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Molecular diversity in sweet sorghum genotypes (*Sorghum bicolor* L.) using SSR markers

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Abstract
The present investigation was undertaken to study the genetic diversity in selected sweet sorghum genotypes at molecular level. The genomic DNA isolated from thirty sweet sorghum genotypes *viz.*, CSV -313, RSSV-269, RSSV-260, RSSV-167, RSSV-350, RSSV-355, RSSV-453, RSSV-430, RSSV-417, RSSV-404, RSSV-397, RSSV-386, RSSV-494, RSSV-493, RSSV-483, RSSV-466, RSSV-454, RSSV-508, RSSV-503, RSSV-502, RSSV-498, RSSV-499, RSSV-500, RSSV-513, CSV-1955, RSSV-512, SPV-2191, RSSV-509, RSSV-495 and SSV-84 was subjected to PCR amplification using 13 SSR primers. Out of these 13 SSR primers, 10 yielded amplification and showed polymorphism. Total of 45 loci were generated by amplification with 09 polymorphic primers, of which 43 loci were polymorphic *i.e* 90% polymorphism. The maximum number of bands were observed in RSSV-355, RSSV-498, RSSV-499, SPV-2191(23 bands), whereas minimum number of bands were present in RSSV-500 (14 bands) and RSSV-313 (16 bands), CSV-19SS (17 bands). The Dice similarity coefficient values ranged from 0.10 to 0.95. Out of total 13 SSR primers used, 4 primers found to be more informative based on PIC values irrespective of per cent polymorphism. The Dice similarity coefficient values for SSR primers indicated that moderate diversity among sweet sorghum genotypes. The identical clustering pattern was observed by SSR primers. Unique loci produced by SSR primers may be the variety specific.

Keywords: Sweet sorghum, simple sequence repeats, primers, amplification, genotypes

Introduction
Sorghum [*Sorghum bicolor* (L.) Moench] (2n=20) is cultivated as a major food crop in several countries in South Asia, Africa and Central America. It is a self-pollinating annual plant and is the fifth most important cereal of the world after wheat, maize, rice and barley (Dhillon *et al*., 2007) [4]. Sweet sorghum and high biomass sorghum are of two types which have gained renewed interest in biofuel production for their large biomass yields and high stem sugar content. Traditionally, these sorghums have been mainly used for syrup and forage production (Almodares and Sharif, 2007) [2]. Sweet sorghum was developed primarily for syrup production from the higher sugar content in the stalks. The first sweet sorghum cultivars were brought to the U.S. in the 1850s. Sweet sorghum is an efficient source of ethanol as it's C4 photosynthesis pathways produce sucrose for energy storage, which is easily convertible to ethanol (Ali *et al.,* 2008) [1]. Since then, sweet sorghum research has turned to develop the plant for emphasis in high biomass production, since it out-produces maize on an average by almost 3 tons/ha dry matter.

Sweet sorghum is a special purpose sorghum with a sugar-rich stalk, almost like sugarcane. Besides having rapid growth, high sugar accumulation, and biomass production potential, sweet sorghum has wider adaptability. Water availability is poised to become a major constraint to agricultural production in coming years, cultivation of sugarcane becomes difficult. Sweet sorghum would be a logical crop option in lieu of sugarcane in such situations. It can be grown with less irrigation and rainfall and purchased inputs compared to sugarcane. The sugar content in the juice extracted from sweet sorghum varies from 16–23% Brix. It has a great potential for jaggery, syrup and most importantly fuel, alcohol production. The silage after extraction of juice from sweet sorghum can be used for co-generation of power.

Self-sufficiency in energy requirements is critical to the success of any emerging economy. Renewable sources of energy are considered to be one of the major pillars of energy security that reduces dependence on fossil fuels besides negating the negative effects on the environment. Agriculture has always been a source of fuel for energy production such as feed for draught animals and more recently juice for biofuels, e.g., bioethanol (blended with fossil fuels) or biodiesel. Production of fuels, especially bio-ethanol from lignocellulosic biomass, holds remarkable potential to meet the current energy demand as well as to mitigate

greenhouse gas emissions for a sustainable clean environment. Sweet sorghum is a widely adapted sugar crop with high potential for bio energy and ethanol production. Sweet sorghum can yield more ethanol per unit area of land than many other crops especially under minimum input production. Sweet sorghum is well-adapted to marginal growing conditions such as water deficits, water logging, salinity, alkalinity, and other constraints.

