*Applied Biological Research* 19 (3): 315-323; (2017)
DOI: 10.5958/0974-4517.2017.00045.3

# GENETIC DIVERSITY ASSESSMENT AMONG EARLY GENERATION SUGARCANE (*Sacccharum officinarum* L.) CLONES THROUGH MICROSATELLITE MARKERS

Rashmi Bisht, A.S. Jeena, M.R. Meena[1], Deepak Koujalagi, S.P. Singh and K.A. Khan

*Department of Genetics & Plant Breeding, G.B. Pant University of Agriculture and Technology, Pantnagar – 263 145, Uttarakhand (India)*
*[1]Scientist (Plant Breeding), ICAR-SBIRC, Karnal - 132 001, Haryana (India)*
*e-mail: *dr.asjeena@gmail.com*

(Received 14 March, 2017; accepted 26 October, 2017)

## ABSTRACT

**The present study was aimed to characterize the early generation sugarcane (*Saccharum officinarum*) clones. Thirty-four sugarcane genotypes from different cross combinations representing six bi-parental crosses, twenty-four general crosses, three poly-crosses and one-selfed were planted along with two checks. A total of 20 SSR markers were used to assess the gen etic diversity of which 93.5% were polymorphic. Polymorphic information content was used to determine allele diversity at each locus ranged from 0.141 (SOMS118) to 0.314 (UGSM585*)*. The average expected gene diversity ranged from 0.105 (SOMS120) to 0.396 (UGSM-585). Genetic similarity based by Jaccard's similarity coefficient ranged from 0.419 to 0.96. Dendrogram constructed using UPGMA cluster analysis revealed two distinct groups among *S. officinarum* species clones and two commercial checks and ordered the populations of 36 genotypes into five clusters with varying number of members proved SSR marker to be an effective tool for assessing genetic diversity among sugarcane clones.**

**Key words:** Genetic diversity, simple sequence repeat, polymorphism, saccharum, sugarcane

## INTRODUCTION

Sugarcane (*Saccharum officinarum*) is a perennial monocotyledonous crop cultivated in tropical and subtropical regions of the world. It is primarily cultivated for sugar as it posses high concentrations of sucrose in its internodes but recently it gained attention of many stakeholder due to recent emphasis on ethanol as an alternative sources of future growing energy demands and production of bio-fuels. Considering the current needs of cane industry, it is imperative to breed high sugar producing varieties with desired agronomic traits. However, considerable difficulties have been encountered in the improvement of sugarcane through hybridization due to narrow base of variation available. In sugarcane frequently aneuploid is impeded by its narrow gene pool, complex genome, and poor fertility caused by genetic recombination as well as long breeding selection cycle (Kang *et al.*, 2013). The success of sugarcane breeding program, therefore, lies in the proper choice of rich and genetically diverse parents. The genetically diverse parents may be selected on the basis of diverse geographical distribution of genotypes and information on

agronomic characters or diversity analysis through molecular markers (Melchinger, 1998). "Nobilization" of *Saccharum* spp. was a major breakthrough in sugarcane varietal improvement programs in terms of improved sugar productivity, high disease resistance and high ratooning ability (Singh *et al.*, 2010). Although nobilization was highly successful, due to limits of the gene pool exploited during traditional breeding programs, very limited progress has been achieved in increasing sugar content (Lima *et al.*, 2002; Pan *et al.*, 2004).

Divergence analysis is a powerful tool in quantifying the degree of divergence at genotypic level which is based on phenotypic observations in different crops. It is useful in selecting desirable parents for breeding programme. Exploitation of heterosis and success in obtaining desirable recombinants is dependent on degree of divergence of parents. The use of diverse germplasm as significant factor contributing to high yield has been stressed by many workers (Singh *et al*., 2004; Kang *et al.*, 2013). Evaluation of genetic diversity based on morphological characters is very limited and influenced by the environmental effects (Afghan *et al*., 2005). Although morphological (agronomic) parameters are simple to use but they are time consuming, expensive and inaccurate, similarly physiological characters are also influenced by external environment. Therefore, techniques which measure genetic relationship without any influence of environmental factors and phenotype properties are the need of future breeding programs. Molecular marker analysis offers an efficient measure of genetic relationships on the basis of molecular profiling from coding and non-coding regions of DNA. With the advent of molecular markers, it is now possible to make direct comparison of genetic diversity at DNA level without some of the over-simplifying assumptions associated with calculating genetic diversity based on pedigree history (McIntyre *et al.*, 2001).

