Genetic Variability in the Medicinal Plant *Hellenia speciosa* (J. Koenig) S.R. Dutta (Costaceae)

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ABSTRACT

Hellenia speciosa (J. Koenig) S.R. Dutta is an important medicinal plant used to cure different diseases. It has, antifungal, anticholineesterase, antioxidant, antihyperglycemic, antiinflammatory, analgesic, antipyretic, antidiuretic, larvicidal, antistress and estrogenic activities. It is also used for ornamental purposes. It is a source of diosgenin (a precursor of contraceptive drugs). The species exhibits enormous phenotypic variations. Here, genetic diversity studies have been carried out in 25 accessions of *H. speciosa*, through ISSR analysis using 16 primers. These accessions clustered into seven major groups with respect to their genetic similarity. Estimation of genetic parameters of heterozygosity such as number of alleles per locus (*Na*), number of effective alleles (*Ne*), Heterozygosity (*H*) and Shannon's Information Index (*I*) revealed that the 25 accessions vary genetically. The study revealed that the gene pool of *H. speciosa* is highly diverse, with 63.11% of the alleles showing polymorphism. This finding suggests that the enormous phenotypic variations exhibited by the species may have a genetic basis. The intraspecific variation available in *H. speciosa*, as revealed through the study, would be useful for conservation, cultivation and genetic improvement of the species and its effective utilization in pharmaceutical industry.

Key words: *Costus speciosus*, Genetic diversity, *Hellenia speciosa*, Heterozygosity, ISSR, Polymorphism

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Introduction

Hellenia speciosa (J. Koenig) S.R. Dutta (*Costus speciosus*) is a rhizomatous, perennial, herbaceous species belonging to the family Costaceae. This species, known as, 'Channakkuva', 'Anakkuva' (Malayalam) and 'Kottam' (Tamil), is also known as 'Crepe ginger', 'Cane reed', 'Spiral flag', 'Spiral ginger' and 'Elegant ginger'. The species is pantropical in distribution. Owing to its hardiness, invasive potential and adaptability to a wide range of habitats, *H. speciosa* has become naturalized in many parts of the world (Chattopadhyay & Sharma, 1983; Govaerts, 2013). This species is widely distributed in countries of South and South-East including India. In India the plant has naturalized in Sub-Himalayan tract, in parts of central India and in the Western Ghats of Maharashtra, Karnataka and Kerala (Sarin et al., 1974). In Kerala, *H. speciosa* is distributed extensively and occurs in all parts of the State (Sasidharan, 2004). It is seen growing in a wide range of habitats such as moist deciduous, semi evergreen forests and thickets from plains up to 1800 m. The plant also occurs in boundaries of cultivated fields, in depressions along roadsides, rail tracks and forest margins situated close to human habitation.

In India, *H. speciosa* is used in traditional systems of medicine such as Ayurveda, Siddha and Unani for curing diverse ailments such as pneumonia, rheumatism, urinary diseases and jaundice. Its rhizomes possess bitter, astringent, cooling, aphrodisiac, purgative, anthelmintic, depurative and febrifuge properties and thus have immense therapeutic potential (Daisy et al., 2008; Warrier et al., 1995). Pharmacological studies revealed that rhizomes of *H. speciosa* have antidiabetic, hypolipidemic, anticholinesterase, hepatoprotective, antioxidant, antibacterial, antifungal, antifertility, and spasmolytic activities (Singh et al., 2014). Moreover, *H. speciosa* is a potential source of the bioactive compound diosgenin, a precursor of contraceptive drugs (Dasgupta & Pandey, 1970, George et al., 2012).

Utilization of *H. speciosa* in traditional systems of medicine and importance as a source-plant of diosgenin (George et al., 2012) have resulted in erratic and disorganized collection of the species from the wild for commercial purpose, leading to gene erosion

(Zahoor et al., 2012). Extinction of genotypes of a species is an irreversible setback to the richness of its germplasm, since genotypes once lost is lost forever. At present the genetic variability of the species is not known; therefore we have undertaken this study.

Materials and Methods

Plant materials of *Hellenia speciosa* were collected from different parts of India such as Kerala, Karnataka, Maharashtra, Andhra Pradesh, Nagaland and Andaman Islands (Table 1). The accessions were conserved under field conditions at Jawaharlal Nehru Tropical Botanic Garden & Research Institute (JNTBGRI), Palode, Thiruvananthapuram. Genomic DNA from 25 accessions of *H. speciosa* was isolated using the Plant Genomic DNA Purification Kit (Origin, Kerala) and the manufacturer's protocol was followed for the purpose.

The concentration and purity of isolated DNA were analyzed using Bio Photometer (Eppendorf, Germany). The DNA was then diluted according to the yield to a concentration of 50 ng/µl using sterile distilled water. For the assessment of purity of extracted genomic DNA, the absorbance level at 750 nm, 260 nm and 230 nm were recorded. The integrity of total DNA isolated was analyzed through agarose gel electrophoresis (0.8% agarose gel containing 0.5 ng/ml ethidium bromide). The gel was visualized and bands were observed using UVP gel documentation system.

