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Research Article

Assessment of diversity using RAPD and ISSR markers in *Sorghum* varieties across Gujarat, India

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Abstract:
Molecular characterization of twelve varieties of *Sorghum bicolor* L. was carried out with 11 RAPD and three ISSR primers. Out of the different anchored primer combinations, ISSR primers with TC and GA motifs produced clear and maximum scorable bands, thus revealing a better coverage of the genome. Genetic diversity parameters [viz., average and effective number of alleles, polymorphic information content (PIC)] were calculated for both RAPD and ISSR markers. UPGMA clustering based on Jaccard's coefficient was calculated. RAPD markers were more efficient than ISSR markers with regards to detection of polymorphism, number of bands scored and PIC values. Genetic variations detected among the geographically different populations of *Sorghum bicolor* L. could be of much use for the introgression of new characters from wild counterparts to the cultivars, isolation of stable segregating markers and selection of improved varieties and conservation of germplasm resources.

Keywords: *Sorghum bicolor* L., RAPD, ISSR, genetic diversity.

Introduction

Sorghum (*Sorghum bicolor* L. Moench) is the fifth most important cereal crop in the world after wheat, rice, maize and barley. It is found in the arid and semi arid parts of the world, due to its feature of being extremely drought tolerant. It has gained importance as a fodder and feed crop in the last decade. Besides being an important food, feed and forage crop, it provides raw material for the production of starch, fiber, dextrose syrup, biofuels, alcohol and other products (Jeyaprakash *et al.,* 2006). Most of the area under high yielding cultivars in India is planted with about 70 hybrids. These hybrids provide a wide range of diversity for maturity and several morphological and grain quality traits offering farmers much wider options than was possible before (Akhare *et al*., 2008). Thus profound genetic variations are prevalent in the species that always demand better germplasm management and conservation practices.

Genetic diversity assessment is one of the key step in any plant breeding programmes. Knowledge of the genetic relationships among different accessions is essential for developing appropriate strategies for breeding, germplasm management (Bhattacharya *et al*., 2010; Nagaral *et al.,* 2009). Most complex quantitative characters related to habit adaptation and end use, significantly contribute towards phenotypic variation, but can not be accurately phenotyped. The study of polymorphism is best done at the level of arrangement of nucleotide bases in DNA, the primary source of all biological information (Nagaral *et al.,* 2009). Marker assisted selection (MAS) has been the mainstay of any modern breeding programmes as can be used at early stages of plant development and is independent of the growth conditions (Soller and Beckmann, 1983).

Randomly Amplified Polymorphic DNA (RAPD) is one such method of identifying polymorphism that can be used to elicit information on divergence, variation, diversity analysis, phylogeny, quantitative traits, marker assisted selection etc (Cooke, 1995). The enormous attraction of RAPDs is that there is no requirement for DNA probes, or for any sequence information for the design of specific primers (Williams *et al.,* 1990). Another molecular system, Inter Simple Sequence Repeat (ISSR) markers, developed by Zietkiewicz and Labuda (1994) based on the amplification of a single primer containing a microsatellite 'core' sequence anchored at the 5' or 3' end by a set of 2-4 purine or pyrimidine residues, offers a high degree of reproducibility with the

detection of rich level of polymorphism in a relatively simple procedure. Hence, it has been widely used in assessment of genetic diversity and cultivar identification (Bhattacharya *et al*., 2010). ISSR have been successfully used to estimate the extent of genetic diversity at inter and intra specific level in a wide range of crop species which include rice, wheat, fingermillet, Vigna, sweet potato and Plantago (Reddy *et al*., 2002).

Considering the potentials of the DNA marker based genetic diversity analysis, the present study aimed to evaluate the congruency of molecular markers system viz. RAPD and ISSR, in assessing and analyzing the nature and the extent of genetic diversity among the different varieties of Sorghum. The present study was undertaken to assess the genetic diversity among sorghum lines using molecular markers.

