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Analysis of genetic diversity in coconut and its conservation in root (wilt) disease affected areas of Kerala: A community participatory approach

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Abstract

Root (wilt) disease is a major constraint to coconut production in Kerala State. Conserving ecotypes with resistance or tolerance to the disease on a community basis is essential to sustain coconut production in the root (wilt) disease prevalent areas. Three communities' *viz.*, Pathiyoor and Devikulangara (Alappuzha District) and Thodiyoor (Kollam District) were selected and a survey was conducted with the participation of stakeholders, to characterize the local coconut ecotypes. Six ecotypes comprising of four talls and two dwarfs were identified and morphological data revealed that the local 'Jappanan' ecotype closely resembled Evoor Green Tall ecotype. Simple Sequence Repeat (SSR) analysis in 90 selected coconut palms representing the six ecotypes using 14 markers indicated that the observed heterozygosity was higher in tall ecotypes (0.179-0.365) compared to the dwarfs (0.03-0.07). Lower values for observed heterozygosity compared to the expected heterozygosity in tall ecotypes are indications of genetic basis for disease resistance observed in disease-free mother palms. Molecular characterization helped in identifying diverse coconut ecotypes having application in production of vigorous hybrids. In the dendrogram constructed using nut character data, three of the tall ecotypes (Green Tall, Brown Tall and Brick Red Tall) clustered together whereas 'Jappanan' clustered separately. Mantel's correlation test using the ZT software revealed significant correlation (0.96) between the SSR data and morphological data.

Key words: Coconut, ecotypes, gene flow, genetic diversity, microsatellite, root (wilt) disease

Introduction

Coconut (*Cocos nucifera* L.) is one of the important tropical plantation crops. It is cultivated in 11.2 million hectares worldwide [1] and is closely integrated into the livelihood of millions of people, especially in the developing countries. Traditional coconut cultivars that have adapted to specific locations are known as ecotypes, of which 1416 accessions are currently recognized by the Coconut Genetic Resources Network [2]. Commercial cultivation of high yielding varieties has led to loss of precious landraces/ecotypes [3]. A better understanding of coconut diversity would enable the farmers to understand the value of various ecotypes, which in turn will increase their income from coconut farming. With this background, CPCRI implemented an IFAD/COGENT/Bioversity International project entitled "Overcoming poverty in coconut growing communities: coconut genetic resources for sustainable livelihoods in India" in three project sites located in the root (wilt) disease prevalent tract of Kerala during 2005-08. Root (wilt) disease is contiguously prevalent in eight southern districts of Kerala and has also been reported from the remaining six districts. The objective was to promote farmer participatory activities at three sites (Pathiyoor, Devikulangara and Thodiyoor) located in two districts of Kerala (Alappuzha and Kollam), for *in situ* conservation and characterization of coconut ecotypes using morphological characters and microsatellite markers.

Materials and methods

Farmer participatory surveys were conducted in the project sites to identity the different ecotypes, based on parameters identified by farmers, and also to collect

