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Original Research Paper

Exploration of genetic variability in Garcinia [*Garcinia gummi-gutta* L. (Robson)] germplasm based on growth, yield and quality traits

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ABSTRACT

Eleven *Garcinia* germplasm along with local check of Pechiparai were evaluated and underwent principal component analysis to assess genetic divergence and variation patterns during 2019 to 2022. The first two principal components, contributing significant Eigen values, explained 71.20% of the total variability. The Acc. Gg 9 was the top performer, exhibiting favourable yield and growth traits with lower pest and disease incidence, high biochemical compounds *viz.*, hydroxy citric acid and tartaric acid compared to local check. Cluster analysis revealed four major clusters, offering diversity for breeding programs. Correlation studies highlighted traits such as number of fruits per tree, rind thickness, and tartaric acid showing significant positive correlations with yield per tree. Selection based on identified key traits was deemed crucial for enhancing effectiveness. Additionally, DNA fingerprinting analysis indicated the potential use of RAPD markers (OPA03570) for differentiating Kudampuli cultivar PPI (K) 1 from the local check. Overall, the present investigation provides insights into optimizing *Garcinia* breeding programs, emphasizing trait-based selection and DNA fingerprinting for varietal differentiation.

Keywords : Correlation analysis, DNA fingerprints, *garcinia*, principal component analysis

INTRODUCTION

A tropical species of Garcinia (Malabar tamarind/ Kudampuli) called *Garcinia gummi-gutta* L.(Robson) a native of South and Southeast Asia, belongs to the family Clusiaceae, is grown all over the world (Hemashekhar 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 2000 m (Angami et al., 2021). It is an important fruit crop for Kerala's economy. Its fruit has medicinal benefit and has commercial possibilities for sauces. It yields a small, tasty exotic fruit whose dried pericarp is used in Keralan curries as a condiment and tamarind substitute to give unique flavour (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., 2011a; Mathew et al., 2011b). Its fruits are rich source of highly prized anti-obesity phytochemical hydroxy citric acid (Shivakumar et al., 2013). As *Garcinia* comes under underutilized medicinal fruit tree, in order to conserve the germplasm, effective characterization of the genotypes using morphological and molecular markers would aid in greater way. Hence, the present investigation was taken up.

MATERIALS AND METHODS

The current investigation was conducted at the Horticultural Research Station, Pechiparai, Kaniyakumari districts, under Tamil Nadu Agricultural University Tamil Nadu during 2019 to 2022 on eleven *Garcinia gummi gutta* accessions (treatments) designated as Gg 1, Gg 2, Gg 3, Gg 4, Gg 5, Gg 6, Gg 7, Gg 8, Gg 9, Gg 10 and Gg 11 and native check Pechiparai. The observations were recorded on tree height, number of fruits per tree, yield per tree, rind thickness, rind dry recovery, hydroxy citric acid (Satish & Thakur, 2012) and tartaric acid (Roopa & Kasiviswanatham, 2013) content analysis. For analysis five samples, were taken from each accession during the peak season *i.e.* July to September. The experiment was carried out in randomized block design with 3 replications. Principal component analysis (PCA), cluster analysis, and correlation coefficient analysis of biometrical traits were performed using PAST 3 software.

Molecular studies

DNA fingerprinting analysis was conducted for the elite type Gg 9 which was released from the Institute as Garcinia cultivar PPI (K) 1 and a local check (Pechiparai). DNA extracted from the leaf samples were isolated as per the procedure developed by Gawel & Jarret (1991). An increased amount of DNA was extracted from the ground samples after >12 hours of incubation at 60°C. Twenty-three RAPD markers were used in total for the marker analysis. DNA fragments known as RAPD (Random Amplified Polymorphic DNA) markers are produced when a single primer with an arbitrary nucleotide sequence is used to amplify random genomic DNA segments. A prominent marker called RAPD was employed to analyse the genomes for variation and to characterize cultivars molecularly (Gawel & Jarret, 1991).

