

## Characterization of flower phenology in *Garcinia indica*

### **Abstract:**

Characterization flowering pattern in *Garcinia indica* was studied using different age groups. Flowering commenced in five-year-old trees (5YP) in all groups studied, followed by four-year-old trees. The first flowering was recorded during 3-4th week of November and continued for ten weeks among the plants. *G. indica* accessions with mixed sexuality were recorded where, the majority of bisexual flowers with female and male flower were there in the same plant. However, flower appearance started in 3rd week of November and extended up to December 3rd week and the maximum number of trees flowered in 1st week of December. Most of the accessions recorded 50 per cent flowering on 2nd and 3rd week of January. The duration of flowering varied from 8 to 10 weeks. Majority of the flowers were observed to be auxiliary and some of them were terminal. Traits such as petal length, sepal length, stamen length and style length did not exhibit significant differences among the accessions studied. However, pollen viability varied. Maximum per cent (88.33%) of viable pollen were recorded in 5YP-1 and minimum (82%) in 5YP-3 & 15 with the mean value of 45.68%. (Table 5&6). The stigma was receptive after the opening of the flower from 6:00 AM to 8:00 AM (Table 5-&6)

Keywords: Characterization, Flower, *Garcinia indica*

UNDE

## Introduction

*Garcinia* species are generally evergreen trees and shrubs which thrive well in high-rainfall areas of the tropics. Sexually the trees are dioecious and hence, largely are cross-pollinated. Morphological variability exhibited by the trees in forest ecosystems is very high and diversity is obvious for all observable traits. The fruits of *Garcinia* species are known to have anti-obesity properties which is largely owing to the presence of the compound called (-)-Hydroxycitric acid (HCA). This property made *Garcinia* species popular in the international and respective local markets. Other phytochemicals present in the species are isoprenylated xanthenes, a class of secondary metabolites with multiple reports of biological effects, such as antioxidant, pro-apoptotic, anti-proliferative, antinociceptive, anti-inflammatory, neuroprotective, hypoglycemic and anti-obesity (Han *et al.*, 2004, Mathew *et al.*, 2011 and Shivakumar *et al.*, 2013). The most popular species of the genus *Garcinia* is *Garciniamangostana*, which is commonly known as mangosteen and has been named as 'queen of tropical fruits' for its unique pleasant taste and visual appearance of a crown-like structure (Chinavat and Subadrabuddhe, 2004). The seeds and pericarps of the fruit have a long history of use in the traditional medicinal practices of the region, and beverages containing mangosteen pulp and pericarps are sold worldwide as nutritional supplements (Chinavat and Subadrabuddhe, 2004).

*Garcinia indica* (Choisy.), commonly known as *kokum*, *murugalu* and *punarpuli* is an evergreen tree with a conical crown shape. This species is cultivated on a small scale in coastal regions and hilly areas of the *Western Ghats* of India, which covers the *Konkan* region of Maharashtra, Goa, coastal and hilly areas of Karnataka and similar ecosystems of Kerala state. The *kokum* fruit is spherical in shape, purplish orange to pinkish-red in colour, fleshy and has an acidic flavour. The *kokum* has mainly two different types - red and yellow, particularly spread over the *Western Ghat* central region in Uttara Kannada district. Red *kokum* is the common type whereas, yellow *kokum* is a unique type of *kokum*, it is normally called '*bilimurughi*' (white *kokum* by native people) (Hegde, 2019). The *kokum* fruit has been used for culinary and medicinal purposes since the age-old days. The rind of the *kokum* fruit is used to make fresh juice which is a natural coolant (Nayak *et al.*, 2010).

The dried rind is used for cooking, as a substitute for tamarind and as a food preservative. The fatty substance present around the seed of the *kokum* is called *kokum* butter, and is extensively used in the cosmetic and pharmaceutical industries. The *kokum* seed contains a fair amount of oil which has its use in pharmaceutical and cosmetic industries. Some of the pharmaceutical activities of *kokum* fruit are anti-bacterial, anti-fungal, anti-ageing, neuroprotective, anti-ulcer, hyperglycemic, anti-obesity, anti-inflammatory and anti-oxidant activities (Milind *et al.*, 2013).

## **Material and Method**

The present study was carried out at the Department of Biotechnology and Crop Improvement, College of Horticulture, Bengaluru, UHS Campus G.K.V.K. Post, Karnataka state. Early growth dynamics of plants play a crucial role in their survival and establishment under field conditions. The details of the material used and the methods adopted during the study in the following four experiments are described in chapter.

Flower phenology, reproductive traits and yield-related observations were studied only in *Garcinia indica* as a few plants with flowering and fruiting were available. The relative plasticity for these traits was attempted to gain an insight into the kind of genetic diversity prevailing among the plants present in *ex situ* field gene bank. A set of 10 traits pertaining to flower and fruit yield were recorded during the years 2019-20 and 2020-21. The observations were recorded according to the Descriptor of *Garcinia* species by Bioversity International, Roam, Italy. The following observations were recorded.

### **Flower phenology traits**

**First flower bud appearance:** The time required for flower bud appearance since initiation was observed and recorded as the particular week of the month.

**Sexuality of the flower:** In each selected plant 20 flowers were randomly selected on different branches of a tree to know the sexuality of the flower and expressed as male, female or bisexual.

**Time of 50% flowering:** The time from flower initiation up to the end of flowering was recorded weekly and expressed as 50 per cent flowering.

**The span of flowering:** The observation of flowering was recorded from the time of initiation of the flower up to the last flowering time the observation was recorded at the particular week of the month

**Flowering habit:** In each selected plant 20 flowers were randomly selected on different branches to know the flowering habit and expressed as a flowering habit.

### **Scanning Electron Microscope analysis**

Male, female and bisexual flowers of *Garcinia indica* were studied for the ultra-fine structure of anther and ovary. The facilities at the Centre for Nanotechnology, University of Agricultural Science, Raichur were utilized. The flowers were cross-cut into two-half for anther and ovary size and anther surface and the sample was dried in the vacuum chamber of SEM for 15 min to remove the moisture from the flower. Later, the SEM images were recorded at 3,300x to 20,000x. The pollen image was scanned from the top of the flower and the stigma and ovary were scanned from the top.

