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Genetic diversity revealed utility of SSR markers in classifying parental lines and elite genotypes of sorghum (*Sorghum bicolor* L. Moench)

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Abstract

Genetic diversity among 82 rainy and post-rainy sorghum genotypes from India was studied using a set of 35 SSR markers distributed across all the linkage groups. A total of 198 alleles were recorded with an average of 5.71 per primer pair. The polymorphism information content (PIC) values ranged from 0.02 (Xisep 0310) to 0.86 (sb5-206) with a mean of 0.49, indicating high discriminating ability of the SSR markers used. Jaccard's similarity coefficients and cluster analysis revealed substantial diversity among the genotypes. Very high estimate of fixation index (F_{ST} = 0.35, P= 0.001) was obtained when genotypes were structured as rainy and post-rainy season adaptation, and a much higher estimate (F_{ST} = 0.40, P= 0.001) was obtained when the genotypes were classified as varieties, maintainers, restorers and germplasm lines. This indicates strong distinction based on usage groups. Pairwise F_{ST} values based on usage groups corresponded well with the Nei's genetic distances. We were able to distinguish parental lines of hybrids based on their fertility groups, especially lines from rainy season sorghum, using SSR markers. To the best of our knowledge, this is the first report in sorghum demonstrating the utility of SSR markers in classifying lines based on their fertility groups. Our results have significant implications in classifying parental lines into heterotic groups for their use in heterosis breeding. The divergent maintainer and restorer lines identified based on Jaccard's similarity coefficients could serve as effective candidates for hybrid development.

Keywords: genetic variability, heterotic groups, parental lines, rainy, post-rainy, sorghum.
Abbreviations: SSR- Simple Sequence Repeats, AMOVA- Analysis of Molecular Variation, PCA- Principal Component Analysis, PIC- Polymorphism Information Content, F_{ST}- Fixation Index.

Introduction

Sorghum (*Sorghum bicolor* L. Moench) is the staple food for millions of people in arid and semi-arid tropics of the world including India (Rakshit et al., 2012b). Sorghum in India is cultivated in rainy and post-rainy seasons with cultivars specifically adapted to each seasons. The area under rainy season sorghum is 2.89 million hectares with a production of 3.05 million tons, while post-rainy sorghum is grown on 4.88 million hectares producing 4.18 million tons (Rakshit et al., 2012a). The cultivated sorghum taxa of the world are classified into five races and fifteen intermediate races based on inflorescence pattern (Harlan and de Wet 1972). The rainy season cultivars are predominantly *caudatum*, *kafir* and *bicolor* races, while post-rainy cultivars are mainly *durra* types. Rainy season sorghum is mostly utilized for industrial purposes such as poultry and distilleries, while post-rainy sorghum is predominantly used for food purposes (Audilakshmi et al., 2007; Rakshit et al., 2012a). In India, heterosis in sorghum has been best exploited in rainy season cultivar development but not to a greater extent in post-rainy sorghum mainly due to the narrow genetic base of the post-rainy genotypes (Sajjanar et al., 2011). Attempts were made to introgress heterosis from rainy sorghum to post-rainy cultivars, however, significant progress could not be achieved due to problems associated with grain quality and adaptation in such crosses. In heterosis breeding, understanding genetic relationship among parental lines is of paramount importance and DNA markers such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) play an important role. Among different DNA markers, SSRs are most commonly used because they are hypervariable, co-dominant, robust, chromosome specific and multi-allelic in nature (Rakshit et al., 2012a). SSR markers are widely used for assessment of diversity in several cultivated crop species including sorghum (Dje et al., 2000; Ghebru et al., 2002; Agrama and Tuinstra, 2003; Anas and Yoshida, 2004; Mutegi et al., 2011; Rakshit et al., 2012a) but little information is available on classification of parental lines of hybrids using DNA markers (Menz et al., 2004). To address this knowledge gap and to facilitate the utilization of parental lines in Indian sorghum hybrid breeding programme, we report the use of SSR markers in assessing the diversity and genetic relationships among the parental (rainy and post- rainy) and elite lines (varieties and germplasm lines) of sorghum. Our results

demonstrate the potential of SSR markers in grouping genotypes based on their fertility reaction.

