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Morphological Diversity and Isolation of Genomic DNA of *Garcinia pedunculata* (Roxburgh ex Buchanan-Hamilton) from selected districts of Brahmaputra Valley of Assam, India

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ABSTRACT: In the present study, the morphological features and isolation of genomic DNA of the Garcinia pedunculata was conducted from five sub-sites each from three Districts namely Dibrugarh, Sivasagar and Tinsukia from Brahmaputra valley of upper Assam for a period of one year, from June 2014 to May 2015. Morphological characterization were studied for mature and bearing trees with respect to leaf size, size of leaf petiole, length of leaf vein, size of petiole and pedicels, fruit size etc. Extraction and isolation of genomic DNA from leaf tissue was done by Modified Doyle & Doyle (1990) methodology using CTAB extraction buffer. Results of the study reveals that the morphological features of the Garcinia pedunculata from different geographical locations showed a significant variation, particularly for leaf and fruit characteristics. Isolation and purification of genomic DNA from leaf tissue by Modified Doyle & Doyle (1990) methodology using CTAB extraction buffer indicates that the isolated genomic DNA may have more than 5000 bp. Isolation and purification of genomic DNA by using Modified Doyle & Doyle (1990) methodology using CTAB extraction buffer is a reliable measurement of DNA concentration and its purity. The research involve collecting plant samples from various districts, documenting and analyzing their morphological traits, and then isolating the genomic DNA from these samples. Due to limited access to specialized equipment or consumables necessary for the CTAB method, the extraction process was hindered.

Keywords: Assam, Brahmaputra Valley, *Garcinia pedunculata*, Genomic DNA, Morphological diversity.

INTRODUCTION

Out of the 12 mega diversity nations, India holds a major position in the world. Its climatic and allied features houses a high level of ecosystem diversity including forests to marine ecosystems each having unique sets of species. The Western Ghats in India is a treasure house of biodiversity and floristic wealth. The Western Ghats is recognized as a UNESCO World heritage Site. Also among the 36 global biodiversity hotspots, it holds its name in the world. The region reported about 7,500 species of flowering plants accounting for 27% of the Indian flora out of which 1250 are endemic to the region (Anonymous, 2014). Gogoi *et al.* (2015) conducted a study on 'Morpho-biochemical characterization of Garcinia species of Assam' by studying comparative morphological characterization of five varieties of Garcinia species found in five districts of Assam i.e, Dibrugarh, Sivasagar, Jorhat, Golaghat and Nagaon. The ethno-botanical study revealed wide variations in fruit weight and volume. The *G. pedunculata* exhibited highest (620.80 g) fruit weight whereas *G. lanceaefolia* exhibited lowest (22.51 g) fruit weight. In respect of number of seeds per fruit *G. pedunculata* had 9.35 numbers of seed whereas *G. xanthochymus* showed 2.50 numbers of seed

Garcinia L. is largest genus in the Clusiaceae Lindl. (Guttiferae Juss.) with 404 species distributed in the tropical and subtropical regions. It also has its centre in Southeast Asia and Madagascar (Gogoi *et al.*, 2016 and Ngernaengsaruay *et al.*, 2022). About 200 species were distributed in the South East Asian region ranging from southern parts of the Thailand and Peninsular Malaysia to Indonesia whereas of the 35 species reported in India, of which, about 15 species were distributed in North East India (Gogoi *et al.*, 2016) In India, states like Assam, Goa, Maharashtra, Karnataka, Kerela, West Bengal, Gujarat, Khasi and Jantia hills have shown extensive growth of Garcinia in semi wild state (Kar *et al.*, 2008). 15 species are reported in North-East India whereas 9 species in undivided Assam (Gogoi *et al.*, 2016). Out of 41 species reported to occur in India, 35 species occur in natural environments (Sabu *et al.*, 2013). The rest are introduced into cultivation (Islam *et al.*, 2021). Reports suggest that there are 5 recognized varieties

