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Genetic Relationship and Diversity Analysis in Arecanut (*Areca catechu* L.) Genotypes using Molecular Markers

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ABSTRACT

Background: Arecanut (*Areca catechu* L.) is an important plantation crop of India belongs to the family Arecaceae. As it is a highly cross-pollinated crop there is a significant difference among the genotypes. Morphological traits used for determining diversity and relationships among genotypes or variety are prone to environmental influences. Thus, the usefulness of molecular markers has been investigated as a means of characterizing and discriminating difference more precisely. Therefore, the present study was carried out to assess the genetic relationship and diversity among the arecanut genotypes by employing RAPD, ISSR and SSR markers.

Methods: A total of 11 genotypes are being grown in University of Agricultural and Horticultural Sciences (UAHS), Shivamogga, Karnataka, India, which includes both local and exotic collections. These 11 genotypes were evaluated for genetic variations using molecular markers *viz.*, random amplified polymorphic DNA (RAPD), inter-simple sequence repeats (ISSR) and simple sequence repeats (SSR).

Result: All the markers studied showed significant polymorphism among the genotypes. Jaccard's genetic similarity coefficient values of RAPD, ISSR and SSR markers were found in the range of 0.56-0.76, 0.55-0.74 and 0.52-0.91. The higher similarity was observed between the genotypes Mohitnagar and Mangala, as well as Theerthahalli Local and Hirehalli Dwarf. The genotypes Maidan Local and Cameroon were placed in very distinct clusters, showing that they are having very distinct traits. The dendrogram prepared using SSR data separately and combining data of RAPD, ISSR, SSR markers showed similar results. Therefore, employing SSR markers for further studies will be more useful for identifying germplasm for varietal improvement programmes.

Key words: Arecanut, Diversity, ISSR, Molecular markers, RAPD, SSR.

INTRODUCTION

Arecanut is a monocot, belongs to Arecaceae family. It is the most profitable plantation crop grown in the humid tropics of India with higher economic returns per unit area (Raja *et al.*, 2007). The genus *Areca* includes 76 species and *Areca catechu* is the only cultivated species. It is highly cross-pollinated crop. Bavappaa and Pillai (1976) found highly significant differences in respect of number of leaves shed, spadices and female flowers produced, nuts set, number of nuts harvested, weight and size of nuts among 13 cultivars of *A. catechu* from eight countries. So far, arecanut germplasms have been characterized and evaluated utilizing morphological and yield parameters (Ananda *et al.*, 2000; Rajesh, 2007). Few studies have also been done on characterising arecanut using molecular markers (Purushotham *et al.*, 2008 and Manimekalai *et al.*, 2012). Morphological traits used for determining diversity and relationships among plant species are prone to environmental influences. Thus, the usefulness of molecular markers has been investigated as a means of characterizing and discriminating different species more precisely (Benharrat *et al.*2002). DNA markers would help the breeders to detect genetic drift, maintain pure seed stocks and develop strategic breeding programmes. Molecular differences, which can be detected using DNA and protein-based markers, are more authentic and unaffected by environmental factors (Dhanraj *et al.* 2002). Hence, the genetic characterisation of genotypes provides the first step towards more efficient conservation, maintenance and utilisation of existing genetic diversity (Prakash *et al.*, 2002). Markers like random amplified polymorphic DNA(RAPD), simple sequence repeats (SSR) and inter simple sequence repeats (ISSR) proved as excellent tools to estimate genetic diversity and relationships among the plant genotypes. Therefore, the present study was carried out to assess the

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genetic relationship and diversity among the arecanut genotypes by employing RAPD, ISSR and SSR markers.

MATERIALS AND METHODS

The genetic relationship and diversity analysis was conducted during 2019-20 atUniversity of Agricultural and Horticultural Sciences (UAHS), Shivamogga, Karnataka, India.

Plant materials

Spindle leaves of 11 arecanut varieties or genotypes which include Maidan Local (ML), Sarwamangala (Sw.M), Mohitnagar (Mn), Mangala (M), Sirsi Arecanut Selection-1 (SAS-1), Sagara Local (SL), Sreemangala (Sh.M), Sumangala (Su.M), Theerthahalli Local (TL), Hirehalli Dwarf (HD) and Cameron (Cam) were collected for genomic DNA isolation.

DNA isolation

From each genotype, leaf samples were collected from four different palms representing each genotype. Tender and soft textured leaf samples (1 g) were cut into small pieces and macerated into fine powder using liquid nitrogen. DNA was extracted by using the standardized protocol (Rajesh *et al.*, 2007). The DNA purity and intactness were checked on 0.8 per cent agarose gel stained with ethidium bromide following the standard protocol (Sambrook *et al.* 1989). Genomic DNA was quantified using the NANODROP instrument and finally the samples were diluted to a concentration of 50 ng μL^{-1}. After extraction of genomic DNA, equal quantities of DNA from four samples representing, each accession, were pooled together and used for analysis.