Molecular markers and their utility have been demonstrated in studies of genetic diversity (Morand *et al.,* 2002; Zeid *et al.,* 2003) [6, 12] mating systems (Durand *et al.,* 2000), pollination biology (White *et al.,* 2002). During plant evolution under domestication yield of crops like wheat has been increased but this has reduced the genetic base of crops. So, it is necessary to have programme which will increase genetic base of a crops. The genetic diversity is base for biodiversity. Genetic diversity is essential for conservation and utilization of genetic resources of targeted species and population. It is necessary to reveal genetic diversity to widen genetic base for crop improvement. The purpose of revealing genetic diversity is served by the use of markers. The knowledge of diversity also helps to develop strategies to incorporate useful diversity in breeding programs. Characterization of crop by markers reveals similarities (i.e. shared alleles) and diversity (i.e. typical alleles) among cultivars of a crop. This also helps in identifying gene pool or origin of cultivar. For development of elite cultivar the genetic base has to be enlarged. The species whose gene pool is identified through markers are used for developing elite cultivars by exchange in germplasm. The use of markers characterizes and develops a DNA profile. These DNA profiles of crops are used in management of genetic resources of a crop in gene bank. DNA profiles help in selection of distinct parents for obtaining higher genetic variation.

Materials and Methods

The seed material for the study comprised of thirty sweet sorghum genotypes, which were collected from Sorghum Improvement Project, M.P.K.V., Rahuri and used for research work. The seeds thus obtained were sown in portrays inside poly house for genomic DNA isolation. The clean and bold seeds of each genotype were planted in portrays in green house and labelled properly. Young 15 DAS (Days After Sowing) healthy tender leaves for DNA extraction were collected from individual plants. Genomic DNA was isolated from 30 sweet sorghum genotypes following CTAB (Cetyl Tri methyl Ammonium Bromide) extraction method with some modifications as described by (Helguera *et al.,* 2005) [5]. Genomic DNA was purified by given RNase treatment. Concentration of purified DNA was measured both spectrophotometrically by using UV visible Spectrophotometer (Nanodrop, ND-1000 USA) at 260 and 280 nm was calculated as well as by gel electrophoresis. Two µl of all DNA extracts were electrophoresed in electrophoresis system in 0.8% (w/v) agarose gel containing 0.5 µl /ml ethidium bromide at 6v/cm in 1XTBE buffer. Gradient PCR amplification for different gene specific primers was carried out to determine the annealing temperature of each primer. The PCR programme was set in thermal cycler. (Eppendorf tube, Master Cycler Gradient, Germany). Amplification reaction mixture was prepared in 0.2ml thin walled flat capped PCR tubes, containing the following components. The total volume of each reaction mixture was 20 µl (Table1).

Table 1: Composition of SSR - PCR reaction mixture

PCR reaction component	Stock concentration	Final Concentration	Volume for one tube
Taq buffer B (Genei)	10 X	1 X	2 µl
$MgCl_2$	25 mM	1 mM	1.2 µl
dNTP mix	10 mM	3.2 mM	1.6 µl
Primer (F)	0.2 picomole /µl	0.32 picomole	1.6 µl
Primer (R)	0.2 picomole /µl	0.32 picomole	1.6 µl
Taq DNA polymerase	3 U	1 U	0.33 µl
Sterilized distilled water	-	-	9.67 µl
Template DNA	25 ng/ µl	50 µl	2 µl
Total volume			20 µl

Thirteen SSR primers were used for PCR amplification. The details of primers and their sequences are given in table 2.

Table 2: Sequences and fixed optimum annealing temperature for SSR primers

S. No.	Primers	Sequences of Primers (5`-3`)	Annealing $Temp_r$
1	Xtxp-8	F-ATATGGAAGGAAGAAGAAGCCGG R-AACACAACATGCACGCATG	50 °C
2	Xtxp-31	F-TGCGAGGCTGCCCTACTAG R-TGGACGTACCTATTGGTGC	55 °C
3	Xtxp-33	F-GAGCTACAGAGGGTTCAAC R-CCTAGCTATTCCTTGGTTG	55 °C
4	Xtxp-40	F-CAGCAACTTGCACTTGTC R-GGGAGGAATTTGGCACTAG	55 °C
5	Xtxp-61	F-GATGCCCATGCCTTGC R-CCCACTAAACTAAAGCGGACA	55 °C
6	Xtxp-104	F-TAACCTATGCGGATAAAACAG R-GAATCGCTGCCAAATAAA	52 °C
7	Xtxp-141	F-TGTATGGCCTAGCTTATCT R-CAACAAGCCAACCTAAA	55 °C
8	Xtxp-270	F-AGCAAGAAGAAGGCAAGAAGA R-GCGAAATTATTTTGAAATGGAGTTGA	55 °C
9	Xtxp-312	F-CAGGAAAATACGATCCGTGCCR-GTCAACTATTCGGAAGAAGTTTGGAGGAAA	55 °C
10	Xtxp-340	F- AGAACTGTGCATGTATTCGTC R-AGAATCATCCAAACTCCAATT	55 °C
11	Xtxp12	F-CAGCAACTTGCACTTGTC R-GGGAGGAATTTGGCACTAG	60 °C
12	Xtxp15	F-AGTCACAGCACACTGCTTGTC R-AATTTACCTGGCGCTCTGC	50 °C
13	Xtxp207	F-ACACATCTACTACCCTCTCAC R-TGATGAACTTGTGAGCAGCTC	60 °C

The 20 µl reaction mixture was gently vortexed and spun down. The DNA amplification was carried out in a Thermal Cycler (Eppendorf, Master Cycler Gradient, and Germany). The temperature profiles set for PCR amplification of different primers are mentioned in Tables 3.