Results

SSR polymorphism

Polymorphism among the 82 sorghum genotypes was investigated with 48 SSR markers. Out of these, 35 showed reliable polymorphism among the genotypes under investigation (Table 1). A total of 198 alleles were detected with 35 SSRs markers. The number of alleles varied from 2 (Xtxp 114, Gpsb148, Xcup11, Xcup61, Xcup62, Xisep0310, Xtxp136, Xtxp339) to 15 (Xtxp012 and Xtxp295) with an average of 5.71 per primer. PIC values ranged from 0.02 (Xisep0310) to 0.86 (sb5-206) with a mean of 0.49. Eighteen SSR markers revealed PIC values of more than 0.5 indicating their usefulness in discriminating the genotypes. Observed heterozygosity (H_o) ranged from 0.0 (mSbCIR248, Xisep0310) to 1.0 (Xtxp 339), with a mean of 0.09. Mean expected heterozygosity/gene diversity (H_e) was observed to be 0.55 with maximum and minimum H_e values recorded by SSR markers, sb5-206 (0.87) and Xispep 0310 (0.03) respectively.

Cluster and principal component analysis

Cluster analysis was carried out independently for both rainy and post-rainy season genotypes. Neighbour joining cluster analysis put the 43 rainy season sorghum genotypes into two major groups, A and B (Fig 1). Group A, referred to as maintainer group, consisted of 11 maintainer lines, 6 germplasm lines and 2 varieties. Group B referred to as the restorer group, was represented by 14 restorer lines, 4 varieties, 3 maintainer and one germplasm line. The maintainer group generally was more diverse than the restorer group. In general, the varieties and germplasm lines from ICRISAT were closely associated with maintainer group, while the varieties and germplasm from Indian breeding programme were more related to the restorer group. The germplasm lines, though clustered close to the maintainer group, formed a separate sub-group within the maintainer group. Among the germplasm lines, IS 18551, a donor of shoot fly resistance, was distinct from rest of the lines. The genetic relationships among the rainy season genotypes was further investigated using principal component analysis (PCA) (Fig 2). The first two major axis of differentiation (PC1 and PC2) explained 36.65% of the total variation. PCA also classified the 43 genotypes into two major groups, *viz*., maintainer and restorer groups indicated by A and B, respectively. Groupings were similar to those detected by cluster analysis except that the two germplasm lines (IS 24995 and IS 24996) remained distinctly out-group in the PCA. This indicated distinctive nature of these two lines over remaining genotypes. The neighbour joining cluster analysis of 39 post-rainy sorghum genotypes revealed high diversity between the maintainers, restorers and varieties (Fig 3). These genotypes were also clustered into two major groups (A and B). Group A was comprised of 12 restorers, 4 maintainers and 3 varieties, while Group B was represented by 6 restorers, 5 maintainers and 7 varieties. Most of the restorers clustered together forming a solitary group. Like rainy season genotypes, post-rainy genotypes could not be clearly classified into maintainers and the restorers and were found to be interspersed with each other. The PC1 and PC2 of 39 post-rainy genotypes together explained 41.9% of the total variation. Unlike cluster analysis, the PCA classified the post-rainy genotypes into three groups, *viz*., restorers (A) maintainers (B), and varieties (C) with few exceptions (Fig 4). The restorers SLR 62, SLR 65, SLR 66 and SLR 73 were interspersed between maintainers and varieties. PCA did not reveal much diversity among the post-rainy genotypes studied (Fig 4), though two varieties (M 35-1 and CSV 14R), two restorers (SLR 70 and TNS 30) and one maintainer (SLB 55) clustered far apart from all other genotypes indicating their dissimilarity with others.

Analysis of molecular variation (AMOVA) and fixation indices

For the purpose of AMOVA and F_{ST} estimates, the rainy and post-rainy season genotypes were further sub-divided into seven sub-groups as maintainers, restorers, varieties and germplasm lines in case of rainy season genotypes; and maintainers, restorers and varieties for post-rainy season genotypes. AMOVA has clearly brought out significant differences among various genotypes evaluated (Table 2). It is observed that greater variance (59.51%) was represented by individuals, while between group (i.e. rainy and post-rainy) variance was less (33.95%) with least variance (6.55%) explained by the seven sub-groups as mentioned above. Pairwise fixation indices (F_{ST}) among the main groups, i.e. rainy and post-rainy and sub-groups along with Nei's genetic distances are presented in Table 3. Fixation indices between rainy and post-rainy genotypes was high (F_{ST} = 0.35, P = 0.001), indicating presence of strong population structure. Much higher estimate (F_{ST} = 0.40, P = 0.001) was obtained when the genotypes were classified into sub-groups, *viz*., varieties, maintainers, restorers and germplasm lines. The highest pairwise F_{ST} value within sub-groups was observed between rainy season restorers and post-rainy maintainers (0.537), while the lowest was recorded between post-rainy restorers and post-rainy varieties (0.007) (Table 3). Pairwise F_{ST} values matched well with Nei's genetic distance. Minimum Nei's genetic distance was observed between post-rainy restorers and post-rainy varieties (0.07), while the maximum distance was recorded between rainy season maintainers and post-rainy restorers (0.54). Among the 82 genotypes studied the maintainer and restorer lines are of immediate use in hybrid breeding programme. Hence, the Jaccard's similarity indices among these parental lines (maintainers and restorers) of both rainy and post-rainy genotypes were calculated and presented in Table 4 and 5, respectively. The similarity values for rainy season parental lines ranged from 0.20 (PMS 74 and KR 191) to 0.83 (Indore 13 and PMS 71B, 11B2). The values for post-rainy ranged from 0.13 (TNS 30 and SLB 50) to 0.80 (RS 585 and SLB 9). Sixty seven rainy and 25 post-rainy parental line combinations showed very low similarity values of less than 0.30.

