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SSR BASED POPULATION BOTTLENECK STUDIES ON COCONUT ACCESSIONS FROM SOUTH PENINSULAR INDIA

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ABSTRACT

The fate of alleles after population bottleneck is important for studying the genetic variability of population. For conducting this study, samples from three coconut populations with varied yield traits were collected from different agro climatic regions of south peninsular India. SSR analysis was carried out using thirty- two primer pairs to evaluate genetic variation. Using the software program BOTTLENECK, fluctuations in the population sizes have been identified by detecting the deviations from mutation drift equilibrium. Three well known microsatellite mutation models SMM, TPM and IAM have been used to assess the genetic bottleneck. Three statistical tests - sign test, standardized difference test and wilcox rank test were performed for each mutation model. The results revealed that high yielding and medium yielding populations showed heterozygosity excess, as most of the DH/SD values were positive under IAM, TPM and SMM. However low yielding population showed a heterozygosity deficiency. This clearly shows that the high and medium yielding coconut populations experience genetic bottleneck, however the low yielding population seems to be undergoing rapid population expansion.

KEY WORDS: Coconut, SSR, mutation model, genetic bottleneck

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INTRODUCTION

Coconut is the most widely grown plantation crop in southern peninsular India. The food and industrial products from coconut palm such as oil, milk, desiccated coconut, copra, coir and timber have a key role in the economy of rural communities[1]. Large numbers of varieties have been identified in coconut based on growth habit, fruit shape, color and other morphological characters. Traditional coconut cultivars are populations, which are generally referred as ecotypes. Genetic diversity study on ecotypes is of special importance in breeding program, especially for developing hybrids. Two different types of coconuts (tall and dwarf) are found commonly. Tall coconut trees are fast growing and predominantly cross-pollinated, hence highly heterozygous and show a wide genetic diversity due to its out-crossing nature. However, dwarf trees are mainly self-pollinated, less heterozygous, and have reduced growth habit[2]. According to Lamothe and Benard[3]hybrids of tall and dwarf varieties show substantial heterosis and allow us to search for characters of adaptation to soil, climatic conditions, pests and diseases[4]. Morphometry has been a major tool to study variation among coconut populations. Although it discriminates populations and environmental influence, their polygenic determinism hinders the use of these parameters in the study on their population genetics. Recently molecular markers were being used to determine the genetic relationships between/ within the populations. Molecular markers like RFLP[5], RAPD[2, 6, 7], AFLP[8] and microsatellites or SSRs[9-11] have been successfully employed for assessing genetic diversity and tagging genes associated with agronomic traits in coconut and other crops. SSR being co-dominant and reproducible with high polymorphism, are being used for DNA fingerprinting, parentage identification, genetic mapping, conservation, and population genetics[12-15]. SSRs are turned out to be ideal tools for identifying individuals and to establish genetic diversity within them, since they allow the detection of alleles at high frequency. Size homoplasy is very common in SSRs due to their mutational modalities [16-19]. Homoplasy has been identified at increased rates at various levels of frequency in number of organisms [20-21]. Due to high mutation rate, SSR markers possess a large number of alleles, which make them particularly suited for genome mapping and paternity analysis. Since the mechanism that produces variation at microsatellite loci are unusual, the use of microsatellite loci for evolutionary purposes has been a subject of intense research. As per previous reports, the process and patterns of mutations at different loci having tandem repeat, differ from locus to locus depending on motif and size of alleles at each locus[23]. Mutation models have been proposed to explain mutational process of the microsatellites. The frequently adapted models in SSR analysis are - IAM-Infinite Allele Model [18], SMM-Stepwise Mutation Model[24] and TPM-Two Phased Mutation Model[25]. IAM for allozyme data and the infinite sites model of DNA substitution mutation are being widely used in the study of population genetics[26]. As per this a unique allele resulted from each mutational event at a given locus happens only once[27]. Whereas the SMM model explains the gain or loss of a single repeat unit due mutation in SSR loci [27, 28] .The two-phase models like, generalized stepwise model and K-allele model are among the frequently used models to study population genetics [14,25,27,29].The genetic diversity in populations is lost through random drift whenever they undergo temporary and large reduction in size, a population bottleneck[30, 31]. Such natural variations in the diversity occurs due to events like heterozygote deficiency in natural populations[32], speciation events [28,33,34] , reduced reproductive function[35, 36] and low levels of genetic variation [37-39]. By carrying out different statistical model analysis, in the present study we discuss the allelic variation in coconut accessions in relation to yield traits using SSR markers.

MATERIALS AND METHODS

Plant sample collection and DNA isolation

Based on the yield trait (number of nuts per year), coconut populations used in this study are classified as high, medium and low yielding groups (Table. 1).

