ISSN (Online): 2349-1183; ISSN (Print): 2349-9265
TROPICAL PLANT RESEARCH
The Journal of the Society for Tropical Plant Research

7(2): 406–414, 2020
DOI: 10.22271/tpr.2020.v7.i2.048

Research article

Phenology and seed development in *Mesua ferrea* L., a rare medicinal tropical tree species

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Abstract: This paper, deals with phenology, seed set, seed development in two populations of *Mesua ferrea* together with biochemical changes during seed development. Though there was a slight variation in leaf and flower flushing among the two populations, further fruit/seed development did not show significant variation. Pollinating agents were also been identified. After pollination seed development was initiated with an increase in seed moisture content and fresh seed and dry weight. The moisture content started decreasing 60 days after anthesis and recorded 44.58% by 160 DAA. Total sugar, starch, protein and lipid content increased during the seed development and its role has been discussed.

Keywords: *Mesua ferrea* - Phenology - Seed development - Germination - Metabolite changes.

[Cite as: Mithun V, Babu KP, Pradeep NS & Krishnan PN (2020) Phenology and seed development in *Mesua ferrea* L., a rare medicinal tropical tree species. *Tropical Plant Research* 7(2): 406–414]

INTRODUCTION

Globally, climate change may force variation in timing, duration and synchronization of phonological events in tropical trees (Reich 1995). In tree species under monsoonic climate variable reproductive and survival strategies like wide diversity of seasonal flowering, fruiting, leaf flush and leafless periods (Singh & Kushwaha 2006, Ashwini *et al.* 2014, Bajpai *et al.* 2017). Seasonal duration of leafing, flowering and fruiting determine physiological condition and behaviour of tropical trees. These phenological events are mutually independent in woody species and flowering may be partly or wholly dependent on leafing activity (van Schaik *et al.* 1993). Reproductive events generally occur during the period of low photosynthetic activity or after the period of high rates of reserve accumulation (Fenner 1998).

Mesua ferrea L. is a rare medicinal tree species distributed in Indo-Malesian regions (tropical parts of Sri Lanka, India, southern Nepal, Burma, Thailand, Indochina, Philippines, Malaysia and Sumatra). The species is an evergreen one going up to 20–30 meters high but it is slow-growing. It is commonly known as iron wood tree. The phenolic compounds present in seeds *viz.* Mesuol ($C_{23}H_{22}O_5$) and Mesuone ($C_{29}H_{42}0_4$) have anti-bacterial properties (Anonymous 1952) other than glycosides, flavonoids, xanthones, triglycerides, and resins. The plant is used as antimicrobial, antibacterial, and anti protozoal (Kar & Jain 1971, Mazumder *et al.* 2004, Chanda *et al.* 2013).

Seeds of many tropical and sub-tropical tree species are characterized with high moisture content on maturation and are intolerant to desiccation and they have termed as recalcitrant by Roberts (1973). In many of the endemic tree species of Western Ghats of India, seeds will mature before the onset of monsoon and germinate with the start of rains. The flowering process, seed formation and maturation are critical phases in the life cycle, of which are highly specific to the environmental factors, disturbances and final viability and germination of the seeds they produce. There are some studies in *M. ferrea* related to fruit size variation, germination, seedling fitness and biomass accumulation during early seedling growth was reported (Khan *et al*. 2002, Arunachalam *et al*. 2003). The objective of the present study was to investigate phenology, seed set and seed development in two populations of *M. ferrea*, grown different geographical locations in Kerala State, India which is a prerequisite for developing sustainable conservation strategies.

Received: 28 February 2020 Published online: 31 August 2020
https://doi.org/10.22271/tpr.2020.v7.i2.048

MATERIALS AND METHODS

Study site

The study was conducted in two geographically different areas, one was at Calicut University Campus, Malappuram District (Latitude: 11.1359, Longitude: 75.8894, Altitude: 48 meters) and another was Iringole Sacred grove, Perumbavoor, Ernakulam District (Latitude: 10.108641, Longitude: 76.500463, Altitude: 32 meters) India (Fig. 1). Iringole sacred grove is a remnant of once existing tropical evergreen forest vegetation spread about 10 hectares of land preserved due to the religious belief and environmental concern of the local population. The climate is tropical evergreen with rich flora similar to the Western Ghats forests. There is a good natural population of *M. ferrea* extensively flourish in this grove. The second site is the Calicut University Campus spread over an area of 500 acres (2.0 km^2) where domesticated populations of the *M. ferrea* is found as well as they are conserved in the Botanical Garden of the University.