The use of molecular markers allow the assessment of genetic diversity at DNA level. Among these molecular marker techniques, DNA based markers which include restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellite or simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) are of significance in crop improvement (Ali *et al.*, 2008). These markers are extensively used to increase the understanding of genetic and taxonomic complexity of various agricultural crops (Singh *et al.*, 2010). The desirable attributes of these markers encouraged its development (Cordeiro *et al.*, 2000) and utilization to achieve agronomical desirable traits in sugarcane (Aitken *et al.*, 2005). Microsatellites have developed as the marker system of choice due to their high reproducibility, abundant genome, hyper variability and co-dominance (Mittal and Dubey, 2009). Due to their hyper variability and efficiency in polymorphism detection, SSR markers have become suitable for diversity analysis, genetic map construction **(**Devey *et al.*, 1996; Paglia *et al.*, 1998), identification of clones (Dayanandan *et al*., 1998), identification of species and hybrids, determination of paternity (Van de Ven and McNicol, 1996) and marker-assisted selection (Weising *et al.*, 1997).

In complex polyploidy of sugarcane, the analysis of data generated by the amplification of SSRs is technically more challenging than for more simple organisms due to banding patterns obtained as a result of multiple copies being present on homeologous chromosomes. This difficulty can be solved to some extent through the use of those markers whose products are easily analyzed and interpreted, while maintaining a sufficient level of polymorphism to permit the discrimination of different genotypes. Due to the abundance of SSR markers available for sugarcane, a key component of their efficient application is the identification of those markers that meet these criteria. Identifying useful SSRs is critical but in sugarcane this can be a lengthy and difficult process due to their abundance and the complexity of sugarcane genome (Smiullah *et al.*, 2013). Less information is available on the genetic diversity within and between *Saccharrum* cultivars which has been based mainly on morphological characteristic. Thus, estimates of genetic similarity based on molecular markers may provide more accurate information to plant breeder. The present investigation was aimed to assess the genetic diversity of sugarcane clones with the help of SSR markers.

## MATERIALS AND METHODS

***Plant material and experimental site***

Thirty four early generation sugarcane clones (*Saccharum* spp. hybrids), obtained from different cross combination representing six bi-parental crosses, twenty-four general crosses (GC), three poly-crosses (PC) and one selfed, used as experimental material in the present study were as under:

| S. No. | Clone number | Parentage | S. No. | Clone number | Parentage |
|---|---|---|---|---|---|
| G1. | PC 2007-08- 5 | CoS 8436 x Co Pant 97222 | G19. | PC 2007-08- 124 | Co Pant 1216 GC |
| G2. | PC 2007-08- 21 | Co 98010 GC | G20. | PC 2007-08- 126 | Co Pant 1216 GC |
| G3. | PC 2007-08- 33 | Bo 91 GC | G21. | PC 2007-08- 128 | Co Pant 1216 GC |
| G4. | PC 2007-08- 44 | CoSe 92423 x CoS 8436 | G22. | PC 2007-08- 253 | Co Pant 1216 self |
| G5. | PC 2007-08- 51 | CoLk 8002 GC | G23. | PC 2007-08- 159 | CoS 8436 x Co 89003 |
| G6. | PC 2007-08- 68 | CoS 97264 GC | G24. | PC 2007-08- 165 | CoS 8436 x Co 89003 |
| G7. | PC 2007-08- 75 | CoS 97264 GC | G25. | PC 2007-08- 182 | Co 239 GC |
| G8. | PC 2007-08- 78 | CoH 114 GC | G26. | PC 2007-08- 192 | Co Pant 97213 x Co 62198 |
| G9. | PC 2007-08- 87 | CoH 114 GC | G27. | PC 2007-08- 214 | ISH100 x Co86002 |
| G10. | PC 2007-08- 90 | CoH 114 GC | G28. | PC 2007-08- 223 | CoS 8432 GC |
| G11. | PC 2007-08- 92 | CoH 114 GC | G29. | PC 2007-08- 253 | CoJ 77 GC |
| G12. | PC 2007-08- 96 | CoH 114 GC | G30. | PC 2007-08- 269 | Co Pant 99214 GC |
| G13. | PC 2007-08- 100 | CoH 114 GC | G31. | PC 2007-08- 294 | Co Pant 90223 GC |
| G14. | PC 2007-08- 111 | CoS 8436 PC | G32. | PC 2007-08- 297 | CoJ 99192 GC |
| G15. | PC 2007-08- 114 | CoS 8436 PC | G33. | PC 2007-08- 264 | CoLk 8102 GC |
| G16. | PC 2007-08- 115 | CoS 8436 PC | G34. | PC 2007-08- 295 | CoJ 99192 GC |
| G17. | PC 2007-08- 117 | Co Pant 1216 GC | G35. | Co Pant 3220 | Standard variety |
| G18. | PC 2007-08- 120 | Co Pant 1216 GC | G36. | Co Pant 97222 | Standard variety |

The experiment was planted during autumn 2011-2012 along with two popular checks in a randomized block design with two replications. Each experimental plot consisted of four rows, each of 5 m with 75 cm row to row distances. These clones were selected from $C_2$ generation and planted as $C_3$ generation. The investigation was carried out at Sugarcane Breeding Block, Norman E. Borlaug Crop Research Centre, GBPUAT Pantnagar (India). The location of trial was at $29^0$ N latitude,$79.3^0$E longitudes and at an altitude of 243.84 m amsl near the foothills of Himalayas (Shiwalik range) and geographically situated in the humid subtropical climate zone.This region is popularly known as '*Tarai*' due to subtropical humid climate.

