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Assessment of RAPD and ISSR marker systems for establishing distinctiveness of forage Sorghum (*Sorghum bicolor* L. Moench) varieties as additional descriptors for plant variety protection

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Abstract

Twenty six varieties of forage sorghum which included 20 released or notified and 6 indigenous local varieties were characterized with 40 morphological DUS descriptors as prescribed by DUS guidelines of PPV &FR Authority and ICAR. RAPD and ISSR markers were also studied to complement the morphological DUS descriptors for establishing distinctiveness of a variety. When all the 33 morphological descriptors of PPV & FR Authority and 7 morphological descriptors of ICAR were studied distinct morphological profiles could be obtained only for 11 out of a total of 26 varieties. Thirteen primers out of a total of 14 RAPD primers were able to establish unique molecular identification profiles (MIPs) for a total of 14 varieties. Fourteen primers out of a total of 20 ISSR primers were able to establish distinctiveness of 19 varieties by the amplification of different genotype specific bands in these varieties. Both the molecular markers revealed a very high level of polymorphism, enabling genetic discrimination of the varieties analyzed by using 121 informative RAPD and 178 ISSR bands. UPGMA cluster analysis of both the markers could distinguish all the twenty six varieties. Higher Mean polymorphic information content (PIC), average expected gene diversity, average resolving power (Rp) and diversity index (DI) were higher for ISSR marker as compared to RAPD one which reflected that ISSR marker is more efficient tool to establish distinctiveness amongst the present set of experimental material. Out of a total of 26 varieties unique identification profiles were developed for 25 varieties by a combination of morphological DUS descriptors and both the RAPD and ISSR markers. However, the variety Pusa Chari 121 was not delineated by any of the morphological and molecular markers. Thus from the present study it could be concluded that in situations where the morpho-physiological DUS descriptors are not able to establish distinctiveness of a variety then unique molecular fingerprints generated by molecular markers may be used as additional or complement descriptors for resolving distinctiveness of varieties.

Key words: DUS, RAPD, ISSR, morphological descriptors

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop providing food and fodder throughout the world [1]. Indian sub-continent is the secondary center of origin for this important cereal [2]. India has enormous diversity of millets including sorghum also called great millet in both cultivated and wild [3]. Obviously there is a need in the country to protect such a vast variability present in the species, which is conserved by farmers as local indigenous varieties.

The issue of ownership over the varieties became alive only after an international body UPOV [Convention of the Union for the Protection of New Varieties of Plants, original in French 'Union Internationale pour la Protection des Obtentions Vegetales] was established in Paris in 1961 and it entered into force in 1968. The UPOV aimed to ensure protection of varieties by the grant of an exclusive right on the protected new plant variety on the basis of a set of uniform and clearly defined principles [4]. India has enacted a *sui generis* form of protection as Protection of Plant Varieties and Farmers Right Act (PPV & FR Act), 2001. Like UPOV, in PPV&FR Act a variety must fulfill the criteria of distinctiveness, uniformity, stability (DUS) and novelty

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(if new) so as to get protection under this Act [5]. There are 33 morpho-physiological DUS descriptors for sorghum which are species specific and recommended procedures for conducting DUS trials [6]. Plant morphological DUS descriptors have been the universally undisputed descriptors applied for DUS testing of crop varieties but serious problems may arise in future for establishing distinctiveness of variety only on morpho-physiological DUS descriptors as the number of candidate varieties are growing with decreased variability [7]. Besides, morphological descriptors are limited in number and are affected by environmental factors. The working group on Biochemical and Molecular Techniques (BMT) of the UPOV has in fact identified isozyme protein markers that could be used as complementary descriptors in soybean [8], maize [9, 10] and barley [11]. But results obtained from these may also be biased by the general consideration that only a minor portion of the genome is represented by these markers [12].

It was anticipated that in the event of failure of morpho-physiological DUS descriptors to discriminate varieties, then molecular markers can be considered as additional descriptors for establishing the distinctiveness of a variety. Availability of a large number of polymorphic DNA-based markers has created an interest in their use for varietal identification. Notwithstanding doubts about reproducibility and intra-varietal uniformity of RAPD (random amplified polymorphic DNA), the technique is found to be highly discriminatory, and having considerable advantage over morphological characteristics as currently used in varietal identification [13]. Likewise, ISSR (Inter simple sequence repeat) reveals high degree of polymorphism, generating reliable information for DNA analysis and with the necessary sensibility to distinguish genetically related individuals [14]. ISSR have been used for cultivar identification in maize [15] and wheat [16]. The present study was conducted to establish distinctiveness of 26 forage sorghum varieties by RAPD and ISSR markers as complementary descriptors to the standard morphological descriptors.

