



Short Communication

Karyomorphology of *Garcinia indica* (Thomas-Dupetite) ChoisyJayesh Anerao¹, Neetin Desai², Behnaz Patel³ and Manjushri Deodhar^{1,*}¹K.E.T's V.G.Vaze College, Mulund (East), Mumbai - 400 081; ²Padmashree Dr. D.Y.Patil University, CBD Belapur, Navi Mumbai – 400 614; ³Ramnarain Ruia College, Matunga (East), Mumbai 400 076.

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Abstract

Garcinia indica (Clusiaceae) is a tree species endemic to Western Ghats of Maharashtra. Fruits are important as they are the rich source of hydroxy citric acid. Being polygamodioecious, plants in different locations show a lot of variation in height of plant, branching pattern, canopy, leaf and fruit morphology. Hence a cytological study was undertaken to see whether the polymorphism also exists at genetic level. Roots from germinating seeds were pre-treated with saturated p-dichlorobenzene. Squash was made by propionic acid orcein method. Chromosome number reported was $2n=54$. The karyotype of *G. indica* consists of 7 pairs of metacentric chromosomes, 17 pairs of submetacentric chromosomes, 1 pair of submetacentric chromosomes with satellites, 1 pair of submetacentric chromosomes with secondary constriction and one asymmetric pair of chromosomes.

Keywords: Propionic acid, Orcein, Karyotype, Satellite, Secondary constriction, Genetic polymorphism

Genus *Garcinia* L. belongs to the family Clusiaceae. The genus is distributed in South-East Asia and Africa. It includes 200 species found in the tropical regions of Asia and Africa. Prominent characters of Genus *Garcinia* are monopodial growth, coriaceous texture of leaves, oil glands or cavities containing yellow or bright coloured resins present in all parts of the plants and polygamodioecious condition. *Garcinia mangostena* is known as the queen of fruits and is well studied for morphological variability, flowering method, fertilization and fruit variation (Ramage et al., 2004). *Garcinia atroviridis* is a well studied endemic species of Malaysia (Pangsuban et al., 2007).

In India about 35 species of *Garcinia* are reported which include *Garcinia combogia*, *Garcinia xanthochymus*, *Garcinia cowa* etc. *G. indica* is endemic and restricted to Western Ghats of Maharashtra. It is used by local people for preparing

pleasant attractive pink coloured soft drinks with bilious action. Recently some industries have started extracting hydroxy-citric acid (HCA) from the rind of the fruit which is an important constituent used as a hypocholesterolaemic and anti-obesity agent (Heymsfield et al., 1998). Fruit and fruit syrup has antioxidant activity (Mishra et al., 2006). As the plants are cross pollinated, plants show a lot of variations in yield and quality. During 1995, a study was initiated in Kelkar Education Trust's Vinayak Ganesh Vaze College, Mulund (East) in Maharashtra to identify the clones of *G. indica* with characteristics like early fruiting, high yield and fruits rich in HCA content in the fruit rind. Method of clonal propagation for elite mature tree species was standardized for *G. indica* (Chabukswar and Deodhar, 2006). As the plants are insect pollinated and polygamodioecious, a lot of morphological variations occur in plant height, leaf morphology and fruit characters. Hence a systematic study was

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undertaken to see whether the polymorphism also existed at genetic level, and a karyomorphological study was initiated. The first cytological observation in *G. indica* was made by Krishnaswamy and Raman (1949) where they documented the chromosome number as $2n=54$. However Razdan (1972) reported 48 chromosomes. The discrepancy in the chromosome number led to the investigation in the karyomorphology of *G. indica*.

Seeds of *G. indica* were collected from Deorukh, district Ratnagiri, Maharashtra in the month of June in 2009 and 2010. The seeds were germinated in plastic trays in polyhouse, K.E.T's V. G. Vaze College. The mitotic index was determined by analyzing the maximum number of dividing cells in the root tip of the seedling of *G. indica*. Maximum number of dividing cells were observed in the morning at 10.45 AM. Root tips were excised at 10.45 AM and pre-treated with p-dichlorobenzene for 3 hours at 4°C. After pre-treating the roots were transferred in distilled water and again kept at 4°C for 1 hour. Then the root tips were fixed in 10% acetic acid for ten minutes, washed and hydrolysed in 1N HCl at 60°C for 5 minutes in thermostat. Squash was made in 2% propionic orcein. Images of chromosomes were taken with Olympus microscope reflected light fluorescence attachment (Olympus CXRFA - 2 model) equipped with Pinnacle software.

Measurement of chromosomes was done by Biovis image plus V4.11 software. The length of chromosomes were measured in micrometer. The number of chromosomes, karyotype formula, ratio of longest to shortest chromosome, mean and median of long arm length, mean and median of short arm length, total chromosome length, total form per cent, mean centromeric index were determined from seven well spread metaphase cells. Chromosome homology was assigned according to similarities in length, morphology and centromere position. Chromosome nomenclature was based on the centromere location as proposed by Levan et al. (1964), that is metacentric (M), submetacentric

(SM), telocentric (T) and acrocentric (AC). Chromosomes were paired with their respective chromosomes and were arranged as 1 to 54 in descending order of length. When all measurements had been compiled, the total chromosome length and total form per cent (TF%) were calculated.

