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Stalk strength and reaction to infection by *Macrophomina phaseolina* of brown midrib maize (*Zea mays*) and sorghum (*Sorghum bicolor*)

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ABSTRACT

Reduced lignin concentration in brown mid-rib mutants in both maize and sorghum have resulted in improved dry matter digestibility, increased milk yield and higher energy in lactating cows. However, the mutations were not widely deployed due to concern that reduced lignin concentration might increase vulnerability to lodging and stalk rot incidence. The objective of this study was to determine the effects of the mutations on stalk strength and stalk rot resistance in both sorghum and maize. Six brown midrib (*bmr*) sorghum, four brown midrib (*bm*) maize, and their normal isolines were evaluated for stalk strength and stalk rot disease reaction at two locations in four replications. Three randomly selected plants in each plot were inoculated with *Macrophomina phaseolina* at 14 d after flowering by using the toothpick inoculation technique. On 28 d after inoculation, the plants were rated for disease severity by measuring the length of necrotic lesions in the stalks. Stalk strength was determined from another three random plants in each plot by using a rind penetrometer. The mutations had no effect on stalk rot disease severity in either sorghum or maize though stalks strength was markedly affected by the mutations in both species. While maize *bm* entries broke easily in response to mild mechanical stress, *bmr* sorghums did not exhibit sign of stalk collapse in all backgrounds. The result suggests that the *bmr* genes in both maize and sorghum can be deployed without incurring losses to stalk rot disease.

1. Introduction

The evolutionary course that granted plant species the ability to synthesize and polymerize monolignol residues into complex lignin has been crucial for development of erect plants and their adaptation to a terrestrial habitat (Raven et al., 1999; Boudet, 2000). Monolignols are synthesized through the phenyl propanoid pathway, and enzymes catalyzing their synthesis have been identified and characterized (Hennion et al., 1992; Baucher et al., 1998; Whetten et al., 1998). Besides providing mechanical support and aiding in water and mineral transport by consolidating the treachery xylem elements (Campbell and Sedroff, 1996; Ye et al., 2001; Peter and Neale, 2004), lignin confers structural defense against mechanical stress and enhances fitness (Casler et al., 2002; Pedersen et al., 2005).

Mutations in lignin biosynthetic pathways have been reported in several crop species (Porter et al., 1978; Hartley et al., 1992; Halpin et al., 1998). These are the results of altered activity of enzymes that polymerize monolignol residues into complex lignin (Pillonel et al., 1991; Halpin et al., 1998; Ralph et al., 1998). Variants of sorghum, maize, and pearl millet genotypes with low lignin concentration in stems, leaf sheaths, and leaf blades have been reported (Porter et al., 1978; He et al., 2003; Vogler et al., 2009).

To date five *bm* loci (*bm*1, *bm*2, *bm*3, *bm*4 and *bm*5) have been identified in maize (Kuc et al., 1968; Haney et al., 2008). More than two dozens of *bmr* alleles have been identified in sorghum, but alleleism tests indicate that most of the mutations are allelic and only four *bmr* loci are known (Saballos et al., 2008). Reduction in the activity of lignin biosynthetic enzymes Caffeic acid O-Methyltransferase (COMT) and Cinnamyl Alcohol Dehydrogenase (CAD) was responsible for reduced lignin concentration and altered lignin composition in the mutants. The *bm*3 mutation in maize and *bmr*7, *bmr*12 and *bmr*26 mutations in sorghum have been reported to have resulted from reduced activity of COMT (Vignols et al., 1995; Bout and Vermerris, 2003; Saballos et al., 2008). On the other hand, the *bm*1 mutation in maize and *bmr*6 and *bmr*28 mutations in sorghum were associated with reduced activity of CAD (Bucholtz et al., 1980; Pillonel et al., 1991; Halpin et al., 1998). The *bm*2 and *bm*4 in maize and several *bmr* loci in sorghum have also been reported to cause reduced lignin concentration. Nevertheless, specific pathways leading to these mutations have not been determined yet. These mutations, which are expressed by a shared brown midrib phenotype in both maize and sorghum, have important biological and economic implications, especially in the feedlot

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Table 1
Analysis of variance for lesion length and resistance to rind penetration among low-lignin and normal sorghum and maize genotypes tested at two locations Purdue Agricultural Center for Research and Education (ACRE) near West Lafayette and Piney Purdue Agricultural Center (PPAC), Piney, Indiana.