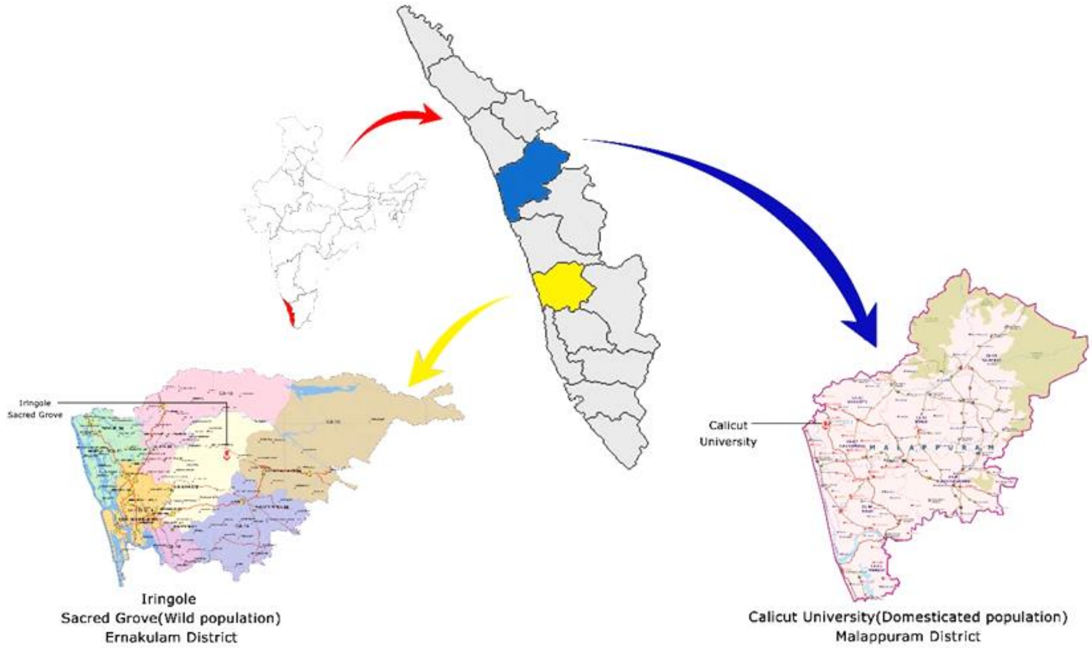

Figure 1. Location of study site.

Climate of the study sites

Both the study sites have marked variation in climate. In the case of Calicut University Campus, there was only one peak of rainy season extending to June, July and August (Fig. 2A). While in Iringole has three peaks, one in June, August and October and rainfall is distributed from May to November (Fig. 2B), from May to September at Calicut University. Here it is to be mentioned that South-West monsoon is prominent in Calicut University while at Iringole, both South-West monsoon and North-East monsoon are evenly distributed (Fig. 2A & B).

Selection of trees and phenological studies

Ten to fifteen healthy trees of *M. ferrea* were selected from both the locations and the phenology of the trees were observed regularly at 15-day intervals from March 2017 to March 2019. New leaf flush time, longevity and peculiarity were recorded. Reproductive phenological events like flowering fruiting were recorded. Upon flowering, each flowers were tagged on the day of opening considered as day of anthesis and further development age was designated as Day After Anthesis (DAA). The flowers were observed to identify the pollinators and they were caught by insect traps and identified with the help of Mr. Muhammed Shameem (Assistant Professor, Department of Zoology) of Government College, Chitoor and insect illustration charts (Distant 1904).

Data on development, dry matter accumulation, and biochemical changes during the fruit and seed development in *M. ferrea* were started after 20 DAA till 160 DAA. Diameter, fresh weight, number of seeds per fruit, dry weight and percentage moisture contents of fruits and fresh weight, dry weight and percentage moisture contents of the seeds were recorded at 20 day interval till maturity at 160 days followed with ISTA standards.

Sample materials after taking dry weights at different periods were used as a source material for estimating metabolites like total sugar, phenols, amino acids, proteins lipids and starch. Three samples of each stage were sampled for biochemical analysis. Tissue samples was ground in known volume of 80% ethanol (v/v) in distilled water and centrifuged at 4000 rpm for 10 minutes. The residue was washed thrice and part of the combined supernatant used for the estimation of total sugar, phenol and amino acids. The rest of the supernatant was kept in a china dish and evaporated in a hot air owen at 60ºC and the residue dissolved in distilled water, centrifuged and served as the source for soluble sugar. The left over residue was ground in 30% Perchloric acid centrifuged, re- extracted and the combined supernatant is used for starch estimation. Total soluble sugar was estimated using phenol sulphuric acid method (Montgomery 1957), total phenols by following method of Swain & Hillis (1959), Protein content by the method of Lowry *et al.* (1951), starch using Mc Cready *et al.* (1950), amino acid following Sadasivam & Manickam (1996) and lipids by the method of Bligh & Dyer (1959).