Discussion

Several studies have been conducted to estimate the genetic diversity using DNA markers in several crops including sorghum (Anas and Yoshida, 2004; Wang et al., 2009; Mutegi et al., 2011; Rahimmalek, 2012). However, few attempts have been made to study the diversity among parental lines to classify them based on heterotic groups with limited success (Menz et al., 2004).

Diversity estimates using SSR markers

In the present study, the 35 SSRs used for genetic diversity studies generated a total of 198 alleles with an average of 5.71 alleles per primer pair (Table 1). Our results are in

Table 1. Features of the 35 SSR markers used in the study.

SSR locus	LG	*N*	*A*	H_o	H_e	*PIC*
gpsb067	8 (H)	5	0.51	0.58	0.03	0.49
gpsb089	1 (A)	3	0.56	0.58	0.06	0.50
gpsb123	8 (H)	4	0.50	0.59	0.11	0.51
gpsb148	5 (E)	2	0.72	0.41	0.09	0.32
mSbCIR240	8 (H)	4	0.39	0.67	0.06	0.60
mSbCIR246	5 (E)	4	0.95	0.11	0.01	0.10
mSbCIR248	10 (J)	3	0.95	0.10	0.00	0.09
mSbCIR262	7 (G)	5	0.51	0.56	0.09	0.47
mSbCIR283	7 (G)	10	0.45	0.70	0.06	0.66
mSbCIR300	5 (E)	5	0.43	0.66	0.05	0.59
mSbCIR306	1 (A)	3	0.52	0.60	0.07	0.52
mSbCIR329	10 (J)	4	0.54	0.60	0.05	0.54
sb4-72	9 (I)	6	0.45	0.62	0.03	0.55
sb5-206	6 (F)	12	0.18	0.87	0.16	0.86
sb6-84	2 (B)	10	0.37	0.77	0.10	0.74
SbAG-B02	5 (E)	6	0.76	0.41	0.05	0.39
Xcup02	6 (F)	6	0.46	0.67	0.01	0.62
Xcup11	3 (C)	2	0.54	0.50	0.05	0.37
Xcup14	3 (C)	4	0.47	0.61	0.09	0.53
Xcup61	3 (C)	2	0.74	0.38	0.05	0.31
Xcup62	1 (A)	2	0.56	0.49	0.04	0.37
Xcup63	2 (B)	3	0.97	0.06	0.01	0.06
Xisep0310	2 (B)	2	0.99	0.03	0.00	0.02
Xtxp010	6 (F)	7	0.48	0.69	0.05	0.66
Xtxp012	4 (D)	15	0.33	0.84	0.13	0.82
xtxp015	10 (J)	11	0.32	0.79	0.12	0.76
Xtxp021	4 (D)	6	0.67	0.51	0.05	0.47
Xtxp114	3 (C)	2	0.54	0.50	0.05	0.37
Xtxp136	10 (J)	2	0.88	0.21	0.07	0.19
Xtxp141	7 (G)	10	0.54	0.67	0.10	0.64
Xtxp265	9 (I)	9	0.20	0.85	0.26	0.84
Xtxp273	8 (H)	9	0.54	0.65	0.05	0.61
Xtxp278	5 (E)	3	0.56	0.52	0.07	0.42
Xtxp295	5 (E)	15	0.39	0.79	0.07	0.78
Xtxp339	6 (F)	2	0.50	0.50	1.00	0.38
Mean		5.66	0.56	0.55	0.09	0.49

N number of alleles, ***A*** major allele frequency, $\boldsymbol{H_o}$ observed heterozygosity, $\boldsymbol{H_e}$ expected heterozygosity, ***PIC*** polymorphism information content.

