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RESEARCH ARTICLE

Cross section of genetic diversity in mainland and insular populations of *Costus speciosus* (Koen ex. Retz.) Sm. using SPAR markers reveal patterns linked to allopolyploidy and biogeography

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Abstract

Costus speciosus (Koen ex. Retz.) Sm. is a major source of diosgenin, used for the commercial synthesis of *cortisone*, sex hormones and contraceptives. The genetic diversity analysis in wild populations of *C. speciosus* from 3 biogeographic regions *viz.*, Western Ghats (WG), Eastern Ghats (EG) and Andaman and Nicobar Islands (AN) were done using 2 different Single Primer Amplification Reaction (SPAR) methods. A total of 70 accessions spanning these regions were used in the present study. The assay yielded a total of 314 amplicons of which 268 were polymorphic, exhibiting 85.35% of polymorphism. The prevalence of high rate of genetic differentiation (mean Gst = 0.90) and low gene flow (mean Nm= 0.06) are the main attributes of the observed low diversity in these populations. The accessions clustered broadly under 2 major groups corresponding to the three biogeographic zones with insular populations diverse from the mainland. This was further resolved by AMOVA analysis. *C. speciosus* is found to exist in different cytotypes exhibiting allopolyploidy. The differences in distribution and genetic fitness of the population from EG and WG may be attributed to the allopolyploid nature of the taxa. In the present study, Island populations comprise very low heterozygosity (Ht = 0.10) suggesting that the rate of fixation is more in these populations. Similarly, the rate of gene flow was almost absent (Nm = 0.02). The higher levels of genetic similarity (0.99) may be due to an increase in fixation of the genes resulting from allopolyploidy. This is the first study on comparative genetic diversity of *C. speciosus* using SPAR markers.

Keywords

Andaman and Nicobar, Eastern Ghats, Diosgenin, Island population, Western Ghats

Introduction

Costus speciosus (Koen ex. Retz.) Sm. is a member of the family *Costaceae* and genus *Costus*. The distribution of this tropical plant species is mostly confined to Southeast Asia, Malaysia and New Guinea. However, it is an introduced species in many islands and primarily cultivated in India for its therapeutic uses and horticultural value. It is an important medicinal plant, which stores appreciable amount of diosgenin, a steroidal sapogenin, in its rhizome (1). Diosgenin is used for the synthesis of cortisone, sex-hormones and contraceptives (2, 3). It grows wild throughout India and its populations are distributed at diploid, triploid and tetraploid levels (4-8). The species is reported to house high variation and many of them were ascribed to new

species prior to Schumann's monograph in 1899 (9). The plant has been classified as being in the near threatened category as a result of overexploitation and other anthropogenic pressures (10).

PCR-based markers have been used extensively for measuring genetic variation at the intraspecies level (11, 12). Due to their ubiquity, reliability and ease of designing the primers, PCR based markers are frequently employed in genetic diversity analysis in plants in which morphological markers are few (13, 14).

Genetic diversity is essential for the long-term survival of species and therefore plays pivotal role in formulating effective conservation strategy for the species (15, 16). Also, genetic diversity helps to ensure survival of species, as it provides better adaptability to the individuals. Therefore, identification of those adapted variants at inter and intrapopulation levels is key to their long-term conservation. The advent of diverse molecular markers accelerated the inquisitiveness to understand the mechanism of evolution occurring in islands by measuring the quantum and distribution of genetic variation within and among populations (17-21). To this end, several novel DNA markers, both dominant and co-dominant, have been used for genome analysis to study genetic relationship (22, 23) at the inter-and intra-specific levels.

Biogeographical Zones included in the study

Being a tropical plant, *C. speciosus* is distributed throughout the wet evergreen forests of India. Three biogeographical zones viz., Western Ghats, Eastern Ghats and Andaman and Nicobar Islands comprising of wet evergreen forest vegetation were selected for the present investigation.

Western Ghats

It is one of the global biodiversity hotspots comprising large variety of flora and fauna located (24-26) harbours more than 5800 species of plants, of which 2100 are endemics. The southern part of Western Ghats, comprising semi evergreen and evergreen forests, has maximum endemic flora. It harbours most of the rhizomatous and succulent plant vegetation (27).

Eastern Ghats

Eastern Ghats is a discontinuous range of mountains set along eastern coast of India. It measures 1700 Km in the north-east south-west strike in the Indian Peninsula. The mountain ranges are rich in biodiversity comprising diverse vegetation's like dry deciduous mixed forest to semi evergreen rain forest (28).

Andaman and Nicobar Islands

These islands are immensely rich in biodiversity. It comprises of 2500 species of angiosperms of which 10% are known to be endemic (29). These islands constitute the largest archipelago in Bay of Bengal, consisting 306 islands and 206 islets (30).

Materials and Methods

Sampling sites and collection of plant material

Seventy accessions of *C. speciosus* were collected from different sites across Southern Western Ghats, Eastern Ghats and Andaman and Nicobar Islands (Fig. 1) (Table 1). The minimum distance between sampling sites is 35 km and within each population, the accessions were collected at an average distance of 5-8 km subject to availability.