Material and Methods:-

Twelve varieties of *Sorghum* spp. viz., S-1049, SSG-59-3, AFS-28, AFS-30, GFS-3, CoFs-29, GJ-38, C-10-2, GJ-42, GJ-40, KH-10, IS-4776 belonging to grain, forage and dual purpose types were used in the study (Table:-1). Genomic DNA was extracted from young leaf by CTAB method and the concentration of the extracted DNA was confirmed through Nanodrop method (Nanodrop 1000 Spectrophotometer, Thermo Scientific). The extracted DNA samples were then used for RAPD and ISSR analysis. The details of RAPD and ISSR marker used in the study were presented in Table 3. The Polymerase Chain Reaction (PCR) was performed in a 25µl mixture containing 2.5 µl of assay buffer, 1 µl of Primers (Fermentas), 0.5 µl of dNTP mix (Biolabs), 0.5 µl of *Taq* DNA polymerase, 19.5 µl of Nuclease free water and DNA sample 1 µl. Amplification was performed in Thermal Cycler (Applied Biosystems, Veriti thermal Cycler).

The RAPD and ISSR gel images and marker data were processed using Gene Snap Software from Syngene. The bands were sized and then binary coded as 1 or 0 for their presence or absence in each genotype. The data analysis was performed using the software package NTYSYS-pc (Ver.2.0). Polymorphism information content (PIC) for RAPD and ISSR set was determined.

Results and Discussion:-

Twelve varieties of *Sorghum bicolor* L. Monech were amplified with eleven RAPD and three ISSR markers with reproducible results to ascertain the level of genetic diversity within different varieties (Table 2). Since high reproducible characters were recorded, the RAPD and ISSR showed more variation.

In image analysis, high resolution and only reproducible characters were recorded so that more genetic variation could be seen in RAPD and ISSR PCR bands. Characterization of these released fodder *Sorghum* varieties was carried out using RAPD markers of Operon series. More than 35 RAPD primers from OPA, OPE, OPF, OPG and OPH series were used, out of which 11 gave reproducible results. The highest PIC value of 0.94 was recorded in the forage sorghum variety AFS 28. The highest similarity coefficient of RAPD was observed as 0.68 (Table 4 a). The present result is in agreement with the results of Mehmood *et al*., (2008) where the similarity coefficient of 10 Sorghum varieties from Pakistan was found to be 0.67. Agrama *et al*., (2003) also reported similar results in RAPD studies on 22 Sorghum varieties, where the genetic similarity was found to be 0.612. During ISSR study, the similarity coefficient was recorded highest as 0.57 and PIC value was recorded as 0.90 (Table 4 b). The values were lower as compared to those obtained using RAPD primers. The ISSR study depicted three major clusters A, B, C. However, one genotype, AFS-28 did not fall into any cluster. Cluster A consisted of IS-4776 and GJ-42. GJ-38, GJ-40, KH-10 and C-10-2 grouped into cluster B. Cluster C consisted of two sub clusters C1 and C2. Sub cluster C1 contained CoFS-29, GFS-3, AFS-30, whereas SSG-59-3 and S-1049 formed sub cluster C2. For the ISSR primers the dendrogram generated for the genotypes using molecular data depicted the presence of two major clusters A and B, while genotypes AFS-28 and AFS-30 did not cluster. Only two genotypes constituted cluster A viz., CoFS-29 and GFS-3. IS4776, SSG-59-3, GJ-40, KH-10, GJ-42, Co-10-2, GJ-38 and S-10-49 grouped into cluster B. The present result is in accordance with the work of Bhattacharya et al. (2010) where the PIC and similarity coefficient of RAPD markers were higher than the ISSR markers used. The difference in the resolution of RAPD and ISSR might be because of the fact that the two marker techniques target different portions of the genome. Similar work was carried out by Ayana *et al*. (2000) wherein they assessed the extent of genetic variation among 80 sorghum accessions from Ethopia and Eritrea using 20 oligonucleotide primers and found limited variation among the accessions. Dahlberg *et al*. (2002) assessed variation among *Sorghum* germplasm using seed morphology and RAPD.

