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Studies on the Inheritance of Brown Midrib Trait and Allelic Relationships among BMR Mutants in Sorghum [*Sorghum bicolor* (L.) Moench]

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ABSTRACT

Brown midrib (bmr) mutants of sorghum have reddish brown vascular tissues in their leaves and stem with reduced lignin content that increases the bioconversion efficiency and digestibility. Earlier studies showed that bmr trait in sorghum is caused by single recessive mutation and there are many independent loci responsible for brown pigmentation. In this study, three novel spontaneous bmr mutants viz., IS 23253, IS 21549 and IS 11861 were used to establish the allelic relationship with 10 bmr mutants belonging to three known bmr groups i.e., bmr 2, bmr 6 and bmr 12. Developed 78 F_1s from 13 selected parents using half-diallel mating design. Based on the allelism test, IS 23253 and IS 21549 grouped with bmr 6 group. Also, all F_2 populations involving IS 11861, IS 23253 and IS 21549 and lines belonging to three known bmr allelic groups, i.e., bmr2, bmr 6 and bmr 12 showed 3:1, 13:3, 49:15, 193:63, 195:61, 189:67, 45:19, 11:5 segregation ratios for normal: brown mid-rib phenotypes indicating that single recessive gene controlling the brown midrib trait.

Keywords: *Brown midrib, CAD ,COMT, 4 CL, Sorghum.*

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] a member of Gramineae family is mainly grown for food, feed, forage, and fuel purposes, globally on 42 m ha in all six continents (Kumar, 2013). It is the fifth most important cereal crop and is the staple food for the people living in the Semi-Arid areas of Africa and Asia. While sweet sorghum is favored for sugar based (1G) ethanol production the biomass sorghums are highly suited to lignocellulosic (2G) biofuel production. Sorghum is considered a model biomass feedstock because of its quick growth, high biomass yield, drought tolerance and effective nutrient usage (Mathur et al., 2017). Brown midrib mutations in sorghum, like in maize, are characterized by the presence of brown vascular tissue in leaf blade and sheath as well as in the stem. The brown midrib phenotype was found to be associated with altered lignin content/composition that increased the bioconversion efficiency and digestibility (Poovaiah et al., 2014).

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The reddish-brown coloration of leaf midrib is a morphological marker to identify this popular genetic mutation *(bmr)* in C_4 grasses. These mutants are characterized by substantial reduction, in amorphous hydrophobic polymer and a component of plant cell wall whose content and composition is phenolic in nature. The gene responsible for this is named as "brown midrib". Brown midrib (*bmr*) mutations have been found naturally but can be induced. In sorghum, all the *bmr* mutants (*bmr* 1 to *bmr* 19) were generated by using diethyl sulfate (DES) mediated chemical mutagenesis from two-grain sorghum lines 954114 and 954104 (Porter et al., 1978; Bittinger et al., 1981). Later, many other spontaneous and chemically induced *bmr* lines were identified (Vogler et al., 1994). These mutants were numbered serially from 1 to 28. Recently, 10 more *bmr* mutants were generated through chemical mutagenesis from genotype BTx623, and they were numbered from 29 to 38 (Xin et al., 2009). The brown midrib (*bmr*) mutation in sorghum significantly reduces the lignin content and increases the digestibility of stover. It improves the ethanol conversion efficiency reducing the cost of biofuel production (Cherney et al., 1991; Oliver et al., 2005; Srinivasa et al., 2009; Srinivasa et al., 2010).

Most of the naturally occurring or induced *bmr* mutants in sorghum have been designated into four allelic groups, i.e., *bmr* 2, *bmr* 6, *bmr* 12 and *bmr* 19 (Sattler et al., 2014; Saballos et al., 2008). Based on the allelic test few mutants in each group have been characterized at the molecular level. Out of the four allelic groups, the genes representing three allelic groups were identified and characterized at the molecular level, i.e., *bmr* 2, *bmr* 6 and *bmr* 12 loci encoding 4-coumarate CoA ligase (*4CL*), cinnamyl alcohol dehydrogenase-2 (*CAD2*) and caffeic acid O-methyltransferase (*COMT*) respectively (Saballos et al., 2008; Bout & Vermerris, 2003; Saballos et al., 2012). The *bmr*19 mutant is not publicly available (Sattler et al., 2014) (effectively reducing the available sorghum brown midrib mutants to a set of three independent loci: *bmr* 2, *bmr* 6, and *bmr* 12). However, *bmr*19 appears to be of limited value for forage and bioenergy applications, because it did not significantly reduce lignin concentration and did not markedly alter lignin subunit composition (Saballos et al., 2008). At ICRISAT we identified three new brown midrib lines IS 23253, IS 21549 and IS 11861 in International Sorghum (germplasm) lines whose genetic control and allelic reaction not known for their utilization. In this context, we studied the inheritance and allelic relationships using a set of established 10 known *bmr* lines and these three unclassified *bmr* mutants.