of *Garcinia* in India. The edible fruits of Garcinia holds its economic importance and hence cultivated as fruits , vegetables and for traditional medicine. *Garcinia* are dioecious with its occurrence in evergreen and semi-evergreen forest with a relatively wild monsoon (Gogoi *et al.,* 2020). *Garcinia pedunculata* is commonly known as "Borthekera" or "Amlavethasa" in India (in Assamese). The Nyishi tribe of Arunachal Pradesh refers it as 'Meba/Mibia' and is a large evergreen tree with fluted trunk, short spreading branches, leaves with stout mid vein and prominent lateral veins (Biswas *et al.,* 2017). In Bangladesh, *G. pedunculata* is referred to as "Taikor", which displays a picture of evergreen tree displaying fluted trunk and branches that are short. It is endemic to the parts of Myanmar and North-Eastern parts of India (Islam *et al.,* 2021). In India, Assam's evergreen forests, Kerela, Karnataka, Goa, Maharashtra, Nagaland etc shows extensive growth of *Garcinia* (Parthasarathy *et al.,* 2014).

Taxonomically, *G. pedunculata* (Roxb. ex Buch.-Ham.) belongs to Kingdom: Plantae, Division: Tracheophyta, Class: Magnoliopsida, Superorder: Rosanae, Order: Malpighiales, Family: Clusiaceae (Guttiferae, latex bearing plants), Genus: Garcinia L. and species: *Garcinia pedunculata* (Roxb. ex Buch.-Ham, 1824) (Gogoi *et al*., 2020).The light green flowers with sparse panicles represents the male flower of the species and the solitary ones are the female flowers. *Garcinia* has globose fruits having a diameter of 8-12 cm. The fruits are juicy adding to the arils which are edible too. The ripe fruit is golden yellow in colour and is usually eaten raw or cooked with pulses or with other vegetables (Islam *et al*., 2021 and Devi, 2021).

Nutritional value of fruits of *G. pedunculata* was well documented by (Islam *et al.,* 2021). Ripe fruits of *Garcinia* in Assam are sometimes preferred cooked and sometimes raw. The slices of ripe or raw fruits of *Garcinia* are made into pickles which can be preserved for a good number of time. In Assam, sun dried sliced fruits of *G. pedunculata* are used for most popular traditional preparation of 'tenga diya masor jol'. Slices of fruits enhancing the flavour of 'kharisa'- fermented bamboo shoot (Gogoi *et al.,* 2020).

Fruits of *G. pedunculata* are valuable and underutilized in Assam, though they possess the medicinal and antioxidant activity. Health benefits of *Garcinia* species were studied by many investigators. Work of following investigators is worth to mention here. (Deore *et al.,* 2011) argued that *Garcinia* have long been used to treat various diseases as it possesses medicinal value. Medicinal benefits of *G. pedunculata* for many ailments were reported by many investigators such as, (Kagyung *et al.,*2010) to cure chronic cough, cataract, fever, bronchitis, dysentery, asthma and as a cardiotonic; (Gogoi *et al.,* 2012) in their study suggested that *Garcinia* helps in decreasing body weight because it curb appetite, reduce fatty acid synthesis and increase glycogen synthesis. (Sharma *et al.,* 2015) for treatment of different types of stomach related disease; (Ali *et al.,* 2017) in their study found *Garcinia* to ameliorate hyperglycemia, reduce diabetic complications and protect against oxidative stress-induced damage; (Biswas *et al.,* 2017) to lower degenerative diseases like heart disease, arthritis, brain dysfunction and cataract. It also helps with decline in immune system and dreadful diseases like cancer. (Islam *et al.,* 2021) for treatment of female obesity and cure diabetes, jaundice, and liver diseases; and (Baruah *et al.,* 2021) to treat dysentery, digestive and cooling (Anonymous 2014).