Table 3: SSR –PCR programme set in thermal cycler

Name of the steps followed	Temperature	Time	Cycle(s)
Initial Denaturation	94 °C	5 min	1
Denaturation	94 °C	45 sec	35
Annealing	55 °C	45 sec.	
Extension/Elongation	72 °C	1 min.	
Final extension	72 °C	10 min	1
Final hold	4° C	-	-

Amplified PCR products were electrophoresed on agarose gel electrophoresis and were observed under UV transilluminator or gel documentation unit and image was captured.

The clearly resolved PCR amplified bands of sweet sorghum genotypes with different SSR primers were scored manually as binary matrix for their presence (1) and absence (0) in the data sheet. The polymorphism information content (PIC) value was calculated. The binary data was analysed under the SIMQUAL module of NTSYSpc 2.0i software (Rohf, 1998) [9] by using Dice similarity coefficient (Nei and Li, 1979) [7]. SAHN module based on UPGMA (Unpaired Group Mean Algorithm) based clustering method (Sneith and Sokal 1973) [11] was used to generate a tree (dendrogram).

Results and Discussion

The diversity observed in the thirty sweet sorghum genotypes was mainly attributed to the genetic dissimilarities. The Dice similarity coefficient values among 30 sweet sorghum genotypes investigated are presented in Table 8. The pair wise similarity coefficient values ranged from 0.10 to 0.95. Maximum similarity coefficient value of 0.95 was noticed 6 times in the genotypes between RSSV-453 and RSSV-355, RSSV-350 and RSSV-269, RSSV-350 and RSSV-260, RSSV-355 and RSSV-350, RSSV-453 and RSSV-269 and between RSSV-453 and RSSV-167 while minimum similarity value of 0.10 was observed in between RSSV-355 and RSSV-269. From these studies it is revealed that sweet sorghum genotypes are more divergent indicating that large part of the genome may be dissimilar among themselves. However, genetic diversity detected using molecular markers in the present investigation indicates the high discrimination capacity of SSR markers. To visualize the genetic relationship among 30 sweet sorghum genotypes, a dendrogram is constructed based on the UPGMA method from similarity matrix using NTSYSpc 2.02i Programme was presented in (Fig 1).

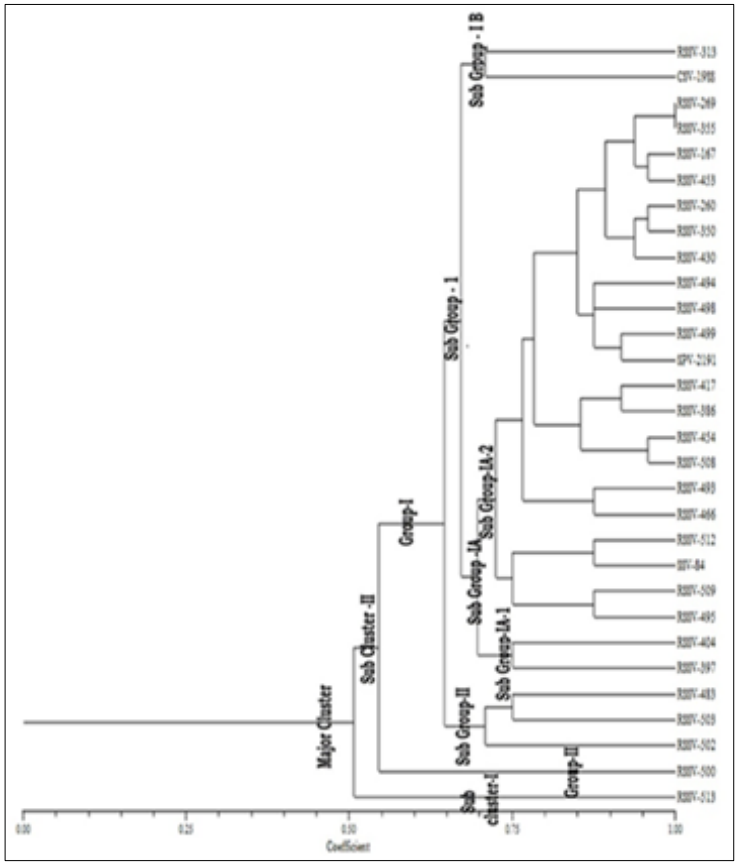


Fig 1: Consensus Tree Showing Clustering of Thirty Sweet Sorghum Genotypes Usimg SSR Markers

