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RESEARCH ARTICLE

Genetic Diversity in Drumstick of Andaman Islands and Their Relatedness with Probable Introduction Sites from Mainland India

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Abstract The study investigated genetic diversity in *Moringa oleifera* Lam. samples (63) from nine islands in Andaman and Nicobar Islands (India) and their relatedness with samples (5) from their probable source regions in mainland India. The polymerase chain reaction analysis with polymorphic random fragment polymorphic DNA (RAPD) (5) and inter simple sequence repeat (ISSR) (20) markers generated 987 and 4190 amplicons, respectively. The RAPD analysis resulted into two main and eight sub-clusters and ISSR markers generated two main and five sub-clusters. Extent of similarity in 68 samples was revealed to be 49% by RAPDs to 53% by ISSR markers. Average polymorphism information content (PIC) value ranged from 0.47 to 0.50 for the studied markers. Maximum collections from the islands were grouped along with samples from Tamil Nadu, Andhra Pradesh and Kerala which suggest high level of genetic relatedness. The information on influence of 'isle factor' in genetic divergence of introduced drumsticks is useful for continuance of studies on genetic changes for adaptation.

Keywords Drumstick · Genetic diversity · Molecular markers · Andaman Islands

Significance Statement The study revealed 47–51% genetic diversity in 68 drumstick collections from Andaman Islands (63) and mainland India (5). Distinct lines were noticed for dispersal of drumstick materials. 'Isle effect' helped in genetic changes for adaptation in stressful island situation hence these genetic changes can explored for breeding use.

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Introduction

Moringa oleifera Lam. (drumstick) is a highly nutritive and multipurpose plant grown for fresh leaves, green fruits and flowers. These parts are used for vegetable and other local food preparations while their dry powders are ingredients in different fortified items. Drumstick originated from Himalayan tract in north-western India [1] and spread all across the Indian sub-continent, East Asia, China, Africa and other parts of the world. The leaves and fruits are good source of iron, calcium, vitamin A, vitamin B and amino acids [2]. It is useful source in food based approach to fight against micronutrient and vitamin deficiencies in rural and tribal communities in tropical regions [3]. It has got medicinal properties to improve immune system, stimulate cardiac and circulatory system and treat ascites, rheumatism and bites [4].

The family Moringaceae is a monogeneric and genus '*Moringa*' has more than 13 species, of them *Moringa oleifera* Lam. (syn. *M. pterygosperma* Gaertn.) and *M. concanensis* Nimmo occur in India [1]. *Moringa oleifera* is a predominant species in India and sporadic breeding efforts resulted few improved local types. For systematic conservation and breeding, it is prerequisite to know the extent of genetic diversity viz *a* viz relatedness in genetic resources, hence efforts were made in India [5–8]. But, till

date none of the reports included drumstick samples from biodiversity rich Andaman and Nicobar Islands (India) which could have provided new source of certain climate resilient or stress tolerant gene(s) and also throw light on adaptive changes in evolutionary process under the 'isle effect'.

The archipelago of Andaman and Nicobar Islands in Bay of Bengal is located amidst of two hot spots of biodiversity of Indo–Myanmarese–Thai and Malaysia–Indonesia [9]. This archipelago in Indian Ocean is situated around 1100–1300 km away from continental India and also fragmented from each other by sea. The islands have typical tropical maritime climate with prolonged rainy season spanning from May to December and short dry twentperiod from January to April. Minimum and maximum temperature in islands ranges from 25 to 32 °C, relative humidity from 65 to 92% and wind movement from 15 to 55 km per hour and brighter sunlight with high ultraviolet rays [10]. The tropical climatic factors along with geographical speciation favours comparatively rapid evolution [11]. Since, its introduction from mainland India and adjoining regions during nineteenth and twentieth century, the drumstick has been distributed to all across the inhabited islands. Seed multiplication and cross-pollination behaviour could be the factors for genetic changes in drumstick which favoured its acclimatization in local situation. But, no systematic study was done on available diversity of drumstick in the islands and also not report exist to establish the level of genetic relatedness with source centres in mainland India which otherwise could help in conservation and improvement for stress tolerance traits in adapted germplasm.

The DNA markers used for dissecting the diversity in drumstick were amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR), inter simple sequence repeats (ISSR) and random amplified polymorphic DNAs (RAPD). The RAPDs and ISSRs are still very commonly used tools for diversity analysis in less investigated or orphan plant species [12–14]. Further, both RAPD and ISSR markers are in routine use in ecological, evolutionary, phylogenic and genetic diversity studies in plant sciences [15]. Therefore, the study aimed to assess the genetic diversity of drumstick in Andaman and Nicobar Islands and its relatedness with samples from mainland India.

Material and Methods

Sample Collection

Fresh healthy leaf samples of drumstick comprised of 63 representative collections of *Moringa oleifera* Lam. from random sites in nine inhabited islands of Andaman and Nicobar Islands and five representative samples from Tamil Nadu, Kerala, Andhra Pradesh, Karnataka and Maharashtra in mainland India (Table 1; Fig. 1). Mature green fruits were also collected from these sites and five random fruits were observed for morphological parameters such as fruit length (cm), fruit diameter (cm), fruit weight (g), number of seeds per fruits, fresh seed weight (g/fruit) and pulp weight (g/fruit) using standard measuring scale and weighing machine. Percentage recoveries of fresh seeds and fresh pulp from fruits were calculated from the observed values.