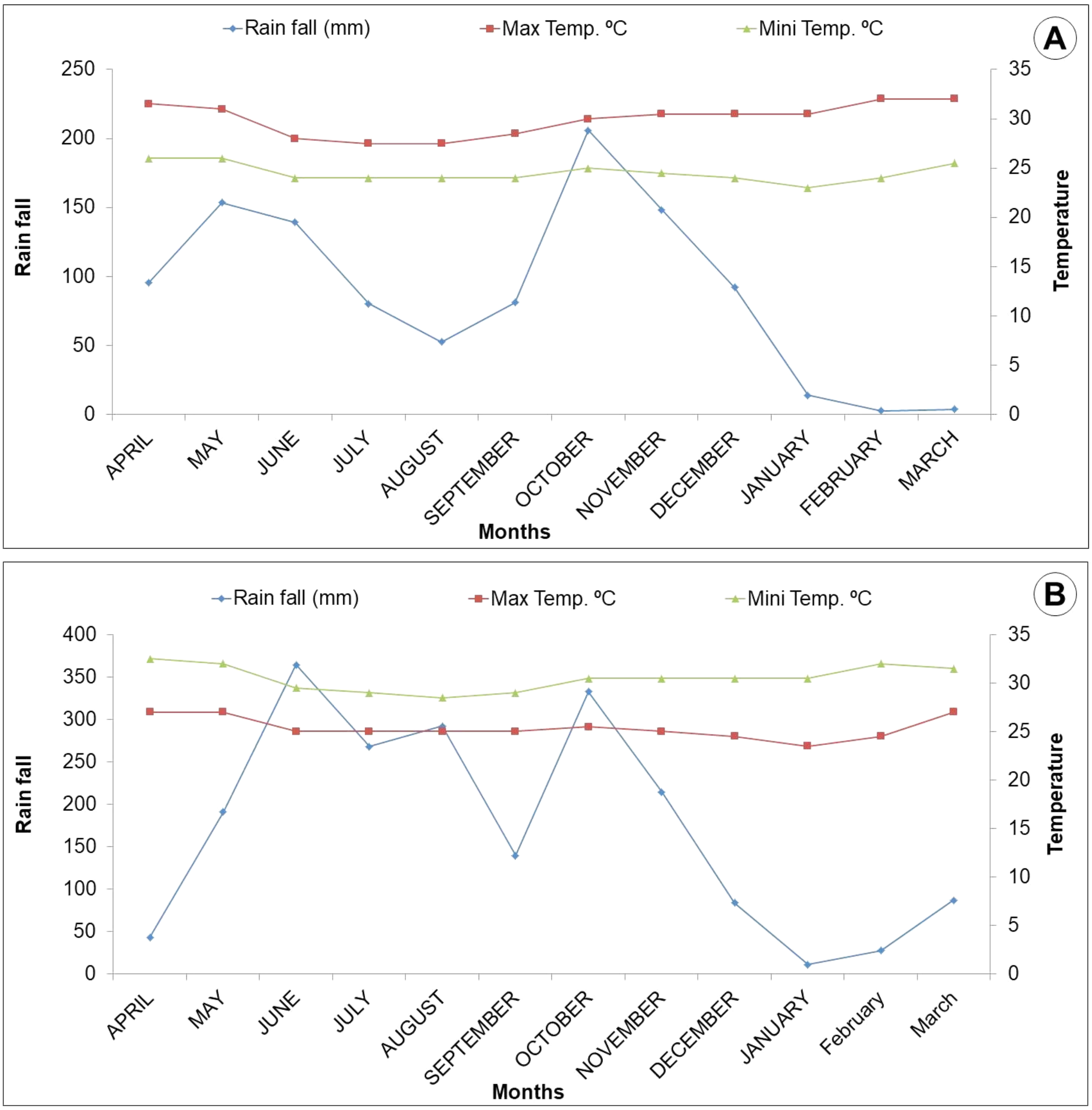

Figure 2. A, Rainfall, maximum and minimum teperature of Calicut University 2017–2019; **B**, Rain Fall, maximum and Minimum teperature of Iringole sacred grove Average of 2017–19.