Table 1
Yield category and genotypes of coconut from each category

Yield category (number of nuts per year)	Range,Mean±SE	Genotypes
Low yielding (1-80)	64, 41.8 ± 5.40	KA210, GU21F1, GU2F1, BR39 , KA244, BR59, GU14MP, KA403, BR245, GU9F1
Medium yielding (81-160)	79, 109.5 ± 9.13	AN32, BR48, BR80 , KA520, AN2/1, HU2DxT, KA402, GU3MP, BRX1, KA552
High yielding(161-250)	75,219.27 ± 9.43	AN1/32, AN38, GU1MP, HUHY1, KA137, HUMPGA3, GU17MP, KA248, GUHY2, GU1MPGR, GU24F1

Leaf samples from coconut genotypes grown in different agro-climatic regions of south peninsular India were collected, which includes coconut germplasm maintained by the Research Stations at Andaman, Arsikere, Brahmavar, and from some local orchards **(**Table. 2). The spacing between the plants was 7.5 x 7.5 (length x breadth) in all the plantations and maintained following the recommendations of the package of practices for the coconut. Samples were dried at 40^0C, powdered to pass through 40 mm-mesh sieve and stored at room temperature in dry plastic bags. DNA was extracted using a CTAB method with minor (addition of 2% polyvinyl pyrrolidone and 1% β-mercaptoethanol) modifications[10, 40, 41]

SSR analysis

SSR primers listed in Table 3 were used for amplification of coconut accessions. Reactions were carried out in total volume of 10 µl containing 12.5 ng of template DNA, 1X*Taq* polymerase assay buffer, 200 µM each of the dNTPs, 0.2 µM each of forward and reverse primer and 0.5 U *Taq* polymerase using PCR (MJ-Research ThermocyclerModel PTC-100). 2mM $MgCl_2$ concentration provided optimum results during amplification. The forward SSR primer in each reaction was radio-labeled with γP^{33}-ATP using T_4 DNA kinase. PCR amplification was carried out with an initial denaturation at 94ºC for 5 min followed by 35 cycles, each consisting of denaturation at 94ºC for 30 sec, primer annealing at 51ºC for 1 min, and extension at 72ºC for 1 min with a final extension step at 72ºC for 8 min. The amplicons were mixed with equal volume of 10X DNA loading buffer [100mM Tris (pH 8.0), 100mM EDTA, 0.25% Xylene cyanol, 0.25% Bromophenol blue and 50% Sucrose] and denatured at 94ºC for 3 min. 4µl of the reaction mixture was applied on 5% denaturing polyacrylamide gels and electrophoresed at 55W constant power for 2 hr using 1X TBE buffer. The gels were dried using a gel drier (Bio-Rad, USA) at 80ºC for 30 min and were exposed to Kodak Biomax MR-2 film for 18 hr (Figure 1).

Table 2
Geographical description of different agro climatic parameters of the regions from where the coconut samples were collected

Region	Latitude	Longitude	Altitude MSL (meter)	Topography	Soil profile	Annual rainfall (mm)
Agricultural Research Station, Brahmavar (BR)	12º30´ 15º00´	74º05´, 76º00´	9.00 - 13.51	Coastal	Laterite	3893.00
Agricultural Research Station, Arasikere (AR)	12º50´ 14º55´	75º30´ 77º20´	240.24 - 270.27	Plain	Granite	794.40
Farmer's field, Gubbi (GU)	12˚10´ 14º00´	76º35´ 78º45´	240.24 - 270.27	Plain	Laterite/ Granite Gneiss	646.00
Farmer's field, Hunsur (HU)	11º30´ 13º05´	76º05´ 77º45´	240.24 - 270.27	Plain	Laterite/ Granite Gneiss	734.00
Coconut development Board Research station, Kanakpura (KA)	12˚10´ 14º00´	76º35´ 78º45´	800.00-900.00	Plain	Laterite/ Granite Gneiss	776.0
World Coconut Germplasm collection center, Andaman (AN)	06º00´ 14º05´	92º00´ 94º05´	35.00-60.00	Coastal	Sand/Silty clay /Diluvial	3180.0

Table 3
Characteristics of SSR Primers and their location on chromosome number (Cn).

No	SSR	Number of alleles Scored*	Size range	Chromosome number
1	CnCir A3	4	224-240	NM
2	CnCir A4	4	196-204	12[#]
3	CnCir A8	5	274-282	NM
4	CnCir A9	4	89-103	14[c]
5	CnCir B5	4	270-278	NM
6	CnCir B6	6	196-208	NM
7	CnCir B12	9	153-177	8[#]
8	CnCir C3	10	174-206	NM
9	CnCir C7	5	157-167	NM
10	CnCir C11	3	217-225	15[¶]
11	CnCir C12	4	167-183	NM
12	CnCir D8	9	241-259	8[#]
13	CnCir E2	15	115-175	NM
14	CnCir E4	5	224-238	1[¶]
15	CnCir E7	4	214-220	13[¶]
16	CnCir E10	5	226-246	NM
17	CnCir E11	7	180-218	11[¶]
18	CnCir E12	2	164-174	NM
19	CnCir F2	4	193-205	NM
20	CnCir G4	3	166-171	13[¶]
21	CnCir G11	6	188-208	NM
22	CnCir H4	5	218-236	3[¶]
23	CnCir H7	5	131-139	NM
24	CnCir R11	3	136-150	NM
25	CnCir S1	4	242-262	NM
26	CnCir S5	3	224-228	NM
27	CnCir S7	9	189-219	NM
28	CnCir S8	3	84-102	NM
29	CnCir S12	9	149-183	NM
30	mCnCir 47	8	122-172	13[¶]
31	mCnCir86	9	172-200	9[¶]
32	mCnCir119	9	187-217	1[¶]

NM: Not mapped.