ISSR markers were used for assessing genetic diversity between and within the accessions. PCR amplification was performed in a 25 µl reaction volume, containing 50 ng template DNA. Thirty one primers were tested; details are given in Table 2. Bands were scored manually as 1 and 0 where 1 denotes presence and 0 denotes absence of band. The binary data coded were used for genetic data analysis.

Population genetic parameters were estimated for multiple populations based on polymorphic loci. Parameters estimated were: number of alleles per locus (*Na*), Shannon's Information Index (*I*), heterozygosity (*H*) and percentage of polymorphism (*P*). Estimations were done using POPGENE software (Yeh and Boyle, 1997). Cluster analysis was performed on the ISSR data using UPGMA, and a dendrogram showing overall genetic relatedness between the accessions

Table 1. Collection details of 25 accessions of *Hellenia speciosa*

S. No.	Acc. Code	Locality	District/Union territories	Latitude	Longitude	Altitude (m)
1	HS 1	Braemore	Thiruvananthapuram	8º 46' N	77º 4' E	161
2	HS 2	Sasthanada	Thiruvananthapuram	8º 54' N	77º 3' E	132
3	HS 3	Karipooru	Thiruvananthapuram	8º 35' N	77º 1' E	63
4	HS 5	Peringamala	Thiruvananthapuram	8º 25' N	77º 1' E	71
5	HS 6	Ponmudi	Thiruvananthapuram	8º 45' N	77º 7' E	915
6	HS 7	Rosemala	Kollam	8º 55' N	77º 10' E	355
7	HS 10	Kulirmala	Kollam	8º 58' N	77º 8' E	245
8	HS 11	Kadammanitta	Pathanamthitta	9º 19' N	76º 46' E	141
9	HS 13	Anakkulam	Idukki	10º 9' N	76º 54' E	319
10	HS 14	Neryamangalam	Idukki	10º 3' N	76º 46' E	51
11	HS 16	Thattekkadu	Ernakulam	10º 5' N	76º 43' E	47
12	HS 17	Thattekkadu	Ernakulam	10º 5'	76º 43' E	47
13	HS 18	Inchathitti	Ernakulam	10º 5'	76º 43' E	47
14	HS 19	Meechuruchimala	Ernakulam	10º 5'	76º 43' E	47
15	HS 22	Mukkali	Palakkad	11º 3' N	76º 32' E	530
16	HS 23	Panthanthode	Palakkad	11º 3' N	76º 32' E	530
17	HS 24	Mukkali	Palakkad	11º 3' N	76º 32' E	530
18	HS 31	Port Blair	Andaman Islands	11º 44' N	92º 39' E	86
19	HS 32	Ross Islands	Andaman Islands	11º 44' N	92º 39' E	86
20	HS 33	Port Blair	Andaman Islands	11º 44' N	92º 39' E	86
21	HS 34	Pushpagiri	Kodagu	12º 39' N	75º 41' E	1469
22	HS 35	Chizani	Phek	25º 35' N	94º 22' E	1452
23	HS 36	Kolhapur	Kolhapur	16º 41' N	74º 14' E	577
24	HS 37	Maredumalli 1	East Godavari	17º 35' N	81º 43' E	421
25	HS 38	Maredumalli 2	East Godavari	17º 35' N	81º 43' E	421

was generated employing and viewed using Treeview software (Page, 1996).

Results

Twenty five accessions of *H. speciosa* were subjected to genetic analysis using ISSR markers. Out of the 31 ISSR primers tested (Table 2), 16 provided reproducible polymorphic patterns. The number of amplified fragments of these primers ranged from 4 to 9 (Figs. 1, 2). Summary statistics of the 16 ISSR primers (loci) with regard to multiple populations were estimated (Table 3). The number of polymorphic bands ranged from 1 to 7 and the percentage of polymorphism ranged from 11.11 to 100 with a mean value of 63.11.

The values of other parameters employed for assessing overall genetic diversity among the 25 accessions were: number of alleles/locus (*Na*) showed a constant value of two, Shannon's Information Index ranged from 0.21 to 0.69 (mean - 0.59). Heterozygosity (*H*) ranged from 0.10 to 0.58 (mean – 0.41) and number of effective alleles (*Ne*) ranged from 0.12 to 1.99 (mean – 1.75).

POPGENE software can be used to analyse data of population sample individually so that genetic variability parameters can be obtained for all the samples separately (single population mode) or it can be analyzed all the samples together which results variability parameters of all the samples as mean values (multi- population mode). Summary statistics of the genetic variability parameters (*Na*, *Ne*, *H*, *I* and *P*) based on variation in ISSR data with respect to all the 25 accessions are given in Table 4. The values of *Na* ranged from 1.44 (HS 1) to 1.88 (HS 23 and HS 24) with a mean value of 1.75 and out of the 25 accessions 10 showed higher *Na* value than the mean. Shannon's Information Index (*I*) ranged from 0.25 (HS 1) to 0.57 (HS 8) and the mean value was 0.48 and out of the 25 accessions 10 showed higher *I* value than the mean. Genetic diversity estimates (*H*) ranged between 0.17 (HS 1) to 0.40 (HS 11). Percentage of polymorphism (*P*) ranged

Table 2. Details of 31 ISSR primers tested in accessions of *Hellenia speciosa.* Numbers as assigned by us.