Characterization of morphological features of mature and bearing trees, particularly features of plant leaf and flowers helps to minimize the genetic erosion of this forest resource, prevents threatening of many species, create awareness regarding many species and reduce habitat destruction (Parthasarathy and Nandakishore 2014) Morphological features such as the plant height, dimensions of the plant leaves, flower characteristics and sizes of fruit and seed were important parameters used to assess the morphological diversity. Morphological characterizations of the species of from different ecosystems indicate variation within the species of the same ecosystem and similarities in the species of two different ecosystems (Beals *et al.,* 2000).

Santosh and Arakera stated in the study of evolution and population study, genetic markers are important tools which are used to construct genetic maps. Applied breeding programs also use genetic markers (Arakera *et al.,* 2017). They are an aid to assess inter and intra genetic variation of out crossing and inbreeding. They also aid in genetic identification of varieties or pedigrees. In India, Indian Institute of Spices Research, Calicut works on survey by collecting the genetic resources of *Garcinia*; They develop GIS (Geographic Information System) prediction models to locate the new areas of diversity. They also work on biochemical and molecular characterization in order to estimate the genetic diversity; genotypes having high HCA content are identified; and micro propagation protocols are developed with the aim to multiply the endangered species leading to their proper utilization and conservation (Parthasarathy *et al.,* 2013) and (Bhuyan *et al.,* 2020). (Gupta *et al.,* 2018) conducted a study on "Morpho-Anatomical and Physicochemical Evaluation of *Garcinia Pedunculata* Roxb.Ex. Buch.-Ham." in which the pharmacognostic and phytochemical details about the plant was established. The presence of simple, petiolate leaf obovate-oblong in general, some elliptic and oblong with obtuse and sub-acute tip and paracytic stomata was revealed by macro and microscopical studies of leaf. Some diagnostic features noted from the anatomical study of the plantare bicollateral vascular bundle covered with sclerenchymatous fibers in leaf and 8-10 layer of collenchyma, scattered pericyclic fiber, the arrangement of phloem in the ring form in stem. The presence of palisade parenchyma with the epidermis,

parenchyma fiber and scalariform vessels was revealed by powder microscopy.

Literature review indicates that, plenty of scientific data is generated on the *G. Pedunculata* with reference to antihyperglycemic, antidiabetic, and antioxidant effects; diversity and ethnobotany; diversity in the Western Ghats; ethno-botanical survey; ethnomedicobotany and phytochemical screening; floral morphology; genetic diversity analysis; lectotypifications; medicinal & antioxidant activity; medicinal potential; nephroprotective effect; novel value added products prepared from fruits; nutritional quality; phylogenetic analysis; polyphenol rich extract; and spice crop.

With reference to India, comparatively meagre data is available on *G. pedunculata* with reference to intra-specific morphological characterisation. Further, no scientific studies have been carried out on the extraction and isolation of genomic DNA; hence, the present study is undertaken. Objective of the study is to evaluate the intra-specific morphological characterisation and extraction and isolation of genomic DNA of *G. pedunculata*.

MATERIALS AND METHODS

Study Area: The hill ranges of the eastern and north-eastern Himalaya harbours the Brahmaputra valley (Lat. 27° 7' 38.4996"N &Long. 94° 44' 23.7192"E). The subdivision of the valley, the Western Brahmaputra Valley harbours Goalpara region and Kamrup region; Darrang, Nagaon & the North Bank are harboured by central Brahmaputra valley; Dibrugarh, Sonitpur, Sivasagar and Lakhimpur by the Eastern Brahmaputra valley. The Teesta River in North Bengal also drains into Brahmaputra River (Fig. 1).

