Molecular diversity among South East Asian coconut (*Coços nucifera* L.) germplasm accessions based on ISSR markers

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Abstract

Central Plantation Crops Research Institute at Kasaragod maintains largest collection of coconut germplasm accessions representing different geographical regions. So far, morphological and isozyme markers were employed for germplasm characterization. To overcome the difficulties associated with the morphological markers, DNA based molecular markers are presently employed for germplasm characterization. In the present study, Inter Simple Sequence Repeat (ISSR) markers are utilized to estimate the genetic diversity among South East Asian germplasm accessions. Nineteen ISSR primers were used to amplify 8 germplasm accessions (4 palms per accession referred as population). The PCR products were electrophoresed in 1.80 % agarose gels. Binary data were analyzed using the software POPGENE ver. 1.32. Dendrogram was constructed based on Nei's unbiased measure of genetic distance. Nineteen ISSR primers detected a total of 85 polymorphic markers across 32 individuals belonging to eight populations. The average genetic distance among the eight populations ranged from 0.7908 to 0.9327 with a mean of 0.8540. Of the 28 pair wise comparisons the accessions Laguna Tall and Kongthienyong Tall (KTYT) showed the highest genetic identity (0.9327). The lowest identity (0.7908) was between San Roman Tall (SNRT) and Philippines Dalig Tall (PDLT). The Shannon's index ranged from 0.1615 to 0.299. Diversity parameters viz. number of effective alleles, gene diversity, number of polymorphic markers were calculated for each population. The dendrogram constructed based on the genetic distance revealed clustering of Kongthienyong Tall (KTYT) and Laguna Tall (LAGT) while Philippines Dalig Tall (PDLT) was positioned separately. In the genetic improvement programmes the diverse accessions can be used in hybridization for increased heterosis.

Key words : coconut, Inter Simple Sequence Repeat, ISSR, genetic diversity

Introduction

Cocos nucifera (*2n = 2 x* = 32) belongs to the monocotyledonous family Arecaceae (Palmaceae). Coconut oil, desiccated coconut, tender nut play an important role for the rural communities and economies of many developing countries. Coconut has a pantropic distribution mainly in coastal regions at 20 Ú either side of the equator. Grown in 11.6 million hectares in 86 countries of tropics.

International Coconut Gene bank for South Asia (ICG-SA) was established at Central Plantation Crops Research Institute (CPCRI), Kidu, India, where a large collection of coconut germplasm accessions are maintained. The coconut germplasm bank at Kidu contains more than 300 accessions representing eco-geographical regions and genetic diversity of coconut. And further collecting of coconut germplasm accessions is underway.

The estimation of genetic diversity between different genotypes is the first and foremost process in any plant breeding programme. For a plant breeder, reliable knowledge of the genetic diversity of his breeding material is important in order to select parents for a new breeding cycle. Genetic diversity is desirable for long term crop improvement and reduction to vulnerability to important crop pest and pathogens (Liu *et al.*, 2000). The assessment of genetic diversity or genetic variation that may exist among a set or sets of germplasm reveals genetic and evolutionary relationships.

Inter simple sequence repeats (ISSR) technique is a PCR based method, which involves amplification of DNA segment present at an amplifiable distance inbetween two identical microsatellite repeat regions oriented in opposite direction. The technique uses microsatellite, usually 16-25 bp long as primers in a single primer PCR reaction targeting multiple genomic loci to amplify mainly the inter-SSR sequences of different sizes. ISSR PCR is a technique which overcomes the problems like low reproducibility of RAPD, high cost of AFLP, the need to know the flanking sequences to develop species specific primers for SSR polymorphism (Goodwin *et al.*, 1997). ISSR amplification is a relatively recent technique which can differentiate closely related genotypes and helps to estimate genetic diversity both at inter and intra species level. ISSR have high reproducibility possibly due to the use of longer primers (16 - 25 mers) as compared to RAPD primers (10 mers) which permits the subsequent use of annealing temperature (45 - 60 °C) leading to higher stringency.

The coconut germplasm held at CPCRI have been characterized based on morphological (Kumaran *et al.*, 2000), isozyme (Geethalakshmi, 2003) and RAPD markers. In the present paper, inter simple sequence repeat (ISSR) markers were used to assess the molecular diversity among South East Asian coconut germplasm accessions. This is the first report of use of ISSR markers in genetic diversity analysis in coconut.

Materials and methods

Leaf materials from germplasm accessions conserved ex situ were used for the analysis. DNA was extracted from sprouting leaflets (pale

yellow color) from accessions of Kong Thienyong Tall (KTYT), Straight Settlement Green Tall (SSGT), Straight Settlement Apricot Tall (SSAT), Philippines Kalambahim Tall (PKBT), Laguna Tall (LAGT), Philippines Palawan Tall (PPWT), Philippines Dalig Tall (PDLT), San Roman Tall (SNRT) using Plant DNA extraction kit (Invitrogen, Nucleon Phyto Pure™)

ISSR Analysis:

Primers targeting simple sequence repeats sequences were obtained from the University of British Columbia, USA.

PCR amplifications were carried out in 10ml reactions containing 30·g of template DNA, 200mM of each dNTPs, 0.45U of Taq DNA polymerase (Bangalore Genei Pvt. Ltd. India) and 0.8mM of primer. The PCR products were separated in a 1.80 per cent agarose gel in 1 x TBE buffer by electrophoresis at 90 volts for 3h in Bio-Rad submarine gel electrophoresis unit. Gels were stained with ethidium bromide and documented using the Alpha Imager™ 1200 Documentation and Analysis system of the Alpha Innotech Corporation, USA, as per the instruction given by the manufacturer.

