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Phenology of *Gymnacranthera canarica* (King) warb in the *Myristica* swamps of Southern Western Ghats

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Abstract

Gymnacranthera canarica is a dioecious habitat-specific species restricted to the critically endangered freshwater *Myristica* swamps of the Western Ghats, India. Phenology and phenotypic variations of this wild nutmeg fruits and seeds along with the morphology and moisture content of different developmental stages were documented. For phenology studies, individual trees were tagged and their vegetative and reproductive phases have been monitored from 2018 -2020. The leafing phenophase – simultaneous flushing and leaf fall before the onset of rain makes *G. canarica* trees appear to be always leafy. Leafing, flowering, and fruiting phenophases were significantly seasonal and fruiting phenophase occurs in the months of high relative humidity (April – September). Fruit maturation occurs at the end of August and fruit falls during the first two weeks of September. High synchrony index was found in flowering, fruiting, and leafing phenophases among the population. *G. canarica* fruits (17.88 ± 0.16g) are large and major portion is constituted by the the rind (13.62 ± 0.14g). Single seed weighs 4.18± 0.03g with the least weighing portion represented by that of the aril (0.63 ± 0.05g). There was a significant ($P<0.001$) variation among fruit and seed traits among all developmental stages of fruit. Highest coefficient of variation was recorded for aril weight (98%) being ranged 0.13 to 0.61g. However, there were significant ($P<0.001$) and strong positive correlations between fruit weight and rind weight ($r = 0.994$) and seed weight and seed length ($r = 0.964$). There was an overall loss in seed moisture content, dropped from 8.15% by 30 DAF to 28.41% by 180 DAF with 41% coefficient of variation. The linear multiple regression model analysis indicated that seed weight, length, and width had a considerable effect on seed moisture content.

Keywords: *Gymnacranthera canarica*, *Myristica* swamp, phenology, seasonality, seed morphology

Introduction

Phenology is defined as the study of the seasonal timing of life cycle events that determine the behaviour of tropical tree species. The timing, duration, and synchronicity of plant life cycles are the fundamental aspects of ecosystem functioning (Lieth 1974) [35]. In plants, it is related to germination, leaf flushing, leaf maturation, flowering, and fruiting. Plant biologists have started examining how these phenological patterns influence the reproductive success of tree species (Okullo *et al.* 2004) [40]. For better survival and reproduction capacity of plants, the seasonality of different phenophases are crucial and the seasonality of tropical tree phenology is mainly determined by the duration and intensity of seasonal drought (Cleland *et al.* 1995). The synchronies of different phenophases have various ecological consequences for individual survival, species fitness, and ecosystem stability (Scariot *et al*, 1991; Ims, R.A, 1990; lessard– Therrien *et al.* 2014) [48, 27, 34]. In evergreen tropical tree species, leaf flush and flowering occurs simultaneously on the same new shoot and vegetative phenology strongly influence the flowering period. Hence, flowering period indirectly depend upon the environmental periodicity (Brochert *et al.* 2002) [10]. Tropical trees show a short flowering period during a particular time of year, coinciding with leafing phenophases, and mostly occurs during a photosynthetically low active periods (Fenner 1998) [20]. The water logged conditions in *Myristica* swamp forests may influence the predominant tree species to be evergreen, showing a brief period of leaf fall so as leaf flushing and flowering occur during the early periods of the dry season.

Myristicaceae (nutmeg family) is considered as the most ancient primitive family with pantropical distribution, having 5 genera and 300 species in tropical Asia. Three genera are found within the Western Ghats of India, namely, *Gymnacranthera* (1 species), *Myristica* (5 species), and *Knema* (2 species) (Chetana and Ganesh 2013) [15]. Myristicaceae are characterized by small, unisexual flowers (Armstrong and Tucker 1986) [3]. Genus *Gymnacranthera* contains 7 species distributed in the Indo- Malayan region, *G. canarica* is only present in India and is endemic to the *Myristica* swamps of the southern Western Ghats. Indiscriminate and steady destruction of its natural habitats, compounded by selective felling of *G. canarica* in the past causes population decline in Western Ghats. Besides that, the narrow distribution of this tree species in Western Ghats has undergone various levels of anthropogenic disturbances and habitat disturbance (Prasad 1998) [43]. These disturbances negatively impact natural regeneration and evolutionary potential of the species by affecting the pollination success, fruit setting, and gene flow. Myristica swamps require effective conservation and management practices through large-scale restoration efforts. In most of the successful tree improvement efforts, proper seed source research causes vital significance. Success of a tree species in an ecosystem will largely depend on its ability to achieve reproductive and vegetative growth. However, knowledge about *G. canarica* phenology, seasonality of various phenophases, and fruit and seed development require more inputs to infer optimal photosynthetic productivity requisites of vegetative and reproductive phases. The phenological events, seasonality of different phenophases, synchronicity between vegetative and reproductive phenophases of *G. canarica* within *Myristica* swamps and phenotypic variations during fruit and seed development and were observed to answer: (1) To vegetative and reproductive phenohases shows synchrony within and between sexes? (2) does *G. canarica* possess strong seasonality in *Myristica* swamp forests? (3) does anthropogenic disturbances cause any phenological shift in *G. canarica*?