***DNA isolation***

The DNA samples from above genotypes along with checks were extracted from the young leaves of sugarcane to estimate the genetic divergence and their distinctiveness. Approximately 2-3 g young plant leaf material of each clone was used to extract and purify genomic DNA. The genomic DNA was extracted for molecular characterization by CTAB method (Doyle and Doyle, 1990). Agarose-based gel electrophoresis was used to check the quality of DNA fragments and quantification was done using UV-visual spectrophotometer (Eppendorf Bio-photometer) at 260 nm.

***Microsatellite marker (SSR) analysis***

A total of twenty microsatellite based markers was used for PCR amplification of sugarcane DNA. Polymerase chain reaction (PCR) is a powerful, extremely sensitive technique based on the enzymatic amplification of DNA fragments that is flanked by oligo-nucleotide primers hybridizing to opposite strands of the target sequence. A reaction mixture of 25 µL was used to amplify genomic DNA in a thermal cycler (Eppendorf, DNA Thermal Cycler 9600). To confirm that the observed bands were amplified genomic DNA and not the primer artifacts, the genomic DNA was omitted from control reaction. A negative control was also run to confirm whether the master/

reaction mixture is correctly prepared or not. The PCR products were electrophoresed at 90 V, in 2% agarose gel for approximately 2 h, using 0.5 × tris-boric acids EDTA (TBE) buffer, along with a DNA molecular size marker. The gels were stained with ethidium bromide and observed on a UV light and photographed using gel documentation unit.

Details of the simple sequence repeats (SSR) primers used to differentiate the sugarcane clones

| S. No | Primer name | Sequence (forward primer) | Sequence (reverse primer) |
|---|---|---|---|
| 1. | UGSM12 | CAGAACTCCATCATTACA | TGGCAGGTTTCGGTTTCC |
| 2. | UGSM73 | GAACCTGGCGATTTATGA | TATGATTGAAAGACGGAA |
| 3. | UGSM96 | TACTTGACCCTTCTTCTT | GCCGATGGACACCTTGAC |
| 4. | SEGM2 | TTGTCCCTTTATGTGTCT | TTGTAGCATAATGGTTGT |
| 5. | SGM6 | TACGGCAAGACAAATCAA | TAGTATGTTTCGGTTCAA |
| 6. | SGM10 | AAGACCTCAACCCAGACA | ATACAAACGAAATGCTAC |
| 7. | UGSM60 | CGACTCCACACTCCACTC | CCGAACACCACCTTCTTG |
| 8. | SEGM291 | ACACCGACCCAATCAACA | CTGTGAGGGTTGTGATTC |
| 9. | SCM4 | CATTGTTCTGTGCCTGCT | CCGTTTCCCTTCCTTCCC |
| 10. | SCM16 | GTGCGAGAGGAACTGTGT | AGCCCTGCCTAACAAGGA |
| 11. | SCM15 | GGAGATGTTTGAGAGGGAA | AGAGTAGCATAAAGGAGGCAG |
| 12. | SCM18 | CATCAGTATCATTTCATCTTGG | CAGTCACAGTCGGGTAGA |
| 13. | SOMS118 | GAGGAAGCCAAGAAGGTG | TAGAGCGAGGAGCGAAGG |
| 14. | SOMS120 | GCATCTATCGGTCTTCTGG | ATCCAATCCTTCATCTTCTTC |
| 15. | UGSM574 | GCTTCCTCGCTCCTCCTC | TACTTCTACCTCGTCTGCTTC |
| 16. | UGSM575 | CTGTTTCCTTCCTTCTCGT | CAATCATAGCCCAGACACC |
| 17. | UGSM585 | GAAGAGGAGGAGAGGAGAAG | TGGGATGGTTGTTGACTG |
| 18. | UGSM665 | GTTACCATCCCATCCCAC | TGTCCCTCGTTCACAGAC |
| 19. | UGSM667 | CTATCCTCTTGTTGGGTCCT | TCCGCACCTCCGTTCACC |
| 20. | UGSM13 | ATCTCCGCCCACCTCTCC | ACCTTGCTTACATCTTCC |

UGSM – Sugarcane unigene-derived microsatellite markers, SCM - cDNA derived microsatellite markers, SGM - sugarcane genomic microsatellite markers; SOMS - sugarcane genomic markers