Statistical analysis

The data collected from the experiments were analysed by Analysis of Variance of (ANOVA) and the ratio obtained were checked for significance at 0.05% level. The means of each treatment were separated following Student T test and significance tested at 0.05% level.

RESULTS

Phenology and seed development

Mesua ferrea L. Ceylon Ironwood, Indian rose chestnut or cobra's saffron is a slow-growing tree belongs to the family Calophyllaceae. Through the plant is a wild one, but now widely cultivated as an ornamental due to its crimson colored new flushed foliage and white fragrant flowers. Leaf flushing occurs in September in Calicut University and October in Iringole (Table 1). The emerging young leaves are red to yellowish pink and drooping then turns green slowly (Fig. 3). Flowering started in the month of March in Calicut University and April in Iringole (Table 1; Fig. 3). Flowers are off-white in colour with fragrant stamens. Sepals 4 decussate, sub orbicular, persistent and variously enlarged and thickened. Calculated reproductive efficiency recorded almost similar in both conditions with a slight variation in ovule- seed ratio (Table 2).

Table 1. Phenological sequence of *Mesua ferrea* L. in two plots studied.

Parameters	Iringole	Calicut University
Leaf flushing	October	September
Flowering	April	March
Fruiting	September–October	August–September

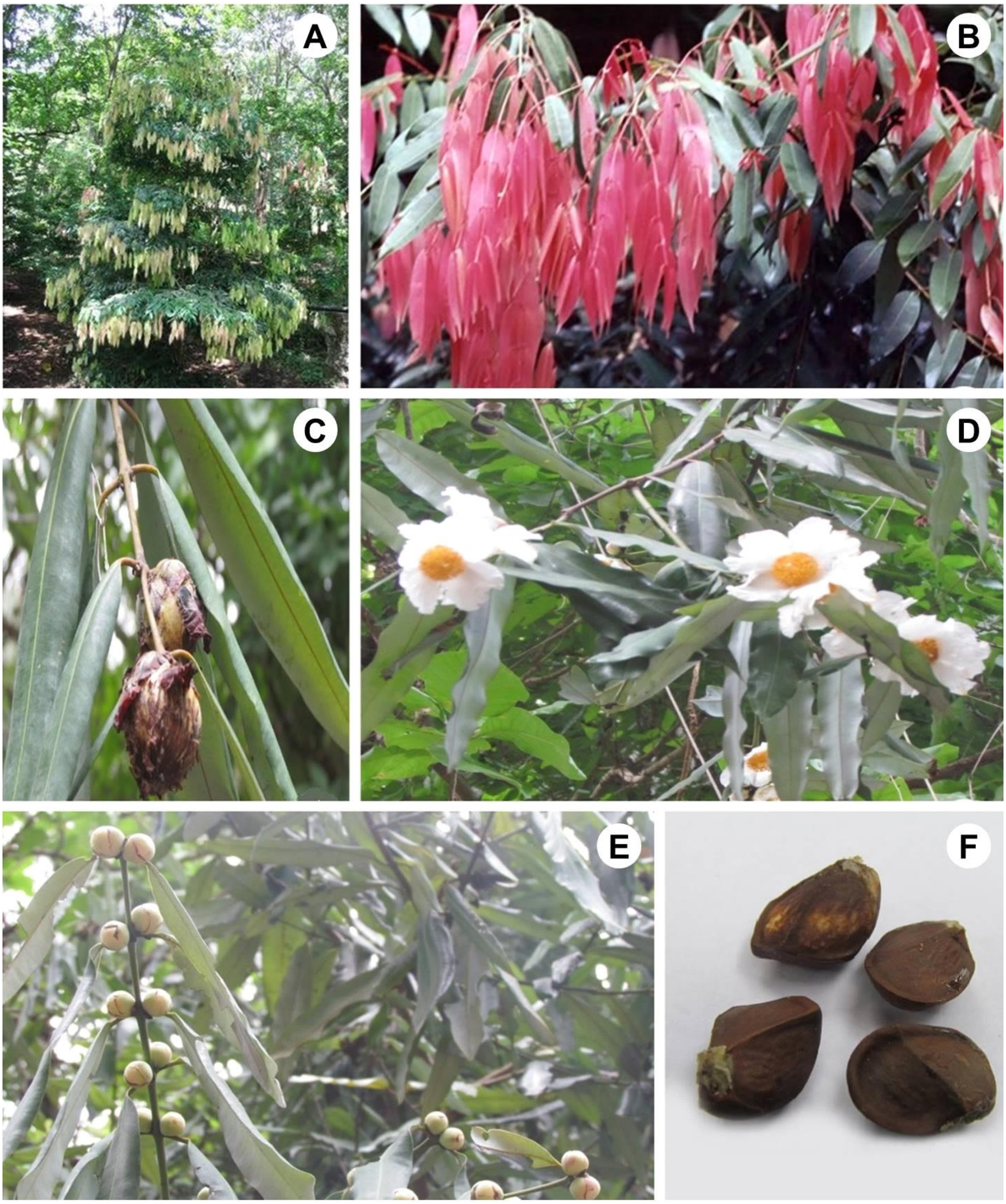

Figure 3. *Mesua ferrea* L.: **A**, Habit; **B**, Leaf flushing; **C**, Fruits; **D**, Flowers; **E**, Floral buds; **F**, Seeds.

Table 2. Reproductive Efficiency of *Mesua ferrea* L.

	Iringole	**Calicut University**
Life span of the flower	1 day	1 day
Ovules/ flower	4 ±1	4 ±1
Anther/ flower	228 ±26	252 ±26
Flower-Fruit ratio	4:1	4:1
Ovule-Seed ratio	1786:432	1841:469
Seed germination rate	65%	60%
Seedling survival rate	10%	10%

Pollinators

A wide range of visitors including bees, wasps, ants and butterflies visit flowers, but only a few of them were effective pollinators. When insects come in contact with the dehisced anthers, the pollen grains stick on their ventral body surface and appendages. *Apis dorsata*, *Apis cerana*, *Trigona irridepennis*, *Tetragonula* spp. are the most frequent visitors (Fig. 4). These bees land on new flowers for nectar, their body comes in contact with the stigmatic surface and transfer pollen grains. They visit the flowers throughout the day. A wide variety of butterflies like *Rapala manae*, *Euploea core* also visit flowers for nectar, spending 2–3 seconds. These butterflies are nectar robbers. Based on the frequency of visits and pollination efficiency (based on pollen load reaching on the stigma and percentage of fruit set - data not provided) *Apis dorsata* and *Apis cerana* are the effective pollinators.