The genetic diversity revealed in the present study might be due to naturally occurring variation and usage of random primers in the study. The genetic variability of plants results from interaction of mutation, genetic recombination, selection, random

migration. Mutation and selection pressure are major factors changing the level of genetic equilibrium. Geographical, ecological and reproductive isolation have all had marked effects on level of genetic diversity within species (Mehmood *et al.,* 2008). Molecular techniques provide an alternative approach for evaluating genetic diversity in crop plants since they are not subjected to environmental effects and are independent of the developmental stage of the plant; these methods have been used to identify cultivators in a wide range of plants. The development of molecular techniques has resulted in DNA based alternative procedure of detecting polymorphism. RAPD techniques reveal an extensive amount of variation leading to clear cultivar identification (Mehmood *et al.,* 2008). The ability to resolve genetic variation among different genotypes may be more directly related to the number of polymorphisms detected with each marker technique rather than a function of the technique employed. With this study, it can be conclude that the molecular analysis of different geographically scattered varieties of *Sorghum* across Gujarat through RAPD and ISSR fingerprinting provides a powerful tool for cultivar analysis. Also, the phylogenetic analysis on the basis of RAPD and ISSR derived dendrogram 1 and 2 supports the fact that region specific variations are there, which is because of the multiple generations of selection carried out after their introduction (Bhattacharya *et al*., 2010). The study also emphasized the fact that as compared to PCR based markers such as SSRs and AFLPs or hybridization based markers such as RFLPs, RAPD may lack in accuracy due to their randomness; but they can, in conjunction with ISSRs, prove to be an effective tool for assessing inherent genetic diversity present in different crop varieties.

References

Agrama,H.A. and M.R. Tuinstra. 2003. Phytogenetic diversity and relationships among Sorghum accessions using SSRs and RAPDs. *African J.Biotech.,* **2**(10): 334-340.

Akhare,A. A., S.B. Sakhare, S.B., P.L. Kulwal., D.B. Dhumale. and A. Kharkar. 2008. RAPD profile studies in Sorghum for identification of hybrids and their parents. *Int. J. Inte. Biol.,* **3**(1): 18-24.

Ayana,A., E.Bekele. and T.Bryngelsson. 2000. Genetic variation in wild sorghum (*Sorghum bicolor* ssp.*Verticilliflorum* L. Monech) germplasm from Ethopia assessed by random amplified polymorphic DNA (RAPD). *Hereditas*, **132**: 249-254.

Bhattacharya,S., Bandopadhyay,T.K. and Ghosh,P.D. 2010. Efficiency of RAPD and ISSR markers in assessment of molecular diversity in elite germplasms of *Cymbopogon winterianus* across West Bengal, India. *Emir.J. Food Agric*., **22**(1): 13-24.

Cooke, R.J. 1995. Gel electrophoresis for the identification of plant varieties. *J. Chromatog*., **698**: 281-291.

Dahlberg,J.A., X.Zhang., G.E.Hart. and J.E. Mullet. 2002. Comparative assessment of variation among sorghum germplasm accessions using seed morphology and RAPD measurement. *Crop Sci*. **42**: 291-296.

Jeyaprakash, S. P., K.R. Biji., S. M. Gomez., K.G. Murthy and R. C. Babu. 2006. Genetic diversity analysis of sorghum (*Sorghum bicolor* L. Moench) accessions using RAPD markers. *Indian J. Crop Sci*., **1** (1-2): 109-112.

Mehmood,S., A.Bashir., A.Ahmad., Z.Akram., N.Jabeen. and M.Gulfraz. 2008. Molecular characterization of regional *Sorghum bicolor* varieties from Pakistan. *Pakistan J. Bot*., **40**(5): 2015-2021.

Nagaral, M.S., V.C. Patil and P. Biradar.2009. RAPD based assessment of genetic diversity in pop sorghum. *Karnataka J. Agric. Sci*., **22**(2): 261-263.

Reddy,M.P., N. Sarla. and E.A. Siddiq. 2002. Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. 2002. *Euphytica,* **128**: 9-17.

Soller, M. and J.S. Beckmann. 1983. Genetic polymorphism in varietal identification and genetic improvement. *Theor. Appl. Genet*. **67**: 25-33.

Williams, J.G.K., A.R. Kubelik., K.L. Livak., J.A. Rafalski. and S.V. Tingey. 1990. DNA polymorphism amplified polymorphic DNA markers. *Nucl. Acids Res*., **18**:6531-6535.

Ziekiewicz, E.A.R. and D. Labuda. 1994. Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. *Genomics*, **20**: 176-183.