### **Result**

In India, the genus *Garcinia* has a rich diversity with over 35 species spread across *Western* and *Eastern Ghats*. *Garcinia indica* locally known as *kokum*, are economically important to the local farmers as non-timber forest products (NTFP) both in the *eastern* and *western ghats* of India. In some regions of the *Western Ghats* of Maharashtra, Karnataka, Goa and Kerala farmers do take up systematic cultivation of these species as a source of additional income (Swami *et al.*, 2014). Characterization of *Garcinia indica* accession for growth and related traits revealed high level of variability.

### **Time of initiation of flower**

In general, most of the trees started flowering during 3<sup>rd</sup> week of December (Table1). Flower appearance started in 2<sup>nd</sup> week of November and extended up to December 3<sup>rd</sup> week and a maximum number of trees flowered in 1<sup>st</sup> week of December in the initial year of the study. Three plants started flowering during 3<sup>rd</sup> and 4<sup>th</sup> week of November, two plants started flowering during the 2<sup>nd</sup> week of December and one tree started in December during 2019-20. During the second year (2020-21) most of the trees started flowering in 3<sup>rd</sup> and 4<sup>th</sup> week of November.

Flower appearance started in 1<sup>st</sup> week of November and extended up to 4<sup>th</sup> week of November; one plant started flowering during 2<sup>nd</sup> week of November and 4 plants started flowering during 2<sup>nd</sup> week of December. Similarly, one plant started flowering on 1<sup>st</sup> and another plant started flowering on 3<sup>rd</sup> week of December (Table1).

UNDER PEER REVIEW

**Table1: Behavior of *G. indica* individual plant for flower-related traits**

Sl. No.	Plant Number	Age (Year)	Sexuality of the flower	Time of initiation of flower	Time of 50 % flowering	Span of flowering (Week)	Flowering habit
1	5YP-1	5	B	4 <sup>th</sup> Week Nov.	1 <sup>st</sup> Week Jan.	10	Auxiliary
2	5YP-2	5	F	3 <sup>rd</sup> Week Nov.	4 <sup>th</sup> Week Dec.	9	Auxiliary
3	5YP-3	5	F	3 <sup>rd</sup> Week Nov.	4 <sup>th</sup> Week Dec.	10	Auxiliary
4	5YP-4	5	F	1 <sup>st</sup> Week Dec.	2 <sup>nd</sup> Week Jan.	9	Auxiliary
5	5YP-5	5	B	3 <sup>rd</sup> Week Dec.	3 <sup>rd</sup> Week Jan.	10	Terminal
6	5YP-6	5	B	3 <sup>rd</sup> Week Dec.	3 <sup>rd</sup> Week Jan.	10	Auxiliary
7	5YP-7	5	F	4 <sup>th</sup> Week Nov.	4 <sup>th</sup> Week Dec.	9	Auxiliary
8	5YP-8	5	F	2 <sup>nd</sup> Week Dec.	2 <sup>nd</sup> Week Jan.	8	Auxiliary
9	5YP-9	5	B	4 <sup>th</sup> Week Nov.	2 <sup>nd</sup> Week Jan.	9	Auxiliary
10	5YP-10	5	F	2 <sup>nd</sup> Week Dec.	2 <sup>nd</sup> Week Jan.	10	Auxiliary
11	5YP-11	5	B	2 <sup>nd</sup> Week Nov.	2 <sup>nd</sup> Week Jan.	9	Terminal
12	5YP-12	5	F	4 <sup>th</sup> Week Dec.	4 <sup>th</sup> Week Jan.	10	Terminal
13	5YP-13	5	B	3 <sup>rd</sup> Week Dec.	3 <sup>rd</sup> Week Jan.	10	Auxiliary
14	5YP-14	5	F	3 <sup>rd</sup> Week Dec.	3 <sup>rd</sup> Week Jan.	9	Auxiliary
15	5YP-15	5	B	3 <sup>rd</sup> Week Nov.	1 <sup>st</sup> Week Jan.	9	Terminal

5 YP = five year old plan

**Table2: Behavior of *G. indica* Choisy individual plant for flower related traits**

Sl. No.	Plant Number	Age (Year)	Sexuality of the flower	Time of initiation of flower	Time of 50 % flowering	Span of flowering (Week)	Flowering habit
1	5YP-1	6	B	3 <sup>rd</sup> Week Nov.	4 <sup>th</sup> Week Dec.	9	Auxiliary
2	5YP-2	6	F	4 <sup>th</sup> Week Nov.	4 <sup>th</sup> Week Dec.	10	Auxiliary
3	5YP-3	6	F	4 <sup>th</sup> Week Nov.	2 <sup>nd</sup> Week Jan.	11	Auxiliary
4	5YP-4	6	F	2 <sup>nd</sup> Week Dec.	4 <sup>th</sup> Week Jan.	10	Auxiliary
5	5YP-5	6	B	3 <sup>rd</sup> Week Nov.	1 <sup>st</sup> Week Jan.	10	Terminal
6	5YP-6	6	B	3 <sup>rd</sup> Week Nov.	4 <sup>th</sup> Week Dec.	9	Auxiliary
7	5YP-7	6	F	2 <sup>nd</sup> Week Dec.	3 <sup>rd</sup> Week Jan.	9	Auxiliary
8	5YP-8	6	F	4 <sup>th</sup> Week Nov.	4 <sup>th</sup> Week Dec.	8	Auxiliary
9	5YP-9	6	B	2 <sup>nd</sup> Week Dec.	3 <sup>rd</sup> Week Jan.	9	Auxiliary
10	5YP-10	6	F	1 <sup>st</sup> Week Nov.	4 <sup>th</sup> Week Dec.	9	Auxiliary
11	5YP-11	6	B	2 <sup>nd</sup> Week Nov.	4 <sup>th</sup> Week Dec.	10	Terminal
12	5YP-12	6	F	3 <sup>rd</sup> Week Dec.	3 <sup>rd</sup> Week Jan.	10	Terminal
13	5YP-13	6	B	3 <sup>rd</sup> Week Nov.	1 <sup>st</sup> Week Jan.	11	Auxiliary
14	5YP-14	6	F	4 <sup>th</sup> Week Nov.	4 <sup>th</sup> Week Dec.	9	Auxiliary
15	5YP-15	6	B	2 <sup>nd</sup> Week Dec.	3 <sup>rd</sup> Week Jan.	9	Terminal
16	4YP-1	5	M	4 <sup>th</sup> Week Nov.	1 <sup>st</sup> Week Jan.	9	Terminal
17	4YP-2	5	F	3 <sup>rd</sup> Week Nov.	4 <sup>th</sup> Week Dec.	8	Auxiliary
18	4YP-3	5	B	1 <sup>st</sup> Week Dec.	2 <sup>nd</sup> Week Jan.	8	Auxiliary
19	4YP-4	5	M	3 <sup>rd</sup> Week Nov.	4 <sup>th</sup> Week Dec.	8	Auxiliary
20	4YP-5	5	F	4 <sup>th</sup> Week Nov.	4 <sup>th</sup> Week Dec.	9	Auxiliary

