*Plant Archives* Vol. 19 No. 2, 2019 pp. 2481-2486 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

# RAPD ANALYSIS OF SUGARCANE CULTIVARS FOR EARLY MATURATION AND YIELD IMPROVEMENT

**Rakesh Tawadare[1]*, Devarajan Thangadurai[1], Rayappa Khandagave[2], Jeyabalan Sangeetha[3], and Ramachandra Pandhari[1]**

[1*]Department of Botany, Karnatak University, Dharwad, Karnataka 580003, India.
[2]S. Nijalingappa Sugar Institute, Belagavi, Karnataka 590009, India.
[3]Department of Environmental Science, Central University of Kerala, Kasaragod, Kerala 671316, India.

## Abstract

Sugarcane (*Saccharum* sp.) is one of the world's most commercial crops as it is the main source of sucrose. Sugarcane breeding programs are concentrated to improve the sucrose content and cane yield. Sucrose content is important for sugar industries and cane yield is profitable concern for farmers. Selection of genetically rich and diverse genotypes is necessary to achieve both the improvements. In the present study, initially physicochemical analysis performed for 24 promising sugarcane cultivars, mainly for sucrose accumulation and cane yield. Among the 24 cultivars, 10 cultivars were selected for further RAPD molecular screening of potential genotypes. PCR analysis was done using 20 RAPD primers. The genetic similarity and UPGMA clustering were performed for all 10 sugarcane cultivars and compared the analysed data of both physicochemical and RAPD analysis. The study showed S1, S8, S11, S18 and S23 as closest ones and S2, S24, S15 as distant ones. This evaluation of genetic relatedness among 10 cultivars revealed primary information for the selection of high sucrose accumulative and cane yielding parental genotypes for further sugarcane breeding.

***Key words***: Cane yield, genetic similarity coefficients, RAPD markers, sucrose accumulation, Sugarcane.

## Introduction

Sugarcane (*Saccharum* sp.) is the main source for sucrose production followed by sugar beet in the world (Zucchi *et al.,* 2002). The basic objective of the sugarcane breeding programs is to enlarge its genetic base by transferring traits of economic value from wild species (Burner and Legendre, 1993). Morphological distinction in *Saccharum* species is very lower due to high levels of genetic polymorphism and gene-environment interactions. Seasonally changing environmental factors affect variations in the phenotypic traits. Hence, morphological characters will not stand as reliable markers for genetic diversity and phylogenetic studies (Harvey and Botha, 1996; Burner *et al.,* 1997). Commercially important Sugarcane varieties are poly-aneuploid hybrid results of unequal composition from *S. officinarum* (80–90%) and *S. spontaneum* (10–20%) as parental genomes with minimum recombination (Jisen *et al.,* 2013). The large genomic size and more complexity have made sugarcane breeding attempts difficult (Cunff *et al.,* 2008). Use of molecular techniques from last two decades has made understanding of complexity in genome easier (Rossi *et al.,* 2003). Genes from *S. officinarum* are responsible for the high cane yield (cane weight) and sucrose production in the new varieties (Sreenivasan *et al.,* 1987). Genetic diversity information obtained by the molecular marker studies has considerable impact on selection of parent materials for crop improvement (Mohammadi and Prasanna, 2003). Molecular markers reveal complete information about genetic diversity, as they are independent of the effects of environmental factors. Use of PCR based DNA markers such as RAPD, SSR, ISSR and AFLP is advantageous than other methods in studying polymorphism and genotypic variability in plants (Rani *et al.,* 1995; Munthali *et al.,* 1996; Devarumath *et al.,* 2002). Development of RAPD markers was an important turning point regarding DNA marker technology based on the use of PCR to amplify