S. No.	Primers	Sequence of nucleotides (5' to 3')	References
1	816	CTCTCTCTCTCTCTCTG	Ahmad et al., 2019
2	817	CACACACACACACACAA	
3	820	GTGTGTGTGTGTGTGTT	
4	847	CTCTCTCTCTCTCTCTRG	
5	808	AGAGAGAGAGAGAGAGT	Felix et al., 2020
6	809	AGAGAGAGAGAGAGAGC	
7	811	GAGAGAGAGAGAGAGAT	
8	813	CTCTCTCTCTCTCTCTT	
9	824	TCTCTCTCTCTCTCTCC	
10	826	TCTCTCTCTCTCTCTCC	
11	829	ACACACACACACACC	
12	866	CTCCTCCTCCTCCTCCTC	
13	873	GACAGACAGACAGACA	
14	880	CTTCACTTCACTTCA	
15	868	GAAGAAGAAGAAGAAGAA	Giridhari et al., 2020
16	854	TCTCTCTCTCTCTCTCRG	Kumar et al., 2015
17	855	TCTCTCTCTCTCTCTCRG	
18	849	CACACACACACACACARG	Lee et al., 2019
19	834	TGTGTGTGTGTGTGC	Mamatha et al., 2017
20	835	AGAGAGAGAGAGAGAGYT	
21	841	AGAGAGAGAGAGAGAGYA	
22	842	GAGAGAGAGAGAGAGAYC	
23	843	GAGAGAGAGAGAGAGATG	
24	844	CTCTCTCTCTCTCTCTRA	
25	845	CTCTCTCTCTCTCTCTRC	
26	850	GTGTGTGTGTGTGTGTCA	
27	851	GTGTGTGTGTGTGTGTCG	
28	856	ACACACACACACACACTT	
29	860	TGTGTGTGTGTGTGTGGA	
30	864	ATGATGATGATGATGATG	Muthusamy et al., 2008
31	848	CACACACACACACACARC	Wang, 2010