PCR Analysis

The genomic DNA of 68 collections of drumstick was extracted by CTAB method [16] with slight modifications. Purified DNA was visualized under (UV) light after electrophoresis using 0.8% (w/v) agarose gel and quantified using DNA ladder and UV spectrophotometer (ELICO Ltd, Hyderabad, India). The DNA was diluted in autoclaved ddH_2O and stored at − 20 °C for further use in polymerase chain reaction (PCR) analysis. Forty-eight ten-mer-oligonucleotide RAPD primers of Operon series (Operon technologies, Alameda, CA, USA) and 68 ISSR primers (Clonitec Inc., USA) were surveyed in genomic DNA of drumstick samples. Only five RAPDs and 20 ISSRs were polymorphic. The PCR analysis for RAPD and ISSR was performed in thermal cycler (Bio-Rad Laboratories, Inc., CA, USA) in a final volume of 20 µl as described by Singh et al. [14]. The PCR reaction mixture contains 1 µl template DNA (30 ng/µl), 2 µl of dNTP mix containing 100 µM of each of the four deoxynucleotide triphosphate, 2 µl of decanucleotide primer, 1.6 µl $MgCl_2$, 2 µl *Taq* buffer (10 mM Tris–HCl pH 9.0, 50 mM KCl), 0.5 U *Taq* DNA polymerase (Bangalore Genei, Bangalore, India) and 10.9 µl millipore water (Heal Force Water Purification System, Sanghai Canrex Analytic Instruments Co. Ltd, Shangai, China). The PCR products from both RAPD and ISSR analysis were resolved on 1% agarose gel prepared in 1X TAE buffer containing 0.5 µg/ml of the ethidium bromide (10 mg/ml). Amplified PCR product (5 µl) mixed with 6X bromophenol blue (5 µl) and electrophoresis was performed on at 100 V for 2.5 h. Gel photography was done with UVP MultiDoc-IT Digital Imaging System (UVP LCC, California). Amplicon size on agarose gel was established by comparing with reference bands of 100 bp DNA ladders.

Statistical Analysis

The bands from PCR analysis of 68 collections were scored as '1' for presence and '0' for absence and created a binary

Table 1 Details of samples of drumstick and their morphological traits

Code No.	Accession	Locations	Fruit length (cm)	No. of seeds	Seed weight (g/ fruit)	Pulp Weight (g/ fruit)	Fruit diameter (cm)	Av. fruit weight (g)	Fresh seed recovery (%)	Fresh pulp recovery (%)
1	CARI-1	Garacharma, SA	38.0	21.0	18.3	67.5	1.5	135.7	13.5	49.7
2	CARI-2	Garacharma, SA	25.3	17.0	15.8	65.2	1.8	132.5	11.9	49.2
3	CARI-3	Garacharma, SA	18.5	12.0	10.0	45.2	2.0	120.0	8.3	37.7
4	Garacharma-1	Garacharma, SA	24.1	16.5	12.0	40.0	1.9	132.0	9.1	30.3
5	Hutbay-1	Little Andaman, SA	26.0	16.0	11.8	64.8	1.7	131.6	9.0	49.2
6	Hutbay-2	Little Andaman, SA	30.2	18.0	17.2	62.0	1.5	140.2	12.3	44.2
7	Hutbay-3	Little Andaman, SA	26.2	15.5	14.0	65.0	1.6	135.0	10.4	48.1
8	Diglipur-1	Diglipur, NA	30.0	18.2	16.5	68.0	1.5	138.5	11.9	49.1
9	Garacharma-2	Garacharma, SA	25.0	15.0	12.8	66.0	1.4	110.0	11.6	60.0
10	Sipighat-1	Sipighat, SA	28.0	16.2	14.0	67.2	1.7	120.0	11.7	56.0
11	Kadamtala-1	Kadamtala, MA	31.0	17.0	15.0	67.0	1.4	115.0	13.0	58.3
12	Bambooflat-1	Bambooflat, SA	28.0	16.5	14.0	65.0	1.5	112.0	12.5	58.0
13	Dollygunj-1	Dollygunj, SA	30.0	18.0	15.0	70.0	1.0	120.0	12.5	58.3
14	Calicut-1	Calicut, SA	38.0	20.0	16.0	75.0	0.9	125.0	12.8	60.0
15	Mayabunder-1	Mayabunder, MA	35.0	17.0	14.5	73.0	1.8	122.0	11.9	59.8
16	Diglipur-1	Diglipur, NA	34.2	16.0	13.5	75.0	1.2	114.0	11.8	65.8
17	Diglipur-2	Diglipur, NA	30.0	18.0	14.0	71.5	1.3	120.0	11.7	59.6
18	Diglipur-3	Diglipur, NA	32.0	15.0	12.0	70.8	1.5	130.0	9.2	54.5
19	Rangat-1	Rangat, MA	38.2	16.0	12.5	65.8	0.9	140.0	8.9	47.0
20	Havelock-1	Havelock, SA	34.0	14.8	13.2	65.0	1.8	142.0	9.3	45.8
21	Havelock-2	Havelock, SA	31.0	14.0	10.0	55.0	1.6	128.0	7.8	43.0
22	Collinpur-1	Collinpur, SA	28.0	14.0	12.0	50.0	1.5	125.0	9.6	40.0
23	Wandoor-1	Wandoor, SA	32.0	13.0	10.5	47.8	1.7	98.5	10.7	48.5
24	Neil Island-1	Neil Island, SA	25.0	10.5	9.6	48.0	1.6	90.0	10.7	53.3
25	Neil Island-2	Neil Island, SA	30.0	12.0	9.6	46.2	1.8	95.8	10.0	48.2
26	Nicobar-1	Car Nicobar, NC	25.0	12.0	10.0	55.0	2.0	90.0	11.1	61.1
27	Nicobar-2	Car Nicobar, NC	23.0	10.0	9.5	40.0	2.2	105.0	9.0	38.1
28	Campbell Bay-1	Campbell Bay, GN	35.0	13.0	11.0	60.0	1.4	115.0	9.6	52.2
29	Brichganj	Brichganj, SA	27.0	14.0	10.0	45.0	2.1	90.0	11.1	50.0
30	Dilanipur	Dilanipur, SA	40.0	17.5	16.0	50.0	1.4	130.0	12.3	38.5
31	Srikakulam	Andhra Pradesh	38.0	16.4	15.0	44.0	1.3	120.0	12.5	36.7
32	Baratang	Baratang, SA	36.0	15.0	12.8	42.0	1.6	122.0	10.5	34.4
33	Ferarganj	Ferarganj, SA	35.0	16.5	15.0	55.0	1.7	105.0	14.3	52.4
34	Guptapara	Guptapara, SA	30.0	14.3	12.5	44.0	1.5	110.0	11.4	40.0
35	Nagarcoil	Tamil Nadu	45.0	20.0	20.5	68.0	0.9	160.0	12.8	42.5
36	*Thiruvananthapuram*	Kerala	42.0	19.5	18.0	55.0	1.1	142.0	12.7	38.7
37	Shaitankari	Shaitankari, SA	31.0	20.0	14.2	50.0	1.2	136.0	10.4	36.8
38	Bathupasthi	Bathubasthi, SA	34.0	14.5	12.0	53.0	1.2	110.0	10.9	48.2
39	Kamorta	Kamorta, NWI	25.0	9.8	8.0	40.0	2.0	95.0	8.4	42.1
40	Mayabandar-2	Mayabunder, MA	31.0	15.0	12.2	56.0	0.8	115.0	10.6	48.7