Primer sequences were obtained from CIRAD (Centre de CoopérationInternationale en Recherche Agronomique pour le Développement), Montpellier, France.

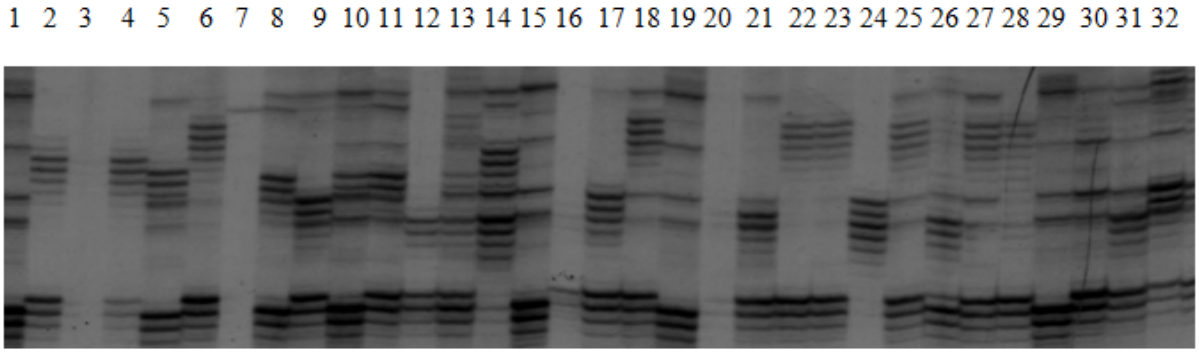

Figure 1

Representative SSR profile of representatives of coconut genotypes (high, medium and low) using the primer CnCirC3'. Lane 1. AN32; Lane 2.BR48; Lane 3.AN1/32; Lane 4.BR80; Lane 5. KA210; Lane 6.KA520; Lane 7.AN38; Lane 8.GU1MP; Lane 9.GU21F1; Lane 10.HUHY1; Lane 11.GU2F1; Lane 12.KA137; Lane 13.AN2/1; Lane 14.HU2DxT;Lane 15.HUMPGA3; Lane 16.KA402; Lane 17.GU17MP; Lane 18.WAT; Lane 19.GU24F1; Lane 20.BR39; Lane 21.KA552; Lane 22.KA244; Lane 23.BR59; Lane 24.GU14MP; Lane 25.BRX1; Lane 26.KA403; Lane 27.BR245; Lane 28.KA248; Lane 29. GUHY2; Lane 30.GU3MP; Lane 31.GU1MPGR; Lane 32.GU9F1.

Data analysis

The fluctuations in the population sizes were identified by detecting the deviations from mutation drift equilibrium using BOTTLENECK[42] software. The mutation models SMM, TPM and IAM were used to assess the genetic bottleneck. SMM allows only single step mutations, whereas TPM allows 10% multistep mutations and 90% single step mutations. For each population sample and the locus, BOTTLENECK software allows the computation of the heterozygosity expected from the observed number of alleles (k), given the sample size (n) under the assumption that the population is at mutation drift equilibrium. The program simulates the coalescent process of (n) loci under the three mutational models. This allows the computation of the expected heterozygosity (Heq) and compared to observed heterozygosity (He) to determine whether there is heterozygosity excess or deficiency[42] (DH difference between the expected and the observed heterozygosity). Further, standard deviations (SD) of the mutation drift equilibrium of the heterozygosity, was also used to compute the standardized difference in each locus (He-Heq)/SD. P value was calculated for the observed heterozygosity. Allowing a single mutation at a time and computing the number of alleles produced simulate the coalescent process under the IAM and TPM. The process is repeated until the number of alleles computed is equal to 'k'. The likelihood distribution of θ (4Neμ) was evaluated based on the number of alleles (k) and sample size (n) as percentage of trials that produce exactly 'k' alleles for various values of θ using SMM. After evaluating all loci, three statistical tests - sign test, standardized difference test[42] and Wilcox rank test[43]were performed for each mutation model. A positive value in the sign test (He-Heq) indicates heterozygosity excess. If the number of loci with heterozygosity excess is significantly larger than half, it suggests a bottleneck. The standardized difference test is similar to the sign test, but it considers the magnitude of heterozygosity excess or deficiency. The Wilcox rank test is a non-parametric test that is similar to matched pairs t-test. It is used to determine differences between groups of paired data when the data do not meet the standards associated with a parametric test, as well as the magnitude of difference between matched groups. This test is statistically powerful to analyse large number of individuals with few loci. A mode shift indicator is used to describe the allele frequency distribution. This discriminates stable population from populations having bottle necks[42].

