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Pattern of genetic relationship as revealed by AFLP markers in Indian sorghum [*Sorghum bicolor* (L.) Moench]

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Abstract

Genetic relationships were assessed in 24 Indian sorghum accessions including released varieties, hybrids and their parental lines using fluorescent based Amplified Fragment Length Polymorphism (AFLP) technique. Nine AFLP primer combinations resulted in a total of 1246 amplification products. The number of polymorphic markers per primer combination ranged from 87 (E-ACT/M-CTG) to 167 (E-ACT/M-CTA). The markers were scored for the presence or absence in binary matrix and relationships among the accessions assessed using Jaccard's similarity coefficient. Mean genetic similarity was estimated at 0.538 based on this set of AFLPs. The most distant two accessions were 104 A and SPV 462 and the closest two were CSH 16 and 27 A at Jaccard's similarity coefficient values of 0.261 and 0.731 respectively. The relationships, among released varieties, hybrids and their parental lines were in accordance with their known pedigree information. Except 104 A, C43 and SPV 462, all the accessions were more or less uniformly distributed because of interrelated pedigree and common ancestors.

Key words: Sorghum, AFLP, genetic relationships

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench, 2n = 2x = 20, family "Poaceae"] is the fifth most important cereal crop in the world after wheat, rice, maize and barley. It is a staple food in arid and semi arid parts of the world, due to its feature of being extremely drought tolerant. Sorghum is also used for the production of ethanol, grain alcohol, starch, adhesives and paper other than being used as food and feed. It is also known with different names depending upon the geographical area including Durra, Egyptian millet, Guinea corn, Jowar, Juwar, Milo, Shallu and Sudan grass.

Sorghum is popularly known as "Jowar" in India. The crop in the country stands at the third place in context of importance after wheat and rice. India accounts for around 20% of the world total area used for the crop production. The crop was introduced in India in the first millennium and since then it has been actively cultivated in the subcontinent. In India, sorghum is produced both as a summer and a winter crop i.e. *kharif* and *rabi* crops. It is grown on a significant scale in states like Maharashtra, Andhra Pradesh, Karnataka, Tamil Nadu, Gujrat, Rajasthan and Madhya Pradesh. Many high yielding hybrids and varieties have been released in India at the National and the State levels and are now in the seed production chain. The plant material used in this study contain hybrids released from 1977 (CSH 6) to 1998 (CSH 17) and varieties released as early from 1930s (M-35-1) to 1996 (CSV 15), which are both kharif and rabi types. These are also the resistant sources for different pests like shoot fly (CSV 14R, 104 A, SPV 1155), midge (CSH 6), corn plant hopper (C 43, RS 29) and diseases like grain molds (SPV 462, CVS 13, CSV 15, CSH 9, CSH 13, CSH 14, CSH 16, CSH 17, RS 29, CS 3541, C 43), downy mildew (AKMS 14A, 27 A, SPV 462, CSV 13, CSV 15, RS 29, C43, CSH 6, CSH 14, CSH 17), sugary disease (SPV 462, CSV 13, AKMS 14 A, RS 29, C 43), rust (SPV 462, CSV 13, CSV 15, CS 3541, RS 29), charcoal rot (SPV 462, CSV 15, CSH 6, RS 29, CS 3541) etc. The varieties *viz*., 'CSV 15', 'SPV 462' and the hybrids 'CSH 13' and 'CSH 15R' serve dual purpose for grain as well as forage. Knowledge of genetic variation existing among such an important material is essential for genetic research, improvement in plant breeding strategies, conservation and effective utilization of genetic resources and intellectual property protection.