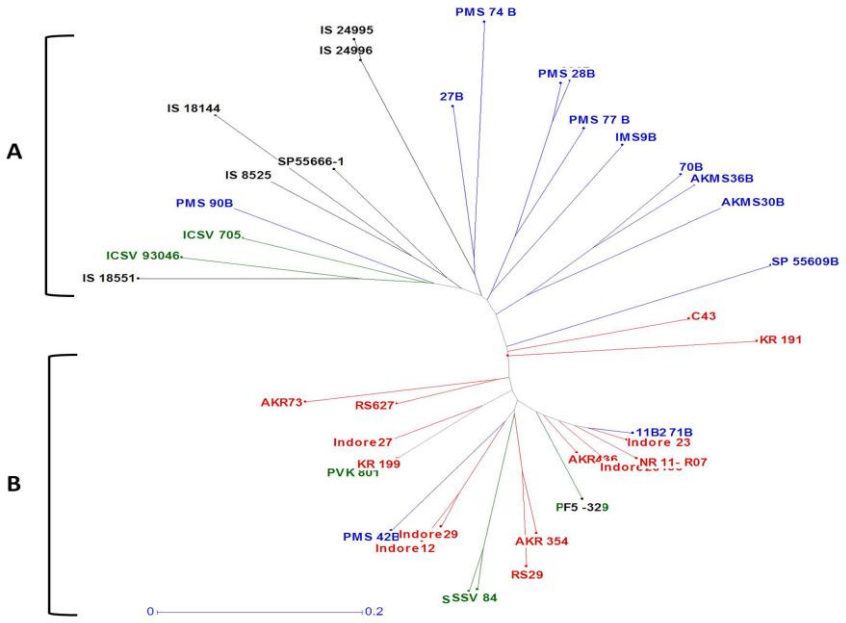

Fig 1. Unrooted neighbor joining tree showing genetic relationship among 43 rainy sorghum genotypes using 35 SSR markers The different working groups are identified by specific colours (blue for maintainers, red for restorers, green for varieties and black for germplasm lines).

congruence with the earlier results of Smith et al. (2000), but lower than reported by Menz et al. (2004) and Muraya et al. (2011) and slightly higher than those of Anas and Yoshida (2004). Eighteen SSR primers recorded PIC values more than 0.5 suggesting the discriminating nature of these markers. Similar results showing high PIC values were reported by others (Smith et al., 2000; Agrama and Tuinstra, 2003; Muraya et al., 2011). Markers with PIC more than 0.5 are efficient in discriminating genotypes and extremely useful in detecting the polymorphism rate at a particular locus (DeWoody et al., 1995).

Genetic relationship and grouping of genotypes

Cluster analysis clearly classified the rainy season sorghum lines into two groups based on the fertility reaction: group A (maintainers) and group B (restorers) (Fig. 1), which are heterotic in nature. This distinct grouping of maintainers and restorers is largely due to the fact that separate breeding programmes are being followed for seed parents and their restorers, and more importantly separate gene pool are being maintained to maximize the level of heterosis (Rooney and Smith, 2000). Differential selection for certain characters like plant height and flowering time contributed to the distinctive nature of the parental lines. Unlike our findings Smith et al. (2000) and Menz et al. (2004) could not classify the genotypes based on the heterotic groups using SSR markers. The clustering pattern of genotypes obtained in the present study is in agreement with their pedigree information. Substantial diversity revealed between B (27B and PMS 28B) and R (C43) lines is reflected in the heterosis level of their hybrids such CSH16 and CSH 25. These two hybrids were released during 1997 and 2007 respectively, and are still popular among the rainy sorghum growing regions in India.
Unlike the rainy season parental lines, the post-rainy parental lines did not separate clearly between maintainer and restorer lines (Fig. 3). This could be attributed to the narrow diversity existing among the post-rainy sorghum genotypes which mostly belong to a single race, *durra* (Sajjanar et al., 2011). In general, both rainy and post-rainy sorghum varieties were diverse with respect to each other. The varieties were more closely associated with the restorer group indicating high gene flow between these groups which share a common gene pool. Tall lines with complete fertility restoration and longer pollen shedding duration are preferred as R lines, while those R lines with high yielding ability and superior grain quality traits are used as varieties for commercial cultivation (Andrews et al., 1997; Rooney and Smith, 2000). The germplasm lines (IS 24995, IS 24996, IS 8525, IS 18144 and IS 18551) clustered separately indicating that they are substantially diverse. IS 18551 is a shoot fly resistant donor and frequently used in most of the resistance breeding programmes (Aruna and Padmaja, 2009; Apotikar et al., 2011). The grouping pattern of the cluster analysis corresponded well with principle component analysis. PCA could classify post-rainy parental lines more effectively based on their fertility and usage groups (Fig. 4). Both in rainy and post-rainy clustering, the first two major axis of differentiation (PC1 and PC2) explained only about 40% of the total variation on an average. This indicates the complexity of the variations in different directions which could not be explained in simple two-dimensional graph. For getting a clear two dimensional graphical representation, the contribution of first two PCs should be more than 80 per cent (Patel et al., 1989).