***Markers data analysis***

Gel photographs were used to score the data manually and independently. Scoring of gels on 0-1 pattern was done for further analysis (Koujalagi *et al.,* 2017). The presence of amplified products was scored as 1 and its absence as '0' for all the genotypes and primer combinations. Only clear resolved bands were used in the analysis. All tactical analyses were done using software NTSYSpc version 2.11 (Rohlf, 2000). The data matrix was entered into NTSYSpc (numerical taxonomy and multivariate analysis system programme) and analyzed using SIMQUAL (similarity for qualitative data) to generate Jaccard's similarity coefficients. These similarity coefficients were used to construct dendrogram using the unweighted pair-group method with arithmetic average (UPGMA) using NTSYSpc program. Polymorphism information content (PIC) was calculated as 1 - p2 - q2, where, p is presence of band frequency and q the absence of band frequency (Mondal *et al.,* 2009). PIC provides estimate of 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 which was estimated using the formula: PIC = 1- 1/n $\sum_{i=1}^{n} Pij$ where $P_{ij}$ is the frequency of $j^{th}$ allele in $i^{th}$ primer.

## RESULTS AND DISCUSSION

Molecular diversity analysis by using sugarcane microsatellite markers is a potential cost-effective technique in allo-polyploidy crop like sugarcane. The study was undertaken to assess the genetic diversity among the 34 early generation sugarcane clones along with two checks varieties. This was

**Table 1: SSR Primers data on DNA profile and polymorphism generated in 36 genotypes of sugarcane**

| Marker ID | Total amplified loci | Mono-morphic loci | Poly-morphic loci | Unique loci | Percent polymorphism | PIC | Gene diversity | Allele size range (bp) |
|---|---|---|---|---|---|---|---|---|
| UGSM60 | 5 | 0 | 5 | 0 | 100 | 0.209 | 0.245 | 180 - >1000 |
| SCM4 | 9 | 2 | 6 | 1* | 66.66 | 0.186 | 0.237 | 100-850 |
| SOMS118 | 2 | 0 | 2 | 0 | 100 | 0.141 | 0.153 | 140-200 |
| UGSM575 | 2 | 0 | 2 | 0 | 100 | 0.258 | 0.326 | 600 - >1000 |
| SGM10 | 4 | 0 | 3 | 1** | 75 | 0.221 | 0.263 | 100-1000 |
| UGSM585 | 3 | 0 | 3 | 0 | 100 | 0.314 | 0.396 | 120-300 |
| UGSM667 | 4 | 0 | 4 | 0 | 100 | 0.279 | 0.339 | 425 - >1000 |
| SGM6 | 1 | 0 | 1 | 0 | 100 | 0.239 | 0.278 | 350 |
| SOMS120 | 1 | 0 | 1 | 0 | 100 | 0.099 | 0.105 | 700 |
| Average | 3.4 | 0.2 | 3 | 0.2 | 93.5 | 0.216 | 0.260 | |

*One unique loci was observed for genotype PC 2007-08- 223 at 450 bp

**One unique loci was observed for the genotype PC 2007-08- 117 at 1000 bp

undertaken to obtain the molecular profiling with the help of 20 SSR markers. The data observed for each marker analysis is presented in Table 1. Out of 20 SSR markers used, 11 SSR markers were found to be mono-morphic and 9 SSR markers were polymorphic. The information obtained from diversity analysis is useful in making the diverse genetic pool and selection of divergent parents in hybridization program mes to maximize heterosis.

***Diversity analysis based on SSR markers***

For determining the genetic relationship among genotypes, the profile of SSR markers were scored on the basis of their band size, either present (1) or absent (0) for each SSR loci. The representative gel image is presented in Fig. 1 for the primer SCM4. Amplification profile generated by each primer was compared with 100 and 50 bp DNA ladder. The totals of 31 loci were amplified from 9 primers. The polymorphism percent ranged from 66.6 to 100% with an average of 93.5% polymorphism. PIC was used to determine allele diversity at each locus. All the nine primers based on their polymorphic information content value and unique band amplification was considered moderately informative primers. PIC value ranged from 0.099 (SOMS-120) to as high as 0.314 (UGSM585) with an average of 0.216 for all the nine primers. The level of polymorphism indicates that distinction between any two varieties is possible with appropriate SSR primer pair. The average expected gene diversity ranged from 0.105 for primer SOMS120 to a maximum of 0.396 for primer UGSM-585 and an average genetic diversity observed was 0.260 for all the primers, in all 36 clones and at all the loci studied. The band profile of PCR amplified products is presented in Fig. 1. Similar results were observed by Singh *et al.* (2010) observed that UGSM and SCM yielded a higher mean number of alleles per locus and PIC values. The level of polymorphism detected by UGSM and SCM markers was comparatively higher than the micro-satellite markers derived from the genomic sequences (SGM). It could be due the use of highly conserved coding sequences as against less conserved genomic sequences in their development.