Source	df[a]	Lesion length (mm)			Rind penetration resistance		
		ACRE	PPAC	Combined	ACRE	PPAC	Combined
Environment (E)	1	–	–	1669.86	–	–	5.31*
E (Block)	6(3)	420.43	285.14	352.78	6	3.1	4.55
Main Plot (MP)	1	110.02	87.01	3.72	4.58*	0.3	3.62*
MP × E	1	–	–	90.61	–	–	1.02
Error[b]	6(3)	63.31	364.92	514.11	0.59	1.41	1
Genotypes (G)	9	6047.87*	1112.21*	2977.7	5.16*	5.15*	9.82*
Sorghum(S)	5	10,273.48*	1235.46*	4937.37	6.43*	6.10*	11.98*
Maize (C)	3	219.25	346.7	170.49	4.04*	3.96*	7.59
S vs. C	1	648.3	3341.87*	632.76	1.77	1.96	5.5
G × MP	9	119.44	275.4	320.11	3.76*	1.02	3.74
S × MP	5	62.46	126.88	262.8	4.00*	0.66	3.09
C × MP	3	119.81	621.44	485.54	4.32*	1.69*	5.56
S vs. C × MP	1	275.17	62.57	291.39	1.09	0.79	1.87
G × E	9	–	–	2968	–	–	0.51
S × E	5	–	–	3771.62	–	–	0.55
C × E	3	–	–	388.47	–	–	0.58
S vs. C × E	1	–	–	3567.61	–	–	0.21
G × MP × E	9	–	–	80.29	–	–	1.04
S × MP × E	5	–	–	218.67	–	–	1.45
C × MP × E	3	–	–	66.93	–	–	0.5
S vs. C × E	1	–	–	29.75	–	–	0.01
Error[c]	107(53/54)	432.72	509.62	466.01	0.84	0.64	0.74

* Statistically significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.
[a] Degrees of freedom in the parentheses are for individual location analysis.

industry, because both maize and sorghum are significant components of animal feed. Most of the brown midrib mutations reduce the level of lignin in plants and remarkably increase intake and dry matter digestibility (Frenchik et al., 1976; Rook et al., 1977; Cherney et al., 1991). Brown midrib maize silage alone and mixed with protein sources has significantly increased milk yield in lactating cows (Keith et al., 1979; Stallings et al., 1982; Oba and Allen, 1999). Similarly, the rates of *in vitro* dry matter digestibility and cell wall degradation by rumen bacterium of leaf blades from *bmr*12 sorghum were shown to be significantly higher than those from its wild-type isoline (Akin et al., 1986a,b). Besides affecting feed values, high lignin concentration in pulp woods has also been detrimental in the paper industry because lignin removal is a major step in paper production (Campbell and Sedroff, 1996). Likewise, in view of the growing interest in bioenergy development, high lignin concentration in biofuel feedstock sources is obviously undesirable because of its negative effect on conversion of biomass into fermentable sugars (Grabber, 2005; Vermerris et al., 2007).

Despite the drawbacks of high-lignin, production of low-lignin cultivars has not been widely adopted, partly because of the concern that altered lignin structure in the mutants may reduce standability and predispose plants to attack by pests and diseases. However, few studies have been conducted to quantitatively describe the effect of these mutations on stalk rot disease incidence in both maize and sorghum. The objectives of this study were to evaluate the effect of the brown mid-rib mutations on mechanical strength of stalks and determine reaction of low-lignin maize and sorghum mutants to the major stalk rotting pathogen, *Macrophomina phaseolina*.

2. Materials and methods

2.1. Genetic materials

Six brown midrib sorghum mutants (*bmr*2, *bmr*6, *bmr*7, *bmr*12, *bmr*26, *bmr*28) and four maize mutants (*bm*1, *bm*2, *bm*3, and *bm*4) along with their normal (*Nor*) isolines were used in this study. The *bmr*2, *bmr*6, *bmr*7 and *bmr*12 mutants were derived by artificial mutagenesis of a breeding line 954114 while *bmr*12 was derived from 954104 in the same way (Porter et al., 1978). *Bmr*26 and *bmr*28 are spontaneous mutations discovered in backgrounds not related to the other mutants. Allelism tests and characterization of the mutations revealed that the six mutants occupy three loci on sorghum genome namely the *bmr*2 group, the *bmr*6 group which includes *bmr*6 and *bmr*28 and the *bmr*12 group which includes *bmr*7, *bmr*12 and *bmr*26. The genotypes were evaluated under two environments at the Purdue University Agronomy Center for Research and Education (ACRE) near West Lafayette, IN, and at Piney Purdue Agricultural Center (PPAC) in Piney, IN. The experiment was conducted in a split-plot design with randomized complete blocks in four replications. The genotypes (six sorghum and four maize) were assigned to the whole plot unit, and their brown mid-rib and normal versions were assigned to the subplots. The whole plot consisted of four 6-m-long rows (two rows each for the two subplot treatments within each whole plot unit). Seeds were sown following the standard procedure for both crops, and optimum agronomic practices were used throughout the growing period. For treatment application, six plants from each subplot unit were marked with tagging tapes at half bloom stage. At 14 d after flowering, three of the marked plants were inoculated on the basal stalk with *M. phaseolina*, a causal organism of charcoal rot, by using a toothpick inoculation technique. Charcoal rot is an aggressive stalk rot pathogen that is particularly devastating when hot, dry weather overlaps with crop maturation in both sorghum and maize (Mughogho and Pande, 1984). The remaining three plants were used to determine stalk strength using digital rind penetrometer, a modified AccuForce Cadet (Ametek, Hatfield, PA) powered by alkaline battery.

2.2. Inoculum preparation and inoculation

Pure culture of *M. phaseolina* was provided by Dr. Mitch Tuinstra from Kansas State University. The strain was originally collected from infested sorghum fields in Texas in summer 2001 by Dr. Garry Odvody and shipped to Kansas State in water agar. In 2003, the strain was grown on fresh water agar and shipped to Purdue University, where it was sub-cultured on potato dextrose agar and