Population genetic estimates of different groups

AMOVA indicated higher variation among the individuals within population than among groups and sub-groups (Table 2). Estimates of the fixation indices and Nei's genetic distance revealed a strong genetic structure between the rainy and post-rainy genotypes (Table 3). This could be attributed to the racial specificity of each group, in which rainy season genotypes mostly belong to *kafir, bicolor* and *caudatum* races, while the post-rainy genotypes predominantly belong to *durra* race. The presence of strong genetic structure indicates that these two groups are reproductively and genetically isolated from each other. Two sub-populations derived from original population are said to be isolated from each other to allow selection and fixation of unique alleles which will then account for divergence and genetic structures among populations (Hartl and Clark, 1997). Further, the results of Jaccard's diversity estimates and F_{ST} values indicated existence of high diversity between rainy season maintainers and post-rainy restorers which could be best utilized for exploitation of heterosis. Hybrids developed prior to 1980s developed using post-rainy landrace based pollinator parents and few female parents lacked significant heterosis and found to be tall statured and experienced problems with threshing (Reddy et al., 2008). In recent years, efforts were made to develop hybrids showing higher levels of heterosis for both grain and fodder yields when post-rainy landrace based pollinators were used. For instance, the male parent RS 627 of the released rainy hybrid CSH 23 (105 days maturity) was derived from crosses between rainy and post-rainy season adapted lines. Again, the R line RS 585 of the released rainy hybrid CSH 15 was derived from crosses involving post-rainy variety and elite rainy line CS 3541. Detailed studies are required by test crossing rainy season male sterile lines with post-rainy restorer lines to exploit the diversity available in the rainy and post-rainy lines. Based on Jaccard's genetic similarity coefficients, combinations showing high dissimilarity between maintainer and restorer lines were identified. An experiment to evaluate the heterotic performance of the divergent parental pairs of both rainy and post-rainy seasons is underway. Rainy season maintainer lines such as PMS 74B, PMS 90B, 27B, 296B, PMS 28B and among post-rainy SLB 55 were found to be more distinct. KR 191, Indore 12 and RS 29 among rainy season genotypes and TNS 30 among post-rainy genotypes were found to be divergent restorers (Table 4 and 5). These lines could be used as candidate parental genotypes in hybrid development.

Material and methods:

Plant material and DNA isolation

Eighty two genotypes, 43 belonging to rainy season and 39 to post-rainy season adaptation were used in the study (Supplementary table 1). The parental lines (maintainers and restorers), varieties and germplasm lines were chosen as promising lines developed under Indian sorghum breeding programmes and also some key lines from international programme (IS 18144, IS 1130, IS 24996, IS 24995, IS 8525, IS 18551, SP55666-1, SP 55609B, ICSV 705 and ICSV 93046). The 43 rainy genotypes included 14 maintainer lines, 15 restorer lines, 6 varieties and 8 germplasm lines. The 39 post-rainy genotypes included 8 maintainer lines, 21 restorer lines and 10 varieties. The genomic DNA was extracted using CTAB (Cetyl trimethyl ammonium bromide) protocol given by Saghai-Maroof et al. (1984)

Table 2. Analysis of molecular variation (AMOVA) of 82 sorghum genotypes based on 35 SSRs.

Source of variation	d.f.	Sum of squares	Variance of components	Percentage of variation
Among groups	1	358.950	7.91777	33.95
Among populations within groups (sub-groups)	5	152.392	1.52651	6.55
Within populations	75	1040.914	13.87886	59.51
Total	81	1552.256	23.32314	

Table 3. Pairwise F_{ST} estimates (above diagonal) and Nei's genetic distance (below diagonal) among rainy and post-rainy genotypes.

Type	Rainy-germplasm	Rainy-maintainer	Rainy-restorer	Rainy-variety	Post-rainy-maintainer	Post-rainy-restorer	Post-rainy-variety
Rainy-germplasm		0.068	0.088*	0.140*	0.388*	0.418*	0.382*
Rainy-maintainer	0.210		0.128*	0.104*	0.297*	0.336*	0.313*
Rainy-restorer	0.269	0.164		0.274	0.537*	0.524*	0.506*
Rainy-variety	0.251	0.170	0.139		0.297*	0.345*	0.319*
Post-rainy-maintainer	0.356	0.356	0.553	0.433		0.022	0.051
Post-rainy-restorer	0.318	0.301	0.456	0.366	0.097		0.007
Post-rainy-variety	0.330	0.313	0.441	0.367	0.124	0.056	

