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A Comparative Study of Karyomorphology among Three Populations of *Garcinia indica* (Clusiaceae) (Thomas-Dupetite) Choisy

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Abstract: *Garcinia indica* is a tree species of the family Clusiaceae. This species is endemically distributed only in Ratnagiri and Sindhudurga district of Maharashtra. Plants collected in particularly Dive Agar and Sawantwadi show morphological variations. Mitotic chromosomes from three different population from the Konkan region were studied using propionic orcein stain. Chromosome numbers 2n = 54 is reported for all the populations. Three populations differed from each other in some aspects. Karyotype analysis showed that Deorukh population from Ratnagiri region showed one pair of satellite and one pair of secondary constrictions while Otawane population from Sawantwadi showed one satellite chromosome but differ in pattern of secondary constriction. Third, Diveagar population from Raigad district showed three pairs of satellite chromosomes and one pair of secondary constriction. So, it can be concluded that, plants from Bapat Garden of Dive Agar and Otawane region can be considered as cytotypes.

Key words: *Garcinia indica*, polymorphism, karyotype analysis

INTRODUCTION

The genus *Garcinia* L. belongs to the family Clusiaceae, having approximately two hundred species distributed in Asia. Thirty six species are found in India among them *G. gummigutta* and *G. indica* are more important ones. *G. indica* is an evergreen tree with drooping branches found endemic in tropical rain forests of the western ghats restricted to Konkan region of Maharashtra. Some industries have started extracting Hydroxy-Citric Acid (HCA) from the rind of the fruit which is an important constituent used as an hypocholesterolaemic and anti-obesity agent (Heymsfield *et al*., 1998). Fruit extract is traditionally used as soft drink with attractive colour, recently has shown to have antioxidant activity (Mishra *et al*., 2006). The fruit rinds of *G. indica* contain cyanindin-3-glucoside and cyanindin-3- sambubioside (Nayak *et al*., 2010). Various pigments of *G. indica* were screened for inhibition of enzymes hyaluronidase and elastase, thus, contributing to antiageing activity (Sahasrabudhe and Deodhar, 2010).

Since 1995, in K.E.T's V.G. Vaze college, a study was intiated to identify the early fruiting, high yielding and HCA rich clone of *G. indica*. Method of clonal propogation for elite species was standardize for *G. indica* (Chabukswar and Deodhar, 2006). *G. indica* is dioecious in nature and lot of morphological variations in leaf shape, branching pattern, thickness of fruit rinds, colour of fruit, tree height was observed in various locations of *G. indica*. Hence systematic study was undertaken to collect the plants from various populations, and to see whether the polymorphism also exists at genetic level. Various populations were screened with molecular markers (Thatte *et al*., 2012) and karyomorphological studies at different locations were also undertaken. The present study aims at karytomorphological study at different locations.

There are only two reports on karyomorphology of *G. indica*. Much earlier in 1949, while study the karyomorphology of some fruit trees, Krishnaswamy and Raman reported the chromosome number of *G. indica* to be 2n = 54. Razdan (1972) reported diploid chromosome number of *G. indica* to be 2n = 48.

MATERIALS AND METHODS

Collection of plant material: Seeds of *G. indica* were collected from various regions (Fig. 1), Deorukh (Dist. Rantagiri), Otawane (Dist. Sindhudurg) and Diveagar (Dist. Raigad) in June 2009, 2010, 2011 and 2012. The seeds were germinated in plastic trays in polyhouse of K.E.T's V.G. Vaze college, Mulund (E.), Mumbai-400081, Maharashtra.