Sorghum bicolor is a relatively small genome species (700Mb based on Cot analysis, 772 Mb based

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on flow cytometry) closely related to important large genome tropical grasses such as maize and sugarcane. Thus, it provides a better roadmap for study of these crops at DNA level and facilitates its use as tropical grass model. Any type of molecular marker studies will produce information beneficial to those interested in sorghum crop improvement. AFLPs are useful tools for genetic analysis of plant but only limited references are available using this technique for cultivar identification and genetic relatedness studies in sorghum [6, 7]. Amplified fragment length polymorphism (AFLP) is a powerful and reliable DNA fingerprinting technique, based on selective PCR amplification of restriction fragments from a total digest of genomic DNA and can be used for DNA of any origin or complexity [1]. AFLP is preferred over other DNA based markers mainly because of its high multiplex ratio, non-requirement of prior sequence information and wide genome coverage [2, 3]. This technique has been successfully used for DNA fingerprinting and genetic relatedness studies [4, 5]. The present investigation on AFLP fingerprinting using fluorescently labeled primer combinations was conducted with the objective of studying genetic relationship among Indian sorghum accessions.

Materials and methods

Twenty four sorghum accessions used in this study included seven hybrids, ten parental lines (variety CS 3541 is parental line of hybrid CSH 6 and CSH 9 and is considered in parental line only), six varieties and one germplasm line (SPV 1155) (Table 1). These were procured from National Research Centre for Sorghum, Hyderabad. Total genomic DNA was isolated from young leaves of green house grown plants according to the modified CTAB procedure [8]. Twenty plants of each accessions were pooled for extraction of DNA. Quantity of isolated DNA was estimated using H33258 dye in a fluorometer (Hoeffer Scientific, San Francisco, USA) with calf thymus DNA as standard.

The analysis was performed using AFLP^{TM} plant mapping kit (PE Applied Biosystems). A 0.25 µg quantity of genomic DNA was digested with 1.2 U of *Mse*I and 5.2 U of *EcoR*I restriction endonucleases. *Mse*I and *EcoR*I adaptors were ligated with T4 DNA ligase. The resulting restriction- ligation mixture was then incubated at 37^{o}C for 4.0 hours and diluted for pre-selective amplification (5.5 ml of Restriction-ligation mixture and 94.5 µl of $TE_{0.1}$).

Pre-selective and selective amplifications were carried out as per the instructions provided in the kit. PCR amplification was carried out in Gene Amp PCR system 9600 (PE Applied Biosystems). Selectively amplified products (3.0 µl) were mixed with 0.25 µl of Gene Scan 500 ROX internal size standard, 19.75 ml of de-ionized formamide and were denatured at 96^{o}C for 6.0 minutes. The denatured PCR products were run on ABI PRISM 310 Genetic Analyzer and data collected were further analysed by Gene Scan^{R} 3.1 analysis software to size and quantitate DNA fragments. A total of 64 primer combinations of *EcoR*I and *Mse*I from the kit (Applied Biosystems, Foster city, CA) were screened and finally nine primer combinations were selected for the analysis. The results obtained as electropherograms were converted to binary unit characters (1.0 = present, 0 = absent) using Genotyper (Version 2.5, Perkin-Elmer/ ABI) software. Then the binary data was used for calculating Jaccard's similarity coefficient [9] and for creating the dendrogram based on UPGMA algorithm using NTSYS-pc version 2.1 [10].

Results and discussion

In the present study, 1246 bands were generated by nine selected primer combinations (Table 2) among 24 sorghum accessions. Of these, 1168 bands were polymorphic. The number of polymorphic fragments detected by individual primer combination (Fig. 1) ranged from 87 (E-ACT/M-CTG) to 167 (E-ACT/M-CTA). The percentage polymorphism for each primer combination varied from 88.88% (E-AGC/M-CAT) to 100% (E-ACT/ M-CTA) with an average of 93.73%. The level of polymorphism obtained is higher than earlier reported value of 61.76% among 46 sorghum landraces and cultivars from Southern Africa using AFLP markers [6].