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information about the frequency of different ecotypes among the total coconut population. Besides, a Coconut Diversity Fair was conducted at CPCRI, Regional Station, Kayamkulam to showcase the available diversity and provide an opportunity for the stakeholders to understand the features of different ecotypes available at the other project sites. A total of six ecotypes were identified for characterization of morphological and nut characters. Farmers were also trained to identify healthy palms from among the different tall ecotypes for use as mother palms. Accordingly, the mother palms identified by stakeholder farmers were verified by the project team of scientists, based on the selection criteria developed by Nair *et al.* [4]. Observations on morphological characters were recorded from ten palms of each ecotype based on standard procedures [5, 6]. Two nuts each from ten palms were collected for nut character studies. The data on various characters were statistically analyzed for estimation of mean and standard error. A total of 90 palms (18 each from four tall ecotypes and nine each from two dwarf ecotypes) comprising of six ecotypes were used for molecular marker studies. Tissue for DNA extraction was taken from the youngest leaf (unopened spindle) of each selected palm. A total of 14 highly polymorphic SSR primer pairs from the microsatellite kit developed by Baudouin and Lebrun [7] were used. PCR reaction was conducted following the procedure described by Rajesh *et al*. [8]. The pattern of amplified products across the samples was resolved by silver staining following the standard procedure [9]. Microsatellite data were analyzed using version 1.31 of the software POPGENE [10] and homozygozity and heterozygozity values were calculated using the software GDA [11] as per Levene [12]. The observed and effective number of alleles was worked out as per Kimuara and Crow [13] and Shannon's Information Index as per Lewontin [14]. Euclidean distance between ecotypes was estimated using SPSS 10.0 for morphological, nut characters and SSR analysis data. Distance matrices for morphological characters, nut characters and SSR analysis were compared using Mantel's correlation test using the ZT software developed by Bonnet and Peer [15]. Simple Mantel tests were conducted with 10000 permutations and the significance of the results compared using the probability values obtained from one-tailed tests.

Results and discussion

The coconut ecotypes were classified by farmer's based on visual characters like nut colour and growing habit of the palm. Survey based on nut colour and growing habit revealed that six ecotypes were available in the three project sites. Tall ecotypes constituted 98.66% of the coconut population while dwarf types and hybrids constituted only 1.34% of the total population (Table 1). Among the tall types, Evoor Brown Tall was the dominant ecotype (85.65%) in all the sites making brown the dominant colour type in the natural tall population of the project site. Green Tall and Brick Red Tall accounted for only 10.16 and 1.58% of the population respectively. Other tall types, including a distinct local ecotype 'Japannan' constituted only 1.27% of the total population and dwarf/hybrid types were only a minority. A total of 73 Brown Talls (3.37 % of the total Brown Tall population), six Green Talls (2.33% of total Green Tall population) and one Brick Red Tall (2.50% of total Brick Red Tall population) were selected as mother palms for seed nut collection.

With respect to most of the morphological characters, Tall ecotypes in the project site were superior to dwarf ecotypes (Table 2). Among the tall types, 'Jappanan' had higher values for reproductive characters like length of inflorescence, length of spikelet bearing portion and bunches with nuts. These may be the unique traits which makes 'Jappanan' distinct. This shows that 'Jappanan' was selected separately from introductions made from other coconut growing countries, whereas the other three tall ecotypes were more related. 'Jappanan' is believed to have been introduced from Srilanka [16] during the period when trade was common between the erstwhile Travancore (part of present Kerala State) and Ceylon (Sri Lanka). With regard to nut characters (Table 3), dwarf and tall ecotypes differed significantly. However, among the tall types, 'Jappanan' had significantly higher values for all nut characters compared to other tall ecotypes. 'Jappanan' also recorded the maximum fruit weight (1847 g) and the maximum content of husk (61.31 %). In addition, its dehusked fruit weight (696.5

Table 1. Details of coconut ecotypes available at the project site

Ecotype	Thodi-yoor	Pathi-yoor	Deviku-langara	Total (all sites)	Density (%)
Evoor Brown Tall	810	565	792	2167	85.65
Evoor Green Tall	55	95	107	257	10.16
Evoor Brick Red Tall	13	12	15	40	1.58
Other tall types including Jappanan	05	18	09	32	1.27
Dwarfs/hybrids	14	13	7	34	1.34