Chromosome preparation: The mitotic index was determined by analyzing maximum number of dividing

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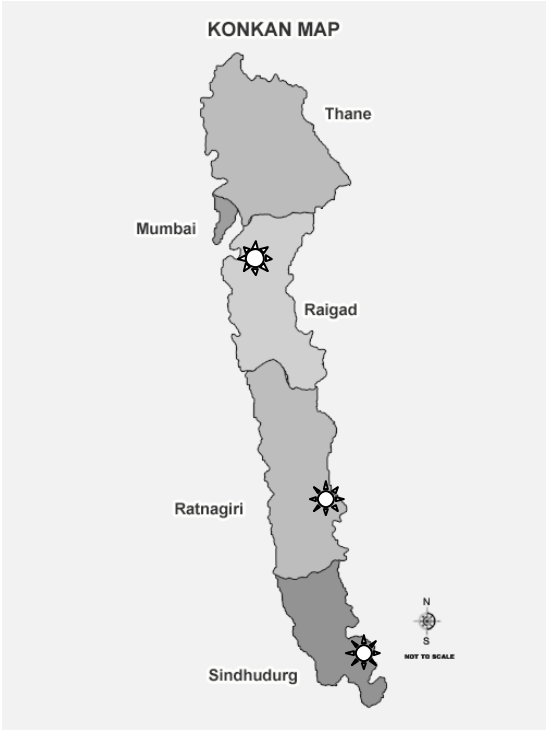

Fig. 1: Geographic locations of *G. indica* populations used in this study. ✸ Indicates collection sites

cells in the root tip of the seedling of *G. indica*. The maximum number of dividing cells were observed in the morning at 10:45 am; so root tips were excised at this time and pretreated with p-Dichlorobenzene for 3 h at 4°C. Then roots were transferred in distilled water and kept at 4°C for 1 h. The root tips were fixed in 10% acetic acid for ten minutes, washed and hydrolysed in 1N HCl at 60°C for 5 min in thermostat. Squash were made in 2% propionic orcein. Chromosomes images were taken with Olympus microscope reflected light fluorescence attachment (Olympus CXRFA -2 model) equipped with Pinnacle software.

Chromosome analysis: Measurement of chromosomes were done by Biovis image plus V4.11 software. The length of chromosomes were measured in micrometer. The number of chromosome, ploidy level, 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 percent, mean centromeric index and symmetry classes determined from seven well spread metaphase cells. Chromosome homology was assigned according to similarities in length, morphology and centromere position. Chromosome nomenclature based on the centromere location followed that proposed by Levan, i.e., 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, total form percent (TF%) and Centromeric Index (CI) were calculated. Karyotype formula of the population investigated were summarized.

RESULTS

In present communication, the plant material was collected from different Konkan region of Maharashtra. At least seven well spread metaphase cells were considered for karyomorphological studies. The karyotypes of all studied plants correspond to the different formula. The study was based on three population of *G. indica* maintaining chromosome number 2n = 54 (Fig. 2a-c). The karyotype formula as well as respective karyotypes (Fig. 3a-c) obtained and the parameters analysed were summarised in Table 1.

For constructing the karyotype and calculations, the chromosomes were arranged in order of decreasing size (Fig. 4a-c). Chromosome number (2n), karyotype formula, the Longest Chromosome Length (LCL), the Shortest Chromosome Length (SCL), long arm/short arm ratios (L/S) and centromeric index (CI) of *G. indica* are listed in Table 1.

All the populations of *G. indica* were diploid, with 2n = 54.

The somatic chromosome in all the population was constant 2n = 54 but differ in some morphological aspects. In Deorukh population (Fig. 2a) chromosome number was 54. Karyotype analysis showed one pair of satellite, one pail of secondary constriction and one pair of asymmetric chromosome. Otawane population (Fig. 2b) from Sawantwadi region was showing similar ploidy level having one pair of satellite chromosome but differ in pattern of secondary constriction. The secondary constriction appeared in rod shape. It also showed one pair of asymmetric chromosome. Where as Dive agar population (Fig. 2c) from Raigad district was showing 54 chromosomes with three pairs of satellite chromosomes, one pair with secondary constriction and one pair with asymmetric chromosome.