**B**=Bisexual Flower; **M**= Male flower; **F**= Female Flower

### **Time of 50 per cent of flowering**

Most of the trees recorded 50 per cent flowering on 2<sup>nd</sup> and 3<sup>rd</sup> week of January (Table1) and they took 4-5 weeks from initiation of 1<sup>st</sup> flowering in the initial year of the study. Three plants recorded 50 per cent flowering during 3<sup>rd</sup> week of December and remaining two plants were observed to be 50 per cent flowering during 1st week of January of 2019-20. During second year of study, 2020-21, most of the trees recorded 50 per cent flowering on 4<sup>th</sup> week of December and they took 4-5 weeks from initiation of 1<sup>st</sup> flowering. Four plants recorded 50 per cent flowering on 3<sup>rd</sup> week of January and 3 plants observed 50 per cent flowering during 1st week of January and only one plant given 50 per cent flowering during 1<sup>st</sup> week of January (Table2). The duration of flowering was recorded from 8 to 10 weeks during initial year of the study the maximum flowering habit was observed to be auxiliary and some of them were Terminal. And the selected plants for the study were mostly bisexual and female during the initial year of the study

### **Petal length (mm)**

The data obtained on petal length measurement showed that maximum petal length (4.58mm) was observed in 5YP during 2019-20, while during 2020-21, the maximum petal length (5.29mm) was ded in 5YP and followed by 4YP (4.86mm) (Table3). The data five-year-old old plants was individually analyzed during 2019-20, maximum petal length (5.0mm) was recorded in 5YP-4<sup>th</sup>, 7<sup>th</sup>, 11<sup>th</sup> and 13<sup>th</sup> plants and minimum petal length (3.60 mm) was recorded in 5YP-8<sup>th</sup> plant with the mean petal length of 4.59 mm. During 2020-21, the maximum petal length (6.03mm) was recorded in 5YP-1<sup>st</sup> and 10<sup>th</sup> plants and minimum petal length (4.50mm) was recorded in 4YP-1<sup>st</sup> plant with the mean value of 5.28mm (Table5-6).

### **Sepal length (mm)**

The length of sepal was recorded in five years old flowering plant, the maximum length of sepal was (5.07 mm) during 2019-20. While, during 2020-21, the maximum sepal length (4.77mm) was recorded by 5YP and 4.4 mm was recorded by 4YP (Table3). The data was recorded for individual plant, the maximum sepal length (5.90 mm) was recorded by 5YP-1 and minimum sepal length (4.56 mm) by 5YP-14 with the mean value of 5.08 mm during 2019-20.

During 2020-21, the maximum sepal length (6.01 mm.) was recorded by 5YP-14 and minimum sepal length (4.00 mm) was recorded by 4YP-11 with the mean value of 4.72 mm (Table5-6).

### **Stamen length (mm)**

The data of stamen length was recorded in five year old flowering plants. The maximum length of stamen was (4.52mm) by 5YP during 2019-20. While during 2020-21, the maximum stamen length (4.44mm) was recorded by 5YP and 4.2 mm by 4YP. (Table3). The data in five year old plant was analyzed for individual plant during 2019-20. The maximum stamen length (5.06 mm) was recorded by 5YP-1 and minimum (4.20 mm) by 5YP-3 with the mean value of 2.41 mm. During 2020-21, the maximum stamen length (5.20mm) was recorded by 5YP-10 and minimum (4.04 mm) by 5YP-1 with the mean value of 1.55 mm (Table5-6 and plate 1).

### **Style length (mm)**

The style length was measured in five-year-old flowering plant. The maximum style length (4.17 mm) was recorded by 5YP during 2019-20. During 2020-21, the maximum style length was (4.47 mm) by 5YP and (4.06 mm) by 4YP (Table4). The data in five-year-old plants was individually analyzed during 2019-20. The maximum style length (4.72mm) were recorded in 5YP-13 and minimum stamen length (4.02mm) was recorded in 5YP-7 with the mean value of 3.10 mm. During 2020-21 the maximum style length (5.06 mm) was recorded in 5YP-5 and minimum length (4.01 mm) of style was recorded by 5YP-11 with the mean value of 4.18 mm (Table5-6).

### **Pollen viability (per cent)**

The pollen viability analysis was done for flowering tree. The maximum number of viable pollen (84.62 %) was recorded in 5YP during 2019-20. During 2020-21 the maximum per cent of viable pollen (81.72 %) was preceded by 5YP and (83 %) in 4YP (Table4). The data in five-year-old was analyzed for individual plant during 2019-20 the maximum per cent (88.33 %) of viable pollen were recorded in 5YP-1 and minimum (82 %) in 5YP-3& 15 with the mean value of 45.68 %. During 2020-21 the maximum pollen viability (85 %.) was recorded by 5YP-1 and 4YP-3 and minimum pollen viability (75 %) was recorded in 5YP-13 with the mean value of 28.40 % (Table5-6). The stigma was receptive after the opening of the flower. The flower opens in the mornfromring 6:00 AM to 8:00 AM.

## Scanning Electron Microscope analysis

The pollen grain of *Garcinia indica* was examined under Scanning electron microscope. The results revealed the surface texture with protuberances of different shapes and sizes, including vertical and horizontal protuberances, often curved, some blunt-ended, some with grooves and some without grooves (Plate.2).

