REPRODUCTIVE BIOLOGY AND EVALUATION OF KOKUM (*GARCINIA INDICA* (THOUARS) CHOISY) GENOTYPES

By

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(2019-12-003)

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VELLANIKKARA, THRISSUR – 680656
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THESIS

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DECLARATION

I hereby declare that this thesis entitled **"Reproductive biology and evaluation of kokum (*Garcinia indica* (Thouars) Choisy) genotypes"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara
24-11-2021

Manjunath Sharanappa Tondihal
(2019-12-003)

CERTIFICATE

Certified that this thesis entitled **"Reproductive biology and evaluation of kokum (*Garcinia indica* (Thouars) Choisy) genotypes"** is a record of research work done independently by **Mr. Manjunath Sharanappa Tondihal (2019-12-003)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to him.

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We, the undersigned members of the advisory committee of **Mr. Manjunath Sharanappa Tondihal (2019-12-003)** a candidate for the degree of **Masters of Science in Horticulture**, with major field in **Fruit Science**, agree that the thesis **"Reproductive biology and evaluation of kokum (*Garcinia indica* (Thouars) Choisy) genotypes"** may be submitted by **Mr. Manjunath Sharanappa Tondihal (2019-12-003)**, in partial fulfillment of the requirement for the degree.

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MANJUNATH SHARANAPPA TONDIHAL

DEDICATED TO MY PARENTS & GUIDE

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INTRODUCTION

1. Introduction

Kokum (*Garcinia indica* (Thouars) Choisy) belonging to the family Guttiferae or Clusiaceae which is endemic to tropical humid forests of Western Ghats, India. Kokum is an evergreen tree that grows to a height of about 10 to 15 m and having drooping canopy. Leaves are characterized by its dark green and glabrous surface. Kokum tree is found naturally in Northern parts of Kerala, Coastal and Southern interior Karnataka, Goa and Konkan region of Maharashtra. Apart from this, species also found to be rarely in Andaman and Nicobar Islands, Odisha and N-E regions (Rema and Krishnamurthy, 2000). Kokum tree is traditionally a part of homestead crop in Konkan region.

Kokum is a valuable yet underexploited perennial fruit tree gaining importance due to its multifarious uses mainly for preparation of pleasant and attractive beverages with rich antioxidant and antibacterial properties (Negi *et al*., 2006); presence of hydroxycitric acid (HCA) in fruit rind got the properties of antiobesity, anticholesterol and protection against UV rays (Patil, 2005). Kokum is also rich source of anthocyanin which has great demand in export market as a natural colourant which can blend well with acidic type of foods. Traditionally fruit rind is used in preparation of ayurvedic medicine having magical effects in curing various ailments. The edible fat present in the seed is commonly called as kokum butter which can be utilized in the cosmetic industries as emollient as well as manufacture of soap (Kirtikar and Basu, 1999). Kokum butter has the quality that of vanaspati ghee.

Kokum is one of the native species which depict dioecious nature. The reproductive system of *G. indica* is highly debatable; Rawat and Bhatnagar (2005) reported kokum as gynodioecious. However, Rajasekharan and Ganeshan (2002) described this species as polygamodioecious, and Thatte and Deodhar (2012) identified four different types of flowers in *G. indica*, showing trioecy nature. Reviewing the previous studies findings, it was observed that *Garcinia indica* is a good candidate specimen for studying dioecy evolution. The cross pollination coupled with the seedling populations of kokum created a large genetic diversity throughout its natural distribution; which has led to the wide adaptability of the species.

Variation among the populations could be seen for flowering and fruiting characteristics in kokam. Generally flowering in kokam begins in the end of October and it continues up to mid of February. Peak harvesting season in the month of April to June. Harvesting coincides with the onset of monsoons results into more fruits get damaged and require artificial structures for drying.

The study on the sequence of growth events of the annual growth cycle of the plant is called phenology. It is very important to study crop phenology to understand the various stages of growth under change in climatic regimes in different environmental conditions. The study of phenology is prerequisite for better planning of crop management methods, characterization of germplasm and preparing for crop improvement programmes as well as plant protection measures. Plant growth stages like bud burst, leaf development, abscission, flowering, fertilization, seed set and development occurs in the right time as well as season in plants. Record of each stage of these events would give an appropriate idea on the crop plants. Based on this, pruning, fruit drop management, irrigation, nutrient, and pests and diseases management could be developed. All these crop management measures depend on the critical phenophases of the crop plant. The yield and quality of crop produce depends upon the right time and stage of the harvest (Chmielewski, 2003; Kishore *et al*., 2017). Understanding flowering phenology is very crucial for better planning of hybridization programmes. Since the phenological growth stages of kokum have yet to be distinguished and explained, the current study aimed to develop a standardized extended BBCH phenological scale. The creation and validation of a phenological scale like this crop is essential because kokum is an emerging crop.

In Kerala, natural distribution of kokum is restricted to northern districts such as Kasaragod and Wayanad. As a result, cultivation and use of this crop is virtually non-existent. Though Kerala climatic conditions are ideal for cultivation of kokum it is unfortunate that not much importance is being given for this crop. Demand for the kokum fruit rind is steadily increasing in Northern and Central parts of Kerala due to awareness of medicinal properties present in it. Considering its importance, Department of Fruit Science, College of Agriculture, Vellanikkara, Kerala Agricultural University and ICAR-National Bureau Plant Genetic Resource, Regional

Station, Vellanikkara have made an intervention on germplasm conservation, characterization and promotion for value addition which would pave the way for attraction of small scale entrepreneurs towards this crop. But information on the performance of conserved germplasms under central Kerala is not available. Identification of ideal genotypes for yield and quality as well as suitability for processing is a long felt need.

In this challenge the present study entitled "Reproductive biology and evaluation of kokum (*Garcinia indica* (Thouars) Choisy) genotypes" was undertaken with the specific objectives probe systematically into the phenological stages and reproductive biology of kokum, and to evaluate genotypes for yield and quality grown under the humid tropical conditions of central Kerala.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

In recent days, demand for dried rind of kokum fruit has been steadily increasing due to its well-known medicinal values. Previously there were many studies concentrated on collection and conservation of kokum genotypes, evaluation of those genotypes for variability and yield as well as utilisation in value addition. The present investigation is pertaining to the phenological studies and floral biology, pollination behaviour and evaluation of genotypes for yield and quality which is being ignored in the past studies.

In this chapter highlighted the information on the phenological events that occurring in the crop growth cycles, floral, morphological characters and fruit parameters, and quality characters of kokum as well as other species of genus *Garcinia.* Wherever adequate information is lacking on kokum, research pertinent on other tree crops is also included. The contents are presented sequentially with suitable subtitles.

2.1. Genus *Garcinia*

The Genus *Garcinia* consists of large a number of species belonging to the family Guttiferae or Clusiaceae. The species of *Garcinia* are mostly concentrated in America, Australia, Asia and South Africa. The uniqueness of species of this family is many of the species that produce resinous gum, so the family is named as Guttiferae. There are about 50 to 300 species reported in the genus *Garcinia*, majority of the species were distributed throughout the tropics. According to Praneetha and Balamohan (2014), India has got nearly 35 species, of which 17 species are indigenous and economically valuable, and have extensive therapeutic qualities. Seven species are endemic to Western Ghats, six are from Andaman and Nicobar Islands and four reported from the North Eastern region of India.

The *Garcinia* species that are found in the Western Ghats regions of India namely, *Garcinia indica, G. gummi-gutta, G. imberti, Garcinia pushpangadaniana, G. rubro-echinita, G. talbotii, G. travancoria, G. Morella and G. wightii.*

2.2. *Garcinia indica*: A summary

2.2.1. Distribution of species

The *Garcinia indica* Choisy (Thouars) is commonly called as kokum, is one of the most valued and remained as underutilized perennial tree species endemic to the Western Ghats of India. Kokum belonging to the family Clusiaceae in the order Theales and subclass Dilleniidae. The *Garcinia*, *Hypericum*, *Vismia*, *Cratoxylon*, *Triandenum*, *Pentadesma*, *Mammea*, *Allenblackia*, *Calophyllum*, *Mesua*, *etc*. are the few important genera that belonging to same family (Robson and Adams, 1968).

Kokum is an evergreen, perennial, monopodial, straight growing tree with medium length lateral branches. Distribution of species can be seen on the West coast part of India from extreme Northern part of Kerala, coastal and interior Karnataka, Goa and Konkan region of Maharashtra. The species also found in a lesser extent in Islands of Andaman and Nicobar, Orissa and North Eastern regions of India (Rema and Krishnamurthy, 2000).

2.2.2. Utilization

Kokum has a wide range of applications, as a result, it is an inextricable part of the human lifestyle. The fruit juice is collected from dried rind is used for preparation of syrup, squash, RTS and agal (salted juice), *etc*. In kokum growing regions, dried rind is utilised as a sour ingredient in food preparations. Kokum butter, which is nutritious and have the properties of demulcent, smoothening, softening skin, is present in seeds and used for cosmetic, confectionery and culinary applications (Kirtikar and Basu, 1999). Raw fruits, young leaves and plant bark are also used to treat various ailments. It is been used in preparations of herbal therapy for centuries to treat diarrhoea, dermatitis and digestive issues. It also acts as an antihelmintic and cardiotonic as well as reducing hypertension.

The fruit rind is rich source of hydroxycitric acid (HCA), which reduces fat deposition in human cells and hence serves as natural source of antiobesity drugs (Patil *et al*., 2005).

2.3. Phenology of genus *Garcinia* and other fruit crops

Awachare and Upreti (2019) described phenological growth stages in mangosteen and they had observed seven primary growth stages and thirty six secondary growth stages. Primary growth stages include bud development (stage 0), leaf development (stage 1, takes 6-7 weeks), shoot development (stage 3, takes 7-8 weeks), reproductive development (stage 5, takes 5-6 weeks), flowering (stage 6, takes 30-35 days), fruit development (stage 7, takes 120-180 days) and fruit maturation (stage 8). Adiga *et al*. (2019) reported seven principal growth stages and 37 secondary growth stages in cashew. Primary growth stages include bud development (stage 0, started in the month of September-October), leaf development (stage 1), shoot development (stage 3, takes 5-7 weeks), inflorescence development (stage 5, began in the month of November and peak period in November and December), flowering (stage 6, began in 2nd week of November and peak in January and February), fruit development (stage 7) and fruit maturation (stage 8, takes 8-10 days). Zhange *et al*. (2016) reported ten principal growth stages and 48 secondary growth stages in pineapple. Primary growth stages include bud development (stage 0), leaf development (stage 1), sucker formation (stage 2), pseudo stem elongation (stage 3), leaf development of the sucker (stage 4), inflorescence development (stage 5), flowering (stage 6), fruit development (stage 7) and fruit ripening (stage 8) and senescence (stage 9). In another study by Kishore *et al*. (2017) reported eight principal growth stages and 37 secondary growth stages in bael. Primary growth stages in bael include, bud development (stage 0, began in the month of April-May), leaf development (stage 1, began in the month of May-June, takes 4-5 weeks), shoot development (stage 3), reproductive development (stage 5, began in the month of May-July, takes 4-5 weeks), flowering (stage 6, began in the month of May-June, takes 5-6 weeks), fruit development (stage 7) and fruit maturation (stage 8, began in the month of February-March, takes 5-6 months), senescence (stage 9, after February-March, takes 4-6 weeks).

The *Garcinia imberti* produces vegetative shoots every year but leaf initiation could be seen throughout the year. Emergence of new leaf began in the month of January-March and leaf fall started in the fourth week of September and completed in

the month of December. First male floral bud primordia formed in the inflorescence in February followed by flowering in late February and early March, and end of flowering in the last week of April. The female floral bud development observed in late February. Flowered in the month of March-April and it was completed in late May. Bud primordia gradually turned into green colour and finally buds turned into complete yellow colour. Fruiting was at the end of the dry season and continued till on set of rainy seasons. The average number of days required for initiation of floral bud primordia to flowering of both male and female flowers about 18 to 26 days. In female flowers, average time required from flowering to fruiting about 26 to 36 days, while in case of time from fruit initiation to maturity takes about 6 to 8 months. Fruit development commenced during the peak of flowering in the month of March-April and it extended until the end of December (Kandasamy *et al*., 2017).

2.4. Flower characteristics of *Garcinia indica*

Kokum produces female flowers solitarily and mostly bear in terminal branches. Staminodes of 20 phalanges whereas stigma 4-8 rays (Godbole and Das, 2000).

The reproductive behaviour of *Garcinia indica* is complex, as Rawat and Bhatnagar (2005) opinioned that as gynodioecious, whereas Rajasekharan and Ganeshan (2002) reported it as polygamodioecious. In another study, Joseph and Murthy (2014) observed significant diversity in sex forms among the kokum populations.

In kokum, male flowers had long pedicellate, clustered and green coloured sepals, measuring approximately about one cm in length. The petals 4 to 5 in number have yellow-red shades. Several fertile stamens located on central hemisphere of the receptacle. Male flowers have no carpel. Another kind of male flower has several fertile stamens as well as a non-functional or rudimentary carpel located at the receptacles centre whereas the female flowers are short pedicellate containing four yellowish petals. Female flowers have functional pistil *i.e.,* 1.50 to 2.50 mm in diameter which encircled by four tufts of staminodes. The other form of female flower

found in Dapoli, where it has 3.50 mm diameter pistil encircled by only two staminode tufts. While bisexual flowers have both red coloured petals and sepals, and contain eight tufts of stamens arranged in a circular manner around the pistil (Thatte and Deodhar, 2012).

Devi *et al*. (2013) identified four types of flowers in kokum namely, male type-1 with long pedicels, 40 to 60 number of functional stamens and no pistil; male type-2 with large pedicels produces numerous functional stamens and rudimentary pistil. Bisexual flowers with freely arranged 20.00 to 30.00 functional stamens, fertile pistil and four lobed stigma. Whereas female flowers were characterised by globose and finely developed ovary attached to an eight lobed stigma, 8.00 to 20.00 sessile staminodes grouped in four tufts around the stigma in a decussate fashion. In another study by Joseph and Murthy (2014) also reported that four types of flowers in kokum such as staminate flowers, staminate flowers with pistillodes, pistilate flowers with staminodes and hermaphrodite flowers. They had described length, breadth and pedicel length of the male (1.05 cm, 0.52 cm and 0.56 cm, respectively), male with pistillode (1.04 cm, 0.54 cm and 0.54 cm, respectively), female with staminodes (0.78 cm, 0.54 cm and 0.34 cm, respectively) and bisexual flowers (1.14 cm, 0.66 cm, 0.57 cm, respectively). The number of stamens produced in the male of 57.00, male with pistillode of 49.00 and bisexual flowers of 58.00.

Kapatia (2019) reported the average length (6.67 mm) and diameter (5.57 mm) of kokum flowers. Whereas the average pedicel length was 0.90 cm under the Thrissur conditions of central Kerala.

The polygamodieocious flower habit of kokum was described by Dike *et al*. (2020). There were three types of flowering plants in kokum *viz*., plants with no fruits are males, plants with more fruits are functionally females and plants with fewer fruits are bisexuals. The male flowers have a long receptacle with four reddish cream petals and four sepals. Stamens are abundant, fertile and make anthophore when they cohere at the base. Carpels are either non-existent or tiny with rudimentary pistils. The flowers produced in female plants were relatively wider and have shorter pedicel than male flowers which has four sepals and a light yellowish-green corolla. There were

few stamens or staminodes in male flower, which grouped into two, four or eight tufts around the pistils. The bigger ovary has four or eight active ovules arranged on axile placentation. The bisexual plants produce typical female flowers and also produce flowers in which the carpel is surrounded by a ring of stamens or staminodes. Carpel is functional and the petals are reddish in colour. The fruits are spindle shaped and produces minimum number of fruits.

Other species of *Garcinia*

Te-chato (2007) reported that petal colour in *Garcinia cowa* has light yellow, *G. speciosa* produces cream-light yellow, *G. atroviridis* has a purple colour flower, *G. mangostana* produce a red colour, *G. dulcis* petals light green and *G. schomburgkiana* exhibit pink petals.

Female flowers in *Garcinia capuronii* were tetramerous, measuring about 4.00 to 5.00 mm long and 5.00 mm wide. Free sepals were present, measuring about 2.80 to 3.80 mm long, 2.80 to 4.20 mm wide (outer pair slightly larger) with imbricate aestivation and free petals were present, measuring 3.00 to 3.50 mm long, 3.00 to 4.00 mm wide with imbricate aestivation. The staminate flowers in *Garcinia lowryi* were tetramerous, measuring about 23.00 mm long and 2.70 to 5.00 mm wide. It also had free sepals which is measuring about 1.00 to 2.50 mm long and 1.00 to 2.50 mm wide with imbricate aestivation. Free petals measuring about 2.00 to 4.00 mm long and 2.50 to 3.50 mm wide with imbricate aestivation (Rogers and Sweeney, 2007).

Flower appear on the tip of juvenile shoots in *Garcinia mangostana*, it may be produces single or in groups, ranged from one to three. The flower has a diameter of 4.00 to 6.00 cm and produces 14.00 to 18.00 stamens that are 5.00 to 6.00 mm long, but do not carry any functional pollens. Obligate apomixis responsible for fruit set (Sinaga *et al*., 2007). *Garcinia atroviridis* is a gynodioecious species produces both hermaphrodite as well as female (pistillate) plants. The stamens in the hermaphrodite flowers were long with plenty of functional pollen grains. Female plants have pollen free anthers and produces more number of fruits than that of hermaphrodites (Pangsuban, 2007).

According to Baruah and Borthakur (2012), flowers of *Garcinia pedunculata* Roxb. were polygamous, tetramerous. Male flowers bold in size with a pale green colour which consists of numerous stamens. *Garcinia paniculata* Roxb. flowers were also polygamous and tetramerous. Whereas male flowers were white having four sepals as well as four petals with many stamens. *Garcinia morella* Desr. flowers were described as polygamous, tetramerous and male flowers were borne on the axil of fallen leaves in a group. While hermaphrodite flowers were solitary. *Garcinia cowa* Roxb. flowers were polygamous, tetramerous, produces in the axil of fallen leaves and male flowers present in dense terminal or axillary cluster. *Garcinia lanceaefolia* Roxb. flowers were polygamous as well as tetramerous in nature. Male flowers could be observed in one to two number which were borne on terminal inflorescence and these male flowers were characterised by thick sepals, small petals with numerous stamens. Whereas hermaphrodite flowers were borne on either terminal or axillary. *Garcinia xanthochymus* Hook. flowers were polygamous and pentamerous. Male flowers were produced in the axils of fallen leaves with five sepals, petals and stamens. There is an oblique stigma present. They also reported the pedicel sizes of flowers in different species of *Garcinia,* such as *Garcinia pedunculata* Roxb (3.00 to 5.00 by 4.00 to 4.50 cm), *G. paniculata* Roxb (0.40 to 0.60 by 1.00 to 1.20 cm) and *G. lanceaefolia* Roxb (0.40 to 0.50 by 0.50 to 0.80 cm).

Flowers in *Garcinia xanthochymus* were borne on corymbose cyme inflorescences in group of 4.00 to 10.00 which was produced in leafless axils, peduncle of the inflorescence measuring about 6.00 to 12.00 mm. The flowers were bisexual, complete, pentamerous with five sepals with three big and two small, arranged in an imbricate aestivation. The imbricate aestivated petals measure one cm in all direction (Sharma *et al*., 2012).

The staminate flowers of *Garcinia pushpangadaniana* was described by Sabu *et al*. (2013). Flowers were pedicellate, size ranged from 7.00 to 9.00 mm in length, ciliate margined with five sepals of size 6.00 by 5.00 mm, fleshy in the centre. Five glabrous petals, 1.00 cm in length and 0.80 cm in breadth, fleshy in the centre, 12.00 to 15.00 stamens with 1.00 mm filaments, brown anthers 0.50 mm long, and a rudimentary pistil and the pistillate flowers as pedicellate of size 1.00-1.50 cm long,

orbicular to suborbicular sepals of size 6.00 by 5.00 mm, ciliate margined membranous petals of size 1.00-1.20 by 1.20 cm, fleshy in the centre, 3.00 to 5.00 slender staminodes with globular ovary of 6.00 by 6.00 mm, very short style with 6.00-8.00 rayed stigma surface.

The male flower in *Garcinia imberti* present in a group ranged from 1.00 to 9.00. The flower length of 0.54 cm and breadth of 0.42 cm. It consists of four sepals having green in colour and measured 0.23 cm in length and 0.22 cm in breadth. The four free petals on the other side were yellowish in colour and measured 0.28 cm in length and 0.26 cm in width. The male trees had typical flowers with long pedicels, each flower consists of 16.00 to 20.00 stamens, all were functional, centrally clustered and hemispherical rudimentary, receptacle, and stamens formed round circle. In this form, the pistils were not present and pollen grains were present. When compared to female plants, male trees had dense flowering. Within a few days, the shrivelled male flowers were visible on the tree branches. Female flowers were solitary and can be found singly or rarely in groups (1.00 to 2.00). Female flowers were bigger when compared to male flowers. Both terminal as well as auxiliary buds produce flowers. The four petals were yellow, measured 0.32 cm in length and 0.30 cm in breadth, and four sepals were green, measured 0.26 cm in length and 0.24 cm in breadth. The female flowers had a pedicel, with length of 0.60 cm and breadth of 0.45 cm, and rudimentary structure. The conical form in place of the pistil and length of 0.28 cm, but produce no functional pollen grains. The ovary was globose, pistils were fully grown and there were two locules visible in the cross section (Kandasamy *et al*., 2017).

According to Sarma (2016) *Garcinia assamica* female flowers were sessile and produce 2.00 to 5.00 numbers at each node. The four light green sepals were 6.00 by 5.00 mm in size and had a membranous margin. Four light yellow petals of 8.00-10.00 by 6.00-8.00 mm; fleshy in the centre and membranous towards the margin. Staminodes were slender and brownish in colour, number of staminodes ranged from 4.00 to 5.00; locular globose ovary of 4.00 to 5.00 numbers; 5.00 by 5.00 mm size with vertical grooves. Pale yellow warty lobed 8.00 to 10.00 stigmatic rayed stigma was present. Raysad (2016) described *Garcinia gummi-gutta* flowers as superior,

regular petals, polypetalous and tetramerous, and also observed pentamerous and hexamerous flowers. Most of the flowers were yellow rarely pinkish red.

Male flowers of *Garcinia gummi-gutta* appears in clusters of 2.00 to 20.00 numbers which produce at terminal or axillary inflorescences. Female flowers appear as solitary or clusters of 2.00 to 6.00 numbers emerged at terminal or axillary branches. The male flowers were 1.70 to 2.30 cm long, yellow with red base, sessile, actinomorphic and had four or five sepals, two of which are seriate. Four light yellow petals. Male flower produces 15.00 to 25.00 stamens have grey colour, straight and linked to a pistillode with stigma that was not functioning. The female flowers were actinomorphic with four or five sepals, pale green or pale yellow and sessile and two cm long. Petals of 4.00 or 5.00 in numbers with pale yellow colour. Staminodes (6.00-20.00) were seen in the ovary as grooved, stigma was broad and moist, ovary is globose had 8.00 locular with a single ovule in each locule and axile placentation (Aswathi *et al*., 2018).

2.5. Anthesis in *Garcinia* species

Bornali (2015) reported that the time of anthesis in *Garcinia pedunculata at* 3.00 am to 5.00 am; *Garcinia cowa* at 2.00 am to 4.00 am; *Garcinia lanceaefolia* at 4.00 am to 6.00 am; *Garcinia xanthochymus* at 1.00 am to 3.00 am. The anthesis in *Garcinia gummi-gutta* male and female flowers reported to be on the same time, it occurs between 4.30 and 6.30 pm (Aswathi *et al*., 2018).

2.6. Pollen morphology and viability

Dike *et al*. (2020) reported that male flower pollen grains of *Garcinia indica* were spheroidal and tetrazonocolporate with a diameter of around 20.00 µm and the exine was ornate and coarsely granulated with 2.00 µm thick. Female flower pollen grains were smaller, measured about 10.00 µm in diameter, the exine was much thicker (3.00 µm thick) and the apertures were barely visible. They had also worked out the pollen viability by following tryphan blue method, it was ranged from 63.00 to 66.00 per cent in male flowers and 1.00 to 2.00 per cent in female flowers.

Other species of *Garcinia*

Te-chato (2007) reported that pollen viability in *Garcinia cowa* of 96-100 per cent; *G. speciosa* of 93 to100 per cent; *G. atroviridis* of 3 to 5 per cent; *G. mangostana* of 0.1 to 1.0 per cent; *G. dulcis* produces no viable pollen and *G. schomburgkiana* of 95 to 100 per cent.

Kandhasamy *et al*. (2017) opinioned that the maximum pollen viability in *Garcinia imberti* of about 89.16 per cent following a 0.20 per cent tryphenyl tetrazolium chloride test and the lowest viability of 84.35 per cent was recorded following one per cent acetocarmine test.