* $P < 0.05$

SSR analysis

For SSR analysis, 48 SSR primer pairs representing all the 10 sorghum linkage groups were selected from SSR diversity kit (http://sat.cirad.fr/sat/sorghum_SSR_kit/) and used in the present study. Genotyping was carried out at Genotyping Services Laboratory of ICRISAT, Patancheru, India. For capillary electrophoresis, a M13-tagged forward primer method (5'CACGACGTTGTAAAACGAC3') was used at the 5'end of each primer. PCR analysis were carried out with 5 ng of DNA, 2.5 mM $MgCl_2$, 0.2 mM of dNTPs, 1× PCR buffer, 0.006 pM of M13-tailed forward primer, 0.09 pM of M13-Forward primer labelled with either 6-Fam or Vic or Ned or Pet (Applied Biosystems), 0.09 pM of reverse primers and 0.1 U of *Taq* DNA polymerase (SibEnzyme Ltd., Russia) in a total of 5 µl reaction volume and amplified using GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems, USA). The reaction conditions were as follows: initial denaturation (94˚C for 3 min) followed by 10 cycles of denaturation (94˚C for 1 min), annealing at 61˚C for 1 min (temperature reduced by 1˚C for each cycle) and primer extension (72˚C for 1 min). This step was followed by 40 cycles of denaturation at 94˚C for 1 min, annealing at 54˚C for 1 min and extension at 72˚C for 1 min with the final extension (72˚C for 10 min). Based on their expected amplicon size and dye, PCR products were pooled together along with internal size standard (GeneScan™ 500 LIZ® from Applied Biosystems, USA). Capillary electrophoresis was carried out using ABI 3730*xl* Genetic Analyzer (Applied Biosystems, USA). The data generated was then analysed using GeneMapper software (Applied Biosystems, USA) and fragment size was scored in base pairs (*bp*) based on the relative migration of the internal size standard.

Data analysis

The allelic data obtained from GeneMapper software were used for estimating diversity parameters including the number of alleles (N), major allele frequency (A), observed heterozygosity (H_o), expected heterozygosity/gene diversity (H_e) and polymorphism information content (PIC) for each

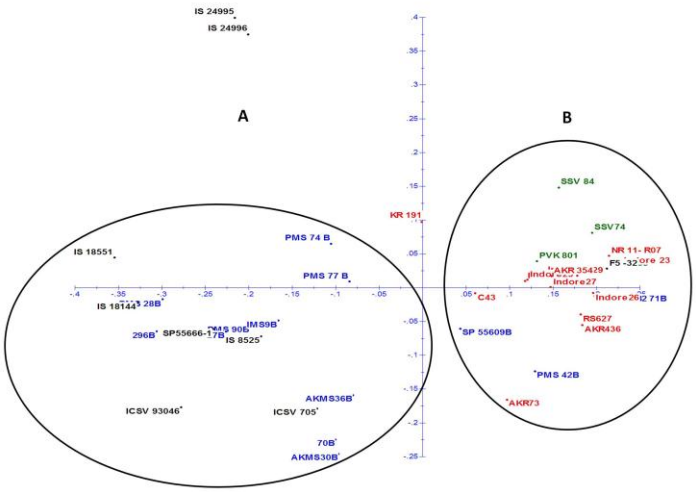

Fig 2. Scatter plot of 43 rainy sorghum hybrid parental lines using first two principal components using 35 SSR markers (maintainer lines indicated in blue, restorers in red, varieties in green and germplasm lines in black).

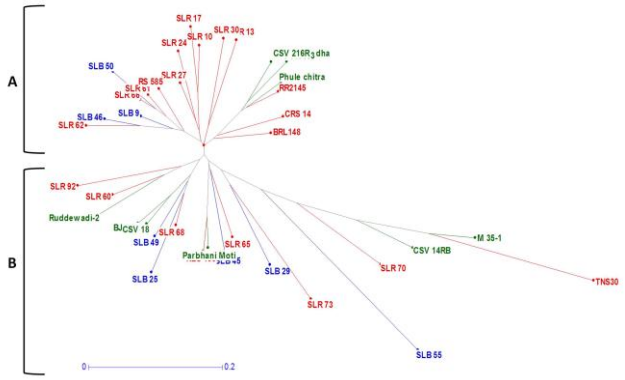

Fig 3. Unrooted neighbor joining of 39 post-rainy genotypes based on 35 SSRs (maintainers indicated in blue, restorers in red and varieties in green).

Table 4. Pairwise similarity coefficients among 29 rainy sorghum hybrid parental lines.