## Discussion

The flower characteristics of trees of different age groups in both accessions were studied. In *Garcinia indica*, flowering commenced in five-year-old trees (5YP) followed by four-year-old trees. The flowers began appearing in the 1<sup>st</sup> week of November and blooming extended up to 4<sup>th</sup> week of November which agrees with the findings of Tripathi (2021) in this species. It was noted that the maximum number of trees flowered during 3<sup>rd</sup> week of December followed by 3<sup>rd</sup> and 4<sup>th</sup> week of November (Table1). *G. indica* is known to have different sex forms of flowers in the population. In the present study male, female and bisexual flowers were observed in different accessions. The phenomenon of different sex forms of flowers in *G. indica* is known and well-recorded by Thatte and Deodhar (2012). described *Kokum* trees with a wide variety of flower patterns, such as separate trees for male and female flowers, as well as trees with bisexual flowers or same tree producing male and female flowers, trees with bisexual flowers along with male and female flowers. George *et al.* (1992) reported the androdiecious character of this plant.

The flowers were mostly axillary, but some were terminal across accessions studied. This well corroborates with the reports of Shameer *et al.* (2016) in *G. indica*. report by Nayar *et al.* (2014) discerned that the genus *Garcinia* has small clusters of flowers that are produced in cymes or fascicles. Further, the flowers can also be sessile, solitary or in few clusters, terminal or axillary and diversely colored. The flowers are also found with numerous stamens and four to five petals.

**Table3: Petal length, Sepal length and Stamen length in *G. indica* accessions**

Treatment	Petal length (mm)		Sepal length (mm)		Stamen Length (mm)	
	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21
2-YP	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)
3-YP	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)
4-YP	0 (1.00)	4.86 (2.42)	0 (1.00)	4.4 (2,32)	0 (1.00)	4.2 (1.51)
5-YP	4.58 (2.36)	5.29 (2.50)	5.07 (2.46)	4.77 (2.40)	4.52 (2.27)	4.44 (2.33)
<b>S.Em.±</b>	<b>0.01</b>	<b>0.01</b>	<b>0.009</b>	<b>0.02</b>	<b>0.03</b>	<b>0.15</b>
<b>C.D. (5%)</b>	<b>0.04</b>	<b>0.05</b>	<b>0.2</b>	<b>0.06</b>	<b>0.10</b>	<b>0.49</b>

**2YP=** Two-year plant

**3YP=** Three-year plant

**4YP=** Four-year plant

**5YP=** Five-year plant

**Table4: Style length, pollen viability and stigma receptivity in *G. indica* accessions**

Treatment	Style length (mm)		Pollen viability (%)		Stigma receptivity	
	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21
2-YP	0 (1.00)	1.00 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)
3-YP	0 (1.00)	1.00 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)
4-YP	0 (1.00)	4.06 (2.24)	0 (1.00)	83 (9.16)	0 (1.00)	2 (1.73)
5-YP	4.17 (2.25)	4.47 (2.26)	84.62 (9.25)	81.72 (9.09)	2.19 (1.78)	2.26 (1.80)
<b>S.Em.±</b>	<b>0.004</b>	<b>0.03</b>	<b>0.02</b>	<b>0.05</b>	<b>0.01</b>	<b>0.01</b>
<b>C.D. (5%)</b>	<b>0.01</b>	<b>0.12</b>	<b>0.06</b>	<b>0.16</b>	<b>0.03</b>	<b>0.05</b>

