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MOLECULAR BIOLOGY

Biochemical and Molecular Characterization of Sweet Sorghum Varieties and Hybrids

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ABSTRACT

The present investigation entitled, "Biochemical and molecular characterization of sweet sorghum varieties and hybrids" was undertaken to study the sugar and juice quality parameters of promising sweet sorghum varieties and hybrids for the ultimate ethanol production. Genetic diversity of these varieties and hybrids were also studied using ISSR primers. Fifteen promising sweet sorghum varieties and three hybrids were grown in *Kharif* 2017 at All India Co-ordinated Sorghum Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri. The experiment was laid out in Randomized Block Design with three replications. The juice from stalks of each variety and hybrid was extracted and analyzed for ^{0}brix, reducing sugar, non-reducing sugar, total sugar, and juice yield. The juice yield ranged from 4011-10661 l/ha with highest 10661 l/ha in sweet sorghum hybrid RSSH 50. The total sugar content in the stem juice of sweet sorghum varieties and hybrids varied from 11.01 to 13.47, with a mean value of 12.18 percent. Seven ISSR primers were used for the genetic diversity studies of sweet sorghum varieties and hybrids. The consensus tree divided ten varieties into three major clusters. One sub-cluster included five sweet sorghum varieties namely RSSV 517, RSSV 520, RSSV 542, CSV 19 SS, SSV 84 and one hybrid CSH 22 SS, while another sub cluster included three varieties *viz*., RSSV 527, RSSV 533 and RSSV 540.

HIGHLIGHTS

- As per the present investigation during kharif season sweet sorghum genotype RSSV 542 and RSSV 522 are suitable for further breeding programme because they recorded maximum juice yield as well as total sugars which ultimately gives higher ethanol recovery.
- The diversity analysis of eight sweet sorghum varieties and two hybrids by ISSR primers revealed that RSSV 540 and RSSV 517 to be diverse genotypes and could be used for juice quality parameter improvement programme.

Keywords: Genetic diversity, sweet sorghum, hybrids, juice quality, sugars

Sweet sorghum [*Sorghum bicolor* (L.) Moench] is a grass of world origin belonging to the family *Gramineae*. It is a C_4 plant having sugar rich stalks, characterized by high biomass and sugar yield (Gnansounou *et al*. 2005). Sweet sorghum is similar to grain sorghum but accumulates a high amount of sugar in the stems that can be used for various uses such as food, feed, fodders, fuel, and tuber, benefiting the sobriquet "SMART CROP". Besides having rapid growth, high sugar accumulation, and biomass production potential, sweet sorghum has wider adaptability (Reddy *et al*. 2005). The stalk yield of sweet sorghum ranges from 29.4 to 46.5 t/ha with a mean of 40.2 t/ha in *Kharif* season with a mean of 16.8 ^{o}brix (Anonymous, 2007). The dual purpose nature of sweet sorghum, which produces grain and sugar rich stalks, offers new market opportunities for smallholder farmers and does not threaten the food, feed, and fodder value of sorghum. Sweet sorghum requires one-fourth amount of water than that required for sugarcane. The plant matures between 115-125 days after

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plantation. The growth period of sweet sorghum is a short day plant. It can be harvested three times a year. The ability of sweet sorghum to resist drought, saline, and alkaline soils, and water logging has been proven by its wide prevalence in various regions of the world.

Biofuel (Bioethanol and biodiesel) produced from renewable energy sources are gaining importance in light of rising fossil fuel prices, depleting oil reserves, and increasing 'greenhouse effect' associated with the use of fossil fuels. Sweet sorghum is best suited for ethanol production because of its higher total reducing sugar content and sugar than sugarcane juice (Huligol *et al.* 2004). The presence of reduced sugar in sweet sorghum prevents crystallization, and the sweet sorghum cultivar has 90 percent fermentation efficiency (Ratnavathi *et al.* 2004). Genetic variations detected among the geographically different populations of sorghum could be of much use for introducing new characters from wild counterparts to cultivars, isolating stable segregating markers, selecting improved varieties, and conserving of germplasm resources (Chakraborty *et al*. 2011). Assessment of the genetic variability within cultivated crops and varieties has a substantial impact on plant breeding strategies and conservation of genetic resources. It is particularly useful in characterizing individuals, accessions, and cultivars in germplasm collections and for choosingarental genotypes in breeding programs (Li, 2004).The selections of RAPD and ISSR were based on their relative technical simplicity, level of polymorphism they detect, cost-effectiveness, easily applicable to any plant species, and target those sequences which are abundant throughout the eukaryotic genome and are rapidly evolved.