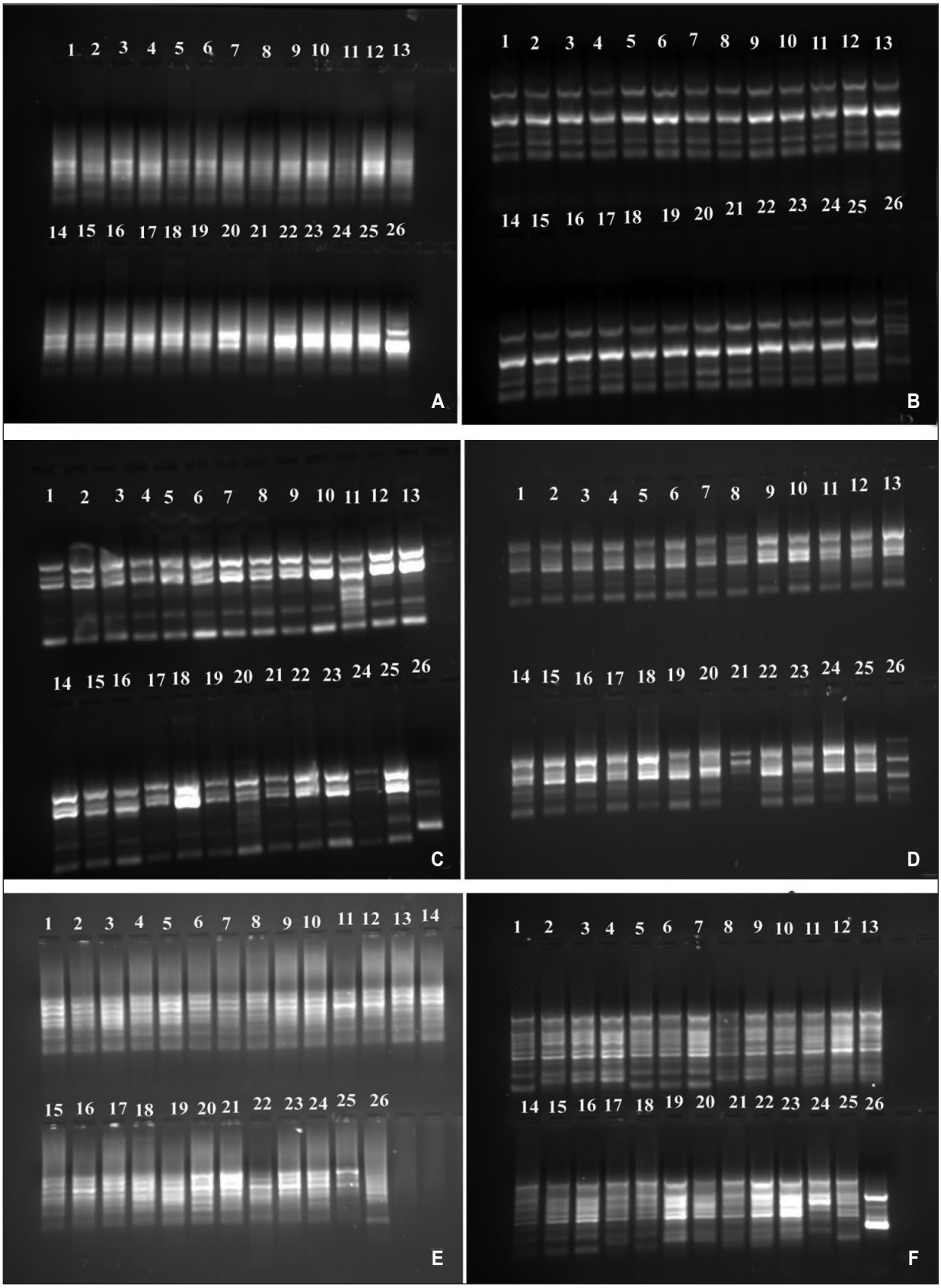

Figure 1: ISSR profiles of 25 accessions of *Hellenia speciosa* in respect of primers (A) 809, (B) 811, (C) 816, (D) 824, (E) 829, & (F) 835

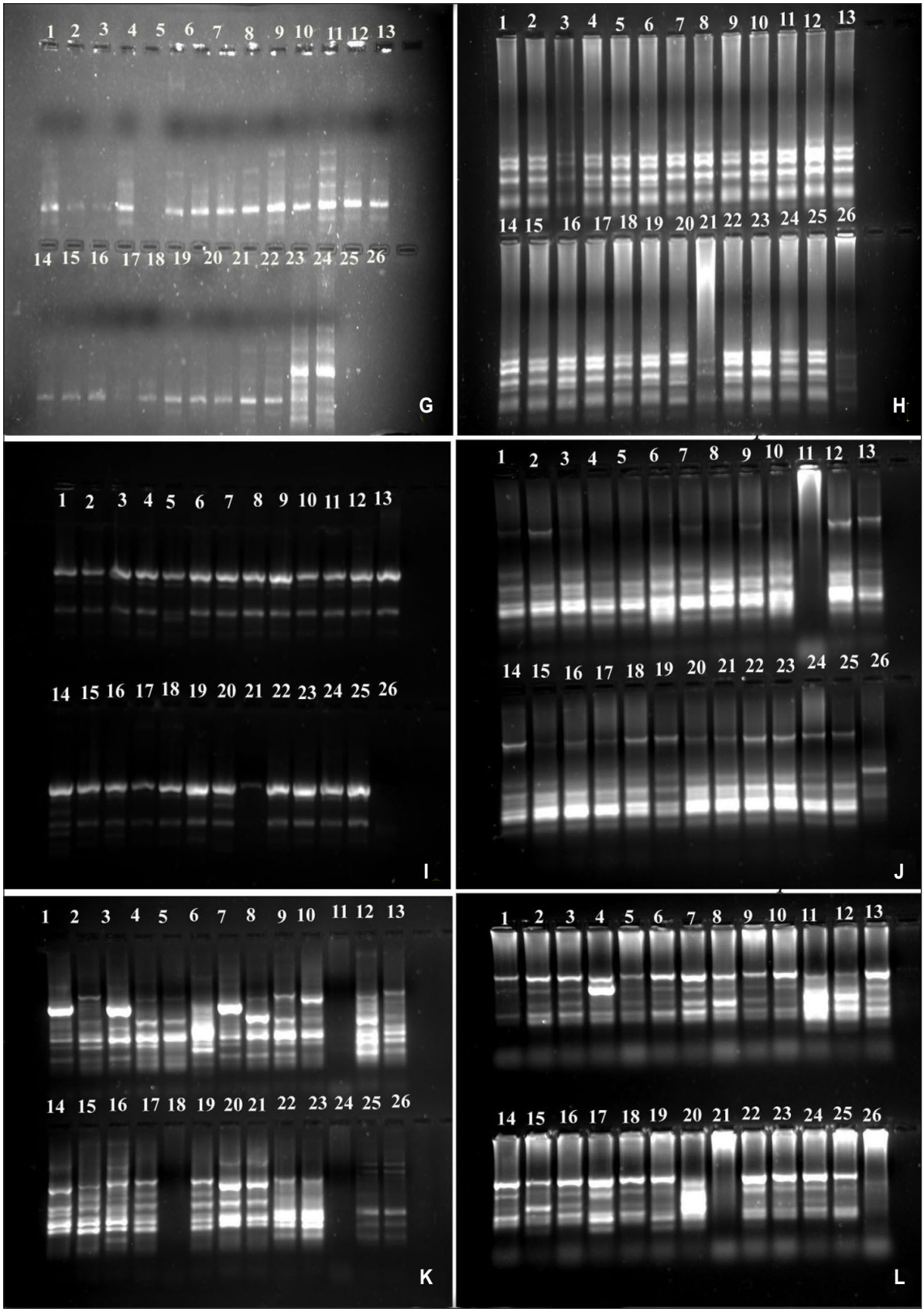

Figure 1: ISSR profiles of 25 accessions of *Hellenia speciosa* in respect of primers (G) 842, (H) 843, (I) 844, (J) n 849, (K) 850, & (L) 855

Table 3. Genetic variability in 25 accessions of *Hellenia speciosa* using 16 ISSR primers that yielded polymorphic bands.

