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Genetic Variation of Selected Progeny Lines of Coconut (*Cocos nucifera* L.) Based on Simple Sequence Repeat Markers

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ABSTRACT. *Genetic variation of selected coconut progeny lines was estimated using simple sequence repeat (SSR) markers. A total of 15 progenies derived from selfing and reciprocal crossing of Laccadive Tall (LCT) and Gangobondam Dwarf (GBD) were used. Cluster and principal coordinate analysis were performed using the software NTSYS pc. Ten coconut specific primer pairs detected a total of 42 alleles. Jaccard's similarity coefficient varied from 0.136 to 1.000 with a mean of 0.590. The dendrogram based on UPGMA resulted in two clusters. Cluster I comprised of individuals of LCT and LCT x GBD and cluster II comprised of individuals of GBD and GBD x LCT. The principal coordinate plot exhibited close grouping of individuals of GBD and GBD x LCT than LCT and LCT x GBD. It showed that there were more genetic variation present among LCT and LCT x GBD progenies than among GBD and GBD x LCT.*

INTRODUCTION

Cocos nucifera (*2n=2x=*32) belongs to the family Arecaceae. Its products, namely, coconut oil, desiccated coconut, tender nut play an important role for the rural communities and economies of many developing countries. It has been grown in 86 countries including India, Sri Lanka and many other South Asian countries.

Genetic diversity estimation based on molecular markers overcomes the limitations encountered in the estimations based on morphological diversity. Progeny lines of coconut have also been studied using isozyme markers. (Cardena *et al.*, 1998) used leaf peroxidase (PRX), endopepdidase (END) and Coomassie Blue stained proteins for the analysis of cultivars of coconut West African Tall (WAT), Rennell Tall (RT), Malayan Yellow Dwarf (MYD), Cameroon Red Dwarf (CRD) and in the hybrids PB121 (MYD x WAT) and PB111 (CRD x WAT) Parthasarathy *et al.* (2004) used isozyme markers to study the genetic variation among the hybrids of West Coast Tall (WCT) x Chowghat Orange Dwarf (COD), COD x COD, WCT x WCT and COD x WCA. Various DNA molecular marker techniques such as RFLP (Lebrun *et al.*, 1998), RAPD (Ashburner *et al.*, 1997; Everard, 1999; Upadhyay *et al.*, 2004), AFLP (Perera *et al.*, 1998; Teulat *et al.*, 2000), ISTR (Rohde *et al.*, 1995) and SSR (Perera

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et al., 1999; 2003; Rivera *et al.*, 1999; Meerow *et al.*, 2003; Dasanayake *et al.*, 2003) have been used to analyze the genetic diversity in coconut.

Microsatellites or simple sequence repeats (SSRs) consist of tandemly repeated multiple copies of mono-, di-, tri-, or tetra-nucleotide motifs, which are hypervariable and ubiquitously distributed throughout the eukaryotic genome. The technical efficiency and multiplex potential of SSRs makes them preferable for high throughout mapping, genetic analysis and marker aided selection. SSR markers are co-dominant, multi-allelic and can be readily used to analyze various genome of crops and facilitates the integration of results from independent studies. In addition, the polymorphic nature of many microsatellites is of particular value when analyzing closely related genotypes. Microsatellite DNA markers, which can be directly amplified by PCR, have been developed using the unique sequences that flank microsatellites (Weber and May, 1989).

The objective of the present study is to analyze the genetic diversity of selected coconut progeny lines derived from selfing as well as reciprocal crossing of Laccadive Tall (LCT) and Gangobondam Dwarf (GBD) and to identify genetic relationship among them using SSR markers.

MATERIALS AND METHODS

Fifteen progenies of two years old coconut plants derived from crossing and selfing of (LCT and GBD) were used in the study (Table 1).

DNA extraction

DNA was extracted using Plant DNA extraction kit (Nucleon Phyto Pure, Amersham Bioscience) as per the manufacturer's instructions.