2.7. Compatibility studies in *Garcinia indica*

Dike *et al.* (2020) studied the mode of reproduction and fruit development in *G. indica*. Fruit set was observed both in natural and artificially pollinated flowers but no fruit set was noticed in flowers without pollination. Further molecular analysis revealed that kokum reproduces through facultative apomixis.

2.8. Season of flowering in *Garcinia indica*

According to Raorane (2003), flowering in kokum began in the last week of September and continued until the third week of November, but it varies with the genotypes. Under the Dapoli conditions of Maharashtra maximum flowering was recorded in the month of November. In another study Korikanthimath and Desai (2005) reported that flowering season in kokum from November to February under the Goa conditions. Patil *et al*. (2005) recorded the initiation of flowering in the kokum variety Konkan Amruta from 10th November in Ratnagiri district of Maharashtra. Hegde (2005) observed that flowering season in kokum from November to February under the Konkan region of Maharashtra.

Devi *et al*. (2013) observed flowering initiation time in kokum varies with cultivars and they categorised into early, mid and late bearing types. The early bearing types flower can be seen on first and second week of November, mid types flowers on third and fourth week of November and late types flowers first and second week of December under Goa conditions. Kapatia (2019) reported that flowering duration in kokum from October to January in Thrissur conditions of central Kerala. As per Dike *et al*. (2020) the male and female trees of kokum flowers from November to January and December to February, respectively under the Dapoli conditions of Maharashtra.

Other species of *Garcinia*

Te-chato (2007) reported the flowering season of a few species of *Garcinia* collected from the Plant Science Research Station, Prince of Songkla University, Hat Yai campus and private orchards in the Narathiwat province, Thailand. Flowering in *Garcinia cowa* during July-September; *G. speciosa* flowers twice in a year during June-September and January-March; *G. atroviridis* in July-September; *G. mangostana* flowering in March-April and continued in July-September; *G. dulcis* in July-September; *G. schomburgkiana* in January-March and July-September. Sharma *et al*. (2012) reported that flowering season in *Garcinia xanthochymus* was from March to April under Manipur conditions. Thatte and Deodhar (2012) recorded that staminate flowers in kokum could be seen in the month of December to February, whereas pistillate flowers was from February to April in Dapoli region of Maharashtra.

Baruah and Borthakur (2012) described flowering season in six species of *Garcinia* such as *G. pedunculata* Roxb. flower throughout the year; *G. paniculata* Roxb. in December-February; *G. Morella* Desr. in February-March; *G. cowa* Roxb. in March-April; *G. lanceaefolia* Roxb. in February-March and *G. xanthochymus* Hook. in March-May under Brahmaputra valley of Assam conditions.

Sarma (2016) reported that flowering season of *Garcinia assamica* from February to May under Karbi Anglong district of Assam conditions. In another study by Raysad (2016) described the month of flowering in Malabar tamarind from February to March under Thrissur district of central Kerala. Shameer *et al*. (2017)

noticed December to January flowering in *Garcinia gamblei* under Thiruvananthapuram district of Southern Kerala. According to Aswathi *et al*. (2018) flowering season of *Garcinia gummi-gutta* from March to April under the Calicut district of central Kerala.

2.9. Plant growth characters

2.9.1. Plant height, girth and canopy spread

Raorane (2003) reported that the height of bearing kokum tree of about 25-year-old varied from 8.85 to 14.56 m. The trunk girth ranged from 32.75 to 50.20 cm under Dapoli conditions of Maharashtra. Korikanthimath and Desai (2005) reported the height of kokum tree ranged from 6 to 12 m. In another report from Patil *et al*. (2005), recorded that the average height of the kokum variety Konkan Amruta was 10.30 m, trunk girth of 1.05 m and spread of North South direction was 3.10 and East West was 2.90 m, canopy volume was 48.58 m^3 under the Ratnagiri conditions of Maharashtra.

Kadam *et al.,* (2012) reported that the Kokum tree is dioecious and can reach a height of 12 to 20 meters. Niveditha (2013) described the tree height and GBH in different fruit morpho types of kokum, such as the red morpho type (8.50 m and 50 cm, respectively), green morpho type (10.50 m and 53.80 cm, respectively), orange morpho type (8.60 m and 53.60 cm, respectively) and yellow morpho type (13 m and 78.50 cm, respectively). Kapatia (2019) reported the average growing height of 11.10 m in *Garcinia indica* in Thrissur conditions of central Kerala.

Other species of *Garcinia*

Garcinia xanthochymus is a bushy type it can grow up to a height of 5.00 to 15.00 m and diameter of 40.00 to 130.00 cm. Trunk is straight and cone shaped canopy with drooping branches (Sharma *et al*., 2012). *Garcinia imberti* grows to an average height of 15.00 m (Kandasamy *et al*., 2017).

Among the different species of *Garcinia,* the tree height was found to be the highest in *Garcinia pedunculata* (24.00 m) followed by *Garcinia cowa* (18.00 m), *Garcinia lanceaefolia* (6.30 m) and *Garcinia lanceaefolia* (10.50 m), (Bornali, 2015).

Raysad (2016) evaluated thirty two accessions of *Garcinia gummi-gutta* which are being maintained at NBPGR Regional Station, Vellanikkara. The height of the accessions ranged from 3.00 m to 19.00 m and trunk girth ranged from 31.00 cm to 107.00 cm.

The *Garcinia gamlei* can be grow to an average height of 15.00 m under Thiruvanantapuram conditions of Kerala (Shameer *et al*., 2017). In another study by Sarma (2016) recorded that an average height of *G. assamica* about 15.00 m, which also produces glabrous leaves with horizontal branches.

2.9.2. Leaf characters

The length and breadth of leaves of seventeen kokum genotypes ranged from 10.24 to 12.81 cm and 3.84 to 4.58 cm, respectively. The leaf area was ranged from 27.70 to 40.89 cm^2 under the Dapoli conditions of Maharashtra (Raorane, 2003).

In a study by Devi *et al*. (2013), it was reported that the length and breadth of kokum leaves of 268 genotypes varied from 6.24 to 11.95 cm and 2.42 to 5.25 cm, respectively under the Goa conditions. Kapatia (2019) recorded the average leaf length (8.62 cm), breadth (3.73 cm) and area (20.41 cm^2) of *Garcinia indica* under the Thrissur conditions of central Kerala.

Other species of *Garcinia*

The *Garcinia capuronis* leaf blade length ranged from 26.00 to 41.40 cm and width ranged from 8.40 to 17.20 cm. Petiole length varied from 1.30 to 1.70 cm. The *Garcinia lowryi* leaf length measured between 1.00 to 10.50 cm and width between 0.50 to 4.60 cm, and petiole length measured from 3.00 to 10.00 mm and width of petiole from 1.00 to 2.00 mm (Rogers and Sweeney, 2007).

Sharma *et al*. (2012) described *G. xanthochymus* petiole size of 1.50-2.50 cm and leaf blade length of 20-35 cm and breadth of 6-12 cm. Baruah and Borthakur (2012) characterized leaves size of different *Garcinia* species such as *Garcinia pedunculata* Roxb. leaves were 13.00 to 30.00 cm long and 15.00 to 21.00 cm wide; *Garcinia paniculate* Roxb. leaves length of 11.00 to 15.00 cm and width of 5.00 to 7.00 cm; *Garcinia morella* Desr. leaves 7.00 to 11.00 cm long and 4.00 to 7.00 cm wide; *Garcinia lanceaefolia* Roxb. measured from 4.50 to 6.00 cm long and 2.50 to 3.50 cm wide; *Garcinia xanthochymus* Hook. leaves were 25.00 to 32.00 cm long and 4.00 to 8.00 cm wide under the Brahmaputra valley of Assam conditions.

Sabu *et al*. (2013) measured leaf length (14-20 cm) and breadth (6-8 cm) *Garcinia pushpagandaniana* under its native, Western Ghats conditions of India. In a study by Bornali (2015) reported that the highest leaf length, breadth and area of *Garcinia pedunculata* (27.05 cm, 16.45 cm and 429.98 cm^2, respectively); *Garcinia cowa,* (12.88 cm, 7.62 cm and 94.72 cm^2, respectively); *Garcinia lanceaefolia* (11.62 cm, 7.82 cm and 30.56 cm^2, respectively); *Garcinia xanthochymus* (30.25 cm, 7.82 cm and 223.95 cm^2, respectively).

According to Sarma (2016), leaves of *Garcinia assamica* were 7.00 to 11.00 cm long, 2.50 to 5.00 cm wide. Raysad (2016) reported that length, breadth and area of *Garcinia gummi-gutta* leaves ranged from 9.47 to 14.97 cm, 3.60 to 7.11 cm and 22.78 to 56.39 cm^2. Shameer *et al*. (2017) reported that the size of leaves of *Garcinia gamblei*, it varied from 22.00 to 28.00 cm long and 10.00 to 12.00 cm wide.

2.10. Fruit and yield parameters

Raorane (2003) recorded fruit characters of seventeen kokum genotypes. The average fruit weight of 30.18 g, fruit volume of 29.17 ml, rind weight of 15.53 g, rind thickness of 3.40 mm and average fruit yield per tree was of 55.00 kg under the Dapoli conditions of Maharashtra.

Hegde (2005) reported that the average yield of kokum was about 70.00 kg in the Konkan region of Maharashtra. Korikanthimath and Desai (2005) reported that average fruit weight 21.00 to 85.00 g and rind thickness between 0.20 to 0.80 mm.

Patil *et al*. (2005) studied various fruit characters of kokum variety Konkan Amruta. They recorded average fruit weight of 34.55 g, rind weight of 17.55 g, rind thickness of 4.45 mm and yield per tree about 138.28 kg under the Ratnagiri regions of Maharashtra. In another study by Patil and Kattimani (2009) recorded the maximum weight of a kokum rind was 17.87 g.

Devi *et al*. (2013) reported that thickness of kokum fruit rind varied from 0.14 to 0.48 cm. Niveditha (2013) revealed that fruit characters of different morphotypes of kokum under Uttara Kannada and Shivamoga conditions of Karnataka. The fruit morphotypes such as red, green, orange and yellow varied for the average fruit weight (33.30 g, 28.15 g, 22.35 g and 34.00 g, respectively), fresh rind weight (17.50 g, 13.86 g, 10.26 g and 18.47 g, respectively), dry rind weight (2.30 g, 1.80 g, 1.60 g and 2.10 g, respectively) and rind thickness (3.14 mm, 2.66 mm, 2.16 mm and 2.40 mm, respectively).

Tripathi *et al*. (2015) recorded varied fruit weight and volume in *Garcinia indica* (52.63 g and 51.80 ml, respectively), *Garcinia gummi-gutta* (60.30 g and 56.10 ml, respectively) and *Garcinia xanthochymus* (127.78 g and 127.80 ml, respectively).

Kapatia (2019) reported that average fruit and rind weight of kokum were 34.39 g and 14.32 g, respectively. The tree produced average number of fruits was 608 per year.

Other *Garcinia* species

Sharma *et al*. (2015) described fruit weight of *Garcinia pedunculata* and *Garcinia xanthochymus* as 460.49 g and 57.26 g, respectively. Bornali (2015) reported the highest fruit weight in *Garcinia pedunculata* (744.75 g) followed by *Garcinia cowa* (183.75 g), *Garcinia lanceaefolia* (30.28 g) and *Garcinia xanthochymus* (144.00 g) and the highest fruit volume of *Garcinia pedunculata* (720.00 cc), *Garcinia cowa*

(183.25 cc), *Garcinia lanceaefolia* (33.00 cc) and *Garcinia xanthochymus* (127.75 cc).

Raysad (2016) reported the weight of fruit and of rind of thirty two accessions in Malabar tamarind, it ranged from 51.40 to 148.50 g, and 29.60 to 83.50 g, respectively. The number of fruits per tree was ranged from 70.00 to 985.00 per year under the Thrissur district of central Kerala.

The average weight of *Garcinia lanceifolia* fruit was 57.53 g, average volume of fruit was 53.86 ml and average rind weight of the fruit was 2.61 g (Hazarika and Lalnunsangi, 2019). According to Berame *et al*. (2020), average weight of pointed and rounded mangosteen was 69.91g and 42.72g, respectively.

2.10.1. Fruiting season

Season of flowering will have impact on season of fruiting. It may vary from region to region and depending on characteristic features of climate of those growing conditions. In Goa, fruiting season observed for kokum varied from April to May (Korikanthimath and Desai, 2005). Under the Ratnagiri regions of Maharashtra conditions, Patil *et al*. (2005) reported the fruiting season in kokum variety Konkan Amruta, it varied from March to April. Hegde (2005) reported that in Konkan region of Maharashtra, the fruiting season in kokum from May to June.

Devi *et al*. (2013) elaborated the harvesting time in different types of cultivars and they categorised into early (last week of April to the 2nd week of May), mid (4th week of June) and late (1st week of June) bearing cultivars.

According to Tripathi *et al*. (2015), fruiting season of *Garcinia indica* from March to May; *Garcinia gummi-gutta* from August to October; *Garcinia xanthochymus* from December to March. Shameer *et al*. (2017) reported that fruiting season of *Garcinia gamblei* from June to August under Thiruvantapuram district of Southern Kerala.

Other *Garcinia* species

Baruah and Borthakur (2012) reported fruiting time in six species of *Garcinia* such as *Garcinia pedunculata* Roxb. in January-April; *Garcinia paniculate* Roxb. in March-April; *Garcinia morella* Desr. in April-June; *Garcinia cowa* Roxb. in June-August; *Garcinia lanceaefolia* Roxb in June-July and *Garcinia xanthochymus* Hook. in October-February under the Brahmaputra valley of Assam conditions.

2.11. Quality characters

Raorane (2003) revealed the contents of moisture (83.13 %), acidity (4.09 %), total sugar (9.72 %), TSS (14.28 °B) and anthocyanin (2.37 g per 100 g) in fruits of *Garcinia indica*. In another study Joshi (2005) analysed the contents of moisture (87.50 %) and TSS (16.44 °B) in ripened fruits of analysed kokum.

Korikanthimath and Desai (2005) observed the acidity in kokum fruit rind varied from 1.10 to 3.20 per cent, TSS in fruits from 6.00 to 12.00 °B and anthocyanin content ranged from 7.87 to 17.03 mg/100 g under Goa conditions. Kokum variety Konkan Amruta exhibited acidity content of 5.12 per cent, total sugars of 4.52 per cent and TSS of 9.08 °B (Patil *et al*., 2005).

Niveditha (2013) reported that percentage of moisture, acidity, total sugars and anthocyanin content in different fruit morpho types of kokum. These contents varied in different morpho types, red morpho type (8.20 %, 18.00 %, 10.00 % and 70.51 mg/100 g of dry weight, respectively), green morpho type (7.00 %, 18.50 %, 9.00 % and 48.56 mg/100 g of dry weight, respectively), orange morpho type (7.10 %, 15.10 %, 9.00 % and 22.41 mg/100 g of dry weight, respectively) and yellow morpho type (6.30 %, 16.80 %, 9.50 % and 37.33 mg/100 g of dry weight, respectively).

Devi *et al*. (2013) reported the moisture content in kokum varied from 69.71 to 91.93 per cent. Tripathi *et al.* (2015) reported the acidity percentage of *Garcinia indica* (4.16 %) and *Garcinia xanthochymus* (7.20 %) and TSS content of *Garcinia indica* (14.78 °B), *Garcinia gummi-gutta* (8.90 °B) and *Garcinia xanthochymus* (12.70 °B).

Ramachandran (2014) reported that anthocyanin content in fresh kokum fruit ranged from 1000 to 2400/100g. Vasundhara *et al*. (2016) opinioned that kokum fruit rind is good source of anthocyanin. The average total anthocyanins content in fresh fruit rind had 79.93 mg/100g and in dry fruit rind 85.03 mg/ 100g and syrup 7.83 mg/100g.

Fruit rind of *Garcinia indica* is of commercial importance due to the presence of hydroxycitric acid (HCA) which is having antiobesity, anticholesterol as well as UV protecting properties (Patil, 2005).

Garcinia gummi-gutta is another species belonging to the same genus, which also contains hydroxy citric acid, the species is distributed throughout Kerala. This species has got more importance in culinary usage. Morphological and physiological diversity studies exhibited variation for biochemical parameters among the germplasm conserved at College of Forestry, KAU and NBPGR Regional Station Vellanikkara (Kavya, 2016).

Parthsarathy and Nandakishore (2014) identified organic acids present in the *Garcinia* species like *Garcinia indica*, *Garcinia mangostana* and *Garcinia gummi-gutta* through HPLC method. The rind of *Garcinia indica* contains HCA (7.43 %), malic acid (2.67 %), oxalic acid (0.63 %), citric acid (0.79 %), tartaric acid (0.51 %) and acetic acid (0.31 %). *Garcinia mangostana* consists of HCA (0.26 %), malic acid (0.54 %), oxalic acid (0.73 %), citric acid (1.42 %), tartaric acid (1.66 %) and acetic acid (0.26 %). *Garcinia gummi-gutta* had HCA (15.48 %), malic acid (4.62 %), oxalic acid (0.18 %), citric acid (0.62 %), tartaric acid (0.11 %) and acetic acid (0.07 %).

Jagtap *et al*. (2015) reported that kokum rind contains many organic acids *viz*., citric acid, acetic acid, malic acid, ascorbic acid, hydroxy citric acid and garcinol. Garcinol and hydroxy citric acid were the most abundant organic acids in the rind.

Other *Garcinia* species

Sharma *et al*. (2015) reported that the contents of moisture (85.00 % and 80.00 %, respectively), acidity (2.53 % and 14.40 %) and TSS (5.00 °B and 1.30 °B) of fruits of *Garcinia pedunculata* and *Garcinia xanthochymus*.

In the species of *Garcinia pedunculata, Garcinia cowa, Garcinia lanceaefolia* and *Garcinia xanthochymus* had the highest moisture (90.29, 94.57, 87.96 and 86.52 %, respectively), highest acidity (2.29, 5.74, 3.74 and 4.70 %, respectively), highest total sugars (8.97, 17.07, 6.39 and 5.09 %, respectively), highest TSS (7.95, 7.95, 5.40 and 10.00 °B, respectively) and the highest anthocyanin (2.80, 4.72, 2.55, and 2.07 g/100 g, respectively) as reported by Bornali (2015).

Hazarika and Lalnunsangi (2019) reported the average percentage of moisture, average acidity, average total sugars and average TSS in the fruits of *Garcinia lanceifolia* was 89.61 per cent, 4.76 per cent, 6.03 per cent, and 7.59 °B, respectively.

2.12. Incidence of pests and diseases

No major pests and diseases have been reported in kokum. In rare cases pink disease appears on the branches. Drying back of twigs is the other minor disease of kokum.

Leaf spot disease caused by *Colletotrichum gloesporoides* on kokum seedlings was reported by Jadhav *et al*. (2008). They also recommended management practices for kokum leaf spot following biological as well as chemical methods.

In kokum plants rarely noticed incidence of pests *viz*., mealy bugs damage on tender leaves and fruit fly cause damage on mature fruits.

Other *garcinia* species

According to Nair *et al*. (2005), no severe pests or diseases were found in nursery or early planted seeds or seedlings of *Garcinia cambogia*. Sivakumar *et al*. (2013) reported the incidence of leafhopper in *Garcinia cambogia*.

MATERIAL AND METHODS

3. Materials and Methods

The present study on "Reproductive biology and evaluation of kokum (*Garcinia indica* (Thouars) Choisy) genotypes" was conducted at Department of Fruit Science, College of Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur during 2019 to 2021. The details of the germplasms used and methodology adopted during the period of investigation are elaborated sequentially in this chapter.

3.1. Geographical location and climatic condition

The Vellanikkara is situated in Thrissur district of central part of Kerala state, India at 100 31' N latitude and 760 17' E longitude. The region has tropical humid climatic conditions and an altitude of 40 m above mean sea level. The region enjoys both South West as well as North South Monsoons; the mean annual rainfall of Vellanikkara is about 3000 mm that distributed over a period of nine months (April to December). The highest mean monthly temperature was recorded in the month of March (36.80 ^{0}C) and the lowest mean monthly temperature was in the month of December (32.00 ^{0}C). The variation in relative humidity could be observed between 54.00 per cent (February) to 84.00 per cent (May), as per meteorological data recorded at College of Agriculture, Vellanikkara during 2019 to 2020. The soils are acidic in nature and mostly of laterite types.

3.2. Study material

The total of twenty nine kokum genotypes were selected for the study which is being maintained as *ex situ* at College Orchard, Department of Fruit Science, College of Agriculture, Vellanikkara and ICAR-National Bureau of Plant Genetic Resources, Regional Station, Vellanikkara. These genotypes were procured from their natural habitat as well as from custodian farmers in states of Karnataka and Kerala. The trees were of bearing stage from 14 to 32 years old seedlings origin. Among the twenty nine genotypes included in the study, nine genotypes were selected from College Orchard and twenty form ICAR-NBPGR, Regional Station. Each genotype consisting of single

tree and observations on phenology, floral and fruit characters were recorded from single tree. The details of geo-tagging of the genotypes are presented in Table 3.1.

3.3. Phenological growth stages

The observations on different phenological stages were recorded in yielding kokum trees. Phenological growth stages were recorded from twenty five tagged shoots on all the four sides of canopy in all the genotypes selected for the study. The observations were recorded at weekly intervals from bud emergence to complete maturity of leaves, reproductive bud initiation to complete opening of flowers and continued recording observations form fruit set to fruit development till the maturity of fruits. Method of recording phenological observations is depicted in Plate 3.3.

Phenology is defined as the analysis of the sequence of events that occur over an annual growth cycle. The three digit extended Biologische Bundesantalt, Bundessortenamt and Chemische Industrie (BBCH) scale system (Meier, 2001) was followed for elaborating developmental stages in kokum and the phenological stages were defined within the code. The first digit denotes the principal growth stage, the second digit for the mesostage and third digit represents the secondary growth stage.

The extended BBCH scale comprises of ten stages of which seven principal growth stages were identified in kokum. The principal growth stages were coded and values given from 0 to 8, starting from vegetative bud development (stage 0), leaf development stage (stage 1), shoot development (stage 3), reproductive bud development (stage 5), flowering (stage 6) and development and maturity of fruits (stage 8). Apart from these, there were 35 secondary growth stages identified and described in the principal growth stages. The stage 2 (formation of side shoots or development of tillers) and stage 4 (development of harvestable vegetative plants parts or vegetatively propagated organs) were not recorded, because, those two stages were not noticed in kokum tree. To explain the related percentiles (leaves, shoots, flower buds and fruit) of elaborated primary growth stage, each principal growth stage was subdivided into ten secondary growth stages and code given as 0 to 9.

College Orchard, Department of Fruit Science

ICAR-NBPGR Regional Station, Vellanikkara

Plate 3.1. View of the kokum germplasm block

Plate 3.2. Labelling of selected kokum genotypes

Table 3.1. List of selected kokum genotypes for the study

Genotypes	Date of collection	Collection source	Biological status	Place of collection
ACC.FSC-1	-	Farmer's field	Traditional	-
ACC.FSC-2	-	Farmer's field	Traditional	-
ACC.FSC-3	-	Farmer's field	Traditional	-
ACC.FSC-4	-	Farmer's field	Traditional	-
ACC.FSC-5	-	Farmer's field	Traditional	-
ACC.FSC-6	-	Farmer's field	Traditional	-
ACC.FSC-7	-	Farmer's field	Traditional	-
ACC.FSC-8	-	Farmer's field	Traditional	-
ACC.FSC-9	-	Farmer's field	Traditional	-
IC136684-3	10-05-1989	Forest	Wild	Kanchika, Siddhapura, Managalore, Karnataka
IC136685-3	10-05-1989	Farmer's field	Cultivated	Hebbegada, Siddhapura, Mangalore, Karnataka
IC136687-1	11-05-1989	Farmer's field	Cultivated	Sonagu, Siddhapura, Mangalore, Karnataka
IC136687-2	11-05-1989	Farmer's field	Cultivated	Sonagu, Siddhapura, Mangalore, Karnataka
IC136687-3	11-05-1989	Farmer's field	Cultivated	Sonagu, Siddhapura, Mangalore, Karnataka
IC342296-1	25-04-2002	Disturbed	Wild	Mainagundi, Shimoga, Karnataka
IC342298-1	25-04-2002	Disturbed	Wild	Adluru, Uttara Kannada, Karnataka
IC342301-3	27-04-2002	Disturbed	Wild	Kukkana Mane, Theretana halli, Kuluru Panchayat, Uttara Kannada, Karnataka
IC342304-1	27-04-2002	Disturbed	Wild	Kukkana Mane,Theretana halli, Kuluru Panchayat, Uttara Kannada, Karnataka
IC342306-1	27-04-2002	Disturbed	Wild	Kukkana Mane,Theretana halli, Kuluru Panchayat, Uttara Kannada, Karnataka
IC342319-1	28-04-2002	Disturbed	Wild	Kakkali, Uttara Kannada, Karnataka
IC342319-2	28-04-2002	Disturbed	Wild	Kakkali, Uttara Kannada, Karnataka
IC552517	21-03-2007	Farmer's field	Traditional	Golithadukka, Kasaragod, Kerala
IC552523-1	22-03-2007	Farmer's field	Traditional	Paraldukka, Puttur, Dakshina Kannada, Karnataka
IC552514-2	21-03-2007	Farmer's field	Traditional	Karangi, Kasaragod, Kerala
IC552513	21-03-2007	Farmer's field	Landrace	Karimbila, Kasaragod, Kerala
IC552526-1	22-03-2007	Farmer's field	Traditional	Kepu, Kuntrody, Dakshina Kannada, Karnataka
IC552522-2	22-03-2007	Farmer's field	Traditional	Sadiappu, Dakshina Kannada, Karnataka
IC552522-1	21-03-2007	Farmer's field	Traditional	Sadiappu, Dakshina Kannada, Karnataka
IC552528-3	27-03-2007	Farmer's field	Landrace	Ganjagadde, Kodagu, Karnataka

Numerous mesostages were also employed to classify the season's successive vegetative and floral flushes. For example, code 118 stands for the principal growth stage 1 that is leaf development stage, mesostage 1 defines the first vegetative flushing and secondary stage 8 shows 80 per cent of final length of shoot. The observations on different growth stages of kokum were captured using digital camera (Canon EOS 1500D).