RESULTS AND DISCUSSION

In case of loci that are not influenced by selection, allele frequency and diversity in a population result from the balance between genetic drift and mutation or mutation drift equilibrium. Rate of mutation and the effective population size are the parameters of this balance[42]. Dramatic drop in effective population size, usually results in decrease in heterozygosity and allele frequency[31]. Population that is undergoing a genetic bottleneck will experience reduction in allele number, which is more rapid than the reduction in heterozygosity. Hence, these populations will show heterozygosity excess. Using BOTTLENECK software, heterozygosity excess in the three coconut populations was assayed. The null hypothesis here is that the populations are at mutation drift equilibrium and have remained at a constant size in the past. Since the program tests only for the presence or absence of mutation drift equilibrium, but not heterozygosity excess. The program tested for a deviation from mutation drift equilibrium for each locus individually and DH/SD value was generated for each locus. This value is negative for heterozygosity deficiency, where as positive for heterozygosity excess. P value generated for each DH/SD value determines its significance. When population is at mutation drift equilibrium, there is a probability that loci showing negative and positive DH/SD value are equal. Genetic bottleneck for three coconut groups (high, medium and low yielding group) were tested in this study. The probability of observed heterozygosity (He) being larger than the expected heterozygosity (Heq) was assessed by sign test, standardized differences test and Wilcox rank test. Under each mutation model, assumption was made that all loci fit that model and population was at mutation drift equilibrium.Observed number of alleles in the high yielding group ranges from two to eight with an average of 4.50 (Table 4). In high yielding population out of thirty-two loci, seven in IAM, twelve in TPM and thirteen in SMM showed negative DH/SD values. Sign test, Standardized differences test and the Wilcox rank test were used to determine whether the population deviated from mutation drift equilibrium with any of the three mutation models. In Sign test, 'P' values were found to be significant under IAM and non-significant under TPM and SMM (Table 5). So the null hypothesis is rejected under IAM and can say that population is not at equilibrium. But null hypothesis is accepted under TPM and SMM, suggests that these populations are at mutation drift equilibrium. Similar results were also found with standardized differences test and Wilcox rank test. Most of the DH/SD values were positive under IAM, TPM and SMM, which indicates that this population is showing heterozygosity excess. The observed number of alleles in the medium-yielding group ranges from one to eight with an average of 4.20. In the sign test, the P value was found to be less than 0.05 for IAM, hence fails to support null hypothesis. Under standardized differences test, *P* values were significant for IAM and TPM and non-significant for SMM. Under Wilcox rank test *P* values were found to be significant for all of the mutation models (Table 6). Null hypothesis for TPM and SMM under sign test and for IAM and TPM under standardized differences test holds good. Under Wilcox rank test, null hypothesis was rejected for IAM, TPM and SMM. This shows that the medium yielding population is not at mutation drift equilibrium. In this population, locus G4 was monomorphic, so was not included in the analysis. Out of thirty-one loci, four in IAM, eight in TPM and nine in SMM showed negative DH/SD values. This indicates that the medium yielding population shows heterozygosity excess. The observed number of alleles in the low yielding group ranges from one to ten with an average of 3.62. Under sign test and Wilcox rank test *P* values were non-significant for IAM, TPM and SMM, but in the case of standardized differences test, *P* values were found to be non-significant for IAM and TPM but significant for SMM (Table 7). However, in case of low yielding population, null hypothesis was accepted for IAM, TPM, and SMM by sign test and Wilcox

rank test. But for standardized differences test, null hypothesis was accepted for IAM and TPM but not for SMM. Hence, low yielding populations are at mutation drift equilibrium. Two loci, F2 and S8 were monomorphic in this population. Out of thirty loci, twelve in IAM, fourteen in TPM and fifteen in SMM showed negative DH/SD values. This shows that low yielding population is experiencing a heterozygosity deficiency.Under IAM, TPM and SMM, most of the loci in high yielding and medium yielding population showed positive DH/SD values, indicating that they are undergoing heterozygosity excess. At the same time in low yielding population it was found that they are undergoing heterozygosity deficiency as most of the loci showed negative DH/SD values under IAM, TPM and SMM. High and medium yielding populations showed significant heterozygosity excess, which strongly suggest that they are not undergoing a population expansion. However, low yielding population shows significant deficiency in gene diversity, which indicates that they are undergoing a rapid population expansion. Microsatellite loci follow SMM more closely[25]. Evolutionary dynamics of most SSR loci are best explained by SMM than IAM[16, 17, 43, 44]. Under SMM, new alleles evolve in steps with an increase or decrease by one repeat unit. When a population is at mutation drift equilibrium, allele sizes are usually contiguous[42]. If these populations experience genetic bottleneck, some of these alleles are eliminated. This produces gaps in allele sizes due to sharp decrease in number of individuals. At this point, the population would exhibit heterozygosity excess. As the population recovers and begins to expand, these gaps in allele sizes are filled in eventually with new alleles. As long as the population regains to its original size, formation of new alleles becomes rare. This results in an excess of alleles and a deficiency in gene diversity[42]. From the analysis it is clear that the high and medium yielding coconut populations experience genetic bottleneck. However, the low yielding populations are undergoing a rapid population expansion and could be that they experienced a genetic bottleneck in the recent past from which they are recovering.

