Sunflower Genome-Wide Association Study for Sclerotinia Basal Stalk Rot Resistance.

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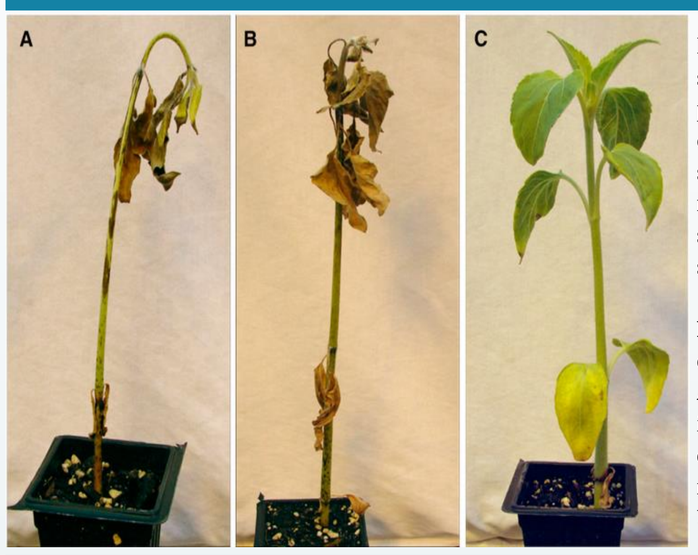
Abstract

Sclerotinia basal stalk rot (BSR) is a significant disease affecting worldwide sunflower production caused by the necrotrophic fungal pathogen *Sclerotinia sclerotiorum*. Resistance to this disease is quantitative and genetically complex. In this study, genome-wide association mapping was conducted to identify quantitative trait loci (QTL) influencing sunflower resistance. A population of 287 sunflower genotypes was inoculated with *S. sclerotiorum* isolate NEB-274 and BSR response data were collected using two disease descriptors: mean days to death (MD) and area under the mortality curve (AUMC). The genotyping data consisted of 3.2 million single nucleotide polymorphisms (SNP) markers identified by re-sequencing of the association mapping population. The phenotypic data were correlated with SNP marker data to identify loci associated with BSR resistance. This study identified 10 loci significantly associated with resistance and candidate genes in linkage disequilibrium with associated markers were identified based on the sunflower reference genome assembly. These identified loci will be used in the future breeding efforts to develop sunflower hybrids with improved resistance to BSR.

Sclerotinia basal stalk rot



BSR of sunflower is an economically significant disease limiting sunflower production in the Northern Great Plains region of the USA. Infection leading to BSR begins in sunflower roots and the fungus subsequently moves into the base of the stem, causing basal stem lesions, plant wilting, and premature senescence or death, resulting in yield loss.

Figure 1: Sunflower BSR symptoms observed in the greenhouse.
A. Plant exhibiting terminal wilt at 14 days post inoculation (dpi). **B.** Plant exhibiting desiccation at 14 dpi. **C.** Plant exhibiting few or no symptoms at 14 dpi. Adapted from Underwood et al. 2021.

Materials and Methods

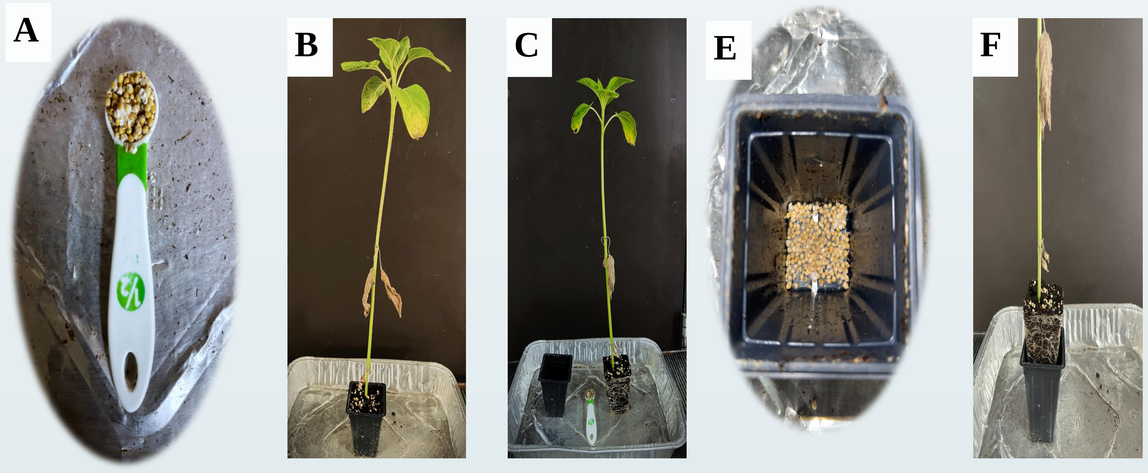


Figure 2. Five-week-old sunflower plants inoculation with millet inoculum.
A. Half teaspoon (0.38g) of millet inoculum. **B**. Five-week-old sunflower plants. **C.** A five-week-old root-bound plant was removed from the sheet pot. **D**. Millet inoculum was placed at the bottom of the sheet pot. **E**. Plant was placed back in inoculated sheet pot.