Restorers	Maintainers													
	PMS 71B	PMS 74 B	PMS 42B	PMS 77B	PMS 90B	11B2	27B	296B	AKMS 30B	AKMS 36B	IMS9B	70B	PMS 28B	SP 55609B
KR 191	0.37	0.20	0.34	0.33	0.30	0.37	0.28	0.28	0.26	0.25	0.31	0.25	0.36	0.34
KR 196	0.76	0.30	0.46	0.37	0.22	0.76	0.33	0.25	0.31	0.45	0.34	0.40	0.23	0.37
KR 199	0.64	0.25	0.43	0.39	0.31	0.64	0.31	0.31	0.39	0.42	0.39	0.37	0.35	0.40
AKR436	0.78	0.31	0.57	0.44	0.31	0.78	0.38	0.29	0.38	0.49	0.38	0.44	0.27	0.39
AKR73	0.48	0.22	0.44	0.37	0.22	0.48	0.26	0.34	0.40	0.36	0.34	0.40	0.32	0.35
C43	0.46	0.25	0.37	0.36	0.36	0.46	0.33	0.31	0.38	0.35	0.38	0.34	0.29	0.39
Indore12	0.57	0.30	0.52	0.39	0.34	0.57	0.31	0.29	0.27	0.35	0.29	0.30	0.32	0.32
Indore 23	0.83	0.27	0.51	0.38	0.26	0.83	0.30	0.26	0.31	0.39	0.36	0.36	0.27	0.37
Indore26	0.75	0.33	0.54	0.41	0.33	0.75	0.35	0.25	0.33	0.44	0.33	0.38	0.26	0.36
Indore27	0.61	0.31	0.50	0.46	0.30	0.61	0.32	0.28	0.38	0.46	0.33	0.36	0.29	0.38
Indore29	0.57	0.30	0.52	0.39	0.34	0.57	0.33	0.29	0.29	0.38	0.32	0.35	0.35	0.30
RS29	0.47	0.25	0.46	0.27	0.30	0.47	0.27	0.25	0.35	0.35	0.33	0.29	0.24	0.28
NR 11- R07	0.76	0.30	0.46	0.37	0.22	0.76	0.33	0.25	0.31	0.45	0.34	0.40	0.23	0.37
RS627	0.65	0.28	0.51	0.42	0.29	0.65	0.33	0.27	0.34	0.38	0.36	0.33	0.26	0.38
AKR 354	0.51	0.32	0.53	0.36	0.38	0.51	0.31	0.25	0.33	0.37	0.33	0.30	0.25	0.28

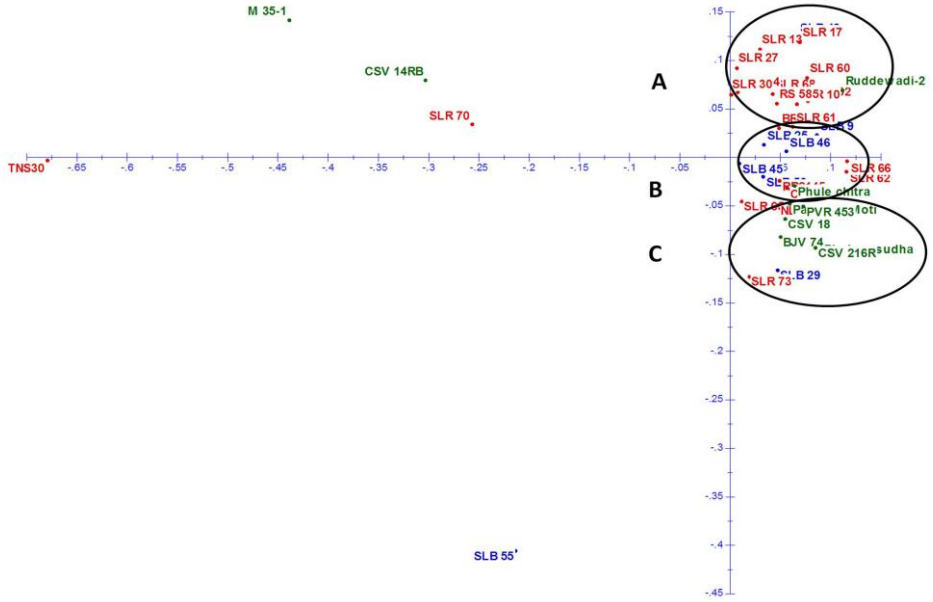

Fig 4. Genetic relationships depicted among 39 post-rainy hybrid parental lines based on first two principal components using 35 SSR markers (maintainers lines indicated in blue, restorers in red and varieties in green colour).

