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EVALUATION OF FORAGE SORGHUM BROWN MIDRIB LINES FOR QUALITY BIOMASS PRODUCTION

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ABSTRACT
The characteristic brown coloration of the leaf mid veins is associated with reduced lignin content and altered lignin content is a trait useful to improve forage digestibility for livestock. Therefore, keeping this in view fourteen brown midrib (*bmr*) lines of sorghum along with three checks were evaluated for agronomic and quality traits for two successive years. On the basis of pooled data of two years significant differences were observed among the bmr genotypes for green fodder yield, plant height, *in vitro* dry matter digestibility (IVDMD) and crude protein. Principal component analysis was carried out and 5 major component traits were find out which are explaining 80% of total variation among genotypes. All genotypes were clustered into 3 major clusters, cluster 1 had 9 genotypes, cluster II had 7 genotypes and cluster III had one genotypes i.e. only SSG 59-3 (multicut variety used as check). The *bmr* genotypes such as DSRBMR 1, PBMR-4 and BMR 233691 had high green fodder yield coupled with better forage quality, hence these can be exploited in future *bmr* hybrid breeding programs.

KEYWORDS: Lignin, fodder, *bmr*, variability and quality.

INTRODUCTION

Sorghum being a versatile crop is used as food, feed and a potential source of fodder for livestock in northern India. There is continuous increase in demand of milk, milk products and meat with the increasing population. Thus, livestock is a sector which plays a critical role in livelihood security. India supports 20% of the livestock population of the world on 2.3 % geographical area only. The country faces a net deficit of 61.1 % green fodder, 21.9 % dry crop residues and 64 % feeds (Vision, 2030). Thus sorghum being a major fodder crop in northern India has the potential to fulfill the growing demand of green fodder. In addition to quantity, quality of feed also is a matter of concern because good quality feed provide better nutrition to the livestock and intern also affect quality of by products. Sorghum feed quality is mainly determined by TSS%, CP% and IVDMD%. There are some morphological markers which indicate sorghum feed quality. In recent years, introduction of sorghum plants containing the *bmr* gene generated much interest because plants with this trait have lower lignin concentrations than conventional types. The BMR mutant of forage sorghum contained substantially less cell wall content than other sorghum types and resulted in greater fiber digestibility (Kotasthane *et al.,* 2010). Green color of leaf midrib in sorghum is an indicator of sweetness in sorghum and where as brown color mutants indicates reduced level of enzyme resistant polymer 'lignin' in plants and increase in palatability and digestibility (Rook *et al.,* 1977; Cherney *et al.,* 1991). Brown midrib (*bmr)* is a visible marker associated with the reduction of lignin in corn (Kue and Nelson, 1964), sorghum (Porter *et al.,* 1978) and pearl millet (Chernery *et al.,* 1988). The brown midrib mutants were isolated in maize, sorghum and pearl millets either by spontaneous and chemical mutagenesis (Sattler *et al.,* 2010). Teshome *et al.,* 1997 reported that farmers also use leaf midrib colour as an important character to identify the important sorghum landraces for *e.g.* juicy and non juicy. Although the intensity of the coloration cannot be taken as a measure of reduction in lignin, it is a clear indicator that *bmr* gene (s) are present.

Chemical and genetic approaches have been employed to improve forage fiber digestibility by reducing the amount of lignin or the extent of lignin cross linked with cell wall carbohydrates. Brown midrib forage genotypes usually contain less lignin and may have altered lignin chemical composition (Vogel and Jung, 2001). Activities of two enzymes involved in lignin synthesis are reduced as the result of the bmr mutations (Bout and Vermerris, 2003). Genetic control of the lignification process by the use of bmr genes is the major key for increasing sorghums digestibility (Gerhardt *et al.,* 1994). Thus keeping this in view we have evaluated *bmr* sorghum genotypes for fodder yield and quality related traits because easily observable quantitative morphological traits are useful tool for preliminary evaluation, and they also offers a fast and useful approach for assessing the extent of diversity.