SSR analysis

The list of coconut specific SSR primer pairs and their sequences used in the study is given in Table 2. These primers were synthesized based on the DNA sequence obtained from CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Dévelopement Montpellier, France) by Microsynth GmbH, Switzerland. The dilution of the primers was done according to the manufacturer's (Microsynth GmbH, Switzerland) instructions. Initially, Gradient PCR was used to standardize the annealing temperature for all the primer pairs used in the study. SSR analysis was carried out as described by Perera *et al.* (2000). The PCR products were separated using 3% Agarose (Agarose 1000™, Invitrogen). The ethidium bromide stained gels were documented using the Alpha Imager™ 1200.

Table 1. List of progeny lines used for genetic diversity analysis of selected progeny lines of coconut

Progeny lines	
LCT1	Selfed progenies
LCT2	
LCT3	
LCT4	
LCT x GBD1	Cross derivatives
LCT x GBD2	
LCT x GBD3	
GBD1	Selfed progenies
GBD2	
GBD3	
GBD4	
GBD x LCT1	Cross derivatives
GBD x LCT2	
GBD x LCT3	
GBD x LCT4	
LCT	
GBD	

Table 2. List of Simple Sequence Repeats forward and reverse primers used in the study

S. No.	Locus name	Forward primer (5'-3')	Reverse primer (5'-3')
1	CnCirA3	AATCTAAATCTACGAAAGCA	AATAATGTGAAAAAGCAAAG
2	CnCirA9	AATGTTTGTGTCTTTGTGCGTGTGT	TCCTTATTTTTCTTCCCCTTCCTCA
3	CnCirB6	GAGTGTGTGAGCCAGCAT	ATTGTTCACAGTCCTTCCA
4	CnCirB12	GCTCTTCAGTCTTTCTCAA	CTGTATGCCAATTTTTCTA
5	CnCirC3	AGAAAGCTGAGAGGGAGATT	GTGGGGCATGAAAAGTAAC
6	CnCirC7	ATAGCATATGGTTTTCCT	TGCTCCAGCGTTCATCTA
7	CnCirC12	ATACCACAGGCTAACAT	AACCAGAGACATTTGAA
8	CnCirE2	TCGCTGATGAATGCTTGCT	GGGGCTGAGGGATAAACC
9	CnCirE10	TTGGGTTCCATTTCTTCTCTCATC	GCTCTTTAGGGTTCGCTTTCTTAG
10	CnCirE12	TCACGCAAAAGATAAAACC	ATGGAGATGGAAAGAAAGG

Data analysis

Microsatellite loci were scored individually and the different alleles were recorded for each individual and assigned "1" for the presence and "0" for the absence of an allele. The binary data matrix was entered into the NTSYS pc package (Exeter Software, New York). The data were analysed using Qualitative routine to generate Jaccard's similarity coefficient. Similarity coefficients were used to construct a dendrogram using UPGMA (Unweighted Pair Group Method with arithmetic Average) and the SHAN (Sequential

Hierarchical and nested clustering) routine in the NTSYS program. The principal coordinate analysis was also conducted with NTSYS software.

RESULTS

Ten coconut specific primer pairs detected a total of 42 alleles among the progeny lines (Table 3). All the alleles were polymorphic. The number of alleles per primer varied between two (CnCirE12 and CnCirC7) to seven (CnCirB6 and CnCirE2) with an average of 4.2 alleles per primer pair. SSR profile of the selected progeny lines using the primer pairs CnCirE2 and CnCirB6 are shown in Plates 1 and 2, respectively.

Table 3. Details of Simple Sequence Repeat markers produced across coconut progeny lines

Serial. No.	Locus	Number of alleles recorded
1	CnCirA3	4
2	CnCirA9	3
3	CnCirB6	7
4	CnCirB12	4
5	CnCirC3	5
6	CnCirC7	2
7	CnCirC12	4
8	CnCirE2	7
9	CnCirE10	4
10	CnCirE12	2
	Total	42
	Mean	4.2

Similarity index

Similarity index obtained for each pair wise comparison among progeny lines is given in Table 4. The Jaccard's similarity coefficient based on 42 SSR markers ranged from 0.136 to 1.000 with a mean of 0.590. The lowest pair wise similarity was found among individuals GBD and LCT; GBD x LCT and LCT. The highest pair wise similarity was found between GBD and GBD; GBD x LCT and GBD; GBD x LCT and GBD x LCT. The calculated average similarity coefficient among the individuals of LCT was 0.377; among the individuals of LCT x GBD was 0.272; among the individuals of GBD was 0.970; among individuals of GBD x LCT was 1.000.