***Genetic relationship among sugarcane genotypes using SSR markers***

The genetic similarities among the accessions were calculated according to Jaccard's coefficient (Jaccard, 1908) using NTSYSpc software package version 2.11 V (Rohlf, 2000). The data scored from SSR analysis consisted all genotypes of early generation sugarcane using 9 SSR primers, to generate pair wise matrix based on Jaccard's similarity coefficient. The Jaccard's similarity coefficient (JSC) ranged from 0.419 to 0.968. The lowest value of similarity coefficient was observed between PC 2007-08-44 and PC 2007-08-68, PC 2007-08-33 and Co Pant 97222, PC 2007-08-92 and Co Pant 97222 i.e. 0.419, hence these genotypes were genetically more diverse. While highest value for genetic similarity was found between PC 2007-08-90 and PC 2007-08-115,

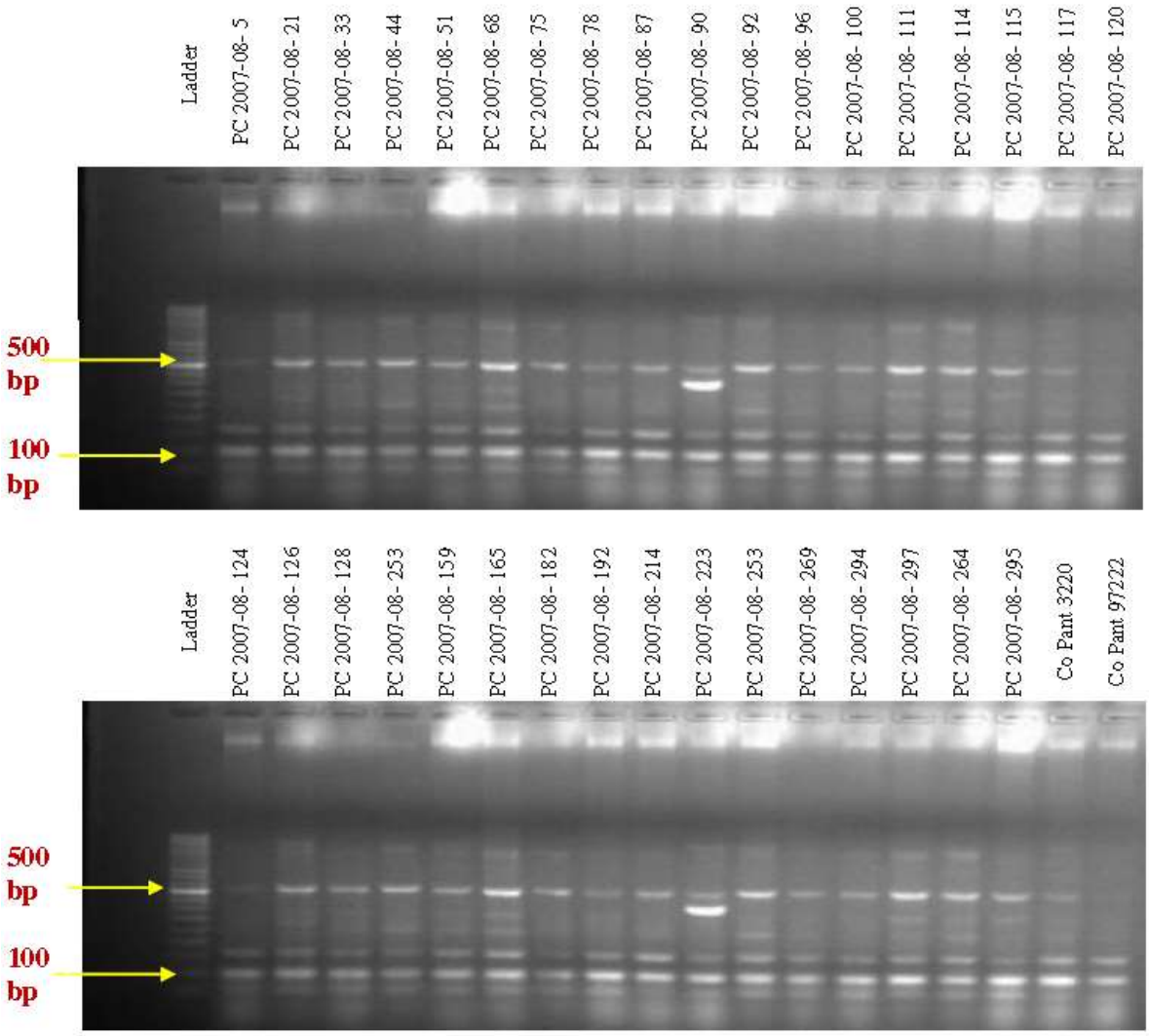


**Fig. 1: Ethidium bromide stained molecular profiling using SSR primer SCM 4 in 36 early generation sugarcane clones.**

PC 2007-08-111and PC 2007-08-297, PC 2007-08-114 and PC 2007-08-297 i.e. 0.968. It revealed that genetic diversity is more important than geographical diversity. On the basis of similarity value, the diverse parents can be identified and such parents can be utilized in hybridization programme to have more heterotic crosses. Therefore, this study will also be helpful in developing diverse gene pool. The similarity values found in this study were similar to those obtained by Filho *et al.* (2010) using SSR markers in sugarcane, indicating that results of this study are consistent. Selvi *et al.* (2003) have revealed a broad range (0.324-0.8335) of pair-wise similarity values when tested on 30 or 40 commercial sugarcane cultivars. The necessity of a high number of the marker for the complete mapping of sugarcane genome is justified by the complex genome, polyploidy structure and relatively narrow genetic base.

***Cluster analysis***

Genetic diversity and relationships among the early generation sugarcane clones were depicted by cluster analysis (Fig. 2). The UPGMA was used to construct dendrogram using JSC based on SSR marker data generated on 36 genotypes. Cluster analysis was performed to divide the experimental material in distinct groups on the basis of similarity values. After that, similarity matrix was calculated and used to construct the dendrogram. The dendrogram from cluster analysis revealed