Jaccard's similarity coefficient between accessions ranged from 0.261 to 0.731 with a mean of 0.538 whereas mean genetic similarity of 0.85 was reported among 46 sorghum accessions from Southern Africa using 28 AFLP primer combinations [6]. This high genetic similarity compared to our studies may be attributed to the reason that most of the accessions used in their study belonged to the same agro-ecological zone and landraces have been sampled in a restricted area. Geleta *et al.* [5] reported 62% genetic variation using AFLP markers in 45 sorghum germplasm lines which was higher than our reported value. The reported AFLP markers were able to assess 46% variability in the material under investigation whereas the same 24 accessions were also studied with RAPD markers but could detect only 22% of variation [13]. This may be due to the reason that AFLPs are more sensitive and efficient markers and could assess the inherent

Table 1. List of 24 Indian sorghum accessions analyzed

Accessions (*K/R*)	Pedigree	Race/origin
AKMS14A (*K*)	(MR 707 x BTx623) AKMS 2B	BTx623=USA
CSH 14 (*K*)	AKMS 14A x AKR 150	
2219 A	IS 2219	IS 2219= Kafir-shallu, yellow endosperm type (6323), Nebraska, USA
CSH 6 (*K*)	2219 A x CS 3541	
CS 3541 (*K*)	IS 3675 x IS 3541 (SA 887 x Nyithin)	IS 3675= Kafir-durra, USA IS 3541= conversion product of Zerazera sorghum from Sudan -Ethiopia border
CSH 9 (*K*)	296 A x CS 3541	
296 A (*R*)	IS 3922 x Karad local	IS 3922= Kafir-durra, USA Karad Local = Nandyal selections from Maharastra, India
CSH 13 (*K&R*)	296 A x RS 29	
RS 29 (*K*)	SPV 126 (CSV 9, mutant of CS 3541) x SC 108	CS 3541 = [IS 3675 (Kafir-durra, USA) x IS 3541 (conversion product of Zerazera sorghum from Sudan - Ethiopia border)] SC 108 = Purdue, USA
27 A (*K*)	(83 B x 199 B)	83 B= [(IS 3687 (Durra- caudatum) x IS 3922 (Durra-caudatum)] 199 B= [2219B (Kafir-shallu) x CS 3922 (Durra-caudatum)]
CSH 16 (*R*)	27 A x C 43	
C 43(*K*)	CS 3541 x IS 23549	CS 3541= [IS 3675 (Kafir-durra, USA) x IS 3541 (conversion product of Zerazera sorghum from Sudan - Ethiopia border)] IS 23549 = Guinea-caudatum, Ethiopia
104 A (*R*)	296 B x Swati	296 B= [(IS 3922 (Kafir-durra) x Karad Local (Kharif local)] Swati = [SPV 86 x M 35-1 (Local Maldandi selection)]
CSH 15R (*R*)	104 A x RS 585	
RS 585	(CS 3541 x M 35-1) x Nandyal Rabi Local	Kafir-durra, NYITHIN, local selection from Maldandi
RS 673 (*K*)	[(CS 3541 x CO 18) x (CO 27 X 1022)] x K-24-1	Kafir-durra, NYITHIN
CSH 17 (*K*)	AKMS 14A x RS 673	
SPV 462 (*K*)	(2947 x 232) x 1022	IS 2947= Caudatum, USA SPV 232= 148x512 [148= IS 3687 (USA) x Aispuri 1151 local of Maharastra) x BP 53 (Locals of Gujrat)] Durra- caudatum x Durra - India
SPV 1155 (*R*)	(SPV 86 x E-36-1) x Local selection	
CSV 13 (*K*)	(IS 12622C x 555) x IS 3612C x 2219 B x E-35-1-5-2	IS 12622C= Durra-bicolor Ethiopia 555 = IS 3687 x Aispuri = Durra- caudatum x Durra- India IS 3612C = Caudatum (Nigeria) E-35-1-5-2 = Ethiopian early line
CSV 14R (*R*)	M 35-1 (CS 2947 x CS 2644) x M 35-1	CS 2947 = Kafir USA CS 2644 = Durra- India M 35-1 = Local Maldandi selection
CSV 15 (*K*)	SPV 475 x SPV 462	Same as given for CSV 13 and CSV 15
M 35-1 (*R*)	Selection from local Maldandi	Selection from local Maldandi
Swati (*R*)	SPV 86 x M 35-1	Local Maldandi selection

