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Assessment of genetic diversity in guava (*Psidium guajava*) germplasm using microsatellites

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ABSTRACT

Although the varietal diversity is fairly rich in guava, most varieties lack one or more desirable characters. Hence, attempts were made for improving specific traits, viz., attractive pink pulp colour, soft seeds, medium fruit size, high TSS and high ascorbic acid. Genetic diversity analysis is a prerequisite for identifying potential parents in breeding programs and germplasm conservation. Molecular characterization helps discriminate closely-related genotypes, as, this technique is unaffected by environment, rendering it more reliable. In this study, 48 polymorphic SSRs screened from a total of 115 SSR markers were used for analyzing marker segregation in 72 guava accessions. Statistical analysis was done using IDENTITY1.0 and CERVUS 3.0 software. Cluster analysis was done with DARwin 5.0 software, using Wards Minimum Variance method, and weighted group neighbour joining method, to check reliability of grouping among clusters. The trend in grouping was found to be similar in both methods. Dendrograms generated showed that the hybrids clustered with their parents; exotic collections fell into two different sub-groups based on productivity; the wild species formed one group; and Navalar cultivars from Dharwad clustered together, reflecting similar origin.

Key words: Guava, genetic diversity, dendrogram, simple sequence repeats

INTRODUCTION

Guava (*Psidium guajava*) belongs to the family *Myrtaceae* and has a diploid chromosom number 2n = 22. Guava is believed to have originated in Central America (Hayes, 1953) and is now well-adapted to India, being widely grown throughout the country. Although guava is self pollinated, 35-40% cross pollination takes place in some cultivars, providing a heterogeneous, open-pollinated seedling population with adequate genetic variation (Pathak and Ojha, 1993). Most guava varieties have evolved through selection from seedling variants. Guava fruit is a rich source of vitamins (A and C), dietary fiber, carotenoids, essential oils and pectin. Besides its nutritional properties, the leaves and bark of *P. guajava* have a long history of medicinal use that still continues today (Joseph and Mini Priya, 2011). The present study aims to use microsatellite markers to measure genetic diversity in guava germplasm. This would help breeders choose genetically diverse germplasm for use as parents in breeding programmes to develop superior hybrids. Microsatellites consist of tandemly repeated units of DNA, show high levels of allelic diversity per locus, are co-dominant in nature and highly reliable. Thus, these can be used for infering genotyping information, and are best suited for genetic diversity studies in both plants and animals (Cholastova and Knotova 2012).

MATERIAL AND METHODS

a. Plant material and molecular markers

Guava germplasm maintained in the Field Gene Bank (FGB) at Indian Institute of Horticultural Research, Bengaluru, India, was selected for the study. The germplasm consisted of hybrids, exotic collections, local collections and wild species used as rootstocks or as parents in the disease resistance breeding program (Vasugi and Dinesh, 2007). Total genomic DNA of the 72 guava accessions was extracted from leaf material by *Doyle and Doyle method* (1990) with minor modifications. The precipitated DNA was dissolved in TE buffer and integrity of genomic DNA isolated was determined by electrophoresis in 0.8% agarose gel. DNA quantification

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was done using a Gene Quant UV- Spectrophotometer (GE Health Care Bio-sciences Ltd; England), and diluted accordingly.

Out of a total number of 72 accessions (Table 1), 24 guava accessions (diverse with respect to fruit characteristics, viz., fruit weight, TSS-Total Soluble Solids, ascorbic acid, pulp colour, seed hardness and fruit weight) were initially selected. Genotyping was performed using the following polymerase chain reaction profile: Volume of the reaction mixture was 20µl which contained 1X buffer (10 mM Tris HCl of pH 8, 50mM KCl, 1.5mM $MgCl_2$), 0.5µM of each primer, 200µM of dNTPs, 0.5 units of Taq DNA polymerase (GeNei, Bangalore) and 50ng genomic DNA. DNA amplification was done as per the PCR program of Risterucci *et al* (2005). Amplified PCR products were separated on 3% agarose gel (3B Black Bio Biotech India, Ltd.) loaded with 100bp ladder (Fermentas). The gels were stained with ethidium bromide and were photographed using UVIPRO platinum gel documentation unit. Molecular weight analysis of the amplified alleles was made in comparison with a 100 bp ladder loaded along with the samples by using UVITEC platinum ID software (ver.12, Cambridge, UK). Genotyping was done to screen 115 SSR (simple sequence repeats) markers, of which only 48 were found to be polymorphic. PCR amplification of the remaining accessions was performed using the 48 shortlisted polymorphic markers (Table 2) to obtain allelic molecular weight data of all the 72 accessions.