Table 1 continued

Code No.	Accession	Locations	Fruit length (cm)	No. of seeds	Seed weight (g/ fruit)	Pulp Weight (g/ fruit)	Fruit diameter (cm)	Av. fruit weight (g)	Fresh seed recovery (%)	Fresh pulp recovery (%)
41	Rangat-2	Rangat, MA	25.2	15.0	12.0	45.0	1.3	100.0	12.0	45.0
42	Hutbay-4	Little Andaman, NA	23.0	10.0	8.5	40.0	1.5	84.0	10.1	47.6
43	Diglipur-4	Diglipur, NA	37.0	15.0	12.8	55.0	1.3	110.0	11.6	50.0
44	Garacharma-3	Garacharma, SA	39.0	20.0	18.0	50.2	2.0	132.0	13.6	38.0
45	Sipighat-2	Sipighat, SA	42.0	20.0	15.8	60.0	1.7	131.0	12.1	45.8
46	Pursakattai	Sipighat, SA	39.0	17.0	14.8	52.0	1.6	125.0	11.8	41.6
47	Chholdhari-2	Chholdhari, SA	40.0	20.0	18.5	55.0	1.3	140.0	13.2	39.3
48	Burmanallah	Burmanallah, SA	28.0	12.0	10.0	42.0	1.2	105.0	9.5	40.0
49	Jirgatang	Jirgatang, SA	35.0	15.0	12.8	58.0	1.2	125.0	10.2	46.4
50	Middle point	Middle point, SA	28.0	13.0	11.5	55.0	1.5	122.0	9.4	45.1
51	Kadamtala-2	Kadamtala, MA	30.0	15.0	12.0	50.0	1.6	122.0	9.8	41.0
52	Bambooflat-2	Bambooflat, SA	28.0	13.0	10.0	40.0	1.7	95.0	10.5	42.1
53	Dollygunj-2	Dollygunj, SA	42.0	22.0	18.0	61.0	1.2	138.0	13.0	44.2
54	Ranchibasthi	Ranchibasti, SA	34.0	16.0	13.0	54.0	1.4	120.0	10.8	45.0
55	Campbell Bay	Campbell Bay, GN	28.0	13.0	11.2	52.0	1.4	95.0	11.8	54.7
56	Chidiyatapu	Chidiyatapu, SA	30.0	15.0	10.8	53.0	1.6	100.0	10.8	53.0
57	Calicut-2	Calicut, SA	34.0	14.0	12.2	46.0	1.4	115.0	10.6	40.0
58	Mayabunder-2	Mayabunder, MA	36.0	16.0	15.0	53.0	1.3	105.0	14.3	50.5
59	Havelock-3	Havelock, SA	38.0	17.0	15.6	65.0	1.5	140.0	11.1	46.4
60	Collinpur-2	Collinpur, SA	31.0	15.0	12.3	53.0	1.4	105.0	11.7	50.5
61	Wandoor-2	Wandoor, SA	28.0	14.0	11.0	45.0	2.0	90.0	12.2	50.0
62	Mittakhadi	Mittakhadi, SA	30.0	12.0	10.5	40.0	1.6	95.0	11.1	42.1
63	Manpur	Manpur, SA	34.0	15.0	12.5	50.0	1.4	120.0	10.4	41.7
64	Nimbutala	Nimbutala, SA	33.0	18.2	14.5	55.0	1.5	115.0	12.6	47.8
65	Neil Island-3	Neil Island, SA	40.0	20.0	18.5	50.3	1.6	124.0	14.9	40.6
66	Ferrargunj	Ferrargunj, SA	34.0	18.0	15.0	46.0	1.5	115.0	13.0	40.0
67	Maharashtra-I	Maharashtra	42.0	18.5	18.0	54.5	1.4	138.0	13.0	39.5
68	Karanataka-1	Karnataka	37.0	20.0	17.0	60.0	1.2	140.0	12.1	42.9
		Mean	32.2	15.8	13.4	55.2	1.5	118.6	11.3	46.8
		STDEV	5.7	2.8	2.8	9.9	0.3	16.5	1.5	7.4
		Range	18.5–45.0	9.8–22.0	8.0–20.5	40.0–75.0	0.8–2.2	84.0–160.0	7.8–14.9	30.3–65.8

SA South Andaman, *NA* North Andaman, *MA* Middle Andaman, *NC* Nicobar, *GN* Great Nicobar, *NWI* Nancowry Islands

