Erwerbs-Obstbau (2019) 61:217–223
https://doi.org/10.1007/s10341-019-00419-3

ORIGINAL ARTICLE

Morphological and Biochemical Studies in *Garcinia gummi-gutta* (L.) Roxb.

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Received: 22 September 2017 / Accepted: 12 January 2019 / Published online: 7 February 2019

Abstract
Garcinia gummi-gutta, popularly known as Malabar tamarind or *kodampuli* is a tropical fruit tree species of high potential. Acidic fruits are used either in raw or pickled form. Fruit is highly prized for its anti-obesity property owing to high hydroxycitric acid content in its rind. Though generally cultivated for its fruits, the plant has multifaceted uses in paint manufacturing industry, as medicine, source of edible fat etc. The present study concerned comparative morphological and physicochemical analysis of two morphotypes of Malabar tamarind, which revealed significant differences among them. Fruits and leaves of these morphotypes had distinctive morphological features. A morphotype with higher rind recovery and fruit weight was identified for further crop improvement programmes. Further, dimethylsulphoxide was identified as better solvent for extraction of photosynthetic pigments, and hence could be recommended for future physiological studies.

Keywords Diversity · DMSO · Malabar tamarind · Photosynthetic pigments · Tropical islands

Morphologische und biochemische Analysen bei *Garcinia gummi-gutta* (L.) Roxb.

Schlüsselwörter Diversität · DMSO · Malabar Tamarinde · Photosynthetische Pigmente · Tropische Inseln

Introduction

Garcinia is an important genus belonging to Clusiaceae family with about 200 species found in tropical and subtropical countries of which ca. 36 species are reported from India (Abraham et al. 2006), especially in the Western Ghats, North Eastern India and Andaman and Nicobar Islands (ANI). A number of *Garcinia* species are being brought under cultivation due to regional preferences for their fruits. In India, species such as *G. mangostana* and *G. indica* are gaining commercial importance in the western and southern states, while *G. gummi-gutta* and *G. xanthochymus* are semi-domesticated species grown in the home gardens of Kerala and parts of Karnataka (Abraham et al. 2006; Kalia et al. 2012).

G. gummi-gutta (syn. *G. cambogia* (Gaertn.) Desr.), popularly known as Malabar tamarind, is a tropical fruit bearing species native to Southeast Asia. Fruits are valued for their dried rind, which is used as an acidulant in traditional curries and other cuisines. Though pulp is also edible, it is generally discarded due to its highly acidic taste. Traditionally, the species is used for making paints, varnishes, seed butter, vinegar and polishing of gold ornaments (Abraham et al. 2006; Karun et al. 2014). In recent days, *G. gummi-gutta* has gained commercial importance due to the presence of (–) hydoxycitric acid (HCA) in it, known for its anti-obesity properties. Though there have been some controversies regarding the efficacy of this species against obesity (Saito et al. 2005), it remains one of the most valuable resources with practical utility (Burdock et al. 2005). Valsaraj et al. (1997) suggested efficacy of *G. gummi-gutta* as a promising antimicrobial species against diverse pathogenic bacteria. Recent review by Semwal et al. (2015) highlighted various activities of *G. gummi-gutta* including its appetite suppressing, hypo-lipidaemic, anti-diabetic, anti-inflammatory, anti-oxidant, hepato-protective, anti-carcinogenic, anti-ulcer, anti-cholinesterase, anti-microbial, anthelmintic and diuretic activities besides others.