***Author for correspondence :** E-mail : rakeshtavadare@gmail.com

random sequence (Williams *et al.,* 1990). Low technical complexity in employing large number of RAPD markers reduces labour and cost of experiments for the determination of genetic relationships among various cultivars (Leon *et al.,* 2001; Pan *et al.,* 2004). Application of the microsatellites and RAPD fingerprints helps marker-assisted selection of high yielding and potential parents for breeding (Stuber *et al.,* 1999). RAPD markers have been widely used in sugarcane germplasm characterization, inter-specific relationship studies and in phylogenetic analysis for breeding programmes (Sharma *et al.,* 2014; Ahmed and Gardezi, 2017). Taking the above points into consideration, phylogenetic and genetic variability analysis was performed using RAPD markers among the ten special varieties categorized in five distinct groups for identification of high sucrose accumulative and cane yielding cultivars for optimizing hybridization and selection procedures.

## Materials and Methods

### Plant materials, field experiments and data analysis

All the 24 sugarcane varieties table 1 were taken from Sugarcane Germplasm Collection of S. Nijalingappa Sugar Institute (22°50' N; 108°14' E), Zadshapur, Belagavi, Karnataka, India. These varieties were planted in a randomized block design with three replications in single rows of 2.5m length, with 1.2m row-spacing. Physicochemical analysis was performed and observations were recorded. Weight of individual plant was measured for single cane weight (kg). Juice volume per individual plant for single cane volume of juice (l), Average weight of cane yield per hectare for cane yield (metric ton/ acre) and sugar concentration for recovery (%) respectively were measured after sugarcane maturity, *i.e.*, after 12 months (365 days) post-germination.

Data obtained from the physicochemical analysis was tabulated and were compared for the relativeness of sucrose accumulation and cane yield parameters for all 24 cultivars. Based on the physicochemical data with respect to the relatedness between sucrose accumulation and cane yield parameters, five groups of ten sugarcane varieties were categorized for further RAPD analysis.

### Isolation of DNA, RAPD analysis and scoring of DNA bands

The ten varieties selected were used for further RAPD molecular phylogenetic and genetic analysis experiments. The Plant genomic DNA was isolated using kit method. The genomic DNA isolation kit used is GeneJET genomic DNA purification mini kit, Thermo-Scientific (Lot No. 00503105). The DNA quality was confirmed by agarose gel electrophoresis (0.8%) and quantified with the aid of Nano-Drop Spectrophotometer (ND-1000, version 3.1.1, USA). The DNA samples were diluted to 20ng $\mu-l^{-1}$ for polymerase chain reaction (PCR) amplification. The isolated DNA samples were analyzed using gel electrophoresis and further used for PCR.

Twenty decamer RAPD markers were selected based on the previous studies of Singh *et al*., (2010, 2017) (Table 4). PCR reactions were carried out in a 10µl