MATERIALS AND METHODS

The seeds of thirteen sweet sorghum genotypes *viz.* RSSV 514, RSSV 515, RSSV 517, RSSV 520, RSSV 522, RSSV 525, RSSV 527, RSSV 529, RSSV 533, RSSV 534, RSSV 535, RSSV 540, RSSV 542, three hybrids CSH 22SS, RSSH 18, RSSH 50 and two checks SSV 84 and CSV 19SS were grown in a randomized block design with three replication during *kharif* season in 2018 on medium deep soil with spacing 45 × 15 cm with application of 100 kg N/ha and 50 kg P_2O_5/ha at All India Coordinated Sorghum Improvement Project, MPKV, Rahuri. Ten random plants having equal height and biomass from the net plot in triplicate were selected carefully. Total fresh cane weight after removing leaves and ear heads from the cane was recorded with the help of electronic balance, and cane yield per hectare was calculated with the hectare factor. These defoliated canes were crushed in three roller crushers, and juice yield was measured with measuring cylinder and further calculated as juice yield L/ha with the help of net plot plant stand and hectare factor. The ^{o}brix was measured with the help of a hand refractometer. The estimation of reducing sugar in sweet sorghum juice was carried by the 3, 5 Dinitrosalycylic acid method (Miller 1959). Estimating total sugar in sweet sorghum juice was carried out by the phenol sulfuric acid method (Dubois *et al*. 1956). Ethanol was estimated using the fermentation and distillation method with a specific gravity chart given by Amerine and Ough, 1974.

The isolation and purification of genomic DNA from fresh young leaves of sweet sorghum varieties and hybrids were carried out by N-lauryl Sarkosyl method described by Dehestani and Tabar (2007). PCR amplification was performed with a reported fifteen ISSR primers obtained from Bangalore GeNei™. PCR amplification was done in oil-free thermal cycler (Eppendorf, Master cycle gradient, Germany) following the PCR program of 94 ^{o}C for 4 min. (initial denaturation) followed by 35 amplification cycles of 45 sec. Denaturation at 94 ^{o}C, followed by annealing temp. (T_a) for 45 sec and elongation or extension at 72 ^{o}C for 90 sec. After the last cycle, a final step of 10 minutes at 72^{o}C was added to allow the complete extension of all amplified fragments. After completion of the cycling program, the reaction was held at 4^{o}C. The PCR condition was standardized, particularly the annealing temperature (varying from 41.7 ^{o}C to 60.2 ^{o}C) for each primer. PCR amplification was carried out with 2 µl Buffer E (10X) with 15 mM $MgCl_2$, 0.5 µl 2.5 mM dNTPs, 50 ηg template DNA, 0.5 µl Taq DNA polymerase (3U/ µl) (Bangalore Genei Pvt. Ltd., India), suitable amount (9.5 µl) of sterile deionized distilled water and 2 µl of ISSR primer from 10 µM working solution. PCR products from each sample were confirmed by running on 1.2% agarose gel containing 6 µl ethidium bromide (10 mg/ml) in 100ml 1X TBE buffer at 60V for 3 hours.

Loading dye (2.5 µl) was added to the PCR products and loaded into the wells. A molecular weight marker, *Eco* R I / Hind *III* double digest, was also loaded on either side of the gel. Dendrogram was constructed using UPGMA software on ISSR data generated from eight sweet sorghum varieties and two hybrids.

RESULTS AND DISCUSSION

In the present investigation, fifteen varieties and three sweet sorghum hybrids were evaluated for cane yield, juice yield, °brix, reducing sugar, non-reducing sugar, total sugar, %, and ethanol in *Kharif* season.

