

Genetic variability Studies and Molecular Characterization of *Garcinia (Garcinia gummi-gutta L.)* Germplasm for Yield and Quality Traits

S.T. Binisundar^{1*}, G. Ashokkumar¹, P. Rajarathinam², A. Jaya Jasmine³ and S. Vasanth⁴

¹Assistant Professor (Horticulture), TNAU, Coimbatore (Tamil Nadu), India.

²Associate Professor (Agronomy), Tamil Nadu Rice Research Institute, Aduthurai (Tamil Nadu), India.

³Professor and Head, Horticultural Research Station, Pechiparai, Kanyakumari (Tamil Nadu), India.

⁴Ph.D. Scholar (Horticulture), HC&RI TNAU, Coimbatore (Tamil Nadu), India.

(Corresponding author: S.T. Binisundar*)

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ABSTRACT: At the Horticultural Research Station, located in Pechiparai, Kanyakumari district, an investigation was conducted during 2018 to 2021 to assess the *garcinia* germplasm maintained at the station along with the local check of Pechiparai. They were assessed for their growth character *viz.*, tree height, fruit characters, major pest and disease infestation. The study showed that the accession Acc. Gg 9 was identified as best performer as it exhibited favourable characters for yield and growth when compared with the local check and also recorded lower level of pest and disease infestation. In the current investigation, 11 *garcinia* accessions were analysed through principal component analysis to assess genetic divergence, pattern of variation present in the germplasm and relationship between the tested individuals. The first two principal components contributed desired eigen values and exhibited 71.20 % of the total variability in the observed characters. The genotypes ACC. Gg 9 showed high positive values. The cluster analysis displayed totally four major clusters *viz.*, I, II, III and IV consisting of 3, 1, 6 and 1 accessions accordingly. For a breeding programme in *garcinia* accessions, it can utilise the accessions from the diverse clusters. With regard to correlation studies, the traits *viz.*, number of fruits per tree, rind thickness, tartaric acid have expressed significant and positive correlation values with yield per tree. Challenges in this investigation include addressing the complex analysis of multiple growth and genetic parameters in 11 *garcinia* accessions, ensuring effective utilization of diverse clusters for breeding programs, and navigating the intricacies of correlation studies to enhance the selection process based on key traits. Therefore, selection based on these characters will increase the effectiveness. With respect to the DNA fingerprinting analysis concluded that RAPD markers can be used for DNA fingerprinting of Kudampuli cultivar PPI (K) 1 and local accession. Among the RAPD markers, OPA03570 can be used to differentiate PPI (K) 1 from the local check.

Keywords: *Garcinia*, principal component analysis, cluster analysis, correlation analysis, DNA Fingerprints.

INTRODUCTION

A tropical species of *Garcinia* called *Garcinia gummi-gutta* is a native of South and Southeast Asia. *Garcinia cambogia* is one of the common names. *Garcinia*, sometimes referred to as Malabar tamarind or Kodampuli, belongs to the Clusiaceae family and is grown all over the world (Hemshekhar *et al.*, 2011). The species is indigenous to the western ghats of India and is found from Konkan to Kerala and in the shola forests of Nilgiri hills up to an altitude of 2000m (Angami *et al.*, 2021). It has a big impact on the local forest's vegetation. The *gummi-gutta* (L.) Robs., the most prevalent of these species is a crucial fruit crop for Kerala's economy. One of the productive fruit trees, with significant therapeutic value and great commercial potential as condiments. It produces a tiny, delicious exotic fruit whose dried pericarp is used as a condiment and as an alternative of tamarind to impart a special

flavour and taste to curries in Kerala (Singh *et al.*, 2022). It functions as a diuretic, hydragogue, anthelmintic, antibiotic, antioxidant, hypolipidemic, and weight-reduction agent (Shara *et al.*, 2004; Mathew *et al.*, 2011 a; Mathew *et al.*, 2011 b). The fruits are also commercially important as a rich source of the much-valued anti-obesity phytochemical hydroxy citric acid (Shivakumar *et al.*, 2013).