**Table 1:** Physicochemical analysis of 24 sugarcane varieties used in the present study.

| Sample Code | Variety | Single cane weight (Kg) | Single cane volume of juice (l) | Cane yield (metric ton/ Acre) | Recovery (%) |
|---|---|---|---|---|---|
| S1 | Co 2012-109 | 1.65±0.04 | 1.43±0.05 | 69.48±0.72 | 9.82±0.25 |
| S2 | Co 06027 | 1.72±0.03 | 1.60±0.05 | 69.93±0.88 | 9.85±0.31 |
| S3 | Co 11024 | 1.52±0.03 | 2.08±0.06 | 61.80±0.90 | 9.38±0.24 |
| S4 | Co 10023 | 1.40±0.02 | 1.54±0.03 | 52.85±0.66 | 9.20±0.23 |
| S5 | Co 10024 | 1.78±0.04 | 1.47±0.03 | 69.78±0.65 | 9.31±0.21 |
| S6 | Co 2001-15 | 1.43±0.04 | 1.37±0.05 | 60.21±0.45 | 9.80±0.13 |
| S7 | CoC 671 | 1.51±0.04 | 1.73±0.05 | 54.81±0.98 | 10.07±0.71 |
| S8 | Co SNK 0632 | 1.53±0.03 | 2.18±0.02 | 48.87±0.42 | 9.85±0.32 |
| S9 | Com 0265 | 1.92±0.04 | 1.46±0.04 | 80.85±0.39 | 9.30±0.61 |
| S10 | Co SNK 09268 | 1.55±0.04 | 2.29±0.03 | 63.68±0.87 | 9.01±0.12 |
| S11 | Co 13006 | 1.58±0.05 | 1.64±0.04 | 71.12±0.35 | 9.91±0.43 |
| S12 | Co 10027 | 1.57±0.03 | 1.73±0.07 | 59.27±0.36 | 8.99±0.23 |
| S13 | Co 2012-23 | 1.60±0.03 | 1.15±0.03 | 62.73±0.41 | 9.27±0.11 |
| S14 | Co 2012-24 | 1.59±0.04 | 0.99±0.04 | 64.64±0.78 | 9.79±0.19 |
| S15 | Co 11023 | 1.39±0.04 | 0.89±0.04 | 58.53±0.92 | 9.81±0.43 |
| S16 | Co SNK 7658 | 1.48±0.04 | 1.10±0.05 | 55.87±0.56 | 9.75±0.32 |
| S17 | Co SNK 07337 | 1.57±0.06 | 1.01±0.06 | 54.71±0.62 | 9.63±0.19 |
| S18 | Co SNK 07680 | 1.62±0.03 | 1.01±0.06 | 65.86±0.84 | 9.94±0.12 |
| S19 | Co SNK 09227 | 1.43±0.04 | 0.90±0.06 | 64.37±0.69 | 9.39±0.36 |
| S20 | Co SNK 09293 | 1.73±0.06 | 0.85±0.05 | 77.87±0.66 | 9.91±0.44 |
| S21 | Co SNK 09232 | 1.40±0.03 | 0.81±0.03 | 63.31±0.55 | 9.52±0.29 |
| S22 | CO SNK 0811324 | 1.33±0.03 | 0.80±0.02 | 65.54±0.53 | 9.47±0.61 |
| S23 | Co SNK 83495 | 1.20±0.05 | 0.79±0.04 | 47.52±0.89 | 9.83±0.81 |
| S24 | Co 86032 | 1.88±0.06 | 1.44±0.04 | 79.16±0.67 | 9.97±0.74 |

reaction volume comprising template DNA (2µl), primers (1.5µl), *Taq* buffer 2X (5µl), *Taq* DNA polymerase ($MgCl_2$ Ampliqon, Batch No. 16K1601) and finally making the volume to 10µl using HPLC water (1.5µl). The PCR amplification was performed using Mastercycler gradient (Eppendorf) with following conditions; the cycles of PCR had initial denaturation at 94! for 5 min followed by 44 cycles of 60 s at 94!; annealing condition was set as per standardized annealing temperature table 2 of each RAPD primer 30 s at $37^0$, and 60 s at $72^0$ and finally an extension of 7 min at $72^0$. The amplified products were separated by the horizontal electrophoresis on a 1.5% (w/v) agarose gel using 1X TBE buffer (pH 8.0) and 0.5 µg/ml ethidium bromide. Samples were electrophoresed at 100 V current for 4 h and photographed under UV (Singh *et al.,* 2017). MAGB and 1 kb (0.25-10 kb, APS labs) DNA Ladder was used. The PCR reactions were repeated thrice for each primer for better reproducibility. Only highly reproducible and polymorphic primers were chosen for the data analysis.

Fragments that were clearly readable were considered for data analysis. Each amplified products were considered to be a unit character and populations were scored for their presence (1) or absence (0) of band on gel (Botstein *et al.,* 1980; Anderson *et al.,* 1993) and cluster analysis was performed. Dendrogram was plotted with the aid of Dendro UPGMA online server and similarity matrix was calculated using Jaccard's coefficient.