Material and methods

Phenotypic characterization

The experimental material consisted of 26 forage sorghum varieties (Table 1). This was a diverse group comprising 20 released and notified varieties and six indigenous local varieties collected from different parts of the country. The trials were conducted during the *kharif* seasons of 2006 and 2007 in the net wire fenced DUS test plot as per DUS guidelines, in a randomized block design with four replications. Each variety was grown in plot of 6 rows of 6m length spaced at 60 cm row to row and 15 cm plant to plant. Observations were recorded on 33 characteristics at different stages with appropriate procedures as per the DUS test guideline of PPV & FR Authority. Besides, 6 visually assessed characteristics *viz*. flag leaf: extension of discolouration of mid rib, flag leaf: intensity of green colouration of mid rib, glume: anthocyanin colouration of pubescence, stalk: juiciness, grain: shattering, grain: form, and one measurable character as stalk: sweetness were adopted from the ICAR national guidelines for the conduct of DUS test [17]. Thus observations were recorded for a total of forty morphological characteristics which included 26 visually assessed characteristics and fourteen measurable characteristics. Characterization of varieties was done according to five morpho-physiological grouping characteristics reported in the DUS test guidelines.

Molecular marker analysis

The genomic DNA was extracted from 14 days old etiolated seedlings by using the method of Doyle & Doyle [18] with slight modifications. The quantification of DNA in RNA free sample was done using a UV visible spectrophotometer (ELICO Ltd.).

RAPD amplification

PCR reactions were performed in 25µl volume containing 10 x Assay Buffer, 0.5 unit of *Taq* DNA polymerase, 200 µm each of dNTPs, 50 ng/µl reaction of random primers and 50 ng of template DNA. Fourteen random primers obtained from Life Technologies India Ltd were used in the study. PCR was performed in 'Eppendorf Thermocycler' by initial denaturation at 94°C for 5 min followed by 43 cycles of denaturation at 94°C for one min, annealing at 39°C for one min, extension at 72°C for two min and final elongation at 72°C for 7 min. The PCR products were electrophoresed on 1.5% agarose gel, prepared in 1X TBE buffer containing 0.5µg /ml of the ethidium bromide at 80V for 3h with cooling. The gel was photographed under UV transilluminator.

ISSR amplification

ISSR amplification reactions were carried out in 25 µl volume containing 50ng template DNA, 0.5 U *Taq* DNA polymerase, 10 mM dNTP mix (Life Technologies India Ltd.), 50 ng/µl primer in 1X reaction buffer that containing 10 mM Tris.HCl (pH 8.0), 50 mM NaCl and 2.5 mM

Table 1. Details of sorghum varieties studied with their Origin.

S.No.	Genotype	Pedigree/parentage	Origin / Source
1	2219B	-	Pantnagar
2	Pusa Chari 121	PC 7 X CSV-1	IARI (Delhi)
3	Pusa Chari 615	PC 40 X PC 67	IARI (Delhi)
4	Rampur local	Farmers collection	Uttar Pradesh, Uttarakhand
5	Gwalior local	Farmers collection	Madhya Pradesh
6	Golden local	Farmers collection	Vidhisa, Madhya Pradesh
7	Jalana local	Farmers collection	Maharashtra
8	Rajasthan local	Farmers collection	Rajasthan
9	MP Chari red	Farmers collection	Madhya Pradesh
10	CSV 15	SPV 475 × SPV 462	NRCS, Hyderabad
11	UPFS 38	Rio× UPFS-22	Pantnagar
12	S 437-1	Selection from a cross (S153/V60-1 X *Sorghum roxburghii*/ P-1-3-7-1-1)	Haryana
13	UP Chari 2	Vidhisa 60-1 ×IS 6593	Pantnagar
14	Pant Chari 3	Vidhisa 60-1 ×IS 6953	Pantnagar
15	Pant Chari 4	IS 4776 × Rio	Pantnagar
16	Pant Chari 5	CS 3541 × IS 6953	Pantnagar
17	Pant Chari 6	Selection from SDSL 2140	Pantnagar
18	CSH-20 MF	2219 B × UPMC 503	Pantnagar
19	GFS 4	GJ 37 X Sudan type	Gujarat
20	GFS 5	SPV 1087 X GSSV-148	Gujarat
21	SSG 59-3	Non sweet Sudan grass × JS 263	Haryana
22	HC 136	IS 3214(bicolor) × PC7R	Haryana
23	HC 171	SPV8× IS 4776 (Durra)	Haryana
24	HC 260	SPV 103 × PC 9	Haryana
25	HC 308	SPV 8 × IS 4776 (Durra)	Haryana
26	HJ 513	Selection from a cross (S305 X PJ7R X SPV 80) X HC 136	Haryana

$MgCl_2$. A combination of 22 anchored and non anchored primers obtained from Life Technologies India Ltd were used in the study. Amplification was performed in an 'Eppendorf Thermocycler' by initial denaturation at 94°C for 4 min followed by 45 cycles of denaturation at 94°C, annealing temperature was maintained at 52°C for one min, extension at 72°C for two min and final elongation at 72°C for 5 min. The amplification products were loaded on 2% agarose gel and run at 75 V. The gels were visualized under UV after staining with ethidium bromide and documented using a gel documentation and image analysis system (Syngene, UK).

Data analysis

RAPD and ISSR reactions were repeated at least three times to test reproducibility. The non reproducible bands were not used for the comparative analysis of the techniques. The percentage reproducibility was determined by dividing the number of reproducible bands by the total number of bands observed. The intensity of banding was not taken into account for reproducibility and for general scoring. Profiles for each cultivar and marker system were constructed by scoring 0 and 1 for the presence/absence of fragments respectively and the final data sets included both polymorphic and monomorphic fragments.