RAPD and ISSR analysis

Six RAPD and six ISSR primers (Table 2) were used for analysis of arecanut genotypes. PCR amplification was carried out using 15 µl PCR mixture containing 20 ng DNA, 200 µM each dNTPs, 20 picomoles primers, 1X *Taq* buffer, *Taq polymerase* (0.5 units) and 2.5 mM $MgCl_2$. The thermocycler (VWR Peqlabthermocycler) was programmed as: Initial denaturation at 94°C for 5 minutes followed by 35 cycles of 1 minute denaturation at 94°C, 1 minute annealing at 38°C and 30 seconds primer extension at 72°C followed by a final extension at 72°C for 10 minutes. For ISSR primers, annealing temperature was kept at 42°C for 1 min.

SSR analysis

Eight SSR primers (developed by Hu *et al.*, 2009) were used for analysis of arecanut genotypes. PCR amplification was carried out using 20 µl PCR mixture containing 35ng DNA, 200 µM each dNTPs, 20 picomoles each primer (forward and reverse), 1X Taq buffer [10 mM Tris-HCl (pH 8.3)], 50 mM KCl, *Taq polymerase* (0.3 unit) and 1.5 mM $MgCl_2$. The thermocycler (VWR Peq lab thermocycler) was programmed as follows: Initial denaturation at 94°C for 5 min. followed by 40 cycles for 1 min. denaturation at 94°C, 1 min. at different annealing temperatures (Table 1) and primer extension at 72°C for 2 min. followed by a final extension at 72°C for 5 min.

Data analysis

Amplified bands generated from RAPD, ISSR and SSR PCR amplification were scored based on the presence (1) or absence (0) of bands for each primer and used to calculate genetic similarity matrix using software NTSYS-pc version 2.1. Cluster analysis was performed for molecular data using the "unweighted pair group method using arithmetic means" (UPGMA) algorithm, from which dendrograms depicting similarity among genotypes were drawn and plotted using NTSYS-pc software.

RESULTS AND DISCUSSION

RAPD analysis

The simplicity of laboratory assay for RAPD markers makes them an attractive method for obtaining intraspecific distinctions. This technique is already used for cultivar identification and genetic variability analysis of arecanut by Purushotham *et al.* (2008), Manimekalai *et al.* (2012) and Bharath *et al.* (2015). In the present study RAPD primers were used for distinguishing the arecanut genotypes. Comparatively higher percentage of polymorphic bands were detected indicating RAPD fragments are moderately polymorphic and particularly informative in the estimation of the genetic relationship of arecanut genotypes.

The polymerase chain reaction of arecanut genomic DNA using 6 selected RAPD primers generated a total of 368 amplified bands (Table 2). The highest number of bands (70) was observed with primers OPAH-18 and OPAH-01 separately and the lowest number of bands (39) was observed with primer OPBA-20. The size of amplified fragments ranged between 150 bp-1500 bp. The highest number of polymorphic loci (5) was observed with OPAF-15 and lowest polymorphic locus (1) was observed with OPBA-20. Comparatively, moderate to higher level of polymorphic information content (0.66 to 0.90) value was shown by selected polymorphic primers. The highest PIC value (0.90) was observed for the primer OPAF-15 (Fig 1) whereas, the lowest PIC value (0.66) was observed for OPAH-01. It was observed that RAPD primers showed an average PIC value of >0.5, which confirms that the primers are highly informative. The average number of bands across genotypes was found maximum in primers OPAH-18 and OPAH-01(6.36 each) while minimum in primer OPBA-20 (3.54). The highest genetic similarity coefficient of 0.78 was found between the genotypes Mohitnagar and Mangala. The genotypes Maidan Local and Sagar Local showed the lowest similarity coefficient (0.56). The molecular diversity was not in agreement with most of the morphological diversity as reported in *Colocasia esculenta* by Singh *et al.* (2012). Comparatively high to moderate amplitude of the genetic similarity coefficient established in the present study confirms the occurrence of considerable genetic variability among arecanut genotypes. However, variation was slightly

Table 1: List of SSR primers and their annealing temperatures.

