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Molecular characterization of parental lines of post-rainy sorghum using simple sequence repeat markers

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Article info:
Received: 2017
Accepted: 2017

ABSTRACT

Heterosis is related to genetic diversity among the parental lines, which is considered to be important for the development of superior hybrids. The objective of this study was to characterize 145 genotypes of post-rainy sorghum comprising of 40 maintainer and 105 restorer lines using 46 simple sequence repeat (SSR) markers, and assess the extent of genetic diversity among them. Among the 244 polymorphic alleles, 164 (67.21%) were common to both maintainer and restorer lines while 24 (9.84%) and 56 (22.95%) were unique to maintainer and restorer lines, respectively. Average relative gene diversity was 0.35 within maintainer lines, 0.32 within restorer lines, and 0.33 between these two groups. The cluster analysis following the UPGMA algorithm grouped the genotypes into four major clusters with an average similarity of 53%. Intermingling of maintainer and restorer groups was observed and more than 50% of the maintainer lines were grouped closer to restorer lines. The lines AKRB302, AKRB306, AKRB307, AKRB311, AKRB318, AKRB324, AKRB354, AKRB388, AKRB413 and SLR43 were distinct among the restorers with minimum similarity. These restorer lines along with the set of male sterile lines corresponding to the maintainer lines identified in this study could be employed in hybrid development. Even though diverse restorers were identified for exploitation in hybrid development, the study clearly exposes the narrow genetic base of the parental lines of post-rainy sorghum, underlining the need to use parental lines from a broader gene pool for the development of heterotic hybrids suited for the post - rainy situation.

Key words: post-rainy sorghum, genetic diversity, maintainer, restorer, simple sequence repeats

Introduction

Sorghum (*Sorghum bicolor* L. Moench) is the fifth most important cereal crop of the world grown mostly in the semi-arid regions. It is also one of the important cereal crops cultivated in India, predominantly under rain-fed conditions in marginal drylands. Sorghum grown in post-rainy season is popular among the farmers for its bold lustrous grains and excellent grain quality for food use and good quality fodder for livestock feed. In India, based on the average of 2007-08 to 2011-12, the production of sorghum grain from 4.28 million ha during the post-rainy season is 3.64 m tons with the productivity of 851 kg ha^{-1} (http://agricoop.nic.in/agristatistics.htm). The area of sorghum under post-rainy season is fairly consistent over the years but the improvement in productivity is much slower compared to the sorghum grown in rainy season. The major reason for this is the large-scale cultivation of highly heterotic hybrids in the rainy season while only varieties and local landraces are predominantly cultivated in the post-rainy season. Narrow genetic base, low variability for yield and grain quality traits with post-rainy season adaptation are considered to be the important constraints. Hence, the development of high yielding hybrids with superior grain quality for the post-rainy season is important. It can be hypothesized that for effective exploitation of heterosis, parents should be derived from genetically divergent germplasm pools, commonly referred to as heterotic groups (Melchinger and Gumber, 1998). However, reports on genetic diversity at the molecular level among

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DOI: 10.69085/jbb20171049

sorghum grown in post-rainy season are very limited. This calls for a clear understanding of genetic diversity among parental lines developed under breeding programmes as it will help the breeders to choose the best lines to maximize heterosis.