Table 1: Green cane yield and juice yield of sweet sorghum varieties/hybrids grown in *kharif* season

Sl. No.	Varieties/ hybrids	Green cane yield (T/ha)	Juice yield (L/ha)
1	RSSV 514	35.87	5806
2	RSSV 515	41.56	7744
3	RSSV 517	39.36	7006
4	RSSV 520	29.47	4067
5	RSSV 522	52.38	9717
6	RSSV 525	34.80	6278
7	RSSV 527	45.32	8233
8	RSSV 529	36.27	6044
9	RSSV 533	31.53	5444
10	RSSV 534	43.26	8111
11	RSSV 535	28.92	4011
12	RSSV 540	45.63	8756
13	RSSV 542	47.11	9028
14	CSH 22 SS	40.42	5020
15	RSSH 18	41.17	7789
16	RSSH 50	57.78	10661
17	SSV 84	31.11	4861
18	CSV 19 SS	41.23	5466
	Range	28.92- 57.78	4011-10661
	Mean	40.18	6891
	SE ±	2.5	350
	CD at 5%	7.6	1054

The cane yield ranged between 31.11- 57.78 t/ha with a mean of 40.18 t/ha. The highest cane yield t/ha was recorded in hybrid RSSH 50 followed by RSSV 522, while RSSV 520 recorded the lowest cane yield of 29.47 t/ha (Table 1). The juice yield l/ha ranged from 4011-10661 l/ha. The highest juice yield l/ha was recorded in hybrid RSSH 50 followed by RSSV 522, while RSSV 520 recorded the lowest juice yield of 4067 l/ha (Table 1). In *kharif* season, the cane yield varied within sweet sorghum varieties and hybrids. Similar observations were made by several investigators. Oyier *et al*. (2017) reported that the green cane yield of sweet sorghum genotypes ranged from 35 to 55 t/ha. Juice volume recorded for the sweet sorghum variety Topper 76-6 was 13094 l/ha (Kering *et al*. 2017). Oyier *et al*. (2017) reported the average juice yield in sweet sorghum canes to be 10000 to 12000 l/ha. The brix ranged between 19-20°. °Brix recorded with 20 ^{0}brix was highest in sweet sorghum genotype RSSV 534. Oyier *et al*. (2017) reported stage wise increase in the °brix from grain filling to the physiological maturity stage. Sweet sorghum genotypes showed an increase in °brix from 15-20 °brix. Sweet sorghum genotypes recorded mean °brix ranged from 13-16° (Ravella *et al*. 2016).

Table 2: 0Brix and total sugar% of sweet sorghum varieties/hybrids grown in *kharif* season

Sl. No.	Varieties/hybrids	°Brix	Total sugar (%)
1	RSSV 514	19.5	13.47
2	RSSV 515	19.0	12.71
3	RSSV 517	19.0	12.97
4	RSSV 520	19.0	12.94
5	RSSV 522	19.0	12.74
6	RSSV 525	19.0	11.31
7	RSSV 527	19.0	12.28
8	RSSV 529	19.5	13.01
9	RSSV 533	19.5	13.08
10	RSSV 534	20.0	11.02
11	RSSV 535	19.0	13.05
12	RSSV 540	19.0	12.46
13	RSSV 542	19.5	12.56
14	CSH 22 SS	19.0	12.14
15	RSSH 18	19.0	11.01
16	RSSH 50	19.0	11.14
17	SSV 84	19.0	11.40
18	CSV 19 SS	19.0	11.77
	Range	19-20	11.01 to 13.47
	Mean	19.17	12.28
	SE ±	0.52	0.15
	CD at 5%	1.56	0.47

The results of the total sugar % of sweet sorghum varieties and hybrids are depicted in Table 2. The total sugar content in the stem juice of sweet sorghum varieties and hybrids varied from 11.01 to 13.47 %, with a mean value of 12.18 percent. The genotypes RSSV 514, RSSV 533, RSSV 535, RSSV 529 recorded the highest total sugar (13.47,13.08,13.05 and 13.01 percent, respectively) while the sweet sorghum hybrid CSH 22SS had the lowest (10.63%)

total sugar percent. Ratnavathi *et al*. (2011) reported total sugar content in sweet sorghum of different cultivars ranged from 11.28 to 14.28 %. Dalvi *et al*. (2011) reported that sweet sorghum varieties had total sugar between 11.5 to 12.7 percent. The reducing sugar contained in the stem of sweet sorghum varieties and hybrids varied from 1.41 to 2.12 percent with a mean value of 1.80 percent (Table 3).