Data analyses were performed using the NTSYS-pc (Numerical Taxonomy System, version 2.0) [19]. The SIMQUAL program was used to calculate the Jaccard's coefficient, a common estimator of genetic identity and

was calculated as follows: Jaccard's coefficient: NAB/ (NAB+ NA+ NB) where, NAB is the number of bands shared by both the samples, NA represents amplified fragments in sample A and NB represents amplified fragments in sample B.

Polymorphic information content (PIC) that provides an estimate of the discriminatory power of a locus or loci, by taking into account, not only the number of alleles that are expressed, but also relative frequencies of those alleles was calculated using the formula PIC = $1-\Sigma p_i^2$, where p_i is the frequency of the i^{th} allele [20].

The Resolving Power (Rp) of a primer was calculated using the formula Rp = Σ Ib, where Ib (band informativeness) takes the value of: 1 – [2 × (0.5 – p)], p being the proportion of the 26 genotypes (sorghum cultivars) containing the band [21].

Cluster analysis was performed using the computer package NTSYS-PC versions 2.0.Similarities between varieties were estimated using Jaccard's coefficient of similarity. Cluster analyses were conducted on similarity estimates using the Unweighted Pair Group Method on Arithmetic Averages (UPGMA).

Results and discussion

The accurate description of sorghum varieties is crucial for registration under PPV&FR Act. The identity/profile of a sorghum variety is to be established by using a set of morphological characteristics prescribed in the DUS test guidelines on sorghum. In general the six indigenous local cultivars under the study revealed narrow genetic diversity for these descriptors as they were monomorphic for thirteen characters, dimorphic for twenty characters and polymorphic for only seven characters out of a total of forty characters. However, the twenty released and notified cultivars revealed sufficient genetic diversity for morphological DUS descriptors showing monomorphism for only three characters, dimorphism for fourteen characters and polymorphism for twenty three characters. This might be due to the fact that the local cultivars were domesticated in their respective ecological zones with narrow genetic base while the released and notified cultivars have different endemic and exotic sources in their pedigree that might have diverse ecological ranges. Similar attempts for germplasm characterization through qualitative and quantitative characters have been made in sorghum [22]. The dimorphic and polymorphic characters among the sorghum varieties indicated their potential for varietal characterization.

A major objective of varietal characterization is to establish the distinctiveness among the varieties so that official regulatory bodies have a basis on which they can assign rights and protect the interests of plant breeders and farmers [23]. Keeping this in view, varieties were characterized to establish their unique identification profiles on the basis of grouping characteristics prescribed for sorghum by DUS guidelines of PPV & FR Authority. Out of the five grouping characteristics one character *viz*. *kharif* or *rabi* adaptation was found to be monomorphic since all the varieties under study exhibited *kharif* adaptation. Hence grouping of varieties was based on rest of the four characteristics *viz*., time of panicle emergence, total height at maturity, panicle shape at maturity and caryopsis colour after threshing. On the basis of grouping characters distinct morphological profiles were obtained for nine varieties *viz*. GFS 4, CSH 20 MF, HC 260, 2219B, Pant Chari 6, Pant Chari 4, Rajasthan local, UP Chari 2 and HC 136 (Table 2). When all the 33 morphological descriptors of PPV & FR Authority and 7 morphological descriptors of ICAR were considered, distinctiveness of two more varieties *viz*. SSG 59-3 and UPFS 38 could also be established. The grouping characters and morpho-physiological DUS descriptors were able to establish distinctiveness of only 11 out of a total of 26 varieties. Thus morpho-physiological DUS descriptors alone were not sufficient for establishing the distinctiveness, especially in related varieties or similar indigenous varieties grown in a particular niche. Hence molecular markers may be considered for establishing the distinctiveness of a particular variety.

RAPD Analysis

RAPD marker analysis was conducted on DNA extracted from each of the 26 cultivars using 14 oligonucleotide primers. The total number of bands (TNB), number of polymorphic bands (NPB), percentage of polymorphic bands (P%), number of distinguished genotype(s) (NG), polymorphic information content (PIC), Resolving Power (Rp) and number of exclusive bands (NEB) obtained from each primer and diversity index (DI) are shown in Table 3.