Earlier, diverse data sets such as morphology (Dje et al., 1998), pedigree records (Jordan et al. 1998) and isozymes (Danquah et al., 2000) have been employed for assessing the genetic diversity in sorghum. Later, with the advent of PCR-based molecular markers, rapid and efficient assessment of genetic diversity among the parental lines became possible. Restriction fragment length polymorphisms (RFLPs) (Tao et al., 1993; Ahnert et al., 1996), randomly amplified polymorphic DNA (RAPDs) (Ayana et al., 2000; Uptmoor et al., 2003), inter-simple sequence repeats (ISSR) (Tadesse and Feyissa, 2013), amplified fragment length polymorphisms (AFLPs) (Geleta et al., 2006; Ritter et al., 2007) and simple sequence repeats (SSRs) (Manzelli et al., 2007; Deu et al., 2008) have all been successfully used for the assessment of genetic diversity in sorghum. These markers can be applied for classification of germplasm, identification of cultivars, assisting in the selection of parental lines for hybridization and reducing the number of accessions needed to ensure sampling a broad range of genetic variability. Identification of unique alleles and genetic diversity assessment of post-rainy sorghum accessions were reported by Thudi and Fakrudin (2011). Recently, SSR markers were used to reveal the genetic diversity among the parental lines and elite genotypes of sorghum (Ganapathy et al., 2012), Maldandi landraces in India (Rakshit et al., 2012), sweet sorghum parental lines (Wang et al., 2013), sorghum accessions of Sudan (El Hussein et al., 2014) and sorghum accessions of Botswana (Motlhaodi et al., 2014). Efforts were also made in rice (Jaikishan et al., 2010), maize (Mohammadi et al., 2008) and other crops in assessing the utility of SSR markers for heterosis prediction based on the relationship between molecular diversity of parental lines and heterosis of the resultant hybrid.

Molecular markers are advantageous over morphological markers and quantitative traits as they are able to sample the variations at the different loci spread across the genome of parental lines to determine the genetic distance. Hence, the assessment of genetic diversity of the available gene pool of parental lines using molecular markers will help to identify the diverse lines, which can be employed in the development of heterotic hybrids. Molecular marker-based genetic diversity assessment will also be helpful to know whether the set of parental lines analyzed constitute different heterotic groups. SSRs are the most favored markers for various applications in plant genetics and breeding due to their multi-allelic nature, high reproducibility, co-dominant inheritance, abundance and extensive genome coverage (Gupta and Varshney, 2000). The objectives of this study were to assess the extent of genetic diversity among parental lines of the post-rainy season developed for use in hybrid sorghum breeding programmes and to identify diverse parental lines for making crosses and testing for heterosis.

Materials and Methods

The plant material used in this study consisted of 145 parental lines, comprising 40 maintainer lines and 105 restorer lines of post-rainy sorghum (Table 1 and Table S1). Genomic DNA was isolated from the leaf samples as per the procedure of Dellaporta et al. (1983). A set of 48 SSR markers of the reference microsatellite kit for the assessment of genetic diversity (Billot et al., 2012) distributed across all 10 chromosomes were used for genotyping. SSR genotyping was performed through capillary electrophoresis at the Genotyping Services Laboratory, ICRISAT, Patancheru, India. Forward primer was synthesized by adding M13-forward primer sequence (5’ CACGACGTTGTAAAACGAC 3’) at the 5’ end of each primer. PCR was performed in 5 µl reaction volume with a final concentration of 5 ng DNA, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 1x PCR buffer, 0.006 pM of M13-tailed forward primer, 0.09 pM of forward primer labeled with either 6-FAM or VIC or NED or PET (Applied Biosystems), 0.09 pM of reverse primer and 0.1 U of *Taq* DNA polymerase (SibEnzyme Ltd., Russia) in a Gene Amp® PCR system 9700 thermal cycler (Applied Biosystems, USA) with the following cyclic conditions: initial denaturation at 94ºC for 3 min., then 10 cycles of denaturation at 94ºC for 1 min., annealing at 61ºC for 1 min. (temperature reduced by 1ºC for each cycle) and an extension at 72ºC for 1 min. This was followed by 40 cycles of denaturation at 94ºC for 1 min., annealing at 54ºC for 1 min. and an extension at 72ºC for 1 min. with a final extension of 10 min. at 72ºC. Based on their expected amplicon size and dye, PCR products were pooled together along with the internal size tstandard (Gene Scan™ 500 LIZR from Applied Biosystems, USA) and capillary electrophoresis was carried out using ABI 3730xl Genetic Analyzer (Applied Biosystems, USA). Raw data produced from ABI 3730xl Genetic Analyzer was analyzed using Genemapper software (Applied Biosystems, USA) and fragment sizes were scored in base pairs based on the relative migration of the internal size standard. The alleles were scored individually as ‘1’ and ‘0’, respectively, for their presence or absence. Molecular data generated by SSR was analyzed by using NTSYSpc ver. 2.20q (Rohlf, 2000). Genetic similarities between parental lines were measured by the DICE similarity coefficient based on the proportion of shared alleles (Dice, 1945; Nei and Li, 1979) using SIMQUAL module. The similarity matrix was subjected to multi-dimensional scaling (MDS) (Kruskal and Wish, 1978) to ascertain whether the molecular diversity indicated any clustering pattern among accessions. Dendrogram was constructed by the clustering of accessions based on the

