

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 will use advanced molecular techniques to identify 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 the mechanisms that mitigate pathogenic growth have yet to be identified. Our goal is to develop 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, 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 within sorghum plants. When sorghum plants experience water stress, host changes often trigger a developmental switch causing the fungi to become pathogenic. 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 (nonsenescence), 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 sorghum *brown midrib (bmr) 6* and *12* lines with altered lignin content and composition, which were previously demonstrated to have increased resistance or tolerance to sorghum stalk pathogens (1,3,4,5). 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. To increase energy content, we have engineered sorghum plants overexpressing a Myb transcription factor that induces synthesis of monolignols, the lignin subunits, and a gene encoding caffeoyl-CoA *O*-methyltransferase, a monolignol pathway enzyme. Both the transgenic and *bmr* plants accumulate phenolic intermediates from monolignol biosynthesis that inhibit stalk pathogens *in vitro* (4). We have identified a procedure to determine pathogen survival in lesions and asymptomatic tissues of sorghum peduncles (top of the stalk, below the head; 3) and have recently developed a controlled-environment, water-stress assay, which reliably induces the developmental switch from endophytic to pathogenic growth of sorghum stalk rot fungi.

Our recent research may have identified sources of resistance in *bmr6* and *bmr12* lines, relative to the wild-type, to two 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 (2). In the current research, there were significant differences in mean lesion lengths resulting on *bmr6* and *bmr12* plants at 13 dpi with each fungus under adequate water or water deficit conditions as compared to wild-type plants with these treatments. In particular, *bmr6* plants under the adequate water treatment and both *bmr6* and *bmr12* plants under water deficiency had significantly smaller mean lesion lengths than wild-type plants after inoculations with *M. phaseolina*. No significant differences were apparent after inoculations with *F. thapsinum* under adequate water, but both *bmr* lines had significantly smaller mean lesion lengths than wild-type under water deficit conditions. Interestingly, *bmr12* plants had significantly smaller mean lesion lengths under water deficit than under adequate water when inoculated with either pathogen, counter to expected response to stalk pathogens under water stress. Across both water conditions, *bmr12* plants had reduced *F. thapsinum* survival within lesions. At 3-cm beyond the lesion border, there was reduced pathogen survival in both *bmr6* (*F. thapsinum* and *M. phaseolina*) and *bmr12* (*M. phaseolina*) plants as compared with wild-type. These results suggest that reduced survival of the pathogens within *bmr6* and *bmr12* stalks is likely due to changes induced by impaired monolignol biosynthesis.

These results strongly indicate that *bmr6* and *bmr12* lines, and near-isogenic wild-type, are promising for identification of genomic and metabolic markers for increased resistance to stalk pathogens under water deficit conditions.

References

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