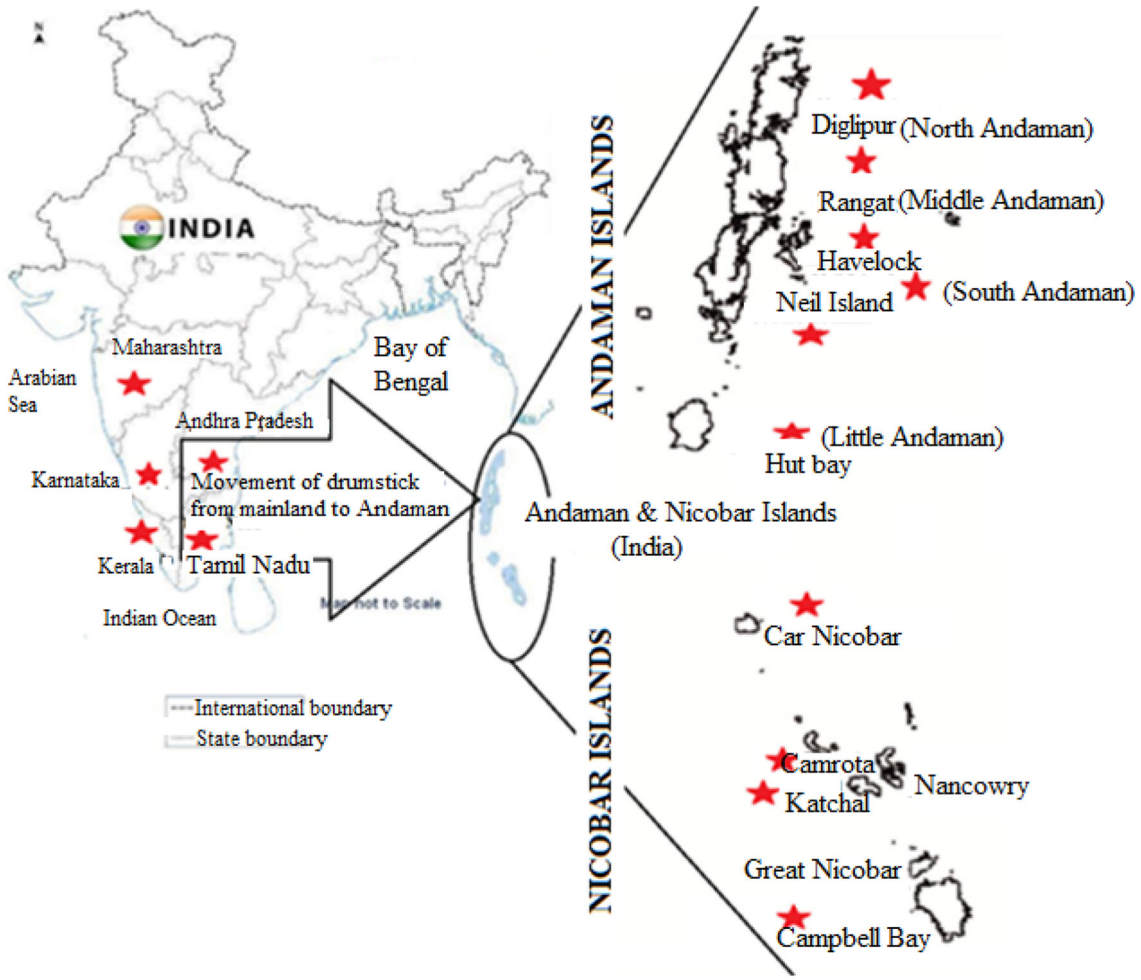

Fig. 1 Drumstick sample collection locations in the Andaman and Nicoabr Islands and mainland India

data matrix. The matrix subjected to similarity analysis by Jaccard's coefficient and employed for clustering the germplasm by sequential hierarchical agglomerative non-overlapping (SHAN) based un-weighted pair group method with arithmetic averages (UPGMA) method using NTSYS-pc, version 2.02 [17]. The polymorphism information content (PIC) was calculated using formula: $PICi = 2fi(1 - fi)$, where, PICi is the polymorphic information content of ith marker, fi is the frequency of the marker band present, $(1 - fi)$ is the frequency of marker band absent [17, 18]. Percentage of expressivity, average number of bands by a marker, range of amplicon size and percent polymorphism were calculated using Microsoft EXCEL software.

Results and Discussion

The PCR analysis of 48 RAPD and 68 ISSR primers with genomic DNA of 68 samples of drumstick resulted into 5 and 20 polymorphic markers, respectively (Table 2). In total, 987 amplicons were generated in 68 samples by five RAPDs and 4190 amplicons by 20 ISSRs, of them polymorphic markers were 230 and 2375, respectively. The ISSR markers showed higher polymorphism (38.1%) than RAPD markers (21.5%). However, it had wide variation for individual markers which ranged from 17.6% (OPX 8) to 25.8% (OPX 15) for RAPD and 12.8% (IISR 23) to 50.2% (UBC 851) for ISSR markers. Number of bands generated in 68 samples by an individual RAPD marker in total 68 samples was ranged from 130 (OPX-08) to 330 (OPN-03) and with ISSR markers, it varied from 109 (UBC855) to 408 (UBC809). Amplicon size from RAPD analysis was ranged from 0.2 to 1.0 kb while it was 0.2–1.5 kb from ISSR analysis. Amplicons of 100–200 bp size were observed but not included in analysis due to their poor reproducibility. Individually, RAPD primer OPN3 generated maximum bands (9.0) while minimum by OPN 15 (6.0). Polymorphic bands from RAPD analysis ranged from 5.0 (OPN-15) to 8.0 (OPN-03). In ISSR analysis, number of amplicons by an individual primer ranged from

Table 2 Description of RAPD and ISSR markers and their PCR analysis for drumstick