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DOI: 10.69085/jbb20171049

Table 1. *List of parental lines of post-rainy sorghum used in the study.*

Maintainer lines		Restorer lines				
DNB1	SLB5	SPV570	AKRB301-4-1	RR20103	AKRB345	SLR34
DNB2	SLB6	PVR489	AKRB302	RR9825	AKRB349-2	SLR35
DNB4	SLB7	BRJ182	AKRB304	RR9826	AKRB350	SLR36
DNB5	SLB8	BRJ104	AKRB306	RR9828	AKRB351	SLR37
PMS20B	SLB9	BRJ62	AKRB307	RS585	AKRB352	SLR38
BJMS1B	SLB10	BRJ67	AKRB397	AKRB309	AKRB353	SLR39
BJMS2B	SLB11	BRJ356	AKRB400	AKRB311	AKRB354	SLR40
BJMS3B	SLB12	BRJ357	AKRB413	AKRB314	AKRB356-1	SLR42
AKRMS69B	SLB13	BRJ358	AKSV13R	AKRB316	AKRB361	SLR43
1409B	SLB14	SPV1380	SLR1	AKRB316-1	AKRB364	SLR44
127B	SLB15	DSV5	SLR5	AKRB317	AKRB368	SLR45
49B	SLB16	BRJ176	SLR8	AKRB318	AKRB369	SLR46
AKRMS82B	SLB17	R2322	SLR10	AKRB324	AKRB370	SLR47
AKRMS45B	SLB18	R2325	SLR13	AKRB325	AKRB371	
AKRMS47B	SLB19	R2330	SLR17	AKRB332	AKRB374	
AKRMS66B	SLB20	R2305	SLR24	AKRB335-2	AKRB378	
AKRMS67B	SLB21	R2369	SLR25	AKRB335-3	AKRB379	
AKRMS63B		R2311	SLR26	AKRB335-4	AKRB388	
104B		R2350	SLR27	AKRB336	AKRB392	
SLB1		AKRB287	SLR28	AKRB336-3	SLR30	
SLB2		AKRB301-1	SLR29	AKRB342	SLR31	
SLB3		AKRB301-2	RR2212	AKRB342-2	SLR32	
SLB4		AKRB301-4	RR2145	AKRB344	SLR33	

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DOI: 10.69085/jbb20171049

Table 2. *Gene diversity among parental lines of sorghum as revealed by SSR markers*.*