Data analysis

Only the clear, unambiguous and reproducible bands were considered for scoring. Each band was considered to be a single locus. Data were scored as "1" for the presence and "0" for the absence of a DNA band of each accession. The binary data matrix was entered into the software POPGENE version 1.32 for individual population number of Observed alleles, Effective number of alleles, Gene diversity, number of polymorphic markers, per cent polymorphic markers and Shannon's information index were calculated.

Results and Discussion

Variability among the populations

ISSR primers detected a total of 85 polymorphic markers across 32 individuals. Philippines Dalig Tall (PDLT) had highest number of observed alleles (1.5000), number of effective allels (1.3716), gene diversity (0.2060), number of polymorphic marker (60) and percent polymorphic markers (50.00) among the accessions. Lowest values were recorded by Philippines Palawan Tall. (Table 1)

Shannon's Information index

Shannon's index provided information regarding within accession diversity. The Shannon's index for individual accession is given in Table 2. The accession, Philippines Dalig Tall (PDLT) had the highest index (0.2990). While, Philippines Palawan Tall (PPWT) had the lowest index (0.1615) followed by Strait Settlement Apricot Tall (SSAT) and San Roman Tall (SNRT). The mean Shannon's index among the accessions was 0.226.

Table 2. Shannon's index based on ISSR markers for the coconut populations

S.No.	Population	Shannon's index
1	KTYT	0.2673
2	SSGT	0.2439
3	SSAT	0.1760
4	PKBT	0.2342
5	LAGT	0.2350
6	PPWT	0.1615
7	PDLT	0.2990
8	SNRT	0.1890
	Mean	0.2260

Genetic identity / genetic distance and clustering

The average genetic distance/identity among populations within six groups were calculated using the software POPGENE ver. 1.32. Dendrogram was also constructed based on Nei's unbiased measure of genetic distance. The average genetic distance among the eight populations is presented in table 3. The pair wise genetic identity among populations ranged from 0.7908 to 0.9327 with a mean of 0.8540. Of the 28 pair wise comparisons Laguna Tall (LAGT) and Kong Thienyong Tall (KTYT) showed the highest genetic identity (0.9327). The lowest identity (0.7908) was between San Roman Tall (SNRT) and Philippines Dalig Tall (PDLT). The dendrogram constructed based on the genetic distance revealed clustering of Kong Thienyong Tall (KTYT) and Laguna Tall (LAGT) while Philippines Dalig Tall (PDLT) was positioned separately. The probable centre of origin for coconut is Far East where rich diversity exists among the coconut accessions. Earlier reports suggest the presence of high diversity in South East Asian accessions (Lebrun *et al.*, 1998 ;Teulat *et al.*, 2000). Among the eight populations belonging to South East Asian accessions studied here, the Philippines Dalig Tall (PDLT)

Table 1. Details of variability parameters generated among coconut populations based on ISSR markers

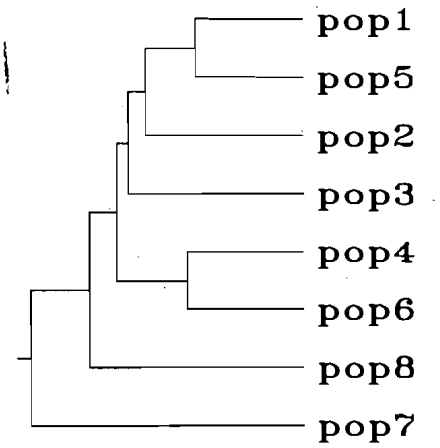
Population	Observed alleles (No.)	Effective allels (No.)	Gene diversity	Polymorphic markers (No.)	Polymorphic markers (%)
KTYT	1.4333	1.3483	0.1865	52	43.33
SSGT	1.4167	1.2996	0.1672	50	41.67
SSAT	1.3250	1.2057	0.1180	39	32.50
PKBT	1.3917	1.2975	0.1619	47	39.17
LAGT	1.3833	1.2987	0.1632	46	38.33
PPWT	1.2750	1.2007	0.1110	33	27.50
PDLT	1.5000	1.3716	0.2060	60	50.00
SNRT	1.3250	1.2317	0.1294	39	32.50

Table 3. Genetic identity and genetic distance among coconut populations

	KTYT	SSGT	SSAT	PKBT	LAGT	PPWT	PDLT	SNRT
KTYT	****	0.8760	0.8901	0.8908	0.9327	0.8662	0.8667	0.8963
SSGT	0.1324	****	0.8757	0.8651	0.9303	0.8719	0.8377	0.8758
SSAT	0.1164	0.1328	****	0.8874	0.9113	0.8637	0.8142	0.8399
PKBT	0.1156	0.1449	0.1195	****	0.9154	0.9270	0.8417	0.8447
LAGT	0.0697	0.0722	0.0929	0.0884	****	0.9280	0.8771	0.8813
PPWT	0.1436	0.1371	0.1465	0.0758	0.0747	****	0.8386	0.8822
PDLT	0.1431	0.1771	0.2055	0.1723	0.1311	0.1760	****	0.7908
SNRT	0.1095	0.1326	0.1745	0.1688	0.1263	0.1253	0.2347	****

Genetic identity (above diagonal) and genetic distance (below diagonal)

exhibited much diversity interms number of observed alleles, Shannon's index, number of polymorphic markers. In the dendrogram also the Philippines Dalig Tall was separated from other accessions showing its diversity. In future crop coconut improvement programmes the accessions Philippines Dalig Tall (PDLT) and San Roman Tall (SNRT) could be used to increase the heterosis.



pop1 = KTYT, pop2 = SSGT, pop3 = SSAT, pop4 = PKBT, pop5 = LAGT, pop6 = PPWT, pop7 = PDLT, pop8 = SNRT

Figure 1. Dendrogram of South East Asian coconut populations based on Nie's genetic distance

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