

# Breeding Charcoal Rot Resistance into Soybeans

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### Introduction

Charcoal rot (CR) disease caused by *Macrophomina phaseolina* is responsible for significant yield losses in soybean production. It is ranked among the top 10 soybean diseases in the United States with an average annual yield loss of one million tons. CR is a soil- and seed-borne polyphagous fungus. The abundant production of minute black microsclerotia causes the infected plant tissues to blacken, and therefore, the disease is known as charcoal rot.

Charcoal rot management strategies in soybean have not been overly effective or widely adopted and have provided limited control, leaving genetic resistance as one of the most feasible and sustainable methods to manage CR. Complete resistance to *M. phaseolina* is not reported in any of the 500 plant species it infects, but identification of partial resistance has been reported in soybean. Breeding for resistance is difficult because most diseases are quantitatively inherited and controlled by multiple genes. CR is a greater concern in the southern United States due to frequent hot and dry conditions that tend to occur during important soybean developmental growth stages. However, the identification of CR in soybean growing regions in the northern latitudes is on the rise and more pronounced in Iowa's warm and dry growing seasons.

The objectives of this study were to identify new sources of resistance to CR in a collection of 459 diverse plant introductions from the USDA Soybean Germplasm Core Collection.

Field and greenhouse screenings, and genome-wide association studies (GWAS) were used to identify candidate genes and associated molecular markers. These genes and markers can be used in subsequent breeding applications to develop resistant cultivars for the northern growing region.

This field study took place in the 2015 and 2016 growing seasons at the Muscatine Island Research Farm.

### Material and Methods

The Muscatine Island Research Farm was chosen for the field study because of the unique sandy soil and the ability to control the amount of water the plots received through irrigation, creating a favorable climate for CR.

Four-hundred sixty-five soybean genotypes, including 459 PI lines, four maturity checks, one moderately resistant check, and one susceptible check were grown at the Muscatine Island Research Farm in 5-ft long, single-row plots with 30-in. row spacing and 3-ft alleys. Maturity and disease checks were spaced every 100 and every 50 entries, respectively. Genotypes were arranged in a randomized complete block design with three replications. At planting, the planter was calibrated to apply three grams of charcoal rot-infected sorghum seed/linear ft in-furrow with a soybean seeding rate of eight seeds/ft.

Stem collection and charcoal rot ratings were based on the evaluation of root and stem severity rating. For each replication, five plants of each genotype were randomly harvested between the R7 and R8 growth stages from each row. Stem and top of the taproot portion of each plant was obtained by gently uprooting each plant and clearing it of the soil and other debris. Each plant stem was longitudinally split and visual ratings were

given on a scale of 1 to 5 (Figure 1). The entire experiment also was conducted at ISU's Agronomy Greenhouse to determine correlation between greenhouse and field charcoal rot disease expression, with the ultimate aim to develop rapid large-scale seedling tests in greenhouse for breeding selections.

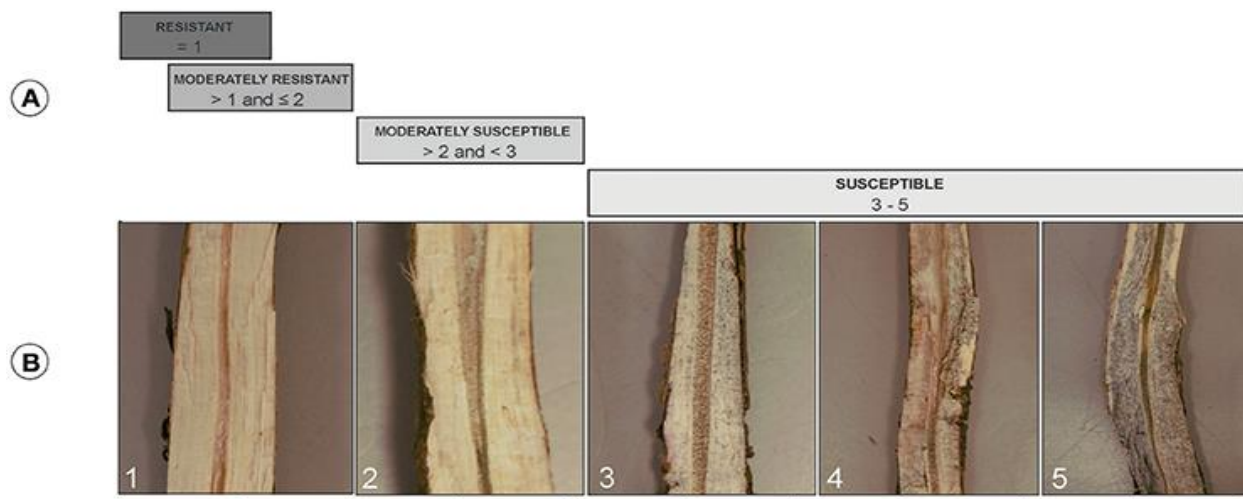
### Results and Discussion

New sources for CR resistance were identified from both field and greenhouse screening from maturity groups I, II, and III, and these have been used as parents to develop commercial cultivars with charcoal resistance for soybean maturity groups late I to early III. In 2017, through the use of Chile winter nurseries in 2015 and 2016 (to fast track cultivar development), F<sub>5</sub> plants (breeding lines bred with resistant lines and selfed for four generations) were harvested for use in first-year yield trial screening in 2018. Five significant single nucleotide polymorphisms (SNP) and putative candidate genes related to

abiotic and biotic stress responses were reported from the field screening. Greenhouse screening revealed eight loci associated with eight candidate gene families, all associated with functions controlling the plant defense response. No overlap of markers or genes was observed between field and greenhouse screenings, which suggests a complex molecular mechanism underlying resistance to CR in soybean with varied response to different environments. Our findings provide useful information for advancing breeding for CR resistance as well as the genetic mechanism of resistance.

### Acknowledgements

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**Figure 1. (A) Classes of charcoal rot resistance: resistant (values of 1), moderately resistant (values >1 and ≤2), moderately susceptible (values >2 and <3), and susceptible (values 3–5). (B) Split stem showing symptoms associated with the scores: 1, no microsclerotia visible in vascular tissue; 2, very few microsclerotia visible and vascular tissue is not discolored; 3, microsclerotia partially covering the vascular tissue and minimal discoloration; 4, numerous microsclerotia in the tissue, and also visible under the outside epidermis, and discolored vascular tissue; 5, darkened vascular tissue due to high numbers of microsclerotia both inside and outside of stem.**

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