SSR primers (Primer name)	Repeat motif	Primer sequence (5′-3′) Forward primer/Reverse primer	Annealing temperature (°C)
AC01	$(AG)_{12}$	GCGTGATCCACATGTACCTT	50
		TTCGGGGAATACTGAGATGG	
AC06	$(TC)_7G(GT)_4$	AGCCTGGAAGGGTTCTCTTT	50
		TGGGAAACGAGTGAAGAAGG	
AC07	$(TC)_{10}(TG)_3$	CCCATATGTTTGGGAGCAAC	50
		AAACATGACACATGGGGTGA	
AC08	$(TC)_8(TG)_5$	TGAAGACAGAAGACCCAGCA	50
		TGCATCCATGGAGTTGTGTT	
AC23	$(CT)_{17}$	TCACCCATCCTTTTTGAATA	50
		CCGGTACCAAGACGGTGAT	
AC28	$(A)_{11}$.... $(AG)_{10}$	CTTAAGCTGTGAAGGTGCAT	50
		CCAAACCCTCTCTTCTTTTT	
AC29	$(CT)_{14}(GT)_{20}$	GCAATGCAGTCCTTTTGTAT	55
		CTCGTAGTTTGGGTGGATTA	
AC30	$(AC)_{16}(AG)_{38}$	ATTGGCCGATCAGCAAGTAA	55
		TGCAATGCAGTCCTTTTGTA	

Table 2: List of RAPD, ISSR primers, generated bands and their PIC values.

Primer	Range of amplicon size (bp)	Total no. of bands	Average no. of bands across genotype	Total no. of loci	No. of polymorphic loci	PIC value
OPAH-18	150-1200	70	6.36	18	3	0.85
OPAF-15	250-1500	58	5.27	18	5	0.90
OPBA-20	220-1000	39	3.54	12	1	0.89
OPE-13	180-1200	66	6.00	16	4	0.79
OPAH-01	200-1300	70	6.36	13	2	0.66
OPAF-06	280-1500	65	5.90	15	0	0.80
UBC-351	250-1200	38	3.45	8	2	0.70
UBC-321	250-1500	69	6.27	15	2	0.75
UBC-72	200-900	49	4.45	14	2	0.88
UBC-52	120-1200	61	5.54	15	1	0.84
UBC-2	300-1100	52	4.72	12	0	0.81
UBC-84	300-1000	38	3.45	11	1	0.88

higher than that reported for 25 cultivars of mango (range 0.69-0.89) as reported by Rajwana *et al.* (2008). The dendrogram (Fig 2) was constructed from values of similarity coefficients generated from RAPD data. The genotypes were divided into three major genotypic groups at 0.597 similarity coefficient, containing 3 clusters respectively, based on the UPGMA cluster analysis. The genotype Cameron was placed in a distinct cluster while other clusters subdivided into sub-clusters. Cluster 'a' consists of 5 genotypes, where these genotypes separated from each other at 0.60 similarity coefficient forming a two sub clusters a_1 and a_2. Further, a_2 cluster was divided into 2 cluster forming a distinct cluster for Hirehalli Dwarf. Cluster 'b' comprised of two sub clusters b_1 and b_2 at similarity co-efficient of 0.672. The cluster b_1 was divided into sub cluster forming a distinct cluster for Sarwamangala. The genotypes Mohitnagar and Mangala were placed at closer distance.

ISSR analysis

ISSR technique provides a quick, reliable and highly informative system for DNA fingerprinting. ISSR markers are inherited in Mendelian mode and segregated as dominant markers. This technique has been widely used in the studies of cultivar identification, genetic mapping, gene tagging, genetic diversity, evolution and molecular ecology. This technique is already used for cultivar identification and genetic variability analysis of arecanut by Manimekalai *et al.* (2012). In this study 6 ISSR primers were used for the analysis (Table 2).

The polymerase chain reaction of arecanut genomic DNA using 6 selected ISSR primers generated a total of 307 amplified bands (Table 2). The highest number of bands (69) was observed with primers UBC-321 and the lowest number of bands (38) was observed with primers UBC-351 (Fig 3) and UBC-84. The size of amplified fragments ranged

between 120 bp-1500 bp.Comparatively higher level of polymorphic information content (0.70 to 0.88) value was shown by selected polymorphic primers. The highest PIC value (0.88) was observed for primers UBC-72 and UBC-84 whereas, the lowest PIC value (0.70) was observed for UBC-351. It was observed that ISSR DNA primers showed an average PIC value of >0.5 indicating that the primers are highly informative. The highest similarity co-efficient (0.74) was observed between Teerthahalli Local and Hirehalli Dwarf. The genotypes Maidan Local and Cameron showed the lowest similarity coefficient (0.55). Comparatively, high to moderate amplitude of the genetic similarity co-efficient

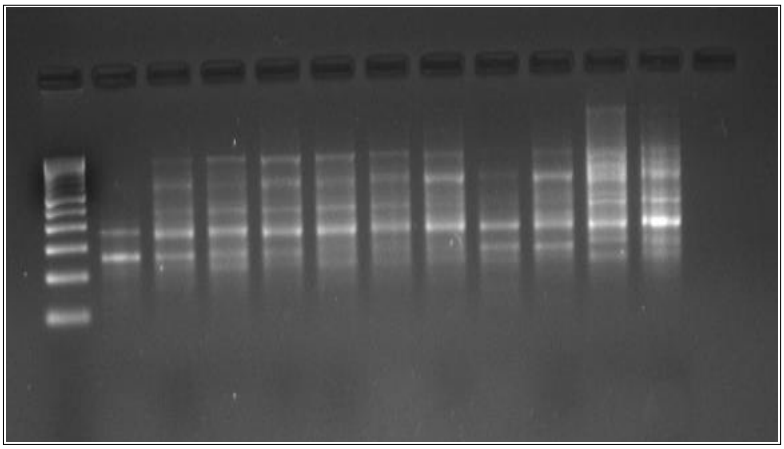

Fig 1: RAPD marker profile of all 11 arecanut samples produced using primer OPAF-15.