Based on cluster analysis using SSR markers, genotypes were grouped into one major cluster. Major cluster consisted of two sub clusters (I and II). First sub cluster (I) consisted of only one genotype RSSV-513, which is entirely distinct among the given genotypes. Second sub cluster comprised of 29 genotypes. Sub cluster (II) is divided into Group-I and Group-II. Group –II comprises only one genotype RSSV-500, which is different among the genotypes in Sub Cluster-II. And Group-I is again sub divided into Sub Group-I and Sub Group-II. And sub group-II consists of 3 genotypes namely RSSV-483, RSSV-503 and RSSV-502. And sub group-I is divided into sub group-I (A) and sub group-I (B). Sub group-I (B) comprises only 2 genotypes namely RSSV-313 and CSV-19SS, which are very distinct from each other. Again subgroup-I (A) is sub divided into sub group-I (A-1) and sub group-I (A-2). Sub group-I (A-1) comprises of two genotypes namely RSSV-404 and RSSV-397. And Sub group-I(A-2) consists of 21 genotypes namely RSSV-269, RSSV-355, RSSV-167, RSSV-453, RSSV-260, RSSV-350, RSSV-430, RSSV-494, RSSV-498, RSSV-499, SPV-2191, RSSV-417, RSSV-386, RSSV-454, RSSV-508, RSSV-493 and RSSV-466, RSSV-512, SSV-84, RSSV-509 and RSSV-495. And among these genotypes, RSSV-269 and RSSV-355 are having more and more similarity (Table 4).