*Accessions in bold letters are hybrids; K = *Kharif*; R = *Rabi*

variability existing in the material as shown by detailed pedigree analysis in Table 1. On the other hand since the material under study share common ancestors and are of interrelated pedigree, are therefore not getting clustered in to conspicuous groupings.

Based on Jaccard's similarity coefficient values, CSH 16 and 27 A were the accessions showing highest similarity coefficient value of 0.731 while SPV 462 and 104 A were having lowest similarity coefficient value of 0.261. Among the CMS lines 27 A and 2219 A (0.672) were the most similar and 104 A and 27 A were the most diverse (0.420). Among restorer lines, RS 585 and C 43 were most distant (0.380) and RS 673 and RS 29 were most similar (0.665). The Jaccard's similarity coefficient values ranged from 0.371 to 0.649 among the six released varieties studied, with Swati and M 35-1 as the most similar and SPV 462 and CSV 14R as the most distant varieties.

UPGMA dendrogram revealed SPV 462, 104 A and C 43 as diverse from rest of the more or less uniformly distributed material i.e. aI to aIV (Fig. 2). Sub-cluster aI comprised of eight accessions including three CMS-lines, one restorer line and four hybrids. Second sub-cluster aII consisted of RS 29 and RS 673. The released varieties CSV 13 and CSV 15 were grouped together in sub-cluster aIII. The hybrid CSH 15R, its restorer parent and three varieties were present in sub-cluster aIV and the variety CSV 14R was present as an outlier of aIV. The accessions SPV 462 and C 43 were also present as outliers.

Among the five CMS lines studied, 104 A stood separately, may be because of having certain distinct morphological traits (purple plant colour, white leaf midrib, brown with reddish base glume colour, slightly flat seed shape and bold seed size), compared to other CMS lines. Similarly, among the five restorer lines studied C 43 was divergent because of different morphological characters like earhead exsertion, cylindrical earhead shape and pearly white endosperm colour. Thus, for these two lines morphological diversity could be correlated with molecular diversity. Among varieties SPV 462 stood separately may be because of its different geographic region (Coimbatore) of adaptation. It was developed by multiple cross involving IS 2947, IS 3687 from USA and IS 1151 and BP 53 locals of Maharastra and Gujarat in India, respectively. The variety is high yielding for grain and fodder with good grain quality.

Out of seven hybrids studied, two hybrids (CSH 14 and CSH 16) were closely grouped with their respective female parent and five (CSH 6, CSH 9, CSH 13, CSH 15R and CSH 17) were grouped with their respective male parent. CSH 6 and CSH 9 having CS 3541 as common parent were closely grouped. Five of these hybrids as studied earlier with RAPD markers [13] showed different pattern *viz*., CSH 17, CSH 9, CSH 13 and CSH 15R were found close to its female parent whereas CSH 16 was grouped with its male parent. RS 585, was grouped separately from RS 29 and RS 673, which had been reported earlier based on seed protein, isozymes and RAPD data, similarly, CSH 15R was found close to 104 A and 104 B [11, 12, 13], contrary to our results where CSH 15R was closer to other parent i.e. RS 585. SPV 1155, M 35-1, RS 585 and Swati, were present together in subcluster aIV and CSV 14R was present as an outlier of sub-cluster aIV, this clustering may be attributed to their interrelated pedigree (Table 1). This may be explained as M 35-1 is, one of the parent of RS 585, Swati as well as CSV 14R. Similarly SPV 1155 and Swati have SPV 86 as one of the common parent. Earlier, RAPD markers [13] showed similar clustering for the accessions SPV 1155, RS 585, M 35-1, Swati and CSV 14R. CSV 15 having CSV 13 as one of its parents was present together in sub-cluster aIII, thereby showing congruence with pedigree relationships, whereas in the studies reported earlier [13], CSV 15 was closely grouped with SPV 462, which is the other parent of CSV 15. So it is apparent that some accessions showed same clustering pattern both with RAPD [13] and AFLP whereas other accessions showed different clustering for RAPD and AFLP markers. This may be due to the reasons that both the