Table 1. List of the Twelve genotypes of *Sorghum* varieties

Sr. No	Name of the Varieties	Pedigree
1.	S-1049	Selection from local *Sundhia Jowar*
2.	SSG-59-3	Non Sweet Sudan grass x Sweet Jowar JS-263
3.	AFS-28	Derivative of the cross Piper-83 x SSG 59-3
4.	AFS-30	Derivative of the cross PB-19 x SSG 59-3
5.	GFS-3	Germplasm Line IS-5026
6.	CoFS-29	Derivative of the cross TNS-30 x *S.sudanense*
7.	GJ-38	GJ35 x E 35-1
8.	C-10-2	Selection from local cultivar *Chhastio*
9.	GJ-42	Gujarat Jowar – 42 (M.S.R.S, Surat)
10.	GJ-40	(2077AA x M25) xMalvan
11.	KH-10	Local Selection
12.	IS-4776	Selection from *Durra caudatom*

Table 2. PCR amplification performance of twelve varieties of *Sorghum bicolor* L. Moench using RAPD and ISSR primers

Sr. No.	Primer	No. of Bands		Size (bp)	PIC Value
		Lowest	Highest		
		RAPD			
1.	OPA-08	01(AFS-28)	11(SSG-59-3, GJ-38)	434-3052	0.90
2.	OPA-21	02 (AFS-28)	07 (CoFS-29)	366-2734	0.85
3.	OPA-34	07(AFS-28)	17(GJ-38)	290-3559	0.94
4.	OPA-38	02(CoFS-29, AFS-28)	12 (GJ-38)	280-4091	0.90
5.	OPF-05	01(AFS-28, GJ-42, IS-4776)	15(C-10-2)	391-6000	0.93
6.	OPF-06	02 (GJ-42)	09 (CoFS-29)	364-6000	0.92
7.	OPG-06	0 (IS-4776)	03 (S-10-49, SSG-59-3, AFS-30)	156-5588	0.68
8.	OPH-03	02(AFS-30)	11(GJ-38, GJ-40)	214-5844	0.89
9.	OPH-13	03 (CoFS-29)	08 (GFS-3)	299-2229	0.86
10.	OPH-15	02(GFS-3, GJ-42)	08(GJ-38)	327-5075	0.90
11.	OPH-18	05(AFS-28)	13(KH-10)	110-4580	0.92
		ISSR			
1.	ISSR-809	0(CoFS-29, AFS-28)	04(GJ-38, C-10-2)	644-2881	0.78
2.	ISSR-823	0(AFS-28, GFS-3, CoFS-29)	12(IS-4776)	764-4057	0.90
3.	ISSR-886	0(AFS-28,AFS-30,GJ-40)	11(IS-4776)	464-4645	0.86

Table 3. List of Sequence of RAPD and ISSR primers used for PCR amplification for twelve varieties of *Sorghum bicolor* L. Moench

Sr. No.	Name of the Primer	Sequence (5'- 3')
	RAPD	
1.	OPA-08	GTC ACG TAG G
2.	OPA-21	GAG GAC TAG G
3.	OPA-34	GCG ATT CTA G
4.	OPA -38	GAG GGG CTA G
5.	OPF-05	CCG AAT TCC C
6.	OPF-06	GGG AAT TCG G
7.	OPG-06	GTG CCT AAC C
8.	OPH-03	AGA CGT CCA C
9.	OPH-13	GAC GCC ACA C
10.	OPH-15	AAT GGC GCA G
11.	OPH-18	GAA TCG GCC A
	ISSR	
1.	ISSR 809	AGA GAG AGA GAG AGA GG
2.	ISSR 823	TCT CTC TCT CTC TCT CC
3.	ISSR 886	VDV CTC TCT CTC TCT CT

Table 4a. Similarity Matrix among genotypes using RAPD

	S-10-49	SSG-59-3	AFS-28	AFS-30	GFS-3	CoFS-29	GJ-38	C-10-2	GJ-42	GJ-40	KH-10
SSG-59-3	0.68										
AFS-28	0.32	0.32									
AFS-30	0.52	0.62	0.4								
GFS-3	0.54	0.58	0.35	0.62							
CoFS-29	0.46	0.48	0.33	0.55	0.62						
GJ-38	0.49	0.48	0.23	0.48	0.52	0.44					
C-10-2	0.46	0.46	0.23	0.49	0.5	0.4	0.59				
GJ-42	0.38	0.38	0.28	0.43	0.46	0.39	0.53	0.43			
GJ-40	0.43	0.44	0.24	0.44	0.42	0.45	0.64	0.44	0.4		
KH-10	0.47	0.5	0.28	0.49	0.52	0.48	0.6	0.58	0.43	0.62	
IS-4776	0.39	0.4	0.28	0.41	0.42	0.43	0.49	0.41	0.53	0.46	0.48