MATERIALS & METHODS

This experiment was conducted during *kharif* 2014 and 2015 at Research area of Forage Farm at CCS HAU, Hisar. Research material consisted of fourteen *bmr* forage sorghum genotypes and checks SSG 59-3 (a multicut variety), CSV21F and HC 308 was sown in randomized block design to estimate overall performance of genotypes for various agronomic traits that affect the biomass

production directly or indirectly for two successive years Table: 1.

Hisar is located at 29.09°N 75.43°E in western Haryana. Hisar has very hot summers and relatively cool winters. The maximum day temperature during the summer varies between 40 to 46°C. Relative humidity varies from 5 to 100 per cent. The average annual rainfall is around 350 mm most of which occurs during the months of July and August. Dew is observed in December and January. This trial was sown in randomized block design having plot size 4m x 2r with row to row and plant to plant spacing 45cm and 30 cm, respectively for evaluating them along with check for various yields and forage quality related traits.

For agronomical evaluation we have recorded data on five randomly chosen plants for plant height (cm), leaf length (cm), leaf breadth (cm), no. of leaves/plant, no. of tillers/plant, early vigour, regeneration potential, plant population per meter row, stem girth(cm), leaf stem ratio, green fodder yield q/ha, dry fodder yield q/ha. Among quality parameters HCN µg/g on fresh wt. basis, *in vitro* dry matter digestibility % (IVDMD %) and crude protein % (CP %) were estimated from dry fodder by the method proposed by Gilchrist *et al.* 1967; Tilley and Terry (1971) and Micro-Kjeldhal's method respectively. For DFY, 500 g of green fodder was dried and then weighed to calculate DFY q/ha and crude protein and *in vitro* dry matter digestibility were also estimated from dry matter. The data recorded were analyzed by OPSTAT.

TABLE 1: List of bmr genotypes evaluated for biomass production

S. No.	Genotypes	Pedigree
1	PBMR 1	UP Chari 2 x EC 582506
2	PBMR 2	Pant Chari 5 x EC 582508
3	PBMR 3	EC 582506 x Pant Chari 5-1
4	PBMR 4	EC 582506 x Pant Chari 5-2
5	PBMR 5	EC 582506 x UP Chari 2
6	PBMR 6	EC 582506 x UP Chari 3-1
7	PBMR 7	EC 582506 x UP Chari 3-2
8	PBMR 8	EC 582506 x UP Chari 3-3
9	EC 582508	Germplasm
10	BMR 233691	-
11	BMR 23150	-
12	BMR 231581	-
13	DSRBMR 1	-
14	DSRBMR 2	-
15	SSG 59-3	Non sweet Sudan Grass x JS 263
16	HC 308	SPV 8 x IS 4776
17	CSV 21F	GSSV-148 x SR-897

RESULTS & DISCUSSIONS

The analysis of variance indicated significant variation for all 14 quantitative traits investigated indicating that there was a high level of genetic diversity among the *bmr* sorghum accessions. Maximum plant height and no. of leaves/plant was observed in DSRBMR-1among *bmr* genotypes excluding check SSG 590-3, maximum leaf length 70.4 cm in PBMR-1, no. of tillers EC 582508, maximum TSS% in PBMR-3, high green fodder yield was recorded in DSRMBR 1 (292.2 q/ha) after SSG 59-3 (390.2 q/ha) a multicut variety used as check high, maximum CP% was recorded in PBMR-3, IVDMD% was more than 60% in PBMR-4 and PBMR -6 on the basis of pooled data of two years. Mean sum of squares due to genotypes were recorded to be highly significant between the genotypes for all agronomic and quality traits for both years. This indicates prevalence of enough genetic variability in the material under study for selection and improvement of genotypes and further its suitability for statistical analysis for all the characters under study. Similar results were observed in an experiment of evaluation of brown-midrib genotypes for fresh and dry biomass suitable for bio fuel production by Kotasthane *et al.,* 2010. Traits like plant height, no. of leaves/ plant, no. of tillers, leaf length, leaf breadth they have shown heritability more than 80%. So on the basis of genetic diversity studies in *bmr* genotypes for fodder yield and their component traits selection can be done for high yielding genotypes with good quality.