RAPD primer sequences used in the study

Primer Name	Sequence 5' > 3"
OPA-03	AGTCAGCCAC
OPB-03	CATCCCCCTG
OPC-03	GGGGGTCTTT
OPA-04	AATCGGGCTG
OPB-04	GGACTGGAGT
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	GGTGATCAGG
OPD-05	TGAGCGGACA
OPE-05	TCAGGGAGGT

RESULTS AND DISCUSSION

Evaluation studies

All of the characters were significantly different from each other. The mean performance of *Garcinia* germplasm is presented in Table 1. Among the accessions evaluated, the maximum plant height was recorded in Gg 1 (4.68 m), followed by Gg 3 (4.50 m) and Gg 2 (4.41 m). The maximum number of fruits per tree was recorded in Gg 9 (721.16) followed by Gg 5 (666.99). The maximum marketable yield (128.00 kg/tree) was observed in Gg 9 followed by Gg 7 (107.43 kg). The differences in the plant growth and yield may be due to inherent genetic makeup of the different accessions.

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 (Priya devi et al., 2012). The thickness of fruit rind showed a wide variation among the accessions studied. The rind thickness varied from 2.14 cm (Gg 3) to 3.39 cm (Gg 9). Higher dry recovery (15.12 %) was recorded in Gg 9.

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 (10.2%) was recorded in Gg 9, while, highest concentration of HCA was recorded in Gg 9 (28.67%). Similar variations in fruit quality traits have been observed in other fruit trees like *Cordiamyxa* (Sivalingam et al., 2012) and *Arbutus unedo* (Aysen et al., 2000).

Based on the results of hierarchical cluster analysis it can be interpreted from the dendrogram that the Gg 9, Gg 5 and Gg 7 are grouped together in the cluster 3 which reveals significant variations among the collection and can be effectively utilised for further studies (Fig. 1).

Table 1 : Mean performance of *Garcinia gummi-gutta* accessions

Accession	Tree height (m)	Fruits/ tree (Nos.)	Yield (kg/ tree)	Rind thickness (cm)	Rind dry recovery (%)	HCA (%)	Tartaric acid (%)
Gg 1	4.68	649.86	105.57	2.29	10.37	23.63	8.81
Gg 2	4.50	623.27	103.70	2.37	10.12	25.11	8.02
Gg 3	4.41	626.36	98.88	2.14	10.50	22.66	9.56
Gg 4	4.20	625.40	98.12	2.26	13.82	24.41	9.11
Gg 5	3.84	666.99	105.00	2.59	10.32	23.11	9.53
Gg 6	3.98	640.62	104.00	2.78	12.66	26.53	8.64
Gg 7	3.69	663.92	107.43	2.49	11.32	24.54	9.69
Gg 8	3.53	651.47	104.22	2.54	12.14	23.01	8.81
Gg 9	4.02	721.16	128.00	3.39	15.12	28.67	10.20
Gg 10	3.71	635.69	111.22	2.77	10.32	24.32	9.37
Gg 11	4.11	637.31	105.04	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