**2YP=** Two-year plant

**3 YP=** Three-year plant

**4YP=** Four-year plant

**5YP=** Five-year plant

**Table5: Behavior of *G. indica* individual for flower-related traits**

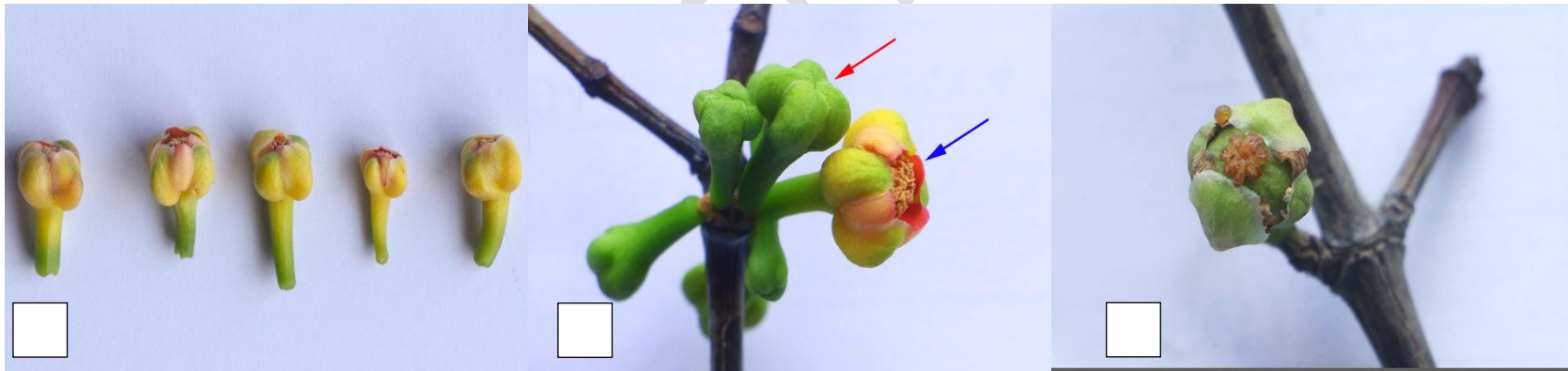
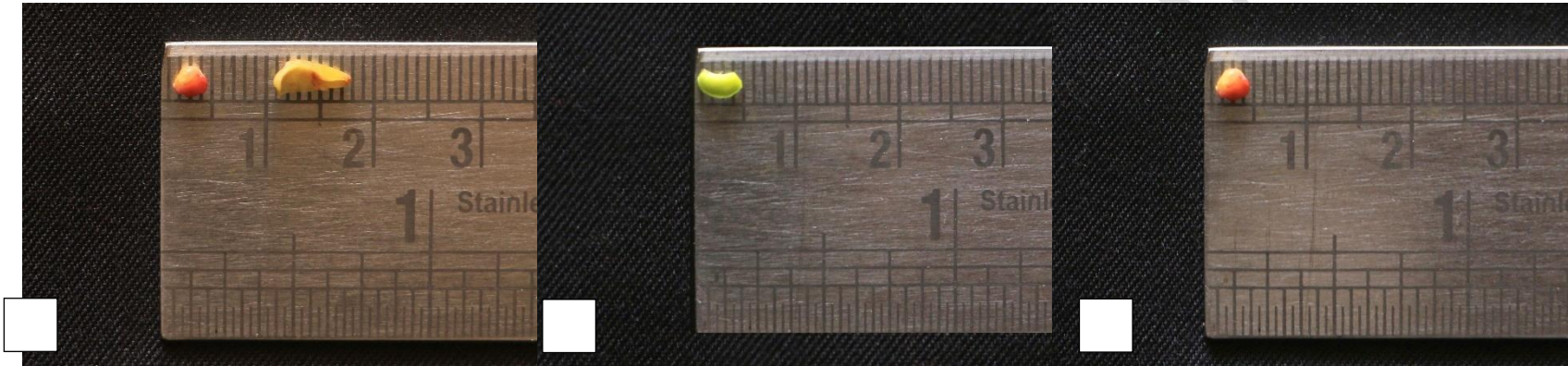
Sl. No.	Plant Number	Age (Years)	PL (mm)	SL (mm)	Sta L (mm)	Sty L (mm)	PV (%)	SR (DAY)	AT
1	5YP-1	5	4.11	5.90	5.06	-	88.33	-	6: 30 AM
2	5YP-2	5	4.50	5.00	-	4.24	-	2	7:15 AM
3	5YP-3	5	4.06	4.80	4.20	4.08	82.00	2	6:00 AM
4	5YP-4	5	5.00	4.70	4.46	4.04	87.00	2	6:10 AM
5	5YP-5	5	4.90	5.00	-	4.44	-	2	7:30 AM
6	5YP-6	5	4.60	4.90	-	4.27	-	2	5:50 AM
7	5YP-7	5	5.00	5.23	4.63	4.02	86.00	2	5:30 AM
8	5YP-8	5	3.60	5.60	4.50	-	88.00	-	5:45 AM
9	5YP-9	5	4.30	5.23	4.04	-	84.00	-	6:00 AM
10	5YP-10	5	4.70	5.06	-	4.20	-	2	6:45 AM
11	5YP-11	5	5.00	5.21	-	4.39	-	2	5:45 AM
12	5YP-12	5	4.90	4.80	4.43	4.13	88.00	2	6:45 AM
13	5YP-13	5	5.00	5.10	-	4.72	-	2	6:30 AM
14	5YP-14	5	4.70	4.56	-	4.06	-	2	5:50 AM
15	5YP-15	5	4.60	5.23	4.83	-	82.00	-	7:30 AM
<b>Mean</b>			<b>4.59</b>	<b>5.08</b>	<b>2.41</b>	<b>3.10</b>	<b>45.68</b>		
S.Em.±			0.18	0.23	0.13	0.19	2.36		
C.D. (5%)			0.52	0.68	0.38	0.57	6.83		