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Commercial scale cultivation of lesser known but potential species such as *G. gummi-gutta* could be promoted if systematic efforts are taken up. Photosynthetic pigments are the prime constituents of plant's primary metabolism and hence they are assumed as key parameters in various physiological studies. Though a number of solvents and methods are available for determining these pigments (Wellburn 1994), species specific suitable solvent/method need to be identified (Coastache et al. 2012). We compared two methods for identifying more efficient one for quantification of photosynthetic pigments in this species. The identified method could be helpful for carrying out further investigations pertaining to crop production, improvement and protection in this species. Reports on diversity in *G. gummi-gutta* have suggested considerable variations for morphological parameters amongst collections made from Indian states of Kerala and Karnataka (Muthulakshmi et al. 1999; Abraham et al. 2006). Crop improvement being a continuous process, identification of superior genotypes is desirable for sustainable utilization of medicinally important species (Waman and Bohra 2016). Considering this, present experiment was undertaken to study the morphological and physico-chemical parameters of two distinct morphotypes of *G. gummi-gutta*.

Materials and Methods

The studies were conducted at Division of Horticulture and Forestry of ICAR-Central Island Agricultural Research Institute, Port Blair, ANI, India during 2016 and 2017. Leaf and fruit samples of *Garcinia gummi-gutta* were collected from the *Garcinia* conservation block of the Institute and used for various studies.

Comparison of Methods of Leaf Pigments Determination

Two methods of leaf pigments determination were compared. For this experiment, leaf tissues were pooled from different plants of *G. gummi-gutta* in the germplasm block. In method I, one gram of leaf sample was extracted with pre-chilled acetone (80%) till the residue became colourless. Volume was made up using 80% acetone, extract was centrifuged (4 °C; 5000 rpm) in a cooling centrifuge for 5 min and supernatant was used for recording the absorption. In method II, 125 mg of fresh leaf sample was incubated in dimethyl sulphoxide (DMSO, 6.25 ml) for 48 h at ambient temperature and the resultant supernatant was used for estimation. Biospectrometer (Eppendorf) was used for recording the absorbance and calculations were done following the formulae given by Wellburn (1994).

Studies on Morphotypes

Fully developed fresh fruits and leaves were collected from two distinct morphotypes *viz.* GG-02 and GG-05 identified from *Garcinia* conservation block of the Institute and were brought to the laboratory for analyses.

Leaf Morphological and Biochemical Analysis

Leaf morphological parameters such as leaf length (cm), leaf width (cm), leaf thickness (mm), leaf weight (g), petiole length (cm), petiole width (mm) and petiole thickness (mm) were recorded for ten fully matured leaves following standard procedures. Known quantity of leaf tissues was kept in oven at 60 °C till constant weight was obtained and

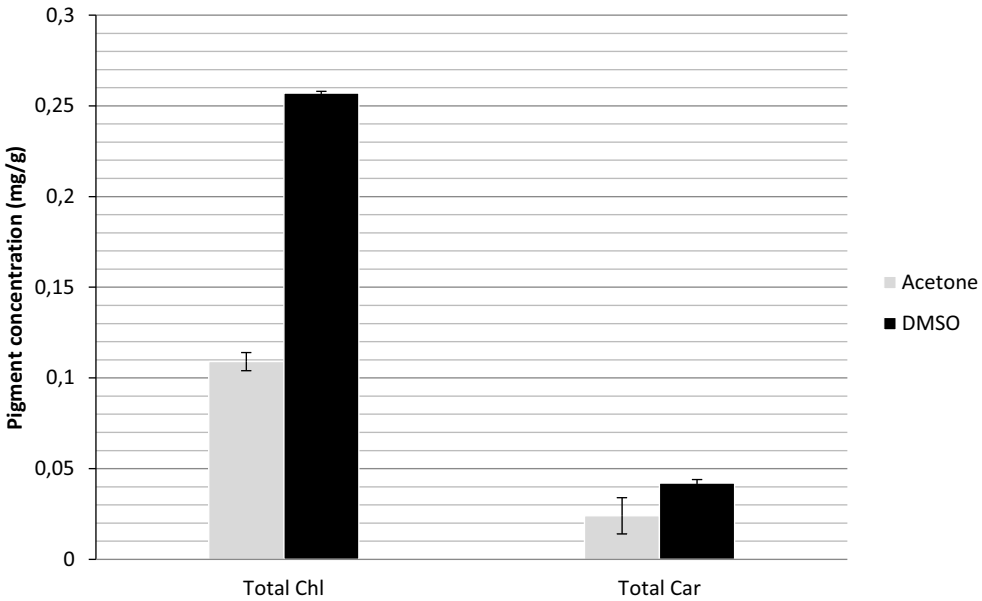

Fig. 1 Effect of acetone and DMSO methods of extraction on total chlorophyll (mg/g) and total carotenoids (mg/g) content in leaves of *G. gummi-gutta*