3.4. Flower characteristics

The following flower characters were recorded in all the kokum genotypes. The various flower characters were recorded during the peak flowering season. From each genotype, quantitative floral observations were recorded from randomly selected ten flowers.

3.4.1. Length and breadth of flower (mm)

The length and breadth of the flowers were recorded from maximum length and width position and were recorded using a digital vernier calliper and expressed in millimetres (mm). Floral measurement methods followed in the study is depicted in Plate 3.4.

3.4.2. Pedicel length (mm)

The distance between point of attachment of flower stalk to shoot and base of flower sepal was measured with a digital vernier calliper and was expressed in millimetres (mm).

3.4.3. Position of inflorescence

The male, female and bisexual flowers were observed separately in the trees for their position of inflorescence.

3.4.4. Calyx and corolla colour

The colour of calyx (sepals) and corolla (petals) of the flowers was observed following the standard minimal descriptors of kokum developed by ICAR-NBPGR, New Delhi.

3.4.5. Nature of stamen

The stamen of male and bisexual flowers was observed for their nature and it was recorded as per the guidelines of minimal descriptors of kokum.

3.4.6. Presence of pistillode

The male and bisexual flowers were observed for the presence of non-functional pistil or pistillode.

3.4.7. Number of staminodes

Total number of staminodes in female flowers was noticed and the this number counted manually.

3.4.8. Type of stigmatic rays

The female and bisexual flowers were observed using electronic microscope for the type of stigmatic rays present on stigma.

3.4.9. Number of flowers in cluster

The number of flowers per inflorescence was counted and recorded.

3.4.10. Number of stamens per flower

The male and bisexual flowers were observed for number of stamens in each flower, counted manually and recorded.

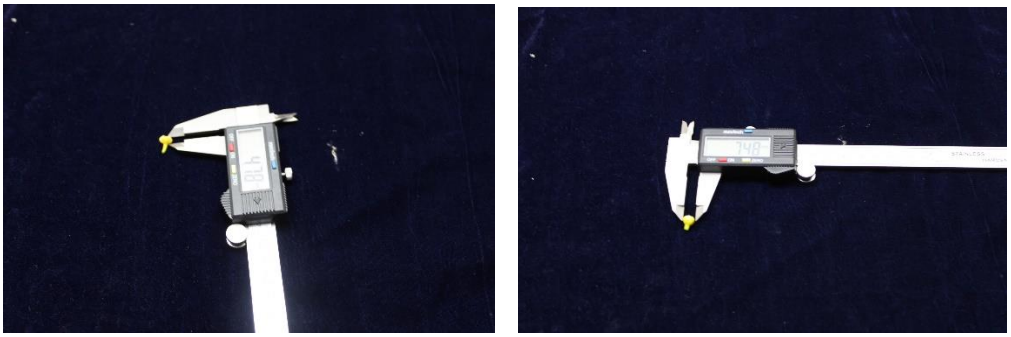
3.4.11. Number of stigma per flower

Both female and bisexual flowers were observed for presence of stigma and number was counted.

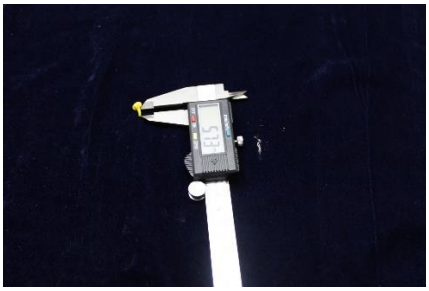
3.4.12. Number of flowers per 0.25 m^2

The number of flowers within wooden frame area was counted and expressed as number of flowers per 0.25 m^2.

Plate 3.3. Labelling of shoots for recording phenological stages



Length of flower **Breadth of flower**



Length of pedicel

Plate 3.4 Floral measurements in kokum

3.4.13. Anthesis

For understanding the time of anthesis in kokum, 100 completely developed flower buds in male and female plants and 75 flower buds in bisexual plants were tagged and observations were made at half hour intervals.

3.4.14. Anther dehiscence

Nearly 50 matured male and bisexual flowers were tagged and observed frequently for anther dehiscence by using a hand lens. A few flowers at various stages of development were also collected and examined under a microscope for anther dehiscence and recorded in hours.

3.4.15. Stigma receptivity

The stigma receptivity of female flowers in kokum was determined by the presence of exudates and the shiny surface of stigma. Flowers are at different stages of development, from unopened flower buds to completely opened flowers, and these were observed under electronic microscope. Controlled pollination was done for further confirmation of stigma receptivity.

3.4.16. Size and shape of pollen

The morphology as well as size and shape of pollen grains were observed using Tescan Vega 3 model scanning electron microscope (Plate 3.5).

The pollen samples were taken in centrifuge tubes (1 ml) and this centrifuged at 3000 rpm for 10 minutes. The supernatant was decanted. The samples were subjected to glacial acetic acid wash and once again centrifugation was carried out at 3000 rpm for 10 minutes. The supernatant was decanted and washed the samples with distilled water in three times. This was followed by centrifugation was done at 3000 rpm for 10 minutes. Later the pollen samples were washed and dehydrated in ascending hydroethanolic series of 50, 70, 90 and 100 per cent for 10 minutes. The ethanolic dried samples were used for scanning.

3.4.17. Pollen viability

Pollen grains were collected from male and hermaphrodite flowers and dusted on glass slide, it was then stained with one per cent acetocarmine solution and covered. Samples were kept for 30 minutes to allow the pollen grains for staining. Then the slides were observed under the electronic microscope and the viability percentage was calculated as the percentage of stained pollen grains to the total number of pollen grains in each microscopic field view. Total of 10 such fields were observed in each slide.

3.4.18. Compatibility

In kokum, 4 types of flowers were found, such as male type-I, male type-II, bisexual and female flowers. Controlled pollination was performed under different combinations in these trees to determine their compatibility (given below). About to open female flowers were bagged with butter paper bags to avoid the chance of cross pollination and about to open male flowers were collected for fresh pollen source. Then pollen from freshly opened flowers was collected in a petridish and the previously bagged female flowers were pollinated by using fine hair brush. After pollination, flowers were bagged with same butter paper bags. Method followed for compatibility studies is shown in Plate 3.6.

Female*Male type-I

Female*Male type-II

Female*Bisexual

Bisexual selfing

Apart from these, female flowers were also bagged without pollination to check the parthenocarpic fruit development.

3.5. Morphological characters

The tree growth, fruit and yield parameters were recorded during the study period are described in the following subheads.

3.5.1. Plant height (m)

The height of kokum plant was recorded from the ground level to the tip of the plant using clinometer instrument and expressed in meter (m).

3.5.2. Girth of the plant (cm)

Girth of the plant at 140 cm height was recorded by using a measuring tape and expressed in centimetres (cm).

3.5.3. Canopy spread (m)

The spread of kokum plants was recorded with the help of measuring tape in both direction from East to West and North to South and was expressed in meters (m).

3.5.4. Canopy volume (m^3)

The canopy volume was computed by adding the recorded values of canopy spread from both the direction (North to South and East to West) using the following formula and expressed in $meter^3$.

$$\text{Canopy volume: } \frac{1}{K\pi r^2 h}$$

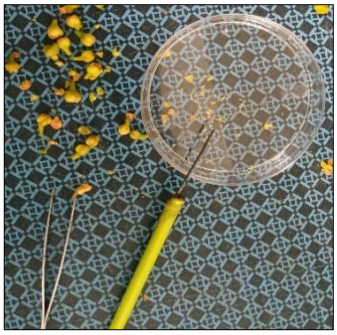
K: constant value (0.3)
π: 3.14
r: canopy spread in both direction
h: height of the tree

3.5.5. Leaf length and breadth (cm)

A total of ten matured leaves were collected from each genotype for measuring length (the vertical distance between apex and base of leaves) and breadth (maximum horizontal distance) of the leaves. The centre of the leaves was measured using scale and expressed in centimetres (cm).

Plate 3.5. Scanning electron microscope instrument used for pollen studies (Tescan Vega 3 model)

Bagging of female flowers using butter paper bags



Collection of pollen grains

Hand pollination of kokum flowers

Plate 3.6. Controlled pollination of kokum

3.5.6. Leaf area (cm^2)

The leaf area was recorded by using leaf area meter and value was expressed in centimetre square (cm^2).

3.5.7. Fruit characters and yield

The observations on fruit characters and yield were recorded during the peak period of fruiting are described below. A total of ten fruits per tree were collected and observations of physical characters of fruits were recorded as per available standard minimal descriptor of kokum.

3.5.8. Fruit weight (g)

The weight of fruits was recorded individually from each genotype that was collected randomly and mean weight was calculated, and expressed in grams (g).

3.5.9. Fruit volume (cm^3)

The volume of each individual fruits of all fruiting genotypes was measured by water displacement method and was expressed in centimetre cube (cm^3).

3.5.10. Fresh and dry weight of rind

The fresh weight of fruit rind was recorded after removing the seeds and weighed using an electronic balance. The same rinds were kept for drying in a hot air oven at 55 to 60 ^{0}C until two consecutive constant weights. The unit expressed as grams (g).

3.5.11. Rind to seed ratio

The ratio of rind to seed was calculated by dividing the fresh weight of the rind with fresh weight of the seed.

3.5.12. Rind thickness (mm)

The thickness of fruit rind was recorded with the help of digital vernier calliper and value was expressed in millimetres (mm).

3.5.13. Number of fruits per meter square

The number of fruits counted inside the one-meter square wooden frame area was expressed as number of fruits per one meter square and such multiple count was recorded in same tree at different positions.

3.5.14. Number of fruits per tree

The tree was divided into different sections based on the height and number of fruits in each section was recorded by manual count and summed up number was expressed as number of fruits per tree.

3.5.15. Fruit yield per tree

The mean weight of fruits harvested periodically from each genotype was recorded and their sum value was expressed as kg per tree.

3.6. Variation in colour of leaf and fruit

The colour of young flush, developing leaf and matured leaf as well as ripen fruit were compared with the Royal Horticultural Society (RHS) colour chart and observations were recorded.

3.7. Quality parameters

3.7.1. Moisture (%)

Moisture content of kokum rind was analysed by oven dry method (Ranganna, 1997).

3.7.2. Titratable acidity (%)

Acidity was determined by titration with standard NaOH solution method as suggested by Ranganna (1997).

3.7.3. Total sugars (%)

Total sugars were estimated by Lane and Eynon method (Ranganna, 1997).

3.7.4. Total soluble solids (°Brix)

TSS content of kokum rind was determined by using hand refractometer and expressed as °Brix.

3.7.5. Anthocyanin (mg/100g)

Anthocyanin was determined by spectrophotometric method (Ranganna, 1986).

3.7.6. Profiling of bioactive compounds

Based on the yield and quality parameters, nine superior genotypes were identified. These genotypes were subjected to profiling of organic acids. Dried kokum rind was used for the profiling of organic acids. Extraction from the sample was done as per the standard methodology. A two ml of extract collected and employed for LC-MS analysis. The pressure was developed at 15000 psi, flow of the sample in the system was 0.05 ml to 0.5 ml per minute, PDA detector was 190 nm to 900 nm. The MS/MS range was 20 amu to 2000 amu with electro spray ionization of ESI+, ESI-, APC+ and APC-. The identified acids were compared with the available standard library.

3.8. Pests and diseases

In a study period the occurrence of pests and diseases in genotypes were observed and their incidence was recorded.

3.9. Statistical analysis

The data recorded from the study was subjected to statistical analysis. The data of floral characters, growth and fruit parameters were subjected to analysis of variance (ANOVA). Biochemical constituents were analysed using the Duncan Multiple Range Test (DMRT). The organic acid values was subjected to multivariate analyses such as agglomerative hierarchical cluster analysis [AHC] as well as principal component analysis [PCA].

RESULTS

4. Results

An investigation was carried out to understand the floral biology and yield and quality of *Garcinia indica* genotypes maintained at the orchard, Department of Fruit Science, College of Agriculture, Vellanikkara, and ICAR-NBPGR, Regional station, under Thrissur conditions of Kerala. The study entitled "Reproductive biology and evaluation of kokum (*Garcinia indica* (Thouars) Choisy) genotypes" revealed tree characters, floral characters, fruit characters and yield characters, and the results obtained are presented in this chapter under various headings and subheadings.

4.1. Phenological observations

Phenology is the study of the sequence of all periodical events involved in a plant life cycle such as shoot, flower and fruit *etc*. It helps to know the influence of weather dynamics on vegetative and reproductive growth period there by adopting the better management of crop. Hence, in the present study, we identified and described the different phenological stages which occur in kokum plant as per the extended three digit BBCH scale.

In kokum, we have identified and described the seven principal growth stages and thirty five secondary growth stages and are presented in Table 4.1.

4.1.1. Principal growth stage 0: Vegetative bud development

The vegetative bud development in kokum was observed throughout the year, but the major season was observed from December to March. The vegetative bud under goes several stages of development before turning into shoots. The beginning of vegetative bud development stage starts with the appearance of pinkish colour tinge and ends with dark pinkish leaflets (Plate 4.1). This stage was completed in 15 to 20 days. The stages are described below.

010. Dormant stage: Buds are dormant and slight pinkish tinge colour visible on shoot tip or between two leaves.

011. Beginning of bud swell: Swelling of buds started, pinkish colour buds clearly visible.

013. End of bud swell: Pair of pinkish scales appears and begins to separate.

017. Beginning of bud break: Pinkish leaflets visible and lamina is closed.

019. End of bud break: Leaves start to separate, two clearly separated pinkish leaves are visible.

4.1.2. Principal growth stage 1: Leaf development

The emergence and development of leaves in kokum was observed throughout the year, but the mainstream of leaf development was observed during end of December to last week of March. The leaves pass through several colour changes during their development process *viz*., dark pinkish, light pinkish, coppery, yellowish green, light green, and green. The entire process of leaf development took 45 to 55 days. The stages are described as below.

111. First leaves separate: Separation of first pair of leaves clearly visible, leaves are dark pinkish in colour.

113. First leaves unfolded: Leaves turn to dark pinkish to light pinkish colour; leaves completely unfolded, the first leaves attain 30 % of their final size. The leaves are shiny and have a soft texture.

114. Few leaves unfolded: Fading of pink colour and developing of green colour; leaves attain 40 % of their final size.

115. More leaves unfolded: Leaves attain 50 % of their final length.

117. All leaves unfolded and fully expanded: Leaves turn green colour; leaves attain 70 % their final size; become slightly rough.

119. Leaves mature: Leaves turn dark green colour and reach their final size; become dull and texture becomes rough.

4.1.3. Principal growth stage 3: Shoot development

The shoot development stage continues with leaf development stage and took 60-70 days to complete and was observed from December to April.

311. Initiation of shoot growth: Slight visible of dark pinkish shoot axes; shoots attain 10 % of their final maturity length.

313. 30 % of shoot maturity: Leaves turn light pink; shoots about 30 % of final maturity.

315. 50 % of final shoot maturity: Again, one pair of leaves emerge from the basal leaves; basal leaves turn yellowish green and new pair of leaves are dark pinkish; shoots about 50 % of their final maturity. Shoot is tender and greenish.

317. 70 % of final shoot maturity: Basal leaves fully expanded and turn light green; new pair of leaves turn a coppery colour; shoots about 70 % of their final maturity. Shoot is become little sturdier, turns green to slightly brownish.

318. 80 % of final shoot maturity: Shoots attain 80 % of their final maturity

319. 90 % of final shoot maturity: Shoots completely matured, two of leaves turn to green and leathery, shoot become sturdy and become brownish.

4.1.4. Principal growth stage 5: Reproductive bud development

In kokum, flower buds emerge in both axillary as well as terminal positions on both old as well as current season growth. The emergence of reproductive buds began in the 2nd week of October and continued up to January. Bud emergence to bud maturity recorded 30-36 days, as described below;

510. Reproductive bud development: Buds are dormant; buds are covered with brownish scales.

511. Emergence of reproductive bud: Emergence of small yellowish coloured reproductive bud.

513. Initiation of reproductive bud swell: Flower buds bulge, yellowish in colour and clearly visible.

515. Elongation of reproductive bud: Flower buds turn shiny green colour, elongation of buds and starts forming lobes in tip of flower.

519. End of flower bud development: Lobes developed completely; flower buds turn to dull green colour. Flower is closed.

4.1.5. Principal growth stage 6: Flowering

After bud development, the bud takes 9-12 days to open. During the period, colour of the bud changed from dark green to slightly yellowish colour to a yellowish orange colour towards the anthesis time. Sepals are 4 in number, exterior and yellowish; petals are 4, interior and yellowish orange.

610. First flower open: Slight pinkish colour develops on flower bud; floral parts are well developed.

615. Full flowering: More than 50 % of flower open; well-developed colour; sepals and petals become widen.

619. End of flowering: Fruit set complete; persistent sepals and stigma; dried petals.

4.1.6. Principal growth stage 7: Fruit development

Fruit development in kokum recorded 90–110 days from fruit set to complete development. The stigma was persistent throughout the development of fruit. Fruit development stage was observed from December to May.

710. Fruit set: Ovary start to bulge; sepals are persistent; dark brown stigma visible.

711. Initial growth of the ovary: fruits continue to grow; sepals still attached with fruit; prominent dark brown stigma; fruit attains 10 % its final size; dark green fruit.

715. 50 % of final fruit size: fruit continue to grow; stigma is still clearly visible; sepals fall off; fruit attains 50 % its final size; fruit still dark green.

717. 70 % of final fruit size: stigma becomes less prominent; fruit turn light green; fruit attains 70 % its final size.

719. 90 % of final fruit size: fruit development is complete; persistent stigma. Fruit turns to lighter green or slight yellowish.

4.1.7. Principal growth stage 8: Fruit maturity

During this period, the fruit passes through several colour changes, beginning with a light reddish colour and progressing to a complete red ripe colour at the final stage. The process takes 5-7 days to complete; The stages are described below.

810. Physiological maturity: fruit attains complete size; the skin start turns to light reddish; 10 % colour change.

812. Fruit maturation: more development of reddish colour; 20 % colour change.

815. Beginning of ripening: The pulp of the fruit begins to soften; more than 50 % fruit skin turns red.

819. Horticultural maturity: The fruit attains harvestable maturity; complete fruit attains dark reddish colour.

Table 4.1. Description of the phenological growth stages of kokum (*Garcinia indica* (Thouars) Choisy) according to the extended BBCH scale

BBCH code	Description
Principal growth stage 0:	**Vegetative bud development**
010	Dormant stage
011	Beginning of bud swell
013	End of bud swell
017	Beginning of bud break
019	End of bud break
Principal growth stage 1:	**Leaf development**
111	First leaves separate
113	First leaves unfolded
114	Few leaves unfolded
115	More leaves unfolded
117	All leaves unfolded and fully expanded
119	Leaves mature
Principal growth stage 3:	**Shoot development**
311	Initiation of shoot growth
313	30% of final shoot length
315	50% of final shoot length
317	70% of final shoot length
318	80% of final shoot length
319	90% of final shoot length
Principal growth stage 5:	**Reproductive bud development**
510	Reproductive bud development
511	Emergence of reproductive bud
513	Initiation of reproductive bud swell
515	Elongation of reproductive bud
519	End of flower bud development
Principal growth stage 6:	**Flowering**
610	First flower open
615	Full flowering
619	End of flowering
Principal growth stage 7:	**Fruit development**
710	Fruit set
711	Initial growth of the ovary
715	50% of final fruit size
717	70% of final fruit size
719	90% of final fruit size
Principal growth stage 8:	**Fruit maturity**
810	Physiological maturity of fruit
812	Fruit maturation
815	Beginning of ripening
819	Horticultural maturity

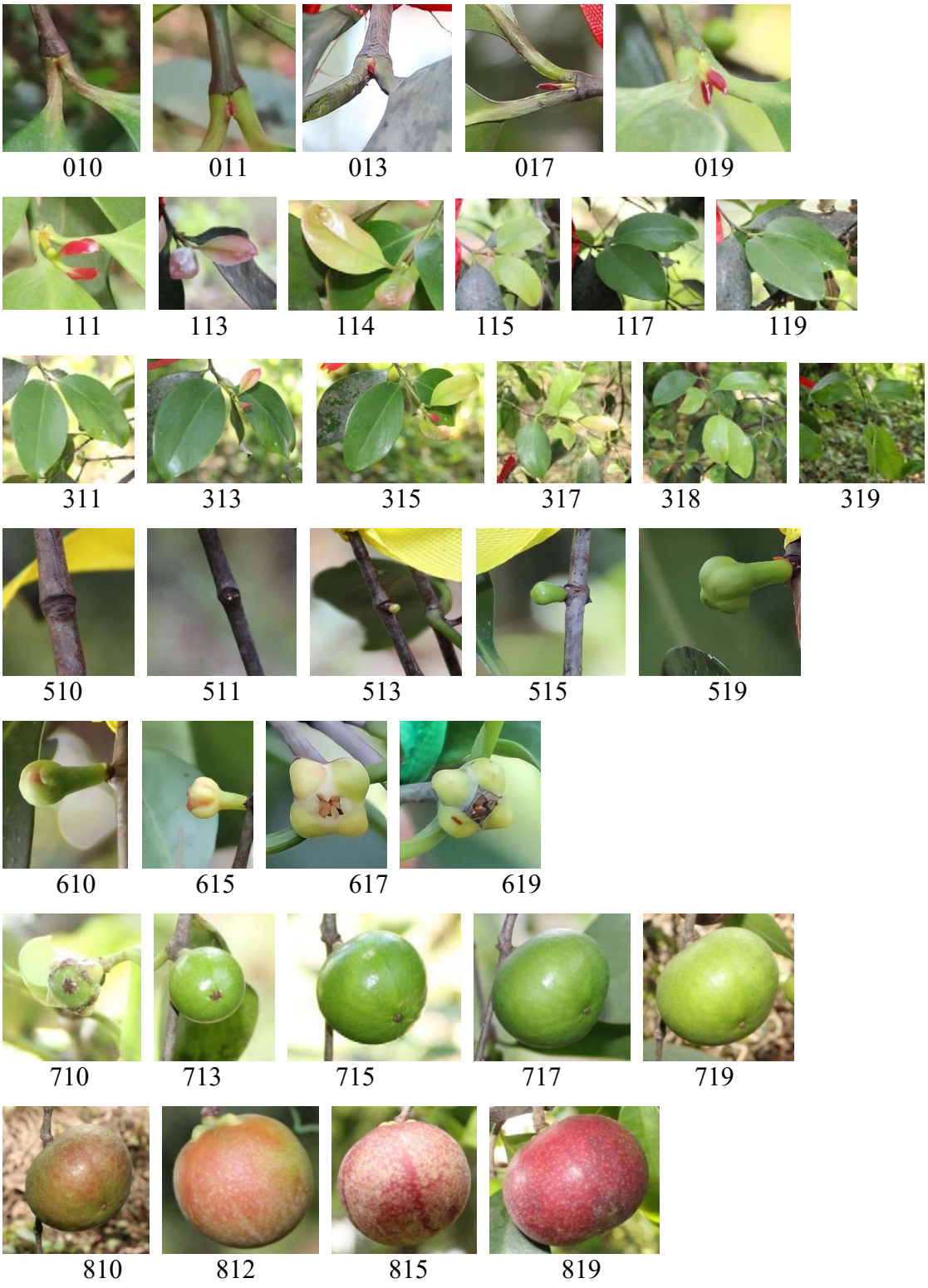


Plate 4.1 Phenological growth stages of kokum according to the extended BBCH scale

4.2. Flower characters

The observations on flower characters were recorded in the select genotypes of kokum. Genotypes with different sex form were observed meticulously for flower parameters. Flower characters were recorded as per standard descriptor and results obtained from the study are presented in various tables.