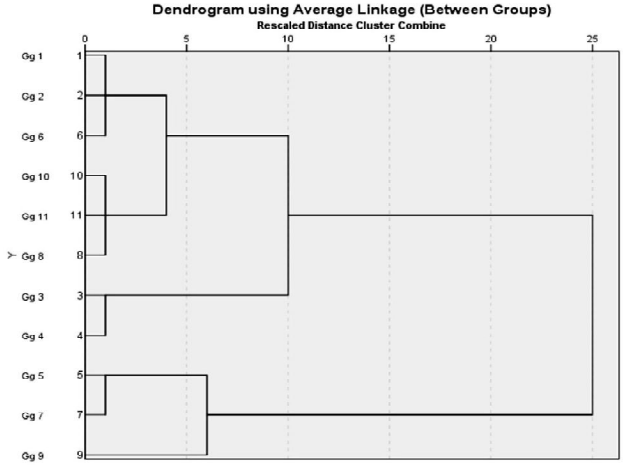

Fig. 1 : Hierarchical cluster dendrogram of *Garcinia gummi-gutta* accessions

Principal component analysis

The various characters *viz*., tree height, fruit count per tree, yield per tree, rind thickness, rind dry recovery, HCA, and tartaric acid content were examined based on the main component analysis. Using STAR software, principal component analysis was used to examine the divergence between the *Garcinia* accessions. The amount of variance that PCs can explain determines how many PCs need to be kept on hand. Rencher et al. (2022) stated that PCs must account for at least 70% of the variation.

The eigen values (λ), proportion of variance, cumulative proportion are presented in the Table 2. Out of 7 PCs, two had shown the eigen value 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 eigen values 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. 2 & 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, while, PC2 indicates positive values for tree height, number of fruits per tree, yield per tree, rind thickness and tartaric acid content (Machamangalath et al., 2016).

Cluster analysis

Eleven *Garcinia* accessions were grouped into 4 groups based on the Euclidean distance technique of agglomerative cluster analysis (Table 3). The four main groupings were created as clusters, with I, II, III, and IV having 3, 1, 6, and 1 germplasm in each group, respectively.

Table 4 displays cluster means for different features in *Garcinia* accessions. The characters with the greatest mean values, tree height (20.49 m), were found in Cluster I. There were no greatest mean values in clusters II or III. The greatest mean values for the

Table 2 : Principal component analysis for *Garcinia gummi-gutta* accessions

Character	PC1	PC2
Tree height (m)	-0.1469	0.6086
Fruits per tree (Nos.)	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
Eigen values	3.9263	1.0577
Total variance (%)	56.09	15.11
Cumulative variance (%)	56.09	71.20

Table 3 : Clustering in *Garcinia gummi-gutta* 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