Correlation analysis

Correlation is measure of strength of linear relationship in between the characters. In the present investigation correlation study indicates plant height, no. of leaves/ plant, no. of tillers, leaf length, leaf breadth are directly correlated to biomass production in forage sorghum. Plant height is positively correlated to no. of leaves/plant (0.705^{**}), green fodder yield (0.741^{**}) and dry fodder yield (0.849**), early vigor is correlated with no. of tillers per plant (0.557*), leaf length was correlated to plant height (0.705*) and dry fodder yield (0.505*) and with green fodder yield exhibit positive correlation but non-significant. Among quality traits IVDMD % had positive but non-significant correlation was observed with dry fodder yield this may be due to less no. of genotypes involved in the present investigation.

TABLE: 2 Pearson phenotypic correlation coefficient between sixteen quantitative traits in *bmr* sorghumgenotypes

Characters	Plant Height (cm)	Early Vigour	No. of Leaves/plant	Leaf Length (cm)	Leaf Breadth (cm)	Leaf Stem Ratio	Plant Pop./m row	Stem Gitrh (cm)	No. of tillers/plant	TSS %	Protien %	IVDMD %	HCN	Reg. Potential	Green fodder yield q/ha	Dry FYQ/ha
Plant Height (cm)		$.219^{NS}$	0.705^{**}	$.301^{NS}$	-0.603^{*}	0.640^{**}	$.607^{**}$	0.157^{NS}	0.269^{NS}	0.219^{NS}	0.255^{NS}	$.002^{NS}$	$.252^{NS}$	0.195^{NS}	0.741^{**}	$.840^{**}$
Early Vigour	0.219^{NS}		0.148^{NS}	$.435^{NS}$	-0.378^{NS}	0.129^{NS}	$.463^{NS}$	0.100^{NS}	0.557^{*}	$.099^{NS}$	0.223^{NS}	$.442^{NS}$	$.100^{NS}$	0.106^{NS}	0.422^{NS}	$.391^{NS}$
No. of Leaves/plant	0.705^{**}	$.148^{NS}$	1	$.010^{NS}$	-0.359^{NS}	0.532^{*}	$.427^{NS}$	0.051^{NS}	0.303^{NS}	0.206^{NS}	0.198^{NS}	0.078^{NS}	$.237^{NS}$	-0.005^{NS}	0.408^{NS}	$.505^{*}$
Leaf Length (cm)	0.301^{NS}	$.435^{NS}$	0.010^{NS}		-0.006^{NS}	0.157^{NS}	$.471^{NS}$	$.090^{NS}$	0.154^{NS}	$.120^{NS}$	0.170^{NS}	$.083^{NS}$	$.347^{NS}$	0.325^{NS}	0.551^{*}	$.525^{*}$
Leaf Breadth(cm)	-0.603^{*}	0.378^{NS}	-0.359^{NS}	0.006^{NS}	1	$.468^{NS}$	0.448^{NS}	$.646^{**}$	-0.505^{*}	$.035^{NS}$	$.212^{NS}$	0.285^{NS}	0.273^{NS}	-0.167^{NS}	0.564^{*}	$.599^{*}$
Leaf Stem Ratio	-0.640^{**}	0.129^{NS}	-0.532^{*}	0.157^{NS}	0.468^{NS}		0.673^{**}	0.103^{NS}	-0.236^{NS}	0.138^{NS}	$.020^{NS}$	$.126^{NS}$	0.084^{NS}	-0.711^{**}	-0.653^{**}	0.635^{**}
Plant Pop./m row	0.607^{**}	$.463^{NS}$	0.427^{NS}	$.471^{NS}$	-0.448^{NS}	0.673^{**}		0.188^{NS}	0.575^{*}	$.106^{NS}$	0.074^{NS}	$.188^{NS}$	$.137^{NS}$	0.576^{*}	0.913^{**}	$.828^{**}$