MATERIALS AND METHODS

Genetic material and field evaluation

The field experiments were conducted at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India (78°12' E, 17°24' N, and 545 m). The three newly identified *bmr* sources (IS 11861, IS 21549 and IS 23253) and known 10 *bmr* lines from three known allelic groups (*bmr* 2, *bmr* 6 and *bmr* 12) were crossed in half-diallel fashion and 78 F_1 were produced in the 2013 rainy season **(Table 1).** These F_1s were evaluated during 2013 postrainy season and scored phenotypically based on the presence of brown coloration of the midrib. All the F_1 were selfed to produce F_2 seeds. In 2014 postrainy season, F_2 progenies were planted to record segregation pattern of the *bmr* alleles (normal midrib- brown midrib). Each F_2 was planted in four rows of 4 m length, spaced at 0.60 m between the rows and a plant-to-plant distance was maintained at 0.15 m. Each individual brown midrib and normal (white) midrib plants were identified, tagged and selfed. Classification of normal/white midrib and brown midrib plants was done at the seven-leaf stage, during the pre-flowering (boot leaf stage) in both the F_1 and F_2 populations. Chi-square test was performed to test the data for the goodness of fit for F_2 progenies (Steel et al., 1980).

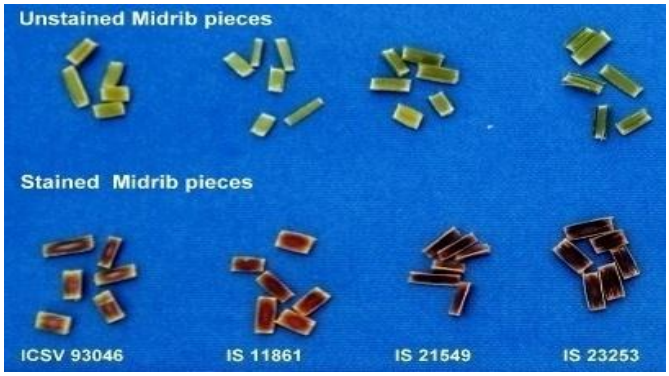
RESULTS AND DISCUSSION

Allelism test establishes the relationship between mutants. When a cross between any two brown mid-rib lines shows brown midrib phenotype in their F_1, then the parents are said to be allelic to each other for brown mid-rib trait. In this study, to identify allelic nature of three new spontaneous brown mid-rib sources IS 11861, IS 21549 and IS 23253, crosses were made in half diallel fashion using 10 well-characterized brown mid-rib lines and all the 78 F_1s were scored for presence of brown mid-rib traits. Of the 78 F_1s evaluated, 25 F_1s showed brown-midrib and remaining 53 F_1s had normal mid-rib (**Table 1**).

The F_1s between IS 21549 and IS 23253 had brown mid-rib, indicating that both have the similar allele for the trait (**Table 1**). Further, the phenotypic evaluation of the F_2 population of this cross-showed all plants with brown mid-rib phenotypes; additionally, confirming that same allele is present in both the genotypes. Similarly, all the F_1s and F_2s derived from cross between these two lines IS 21549 and IS 23253 with four known lines belonging to *bmr* 6 groups (IS 21888 *bmr* 3, N 592, N594, N 596) showed brown mid-rib trait indicating that both genotypes having similar alleles of that of *bmr 6* group (**Table 2**). Similar reports on unknown mutants were made earlier using allelic tests and molecular marker based candidate gene study (Gupta et al., 1995a; Gorthy et al., 2013). The F_1s between IS 21549 and IS 23253 with *bmr* 2 and *bmr* 12 group genotypes showed normal mid-rib. Indicating that these both genotypes were non-allelic to *bmr* 2 and *bmr* 12.