All the selected primers amplified 121 RAPD loci (average of 8.7 bands per primer) across the 26 genotypes studied, out of which 111 loci were polymorphic. Nine primers out of 14 produced 100 % polymorphism which together accounted for a very high level (92.4%) of polymorphism (Fig. 1a). Jeya Prakash

et al. [24] also reported very high level of polymorphism (97.4%) among the 32 sorghum accessions utilizing RAPD marker. Unique bands were amplified from different RAPD primers, which could identify the varieties- Pant Chari 5, Pant Chari 6, UPFS 38, GFS 5, HC 136, HC-171,HC 308, SSG 59-3, Rampur local, Gwalior local, Golden local, Jalana local and MP Chari red. The size and number of these exclusive or genotypic specific bands amplified in the mentioned varieties are presented in Table 4. These unique molecular fingerprints generated in different varieties could be incorporated as additional or complementary descriptors to the standard morphological DUS descriptors for their registration and protection. It was observed that some closely related varieties, or varieties derived from common ancestor did not show differences on the basis of morphological descriptors but revealed differences in banding pattern. The PIC value ranged from 0.27 for primer OPH-4 to 0.55 for primer LC-74 with an average of 0.37 for all the fourteen primers which showed the ability of different primers to discriminate among the sorghum cultivars. Prevost and Wilkinson [21] reported the Rp as the capacity of a given primer to discriminate among different genotypes. Four RAPD primers *viz.* LC-72, OPB-1, OPH-19 and LC-78 having high resolving power of 9.56, 8.85, 8.66 and 8.31, respectively were able to discriminate majority of the varieties.

The dendrogram based on UPGMA analysis grouped the 26 varieties into two major clusters and separated all the varieties (Fig. 2a) . The clustering obtained through this molecular profiling roughly coincided with the pedigree of the varieties as observed in UP Chari 2 and Pant Chari 3 as both of them share common female parent (Vidhisa 60-1) in their pedigree. Similarly two cross-based single cut forage sorghum varieties *viz.* HC 171 and HC 308 which have evolved from the same cross SPV 8 X IS 4776 (Durra) and hybrid CSH 20MF and its parental line 2219 B were clustered together. However, some varieties which are related by their pedigree *viz*. Pant Chari 3 and Pant Chari 5, Pant Chari 4 and HC 171, and Pant Chari 4 and UPFS 38 are in different clusters, groups and subgroups (Fig. 1a). This could be attributed to the fact that after every parental cross there are always several generations of selection and during this selection process it is possible that two sorghum varieties with a common origin accumulated many differences in a short period of time.

Consistency of RAPD marker is debatable. For example when sorghum germplasm consisting of 42,000 accessions were analyzed with RAPD markers, the clusters developed were not even close to those obtained on the basis of morphological and agronomic data [25]. Likewise when RAPD marker profile obtained in tomato was converted into SCAR (sequence characterized amplified region), it was unable to differentiate two parental cultivars under a variety of PCR conditions [26]. Thus, fingerprinting with RAPD markers is conditional; in most cases it is not reliable.

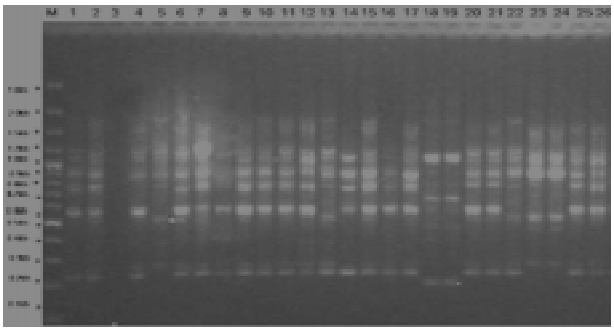

Fig. 1a. Molecular diversity generated among the 26 sorghum varieties by RAPD primer OPD 6. The lane No. corresponds to the No. of genotypes listed in Table 1

ISSR Analysis

ISSR involves amplification of DNA segments present at an amplifiable distance in between two identical microsatellite repeat regions oriented in opposite direction. ISSR uses long primers (15-30 mers) as compared to RAPD primers (10 mers), which permit the subsequent use of high annealing temperature leading to high stringency and reproducibility [27].

The PCR amplification using a combination of 20 anchored and nonanchored dinucleotide repeat primers gave rise to reproducible amplification products. The 20 primers produced 178 bands across 26 varieties of which 168 were polymorphic, accounting for a very high level (94.0%) of polymorphism (Fig. 1b). Thirteen primers revealed 100% polymorphism showing their ability to discriminate the varieties (Table 5). This result is not unexpected, because the technique amplifies microsatellite areas that are potentially highly polymorphic and concurs with previous studies on sorghum [28].

On the basis of genotype specific band(s) amplified by different primers, 13 varieties were discriminated *viz*. 2219 B, CSH 20MF, Pusa Chari 615, CSV 15, S437-1, UP Chari 2, Pant Chari 3, Pant Chari 5, Pant Chari 6, HC 171, HC 260, HC 308 and HJ 513 (Table 4). The 20 ISSR primers collectively yielded 41 unique genotype specific bands which were able to

Table 2. Distinctive morphological profiles of sorghum varieties based on grouping characteristics and other morphological descriptors