MATERIALS AND METHODS

A. Collection and maintenance of *garcinia* accessions

The current investigation was conducted from 2018 to 2021 at the Horticultural Research Station at Pechiparai, Kanyakumari districts, Tamil Nadu and several *garcinia* germplasm collections are maintained. These groups have been designated as *garcinia gummi gutta* accessions ACC. Gg 1, ACC. Gg 2, ACC. Gg 3, ACC. Gg 4, ACC. Gg 5, ACC. Gg 6, ACC. Gg 7, ACC. Gg 8, ACC. Gg 9, ACC. Gg 10 and ACC. Gg 11. To

serve as a point of reference, Pechiparai native check garcinia plants sutilised in this research.

B. Characters observed

For the attributes, the observations were recorded *viz.*, Tree height, number of fruits per tree, yield per tree, rind thickness, rind dry recovery, hydroxy citric acid, tartaric acid content, pest and disease incidence in garcinia accessions.

C. Biometrical analysis

The Biometrical studies such as Principal component analysis (PCA) to assess the genetic divergence, cluster analysis for grouping the germplasm into different clusters and correlation coefficient analysis to understand the relationship between the various traits with the yield were performed among the 11 garcinia germplasm.

D. Molecular studies

DNA fingerprinting analysis was conducted for the garcinia cultivar PPI (K) 1 and a local check in pechiparai. DNA extracted from the leaf samples were isolated as per the procedure laid out in (Gawel and Jarret 1991). Better quantity of DNA was obtained when the ground samples were incubated at 60°C for ≥ 12 hours. In the marker analysis totally, 23 RAPD markers were employed. RAPD (Random Amplified Polymorphic DNA) markers are DNA fragments obtained from PCR amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequence. RAPD is a dominant marker and it was used for molecular characterization of cultivars and analyse the genomes for variation.

E. RAPD Primer Sequences

RAPD Primer Sequences given in Table 1.

Table 1

Primer Name	Sequence 5' > 3'
OPA-03	AGTCAGCCAC
OPB-03	CATCCCCCTG
OPC-03	GGGGGTCTTT
OPA-04	AATCGGGCTG
OPB-04	GGA CTGGAGT
OPC-04	CCGCATCTAC
OPA-05	AGGGGTCTTG
OPB-05	TGCGCCCTTC
OPC-05	GATGACCGCC
OPA-06	GGTCCCTGAC
OPB-06	TGCTCTGCCC
OPC-06	GAACGGACTC
OPD-02	GGACCCAACC
OPE-02	GGTGCGGGAA
OPF-02	GAGGATCCCT
OPD-03	GTCGCCGTCA
OPE-03	CCAGATGCAC
OPF-03	CCTGATCACC
OPD-04	TCTGGTGAGG
OPE-04	GTGACATGCC
OPF-04	GGTGTATCAGG
OPD-05	TGAGCGGACA
OPE-05	TCAGGGAGGT

RESULTS AND DISCUSSION

According to the present studies, all of the characters were significantly different from each other. The

recorded observations were assessed statistically and the values were tabulated and provided in Table 2-7.

Among the accessions evaluated the maximum plant height of 20.49 m was recorded in Acc. Gg 1, followed by Acc. Gg 3 and Acc. Gg 2 with 19.76 and 19.52m respectively. The maximum tree height 20 meters was also reported by (Abraham *et al.*, 2006). The maximum number of fruits per tree was recorded in Acc. Gg 9 with 744.48 followed by ACC. Gg 5 (696.78). The maximum marketable yield of 119.94 kg per tree was observed in Acc. Gg 9 followed by ACC. Gg 7 (103.9 kg) (Table 2, Fig. 2).

Fruit rind thickness is an important trait that contributes to dry rind mass/tree/year, and also recognized as desirable in local markets, achieving a higher price than normal quality dry rind. Rind is the most sought-after part of the fruit. Dried rind is an important commercial product. Besides, fruit juice purely extracted from locules bear no colour. Only when juice is extracted from both rind and locules, it bears an appealing colour and flavour. Therefore, rind composition in fruit is very crucial. The thickness of fruit rind showed a wide variation among the accessions studied (Table 2). It varied from a minimum of 2.14 cm in Acc. Gg 3 to a maximum of 3.39 cm in Acc. Gg 9. The highest rind thickness of 3.39 was recorded in ACC. Gg 9 followed by ACC. Gg 6 (2.78). Higher dry recovery was recorded in ACC. Gg 9 with a recovery percentage of 15.12 % (Fig. 8).