Table 4

Observed and expected number of the alleles and heterozygosities. Sample size (n),number ofalleles (Ko), expected heterozygosity (Heq), observed heterozygosity (He), difference in the expected and the observed heterozygosity(DH), standard deviations(SD),probability (Prob)

Population	locus	n	Ko	He	IAM				SMM				TPM			
					Heq	S.D	DH/SD	Prob	Heq	S.D	DH/SD	Prob	Heq	S.D	DH/SD	Prob
High yielding	CnCirE12	9	2	0.523	0.280	1.154	1.577	0.086	0.328	0.152	1.284	0.131	0.309	0.152	1.411	0.110
	CnCirA9	9	4	0.706	0.589	0.124	0.939	0.191	0.670	0.087	0.412	0.430	0.656	0.092	0.548	0.366
	CnCirB12	8	6	0.783	0.776	0.065	0.105	0.611	0.816	0.045	-0.710	0.243	0.807	0.049	-0.480	0.326
	CnCirC3	9	6	0.817	0.750	0.078	0.850	0.196	0.805	0.045	0.263	0.499	0.792	0.056	0.447	0.408
	CnCirA3	10	3	0.484	0.460	0.143	0.167	0.515	0.531	0.123	-0.379	0.303	0.518	0.127	-0.269	0.332
	CnCirC7	9	4	0.725	0.600	0.114	1.095	0.138	0.667	0.086	0.688	0.311	0.653	0.092	0.786	0.268
	CnCirH4	8	4	0.592	0.613	0.112	-0.194	0.391	0.679	0.085	-1.030	0.167	0.666	0.090	-0.823	0.219
	CnCirE2	10	8	0.837	0.830	0.053	0.123	0.473	0.864	0.030	-0.906	0.173	0.858	0.036	-0.596	0.229
	CnCirF2	9	4	0.399	0.597	0.119	-1.665	0.128	0.671	0.0830.063	-3.295	0.020	0.657	0.092	-2.801	0.027
	CnCirH7	9	5	0.797	0.688	0.097	1.133	0.099	0.749	0.120	0.765	0.242	0.734	0.075	0.845	0.199
	CnCirB6	10	3	0.695	0.453	0.146	1.663	0.009	0.535	0.084	1.327	0.030	0.520	0.128	1.362	0.024
	CnCirE10	9	4	0.647	0.594	0.119	0.445	0.415	0.668	0.051	-0.250	0.325	0.649	0.099	-0.022	0.407
	CnCirG11	9	6	0.797	0.757	0.076	0.531	0.402	0.802	0.083	-0.095	0.380	0.792	0.055	0.101	0.461
	CnCirC12	8	4	0.517	0.615	0.116	-0.848	0.217	0.681	0.078	-1.985	0.056	0.665	0.090	-1.641	0.088
	CnCirA4	7	4	0.714	0.636	0.106	0.739	0.294	0.695	0.144	0.250	0.520	0.679	0.087	0.406	0.456
	CnCirC11	7	2	0.440	0.305	0.150	0.901	0.331	0.351	0.023	0.616	0.432	0.342	0.147	0.667	0.410
	CnCirD8	7	8	0.923	0.886	0.032	1.153	0.104	0.900	0.047	1.029	0.205	0.897	0.026	0.993	0.185
	CnCirE11	9	6	0.843	0.754	0.075	1.183	0.058	0.806	0.115	0.786	0.219	0.794	0.054	0.914	0.175
	CnCirG4	10	3	0.279	0.451	0.150	-1.147	0.237	0.540	0.052	-2.274	0.057	0.515	0.129	-1.826	0.101
	CnCirE4	7	5	0.780	0.725	0.083	0.663	0.310	0.775	0.086	0.097	0.573	0.763	0.065	0.270	0.497
	CnCirE7	9	4	0.608	0.602	0.119	0.051	0.468	0.672	0.120	-0.742	0.221	0.654	0.090	-0.515	0.288
	CnCirS8	9	3	0.386	0.472	0.142	-0.608	0.320	0.541	0.110	-1.291	0.143	0.529	0.125	-1.147	0.179
	CnCirS1	7	3	0.484	0.509	0.130	-0.196	0.440	0.569	0.022	-0.774	0.242	0.552	0.116	-0.594	0.302
	CnCirS12	7	8	0.901	0.885	0.032	0.489	0.447	0.901	0.040	0.008	0.666	0.896	0.027	0.203	0.606
	CnCirS7	7	6	0.846	0.800	0.057	0.802	0.238	0.830	0.119	0.406	0.433	0.822	0.046	0.522	0.377
	CnCirB5	8	3	0.675	0.476	0.144	1.379	0.072	0.547	0.114	1.075	0.146	0.537	0.126	1.091	0.142
	CnCirS5	7	3	0.670	0.502	0.132	1.273	0.088	0.567	0.108	0.901	0.218	0.559	0.115	0.967	0.180
	CnCirR11	7	3	0.692	0.506	0.132	1.418	0.069	0.571	0.067	1.117	0.136	0.553	0.117	1.194	0.105
	CnCirA8	5	4	0.644	0.696	0.083	-0.621	0.366	0.736	0.083	-1.378	0.181	0.724	0.074	-1.073	0.239
	mCnCir119	7	4	0.758	0.638	0.107	1.128	0.114	0.688	0.070	0.846	0.213	0.682	0.085	0.894	0.189
	mCnCir47	5	4	0.822	0.694	0.085	1.518	0.036	0.734	0.022	1.274	0.070	0.726	0.074	1.307	0.073
	mCnCir86	7	8	0.901	0.888	0.031	0.441	0.458	0.901		0.012	0.497	0.898	0.023	0.140	0.607