## Results and Discussion

The study was aimed to evaluate genetic relatedness within 10 selected cultivars of *Saccharum* sp. from five distinct groups. Initially, the physicochemical analysis was carried out for all the 24 selected sugarcane varieties. During the physicochemical analysis, it was noted that ten sugarcane varieties showed fluctuations in sucrose accumulation and cane yielding parameters. Some sugarcane varieties were high in sucrose production but same were medium or low in cane yield and these results were systematically compared with standard values. Depending on the relatedness in the values of two prime important parameters (1) sucrose accumulation and (2) cane yield, ten sugarcane cultivars were identified in five distinct combinations (Table 3). Further, these ten cultivars were subjected to RAPD marker-assisted genetic diversity analysis to construct the phylogenetic tree.

**Table 2:** List 20 RAPD primers used in the present study with annealing temperatures.

| Name ofPrimers | Sequence 5'-3' | Annealing temperature in ˚C |
|---|---|---|
| A1 | AGT CAG CCA C | 52 |
| A6 | GGT CCC TGA C | 52 |
| A10 | GTG ATC GCA G | 53 |
| B 1 | GTT TCG CTC C | 52 |
| B 8 | GTC CAC ACG G | 53 |
| J 4 | GAA TGC GAC C | 53 |
| J 14 | ACC GAT GCT G | 55 |
| J17 | ACC CCC TAT G | 53 |
| J18 | ACA GTG GCC T | 53 |
| J 19 | ACA GTG GCC T | 55 |
| J20 | ACA CGT GGT C | 52 |
| K8 | CTG TCA TGC C | 54 |
| OPA-04 | AATCGGGCTG | 55 |
| OPA-17 | GACCGCTTGT | 53 |
| OPAB-17 | AGGGAACGAG | 51 |
| OPC-08 | TGGACCGCTG | 54 |
| OPC-16 | CACACTCCAG | 53 |
| OPG-05 | CTGAGACGGA | 55 |
| OPG-17 | ACGACCGACA | 53 |
| OPK11 | AATGCCCCAG | 53 |

**Table 3:** List of 10 selected sugarcane cultivars used for further RAPD analysis.

| Group | Cultivar code | Name |
|---|---|---|
| Sucrose medium and Yield medium | S1 | Co 2012-09 |
| | S2 | Co 06027 |
| | S4 | Co 10023 |
| | S12 | Co 10027 |
| | S15 | Co 11023 |
| Sucrose high, Yield medium | S11 | Co 13006 |
| | S18 | Co SNK 07680 |
| Sucrose high, Yield low | S23 | Co SNK 83495 |
| Sucrose and Yield low | S8 | Co SNK 0632 |
| Sucrose and Yield high | S24 | Co 86032 |

Sugarcane is a heterozygous and complex aneu-polyploid species. Hence, sugarcane readily undergoes inbreeding depression upon selfing (Stevenson, 1965). It is very essential to understand genetic variability among *Saccharum* sp. varieties before going for breeding experiments for commercial purpose. Thus, in this study, an effort has made to analyze the genetic diversity of selected varieties using RAPD markers. A total of 20 RAPD primers were used which are specific for *Saccharum* sp. All twenty primer sets showed reproducible bands (Fig. 2). The detailed information about the primers used in this study is tabulated

**Table 4:** Similarity Index based on Jaccard coefficient of RAPD analysis of 10 selected sugarcane cultivars.

| | S1 | S2 | S4 | S8 | S11 | S12 | S15 | S18 | S23 | S24 |
|---|---|---|---|---|---|---|---|---|---|---|
| S1 | 1 | 0.500 | 0.750 | 1.000 | 1.000 | 0.750 | 0.250 | 1.000 | 1.000 | 0.300 |
| S2 | | 1 | 0.400 | 0.500 | 0.500 | 0.400 | 0.000 | 0.500 | 0.500 | 0.300 |
| S4 | | | 1 | 0.750 | 0.750 | 1.000 | 0.500 | 0.750 | 0.750 | 0.400 |
| S8 | | | | 1 | 1.000 | 0.750 | 0.250 | 1.000 | 1.000 | 0.300 |
| S11 | | | | | 1 | 0.750 | 0.250 | 1.000 | 1.000 | 0.300 |
| S12 | | | | | | 1 | 0.500 | 0.750 | 0.750 | 0.400 |
| S15 | | | | | | | 1 | 0.250 | 0.250 | 0.200 |
| S18 | | | | | | | | 1 | 1.000 | 0.300 |
| S23 | | | | | | | | | 1 | 0.300 |
| S24 | | | | | | | | | | 1 |