Table 4: The Dice Similarity Co-Efficient Values based on SSR Markers Data

Geno- types	RSSV 313	RSSV 269	RSSV 260	RSSV 167	RSSV 350	RSSV 355	RSSV 453	RSSV 430	RSSV 417	RSSV 404	RSSV 397	RSSV 386	RSSV 494	RSSV 493	RSSV 483	RSSV 466	RSSV 454	RSSV 508	RSSV 503	RSSV 502	RSSV 498	RSSV 499	RSSV 500	RSSV 513	CSV 19SS	RSSV 512	SPV 2191	RSSV 509	RSSV 495	SSV 84
RSSV 313	1.00																												
RSSV 269	0.70	1.00																											
RSSV 260	0.79	0.91	1.00																										
RSSV 167	0.70	0.91	0.83	1.00																									
RSSV 350	0.75	0.95	0.95	0.87	1.00																								
RSSV 355	0.70	0.10	0.91	0.91	0.95	1.00																							
RSSV 453	0.75	0.95	0.67	0.95	0.91	0.95	1.00																						
RSSV 430	0.75	0.87	0.95	0.87	0.91	0.87	0.83	1.00																					
RSSV 417	0.79	0.83	0.91	0.75	0.87	0.83	0.79	0.87	1.00																				
RSSV 414	0.70	0.83	0.75	0.83	0.79	0.83	0.79	0.79	0.75	1.00																			
RSSV 397	0.62	0.66	0.75	0.66	0.70	0.66	0.62	0.79	0.75	0.75	1.00																		
RSSV 386	0.79	0.75	0.83	0.75	0.79	0.75	0.79	0.79	0.91	0.66	0.75	1.00																	
RSSV 494	0.66	0.87	0.87	0.79	0.91	0.87	0.83	0.83	0.87	0.70	0.62	0.79	1.00																
RSSV 493	0.66	0.79	0.79	0.79	0.83	0.79	0.83	0.75	0.70	0.62	0.62	0.79	0.75	1.00															
RSSV 483	0.58	0.79	0.70	0.79	0.75	0.79	0.83	0.66	0.62	0.62	0.54	0.70	0.66	0.75	1.00														
RSSV 466	0.62	0.83	0.75	0.75	0.79	0.83	0.79	0.70	0.75	0.75	0.66	0.75	0.70	0.87	0.70	1.00													
RSSV 454	0.66	0.79	0.70	0.79	0.75	0.79	0.83	0.66	0.79	0.70	0.62	0.87	0.75	0.75	0.75	0.79	1.00												
RSSV 508	0.70	0.75	0.75	0.75	0.70	0.75	0.79	0.70	0.83	0.66	0.66	0.91	0.70	0.70	0.70	0.75	0.95	1.00											
RSSV 503	0.58	0.62	0.62	0.54	0.58	0.62	0.58	0.58	0.62	0.62	0.62	0.62	0.50.	0.50	0.75	0.62	0.58	0.62	1.00										
RSSV 502	0.50	0.70	0.62	0.62	0.66	0.70	0.66	0.58	0.70	0.62	0.45	0.62	0.66	0.66	0.75	0.79	0.66	0.62	0.66	1.00									
RSSV 498	0.70	0.83	0.83	0.75	0.87	0.83	0.79	0.79	0.83	0.66	0.66	0.83	0.87	0.79	0.70	0.75	0.79	0.75	0.54	0.62	1.00								
RSSV 499	0.70	0.91	0.83	0.83	0.87	0.91	0.87	0.79	0.83	0.75	0.58	0.75	0.87	0.70	0.70	0.75	0.79	0.75	0.54	0.70	0.91	1.00							
RSSV 500	0.50	0.54	0.45	0.62	0.50	0.54	0.58	0.50	0.45	0.62	0.54	0.54	0.41	0.58	0.66	0.62	0.58	0.54	0.66	0.58	0.45	0.45	1.00						
RSSV 513	0.45	0.54	0.58	0.54	0.58	0.54	0.50	0.62	0.58	0.62	0.66	0.58	0.50	0.41	0.45	0.45	0.41	0.41	0.41	0.45.	0.45	0.45	0.45	1.00					
CSV 19SS	0.70	0.66	0.75	0.58	0.70	0.66	0.62	0.70	0.83	0.58	0.58	0.75	0.70	0.62	0.54	0.66	0.62	0.66	0.62	0.70	0.75	0.75	0.54	0.50	1.00				
RSSV 512	0.50	0.75	0.70	0.75	0.70	0.75	0.70	0.75	0.70	0.66	0.62	0.62	0.71	0.54	0.70	0.58	0.62	0.62	0.66	0.70	0.66	0.75	0.45	0.50	0.54	1.00			
SPV 2191	0.62	0.91	0.83	0.83	0.87	0.91	0.87	0.79	0.83	0.75	0.58	0.75	0.87	0.70	0.70	0.75	0.79	0.75	0.62	0.70	0.83	0.91	0.54	0.50	0.75	0.75	1.00		
RSSV 509	0.45	0.75	0.66	0.75	0.70	0.75	0.70	0.70	0.66	0.66	0.58	0.58	0.79	0.62	0.54	0.66	0.62	0.58	0.55	0.62	0.66	0.75	0.62	0.50	0.66	0.66	0.83	1.00	
RSSV 495	0.50	0.79	0.70	0.79	0.75	0.79	0.75	0.75	0.70	0.70	0.62	0.62	0.83	0.58	0.66	0.62	0.66	0.62	0.50	0.66	0.70	0.79	0.58	0.54	0.54	.0.79	0.79	0.87	1.00
SSV 84	0.58	0.87	0.79	0.79	0.83	0.87	0.83	0.75	0.79	0.70	0.62	0.70	0.83	0.66	0.75	0.70	0.75	0.70	0.66	0.75	0.79	0.87	0.50	0.45	0.62	0.87	0.87	0.70	0.83	1.00

Table 5: Unique SSR fragments amplified in Sweet Sorghum genotypes

Sr. No.	Genotypes	Primers revealing unique size SSR size of bp of amplified fragments
1.	RSSV-167	Xtxp 31 (400bp), Xtxp 312 (600 bp)
2.	RSSV-350	Xtxp 12 (280 bp)
3.	RSSV-430	Xtxp 270 (620 bp)
4.	RSSV-417	Xtxp 61 (800 bp)
5.	RSSV-404	Xtxp 61 (310 bp)
6.	RSSV-386	Xtxp 31 (500 bp)
7.	RSSV-494	Xtxp 270 (550 bp)
8.	RSSV-493	Xtxp 312 (500 bp)
9.	RSSV-508	Xtxp 61 (750 bp)
10.	RSSV-499	Xtxp 312 (500 bp)
11.	RSSV-513	Xtxp 340 (450 bp)
12.	SPV-2191	Xtxp 312 (380 bp)
13.	RSSV-499	Xtxp 312 (500 bp)
14.	CSV-19SS	Xtxp 31 (400 bp)
15.	RSSV-509	Xtxp 31 (500 bp)