S. No.	Primer code	Number of bands	Number of polymorphic bands	*Na*	*Ne*	*H*	*I*	*P* (%)
1	809	6	3	2	1.99	0.49	0.69	50
2	811	6	1	2	1.92	0.48	0.67	16.67
3	816	8	4	2	1.98	0.49	0.69	50
4	824	6	3	2	1.94	0.49	0.68	50
5	829	7	7	2	1.66	0.39	0.59	100
6	835	7	5	2	1.18	0.15	0.28	71.43
7	842	6	5	2	1.33	0.25	0.41	83.33
8	843	5	2	2	1.12	0.10	0.21	40
9	844	6	5	2	1.86	0.46	0.65	83.33
10	849	6	6	2	1.53	0.34	0.53	100
11	850	7	7	2	1.99	0.58	0.69	100
12	855	7	6	2	1.93	0.48	0.67	85.71
13	856	9	1	2	1.74	0.43	0.62	11.11
14	866	5	4	2	1.89	0.47	0.66	80
15	873	4	1	2	1.99	0.49	0.69	25
16	880	6	3	2	1.99	0.50	0.69	50
	Mean	6.31	3.94	2	1.75	0.41	0.59	63.11

Table 4. Estimates of genetic diversity parameters (*Na, Ne, H, I* and *P*) with respect to 25 accessions of *Hellenia speciosa*

S. No.	Acc. Code	*Na*	*Ne*	*H*	*I*	*P* %
1	HS 1	1.44	1.23	0.17	0.24	43.75
2	HS 2	1.81	1.67	0.36	0.51	81.25
3	HS 3	1.75	1.64	0.34	0.47	75.00
4	HS 5	1.75	1.66	0.35	0.49	75.00
5	HS 6	1.81	1.71	0.37	0.52	81.25
6	HS 7	1.81	1.69	0.37	0.52	81.25
7	HS 10	1.75	1.64	0.34	0.48	75.00
8	HS 11	1.88	1.77	0.40	0.57	87.50
9	HS 13	1.75	1.66	0.34	0.48	75.00
10	HS 14	1.81	1.72	0.37	0.53	81.25
11	HS 16	1.50	1.40	0.22	0.31	50.00
12	HS 17	1.63	1.58	0.30	0.42	62.50
13	HS 18	1.69	1.58	0.30	0.44	68.75
14	HS 19	1.69	1.57	0.30	0.43	68.75
15	HS 22	1.88	1.75	0.39	0.56	87.50
16	HS 23	1.88	1.74	0.39	0.56	87.50
17	HS 24	1.81	1.64	0.35	0.49	81.25
18	HS 31	1.75	1.61	0.33	0.47	75.00
19	HS 32	1.75	1.61	0.33	0.47	75.00
20	HS 33	1.81	1.69	0.37	0.52	81.25
21	HS 34	1.88	1.64	0.35	0.51	87.50
22	HS 35	1.75	1.65	0.34	0.48	75.00
23	HS 36	1.75	1.65	0.34	0.48	75.00
24	HS 37	1.75	1.63	0.33	0.47	75.00
25	HS 38	1.69	1.56	0.29	0.43	68.75
	Mean value	1.75	1.63	0.33	0.48	75.00

from 43.75 (HS 1) to 87.50 (HS 1, HS 22, HS 23, HS 34) (mean 75%). Estimates of the genetic parameters revealed moderate to high level of variation.

A pair wise genetic similarity and distance matrix was prepared on the basis of ISSR data (Table 5). The genetic similarity values varied from 0.67 to 0.99. The observed maximum value has to be treated as 1 and the values ranged from '0' to 1, '0' refers no similarity and 1 maximum similarity. The mean genetic identity among the samples was 0.91. The highest genetic identity (0.99) was found between 10 pairs of accessions and lowest genetic identity (0.65) was between HS 1 and HS 16, HS 16 and HS 34.