Primer	Sequence (5′–3′)	Total bands (no.)	Polymorphic bands (no.)	Total scorable bands (no.)	Avg. no. of amplicon	Amplicon size (kb)	% of polymorphism	PIC value
OPA-02	TGCCGAGCTG	7	6	211.0	30.1	0.2–1.0	18.5	0.5
OPN-03	GGTACTCCCC	9	8	330.0	36.7	0.2–1.0	22.0	0.5
OPN-05	ACTGAACGCC	9	8	160.0	17.8	0.2–1.0	24.0	0.4
OPX-8	CAGGGGTGGA	8	7	130.0	16.3	0.2–1.0	17.6	0.4
OPX-15	CAGACAAGCC	6	5	156.0	26.0	0.2–1.0	25.3	0.5
UBC836	$(AG)_8$ YA	10	7	383.0	38.3	0.2–1.5	14.6	0.5
UBC857	$(AC)_8$YG	5	4	125.0	25.0	0.2–0.5	44.0	0.4
ISSR 24	$(GA)_6$CC	5	5	170.0	34.0	0.2–0.6	41.2	0.5
UBC851	$(GT)_8$YT	6	5	209.0	34.8	0.2–0.5	50.2	0.5
UBC809	$(AG)_8$G	9	5	408.0	45.3	0.2–1.0	42.9	0.5
ISSR23	$(GA)_6$GG	6	3	296.0	49.3	0.2–0.8	12.8	0.4
ISSR22	$(CA)_6$AC	6	4	224.0	37.0	0.2–0.55	38.4	0.5
UBC840	$(GA)_8$YT	5	4	211.0	42.2	0.2–0.6	37.4	0.5
ISSR18	$(AAC)_5$	6	5	144.0	24.0	0.2–0.6	41.0	0.4
ISSR14	(GACA)4	5	4	179.0	35.8	0.2–0.7	34.1	0.4
UBC827	$(AC)_8$G	8	6	231.0	28.9	0.2–0.7	31.6	0.5
UBC812	$(GA)_8$A	6	3	268.0	44.7	0.3–0.7	40.3	0.5
UBC841	$(GA)_8$YC	5	3	162.0	32.4	0.2–0.7	46.9	0.5
UBC866	$(CTC)_6$	6	3	205.0	34.2	0.3–0.8	38.0	0.5
UBC810	$(GA)_8$T	6	3	188.0	31.3	0.2–0.8	43.6	0.5
UBC845	$(CT)_8$RG	6	6	111.0	18.5	0.2–0.7	37.9	0.4
UBC855	$(AC)_8$YT	5	4	109.0	21.8	0.2–0.5	42.2	0.4
UBC811	$(GA)_9$C	7	4	295.0	42.1	0.2–0.7	37.3	0.5
UBC808	$(AG)_8$C	5	4	138.0	27.6	0.2–0.45	46.4	0.5
UBC868	$(CCG)_6$	3	3	134.0	44.7	0.3–0.7	41.0	0.5
	Range	3–10	3–8	109–408	16.349.3	0.2–1.5	12.8–50.2	0.4–0.5

3.0 (UBC 868) to 10.0 (UBC 836) of them, polymorphic bands ranged from 3.0 to 7.0. Level of polymorphism at individual RAPD and ISSR markers ranged from 83.3 to 88.8% and 50 to 100%, respectively. Number of alleles varied greatly with all markers having average PIC value of 0.4–0.5 for both RAPD and ISSR primers. Level of polymorphism in RAPD and ISSR markers in drumstick samples indicates fair extent of variability in the islands [Supplementary Figs. 1(a, b), 2(a, b)], respectively. The findings confirm indicative reports of diversity in drumstick reported by Abraham et al. [19] alongwith other horticultural crops in these islands. Singh et al. [14] reported significant level of diversity in introduced plant species in the islands which might occurred due to natural mutations favoured by geographical speciation, selection pressure by biotic and abiotic stresses and to some extent by incidence of ultra-violet rays in tropical location of islands. The RAPD and ISSR markers are still in routine use in ecological, evolutionary, phylogenetic and genetic studies of plant sciences [5–7], hence the observations appears more useful for drumstick conservation and improvement.

RAPD Analysis

Cluster analysis using Jaccard's genetic similarity coefficient showed significant level of genetic variation within 68 samples of drumstick. No separate clusters were observed for samples from two geographical regions. However, relative proximity was seen within the sample groups from mainland as well as the islands. Variation in the samples could be attributed by the prevalent diversity in drumstick in source states in mainland India [5–8, 20] and also within the islands [19]. Similarity, coefficient for RAPD analysis varied from 0.49 to 0.97, with Campbell Bay-2 and Chidiyatapu-1 collections found to have highest genetic similarity while CARI-1 (from Karnataka) had least similarity with other collections (Fig. 2). Dendrogram from RAPD analysis revealed two main-clusters at a coefficient of 0.56, wherein cluster-1 had 62 samples and cluster-2 had only five samples. The CARI-1 formed mono-genotype cluster with a coefficient value of 0.49. The main-cluster-1 had three sub-clusters with 17 in sub-cluster-1, 35 in sub-cluster-2, and three in sub-cluster-3