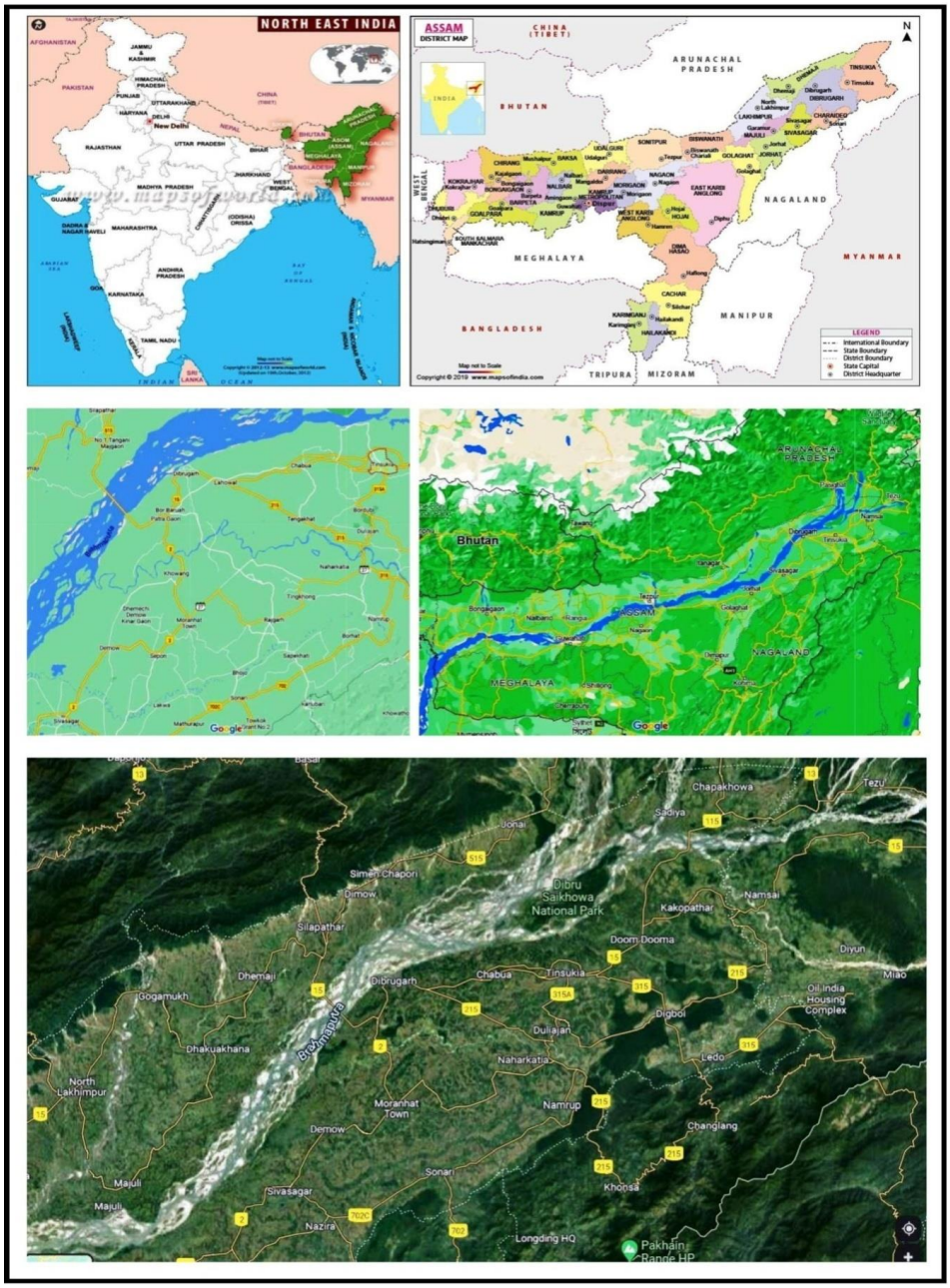

Fig. 1. Map and Satellite image of the study area (Source: Google map)

30 districts lie within the heart of the Brahmaputra Valley covering an area of 71,516 km² in total. Its soil is considered as the most productive soils in the world. The flow of Brahmaputra River starts from Assam and flows to Bangladesh, there the river meets river Ganges here the world's largest delta is created. Finally, the river flows into the Bay of Bengal in the south. Start of the Monsoon months i.e. May- September brings 85% of the total annual rainfall. The valley experiences average temperature range of 9.2°C - 19.6°C in the winter and summer respectively, whereas humidity ranges in Brahmaputra valley of Assam is 84% to 95%.