There are 4 half sib parents in the given 30 genotypes of sweet sorghum genotypes namely NSS-223, SSV-84, RSSV-82 and NSSV-258 parents. NSS-223 is one of the common parents for 3 genotypes namely RSSV-167, RSSV-313 and SPV-2191s. RSSV-313 is coming in a distinct cluster *i.e* Sub group-IB while RSSV-167 and SPV-2191 falling under Sub group-IA-2. RSSV-82 is most common half sib parent found in 11 genotypes namely RSSV-454, RSSV-508, RSSV-503, RSSV-502, RSSV-498, RSSV-499, RSSV-500, RSSV-513, RSSV-512, RSSV-509 and RSSV-495. Among these genotypes RSSV-502 and RSSV-RSSV-503 fell under cluster, Sub group-II and rest of the 9 genotypes fell in the cluster, Sub group-IA-2. RSSV-167 and RSSV-453 have dice similarity coefficient value of 0.95 so they fell under same Sub group-IA-2. Some genotypes even though having half sib parent, they fell under different clusters this is because of natural genetic variation arising due to often cross pollination behavior of sorghum, spontaneous mutation and natural hybridization. Even though some genotypes fell under different clusters, they are having very narrow range of difference in the genetic variability. From the Dice Similarity Coefficient values it can be understood that the genotypes having the higher Similarity Coefficient values are less divergent than the genotypes having lower Similarity Coefficient values. It means that genotypes with the higher Similarity Coefficient values are very similar and they can't be distinguished from one another. There is always requirement of the genotypes having less Similarity Coefficient values for plant breeding programmes. The Dice Similarity Coefficient value of the genotypes in the range of 0.71-1.0 indicates that the genotypes are less divergent. The genotype with similarity coefficient values in the range of 0.51-0.70 indicates the moderate diversity and the genotypes with 0 to 0.5 indicating the high divergent genotypes. The genotypes with moderate and high diversity can be utilized in hybridization programmes. The diversity observed in the thirty sweet sorghum genotypes was mainly attributed to the genetic dissimilarities. The Dice similarity coefficient values among 30 sweet sorghum genotypes investigated are presented in Table 4. The pair wise similarity coefficient values ranged from 0.10 to 0.95. Maximum similarity coefficient value of 0.95 was noticed 6 times in the genotypes between RSSV-453 and RSSV-355, RSSV-350 and RSSV-269, RSSV-350 and RSSV-260, RSSV-355 and RSSV-350, RSSV-453 and RSSV-269 and between RSSV-453 and RSSV-167 while minimum similarity value of 0.10 was observed in between RSSV-355 and RSSV-269. From these studies it is revealed that sweet sorghum genotypes are more divergent indicating that large part of the genome may be dissimilar among themselves. However, genetic diversity detected using molecular markers in the present investigation indicates the high discrimination capacity of SSR markers. To visualize the genetic relationship among 30 sweet sorghum genotypes, a dendrogram is constructed based on the UPGMA method from similarity matrix using NTSYSpc 2.02i Programme was presented in (Fig 1). Chauhan *et al*., (2016) [3] studied that the five SSR markers were used for genetic diversity studies generated a total of 34 alleles with an average of 6.8 alleles per primer.

Out of the 9 polymorphic SSRs, 6 markers showed unique amplification in sweet sorghum genotypes. Out of 30 genotypes, these 6 primers amplified specific unique bands in 15 genotypes. The details of these unique markers are given in (Table 5). These primers amplify unique bands can be very useful in characterization and identification of specific sweet sorghum genotypes. The SSR primer Xtxp 270 amplified 10 loci out of which all 10 are polymorphic bands having size range of 150-350 bp (Fig.2). There are 2 unique bands found in the genotype RSSV-430 with size of 620 bp and another unique band found in the genotype RSSV-494 with size of 530 bp. The SSR primer Xtxp 312 amplified 5 loci out of which 4 are unique bands (Table 6, Plate 5). The unique bands having sizes of 500bp, 600bp, 380bp and 310bp are present in RSSV-499, RSSV-167, SPV-2191 and RSSV-404 genotypes respectively (Fig.3). The SSR primer Xtxp 340 amplified 4 loci out of which 4 were polymorphic bands having the size in the range of 210-900 bp and among the 4 bands, 2 were unique band. (Fig.4). The unique bands having size of 450bp and 400bp are present in RSSV-508 and RSSV-513 genotypes.

The PIC values were calculated to find out the efficiency of primers in distinguishing individual genotypes. The Polymorphism Information Content (PIC) values of SSR primers ranged from 0.13 in SSR primer Xtxp 15 to 0.71 in SSR primer Xtxp 40. The four SSR primers *viz.* Xtxp 270, Xtxp 33, Xtxp 40 and Xtxp 61 showed PIC values 0.5 or more indicating that these primers may be considered as more informative. The primers showed the PIC values between 0.26 and 0.50 may be grouped in moderately informative primers. The primers Xtxp 15 and Xtxp 31 showed less PIC values than 0.25 may be grouped in less informative primers. Further, it was observed that there was no correlation between percent polymorphism and PIC values as SSR primers Xtxp 15, Xtxp31, Xtxp 312 and Xtxp 340 showed minimum PIC value but, it has 100% polymorphism (Table 6).