Population	Locus	n	Ko	He	IAM				SMM				TPM			
					Heq	S.D	DH/SD	Prob	Heq	S.D	DH/SD	Prob	Heq	S.D	DH/SD	Prob
Medium yielding	CnCirE12	7	2	0.440	0.315	0.148	0.840	0.344	0.346	0.147	0.638	0.422	0.331	0.150	0.722	0.393
	CnCirA9	8	3	0.508	0.488	0.140	0.149	0.523	0.558	0.112	-0.441	0.299	0.535	0.127	-0.209	0.386
	CnCirB12	8	7	0.892	0.826	0.051	1.289	0.026	0.856	0.035	1.044	0.123	0.848	0.039	1.110	0.092
	CnCirC3	8	8	0.808	0.867	0.036	-1.635	0.112	0.886	0.026	-3.018	0.030	0.878	0.032	-2.205	0.065
	CnCirA3	7	2	0.538	0.299	0.145	1.644	0.034	0.350	0.147	1.288	0.053	0.339	0.149	1.337	0.055
	CnCirC7	8	4	0.733	0.607	0.118	1.073	0.134	0.677	0.085	0.668	0.282	0.667	0.092	0.719	0.266
	CnCirH4	8	3	0.508	0.484	0.137	0.179	0.527	0.556	0.111	-0.431	0.323	0.550	0.115	-0.359	0.321
	CnCirE2	8	8	0.892	0.864	0.037	0.741	0.303	0.885	0.028	0.248	0.561	0.880	0.029	0.390	0.501
	CnCirF2	8	2	0.500	0.293	0.154	1.344	0.192	0.336	0.151	1.085	0.259	0.329	0.150	1.146	0.231
	CnCirH7	9	4	0.712	0.587	0.121	1.034	0.162	0.670	0.082	0.516	0.373	0.657	0.090	0.609	0.318
	CnCirB6	9	5	0.752	0.692	0.095	0.631	0.343	0.749	0.066	0.047	0.452	0.734	0.070	0.248	0.514
	CnCirE10	8	4	0.592	0.611	0.115	-0.165	0.410	0.679	0.082	-1.062	0.144	0.664	0.087	-0.829	0.216
	CnCirG11	8	5	0.700	0.710	0.088	-0.113	0.361	0.756	0.063	-0.884	0.153	0.746	0.070	-0.656	0.204
	CnCirC12	8	4	0.642	0.623	0.112	0.165	0.589	0.679	0.086	-0.428	0.288	0.663	0.092	-0.227	0.362
	CnCirA4	8	2	0.525	0.288	0.149	1.590	0.095	0.325	0.150	1.332	0.133	0.332	0.149	1.300	0.135
	CnCirC11	7	2	0.440	0.316	0.150	0.823	0.358	0.350	0.145	0.616	0.439	0.338	0.143	0.711	0.381
	CnCirD8	7	6	0.868	0.795	0.059	1.232	0.055	0.829	0.041	0.964	0.183	0.821	0.046	1.013	0.140
	CnCirE11	8	5	0.792	0.704	0.089	0.991	0.169	0.761	0.059	0.527	0.41	0.744	0.069	0.687	0.318
	CnCirG4	8	1	0.000	MML	MML	MML	MML	MML	MML	MML	MML	MML	MML	MML	MML
	CnCirE4	8	6	0.800	0.778	0.068	0.321	0.491	0.816	0.044	-0.377	0.289	0.806	0.053	-0.105	0.365
	CnCirE7	7	4	0.714	0.640	0.104	0.721	0.306	0.691	0.085	0.278	0.535	0.678	0.086	0.420	0.452
	CnCirS8	8	2	0.458	0.292	0.149	1.115	0.257	0.331	0.152	0.839	0.369	0.325	0.148	0.903	0.318
	CnCirS1	8	3	0.625	0.481	0.138	1.039	0.182	0.556	0.110	0.621	0.343	0.541	0.120	0.701	0.303
	CnCirS12	8	8	0.917	0.868	0.036	1.371	0.041	0.886	0.026	1.200	0.092	0.882	0.027	1.274	0.068
	CnCirS7	8	6	0.767	0.773	0.069	-0.096	0.385	0.816	0.044	-1.114	0.134	0.807	0.050	-0.804	0.192
	CnCirB5	8	4	0.692	0.610	0.117	0.700	0.334	0.683	0.078	0.112	0.590	0.663	0.089	0.323	0.510
	CnCirS5	8	3	0.700	0.489	0.138	1.533	0.021	0.552	0.114	1.304	0.052	0.541	0.120	1.324	0.031
	CnCirR11	7	3	0.626	0.504	0.134	0.908	0.204	0.556	0.115	0.528	0.350	0.550	0.120	0.639	0.297
	CnCirA8	7	4	0.692	0.641	0.106	0.484	0.455	0.693	0.079	-0.005	0.429	0.683	0.081	0.117	0.491
	mCnCir119	6	4	0.758	0.667	0.097	0.939	0.190	0.710	0.073	0.649	0.324	0.699	0.080	0.724	0.306
	mCnCir47	5	5	0.844	0.796	0.055	0.878	0.270	0.817	0.044	0.636	0.401	0.812	0.046	0.698	0.365
	mCnCir86	5	6	0.889	0.862	0.036	0.735	0.463	0.875	0.029	0.493	0.621	0.873	0.031	0.497	0.610