Marker	No. of alleles	Gene Diversity				Relative Gene Diversity		
		Within B	Within R	Between B & R	Total	Within B	Within R	Between B & R
gpsb089	3	0.18	0.34	0.30	0.82	0.22	0.41	0.37
mSbCIR286	5	0.59	0.60	0.60	1.79	0.33	0.34	0.34
mSbCIR306	3	0.22	0.33	0.30	0.85	0.26	0.39	0.35
Xcup53	5	0.60	0.56	0.58	1.74	0.34	0.32	0.33
Xcup62	2	0.29	0.33	0.32	0.94	0.31	0.35	0.34
Xtxp320	8	0.78	0.79	0.80	2.37	0.33	0.33	0.34
Xisep0310	2	0.05	0.00	0.01	0.06	0.83	0.00	0.17
mSbCIR223	6	0.34	0.33	0.34	1.01	0.34	0.33	0.34
mSbCIR238	8	0.76	0.60	0.68	2.04	0.37	0.29	0.33
Xgap84	11	0.76	0.76	0.76	2.28	0.33	0.33	0.33
Xcup63	4	0.10	0.02	0.04	0.16	0.63	0.13	0.25
mSbCIR276	3	0.41	0.28	0.32	1.01	0.41	0.28	0.32
Xcup11	2	0.47	0.36	0.40	1.23	0.38	0.29	0.33
Xcup14	3	0.52	0.42	0.46	1.40	0.37	0.30	0.33
Xcup61	2	0.14	0.31	0.27	0.72	0.19	0.43	0.38
Xtxp114	4	0.59	0.47	0.51	1.57	0.38	0.30	0.32
Xtxp012	11	0.78	0.78	0.82	2.38	0.33	0.33	0.34
Xtxp021	8	0.30	0.49	0.45	1.24	0.24	0.40	0.36
mSbCIR248	3	0.18	0.00	0.05	0.23	0.78	0.00	0.22
mSbCIR329	4	0.19	0.44	0.38	1.01	0.19	0.44	0.38
Xtxp015	8	0.72	0.61	0.68	2.01	0.36	0.30	0.34
Xtxp136	3	0.40	0.27	0.31	0.98	0.41	0.28	0.32
gpsb069	6	0.59	0.63	0.64	1.86	0.32	0.34	0.34
Xgap72	4	0.58	0.55	0.56	1.69	0.34	0.33	0.33
Xtxp057	7	0.64	0.60	0.62	1.86	0.34	0.32	0.33
Xtxp145	7	0.75	0.76	0.77	2.28	0.33	0.33	0.34
Xtxp265	7	0.64	0.80	0.78	2.22	0.29	0.36	0.35
gpsb148	3	0.52	0.48	0.50	1.50	0.35	0.32	0.33
mSbCIR246	2	0.00	0.11	0.08	0.19	0.00	0.58	0.42
mSbCIR300	4	0.59	0.54	0.57	1.70	0.35	0.32	0.34
SbAG-B02	6	0.54	0.44	0.51	1.49	0.36	0.30	0.34
Xtxp040	2	0.40	0.17	0.25	0.82	0.49	0.21	0.30
Xtxp278	3	0.55	0.48	0.50	1.53	0.36	0.31	0.33
Xtxp295	11	0.78	0.73	0.77	2.28	0.34	0.32	0.34
gpsb067	3	0.53	0.28	0.43	1.24	0.43	0.23	0.35
gpsb123	3	0.37	0.37	0.37	1.11	0.33	0.33	0.33
mSbCIR240	5	0.70	0.55	0.60	1.85	0.38	0.30	0.32
Xtxp273	5	0.69	0.56	0.62	1.87	0.37	0.30	0.33
Xtxp321	11	0.84	0.71	0.76	2.31	0.36	0.31	0.33
Xtxp339	2	0.14	0.31	0.27	0.72	0.19	0.43	0.38
Xgap206	10	0.81	0.84	0.84	2.49	0.33	0.34	0.34
Xcup02	5	0.34	0.35	0.35	1.04	0.33	0.34	0.34
Xtxp010	7	0.23	0.52	0.45	1.20	0.19	0.43	0.38
mSbCIR262	4	0.50	0.58	0.57	1.65	0.30	0.35	0.35
mSbCIR283	7	0.48	0.41	0.44	1.33	0.36	0.31	0.33
Xtxp141	12	0.74	0.80	0.80	2.34	0.32	0.34	0.34
Total	**244**	**22.32**	**21.66**	**22.43**	**66.41**	**16.09**	**14.60**	**15.32**
Average	**5.30**	**0.49**	**0.47**	**0.49**	**1.44**	**0.35**	**0.32**	**0.33**

B - maintainer line **R - restorer line**
***Only those SSR markers which revealed polymorphism were taken into consideration**

similarity matrix using un-weighted pair group method with arithmetic average (UPGMA) algorithm following SAHN module. The reliability of the tree was tested by bootstrap analysis (Felsenstein, 1985).