The F_1s between IS 21549 and IS 23253 with IS 11861 showed normal mid-rib. Similarly, in the crosses made between IS 11861 and lines belonging to three known *bmr* allelic groups, *i.e*., *bmr* 2, *bmr* 6 and *bmr* 12, all the F_1s showed normal mid-rib, indicating that the allele in this mutant (IS 11861) is different from the known mutants used in this experiment. All these F_1 involving IS 11861 were further advanced to F_2 to study the segregation pattern of the brown-midrib allele. All the 19 F_2 populations developed between spontaneous unknown *bmr* lines (IS 1181, IS 21549 and IS 23253) and known *bmr* lines belonging to *bmr* 2, *bmr* 6 and *bmr* 12 groups showed segregation ratios of 3:1, 13:3, 49:15, 193:63, 195:61, 189:67, 45:19, 11:5 for normal midrib: brown-midrib showing good fit to 3:1 segregation ratio with few exceptions (**Table 3**). This data clearly suggested that brown midrib trait is controlled by a single gene and smaller population size in those F_2s, inheritance pattern sometimes show deviation from expected ratios due to or allelic interactions or possibility that some modifying genes affecting the expression of this trait. Earlier studies in sorghum (Saballos et al., 2008; Gupta et al., 1995a) and pearl millet [19] also reported the presence of interaction component and also non-consistency in the interaction ratio different crosses. It was also reported that non-consistency in the interaction ratio might be due to the fitness penalty of the genetic background of *bmr* trait (Saballos et al., 2008). In a similar study, it was reported that these differences in inheritance pattern might be due to the presence of some modifying genes affecting the expression of this trait and this can be confirmed by an extended study using test crosses and progeny performance of F_3 populations (Gupta, 1995a).

Phloroglucinol staining helps in differentiating wild type alleles from brown midrib alleles. The *bmr* 6 ref mutant, a null allele of *CAD2*, shows intense wine-red color with Phloroglucinol staining (Saballos et al., 2008). In our study involving *bmr* and white midrib control, clear differences in the intensity of the staining were observed among dissected midrib samples from the different *bmr*lines **(Fig 1**). The intensity of the color reaction depends on the abundance of hydroxyl cinnamaldehyde end groups and total lignin contents in this tissue. The staining is more intense in *bmr* than non-*bmr* genotypes **(Fig 1**). The midrib of IS 23253 and IS 21549 showed the highest intensity, with a dark wine red color followed by IS11861 (**Fig 1**). The dark red staining of midribs of lines IS 23253 and IS 21549 indirectly supports the presence of the *bmr* 6 allele which may be associated with reduced *CAD2* activity, which needs to be validated.



Non-bmr ***bmr***

Fig. 1: Dissected midribs fromthenew*bmr* sources, IS 11861, IS 21549, IS 23253 along control (non- *bmr*) ICSV 93046priortothe staining (top) and after staining in acidPhloroglucinol. *Bmr* mutants take more stain than control and staining pattern is same in mutants (IS 21549 and IS 23253) belonging to same group (*bmr* 6).

Table 1: Phenotypes of the F_1s generated between three unknown *bmr* lines and 10 lines belonging to known *bmr* groups, evaluated during the 2013 rainy seasons at ICRISAT-Patancheru

Allelic group	Genotype	Unknown *bmr* source			*bmr*2 group	*bmr*6 group				*bmr*12 group				
		IS 21549	IS 23253	IS 11861	*bmr* 2	IS 21888	N 592	N 594	N 596	IS 21890	N 593	N 597	Atlas	IS 40602
Unknown *bmr* source	IS 21549	x	*bmr*	N	N	*bmr*	*bmr*	*bmr*	*bmr*	N	N	N	N	N
	IS 23253		x	N	N	*bmr*	*bmr*	*bmr*	*bmr*	N	N	N	N	N
	IS 11861			x	N	N	N	N	N	N	N	N	N	N
*bmr*2 group	*bmr* 2				x	N	N	N	N	N	N	N	N	N
*bmr*6 group	IS 21888 *(bmr 3)* *(bmr 6)*					x	*bmr*	*bmr*	*bmr*	N	N	N	N	N
	N 592						x	*bmr*	*bmr*	N	N	N	N	N
	N 594							x	*bmr*	N	N	N	N	N
	N 596								x	N	N	N	N	N
*bmr*12 group	IS 21890 (*bmr* 7) (*bmr* 12)									x	*bmr*	*bmr*	*bmr*	*bmr*
	N 593										x	*bmr*	*bmr*	*bmr*
	N 597											x	*bmr*	*bmr*
	Atlas *bmr*12												x	*bmr*
	IS 40602													x