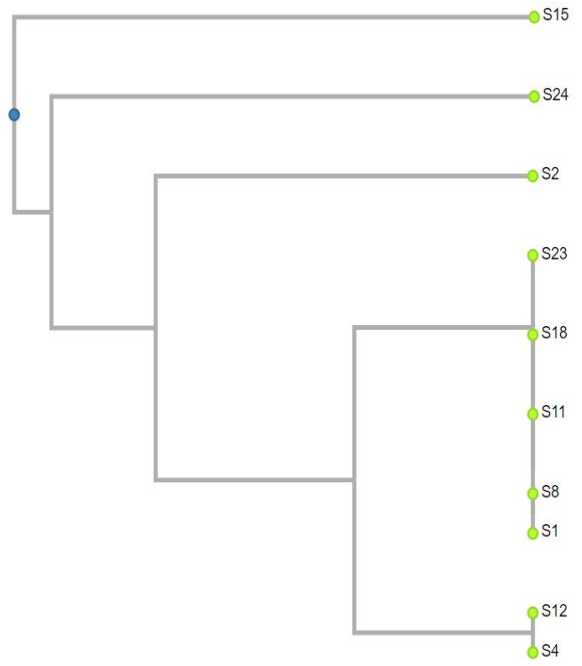


**Fig. 1:** Phylogenetic tree analyses based on Jaccard Coefficient of RAPD analysis of 10 selected sugarcane cultivars (scale: 0.125-0.364).

in table 4. Primer J20 amplified highest polymorphic bands as compared with other selected primers.

Based on the RAPD data generated, cluster analysis was performed using Jaccard Similarity Index and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) to plot a dendrogram representing genetic diversity among 10 varieties (Fig. 1). The genetic similarity indices among cultivars ranged from 0.000 to 1 (Table 4). The cultivar S15 showed least genetic similarity; cultivars S1, S8, S11, S18 and S23 with similarity index 1.000 were 100% similar to each other. Further, cluster analysis categorized the cultivars into four clusters (Fig. 1).

Cluster 1 consists of two groups with a gap of 0.125 where first group consists of S4 and S12 cultivars and second group consist of S1, S8, S11, S18 and S23 cultivars. Cluster 2 consists of S2 cultivar with a distance of 0.139 from S23 cultivar. Cluster 3 consists of S24 cultivar with a distance of 0.073 from S2 cultivar and 0.338 from S23 cultivar. Cluster 4 consists of cultivar S15 with a distance of 0.026 from S24 and 0.364 from S23. All clusters showed a mixture of varieties from all five distinct groups. This suggests the application of large number of RAPD markers for precise differentiation of cultivars.