Materials and methods

Study system and species

G. canarica is a vulnerable endemic canopy tree species limited to fresh water *Myristica* swamp forests dominated by Myristicaceae members (Chandran and Mesta 2001) [13]. The best representatives of this habitat are found in the Sasthanada swamp of Sangili section, Kulathupuzha forest range (Latitude8˚ 45 N, longitude 77˚ 10 E, altitude 155 MSL). The area experiences a mean annual rainfall of 3.94 mm/day and a mean maximum temperature of 23.69˚C and minimum temperature of 27.29˚C (Figure 1).Swamps are confined as small patches less than 20 ha to valleys along streams flowing through evergreen, semi-evergreen forests (Chandran *et al*. 1999; Roby and Jose 2014) [14, 47]. Warm humid forest floor is covered by knee-like bended roots of *G. canarica*. Monocarpellate yellow flowers mature in to large berries, each with a seed enclosed in a bright reddish arils. Aspects of flowering and fruiting phenology, morphological, physiological, and biochemical changes in *G. canarica* seeds from development to maturation are documented as part of this study.

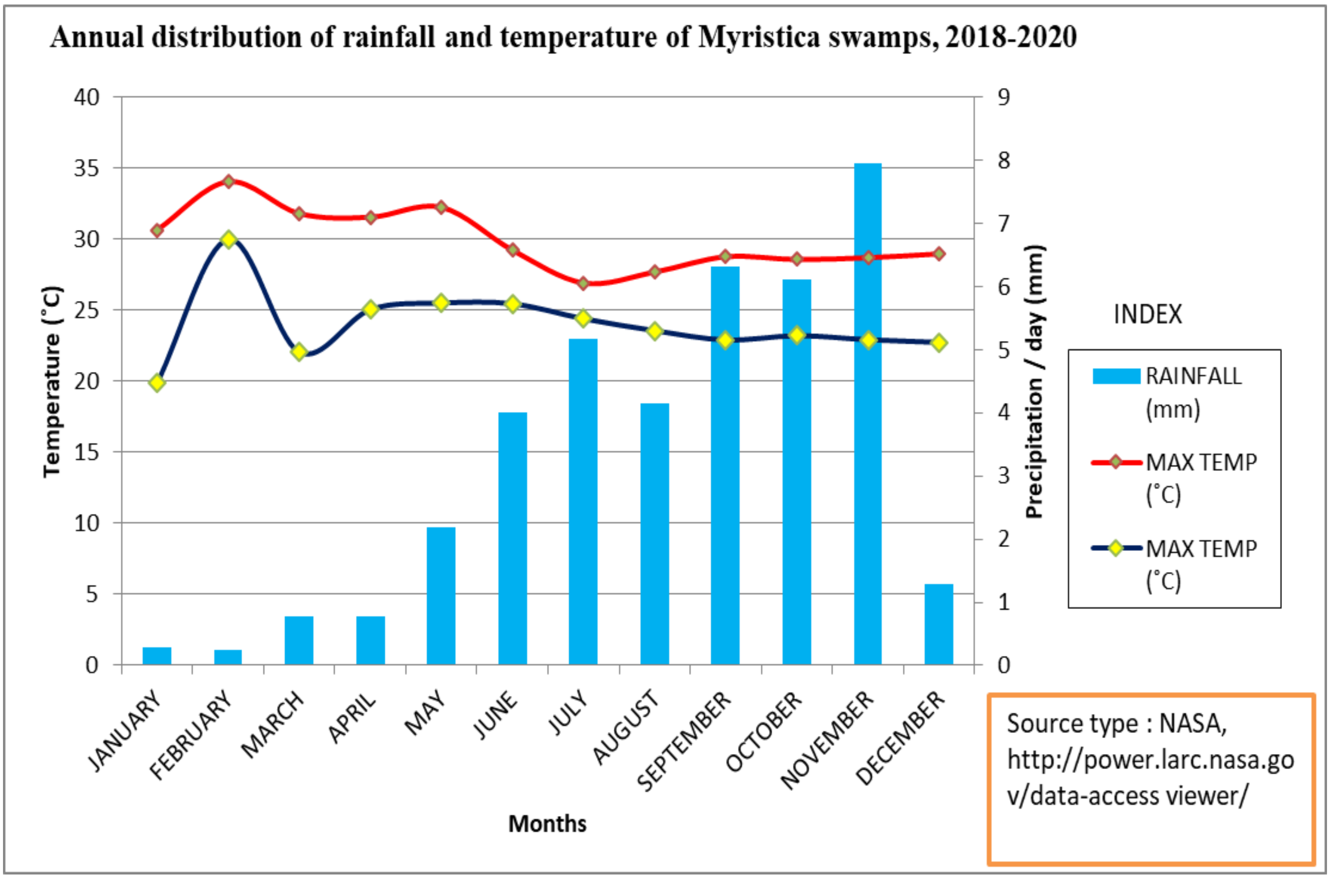

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Fig 1: Annual distribution of rainfall and temperature of *Myristica* swamps, 2018-2020.

Vegetative phenology, flowering phenology, and flower production

Seasonal observations during 2018 to 2020 on male and female leaf initiation (LI), leaf senescence (LS), peak flowering (PFL), peak fruiting (April – September), fruit set from fruit initiation to maturation and fruit/seed dehiscence and seed dispersal (September) in Sasthanada swamps (N= 10 males, N = 15 females) were by following the method by Fourier and Charpentier (1975) [22]; Opler *et al.* (1980) [41]. Augspurger's index of flowering synchrony (Z, where 0 = total asynchrony and 1 = total synchrony; Augspurger 1983) [4] was computed and compared to examine the differences in the degree of synchrony within and between the sexes. To estimate flower production, the number of flowers on branches (N= 50 branches) of male and female trees (N=5 each trees) were counted. Since accurate counting of male flowers on tall trees above 30m height was not easy from the forest floor, it was estimated by counting the number of flowers per inflorescence randomly near the forest floor. Since female trees produced fewer flowers, the number was counted from the forest floor. To determine whether there was a difference in flower production and other characters between sexes, Kruskal –wallis test (χ^2) (Zar 1999) [54] was adopted for the mean values among three-year study period.