Table 3: Reducing and non-reducing sugar % of sweet sorghum varieties/hybrids grown in *kharif* season

Sl. No.	Varieties/ hybrids	Reducing sugar (%)	Non reducing sugar (%)
1	RSSV 514	1.41	12.06
2	RSSV 515	1.48	11.22
3	RSSV 517	1.96	11.00
4	RSSV 520	2.00	10.94
5	RSSV 522	1.95	10.80
6	RSSV 525	1.92	9.38
7	RSSV 527	1.67	10.61
8	RSSV 529	1.68	11.34
9	RSSV 533	1.79	11.29
10	RSSV 534	2.12	8.90
11	RSSV 535	1.85	11.20
12	RSSV 540	1.77	10.70
13	RSSV 542	2.05	10.51
14	CSH 22 SS	1.90	10.24
15	RSSH 18	1.86	9.16
16	RSSH 50	2.03	9.11
17	SSV 84	1.49	9.91
18	CSV 19 SS	1.51	10.26
	Range	1.41- 2.12	9.11- 12.06
	Mean	1.80	10.48
	SE ±	0.11	0.63
	CD at 5%	0.34	1.80

The sweet sorghum genotypes RSSV 534, RSSV 540, RSSH 50, RSSV 520 recorded the highest reducing sugar (2.12, 1.77, 2.03, and 2 percent, respectively) while genotype RSSV-514 showed the lowest reducing sugar content. Bhoyer and Thakare (2009) recorded that at physiological maturity, the reducing sugar was 1.46 % in NSS- 208 followed by BJ-248 (1.58 %). Chavan *et al*. 2009 reported that reducing sugar ranges from 0.69 to 1.79 % in different sweet sorghum genotypes in *Kharif* season. The non-reducing sugar contained in the stem of sweet sorghum genotypes varied from 9.11 to 12.06%, with a mean value of 10.48 percent. The genotype RSSV-514 showed the highest non-reducing sugar (12.06%) followed by RSSV-529, RSSV-533. However, RSSH 50 recorded the lowest non-reducing sugar content. Almodares *et al*. (2013) reported mean sucrose content from 9 to 10.5 % in different sweet sorghum genotypes.

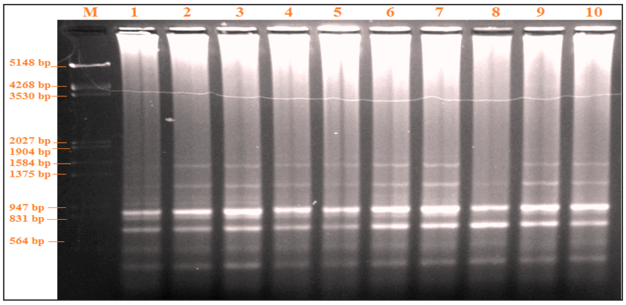
Table 4: Alcohol per cent and ethanol yield of sweet sorghum varieties and hybrids in *kharif* season

Sl. No.	Varieties/ hybrids	Juice yield (L/ha)	Sp. gravity	Alcohol (%)	Ethanol yield (L/ha)
1	RSSV 514	5806	0.98784	9.0	523
2	RSSV 515	7744	0.98806	8.5	658
3	RSSV 517	7006	0.9874	9.0	631
4	RSSV 520	4067	0.98724	9.5	386
5	RSSV 522	9717	0.98756	9.0	875
6	RSSV 525	6278	0.98740	9.0	565
7	RSSV 527	8233	0.98594	10.5	864
8	RSSV 529	6044	0.98596	10.5	635
9	RSSV 533	5444	0.9878	9.0	490
10	RSSV 534	8111	0.98584	10.5	852
11	RSSV 535	4011	0.98686	10	401
12	RSSV 540	8756	0.98760	9.0	788
13	RSSV 542	9028	0.98494	11.5	1038
14	CSH 22 SS	5020	0.98616	10.5	527
15	RSSH 18	7789	0.9850	11.5	896
16	RSSH 50	10661	0.98644	10	1066
17	SSV 84	4861	0.98746	9.5	462
18	CSV 19 SS	5466	0.9865	10	547
	Range	4011- 10661		9- 11.5	386- 1066
	Mean	6891		9.80	678
	SE ±	350		0.43	15.76
	CD at 5%	1054		1.24	45.29