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DOI: 10.69085/jbb20171049

Gene diversities between lines were calculated using an algorithm: $H_k = 1 - \sum P_i^2$, given P_i is the frequency of the ith allele at k^{th} locus. Gene diversity, often referred as Polymorphism Information Content (PIC) was calculated using the web resource PIC Calculator (http://www.liv.ac.uk/~kempsj/pic.html). Total gene diversity was partitioned into its components in a nested manner as suggested by Xu et al., (2002):

$H_T = H_B + H_R + H_{BR}$

where H_T, H_B, H_R, and H_{BR} are weighted gene diversity in total, within maintainer lines, within restorer lines and between maintainer and restorer lines, respectively. The corresponding relative gene diversities are given by dividing each component with H_T, which was calculated using Microsoft Excel utility.

Results and Discussion

Identification of high-yielding heterotic hybrids is expensive under field conditions and involves testing a large number of experimental hybrid combinations in multi-environment trials (Jordan et al., 2003). In this situation, knowledge on the extent of the genetic diversity present in the available gene pool of the parental lines will be useful for the classification of parental lines into heterotic groups. Moreover, the genetic diversity information will be highly helpful in the selection of diverse lines for hybrid breeding programmes. Molecular markers offer an easy and rapid method of analyzing the genetic diversity as compared to an analysis by morphological traits. SSR markers were popularly used for the genetic diversity analysis in sorghum (Kamala et al., 2006; Ali et al., 2008; Shehzad et al., 2009; Wang et al., 2009; Nguni et al., 2011). The markers used in the present study were randomly distributed across the genome, and thus, should provide better estimates of the total diversity between and within the groups as suggested in rice by Xu et al., (2002). The breeding lines included in this study represented an available gene pool of parental lines used for post-rainy sorghum hybrid breeding programmes in India.

Among the 48 SSR markers, 46 were polymorphic exhibiting a total of 244 alleles among the genotypes analyzed. The mean number of alleles per SSR locus was 5.30, which was similar (5.9) to that detected by Smith et al., (2000) but higher than that reported by Schloss et al., (2002) and Agrama and Tuinstra (2003) with mean allele per locus of 3.4 and 4.3, respectively. The number of alleles ranged from 2 (Xcup11, Xcup61, Xcup62, Xisep0310, mSbCIR246, Xtxp040 and Xtxp339) to 12 (Xtxp141). There were 18 loci at which more than five alleles were resolved. Out of the 244 polymorphic alleles, 164 (67.21%) alleles were common to both maintainer and restorer lines, 24 alleles at 18 loci were unique to maintainer lines, while 56 alleles at 27 loci were unique to restorer lines. The relative gene diversity averaged 0.35 within maintainer lines, 0.32 within restorer lines, and 0.33 between the two groups of lines, suggesting a moderate divergence at the molecular level between the parental groups (Table 2). The total gene diversity observed in this parental gene pool was 0.49, which is less than the value of 0.62 and 0.58 reported earlier by Agarma and Tuinstra (2003) and Smith et al., (2000), respectively. Many of the lines shared common parents in their pedigree (Table S1), and this was one of the reasons for lower gene diversity observed in the present study. A similar observation was reported by Ali et al., (2008). The mean genetic similarity between pair-wise maintainer lines, and pair-wise restorer lines was 0.53, indicating that both the maintainer and restorer groups shared moderate within group similarity. Even though the average similarity was moderate among the maintainer and restorer group, a small set of lines exhibited a similarity index as low as 0.19 (Table S2). This may be attributed to genetic differentiation of their unique ancestral materials used, and partially to the differential selection for specific phenotypes (such as plant height, flag leaf length, etc.) in the development of the maintainers and the restorers. For instance, taller genotypes are usually selected in the case of restorers while shorter plants with complete panicle exertion are selected in the case of maintainers to increase hybrid seed production.

