

Genetic mapping of Sclerotinia basal stalk rot resistance and oxalic acid tolerance in two sunflower recombinant inbred line populations

Israt Ansari Zaman¹, Srushtideep Angidi¹, Luis Del Rio Mendoza¹, Julie S. Pasche¹, William Underwood²

¹ North Dakota State University Dept. of Plant Pathology, Microbiology & Biotechnology; ² USDA-ARS Sunflower Improvement Research Unit



Research Background

- Sclerotinia basal stalk rot (BSR) of sunflower (*Helianthus annuus* L.) is a destructive disease incited by the necrotrophic fungal pathogen *Sclerotinia sclerotiorum*, which causes significant yield losses worldwide.
- Oxalic acid (OA) is a key virulence factor in *Sclerotinia sclerotiorum* pathogenesis inducing plant cell death, creating an acidic environment, aiding fungal penetration, and compromising host cell walls.
- Typical symptoms of BSR include- wilting in aerial tissues and basal stem lesions
- Noyes and Hancock identified OA as a wilt-inducing toxin from *Sclerotinia*-infected sunflower tissues
- Previously, while checking the oxalic acid tolerance in several stalk rot-susceptible and resistant lines, the roots of the plants were treated by soil drench with 60 mM potassium oxalate (pH 5.6). This treatment demonstrated that symptoms of OA mirror BSR symptoms, suggesting that OA may play a role in disease development (Figure 1).



Figure 1: Oxalic acid treatment mimics symptoms with *S. sclerotiorum* millet inoculum.
A. Wilting of OA sensitive line HA 89 after OA treatment,
B. Stem lesion caused by OA
C. Tolerant line RHA 801 showing no visible symptoms after OA treatment
D. Susceptible line HA 89 exhibiting symptoms of stalk rot at 11 days after inoculation with *S. sclerotiorum* isolate NEB 274
E. Stem lesion caused by inoculation.
F. Partially resistant line RHA 801 exhibiting no stalk rot symptoms at 11 days post-inoculation
 (Image Source : Underwood ,unpublished)

- Previous research has identified OA tolerance in the sunflower inbred lines HA 61 and RHA 801, which also exhibit partial resistance to BSR (Figure 2).