Variety	Characteristics
GFS 4	Very early (Time of panicle emergence)
CSH 20 MF	Early (Time of panicle emergence) Tall (Plant total height at maturity)
HC 260	Early (Time of panicle emergence) Very tall (Plant total height at maturity) Symmetrical (Panicle shape at maturity)
2219 B	Early (Time of panicle emergence)
	Very short (height of foliage upto to base of flag leaf) Short (Plant total height at maturity *****)
SSG 59-3	Early (Time of panicle emergence)
Pusa Chari 615	Very tall (Plant total height at maturity) Pyramidal (Plant total height at maturity)
GFS 5	Medium (Time of panicle emergence)
CSV 15	
Pant Chari 6	Late (Time of panicle emergence) Tall (Plant total height at maturity)
Pant Chari 4	Late (Time of panicle emergence) Very tall (Plant total height at maturity) Symmetrical (Panicle shape at maturity)
Rajasthan local	Late (Time of panicle emergence) Very tall (Plant total height at maturity) Panicle broader in upper part (Panicle shape at maturity)
	Grayed white (Caryopsis colour after threshing)
Pant Chari 5	Late (Time of panicle emergence)
Jalana local	Very tall (Plant total height at maturity) Panicle broader in upper part (Panicle shape at maturity) Yellow white (Caryopsis colour after threshing)
UP Chari 2	Very late (Time of panicle emergence) Tall (Plant total height at maturity)
HC 136	Very late (Time of panicle emergence) Very tall (Plant total height at maturity) Symmetrical (Panicle shape at maturity) Yellow white (Caryopsis colour after threshing) Compact (panicle density at maturity *****)
Pusa Chari 121	Very late (Time of panicle emergence)
MP Chari red	Very tall (Plant total height at maturity) Symmetrical (Panicle shape at maturity) Grayed white (Caryopsis colour after threshing)
S 437-1	Very late (Time of panicle emergence)
UPFS 38	Very tall (Plant total height at maturity)
HC 308	Pyramidal (Plant total height at maturity) Yellow white (Caryopsis colour after threshing)
Pant Chari 3	Very late (Time of panicle emergence)
Rampur local	Very tall (Plant total height at maturity)
Gwalior local	Pyramidal (Plant total height at maturity) Grayed white (Caryopsis colour after threshing)
UPFS 38**	Very small (size of mark of germ*****)
SSG 59-3**	Very loose(Panicle density of maturity *****) Narrow elliptic (Grain shape in dorsal view *****) Narrow elliptic (Grain shape in profile view *****) Grayed orange (colour of vitreous albumen*****)

*****indicates the morphological DUS descriptor other than the grouping characters; ******Varieties distinguished by all the morphological DUS descriptors rather than the grouping characters.

discriminate 19 out of a total of 26 varieties. These genotype-specific bands could be used as additional descriptors for plant variety protection and can also be converted into STS markers of great value to detect any mix up between the cultivars and as DNA fingerprints. The PIC values ranged from 0.69 for primer LC-64 to 0.94 for primer LC-15, with an average of 0.85 for all the ISSR primers. The resolving power (Rp) of ISSR primers ranged from 2.31 for primer LC-38 to 16.55 for primer LC-64 with an average of 8.29 for all the primers. Furthermore, three primers *viz.* LC-64, LC-7 and LC-409 having resolving power of 16.55 and 12.85 and 8.15 respectively were able to distinguish most of the twenty six sorghum cultivars under study.

Grouping of varieties based on UPGMA cluster analysis was able to distinguish all the twenty six sorghum varieties. The clustering obtained through ISSR profiling roughly coincided with the pedigree of the varieties as majority of the varieties which are related by their pedigree *viz.* UP Chari 2 and Pant Chari 3, Pant Chari 3 and Pant Chari 5, UPFS 38 and Pant Chari 4 clustered together in minor group $a_{2.1}$ of the dendrogram (Fig. 2b). However, varieties HC 171 and HC 260 evolved from the same cross (SPV 8 X IS 4776) clustered in different groups.

The grouping of majority of the cultivars corresponds to their ecogeographic regions or site of collection. However, three local cultivars *viz.* Golden local, Jalana local and Rajasthan local inspite of their distant and different ecogeographic regions remained together. This may be for the fact that human

Table 3. Details of RAPD primers used for the molecular characterization of 26 varieties of sorghum

Primer code	Primer sqeuence	Amplified product	Total bands	Mono-morphic bands	Poly-morphic bands	% poly-morphism	Poly-morphic infor-mation content (PIC)	Average expected gene diversity (H_i)	Resol-ving power (Rp)	Diversity index
OPH-4	GGAAGTCGCC	2 Kb-0.2 Kb	12	2	10	83.3%	0.27	0.20	4.62	0.27
OPD-6	GGGAATTCGG	2.5Kb-0.1Kb	12	-	12	100%	0.53	0.34	5.36	0.53
OPD-8	GTGTGCCCCA	1.5Kb-0.45 Kb	6	-	6	100%	0.33	0.40	5.87	0.33
OPH-19	CTGACCAGCC	1Kb-0.3Kb	4	-	4	100%	0.29	0.17	8.66	0.29
OPK-19	CACAGGCGGA	2.5Kb-0.3Kb	12	-	12	100%	0.32	0.27	7.45	0.32
OPH-20	GGGAGACATC	1.5Kb-0.6Kb	6	-	6	100%	0.35	0.20	4.46	0.35
ADG-4	CCCGCCGTTG	1 Kb-0.3 Kb	7	-	7	100%	0.52	0.23	3.54	0.52
OPK-4	CCGCCCAAAC	2.0Kb-0.3Kb	12	2	10	83.3%	0.34	0.21	6.38	0.34
OPB-1	GTTTCGCTCC	2.5Kb-0.1Kb	9	2	7	77.7%	0.14	0.19	8.85	0.14
LC-71	TGCCGAGCTG	3.5Kb-0.2Kb	11	-	11	100%	0.40	0.28	7.54	0.40
LC-72	AGTCAGCCAG	3.5Kb-.4 Kb	7	2	5	71.5%	0.20	0.13	9.56	0.10
LC-74	GAAACGGGTG	2 Kb-0.4Kb	6	-	6	100%	0.55	0.37	5.57	0.55
LC-78	GTGATCGCAG	1.5Kb-0.1Kb	8	-	8	100%	0.49	0.39	4.47	0.49
LC-80	CAGCACCCAC	2 Kb -0.2Kb	9	2	7	77.7%	0.38	0.27	8.31	0.42
	Average		8.7	-	-	92.39	0.37	0.26	6.47	0.36