Table 2. Morphological characters of different coconut ecotypes

Morphological characters	EBT	EGT	EBRT	Jappanan	GD	OD	Mean	CD (p=0.05)
Vegetative characters								
Total number of leaves	30.67	33.00	30.29	29.13	25.00	27.00	28.54	4.82
Length of petiole (cm)	112.83	118.67	120.14	126.13	105.40	110.00	120.97	-
Length of leaflet (cm)	335.33	332.33	330.29	335.38	302.80	340.50	341.41	-
No. of leaflets	111.50	113.30	107.00	115.75	99.80	101.50	108.47	9.82
Length of leaflets (cm)	117.25	112.00	108.00	112.40	106.20	107.00	109.47	11.75
Breadth of leaflet (cm)	5.74	5.98	5.50	5.97	4.70	4.90	5.44	0.674
Girth of trunk (cm)	93.00	98.00	95.71	95.75	60.20	68.00	86.35	12.08
No. of leaf scars in 1 meter	16.00	13.71	12.71	12.75	51.20	41.30	22.48	3.16
Reproductive characters								
Length of inflorescence (cm)	94.83	95.50	99.29	110.75	69.20	71.30	93.22	15.92
Length of spikelet bearing portion (cm)	62.50	62.00	64.57	70.50	39.60	33.50	57.19	11.56
Length of stalk (cm)	32.33	34.17	33.57	40.25	29.60	35.00	36.32	7.72
Length of spikelet (cm)	38.67	38.83	40.29	47.00	34.60	36.80	39.00	7.97
No. of spikelet	33.33	30.50	33.29	39.50	26.00	34.50	32.14	6.77
No. of female flowers	15.83	22.83	12.90	25.25	11.60	16.80	18.54	-
Bunches with buttons	6.00	7.67	7.00	7.75	3.20	4.20	6.24	2.73
Bunches with nuts	8.00	9.00	9.14	9.75	3.40	3.70	7.73	2.26

EBT-Evoor Brown Tall; EGT-Evoor Green Tall; EBRT-Evoor Brick Red Tall; GD-Green Dwarf; OD-Orange Dwarf

Table 3. Nut characters of different coconut ecotypes

Nut characters	EBT	EGT	EBRT	Jappanan	GD	OD	Mean	CD (p=0.05)
Shape of fruit	oval	oval	oval	oval	oblong	round	-	-
Colour of fruit	Bronze	Green	Brick Red	Green	Green	Orange	-	-
Length of fruit (cm)	20.60	19.94	20.50	23.44	17.33	18.16	19.99	1.05
Breadth of fruit (cm)	15.93	15.49	15.46	18.79	12.18	14.10	15.32	0.86
Weight of fruit (g)	1338	1175	1294	1847	706	768	1188.10	171.55
Thickness of husk (cm)	2.07	1.92	1.95	2.79	4.45	1.31	2.41	0.31
Weight of dehusked fruit (g)	632.00	643.70	536.00	696.50	347.50	544.30	566.66	52.63
Shape of nut	round	round	round	oval	oblong	round	-	-
Husk to whole fruit weight (%)	51.20 *(45.68)	43.52 (41.20)	57.33 (49.25)	61.31 (51.60)	51.11 (45.62)	29.18 (33.67)	48.94 (44.33)	(2.79)
Weight of shell (g)	152.05	140.79	128.45	168.65	65.00	128.33	130.54	12.31
Thickness of shell (cm)	0.47	0.45	0.44	0.50	0.22	0.36	0.41	0.03
Dry weight of copra (g)	184.80	185.9	157.17	191.42	97.05	140.9	159.48	13.61

EBT-Evoor Brown Tall; EGT-Evoor Green Tall; EBRT-Evoor Brick Red Tall; GD-Green Dwarf; OD-Orange Dwarf.
Figures in parenthesis indicate angular transformed data

g) and copra weight (191.42 g) were the highest. 'Jappanan' ecotype closely resembled 'Evoor Green Tall' ecotype. The fruit colour was green in both the ecotypes, nut weight was 696.5 g and 643.7 g, copra weight was 191.4 g and 185.9g in Jappanan and Evoor Green Tall, respectively.