PIC provides an estimate of the discriminatory power of a locus by taking into account, not only the number of alleles that are expressed, but also the relative frequencies of the alleles. PIC values range from 0 (monomorphic) to 1 (very rigidly polymorphic), with many allelic frequencies. The PIC (polymorphic information content) values of markers can provide an estimate of description power in a set of genotypes by taking not only the number of alleles but also the relative frequencies of each alleles (Smith *et al.*, 2000) [10]. The average PIC value of SSRs marker (0.56) was a bit lower in this set of sorghum genotypes in comparison with previous studies using SSR markers for genetic diversity analysis in sorghum with average PIC values observed by Ramu *et al.*, 2013 [8] (0.523). The result indicates discriminating ability of the SSR markers used. The relatively high frequency of SSR polymorphism could be helpful in diversity analyses to better understand the relationship between the genotypes.

Table 6: Information contents of SSR primers used for Sweet Sorghum divergence analysis.

Sr. No.	Primers	No. of bands amplified	Polymorphic bands	Monomorphic bands	Unique bands*	%Polymorphic bands	Size range (bp)	PIC
1	Xtxp 12	5	5	0	1	100	170-550 bp	0.46
2	Xtxp 15	1	1	0	0	100	210 bp	0.13
3	Xtxp 270	10	10	0	2	100	150-350 bp	0.54
4	Xtxp 312	5	5	0	4	100	150 bp	0.26
5	Xtxp 31	5	5	0	4	100	140 bp	0.19

6	Xtxp 33	3	3	0	0	100	120-200 bp	0.58
7	Xtxp 40	4	4	0	0	100	110-410 bp	0.71
8	Xtxp 61	6	6	0	2	100	140-500 bp	0.56
9	Xtxp 340	4	4	0	2	100	210-800 bp	0.31
10	Xtxp 08	2	0	2	0	0	450-515 bp	0
	Total	45	43	2	15	90%	110-800bp	0.316

*Unique bands are also counted under polymorphic bands

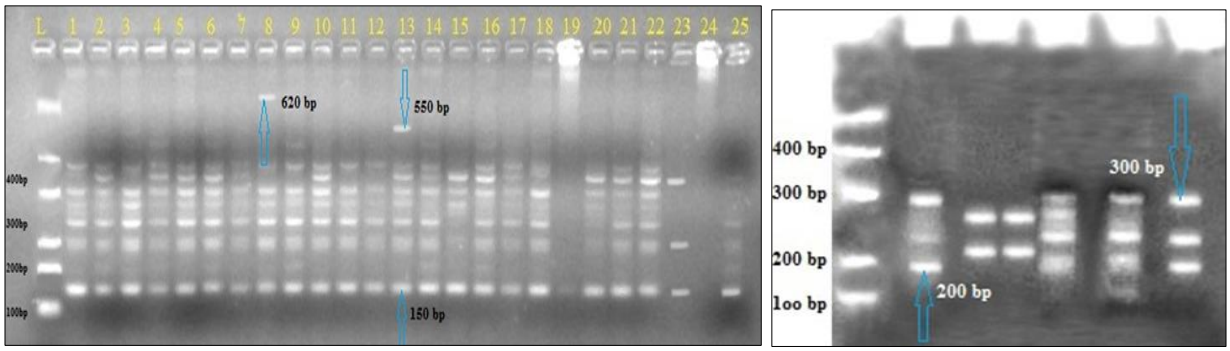


Fig 2: DNA fingerprinting of sweet sorghum genotype RSSV 494 (SPV 2462) obtained using SSR primer Xtxp 270 Lane M= Marker 100 bp

Lane No.	Genotypes	Lane No.	Genotypes	Lane No.	Genotypes
1	RSSV-313	11	RSSV-397	21	RSSV-498
2	RSSV-269	12	RSSV-386	22	RSSV-499
3	RSSV-260	13	RSSV-494	23	RSSV-500
4	RSSV-167	14	RSSV-493	24	RSSV-513
5	RSSV-350	15	RSSV-483	25	CSV-19SS
6	RSSV-355	16	RSSV-466	26	RSSV-512
7	RSSV-453	17	RSSV-454	27	SPV-2191
8	RSSV-430	18	RSSV-508	28	RSSV-509
9	RSSV-417	19	RSSV-503	29	RSSV-495
10	RSSV-404	20	RSSV-502	30	SSV-84