4.2.1. Sex form

Among twenty nine genotypes selected for the study, 12 genotypes were identified as pure female, 15 genotypes were male and one genotype exhibited as bisexual (ACC.FSC-3) whereas, one genotype had not produced flowers and fruits. The different form of sex exhibited by the kokum tree was presented in Table 4.1.

4.2.2. Position of inflorescence

The position of inflorescence in kokum was observed for axillary, terminal and for both positions. There was no variation among the genotypes for position of inflorescence. All genotypes with regard to the position of inflorescence had both axillary and terminal positions which is depicted in Plate 4.3.

4.2.3. Calyx colour

The colour of the calyx varied from greenish yellow to yellowish green. Only a few genotypes *viz.,* ACC.FSC-1, ACC.FSC-3, ACC.FSC-4, ACC.FSC-5, ACC.FSC-9 and IC136684-3 flowers expressed as yellowish green colour calyx and all other genotypes had greenish yellow calyx (Plate 4.4).

4.2.4. Corolla colour

The colour of the corolla varied from yellow to yellowish orange. The genotypes viz., ACC.FSC-6, ACC.FSC-8 and ACC.FSC-9 had yellowish colour corolla and the rest of the genotypes had yellowish orange corolla.

4.2.5. Nature of stamens

Among the genotypes, there was no variation for nature of stamens. Both male and bisexual trees flowers were found free stamens (Plate 4.5).

4.2.6. Presence of pistillode

The presence of pistillode was observed in male type 2 flowers and that was absent in the male type 1 flowers (Plate 4.6).

4.2.7. Type of stigmatic rays

The female flowers were examined for type of stigmatic rays. All the genotypes had tuberculate type of stigmatic rays (Plate 4.7).

4.2.8. Length and breadth of flower (mm)

The kokum genotypes were differed statistically significant for length and breadth of flowers (Table 4.2). Length of the flower was highest in IC552522-2 (6.64 mm) which was followed by ACC.FSC-1 (5.83 mm) and ACC.FSC-6 (5.46 mm). The least length of flower was recorded in ACC.FSC-7 (3.48 mm).

The highest breadth of flower was recorded in IC552522-2 (8.05 mm) and the least breadth of flower was in ACC.FSC-7 (4.78 mm).

4.2.9. Number of flowers in cluster

Among the genotypes evaluated, most of the female flowers produced as solitary or in group of two or three. In female trees number of flowers in cluster ranged between 1.20 (ACC.FSC-8, IC342319-2 and IC552522-1) to 2.20 (IC136687-2). Male and bisexual flowers were present in groups of 3, 4 or 5. The number of male flowers in each cluster varied from 1.60 (IC136687-1) to 7.10 (ACC.FSC-2). The variation in number of flowers per cluster is shown in Plate 4.8.

4.2.10. Pedicel length (mm)

The length of the pedicel varied significantly among the genotypes (Table 4.3). The pedicel was absent or a very small pedicel was present in female flowers, it ranged from 0.18 mm (ACC.FSC-9) to 2.80 mm (ACC.FSC-6). Male and bisexual flowers were pedicellate. The length of the pedicel varied from 3.41 mm (IC136687-1) to 5.73 mm (IC342319-1).

4.2.11. Number of stigma per flowers

There was no variation among the genotypes with regard to the number of stigma per flower. In all the female as well as bisexual trees number of stigma per flower was reported to be one.

4.2.12. Number of flowers per 0.25 m^2

The genotypes found significantly varied for number of flowers per 0.25 m^2 (Table 4.3.) Among the male genotypes, genotype IC342306-1 (25.20) had recorded the highest number of flowers per 0.25 m^2 followed by ACC.FSC-4 (20.30) and ACC.FSC-5 (19.90). The genotype IC136687-1 had the lowest number of flowers per 0.25 m^2 (10.90). In case of female trees IC552528-3 had the highest number of flowers per 0.25 m^2 (18.50) and IC136687-3 had the lowest number of flowers per 0.25 m^2 (13.40).

4.2.13. Number of stamens per flower

Number of stamens per flower differed significantly among the genotypes (Table 4.4). The functional stamens were noticed in male type 1 flowers and male type-2 flowers as well as bisexual flowers. Significantly the highest number of stamens per flower was recorded in ACC.FSC-5 (41.60) which was on par with IC136685-1 (41.30), IC342319-1 (39.20), IC552523-1 (38.50), ACC.FSC-4 (38.30), IC552514-2 (37.00). The lowest number of stamens per flower was recorded in IC342298-1 (25.90).

4.2.14. Number of staminodes

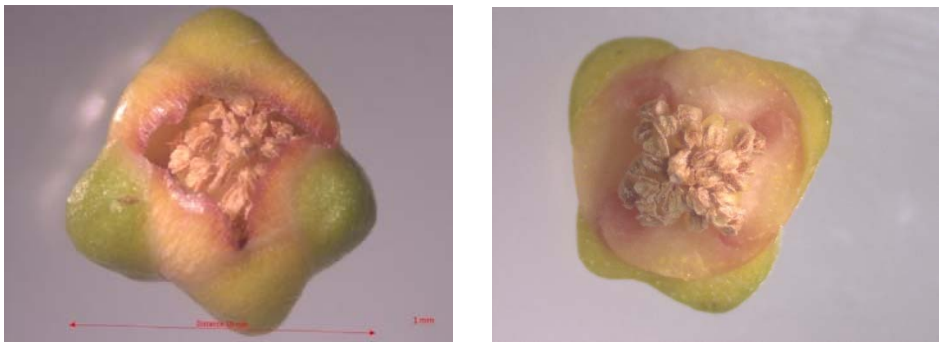
Number of staminodes differed statistically significant among the genotypes (Table 4.4). The staminodes (non-functional stamens) were found in female flowers. The number of staminodes varied from 3.70 (ACC.FSC-8) to 11.80 (IC136687-3).

Table 4.2. Sex form and qualitative flower characters of different genotypes of *Garcinia indica*

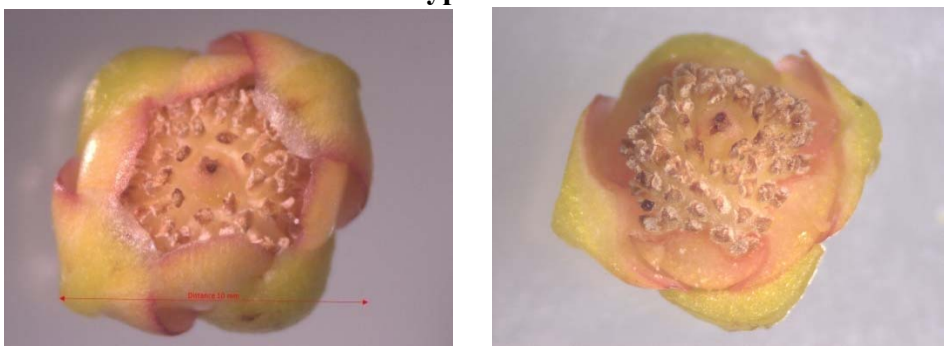
Genotypes	Sex form	Position of inflorescence	Calyx colour	Corolla colour	Nature of stamens	Presence of pistillode	Type of stigmatic rays
ACC.FSC-1	Female	Both	Greenish yellow	Yellowish orange	-	-	Tuberculate
ACC.FSC-2	Male	Both	Greenish yellow	Yellowish orange	Free	Present in type-2	-
ACC.FSC-3	Bisexual	Both	Greenish yellow	Yellowish orange	Free	-	Tuberculate
ACC.FSC-4	Male	Both	Yellowish green	Yellowish orange	Free	Present in type-2	-
ACC.FSC-5	Male	Both	Yellowish green	Yellowish orange	Free	-	-
ACC.FSC-6	Female	Both	Yellowish green	Yellowish orange	-	-	Tuberculate
ACC.FSC-7	Female	Both	Greenish yellow	Yellowish orange	-	-	Tuberculate
ACC.FSC-8	Female	Both	Yellowish green	Yellowish orange	-	-	Tuberculate
ACC.FSC-9	Female	Both	Yellowish green	Yellowish orange	-	-	Tuberculate
IC136684-3	Male	Both	Greenish yellow	Yellowish orange	Free	Present in type-2	-
IC136685-3	Male	Both	Greenish yellow	Yellowish orange	Free	Present in type-2	-
IC136687-1	Male	Both	Greenish yellow	Yellowish orange	Free	Present in type-2	-
IC136687-2	Female	Both	Yellowish green	Yellowish orange	-	-	Tuberculate
IC136687-3	Female	Both	Greenish yellow	Yellowish orange	-	-	Tuberculate
IC342296-1	Female	Both	Greenish yellow	Yellowish orange	-	-	Tuberculate

Continued…

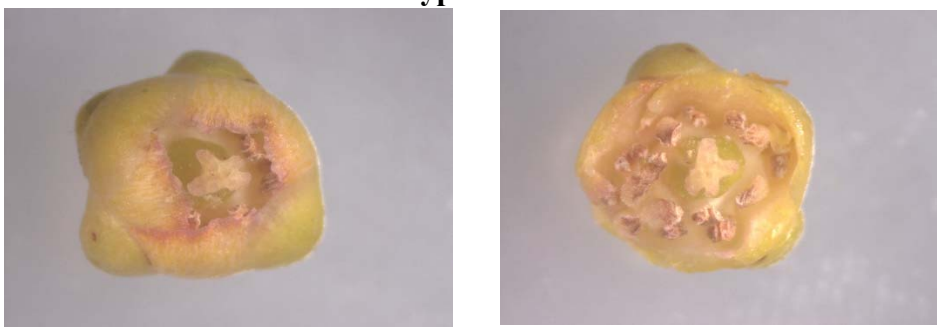
IC342298-1	Male	Both	Greenish yellow	Yellowish orange	Free	-	-
IC342301-3	Male	Both	Greenish yellow	Yellowish orange	Free	-	-
IC342304-1	Male	Both	Yellowish green	Yellowish orange	Free	-	-
IC342306-1	Male	Both	Yellowish green	Yellowish orange	Free	-	-
IC342319-1	Male	Both	Yellowish green	Yellowish orange	Free	Present in type-2	-
IC342319-2	Female	Both	Yellowish green	Yellowish orange	-	-	Tuberculate
IC552517	Male	Both	Greenish yellow	Yellowish orange	Free	-	-
IC552523-1	Male	Both	Greenish yellow	Yellowish orange	Free	Present in type-2	-
IC552514-2	Male	Both	Greenish yellow	Yellowish orange	Free	-	-
IC552526-1	Male	Both	Greenish yellow	Yellowish orange	Free	-	-
IC552522-2	Female	Both	Greenish yellow	Yellowish orange	-	-	Tuberculate
IC552522-1	Female	Both	Greenish yellow	Yellowish orange	-	-	Tuberculate
IC552528-3	Female	Both	Greenish yellow	Yellowish orange	-	-	Tuberculate



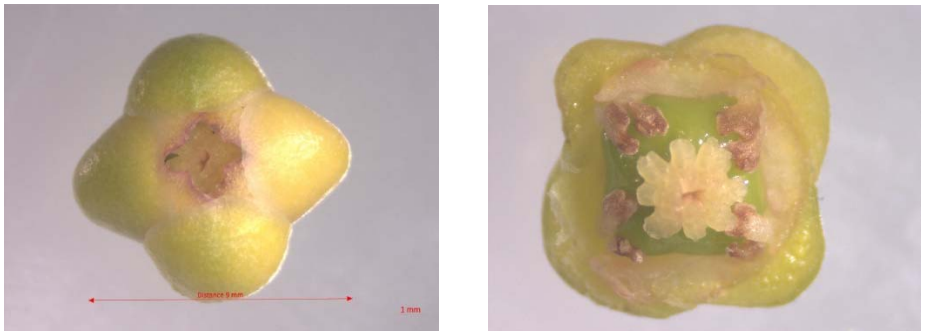
Male type I flower



Male type II flower



Bisexual flower



Female flower

Plate 4.2 Different type of flowers in kokum

Axillary bearing on old season growth growth

Terminal bearing on current season

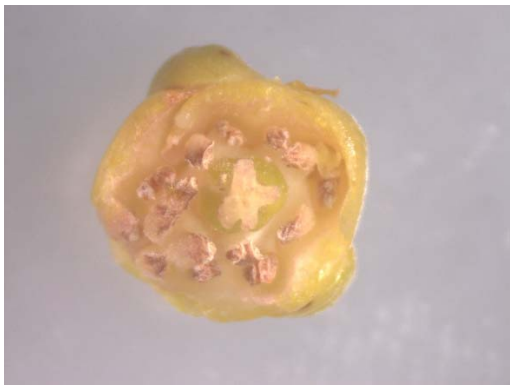
Plate 4.3 Position of inflorescence

Greenish yellow- calyx

Yellowish orange corolla

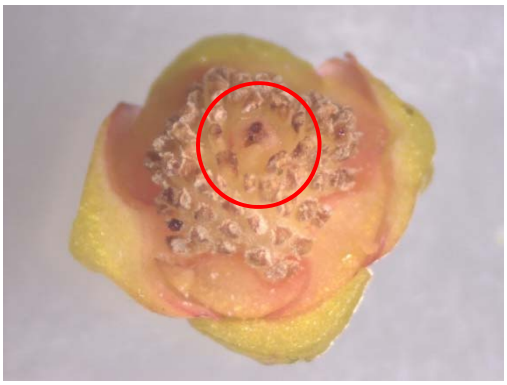
Plate 4.4 Colour of calyx and corolla

Male flower



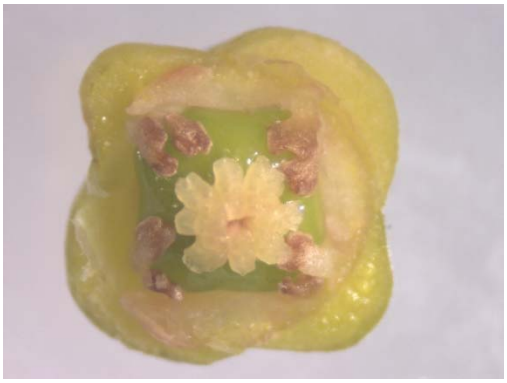
Bisexual flower

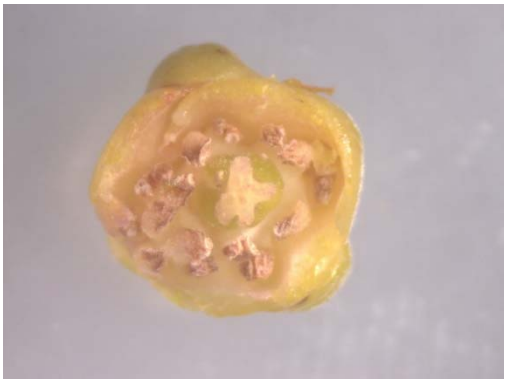
Plate 4.5 Free nature of stamens



Absent- male type I flower **Present- male type II flower**

Plate 4.6 Presence of pistillode





Female flower **Bisexual flower**

Plate 4.7 Tuberculate type of stigmatic rays

Female flower- solitary and sessile

Male- pedicellate and in group of 3 **Male- in group of 4**

Male - more than 4 in a group

Plate 4.8 Number of flowers in cluster

4.2.15. Anthesis

The time of anthesis starts at 19.30 hours and it continued till 21.00 hours in all type of flowers (Table 4.5 and Table 4.6). The peak period of anthesis was from 19.30 hours to 20.00 hours. Most of the males (48 %), females (53 %) and bisexual (52 %) flowers opened at 20.00 hours. All types of flowers take 3 to 4 hours to complete anthesis. After anthesis flowers remain attached to the tree for 2 to 3 days and then female and bisexual flowers set fruit whereas male flower drop off.

4.2.16. Anther dehiscence

The anther dehiscence in both male and bisexual flowers found to be recorded in 30 minutes before flower opening. The anther dehiscence in male and bisexual flowers begin at 19.00 hours and it continued till 20.00 hours in bisexual flowers whereas it was 20.30 hours in male flowers. The peak period of anthesis in male and bisexual flowers from 19.00 hours to 19.30 hours. The highest anther dehiscence in male flowers (54 %) and bisexual flowers (52 %) was found at 19.30 hours.

4.2.17. Stigma receptivity

Stigma receptivity was observed during the period of anthesis in all female and bisexual flowers. The indication of shiny surface stigma found to be the receptive condition of stigma. Stigma receptivity coincides with the anthesis of the flower and the peak period of stigma receptivity was at the time of anthesis. Stigma remained receptive for 12 to 14 hours and thereafter stigma found to be dull in colour.

4.2.18. Pollen studies

The acetocarmine (1 %) test recorded different level of pollen viability among the male and bisexual tree flowers. The percentage of pollen viability was ranged from 84.07 per cent (IC342301-3) to 98.49 per cent (ACC.FSC-2). All the genotypes except IC342301 and IC136684 had exhibited above 90 per cent pollen viability.

The type I and type II male flowers produce round shaped pollen of about 5 µm diameter. No significant difference was found with respect to size and shape of pollen between these two male flower types. The pollen from bisexual flowers was found to

be elongated in shape with prominent ridges on the surface. It measured about 5 µm in diameter (Plate 4.9).

The highest pollen viability was observed in ACC.FSC-2 (98.49 %), followed by ACC.FSC-5 (98.00 %) and IC342319-1 (97.73 %). The lowest pollen viability was obtained in IC342301-3 (84.07 %), (Table 4.9).

4.2.19. Compatibility

Controlled pollination was performed in different combinations of kokum flowers viz., male type I, type II flower, female flower and bisexual flower (Table 4.8), female tree flowers as receptors and male tree flowers (type I and type II) as well as bisexual flowers as donors, and in one case selfing of bisexual flowers. All flowers set fruits when female flowers crossed with male type II flowers and bisexual flowers, and 60 per cent success was reported when female flowers crossed with male type I flowers. Bisexual flowers on selfing exhibit 50 per cent on selfing (Plate 4.10).

4.2.20. Variation in shape of fruit in female and bisexual flower

The fruits produced from female flowers were round in shape whereas fruits produced from bisexual flowers were elongated with beak at the apex (Plate 4.11).

Table 4.3. Quantitative flower characters of different genotypes of *Garcinia indica*

Genotypes	No. of flowers/ 0.25 m^2	Length of flower (mm)	Breadth of flower (mm)	No. of flowers in cluster	Pedicel length (mm)
ACC.FSC-1	18.20	5.83	7.63	1.70	1.24
ACC.FSC-2	17.30	5.26	7.12	7.10	5.00
ACC.FSC-3	15.40	5.21	7.79	2.10	3.84
ACC.FSC-4	20.30	5.27	7.75	6.90	4.12
ACC.FSC-5	19.90	5.42	7.76	5.70	4.49
ACC.FSC-6	15.00	5.46	6.88	1.50	2.82
ACC.FSC-7	14.50	3.48	4.78	1.30	0.52
ACC.FSC-8	15.40	4.09	5.17	1.20	0.32
ACC.FSC-9	17.80	3.59	5.36	1.30	0.11
IC136684-3	18.70	4.05	5.81	3.30	4.14
IC136685-3	17.80	4.54	7.06	4.10	4.58
IC136687-1	10.90	5.29	7.81	1.60	3.40
IC136687-2	17.50	4.32	6.31	2.20	0.78
IC136687-3	13.40	4.47	5.95	1.30	0.51
IC342296-1	15.90	4.95	6.55	1.50	0.69
IC342298-1	14.50	4.15	6.53	3.70	3.61
IC342301-3	14.80	4.73	6.72	4.50	2.25
IC342304-1	13.10	4.05	6.37	2.80	3.65
IC342306-1	25.20	4.16	6.50	5.30	4.58
IC342319-1	18.10	4.50	6.66	5.40	5.72
IC342319-2	16.20	4.88	7.45	1.20	0.53
IC552517	16.30	3.99	5.45	3.10	4.71
IC552523-1	14.50	4.99	6.69	2.50	4.08
IC552514-2	11.60	4.45	6.26	2.90	4.08
IC552526-1	15.90	4.00	5.70	3.20	4.15
IC552522-2	16.60	6.36	8.04	1.40	0.78
IC552522-1	13.70	4.70	6.01	1.20	1.89
IC552528-3	18.50	4.70	5.89	1.40	0.55
SE(m)	0.969	0.224	0.249	0.156	0.139
C.D@ 5%	2.700	0.623	0.695	0.435	0.389
C.V. (%)	18.774	15.122	11.988	16.979	15.987

Table 4.4. Number of stamens and staminodes in different type of flowers of *Garcinia indica*

Genotypes	Number of stamens/flowers	Number of staminodes
ACC.FSC-1	*	7.20
ACC.FSC-2	36.00	**
ACC.FSC-3	27.20	***
ACC.FSC-4	38.30	**
ACC.FSC-5	41.60	**
ACC.FSC-6	*	6.10
ACC.FSC-7	*	4.60
ACC.FSC-8	*	3.70
ACC.FSC-9	*	6.60
IC136684-3	29.70	**
IC136685-3	41.30	**
IC136687-1	29.80	**
IC136687-2	*	6.60
IC136687-3	*	11.50
IC342296-1	*	7.80
IC342298-1	25.90	**
IC342301-3	26.10	**
IC342304-1	26.10	**
IC342306-1	33.20	**
IC342319-1	39.20	**
IC342319-2	*	8.80
IC552517	29.90	**
IC552523-1	38.50	**
IC552514-2	37.00	**
IC552526-1	31.50	**
IC552522-2	*	5.40
IC552522-1	*	14.40
IC552528-3	*	10.20
SE(m)	1.892	0.365
C.D@ 5%	5.295	1.025
C.V. (%)	18.020	14.910

(* indicates female tree, ** indicates male tree and *** indicates bisexual tree)

Table 4.5. Anthesis in male and female flowers of *Garcinia indica*

Male flowers				Female flowers			
Total number of flowers observed	**Time**	**Number of flowers opened**	**Percentage of flowers opened**	**Total number of flowers observed**	**Time**	**Number of flowers opened**	**Percentage of flowers opened**
100	19.00	00	00	100	19.00	00	00
	19.30	39	39		19.30	35	35
	20.00	48	48		20.00	53	53
	20.30	11	11		20.30	10	10
	21.00	02	02		21.00	02	02
	21.30	00	00		21.30	00	00

Table 4.6. Anthesis in bisexual flowers of *Garcinia indica*

Total number of flowers observed	Time	Number of flowers opened	Percentage of flowers opened
75	19.00	00	00
	19.30	24	32
	20.00	39	52
	20.30	09	12
	21.00	03	04
	21.30	00	00

Table 4.7. Time of anther dehiscence in male and bisexual flowers of *Garcinia indica*

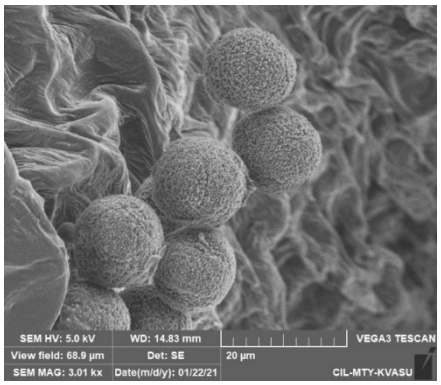
Male flowers				Bisexual flowers			
Total number of flowers observed	**Time**	**Number of dehisced flowers**	**Percentage of dehisced flowers**	**Total number of flowers observed**	**Time**	**Number of dehisced flowers**	**Percentage of dehisced flowers**
50	18.30	00	00	50	18.30	00	00
	19.00	18	36		19.00	20	40
	19.30	27	54		19.30	26	52
	20.00	04	08		20.00	04	08
	20.30	01	02		20.30	00	00
	21.00	00	00		19.00	00	00

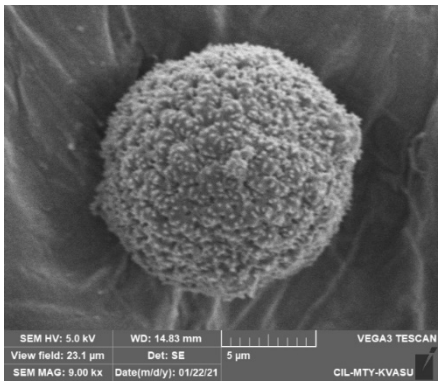
Table 4.8. Crossing between different types of flowers of *Garcinia indica*

Cross	Total flowers pollinated	No. of flowers set fruits	Percentage of fruit set
Female * Male type-I	20	12	60
Female * Male type-II	20	20	100
Female * Bisexual	16	16	100
Bisexual (selfing)	12	06	50