Table1. List of accessions used in the present study

1.	Nasik	37.	Florida Seedling
2.	Local 1	38.	Seedless Triploid
3.	Chittidar	39.	Karela
4.	Sindh	40.	EC-147039
5.	Local 2	41.	Spear Acid
6.	Local 3	42.	Purple Local
7.	Behat Coconut	43.	Red Flesh
8.	G6	44.	Pati
9.	EC-147037	45.	Dharwad
10.	CIW5	46.	Hisar Safeda
11.	Nagpur Seedless	47.	Superior Sour Lucidum
12.	Abu Ishaqwala	48.	White Flesh
13.	Phili Pink	49.	*Psidium molle*
14.	Lucknow- 42	50.	*Psidium cattelianum*
15.	CIW1	51.	*Psidium quadrangularis*
16.	Apple Colour	52.	*Psidium chinensis*
17.	Aneuploid-2	53.	*Psidium friedrichsthalianum*
18.	Dhareedar	54.	S.P.No.6
19.	CIW2	55.	S.P.No.7
20.	GR1	56.	7-12 EC147036
21.	Surkha Chitti	57.	Hafsi
22.	Aneuploid- 1	58.	Bangalore Local
23.	Chakaiya Ruthamnagar	59.	7-39147034
24.	Surkha Chitti Neptuani	60.	9-35147036
25.	Arka Amulya	61.	EC-162904
26.	Arka Mridula	62.	Parker Dessert
27.	Allahabad Safeda	63.	Sabdana Badri
28.	Safed Jam	64.	Kohir Long
29.	C.P.A. White	65.	Kohir Safeda
30.	Ben Dror	66.	Swetha
31.	Smooth Green	67.	CISH-G-1
32.	Benaras	68.	Local White
33.	Portugal	69.	Beaumont
34.	Lalit	70.	Kg Guava
35.	Mirzapur Seedling	71.	Thailand Guava
36.	Sardar Guava	72.	Kamsari

b. Statistical and genetic diversity analysis

Statistical parameters such as number of alleles per locus (k), allele frequencies, expected heterozygosity (EXP.HET.), observed heterozygosity (OBS.HET.) and Polymorphic Information Content (PIC) were analyzed using allele frequency analysis module of Cervus 3.0 (Kilinowski *et al*, 2007). Probability of identity (PI) was calculated using IDENTITY1.0 software. DARwin 5.0 software was applied to construct a dendrogram by both Wards Minimum Variance method and Neighbor Joining method. Factorial analysis was performed using DARwin 5.0 (http://darwin.cirad.fr) (Perrier *et al*, 2003).

RESULTS AND DISCUSSION

1. Allelic diversity

The 48 SSRs exhibited unique amplification fragments in the 72 accessions. Results of statistical analysis are depicted in Table 2. A total of 249 alleles were amplified. Amplicon size varied from 71bp (mPgCIR207) to 386bp (mPgCIR100). Based on results on statistical analysis (Table 2), the number of alleles ranged from 2 (mPgCIR029, mPgCIR236) to 11 (mPgCIR201), with a mean number of 5.33 alleles per locus (which was higher than 4.5 alleles per locus reported earlier) (Rodriguez *et al*, 2007). PIC values ranged from 0.260 (mPgCIR236) to 0.811 (mPgCIR243), with a mean of 0.563. Expected heterozygosity ranged from 0.078 (mPgCIR038) to 0.838 (mPgCIR243), with a mean of 0.616. PI values computed using IDENTITY1.0 software (Wagner and Sefc, 1999) ranged from 0.052 to 0.847 for the loci mPgCIR321 and mPgCIR038, respectively (which was higher than the range 0.031 to 0.487 reported earlier) (Kanupriya *et al*, 2011).

2. Genetic diversity analysis

Cluster analysis was performed using the distance-based clustering method, which takes pair-wise distance matrix as an input for analysis, by a specific clustering algorithm (Johnson and Wichern, 1992).