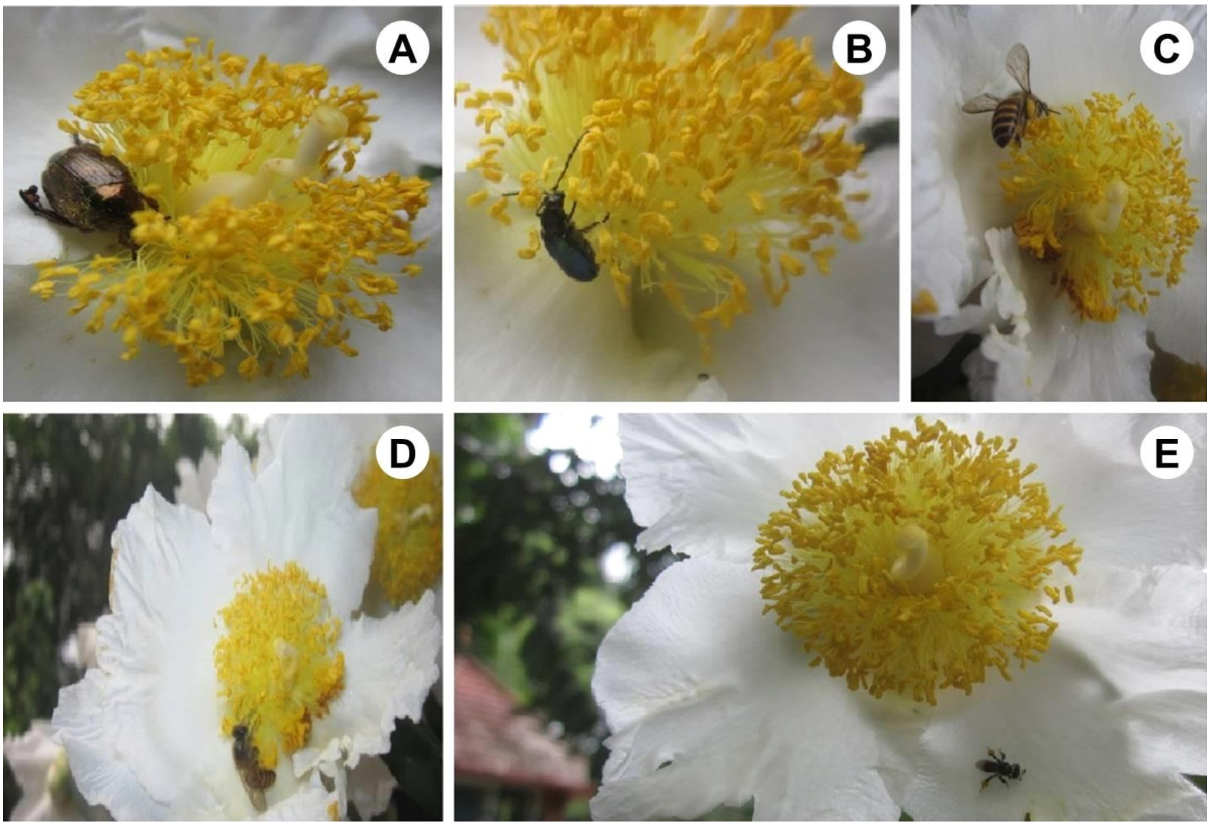

Figure 4. Variour pollinators visiting on the flowers of *Mesua ferrea* L.

Though there was a difference in the phonological sequence and a slight variations in reproductive efficiency among the populations of Calicut University and Iringole, further development of the fruits, seeds and the biochemical parameters did not showed much difference. Hence, data from both the sites were taken and averages calculated with standard deviation.

The fruits during the early days of development possessed a beak-like structure at one end (bottom). The colour of the fruit was greenish-yellow up to 100 DAA, which turned to brown at the harvesting maturity. In the final stage of development, the fruit wall splits longitudinally at the time of maturity thus exposing the seeds.

After pollination and fertilization, seed development begins parallel with an increase in weight because of nutrient and water intake associated with rapidly accelerating cell division and elongation. In *M. ferrea*, measurement of growth parameters started after 20 DAA. Fruit size and seed size increased from 0.96±0.02 cm and 3.39±0.03 cm at 160 DAA and an average of 2 seeds could be found per fruit. At par with the size increase, fresh weight and dry weight of fruits and seeds increased which stabilized at 160 DAA (Table 3). The percentage of Moisture content of fruit increased up to 60 DAA and the subsequent