Seasonality study

Seasonality was defined as the repeated occurrence of phenological events in a cyclic pattern. Different phenophases are found to be cyclic and to assess the strength of cyclicity in a given event, circular statistical analyses were conducted using the phenological variables and dates of observation on the software "STATISTIXL". To calculate the circular statistical parameters, months were converted to angles from 0˚ = January to 360˚ = December at intervals of 30˚. The day of observation in a given month was converted to angles. These angles in a given month in a given phenophase were estimated by Rayleigh's Z. test, which in turn indicates the seasonality. The Rayleigh's score was interpreted based on the significance of P value, so that if P value is significant, then the phenological event is cyclic (Zar 1999) [54].

Fruiting phenology, sex ratio and pollination success

Fruiting individuals (N=5) of the same (age) and girth class (< 10cm DBH) were marked and observed to record the production, maturation, ripening of fruits. The synchrony index for fruiting and fruitfall phenophases was calculated as the ratio between the individual's mean duration of a phenological phase and the overall duration of the phase (Devineau 1999) [18]. Sex ratio was expressed as the proportion of female flowers (number of female/ total number of male + female flowers) per tree. Pollination success was calculated by estimating fruit set (flower: fruit ratio). At the end of the flowering and fruiting period, immature and mature fruits were counted per branch for an approximate number of fruits per tree. The mean number of female flowers, immature fruit number, mature fruit number, percentage of natural fruit set per tree, and premature fruit fall for three years as well as Spearman correlation (correlation coefficient, r) showing the relationship between fruit set of female trees and number of female flowers, sex ratio, and fruit fall for three years (2018 -2020) study period were also calculated.

Variation in morphometric traits during fruit and seed development.

G. canarica fruit and seed development has been divided into 6 consecutive stages (April – September): 30, 60, 90,120, 145, and 160 days after fertilization (DAF). A complete randomized design with a sample size of 30 fresh fruits after immediate harvesting during each developmental stage was used to determine the fruit weight, seed weight, fruit length, fruit diameter, seed length and seed diameter. Developing fruit mass and seed weight were measured by using an electronic weight balance. Length (from bottom to top) and width (the middle of fruit and seed) of the fruits and seeds were measured by using the digital vernier calliper. Morphometric traits measurement during each developmental stage were analyzed by using SPSS Duncan's multiple range test and the amount of variation during fruit and seed development by analyzing the coefficient of variance(%). Pearson's correlation coefficient was used to determine the relationship among fruit traits and seed traits during each developmental stage.

Moisture content analysis.

During each stage of development, the moisture content (MC) of whole fruit, fruit portion (rind and aril), and seed (N =10) was determined gravimetrically in samples using the low constant hot air oven method at 103 ± 2˚C, according to ISTA recommendation for oily seeds. A multiple linear regression was used to develop the relationship between seed MC as well as the seed length, seed breadth, and with fruit MC, fruit length, and fruit width. Test was conducted to check whether the seed MC can be predicted from the above mentioned variables. Pearson's correlation coefficient was used to determine the relation between fruit MC and stage of development, seed MC, rind MC, and aril MC.

Statistical analysis

The data were analyzed by SPSS (Version 23.0; IBM Corp., Armonk, NY, USA) and EXCEL (Version 2013; Microsoft Corp., Redmond, WA, USA) software. Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). Circular statistical procedures were analysed using "STATISTIXL" and excel software. Pearson's correlation coefficient was calculated to determine the relationship between the fruits traits and seed traits during each developmental stage. Simple non-parametric correlations were performed for both corresponding phenophases and phenological events. Multiple linear regressions were used to bring out the most influencing variable on seed moisture content.

Results

Vegetative phenology, flowering phenology, and flower production

G. canarica sets out for bloom at the onset of leaf fall in winter and both male and female trees experience a concomitant leaf transition states -leaf initiation (LI), leaf senescence (LS) (Table 1). Flowering in *G. canarica* extends for a short period of time with high intensity. Peak flowering in males was between February to March with more than 80% in bloom, male trees have a prolonged flowering period than female trees (March) (Kruskal – Wallis test, χ^2= 14.394, df = 1, P <0.001). Male flowers are borne in larger clusters of 10 -14 in an axillary panicle inflorescences, while female flowers occur solitary or in small clusters of 3 -5 in axillary racemes. Female flowers are larger than male flowers. The mean number of male flowers per tree was significantly higher than the number of female flowers per tree. Total number of female flowers rounded to the nearest integer ranged from 21493 to 22127 with a mean value of 22127.58 (± 634.8) within a flowering season (Table 2). The total number of male flowers (44039.91 ± 357.1) produced by male trees was two times greater than female trees. Male and female flowers opened at night with strong aroma and longevity (72-96 h), higher in female flowers than male flowers (48-72 h). Corolla tube depth and diameter were slightly larger in female flowers compared to male flowers (table 2). Mean significant differences did not see in corolla tube diameter and aperture diameter between sexes. (Kruskal –Wallis test, χ^2 =0.37, df =1, P = 0.54 for corolla tube diameter) and (Kruskal –Wallis test, χ^2 =0.48, df =1, P = 0.48 for aperture diameter). Augspurger index of flowering synchrony (Z) ranged from 0.92 to 1 in males and 0.78 to 0.82 in females for three years and showed significant differences between the sexes (Kruskal –Wallis test, χ^2 =17.43, df =1, P = 0.00).

Table 1: Phenological events of *G. canarica* in Sasthanada swamp.