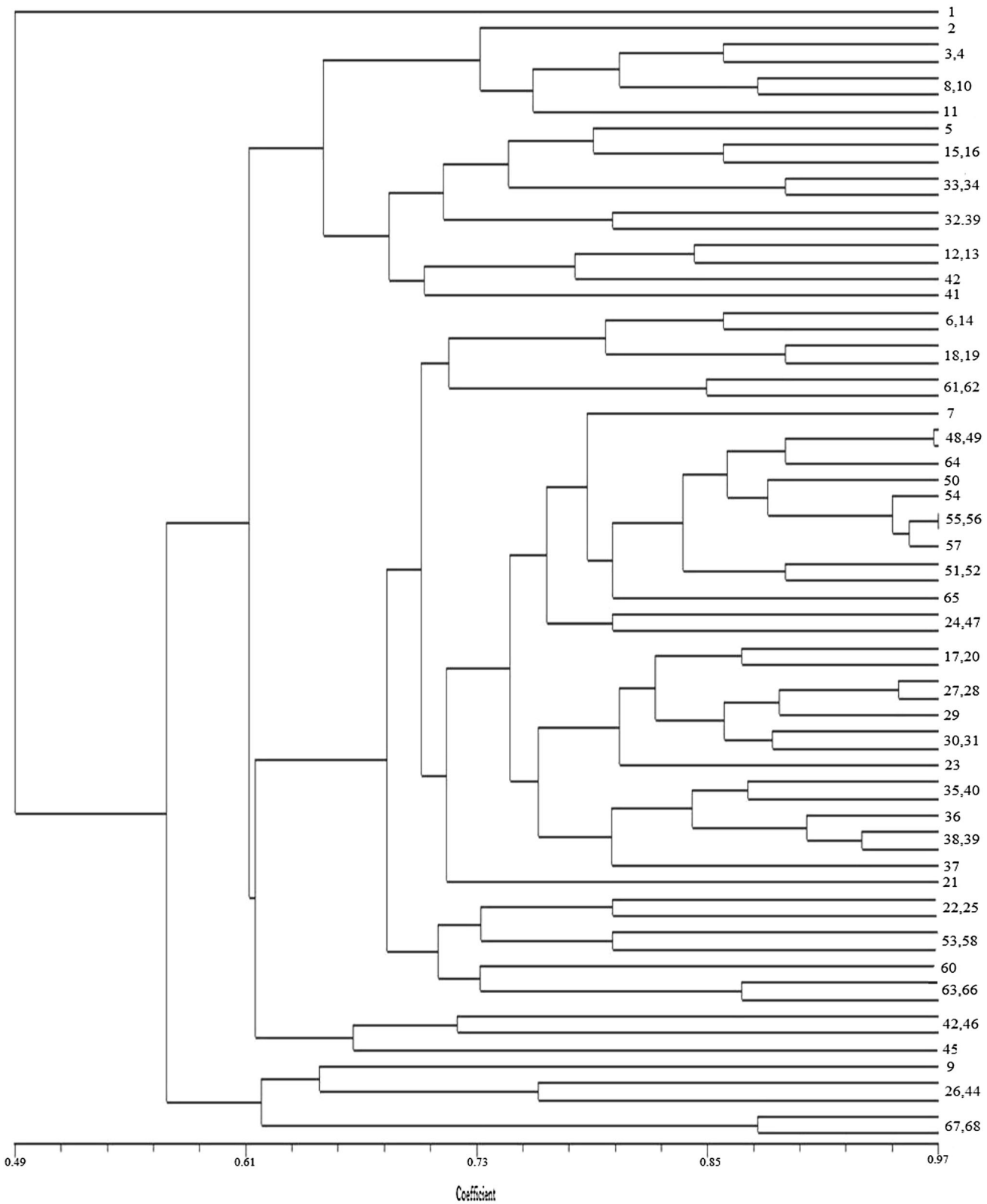

Fig. 2 UPGMA cluster analysis of 68 accessions of drumstick using RAPD markers (samples details in Table 1)

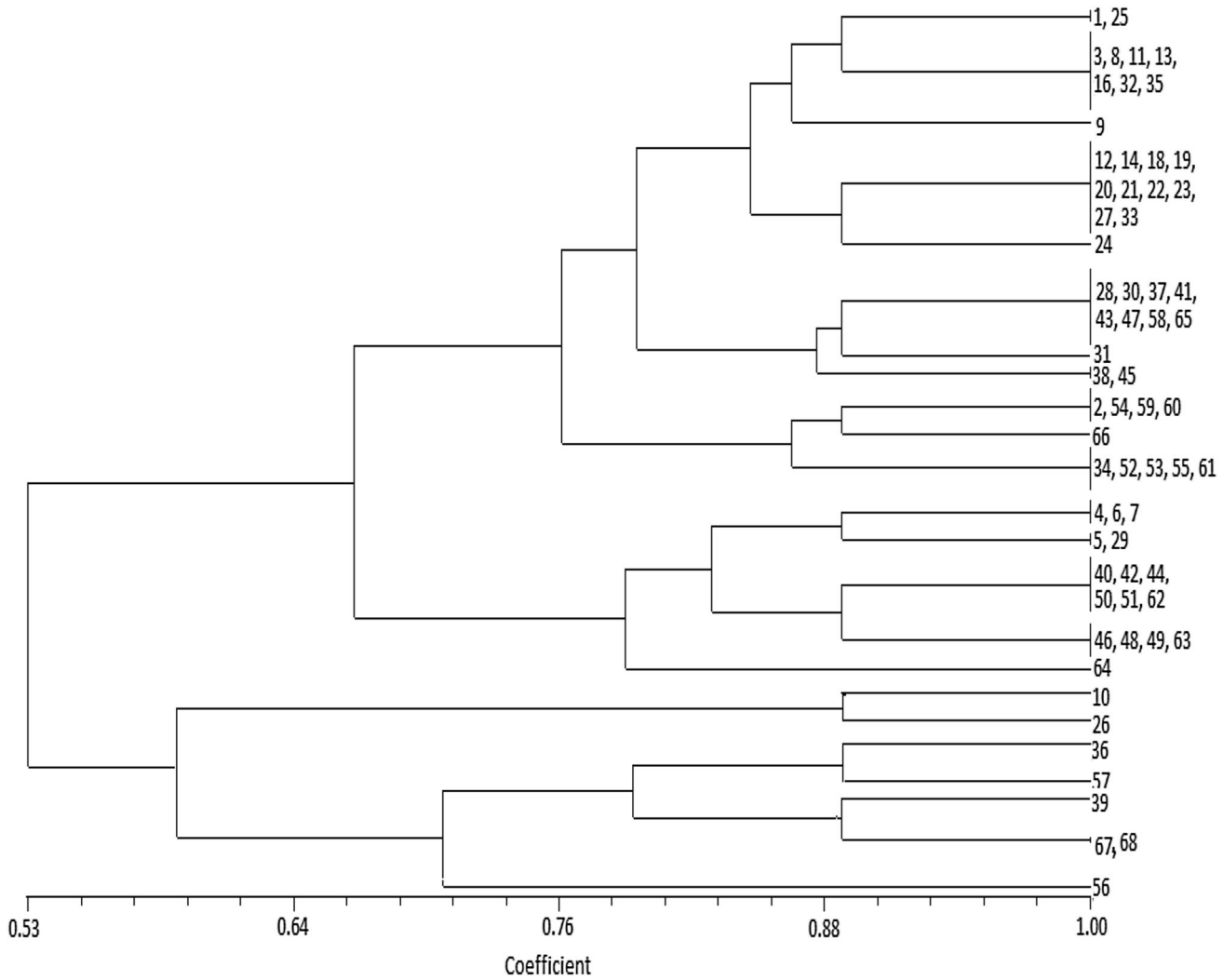

Fig. 3 UPGMA cluster analysis of 68 accessions of Drumstick using ISSR markers (samples details in Table 1)

samples with similarity value in the range of 0.65–0.88, 0.68–0.97 and 0.65–0.70, respectively.