Table 4b. Similarity Matrix among genotypes using ISSR

	S-10-49	SSG-59-3	AFS-28	AFS-30	GFS-3	CoFS-29	GJ-38	C-10-2	GJ-42	GJ-40	KH-10
SSG-59-3	0.3										
AFS-28	0	0									
AFS-30	0.1	0.11	0								
GFS-3	0.36	0.16	0	0.14							
CoFS-29	0.2	0.06	0	0	0.36						
GJ-38	0.46	0.31	0	0.07	0.35	0.15					
C-10-2	0.5	0.33	0	0.16	0.2	0.1	0.56				
GJ-42	0.26	0.28	0	0.2	0.23	0.11	0.43	0.57			
GJ-40	0.23	0.36	0	0.12	0.08	0	0.42	0.46	0.41		
KH-10	0.42	0.35	0	0.18	0.3	0.17	0.5	0.53	0.5	0.28	
IS-4776	0.21	0.17	0	0.03	0.14	0.25	0.31	0.32	0.29	0.23	0.24

Fig. 1 Dendrogram of twelve varieties of *Sorghum bicolor* L. Moench using RAPD

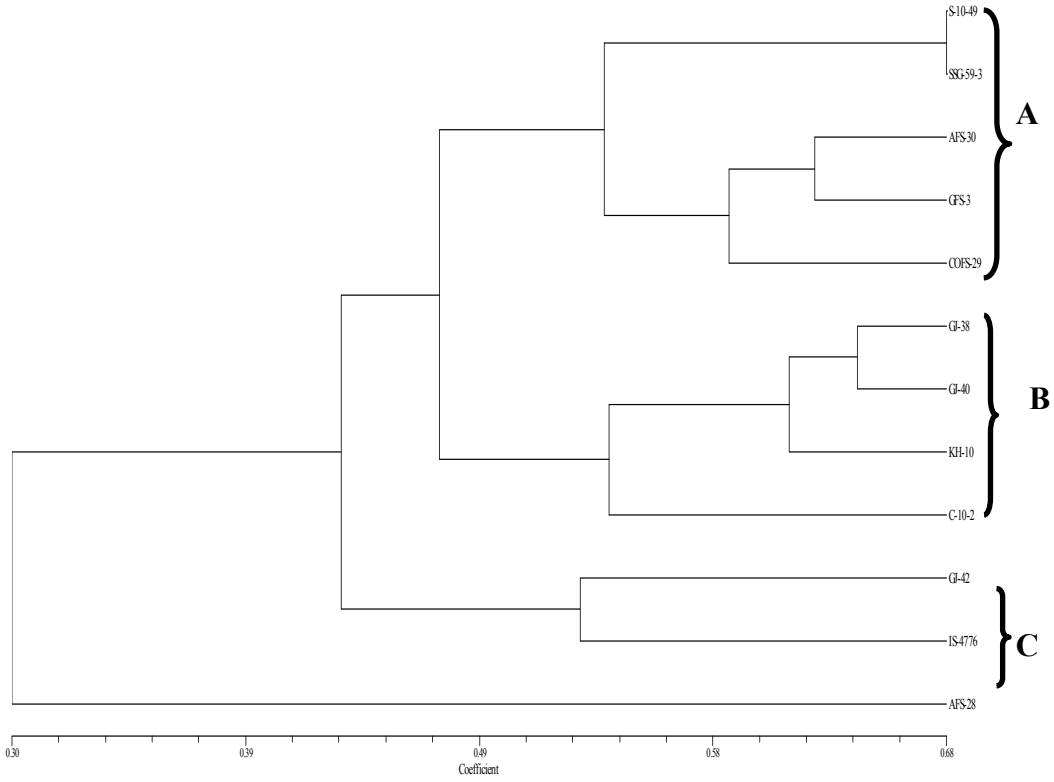

Fig. 2 Dendrogram of twelve varieties of *Sorghum bicolor* L. Moench using ISSR