Male Trees												
Population	2018				2019				2020			
	LI	LFI	PFL	PFR	LI	LFI	PFL	PFR	LI	LFI	PFL	PFR
Sasthanada swamp	March – April	Jan - Feb	Feb - March	-	March - May	January	Feb - March	-	March - April	January	Feb - March	-
Female Trees												
Population	2018				2019				2020			
	LI	LFI	PFL	PFR	LI	LFI	PFL	PFR	LI	LFI	PFL	PFR
Sasthanada swamp	March - April	Jan- March	March	April - September	March - April	Jan- March	March	April - September	March - April	Jan - Apil	March	April – September

LI – leaf initiation, LFI –leaf fall initiation, PFL – peak flowering, PFR- Peak fruiting

Table 2: characteristics of male and female *G. canarica* flowers and flower production.

Characters	Male trees	Female trees	Statistics, χ^2
Inflorescence	Axillary paniculate inflorescence	Axillary racemes	
Number of inflorescence /branch	16.66 ± 2.38	8.66 ± 2.57	16.49
No of flower / inflorescence (initial)	271 ± 08	82.75 ± 12.10	17.31
Number of flower/ inflorescence(mature)	208.5 ± 5.41	39.00 ± 5.59	17.34
Number of flower / tree	44039.91 ± 357.1	22127.58 ± 634.8	45.84
Mean length of flower (mm)	6.341 ± 0.069	8.25 ± 0.23	17.363
Mean width of flower (mm)	4.393 ± 0.068	6.17 ± 0.08	12.01
Mean pedicel length (mm)	1.192 ± 0.136	1.57 ± 0.024	14.699
Mean corolla depth	5.29 ± 1.52	6.86 ± 0.08	17.363
Mean corolla diameter	7.038 ± 0.20	6.97 ± 0.02	0.371
Aperture diameter	4.25 ± 0.07	3.95 ± 0.07	0.48
Mean flowering synchrony	0.94 ± 0.02	0.80 ± 0.02	17.43
Anthesis	At night	At night	
Longevity(h)	24-48	48-72	
Flowering months	February – March	March	
Flowering duration (mo)	2	1	

Differences in male and female floral traits are presented with Chi-square statistics derived from Kruskal–Wallis test (asterisk indicates statistical significance). Z denotes the index of synchrony ranging from 0 to 1 (Augspurger 1983) [4] for complete asynchrony to complete synchrony.

Fig 2: A. Female branches showing axillary raceme inflorescence with leaf flushing, **B**. branches with axillary panicle inflorescence

Seasonality of *G. canarica* male and female trees in *Myristica* swamps

Vegetative and reproductive phases of *G. canarica* male and female trees in *Myristica* swamp habitat presented strong seasonality, Rayleigh's Z values are highly significant (Table 3). Leafing pattern, flowering pattern, fruiting pattern, and seed germination pattern are indicated by the mean angle. Leaf initiation occurs at the first of March (March 10 in males, March 8 in females), leaf senescence happens at the end of January (January 25) in males and the middle of February (February 14) in females. Peak flowering coincides with leaf initiation in both males (March 5) and females (March 2) and peak fruiting in female trees occurs in April (April 2). The strength of seasonality measured by the vector "r" indicates that in male trees, peak flowering (0.99) has strong seasonality followed by leaf initiation (0.78) and leaf senescence (0.59) and in female trees peak flowering and peak fruiting has strong seasonality (0.99) followed by leaf initiation (0.79), leaf senescence (0.66) and seed germination (0.35) among the three year study. Nevertheless, the seasonality test showed that the rhythm of seed germination was not very seasonal in the swamp area.

Table 3 circular statistical analysis testing for the occurrence of seasonality in different phenophases of *G. canarica* male and female trees in Sasthanada *Myristica* swamps.

Male Trees			
Parameters	**Leaf initiation (LI)**	**Leaf senescence (LS)**	**Peak flowering (PFL)**
Mean angle	70.71	25.26	67.61
Mean data	March 10	January 25	March 7
Mean vector r	0.78	0.59	0.99
Angular SD	6.45	6.40	4.38
Rayleigh's Z	41.37	20.88	56.84
P value	$<0.000^{*}$	$<0.000^{*}$	$<0.000^{*}$
N	68	60	58

Female Trees					
Parameters	**Leaf initiation (LI)**	**Leaf senescence (LS)**	**Peak flowering (PFL)**	**Peak fruiting (PFR)**	**Seed germination (SG)**
Mean angle	68.88	44.36	62.50	92.72	328.77
Mean data	March 8	February 14	March 2	April 2	November 28
Mean vector r	0.79	0.66	0.99	0.99	0.35
Angular SD	5.18	15.47	2.08	2.39	6.27
Rayleigh's Z	34.32	49.65	29.40	172.49	4.165
P value	$<0.000^{*}$	$<0.000^{*}$	$<0.000^{*}$	$<0.000^{*}$	$<0.000^{*}$
N	55	114	30	176	34

Significance at <0.05 level

Fruiting synchrony indices, sex ratio, and pollination success

Devineau synchrony indices for fruiting and fruit fall phenophases did not differ considerably within the species for three years (2018 -2020). Fruiting phenophase in 2020 shows the high synchrony index (0.92) and least synchrony index (0.86) seen in fruit fall phenophase for the 2019 year (Table 4). Higher synchrony ratio indicates greater coincidence of the phase among individuals. Mean percent of fruit set was 59.106 (±10.10) in *Myristica* swamp study area and little variation was seen in the percent of fruit set between years among the fixed population (Kruskal –Wallis test, χ^2 =5.18, df =2, P = 0.07). Fruit set on female trees was negatively correlated with sex ratio of the swamp (Spearman rank correlation test, r = - 0.61, P = 0.01) and strongly correlated with fruit fall percentage (Spearman rank correlation test, r = 0.90, P = 0.017) (Table 5).