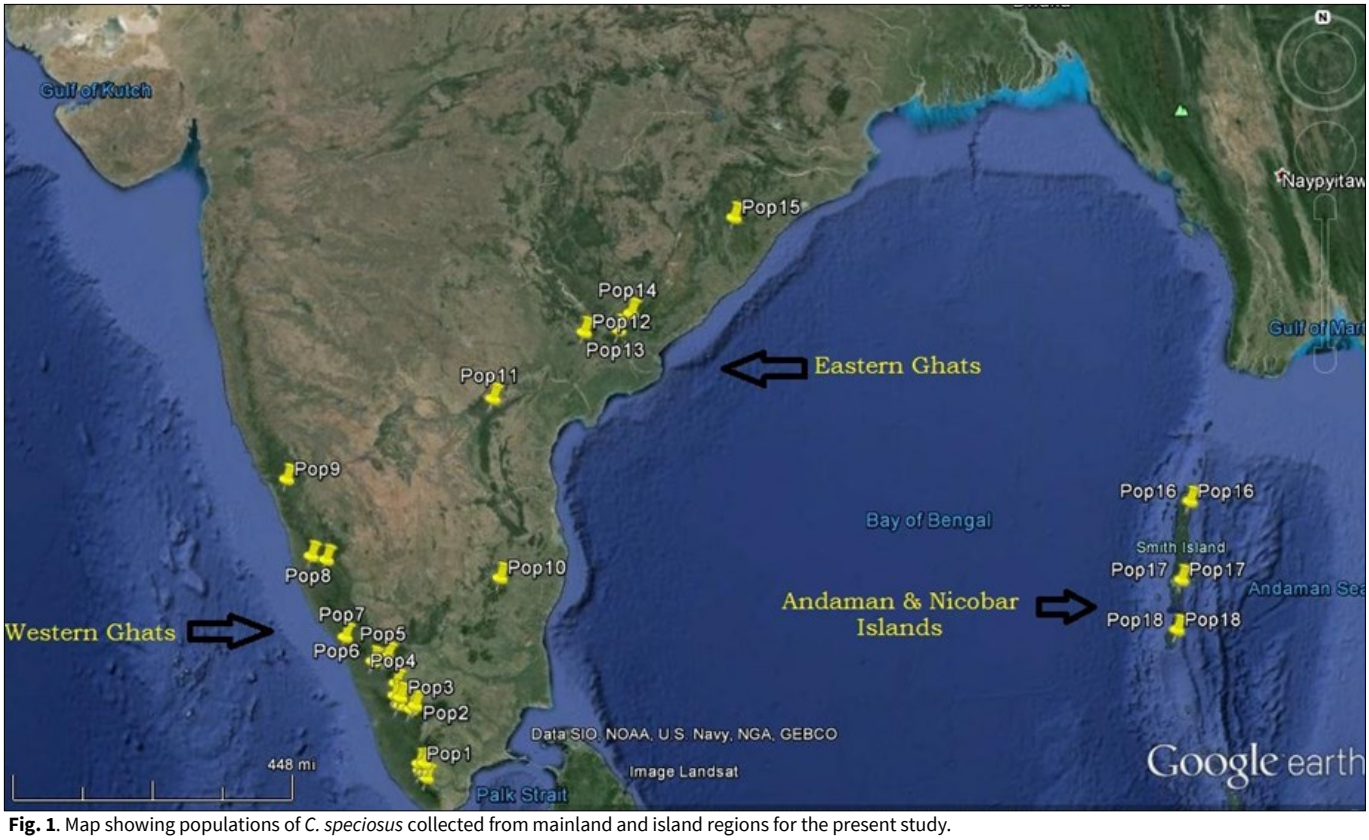

Fig. 1. Map showing populations of *C. speciosus* collected from mainland and island regions for the present study.

Table 1. List of *C. speciosus* collected from different biogeographical zones for genetic diversity analysis

Accessions	Population	Location	Lattitude	Longitude
CS-1	pop1	Braemore	8°45¢54.37¢¢N	77°04¢53.48¢¢E
CS-2	pop1	Braemore	8°45¢52.98¢¢N	77°05¢11.82¢¢E
CS-3	pop1	Kulathpuzha	8°54¢25.04¢¢N	77°03¢41.43¢¢E
CS-4	pop1	Kulathpuzha	8°54¢33.39¢¢N	77°03¢53.28¢¢E
CS-5	pop1	Peppara	8°36¢58.59¢¢N	77°11¢35.37¢¢E
CS-6	pop1	Peppara	8°37¢27.50¢¢N	77°11¢19.66¢¢E
CS-7	pop1	Rosemala	8°54¢59.21¢¢N	77°10¢09.14¢¢E
CS-8	pop1	Rosemala	8°55¢07.07¢¢N	77°10¢10.24¢¢E
CS-9	pop2	Adimali	10°01¢03.41¢¢N	76°57¢26.77¢¢E
CS-10	pop2	Adimali	10°00¢48.96¢¢N	76°57¢36.72¢¢E
CS-11	pop2	Munnar	10°05¢25.22¢¢N	77°03¢04.03¢¢E
CS-12	pop2	Munnar	10°05¢41.91¢¢N	77°03¢13.47¢¢E
CS-13	pop3	Thattekkadu	10°06¢26.03¢¢N	76°42¢22.96¢¢E
CS-14	pop3	Thattekkadu	10°06¢39.00¢¢N	76°42¢45.63¢¢E
CS-15	pop3	Thattekkadu	10°06¢30.18¢¢N	76°43¢01.86¢¢E
CS-16	pop3	Thattekkadu	10°06¢07.34¢¢N	76°42¢03.82¢¢E
CS-17	pop3	Vazhachal	10°17¢34.71¢¢N	76°38¢54.94¢¢E
CS-18	pop3	Vazhachal	10°17¢25.22¢¢N	76°38¢49.35¢¢E
CS-19	pop3	Vazhachal	10°18¢08.83¢¢N	76°37¢33.35¢¢E
CS-20	pop3	Vazhachal	10°18¢39.72¢¢N	76°38¢47.98¢¢E
CS-21	pop3	Inchathotti	10°05¢40.37¢¢N	76°45¢14.14¢¢E
CS-22	pop3	Inchathotti	10°05¢03.41¢¢N	76°45¢22.95¢¢E
CS-23	pop4	Nelliampathy	10°32¢17.60¢¢N	76°41¢07.94¢¢E
CS-24	pop4	Nelliampathy	10°32¢37.36¢¢N	76°40¢43.42¢¢E
CS-25	pop5	Silent valley	11°04¢02.11¢¢N	76°31¢37.81¢¢E
CS-26	pop5	Silent valley	11°04¢05.71¢¢N	76°31¢33.58¢¢E
CS-27	pop5	Silent valley	11°04¢12.88¢¢N	76°31¢52.40¢¢E
CS-28	pop5	Silent valley	11°04¢10.55¢¢N	76°31¢58.85¢¢E
CS-29	pop5	Silent valley	11°04¢19.35¢¢N	76°30¢24.72¢¢E
CS-30	pop5	Silent valley	11°04¢21.43¢¢N	76°30¢17.35¢¢E
CS-31	pop5	Silent valley	11°04¢22.45¢¢N	76°30¢33.02¢¢E
CS-32	pop5	Silent valley	11°04¢20.27¢¢N	76°30¢37.25¢¢E
CS-33	pop6	Perinthalmanna	10°58¢36.89¢¢N	76°14¢15.07¢¢E
CS-34	pop6	Perinthalmanna	10°58¢46.47¢¢N	76°13¢52.47¢¢E
CS-35	pop7	Koilandy	11°27¢26.02¢¢N	75°40¢41.66¢¢E
CS-36	pop7	Koilandy	11°27¢42.10¢¢N	75°40¢27.77¢¢E
CS-37	pop8	Moodabidri	13°03¢39.34¢¢N	75°02¢13.25¢¢E
CS-38	pop8	Ujire	13°59¢21.93¢¢N	75°21¢02.72¢¢E
CS-39	pop9	Yana	14°35¢04.74¢¢N	74°33¢52.48¢¢E
CS-40	pop9	Yana estate	14°34¢12.20¢¢N	74°33¢58.48¢¢E
CS-41	pop10	Jamunamarathur	12°35¢50.12¢¢N	78°53¢15.63¢¢E
CS-42	pop10	Jamunamarathur	12°36¢01.83¢¢N	78°53¢09.19¢¢E
CS-43	pop11	Sreesylam	16°03¢35.15¢¢N	78°52¢36.81¢¢E
CS-44	pop11	Sreesylam	16°03¢37.34¢¢N	78°52¢41.27¢¢E
CS-45	pop11	Sreesylam	16°04¢13.29¢¢N	78°52¢44.56¢¢E