Acidity is of prime importance as it largely determines the acceptance of Malabar tamarind in both industrial processing for the extraction of natural HCA and in various traditional cuisines of South India. The highest percentage of tartaric acid was found in the Accession Gg 9 with 10.2 %. The concentration of HCA was quantified and ACC. Gg 9 noted the highest per cent of 28.67. Similar variations in fruit quality traits have been observed in other fruit trees like *Cordia myxa* (Sivalingam *et al.*, 2012) and *Arbutus unedo* (Colak, 2000).

The results of the best performing Acc. Gg 9 out of the collection in comparison with the local check of Pechiparai are given in Table 3 and 4. The Acc. Gg 9 has reported superiority over the local check in terms of major traits and also has less incidence of pests such as scales, hoppers and blight disease when compared to the local check.

Based on the results of hierarchical cluster analysis it can be interpreted from the dendrogram that the Acc. Gg9, ACC. Gg 5 and ACC. Gg 7 are grouped together in the cluster 3 which reveals significant number of variations among the collection and can be effectively utilised for further studies (Fig. 1).

PCA for garcinia: From the principal component analysis, the different characters *viz.*, Tree height, number of fruits per tree, yield per tree, rind thickness, rind dry recovery, HCA, tartaric acid content were studied. Principal component analysis was performed to analysed the divergence between the accessions of garcinia by using STAR software. The number of PCs that must be retained depends on the amount of variance that PCs can explain. According to (Rencher *et*

al., 2022) PCs must explain at least 70% of the variance.

The eigenvalues (λ), proportion of variance, cumulative proportion are shown in the Table 5. Out of 7 PCs, two had shown the eigenvalue more than 1. The first two PCs explicated approximately 71.20% of all the variability in the observed traits (56.09% explicated by PC1, 15.11% by PC2) (Chen *et al.*, 2016). The related principal components (PCs) that should be taken into account for the analysis are presented with the eigenvalues of the principal components in a Scree plot. According to this, the largest variability was seen in the first two PCs (PC1 and PC2) (Fig. 3) (Hossain *et al.*, 2011). The PC1 showed positive values for the characters *viz.*, number of fruits per tree, yield per tree, rind thickness, rind dry recovery, hydroxy citric acid, tartaric acid content. In PC2 indicates positive values for the characters such as Tree height, number of fruits per tree, yield per tree, rind thickness, tartaric acid content (Machamangalath *et al.*, 2016).

Cluster analysis: Based on the Euclidean distance technique of agglomerative cluster analysis, 11 garcinia accessions were divided into 4 clusters in the current study, as shown in Fig. 5 and Table 6. The four major groups were formed as clusters such as I, II, III, IV consists of 3, 1, 6 and 1 germplasm correspondingly. Cluster means of various traits in garcinia accessions are shown in Table 7. Cluster I showed the highest mean values for the characters *viz.*, Tree height (20.49 m). In cluster II and cluster III had no highest mean values for the characters studied. In cluster IV had highest mean values for the traits *viz.*, number of fruits

per tree (744.58), yield per tree (119.94 kg), rind thickness (3.39) (cm), rind dry recovery (15.12 %), hydroxy citric acid (28.67 %) and tartaric acid (10.20 %) (Wang *et al.*, 2019). The cophenetic correlation coefficient in this instance was 0.914, demonstrating the strong clustering pattern. Therefore, the germplasm of the corresponding clusters can be used to develop crops that indicated higher mean values (Utpala *et al.*, 2010).

Correlation analysis: In the current study, number of fruits per tree (0.924), rind thickness (0.883), tartaric acid (0.653) has expressed significant and positive correlation values with yield per tree. In inter correlation among different traits number of fruits per tree expressed significant positive association with rind thickness (0.74) and yield per tree (0.924) (Liyanagamage *et al.*, 2020). Rind thickness showed significant positive association with yield per tree (0.883). Tartaric acid observed significant positive association with yield per tree (0.653) (Table 8 and Fig. 6).

Molecular studies: In the present study, RAPD markers were used for the DNA fingerprinting analysis for the garcinia cultivar PPI (K) 1 and a local check in pechparai. Among the 23 RAPD markers, only one marker *viz.*, **OPA03570** was found to be specific to the Kudampuli variety PPI (K) 1 when compared with its local check. This marker was shown polymorphic between these two investigated Kudampuli accessions as it was reported only in PPI (K) 1 and absent in Kudampuli local check (Fig. 7). So, this marker can be used to differentiate this germplasm.