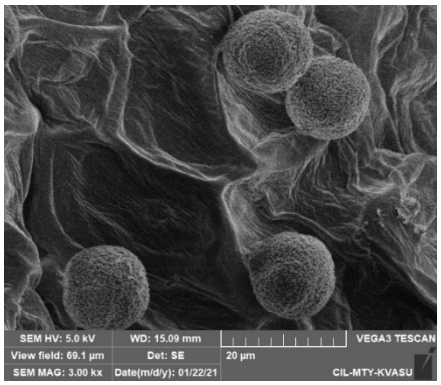
Table 4.9. Pollen viability of male and bisexual genotypes of kokum

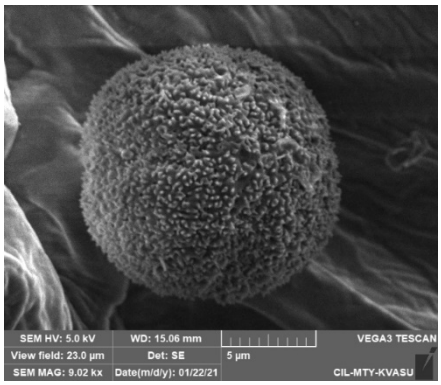
Genotypes	Pollen viability (%)
ACC.FSC-2	98.49
ACC.FSC-3	97.65
ACC.FSC-4	97.37
ACC.FSC-5	98.86
IC136684-3	89.23
IC136685-3	94.28
IC136687-1	95.60
IC342298-1	93.40
IC342301-3	84.07
IC342304-1	90.49
IC342306-1	92.01
IC342319-1	93.86
IC552517	97.26
IC552523-1	96.34
IC552514-2	95.26
IC552526-1	96.45
SE(m)	1.047
C.D. @5%	3.029
C.V. (%)	1.927



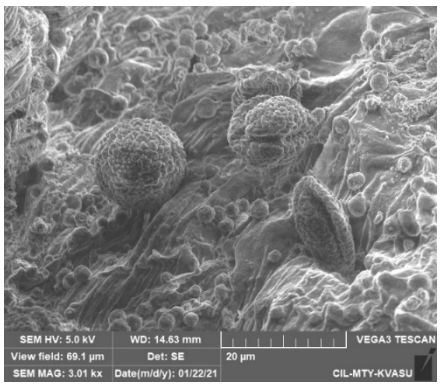


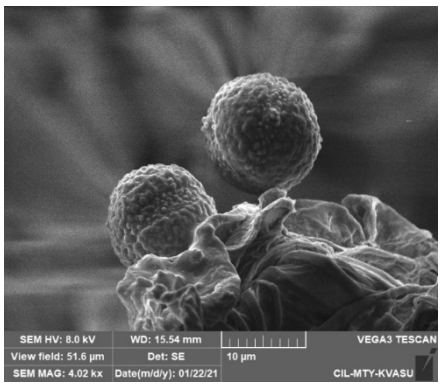
Pollen grains of male type I flower





Pollen grains of male type II flower

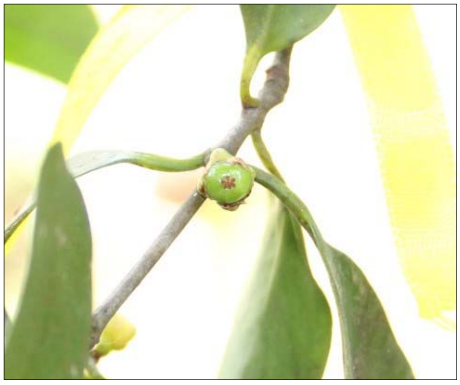




Pollen grains of bisexual flower

Plate 4.9. SEM images of pollens

Female*Male type I



Female*Male type II

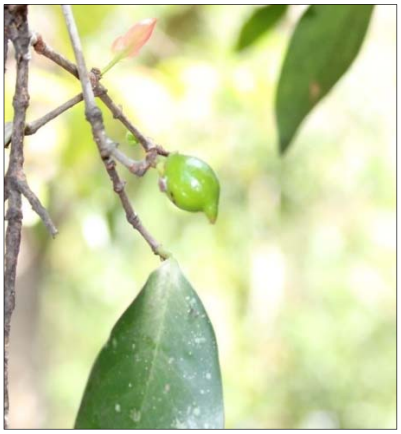
Female*Bisexual

Bisexual on selfing

Dropped flowers without pollination

Plate 4.10. Controlled pollination in kokum

Female flower fruit- round in shape



Bisexual flower fruit- oval with beak at the apex

Plate 4.11. Variation in shape of fruit in female and bisexual flower

4.3. Growth characters

Observations on plant growth characters were recorded in the study and the data was statistically analysed and presented in Table 4.10.

4.3.1. Plant height

Plant height varied from 5.20 m (IC552513) to 15.60 m (IC136687-1). The highest plant height was recorded in IC136687-1 (15.60 m) which was followed by IC136684-3 (13.90 m) and IC136687-3 (13.60 m). The lowest plant height was recorded in IC552513 (5.20 m). The average height of kokum genotypes was 10.82 m of which ACC.FSC.2, ACC.FSC.4, IC552517, IC552523-1, IC552514-2, IC552513, IC552526-1, IC552522-2, IC552522-1 and IC552528-3 genotypes recorded less than the average plant height. The coefficient of variation for plant growth was 0.23 per cent.

4.3.2. Girth at 140 cm height

Among the 29 genotypes of kokum, girth of the plant at 140 cm height varied between 25.10 cm to 156.88 cm. The highest plant girth was recorded in ACC.FSC-1 (156.88 cm) followed by IC136687-3 (141.30 cm) and IC342306-1(133.10 cm). The lowest plant girth was recorded in IC552522 (25.10 cm). The coefficient of variation was calculated 0.36 per cent.

4.3.3. Canopy spread of the tree (N-S, E-W), (m)

Canopy spread of the trees from East to West direction ranged from 3.20 m (IC552513) to 8.10 m (ACC.FSC-4). The average canopy spread from East to West direction was of 5.94 m. The coefficient of variation for East-West direction of canopy spread was 0.23 per cent.

Canopy spread from North to South direction ranged from 3.30 m (IC552513) to 9.42 m (ACC.FSC-9). The average canopy spread in North-South direction was 5.76 m. The coefficient of variation for North-South direction of canopy spread was 0.23 per cent.

4.3.4. Canopy volume (m^3)

Among the 29 genotypes, the highest canopy volume was recorded in ACC.FSC-9 (1030.01 m^3). The lowest canopy volume was in IC552513 (57.12 m^3). The mean canopy volume was calculated of 412.28 m^3. The coefficient of variation for canopy volume was 0.50 per cent.

4.4. Leaf characters

Data on leaf characters were recorded on all the 29 genotypes and the findings are presented in Table 4.11.

4.4.1. Leaf length and breadth (cm)

The genotypes differed significantly for leaf length. The significantly highest leaf length was recorded in IC342319-1 (10.24 cm). The genotypes, ACC.FSC-4, ACC.FSC-6, IC136684-3, ACC.FSC-5, IC342298-1, IC342319-2, ACC.FSC-8, ACC.FSC-7, ACC.FSC-2, IC136687-2, ACC.FSC-1 and IC136685-3 on par with that of IC342319-1.

The kokum genotypes differed significantly for leaf breadth. The genotype ACC.FSC-4 had significantly highest leaf breadth (4.78 cm) which was on par with IC552514-2, IC342306-1, IC552522-2, IC552513, IC552526-1, IC342319-2, IC552523-1, ACC.FSC-1 and IC552522-1. The lowest leaf breadth was recorded in IC342304-1 (3.18 cm).

4.4.2. Leaf area (cm^2)

There was a significant difference was noticed among the genotypes for leaf area. Significantly highest leaf area was recorded in ACC.FSC-4 (34.53 cm^2) which was on par with ACC.FSC-5, ACC.FSC-8, ACC.FSC-1 and IC342319-2. The lowest leaf area was recorded in IC342304-1 (19.87 cm^2).

Table 4.10. Plant growth characters of kokum genotypes

Genotypes	Plant height (m)	Girth at 140 cm height (cm)	Canopy spread (E-W), (m)	Canopy spread (N-S), (m)	Canopy volume (m^3)
ACC.FSC-1	10.80	156.88	7.30	8.00	665.94
ACC.FSC-2	7.80	77.75	7.60	6.40	397.49
ACC.FSC-3	12.20	105.63	7.30	7.10	657.74
ACC.FSC-4	8.20	92.98	8.10	7.42	513.53
ACC.FSC-5	12.80	96.26	5.80	5.10	395.39
ACC.FSC-6	12.70	75.86	6.20	5.86	480.25
ACC.FSC-7	12.40	86.68	6.40	7.60	631.90
ACC.FSC-8	12.30	83.86	8.50	6.50	719.55
ACC.FSC-9	12.20	100.56	7.18	9.42	1030.01
IC136684-3	13.90	84.60	4.60	5.40	361.40
IC136685-3	11.80	111.30	6.10	6.30	471.73
IC136687-1	15.60	123.60	5.20	5.40	404.67
IC136687-2	11.60	65.20	4.00	4.50	216.02
IC136687-3	13.60	141.30	5.60	6.70	534.96
IC342296-1	13.40	101.50	5.50	5.80	444.87
IC342298-1	11.30	55.80	6.70	5.40	430.15
IC342301-3	11.20	60.40	5.60	4.20	284.25
IC342304-1	11.10	62.40	5.50	4.73	302.62
IC342306-1	12.30	133.10	6.90	5.90	523.96
IC342319-1	11.30	70.60	5.50	5.20	336.37
IC342319-2	13.20	101.40	4.30	4.40	259.77
IC552517	8.60	86.20	7.20	6.40	413.56
IC552523-1	8.90	77.20	4.40	3.90	159.41
IC552514-2	8.70	60.10	7.60	6.40	443.35
IC552513	5.20	38.40	3.20	3.30	57.12
IC552526-1	8.10	68.00	6.10	5.10	264.17
IC552522-2	6.90	25.10	4.50	4.70	151.84
IC552522-1	6.60	37.80	4.10	4.30	121.08
IC552528-3	9.00	82.40	5.40	5.60	283.14
Mean	10.82	84.93	5.94	5.76	412.28
SD	2.52	30.42	1.34	1.34	205.07
CV (%)	0.23	0.36	0.23	0.23	0.50

4.5. Fruit and seed characters

Fruit as well as seed characters were recorded in all the yielding 13 genotypes. The findings of the fruit and seed characters are furnished in Table 4.12.

4.5.1. Number of fruits per m^2

There was a significant difference was recorded among the genotypes for number of fruits per m^2. Significantly the highest number of fruits per m^2 was recorded in IC552528-3 (71.10), it was followed by ACC.FSC-1 (59.20) and ACC.FSC-9 (46.80). The other genotypes were recorded below 40 number of fruits per m^2. The lowest number of fruits per m^2 was recorded in ACC.FSC-3 (9.50).

4.5.2. Fruit weight (g)

The fruit weight was varied from 12.17 g to 40.14 g. Significantly highest fruit weight was recorded in ACC.FSC.6 (40.14 g), it was on par with IC342319-2, ACC.FSC-1 and IC342296-1. The lowest weight was recorded in ACC.FSC-3 (12.17 g).

4.5.3. Fruit volume (cm^3)

Fruit volume was ranged from 15.10 cm^3 to 45.60 cm^3. Significantly highest fruit volume was recorded in ACC.FSC-6 (45.60 cm^3), it was on par with IC342319-2, ACC.FSC-1 and IC342296-1. The lowest fruit volume was in ACC.FSC-3 (15.10 cm^3).

4.5.4. Fresh and dry weight of rind (g)

There was a significant difference among the genotypes for fresh weight of rind. Fresh weight of rind ranged from 7.15 g to 21.01 g. Significantly the highest fresh weight of rind was recorded in ACC.FSC-6 (21.01 g), it was on par with IC342319-2. Other genotypes produce medium fresh weight of rind. Whereas genotype ACC.FSC-3 recorded lowest fresh weight of rind (7.15 g).

Dry weight of rind had exhibited significant differences among the genotypes. Significantly highest dry weight of rind was recorded in ACC.FSC-6 (3.07 g), it was closely followed by IC136687-2 and IC342319-2. The lowest dry weight of rind was recorded in ACC.FSC-3 (1.09 g).

Table 4.11. Leaf characters of kokum genotypes

Genotypes	Leaf length (cm)	Leaf breadth (cm)	Leaf area (cm^2)
ACC.FSC-1	9.08	4.30	29.31
ACC.FSC-2	9.21	4.03	25.83
ACC.FSC-3	8.47	4.00	22.99
ACC.FSC-4	10.19	4.78	34.52
ACC.FSC-5	9.50	4.56	31.24
ACC.FSC-6	9.88	3.35	25.41
ACC.FSC-7	9.31	3.72	24.33
ACC.FSC-8	9.39	4.02	29.60
ACC.FSC-9	8.87	4.11	27.89
IC136684-3	9.60	3.76	24.18
IC136685-3	9.07	3.65	22.52
IC136687-1	8.91	4.03	26.59
IC136687-2	9.12	3.70	22.94
IC136687-3	8.54	4.00	23.50
IC342296-1	8.96	3.43	21.40
IC342298-1	9.46	3.63	23.35
IC342301-3	8.96	3.58	21.96
IC342304-1	8.41	3.18	19.87
IC342306-1	8.72	4.72	28.79
IC342319-1	10.24	4.09	28.79
IC342319-2	9.44	4.30	29.21
IC552517	8.85	4.11	24.86
IC552523-1	7.84	4.30	22.93
IC552514-2	8.18	4.74	27.37
IC552513	8.11	4.45	27.56
IC552526-1	8.92	4.32	25.40
IC552522-2	8.53	4.47	26.67
IC552522-1	7.57	4.22	22.40
IC552528-3	8.21	3.77	20.98
SE(m)	0.447	0.212	2.014
C.D@ 5%	1.245	0.591	5.613
C.V. (%)	15.793	16.589	24.879

4.5.5. Rind to seed ratio

Rind to seed ratio had exhibited significant differences among the genotypes. Significantly highest rind to seed ratio was recorded in ACC.FSC-3 (1.77), it was followed by ACC.FSC-8 and ACC.FSC-6. The lowest rind to seed ratio was recorded in IC136687-2 (0.81).

4.5.6. Rind thickness (mm)

Rind thickness ranged from 2.46 mm to 3.41 mm. Significantly the highest rind thickness was recorded in IC552522-2 (3.41 mm), it was on par with ACC.FSC-6, IC136687-3, IC342319-2, IC552528-3, ACC.FSC-7 and ACC.FSC-9. The lowest rind thickness was in IC552522-1 (2.46 mm).

4.5.7. Fresh seed weight (g)

There was a significant difference among the genotypes for fresh seed weight. Fresh seed weight ranged from 4.26 g to 17.90 g. Significantly the highest seed weight was recorded in IC342319-2 (17.90 g), it was on par with IC136687-2, ACC.FSC-1, ACC.FSC-6 and IC342296-1. The lowest fresh seed weight was recorded in ACC.FSC-3 (4.26 g).

4.5.8. Number of seeds per fruit

Number of seeds per fruit was ranged from 2.00 to 5.30. Significantly highest number of seeds per fruit was recorded in ACC.FSC-9 (5.30), it was on par with IC342319-2. The lowest number of seeds per fruit were recorded in ACC.FSC-3 (2.00).

4.6. Yield attributes

Number of fruits per tree and fruit yield per tree were recorded during the fruiting season of kokum. The observations recorded were statistically analysed and depicted in Table 4.13.

4.6.1. Number of fruits per tree

Number of fruits per tree ranged from 252 to 2258. The genotype ACC.FSC-9 had the highest number of fruits per tree (2258) which was followed by IC552528-3 (2055) and ACC.FSC-1 (1335). The genotype ACC.FSC-3, the only bisexual tree

recorded the lowest number of fruits per tree (252). The average number of fruits per tree was 1101.68 and the coefficient of variation was 0.49 per cent.

4.6.2. Fruit yield per tree (kg)

Similar trend was observed in case of fruit yield per tree with number of fruits per tree. The genotype ACC.FSC-9 (60.64 kg) had registered highest fruit yield per tree which was followed by IC552528-3 (51.43 kg) and ACC.FSC-1 (46.52 kg). The genotype ACC.FSC-3, which was bisexual, had recorded lowest fruit yield (3.07 kg). The mean fruit yield per tree was 32.63 kg and the coefficient of variation was calculated as 0.45 per cent.

4.7. Variation in leaf and fruit colour

Young flush, developing leaf and mature leaf as well as ripen fruit were compared with the help of royal horticultural society colour chart. The observations are tabulated and presented in Table 4.14. Young flush colour varied from moderate purplish red to strong purplish red. All the genotypes except ACC.FSC-4, ACC.FSC-7, IC342306-1, IC342319-1, IC552522-1 and IC552528-3 had moderate purplish red colour young leaves whereas these five genotypes had strong purplish red colour young leaves. On developmental stage of kokum leaves exhibited three different colours viz., strong yellow green, strong yellowish green and deep yellowish green. Among the 29 kokum genotypes, 21 genotypes had expressed strong yellow green, 7 genotypes had expressed strong yellowish green and one genotype had deep greenish yellow colour leaf. The matured leaves of kokum had exhibited moderate olive green (20 genotypes), dark yellowish green (7 genotypes), and greyish olive green (2 genotypes). The ripened fruit had exhibited greyish purple as well as dark red colour. Among the fruiting genotypes, dark red colour was observed in five genotypes. The remaining six genotypes had exhibited greyish purple colour (Plate 4.13).

Table 4.12. Fruit characters of kokum genotypes

Treatment	No. of fruits/m^2	Fruit weight (g)	Fruit volume (cm^3)	Fresh weight of rind(g)	Dry weight of rind (g)	Rind to seed ratio	Rind thickness (mm)	Fresh seed weight (g)	No. of seeds/fruit
ACC.FSC-1	59.20	34.85	42.00	16.53	2.27	1.03	2.91	17.52	4.30
ACC.FSC-3	9.50	12.17	15.10	7.14	1.09	1.77	3.07	4.27	2.00
ACC.FSC-6	39.50	40.14	45.60	21.01	3.06	1.32	3.40	16.07	4.90
ACC.FSC-7	26.00	30.75	34.70	15.86	1.83	1.17	3.16	13.70	3.90
ACC.FSC-8	39.80	21.73	25.00	12.25	1.77	1.48	2.87	8.52	3.60
ACC.FSC-9	46.80	26.86	33.30	14.07	1.85	1.31	3.15	11.20	5.30
IC136687-2	29.10	33.19	38.80	14.47	2.66	0.85	2.67	17.68	4.40
IC136687-3	23.60	27.82	32.30	13.19	1.86	1.03	3.34	13.15	3.80
IC342296-1	22.90	34.32	36.70	16.16	2.32	1.17	2.86	15.69	3.80
IC342319-2	35.10	38.08	42.80	18.37	2.38	1.03	3.29	17.90	5.00
IC552522-2	31.10	29.66	38.40	14.98	2.17	1.30	3.41	11.63	4.80
IC552522-1	23.60	29.62	31.70	15.60	2.06	1.15	2.46	13.49	4.00
IC552528-3	71.10	25.02	26.50	11.80	1.50	1.08	3.17	11.08	4.50
SE(m)	2.078	2.471	2.693	1.066	0.132	0.083	0.117	1.408	0.344
C.D@ 5%	5.828	6.930	7.554	2.989	0.371	0.232	0.328	3.949	0.963
C.V. (%)	18.682	26.434	25.000	22.880	20.221	21.566	12.062	33.660	26.010

Table 4.13. Yield characters of kokum genotypes

Treatment	Number of fruits per tree	Fruit yield per tree (kg)
ACC.FSC-1	1335	46.52
ACC.FSC-3	252	3.07
ACC.FSC-6	1022	41.02
ACC.FSC-7	866	26.36
ACC.FSC-8	1196	26.00
ACC.FSC-9	2258	60.64
IC136687-2	1047	34.75
IC136687-3	1160	32.27
IC342296-1	700	24.02
IC342319-2	722	27.49
IC552522-2	960	28.47
IC552522-1	749	22.18
IC552528-3	2055	51.43
Mean	1101.69	32.63
SD	543.86	14.69
C.V. (%)	0.49	0.45

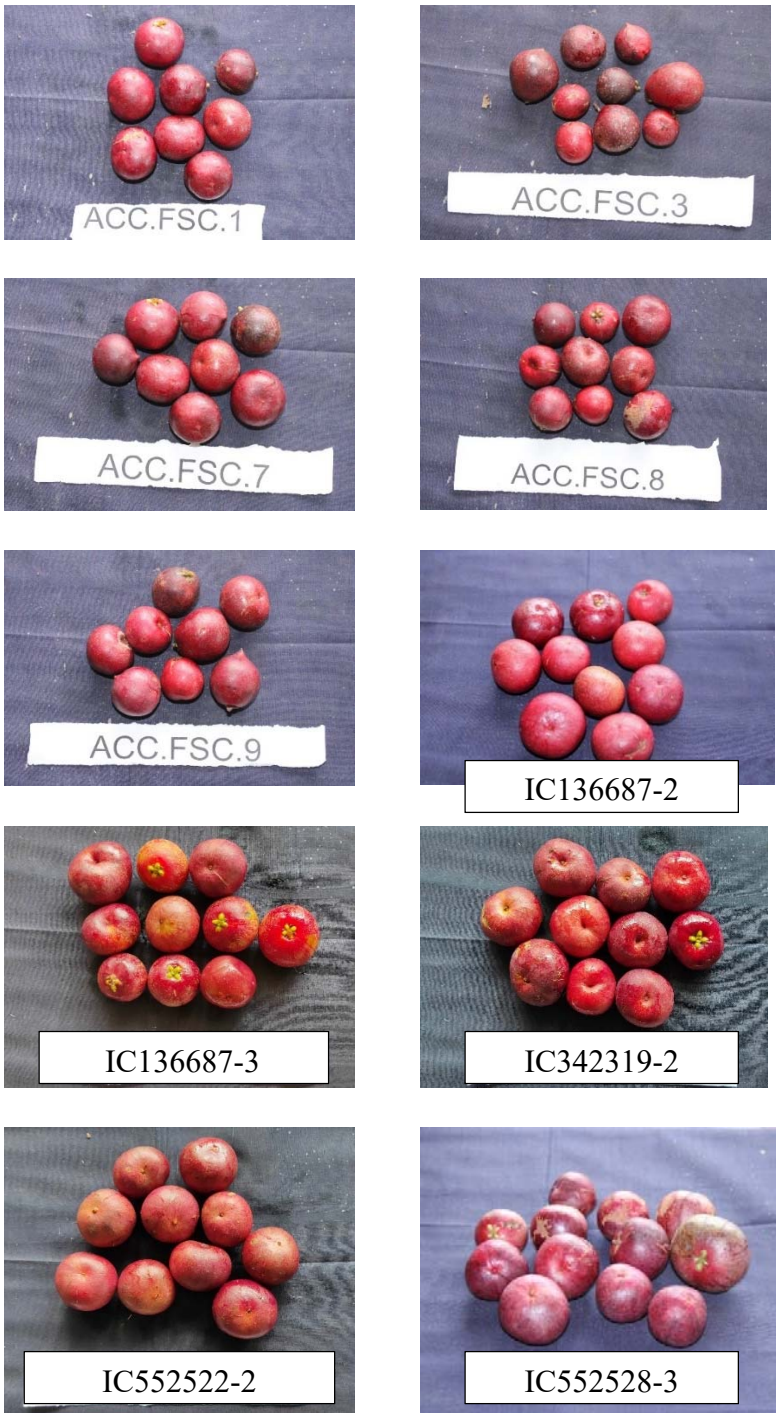


Plate 4.12. Ripened fruits of bearing kokum genotypes

4.8. Fruit quality parameters

The biochemical analysis of fruit rind was estimated in all 13 genotypes. The values were statistically analysed and given in Table (4.15).

4.8.1. Moisture (%)

There was a significant difference was noticed among the kokum genotypes for moisture content in fruit rind. Significantly highest moisture content was recorded in ACC.FSC-9 (90.34 %) and IC552528-3 (89.54 %). It was closely followed by IC552522-1 (86.36 %) and IC342319-2 (86.35 %). All the genotypes had registered above 82 per cent of moisture. The lowest moisture content was recorded in IC136687-2 (82.91 %).

4.8.2. Titratable acidity (%)

The genotypes differed significantly for titratable acidity. Titratable acidity varied from 2.30 per cent to 4.47 per cent. The titratable acidity is intrinsic character of tree. The lowest titratable acidity is of appreciable character, genotype IC136687-3 (2.3 %) had registered lowest titratable acidity. The highest acidic fruit was produced in genotype ACC.FSC-1 (4.47 %).

4.8.3. Total sugars (%)

Genotypes had exhibited significant difference for total sugars. The genotype IC552528-3 (10.60 %) had highest total sugars which was closely followed by IC136687-3 (8.45 %) and IC136687-2 (7.73 %). The lowest total sugar was estimated in ACC.FSC-3 (4.32 %).