Table 4: synchrony indices for fruiting and fruit fall phenological events, sex ratios (M:F), mean percent fruit sets, and mean percent fruit fall in *Myristica* swamp site for three years.

Year	**Synchrony index**		**Sex ratio**	**Fruit set**	**Fruit fall**
	Fruiting phenophase	**Fruit fall phenophase**			
2018	0.91	0.88	0.34 ± 0.02	57.20 ± 9.97	41.87 ±9.55
2019	0.89	0.86	0.32 ± 0.01	67.05 ± 7.97	46.80 ±4.630
2020	0.92	0.90	0.35 ± 0.004	53.05 ± 8.08	38.15 ±7.04

Values are the mean of three annual cycles, synchrony indices ranging from 0 to 1 (Devineau, 1999) [18] for complete asynchrony to complete synchrony.

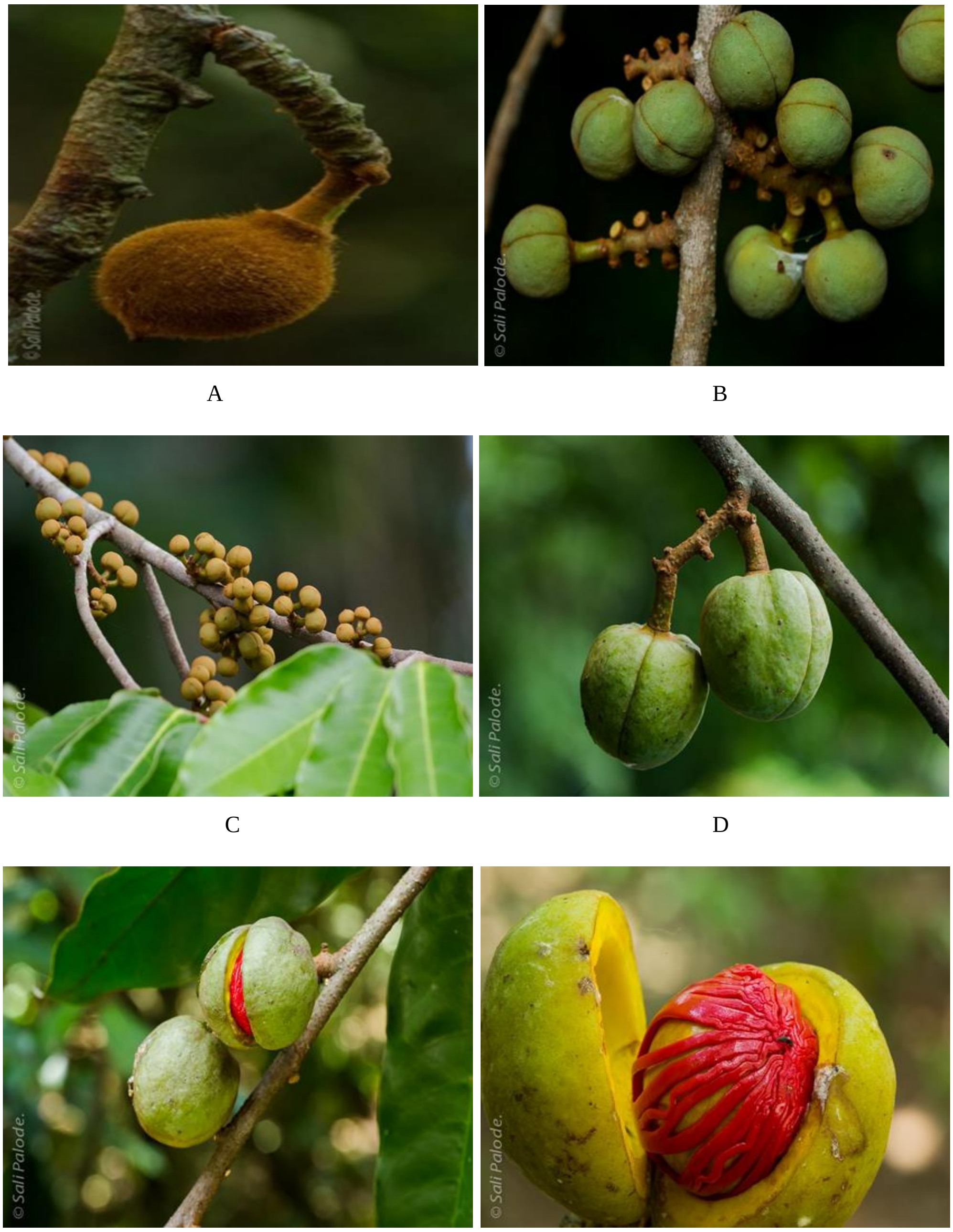
Table 5 Spearman correlations (correlation coefficient, r) showing the relationship between fruit set of female trees and number of female flowers, sex ratio, and fruit fall for three years (2018 -2020).

	Female flowers		**Sex ratio**		**Fruit fall**	
Fruit set	2018	r_s = 0.10 *p =0.95*	2018	r_s = -0.70 *p =0.23*	2018	r_s = 1.0 *p =0.01*
	2019	r_s = -0.30 *p* = 0.68	2019	r_s = 0.54 *p =0.35*	2019	r_s = 0.80 *p =0.13*
	2020	r_s = 0.30 *p =0.68*	2020	r_s = 0.30 *p =0.65*	2020	r_s = 0.90 *p =0.03*

Note: Values in bold are considered as significant.