Diversity measures could not reveal two distinct groups as the restorer group and the maintainer group, and this was clearly reflected in the dendrogram (Fig. 1), which grouped the 145 parental lines into four major clusters with an average similarity of 53%. Cluster I included 13 genotypes in two sub-clusters, I-A (10 restorer lines and 1 maintainer line) and I-B (2 maintainer lines). Cluster II consisted of only a single maintainer line (DNB2) while Cluster III consisted of 17 restorer lines developed from All India Coordinated Sorghum Improvement Programme, Akola Centre, Maharashtra, India. Most of these genotypes shared a common genetic background with regard to pedigree relationship. Cluster IV was the largest, accommodating 114 lines in two sub-clusters, IV-A (79 restorer lines and 22 maintainer lines) and IV-B (13 maintainer lines). Intermingling of maintainer and restorer groups was also confirmed through multi-dimensional scatter (MDS) plot analysis (Fig. 2). An earlier study in public inbreds of sorghum by Menz et al. (2004) using AFLP and SSR markers and a recent study in sweet sorghum by Wang et al., (2013) using SSR markers also failed to clearly differentiate the maintainer lines and the restorer lines, suggesting that these lines do not constitute well-defined heterotic groups. It was noted that between groups differentiation was not much pronounced whereas within group differentiation was more pronounced in the maintainer group as compared to restorer group. This feature is clear from the fact that the maintainer lines were distributed in three different clusters while the majority of the

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DOI: 10.69085/jbb20171049

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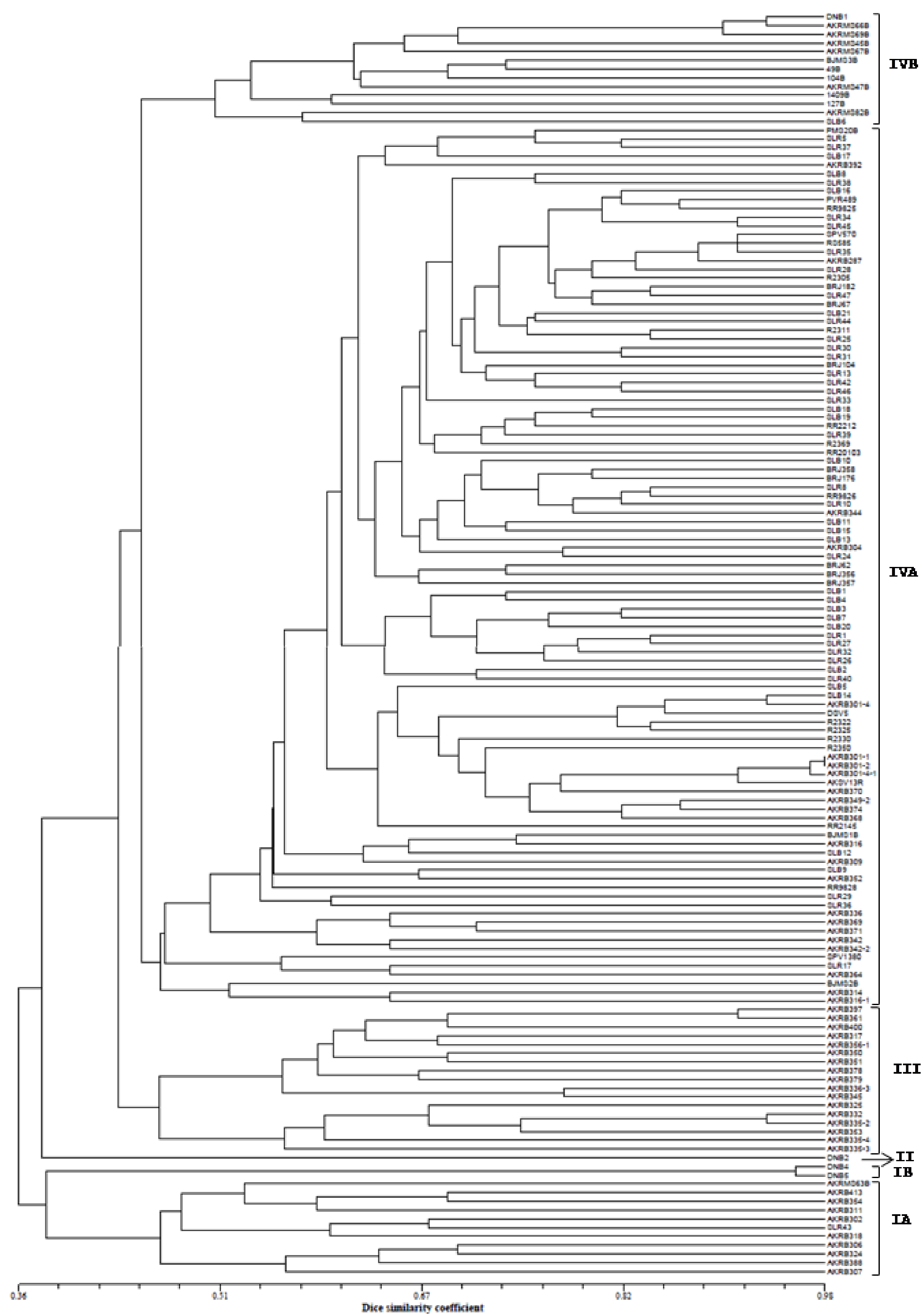