Study sites and Sampling strategy: The present study was carried out for a period of one year, from June 2014 to May 2015. For present investigation, three Districts namely Dibrugarh, Sivasagar and Tinsukia from Brahmaputra valley of upper Assam were selected as primary study sites and among them, five sub-sites were selected on the basis of geographical locations (Table 1).

Table 1: Study sites and sub-sites selected from Brahmaputra valley of upper Assam

Sr. No.	Districts from Brahmaputra valley	Geographical positions	Sub-sites
1	Dibrugarh	Lat. 27° 28' 26.98" N Long. 94° 55' 5.15" E	Jokai, Moran, Namrup, Tengakhat, Tingkhong
2	Sivasagar	Lat. 26° 58' 48.00" N Long. 94° 37' 48.00" E	Betbari, Gaurisagar, Meteka, Patsaku, Sepon
3	Tinsukia	Lat. 27° 29' 31.89" N Long. 95° 20' 48.39" E	Digboi, Doomdooma, Guijan, Kakopothar, Ledo

The study sub-sites were surveyed monthly and at each site, morphological characterisation of *G. pedunculata* was carried out using different morphological markers such as leaf size, size of leaf petiole, length of leaf vein, size of petiole and pedicels, fruit size etc. From each sub-site, young leaves were collected and kept at -20ºC for isolation and quantification of genomic DNA.

All specimens were photographed by Cannon EOS1100D digital camera. In the laboratory, identification of the specimens were done by following the available literature, standard taxonomic keys and reference books of (Hooker 1896) and (Kanjilal *et al.*, 1934-1940) were followed. The herbarium preservation technique given by Jain & Rao in 1977 were used to preserve the voucher specimens.

- **Morphological characterisation:**

Standard linear graph sheet having division 1 cm and subdivision 1mm was used to measure the dimensions of the plant leaves. Vernier calliper was used to measure the size of the fruit. Available literature comparison was done to accurately jot down the morphological features of plant leaf and fruit.

Isolation of Genomic DNA:

Collection of samples: For DNA isolation, leaf tissue is preferred since it can be found easily and available in large amount. In this study, 5 accessions were collected from three districts namely Dibrugarh, Sivasagar and Tinsukia from Brahmaputra valley of upper Assam. After collection and cleaning with distilled water, the leaves were blotted and then to kill the surface microbes, sterilization was carried out with alcohol. Then the leaves were processed further.

Random Amplified Polymorphic DNA (RAPD) (Chaudhry *et al.*, 1999).

Total genomic DNA was isolated from leaf tissue according by Modified Doyle & Doyle (1990) methodology using CTAB extraction buffer.

Extraction of DNA: 0.45 µg of refrigerated leaves were washed thoroughly with distilled water, dried partially under sunlight and partially under the shade. Dried leaves were powdered in mechanical grinder using liquid nitrogen to extract the DNA. 5ml CTAB buffer was added as cationic surfactant and incubated in water bath at 55ºC for 1 hour with regular mixing to avoid aggregation. Some amount of the incubated extract and equal amount of chloroform: isoamylalcohol (24:1) was added and shaked gently to form a uniform emulsion. Material was centrifuged at 14,000 rpm at room temperature for 10 min and aqueous phase were transferred to a new tube. To it 0.08 vol of wash buffer and 0.6 vol of isopropanol was added and incubated at -20º C for 1 hr. After incubation, add 50 µl of 2M NaCl solution and centrifuged at 14,000 rpm at 5ºC. Remove aqueous phase without disturbing the pellets and the pellets were washed with 70% ice cold ethanol. Pellets were dissolved in 300 µl of TE buffer and stored at 4ºC for further work.

Agarose Gel Electrophoresis: Agarose gel electrophoresis is a technique which separates DNA fragments including PCR products on the basis of their size and charge. The DNA in the product filter through itself after being loaded on the gel.