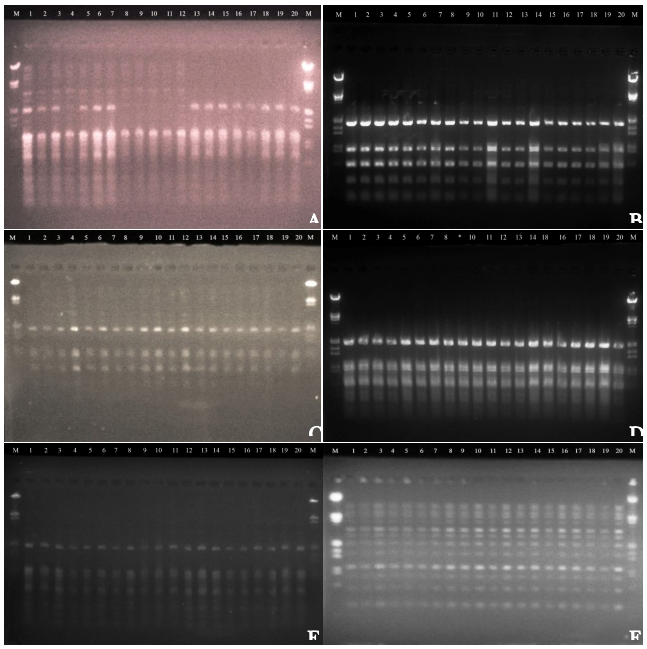

**Fig. 2:** Twenty RAPD marker analyses of sugarcane cultivars (M: Marker, A: S2 – Co 06027, B: S4 – Co 10023, C: S8 – Co SNK 0632, D: S12 – Co 10027, E: S11 – Co 13006, F: S24 – Co 86032).

## Conclusion

In sugarcane cultivation, sucrose accumulation is a very important factor for sugar industries. Sugar production is mainly based on the net sucrose accumulation in the raw material. Sugarcane cane yield is the total cane weight yield per hectare. Farmers are paid as per the cane yield/hectare. Hence, cane yield stands as a profitable parameter for farmers. As all sugarcane cultivars are not equally good in both sugar accumulation and cane yield, breeding programs target the improvement of both parameters. This study is remarkable in selecting good parental genotypes for such breeding plans. Only the results of physicochemical

analysis will not stand as prompt basis for parent selection, as they are under the influence of frequent interactions between gene and environment. Hence, molecular marker-assisted genetic diversity and phylogenetic study can provide confident evidence for the selection of potential parents. This study revealed S24 as highest sucrose accumulative and cane yielding variety and S8 as lowest sucrose and cane yielding variety. Results indicate that S2, S24 and S15 located in different clusters with a minimum distance of 0.073 and 0.026 respectively, can be employed as potential parents for sugarcane breeding programs concentrating sucrose accumulation and cane yield that can benefit both farmers and sugar industries.

## Acknowledgements

First author RT would like to thank S. Nijalingappa Sugar Institute, Belagavi, Karnataka for technical assistance and support.

## References

Afghan, S., M.S. Haider, A.H. Shah, N. Rashid, J. Iqbal, M. Tahir and M. Akhtar (2005). Detection of genetic diversity among sugarcane (*Saccharum* sp.) genotypes using Random Amplified Polymorphic DNA markers. *Sugar Cane International*, **23(6):**17-21.

Ahmed, M.S. and S.D. Gardezi (2017). Molecular characterization of locally adopted sugarcane (*Saccharum officinarum* L.) varieties using microsatellite markers. *Journal of Animal and Plant Sciences*, **27(1):**164-174.

Anderson, A., G. A. Churchill, J.E. Autrique, S.D. Tanksley and M.E. Sorrells (1993). Optimizing parental selection for genetic linkage maps. *Genome*, **36(1):**181-186.

Anderson, N.O. (2006). *Flower breeding and genetics: issues, challenges and opportunities for the 21st century*. Springer Science and Business Media, New York, 586-587.

Botstein, D., R.L. White, M. Skolnick and R.W. Davis (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*, **32(3):** 314-331.

Burner, D.M. and B.L. Legendre (1993). Sugarcane genome amplification for the subtropics: A 20-year effort. *Sugar Cane,* **3:** 5-10.

Burner, D.M., Y.B. Pan and R.D. Webster (1997). Genetic diversity of North American and Old World *Saccharum* assessed by RAPD analysis. *Genetic Resources and Crop Evolution,* **44:** 235-240.