The main-cluster-2 had five samples with similarity value of 0.61–0.87. These five samples were from Maharashtra, Karnataka, Garacharma, Car Nicobar and Garacharma which support drumstick introduction from Maharashtra and Karnataka by the settlers or floating population in Garacharma area, a village near Port Blair city. It suggests that the some of the drumstick materials in Car Nicobar resembles with its samples from Maharashtra and Karnataka suggesting its movement via Garacharma village near Port Blair. Most of the samples grouped in sub-cluster 2 along with samples from Andhra Pradesh, Tamil Nadu and Kerala establish common perception of 'drumstick in the islands was brought in by Tamil, Malayali and Telugu settlers'. The observations of genetic relatedness of drumstick in the islands with its samples from mainland India finds support from earlier report by Singh et al. [14] on *Colocasia esculenta* (L.) Schott and in Capsicum [21] and in Bael [22]. Although, introductions might have taken place from other adjoining regions but this study indicates for mainland India as major source of drumstick in the islands, however variation within local samples suggests occurrence of genetic changes in favour of adaptation.

ISSR Analysis

The ISSR analysis of 68 samples resulted two main clusters with similarity value of 0.39 (Fig. 3). Main-cluster-1 had similarity coefficient from 0.68 to 1.00 and grouped altogether 59 samples from both regions. Sub-cluster-1 had 21 samples from different islands viz a viz mainland region of Tamil Nadu and Kerala. This sub-cluster indicates the route of drumstick material from mainland India (Tamil Nadu and Kerala) to main settlement locations in South Andaman and then in Car Nicobar and Campbell Bay. Sub-

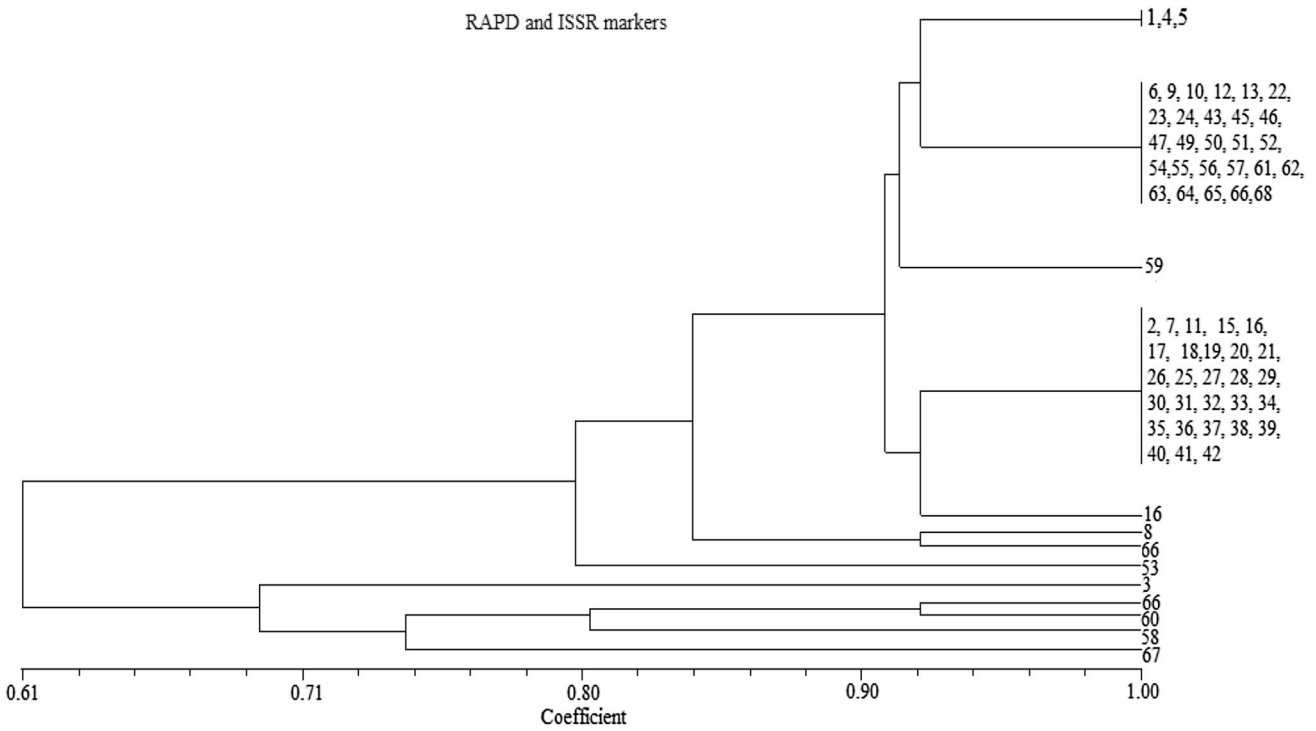

Fig. 4 Dendrogram based on UPGMA cluster analysis of 68 accessions of Drumstick using RAPD and ISSR markers (samples details given serially in Table 1)