Clusters based on dendrogram

The dendrogram based on Jaccard's coefficient and UPGMA clustering resulted two clusters (Figure 1). Cluster I consisted of individuals of LCT and LCT x GBD. Cluster II consisted of GBD and GBD x LCT. Dendrogram clearly distinguished the individuals of LCT, LCT x GBD with, GBD and GBD x LCT. The individuals of GBD x LCT and GBD grouped more closely than the individuals of LCT and LCT x GBD.

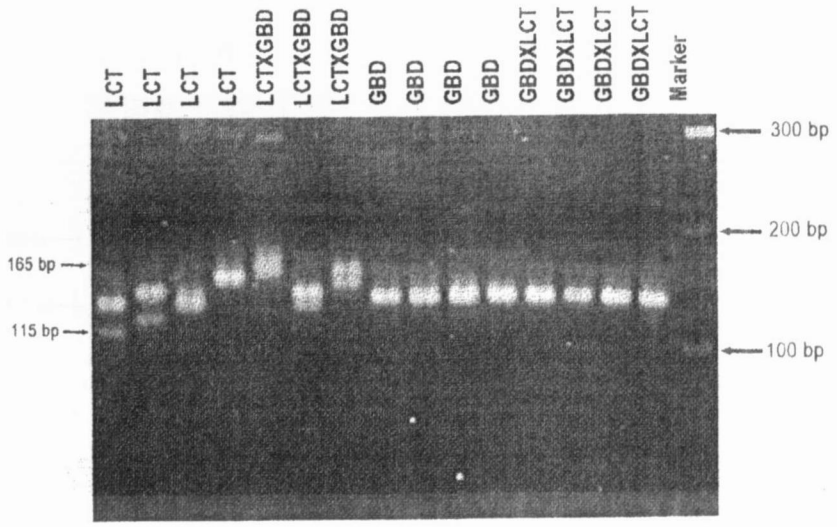

Plate 1. Simple Sequence Repeat marker profile of coconut progeny lines using the primer pair CnCirE2.

Note: Each lane is labeled with the name of the sample at the top of the gel. Alleles size is indicated using arrows at the left hand side of the gel. Standard 100bp ladder marker size is indicated using arrows at the right hand side.

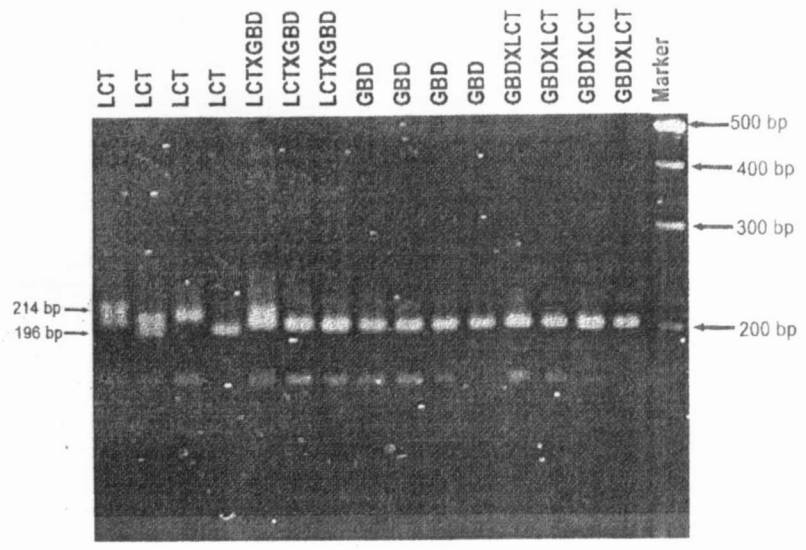

Plate 2. Simple Sequence Repeat marker profile of coconut progeny lines using the primer pair CnCirB6.

Note: Each lane is labeled with the name of the sample at the top of the gel. Alleles size is indicated using arrows at the left hand side of the gel. Standard 100bp ladder marker size is indicated using arrows at the right hand side.