CS-46	pop12	Ramachandrapuram	17°15¢58.98¢¢N	80°45¢16.06¢¢E
CS-47	pop12	Ramachandrapuram	17°16¢04.55¢¢N	80°45¢35.89¢¢E
CS-48	pop12	Ramachandrapuram	17°16¢35.06¢¢N	80°45¢38.51¢¢E
CS-49	pop13	Munjuluru	17°16¢57.71¢¢N	81°27¢17.14¢¢E
CS-50	pop13	Munjuluru	17°17¢04.45¢¢N	81°27¢10.38¢¢E
CS-51	pop13	Munjuluru	17°17¢16.62¢¢N	81°27¢18.17¢¢E
CS-52	pop14	Maredumilli	17°35¢30.32¢¢N	81°42¢45.29¢¢E
CS-53	pop14	Maredumilli	17°36¢08.32¢¢N	81°42¢40.92¢¢E
CS-54	pop14	Maredumilli	17°36¢57.90¢¢N	81°42¢47.85¢¢E
CS-55	pop15	Patanagudi	19°20¢30.71¢¢N	83°53¢35.11¢¢E
CS-56	pop15	Patanagudi	19°20¢34.10¢¢N	83°53¢51.38¢¢E
CS-57	pop15	Patanagudi	19°20¢55.37¢¢N	83°53¢44.83¢¢E
CS-58	pop16	Sippighat	13°09¢32.97¢¢N	92°58¢19.03¢¢E
CS-59	pop16	Sippighat	13°09¢33.97¢¢N	92°58¢22.02¢¢E
CS-60	pop16	Sippighat	13°09¢39.07¢¢N	92°58¢12.99¢¢E
CS-61	pop17	Dhanikhari	11°36¢28.96¢¢N	92°40¢35.05¢¢E
CS-62	pop17	Dhanikhari	11°36¢43.23¢¢N	92°40¢47.62¢¢E
CS-63	pop17	Dhanikhari	11°36¢45.21¢¢N	92°40¢58.01¢¢E
CS-64	pop17	Dhanikhari	11°37¢03.90¢¢N	92°40¢47.48¢¢E
CS-65	pop18	Indira bazar	10°36¢25.27¢¢N	92°31¢34.39¢¢E
CS-66	pop18	Indira bazar	10°36¢26.35¢¢N	92°31¢34.47¢¢E
CS-67	pop18	Indira bazar	10°36¢48.09¢¢N	92°31¢40.54¢¢E
CS-68	pop18	Indira bazar	10°36¢53,85¢¢N	92°31¢42.22¢¢E
CS-69	pop18	Indira bazar	10°36¢29.51¢¢N	92°31¢41.74¢¢E
CS-70	pop18	Indira bazar	10°36¢02.33¢¢N	92°31¢58.75¢¢E

Genomic DNA isolation, RAPD and ISSR

Total genomic DNA was isolated from the tender young leaves following CTAB method (31) with appropriate modifications. The RAPD and ISSR protocols were standardized as reported by the authors earlier (32, 33).

Genetic data analysis

Amplification with each arbitrary primer was repeated 3 times and those primers that produced reproducible and consistent bands were selected for data generation. Reproducible marker products were scored against the presence or absence of a fragment. Dice coefficient of similarity defined as 2a/2a+u, where 'a' is the number of positive matches and 'u' the number of non-matches was computed using the WINDIST software. The scored binary matrix was analysed using the WINBOOT software (34). Cluster analysis by PCA was carried out using GenAlEx (version 6.1) (35). Analysis of Molecular Variance (AMOVA) was performed to describe the genetic structure and variability among 2 populations. The components of Variance partitioned within and among the populations were estimated from a Eulidean distance matrix using GenAlEx 6.1 with 1000 random permutations. Genetic variation in different biogeographical zones were analysed for various parameters. The genotype and allelic frequency data were used to compute the genetic diversity indices *i.e.* observed number of alleles (na), expected number of alleles (ne), Shannon index of genetic diversity (I) and Nei's gene diversity (h) at the population level using the statistical package *POPGENE* 1.3 (36). The sampling populations were assumed to be in Hardy-Weinberg equilibrium implying that the population is at random mating. Based on the above assumption, the bands were scored and estimation of heterozygosity (Ht) was done according to the formula: $Ht = 1-\sum pi^2$ where pi is the frequency of the i^{th} allele in the population.