Table 2: Mean performance for garcinia accessions.

Accessions	Tree height (m)	No. of fruits/tree	Yield kg/tree	Rind thickness (cm)	Rind dry recovery (%)	HCA (%)	Tartaric acid (%)
ACC. Gg 1	20.49	620.36	90.18	2.29	10.37	23.63	8.81
ACC. Gg 2	19.52	621.52	91.81	2.37	10.12	25.11	8.02
ACC. Gg 3	19.76	571.1	90.66	2.14	10.5	22.66	9.56
ACC. Gg 4	16.1	579.68	85.39	2.26	13.82	24.41	9.11
ACC. Gg 5	17.71	696.78	103.2	2.59	10.32	23.11	9.53
ACC. Gg 6	17.13	611.57	95.66	2.78	12.66	26.53	8.64
ACC. Gg 7	15.91	699.7	103.9	2.49	11.32	24.54	9.69
ACC. Gg 8	17.42	666.44	97.88	2.54	12.14	23.01	8.81
ACC. Gg 9	18.35	744.78	119.94	3.39	15.12	28.67	10.2
ACC. Gg 10	17.92	647.9	100.44	2.77	10.32	24.32	9.37
ACC. Gg 11	16.69	648.66	96.5	2.42	9.17	25.48	8.54
SEd	0.15	23.83	3.92	0.09	0.43	0.91	0.34
CD (0.05)	1.38	0.31	8.12	0.20	0.88	1.88	0.69

Table 3: Comparative performance of Gg 9 with Pechparai Local check.

Year	No. of fruits (per tree)				Yield (kg/tree)			
	Accessions		Grafts		Accessions		Grafts	
	Gg 9	Local check	Gg 9	Local check	Gg 9	Local check	Gg 9	Local check
2015	626.22	617.93	681.43	598.99	110.05	92.55	121.92	113.82
2016	727.12	645.68	726.32	647.36	113.17	101.58	128.84	100.01
2017	778.76	695.83	708.49	631.89	118.01	103.51	131.72	104.48
2018	763.88	688.72	753.16	654.39	121.96	104.16	125.65	100.33
2019	827.94	595.16	736.42	653.93	136.51	80.71	131.36	106.54
Mean	744.78	648.66	721.16	637.31	119.94	96.50	128.00	105.04

Table 4: Pest and Disease Incidence of Gg 9 with Pechparai Local check.

Pest and Disease	Gg 9	Local check
Scales	2.1	3.0
Leaf folders	1.1	5.1
Hoppers	6.5	7.2
Twig blight	2.2	3.5

Table 5: Principal component analysis for garcinia accessions.

Characters	PC1	PC2
Tree height (m)	-0.1469	0.6086
Number of fruits per tree	0.4204	0.3274
Yield per tree (kg)	0.4742	0.2955
Rind thickness (cm)	0.4722	0.0158
Rind dry recovery %	0.3274	-0.4966
HCA %	0.3697	-0.3361
Tartaric acid %	0.3315	0.2745
Eigenvalues	3.9263	1.0577
% total variance	56.09	15.11
% cumulative variance	56.09	71.20

Table 6: Clustering in garcinia germplasm.

Cluster	Frequency	Cluster Membership
I	3	Gg 1, Gg 2, Gg 3
II	1	Gg4
III	6	Gg5, Gg6, Gg7, Gg8, Gg10, Gg11
IV	1	Gg9

Table 7: Cluster mean for traits in garcinia accessions.

Variables	Cluster	Min	Max	Mean	Std Dev
Tree height (m)	1	19.52	20.49	19.92	0.51
	2	16.10	16.10	16.10	-
	3	15.91	17.92	17.13	0.74
	4	18.35	18.35	18.35	-
Number of fruits per tree	1	571.10	621.52	604.33	28.78
	2	579.68	579.68	579.68	-
	3	611.57	699.70	661.84	33.38
	4	744.78	744.78	744.78	-
Yield per tree (kg)	1	90.18	91.81	90.88	0.84
	2	85.39	85.39	85.39	-
	3	95.66	103.90	99.60	3.47
	4	119.94	119.94	119.94	-
Rind thickness (cm)	1	2.14	2.37	2.27	0.12
	2	2.26	2.26	2.26	-
	3	2.42	2.78	2.60	0.15
	4	3.39	3.39	3.39	-
Rind dry recovery %	1	10.12	10.50	10.33	0.19
	2	13.82	13.82	13.82	-
	3	9.17	12.66	10.99	1.30
	4	15.12	15.12	15.12	-
HCA %	1	22.66	25.11	23.80	1.23
	2	24.41	24.41	24.41	-
	3	23.01	26.53	24.50	1.36
	4	28.67	28.67	28.67	-
Tartaric acid %	1	8.02	9.56	8.80	0.77
	2	9.11	9.11	9.11	-
	3	8.54	9.69	9.10	0.49
	4	10.20	10.20	10.20	-