Table 4. Similarity matrix among coconut progeny lines based on Simple Sequence Repeat markers

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1.000													
2	0.250	1.000												
3	0.227	0.368	1.000											
4	0.227	0.625	0.400	1.000										
5	0.238	0.316	0.350	0.286	1.000									
6	0.200	0.533	0.316	0.563	0.263	1.000								
7	0.182	0.250	0.350	0.286	0.300	0.263	1.000							
8	0.150	0.222	0.143	0.143	0.150	0.167	0.150	1.0						
9	0.143	0.211	0.190	0.136	0.143	0.158	0.143	0.909	1.0					
10	0.143	0.211	0.190	0.136	0.143	0.158	0.143	0.909	1.0	1.0				
11	0.143	0.211	0.190	0.136	0.143	0.158	0.143	0.909	1.0	1.0	1.0			
12	0.143	0.211	0.190	0.136	0.143	0.158	0.143	0.909	1.0	1.0	1.0	1.0		
13	0.143	0.211	0.190	0.136	0.143	0.158	0.143	0.909	1.0	1.0	1.0	1.0	1.0	
14	0.143	0.211	0.190	0.136	0.143	0.158	0.143	0.909	1.0	1.0	1.0	1.0	1.0	1.0
15	0.143	0.211	0.190	0.136	0.143	0.158	0.143	0.909	1.0	1.0	1.0	1.0	1.0	1.0

Note: 1=LCT, 2=LCT, 3=LCT, 4=LCT, 5=LCTXGBD, 6=LCTXGBD, 7=LCTXGBD, 8=GBD, 9=GBD, 10=GBD, 11=GBD, 12=GBDXLCT, 13=GBDXLCT, 14=GBDXLCT 15=GBDXLCT
See Table 1 for details of crosses.

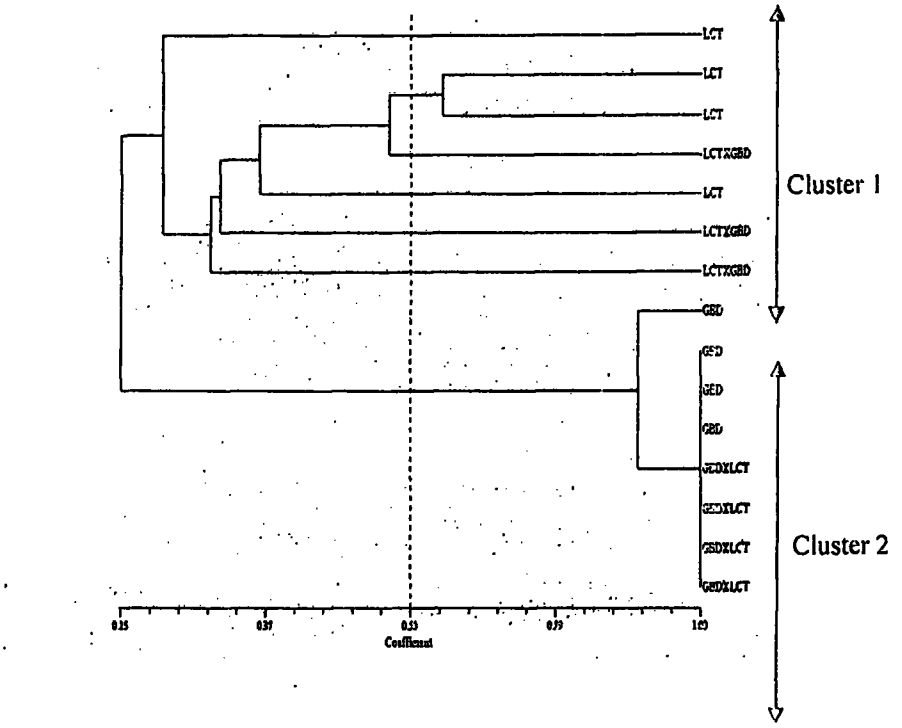

Fig. 1. Dendrogram of coconut progeny lines based on SSR markers

Note: Reference line is shown in the center of the dendrogram. Two Clusters are indicated on the left side. LCT = Laccadive Tall: GBD = Gangobondam Dwarf

Principal coordinate analysis

Three dimensional plot of principal coordinate analysis revealed closer grouping of individuals of GBD and GBD x LCT (Figure 2). The individuals of LCT and LCT x GBD showed some variations among them and dispersed in the plot. The principal coordinates one, two and three encompassed a 54 %, 18 % and 6.7 % of total variation, respectively.