Protocol standardization for Genomic DNA isolation: The DNA isolation protocol was standardized by slight modification of Doyle and Doyle method (1990) and quantification of DNA was carried out by comparing the DNA base pair ladder followed by method of spectrophotometer. The concentration of extracted genomic DNA was determined by following formula;

Genomic DNA (µg/ml) = 50 µg/ml × measured at 260 wavelength × dilution factor

Statistical analysis: The results were analysed statistically by using spss software version 20. Variables were summarised as mean ± standard deviation (SD) and graphical representation done with the help of Microsoft excel.

RESULTS AND DISCUSSION

Morphological characterisation: During present study, morphological characterisation of *G. Pedunculata* was studied using parameters such as leaf size, size of leaf petiole, length of leaf vein, size of petiole and pedicels, fruit size. Results on morphological studies were presented in Table 2 to 4.

Morphological characters of the five accession of *G. pedunculata* collected from five sub-sites. Dibrugarh (GD) district reveals highest average of leaf length in GD-02 and lowest in GD-01, whereas highest leaf width was recorded in GD-05 and lowest in GD-04. Highest petiole length was noted in GD-02 and lowest in GD-03. Highest pedicel size was noted in GD-02 and lowest pedicel size in GD-03. The average diameter of the fruit was found to be highest in GD-05 and lowest in GD-03.

For Sivasagar (GS) district, the highest average leaf length was observed in GS-03 and lowest in GS-02. Highest leaf width was recorded in GS-05 and lowest in GS-01. Highest petiole length was noticed in GS-O2 and lowest in GS-O3; whereas highest pedicel size was reported in GS-02 and lowest in GS-03. Fruit diameter was found to be highest in GS- 03 whereas GS -01 and GS-02 bears equal sized fruits.

Table 2: Morphological analysis of five accession of *G. pedunculata* in Dibrugarh District.

Sr. No.	Acc No.	Source	Leaf length	Leaf width	Petiole size	Pedicel size	Fruit size
1	GD-01	Tingkhong	21.7±5.37	9.85±2.81	2.37±0.25	2.37±0.25	8.3±0.24
2	GD-02	Moran	28.4±1.87	10.2±0.75	2.7±0.20	3.42±0.43	8.2±0.32
3	GD-03	Tengakhat	24.3±5.9	9.92±2.24	2.0±0.21	2.2±0.16	7.5±0.47
4	GD-04	Jokai	23.5±3.45	8.92±0.71	2.05±0.1	3.0±0.16	7.7±0.45
5	GD-05	Namrup	24.7±5.02	11.05±0.82	1.97±0.17	2.82±0.33	8.4±0.64

Table 3: Morphological analysis of five accession of *G. pedunculata* in Sivasagar District.

Sr. No.	Acc No.	Source	Leaf length	Leaf width	Petiole size	Pedicel size	Fruit size
1	GS-01	Sepon	17.7±0.98	8.6±1.60	2.4±0.27	2.37±0.25	8.5±0.54
2	GS-02	Patsaku	16.6±4.20	8.7±1.21	2.8±0.24	3.42±0.43	8.5±0.51
3	GS-03	Gaurisagar	19.37±0.75	9.92±2.24	2.0±0.21	2.70±0.47	10.5±0.43
4	GS-04	Betbari	19.06±2.72	8.95±0.71	2.55±0.52	3.25±0.37	9.8±0.54
5	GS-05	Meteka	19.2±0.98	11.05±0.82	2.45±0.44	3.25±0.27	9.4±0.76

Table 4: Morphological analysis of five accession of *G. pedunculata* in Tinsukia District.