Results

RAPD Polymorphism

The 18 random primers used for diversity measurement in 70 samples of *C. speciosus* (Table 2) provide interesting insights into the prevailing genetic variability in this endangered species. Out of 182 amplicons, 149 were polymorphic (81.86%). On an average, the primers generated

10 amplicons and 8 polymorphisms per primer (Table 2). The number of amplicons generated was found to range

Table 2. List of RAPD primers and their sequence used for diversity analysis of *C. speciosus*

Sl. No:	RAPD Primer series	RAPD Primer sequence	No. of Bands	No. of Polymorphic Bands
1	C61	TGT CAT CCC C	15	13
2	C 62	GTG AGG CGT C	17	17
3	C 63	GGG GGT CTT T	6	4
4	C 64	CCG CATCTA C	15	15
5	C 65	GAT GAC CGC C	8	7
6	C66	GAA CGG ACT C	10	10
7	C 67	GTC CCG ACG A	14	10
8	C 68	TGG ACC GGT G	10	9
9	C 69	CTC ACC GTC C	10	10
10	C 70	TGT CTG GGT G	8	6
11	C 71	AAA GCT GCG G	9	2
12	C 73	AAG CCT CGT C	8	8
13	C 74	TGC GTG CTT G	14	8
14	C76	CACACTCCAG	6	5
15	C77	TTCCCCCCAG	1	1
16	C78	TGAGTGGGTG	12	12
17	C79	GTTCCCAGCC	10	8
18	C80	ACTTCGCCAC	9	4
Total No. of Bands			182	149
Mean per primer			10.11	8.27
Percentage Polymorphism			81.86 %	

from 1 to 17 with primer S62 yielded the maximum (17) whereas primer S77 gave the minimum (1). The various genetic diversity indices like Nei's gene diversity at the population level (h), Shannon index (I), expected number of alleles (ne) etc., were calculated to measure the extent of variation. The accessions included in this study showed relatively low to moderate levels of genetic diversity, *i.e.* $h = 0.29$ and $I = 0.43$. The mean genetic diversity based on Nei's statistics also supports the above data. The mean value of heterozygosity (Ht) observed in the various accessions of *C. speciosus* was found to be 0.29. The mean value of average heterozygosity value was 0.02. The degree of genetic differentiation (Gst) is found to be 0.92. The gene flow (Nm) among all accessions is 0.04, which is too low. RAPD assay revealed relatively less gene flow and higher genetic differentiation rates among the populations collected from three biogeographical zones of peninsular India.

ISSR Profiling

The ISSR analysis was carried out with 18 primers with few of them anchored at the 3' end for increased specificity. Out of 132 products generated, 113 were polymorphic thereby showing 85.6% polymorphism. The primers on an average generated 8 products and the mean number of polymorphism per primer is 6 (Table 3). The accessions

Table 3. List of ISSR primers and their sequence used for diversity analysis of *C. speciosus*

Sl. No:	ISSR Primer series	ISSR Primer sequence	No. of Bands	No. of Polymorphic Bands
1	808	AGAGAGAGAGAGAGAGC	4	3
2	809	AGA GAG AGA GAG AGA GG	4	4
3	811	GAG AGA GAG AGAGAG AC	11	4
4	815	CTC TCT CTC TCT CTC TG	9	9
5	816	CAC ACA CAC ACA CAC AA	9	9
6	817	CAC ACA CAC ACA CAC AA	9	9
7	823	TCT CTC TCT CTC TCT CC	6	5
8	826	ACA CAC ACA CAC ACA CC	10	8
9	827	ACA CAC ACA CAC ACA CG	7	7
10	834	AGA GAG AGA GAG AGA GYT*	11	11
11	835	AGA GAG AGA GAG AGA GYC*	9	9
12	836	AGA GAG AGA GAG AGA GYA*	11	11
13	840	GAG AGA GAG AGA GAG AYT*	4	2
14	841	GAG AGA GAG AGA GAG AYC*	6	4
15	843	CTCTCTCTCTCTCTCTRA**	7	1
16	844	CTC TCT CTC TCT CTC TRC**	3	3
17	847	CAC ACA CAC ACA CAC ARC	8	8
18	848	CAC ACA CAC ACA CAC ARG	6	6
Total No. of Bands			132	113
Mean per primer			7.55	6.27
Percentage Polymorphism			85.6 %	

showed relatively low to moderate levels of genetic diversity, i.e. $h = 0.23$ and $I = 0.36$. The mean value of heterozygosity (Ht) was found to be 0.23. The degree of genetic differentiation (Gst) and gene flow (Nm) among accessions was 0.57 and 0.38 respectively. The diversity measures as indicated reveal less diversity at the inter and intra-population levels.