Table 2. Results of AFLP primer combinations selected for the present study

S.No.	Primer	Total amplification products	Polymorphism (%)
1.	E-ACT/M-CAA	144	97.22
2.	E-ACA/M-CAC	133	90.97
3.	E-AGC/M-CAT	99	88.88
4.	E-ACT/M-CTC	125	89.60
5.	E-ACG/M-CTG	164	92.68
6.	E-ACT/M-CTG	96	90.63
7.	E-AGG/M-CTA	145	89.65
8.	E-ACT/M-CTA	167	100.00
9.	E-ACC/M-CTA	173	98.84

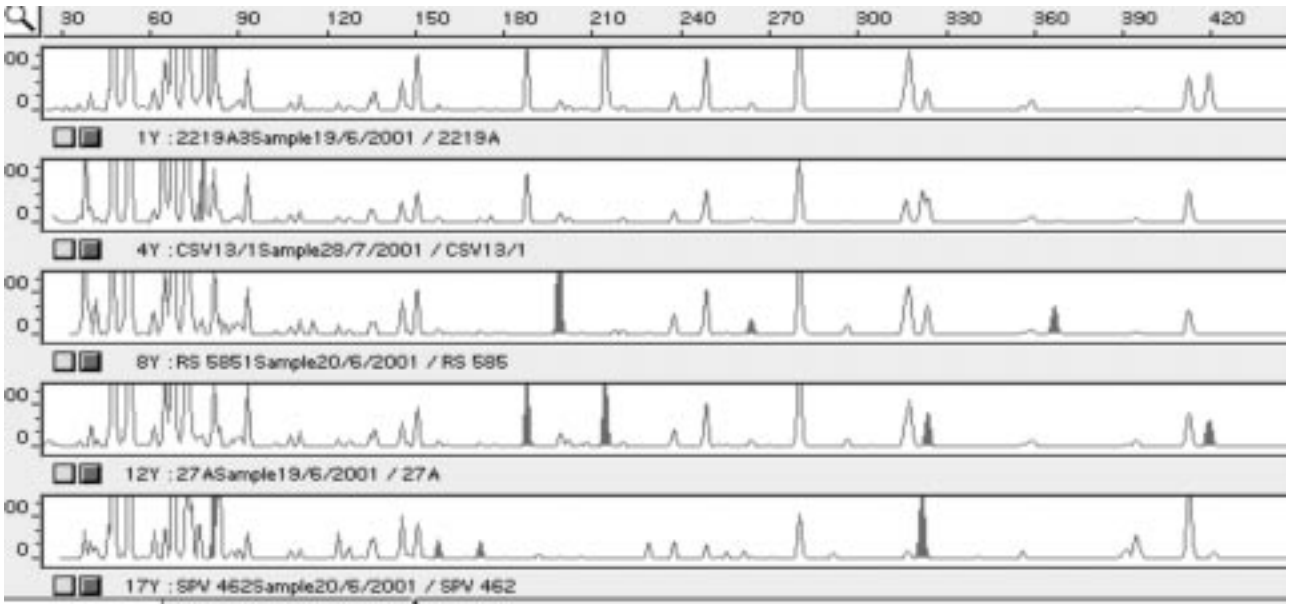

Fig. 1. AFLP electropherogram of five sorghum accessions using primer combination E-ACC/ M-CTA. Filled peaks depict polymorphic amplification products. Scale on the top is fragment size in base pairs and vertical scale on the left depicts fluorescence intensity.