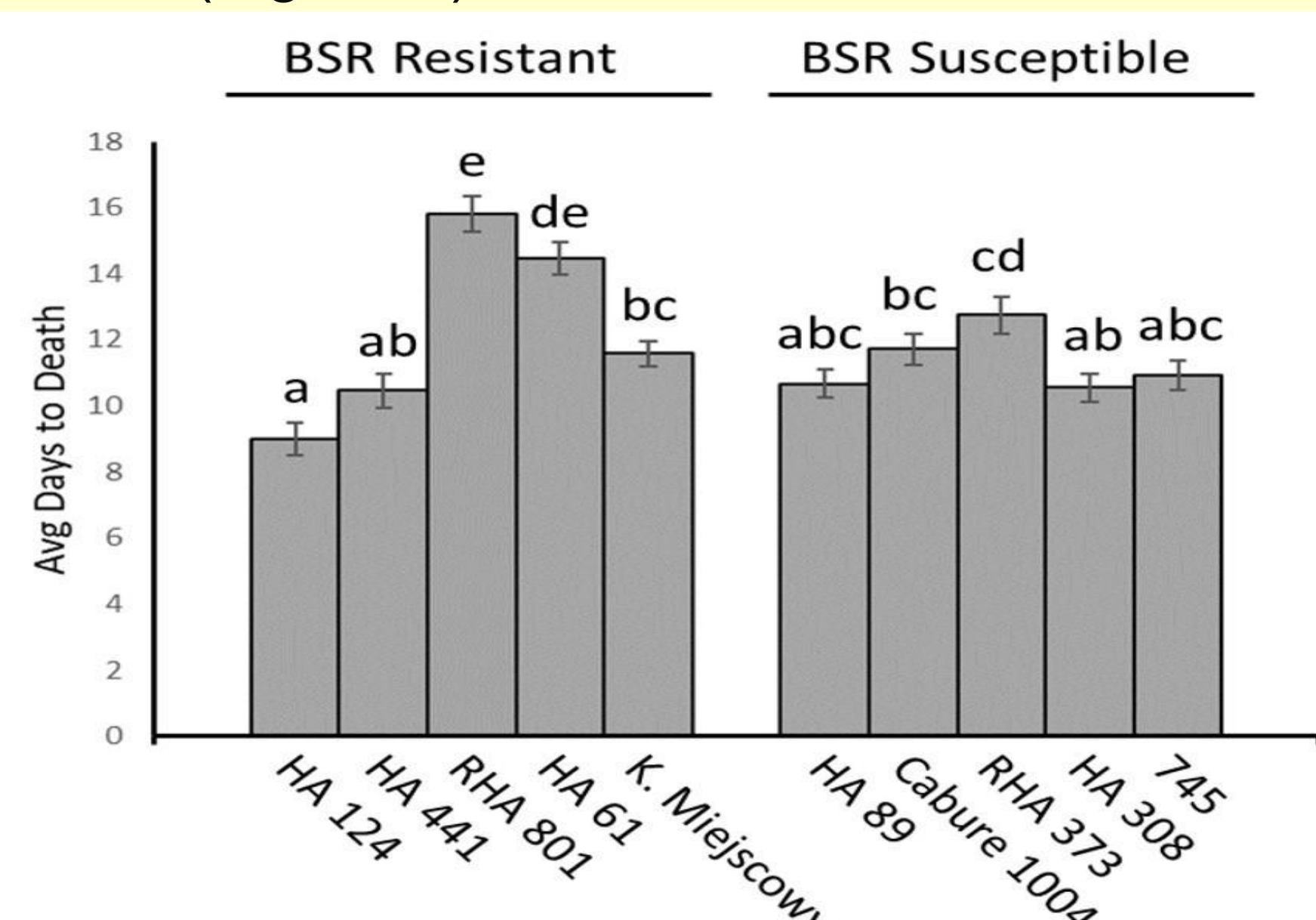


Figure 2: Evaluation of sunflower genotypes with OA

- The degree to which OA tolerance contributes to BSR resistance in HA 61 and RHA 801, if at all, is unknown.
- Therefore, we developed recombinant inbred line populations to facilitate genetic characterization of the BSR resistance and OA tolerance traits in HA 61 and RHA 801.

Rationale

- Understanding the relationship between BSR resistance and OA tolerance and the genetic loci underlying these traits may allow sunflower breeders to improve resistance to this important disease by combining specific resistance mechanisms.

Specific Objectives

- To map QTL for BSR resistance after inoculation with *S. sclerotiorum*.
- To map QTL for OA tolerance using a soil drench assay.
- To assess the correlation between the two traits and determine if any mapped loci are overlapping.

