



# Resistance to *Sclerotinia sclerotiorum* in PI391589A

E. HELLIWELL(1), T. Smith(1), S. A. Berry(1), S. G. Gordon(1), S.K. St. Martin(2) and A. E. Dorrance (1). (1)Dept of Plant Pathology, The Ohio State University, Wooster, OH 44691; (2) Dept of Horticulture and Crop Science, The Ohio State University, Columbus, OH 43210.

## Introduction

Sclerotinia stem rot, caused by the fungus *Sclerotinia sclerotiorum* causes yield reduction in soybeans in the upper-midwest region of the United States and southern Canada (Grau, 2003). The most effective way to manage this disease is by planting soybean cultivars that show high levels of resistance to colonization by *S. sclerotiorum*. As of 2001, 28 QTLs across 15 linkage groups were identified that confer resistance to *S. sclerotiorum* in known cultivars (Arahana et. al., 2001; Kim and Diers, 2000). In addition, 68 plant introductions (PIs) were also identified as having high levels of partial resistance to Sclerotinia stem rot (Hoffman et. al., 2000). Through genotyping these PIs, it may be possible to find additional QTLs that confer resistance to this soybean pathogen.

In this study, a population was developed with Kottman (moderately susceptible) and PI391589A (high levels of partial resistance) as parents. To identify the QTLs associated with partial resistance in PI391589A, the progeny lines were screened for resistance to infection by *S. sclerotiorum*, with the cotyledon assay (Dorrance, 2003; Vuong et al., 2004) and genotyped using simple-sequence repeat (SSR) markers. The primary objective of this study is to determine if any of the resistance QTLs found in this plant introduction are different from those that have been previously identified in current soybean cultivars.



**Figure 1.** Screening for resistance to Sclerotinia with colonized oat grain in a growth chamber. The percentage of dead seedling was measured when the susceptible check is 80 to 90% dead.

## Materials and Methods

**Plant material:** A BC1F2:5 population of 230 families was developed using Kottman x PI391589A as parents.

**Cotyledon assay:** Twelve to 15 plants were inoculated with *S. sclerotiorum*. For each plant a hole was punched in the cotyledon, in the 1/3 closest to the stem and a colonized oat kernel placed in the hole. Plants were placed in a mist chamber for 48 hrs at 20°C. Plants were then removed from the mist chamber and placed in the greenhouse for 24 to 48 hours and data was collected when the control plants (Williams 82) were 70 to 90% dead. Inbred lines were treated as entries and for every 15 entries 2 controls were also inoculated. The

**Table 1.** Statistical analysis to determine the association of the SSR marker with a resistance QTL for *S. sclerotiorum*.

MLG <sup>a</sup>	SSR	ANOVA P value (w/o heterozygotes)	ANOVA P value	K test Statistic
A1	<b>Satt050</b>	<b>0.1435</b>	<b>0.0492*</b>	<b>5.19*</b>
A1	Satt225	0.15	0.6134	0.083
<b>A1</b>	<b>Satt545</b>	<b>0.2758</b>	<b>0.2758</b>	<b>1.391</b>
A1	Satt591	0.3566	0.6093	0.114
A2	Satt233	0.9626	0.8325	0.032
A2	Satt409	0.1122	0.1632	1.113
<b>A2</b>	<b>Satt424</b>	<b>0.6282</b>	<b>0.4258</b>	<b>0.135</b>
A2	Satt437	0.9453	0.8012	0.081
B1	Satt430	0.916	0.6846	0.143
B2	Satt168	0.9397	0.8041	0.46
B2	Satt304	0.8785	0.7196	0.455
B2	Satt577	0.7593	0.6204	0.148
C1	Satt294	0.1452	0.2794	1.463
C1	Satt565	0.4101	0.9747	0.015
C2	Satt281	0.744	0.5964	0.423
C2	Satt307	0.7298	0.4678	0.645
D1b	Satt271	0.3227	0.1544	1.678
D1b	Satt579	0.5381	0.3273	0.38
<b>D2</b>	<b>Satt301</b>	<b>0.5794</b>	<b>0.3715</b>	<b>0.711</b>
<b>D2</b>	<b>Satt458</b>	<b>0.6716</b>	<b>0.5756</b>	<b>1.662</b>
E	Satt212	0.121	0.0609	3.22
E	Satt268	0.9059	0.6882	0.009
E	Satt369	0.5531	0.8082	0.03
F	Satt490	0.3158	0.1866	0.850
F	Satt269	0.4334	0.4832	0.778
F	Satt425	0.7624	0.5577	0.202
F	Sat_240	0.8154	0.9047	0.089
G	Satt288	0.1666	0.2775	0.851
G	Sat_131	0.7954	0.8790	0.055
<b>G</b>	<b>Satt303</b>	<b>0.4138</b>	<b>0.3727</b>	<b>1.598</b>
<b>G</b>	<b>Satt191</b>	<b>0.0991</b>	<b>0.0369*</b>	<b>4.532*</b>
G	Satt472	0.4279	0.6280	0.939
H	Satt353	0.4533	0.2909	1.054