Table 8: Correlation analysis for traits in garcinia accessions.

	Tree height	Number of fruits per tree	Rind thickness	Rind dry recovery	HCA	Tartaric acid	Yield per tree
Tree height	1	-0.232	-0.141	-0.272	-0.172	-0.134	-0.145
Number of fruits per tree		1	0.74**	0.247	0.426	0.514	0.924***
Rind thickness			1	0.578	0.772**	0.473	0.883***
Rind dry recovery				1	0.566	0.447	0.388
HCA					1	0.149	0.587
Tartaric acid						1	0.653*
Yield per tree							1

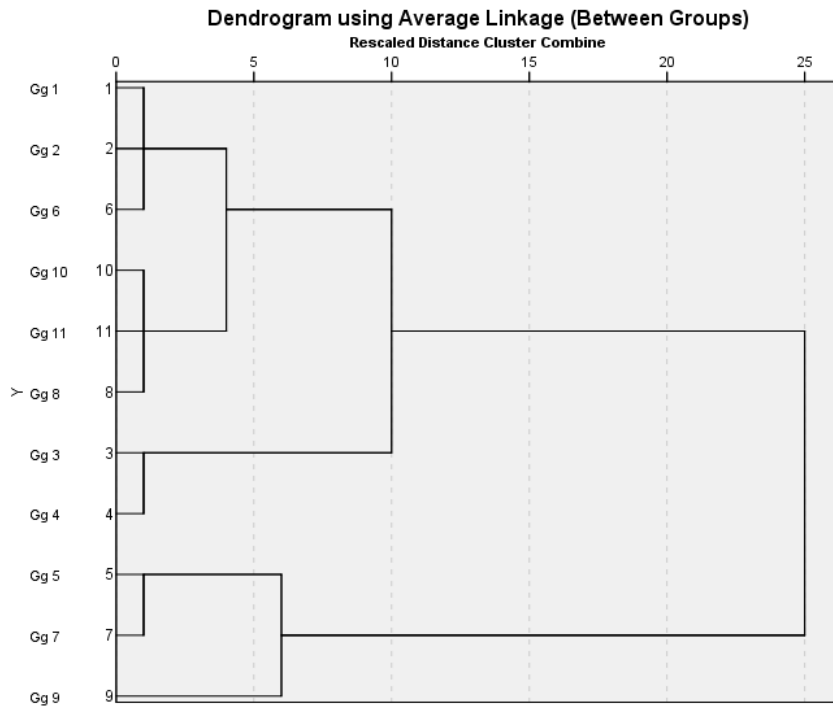
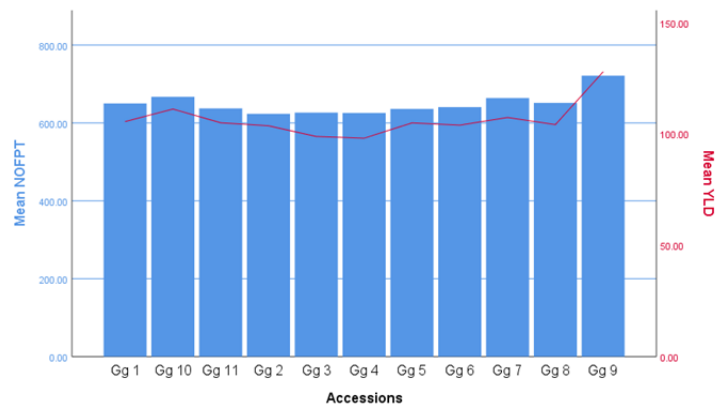


Fig. 1. Hierarchical cluster dendrogram of garcinia accessions.