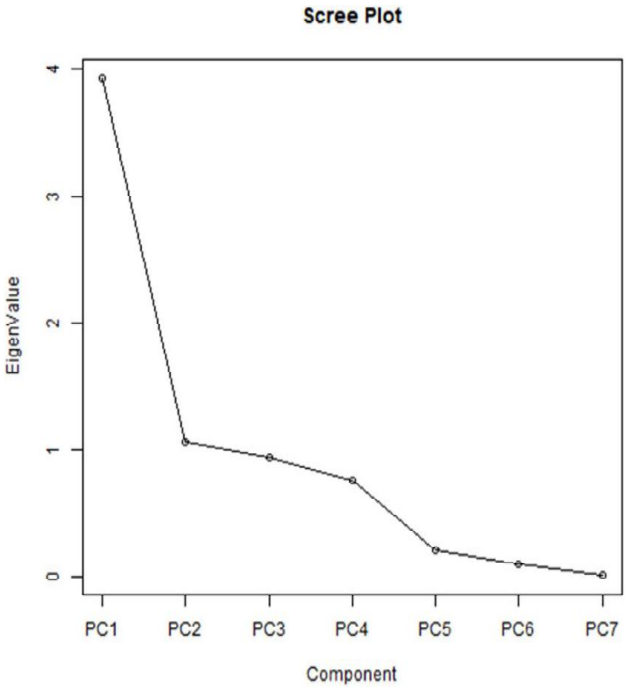

Fig. 2 : Screen plots of eigen values

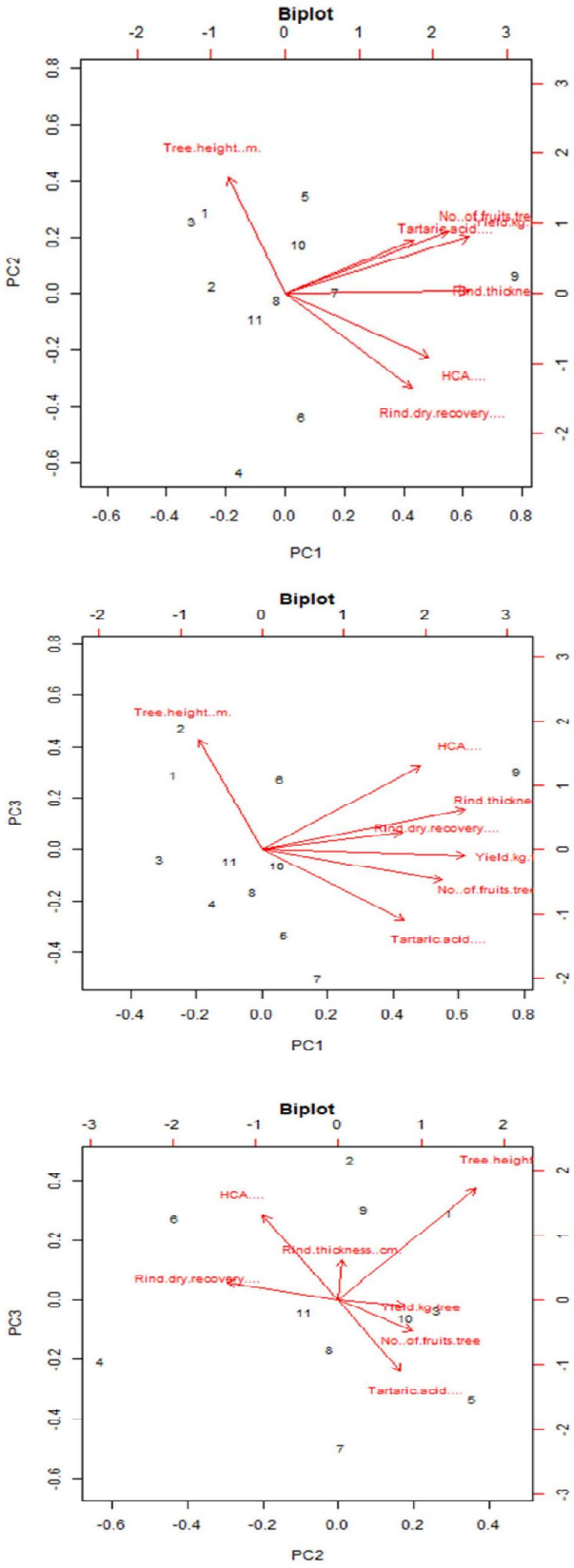

Fig. 3 : Biplots displaying for *Garcinia gummi-gutta* accession

following features were found in cluster IV: hydroxy citric acid (28.67%), tartaric acid (10.20%), rind thickness (3.39) (cm), yield per tree (119.94 kg), number of fruits per tree (744.58), and rind dry recovery (15.12 %) (Wang et al., 2019). In this case, the cophenetic correlation coefficient was 0.914, indicating a significant clustering pattern. Consequently, crops with higher mean values can be produced using the germplasm of the appropriate clusters (Utpala et al., 2010; Wang et al., 2019).

Correlation analysis

In the current study, there was a substantial and positive association found between the quantity of fruits per tree (0.924), rind thickness (0.883), and tartaric acid (0.653) and the yield per tree. According to Liyanagamage et al., (2020), there was a substantial positive link between the number of fruits per tree and both rind thickness (0.74) and yield per tree (0.924) in the inter-trait correlation analysis. There was a strong positive correlation (0.883) between yield per tree and the thickness of the roots. There was a strong positive correlation (0.653) between tartaric acid and yield per tree (Table 5 and Fig. 4).