Population	Locus	n	Ko	He	IAM				SMM				TPM			
					Heq	S.D	DH/SD	Prob	Heq	S.D	DH/SD	Prob	Heq	S.D	DH/SD	Prob
Low yielding	CnCirE12	9	2	0.425	0.276	0.151	0.982	0.280	0.331	0.151	0.624	0.419	0.320	0.157	0.670	0.403
	CnCirA9	8	3	0.342	0.486	0.140	-1.035	0.274	0.557	0.111	-1.947	0.098	0.540	0.117	-1.700	0.113
	CnCirB12	8	3	0.708	0.488	0.138	1.598	0.011	0.551	0.115	1.368	0.015	0.540	0.121	1.394	0.020
	CnCirC3	9	6	0.843	0.753	0.077	1.168	0.063	0.806	0.043	0.867	0.196	0.795	0.053	0.917	0.145
	CnCirA3	9	2	0.111	0.274	0.155	-1.057	0.366	0.324	0.149	-1.430	0.211	0.310	0.153	-1.303	0.249
	CnCirC7	8	3	0.608	0.490	0.138	0.855	0.247	0.553	0.113	0.488	0.388	0.543	0.120	0.545	0.363
	CnCirH4	9	3	0.216	0.471	0.142	-1.802	0.119	0.539	0.121	-2.665	0.033	0.531	0.123	-2.569	0.037
	CnCirE2	9	10	0.902	0.904	0.027	-0.062	0.435	0.917	0.019	-0.754	0.220	0.913	0.021	-0.529	0.279
	CnCirF2	9	1	0.000	MML	MML	MML	MML	MML	MML	MML	MML	MML	MML	MML	MML
	CnCirH7	9	4	0.654	0.596	0.120	0.485	0.382	0.674	0.083	-0.252	0.322	0.654	0.094	-0.008	0.406
	CnCirB6	9	2	0.366	0.283	0.155	0.533	0.400	0.323	0.152	0.283	0.494	0.321	0.154	0.290	0.500
	CnCirE10	9	3	0.451	0.465	0.143	-0.094	0.435	0.545	0.114	-0.818	0.198	0.525	0.130	-0.574	0.275
	CnCirG11	9	4	0.529	0.599	0.121	-0.573	0.255	0.663	0.090	-1.482	0.078	0.650	0.095	-1.272	0.114
	CnCirC12	9	3	0.216	0.469	0.148	-1.714	0.137	0.535	0.122	-2.614	0.033	0.528	0.122	-2.551	0.043
	CnCirA4	8	3	0.567	0.487	0.136	0.583	0.397	0.555	0.110	0.102	0.443	0.535	0.122	0.258	0.540
	CnCirC11	8	3	0.242	0.481	0.141	-1.699	0.140	0.551	0.118	-2.627	0.046	0.536	0.125	-2.357	0.062
	CnCirD8	9	7	0.850	0.807	0.058	0.731	0.278	0.845	0.035	0.147	0.554	0.837	0.040	0.313	0.493
	CnCirE11	9	4	0.784	0.600	0.118	1.557	0.006	0.667	0.088	1.340	0.014	0.654	0.090	1.451	0.016
	CnCirG4	9	2	0.111	0.275	0.153	-1.075	0.344	0.322	0.153	-1.377	0.226	0.309	0.155	-1.281	0.268
	CnCirE4	8	6	0.750	0.772	0.073	-0.303	0.326	0.814	0.044	-1.462	0.093	0.805	0.050	-1.112	0.142
	CnCirE7	9	4	0.686	0.597	0.118	0.751	0.267	0.671	0.086	0.178	0.498	0.653	0.095	0.353	0.447
	CnCirS8	8	1	0.000	MML	MML	MML	MML	MML	MML	MML	MML	MML	MML	MML	MML
	CnCirS1	8	2	0.325	0.293	0.293	0.211	0.476	0.334	0.152	-0.060	0.606	0.333	0.152	-0.053	0.597
	CnCirS12	8	6	0.842	0.774	0.774	1.004	0.143	0.814	0.042	0.643	0.314	0.805	0.049	0.736	0.268
	CnCirS7	8	2	0.500	0.303	0.303	1.262	0.212	0.333	0.152	1.100	0.255	0.329	0.149	1.146	0.241
	CnCirB5	7	3	0.484	0.501	0.501	-0.137	0.468	0.566	0.115	-0.716	0.266	0.548	0.118	-0.548	0.318
	CnCirS5	9	3	0.582	0.463	0.463	0.812	0.270	0.545	0.120	0.308	0.494	0.528	0.128	0.420	0.431
	CnCirR11	8	3	0.658	0.490	0.490	1.222	0.106	0.561	0.113	0.863	0.247	0.543	0.119	0.968	0.186
	CnCirA8	8	5	0.767	0.709	0.709	0.653	0.356	0.759	0.062	0.118	0.48	0.744	0.072	0.318	0.507
	mCnCir119	7	4	0.571	0.641	0.641	-0.685	0.240	0.695	0.080	-1.547	0.085	0.682	0.084	-1.313	0.131
	mCnCir47	7	3	0.582	0.510	0.510	0.558	0.403	0.564	0.108	0.167	0.571	0.557	0.115	0.223	0.562
	mCnCir86	8	6	0.808	0.776	0.776	0.463	0.438	0.814	0.044	-0.134	0.3880	0.808	0.050	0.015	0.431