decrease was noted (Table 3). The increase in seed dry weight noticed up to 100 DAA may be due to the synthesis and deposition of storage materials, supported by the accumulation of sugar, starch, protein and lipids during seed development. It is also noted that the period of reduction in the seed water content coincided with the increase in the dry matter accumulation between 60-120 DAA (Table 3).

Table 3. Fruit and Seed dry matter accumulation during the development of *Mesua ferrea* L.

DAA	Fruit					Seed		
	Diameter (cm)	Fr.Wt. (gm/fruit)	Seeds P.Fr.	Dr.Wt. (gm/fruit)	MC (%)	Fr.Wt. (gm/seed)	Dr.Wt. (gm/seed)	MC (%)
20	0.96±0.020	1.52±0.008	1	0.43±0.02	72.3±0.2	0.057±0.0008	0.010±0.0003	42.53±0.15
40	1.96±0.004	3.19±0.030	2	0.60±0.30	81.23±0.3	0.086±0.0008	0.0246±0.0007	71.47±0.19
60	2.10±0.003	5.13±0.02	2	0.86±0.03	83.40±0.4	0.137±0.0006	0.413±0.0003	69.49±0.21
80	2.41±0.002	5.25±0.02	2	2.47±0.03	53.5±0.30	0.384±0.0006	0.117±0.0007	64.93±0.49
100	2.97±0.011	7.61±0.17	2	3.84±0.04	49.34±0.5	0.415±0.0006	0.196±0.001	51.86±0.23
120	3.08±0.003	7.24±0.02	2	3.91±0.05	48.31±0.60	0.436±0.0005	0.214±0.0005	49.47±0.63
140	3.24±0.004	6.91±.080	2	3.90±0.06	48.50±0.48	0.451±0.0006	0.235±0.0008	47.37±0.20
160	3.39±0.031	6.74±0.03	2	4.5±0.01	48.15±0.16	0.487±0.002	0.266±0.001	44.58±0.12

Note: DAA- Day After Anthesis; Fr.Wt.- Fresh Weight; Seeds P.Fr.- Number of seeds per fruit; Dr.Wt.- Dry weight; MC- Moisture content.