4.8.4. Total soluble solids (^{0}Brix)

Total soluble solids varied significantly among the genotypes. Similar pattern of values was observed for total soluble solids as well as total sugars. IC552528-3 (14.15 ^{0}Brix) had recorded highest total soluble solids which was on par with IC136687-3 (12.75 ^{0}Brix). Genotype IC552522-1 (10.90 ^{0}Brix) and IC136687-2 (10.80 ^{0}Brix) on par with that of IC136687-3. The lowest TSS was recorded in ACC.FSC-3 (5.65 ^{0}Brix).

4.8.5. Anthocyanin content (mg/g)

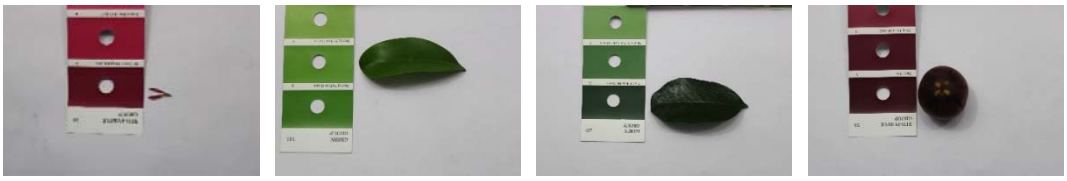
Anthocyanin content in fruits ranged from 11.13 mg/100 g to 25.01 mg/100 g. Significantly highest anthocyanin content was recorded in ACC.FSC-1 (25.01 mg/100 g) as well as IC552528-3 (24.84 mg/100 g). It was on par with ACC.FSC-9 (23.21 mg/100 g). The lowest anthocyanin content was recorded in IC552522-1 (11.13 mg/100 g).

Table 4.14. Variation in leaf and fruit colour of kokum

Genotypes	Leaf colour			Fruit colour
	Young flush	Developing leaf	Matured leaf	
ACC.FSC-1	RHS/2015- Red-Purple group- 58- Moderate purplish red- A	RHS/2015- Yellow-Green group- 144- Strong yellow green- A	RHS/2015- Green group- 139- Dark yellowish green-A	RHS/2015- Red purple group- 59- Dark red- A
ACC.FSC-2	RHS/2015- Red-Purple group- 58- Moderate purplish red- A	RHS/2015- Yellow-Green group- 144- Strong yellow green- A	RHS/2015- Green group- 139- Dark yellowish green-A	-
ACC.FSC-3	RHS/2015- Red-Purple group- 58- Moderate purplish red- A	RHS/2015- Yellow-Green group- 144- Strong yellow green- A	RHS/2015- Green group- 137- Moderate olive green- A	-
ACC.FSC-4	RHS/2015- Red-Purple group- 63- Strong purplish red- A	RHS/2015- Yellow-Green group- 144- Strong yellow green- A	RHS/2015- Green group- 139- Dark yellowish green-A	-
ACC.FSC-5	RHS/2015- Red-Purple group- 58- Moderate purplish red- A	RHS/2015- Yellow-Green group- 144- Strong yellow green- A	RHS/2015- Green group- 137- Moderate olive green- A	-
ACC.FSC-6	RHS/2015- Red-Purple group- 58- Moderate purplish red- A	RHS/2015- Yellow-Green group- 144- Strong yellow green- A	RHS/2015- Green group- 137- Moderate olive green- A	RHS/2015- Red purple group- 59- Dark red- A
ACC.FSC-7	RHS/2015- Red-Purple group- 63- Strong purplish red- A	RHS/2015- Yellow-Green group- 144- Strong yellow green- A	RHS/2015- Green group- 137- Moderate olive green- A	RHS/2015- Purple group- N77- Greyish purple- A
ACC.FSC-8	RHS/2015- Red-Purple group- 58- Moderate purplish red- A	RHS/2015- Yellow-Green group- 144- Strong yellow green- A	RHS/2015- Green group- 137- Moderate olive green- A	RHS/2015- Purple group- N77- Greyish purple- A
ACC.FSC-9	RHS/2015- Red-Purple group- 58- Moderate purplish red- A	RHS/2015- Yellow-Green group- 144- Strong yellow green- A	RHS/2015- Green group- 137- Moderate olive green- A	RHS/2015- Red purple group- 59- Dark red- A
IC136684-3	RHS/2015- Red-Purple group- 58- Moderate purplish red- A	RHS/2015- Yellow-Green group- N144- Strong yellowish green-A	RHS/2015- Green group- 137- Moderate olive green- A	-
IC136685-3	RHS/2015- Red-Purple group- 58- Moderate purplish red- A	RHS/2015- Yellow-Green group- N144- Strong yellowish green-A	RHS/2015- Green group- 137- Moderate olive green- A	-
IC136687-1	RHS/2015- Red-Purple group- 58- Moderate purplish red- A	RHS/2015- Yellow-Green group- N144- Strong yellowish green-A	RHS/2015- Green group- 137- Moderate olive green- A	-
IC136687-2	RHS/2015- Red-Purple group- 58- Moderate purplish red- A	RHS/2015- Yellow-Green group- N144- Strong yellowish green-A	RHS/2015- Green group- 137- Moderate olive green- A	-
IC136687-3	RHS/2015- Red-Purple group- 58- Moderate purplish red- A	RHS/2015- Yellow-Green group- N144- Strong yellowish green-A	RHS/2015- Green group- 137- Moderate olive green- A	RHS/2015- Red purple group- 59- Dark red- A

Continued…

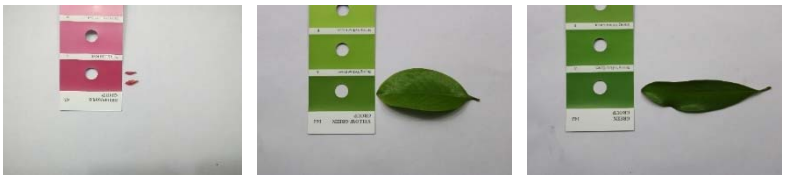
IC342296-1	RHS/2015- Red-Purple group- 58- Moderate purplish red- A	RHS/2015- Yellow-Green group- 144- Strong yellow green- A	RHS/2015- Green group- 137- Moderate olive green- A	RHS/2015- Purple group- N77- Greyish purple- A
IC342298-1	RHS/2015- Red-Purple group- 58- Moderate purplish red- A	RHS/2015- Yellow-Green group- 144- Strong yellow green- A	RHS/2015- Green group- 137- Moderate olive green- A	-
IC342301-3	RHS/2015- Red-Purple group- 58- Moderate purplish red- A	RHS/2015- Yellow-Green group- N144- Strong yellowish green-A	RHS/2015- Green group- 137- Moderate olive green- A	-
IC342304-1	RHS/2015- Red-Purple group- 58- Moderate purplish red- A	RHS/2015- Yellow-Green group- N144- Strong yellowish green-A	RHS/2015- Green group- 137- Moderate olive green- A	-
IC342306-1	RHS/2015- Red-Purple group- 58- Moderate purplish red- A	RHS/2015- Yellow-Green group- 153- Deep greenish yellow-A	RHS/2015- Green group- 137- Moderate olive green- A	-
IC342319-1	RHS/2015- Red-Purple group- 63- Strong purplish red- A	RHS/2015- Yellow-Green group- 144- Strong yellow green- A	RHS/2015- Green group- 137- Moderate olive green- A	-
IC342319-2	RHS/2015- Red-Purple group- 58- Moderate purplish red- A	RHS/2015- Yellow-Green group- 144- Strong yellow green- A	RHS/2015- Green group- 137- Moderate olive green- A	RHS/2015- Purple group- N77- Greyish purple- A
IC552517	RHS/2015- Red-Purple group- 58- Moderate purplish red- A	RHS/2015- Yellow-Green group- 144- Strong yellow green- A	RHS/2015- Green group- 137- Moderate olive green- A	-
IC552523-1	RHS/2015- Red-Purple group- 58- Moderate purplish red- A	RHS/2015- Yellow-Green group- 144- Strong yellow green- A	RHS/2015- Green group- NN137- Greyish olive green- A	-
IC552514-2	RHS/2015- Red-Purple group- 58- Moderate purplish red- A	RHS/2015- Yellow-Green group- 144- Strong yellow green- A	RHS/2015- Green group- NN137- Greyish olive green- A	-
IC552513	RHS/2015- Red-Purple group- 58- Moderate purplish red- A	RHS/2015- Yellow-Green group- 144- Strong yellow green- A	RHS/2015- Green group- 139- Dark yellowish green-A	-
IC552526-1	RHS/2015- Red-Purple group- 58- Moderate purplish red- A	RHS/2015- Yellow-Green group- 144- Strong yellow green- A	RHS/2015- Green group- 139- Dark yellowish green-A	-
IC552522-2	RHS/2015- Red-Purple group- 58- Moderate purplish red- A	RHS/2015- Yellow-Green group- 144- Strong yellow green- A	RHS/2015- Green group- 139- Dark yellowish green-A	RHS/2015- Purple group- N77- Greyish purple- A
IC552522-1	RHS/2015- Red-Purple group- 63- Strong purplish red- A	RHS/2015- Yellow-Green group- 144- Strong yellow green- A	RHS/2015- Green group- 137- Moderate olive green- A	RHS/2015- Purple group- N77- Greyish purple- A
IC552528-3	RHS/2015- Red-Purple group- 63- Strong purplish red- A	RHS/2015- Yellow-Green group- 144- Strong yellow green- A	RHS/2015- Green group- 139- Dark yellowish green-A	RHS/2015- Red purple group- 59- Dark red- A



ACC.FSC-1

ACC.FSC-2

ACC.FSC-3



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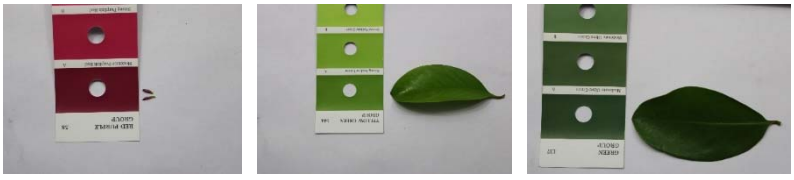
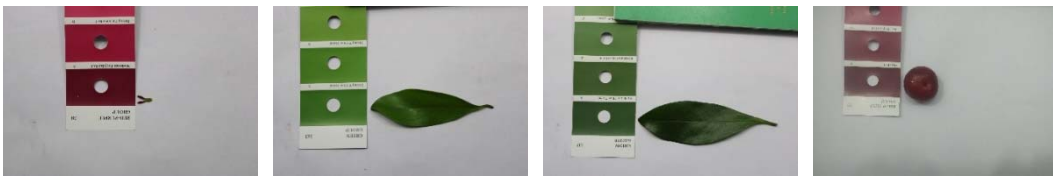
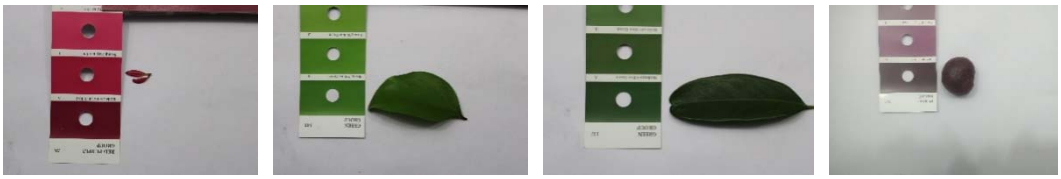


ACC.FSC-5

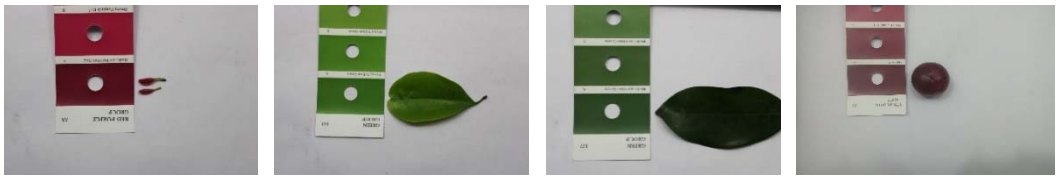


ACC.FSC-6



ACC.FSC-7

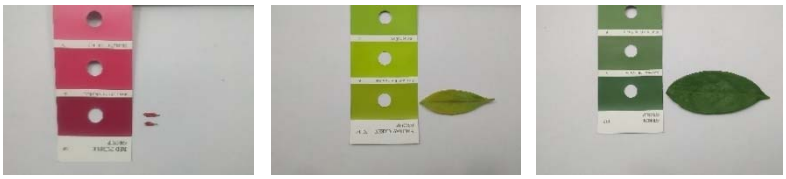
ACC.FSC-8



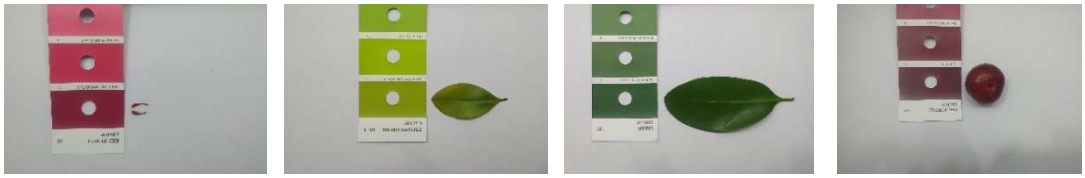
ACC.FSC-9

IC136685-3

IC136687-1

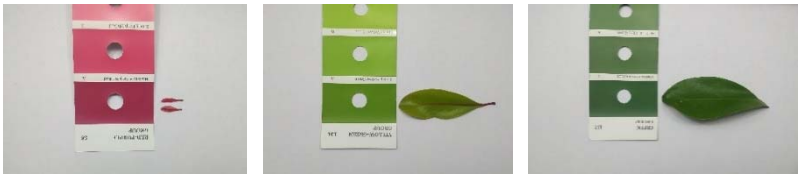


IC136687-2



IC136687-3

IC342296-1



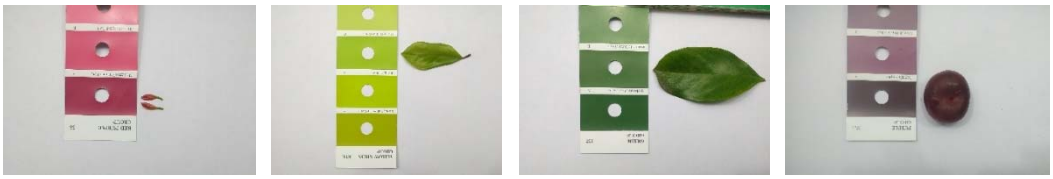
IC342298-1

IC342301-3

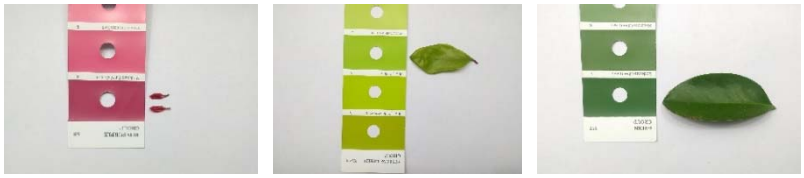
IC342304-1

IC342306-1

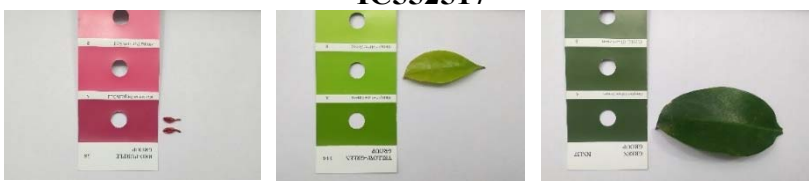
IC342319-1



IC342319-2



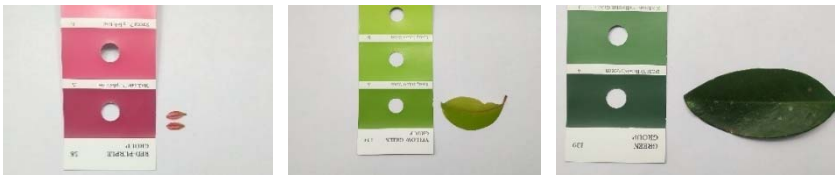
IC552517



IC552523-1

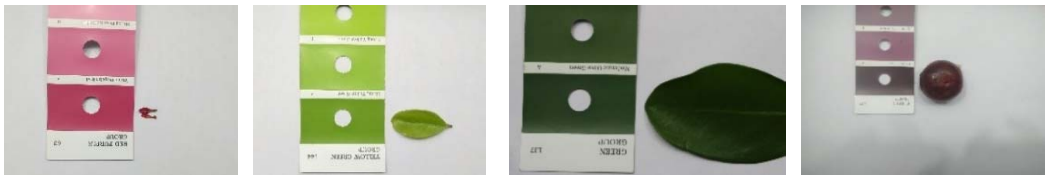
IC552514-2

IC552513



IC552526-1

IC552522-2



IC552522-1

IC552528-3

Plate 4.13. Variation in leaf and fruit colour of kokum genotypes

Table 4.15. Fruit quality parameters of *Garcinia indica* genotypes

Genotypes	Moisture (%)	Titratable acidity (%)	Total sugars (%)	Total soluble solids (^{0}B)	Anthocyanin content (g/100g)
ACC.FSC-1	85.78^{bc}	4.48^{a}	5.32^{ef}	7.50^{def}	25.01^{a}
ACC.FSC-3	84.33^{de}	3.01^{f}	4.32^{f}	5.65^{f}	12.87^{f}
ACC.FSC-6	84.96^{cd}	3.12^{def}	5.21^{ef}	8.00^{de}	16.48^{e}
ACC.FSC-7	83.55^{ef}	3.67^{bc}	6.14^{cdef}	8.50^{d}	18.72^{de}
ACC.FSC-8	85.28^{bcd}	3.96^{ab}	5.32^{ef}	8.05^{de}	21.67^{bc}
ACC.FSC-9	90.35^{a}	3.64^{bcd}	6.64^{bcde}	8.70^{d}	23.21^{ab}
IC136687-2	82.91^{f}	3.22^{cdef}	7.74^{bc}	10.80^{bc}	19.74^{cd}
IC136687-3	85.26^{bcd}	2.33^{g}	8.46^{b}	12.75^{ab}	21.36^{bcd}
IC342296-1	85.21^{bcd}	3.68^{bc}	4.67^{f}	6.40^{ef}	20.99^{bcd}
IC342319-2	86.35^{b}	3.27^{cdef}	5.75^{def}	7.15^{def}	19.42^{cd}
IC552522-2	85.08^{bcd}	3.26^{cdef}	6.93^{bcde}	8.90^{cd}	21.75^{bc}
IC552522-1	86.36^{b}	3.07^{ef}	7.67^{bcd}	10.90^{bc}	11.13^{f}
IC552528-3	89.50^{a}	3.58^{bcde}	10.61^{a}	14.15^{a}	24.84^{a}
C.V. (%)	0.711	7.476	13.792	10.349	6.637

Superscripts with same letters in column represents no significant

4.8.6. Profiling of bioactive compounds

The organic acids profiling using kokum rind extract was done through liquid chromatography mass spectrometry (LCMS) analyser. The profiling of kokum rind from the nine genotypes exhibited eleven compounds. The total bioactive compounds in the genotypes varied from 47.12 mg/g (IC342319-2) to 759.29 (ACC.FSC-1). The findings of the bioactive compounds identified in kokum rind are presented in Table shown the presence of 11 organic acids. The concentration of each organic acid present in the rind is tabulated in the Table 4.16.

There was significant difference was observed for all the constituents in genotypes except lactic acid. Among the different organic acids, hydroxy citric acid (HCA) was more predominant, which comprises about 1.95 mg/g to 655.40 mg/g. Among the different genotypes, ACC.FSC-6 (655.40 mg/g) had exhibited highest HCA followed by ACC.FSC-1 (442.80 mg/g). Whereas lowest HCA was recorded in genotypes IC552528-3 (1.95 mg/g), it was on par with IC552522-2 (8.67 mg/g), IC342319-2 (4.18 mg/g). Citric acid found to be significantly highest in ACC.FSC-7 (388.12 mg/g), it was closely followed by IC13687-2 (339.41 mg/g). Lowest was in IC552528-3 (1.59 mg/g). Malic acid content was significantly highest in IC13687-2 (51.12 mg/g) followed by ACC.FSC-7 (44.89 mg/g). The lowest was in IC13687-3 (7.28 mg/g). Succinic acid was rich in genotype ACC.FSC-9 (66.89 mg/g) which was closely followed by ACC.FSC-1 (58.04 mg/g). The lowest succinic acid was recorded in ACC.FSC-6 (5.87 mg/g). The kokum genotypes had comprised of medium contents of malonic acid (0.28 to 11.85 mg/g), maleic acid (0.01 to 5.90 mg/g), fumaric acid (0.01 to 5.33 mg/g), shikmic acid (0.30 to 0.79 mg/g), pyruvic acid (0.26 to 0.72 mg/g), tartaric acid (0.02 to 0.17 mg/g) and very low lactic acid (0.01 mg/g).

Table 4.16. Organic acid constituents of kokum genotypes

Genotypes	Lactic acid	Pyruvic acid	Malonic acid	Maleic acid	Fumaric acid	Succinic acid	Malic acid	Tartaric acid	Shikmic acid	Citric acid	HCA
ACCFSC-1	0.01^{a}	0.36^{ab}	10.81^{b}	0.86^{e}	1.06^{e}	58.04^{b}	34.56^{d}	0.15^{a}	0.52b^{c}	210.08^{d}	442.8^{b}
ACCFSC-6	0.01^{a}	0.35^{ab}	0.30^{f}	0.02^{f}	0.02^{g}	5.87^{g}	23.34^{e}	0.02^{c}	0.34^{e}	35.26^{e}	652.52^{a}
ACCFSC-7	0.01^{a}	0.28^{b}	5.45^{c}	1.41^{d}	1.76^{d}	36.60^{c}	44.89^{b}	0.08^{b}	0.32^{e}	385.6^{a}	259.38^{d}
ACCFSC-9	0.01^{a}	0.31^{b}	4.05^{d}	0.64^{e}	0.65^{f}	66.89^{a}	41.12^{c}	0.09^{b}	0.35^{e}	243.26^{c}	392.94^{c}
IC342319-2	0.01^{a}	0.39^{ab}	0.29^{f}	3.53^{b}	3.23^{b}	12.76^{e}	20.19^{f}	0.09^{b}	0.47^{cd}	1.96^{h}	4.18^{f}
IC552522-2	0.01^{a}	0.31^{b}	0.39^{f}	5.66^{a}	4.99^{a}	6.58^{f}	21.33^{ef}	0.04^{c}	0.55^{b}	13.26^{g}	8.67^{f}
IC552528-3	0.01^{a}	0.41^{ab}	11.86^{a}	5.88^{a}	5.25^{a}	12.33^{e}	20.11^{f}	0.09^{b}	0.75^{a}	1.64^{h}	1.96^{f}
IC136687-2	0.01^{a}	0.55^{a}	0.44^{f}	3.59^{b}	2.67^{c}	9.82^{ef}	51.12^{a}	0.04^{c}	0.39^{de}	339.41^{b}	142.68^{e}
IC136687-3	0.01^{a}	0.32^{ab}	1.28^{e}	2.7^{c}	2.71^{c}	22.99^{d}	7.28^{g}	0.04^{c}	0.41^{de}	24.41^{f}	3.34^{f}

Superscripts with same letters in column represents no significant

Hierarchial clustering analysis was performed to understand the relationship among the nine kokum genotypes based on the amount of organic acids present in it. Dendrogram was obtained by cluster analysis performed based on the organic acid constituents of nine kokum genotypes and derived into five clusters (Fig. 4.1). Cluster I which composed of four genotypes namely, IC342319-2, IC552528-3, IC552522-2 and IC136687-3. Cluster II consists of two genotypes such as ACC.FSC-1 and ACC.FSC-9. Cluster III, cluster IV and cluster V consists of one genotype each. The genotypes fall under same cluster exhibit more similarity in organic acid constituents.

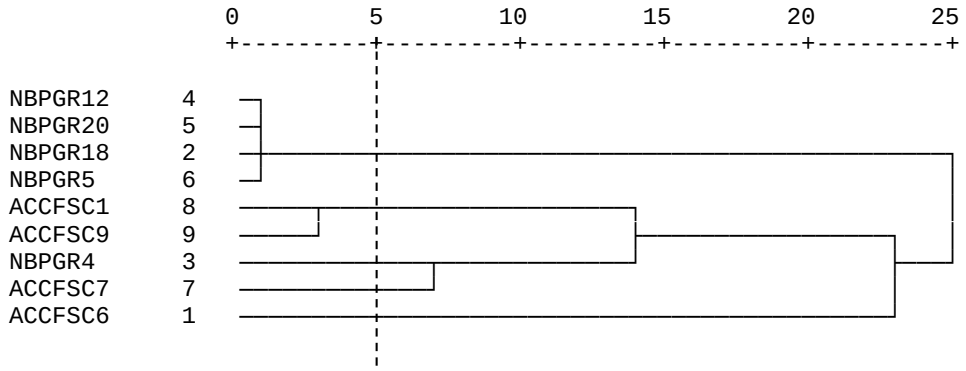


Fig. 4.1. Hierarchial analysis of the organic acid constituents of kokum genotypes based on the rescaled distance using the average linkage between genotypes.