cluster-2 represented 11 samples mainly from North and Middle Andaman with collections from villages in South Andaman. It also had samples from Karnataka which point towards possible spread of this material in North and Middle Andaman via South Andaman. Sub-cluster-3 had 10 samples from Bambooflat, Dollygunj, Havelock, Neil Island and Campbell Bay which might have brought in from Port Blair localities. Sub-cluster-4 had 16 samples South Andaman, Little Andaman and Middle Andaman suggesting widespread distribution of the materials. Main-cluster-2 had eight samples from South Andaman, Nicobar, Thiruananthapuram, Kamorta, Maharashtra and Karnataka with similarity value from 0.60 to 1.00 indicating strong genetic relatedness. The drumstick was introduced by the individual settler communities from their sources in mainland India and grown in and around the Port Blair city which was among the earliest settlement site in Islands. Over the years, the human settlement spread to other parts and drumstick material also moved along with them. Introduction of drumstick from mainland India still continue particularly improved genotypes by the individual or government agencies. There could be various channels through which drumstick spread in islands such as (1) South Andaman–Little Andaman–Car Nicobar–Campbell Bay; (2) South Andaman–Neil Island–Havelock Island; (3) South Andaman–Middle Andaman–North Andaman; and (4) South Andaman–Middle Andaman–Little Andaman. The extent of diversity in the 68 samples was revealed to be in the range of 47% by ISSR and 51% by RAPD markers which are in the close agreement with report of Resmi et al. [5] who investigated 57% genetic diversity using RAPD markers in drumstick 25 samples from the Kerala. High genetic divergence was reported in drumstick using Shannon's information index with value of 1.80 and 0.13 for Malaysian population and 0.30 and 0.19 for international population, respectively [13].

Relatedness Between Island and Mainland India Samples

The pooled data analysis from RAPD and ISSR markers divided samples into two main clusters with similarity ranging from 39 to 92% (Fig. 4). Cluster one had 63 samples distributed in four sub-clusters wherein sub-cluster-1 consist of 31 and sub-cluster-2 had 29 samples from both the islands and mainland India supplementing the proximities between local and mainland based drumsticks. Though, flora of Nicobar Islands has affinity with wild flora in Indonesia-Sumatra region while Andaman flora with flora of North-East India [9] but cultivated flora is close to mainland India particularly to southern and eastern India [23] due to reasonably similar climatic conditions and majority of human settlements from these regions. Hufford et al. [24] also reported fair extent of genetic variation

among the samples of perennial grass *Elymus glaucus* Buckl. from mainland sites in United States of America and Southern Channel Islands. Further, genetic variation in introduced drumstick the islands might be attributed by change at genetic level due to ecological divergence by localized environment and community isolation [25]. The study included only representative samples from states in mainland India which need to be increased for better resolution of relatedness. Resmi et al. [5] reported diversity in drumstick collections from Kerala using phenotypic and molecular parameters and Pandey et al. [1] in collections from Himalayan region. Saini et al. [6], Ganesan et al. [7] and Rajalakshmi et al. [8] also observed diversity in collections from most of the part in mainland India excluding Andaman and Nicobar Islands. Further, close association between drumstick samples of islands with samples from mainland India particularly Tamil Nadu and Kerala revealed by amplified RAPD and ISSR markers suggests these regions as predominant source sites of drumstick materials in the islands. Afterward, it distributed through seeds or cutting in homegardens, community gardens located all across the islands. It was introduced in traditional homegardens of tribal communities in Car Nicobar, Nancowry Island and Campbell Bay where it got acclimatized in prevalent farming situation through genetic adjustments. The study hypothesize that introduction of perennial vegetable plants in geographically isolated region brings changes genetic level for adaptive traits to acclimatize in new region. Hot humid conditions favour various biotic agents and expose to abiotic challenges naturally, hence adaptive genetic changes may serve as source for breeding of stress tolerance traits. Notably, large numbers of duplicities were observed in the samples suggesting targeted collection for conservation plan. For this, representative samples with similarity index of 1.0 in different clusters can be reduced to one sample for conservation purpose.

Morphological Diversity

Morphological parameters were recorded from fruits of 68 samples which showed wide range for fruit length (18.5–45.0 cm), number of seeds per fruit (9.8–22.0), fruit diameter (0.8–2.2 cm) and individual fruit weight (84.0–160.0 g) (Table 1). Fruit length (41.5 ± 2.9 cm) and individual fruit weight (145.0 ± 8.7 g) of samples from mainland India were significantly ($p < 0.05$) higher than the samples from islands (31.6 ± 5.3 cm; 116.9 ± 15.4 g). However, fruit diameter of island samples was higher (0.8–2.2 cm; 1.5 ± 0.3 cm) than mainland India samples (0.9–1.4 cm; 1.15 ± 0.18 cm). Recovery of fresh seeds (7.8–14.9%) and fresh pulp (30.3–65.8%) also had significant variation ($p < 0.05$) among the tested samples. Similarly variation was reported by Resmi et al. [5] and Tak and Maurya [20] in drumstick genotypes from four districts i.e. Jhalawar, Kota, Ajmer and Udaipur of Rajasthan fruit weight (26.37–66.43 g), fruit length (24.43–59.47 cm) and pod girth (7.33–23.67 mm). Raja et al. [26] also observed wide variation for fruit weight (76. 9–285.9 g) in semiarid and arid ecosystem. The variation in fruit traits might be attributed by differences in edaphic and climatic factors in sampling sites which were located all across the islands. Besides, differences in nutrient management, pruning practices and other crop practices also influence fruit parameters in drumstick [27] and these differences are not uncommon in the islands for drumstick. The soil of sampling sites in different homegardens, community gardens or farms located all across the islands reported to have difference in nutrient levels [28]. Hence, the observed morphological diversity in drumstick samples was not compared with genetic diversity however, present study suggest for such investigation in further.

Conclusion

The DNA markers revealed 47–51% genetic diversity in drumstick samples from Bay Islands and also established relatedness with material from mainland India. The ISSR markers were more efficient than RAPD markers to reveal genetic diversity in common geographical backgrounds. The study hypothesize that the culinary plants moves along with human settlement and acclimatize in local environment through appropriate genetic changes. Genetically diverse samples identified from different islands in the study have adaptive traits which can be further evaluated for conservation and use in breeding programme. Further, the findings have future perspective in investigating the level of genetic changes in drumstick population of the islands particularly climate resilient traits because these kind of multi-nutrient perennial crops will have better role in mitigating the impact of climate change on micronutrient malnutrition.

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Compliance with Ethical Standards

Conflict of interest There is no conflict of interest among the authors of the article.

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