Stem Gitrh (cm)	-0.157^{NS}	0.100^{NS}	-0.051^{NS}	$.090^{NS}$	0.646^{**}	0.103^{NS}	0.188^{NS}		-0.408^{NS}	0.027^{NS}	$.083^{NS}$	0.354^{NS}	0.246^{NS}	0.181^{NS}	-0.273^{NS}	0.289^{NS}
No. of tillers/plant	0.269^{NS}	$.557^{*}$	0.303^{NS}	$.154^{NS}$	-0.505^{*}	0.236^{NS}	$.575^{*}$	0.408^{NS}	1	$.232^{NS}$	$.007^{NS}$	$.196^{NS}$	$.418^{NS}$	0.282^{NS}	0.448^{NS}	$.423^{NS}$
TSS %	-0.219^{NS}	$.099^{NS}$	-0.206^{NS}	$.120^{NS}$	0.035^{NS}	0.138^{NS}	$.106^{NS}$	0.027^{NS}	0.232^{NS}		0.303^{NS}	$.006^{NS}$	$.436^{NS}$	0.241^{NS}	-0.021^{NS}	0.084^{NS}
Protien %	-0.255^{NS}	0.223^{NS}	-0.198^{NS}	0.170^{NS}	0.212^{NS}	$.020^{NS}$	0.074^{NS}	$.083^{NS}$	0.007^{NS}	0.303^{NS}		0.396^{NS}	0.495^{*}	0.144^{NS}	-0.211^{NS}	0.249^{NS}
IVDMD %	0.002^{NS}	$.442^{NS}$	-0.078^{NS}	$.083^{NS}$	-0.285^{NS}	$.126^{NS}$	$.188^{NS}$	0.354^{NS}	0.196^{NS}	$.006^{NS}$	0.396^{NS}		0.137^{NS}	-0.150^{NS}	0.291^{NS}	$.297^{NS}$
HCN	0.252^{NS}	$.100^{NS}$	0.237^{NS}	$.347^{NS}$	-0.273^{NS}	0.084^{NS}	$.137^{NS}$	0.246^{NS}	0.418^{NS}	$.436^{NS}$	0.495^{*}	0.137^{NS}		0.072^{NS}	0.226^{NS}	$.229^{NS}$
Reg.Potential	0.195^{NS}	$.106^{NS}$	-0.005^{NS}	$.325^{NS}$	-0.167^{NS}	0.711^{**}	$.576^{*}$	$.181^{NS}$	0.282^{NS}	$.241^{NS}$	$.144^{NS}$	0.150^{NS}	$.072^{NS}$	1	0.511^{*}	$.393^{NS}$
Green FY q/ha	0.741^{**}	$.422^{NS}$	0.408^{NS}	$.551^{*}$	-0.564^{*}	0.653^{**}	$.913^{**}$	0.273^{NS}	0.448^{NS}	0.021^{NS}	0.211^{NS}	$.291^{NS}$	$.226^{NS}$	0.511^{*}	1	$.964^{**}$
Dry FYQ/ha	**$.840^{**}$**	$.391^{NS}$	**0.505^{*}**	**0.525^{*}**	**-0.599^{*}**	**0.635^{**}**	**$.828^{**}$**	0.289^{NS}	0.423^{NS}	0.084^{NS}	0.249^{NS}	$.297^{NS}$	$.229^{NS}$	0.393^{NS}	$.964^{**}$	

Negative but significant correlations were also observed among some traits given in (Table 1). Such strong positive correlations recorded among the genotypes, suggest that they are heritable and genetically controlled traits which could be transmitted into desired genotypes. The finding of present study was agreed with the Jain *et al.,* (2011), Jain and Patel (2016). All the other yield contributing traits were also positively correlated with each other indicated that selection may be done in positive direction based on these traits towards crop improvement programs and also identify the parents which could be used for hybridization programme to produce superior hybrids having high biomass for forage crop improvement.