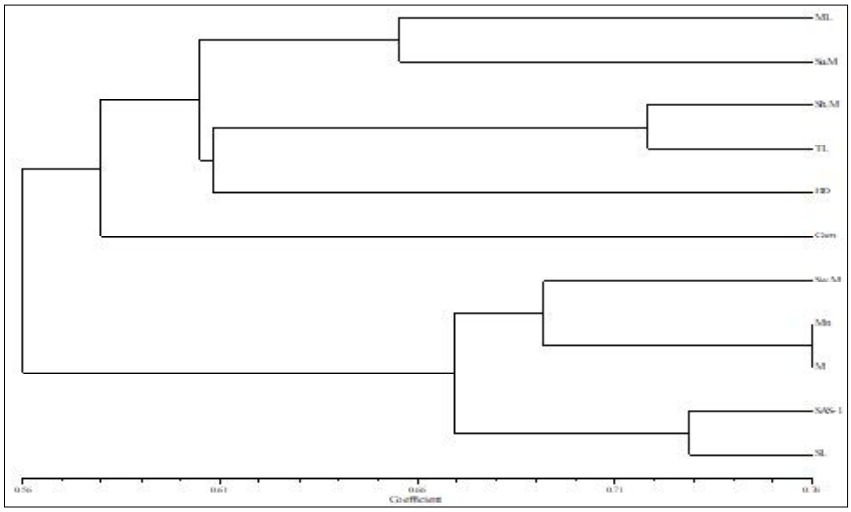

Fig 2: UPGMA dendrogram of arecanut genotypes based on RAPD data.

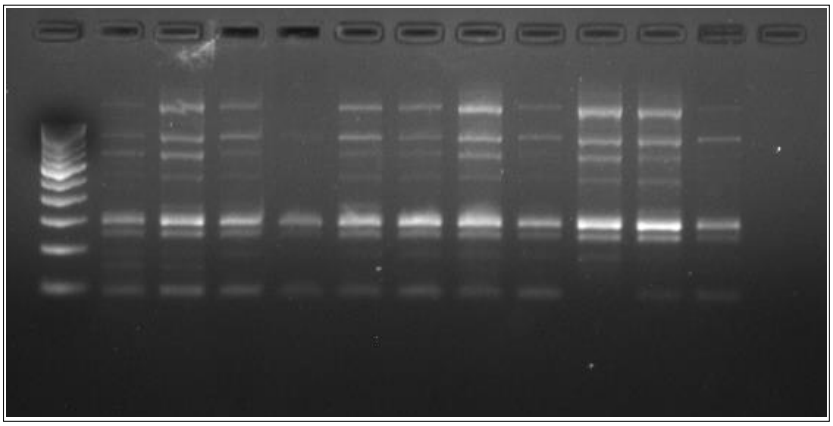

Fig 3: ISSR marker profile of all 11 arecanut samples produced using primer UBC-321.

established in the present study confirms the occurrence of considerable genetic variability among arecanut genotypes studied. The variation was similar to that reported in molecular characterisation of coconut (*Cocos nucifera* L.) varieties (0.657 to 0.775) by Rasam *et al.* (2016) and to that of Manimekalai *et al.* (2012) (0.526-0.855). The dendrogram (Fig 4) was constructed from values of similarity co-efficients generated from ISSR data. The genotypes studied were divided into two major genotypic groups at a 0.587 similarity coefficient, based on the unweighted pair group method using arithmetic average cluster analysis. Cluster 'a' consists of 8 genotypes, where these genotypes separated from each other at 0.59 similarity coefficients forming a two sub clusters a_1 and a_2. Further, a_1 cluster was divided into 2 cluster at 0.62 placing Maidan Local and Sumangala, Sagar Local and Sreemangala. a_2 cluster was divided into 2 groups at 0.663 forming a distinct cluster for Sarnamangala. Cluster 'b' formed a distinct cluster for Cameron.