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 Pechiparai. Among the

Table 4 : Cluster mean for traits in *Garcinia gummi-gutta* accessions

Variable	Cluster	Min.	Max.	Mean	SD
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	-
Fruits per tree (Nos.)	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 5 : Correlation analysis for traits in *Garcinia gummi-gutta* accessions

Parameter	Tree height	Fruits per tree	Rind thickness	Rind dry recovery	HCA	Tartaric acid	Yield/ tree
Tree height	1	-0.232	-0.141	-0.272	-0.172	-0.134	-0.145
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/tree							1

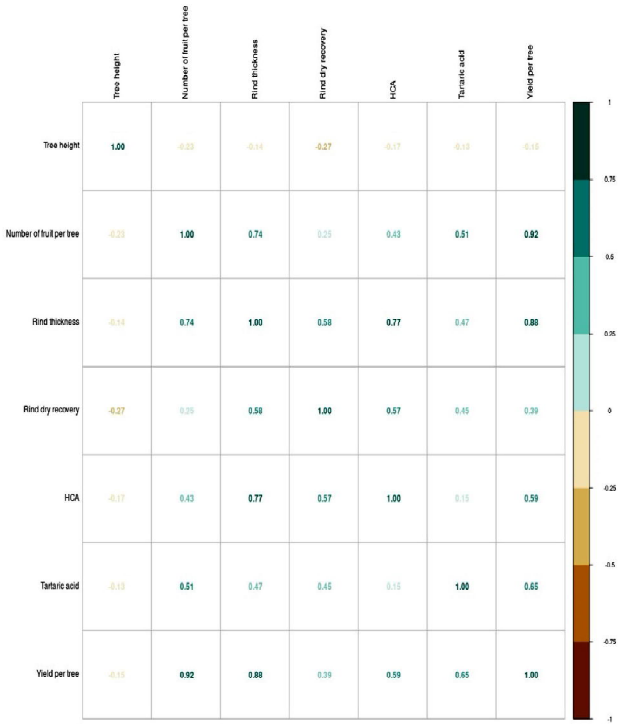

Fig. 4 : Correlogram for *Garcinia gummi-gutta* accessions

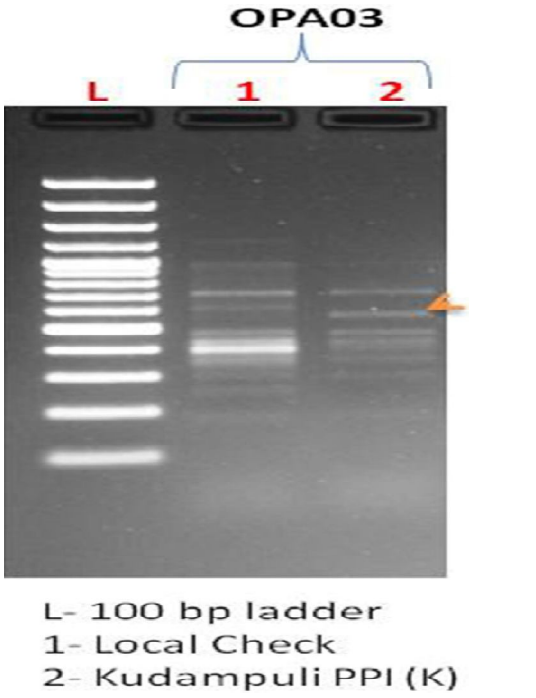

Fig. 5 : RAPD analysis of Local check and PPI (K) 1 of Kudampuli (*Garcinia gummi-gutta*)

23 RAPD markers, only one marker *viz*., $OPA03_{570}$ 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. 5). So, this marker can be used to differentiate this germplasm.

This study aimed to determine the genetic diversity of *Garcinia gummi-gutta* accessions using morphological analysis. The Gg 9 was identified as the best performing *Garcinia* accession due to its desirable yield and fruit characteristics, compact height, low pest and disease incidence, and outcrossing. The study found high tree-to-tree intraspecific variation within the same geographical zone. The STAR software was used for principal component analysis and cluster analysis, with 11 accessions evaluated. The study found significant variations in tree height, fruit number, yield per tree, rind thickness, rind dry recovery, hydroxy citric acid, and tartaric acid.

The study concluded that RAPD markers can be used for DNA fingerprinting of Kudampuli cultivar PPI (K) 1 and local accession, with OPA03570 being the most effective.

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