Table 2: List of polymorphic markers with results of statistical analysis

Sl. No.	Locus	F=Forward primer (5'-3') R=Reverse primer (3'-5')	Expected size (bp)	No. of alleles	Allele size (bp)	OBS. HET	EXP. HET	PIC	PI
1	mPgCIR339	F: CCGAAGACGAGGAGATTA R: TTAAGTGGAAAATCACAGTTG	160	5	140-227	0.070	0.658	0.582	0.181
2	mPgCIR243	F: ACAGCAGGACACAAAGGA R: GCTCTGAGGTGGTTTTCAT	174	7	107-212	0.292	0.798	0.764	0.072
3	mPgCIR182	F: GAGGAAGAAACCCGAAGTTA R:GGTAGAAAGATCGGAAAGAC	181	8	87-202	0.264	0.8	0.769	0.068
4	mPgCIR236	F: ACTCATATTCCGTTTGCATC R:GAATTAACGACGAGTTCCAC	164	2	154-168	0.056	0.301	0.254	0.507
5	mPgCIR316	F: GCTTCATATTACAAACCTTGG R:GATCTAACTGACTTGCCAAAA	232	4	192-281	0.239	0.464	0.416	0.317
6	mPgCIR326	F: AGAACAAGACACGAGAAGAG R:AAAATCTACGCACAAACC	116	6	83-179	0.250	0.787	0.748	0.081
7	mPgCIR207	F: CAAGATTTGCCTCAAGAAAC R:AACTAAATAGCCTGCTGGTG	136	5	71-145	0.306	0.460	0.433	0.319
8	mPgCIR206	F:GGAAGTTTCAAAGTAACAGCAC R:AGAATGAGTCCATGCTCAAA	181	6	174-295	0.111	0.764	0.721	0.096
9	mPgCIR220	F:AGAGCAGTGGTTGCTATTTT R:CCCATCTCTTACTTTTCTTGTG	218	7	145-164	0.083	0.731	0.679	0.121
10	mPgCIR277	F:AGCCGATTATGATTACCTG R:CGATTCACTCCCTCATTACT	173	5	144-191	0.250	0.732	0.689	0.113
11	mPgCIR039	F:GCTCACCTTACTCATTCAGC R:CTGTTGCTAAGAGCTTTCGT	155	4	145-200	0.014	0.524	0.406	0.309
12	mPgCIR222	F:CCAGAATCAGACATAGTTAGAG R:CTGAAGACATCAACATGGAA	166	3	169-213	0.171	0.393	0.337	0.381
13	mPgCIR093	F:GCATCATGTGTTTGAACGAT R:AAGTGTGCGTTCTCCATCT	123	6	102-168	0.194	0.803	0.768	0.070
14	mPgCIR099	F:TCAAAGTCCAAAACTCATGC R:GGGATGGAGTAAAGATGAAA	220	4	194-267	0.208	0.532	0.475	0.276
15	mPgCIR042	F:CTCACCCAAAATCTACACAAG R:AAGGGACTGGACGATGTT	107	3	110-140	0.029	0.322	0.296	0.436
16	mPgCIR100	F:CTAGAAGTCGAAGAATGGAA R:TTTGTTAGTATCGGAGTCGAG	128	5	122-386	0.239	0.671	0.617	0.154
17	mPgCIR185	F:AACGCATCTGGCATTGAT R:CCTTGGTCTCCCTCTTACTC	117	4	97-135	0.141	0.308	0.285	0.476
18	mPgCIR165	F:TAAGGGATTCATTTCCGAGT R:CTGGTGTGACGATGACTTTT	124	3	127-176	0.029	0.523	0.411	0.289
19	mPgCIR029	F:CTCGCTTCAATCTCCATCTA R:AGCGACACAGACTCTTCATT	162	2	166-202	0.521	0.414	0.326	0.409
20	mPgCIR154	F:CTTCAGCTACAGCCTTTCC R:GGAGAAAGCAGAAATTCCA	138	8	102-285	0.903	0.794	0.759	0.074
21	mPgCIR038	F:AGCCTGTTTTACGCCTTC R:CGGCTGCTCTATTGTTATTT	111	3	102-131	0.028	0.081	0.079	0.847
22	mPgCIR194	F:GCAGAGAATCGAAGCACTA R:GCAAGCACAGGTTCTACTTT	172	6	154-208	0.278	0.747	0.695	0.113
23	mPgCIR193	F:GAACGTGGGTTACATACCAT R:ATCACCGTCCTCCTAAATCT	122	4	102-132	0.028	0.594	0.506	0.252
24	mPgCIR027	F:AGCACTTAGGGACAAATTCA R:CTCACTCTCCTCCATTCAAG	292	4	262-337	0.167	0.668	0.598	0.178
25	mPgCIR191	F:GACCCTCCCACTTATATTTTG R:AAGCTGACATAACAGTCGAA	210	6	216-282	0.485	0.766	0.726	0.071
26	mPgCIR091	F:GCGGTGGATTTGAATTTAG R:CCAAGTAACCCACAACAATA	125	3	107-142	0.324	0.552	0.465	0.272
27	mPgCIR031	F:TCTCACTGATGCAACTTTTC R:CCCATTTTCATCTCAAAGTC	128	8	104-191	0.159	0.616	0.580	0.156
28	mPgCIR157	F:AACCACCAAACCATACACC R:CGACCAACCCTACATTCTG	209	4	163-224	0.246	0.692	0.636	0.128

Table 2: Contd.