Table 1 continued next panel

Table 1, Cont'd

MLG <sup>a</sup>	SSR	ANOVA P value (w/o heterozygotes)	ANOVA P value	K test Statistic
I	Satt292	0.8088	0.9720	0.061
I	Satt354	0.5464	0.3588	0.796
I	Satt440	0.2582	0.8869	0.004
J	Satt215	0.5280	0.3181	1.196
J	Satt285	0.6160	0.5254	0.961
J	Satt414	0.8911	0.9262	0.06
L	Satt143	0.5818	0.5833	0.272
L	Satt182	0.6894	0.5802	0.032
L	Satt373	0.1690	0.0631	3.219
<b>M</b>	<b>Satt323</b>	<b>0.0348*</b>	<b>0.4812</b>	<b>0.615</b>
M	Satt463	0.1424	0.1722	1.761
M	Satt540	0.2468	0.1952	0.879
M	Satt551	0.4659	0.2518	0.899
N	Satt257	0.8368	0.5664	0.133
<b>N</b>	<b>Satt387</b>	<b>0.2622</b>	<b>0.1281</b>	<b>3.185</b>
N	Satt549	0.6748	0.5138	0.024
O	Satt173	0.2540	0.1234	3.072
<b>O</b>	<b>Satt243</b>	<b>0.7286</b>	<b>0.4392</b>	<b>0.484</b>
O	Satt420	0.3347	0.1470	2.217
O	Satt581	0.5252	0.3857	0.121

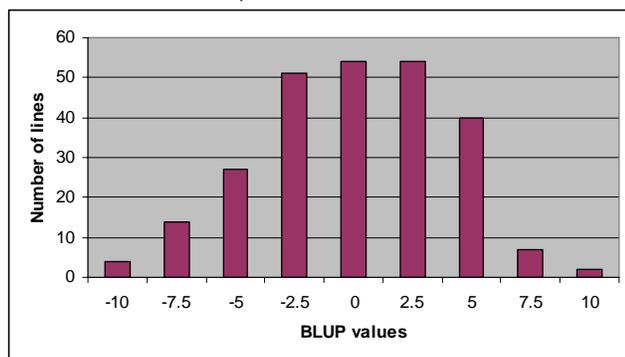
aMLG indicates major linkage group (Grant et al., 2002)

Denotes Sclerotinia QTL identified in previous studies (Arahana et al., 2001, Kim and Diers, 2000)

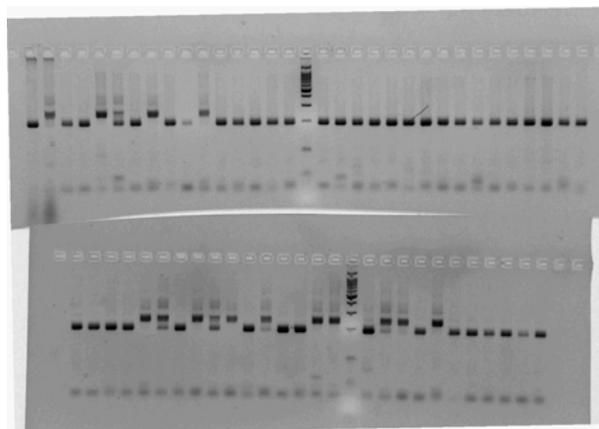
\* Indicates significant at P≤0.05.

experiment was planted as a randomized complete block design with three replications in time. Both an analysis of variance on the percentage of plants that die from Sclerotinia and a mixed models analysis to obtain the best linear unbiased predictor (BLUP) for each inbred line (Stroup, 1989) were completed.