SPAR analysis

SPAR analysis was carried out to estimate the efficiency of SPAR markers (ISSR and RAPD) in estimating the genetic diversity indices of taxa collected from different geographical zones (Table 4). The combined data set from RAPD and ISSR patterns was performed (37). The assay yielded a total of 314 amplicons of which 268 were polymorphic, exhibiting 85.35% of polymorphism. An average of 7.44 products per primer was polymorphic. The % of polymorphic loci, when samples from different biogeographic regions were pooled, ranged from 22.29% (Andaman and Nicobar Islands) to 70.06% (Western Ghats). The samples from Western Ghats showed relatively higher genetic diversity (Na= 1.70±0.46; Ne= 1.34±0.32; h= 0.21± 0.18; I= 0.33±0.25) compared to samples from other biogeographic regions in the study. The degree of genetic differentiation (Gst) is found to be highest in samples from Andaman and Nicobar islands (0.96), followed by Eastern Ghats (0.92) and Western Ghats (0.82). The gene flow (Nm) among all samples was analysed and found to be higher among populations from the Western Ghats compared to

Table 4. Mean value of genetic diversity indices in *C. speciosus* from diverse biogeographical zones *using* SPAR markers (RAPD + ISSR)

Mean	h	I	Nm	Gst	Ht	Hs	PL	% PL
Cluster I Western Ghats	0.21	0.33	0.11	0.82	0.23	0.04	220/314	70.06%
Cluster II Eastern Ghats	0.14	0.19	0.05	0.92	0.14	0.02	80/314	25.47%
Cluster III Andaman & Nicobar Islands	0.10	0.14	0.02	0.96	0.10	0.004	70/314	22.29%

Ht-heterozygosity at the polymorphic loci, **Hs**-average heterozygosity, **Gst**-degree of genetic differentiation, **h**-Nei's gene diversity at population level, **I**- Shannon index of genetic diversity, **PL**- Polymorphic Loci, **%PL**- percentage Polymorphic Loci.

other biogeographical regions. The mean value of gene differentiation (Gst) was found to be 0.90 indicating high level of gene differentiation. While the estimate of gene flow (Nm) is 0.06 indicating very low gene flow among the populations.

Cluster analysis

The genetic relatedness among 70 accessions was revealed by cluster analysis based on Nei's genetic distance (GD) (38) of SPAR. It was observed that mostly all individuals in the biogeographical zone were arranged in the same cluster. The accessions of *C. speciosus* clustered broadly under three major groups corresponding to the 3 biogeographical zones. All accessions from the Western Ghats were grouped and constituted cluster I. Similarly, accessions from the Eastern Ghats formed cluster II and samples from Andaman and Nicobar Islands constituted the cluster III (Fig. 2). Cluster analysis clearly shows that the populations from Andaman and Nicobar Islands are diverse from the mainland populations. This was further resolved by AMOVA analysis carried out to study the variation between the mainland and island populations of *C. speciosus* (Fig. 3). It was found that there is substantial genetic variation among the population harboring the Andaman and Nicobar Islands compared to the populations on the mainland.

Population Structure

PCA analysis separated the populations following their spatial distribution, similar to the phenogram (Fig. 4). The first coordinate (39.98%) separated the Western Ghats population from the island population (Andaman and Nicobar Islands). The second coordinate (29.70%) separated the insular populations from the mainland populations.

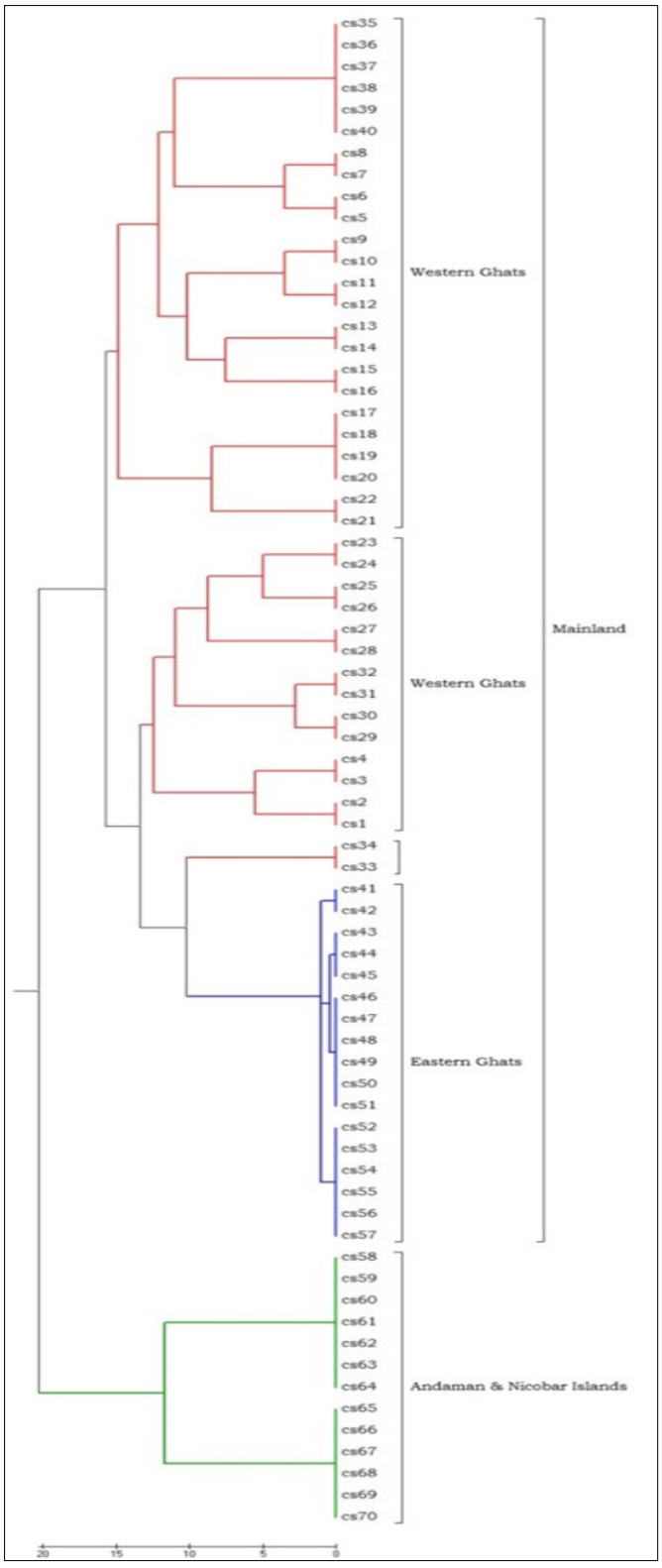

Fig. 2. UPGMA clustering of populations of *C. speciosus* from different biogeographical regions of peninsular India using SPAR markers.