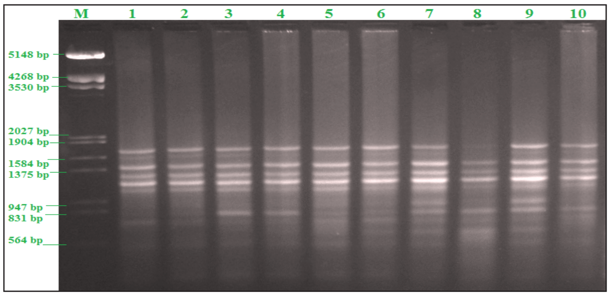
The results on the alcohol % of sweet sorghum varieties and hybrids are presented in Table 4. The alcohol % contained in the stem juice of sweet sorghum varieties and hybrids varied from 9 to 11.5 percent with a mean value of 9.8 percent. Also the ethanol yield varied from 386- 1066 l/ha. The sweet sorghum hybrid RSSH 50 and variety RSSV 542 contained the highest ethanol yield (1066 and 1038 l/ha, respectively), while the genotype RSSV-520 contained the lowest (386 l/ha) ethanol yield. The ethanol percent was varied with sweet sorghum varieties and hybrids. Rutto *et al*. (2013) reported ethanol yield ranged from 750 to 1400 l/ha from different sweet sorghum varieties. Ratnavathi *et al*. (2011) reported that the ethanol yield of different sweet sorghum cultivars ranged from 8 to 11 percent, and residual sugar content was 0.94 to 0.18 percent. Oyier *et al*. (2017) reported 805 –1062 l/ha total ethanol yield in different sweet sorghum genotypes.

Genetic diversity of sweet sorghum varieties and hybrids by ISSR primers

A total of fifteen ISSR were used to amplify the genomic DNA of eight sweet sorghum varieties and two hybrids for genetic diversity analysis. Out of fifteen ISSR primers, seven primers showed polymorphism.



ISSR 810



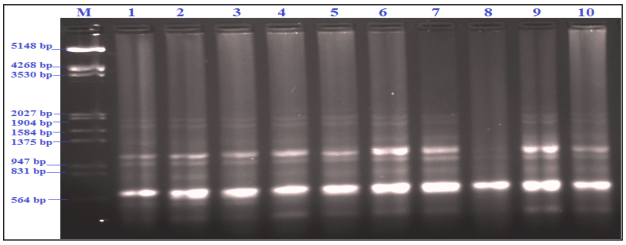
ISSR 823

Plate 1: Amplification pattern of eight sweet sorghum varieties and two hybrids using ISSR 810 and 823 primers for study the genetic diversity

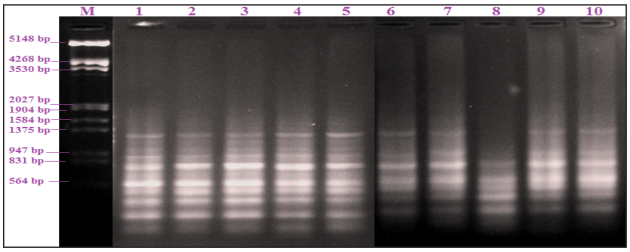
Varieties:

1. RSSV-517
2. RSSV-520
3. RSSV-527
4. RSSV-533
5. RSSV-540
6. RSSV-542
7. CSH 22SS
8. RSSH-18
9. SSV-84
10. CSV 19SS

The total number of bands resolved per primer ranged from a minimum of 8 (ISSR 810- Plate 1) to a maximum of 11 in ISSR 823 (Plate 1). ISSR 841 was the most informative primer, with 8 polymorphic bands and 89 % polymorphism (Plate 2), while ISSR 810 was the least informative, with only 3 polymorphic bands and 38% polymorphism (Table 5). Thus, the results obtained on the polymorphism with different ISSR primers in relation to sweet sorghum varieties and hybrids indicated the usefulness of the ISSR marker in determining genetic diversity in sweet sorghum.



ISSR -841



ISSR 842

Plate 2: Amplification pattern of eight sweet sorghum varieties and two hybrids using ISSR 841 and 842 primers for study the genetic diversity

Varieties

1. RSSV-517
2. RSSV-520
3. RSSV-527
4. RSSV-533
5. RSSV-540
6. RSSV-542
7. CSH 22SS
8. RSSH-18
9. SSV-84
10. CSV 19SS

Table 5: Polymorphism detected by ISSR primers employed in the genetic diversity studies for eight sweet sorghum varieties and two hybrids

Sl. No.	Primer	Sequence	Amplified bands	Polymorphic bands	%of polymorphic bands
1	ISSR-807	5′ -AGAGAGAGAGAGAGAGT	10	8	80
2	ISSR-808	5′ -AGAGAGAGAGAGAGAGC	9	6	67
3	ISSR-810	5′ -GAGAGAGAGAGAGAGAT	8	3	38
4	ISSR-823	5′ -TCTCTCTCTCTCTCTCC	11	8	73
5	ISSR-825	5′ -ACACACACACACACACT	8	5	63
6	ISSR-841	5′ -GAGAGAGAGAGAGAGACTC	9	8	89
7	ISSR-842	5′ -GAGAGAGAGAGAGAGAYG	10	6	60