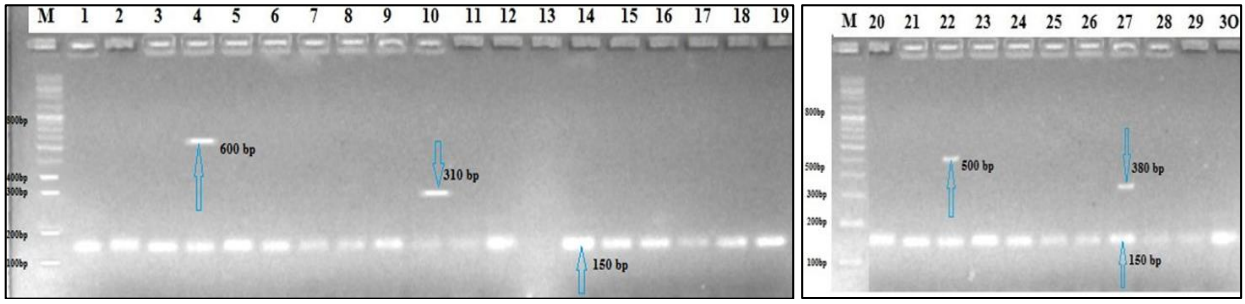
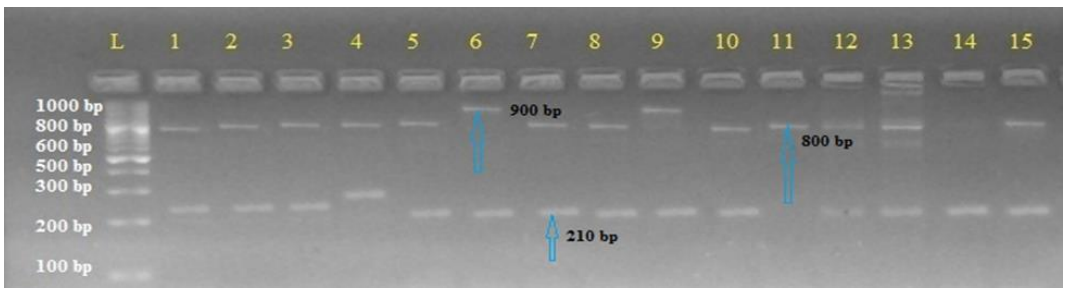


Fig 3: PCR amplification obtained using primer Xtxp 312 Lane M= Marker 100 bp

Lane No.	Genotypes	Lane No.	Genotypes	Lane No.	Genotypes
1	RSSV-313	11	RSSV-397	21	RSSV-498
2	RSSV-269	12	RSSV-386	22	RSSV-499
3	RSSV-260	13	RSSV-494	23	RSSV-500
4	RSSV-167	14	RSSV-493	24	RSSV-513
5	RSSV-350	15	RSSV-483	25	CSV-19SS
6	RSSV-355	16	RSSV-466	26	RSSV-512
7	RSSV-453	17	RSSV-454	27	SPV-2191
8	RSSV-430	18	RSSV-508	28	RSSV-509
9	RSSV-417	19	RSSV-503	29	RSSV-495
10	RSSV-404	20	RSSV-502	30	SSV-84



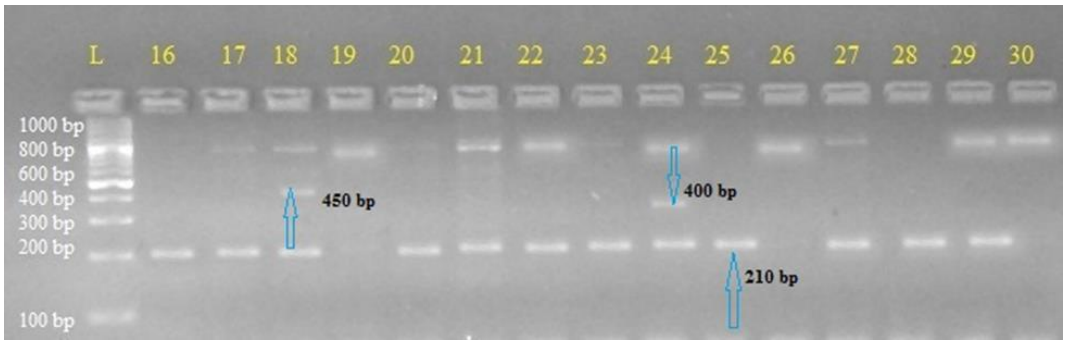


Fig 4: PCR amplification obtained using primer Xtxp 340 Lane M= Marker 100 bp

Lane No.	Genotypes	Lane No.	Genotypes	Lane No.	Genotypes
1	RSSV-313	11	RSSV-397	21	RSSV-498
2	RSSV-269	12	RSSV-386	22	RSSV-499
3	RSSV-260	13	RSSV-494	23	RSSV-500
4	RSSV-167	14	RSSV-493	24	RSSV-513
5	RSSV-350	15	RSSV-483	25	CSV-19SS
6	RSSV-355	16	RSSV-466	26	RSSV-512
7	RSSV-453	17	RSSV-454	27	SPV-2191
8	RSSV-430	18	RSSV-508	28	RSSV-509
9	RSSV-417	19	RSSV-503	29	RSSV-495
10	RSSV-404	20	RSSV-502	30	SSV-84

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