The dendrogram revealed that the 25 accessions belonged to 9 major clusters (Fig. 3; Table 6). The Cluster IV includes seven accessions, HS 17 (Thattekkadu, Ernakulam), HS 31 (Port Blair, Andaman Islands), HS 32 (Ross Island, Andaman Islands), HS 35 (Chizani, Phek), HS 36 (Kolhapur, Kolhapur) and HS 38 (Maredumalii 2, East Godavari), Cluster VI four accessions, HS 10 (Kulirmala, Kollam), HS 13 (Anakkulam, Idukki), HS 18 (Inchathitti, Ernakulam) and HS 24 (Mukkali, Palakkad), Cluster VII two accessions, HS 22 (Mukkali, Palakkad) and HS 23 (Panthanthode, Palakkad); Cluster VIII seven accessions, HS 2 (Sasthanada, Thiruvananthapuram), HS 3 (Karipooru, Thiruvananthapuram), HS 5 (Peringamala, Thiruvananthapuram), HS 6 (Ponmudi, Thiruvananthapuram); HS 7 (Rosemala, Kollam), HS 11 (Kadammanitta, Pathanamthitta) and HS 14 (Neryamangalam, Idukki). The rest of five clusters are single member clusters; Cluster I HS 16 (Thattekkadu, Ernakulam), Cluster II HS 33 (Port Blair, Andaman Islands), Cluster V HS 19 (Meechuruchimala, Ernakulam) and Cluster IX HS I (Braemore, Thiruvananthapuram) (Table 6). In general clustering of all the analyzed accessions did not always followed geographical distances. This is very evident from group IV where samples collected from Kerala, Andaman Islands and other regions of India group together. However certain groups like group VIII consists of samples collected from Kerala only.

Discussion

H. speciosa plants exhibit high amount of phenotypic variation. The present study attempts genetic analysis of the species using ISSR PCR and found that the gene pool is genetically diverse (63.11% alleles show polymorphism). As of now, limited number of attempts were made to analyze the genetic diversity of *H. speciosa*. However, there are clues on the existence of natural genetic variations in this species owing to formation of several structural alterations in chromosomes (Chatopadhyay & Sharma, 1983). Mandal and Thomas (2009) analyzed 14 accessions of *Costus speciosus* from Andaman and Nicobar Islands using 14 RAPD primers and found considerable instraspecific genetic diversity (polymorphism of 73%). This study is on par with the present analysis, but with a greater number of accessions (25 nos.) and 16 ISSR primers which resulted in comparable results (polymorphism of 63%). Yadavu and Saluja (2017) carried out the assessment of genetic diversity studies in *Costus speciosus* and they suggest RAPD markers could reveal genetic relationships among distinct *Costus specious* accessions.

The results of the clustering of the accessions substantiate the outcome of genetic diversity analysis in the gene pool of the species, which showed that the average genetic polymorphism in the genepool is very high (63.11%). Fig 3. clearly shows the genetic relationships among the accessions. Where a conservation effort has been undertaken, it may not be possible to conserve all the population of a species. In this context, clustering of accessions based on their genetic relationship will help to omit certain accessions which are genetically identical (HS2-HS3, HS22-HS23, HS10-HS13) where as preference can be given to accessions such as HS1, HS16, HS33 etc. This gives a clue to the importance of these accessions of *H. speciosa* from the point of view of conservation of genetic resource of the species.

Genetic variations in medicinal plants might affect the secondary metabolite it produces either qualitatively, quantitatively or both. In this contexts the present study suggests further detailed genetic and phytochemical analyses of selected accessions of *H. speciosa* such as HS 1 and HS 16, where the heterozygosity and Shannon's index were extremely low (H = 17 and 22; I = 0.24 and 0.31 respectively in the two accessions). These accessions should be analyzed along with selected accessions with higher genetic variability such as HS11, HS 14, HS 22 and HS 23 where H = 0.40, 0.37, 0.39 and 0.39; I = 0.57,0.53, 0.56 and 0.56 respectively in the four accessions.

This study indicates much variability within the species, and further information may reveal sub-specific taxa. The present study revealed that *H. speciosa* is highly variable genetically, possessing 75% polymorphism

Table 5. Nei's original measures of genetic identity (above diagonal) and genetic distance (below diagonal) estimated using ISSR marker analysis in the 25 accessions of *Hellenia speciosa*