two distinct groups among *S. officinarum* species clones and two commercial hybrids. Clusters I and cluster II contained 34 and 2 accessions, respectively. Major-cluster II has two genotypes i.e. PC 2007-08-21 and PC 2007-08- 294. The major cluster I comprised of 34 accessions and was subdivided into two sub-clusters *viz*., IA and IB (63% similarity) containing 28 and 6 accessions, respectively. Cluster IA was further subdivided into two clusters *viz*., IA1 and IA2 having 27 and 1 accession, respectively, at the demarcation of 66% similarity. Sub-cluster IA2 consisted of single genotype, PC 2007-08- 96 while sub-cluster IA1 consisted of 2 sub-clusters IA1a and IA1b at the demarcation of 75% similarity, cluster IA1a comprised of 17 genotypes and sub-cluster IB consisted of 6 genotypes. However, the selections from the same cross i.e. CoS 8436 x Co 89003 gave 2 offspring (PC 2007-08-159 and PC 2007-08-165) so fall into different clusters and showed great genetic diversity exist within *S. officinarum* spp. clones. As a result of polyploid nature and heterozygosity of sugarcane genome all the clones contained a large number of markers. Poor correspondence between pedigree and molecular markers in sugarcane has been reported by Hemaprabha *et al.* (2005). Selvi *et al.* (2003) could explain the high level of heterozygosity at the parental level, whereby the progenies differ substantially in phenotype and genotype. Hence the right choice of diverse parents for sugarcane varietal improvement would depend on genetic diversity estimates and pedigree records. These findings are in confirmation with Hemprabha *et al.* (2005). The genetic relationship among the *Saccharum* complex species and commercial cultivars has previously been investigated through RAPD profile (Singh *et al*., 2008). A higher level of molecular genetic variation was detected among sugarcane accessions on the basis of JSCs evaluated by others (Pan *et al*., 2004).

The analysis of variations in SSR fragments provides an effective tool for examining diversity. The results of this study suggest that utility of SSR markers for assessing genetic diversity in sugarcane accession. The diverse germplasm can be used as parents in hybridization programme to improve