Figure 1. *Dendrogram revealing the grouping of parental lines of post-rainy sorghum.*

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DOI: 10.69085/jbb20171049

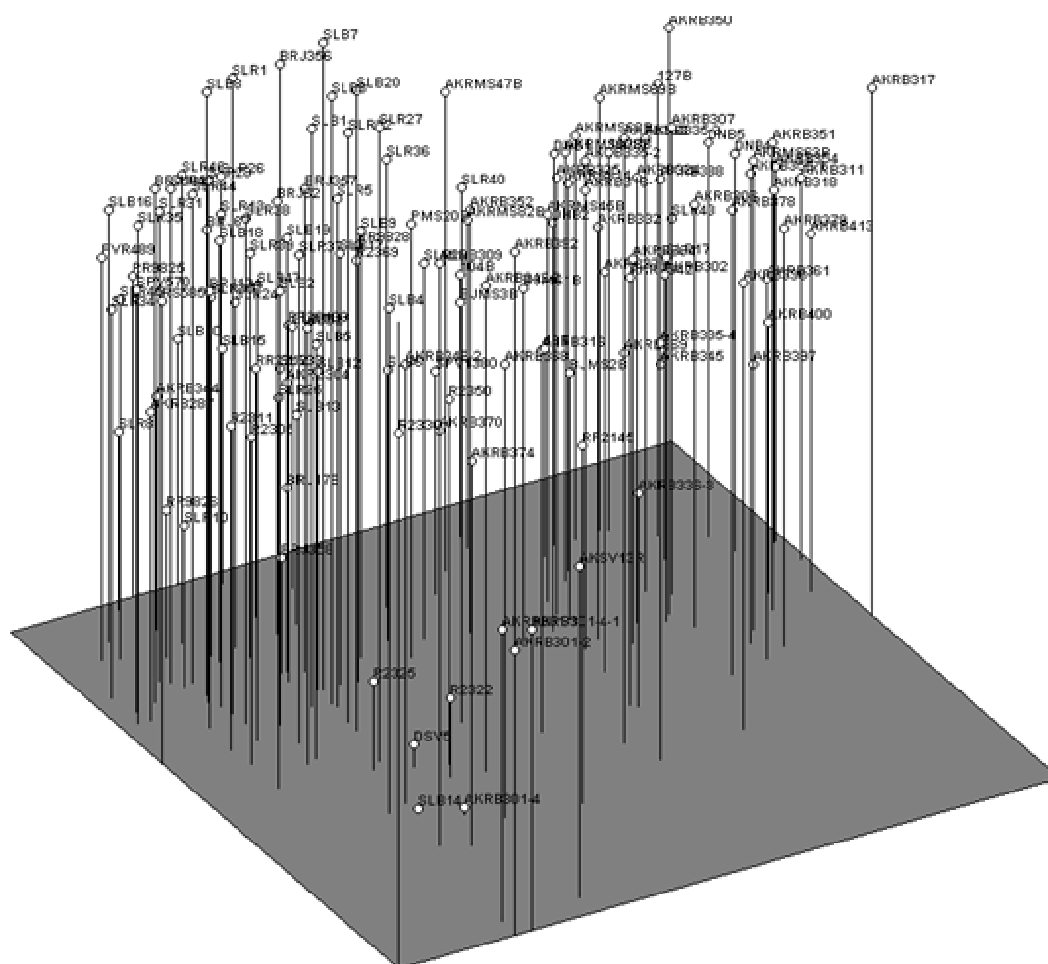

Figure 2. *Multidimensional scatter plot revealing the inter-mingling of parental lines of post-rainy sorghum.*

restorer lines were more similar and grouped into a single cluster. About 50% of the maintainer lines were grouped closer to restorer lines. This may be due to the limited gene pool available for exploitation in the improvement of post-rainy sorghum. The maintainer lines DNB1, AKRMS 66B, AKRMS 69B, AKRMS 45B, AKRMS 67B, BJMS 3B, 49B, 104B, AKRMS 47B, 1409B, 127B, AKRMS 82B and SLB6 were distinctly different from other maintainer lines as well as restorer lines. As shown in Fig. 1, most of the restorer lines (75.2%) were grouped in a highly related cluster (Cluster IVA), which also included 22 maintainer lines (55%). Some genotypes that shared a common parentage were found to be grouped in different clusters instead of being grouped together, a feature similar to that observed by Ali et al., (2008) in sweet sorghum. For example, SLR35 ((CSV-14R × CR-6)-2-4-3) and SLR43 (Dhamangoan local × CR-6)-2-4-7) shared CR-6 in their parentage but did not cluster together. Such un-related grouping of genotypes may be attributed to differential selection exercised on these genotypes. A number of studies (Bohn et al., 1999; Tams et al., 2005; Fufa et al., 2005; Ali et al., 2008) have demonstrated that genetic relationships based on molecular markers do not always agree with those estimated by pedigree information because of unrealistic assumptions for estimating the co-ancestry coefficients. Interestingly, a majority of restorer lines developed at the same research centre were grouped together. This may be because only the germplasm available in the