Discussion

Genetic Diversity of C. speciosus in the Western and Eastern Ghats

In the present analysis, genetic diversity was studied in *C. speciosus* along three biogeographical regions. The heterozygosity level in the Western Ghats was 0.23. Although the heterozygosity level was less in these taxa, the heterozygosity in the Western Ghats population was notably higher compared to the other 2 regions (0.14 and 0.10 for the Eastern Ghats and Andaman and Nicobar Islands respectively). This may be attributed to the allopolyploid nature of this taxon (4, 6, 39, 40). Populations from all the 2 regions showed increased Gst value, which is a charac-

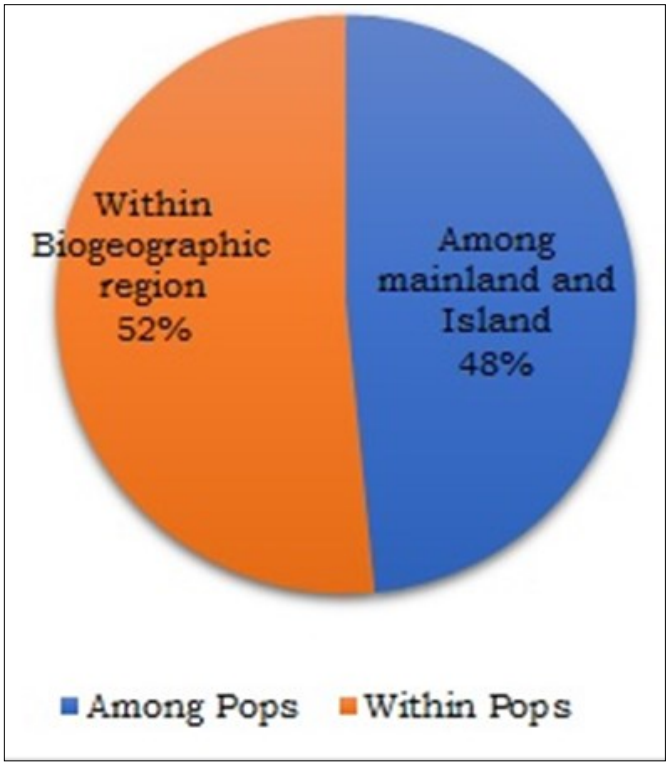

Fig. 3. AMOVA analysis showing the percentage of molecular variance in populations of *C. speciosus* from Mainland (WG and EG) and Island (Andaman and Nicobar Islands) biogeographical regions.

semi-arid and arid tropical zones. The PCA analysis of populations from the Western Ghats and Eastern Ghats revealed genetic relatedness among the populations from these 2 biogeographical regions (Fig. 5). A higher rate of variance is seen in the populations from the Western Ghats compared to the populations from the Eastern Ghats (which have very less genetic distance among the populations). These results were further substantiated with molecular variance analysis (AMOVA) of these 2 populations (Fig. 6). Hierarchical analysis of molecular variance (AMOVA) in GenAlEx was carried out to segregate genetic variation occurring within and among populations in the target sites *i.e* the island and mainland. The AMOVA component of variance includes FPT, an analogue of FST (35, 42-44).

Genetic diversity of C. speciosus on Andaman & Nicobar Islands

Oceanic islands are windows for understanding the pattern and process of evolution. They have served as case studies for formulating and refining hypotheses on biogeography, evolution and ecological processes (7, 45-49). Studies indicate that Island populations contain less variation than their mainland counterparts, a phenomenon usually resulting from the founder effect and genetic drift in these small populations (50-52). Consequently, these

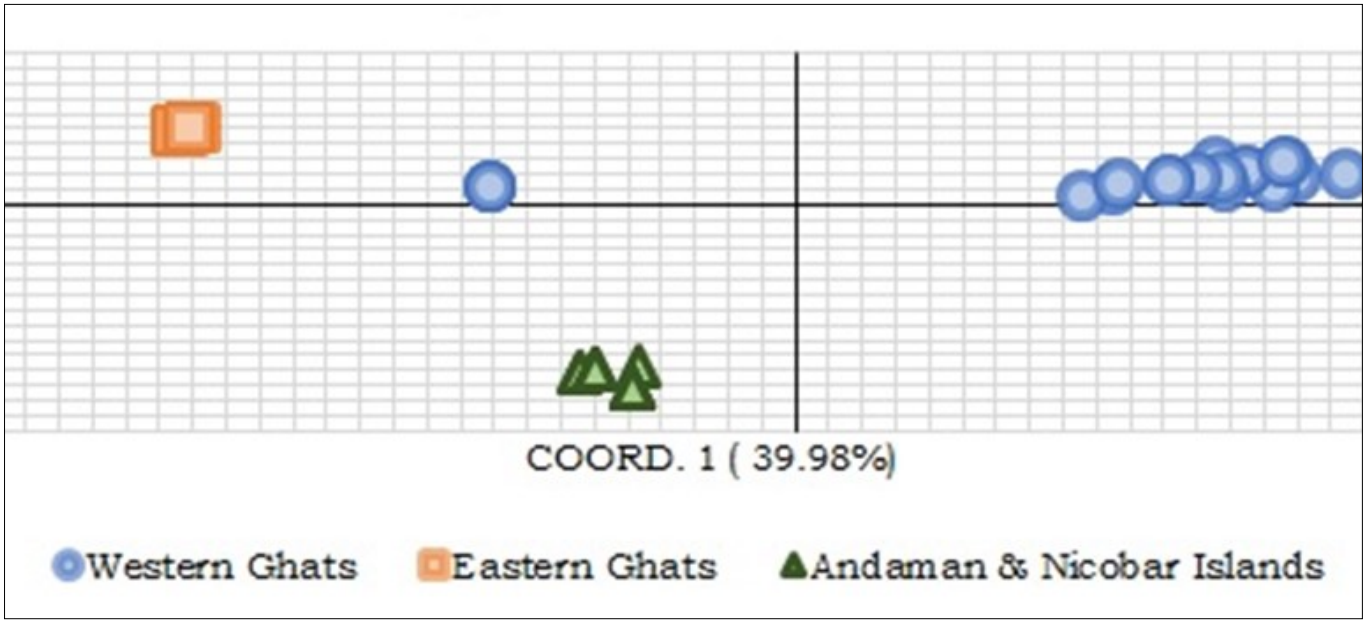

Fig. 4. Two-dimensional Principal Co-ordinate analysis of populations of *C. speciosus* from three biogeographic zones of peninsular India using SPAR markers.

