Title: Resistance to Stalk Pathogens for Bioenergy Sorghum

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Project Goals: This research is focused on discovering molecular and metabolic networks that drive sorghum resistance or tolerance to stalk rot pathogens. We are using advanced molecular techniques with the goal of identifying key factors associated with resistance and tolerance to stalk pathogens in sorghum lines optimized for key bioenergy traits (modified phenylpropanoid metabolism), with enhanced drought tolerance (nonsenescence) or with increased tolerance to stalk pathogens. Some lignin altered lines and drought tolerant lines already have demonstrated increased tolerance to these pathogens but specific mechanisms that mitigate pathogenic growth have yet to be identified. Our long-term goal is development of sorghum lines that withstand increased pathogen loads under reduced water conditions based on knowledge gained through this research.

Sorghum is a promising bioenergy crop with high yield potentials and significant tolerance to both drought and heat. However, under water or heat stress, sorghum is prone to stalk rots, which can significantly limit sorghum biomass yield through growth reductions and lodging. Stalk rot-causing fungi normally grow endophytically (asymptomatically) within sorghum plants. When sorghum plants experience water stress, host changes often trigger a developmental switch causing the fungi to become pathogenic, resulting in decayed stalk tissue. The underlying plant molecular circuits that either limit or exacerbate this fungal transition from endophytic to pathogenic growth are not known and are the focus of this proposal. Several publicly available lines have previously demonstrated resistance or tolerance to sorghum stalk pathogens, including lines with post-flowering drought tolerance (nonsenscence), which appears to suppress pathogenic growth, or a variety of lines that have exhibited increased resistance under field conditions. We have developed several near-isogenic *brown midrib* (*bmr*) *6* and *12* lines with altered lignin content and composition, which were previously demonstrated to have increased resistance or tolerance to stalk pathogens (2,3,5). Lignin, a component of plant cell walls, has been a focus for development of bioenergy sorghums because its presence increases recalcitrance of biomass to cellulosic ethanol conversion, but its presence also increases total energy content of biomass, which is important for thermal conversion technologies. The *bmr* lines have reduced lignin and increased ethanol conversion efficiency (1) due to single mutations in enzymes in the monolignol (lignin subunits) biosynthesis pathway, cinnamyl alchohol dehydrogenase (*bmr6*) or caffeic acid *O*-methyltransferase (*bmr12*). To increase energy content, we have engineered sorghum plants overexpressing a Myb transcription factor that induces synthesis of monolignols and a gene encoding caffeoyl-CoA *O*-methyltransferase, a monolignol pathway enzyme. Both transgenic and *bmr* plants accumulate phenolic intermediates from monolignol biosynthesis that inhibit stalk pathogens *in vitro* (5). We have developed an assay in a controlled environment, with applied water-stress, which reliably induces the developmental switch from endophytic to pathogenic growth of sorghum stalk rot fungi (2).

Our research may have identified sources of resistance in *bmr6* and *bmr12* lines, relative to wild-type, to the stalk rot pathogens, *Fusarium thapsinum* and *Macrophomina phaseolina.* We

have previously shown that following inoculation of peduncles with each of these fungi, a visible lesion is first apparent at 3 days post inoculation (dpi) and lesion expansion is first apparent at 13 dpi (4). In the current research, mean lesion lengths at 3 dpi were not significantly different between near-isogenic wild-type, *bmr6* and *bmr12* lines under either well-watered (100% field capacity) or reduced-water (25% field capacity) conditions. However, at 13 dpi, *bmr12* had significantly reduced lesion lengths, but only under reduced water, as compared with the wild-type; reductions in mean lesion lengths resulting on *bmr6* plants under this condition were not significant. Global gene expression of *bmr6, bmr12* and wild-type plants at 3 dpi under both watering conditions was conducted to identify early abiotic and biotic stress response genes.

The relative expression profile of infected tissues from the three plant genotypes under both watering conditions suggested common and unique host genetic responses influenced by genotype and watering condition. Gene expression analysis suggested that the reduced water condition primed *bmr12* for resistance to infection. Inoculated well-watered *bmr12* plants exhibited similar expression profiles to each fungus and to control reduced-water *bmr12,* but not control well-watered *bmr12*. Expression profiles of reduced water-treated wild-type and *bmr12* plants, and well-watered *bmr12* were mostly similar, suggesting that *bmr12* is stressed under well-watered conditions. Expression modules from *bmr12* well-watered control, but not wild-type, overlaps with reduced-water modules also suggesting priming for resistance to drought and infection. Nonetheless, there does not appear to be a unique expression pattern associated with the reduced lesion length observed in pathogen-infected *bmr12* plants under reduced water. Expression modules associated with reduced water included genes and pathways involved in photosynthesis, protein processing, carbon metabolism, and ubiquitin-mediated proteolysis. Expression modules associated with fungus infection under reduced water included amino acid biosynthesis; phenylpropanoid, glutathione and flavonoid biosynthesis; and, pyrimidine metabolism, DNA replication, homologous recombination and mismatch repair.

These results strongly indicated that *bmr6* and *bmr12* lines, and near-isogenic wild-type, are promising sources to identify genomic and metabolic markers that can be used to develop lines with increased resistance to stalk pathogens under water deficit conditions.

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