Sl. No.	Locus	F=Forward primer (5'-3') R=Reverse primer (3'-5')	Expected size (bp)	No. of alleles	Allele size (bp)	OBS. HET	EXP. HET	PIC	PI
29	mPgCIR161	F:TCTCAAGGACCAACAAGAAG R:AGGACTTAGCTTGGGTTTTC	246	5	218-283	0	0.681	0.622	0.136
30	mPgCIR111	F:CAACCTCGTTTGAGTCTTCT R:AACATCATTGGGACCATTC	115	5	86-156	0.222	0.420	0.394	0.363
31	mPgCIR041	F:AAGTGGTGTCAGCAACTACC R:CTTAGTTTGACCGCTCCAGT	136	4	130-170	0.014	0.579	0.505	0.214
32	mPgCIR174	F:GCCACTGTGTAAGAGGATTG R:ATTGTGGGAGATTGGAGAC	261	4	181-273	0.108	0.626	0.545	0.158
33	mPgCIR184	F:AAGCTACAATCGACGAAAAC R:CACTATTAGCGAACCTGCAT	221	5	171-254	0.171	0.706	0.660	0.117
34	mPgCIR104	F:ATTCCCGTGGATTATGTATC R:ACAACCATTTTCTCCTCATC	120	2	125-141	0	0.423	0.332	0.381
35	mPgCIR109	F:AATTTCCACAGATCACAAGG R:GGCATCTCCATCAAATACAT	110	5	104-147	0.083	0.686	0.620	0.162
36	mPgCIR325	F:AAACGCTCGAATCAGTTG R:CCAAGAAACACAGGGATTAC	172	7	140-202	0.319	0.807	0.773	0.068
37	mPgCIR200	F:CCTTGCTTTGGTGAGGTC R:GCTAATTCAGTCCTTCCAACT	178	8	141-377	0.203	0.685	0.645	0.118
38	mPgCIR321	F:TTTTGGCCTGGGAATATAG R:TAAAACGAAAGCAGAAAACC	129	8	114-191	0.209	0.810	0.778	0.052
39	mPgCIR032	F:CGCCTTTCGTAAAAGAAGT R:TCATATACTCGGACAAAACG	100	5	73-136	0.071	0.672	0.613	0.148
40	mPgCIR102	F:AATTGGTGTAGCATCTGGA R:GCCTACCATGAACAGAGAAA	176	5	181-255	0.403	0.661	0.586	0.188
41	mPgCIR201	F:TTTGCCTTCGAGCTTCTACT R:ACAATTTCGTGGGCTCGT	133	11	120-303	0.471	0.791	0.754	0.070
42	mPgCIR203	F:ATGAAGGCATTACCTAAGAC R:ACCCTATTAACCCTTAGCAA	126	3	127-341	0.086	0.547	0.447	0.274
43	mPgCIR205	F:ACCTCTCCAGCTCTACACG R:GAGGTTGTCGAAGGTTGAT	101	5	89-164	0.458	0.786	0.745	0.084
44	mPgCIR098	F:CATCAACTTTCCAGGCATA R:CCATTCAGTCGGTTTGAC	127	4	116-148	0.0	0.663	0.595	0.154
45	mPgCIR101	F:ATGGCTGTAAGAAGCAAAAG R:GAAGAAATGTAGGTGCGTTC	110	5	100-164	0.074	0.413	0.368	0.317
46	mPgCIR150	F:CCTAGTGACTCGAAGCAATC R:TTGAGCCCTAGCATAGACAG	108	5	106-152	0.153	0.657	0.592	0.182
47	mPgCIR133	F:CGATCTTGGAATGTAAGAGG R:TGGATTTGCAGGTTCTATCT	148	8	134-244	0.181	0.709	0.659	0.133
48	mPgCIR437	F:ACAACAGTTCTGATCCCAAA R:CTCGGAGACACAGAGGTCTA	153	6	155-346	0.099	0.725	0.678	0.113

bp= number of base pairs, OBS. HET= Observed heterozygosity, EXP. HET= Expected heterozygosity, PIC= polymorphic information content , PI= probability of identity

a Wards Minimum Variance method

Wards Minimum Variance method (Ward, 1963) generates a graphic representation such as a tree or a dendrogram, in which clusters can be visually identified. Confidence limits of different clades in the dendrogram were tested by bootstrapping 1000 times to assess repetitiveness of genotype clustering (Felsenstein, 1985) in both the methods. Dendrogram generated by Wards Minimum Variance method is shown in Figure 1. This method showed two clusters: a major cluster (Cluster 1) with 50 accessions, and a minor cluster (Cluster 2) with 22 accessions.