SSR analysis

Simple sequence repeats have become the genetic markers of choice in many plant species because they are PCR-based, highly reproducible, polymorphic and abundant in plant genomes (Powell *et al.*, 1996). They are generally considered as the marker of choice for DNA fingerprinting purposes in perennial trees due to their high levels of polymorphism, high degree of reliability and reproducibility and codominant mode of inheritance. In plants these markers have been used widely for cultivar identification and genetic mapping (Cipriani *et al.*, 1999 and Guilford *et al.*, 1997). Hu *et al.* (2009) isolated and characterized polymorphic microsatellite loci from *Areca catechu* (Arecaceae), these SSR primer pairs were used for microsatellite analysis in the present study. Simple sequence repeats were already used for genetic variability analysis of arecanut by Kiran Kumar *et al.* (2020), Nagaraja *et al.* (2019; 2016a), Bharath *et al.* (2012).

Comparatively moderate to higher level of polymorphic information content (0.58 to 0.89) value was shown by selected polymorphic primers (Table 3). The highest PIC value (0.89) was observed for primer AC23 (Fig 5) whereas, the lowest PIC value (0.58) was observed for AC08. It was observed the SSR primers showed an average PIC value of >0.5, which indicates that the primers are highly informative. The highest similarity co-efficient (0.91) was observed between Mohitnagar and Mangala. The genotypes Maidan Local and Cameron showed the lowest similarity coefficient (0.52). Comparatively, high to moderate amplitude of the genetic similarity coefficient established in the present study confirms the occurrence of considerable genetic variability among arecanut genotypes. The similarity coefficient was ranged between 0.52 to 0.91. The variation was slightly higher than that reported in molecular characterisation of coconut (*Cocos nucifera* L.) varieties (0.037 to 0.304) by Rasam *et al.* (2016) and lower to that of

Table 3: SSR primers and their PIC values.

SSR primers (Primer name)	Repeat motif	PIC value
AC01	$(AG)_{12}$	0.88
AC06	$(TC)_7G(GT)_4$	0.84
AC07	$(TC)_{10}(TG)_3$	0.88
AC08	$(TC)_8(TG)_5$	0.58
AC23	$(CT)_{17}$	0.89
AC28	$(A)_{11}$.... $(AG)_{10}$	0.75
AC29	$(CT)_{14}(GT)_{20}$	0.85
AC30	$(AC)_{16}(AG)_{38}$	0.84

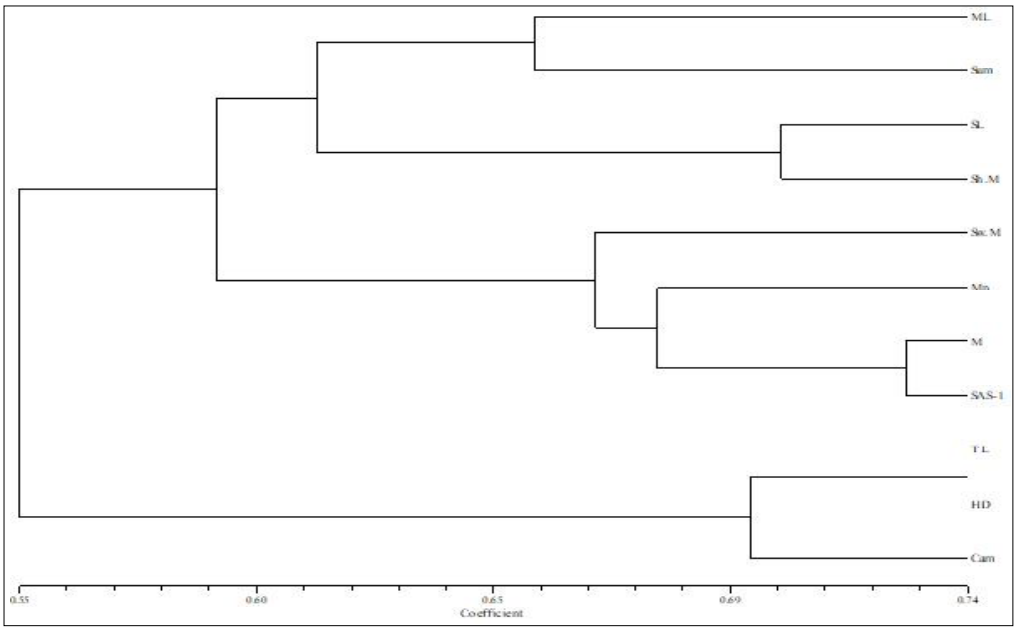

Fig 4: UPGMA dendrogram of arecanut genotypes based on ISSR data.

Liu *et al*. (2008) (0.061-0.896). The dendrogram (Fig 6) was constructed from values of similarity coefficients generated from SSR data. The genotypes were divided into two major genotypic groups at 0.52 similarity coefficient, based on the unweighted pair group method using arithmetic average cluster analysis. Cluster 'a' consists of 5 genotypes, where these genotypes separated from each other at 0.60 similarity coefficient forming a two sub clusters a_1 and a_2. a_1 cluster was comprised of two genotypes Maidan local and Sarnamangala. a_2 cluster was divided into 2 groups at 0.886 forming a distinct cluster for SAS-1. Cluster 'b' was divided into two sub clusters at 0.57 co-efficient. Sub cluster $b_1$comprised of four varieties and b_2 sub cluster consisted of two genotypes.