Karyotype formula of diploid *G. indica* is

$$2n = 54 = 14M + 34SM + 2SM^{SAT} + 2SM^{SC} + 2$$

Unpaired chromosomes

The chromosome number is found to be $2n = 54$ (Fig.1a). The karyotype showed only one pair of chromosomes which had satellites present on short arm and one pair of secondary constrictions on long arm. Three types of chromosomes, namely, seven pairs of metacentric, nineteen pairs of submetacentric, and one pair with secondary constriction on long arm were observed in the study. Out of nineteen submetacentric pairs, one pair of chromosome showed satellites on short arm. Ideogram shows the last pair with asymmetric chromosome (Fig.1b). For constructing the karyotype and calculations, the chromosomes were arranged in the order of decreasing size (Fig.1c). The shortest chromosome was 1.24998 μm and longest chromosome was 3.4027 μm in length. The ratio of longest to shortest chromosome was 2.722. Mean of long arm length was 1.2223 μm and median of long arm length 1.135 μm . The mean of short arm length was 0.9767 μm and median of short arm was 0.978 μm . In *G. indica* the total chromosome length was found to be 120.1384 μm , total form percent (TF%) was 43.90.

Lack of information on chromosome number and chromosome morphology in the family Clusiaceae make it difficult to construct evolutionary trends. There is very wide variation in chromosome number among genera and even among species such as *Clusia*, *Hypericum* and *Garcinia*, for which different haploid numbers have been detected. In *Clusia* $n=30$ seems to be constant for the genus (Cruz et al., 1990) Robson and Adams (1968), suggested the basic chromosome number for *Hypericum* and allied

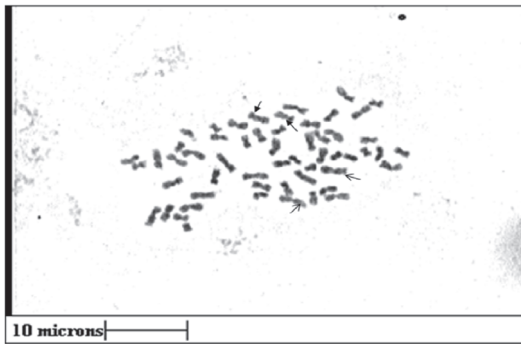


Figure 1. (a) Photomicrograph with measurement scale bar showing well spread somatic chromosomes of *G. indica*



Figure 1. (b) Karyotype showing paired chromosome

Note: → Arrow showing Satellite chromosomes

→ Arrow showing chromosomes with secondary constriction

⇨ Arrow showing asymmetric pair of chromosomes

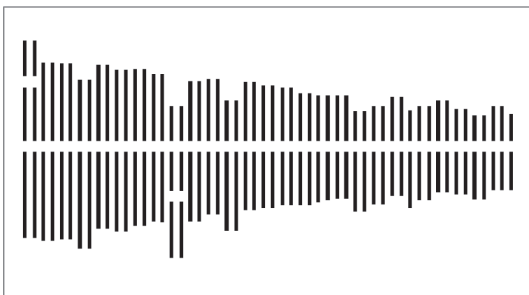


Figure 1. (c) Ideogram of *G. indica*

Note: Chromosomes arranged in order of decreasing size for constructing the karyotype and calculations.

genera to be $x = 8$ or 9 . For other allied species in family Clusiaceae, *Callophyllum* and *Mesua* it is reported to be $n = 8$ and 16 respectively, and the same count applies to Genus *Garcinia*.

Razdan (1972) determined the chromosome number of various species of Genus *Garcinia*. He suggested haploid number for *Garcinia* as $n = 24$. Species of *Garcinia* like *G. morella* and *G. cowa* are diploids representing $2n = 48$. Species like *G. combogia*, *G. spicata* and *G. parviflora* are triploids, that is, $2n = 72$, whereas in *G. mangosteena* $2n = 96$. The exceptions are *G. livinstonii* where $2n = 56$, and *G. xanthochymus* where $2n = 80$. It is possible that these might be hybrids with $n = 24$ and $n = 32$. For karyotyping, Razdan studied eight genotypes collected from different locations of Karnataka state. He reported somatic chromosome number as $2n=48$.

Much earlier Krishnaswamy and Raman in their reports of some important horticultural plants (1949), reported the somatic chromosome number of *G. indica* to be $2n = 54$. Our reports reveals chromosome number of $2n = 54$ having one pair with satellite chromosome, one pair with secondary constriction and one pair of asymmetric chromosomes which was not reported earlier. Razdan (1972) studied about eight genotypes of *G. indica*. There were four pairs of satellite chromosomes and one pair with secondary constriction. Out of four pairs of satellite chromosomes, 3 pairs had satellite on short arm and one pair had satellite on long arm. Apart from this Razdan reported two more cytotypes which differ in number and position of satellite. In cytotype two, there were only three pairs of satellite chromosomes and one pair with secondary constriction. All satellites were on the short arm. In cytotype three also there were three pairs of satellite chromosomes and one pair with secondary constriction. Of three pairs of satellites, two pairs had satellite on short arm and one pair had satellite on long arm. The results of present study showed change in the chromosomal count and structural features of chromosomes. All the plants studied had 54 chromosomes and first pair had satellite located at the end of short arm. Other satellites reported were not observed in present population.

While screening the plants, it was observed that

height of plant, branching pattern, canopy, leaf and fruit morphology varied at various locations. It is necessary to investigate the plants collected from various locations which show some variation in chromosome morphology or satellite position or secondary constriction. Also, in the present study, one pair of chromosomes with unequal length was seen. As the plant is polygamodioecious, it becomes necessary to investigate whether it has some relation with gender of the plant.

Study on the karyomorphology of the polygamodioecious plant *G. indica* indicated that the diploid chromosome number is $2n = 54$. The specific morphological features of the chromosomes can be used to identify the local variety.

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