Fruiting phenology

G. canarica has a large capsules, globose fruit (weight = 4.18 ± 0.03, length = 19.27 ± 0.64, and width = 32.11±0.20, n =30) dehisced longitudinally while still attached to the tree exposing deep bright red lacinated aril (Fig. 3F), that covers a single large globose, pale brown coloured seed (weight = 4.18 ± 0.03, length = 19.27± 0.64 and width = 21.07± 0.077). Fruiting phenology showed a steady pattern in *G. canarica* among three years (2018 -2020) from April to September with six months duration. Fruit maturation duration did not vary among the three years (Kruskal – Wallis test, χ^2 =1.51, df =2, P = 0.48) and only 45 – 50% of individuals fruited over 6 months with high intensity fruiting. The number of fruits produced per panicle at premature stage ranged from 6 – 10 (rounded to the nearest integer) and with a mean value 8.4 ± 2.0 (Fig. 3A) declined to 2.13 ± 0.51(Fig. 3B) at maturity and showed considerable percent of fruit fall in accordance with natural fruit set in female trees (Fig. 3E). The mean number of total fruits produced by a tree is 3564 ± 884.89 (N=15, 2018-2020). *G. canarica* fruits are single seeded and the major portion of the fruit constitute rind (13.62g) and the least portion is aril (0.63g) (Table 6).



A B

C D

E F

Fig 3: Six different developmental stages of *G. canarica* fruit.

Table 6 Characteristics of fruit and fruit production

Characters	Observation
Fruiting months	April – September
Fruiting duration (mo)	6
Mean number of fruits/panicle (premature)	8.4 ± 2.02
Mean number of fruits/panicle (mature)	2.13 ± 0.51
Mean total number of fruits/ tree	3564 ± 884.89
Number of ovules	1
Number of seed per fruit	1
Fruit weight (g)	17.88 ± 0.16
Fruit length (mm)	31.66 ± 0.26
Fruit width (mm)	32.11 ±0.20
Fruit thickness (mm)	30.91 ±0.12
Seed weight (g)	4.18 ±0.034
Aril weight (g)	0.63 ± 0.04
Rind weight (g)	13.62 ± 0.15

Values are the mean of three annual cycles (2018 -2020)

Morphometric variation in fruit and seed traits

There was significant ($P<0.001$) difference among all developmental stages in fruit and seed traits. Results indicated that the fruit weight ranged from 6.16 to 17.88g with coefficient of variation 34% (Table 7). Seed weight ranged from 0.38 to 4.18g with coefficient of variation 52%. The highest coefficient of variation was recorded for aril weight (98%) ranging from 0.13 to 0.61g. Fruit length ranged from 10.44 to 31.66 mm with coefficient of variation 38%, seed length ranged from 7.4 to 22.47 mm with coefficient of variation 37%. The results showed that fruit breadth ranged from 10.28 to 32.1 mm and seed breadth from 5.72 to 20.44 mm showed 43% and 42% coefficient of variation, respectively.

Table 7: phenotypic variation in fruit and seed traits characteristics among different developmental stages.

Stages of development (DAF)	Fruit weight(g)	Seed weight (g)	Aril weight (g)	Rind weight(g)	Fruit length (mm)	Fruit width (mm)	Seed length (mm)	Seed breadth (mm)
30 DAF	6.16 ± 0.21^{a}	0.38 ± 0.03^{a}	0.00 ± 0.00^{a}	5.78 ± 0.20^{a}	10.44 ± 0.16^{a}	10.28 ± 0.28^{a}	7.44 ± 0.16^{a}	5.72 ± 0.18^{a}
60 DAF	10.00 ± 0.05^{b}	1.75 ± 0.06^{b}	0.00 ± 0.00^{a}	8.25 ± 0.08^{b}	14.63 ± 0.45^{b}	13.03 ± 0.17^{b}	10.78 ± 0.17^{b}	9.10 ± 0.24^{b}
90 DAF	13.89 ± 0.43^{c}	3.12 ± 0.06^{c}	0.13 ± 0.008^{b}	10.63 ± 0.41^{c}	19.11 ± 0.05^{c}	17.60 ± 0.44^{c}	18.54 ± 0.19^{c}	17.07 ± 0.20^{c}
120 DAF	16.49 ± 0.31^{d}	3.58 ± 0.08^{d}	0.36 ± 0.007^{c}	12.54 ± 0.28^{d}	25.36 ± 0.62^{d}	28.98 ± 0.26^{d}	19.27 ± 0.64^{c}	19.09 ± 0.26^{d}
145 DAF	17.05 ± 0.05^{d}	3.99 ± 0.05^{e}	0.50 ± 0.003^{d}	12.85 ± 0.08^{d}	28.36 ± 0.27^{e}	30.21 ± 0.23^{e}	21.76 ± 0.25^{d}	20.91 ± 1.01^{e}
160 DAF	17.88 ± 0.16^{e}	4.18 ± 0.03^{f}	0.61 ± 0.005^{e}	13.08 ± 0.14^{d}	31.66 ± 0.26^{f}	32.11 ± 0.20^{f}	22.47 ± 0.53^{d}	20.44 ± 0.077^{e}
CV (%)	34	52	98	28	38	43	37	42

Note: mean values are followed by standard errors; ^{a,b,c,d,e} means with different superscripts within a column significantly differ ($P < 0.001$).

Pearson's correlation coefficient of fruit and seed traits

There was significant ($P<0.01$) correlation between fruit and seed traits during the whole process of fruit and seed development. Fruit weight shows a significant positive correlation between rind weight, fruit length, seed length, seed breadth, fruit breadth, and aril weight. Strong correlation seen between fruit weight and rind weight (r = 0.994). Seed weight shows correlation with seed length (r = 0.964), followed by seed breadth, rind weight, fruit length, fruit breadth, and aril weight (Table 8).