Table 5

Number of loci showing heterozygosity excess or deficiency in high yielding coconut population under the three mutation models IAM, TPM and SMM.

Test	Mutation models		
	IAM	TPM	SMM
Sign test			
H excess-expected # loci	19.0200	19.1100	19.1500
H excess-observed # loci	25.0000	20.0000	19.0000
H deficiency-observed # loci	7.0000	12.0000	13.0000
Probability Heq>He	0.0207	0.4488	0.5451
Standardized differences test			
T2	2.9140	0.5800	-0.3450
Probability Heq>He	0.0017	0.2809	0.3650
Wilcox rank test			
Probability (two tails for H excess and deficiency)	0.0022	0.3217	0.8033

Table 6

Number of loci showing heterozygosity excess or deficiency in medium yielding coconut population under the mutation models IAM, TPM and SMM

Test	Mutation models		
	IAM	TPM	SMM
Sign test			
H excess-expected # loci	17.7900	18.2200	18.5800
H excess-observed # loci	27.0000	23.0000	22.0000
H deficiency-observed # loci	4.0000	8.0000	9.0000
Probability Heq>He	0.0003	0.0556	0.1409
Standardized differences test			
T2	4.0340	2.1950	1.4140
Probability Heq>He	0.0000	0.0141	0.0786
Wilcox rank test			
Probability (two tails for H excess and deficiency)	0.0000	0.0034	0.0233

Table 7
Number of loci showing heterozygosity excess or deficiency in the low yielding population under the three mutation models IAM, TPM and SMM.

Test	Mutation models		
	IAM	TPM	SMM
Sign test			
H excess-expected # loci	16.9400	17.3600	17.2800
H excess-observed # loci	18.0000	16.0000	15.0000
H deficiency-observed # loci	12.0000	14.0000	15.0000
Probability Heq>He	0.4216	0.3720	0.2533
Standardized differences test			
T2	0.9480	-1.3060	-2.0610
Probability Heq>He	0.1715	0.0958	0.0197
Wilcox rank test			
Probability (two tails for H excess and deficiency)	0.3285	0.5159	0.2286

CONCLUSION

In this study three coconut populations-high, medium and low yielding were tested for a genetic bottleneck under three mutation models (IAM, TPM, and SMM). Thirty two SSR primer pairs were used for the analysis. High and medium yielding populations showed genetic bottleneck as they found to be experiencing significant heterozygosity excess. But the low yielding population with a heterozygosity deficiency is seen to be undergoing rapid population expansion. As such these mutational models (IAM, TPM and SMM) were powerful tools in understanding genetics and evolution.

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