The principal component analysis (PCA) was derived for the organic acid constituents of kokum fruit rind. The findings highlighted that there was high variability in the bioactive compounds of kokum fruits with cumulative variance. The PCA constructed for the first two principal axes (Dim 1 and Dim 2) exhibited 63.03 per cent of the total cumulative percentage of variance. The axes Dim 1 consists of 40.20 per cent of the total variance and Dim 2 consists of 23.10 per cent of the total variance.

The PCA distinguished distribution of organic acid constituents in different principal components. The first axes comprised of Hydroxycitric acid, citric acid, malic acid, succinic acid. These characters also had positive correlation with first principal components (Fig. 4.3). Shikmik acid, fumaric acid and maleic acid had negatively correlated with first principal components. The second principal components had

positive correlation with malonic acid and tartaric acid. Principal component analysis III had positive correlation with lactic acid and pyruvic acid.

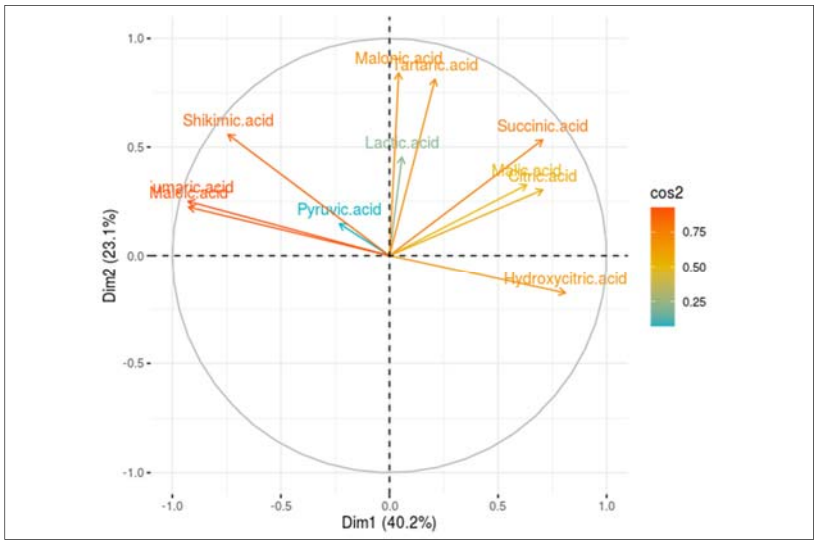


Fig. 4.2. Distribution of organic acid constituents from nine kokum genotypes in the different principal components

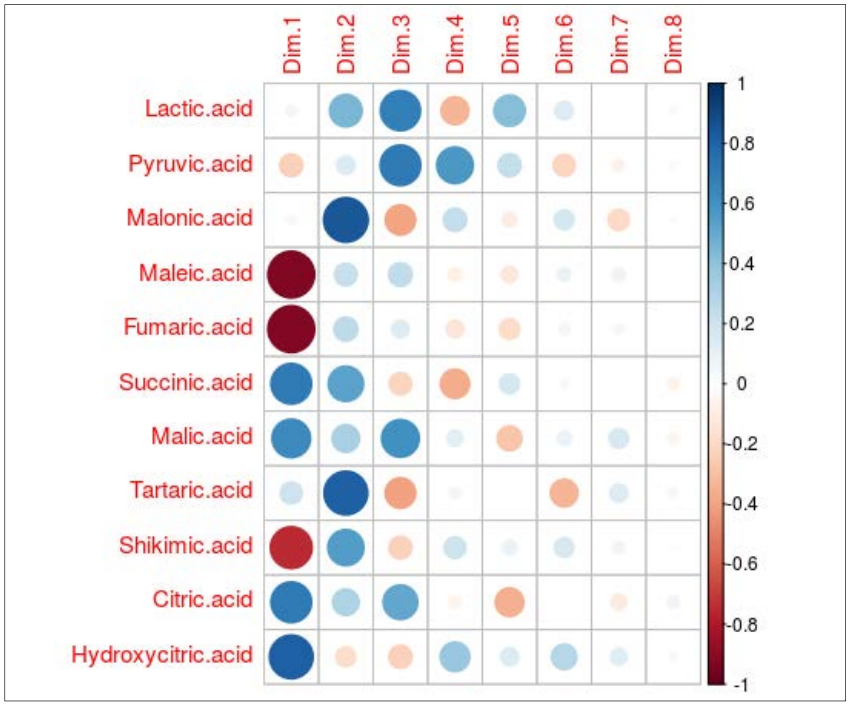


Fig. 4.3. Correlation plot of organic acids vs principal components

4.9. Incidence of pests and diseases

During the period of study, incidence of pests and diseases were observed regularly in kokum genotypes. There were no serious cause of pests and diseases in kokum tree. Fruit fly damage was recorded in all the genotypes, which attacked on ripened kokum fruits. leafhopper incidence was recorded, it causes damages to the leaves and also secretions of leaf hopper was observed. Sooty mould incidence was noticed on the secretion of leaf hoppers. No serious disease incidence was found to be recorded in kokum.

Incidence of pests and diseases observed in kokum

Sl. No.	Pest	Disease
1.	Fruit fly	Sooty mould
2.	Leaf hopper	

Leaf hopper

Sooty mold

Plate 4.14 Pest and disease incidence in kokum

DISCUSSION

5. Discussion

Kokum (*Garcinia indica* (Thouars) Choisy) is one of the important less exploited perennial fruit trees valued for its rind, which is well known for its antioxidant as well as medicinal properties. Kokum is native of India, distributed from konkan region of Maharashtra to Northern parts of Kerala in the Western Ghats region of India. Department of Fruit Science and ICAR-NBPGR Regional Station have collected the germplasms from its native habitat and conserved as ex situ in the field gene bank. In the present investigation, kokum genotypes were studied comprehensively for reproductive biology and fruit, yield and quality parameters. Among the select genotypes, 12 were females, 15 were males and one genotype was bisexual. In the study, phenological growth stages, floral characters, growth, yield and quality parameters were recorded. The result obtained from the study are discussed sequentially in this chapter.

5.1. Phenological studies

In order to recognising and elaborating each growth stages of a crop species is very important for developing the schedule of management practices, germplasm characterisation, crop improvement programmes and for analysing the impact of climate change on growth and development of crop (Chmielewski, 2003; Kishore *et al*. 2017). The described phenological stages could be utilized for sampling the appropriate growth and developmental stages of crop plants in the field experiments. Articulating the scale which consists of even minute details of crop growth characters is precisely described in the developmental stages. The application of common developed scale like extended BBCH is based on decimal coding system, which helped in designing crop growth specific phenological scale in other crops. The coding system which is been included in the scale is highly useful for researchers and crop management practitioners to easily remember the stages. In the present study for the first time, unique phenological growth stages have been defined as well as described in the kokum and modified three digits extended BBCH scale has been proposed to fit in the growth features of kokum.

A total of seven principal growth stages have been in identified in kokum. Apart from primary stages, a total of 35 secondary stages were elaborated and the coding was

done for each mesostage as well as secondary growth stages in kokum. The duration to complete each stage were also recorded for each principal growth stages. The principal growth stage 0- the vegetative bud development recorded 17 days, principal growth stage 1- the leaf development stage took 48 days, principal growth stage 3- the shoot development extended for 65 days, principal growth stage 5- the reproductive bud development took 32 days, principal growth stage 6- flowering lasted for 11 days, principal growth stage 7- which represented the fruit development had the longest duration of 103 days and principal growth stage 8- the fruit maturity and ripening took 5 days.

The phenological growth stages finds its application in crop improvement programmes. Understanding reproductive stages is very important for planning proper hybridization programme in polygamodieocious tree species like kokum. In kokum flowering could be seen during cool seasons in the month of 2nd week of October to January. During the initial days of flowering, male flowers of both type (2nd week of October to January) dominate followed by female as well as bisexual flowers (last week of October to January). The correlation between weather data and phenological growth cycle helps to understand the relationship between the timing of crop growth events affected by rainfall, temperature and other weather parameters. Previously scientists have reported phenological studies in other crops like in mangosteen (Awachare and Upreti, 2019), in mango (Delgado *et al*., 2011), in avocado (Alcaraz *et al*., 2013), in pineapple (Zhang *et al*., 2016) and in ber (Krishna, 2018).

5.2. Flower characters

The reproductive behaviour of kokum is very complex and the studies on floral biology of kokum are limited. The study on floral biology may helpful in the future crop improvement as well as development of hybrids.

The sexual form of kokum was described multiple forms by various scientists and it was mentioned as dioecious (Kadam, 2012), trioecious (Joseph, 2014) and polygamodioecious (Dike *et al*., 2020).

In present study, kokum trees were observed as polygamodioecious with the presence of female trees (12 genotypes), male trees (15 genotypes) and bisexual tree

(one genotype). The frequency of distribution of sex form is presented in Fig 5.1. In male flowers, two types were present, among the evaluated kokum genotypes, type I male flowers were recorded in fifteen genotypes, these male flowers were devoid of pistillode whereas male type II flowers were recorded in eight genotypes, pistillodes were present in this type of male flowers. The findings are supported by previous works of Dike *et al*. (2020) who reported the presence of three types of plants in kokum; plants with no fruits were considered as males, plants with more yield were functional females and plants with less yield were bisexuals. Among the select 29 genotypes, no variation was recorded with respect to position of inflorescence. In all the genotypes position of inflorescence was found to be both terminal and axillary, in the previous or current season of shoots. The colour of calyx and corolla play a pivotal role in insect pollination. As coloured petals and sepals could attract insects. In the case of kokum, colour of petals and sepals had no big role because of the wind pollination. The colour of calyx in kokum genotypes varied from greenish yellow to yellowish green. Most of the genotypes exhibited yellowish green colour calyx. The corolla colour varied from yellow to yellowish orange. The genotypes were dominate in yellowish orange colour corolla. In the past Kandasamy *et al*. (2015) reported green coloured sepals and yellowish coloured petals in *Garcinia imberti*.

Kokum genotypes exhibited free nature of stamens in male as well as bisexual flowers. Stigmatic rays were tuberculate type. The stigmatic rays in female flower varied from eight to ten in number, in case of bisexual flower stigmatic rays are commonly four in number. Kokum genotypes were differed significantly with respect to all quantitative flower characters except number of stigma per flower. Length of flower varied from 3.48 to 6.64 mm. breadth of flower varied from 4.78 to 8.05 mm. female flowers were sessile or very small pedicel present. In male and bisexual flowers, pedicels ranged from 3.41 to 5.73 mm. Female trees produced flowers as solitary and rarely produced in group of two to three, male and bisexual trees produced flowers in cluster, it ranged from group of three, four and more than four. Significantly the highest number of stamens per flower observed in genotype ACC.FSC-5. Number of stamens per flower varied from 25.90 to 41.60. Staminodes were observed only in female flower, it varied from 3.70 to 11.80. In male genotypes, number of flowers per 0.25 m^2 varied

from 10.90 to 25.20. In case of female genotypes, number of flowers ranged from 13.40 to 18.50. Bisexual genotypes produced 15.40 number of flowers per 0.25 m^2. These genotypes were collected from their epic centre natural habitat mainly from Mangalore, Uttara Kannada, Dakshin Kannada districts of Karnataka and Kasaragod district of Kerala. In the previous study, Thatte and Deodhar (2012), Devi *et al*. (2013) and Joseph and Murthy (2014) studied the flower characteristics of kokum under different growing conditions.

The time of anthesis, anther dehiscence, and stigma receptivity are the key factors to be studied thoroughly. These factors play an important role in planning for hybridization programme in crop species. In the present study, time of anthesis in all types of flowers (male, female and bisexual) was observed from 19.30 hr and continued up to 21.00 hr. The peak time of anthesis in male (48 %), female (53 %) and bisexual (52 %) could be seen from 19.30 hours to 20.00 hours (Fig. 5.2). Anther dehiscence in male and bisexual flowers were found to start simultaneously at 19.00 hours and continued till 20.30 hours. The peak period of anther dehiscence in male (54 %) and bisexual flower (52 %) was at 19.30 hour (Fig. 5.3). The maximum receptive condition of stigma was found to be occurred at the time of anthesis and it remained receptive for about another 12-14 hours. The peak period of stigma receptivity in female (53 %) and bisexual flower (52%) found to be at 20.00 hour. In the past, Karnik and Gunjate (1984) reported that anthesis time in kokum from 06.00 hour to 08.00 hour, the present result was contradictory to the very early report from the scientists. They also reported that anther dehiscence in kokum occurs at 15 to 20 minutes before the anthesis and the stigma receptivity was maximum at the time of anthesis, these findings from the previous work supports the present study outcome. In *Garcinia gummi-gutta*, time of anthesis in male and female flowers was recorded from 16.30 hour to 18.30 hour (Aswathi *et al*., 2018). Sherly (1994) reported that *Garcinia cambogia* flowers shed their anther before opening of the flower. Further female flowers were observed for their stigma receptivity, the receptive condition of stigma was recorded 12 hours before the time of anthesis.

In the present research, pollen morphology of male flowers (male type I and male type II) and bisexual flowers were found to be of same size. In case of pollen

grains of male flower type I were found to be round in shape and on the surface of pollen many apertures present in a concentric ring. In each direction of the apertures, white scars could be seen. These found to be ornate in nature. Male type II pollens were round and inaperturate, and exine was coarse and a typical aperture patterns. Bisexual pollens were found to be round to elongated shape with three equally divided apertures from the polar region. Exine was coarsely granulated. In the past, Dike *et al*. (2020) studied pollen morphology of different sex of kokum. They reported that kokum pollens were spheroidal in shape and also other similar characters observed in the present results. Aswathi *et al*. (2018) reported that pollen grains of *Garcinia gummi-gutta* were spherical in shape and pollens measured about 24.60 µm diameter. Acetocarmine (one per cent) test resulted into 84.07 to 98.49 per cent of pollens were found to be viable in male and bisexual flowers of kokum. In the present study it was observed that a very high percentage of pollen viability. Dike *et al*. (2020) reported that a medium range of pollen viability in kokum male flowers. This difference could be due to method followed for testing of pollen grains, stage of selection of flower as well as climatic conditions. Rajkumar *et al*. (2017) followed two different method of pollen viability test in *Garcinia imberti*, triphenyl tetrazolium chloride (0.2 %) revealed 89.16 per cent of pollen viability whereas acetocarmine (one per cent) resulted into 84.33 per cent of pollen viability.

To understand the cross pollination as well as compatibility between different types of flowers in kokum. Controlled pollination was done in different combinations. Female tree as receptors and male (type I and type II flower) and bisexual tree as donors. Female trees crossed with type II and bisexual flowers recorded 100 per cent fruit set. Female trees crossed with type I male flower found only about 60 per cent fruit set. Selfing of bisexual flowers resulted into 50 per cent fruit set. Female flowers without pollination found no fruit set.

Variation in the fruit shape was observed in different sex form. Female flowers crossed with male pollens, the produced fruits were round in shape whereas female trees crossed with bisexual flowers fruits were developed into elongated shape with beak. In case of bisexual flowers on selfing, fruits were produced in elongated shape with beak. In case of bisexual flowers on selfing, fruits were produced in elongated shape with

beak. Elongated fruit shape with beak appearance could be due to influence of bisexual flowers pollen. Further confirmation study may be necessary in these aspects. Dike *et al*. (2019) reported that artificial pollination resulted into 25 to 35 per cent of fruit set in kokum.

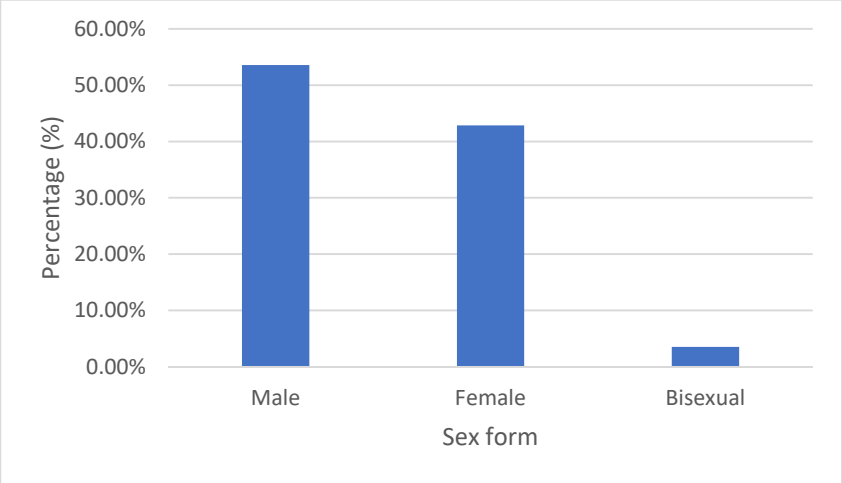


Fig. 5.1. Frequency distribution of sex form among the kokum kokum genotypes

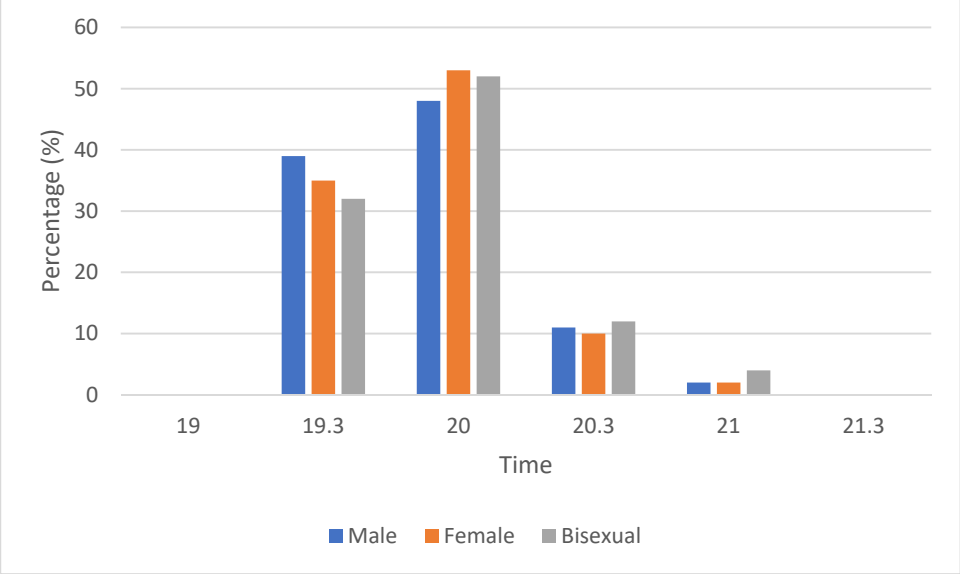


Fig. 5.2. Anthesis time in different types of kokum flowers

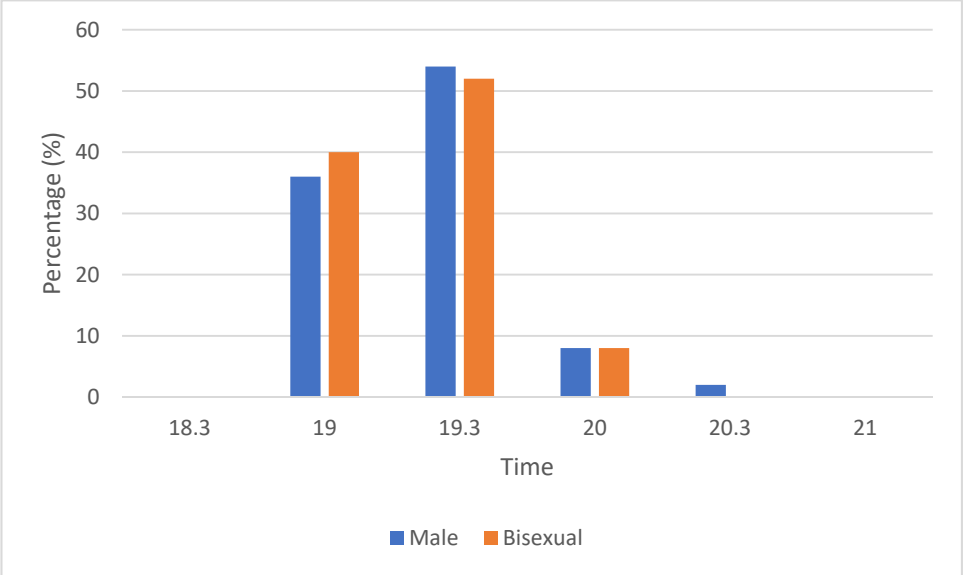


Fig. 5.3. Anther dehiscence in different types of kokum flowers

5.2. Morphological characters of tree

In the natural habitat, kokum trees have wide range of variation for morphological characters. Similarly, such variations had recorded in the genotypes selected for the study. These genotypes were collected from its native habitat might be one of the reasons for such morphological variations. Canopy volume is very important as it determines the yield of trees.

Among the genotypes evaluated, IC136687-1 had the highest height (15.60 m) and the lowest plant height was in IC552513 (5.20 m). The highest plant girth was recorded innACC.FSC-1 (156.88 cm) and the lowest plant girth was in IC552522 (25.10 cm). The coefficient of variation with respect to plant girth was 0.36 per cent. Canopy volume is one of the important criteria that provides bearing area as well as harnessing the sunlight. Canopy spread of the trees East to West direction varied from 3.20 m (IC552513) to 8.10 m (ACC.FSC-4). Canopy spread from North to South direction varied from 3.30 m (IC552513) to 9.42 m (ACC.FSC-9). Canopy volume was highest in ACC.FSC-9 (1030.01 m^3) and lowest was in IC552513 (57.12 m^3). In the present study, genotypes are collected and planted in different occasions. Though these genotypes are stabilized in bearing, the age factor may also influence the growth of the

plant. Apart from the age, the growing environment and acclimatization to the new growing conditions also influence plant morphology. Kapatia (2019) observed variation in morphological characters of kokum genotypes under Thrissur, Kerala conditions. Patil *et al*. (2005) reported that a huge variation in canopy spread in variety Konkan Amrita under Konkan region of Maharashtra.

5.3. Leaf characters

The photosynthetic efficiency of plants determined by leaf area of the plant. It is directly correlated with the plant yield. In the present study, significantly the highest leaf length was recorded in IC342319-1 (10.24 cm) and the lowest was in IC552522-1 (7.57 cm). Significantly the highest breadth was recorded in ACC.FSC-4 (4.78 cm) and the lowest was in IC342304-1 (3.18 cm). leaf area was significantly highest in ACC.FSC-4 (34.53 cm^2) and the lowest was in IC342304-1 (19.87 cm^2). In the previous study Raorane (2003) recorded the leaf length (10.24 to 12.81 cm), leaf breadth (3.84 to 4.58 cm) and leaf area (27.70 to 40.89 cm^2) in kokum genotypes.

5.5. Fruit and yield parameters

Significant difference was recorded with respect to fruit characters among the yielding genotypes. Significantly the highest fruit weight was recorded in ACC.FSC-6 (40.14 g) and it was on par with IC342319-2, ACC.FSC-7 and IC342296-1. Significantly the highest fruit volume was recorded in ACC.FSC-6 (45.60 cm3) and lowest fruit volume was in ACC.FSC-3 (15.10 cm3). Significantly the highest fresh and dry weight of rind were recorded in genotypes ACC.FSC-3. Significantly highest rind to seed ratio was recorded was recorded was recorded in ACC.FSC-3 (1.77) and the lowest was in IC136687-2 (0.81). Significantly the highest rind thickness was recorded in IC552522-3 (3.41) and the lowest rind thickness was in IC552522-1 (2.46). The weight of the rind and rind thickness are of economic importance. The genotype with high rind weight and rind thickness could be given more importance in the crop improvement programme. In the past, Raorane (2003) and Niveditha (2013) also worked on fruit parameters of kokum. Kokum seeds are material for the propagation, it can directly used for seedling production as rootstocks. Seeds of kokum are also used for extraction of kokum butter. Kokum fruits comprises of varied number of seeds per

fruit (2.00 to 5.30). Significant difference was noticed among the genotypes for fresh seed weight (4.26 to 17.90 g).