NOFPT- Number of fruits per tree; YLD- yield in Kg per tree

Fig. 2. Yield characters of garcinia accessions.

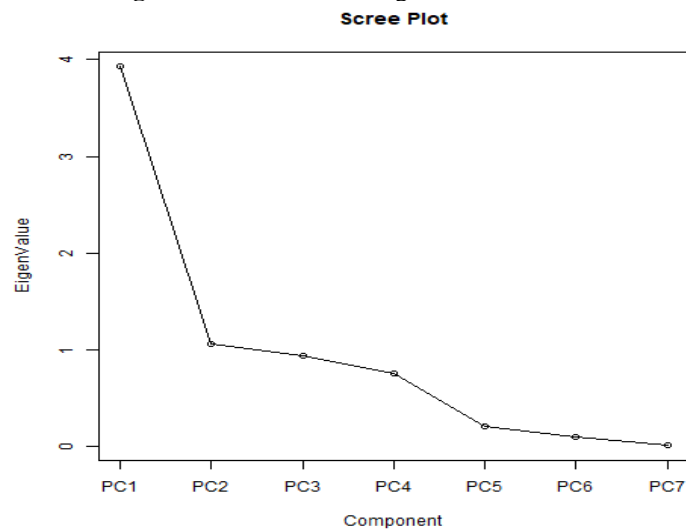


Fig. 3. Screen plots of eigenvalues.

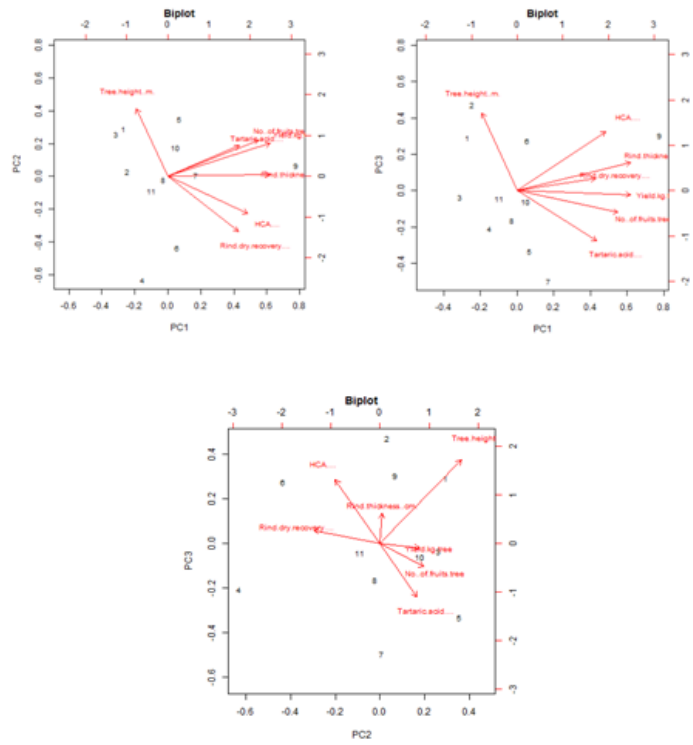


Fig. 4. Biplots displaying for garcinia accession.