Table 1 Comparative leaf morphological analysis in two morphotypes of *G. gummi-gutta*

Parameter	GG-02		GG-05		t-test
	Range	Mean ± SD	Range	Mean ± SD	
Leaf length (cm)	9.0–11.6	10.26 ± 0.282	10.6–13.0	11.58 ± 0.303	**
Leaf width (cm)	4.9–6.2	5.52 ± 0.126	4.0–5.0	4.50 ± 0.111	**
Leaf thickness (mm)	0.33–0.47	0.42 ± 0.014	0.37–0.48	0.44 ± 0.012	ns
Leaf weight (g)	1.52–2.42	1.84 ± 0.093	1.41–1.88	1.60 ± 0.067	ns
Petiole length (cm)	1.1–1.5	1.27 ± 0.050	1.5–2.2	1.84 ± 0.076	**
Petiole width (mm)	2.92–3.73	3.33 ± 0.080	2.35–3.96	3.14 ± 0.173	ns
Petiole thickness (mm)	1.47–2.02	1.67 ± 0.047	1.22–1.69	1.50 ± 0.044	*

ns non-significant, *significant at 0.05 level of significance, **significant at 0.01 level of significance

Fig. 2 Leaf morphology in morphotypes GG-05 (*up*) and GG-02 (*down*) of *Garcinia gummi-gutta*

dry matter content of leaves was determined using formula given below.

$$\text{Dry matter (\%)} = \frac{\text{Final wt. of sample}}{\text{Initial wt. of sample}} \times 100$$

For determining pigment content in the leaves of both the morphotypes, the superior method obtained from previous experiment was used.

Fruit Physico-Chemical Analysis

Fruit polar circumference (cm), fruit equatorial circumference (cm), number of groves per fruit, mean fruit weight (g), pedicel length (mm), pedicel thickness (mm), rind thickness (mm), rind (%), pulp (%), seed (%), number of seeds per fruit, seed length (mm), seed width (mm), seed thickness (mm) and seed weight (g) were recorded in ripe fruits of both morphotypes. Biochemical parameters were studied in rind and pulp of ripe fruits from both morphotypes. Total soluble solids (TSS, °B) content and pH were determined using digital refractometer (Atago, Japan) and digital pH meter (Hanna, USA), respectively. Ascorbic acid content in rind and pulp were estimated using established procedures (Sadasivam and Manickam 2008).

Statistical Analysis

Observations were recorded for different morphological and physico-chemical parameters and presented as range and mean values ± standard deviation (SD). Mean values were compared following t-test using Web Agri Statistical Package (WASP v. 2.0, ICAR-CCARI, Ela, India). Analysis of variance was done in the experiments wherever appropriate.

Results

Comparison of Method of Leaf Pigments Determination

Two methods employing DMSO and chilled acetone were compared for identifying most efficient method for extraction and quantification of chlorophylls and carotenoids (Fig. 1). Results revealed marked differences amongst the methods for both the pigment groups studied, DMSO method being the efficient one. The recovery of total chlorophylls was 0.257 mg/g of tissues in case of DMSO, whereas mere 0.109 mg/g recovery was obtained with chilled acetone method. Similarly, higher recovery of total carotenoids was observed in DMSO method (0.042 mg/g) when compared with chilled acetone (0.024 mg/g).