Combined analysis of RAPD, ISSR and SSR markers data

A dendrogram was constructed using the values of similarity coefficients generated from RAPD, ISSR and SSR data together. As per the dendrogram (Fig 7), the genotypes were divided into two major clusters a and b at 0.55 similarity coefficient, containing 8 and 3 genotypes respectively, based on unweighted pair group method using arithmetic average cluster analysis. At similarity coefficient of 0.595 cluster a was divided placing 8 genotypes in three sub clusters. One sub cluster was comprised of 4 genotypes, other consisted of 3 genotypes and the Maidan Local genotype alone was placed in separate sub cluster. The genotypes Mohitnagar and Mangala were placed very closely at similarity coefficient of 0.76. Cluster b was divided into 2 groups placing Cameron at distinct cluster at similarity coefficient of 0.648. The genotypes Hirehalli Dwarf and Theerthahalli Local were placed in another group.

The molecular markers analysis showed a high degree of variation among the arecanut genotypes studied. The present study revealed that the molecular markers can be

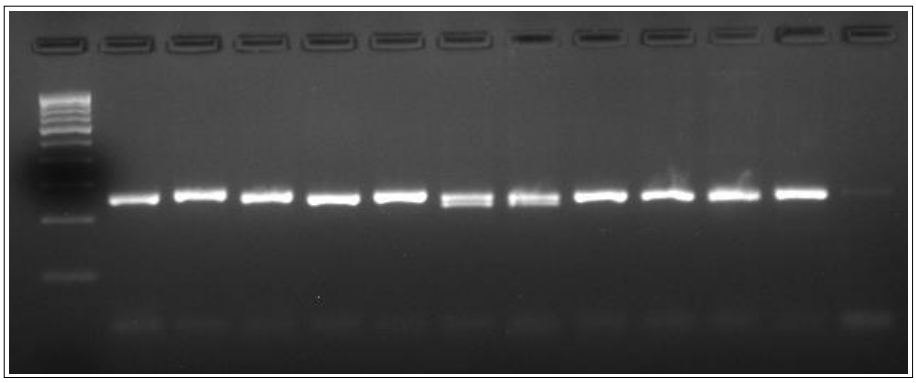

Fig 5: SSR marker profile of all 11 arecanut samples produced using primer AC-23.

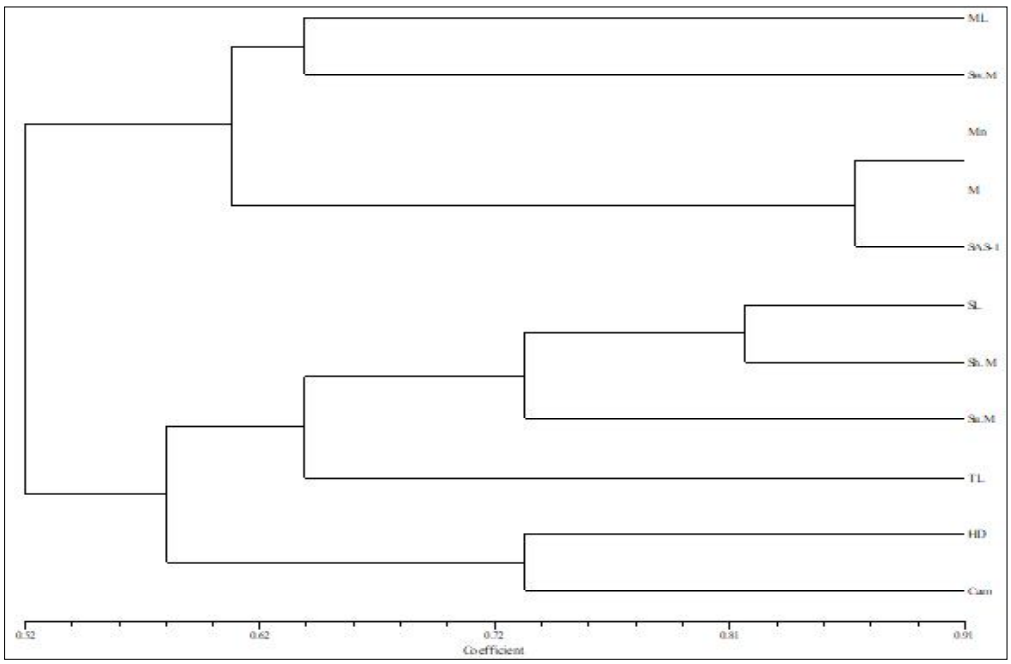

Fig 6: UPGMA dendrogram of arecanut genotypes based on SSR data.