Subgroup-C_1 included genotypes like Sindh, Chittidar, Hafsi, Behat Coconut, Local 1, Nasik, Local 3, Bangalore Local, CIW5, Abu Ishakwala, and Nagpur Seedless. Sub group-C_2 included seven varieties and five wild species, along with Phili Pink and Lucknow-42. Most of the exotic collections grouped with Local-2 in Group-D, leading to the inference that Local-2 is an introduction. Fourteen accessions were clustered in Subgroup-E_1 as shown in Figure 1. Genotypes like Dhareedar, Aneuploid -2 and Apple Colour clustered with CIW2 and CIW1, in Subgroup-E_2. Local - White, S.P. No.7, Bendror, CISH-G-1, Swetha, Beaumont, S.P. No.6 clustered in Group-F of Cluster-1.

Cluster-2 had one Group-G which was divided into Sub-groups G_1 and G_2. In Subgroup-G_1, all the white-pulped varieties clustered with Purple Local, a purple-pulp accession. Many pink-pulp varieties clustered with two white-pulp accessions, Florida Seedling and Superior Sour Lucidum, in Subgroup-G_2.

b. Neighbor Joining method (NJ)

To overcome systemic errors, an alternative method known as Neighbor Joining is used in phylogenetic studies. This removes the assumption that the data are ultrameric (Swofford *et al*, 1996). In this method, bootstrapping values of the allele frequencies can be displayed to assess reliability of the nodes. The dendrogram obtained is shown in Fig. 2. The Neighbor Joining method is discussed in detail, as, it includes bootstrapping. The bootstrap values varied from 2% to 100%. Highest bootstrap value of 100% was observed for the varieties Arka Amulya and Kohir Long. A similar low bootstrapping value, ranging from 3% to 100%, was reported earlier by Hadadinejad *et al* (2011).

Cluster analysis clearly showed that the accessions fell broadly into three clusters. Cluster-1 was divided into Group-A and Group-B. Group-A was subsequently subdivided into A_1 and A_2. A_1 Sub-group clustered 11 accession together showing that these were genetically closer; but morphologically, the similarity was not visible, perhaps due to minor differences in allelic composition. Clustering of five wild species with Phili Pink and Lucknow-42 in Subgroup-A_2 represents their close genetic similarity; it can be inferred indirectly that these are quite dissimilar to the commonly cultivated *Psidium guajava*. Similar clustering of wild species was earlier reported by Rajkumar *et al*, (2011). Group-B consisted of five exotic collections, with higher productivity (EC-162904, G6, 9-35147036, 7-39147034, EC-147037) (Vasugi and Rami Reddy, 2003). Cluster-2 with 26 germplasm accessions was divided into Group-C and Group-D. Group-C was further divided in to Sub-groups C_1 and C_2, that clustered 21 and one genotypes, respectively. Cluster C_1 confirmed the parentage of hybrids (Dinesh and Vasugi, 2010) as Arka Mridula, and the hybrid Arka Amulya clustered with its maternal parent, Allahabad Safeda. Other hybrids (Kohir Safeda and Safed Jam) clustered with their parent, Allahabad Safeda. In C_2, only GR1 was present. Navalur varieties grouped with Dhareedar, Aneuploid-2 and Apple Colour, indicating their genetic similarity within Group-D. Cluster-3 was divided into Sub-groups E_1 and E_2. Subgroup-E_1 was further divided into Group-F and G. Group-F was sub-divided into F_1 and F_2. F_1 was divided into F_{1a} and F_{1b} and contained white and pink pulp varieties in two different clusters, respectively. Subgroup-E_2 clustered three accessions, Hisar Safeda, Purple Local and White Flesh, which separated from the other 19 genotypes. All the remaining 19 genotypes under E_1 were divided into four Sub-groups like F_1 (F_{1a}, $F1_b$, and F_2) and G. Subgroup-F_1 consist of both white and pink pulp varieties in two different clusters. Further clustering resulted in clear differentiation of the remaining pink and white pulp varieties. The white-pulp varieties clustered in Sub-group F_{1a}. Sub-group F_{1b} grouped all pink pulp varieties with Florida Seedling, a white pulp accession. Sub-groups F_2 clustered two pink pulp varieties with Superior Sour Lucidum, a white-pulp accession. This grouping may perhaps be due to their highly acidic nature.