Leaf Morphological and Biochemical Analysis

There were distinct differences in leaf morphology of studied morphotypes (Table 1). Leaf tip was obtuse in case of GG-02, while it was acute in case of GG-05 (Fig. 2). With respect to quantitative parameters, highly significant differences were observed in leaf length, width and petiole length, whereas significant differences were recorded for petiole thickness. Leaves were longer (11.58 cm) with longer petioles (1.84 cm) in case of GG-05, while leaf width was more (4.50 cm) in case of GG-02. There were no significant differences between the morphotypes for leaf thickness, leaf weight and petiole width.

Highly significant differences were noticed between the morphotypes for all the photosynthetic pigments studied. In general, leaves of GG-02 contained significantly higher chlorophyll a (0.933 mg/g), chlorophyll b (0.373 mg/g), total chlorophyll (1.313 mg/g) and total carotenoids (0.203 mg/g), when compared with GG-05 (Table 2). The total chlorophyll and total carotenoids content in GG-02 were about 1.7 and 1.3 times higher than that in GG-05.

Fruit Morphological Parameters

Ripe fruits were used for recording the observations on various morphological parameters, which revealed significant differences amongst fruits obtained from both the morphotypes (Table 3). Fruits of GG-02 had higher polar (19.07 cm) and equatorial circumference (20.69 cm) than those in GG-05. Number of groves did not vary significantly between the

Table 2 Comparative leaf biochemical analysis in two morphotypes of *G. gummi-gutta*

Parameter	GG-02	GG-05	t-test
Chlorophyll a (mg/g)	0.93 ± 0.001	0.58 ± 0.001	**
Chlorophyll b (mg/g)	0.37 ± 0.001	0.21 ± 0.002	**
Total chlorophyll content (mg/g)	1.31 ± 0.001	0.79 ± 0.002	**
Total carotenoid content (mg/g)	0.20 ± 0.000	0.15 ± 0.000	**

**significant at 0.01 level of significance

Table 3 Comparative fruit and seed morphological analysis in two morphotypes of *G. gummi-gutta*

Parameter	GG-02	GG-05	t-test
Fruit parameters			
Fruit polar circumference (cm)	19.07 ± 0.317	18.12 ± 0.199	*
Fruit equatorial circumference (cm)	20.69 ± 0.266	18.83 ± 0.215	**
Number of groves	8.20 ± 0.20	8.30 ± 0.260	**ns**
Fruit weight (g)	109.42 ± 4.765	90.00 ± 2.845	**
Pedicel length (mm)	3.12 ± 0.240	5.90 ± 0.433	**
Pedicel thickness (mm)	6.42 ± 0.144	5.20 ± 0.291	**
Rind thickness (mm)	11.09 ± 0.446	8.40 ± 0.649	**
Rind (%)	75.56 ± 0.818	59.37 ± 1.751	**
Pulp (%)	16.28 ± 1.192	28.26 ± 1.117	**
Seed parameters			
Seed (%)	8.16 ± 0.701	12.06 ± 1.575	ns
Seeds/fruit	6.60 ± 0.34	7.20 ± 0.291	ns
Seed length (mm)	24.16 ± 0.358	24.53 ± 0.574	ns
Seed width (mm)	11.42 ± 0.188	10.56 ± 0.376	ns
Seed thickness (mm)	6.40 ± 0.16	6.31 ± 0.141	ns
Seed weight (g)	1.82 ± 0.084	1.39 ± 0.091	**

ns non-significant, *significant at 0.05 level of significance, **significant at 0.01 level of significance