interventions have played a major role in varietal distribution followed by cross pollination between local and introduced materials [29].

Thus out of a total of 26 varieties unique identification profiles were developed for 25 varieties by a combination of morphological DUS descriptors and both the RAPD and ISSR markers. However, the variety Pusa Chari 121 was not delineated by morphological and molecular markers.

None of the molecular markers either individually or in combination showed significant correlation with morphological descriptors (Table 6). The lack of significant correlation between morphological descriptors and molecular markers could partially be explained by the fact that different coefficients i.e. Euclidean dissimilarity coefficient for morphological descriptors and Jaccard's similarity cofficient for molecular markers were used in the study. Secondly, the molecular markers measures genetic variation mainly in non-coding sequences which probably have a minor impact on the phenotype. Morphological descriptors on the other hand are affected by environmental conditions and show considerable variation.

Based on the foregoing results, it can be concluded that in situations where the morpho-physiological DUS descriptors are not able to establish distinctiveness of a variety then molecular markers may be used as additional descriptors for resolving distinctiveness of forage sorghum varieties for granting plant variety protection under PPV&FR Act. However, efficacy of RAPD markers as compared to ISSR was found to be limited in this case. Inclusion of molecular marker as additional descriptors would offer other

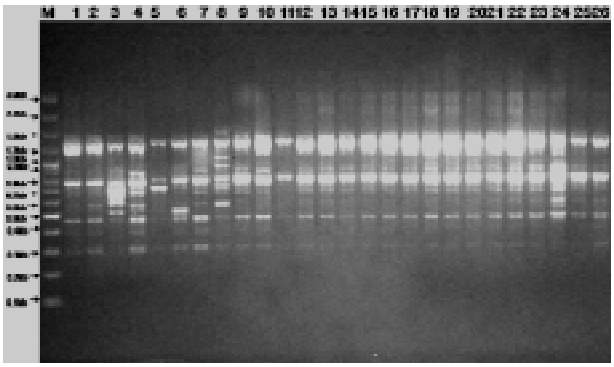

Fig. 1b. Molecular diversity generated among the 26 sorghum varieties by ISSR primer LC-46. The lane No. corresponds to the No. of genotypes listed in Table 1

Table 4. Number and size of genotype specific bands amplified by RAPD and ISSR markers in 26 sorghum varieties

Varieties	RAPD			ISSR		
	Primer	No. of exclusive loci	Size of exclusive loci	Primer	No. of exclusive loci	Size of exclusive loci
2219 B	-	-	-	LC-32	1	1.45 Kb
CSH 20 MF	-	-	-	LC-32	1	0.4 Kb
Pusa Chari 121	-	-	-	-	-	-
Pusa Chari 615	-	-	-	LC-16	1	0.25 Kb
				LC-20	1	0.8 Kb
				LC-46	1	0.7 Kb
				LC-64	1	0.7 Kb
				LC-425	1	0.6 Kb
CSV-15	-	-	-	LC-406	1	0.58 Kb
UPFS-38	OPD-8	1	1.3 Kb	-	-	-
S 437-1	-	-	-	LC-418	1	1.2 Kb
UP Chari-2	-	-	-	LC-16	1	1.5 Kb
Pant Chari 3	-	-	-	LC-38	1	0.8 Kb
Pant Chari 4	-	-	-	-	-	-
Pant Chari 5	OPD-6	1	0.38 Kb	LC-7	1	1.5 Kb
Pant Chari 6	LC-78	1	0.1Kb	LC-6	1	2.1 Kb
				LC-418	1	0.1 Kb
GFS-4	-	-	-	-	-	-
GFS-5	OPH-20	1	0.2 Kb	-	-	-
SSG 59-3	OPH 20	1	0.95 Kb	-	-	-
HC-136	LC-72	1	3.5 Kb	-	-	-
HC-171	LC-71	1	0.8 Kb	LC-59	1	2.0 Kb
	LC-80	1	0.78 Kb	LC-424	1	0.7 Kb
HC-260	-	-	-	LC-46	1	1.0 Kb
				LC-418	1	0.18 Kb
HC-308	LC-71	1	3.2 Kb	LC-20	1	0.8 Kb
	LC-72	1	0.95 Kb			
HJ-513	-	-	-	LC-6	1	1.5 Kb
Rampur local	OPH-19	1	0.5 Kb	LC-6	1	2.0 Kb
				LC-16	1	0.49 Kb
				LC-424	1	1.2 Kb
Gwalior local	OPH-4	1	1.3 Kb	LC-6	1	0.6 Kb
	OPD-6	1	0.52 Kb	LC-15	1	0.4 Kb
	OPK-19	1	1.5 Kb			
	OPB-1	1	0.7 kb			
	LC-74	1	0.6 Kb			
Golden local	LC-78	1	0.3 Kb	LC-405	1	1.5 Kb
Jalana local	LC-71	1	1.5 Kb	LC-14	1	0.4 Kb
				LC-406	1	0.8 Kb
				LC-424	1	1.5 Kb
Rajasthan local	-	-		LC-16	1	0.6 Kb
				LC-46	1	1.6 Kb
MP chari red	LC-78	1	0.3 Kb	LC-7	3	0.6 Kb
						0.5 Kb
						0.4 kb
				LC-14	1	1.5 Kb
				LC-32	1	1.0 Kb
				LC-38	1	0.6 Kb
				LC-49	1	0.7 Kb
				LC-51	2	1.0 Kb
						0.75 Kb
				LC-59	1	0.6 Kb