Table 6: Similarity coefficient of sweet sorghum varieties and hybrids using ISSR primers

	RSSV-517	RSSV-520	RSSV-527	RSSV-533	RSSV-540	RSSV-542	CSH- 22SS	RSSH-18	SSV-84	CSV-19SS
RSSV-517	1.0000									
RSSV-520	0.9090	1.000								
RSSV-527	0.8909	0.9090	1.0000							
RSSV-533	0.8000	0.8181	0.8727	1.0000						
RSSV-540	0.8545	0.8363	0.8909	0.9090	1.0000					
RSSV-542	0.8727	0.9272	0.8363	0.8181	0.8363	1.0000				
CSH- 22SS	0.8363	0.8545	0.8363	0.7818	0.8000	0.9272	1.0000			
RSSH-18	0.7454	0.6545	0.6727	0.6181	0.6727	0.6181	0.6181	1.000		
SSV-84	0.8181	0.8363	0.8181	0.7636	0.8181	0.8727	0.9090	0.636	1.0000	
CSV- 19SS	0.8363	0.8545	0.8363	0.7818	0.8363	0.9272	0.9272	0.654	0.9454	1.00

The similarity coefficient of eight sweet sorghum varieties and two hybrids based on the ISSR analysis varied from maximum similarity and minimum divergence with 0.945 in CSV 19 SS and SSV 84 (Table 6).

Similarity index of sweet sorghum varieties and hybrids showed a minimum similarity of 0.618, confirming that sweet sorghum have a narrow genetic base.

The Dendrogram of eight varieties and two sweet sorghumsweet sorghum hybridwere constructed using UPGMA software. The results indicated that the consensus tree was divided into three significant clusters (Fig. 1). From which one sub-cluster included five sweet sorghum varieties, namely RSSV 517, RSSV 520, RSSV 542, CSV 19 SS, SSV 84, and one hybrid CSH 22 SS, while another sub-cluster included three varieties *viz*., RSSV 527, RSSV 533 and RSSV 540.

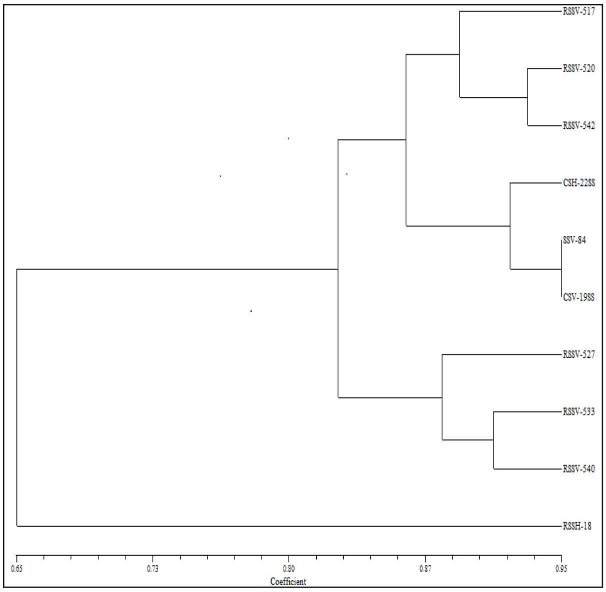


Fig. 1: Dendrogram showing genetic distance of sweet sorghum varieties and hybrids using ISSR primers

Similar genetic diversity studies were taken by Dalvi *et al*. (2012) using RAPD primers in sorghum. Taher *et al*. (2015) worked out genetic diversity in sorghum accessions using ISSR primers. Alhajturki *et al*. (2011) studied genetic variation in ten sorghum varieties using ISSR markers. Genetic diversity of forty Chrysanthemum varieties was evaluated by Mukharjee *et al*. (2013) using ISSR primers.

CONCLUSION

As per the present investigation, during *kharif* season, sweet sorghum genotypes RSSV 542 and RSSV 522 are suitable for further breeding programs because they recorded maximum juice yield and total sugars, which ultimately gives higher ethanol recovery. The diversity analysis of eight sweet sorghum varieties and two hybrids by ISSR primers revealed that RSSV 540 and RSSV 517 are diverse genotypes could be used for juice quality parameter improvement program with an ultimate aim to enhance ethanol production from sweet sorghum.

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