The number of fruits per m^2 and number of fruits per tree are the two important criteria that could decide yield of the tree. The number of fruits per m^2 varied from 9.50 to 71.10. Significantly the highest number of fruits per m^2 was recorded in IC552528-3. Number of productive flowers per unit area is directly correlated with number of fruits per unit area. In the present study, IC552528-3 produced highest number of flowers per 0.25 m^2 and same genotype produced highest number of fruits per m^2. In the bisexual genotype ACC.FSC-3, the ratio of bisexual flowers to male flowers ratio was low, it resulted in minimum number of fruits per m^2. Number of fruits per tree varied from 252 to 2258. Fruit yield per tree varied from 3.07 to 60.64 kg. The genotype ACC.FSC-9 produced maximum number of fruits per tree. The coefficient of variation with respect to number of fruits per tree among the bearing genotypes was high. The genotype ACC.FSC-9 had the highest canopy volume (1030.01 m^3), the maximum canopy volume provided more bearing area. This might be the reason for higher number of fruits and fruit yield in the genotype ACC.FSC-9. The genotype ACC.FSC-3 produced minimum number of fruits per m^2 which resulted into less yield per tree. Though genotype IC552528-3 produced highest number of fruits per m2, due to the less canopy volume tree couldn't produce more yield per tree. In the past, Kapatia (2019), Hegde (2005) and Raorane (2003) reported the yield attributes of kokum under different growing conditions.

5.6. Variation in leaf and fruit colour

Variation in leaf and fruit colour at different growth stages were determined using RHS colour chart. The high variation was recorded with respect to young flush, developing leaf, matured leaf and ripened fruits. In the past, Kapatia (2019) reported variations in the quantitative parameters of kokum genotypes.

5.7. Quality parameters

Kokum genotypes differed significantly for all biochemical characters except for moisture content. The biochemical composition of fruit rind is intrinsic parameter which is mostly depend on genetic character of the mother tree and growing condition

may also influence these parameters. As regard to kokum, biochemical compositions of fruit rind are very important. Based on the compositions it can be utilized in preparation of different value added products. From the study, significantly lowest titratable acidity was recorded in ACC.FSC-5 (2.30 %). Total sugars were ranged from 4.32 to 10.60 per cent. Total soluble solids were ranged from 5.65 to 14.15 ºBrix. Patil *et al*. (2008) evaluated kokum genotypes for processing value under Uttara Kannada district of Karnataka and they described those genotypes low in acidity and high in total sugars as well as TSS were preferred for value addition like kokum syrup. The content of titratable acidity along with total sugars categorise the genotypes into sweet or sour type. In the previous studies, Raorane (2003), Patil *et al*. (2005), Bornali (2015) worked on quality parameters of *Garcinia* species. Kokum is rich source of anthocyanin which has very high demand in export market as natural colourant. In the present investigation, anthocyanin content in kokum rind ranged from 11.13 to 25.01 mg per 100 g. Significantly highest anthocyanin content was recorded in ACC.FSC-1 (25.01 mg/100g). from the study, it was observed that sweet kokum genotypes were poor in anthocyanin content visa-visa sour kokum genotypes were rich in anthocyanin content. Vasundhara *et al*. (2016) evaluated fresh fruit rind, dry fruit rind and kokum syrup for anthocyanin content and they opinioned that kokum dry fruit rind was rich in anthocyanin content followed by fresh fruit rind and kokum syrup. The results of previous study by Korikanthmath and Desai (2005) and Niveditha (2013) are accordance with the present study. Based on the biochemical composition, kokum genotypes were ranked using the DMRT analysis (Table 5.1). Genotypes IC552528-3 and IC336687-3 had significantly high TSS and low acidity were ranked as superior quality genotypes and these genotypes can be utilized for value addition.

Table 5.1. Ranking of kokum genotypes based on biochemical composition

Genotypes	Moisture (%)	Titratable acidity (%)	Total sugars (%)	Total soluble solids (^{0}Brix)	Anthocyanin content (g/100g)	Average	Ranking
ACC.FSC-1	2.5	7	5.5	7.5	1	4.7	8
ACC.FSC-3	4.5	2	6	6	6	4.9	9
ACC.FSC-6	3.5	4.5	5.5	4.5	5	4.6	7
ACC.FSC-7	5.5	5.5	9	4	4.5	5.7	11
ACC.FSC-8	3	6.5	5.5	4.5	2.5	4.4	6
ACC.FSC-9	1	7.5	7	4	1.5	4.2	4
IC136687-2	6	7	2.5	2.5	3.5	4.3	5
IC136687-3	3	1	2	1.5	4.5	2.4	1
IC342296-1	3	5.5	6	5.5	4.5	4.9	9
IC342319-2	2	7	7.5	7.5	3.5	5.5	10
IC552522-2	3	7	7	3.5	2.5	4.6	7
IC552522-1	2	2.5	4.5	2.5	6	3.5	3
IC552528-3	1	9	1	1	1	2.6	2

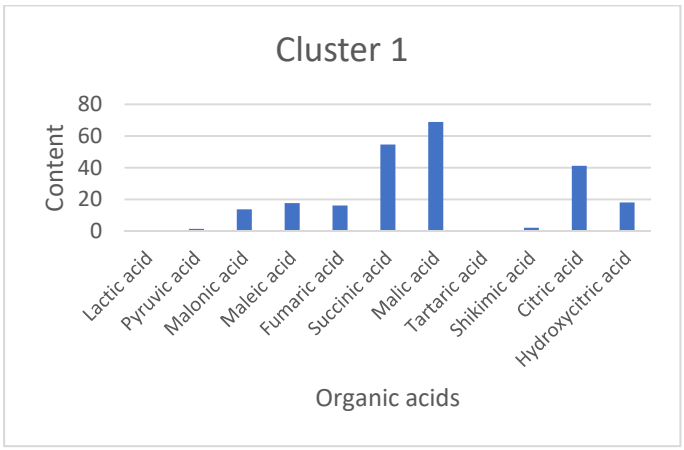
The LCMS analysis of nine kokum genotypes revealed that eleven major constituents are present in kokum rind. The total bioactive compounds in the genotypes ranged from 47.12 to 759.29 mg per g. Among the different organic acids, hydroxycitric acid was predominant (1908.48 mg/g) followed by citric acid (1254.89 mg/g), malic acid (263.48 mg/g) and succinic acid (231.90 mg/g). Significantly the highest HCA was recorded in ACC.FSC-6 (655 mg/g) followed by ACC.FSC-1 (442.80 mg/g). HCA got the properties of antiobesity, anticholesterol and UV protecting activities. Genotypes rich in HCA can be utilized by the pharmaceutical industries. Citric acid was second predominant organic acid present in kokum rind, it ranged from 1.59 mg/g to 388.12 mg/g. Significantly, highest citric acid was recorded in ACC.FSC-7. Citric acid is one of the flavouring agents and present in the many of the citrus fruits. It is also commonly used as food preservative, cosmetics and disinfectants. Malic acid content in kokum rind ranged from 7.28 to 51.12 mg per g. Significantly, the highest malic acid was recorded in IC13687-2. Malic acid is one of the flavouring agents. Malic acids commonly found in temperate like apple, peach, plum, apricot *etc*. Malic acid is commonly found in skin lotions which helps to remove dead skin cells and protect skin from adverse conditions. Similarly succinic acid also used in the skin lotions to get rid

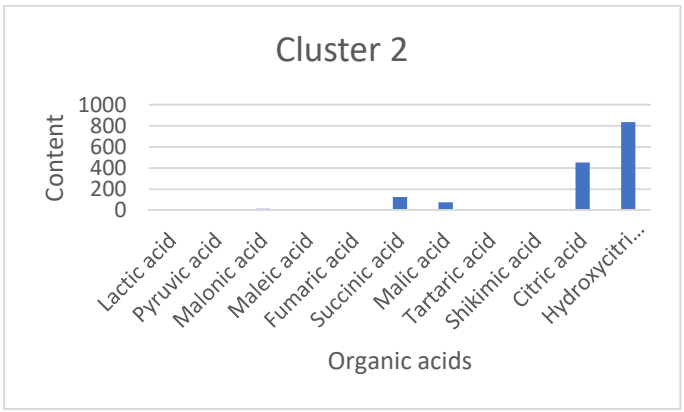
of dead skin cells. In the previous studies, Parthsarathy and Nandakishore (2014) and Jagtap (2015) studied on organic acid constituents of kokum.

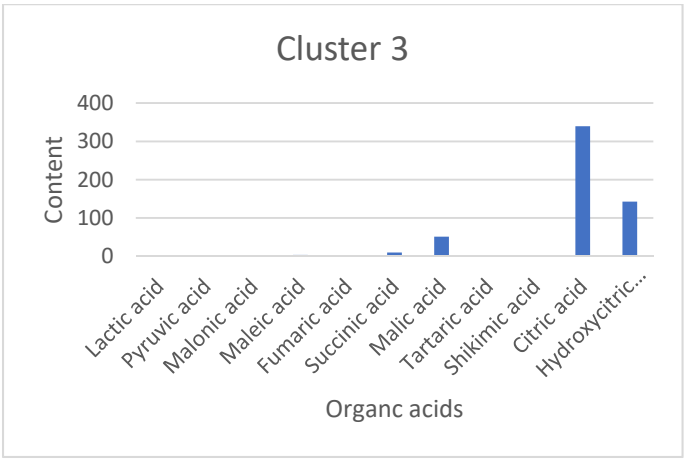
Cluster analysis of kokum genotypes based on the organic acids formed five different clusters (Fig. 5.4). Genotypes under same cluster exhibited more similarity in bioactive compounds. Cluster I consist of four genotypes namely, IC342319-2, IC552528-3, IC136684-3 and IC136687-3. Genotypes in this cluster were rich in malic acid and succinic acid. Genotypes of this cluster were consisted of medium content of citric acid and HCA. Cluster II consist of two genotypes such as ACC.FSC-1 and ACC.FSC-9. Genotypes under this cluster were rich in HCA as well as citric acid. Cluster III, IV and V consist of one genotype each. Genotype in cluster III was rich in citric acid and had below average content of HCA. Cluster IV had one genotype (ACC.FSC-7) which consisted of high citric acid as well as HCA. Genotype under cluster V had highest content of HCA and other bioactive compounds were low.

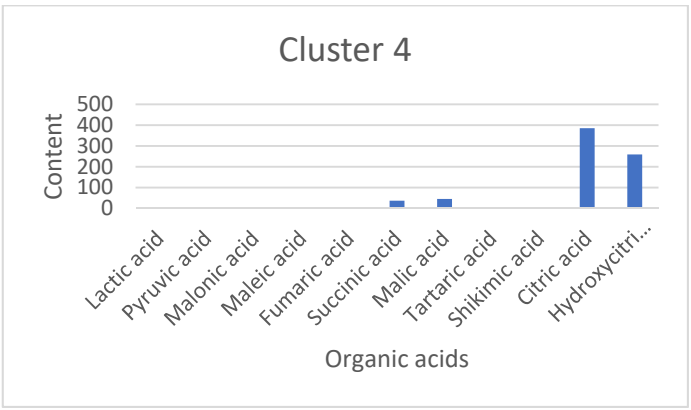
The principal component analysis distinguished the distribution of organic acid constituents into two axes, the first principal axes explained about 40.20 per cent of total variance and second principal axes displayed 23.10 per cent of total variance. HCA, citric acid, malic acid and succinic acid were positively correlated with first principal axes, whereas shikmik acid, fumaric acid and maleic acid were negatively correlated with first principal axes. Genotypes rich in HCA, citric acid, malic acid and succinic acid had less constituents of shikmik acid, fumaric acid and maleic acid. Tartaric and malonic acids were positively correlated with principal axes two.

The knowledge on variability of chemical constituents is key for identification of chemotypes which pay way for investigation on therapeutic potential genotypes. In the past Ashokkumar *et al*. (2020) assessed phytochemical diversity in essential oil of black pepper and they also revealed existence of chemotypes.


Cluster 1
Content
80
60
40
20
0
Lactic acid
Pyruvic acid
Malonic acid
Maleic acid
Fumaric acid
Succinic acid
Malic acid
Tartaric acid
Shikimic acid
Citric acid
Hydroxycitric acid
Organic acids


Cluster 2
Content
1000
800
600
400
200
0
Lactic acid
Pyruvic acid
Malonic acid
Maleic acid
Fumaric acid
Succinic acid
Malic acid
Tartaric acid
Shikimic acid
Citric acid
Hydroxycitri...
Organic acids


Cluster 3
Content
400
300
200
100
0
Lactic acid
Pyruvic acid
Malonic acid
Maleic acid
Fumaric acid
Succinic acid
Malic acid
Tartaric acid
Shikimic acid
Citric acid
Hydroxycitric...
Organc acids



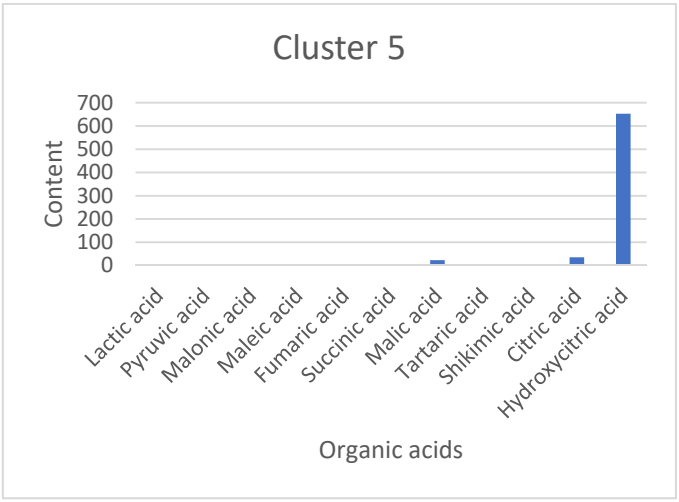


Fig. 5.4. Cluster mean analysis of bioactive compounds

SUMMARY

6. Summary

The present investigation entitled "Reproductive biology and evaluation of kokum (*Garcinia indica* (Thouars) Choisy) genotypes" was undertaken with the objective to study the phenology and reproductive biology as well as to evaluate the genotypes under humid tropical conditions of Kerala. The study was carried out at the Department of Fruit Science, College of Agriculture, Kerala Agricultural University, Vellanikkara, Thrissur during 2019 to 2021. The findings of the study are summarised in this chapter.

I. Phenological studies

Phenological parameters like vegetative, floral and fruit growth and development stages were recorded at different stages of crop growth.

In kokum, 7 principal growth stages and 35 secondary growth stages were identified and described according to the extended BBCH scale in kokum. The principal growth stage 0 (vegetative bud development) was observed throughout the year (major season- December to March) and recorded 15-20 days, the principal growth stage 1 (leaf development) was observed throughout the year (major season- December to March) and took 40-55 days, the principal growth stage 3 (shoot development) was observed throughout the year (major season- December to April) and extended for 60-70 days, the principal growth stage 5 (reproductive bud development) was observed from 2nd week of October to January and took 30-36 days, the principal growth stage 6 (flowering) was observed from November to January and lasted for 9-12 days, the principal growth stage 7 (fruit development) was observed from December to May and took 90-110 days and the principal growth stage 8 (fruit maturity) was observed from January to May and took 5-7 days to complete.

II. Floral characters

Variation was observed among the genotypes for qualitative and quantitative flower characters.

Among 29 genotypes selected for the study, 12 were females, 15 were males and one genotype was bisexual. The position of the inflorescence was found to be both

axillary and terminal in all the genotypes. The nature of stamens was found free in all the male and bisexual flowers. The tuberculate type of stigmatic rays was observed in all the observed female and bisexual flowers. The colour of the calyx varied from greenish yellow to yellowish green, while colour of corolla varied from yellow to yellowish orange. The pistillode was present in the type II male flower and was absent in the type I flower.

All the quantitative floral characters except number of stigma per flower showed significant variation among the kokum genotypes. The length (3.48 to 6.64 mm) and breadth of the flower (4.78 to 8.05 mm), and number of flowers per 0.25 m^2 (10.90 to 25.20) varied significantly among the kokum genotypes. Female flowers were sessile and male flowers were pedicellate. The number of stamens per flower varied from 25.9 to 41.6 and the highest number of staminodes ranged from 3.7 to 11.8.

The time of anthesis in all three types of flowers was observed from 19.30 to 21.00 hours and peak period of anthesis was from 19.30 to 20.00 hours. Stigma receptivity coincides with anthesis and it remained receptive for about 12 to 14 hours, and anther dehiscence occurred 25 to 30 minutes before the anthesis.

Both type I and type II male flowers had round shaped pollen, whereas bisexual flower pollens were elongated with prominent ridges on the surface. Pollens of all types of flowers were measured about 5 µm in diameter. High percentage of pollen viability was recorded, it ranged from 84.07 per cent to 98.49 per cent.

Female tree crossed with type II male and bisexual flowers recorded 100 per cent fruit set, female tree crossed with type I male recorded 60 per cent fruit set, whereas bisexual on selfing recorded 50 per cent fruit set.

III. Evaluation of kokum genotypes

Variation was observed among the kokum genotypes for growth, fruit and yield parameters.

High variation was observed with respect to plant height (5.20 m to 15.60 m), girth of the plant (25.10 cm to 156.88 cm), canopy spread in East-West (3.20 to 8.10

m) and North-South (3.30 to 9.42 m) direction, and canopy volume (57.12 m^3 to 1030.01 m^3) among the kokum genotypes.

The kokum genotypes varied significantly for leaf length, leaf breadth and leaf area.

Significant difference was noticed for fruit characters *viz*., fruit weight (12.17 g to 40.14 g), fruit volume (15.00 ml to 45.60 ml), fresh weight of the rind (7.15 gm to 21.01 g), dry weight of rind (1.09 g to 3.07 g). Rind to seed ratio and rind thickness also exhibited significant differences among the genotypes.

Number of fruits per m^2 ranged from 9.50 to 71.10 and yield varied from 3.07 to 60.64 kg. Genotype, ACC.FSC-9 recorded highest number of fruits per tree and fruit yield per tree.

IV. Biochemical and bioactive constituents

Kokum genotypes differed significantly for biochemical parameters *viz*., moisture content (82.91 % to 90.34 %), titratable acidity (2.30 % to 4.47 %), TSS (5.65 ^{0}Brix to 14.15 ^{0}Brix), total sugars (4.32 % to 10.60 %) and the anthocyanin content (11.12 mg to 25.01 mg per 100 g). The genotypes, IC552528-3 and IC136687-3 had significantly high TSS and low acidity were considered as superior quality genotypes

The profiling of kokum rind for organic acids revealed the presence of eleven organic acids. The hydroxy citric acid was present predominantly, it ranged from 1.95 mg per g to 655.40 mg per g. The genotypes rich in HCA can be utilised by the pharmaceutical industries. The other acids present in kokum rind were citric acid, pyruvic acid, fumaric acid, succinic acid, shikmik acid, malic acid, maleic acid, malonic acid and tartaric acid. Cluster analysis of kokum genotypes based on organic acids formed five different clusters. The genotypes under cluster I were rich in malic acid and succinic acid. The genotypes falls under cluster II were rich in hydroxycitric acid (HCA) as well as citric acid. The genotypes in cluster III and cluster IV were rich in citric acid followed by HCA, whereas the genotype in cluster V was rich in HCA. Principal component analysis distinguished distribution of organic acid constituents into two axes. The axes first two principal components explained 63.03 per cent of total variance.

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REPRODUCTIVE BIOLOGY AND EVALUATION OF KOKUM (*GARCINIA INDICA* (THOUARS) CHOISY) GENOTYPES

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ABSTRACT OF THE THESIS

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Reproductive biology and evaluation of kokum (*Garcinia indica* (Thouars) Choisy) genotypes

Abstract

Kokum (*Garcinia indica* (Thouars) Choisy) is a valuable yet underutilized perennial fruit tree. It is gaining importance due to multifarious uses mainly in the preparation of pleasant and attractive beverages which have rich medicinal properties. Kokum is one of the native species exhibiting a complex nature of flowering and fruiting behaviour. In the present investigation, twenty nine genotypes of kokum maintained at College Orchard, Department of Fruit Science as well as ICAR-NBPGR, Regional Station, Vellanikkara were studied systematically for phenological growth stages, floral characters, growth, yield and quality parameters during the period 2019-2021.

In kokum, a total of 7 principal growth stages and 35 secondary growth stages were identified and described by using extended Biologische Bundesantalt, Bundessortenamt and Chemische Industrie (BBCH) scale (Meier, 2001). The principal growth stage 0- the vegetative bud development recorded 17 days, stage 1- the leaf development stage took 48 days, stage 3- the shoot development extended for 65 days, stage 5- the reproductive bud development took 32 days, stage 6- flowering lasted for 11 days, stage 7- which represented the fruit development had the longest duration of 103 days and stage 8- the fruit maturity and ripening took 5 days.

Among the twenty nine genotypes studied for floral characters, twelve genotypes were female, fifteen were male and one genotype produced bisexual flowers. Male flowers were of two types, type I male flowers were present in all the fifteen male trees, whereas type II male flowers were present in seven male trees. Variation was observed among the genotypes for qualitative characters of flower *viz*., position of inflorescence (axillary, terminal or both), calyx colour (greenish yellow to yellowish green), corolla colour (yellow to yellowish orange), nature of stamens (free in all male and bisexual flowers), presence of pistillode (present in type II male flower and absent in type I male flower), staminodes were observed in female flowers only and it ranged from 3.7 to 11.8 in number, stigmatic rays was tuberculate and it found

to be similar in all the female genotypes. The genotypes varied significantly with respect to quantitative characters of flowers such as number of flowers per m^2 (10.90 to 25.20), length (3.48 mm to 6.64 mm) and breadth (4.78 mm to 8.05 mm) of flower. Majority of the female trees produced solitary or in groups of two to three, whereas male and bisexual trees produced flowers in clusters. Female flowers were sessile or with small pedicels, whereas in male flowers, pedicel length ranged from 3.41 to 5.73 mm. The time of anthesis in all three types of flowers found to occur from 19.30 to 21.00 hours. The anther dehiscence in male and bisexual flowers were found to start simultaneously from 19.00 hours and it continued till 20.30 hours. Stigma receptivity was observed at the time of anthesis and it remained receptive for about 12 to 14 hours. Both type I and type II male flowers had round shaped pollen which measured about 5 µm diameter. The pollens of bisexual flowers were elongated with prominent ridges on the surface and showed same size as that of male pollen. Acetocarmine test revealed that 84.07 to 98.49 per cent of pollen were found to be viable in male and bisexual flowers of kokum. Controlled pollination was performed in different combinations, female tree as receptors and male (type I and type II flowers) as well as bisexual trees as donors for understanding pollination and fruit set behaviour in different sex form of kokum. Female trees crossed with type II male and bisexual flowers recorded 100 per cent fruit set. Female trees crossed with type I male flower recorded 60 per cent of fruit set, whereas bisexual flowers on selfing exhibited 50 per cent fruit set.

In the present study, variation was noticed among the kokum genotypes with regard to the growth and leaf characters. Fruit characters were recorded from thirteen bearing genotypes and all these genotypes were found to vary significantly for the fruit characters. Fruit weight ranged from 12.17 to 40.14 g, fruit volume varied from 34.22 to 45.60 cm^3, fresh weight of rind varied from 7.15 to 21.01 g, dry weight of rind ranged from 1.09 to 3.07 g, rind to seed ratio ranged from 0.81 to 1.77 and rind thickness varied from 2.46 to 3.41 mm. Genotype, IC552528-3 recorded maximum number of fruits per m^2 (71.10), ACC.FSC-9 had produced the highest number of fruits per tree (2258) and fruit yield per tree (60.64 kg).

Kokum genotypes differed significantly for all the biochemical parameters except moisture content. Moisture content ranged from 82.91 to 90.34 per cent, titratable acidity ranged from 2.30 to 4.47 per cent, total sugars varied from 4.32 to 10.60 per cent, TSS ranged from 5.65 to 14.15 ^{0}Brix and anthocyanin content varied from 11.12 to 25.01 mg per 100 g. Genotypes were ranked based on the biochemical composition of the fruit rind. Genotypes, IC552528-3 and IC136687-3 which had significantly high TSS and low acidity were ranked as superior quality genotypes and these genotypes can be utilised for value addition.

Based on the yield and quality parameters nine genotypes were selected for organic acid profiling of kokum rind. The Liquid Chromatography Mass Spectrometry (LCMS) analysis revealed that eleven major constituents are present in kokum rind. Total organic acids in the genotypes varied from 47.12 (IC342319-2) to 759.29 mg/g (ACC.FSC-1). Among the different organic acids, hydroxycitric acid (HCA) was predominant (1908.48 mg/g) followed by citric acid (1254.89 mg/g). Significantly the highest HCA (652.52 mg/g) was recorded in ACC.FSC-6. It has antiobesic, anticholesterol and UV protecting properties and hence, genotypes which are found rich in HCA can be utilised by the pharmaceutical industries.

Cluster analysis of kokum genotypes based on organic acids formed five different clusters. Genotypes under the same cluster showed more similarity in bioactive compounds. Principal component analysis distinguished distribution of organic acid constituents into two axes. The axes first two principal components explained 63.03 per cent of total variance. The knowledge on variability of chemical constituents has vital role in identification of chemotypes which pave way for investigation on therapeutic potential of genotypes.