teristic of selfing species, annuals, vegetatively propagating and early successional species (41). The population from the Eastern Ghats showed relatively low heterozygosity levels compared to the Western Ghats. Although, *C. speciosus* is cultivated for its medicinal utility and as an ornamental plant in these areas, natural wild populations are confined to wet evergreen forest vegetation. Such vegetations are scanty and found only in isolated patches throughout the Eastern Ghats. Random fluctuations in allele frequency in fragmented populations reduce genetic variation which is evident in populations of Eastern Ghats (Ht = 0.14). The distribution of wild populations of *C. speciosus* in the Western Ghats is abundant throughout. However, the distribution tends to reduce towards the

populations facing genetic drift, restricted gene flow and inbreeding exhibit higher rates of genetic differentiation. A previous study on genetic diversity of *C. speciosus* in Andaman and Nicobar Islands using RAPD markers showed relatively high rates of genetic similarity (29, 53). In the present study, a more precise and stringent marker system, ISSR, was used in addition to RAPD to assess the genetic diversity of the Island populations. The study shows the prevalence of high rate of Gst (0.96) in island populations of *C. speciosus* compared to populations in the other two biogeographical zones on the mainland. The heterozygosity level in the island populations of Andaman and Nicobar was estimated to be 0.10, indicating the increased inbreeding in the population. Similarly, reduced % of poly-

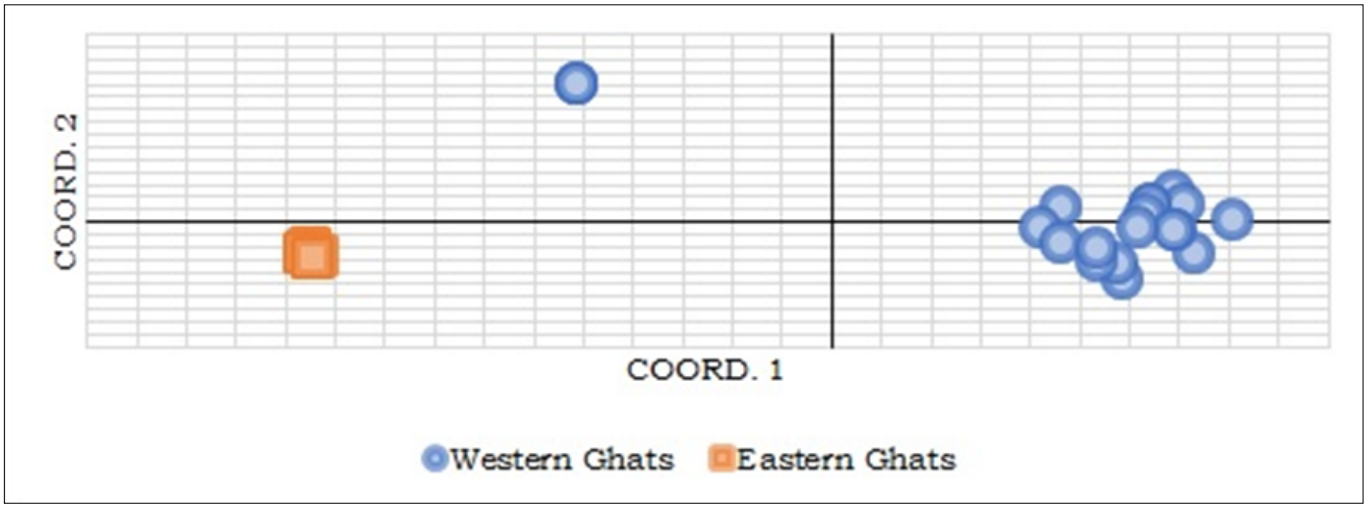

Fig. 5. Two-dimensional Principal Co-ordinate analysis of populations of *C. speciosus* from Western Ghats and Eastern Ghats.

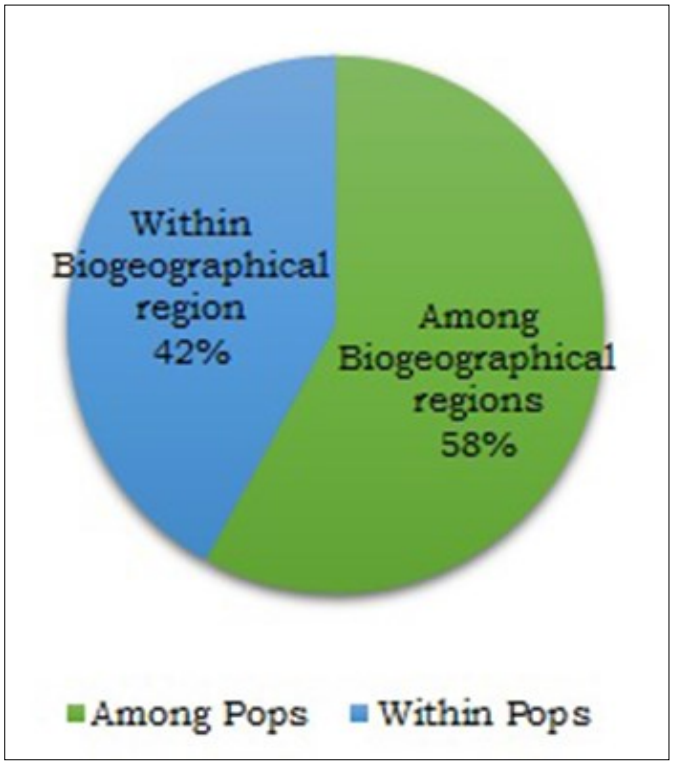

Fig. 6. AMOVA analysis showing the percentage of molecular variance in populations of *C. speciosus* from Western Ghats and Eastern Ghats.