The observed value of total chromosome length of complement in Deorukh population (120.1384 μm) and Otawane population (118.3909 μm) were found

Table 1: The karyotype formula and the parameters analysed

Population	2n	Karyotype Formula	Chromosome length (μm) LCL	SCL	L/S	CI	Karyotype symmetry
Otawane	54	14M+34SM+2SM^{SAT}+2SM^{SC}+ 2 Unpaired chromosome	3.4748	1.1443	3.03668	0.7775	1A
Deorukh	54	14M+34SMO+2SM^{SAT}+2SM^{SC}+ 2 Unpaired chromosome	3.4027	1.2499	2.7222	0.78084	1A
Diveagar	54	14M+30SM+4SM^{SAT}+2SM^{SC}+2T^{SAT} + 2Unpaired chromosomes	4.0934	1.4275	2.8673	0.6960	1A

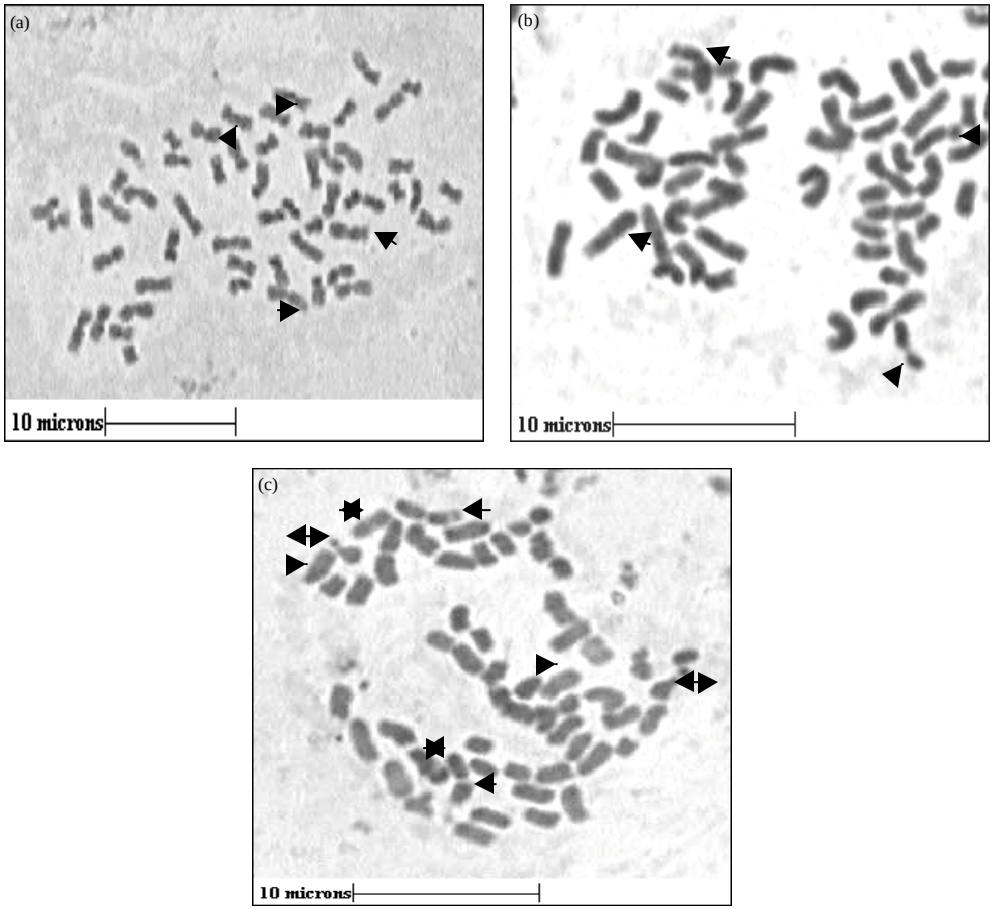

Fig. 2(a-c): Microphotographs of well spread mitotic metaphase plates of *G. indica* (a) Accession from Otawane, (b) Accession from Deorukh and (c) Accession from Diveagar