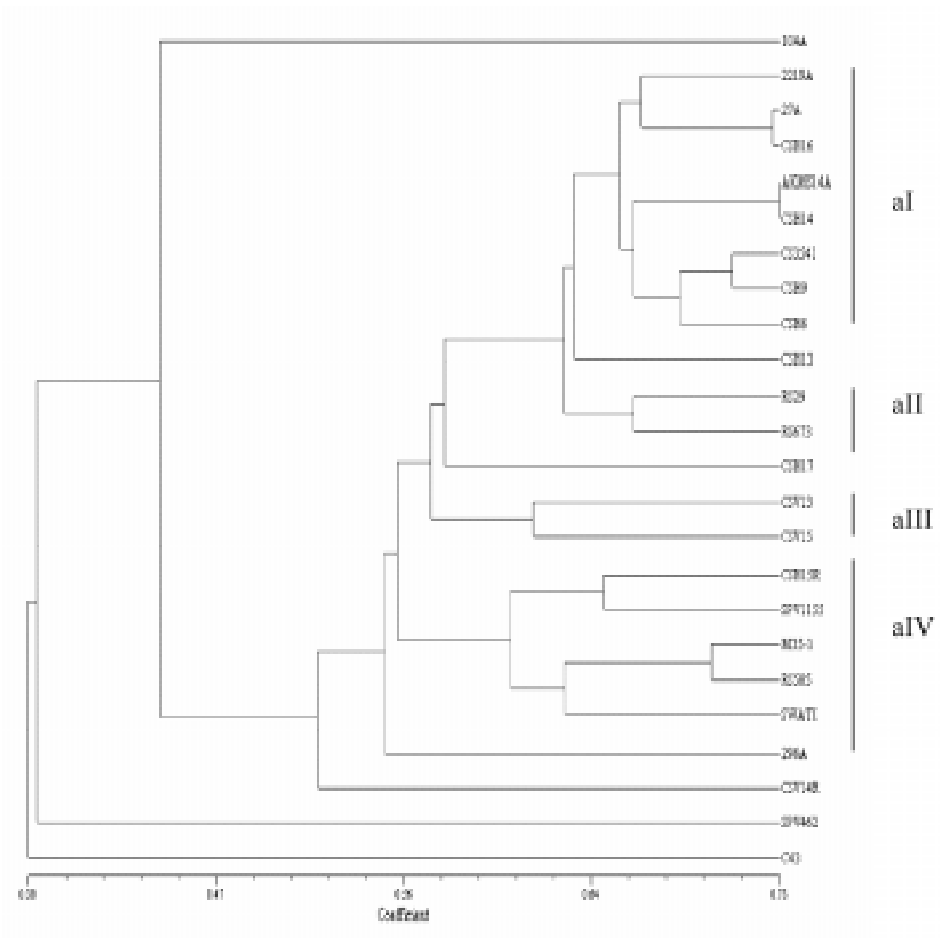

Fig. 2. Dendrogram of 24 Indian sorghum accessions using nine AFLP primer combinations

techniques target different portions of the genome; higher the number of markers and wider genome coverage, more precise is the estimate of genetic relationship [7].

All the varieties except CS 3541 which is a common parental line for the hybrids CSH 6 and CSH 9, were closely grouped in subcluster aIII and aIV with CSV 14R and SPV 462 as outliers. Most of the kharif grown accessions were grouped in subcluster aI, aII and aIII and most of the rabi grown accessions were nested in subcluster aIV with 296 A and CSV 14R as its outlier.

The molecular genetic relationships among the accessions are correlated with their pedigree. These are more or less uniformly distributed, since the material under investigation share common ancestors and have interrelated pedigree. Genetic variation among the sorghum accessions studied is on the higher side as compared to other studies in sorghum. In summary AFLP markers proved to be an efficient tool for precise estimation of genetic relatedness among sorghum accessions.

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