The level of soluble sugar content recorded minimum (34.83±1.2 mg g^{-1} Dr.Wt.) in the developing seed of *M. ferrea* during the initial stages of development (20 DAA) with a sharp significant increase up to 100 DAA (94.65±0.68 mg g^{-1} Dr.Wt.) and later decrease in sugar content was recorded (Table 4). Starch content recorded a significant increase from 20 DAA up to 160 DAA days from 16.43±0.30 mg g^{-1} Dr.Wt. to 142.43±0.58 mg g^{-1} Dr.Wt., where 8 to 9 times of increase was noted. Protein content in developing seeds of *M. ferrea*, recorded gradual increase up to 120 DAA, from 20 DAA was significant and then a slight decrease was noted during maturity. In the case of phenol content recorded minimum (12.34±0.52 mg g^{-1} Dr.Wt.) in seed of *M. ferrea* during 80 DAA after that gradual increase was recorded. The level of amino acids was recorded low during initial seed development at 20 DAA. Later a linear increase in amino acid content was recorded till maturity (Table 4). Lipid content recorded low during the initial stages of seed development at 20 DAA (78.2±0.70 mg g^{-1} Dr.Wt.) and later a linear significant increase recorded till maturity (453.5±0.39 mg g^{-1} Dr.Wt.).

Table 4. Changes in metabolites during the development of *Mesua ferrea* L. seeds.

DAA	Sugar (mg g^{-1} Dr.Wt.)	Protein (mg g^{1} Dr.Wt.)	Starch (mg g^{-1} Dr.Wt.)	Phenol (mg g^{1} Dr.Wt.)	Amino acids (mg g^{-1} Dr.Wt.)	Lipids (mg g^{-1} Dr.Wt.)
20	35.18±1.01^{g}	44.65±0.47^{h}	15.82±0.41^{h}	18.37±0.22^{f}	0.287±0.007^{h}	80.33±0.73^{h}
40	39.79±0.32^{f}	48.90±0.55^{g}	21.90±0.20^{g}	20.95±0.21^{e}	0.486±0.006^{g}	86.72±0.77^{g}
60	68.36±0.41^{c}	54.79±0.43^{f}	32.54±0.38^{f}	15.10±0.20^{g}	0.972±0.006^{f}	123.20±0.75^{f}
80	68.19±0.41^{c}	70.63±0.47^{e}	46.00±0.38^{e}	13.80±0.45^{h}	1.13±0.007^{e}	152.12±0.38^{e}
100	93.02±1.16^{a}	81.66±0.61^{bc}	65.98±0.55^{d}	21.79±0.40^{d}	1.95±0.007^{d}	251.46±0.34^{d}
120	72.00±0.50^{b}	87.29±0.52^{a}	80.47±0.45^{c}	26.43±0.33^{c}	2.75±0.05^{c}	374.42±0.78^{c}
140	64.11±0.53^{d}	82.22±0.99^{b}	114.99±0.47^{b}	35.22±0.48^{b}	3.43±0.07^{b}	406.43±0.26^{b}
150	63.69±0.35^{de}	79.83±0.52^{d}	138.40±0.74^{a}	36.50±0.59^{a}	3.78±0.08^{a}	466.05±0.50^{a}

Note: DAA- Day After Anthesis; Dr.Wt.- Dry weight; *Values followed by the same letter as superscript in a column do not differ significantly based on ANOVA and t-test at $p \leq 0.05$.

DISCUSSION

The phenology of plant communities can be studied by dealing with particular life-history stages separately such as leafing, flowering, fruiting, seed dispersal and germination. In the present study, *M. ferrea* leaf flushing started during September in Calicut University campus, where there was a little rainfall and an increase in maximum temperature and sunny days. In Iringole the flushing started in October, where there was the third peak of rainfall during the early days in the month and then decreased with an increase in maximum temperature during the daytime. The flushing of leaves just after the rain and its maturation before starting of the dry season (February–March).The trees against the impact of seasonal changes and to initiate flowering before the rainy season. Here flowering occurred during March in Calicut University and April in Iringole before the onset of south-west monsoon as the character of tropical species. Usually leafing is linked with some climatic features such as rainfall (Lieberman & Lieberman 1984, Bullock & Solis-Magallans 1990, Bajpai *et al.* 2012, Borah &

Devi 2014, Bajpai *et al.* 2017, Devi *et al.* 2019), temperature or Photoperiod (Bertero 2011, Borah & Devi 2014, Bajpai *et al.* 2017, Devi *et al.* 2019). When water is not a limiting factor, irradiance may play an important factor. In a major study on tropical forests (Wright & Van Shack 1994), showed that leaf and flower production coincides with seasonal peaks of irradiance that might be operated in case *M. ferrea*, where there was sufficient water and optimum temperature and have sunny days.