Cunff, L.L., O. Garsmeur, L.M. Raboin, J. Pauquet, H. Telismart, A. Selvi, L. Grivet, R. Philippe, D. Begum, M. Deu, L. Costet, R. Wing, J.C. Glaszmannand and A.D'Hont (2008). Diploid/polyploid syntenic shuttle mapping and haplotype-specific chromosome walking towards a rust resistance gene (*BruI*) in highly polyploid sugarcane (2n approximately 12x approximately 115). *Genetics*, **180:** 649-660.

Devarumath, R.M., R.B. Doule, P.G. Kawar, S.B. Naikebawane and Y.S. Nerker (2007). Field performance and RAPD analysis to evaluate genetic fidelity of tissue culture raised plants vis-à-vis conventional setts derived plants of sugarcane. *Sugar Technology*, **9(1):**17-22.

Devarumath, R.M., S. Nandy, V. Rani, S. Marimuthu, N. Muraleedharan and S.N. Raina (2002). RAPD, ISSR and RFLP fingerprints as useful markers to evaluate genetic integrity of diploid and triploid elite tea clones representing *Camellia sinensis* (China type) and *C. assamica* spp. *assamica* (Assam type). *Plant Cell Reports*, **21:** 166-173.

Harvey, M., B.I. Huckett, and F.C. Botha (1994). Use of the polymerase chain reaction (PCR) and Random Amplification Polymorphic DNAs (RAPDs) for the determination of genetic distances between 21 sugarcane varieties. *Proceedings of 68th Annual Congress of the South African Sugar Technologists' Association*, Durban and Mt Edgecombe, 36-40.

Jisen, Z., Z. Marvellous, W. James and R. Ming (2013). Sugarcane genetics and genomics. In: *Sugarcane: physiology, biochemistry, and functional biology*, John Wiley and Sons, Hoboken, New Jersey, 623-643.

Kawar, P.G., R.M. Devarumath and Y. Nerkar (2009). Use of RAPD markers for assessment of genetic diversity in sugarcane cultivars. *Indian Journal of Biotechnology*, **8:** 67-71.

Krishnamoorthy, T. S., D. Nandhini and V. Padmavathy (2017). A study on sugarcane production in India. *International Journal of Advanced Research in Botany*, **3**(2):13-17, doi.org/10.20431/2455-4316.0302003.

Lal, M., R.K. Singh, S. Srivastava, N. Singh, S.P. Singh and M.L. Sharma (2008). RAPD marker based analysis of micropropagated plantlets of sugarcane for early evaluation of genetic fidelity. *Sugar Technology*, **10(1):** 99-103.

Leon, F., J. Prodriguez, C. Azurdia, D. Amador, J. Queme and M. Melgar (2001). Use of RAPD markers to detect genetic variability among 39 sugarcane varieties. *Proceedings of International Society of Sugar Cane Technologists*, **24:** 634-636.

Md Noor, N.A., M. Murad and E.M. Esah (2018). Physicochemical, antioxidant and microbial properties of fresh black stem sugarcane juice with addition of calamansi juice. *Sains Malaysiana*, **47(9):** 2047-2054, doi.org/10.17576/jsm-2018-4709-12.

Mohammadi, S.A. and B.M. Prasanna (2003). Review and interpretation analysis of genetic diversity in crop plants salient statistical tools. *Crop Science*, **43:** 1235-1248.

Munthali, M.T., H.J. Newbury and B.V. Ford-Lioyd (1996). The detection of somaclonal variants of beet using RAPD. *Plant Cell Reports*, **15:** 474-478.

Pan, Y.B., D.M. Burner and Q. Wei (2001). Developing species-specific DNA markers to assist in sugarcane breeding. *Proceedings of International Society of Sugar Cane Technologists*, **24:** 337-342.

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

Rani, V. and S.N. Raina (2000). Genetic fidelity of organized meristem-derived micropropagated plants: a critical reappraisal. *In Vitro Cellular and Developmental Biology*, **36:** 319-330.