Table 8: Relationship between fruit and seed characters in *G. canarica* in *Myristica* swamps

	Seed weight	Aril weight	Rind weight	Fruit length	Fruit breadth	Seed length	Seed breadth
Fruit weight	0.979^{**}	0.883^{**}	0.994^{**}	0.946^{**}	0.927^{**}	0.946^{**}	0.943^{**}
Seed weight		0.868^{**}	0.952^{**}	0.931^{**}	0.911^{**}	0.964^{**}	0.953^{**}
Aril weight			0.857^{**}	0.965^{**}	0.969^{**}	0.868^{**}	0.856^{**}
Rind weight				0.926^{**}	0.906^{**}	0.919^{**}	0.920^{**}
Fruit length					0.969^{**}	0.925^{**}	0.910^{**}
Fruit breadth						0.901^{**}	0.900^{**}
Seed length							0.956^{**}

Correlation is significant at the 0.01 level (2 tailed)

Moisture content analysis during different developmental stages

There was an overall loss in seed moisture which dropped from 81.51% at 30 DAF to 28.41% at 180 DAF with 41% coefficient of variation (Fig. 4). Fruit poss high moisture content of 93.87% at the first developmental stage (30 DAF), declined to 41.26% at 160 DAF with coefficient of variation 28%, rind poss 80.17% at 30 DAF increased to 88.56% at 180 DAF with least coefficient of variation 12%. In *G. canarica* seeds, aril development occurs after 60 DAF and MC shows an increase from 50.18% (30 DAF) to 53.78% (160 DAF) with the highest coefficient of variation 77%. The linear multiple regression model of seed MC including seed weight, seed length, and seed width explained 97.3% of the variation ($R^{2} = 0.976$, $P < 0.000$, $d = 2.004$) and for seed MC with fruit MC and fruit weight shows 95% of variance ($R^{2} = 0.95$, $P < 0.000$, $d = 1.77$). The Durbin – Watson "d" value is between two critical values $1.5<d<2.5$, with no first-order linear autocorrelation in our multiple linear regression data.

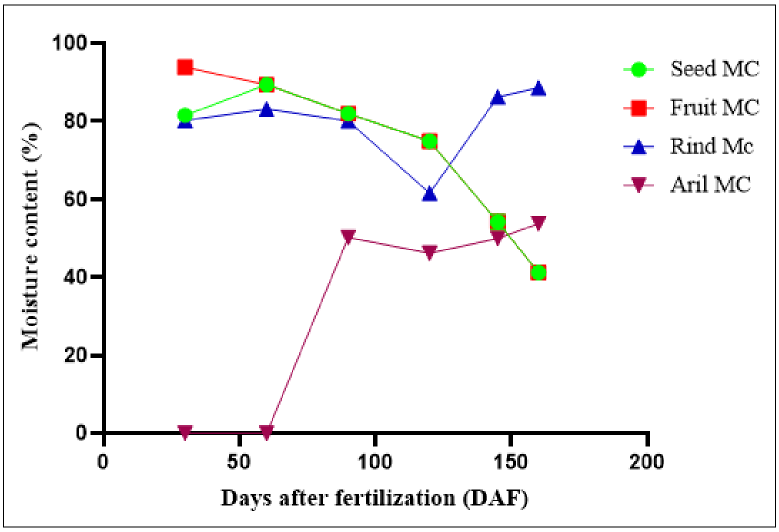


Fig 4: Graph showing the variation in moisture content of fruit, seed, rind and aril during the different developmental stages are expressed in percentage on a fresh weight basis.

Discussion

Phenological studies are important to understand plant growth patterns, plant responses to various environmental, biotic, and abiotic factors (Reich 1995) [45]. The leafing, flowering, and fruiting phenophases do not show any considerable annual variation in *G. canarica*. Leaf initiation begins from March to April (Summer) like the dry period initiation of leaves observed in several forest types of India

(Kushwaha and Singh 2005) [33] as well as in other tropical forest sites (Borchert 1994) [9]. Synchronization between leaf senescence and flushing makes trees evergreen. Production of leaves during the early dry summer on energy grounds seems to be the worst possible time for leaf expansion (Duke *et al.* 1984) [19]. The basic knowledge on reproductive phenology is not only essential for evolutionary and systematic studies (Bernardello 1999) [7] but also important for effective conservation strategies (Holsinger *et al*. 1991). In *G. canarica*, flower initiation was observed simultaneously with leaf flush during the dry season. Almost simultaneous emergence of *G. canarica* flower buds as well as leaves was also been reported by Sundarapandian *et al.* 2005 [51]. Dry season flowering was reported in *Butea monosperma, Melia duba,* and *Bombax malabaricum* from dry forests both in India and elsewhere in the tropics (Nanda, 2009; Nomura *et al*. 2003) [38, 39]. Similarly, in rain forests, dry season flowering was also reported (Hamann A 2004) [24]. Leaf senescence peaks in January in *G. canarica* and similar observations could be seen in most canopy trees. The rate of leaf fall during the dry season was correlated with the decline in soil moisture content and increased water stress of the tree (Reich and Borchert 1982) [46]. The floral characters of *G. canarica* are more generally like other members of the *Myristicaceae* family. Flowers are small, numerous in small clusters with yellow coloured perianth with small lobes that are not showy but are highly fragrant (Manju and Armstrong 2013). Anthesis begins nocturnally similar to *Myristica inspida* and *Myristica fragrans,* and male flowers remain functional for 24-48 hrs while female flowers remain receptive for 48-72 hrs (Armstrong and Irvine 1989). In *G. canarica*, flowering shows greater synchrony among individuals in the *Myristica* swamp forests and between male and female trees. Similar synchrony was also reported in *Myristica fatua,* another dominating *Myristicacean* tree species of *Myristica* swamp forests (Krishna and Somanathan 2018). *G. canarica* fruits mature by September and the maximum number of female flowers became fruits (67%), which is an indication of proactive synchrony. Augspurger (1982) [4] reported the positive effects of synchronous flowering in *Hybanthus prunifolicus,* that bestowed greater fruiting success even in less synchronous individuals irrespective to the phenotypic plasticity Vegetative and reproductive phenophase of *G. canarica* were found to be significantly seasonal at *Myristica* swamps. The strength of seasonality measured by the vector “r” indicated that in *G. canarica,* peak flowering has strong seasonality followed by leaf initiation in male trees and peak flowering, peak fruiting followed by leaf initiation in female trees. Leaf senescence showed the least seasonality as it is an evergreen tree. Reproductive phenophase remained more synchronized compared to vegetative phenophases. Similar pattern was observed in Sarawak (Harrison *et al.* 2000) [25], but relatively high synchrony was reported with vegetative phase in dry forests of Coorg (Patel A 1997) [42].