Table 5. Details of ISSR primers used for the molecular characterization of 26 varieties of sorghum

Primer code	Primer sqeuence	Amplified product (kb)	Total bands	Mono-morphic bands	Poly-morphic bands	% poly-morphism information content (PIC)	Poly-morphic infor-diversity (H_i)	Average expected gene (Rp)	Resol-ving power	Diversity index (Di)
LC-6	AGAGAGAGAGAGAGAGT	1.5-0.3	12	-	12	100	0.86	0.16	6.23	0.90
LC-7	AGAGAGAGAGAGAGAGG	1.5-0.45	5	-	5	100	0.91	0.43	12.85	0.54
LC-14	GAGAGAGAGAGAGAGAT	1.5-0.2	5	-	5	100	0.92	0.44	7.46	0.86
LC-15	GAGAGAGAGAGAGAGAA	0.9-0.3	6	1	5	83.3	0.94	0.28	4.54	0.76
LC-16	CTCTCTCTCTCTCTCTG	1.5-0.25	11	-	11	100	0.78	0.24	4.31	0.79
LC-20	TCTCTCTCTCTCTCTCC	2-0.4	10	3	7	70	0.70	0.10	4.31	0.78
LC-32	TCTCTCTCTCTCTCTCG	1.45-0.35	12	-	12	100	0.88	0.32	5.35	0.60
LC-38	ACACACACACACACACT	1.0-0.45	7	-	7	100	0.92	0.29	2.31	0.28
LC-46	ACACACACACACACACG	1.5-0.3	11	2	9	81.8	0.75	0.24	4.08	0.85
LC-49	TGTGTGTGTGTGTCTGA	1.0-0.3	9	1	8	90	0.86	0.16	5.46	0.69
LC-51	AGAGAGACAGACAGAGGA	1.3-0.45	7	1	6	97	0.86	0.39	7.08	0.69
LC-59	GAGAGAGAGAGAGAGAYT	2.0-0.3	12	-	12	100	0.85	0.31	7.92	0.83
LC-64	TCTCTCTCTCTCTCTCCT	2.0-0.3	10	2	8	80	0.69	0.23	16.55	0.31
LC-405	HBHAGAGAGAGAGAGAG	2.0-0.5	8	-	8	100	0.88	0.40	7.54	0.83
LC-406	BHBGAGAGAGAGAGAGA	2.0-0.2	11	-	11	100	0.82	0.34	5.08	0.84
LC-409	VHVGTGTGTGTGTGTGT	1.2-0.5	7	-	7	100	0.92	0.33	8.15	0.53
LC-418	HVHTGTGTGTGTGTGTG	3.0-0.2	11	-	11	100	0.86	0.34	6.27	0.73
LC-419	ATGATGATGATGATGATG	2.0-0.5	10	-	10	100	0.86	0.41	4.96	0.82
LC-424	CTCCTCCTCCTCCTCCTC	2.0- 0.9	7	-	7	100	0.90	0.45	7.31	0.83
LC-425	TGTGGGCGTAAGCGCAT	3.2-0.5	7	1	6	85.7	0.84	0.22	5.00	0.88
	Average		8.9	-	8.35	94.0	0.85	0.30	8.29	0.72

Table 6. Pearson Correlation (Mantle t-test) between genetic similarity/ dissimilarity matrices based on different marker systems. Euclidean distances were used for morphological descriptors, and Jaccard coefficients of similarity for molecular markers

	RAPD	ISSR	Combined molecular markers	Visually assessed characters	Measurable characters
RAPD	-	0.81268**	0.56600**	0.01417^{NS}	0.03171^{NS}
ISSR	-	-	0.88771**	0.09801^{NS}	0.5294^{NS}
Combined molecular markers	-	-	-	0.59777^{NS}	0.07644^{NS}
Visually assessed characters	-	-	-	-	0.43531*
Measurable characters	-	-	-	-	-