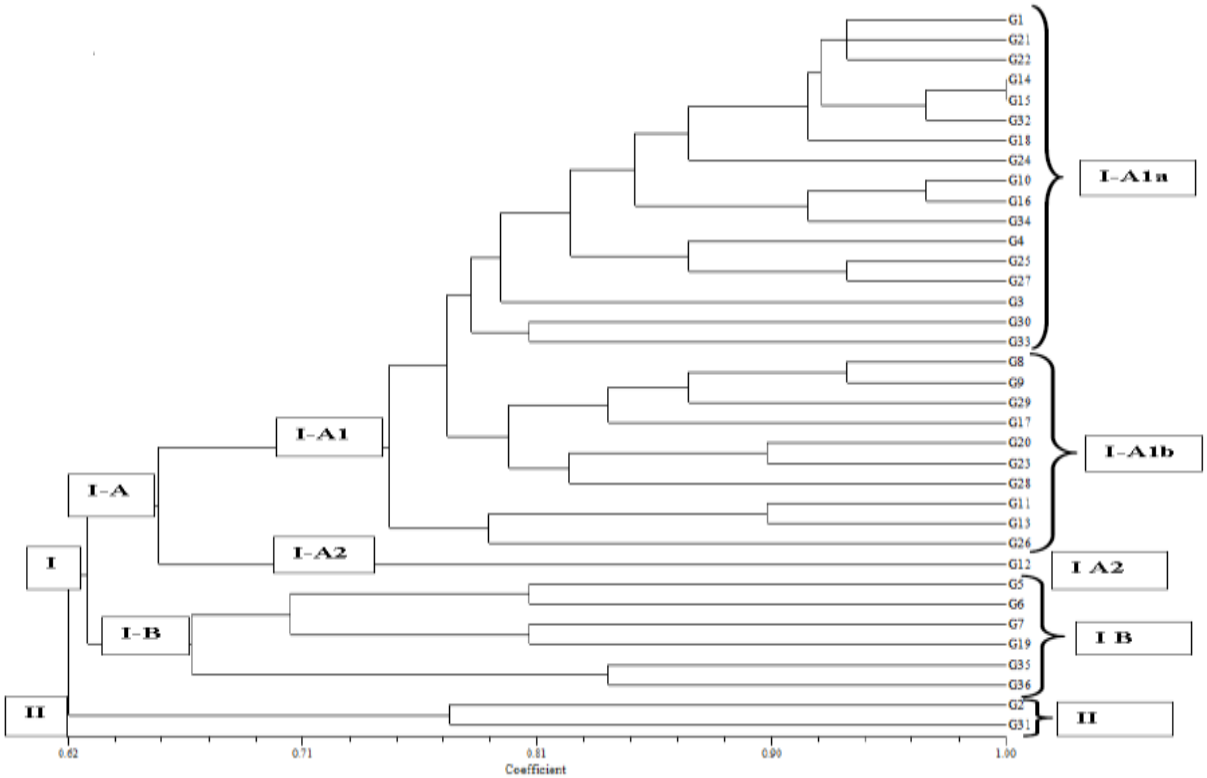


**Fig. 2: Dendrogram depicting the classification of 36 Sugarcane genotypes constructed through UPGMA method and based on SSR markers**

plant breeding strategies which can contribute substantially towards varietal improvement in sugarcane crop, in view of its greater divergence from *Saccharum*. The crosses between more genetically divergent parents provide chance of high transgenic segregates in its offspring's. Hence, it shall be worth to choose parents in hybridization programme on the basis of their genetic diversity based on molecular profiling.

**Acknowledgment:** The authors are thankful to the Head, Genetic & Plant Breeding; Director, Experiment Station and Nodal Officer, Pantnagar Center for Plant Genetic Resources, G.B. Pant Univ. of Agric. & Tech., Pantnagar (India) for providing research facilities during the study.

## REFERENCES

Afghan, S., Haider, M.S., Shah, A.H., Rashid, N., Iqbal, J., Tahir, M. and Akhtar, M. 2005. Detection of genetic diversity among sugarcane genotypes using RAPD markers. *Sugarcane International,* **23**(6): 15-19.

Aitken, K.S., Jackson, P.A. and McIntyre, C.L. 2005. A combination of AFLP and SSR markers provides extensive map coverage and identification of homologous linkage groups in a sugarcane cultivar. *Theory of Applied Genetics*, **110**: 789-801.

Ali, M.A., Seyal, M.T., Awan, S.I., Niaz, S., Ali, S. and Abbas, A. 2008. Hybrid authentication in uplandcotton through RAPD analysis. *Australian Journal of Crop Sciences,* **2**(3): 141-149.

Cordeiro, G.M., Taylor, G.O. and Henry, R.J. 2000. Characterization of microsatellite markers from sugarcane (*Saccharum* sp.), a highly polyploidy species. *Plant Sciences,* **155**: 161-168.

Dayanandan, S., Rajora, O.P. and Bawa, K.S. 1998. Isolation and characterization of microsatellites in trembling aspen (*Populus tremuloides*). *Theory of Applied Genetics,* **96**: 950-956.

Devey, M.E., Bell, J.C., Smith, D.N. and Neale, D.B. 1996. A genetic linkage map for *Pinus radiata* based on RFLP, RAPD, and microsatellite markers. *Theory of Applied Genetics,* **92**: 673-679.

Doyle, J.J. and Doyle, J.L. 1990. Isolation of plant DNA from fresh tissues. *Focus,* **12**: 13-15.

Filho, L.S.C.D., Silva, P.P., Santos, J.M., Barbosa, G.V.S., Ramalho-Neto, C.E., Soares, L., Andrade, J.C.F. and Almeida, C. 2010. Genetic similarity among genotypes of sugarcane estimated by SSR and coefficient of parentage. *Sugar Tech*, **12**(2): 145-149.

Hemprabha, G., Govindaraj, P., Balasundaram, N. and Singh, N.K. 2005. Genetic diversity analysis of Indian sugarcane breeding pool based on sugarcane specific STMS markers. *Sugar Tech,* **7**(2&3): 9-14.