All the 14 SSR markers employed were highly polymorphic (Table 4) and the number of alleles observed at each locus ranged from two (loci CnCir E12 and CnCir A3) to nine (locus CnCir C3 and locus CnCir E2). The highest effective number of alleles was observed in locus CnCir C3 (6.27) as also Shannon's Information Index (1.92). A total of 61 alleles were detected using 14 SSR markers with an average of 4.35 alleles per SSR locus which indicate the potential of the SSR markers developed by CIRAD for diversity studies in coconut as reported earlier by Rajesh *et al*. [8]. The degree of genetic differentiation was high and varied among the loci from 0.19 (CnCir C7) to 0.78 (CnCir E12) with a mean of 0.47 (Table 4) indicating a high level of population differentiation among the coconut ecotypes. F_{IS} values for 11 out of 14 loci were high (value more than 0) indicative of more of homozygotes which would have occurred due to higher level of *inter se* mating in the locally adapted tall ecotypes and also due to inclusion of two dwarf ecotypes which are known to be inbreeders in general.

The Nei's genetic diversity was observed to range from 0.28 for the locus CnCir A3 to 0.84 for locus CnCir C3 with mean genetic diversity of 0.55 (Table 5). Average heterozygosity ranged from 0.10 for the locus CnCir E12 to 0.63 for the locus CnCir C3 with a mean of 0.29. Across the ecotypes, the mean genetic diversity, or expected heterozygosity under random mating, was 0.30 (Table 6). It was the highest in Evoor Green Tall (0.492) and the least in Green Dwarf ecotype (0.038). Similarly, the observed heterozygosity was higher in tall ecotypes (0.179-0.365) compared to dwarf ecotypes (0.03-0.07) as reported earlier [17, 18, 19]. The mean inbreeding co-efficient across the six ecotypes was 0.39. It was the highest for Evoor Brick Red Tall (0.61) and the least for the Green Dwarf (–1.00).

Though tall ecotypes are considered to be highly heterozygous, the observed heterozygosity in the talls was much less in comparison to the expected heterozygosity. The lower values for observed heterozygozity in talls may be due to *inter se* mating that would have occurred in the tall types which are locally adapted in the project sites. Rajesh *et al*. [8] have also reported lower observed heterozygosity

Table 4. Number of alleles, Shannon's Information Index, F-statistics and gene flow for the 14-microsatellite loci

Locus	na	ne	I	F_{IS}	F_{IT}	F_{ST}	N_m*
E12	2.000	1.664	0.588	-0.0170	0.7845	0.7882	0.0672
A9	4.000	3.020	1.212	0.9116	0.9726	0.6905	0.1121
B12	4.000	2.146	1.025	0.1413	0.5243	0.4460	0.3106
C3	9.000	6.279	1.927	-0.1012	0.1608	0.2379	0.8007
A3	2.000	1.389	0.454	-0.1396	0.5430	0.5990	0.1674
C7	5.000	2.604	1.133	0.7162	0.7703	0.1905	1.0626
H4	4.000	1.637	0.756	0.5427	0.8015	0.5658	0.1919
E2	9.000	3.752	1.606	0.2299	0.4620	0.3013	0.5798
F2	3.000	1.972	0.818	0.2871	0.7263	0.6161	0.1558
H7	3.000	2.494	0.980	0.9741	0.9841	0.3852	0.3991
B6	5.000	2.911	1.257	0.4341	0.6222	0.3324	0.5021
E10	3.000	1.775	0.724	0.2323	0.7965	0.7349	0.0902
G11	4.000	3.180	1.248	0.4637	0.7158	0.4700	0.2819
C12	4.000	1.720	0.758	0.3272	0.8366	0.7571	0.0802
Mean	4.357	2.610	1.035	0.3776	0.6710	0.4714	0.2804
S.D	2.170	1.265	0.402	-	-	-	-

na = Observed number of alleles, ne = Effective number of alleles, I = Shannon's Information Index, *Nm = gene flow estimated from F_{ST} as $0.25(1 - F_{ST})/F_{ST}$