Preliminary Results

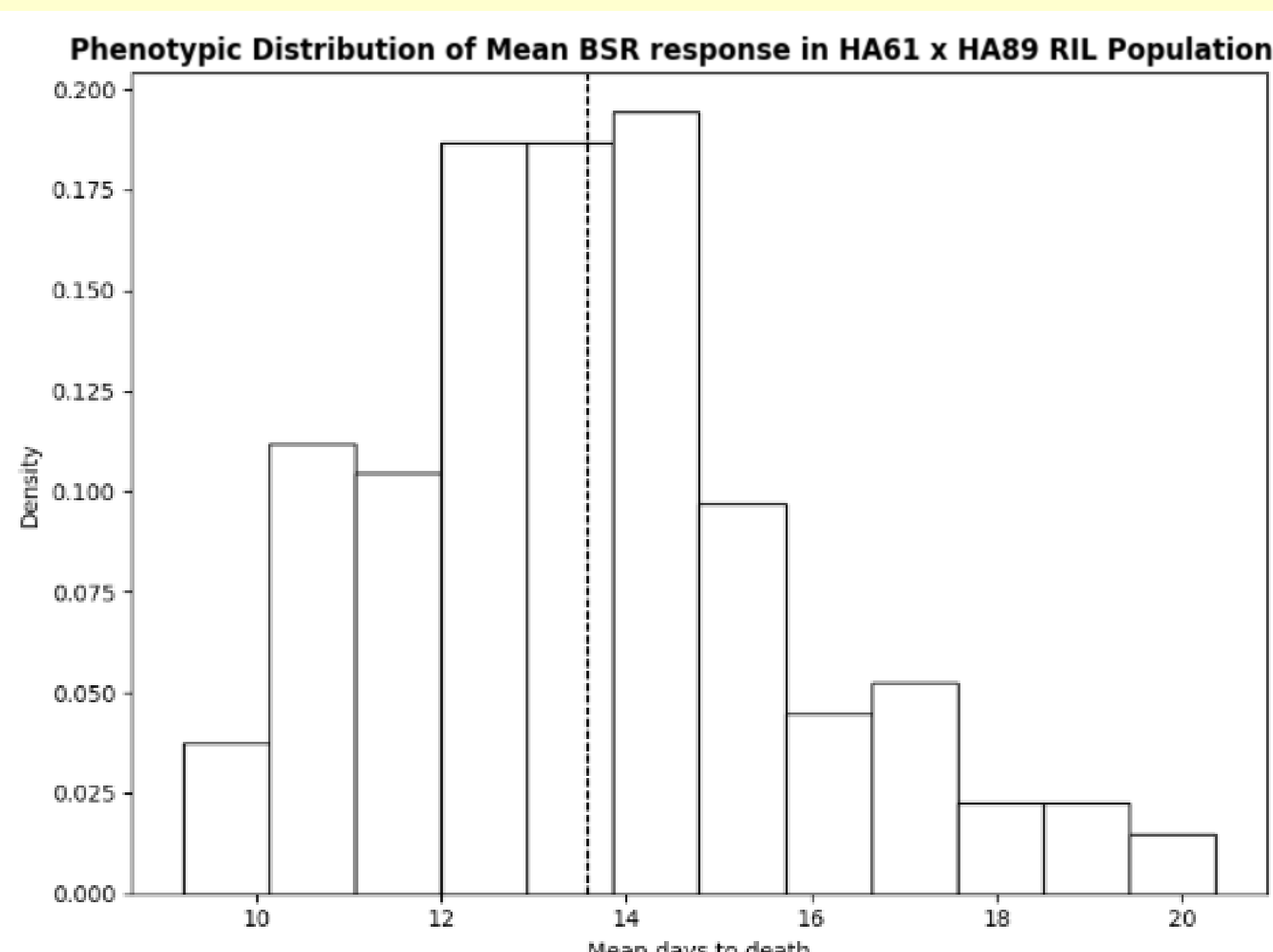


Figure 3 : Distribution of BSR response among RILs of the HA 61 × HA 89 population