- After inoculation, plants were evaluated for 28 days based on aerial wilting and complete plant desiccation.
- Experimental design – Completely randomized design.
- Greenhouse conditions - 16-h photoperiod and 22°Celsius temperature.

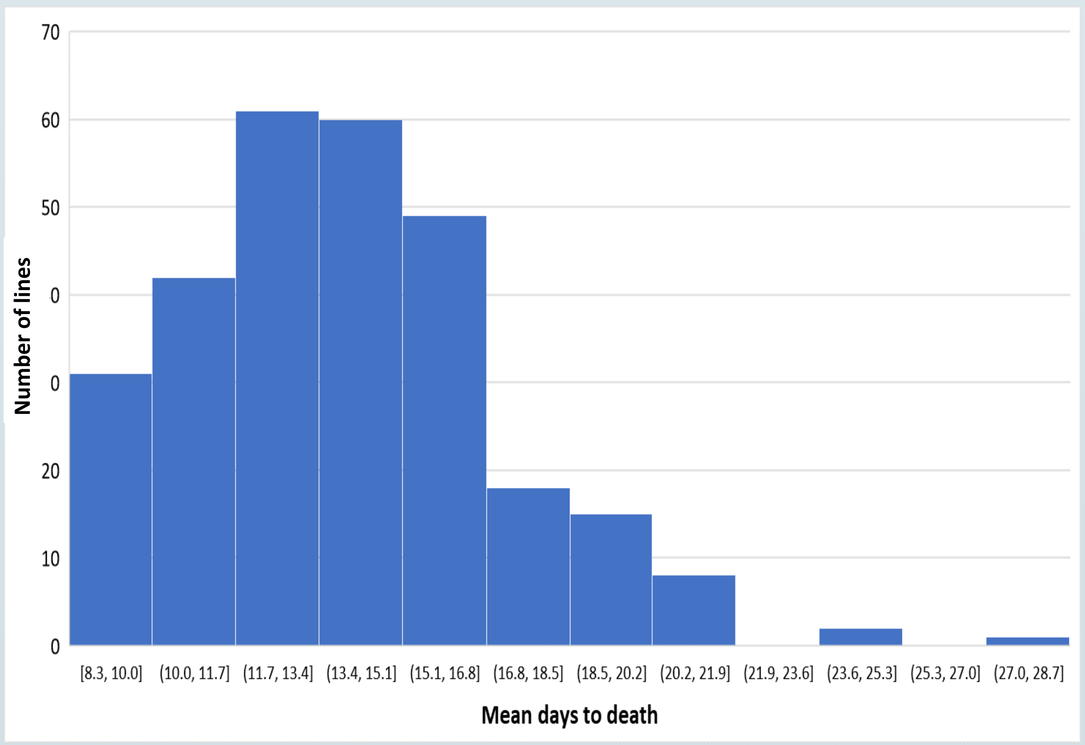


Figure 3. The distribution of BSR mean days-to-death scores was observed in the SAM population, where the Y-axis represents the number of sunflower lines, and the X-axis represents the mean days to death score for the population.