comparatively lesser than that of population from Diveagar (131.0864 μm). Shortest chromosome were observed in Deorukh (1.24998 μm) and in Otawane (1.1443 μm) which was found comparatively lesser than that of Dive agar (1.42757 μm). Longest chromosome found in Dive agar (4.0934 μm) was comparatively higher than Deorukh (3.4027 μm) and Otawane (3.4748 μm) population.

The karyotypes investigated can be summarized as follows:

- The composition of the karyotype gives ground to distinguish three cytotypes
  - Deorukh population 2n = 54 = 14M+ 34SM+ 2SM^{SAT}+2SM^{SC}+2 Unpaired chromosomes
  - Otawane population 2n = 54 = 14M+ 34SM+ 2SM^{SAT}+2SM^{SC}+2 Unpaired chromosomes
  - Diveagar population 2n = 54 = 14M+ 30SM+ 4SM^{SAT}+2SM^{SC}+2T^{SAT}+2 Unpaired chromosomes
- Heteromorphic chromosomes pairs were established in all the populations

Fig. 3(a-c): Karyotypes of *G. indica* (a) Population of Deorukh (b) Population of Otawane and (c) Population of diveagar

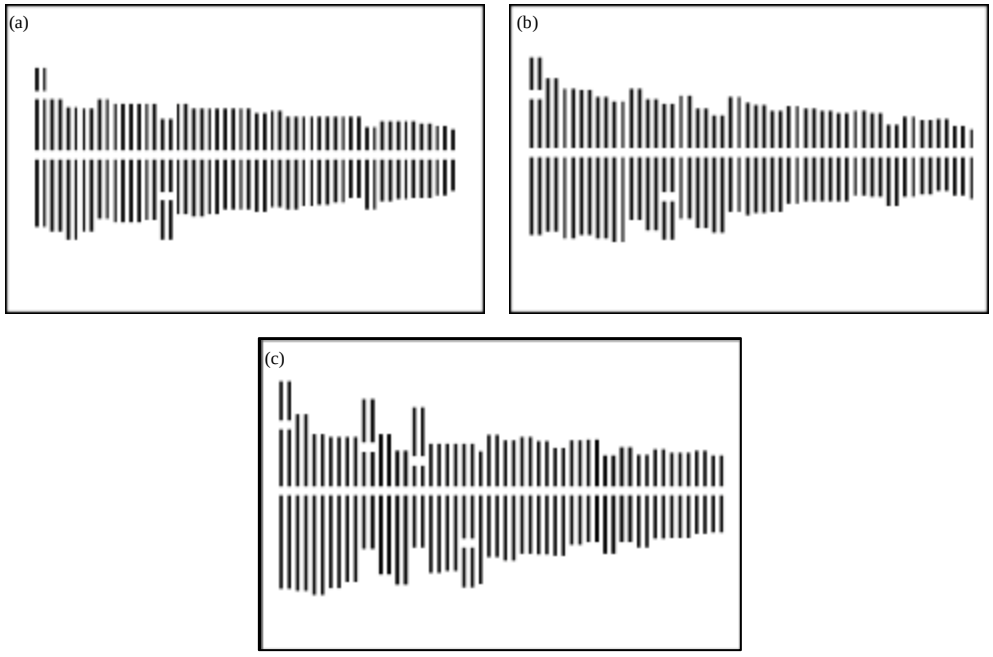

Fig. 4: Ideogram of *G. indica* (a) Deorukh population, (b) Otawane population and (c) Diveagar population

DISCUSSION

From family Clusiaceae in genus *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* 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 has reported haploid number for *Garcinia* is n = 24. Species like *G. morella* and *G. cowa* are diploids representing 2n = 48. for G. in *G. cambogia, G. spicata* and *G. parviflora* are

triploids that is 2n = 72 whereas *G. mangostana* is 2n = 96. The exceptions are *G. livingstonii* 2n = 56 and *G. xanthochymus* 2n = 80. It is possible that these might be hybrids.

