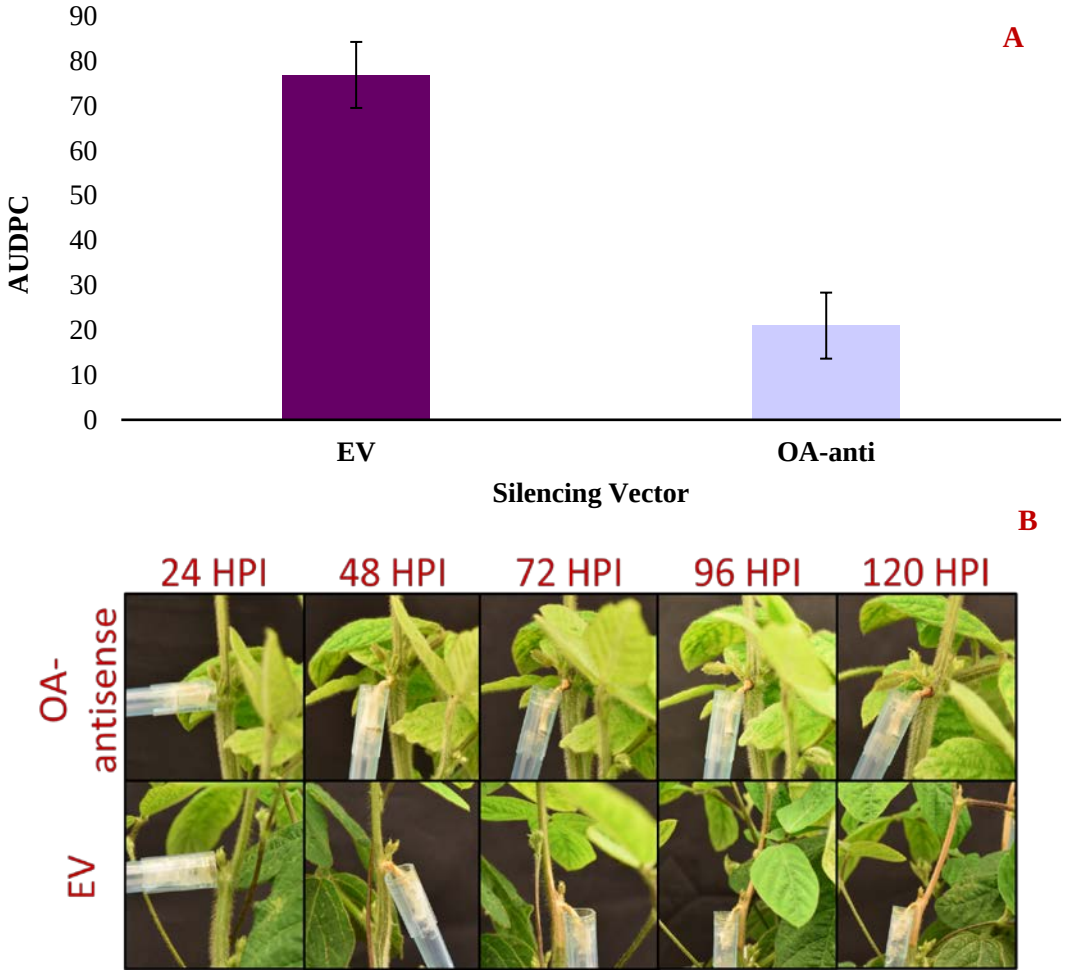

Figure 2. (A) The AUDPC was lower in soybeans transformed with the OA-antisense silencing construct compared to soybeans containing the EV (*P*=0.0035). (B) Lesions measured 24 -120 hours post inoculation (HPI) showed visual differences in the OA-antisense and EV transformed plants. Lesion development was delayed and lesions were smaller in the OA-antisense plants, whereas EV-containing plants had large, often girdling, lesions at 96 HPI.