popID	HS 1	HS 2	HS 3	HS 5	HS 6	HS 7	HS10	HS11	HS13	HS14	HS16	HS17	HS18	HS19	HS22	HS23	HS24	HS31	HS32	HS33	HS34	HS35	HS36	HS37	HS38
HS 1	****	0.88	0.86	0.84	0.83	0.85	0.88	0.86	0.88	0.88	0.67	0.81	0.88	0.87	0.86	0.88	0.91	0.81	0.79	0.75	0.87	0.83	0.82	0.83	0.78
HS 2	0.13	****	0.98	0.98	0.98	0.99	0.98	0.97	0.97	0.99	0.86	0.92	0.96	0.92	0.96	0.96	0.96	0.90	0.88	0.82	0.90	0.90	0.90	0.90	0.86
HS 3	0.15	0.02	****	0.99	0.97	0.99	0.97	0.95	0.97	0.98	0.86	0.93	0.96	0.91	0.96	0.94	0.94	0.90	0.91	0.84	0.89	0.92	0.92	0.91	0.90
HS 5	0.17	0.02	0.01	****	0.98	0.98	0.95	0.95	0.95	0.98	0.88	0.90	0.92	0.89	0.95	0.93	0.92	0.89	0.87	0.80	0.88	0.90	0.89	0.89	0.88
HS 6	0.19	0.02	0.03	0.02	****	0.99	0.96	0.95	0.96	0.99	0.90	0.90	0.92	0.93	0.95	0.94	0.91	0.89	0.88	0.79	0.87	0.90	0.90	0.90	0.86
HS 7	0.16	0.01	0.02	0.02	0.01	****	0.98	0.96	0.98	0.99	0.88	0.94	0.96	0.93	0.96	0.95	0.94	0.90	0.91	0.84	0.89	0.93	0.92	0.91	0.89
HS10	0.13	0.02	0.03	0.05	0.04	0.02	****	0.95	0.99	0.98	0.82	0.96	0.98	0.95	0.94	0.94	0.96	0.92	0.92	0.86	0.91	0.95	0.94	0.93	0.90
HS11	0.15	0.03	0.05	0.05	0.05	0.04	0.05	****	0.95	0.97	0.85	0.89	0.93	0.91	0.93	0.93	0.93	0.87	0.86	0.85	0.91	0.88	0.88	0.88	0.84
HS13	0.13	0.03	0.03	0.05	0.04	0.02	0.01	0.05	****	0.98	0.84	0.95	0.98	0.95	0.94	0.94	0.96	0.93	0.93	0.86	0.90	0.95	0.94	0.94	0.90
HS14	0.13	0.01	0.02	0.02	0.01	0.01	0.02	0.04	0.02	****	0.88	0.93	0.95	0.94	0.96	0.95	0.95	0.92	0.90	0.83	0.90	0.93	0.93	0.93	0.88
HS16	0.41	0.15	0.15	0.12	0.11	0.12	0.20	0.16	0.17	0.13	****	0.85	0.83	0.75	0.82	0.80	0.76	0.81	0.80	0.76	0.67	0.81	0.80	0.83	0.77
HS17	0.21	0.09	0.08	0.10	0.10	0.06	0.04	0.11	0.05	0.08	0.17	****	0.97	0.87	0.88	0.87	0.90	0.91	0.92	0.90	0.80	0.95	0.94	0.93	0.90
HS18	0.12	0.04	0.05	0.08	0.08	0.04	0.02	0.07	0.02	0.05	0.18	0.03	****	0.92	0.92	0.93	0.96	0.90	0.92	0.89	0.86	0.94	0.94	0.93	0.89
HS19	0.15	0.08	0.09	0.12	0.07	0.07	0.06	0.09	0.05	0.07	0.29	0.13	0.09	****	0.91	0.92	0.92	0.84	0.85	0.76	0.86	0.88	0.88	0.87	0.83
HS22	0.15	0.04	0.05	0.06	0.05	0.04	0.06	0.07	0.06	0.04	0.20	0.13	0.08	0.10	****	0.99	0.97	0.93	0.93	0.84	0.95	0.93	0.93	0.92	0.92
HS23	0.12	0.04	0.06	0.07	0.07	0.05	0.07	0.07	0.07	0.05	0.22	0.14	0.07	0.08	0.01	****	0.98	0.90	0.91	0.83	0.93	0.92	0.92	0.91	0.91
HS24	0.09	0.05	0.06	0.09	0.09	0.06	0.04	0.08	0.04	0.06	0.28	0.11	0.04	0.08	0.03	0.02	****	0.92	0.92	0.86	0.95	0.93	0.93	0.92	0.91
HS31	0.22	0.11	0.11	0.12	0.12	0.10	0.08	0.14	0.07	0.09	0.21	0.10	0.11	0.17	0.08	0.10	0.09	****	0.97	0.89	0.90	0.98	0.98	0.99	0.97
HS32	0.24	0.12	0.10	0.14	0.13	0.10	0.08	0.16	0.07	0.10	0.23	0.08	0.09	0.16	0.07	0.09	0.08	0.03	****	0.91	0.88	0.98	0.99	0.97	0.97
HS33	0.29	0.20	0.18	0.22	0.23	0.18	0.15	0.17	0.15	0.19	0.28	0.11	0.12	0.28	0.18	0.18	0.16	0.12	0.10	****	0.81	0.91	0.91	0.90	0.90
HS 34	0.14	0.10	0.12	0.13	0.14	0.12	0.10	0.10	0.10	0.10	0.40	0.22	0.15	0.15	0.06	0.08	0.05	0.11	0.13	0.21	****	0.89	0.88	0.87	0.87
HS35	0.18	0.10	0.08	0.11	0.10	0.08	0.06	0.13	0.06	0.07	0.21	0.05	0.06	0.13	0.07	0.08	0.07	0.02	0.02	0.09	0.12	****	1.00	0.99	0.98
HS36	0.20	0.10	0.09	0.11	0.11	0.08	0.06	0.13	0.06	0.08	0.22	0.06	0.07	0.13	0.07	0.08	0.07	0.02	0.01	0.09	0.12	0.00	****	0.98	0.98
HS37	0.19	0.10	0.10	0.11	0.11	0.09	0.07	0.13	0.06	0.08	0.18	0.07	0.08	0.14	0.08	0.09	0.08	0.01	0.03	0.11	0.13	0.01	0.02	****	0.96
HS38	0.25	0.15	0.11	0.13	0.15	0.12	0.10	0.18	0.10	0.12	0.26	0.10	0.12	0.18	0.08	0.10	0.10	0.03	0.03	0.11	0.14	0.02	0.03	0.04	****