Sr. No.	Acc No.	Source	Leaf length	Leaf width	Petiole size	Pedicel size	Fruit size
1	GT-01	Digboi	17.45±1.80	9.35±2.02	1.87±0.47	3.2±0.21	7.8±.22
2	GT-02	Doomdooma	22.17±1.72	10.27±0.75	2.3±0.35	3.4±0.39	7.5±.32
3	GT-03	Ledo	19.87±1.54	9.92±2.24	2.0±0.21	3.3±0.42	7.9±.42
4	GT-04	Kakopothar	21.06±1.67	8.67±0.71	1.95±0.10	3.1±0.26	7.8±.54
5	GT-05	Guijan	23.02±2.91	10.8±0.70	1.95±0.17	3.0±0.39	7.6±.38

G. pedunculata collected from five sub-sites from Tinsukia district reveals the highest average leaf length in GT-05 and lowest in GT -01; while highest leaf width was recorded for GT-05 and lowest in GT-04. Highest petiole length was noted in GT-02 and lowest in GT-01. Highest pedicel size was found in GT-02 and lowest in GT-05. The highest average fruit diameter was recorded in GT- 03 and lowest in GT-02.

The graphical representation of morphological characterisation with respect to leaf length, leaf width, petiole size, pedicle size and fruits size were presented in Fig. 2. Results of the data suggest that leaf length and Leaf width of *G. pedunculata* in Dibrugarh district is comparatively larger than Sivasagar and Tinsukia district.

Petiole size and fruit size of *G. pedunculata* in Sivasagar district is comparatively larger than Dibrugarh and Tinsukia districts. Further, size of pedicel in Tinsukia district is larger than Dibrugarh and Tinsukia districts.

Results of the present study on morphological characterisation of *G. pedunculata* are in agreement with the findings of Parthasarathy and (Nandakishore 2014), (Rameshkumar 2015), (Gogoi *et al.,* 2016) and (Ngernsaengsaruay 2022). Similar study was also carried out by (Shameer *et al.,* 2016*)* where *Garcinia* species of Western ghats showed diversity in fruit morphology, leaf morphology and floral morphology.