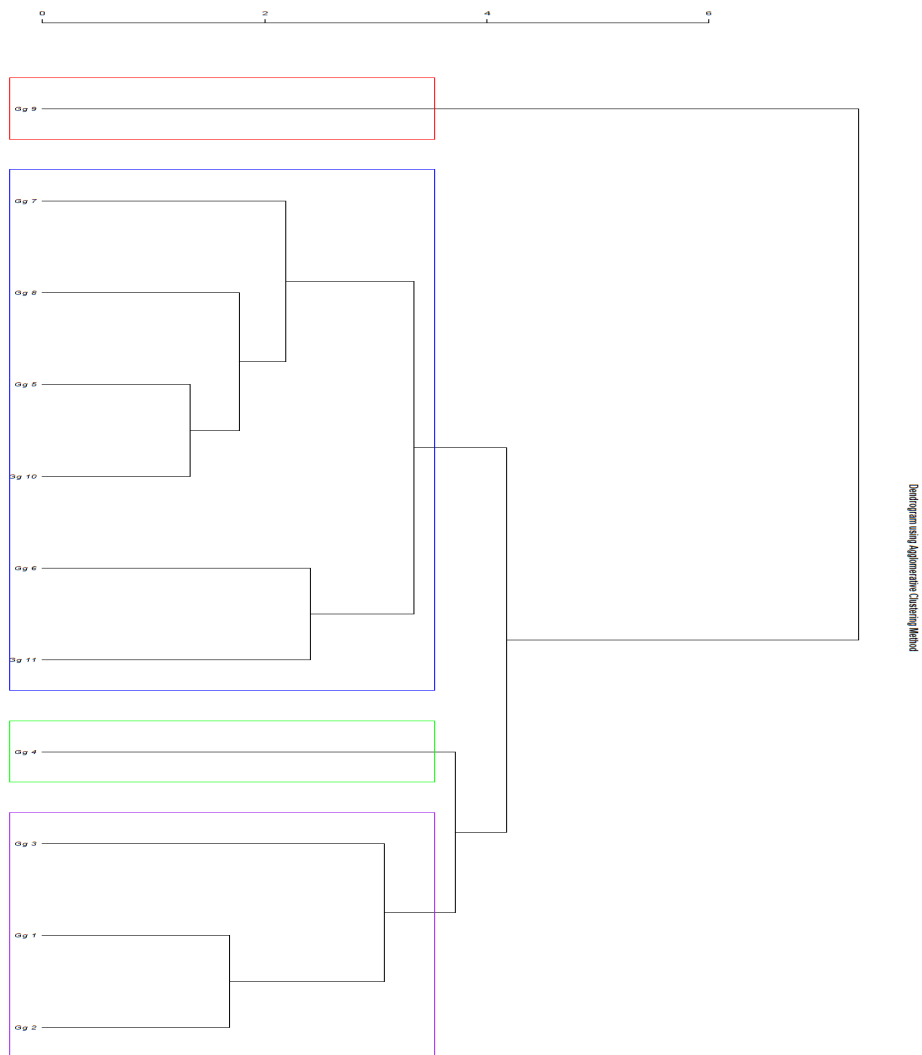


Fig. 5. Dendrogram based on agglomerative cluster analysis for garcinia accessions.

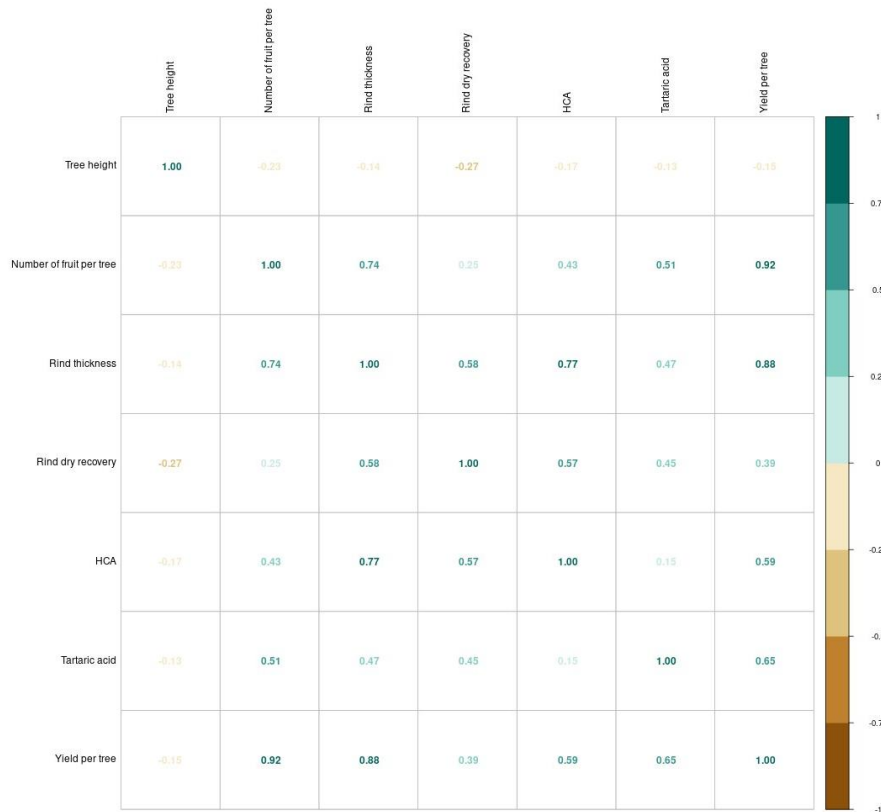
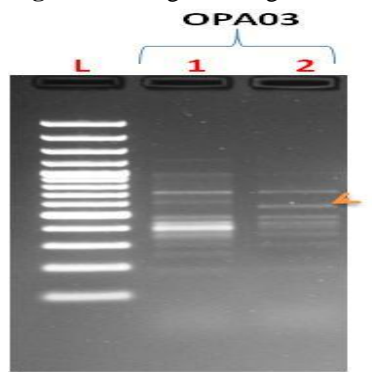


Fig. 6. Correlogram for garcinia accessions.