In the present study the fruits of *M. ferrea* from both the sites contain 1 or 2 seeds and about 90% of seed bearing fruits contained 2 seeds. On the other hand, Khan *et al*. (1999) reported that the presence of 1–4 seeds from the populations of North-eastern region. The moisture content was initially high in fruits and seeds (81.23% and 71.47%) respectively at 40 DAA, remain high until 80 DAA with significant reduction from this point reaching lower values at 160 DAA *i.e.*, 48.15% for fruits and 44.58% for seeds. Attainment of high fresh weight of fruits /seeds indicates the cessation of cell division during maturity (Noggle & Fritz 1991). The increase in seed dry weight noticed up to 100 DAA may be due to the synthesis and deposition of storage materials, supported by accumulation of sugar, starch, protein and lipids during seed development. Photoassimilates like sucrose and amino acids during seed development and maturation phase are converted to seed storage compounds such as starch, lipids and proteins (Ruan & Chourey 2006, Baud *et al*. 2008, Meyer & Kinney 2010). The increase in seed dry weight noticed up to 100 DAA may be due to the synthesis and deposition of storage materials, supported by the accumulation of sugar, starch, protein and lipids during seed development. It is also noted that period of reduction in the seed water content coincided with the increase in the dry matter accumulation between 60–120 DAA. When seed loses Moisture content and reaches a minimum (44.5% in seeds of *M. ferrea*), the vascular connection between the developing seed and mother plant is broken so that no water or solute could enter into the seed. Such type of low seed moisture content was recorded at physiological maturity was also reported by Kameswara Rao *et al.* (1991).

The deposition of storage substances is one of the key process of zygotic embryogenesis providing compounds that will be used from the early stages of the embryonic development until autotrophy, after germination (Merkle *et al.* 1995). Generally, mature seeds contain at least two or three stored reserves (Starch, Protein and Lipids) in appreciable quantities and to a larger extent they are generally synthesized simultaneously during seed development (Bewley & Black 1994). In *M. ferrea* the total sugar increased till 100 DAA and later decreased further which was coincided by a sharp increase in lipid content at 120, 140, 160 DAA. The soluble carbohydrates are an important component involved in desiccation tolerance during seed maturation and storage (Obendorf 1997). Whereas the present study recorded a decrease in soluble sugar content during maturation indicate the recalcitrant nature of *M. ferrea* seeds.

In the present study of *M. ferrea*, a higher rate of accumulation of lipids was noticed during the period where protein content recorded a decrease at 140 and 160 DAA. Murphy (1993) showed the relationship between storage protein and lipid accumulation and concluded that lipid related to mRNA found at an early stage of embryo development. In the present investigation lipid synthesis started during initial stages but a higher rate of accumulation was recorded at later stages.

Phenols are the aromatic compound with hydroxyl groups which found widely distributed in plant kingdom. At lower concentrations of phenolic compounds, induce defence mechanisms in plants, whereas at higher concentrations, they inhibit the germination of seeds (Noggle & Fritz 1991). In *M. ferrea*, during seed development approximately 100% increase in phenol content was recorded (Table 3). Contrary to our results, Renganayaki & Krishna Swamy (2001) could not show any marked variation between phenol content during seed development in Sunflower. Amino acids in the present study recorded an increase during development from 20 DAA to 160 DAA with an enormous increase of approximately 1400%. In *Pinus taeda* L. Silveira *et al.* (2004) showed a progressive increase of amino acid content from early stages to till cotyledonary stage in *Pinus taeda* This may be due to the role of amino acids for special functions in primary and secondary metabolism of developing seeds. Some amino acids are used as a nitrogen source, whereas others are used as precursors of secondary products (Coruzzi & Last 2000).

In general, the increase in dry matter during seed development in *Mesua ferrea* is a result of the synthesis and deposition of storage substances. Here the stored reserve material consists of 58.09% lipids, 18.29% starch, 10.30% protein, soluble sugar 8.5%, phenol 4.33% and amino acids 0.45%.

CONCLUSION

Leafing and flowering phenology of *Mesua ferrea* recorded difference in both the sites indicate that the rainfall, temperature and daylight had an influence in the leafing and flowering pattern and seed setting. The climate change had not effect in the fresh and dry biomass accumulation, primary metabolite accumulation and the maturity of the seeds during development in *M. ferrea*. The low seed yield, longer period required for seed maturity and the damage of seeds due to the infection are some of the major problems of loss in the number of plants and its distribution. In general, the increase in the dry matter during seed development in *M. ferrea* is a result of synthesis and deposition of storage substances. Here the stored reserve materials consist of 58.09% lipids, 18.29% starch and 10.30% soluble sugar.

ACKNOWLEDGEMENTS

Authors acknowledge Director, KSCSTE-MBGIPS for providing facilities and KSCSTE for funding the Emeritus Scientist Fellowship to PNK.

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