morphic loci (22.29%) suggests increased homozygosity in the alleles and the rate of gene flow (Nm = 0.02) is almost negligible, apparently indicating the presence of genetic drift in the island population of *C. speciosus.* It has been observed that the gene flow between the populations from Andaman Island and the Nicobar Islands is very less and negligible. We assume that the absence of potential dispersal agents and the presence of ocean forming a natural barrier between these islands are the elements for the reduction in gene flow. This might have led to widespread selfing and shifting to the vegetative mode of multiplication, for its survival in these islands. This corroborates with the low heterozygosity and high Gst level found in island populations of *C. speciosus.* The low genetic diversity in this species may be due to the recent environmental or anthropogenic habitat deterioration on these islands (54, 55).

Effect of allopolyploidy on evolution and speciation of C. speciosus

It has been suggested that structural and numerical changes in the genetic material bear direct relationship to the evolution of the particular taxa. Karyomorphological data has helped researchers better understand the events occurring in evolution (56-59). The abundance of growth of taxa belonging to Zingiberaceae is suspected to be due to the existence of polyploid cytotype forms (60-63). Incidentally, *C. speciosus* is also found to exist in different cytotypes like diploid, triploid and tetraploid forms (4-6, 64-66). Previous studies revealed that *C. speciosus* is segregated in these different biogeographical zones and exists at different ploidy levels. For instance, as diploids in the Eastern Ghats, triploids and hexaploids in the Andaman and Nicobar islands, and tetraploids in the Western Ghats and Southern part of Indian Peninsula (4, 6, 39, 67). These studies suggest the role of allopolyploidy in the evolution of these cytotypes. Instances of karyomorphology and its effects on events of evolution are reported in some genera like *Chlorophytum* (68, 69), *Smilax* (70), *Vernonia* (71) and *Blumea* (72). Interestingly, the phenomenon of polyploidy has been suggested as the source of evolution in plants (73-80).

The differences in the distribution and genetic fitness of population from the Eastern Ghats and the Western Ghats may be attributed to the allopolyploid nature of the taxa. The wild populations in the Eastern Ghats are predominantly diploid in nature. However, polyploids are also found in the domesticated populations. It may have been introduced from other biogeographical regions for cultivation. The populations from the Western Ghats have a variety of cytotypes, mostly tetraploids along with diploid populations, which are restricted to wet evergreen populations. The formation of tetraploids may be due to the stable heterosis events seen in allopolyploids (81-83). The polyploids usually acquire neo-functionalization or sub-functionalization, which helps them in potential niche expansion by an increase of flexibility and adaptability in the taxa's response to environmental changes (84-86). The predominant distribution of *C. speciosus* in the Western Ghats may be due to the increased adaptability of the taxa to variable microclimatic conditions and varying vegeta-

tion. However, studies reveal that there may be a substantial reduction in fitness in such taxa (82). Polyploidy increases homogeneity in the taxa as increase in the rate of fixation of alleles may lead to decrease in genetic diversity of the populations; which is evident in the populations from all the three biogeographical regions of peninsular India. Tetraploid populations contain twice the number of copies of genes compared to the diploid populations. Therefore, they can harbour a large amount of genetic diversity owing to more mutations and thus lower the impact of genetic drift. A similar rate of migration of individuals between populations will lead to low degree of genetic differentiation in polyploids compared to diploid populations (87-92). Similar results are evident in the Western and Eastern Ghats populations of *C. speciosus* in Peninsular India. The genetic similarity in the Western Ghats (0.72) is less compared to the Eastern Ghats (0.90).

Previous studies reveal the fact that populations of *C. speciosus* on the Andaman and Nicobar Islands mostly comprised of triploids and hexaploids apart from diploids. It was suggested (93, 94) that the triploid individuals are most likely sterile. Later, researchers (95), determined that polyploidy may often lead to extinction than diversification. In the present study, the island populations comprise very low heterozygosity (Ht = 0.10) suggesting the rate of fixation is more in these populations. The gene flow among the populations on islands is almost absent (Nm = 0.02). The higher levels of genetic similarity (0.99) in certain accessions may be attributed to the increase in the fixation of genes resulting from the allopolyploidy. The interaction and gene flow between the populations are minimal suggesting a higher risk of extinction in these island populations. Interestingly, other genetic diversity indices measured also substantiate a similar scenario.

Conclusion

The present study assumes significance as it provides further insight in to the pattern and extent of genetic variation in this medicinal plant. This is the first study on the comparative genetic diversity of *C. speciosus* using SPAR markers. The genetic diversity of *C. speciosus* in all the three biogeographical regions showed relatively very low levels of heterozygosity. Albeit, *C. speciosus* is reported to have wide spread distribution, the density of the population is seen to be higher in the wet evergreen vegetation compared to other vegetation patterns. The prevalence of many micro-climatic zones in the Western Ghats and allopolyploidy in the taxa may have contributed to increased heterozygosity in *C. speciosus* of Western Ghats. The protection of existing patches of natural populations coupled with biotechnological intervention is not only essential but also a viable method for the long-term conservation of this taxa.

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Authors contributions

MK carried out plant sample collection, conducted the experiments, analysed the data and prepared the draft manuscript. KS refined the data analysis, improved the manuscript. PP conceptualized the research problem, designed the experiments and finalized the manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest in the work reported in this paper.

Ethical issues: None.

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