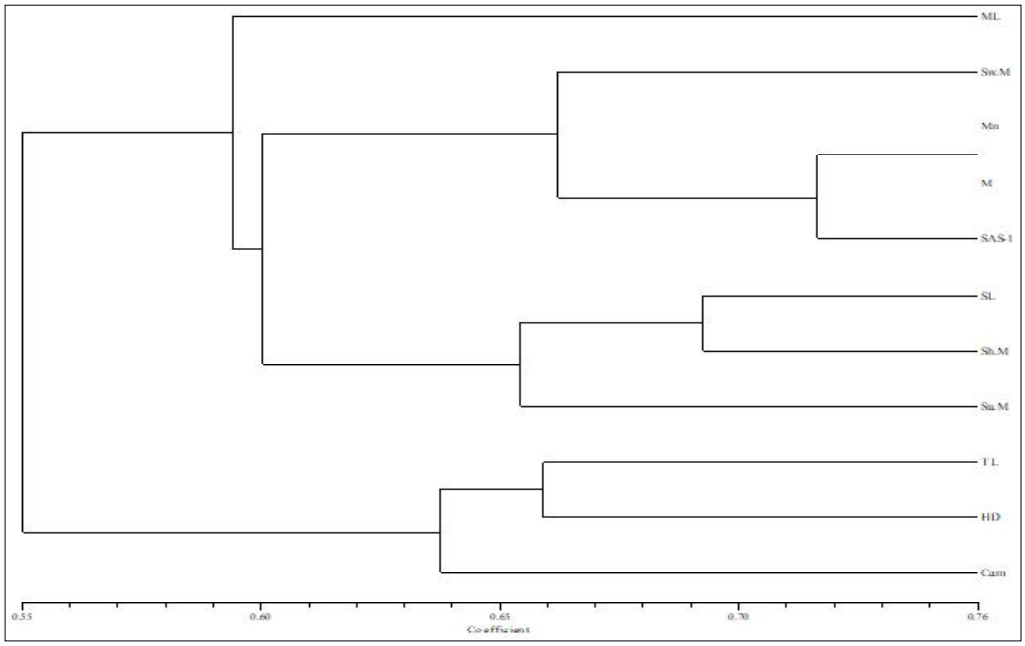

Fig 7: UPGMA dendrogram of arecanut genotypes based on combined analysis of RAPD, ISSR and SSR markers data.

successfully utilized for inferring genetic relationship and diversity in arecanut genotypes. The higher similarity was observed between the genotypes Mohitnagar and Mangala. It was also observed that the genotypes Theerthahalli Local and Hirehalli Dwarf also showed higher similarity. These genotypes were grouped nearer to each other in all the dendrograms of the markers indicaing that they are genetically closer. The genotypes Maidan Local and Cameron were placed in very distinct clusters in all the dendrogram of the markers, showing that they are having very distinct characters. The dendrograms prepared using SSR data and combined data of the all the three markers used showed very similar results. Hence, Using the SSR marker for further studies will be more useful since it is more reliable and repeatable.

Conclusion

Findings of this study indicate that identification of genotypes from various locations mainly based on morphological characteristics may encounter the mismatches and mistakes. This indicates the importance of characterisation of genotypes both at morphological and molecular level for efficient maintenance and exploitation of precious germplasm and to determine groups of high genetic similarity and dissimilarity, which is the key for establishing breeding strategies in genetic improvement programmes of arecanut.

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Conflict of interest: None.

References

Ananda, K.S., Sane, A. and Choudhary, B.S. (2000). Growth and yield performance of arecanut varieties under Malanad condition. J. Plantation Crops. 28: 05-109.

Bavappa, K.V.A. and Pillai, S.S. (1976). Yield and Yield Component Analysis in Different Exotic Cultivars and Species of *Areca*. In: Improvement of Horticulture, Plantation and Medical Plants. [Chadha, K.L. (ed.)], (Vol I) Today and Tommorrow's Printers and publishers, New Delhi. 242-246.

Benharrat, H., Veronesi, C., Theodet, C. and Thalouam, P. (2002). Orobanche species and population discrimination using inter simple sequence repeat (ISSR). Weed Resea. 42: 470-474.

Bharath, B.G., Ananda, K.S., Rajesh, M.K., Nagaraja, N.R. and Rijith, J. (2012). Evaluation of microsatellite markers for genetic diversity analysis among different accessions of arecanut. 22nd Swadeshi Science Congress, CPCRI, Kasaragod, Kerala.

Bharath, B.G., Ananda, K.S., Rijith, J., Nagaraja, N.R., Chandran, K.P., Karun, A. and Rajesh, M.K. (2015). Studies on genetic relationships and diversity in arecanut (*Areca catechu* L.) germplasm utilizing RAPD markers. J. Plantation Crops. 43: 117-125.