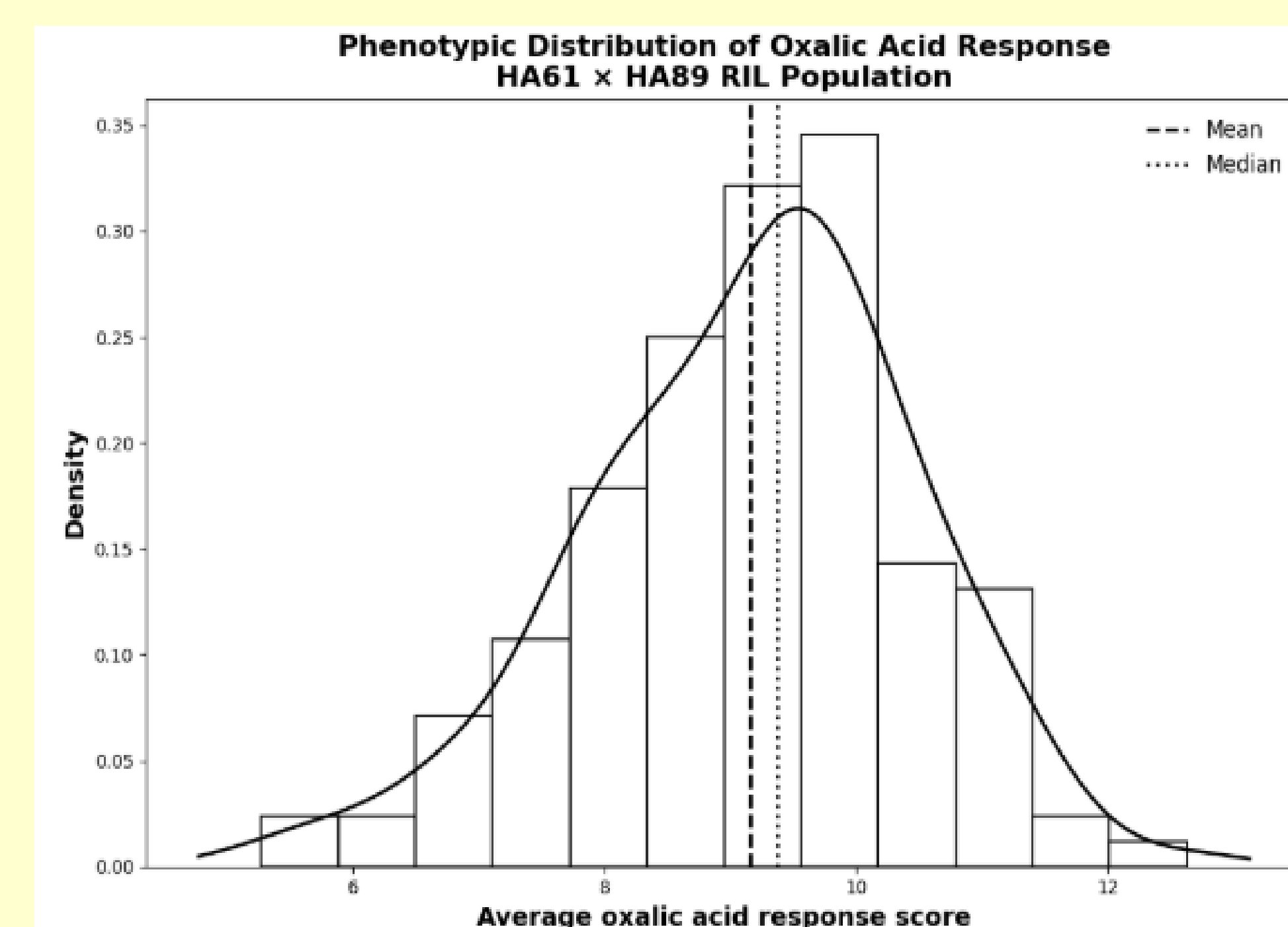


Figure 4: Distribution of OA response among RILs of the HA 61 × HA 89 population

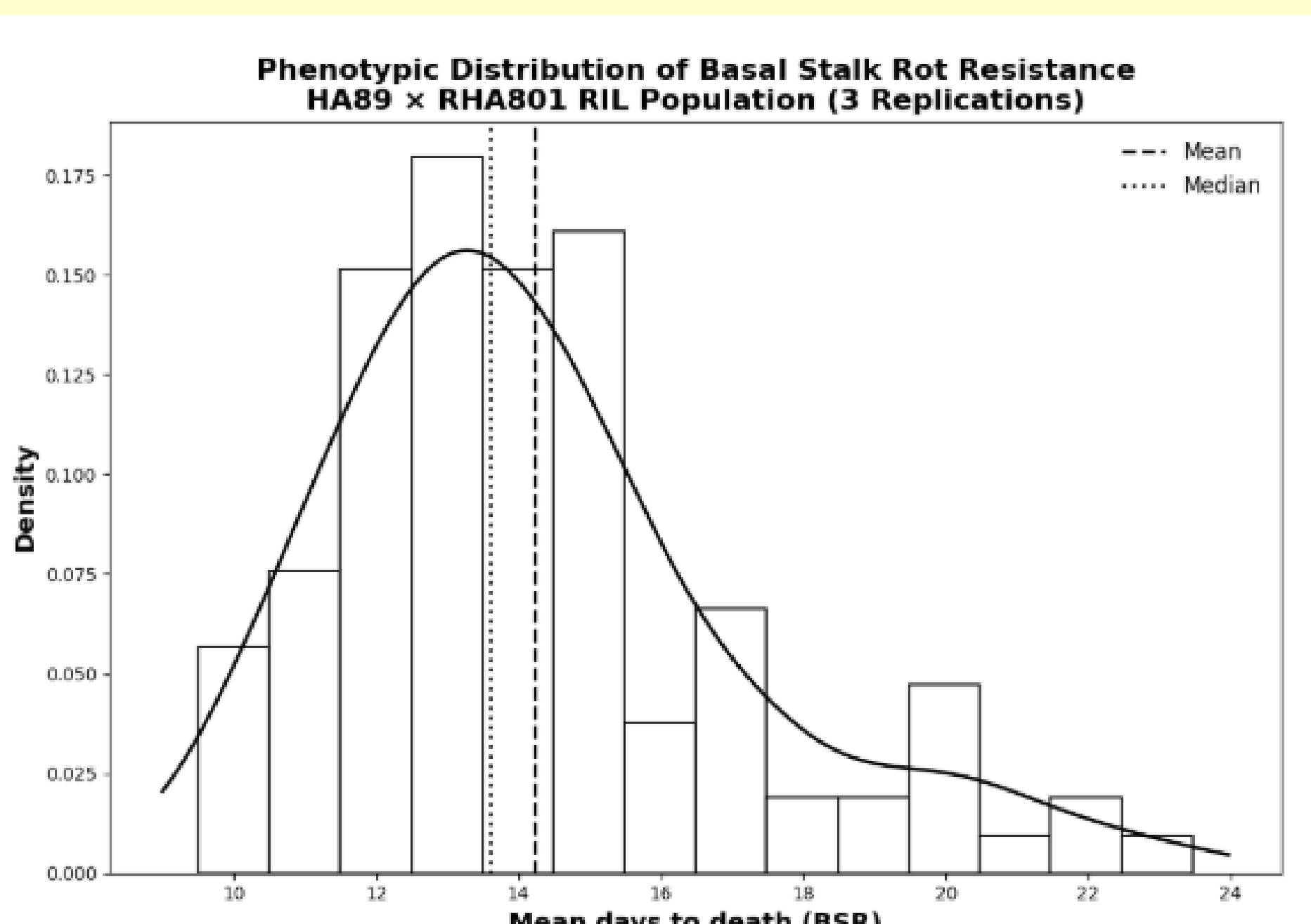


Figure 5 : Distribution of BSR response among RILs of the HA 89 × RHA 801 population

Summary

- In conclusion, previous studies have identified inbred line HA 61 and RHA801 as displaying partial resistance to BSR in field trials and broad-spectrum resistance against diverse *S. sclerotiorum* isolates when evaluated in the greenhouse.
- Preliminary results for BSR phenotyping of the HA 61 × HA 89 and HA 89 × RHA 801 indicated the expected transgressive segregation consistent with quantitative resistance to BSR.
- Additionally, a single replication for Oxalic Acid response of HA 89 × RHA 801 RIL populations has been completed
- The results of this study will provide valuable insights into the genetic mechanisms governing resistance to BSR and the specific genes responsible for OA tolerance.

Future Work

- In the future, our priority will be to complete OA response of HA 89 × RHA 801 RIL populations
- Sequence genetic samples and construct high-density linkage maps to enable robust QTL mapping.
- We aim to identify genomic regions associated with oxalic acid tolerance and BSR resistance while identifying overlapping QTLs to understand host-pathogen interactions.

Acknowledgements

This research is supported by:

- Department of Plant Pathology, North Dakota state University
- USDA-ARS
- **National sclerotinia initiative**