**PL:** Petal length; **SL:** Sepal length; **Sta. L:** Stamen length; **Sty.L:** Style length; **PV:** Pollen viability

**Table6: Behaviour of *G. indica* individual for flower related traits**

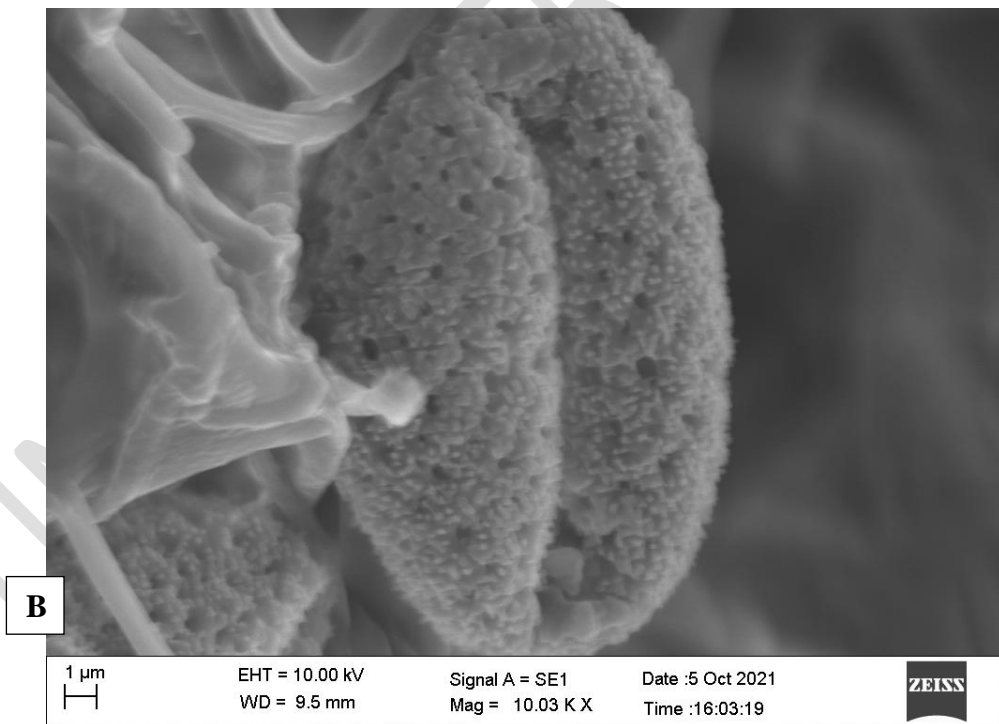
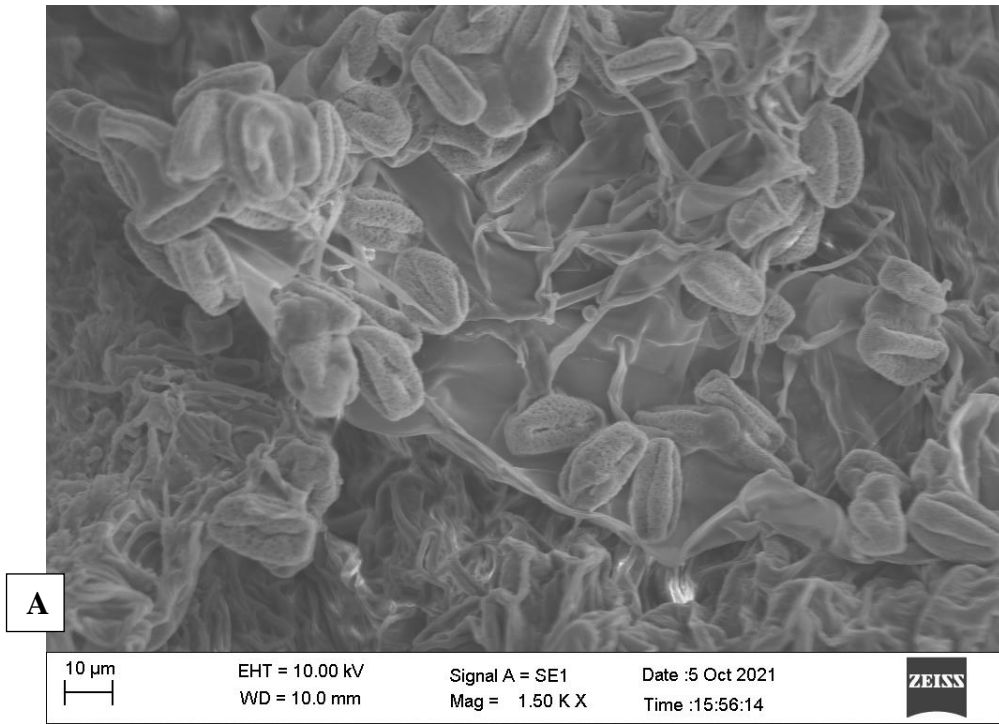
Sl. No.	Plant Number	Age (Years)	PL (mm)	SL (mm)	Sta L (mm)	Sty L (mm)	PV (%)	SR (DAY)	AT
1	5YP-1	6	6.03	5.01	4.04	4.04	85	2	7: 30 AM
2	5YP-2	6	5.00	5.00	-	4.93	-	2	6:45 AM
3	5YP-3	6	6.00	4.93	-	4.73	-	2	8:00 AM
4	5YP-4	6	6.00	5.01	-	4.06	-	3	7:10 AM
5	5YP-5	6	5.03	5.06	-	5.06	-	2	7:30 AM
6	5YP-6	6	5.06	5.06	4.34	4.70	80	2	5:50 AM
7	5YP-7	6	5.05	4.06	-	3.83	-	3	5:30 AM
8	5YP-8	6	5.29	5.01	4.53	4.13	81	3	5:45 AM
9	5YP-9	6	6.03	4.10	-	4.03	-	2	7:30 AM
10	5YP-10	6	5.13	5.05	5.20	-	79	-	6:45 AM
11	5YP-11	6	5.04	4.00	-	4.01	-	2	7:45 AM
12	5YP-12	6	5.52	4.96	-	5.00	-	2	7:15 AM
13	5YP-13	6	5.06	4.90	4.53	4.83	75	2	7:30 AM
14	5YP-14	6	5.05	6.01	-	4.90	-	2	6:50 AM
15	5YP-15	6	5.03	4.06	-	4.80	-	3	7:50 AM
16	4YP-1	5	4.50	4.03	-	3.94	-	2	5:50 AM
17	4YP-2	5	5.00	4.63	-	4.16	-	2	6:30 AM
18	4YP-3	5	4.93	4.53	4.20	4.10	85	2	6:15 AM
19	4YP-4	5	5.00	4.63	-	4.20	-	2	7:30 AM
20	4YP-5	5	4.90	4.30	4.23	4.13	83	2	6:30 AM
<b>Mean</b>			<b>5.28</b>	<b>4.72</b>	<b>1.55</b>	<b>4.18</b>	<b>28.40</b>		
S.Em.±			0.37	0.29	0.16	0.19	1.82		
C.D. (5%)			-	0.85	0.47	0.56	5.23		

**PL:** Petal length; **SL:** Sepal length; **Sta. L:** Stamen length; **Sty.L:** Style length; **PV:** Pollen viability



**Plate 1: Morphology of different flower types and parts in *G. indica*.**

A: Petal & Sepal; B: Petal; C: Sepal; D: Male flower; E: Bisexual flower; E: Male flower; F: Female flower



**Plate 2 SEM image of *G. indica* anther**

**A:** Anther surface **B:** Pollen shape

The flowering is highly influenced by the climatic and edaphic factors of a location, as it appears from the data. Factors such as temperature, rainfall, nutrient and water availability in the soil besides multiple other factors seem to have influence in flowering behaviour of *Garcinia indica* in western ghats of India (Palkar *et al.*, 2020). During the period of flower initiation, the temperature in the location was 28.40 to 26.00 °C. An elevated temperature shortens the growth period of flower clusters, flowering durations, and lifespans of individual flowers, further, it also decreased the number of hermaphrodite and male flowers (Pramanik *et al.*, 2018). A warm temperature, however, increased the rates and percentage of anther dehiscence and fertilization (Shu, 1999). During the present study, temperatures were low during the flowering period, which led to increased flowering duration, lifespan, and growth rate of flower clusters. In addition to those lower temperatures also increased the number of hermaphrodite and male flowers. In contrast to the present study, Sawant *et al.*, (1997) reported the flower bud appearance from September 29 to 8<sup>th</sup> October in different genotypes of *kokum*. This variation in the flowering season might be due to the different sets of genotypes studied by each worker and also as a result of the experimental location of the study conducted. Similar variations were reported by Abraham *et al.* (2002); Patil (2005); Himabindu *et al.*, (2010) and Devi *et al.*, (2012) in *kokum*. Gogoi *et al.* (2015) documented the total span of flowering to be ranged from 42-52 days in case of *Garcinia pedunculata* and in *Garcinia cowa* duration of flowering was from 44-50 days. Whereas, in *Garcinia lanceaefolia* and *Garcinia xanthochymus* the total span of flowering ranged from 38-45 days and 45-56 days respectively. Mansyah (2002) also reported a similar result in *Garcinia mangostana*.