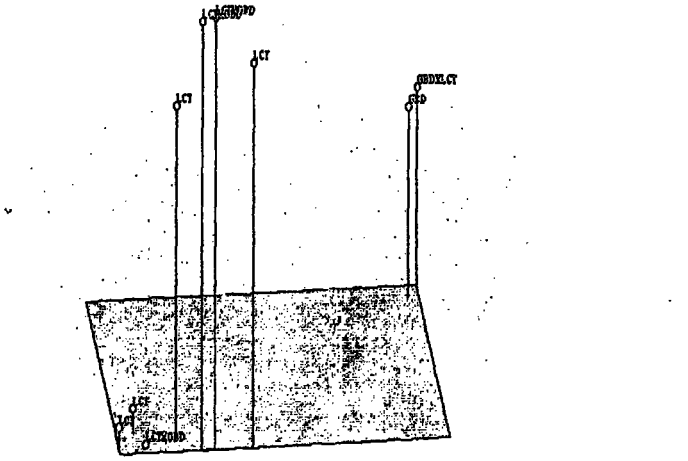

Fig. 2. Three-dimensional plot of principal coordinate analysis of selected coconut progeny lines based on SSR markers

Note: Only distinguishable Simple Sequence Repeat individuals are shown in the Figure.

DISCUSSION

In the present study, SSR markers were successfully used to estimate the genetic diversity among the coconut progeny lines. The progeny lines constituted the selfed and crossed lines of LCT and GBD and also the reciprocal cross derivatives of LCT x GBD and GBD x LCT. Coconut specific primer pairs used in the study detected an average of 4.2 alleles/locus. Earlier, 7.4 alleles/locus (Perera *et al.*, 2003), 4.4 alleles/locus (Dasanayake *et al.*, 2003) and 5.2 alleles/locus (Rivera *et al.*, 1999) were reported in coconut. Comparatively less number of alleles obtained in the present study revealed less variability among the selected progeny lines.

The high mean variability noticed among the progeny lines was due to tall lines LCT and progenies of LCT x GBD. The individuals of GBD and GBD x LCT exhibited similar banding pattern and their mean similarity was very high (>90%). The mean similarity among individuals of LCT and LCT x GBD was less compared to mean similarity among individuals of GBD and GBD x LCT. It was evident from both dendrograms and principal coordinate plot that the individuals of LCT exhibited much variation and they failed to cluster closer as GBD. This observation can be related to the breeding behaviour of coconut palms that tall plants are mostly outbreeding whereas dwarf is inbreeding (Harries, 1978).

The interesting observation noticed was that even though the parental palms were same for LCT x GBD and GBD x LCT progenies, there were lots of differences noticed

among them when reciprocally crossed which exhibited high variability in the progeny line analysis with molecular markers. It reflected the existence of reciprocal cross differences in coconut. Earlier, reciprocal cross differences in the expression of heterosis for oil content was observed by Nallathambi *et al.* (1986), who reported GDB x ECT hybrid with 7.1% heterosis for oil content as against 13.4% in its reciprocal cross hybrid. In the present study, when a dwarf is used as female parent (D x T), the progenies resemble dwarfs and the population is homogenous. On the contrary, when a tall is used as female parent (T x D), progenies were heterogeneous as like talls. Rajagopal *et al.* (1988) drew similar conclusion for leaf water potential data. They reported progenies of WCT x COD were drought tolerant. But, the progenies of reciprocal cross (dwarf as female parent) COD x WCT were drought susceptible. Recently (Parthasarathy *et al.*, 2004) analysed hybrids (WCT x COD, COD x WCT, WCT x WCT and COD x COD) and parents and reported that the clustering of WCT x COD with WCT and clustering of COD x WCT with the COD.

The possible reason for the clustering of hybrids along with the female parent could be given by analyzing more number of progenies. It seems that the dwarf plants are preferentially self-compatible and tall plants are incompatible. Analysis with more number of progenies of reciprocal crosses will provide the information on segregation pattern for the parental types.

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