***** Significant at 5 % level of probability**; ****Significant at 0.1% level of probability

advantages. Molecular markers show better resemblance with the pedigree as compared to morphological markers. Present study also revealed similar results which are in agreement with the results of a study on maize inbred lines [30]. Further advantage of molecular markers is their relatively higher discrimination power generated by more balanced distribution of allele frequencies. This could indicate that

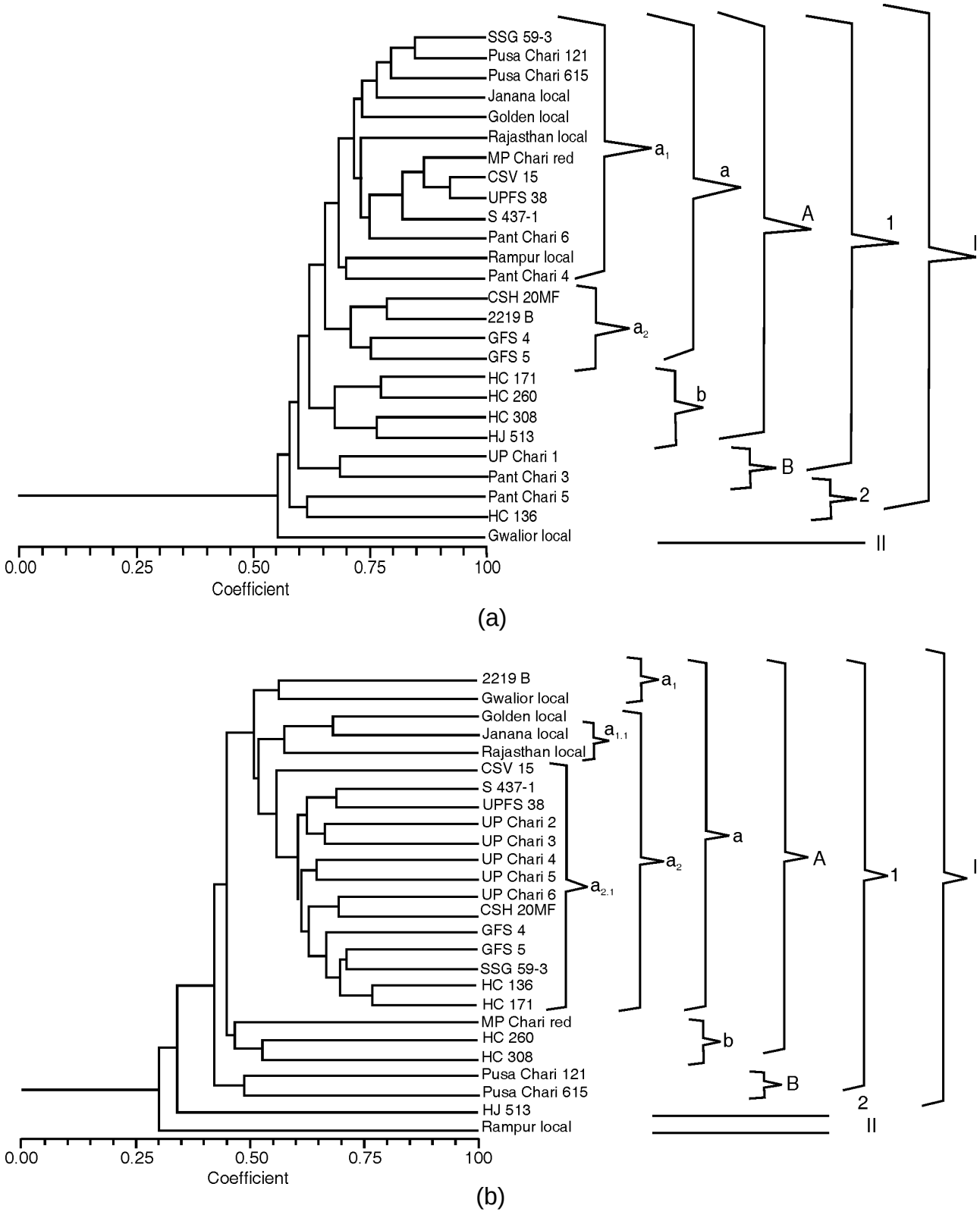

Fig. 2. (a) Dendrogram depicting the classification of 26 sorghum varieties constructed through UPGMA method and based on RAPD (a) ISSR marker (b). The scale at the bottom is Jaccard's coefficient of genetic similarity. I and II: major clusters; 1and 2: groups; A and B: subgroups; a and b: minor groups; a_1 and a_2: sub clusters; $a_{1.1}$ and $a_{2.1}$: minor clusters

erosion of variability introduced through breeding is expressed with higher intensity on morphological than on molecular level. These attributes which have been tested and confirmed, call for the consideration that molecular markers are ideal additional descriptors for establishing distinctiveness of sorghum cultivars, which in turn serve the purpose of granting plant variety protection. However, before using these descriptors in DUS testing, their validity for testing the distinctiveness of varieties and consistency has to be reconfirmed in other sorghum genotypes.

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