Table 5. Homozygosity, heterozygosity and genetic diversity across the 14 SSR loci

Locus	Observed homozygosity	Observed heterozygosity	Expected homozygosity	Expected heterozygosity	Nei* expected heterozygosity	Average heterozygosity
E12	0.8764	0.1236	0.5987	0.4013	0.3990	0.1012
A9	0.9775	0.0225	0.3274	0.6726	0.6689	0.2095
B12	0.6854	0.3146	0.4630	0.5370	0.5340	0.3070
C3	0.2809	0.7191	0.1545	0.8455	0.8407	0.6306
A3	0.8202	0.1798	0.7181	0.2819	0.2803	0.1402
C7	0.8556	0.1444	0.3806	0.6194	0.6160	0.4568
H4	0.8966	0.1034	0.6085	0.3915	0.3893	0.1872
E2	0.5556	0.4444	0.2624	0.7376	0.7335	0.4810
F2	0.8222	0.1778	0.5045	0.4955	0.4928	0.2078
H7	0.9888	0.0112	0.3975	0.6025	0.5991	0.3573
B6	0.7303	0.2697	0.3399	0.6601	0.6564	0.4013
E10	0.8750	0.1250	0.5608	0.4392	0.4367	0.1348
G11	0.7640	0.2360	0.3106	0.6894	0.6855	0.3707
C12	0.9101	0.0899	0.5790	0.4210	0.4186	0.1273
Mean	0.7885	0.2115	0.4433	0.5567	0.5536	0.2938
S.D	0.1862	0.1862	0.1587	0.1587	0.1578	0.1608

*Nei's [21]

levels in tall types compared to expected heterozygosity. This is also evident from the higher values for fixation index (mean F_{IT} = 0.671, table 4). F_{IT} combines contribution from non random mating within populations (F_{IS}) and effects of random drift among population (F_{ST}). Higher F_{IT} values also indicate an excess of homozygotes. Another reason for more homozygotes could be due to the farmer's practice of collecting seed nuts from healthy and high yielding palms located in each project site. Most of the palms in disease prevalent localities are actually half sib/full sib progenies of a few mother palms in each locality [20]. High level of population differentiation among the coconut ecotypes was again supported by low value for gene flow (N_m = 0.28, Table 4). This may also be due to the fact that two of the ecotypes used for SSR analysis were dwarf types and there was little gene flow between the tall and dwarf types. Generally, the breeding behavior in tall types is out crossing (cross pollinated) whereas the dwarf types are inbreeders (self pollinated). The gene flow that would have happened would be among the tall coconut ecotypes. AMOVA was used to partition the total genetic diversity among and within tall populations. Most of the variations were found within populations (88.64%) than among populations (11.36%) indicating a considerable gene flow within the populations, through both seed and pollen flow.

Nei's [21] genetic distances and identities between the ecotypes are given in Table 7. Nei's similarity index between Evoor Brick Red Tall and Evoor Brown Tall was 0.0523 and between 'Jappanan' and Evoor Green Tall was 0.0531. Genetic distance was the greatest between Evoor Brown Tall and Orange Dwarf (1.369). Molecular characterization of the local ecotypes has helped to identify the most diverse coconut ecotypes (Orange Dwarf and Evoor Brown Tall) in the project site and these were selected as female and male parent respectively in the D x T hybrid production programme. Evoor Brick Red Tall with the lowest value for observed heterozygozity (0.178) and highest inbreeding co-efficient (0.612) was identified to be more or less an inbred tall ecotype. This tall ecotype was also selected as the male parent for utilization in the hybrid production.