In Sub-group G, pink-pulp varieties like Thailand Guava and Pati clustered together. Similar differentiation of white and pink pulp varieties was reported by Kanupriya *et al (*2011).

c. Factorial analysis

Factorial analysis represented in Fig. 3 is a type of Principal Co-ordinate Analysis used for deriving a 2-3 dimensional scatter plot of individuals. This method facilitates identification of individuals showing intermediacy between two groups (Lessa, 1990). Individuals belonging to a single plot reveal sets of genetically similar individuals (Karp *et al*, 1997). The picture consists of X axis and Y axis, based on which it is divided into four co-ordinates (Co-1, Co-2, Co-3, and Co-4). Interestingly, the accessions that grouped in Cluster 1 in Neighbor Joining method (NJ) were included in Co-2 (except *P.quadrangularis*, which grouped in Co-1). This alignment of *P. quadrangularis* in Co-1 of factorial analysis may be due to the superior morphological traits like high stamen number, large flower and fruit, and good flavour, compared to the other species used in the study (Vasugi and Dinesh, 2007). Accession G6 showed intermediacy between Co-1 and Co-2. Co-4 included accessions from Cluster-2 in NJ method, except Dhareedar, Aneuploid-2 and Apple Colour (which grouped in Co-2). Co-1 and Co-3 together included all the accessions belonging to cluster 3 in Neighbour Joining method. Irrespective of the method used, pattern of clustering among genotypes was found to be similar. Both the cluster-analysis methods grouped individuals into stringently defined groups or clusters. Finally, factorial analysis clearly confirmed the patterns obtained by cluster analysis.

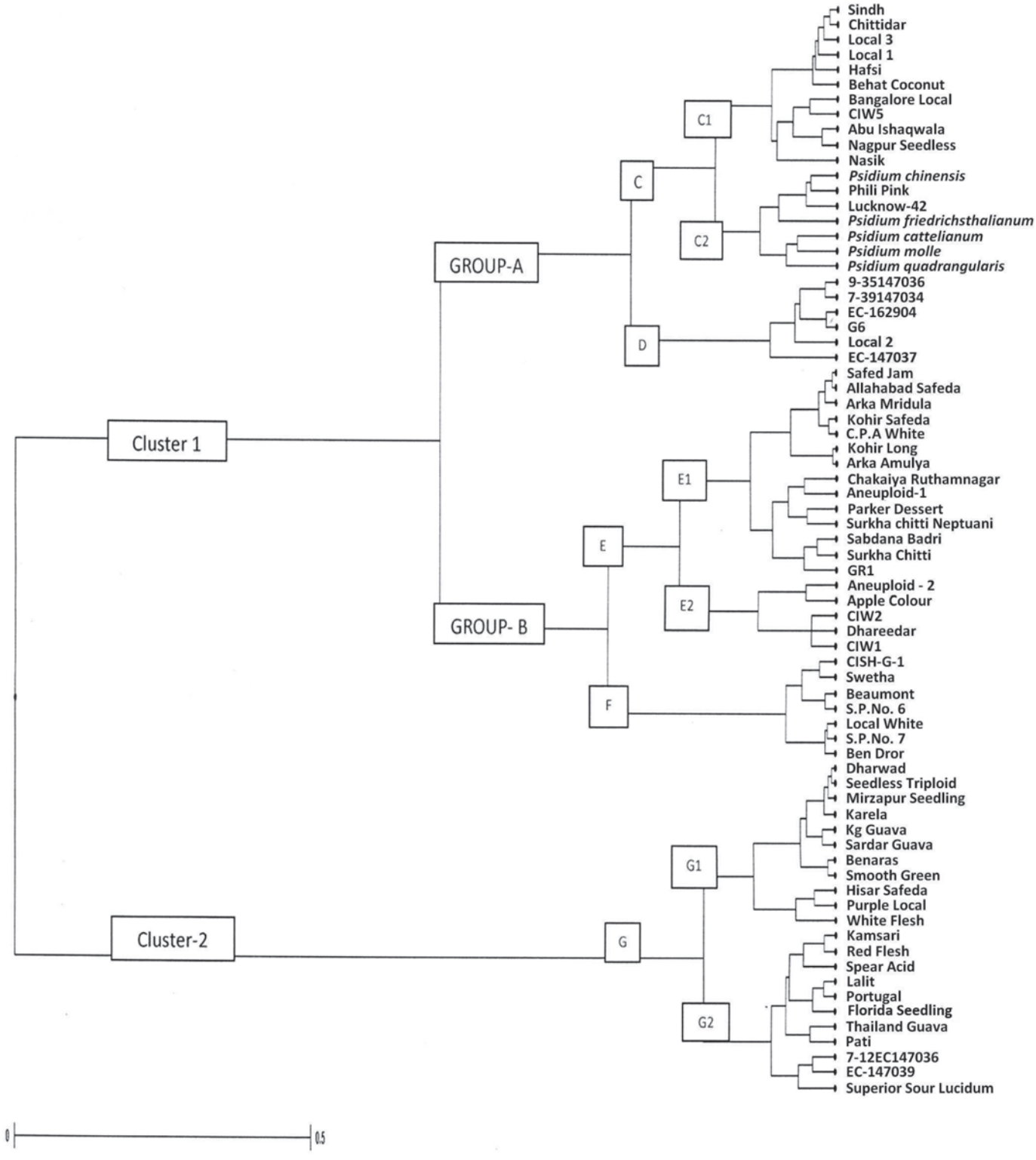

Fig. 1. Dendrogram generated using Wards Minimum Variance method from the computed genetic distances of simple matching coefficient. The black dot on the left of the dendrogram indicates the origin, and the line at the bottom indicates the coefficient of Jaccards Dissimilarity Matrix.