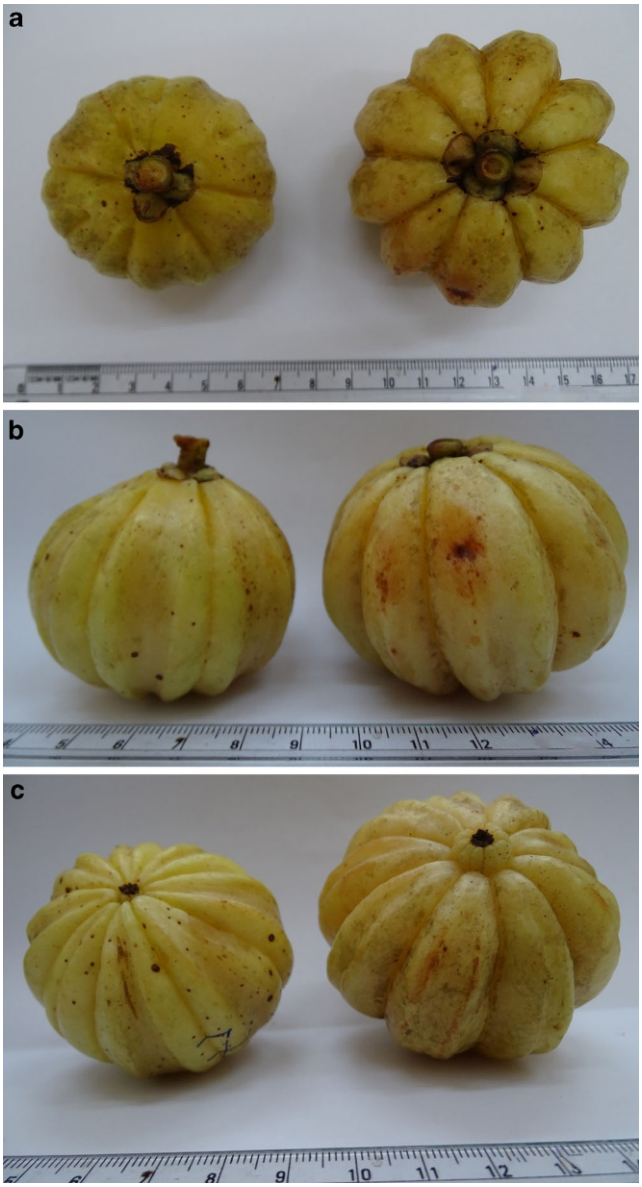

Fig. 3 Fruit morphology in morphotypes GG-05 (*left*) and GG-02 (*right*) of *G. gummi-gutta*: **a** top view, **b** side view and **c** bottom view

fruits of two groups (8.20 and 8.30), though morphologically they looked distinctly different (Figs. 3 and 4). Fruits of GG-02 had shorter (3.12 mm) but stouter (6.42 mm) pedicles than that in GG-05 (5.90 and 5.20 mm). Fruits of GG-02 were heavier (109.42 g) with thicker rind (11.09 mm) and higher rind recovery (75.56%), which is the economic portion of the plant. Interestingly, fruits of GG-05 contained 28.26% pulp compared to 16.28% pulp in GG-02 and the difference was highly significant. Though non-significant differences were observed for seed percentage, number of seeds/fruit and seed dimensions, fruits of GG-02 contained heavier seeds (1.82 g). In general, contribution of various parts of fruits to total fruit weight followed the similar trend (rind > pulp > seed) in both the types (Table 3).

Fruit Biochemical Analysis

There were no significant variations in TSS content and pH of fruit pulp, but these parameters varied significantly in case of fruit rind (Table 4), which is the economic part of the fruit. Significantly higher TSS (8.79 °B) was observed in fruit rind of GG-05 than that in GG-02 (6.55 °B). Though pH was higher in the fruits of GG-02, it was observed to be in highly acidic range in both the types studied. Ascorbic acid content in rind and pulp of both the morphotypes was not significantly different from each other.

Discussion

G. gummi-gutta is one of the multipurpose fruit species with diversified applications in food, medicinal, timber and chemical industries. Considering the potential of this species, it has been regarded as an important horticultural crop for the future (Abraham et al. 2006). Popularity for products, mainly HCA, is increasing as evident from the large number of advertisements on the internet and other mass media options. Existing supply of the raw material comes mainly from the wild, semi-domesticated populations and home gardens (Haldankar et al. 2011). A report on short term surveys suggested a decrease in the wild population of this species over two years without any catastrophic disturbances, indicating natural and slow shift in species composition under natural conditions (Ayyappan and Parthasarathy 2004). Considering these two factors i. e. exploitation of natural sources for meeting the growing demands and reducing natural stands, the species needs to be utilized in a sustainable manner (Waman and Bohra 2016). Report by Pandey et al. (2008) has also categorized the species with medium conservation priority and efforts are required to avoid any permanent loss to valuable germplasm.