L- 100 bp ladder
 1- Local Check
 2- Kudampuli PPI (K) 1

Fig. 7. RAPD analysis of Local Check and PPI (K) 1 of Kudampuli.

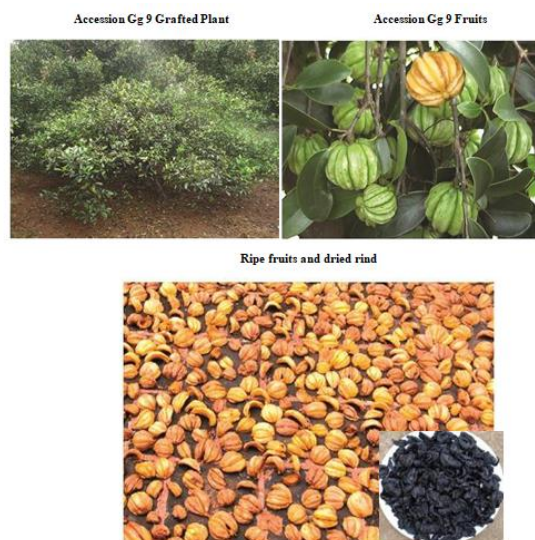


Fig. 8. Higher dry recovery was recorded in ACC. Gg 9.

CONCLUSIONS

The aim of this work was to characterise and evaluate the morphological in order to determine the genetic diversity of *G. gummi-gutta* accessions. For genetic diversity to be conserved, we need a better understanding for its distribution. The results of this investigation showed that the Acc. Gg 9 was identified as the best performing garcinia accession as it exhibited desirable characters in terms of yield and fruit characteristics with a compact height making harvest easy. Also, on comparison it outperformed the local check in most of the characters and had low incidence of pest and disease. Outcrossing, which resulted in gene flow both within and between populations and may have contributed to the high amount of variation shown in these accessions (*G. gummi-gutta*'s method of pollination may include insects), may have occurred. A high tree to tree intraspecific variation even within the same geographical zone has been previously reported for *G. gummi-gutta* (Parthasarathy *et al.*, 2014). This report could be useful in efficient management of *G. gummi-gutta* germplasm for their conservation and optimal utilization. Further studies using larger number of accessions from different geographical locations of India could provide better understanding of the genetic diversity of *G. gummi-gutta*. In garcinia accessions the STAR (Computer software) was used for this principal component analysis and cluster analysis. Total 11 accessions were evaluated in the analysis. From the results, out of 11 accessions ACC. Gg 9 had high diversity. Among the characters tree height, number of fruits per tree, yield per tree, rind thickness, rind dry recovery, hydroxy citric acid and tartaric acid showed significant variations. In cluster analysis, there performed four clusters. Among the four-cluster IV cluster showed ACC. Gg 9 genotypes had high diversity comparing other genotypes. Here, the value of cophenetic correlation coefficient was 0.914 that showed the high efficiency of the clustering pattern. With regard to correlation association analysis in garcinia accessions, the characters such as number of fruits per tree, rind thickness, tartaric acid have expressed significant and positive correlation values with yield per tree. Therefore, selection based on these characters will increase the effectiveness. With respect to the DNA fingerprinting analysis concluded that RAPD markers can be used for DNA fingerprinting of Kudampuli cultivar PPI (K) 1 and local accession. Among the RAPD markers, OPA03₅₇₀ can be used to differentiate PPI (K) 1 from the local check.

FUTURE SCOPE

Current study will provide the characters to utilized for more crop improvement in garcinia accessions for future use.

Acknowledgement. This is to verify that there are no conflicts of interest in the research article that both authors have submitted since all of the data that was collected, aggregated, analysed, reviewed, developed a methodology for, and deduced the results and recommendations from were reliable.

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