In present study all the populations of *G. indica* were diploid, 2n = 54.

There are several reports of chromosome polymorphism observed among populations in several species which reported both numerical and structural variations. Chengqi (2008) studied somatic chromosome number of *Allium przewalskianum* in different populations. They screened 302 individuals from 43 locations, 90 were diploid 2n = 16 and 212 were tetraploid 2n = 32.

Li (2005) studied chromosome number of *Aster ageratoides* an endemic to china and related if geographically. Most of the populations were hexaploid (2n = 6x = 54) an occupied an extensive area of south west china to eastern chile. And diploid and tetraploids were less frequent and limited to narrow regions.

There are several reports including a species in different populations e.g., *Melilotus officinalis* (Pavlova and Toshewa, 2004), *Cicer arietinum* (Kordi *et al*., 2006) and *Impatiens balsamina* (Momtaz *et al*., 2007) exhibited morphological variations in chromosomes. Pavlova and Toshewa (2004) studied karyomorphology of *Melilotus officinalis* in populations of Bulgaria. All *M. officinalis* populations were diploids (2n = 16). On the basis of karyomorphology, the populations can be divided into 3 groups. The group one comprised of 10 metacentric, 4 submetacentric chromosomes with a pair of satellite was on submetacentric chromosome. In group II also there were 10 metacentric, 4 submetacentric chromosomes but the satellite was attached to the metacentric type chromosomes. The group III was having 8 metacentric, 4 submetacentric chromosomes. In this group also the satellite was associated with metacentric chromosomes and there was one pair of intercentric chromosome (Pavlova and Toshewa, 2004). They also observed deviation in macromorphology of these plants in group III. The plants were shorter with less number of inflorescence, larger legumes and hair on lower leaflet surface. They called this taxon as variety arenaria it was locally endemic suggested that it should be under control and protected.

In present study also out of three populations, two populations Deorukh and Otawane had 14 metacentric, 34 submetacentric chromosomes, pair of asymmetric chromosome and one unique pair of submetacentric satellite chromosome. But in Otawane population the secondary constriction appeared to be rod shaped (Fig. 2b). Diveagar populations showed 14 metacentric, 30 submetacentric chromosomes. Satellite appeared on 3 pairs of chromosomes. Out of them two satellite appeared on submetacentric chromosomes (Fig. 4c) In addition to this there was a pair of telocentric chromosomes with satellites which was unique to this populations. They also have comparatively longer chromosomes (Table 1).

Razdan (1972) reported karyomorphology of three different cytotypes of *G. indica*. In cytotype one there were four pairs of satellite chromosome while in cytotype two had three satellite and one chromosome pair with secondary constriction which matches with our observations. But a pair of heteromorphic chromosome was not reported earlier.

G. indica being dioecious insect or cross pollinated plant was expected to show lot of morphological variation in canopy shape, shape of leaves, branching pattern, thickness of fruits etc. hence the plants from various locations were studied morphologically. Also percentage of polymorphism was studied using RAPD and ISSR markers (Thatte *et al*., 2012). To our surprise most of the populations like Dapoli, Chiplun, Shirode of Sawantwadi showed only 4-5% polymorphism. In comparison the plants in Diveagar populations were significantly taller having significantly longer leaf lamina. And these plants also showed higher degree polymorphism (8-10%), showing some unique bands characteristics to this populations (Thatte *et al*., 2012). These plants also differ karyomorphologically.

CONCLUSION

The plants of *G. indica* located in small pocket of Diveagar can be considered as separate taxon. As the plant is highly endemic, there is urgent need for conserve the cytotypes.

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