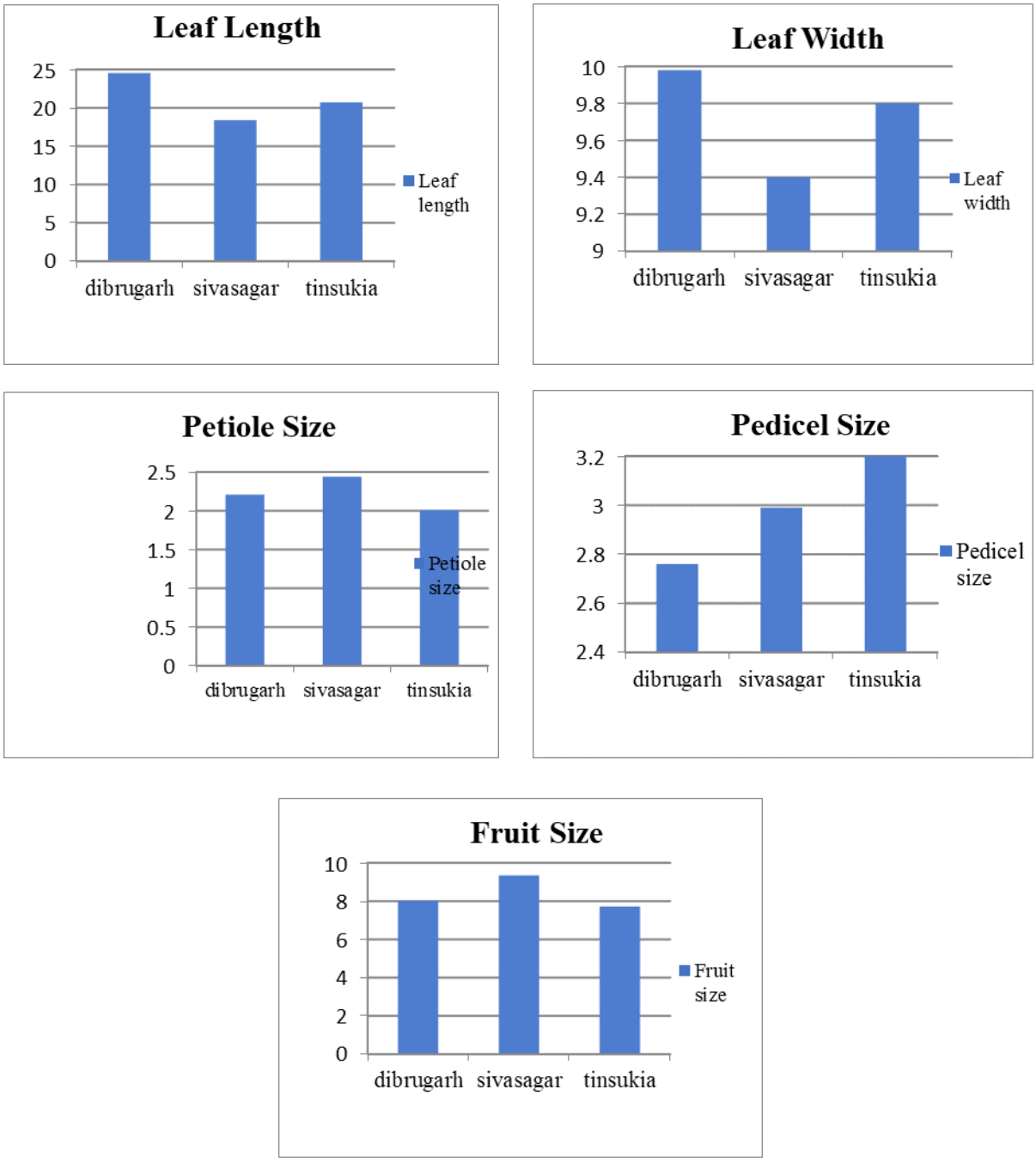

Fig. 2. Comparative account of morphological characterisation of *G. pedunculata*

Extraction and Isolation of Genomic DNA. Agarose gel electrophoresis results showing band intensities of DNA isolated by modified method in comparison with 5000bp. The result shows that the base pair of isolated genomic DNA more than 5000 bp. The method employed in the study proved to be successful and applicable for extraction of DNA with high yield and purity for *G. pedunculata* from 15 sub-sites of three districts of Assam. Electrophoresis separation of DNA extracted by the present protocol showed intense bands very close to the gel wells signifying high degree of purity and intact DNA (Nadia and Oraby 2019).

Results of the present study on extraction and isolation of Genomic DNA of *G. pedunculata* are in agreement with the work of (Asish *et al.*, 2010), (Santosh *et al.*, 2017), (Seethapathy *et al.*, 2018) and (Nadia and Oraby, 2019).

CONCLUSIONS

All molecular and genetic studies rely on accuracy in measurement of DNA concentration and purity. Morphological features of the *Garcinia pedunculata* from different geographical locations showed a significant variation, particularly for leaf and fruit characteristics. Isolation and purification of genomic

DNA from leaf tissue by Modified Doyle & Doyle (1990) methodology using CTAB extraction buffer indicates that the isolated genomic DNA may have more than 5000 bp. Sahasrabudhe *et al*. (2010), worked on standardization of DNA extraction and optimization of RAPD-PCR conditions of *Garcinia indica.* They worked on modified CTAB protocol adding polyvinylpyrrolidone (PVP) in separate tubes and precipitating with 5M Nacl along with chilled alcohol to increase the solubility of polysaccharides. They could isolate pure and sufficient amount of DNA and optimize RAPD conditions. Isolation and purification of genomic DNA by using Modified Doyle & Doyle (1990) methodology using CTAB extraction buffer is a reliable measurement of DNA concentration and its purity. This study will act as a tool for other researchers in this field to continue their work further on *Garcinia* species and understand the morphological diversity. Such studies will help in authentication of the genus *Garcinia.*

FUTURE SCOPE

The morphological study will help researchers explore and locate the importance of the species. Also mitigate ways to preserve it for the future. Moreover, genetic level studies can also be carried out in this field. The extracted DNA could be used for genetic studies, such as molecular markers, genetic diversity analysis, or even potential applications in plant breeding and conservation.

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Conflict of interest. None.

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