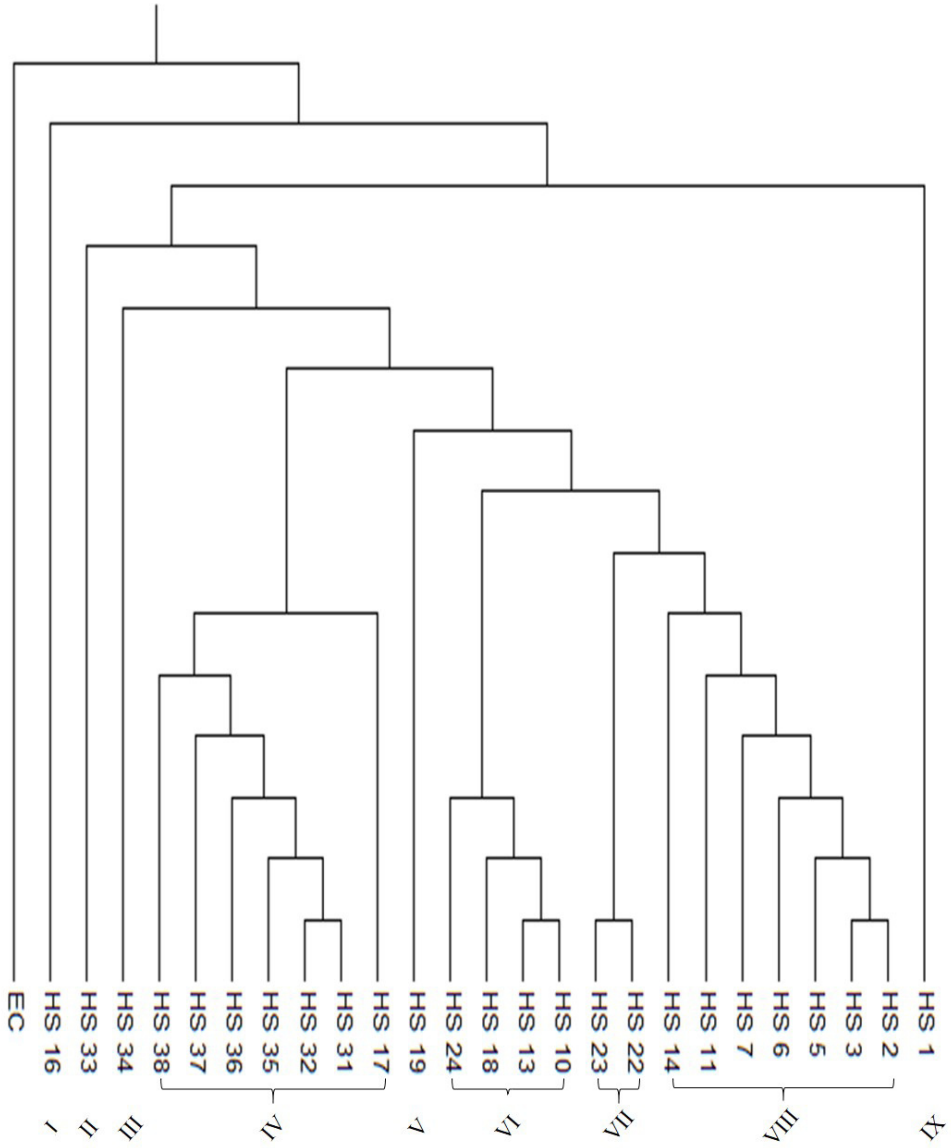

Figure 3: Dendrogram showing the IX clusters of accessions of *Hellenia speciosa* (HS) formed based on POPGENE analysis. (*Elettaria cardamomum* (EC) was used as an outgroup)

Table 6. Composition of clusters (I to IX) formed by cluster analysis based on ISSR data of 25 accessions of *Hellenia speciosa*

Cluster	No. of Accessions	Accessions
I	1	HS 16
II	1	HS 33
III	1	HS 34
IV	7	HS 17, HS 31, HS 32, HS 35, HS 36, HS 37, HS 38
V	1	HS 19
VI	4	HS 10, HS 14, HS 18, HS 24
VII	2	HS 22, HS 23
VIII	7	HS 2, HS 3, HS 5, HS 6, HS 7, HS 11, HS 14
IX	1	HS 1

in the gene pool, and this finding suggests that the enormous phenotypic variations exhibited by the species are genetically determined.

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