'N'-Normal/wild-type midrib, '*bmr*'-brown midrib

Table 2: Segregation for midrib color in F_2 population of the crosses between new *bmr* line IS 21549 and IS 23253 and the *bmr* lines belonging to *bmr*6 group

F_2 population			No of Plants	
			Normal midrib	Brown midrib
IS 21549	x	IS 23253	0	190
IS 21549	x	IS 21888	0	210
IS 21549	x	N 592	0	186
IS 21549	x	N 594	0	180
IS 21549	x	N 596	0	204
IS 23253	x	IS 21888	0	174
IS 23253	x	N 592	0	192
IS 23253	x	N 594	0	178
IS 23253	x	N 596	0	193

Table 3: Midrib phenotypes of F_2 population derived from crosses spontaneous unknown *bmr* genotypes (IS 11861, IS 21549 and IS 23253) with known *bmr* lines belonging to *bmr* 2, *bmr* 6 and *bmr* 12 groups

SN	Cross	Normal	Brown	Test	3:1	13:3	49:15	193:63	195:61	189:67	45:19	11:5
1	IS 11861 x *bmr* 2	87	23	Chi-square	0.982	0.337	0.392	0.812	0.516	1.577	4.061	5.48
				P value	0.322	0.562	0.531	0.368	0.472	0.209	0.044	0.019
				Sig	NS	NS	NS	NS	NS	NS	*	*
2	IS 11861 x IS 21888 (*bmr* 3) (*bmr* 6)	55	20	Chi-square	0.111	3.085	0.436	0.171	0.333	0.010	0.328	0.73
				P value	0.739	0.079	0.509	0.679	0.564	0.922	0.567	0.392
				Sig	NS	NS	NS	NS	NS	NS	NS	NS
3	IS 11861 x N 592 (*bmr* 6)	70	20	Chi-square	0.370	0.712	0.074	0.276	0.128	0.727	2.403	3.41
				P value	0.543	0.399	0.785	0.599	0.721	0.394	0.121	0.065
				Sig	NS	NS	NS	NS	NS	NS	NS	NS
4	IS 11861 x N 594 (*bmr* 6)	82	26	Chi-square	0.049	2.009	0.024	0.017	0.004	0.246	1.630	2.59
				P value	0.824	0.156	0.876	0.897	0.952	0.620	0.202	0.108
				Sig	NS	NS	NS	NS	NS	NS	NS	NS
5	IS 11861 x N 596 (*bmr* 6)	115	38	Chi-square	0.002	3.721	0.167	0.004	0.086	0.141	1.725	2.93
				P value	0.963	0.054	0.683	0.948	0.770	0.707	0.189	0.087
				Sig	NS	NS	NS	NS	NS	NS	NS	NS
6	IS 11861 x IS 21890 (*bmr* 7) (*bmr* 12)	67	13	Chi-square	3.267	0.328	2.303	3.013	2.531	4.076	6.920	8.38
				P value	0.071	0.567	0.129	0.083	0.112	0.043	0.009	0.004
				Sig	NS	NS	NS	NS	NS	*	**	**
7	IS 11861 x N 593	98	36	Chi-square	0.249	5.793	0.878	0.368	0.681	0.033	0.511	1.20
				P value	0.618	0.016	0.349	0.544	0.409	0.855	0.475	0.274
				Sig	NS	*	NS	NS	NS	NS	NS	NS
8	IS 11861 x Atlas *bmr* 12	118	35	Chi-square	0.368	1.710	0.027	0.248	0.076	0.860	3.401	4.99
				P value	0.544	0.191	0.870	0.619	0.782	0.354	0.065	0.025
				Sig	NS	NS	NS	NS	NS	NS	NS	*
9	IS 11861 x IS 40602	102	40	Chi-square	0.761	8.269	1.772	0.970	1.474	0.293	0.157	0.63
				P value	0.383	0.004	0.183	0.325	0.225	0.588	0.692	0.428
				Sig	NS	**	NS	NS	NS	NS	NS	NS
10	IS 21549 X Atlas *bmr* 12 (*bmr*12)	80	27	Chi-square	0.003	2.953	0.192	0.022	0.116	0.049	1.017	1.80