centre has been mostly used in the breeding programme followed by selection of the target trait(s) of interest at that particular centre. However, a little earlier Prabhakar and Raut (2010) had identified the crosses SLB19 × SLR13, SLB19 × SLR17, SLB19 × SLR30 and SLB19 × SLR39 for improving grain yield and the similarity indices of these cross combinations in the present study were 0.67, 0.53, 0.71 and 0.77, respectively. This clearly indicated that the genetic diversity among the parental lines can be used as a criterion in the selection of parental lines for the development of superior hybrids. The restorer lines AKRB302, AKRB306, AKRB307, AKRB311, AKRB318, AKRB324, AKRB354, AKRB388, AKRB413 and SLR43 were distinct among the restorers with minimum similarity.

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DOI: 10.69085/jbb20171049

These restorer lines along with the male sterile lines of the corresponding maintainer lines identified as distinct in this study could be utilized in hybrid development.

In conclusion, the present study has clearly indicated the presence of narrow genetic base of the available parental lines of post-rainy sorghum as revealed by the measures of genetic similarity and relative gene diversity. This study has identified a set of parental lines that can be exploited for hybrid development and also emphasizes the need for the use of parental lines from a broader gene pool that can lead to the development of more heterotic hybrids in post-rainy sorghum resulting in enhanced productivity and production during post-rainy season.

Acknowledgement

The authors thank Director, ICAR – Indian Institute of Millets Research, Hyderabad for providing the facilities and the centres of All India Coordinated Research Project (AICRP) on Sorghum for providing the plant material.

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