Principal component analysis

Principal component analysis was carried out and 5 major component traits were extracted which are explaining 80% of total variation among genotypes. According to Chatfied and Collins (1980), components with an eigenvalue of < 1 should be eliminated so that fewer components are dealt with. Furthermore, Hair *et al.* (1998) suggested that, eigen values greater than one are considered significant and component loading greater than 0.3 were considered to be meaningful. In the present study first three Eigenvectors which has eigenvalues greater than one and cumulatively explained about 80 per cent of the total variation among the bmr genotypes of sorghum (Table 3). Hence PC-I has eigenvalue 6.021 and accounted for 36.62 % of the variations. This represents an equivalent of five variables viz., plant height, early vigour, no. of leaves, leaf length, leaf width, and indicated that were important contributing variables for the variation among the genotypes. Genotypes with high PC 1 score therefore would have high level variability of these quantitative traits. Yadav *et al.,* 2004; Ali *et al.* (2011) and Jain & Patel, 2016 also reported important contribution of the first PCs in total variability while studying different traits. The second and third PC explained 2.19 and 1.8 eigen values and contributing 13.7% and 11.5% variations, respectively. Therefore we can say that the PCA analysis under this study shows that phenotypic markers are useful in genotypes of sorghum and able to identify few key traits that accounted for the largest variability. The present study supported by earlier workers also (Akatwijuka *et al.,* 2016; Jain and Patel, 2016).

Similarly, Sinha and Kumaravadivel, 2016 reported that the first three factors are contributing to 57% of the total variance observed. This showed that PCA is a reliable method in identifying few key traits contributing to the largest variation and to predict the important traits influencing clustering of different cultivars observed in Figure 1 under cluster analysis. According to Chahal and Gosal (2002), characters with largest absolute value closer to unity within the first principal component influence the clustering more than those with lower absolute value closer to zero. Therefore, in the present study, differentiation of the genotypes into different clusters was because of relatively high contribution of few characters rather than small contribution from each character. The fourteen genotypes along with three checks were grouped into three clusters on the basis of average linkage among genotypes presented in Fig. 1. The cluster analysis sequestrates genotypes into clusters which exhibit high homogeneity within a cluster and high heterogeneity between clusters. The cluster 1 is divided into 2 subclusters i.e. sub cluster-I having 9 genotypes were grouped viz., EC 582508, EC 582506 crosses with different single cut varieties, sub-cluster 2 had 7 genotypes viz., DSRBMR 1, DSRBMR 2, Single cut varieties; HC 308, CSV 21F etc. and in cluster II had one genotypes i.e SSG 59-3 a multicut variety used as check. Aruna *et al.,* 2015 reported that EC 582508 is also a good combiner for fodder quality traits.

TABLE 3: Eigen values and extracted sum of squares loadings of 16 quantitative traits in *bmr* genotypes of sorghum

Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	6.021	37.629	37.629	6.021	37.629	37.629
2	2.195	13.719	51.348	2.195	13.719	51.348
3	1.833	11.455	62.802	1.833	11.455	62.802
4	1.516	9.473	72.275	1.516	9.473	72.275
5	1.345	8.409	80.684	1.345	8.409	80.684
6	.932	5.824	86.509			
7	.838	5.240	91.748			
8	.453	2.830	94.578			
9	.333	2.080	96.658			
10	.192	1.200	97.858			
11	.171	1.070	98.928			
12	.079	.496	99.424			
13	.053	.329	99.753			
14	.028	.174	99.927			
15	.011	.070	99.998			
16	.000	.002	100.000			