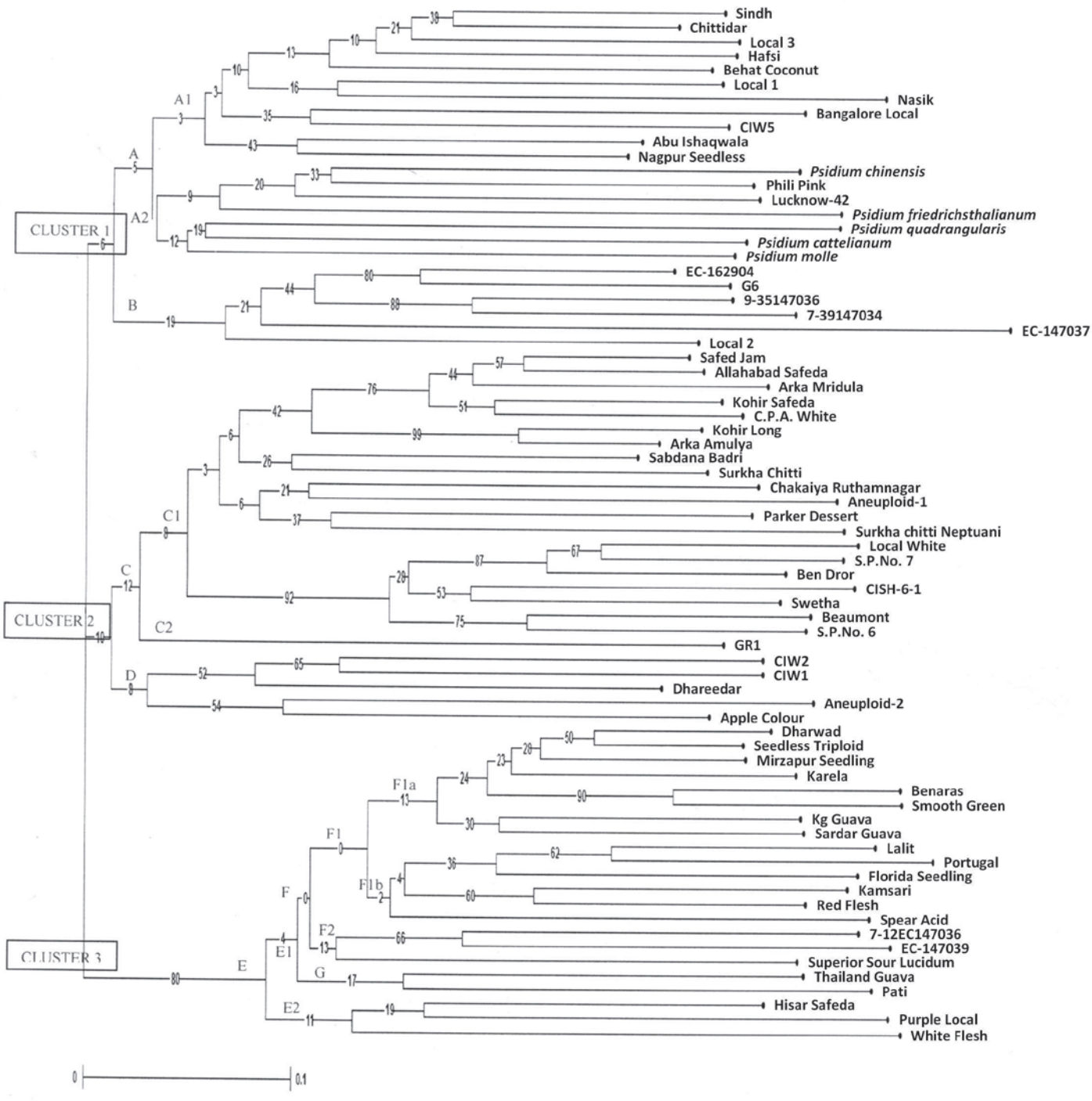

Fig. 2. Dendrogram generated using Weighted Neighbor Joining method from the computed genetic distances of simple matching coefficient. Bootstrap values supporting nodes are shown on the branches. The black dot on the left of the dendrogram indicates origin, and the line at the bottom indicates coefficient of Jaccards Dissimilarity Matrix.