Methodology



Gene Identification through QTL mapping

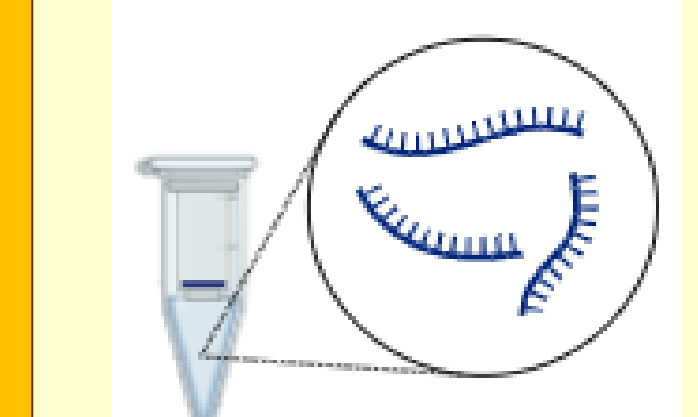
Genotyping



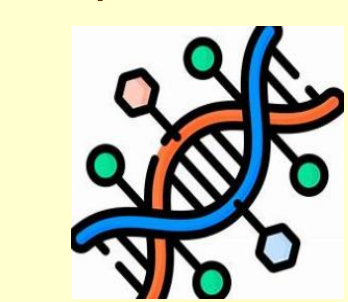
Leaf sample



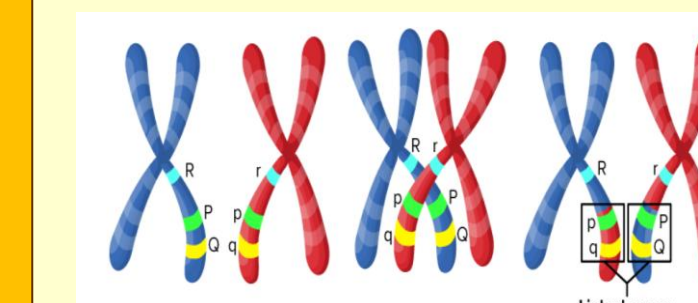
DNA isolation



Genotyping by sequencing (GBS)

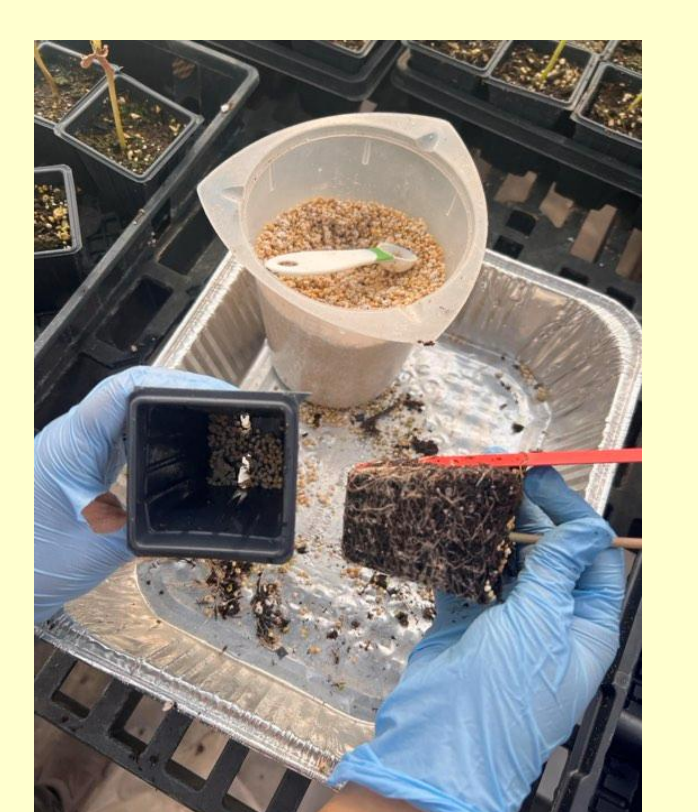


Linkage map



Phenotyping

BSR inoculation



Inoculation of 5-week-old sunflower genotype with *S. sclerotiorum* millet inoculum

OA treatment



Roots treated by soil drench with 60 mM potassium oxalate (pH 5.6)



After inoculation and OA treatment, plants were evaluated for terminal wilting and complete plant desiccation for 28 days

References

- Angidi et al., unpublished.
 Hossain, M. M., Sultana, F., Li, W., Tran, L. S. P., & Mostofa, M. G. (2023). *Sclerotinia sclerotiorum* (Lib.) de Bary: Insights into the pathogenomic features of a global pathogen. *Cells*, 12(7), 1063.
 Misar, C. G. (2022). Root Plate Growth in Sunflower and Its Relevance to *Sclerotinia* Basal Stalk Rot. *American Journal of Plant Sciences*, 13(3), 359-370.
 Noyes, R. D., & Hancock, J. G. (1981). Role of oxalic acid in the *Sclerotinia* wilt of sunflower. *Physiological Plant Pathology*, 18(2), 123-132.
 Talukder, Z. I., Underwood, W., Misar, C. G., Seiler, G. J., Cai, X., Li, X., & Qi, L. (2022). Genomic insights into *Sclerotinia* basal stalk rot resistance introgressed from wild *Helianthus praecox* into cultivated sunflower (*Helianthus annuus* L.). *Frontiers in Plant Science*, 13, 840954.
 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 basal stalk rot caused by *Sclerotinia sclerotiorum*. *Plant Disease*, 105(2), 464-472.
 Underwood et al., unpublished.