The results for pollen viability showed that the highest percentage of viable pollen (84.62 %) was recorded in the 5-year period 2019-20. Sutthinon *et al.* (2018) demonstrated the pollen viability in *G. celebica* through 2,3,5-triphenyl tetrazolium chloride (TTC) assays and found that the viability was 68 per cent. During 2020-21, the maximum per cent of viable pollen (83%) was recorded in the 4-YP (Table 6). Rajkumar *et al.* (2017) also reported maximum pollen viability (89.16 %) when treated with 0.2 per cent TTC. High pollen viability has also been reported in several *Garcinia* male flowers such as *G. cowa* (96-100 %), *G. speciosa* (93-100 %), and *G. schomburgkiana* (95-100 %) (Te-chato, 2007). Meanwhile, *G. atroviridis* had a very low rate

with approximately 3-5% viable pollen, and no viable pollen was found in *G. dulcis* (Te-chato, 2007) and *G. mangostana* (Sutthinonet *et al.* 2013). According to the findings of the harvested pollen grains of many *Garcinia* species have high viability as assessed by their stainability like 99.4% of *Garcinia corymbosa*, 92.5 per cent of *Garcinia forbesii*, and 85 per cent of *Garcinia cf. forbesii* (Ha *et al.*,1988).

Five-year-old plant data were analyzed for individual plants in 2019-20. The highest percentage of pollen viability was found in 5YP-1 (88.33%) and the lowest in 5YP-3 and 15 (82%) with a mean of 45.68 per cent. During 2020-21 the maximum pollen viability was recorded in 5YP-1 and 4YP-3 (85 %) and minimum pollen viability was recorded in 5YP-13 (75%) with the mean value of 28.40 per cent (Table6). The pollen viability was  $89.19 \pm 2.11\%$  as tested by 2% TTC. The *in vitro* pollen germination was  $72.73 \pm 9.20$  per cent with  $67.13 \pm 21.33$  per cent long pollen tubes in 20 per cent sucrose solution. This is consistent with earlier studies demonstrating that sucrose is an important component for *in vitro* pollen germination (Honshoet *et al.*, 2007). This optimal sucrose concentration is due to the essential role sucrose plays in nutrient provision and environmental osmotic maintenance (Baloch *et al.*, 2001). It has been suggested that bicellular pollen requires a low level of sucrose (10-15%) (Shivanna, 2003). In *G. celebica*, the most effective conditions for pollen germination were observed on treatment with 20% sucrose for 10-12 h (Sutthinonet *et al.*, 2018). The stigma was receptive two days after anthesis and the flower opened between 6:00 AM and 8:00 AM (Dike *et al.*, 2020). The stigma became receptive only after the opening of flowers. Aswathi *et al.* (2018) observed that in *G. cambogia* maximum stigma receptivity was found at 7.00 PM. On the day of anthesis high percentage of pollen grains were fertile. Stigma is the wet type and broad umbrella-shaped. Karnik and Gunjate (1984) reported that the maximum stigma receptivity (80%) was observed on the day of anthesis followed by 68, 64 and 44 per cent, 1, 2 and 3 days after anthesis respectively. Rajkumar *et al.* (2017) reported that in *G. imberta*, receptivity of stigma gradually decreased with age and within one or two days as the stigmatic lobes became brown and completely dry, indicating a complete loss of receptivity.

When flower visitors contact with stigmas and anthers, the stigmatic exudates can support them to attach to the body of the biotic pollinator. This corresponds with the prevailing notion that

most *Garcinia* species are pollinated by biotic pollinators such as social bees, diverse insects and *Apis* spp. (Richards, 1997; Momose *et al.*, 1998).

## References

- Abraham, Z, Latha, M., Kumar, R, S., Rathy, K., Shelja, P. B. and Sunanda, C, 2002, Variability studies in kokum (*Garcinia indica*). *Indian. J. Pl. Genet. Resour.*, **15**(2): 333-339.
- Aswathi, P., Aswani, K. and Sabu, M., 2018, Reproductive biology of Malabar tamarind (*Garcinia gummi-gutta* (L.) Rob.: An endemic, medicinal and spice plant from Western Ghats. *The International J. Pl. Reprod. Bio.*, **10**(1), pp.65-68.
- Chinavat, Y. and Subhadrabuddhe, S., 2004, Phylogenetic relationship of mangosteen (*Garcinia mangostana* L.) and several wild relatives revealed by ITS sequence data. *J. American Soc. Hort. Sci.*, **3**(129): 368 -373.
- Devi, P. S., Balamohan, T. N., Thangam, M., Ashok, K. J., Ramachandrudu, K. and Korikanthimath, V. S., 2012, A study on diversity and distribution of kokum ( *Garcinia indica* (Choisy) Thouars) using DIVA-GIS in Goa with respect to fruit characters. *Indian J. Hort.*, **69**(2): 156-162.
- Dike, M. S., Malik.S. K., Sawardekar, S. V. and Deodhar, M. A., 2020, Study of the mode of reproduction and fruit development in *Garcinia Indica*. *Int. J. Fruit Sci* .**20**(1): 20-38.
- George, S.T., Latha, B., Mathew, L. and Geetha, C. K, 1992. The pattern of flowering and flower develop- ment in Kodapuli (*Garcinia cambogia*Desr). *Indian Cocoa Arecanut*. **16**(2): 68-70.
- Gogoi, B., 2015, Morpho-biochemical characterization of *Garcinia* species of Assam (Doctoral dissertation, AAU, Jorhat).
- Ha, C.O., Sands, V.E., Soepadmo, E. and Jong, K., 1988, Reproductive patterns of selected understorey trees in the Malaysian rain forest: the apomictic species. *Bot.J.Linnean Soc.*, **97**(3), pp.317-331