				P value	0.955	0.086	0.661	0.881	0.733	0.825	0.313	0.179
				Sig	NS	NS	NS	NS	NS	NS	NS	NS
11	IS 21549 X IS 40602 (*bmr*12)	118	40	Chi-square	0.008	4.472	0.311	0.043	0.193	0.060	1.446	2.59
				P value	0.927	0.034	0.577	0.837	0.661	0.807	0.229	0.108
				Sig	NS	*	NS	NS	NS	NS	NS	NS
12	IS 21549 X IS 21890 (*bmr* 7) (*bmr*12)	91	29	Chi-square	0.044	2.311	0.036	0.013	0.008	0.250	1.752	2.80
				P value	0.833	0.128	0.850	0.910	0.931	0.617	0.186	0.094
				Sig	NS	NS	NS	NS	NS	NS	NS	NS
13	IS 21549 X N 593 (*bmr*12)	90	29	Chi-square	0.025	2.467	0.058	0.004	0.019	0.200	1.612	2.62
				P value	0.874	0.116	0.810	0.952	0.890	0.655	0.204	0.105
				Sig	NS	NS	NS	NS	NS	NS	NS	NS
14	IS 23253 X Atlas *bmr* 12 (*bmr*12)	160	57	Chi-square	0.186	8.049	0.968	0.321	0.711	0.001	1.216	2.51
				P value	0.666	0.005	0.325	0.571	0.399	0.974	0.270	0.113
				Sig	NS	**	NS	NS	NS	NS	NS	NS
15	IS 23253 X IS 40602 (*bmr*12)	116	36	Chi-square	0.140	2.429	0.005	0.070	0.002	0.487	2.624	4.05
				P value	0.708	0.119	0.943	0.791	0.967	0.485	0.105	0.044
				Sig	NS	NS	NS	NS	NS	NS	NS	*
16	IS 23253 X IS 21890(*bmr* 7) (*bmr*12)	80	32	Chi-square	0.762	7.092	1.645	0.948	1.388	0.334	0.067	0.37
				P value	0.383	0.008	0.200	0.330	0.239	0.563	0.796	0.541
				Sig	NS	**	NS	NS	NS	NS	NS	NS
17	IS 23253 X N 593 (*bmr*12)	80	32	Chi-square	0.762	7.092	1.645	0.948	1.388	0.334	0.067	0.37
				P value	0.383	0.008	0.200	0.330	0.239	0.563	0.796	0.541
				Sig	NS	**	NS	NS	NS	NS	NS	NS
18	IS 21549 X IS 11861	98	36	Chi-square	0.249	5.793	0.878	0.368	0.681	0.033	0.511	1.20
				P value	0.618	0.016	0.349	0.544	0.409	0.855	0.475	0.274
				Sig	NS	*	NS	NS	NS	NS	NS	NS
19	IS 23253 X IS 11861	172	44	Chi-square	2.469	0.372	1.132	2.092	1.423	3.763	8.983	11.90
				P value	0.116	0.542	0.287	0.148	0.233	0.052	0.003	0.001
				Sig	NS	NS	NS	NS	NS	NS	**	**

** Significant at 1% level of significance, *significance at 5% level of significance; NS- Non significant

CONCLUSION

In conclusion, based on the allelism tests, out of the three spontaneous *bmr* mutants, two *bmr* lines (IS 23253 and IS 21549) were found to be allelic to *bmr* 6 group. In the crosses involving the third line, IS 11861 and the *bmr* allelic groups (*bmr* 2, *bmr* 6 and *bmr* 12), all the F_1 plants showed normal (white) midrib, indicating that this mutant (IS 11861) is different from all the known mutants used in this experiment. Brown midrib is controlled by a single gene but there is a strong possibility that some modifying genes may affect the expression of *bmr* trait.

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