Mantel's correlation test using ZT software revealed that there was significant correlation (0.96) between the SSR data and morphological data. The morphological data and nut character data was 65%

Table 6. Expected heterozygosity (He), observed heterozygosity (Ho) and inbreeding co-efficient (F_{IS}) across the coconut ecotypes

Population	He	Ho	F_{IS}
EBT	0.4061	0.2276	0.4467
EGT	0.4922	0.3649	0.2644
EBRT	0.4520	0.1786	0.6119
JPN	0.3865	0.2370	0.3941
GD	0.0378	0.0714	-1.0000
OD	0.0412	0.0337	0.1918
Mean	0.3027	0.1855	0.3956

EBT-Evoor Brown Tall; EGT-Evoor Green Tall; EBRT-Evoor Brick Red Tall; JPN- Jappanan; GD-Green Dwarf; OD-Orange Dwarf

Table 7. Nei's unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal) among coconut ecotypes

Pop ID	EBT	EGT	EBRT	JPN	GD	OD
EBT	-	0.8798	0.0523	0.8413	0.3107	0.2542
EGT	0.1281	-	0.1441	0.0531	0.3542	1.1041
EBRT	0.9490	0.8658	-	0.8229	0.3522	0.3651
JPN	0.1728	0.9483	0.1949	-	0.3613	0.2830
GD	1.1688	1.0378	1.0436	1.0181	-	0.5356
OD	1.3696	0.3315	1.0076	0.2772	0.5853	-

EBT-Evoor Brown Tall; EGT-Evoor Green Tall; EBRT-Evoor Brick Red Tall; JPN-Jappanan; GD-Green Dwarf; OD-Orange Dwarf

correlated. However, the SSR data and nut character data had only 57% correlation as per Mantel's correlation test. This contradicts the general belief that nut character is the most stable character for variability studies. Present studies using 14 highly polymorphic SSR markers clearly points out that morphological data and SSR data are highly correlated (96%).

Dendrograms showing the relationships among the six ecotypes using morphological data, nut character data and microsatellite data are given in Figs. 1, 2 and 3. Dendrogram constructed using morphological data and microsatellite data were showing almost the same pattern. In both dendrograms, two major clusters could be visualized; one cluster representing the tall ecotypes and the other representing dwarf ecotypes. Within the tall types, two sub-clusters could be observed. While the Evoor Brown Tall and Evoor Brick Red Tall clustered together, the 'Jappanan' clustered with Evoor Green Tall. However,

Fig. 1. Dendrogram showing the genetic relationships among the six coconut ecotypes based on morphological character analysis

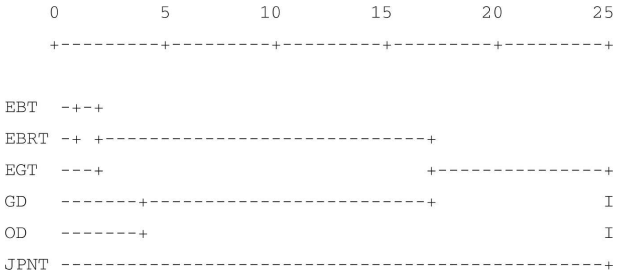

Fig. 2. Dendrogram showing the genetic relationships among the six coconut ecotypes based on nut character analysis

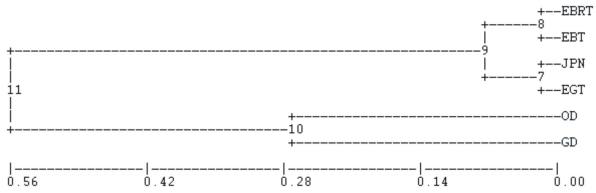

Fig. 3. Dendrogram showing the genetic relationships among the six coconut ecotypes based on SSR analysis

the dendrogram constructed based on nut character data was slightly different from the other two dendrograms. The three tall ecotypes (Green Tall, Brown Tall and Brick Red Tall) clustered together whereas 'Jappanan' clustered separately. The dwarf ecotypes clustered together.

The project was implemented with the objective of identifying the different coconut ecotypes available in the three project sites. The study has practical application as molecular characterization of the local ecotypes has helped to identify the most diverse coconut ecotypes in the project site.

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