Photosynthetic pigments are amongst the basic parameters that are influenced by various internal and external factors and hence, their determination is of prime importance for conducting various physiological studies (Chinthamani 2016). During present study, DMSO based method was found to be more efficient, when compared with traditional acetone method as higher recovery of total chlorophylls and total carotenoids was noticed in the former method. Though acetone (80%) is widely employed, it is less efficient in extracting some less polar components such as chlorophyll a and beta carotene (Lichtenthaler 1987). Also, the process of extraction using acetone mainly involves grinding, centrifugation and filtration, which provide scope for altered efficacy of the solvent through changed ratio of acetone and water (Wellburn 1994). On the other hand, leaf tissues are directly immersed in DMSO and the incubation eventually

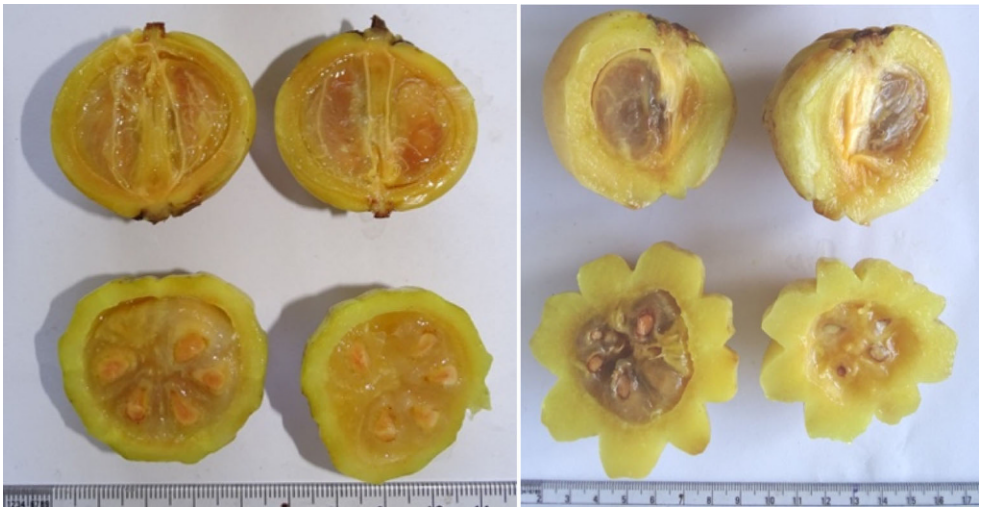

Fig. 4 Fruit morphology in morphotypes GG-05 (*left*) and GG-02 (*right*) of *G. gummi-gutta*: VS (*top*) and TS (*bottom*) of the fruit

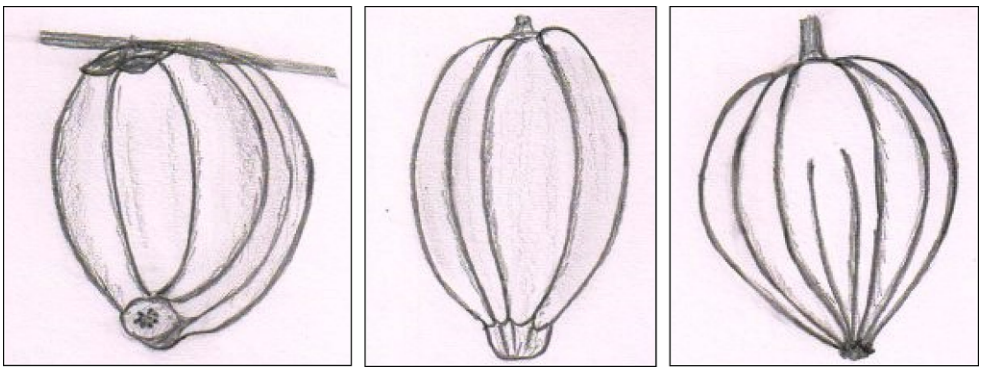

Fig. 5 Few shapes noticed in *G. gummi-gutta*: fruit with curved end (*left*); prominent nipples at the end (*middle*) and drop shape (*right*)