**Genotypic analysis.** 113 of the 230 families have been genotyped with 53 SSR markers to date. The PCR products were visualized on a 4% agarose gel (Ameresco, Solon, OH) (Figure 3). For QTL detection a Kruskal-Wallis test (non-parametric) and a mixed model ANOVA were performed using BLUP values as the dependent variable.



**Figure 2.** Frequency distribution of the best linear unbiased predictors (BLUP) values for percentage of plants that die from Sclerotinia stem rot. -10 are the lines that are highly resistant and 10 are the lines that are highly susceptible. Data presented is from 3 replications. Family mean heritability estimates for this population was 0.29.



**Figure 3.** Visualization of PCR products with SSR marker Satt173

### Conclusions

\*In this cross, resistance to *Sclerotinia sclerotiorum* has a low heritability estimate. This may be due in part to the type of assay and also that the two parents do not have very wide differences in resistance response to this pathogen.

\*These preliminary data identified two QTLs on MLGs A1 (Satt050) and G (Satt191) that contribute to resistance to *S. sclerotiorum*. Satt191 was significantly associated with QTLs for resistance to *S. sclerotiorum* in 4 crosses, while Satt050 is 25cm distal to any previously reported QTL. Two additional disease assays, field and cut stem, as well as genotyping the remaining families are in progress.

**Acknowledgements:** This study is part of a larger study entitled "Characterization of soybean genotypes with partial resistance to Sclerotinia stem rot", led by Dr. Brian Diers at the University of Illinois and funded by the USDA Sclerotinia Initiative.

### Literature Cited

- Arahana, V.S., Graef, G.L., Specht, J.E., Steadman, J.R., and Eskridge, K.M., 2001. Identification of QTLs for resistance to *Sclerotinia sclerotiorum* in soybean. *Crop Sci.* 41:180-188
- Dorrance, A.E. Sclerotinia oat grain inoculation <http://ncsrp.com/whitemold/inoculation/oatgrain.htm>
- Grant, D., Imsande, M.I., and Shoemaker, R.C. 2002 SoyBase, The USDA-ARS Soybean Genome Database [Online]. <http://soybase.agron.iastate.edu>. Verified December 4, 2003
- Grau, C.R., 2003. Management strategies for white mold (Sclerotinia stem rot). <http://www.planthealth.info/whitemgmt.htm>.
- Hoffman, D.D., Diers, B.W., Hartman, G.L., Nickell, C.D., Nelson, R.L., Pederson, W.L., Cober, E.R., Graef, G.L., Steadman, J.R., Grau, C.R., Nelson, B.D., delRio, L.E., Helms, T., Anderson, T., Poysa, B., Rajcan, I., and Stienstra, W.C. 2002. Selected soybean plant introductions with partial resistance to *Sclerotinia sclerotiorum*. *Plant Dis.* 86:971-980.
- Kim, H.S., and Diers, B.W. 2000 Inheritance of partial resistance to Sclerotinia stem rot in soybean. *Crop Sci.* 40:55-61.
- Stroup, W.W. 1989. Why mixed models in applications of mixed models in agriculture and related disciplines. *Southern Coop. Ser. Bull.* 343. P. 1-8. Louisiana Agricultural Experiment Station. Baton Rouge, LA.
- Vuong, T.D., Hoffman, D.D., Diers, B.W., Miller, J.F., Steadman, J.R., Hartman, G.L. 2004. Evaluation of soybean, dry bean, and sunflower for resistance to *Sclerotinia sclerotiorum*. *Crop Sci.* 44:777-783.