J. Kang, S.A., Noor, M., Khan, F.A. and Saeed, F. 2013. Divergence analysis and association of some economical characters of sugarcane *(Saccharum Officinarum* L.). *Plant Breeding & Genetics.* **1:** 1-6.

Koujalagi, D., Jeena, A.S., Singh, S.P., Chourasia, K.N. and Khan, K.A. 2017. Hybridity tests to identify true hybrids in sugarcane. *Journal of Hill Agriculture*, **8:** 10-16. DOI 10.5958/2230-7338.2017.00017.9

Lima, M.L.A., Garcia, A.A.F., Olivira, K.M., Matsuoka, S., Arizono, H., Suza, C.L. and Suza, A.P. 2002. Analysis of genetic similarity detected by AFLP and coefficient of parentage among genotype of sugarcane *(Saccharum* spp.). *Theory of Applied Genetics,* **104**: 30-38.

McIntyre, L., Aitken, K., Berding, N., Casu, R., Drenth, J., Jackson, P., Jordan, D., Piperidis, G., Reffay, N., Smith, G., Tao, Y. and Whan, V. 2001. Identification of DNA markers linked to agronomic traits in sugarcane in Australia. pp. 560-562. **In**: *International Society of Sugar Cane Technologists*. *Proceedings of the XXIV Congress*. 17-21 September, 2001. Australian Society of Sugar Cane Technologists, Brisbane, Australia.

Melchinger, A.E., Kuntze, L., Gumber, R.K., Lubberstedt, T. and Fuchs, E. 1998. Genetic basis of resistance to sSugarcane mosaic virus in European maize germplasm. *Theory of Applied Genetics*, **96**: 1151-1161.

Mittal, N. and Dubey, A.K. 2009. A new practice of DNA based markers in molecular genetics. *Pharmacognosy Review*, **3**: 235-246.

Mondal, S., Sutar, S.R. and Badigannavar, A.M. 2009. Assessment of genetic diversity in cultivated groundnut (*Arachis hypogaea* L.) with differential responses to rust and late leaf spot using ISSR markers. *Journal of Genetics and Plant Breeding*, **63**: 219-224.

Paglia, G.P., Olivieri, A.M. and Morgante, M. 1998. Towards second-generation STS (sequence-tagged sites) linkage maps in conifers: A genetic map of Norway spruce (*Picea abies* K.). *Molecular and General Genetics*, **258**: 466-478.

Pan, Y.B., Burner, D.M., Legendre, M.P., Grisham. and White, W.H. 2004. An assessment of genetic diversity within a collection of *Saccharum spontaneum* L. with RAPD-PCR. *Genetic Resources and Crop Evolution*, **51**: 895-903.

Rohlf, F.J. 2000. *NTSYSpc Numerical Taxonomy and Multivariate Analysis System*. Exeter Software, New York, USA. (*http://www.exetersoftware.com/downloads/ntsysguide21.pdf*).

Selvi, A., Nair, N.V., Balasundaram, N. and Mohapatra, T. 2003. Evaluation of maize micro-satellite markers for genetic for genetic diversity analysis and fingerprinting in sugarcane. *Genome*,**46**: 394-403.

Singh, P.K., Kumar, S. and Singh, J. 2004. Genetic divergence in *Saccharum* spp. germplasm under sub-tropics. *Indian Sugar*, **53**: 903-906.

Singh, P.K., Srivastava, S., Gupta, P.S., Singh, J., Jain, R. and Lal, P. 2008. Identification of genetically diverse *Saccharum spontaneum* genotypes from India using SSR-SSCP and RAPD markers. pp. 273–277. **In**: *Meeting the Challenging of Sugar Crop and Integrated Industries in Developing Countries*. International Association of Professionals in Sugar and Integrated Technologies, Egypt.

Singh, R.K., Mishra, S.K., Singh, S.P., Mishra, N. and Sharma, M.L. 2010. Evaluation of microsatellite markers for genetic diversity analysis among sugarcane species and commercial hybrids. *Australian Journal of Crop Science*, **4**: 116-125.

Smiullah, Khan, F.A., Afzal, A., Abdullah., Ijaz, A. and Ijaz, U. 2013. Diversity analysis of sugarcane genotypes by microsatellite (SSR) markers. *International Journal for Biotechnology and Molecular Biology Research*, **4**(7): 105-110.

Van, D.,Ven, W.T.G. and Mc Nicol, R.J. 1996. Microsatellites as DNA markers in Sitka spruce. *Theory of Applied Genetics*, **93**: 613-617.

Weising, K., Winter, P., Huttle, P.B. and Kahl, G. 1997. Microsatellite markers for molecular breeding. *Journal of Crop Production*, **1**: 113-143.