From the dendrograms, it can be deduced that many accessions were comparable to known superior varieties, and, can be used as parents in future guava breeding programs. Allelic pattern shown by the primer combinations evaluated in the present study confirms the high discriminatory capacity of SSR markers. Therefore fingerprinting of guava accessions can be done using such data. An acceptable level of genetic diversity was detected, as, a number of clusters were formed, allowing for efficient selection of parents in future breeding programs. Parentage was also confirmed through molecular diversity analysis. Exotic collections have a good demand in the processing

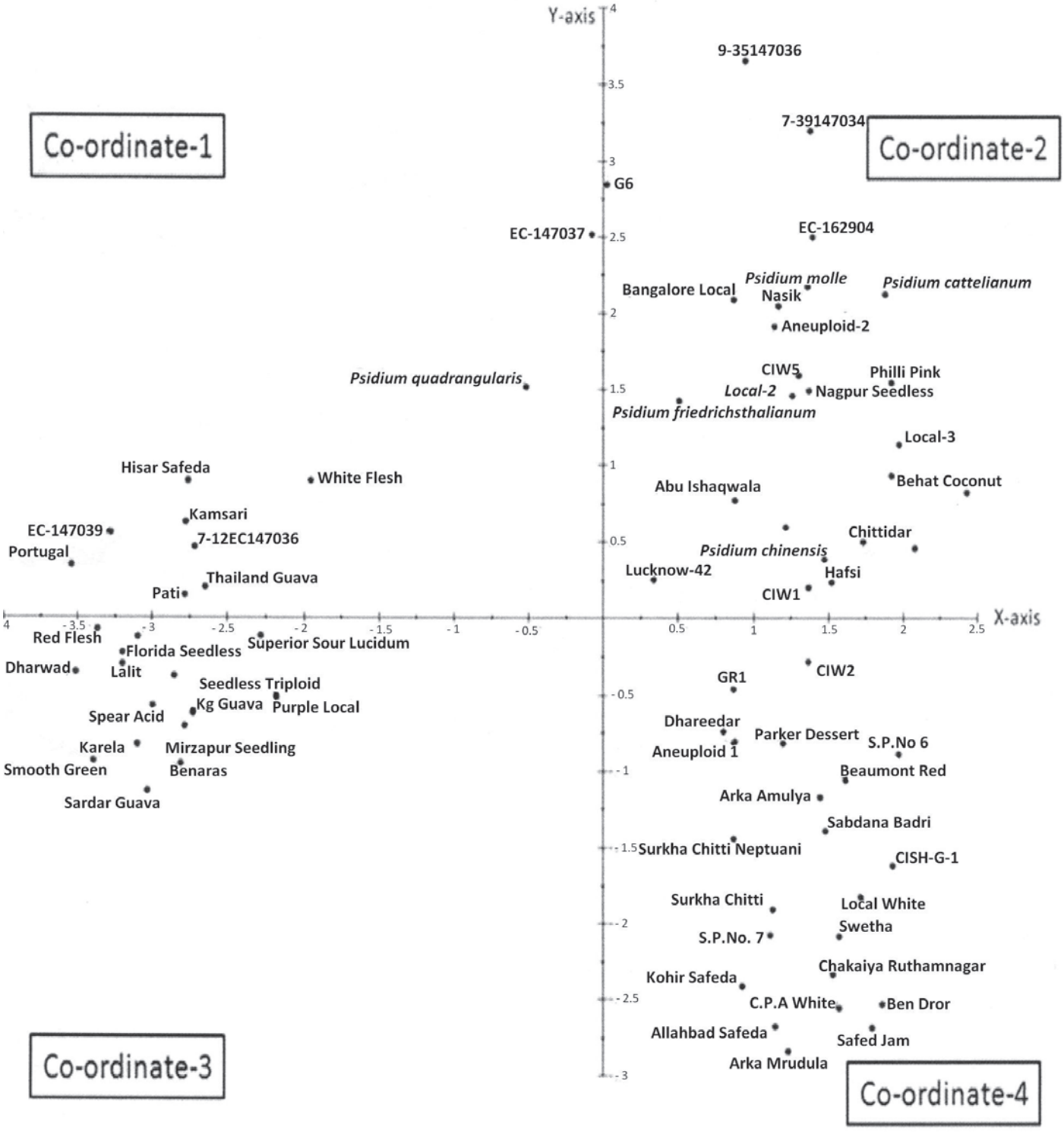

Fig. 3. Factorial analysis generated using DARWin 5.0 software

industry because of their pink pulp, with sweet-acid blend and high productivity. These can be exploited in guava breeding programmes in India.

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