Cipriani, G.G., Lot, W.G., Huang, M.T., Marrazzo, E., Peterlunger and Testolin, R. (1999). AC/GT and AG/CT microsatellite repeats in Peach (*Prunuspersica*): Isolation, characterization and cross species amplification in Prunus. Theo. Appl. Gene. 99: 65-75.

Dhanraj, A.L., Rao, E.V.V.B., Swamy, K.R.M., Bhat, M.G., Prasad, T. and Soundur, S.N. (2002). Using RAPDs to assess the diversity in Indian cashew (*Anacardium occidentale* L.) germplasm. J. Hort. Sci. Biotech. 77: 41-47.

Guilford, P., Prakash, S., Zhu, J.M., Rikkerink, E., Gardiner, S., Bassett, H. and Forster, R. (1997). Microsatellites in *Malusdomestica* (apple): Abundance, polymorphism and cultivar identification. Theo. Appl. Gene. 94: 249-254.

Hu, C., Huang, C., Hung, K., Hsu, T. and Chiang, T. (2009). Isolation and characterization of polymorphic microsatellite loci from *Areca catechu* (Arecaceae) using PCR-based isolation of microsatellite arrays. Mol. Eco. Resour. 9: 658-660.

Kiran Kumar, D.G., Lakshmana, D., Nagaraja, N.R., Sadashiv Nadukeri and Ganapathi, M. (2020). Molecular diversity analysis in arecanut (*Areca catechu* L.) germplasm by employing SSR markers. 29th Swadeshi Science Congress-National Conference on Science and Technology for Sustainable Development, 27th to 29th February 2020, ICAR-CPCRI, Kasaragod, Kerala.

Liu, X., Tang, H., Li, D., Wang, Q., Lin, Y. and Zhou, R. (2008). Genetic diversity of coconut cultivars from Hainan Province based on SSR molecular markers. Acta. Hort. 35: 1199-1204.

Manimekalai, R., Deeshma, K.P., Manju, V.P., Soumya, M., Sunaiba Smitha Nair and Ananda, K.S. (2012). Molecular marker-based genetic variability among Yellow Leaf Disease (YLD) resistant and susceptible arecanut (*Areca catechu*. L.) genotypes. Indian J. Hort. 69: 455-461.

Nagaraja, N.R., Ananda, K.S. and Rajesh, M.K. (2019). Assessment of genetic diversity in arecanutgermplasm using SSR markers. In: Book of abstracts.

Nagaraja, N.R., Ananda, K.S., Sabana, A.A. and Rajesh, M.K. (2016a). Simple sequence repeat marker-based molecular characterization and genetic diversity studies in cultivated and wild species of arecanut. Pp.47. PLACROSYM 22, 22nd Biennial Symposium on Plantation Crops, 15th-17th December 2016, ICAR-Central Plantation Crops Research Institute, Kasaragod, India.

Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingley, S. and Rafalski, A. (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol. Bree. 2: 225-238.

Prakash, D.P., Narayanaswamy, P. and Sondur, S.N. (2002). Analysis of molecular diversity in guava using RAPD marker. J. Hort. Sci. Biotech. 77: 287-293.

Purushotham, B., Narayanaswamy, P., Simon, L., Shyamalamma, S., Hegde, M. and Jaypalgowdu, B. (2008). Genetic relationship between cultivars of arecanut (*Areca catechu* L.) determined by RAPD. AsianAustralian. J. Plt. Sci. Biotech. 2(1): 31-35.

Raja, K.B., Hazarey, V.K., Peters, T.J. and Warnakulasuriya, S. (2007). Effect of arecanut on salivary copper concentration in chronic chewers. Bio Metals. 20(2007) 43-47.

Rajesh, B. (2007). Genetic variability for morphological, biochemical and yield traits in Arecanut (*Areca catechu* L.) accessions. PhD dissertation, University of Mangalore, Karnataka, India. 202.

Rajesh, M.K., Bharathi, M. and Nagarajan, P. (2007). Optimization of DNA isolation and RAPD technique in arecanut (*Areca catechu* L.). Agrotropica. 19: 31-34.

Rajwana, I.A., Tabbasam, N., Malik, A.U. and Malik, A.S. (2008). Assessment of genetic diversity among mango (*Mangifera indica* L.) trees using RAPD markers. Sci. Horti. 117: 297-301.

Rasam, D.V., Gokhale, N.B., Sawardekar, S.V. and Patil, D.M. (2016). Molecular characterisation of coconut (*Cocos nucifera* L.) varieties using ISSR and SSR markers. J. Horti. Sci. Biotech. 77: 287-293.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual. Cold Spring Harbour Laboratory Press, USA.

Singh, S., Singh, D.R., Faseela, F., Kumar, N., Damodaran, V. and Srivastava, R.C. (2012). Diversity of 21 taro [*Colocasia esculenta* (L.) Schott] trees of Andaman Islands. Genetic Resour. Crop Evo. 59: 821-829.