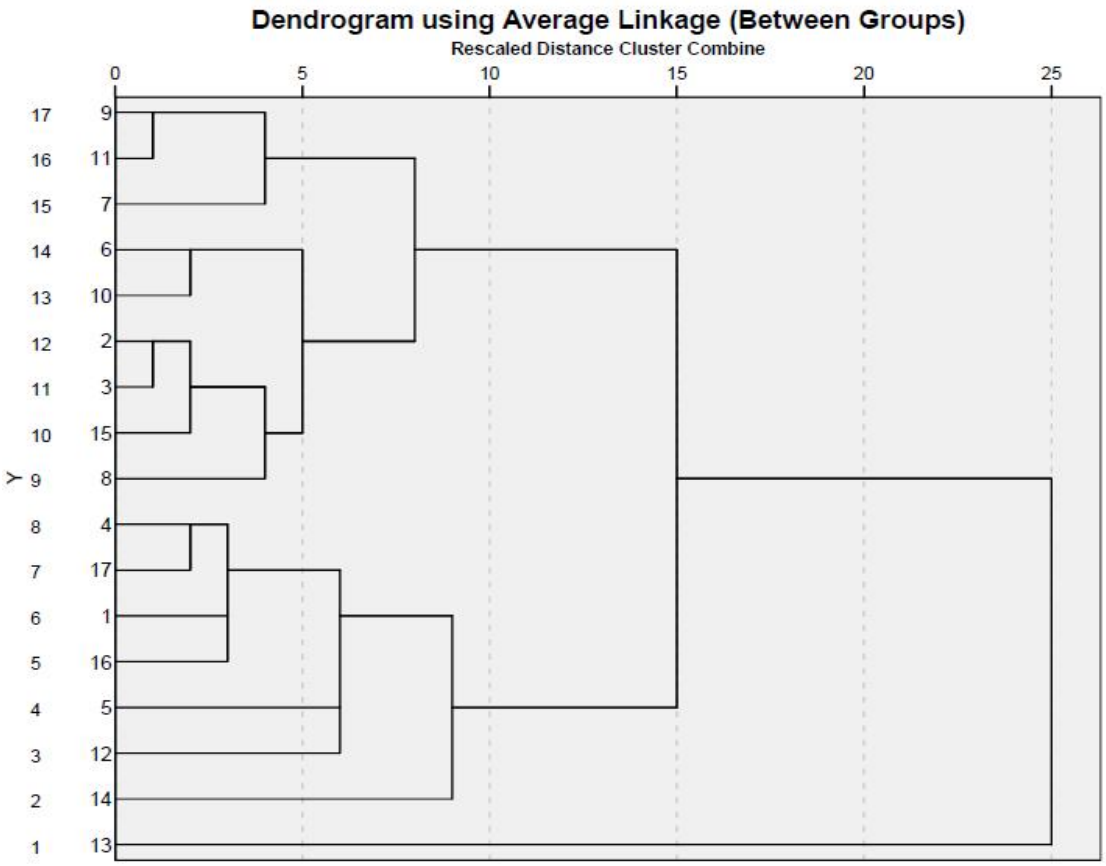

FIGURE 1: The dendrogram of sorghum genotypes resulting from cluster analysis using ward method based on standardized data of all the traits.

Distribution pattern of all the genotypes into two clusters showed the presence of considerable genetics diversity among the genotypes for most of the traits under consideration which indicates the presence of excellent opportunity to bring about improvement through hybridizing genotypes from different clusters and assemble desirable traits with higher heterotic potential. Thus, the PC analysis, cluster analysis and correlation coefficient in this present set of the experiment provided facilitation in the classification of genotypes and identification of the subset of genotypes having quantitative difference between fodder yield and quality parameters. Similar results of superiority in term of quality due to presence of bmr gene was reported by Oliver *et al.*, 2005 that the *bmr*-12 gene appears superior in adding value to grain sorghum for use in grain production.

Various useful correlations and aforementioned information extracted from cluster and PC analysis will be helpful in designing breeding programmes to obtain high yielding *bmr* genotypes in sorghum for high biomass and its related traits.

CONCLUSION

This study supports that quantitative traits are useful tool for preliminary evaluation of genetic diversity and also concludes that the *bmr* genotypes such as DSRBMR 1, PBMR-4 and BMR 233691 had high green fodder yield coupled with better forage quality. Thus, these genotypes can be exploited in future *bmr* hybrid/varieties breeding programs.

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