Table 4 Comparative biochemical analysis in two morphotypes of *G. gummi-gutta*

Parameters	GG-02	GG-05	t-test
TSS of rind (°B)	6.55 ± 0.315	8.79 ± 0.239	**
TSS of pulp (°B)	10.10 ± 0.217	9.67 ± 0.210	ns
pH of rind	1.90 ± 0.015	2.04 ± 0.020	**
pH of pulp	2.67 ± 0.015	2.61 ± 0.012	ns
Ascorbic acid content in rind (mg/100 g)	25.09 ± 3.583	16.13 ± 0.000	ns
Ascorbic acid content in pulp (mg/100 g)	16.13 ± 3.106	10.75 ± 0.000	ns

ns non-significant, **significant at 0.01 level of significance

results in extraction of pigments with reduced possibility of solvent loss in the process (Wellburn 1994). Considering the better recovery of pigments in DMSO solvent, this method could be recommended for *G. gummi-gutta*.

Though considerable scope exists for commercial cultivation of this species, no improved varieties have been released so far and hence, identification of superior genotypes through organized research has been emphasized (Haldankar et al. 2011). Though a few reports are available that describe the diversity of *G. gummi-gutta* from different geographical regions (Muthulakshmi et al. 1999; Abraham et al. 2006), analysis for important morphological and biochemical parameters has not been attempted so far.

During present investigation, highly significant differences were noticed for leaf and fruit related morphological and biochemical parameters. Most of existing populations (both wild and homestead) of this species are seedling progenies and hence considerable diversity has been reported in this species for various morphological parameters (Abraham et al. 2006). Variation in the recovery of chlorophylls and carotenoids noticed among the two morphotypes during present study could be attributed to varied levels of Chlorophyllase enzyme in the leaf tissues as noticed in other species (Gupta et al. 2011). Such variability for photosynthetic pigments amongst ecotypes of medicinally important *Piper sarmentosum* has been reported from ANI

(Chinthamani 2016). Large fruit size, thick rind and high acidity have been identified as targeted traits for crop improvement in *G. gummi-gutta* (Mathew et al. 2011). Considering this, the identified morphotype GG-02 with heavier fruits (109.42 g) and higher rind recovery (75.56%) could be a valuable germplasm for future improvement programmes. The morphotype also had short and stout pedicels, which are advantageous especially in areas such as ANI, wherein heavy velocity winds and cyclones are common.

Variations in the morphological and biochemical parameters have been considered as a boon especially in the species of medicinal importance. Significant variations for leaf morphological, anatomical and active ingredient content have been reported in anti-diabetic species *Gymnema sylvestre* collected from various parts of India (Dhanani et al. 2015). Characterization of germplasm and identification of superior chemotypes are helpful in crop improvement programmes (Singh et al. 2016; Waman and Bohra 2016) and hence, the present study could serve as a preselection activity in *G. gummi-gutta*. The natural population being seedling originated, a number of morphotypes are observed in the wild (Fig. 5). This variability includes differences in fruit shape, size, prominence of nipple and ridges (Abraham et al. 2006). Results noticed in the present study open up opportunities for evaluating such morphotypes for identifying commercially suitable types.

Acknowledgements Authors are thankful to the Director, ICAR-CIARI, Port Blair for providing necessary facilities for carrying out the experiments. Authors are also thankful to Dr. Shameer P., JNTB-GRI, Thiruvananthapuram for his help in identification of the species.

Conflict of interest P. Bohra and A.A. Waman declare that they have no competing interests.

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