Results

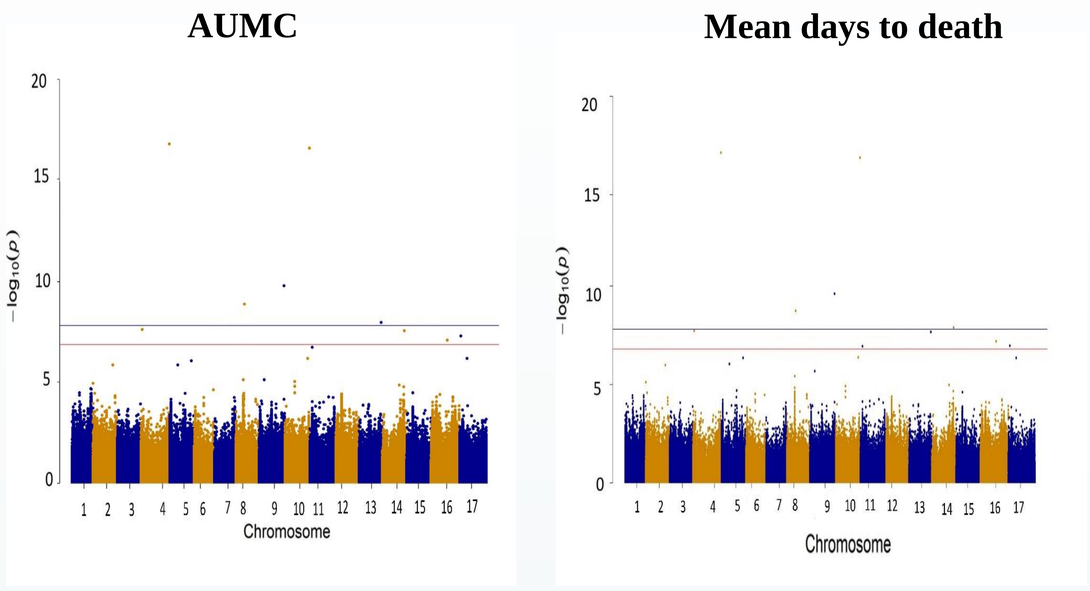


Figure 4. Manhattan plot of genome-wide association for AUMC and mean days to death from BSR using 3.2 million SNP markers. The red line indicates the genome-wide significance threshold at 5% false discovery rate and the blue line indicates the genome-wide significance threshold determined using the Bonferroni multiple comparison correction.

QTL	Trait	Chromosome	Position	Gene designation	Gene Annotation
Qbsr- 4.5	MD, AUMC	4	9808311	Ha412HOChr04g00009550	DNA/RNA polymerase superfamily
Qbsr- 4.6	MD, AUMC	4	217594499	Ha412HOChr04g00012739	Transcription factor TUBBY family / Putative tubulin
Qbsr- 8.6	MD, AUMC	8	67034050	Ha412HOChr08g00046724	Putative transcription factor MYB-HB-like family
Qbsr- 9.6	MD, AUMC	9	190614345	Ha412HOChr09g00023449	Putative glucuronoxylan 4-O-methyltransferase
Qbsr- 10.3	MD, AUMC	10	187831345	Ha412HOChr10g00027498	Putative Arf GTPase activating protein
Qbsr- 11.3	MD	11	17482526	Ha412HOChr11g00027874	Putative salicylate carboxymethyltransferase
Qbsr- 13.4	MD, AUMC	13	168276912	Ha412HOChr13g00037599	Putative disease resistance protein At3g14460 isoform
Qbsr- 14.6	MD, AUMC	14	170169222	Ha412HOChr14g00040747	Putative protein JASON
Qbsr- 16.6	MD, AUMC	16	123751261	Ha412HOChr16g00046262	Putative retrotransposon gag domain, aspartic peptidase domain superfamily
Qbsr- 17.4	MD, AUMC	17	11514759	Ha412HOChr17g00048056	Putative transcription factor Nin-like family

Table 1. Candidate genes potentially associated with BSR resistance within linkage disequilibrium of significantly associated SNP markers, are presented. Blast searches were carried out, and similarities to known proteins are indicated**.**

Conclusion

- The SAM population displayed significant variations ($p<0.0001$) in mean days to death from BSR, ranging from 8 days for highly susceptible genotypes to 28 days for highly resistant lines. The mean score for the panel was 13.41 days.
- Three genotypes, HA 124, HA 1 and RHA 417, displayed a significantly higher resistance level than susceptible genotypes among the SAM panel.
- A total of 10 QTLs were found on nine chromosomes (4, 8, 9, 10, 11, 13, 14, 16, and 17). Significant QTLs were estimated to explain 1.2% to 10.7% of the phenotypic variation in BSR resistance.
- Among the 10 QTLs identified, nine were common to both disease descriptors (MD and AUMC).
- Candidate genes included a predicted NBS-LRR protein, commonly associated with resistance to diseases caused by biotrophic phytopathogens, and a predicted salicylate carboxy methyltransferase, associated with modification of salicylic acid, a plant hormone with an important role in pathogen defence.

References

- Mandel JR, Dechaine JM, Marek LF, Burke JM (2011) Genetic diversity and population structure in cultivated sunflower and a comparison to its wild progenitor, *Helianthus* annuus L. Theoretical and Applied Genetics 123:693–704.
- Underwood, W., Misar, C. G., Block, C., Gulya, T. J., Talukder, Z., Hulke, B. S., & Markell, S. G. (2021). A greenhouse method to evaluate sunflower quantitative resistance to BSR caused by Sclerotinia sclerotiorum. Plant Disease, 105(2), 464-472.

Acknowledgement

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