Table 5. Pairwise similarity coefficients among 29 post-rainy sorghum hybrid parental lines**.**

Restorers	Maintainers							
	SLB 25	SLB 29	SLB 9	SLB 45	SLB 46	SLB 49	SLB 50	SLB 55
SLR 62	0.51	0.48	0.69	0.50	0.77	0.65	0.56	0.29
SLR 65	0.59	0.61	0.66	0.67	0.52	0.59	0.53	0.34
SLR 66	0.49	0.61	0.74	0.59	0.71	0.59	0.78	0.29
SLR 68	0.74	0.49	0.65	0.65	0.59	0.73	0.50	0.27
SLR 70	0.43	0.41	0.46	0.52	0.41	0.41	0.41	0.30
SLR 73	0.44	0.53	0.47	0.43	0.47	0.46	0.39	0.28
SLR 92	0.51	0.47	0.60	0.54	0.54	0.51	0.49	0.24
TNS30	0.18	0.16	0.17	0.21	0.16	0.17	0.13	0.23
BRL148	0.52	0.59	0.67	0.57	0.58	0.60	0.60	0.29
SLR 10	0.51	0.52	0.61	0.58	0.54	0.54	0.63	0.26
SLR 13	0.53	0.47	0.56	0.58	0.53	0.55	0.48	0.24
SLR 17	0.52	0.50	0.59	0.52	0.52	0.59	0.50	0.22
SLR 24	0.59	0.53	0.62	0.59	0.52	0.59	0.47	0.26
SLR 27	0.58	0.56	0.64	0.61	0.54	0.65	0.56	0.26
SLR 30	0.44	0.51	0.53	0.56	0.60	0.56	0.51	0.24
SLR 60	0.50	0.43	0.56	0.59	0.60	0.63	0.55	0.23
SLR 61	0.47	0.61	0.70	0.67	0.67	0.59	0.74	0.30
RR2145	0.50	0.50	0.65	0.55	0.53	0.49	0.54	0.32
CRS 14	0.55	0.60	0.62	0.58	0.52	0.58	0.47	0.30
RS 585	0.53	0.57	0.80	0.59	0.66	0.70	0.68	0.27
NLS 100	0.52	0.62	0.61	0.57	0.49	0.57	0.50	0.33

SSR locus. Gene diversity was defined by Weir (1996) as the probability of two randomly chosen alleles being different from a population. PIC was defined by Botstein et al. (1980) as the measure to calculate the discrimination power and informativeness of the SSR markers. Pairwise genetic distance was calculated as given by Nei and Takezaki (1983). For this purpose the 82 genotypes were classified as maintainers, restorers, varieties and germplasm lines for both rainy and post-rainy seasons. All the above parameters were analyzed using Powermarker 3.25 (Liu and Muse, 2005). Further, the allelic data were subjected to estimation of genetic distances for both rainy and post-rainy genotypes using simple matching coefficients (10,000 bootstraps) and the genotypes were clustered using neighbor joining method. Principal component analysis (PCA) was performed and the first two principal components (major axis of differentiation) were used to represent the genotypes in the graphical form. Both the clustering analysis and principal component analysis were done using DARwin software ver 5.0 (Perrier et al., 2003; Perrier and Jacquemoud-Collet, 2006). For further genetic analysis, the allelic data was treated as haplotypic comprising of a combination of alleles at one or several loci (Schneider et al., 2000). The data were tested for presence of population structure and analysis of molecular variance (AMOVA) was performed to separate the total molecular variance into components between groups, within groups and intra population variation (Excoffier et al., 1992) using Arlequin version 2.0 software (Excoffier et al., 2005). Pairwise genetic differentiation between different groups was assessed with fixation index (Weir and Cockerham, 1984) as implemented in Arlequin software (Weir, 1996; Excoffier et al., 2005). The significance of F_{ST} was tested using a nonparametric permutation approach described by Excoffier et al.(1992). The Numerical Taxonomy Multivariate Analysis System (NTSYS-pc) version 2.02 (Rohlf, 2000) was used to calculate Jaccard's similarity coefficients among different genotypes (Jaccard, 1908).

Conclusions

Genetic diversity studies with a set of 35 SSR markers revealed high variability among 82 sorghum genotypes of interest to sorghum improvement programme in India. Strong genetic structure was observed when the genotypes were classified as rainy and post-rainy genotypes and when classified based on usage groups such as maintainers, restorers, varieties and germplasm lines. Our findings demonstrated the utility of SSR markers in classifying the hybrid parental lines of sorghum based on fertility reaction and will serve as effective tool for classifying parental lines based on heterotic groups. The diverse set of parental lines (maintainers and restorers) identified in the present study based on Jaccard's similarity values will serve as effective candidates for hybrid development.

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