Fruit setting starts in dry summer (April) that mature with the onset of rain (June – August) and subsequent fruit fall in September was also been reported (Sundarapandian *et al.*, 2005; Borah and Devi 2014) [51, 8]. Fruit set in *G. canarica* was higher and was greater than 50% in *Myristica* swamps compared with the general trend for other allied swamp species which have an average fruit set < 15% (Tremblay *et al*. 2005) [52]. Smaller number of pollen grains was sufficient for the fruit set in this single ovuled tree species of *Myristicaceae* (Armstrong 1997) [1]. High synchrony index of fruit flush and fruit fall ranging from 0.92 to 0.88, indicating that dry season flowering showed strong synchrony in reproductive phenophase as also were reported by Mishra *et. al.* (2006) [32]. It was also observed that the more number of fruits per tree results in the low investment per seed (seed weight = 4.18 ± 0.03). This observation is inconsistent with *Myristica fatua*, another swamp specialist *Myristicacean* species (Krishna and Somanathan 2018) [32]. Fruit set is not related to sex ratio in *G. canarica,* as observed in *Aralia nudicaulis* (Barrett and Thomson 1982) [5] and Jacartia dolichaula (Bullock and Bawa 1981) [11]. *G. canarica* has globose fruits and seeds similar to the other species of *Myristica* genus although differ in size, shape, and aril color. *G. canarica* has a red aril, the most common colour of nutmeg arils (Kitamura and Poonswad 2013) [31] associated with bird dispersal (Manju and Armstrong 2013). Bird dispersal of diverse nutmegs has been observed by Davlantes and Howe, 2018. Fruits of *G. canarica* dehisce on the tree upon complete maturity, similar to *Myristica inspida* where fruits first dehisce from a tree presenting the arillated seed, further dropped passively or dispersed by frugivores (Armstrong *et al*. 2013).

In this study, phenotypic variations of *G. canarica* fruits were observed at different developmental stages. Seeds took 160 days to attain physiological maturity. In this study, distinct stages of seed development were identified based on the morphological characters. The colour of the developing seeds was initially creamish white (30 - 60 DAF) that became yellowish green (90 DAF) to pale brown in colour (160 DAF). Similar change in seed has also been observed in *Pongamia pinnata* seeds (Rao *et al.* 2011). Aril development occurs after 60 DAF and the initially the white coloured thin aril turns to pale pink followed by pinkish red to dark red as in other Myristica species (Kitamura and Poonswad 2013) [31]. Length, diameter, and weight of fruits and seeds increased across the developmental stages of *G. canarica* and the observations revealed substantial variation of fruit traits (Fruit weight, seed weight, aril weight, rind weight, fruit length, fruit breadth, seed length and seed width) Similar observations were documented in *Madhuca latifolia* seed development (Chandra and Keshavkant 2016). As far as the best and lowest means between developmental stages are concerned, the following variations of high magnitude were documented, *i.e.* : Aril weight 98%, seed weight 52%, fruit width 43%, seed width 42%, fruit length 38%, seed length 37%, fruit weight 34%, and rind weight 28%. The largest component of fruit weight was constituted by that of the rind weight 13.08g (73%). Pericarp (rind) is the outermost part of nutmeg fruit which is thick and fleshy (Mini *et al.* 2017) which has an astringent taste with aromatic flavour as in *Myristica fragrans.*

A negative relationship among seed moisture content and developmental stage could be seen, a high water content of 0.81g H_2O/ g fresh weight was recorded in 30 DAF seeds, which was tended to decline significantly with developmental stage and reached water content of 0.28 g H_2O/g fresh weight at 160 DAF. During the initial stages of seed development, high water content is crucial for maintaining metabolic activity and adequate seed growth (Westgate and Grant 1989) [53]. When the seed gets fully mature, the loss of moisture content is most probably due to the accumulation of reserves which displaces water from the storage cells (Kermode 1990) [30]. *G. canarica* seeds registered 28% moisture content at the time of shedding from the mother plant which is a typical Myristicacean character seen in *Endocomia, Knema* and *Myristica* species (Mathew *et al.* 2016) [36]. Relative high water content of seeds at the time of shedding indicated the desiccation sensitivity of the seeds (Berjak *et. al*. 1992) [6].

Despite the threats of G. canarica within the Myristica swamps, this species has reported a higher degrees of phenological synchrony and seasonality within the population. Such synchrony may be an evolutionary strategy of the species to ensure a sustainable competitive advantage for an optimal reproductive phase. *G. canarica* faces no barriers in the phenophase or reproductive cycles that hinder reproduction in a swampy habitat, which would otherwise prompt natural selection to enhance genetic diversity. The species vulnerability due to habitat loss may overcome with the present genetic setup, provided no more loss of habitat occurs. Therefore also no stochastic or manmade meddling that are detrimental which in turn demand proportionate cross pollination to check gene erosion.

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