- Han, K. H., Seo, J. A., and Yu, J. H., 2004, Regulators of G-protein signalling in *Aspergillus nidulans*: RgsA downregulates stress response and stimulates asexual sporulation through attenuation of GanB (Gaaaa) signaling. *Mol. Microbiol.*, **53**(2): 529 – 540.
- Hegde, I., 2019, Kokum (*Garcinia indica*)- its status, problems and prospect of cultivation and processing. *Int. J. Agri. Sci.*, **11**(7): 8239-8241.
- Himabindu, A., Subbaramamma, P. and Kumar, V., 2010, Evaluation of *Garcinia indica* Choisy for floral and yield traits. National Symposium on *Garcinia* genetic resources. Linking diversity, livelihood and management. College of Forestry, Sirsi. PP87-90.
- Honsho, C., Somsri, S., Tetsumura, T., Yamashita, K., Yapwattanaphun, C. and Yonemori, K., 2007, Characterization of male reproductive organs in durian; Anther dehiscence and pollen longevity. *J. Jpn. Soc. Hort. Sci.*, **76**(2): 120–124.
- Karnik, A.R. and Gunjate, R.T., 1984, Floral biological studies in kokum [*Garcinia indica*]. *J. Maharashtra Agril. Universities*.
- Mansyah, E. 2002. Genetic variability analysis of mangosteen population in java and Sumatra Island through their phenotypic performance and RAPD technique (Thesis). Grad. Schl. Padjadjaran Univ. p. 108
- Mathew, G. E., Mathew, B. and Nyanthara, B., 2011, Diuretic activity of leaves of *Garcinia cambogia* in rats. *Indian J. Pharm. Sci.*, **73**(2): 228–230.
- Milind, P. and Isha, D., 2013, Golden benefits of drinking kokum cola. *Int. Res. J. Pharm.* **5**(4): 5-9.
- Momose, K., Yumoto, T., Nagamitsu, T., Kato, M., Nagamasu, H., Sakai, S., Harrison, R.D., Itioka, T., Hamid, A.A. and Inoue, T. 1998. Pollination biology in a lowland dipterocarp forest in Sarawak, Malaysia. I. Characteristics of the plant-pollinator community in a lowland dipterocarp forest. *Amer. J. Bot.*, **85**(10): 1477- 1501.
- Nayak, C. A., Srinivas, P. and Rastogi, N. K. 2010, Characterization of anthocyanin from *Garcinia indic* choisy. *Food Chemistry*. **118**: 719-724.
- Nayar, T. S., Beegam, A. R. and Sibi, M., 2014, Flowering plants of the Western Ghats, India.

- Palkar, R.S., Janarthnam, M.K. and Sellappan, K., 2020, Prediction of potential distribution and climatic factors influencing *Garcinia indica* in the *Western Ghats* of India using ecological niche modeling. *Natl.Aca.Sci. Let.*, **43**(6), pp.585-591.
- Patil, B. P., 2005, Fact on kokum-Brochure, western ghats kokum Foundation, Goa. Proceedings and compendium second national conference on kokum, 81-92.
- Pramanik, M., Paudel, U., Mondal, B., Chakraborti, S. and Deb, P., 2018, Predicting climate change impacts on the distribution of the threatened *Garcinia indica* in the *Western Ghats*, India. *Climate Risk Management*, **19**, pp.94-105.
- Rajkumar, K., Keshavanarayan, P., Shubharani, R. and Sivaram, V., 2017, Studies on pollen biology and stigma receptivity of *Garcinia imberti* Bourd. (Clusiaceae)- a critically endangered tree of western ghats, Kerala, *Int. J. Plant Reprod. Biol.*, 9(2): 109-114.
- Rajkumar, K., Keshavanarayan, P., Shubharani, R. and Sivaram, V., 2017, Studies on pollen biology and stigma receptivity of *Garcinia imberti* Bourd. (Clusiaceae)- a critically endangered tree of western ghats, Kerala, *Int. J. Plant Reprod. Biol.*, 9(2): 109-114.
- Richards, A.J. 1997. Plant breeding systems (2nd ed.), Chapman and Hall, London.
- Sawant, D. S., Haldankar, P. M., Nagwekar, D. D. and Rajput, J. C., 1997, Screening of kokum (*Garcinia indica* Choisy) genotypes. *Indian. J. Arecanut Spices Medicinal Pl.*, **2**: 55-58.
- Shameer, P. S., Rameshkumar, K. B., Sivu, A. R., Sabu, T., Pradeep, N. S. and Mohanan, N., 2016, Morphological, chemical and molecular taxonomy of a new *Garcinia* species- *Garcinia pushpangadianiana*, In *Diversity of Garcinia species in the Western Ghats: Phytochemical Perspective*. (Ed) Rameshkumar, K. B., JNTBGRI, Kerala, Pp.196-201.
- Shivakumar, S., Sriraman, S., Subhasree, N. and Dubey, G. P., 2013, *In vitro* assessment of antibacterial and antioxidant activities of fruit rind extracts of *Garcinia cambogia* L. *Int. J. Pharm. Pharm. Sci.*, 5(2):254-257.
- Shivanna, K.R., 2003, Pollen biology and biotechnology. Science Publishers, Inc., Enfield, New Hampshire.

- Shu, Z. H., 1999, Effect of temprature on the flowering biology and fertilization of mango (*Mangifera indica* L.). *J. Appl. Hort.*, **1**(2):79-83.
- Sutthinon, P., Meesawat, U. and Purintavaragul, C., 2013, Developmental anatomy of sporogenesis and embryogenesis in mangosteen (*Garcinia mangostana* L.). *Thai.J. Bot*, **5**(2), pp.119-129.
- Sutthinon, P., Samuels, L. and Meesawat, U., 2018, Male functionality in *Garcinia celebica* L., a candidate ancestor species of mangosteen (*G. mangostana* L.). *Bot.*, 96(10), pp.685-693.
- Swami, S. B., Thakor, N. J., and Patil, S. C., 2014, Kokum (*Garcinia Indica*) and its many functional components as related to the human health. *J. Food Sci. Technol.*, **4**:130-142.
- Te-Chato, 2007, Floral and fruit morphology of *Garcinia* species. *Songklanakarin J. Sci. Technol.*, **29**(2): 245-252.