Rani, V., A. Parida and S.N. Raina (1995). Random amplified polymorphic DNA (RAPD) markers for genetic analysis on micropropagated plants of *Populus deltoids* Marsh. *Plant Cell Reports*, **14:** 459-462.

Rossi, M., P.G. Araujo, F. Paulet, O. Garsmeur, V.M. Dias, H. Chen, M.A. Van Sluys and A.D. D'Hont (2003). Genomic distribution and characterization of EST-derived resistance gene analogs (RGAs) in sugarcane. *Molecular Genetics and Genomics*, **269:** 406-419.

Saengprajak, J. and P. Saensouk (2012). Genetic diversity and species identification of cultivar species in subtribe *Cucurminae* (Cucurbitaceae) using RAPD and SCAR markers. *American Journal of Plant Sciences*, **3:**1092-1097.

Sarid Ullah, S.M., M.A. Hossain, M.M. Hossain, S. Barman, M. M.H. Sohag and S.H. Prodhan (2013). Genetic diversity analysis of chewing sugarcane (*Saccharum officinarum* L.) varieties by using RAPD markers. *Journal of Bioscience and Biotechnology*, **2(2):** 145-150.

Sharma, M.S., U. Dobhal, P. Singh, S. Kumar, A.K. Gaur, S.P. Singh, A.S. Jeena, E.P. Koshy and S. Kumar (2014). Assessment of genetic diversity among sugarcane cultivars using novel microsatellite markers. *African Journal of Biotechnology*, **13(13):** 1444-1451.

Sindhu, R., M. Kuttirajan and P. Binod (2014). Physicochemical characterization of alkali pretreated sugarcane tops and optimization of enzymatic saccharification using response surface methodology. *Renewable Energy*, **62:** 362-368.

Singh, P., S.P. Singh, A.K. Tiwari and B.L. Sharma (2017). Genetic diversity of sugarcane hybrid cultivars by RAPD markers. *3Biotech*, **7(3):** 222.

Singh, P., S.P. Singh, A.K. Tiwari and B.L. Sharma (2017). Genetic diversity of sugarcane hybrid cultivars by RAPD markers. *3 Biotech*, **7:** 222, doi 10.1007/s13205-017-0855-x.

Singh, R.K., S.K. Mishra, S.P. Singh, N. Mishra and M.L. Sharma (2010). Evaluation of microsatellite markers for genetic diversity analysis among sugarcane species and commercial hybrids. *Australian Journal of Crop Science,* **4(2):** 116.

Sreenivasan, T.V., B.S. Ahloowalia and D.J. Heinz (1987). *Sugarcane improvement through breeding*. Elsevier Science, Amsterdam, **11:** 211-253.

Stevenson, G.C. (1965). *Genetics and Breeding of Sugar Cane*. London: Logmans Green, 264.

Stuber, C.W., M. Palocco and M.L. Senior (1999). Synergy of empirical breeding, marker assisted selection and genomic to increase crop yield potential. *Crop Science*, **39:**1571-1583.

Upreti, P. and A. Singh (2017). An economic analysis of sugarcane cultivation and its productivity in major sugar producing states of Uttar Pradesh and Maharashtra. *Economic Affairs*, **62(4):**711-718, doi: 10.5958/0976-4666.2017.00087.0

Weerawatanakorn, M., Y. Asikin, M. Takahashi, H. Tamaki, K. Wada, C. Ho and R. Chuekittisak (2016). Physico-chemical properties, wax composition, aroma profiles, and antioxidant activity of granulated non-centrifugal sugars from sugarcane cultivars of Thailand. *Journal of Food Science and Technology*, **53(11):** 4084-4092, doi 10.1007/s13197-016-2415-5.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J. A. Rafalski and S.V. Tingey (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*, **18:** 6531-6535.

Zucchi, M.I., A. Hidto, A.V. Morais, M.H.P. Fungaro and M.L.C. Viera (2002). Genetic instability of sugarcane plants derived from meristem cultures. *Genetics and Molecular Biology*, **25:**191-196.