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Research Article

Germination, Growth and Biomass Accumulation as Influenced by Seed Size in *Mesua ferrea* L.

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Abstract: *Mesua ferrea* L. was evaluated for its germination, seedling growth and biomass across four seed size classes. The production of two- and three-seeded fruits was high. The viability of the seeds was 58-81%. Germination was positively correlated with seed weight. Heavier seeds showed early and rapid germination. The contribution of leaves to total biomass yield was 27-60% in 1-month old seedlings. Carbon content was also related with plant length and weight in the seedlings. The study concludes that the variations in seed size have a substantial influence on growth and biomass accumulation in *Mesua ferrea*. Such variation also helps in the physiology and regeneration of the tree species.

Key Words: Biomass, Germination, *Mesua ferrea*, Seed weight, Tropical rain forest

Introduction

Variation in seedling fitness may be caused by differences in initial seed mass, microsite characteristics and/or genotypic variations. Tripathi and Khan (1990) argued that a larger reserve in seeds may allow for the better pre-photosynthetic growth of seedlings, and this in turn may contribute to the better growth and survival of seedlings that emerge from heavy seeds. Several reports emphasise that differences in the early juvenile stage could be an important determinant of the relative success of individuals in later phases of the life cycle (e.g. Harper, 1977; Roach, 1987). Forget (1991) reported that there is a continuum of regeneration strategies and niches for forest tree species. Osunkoya et al. (1994) indicated that the ability of plants to establish and grow in these niches depends on species-specific attributes and extrinsic factors. On the whole, studies on the ecological adaptation and growth characteristics of trees, particularly in humid tropical forests, are rather few and far between (e.g. Ashton & Larson, 1996; Singh & Khan, 1998). Such studies are important for the conservation and management of moist tropical forest ecosystems, which are in rich in plant diversity.

Mesua ferrea L. (Assamese – "nahor"; Hindi – "nagkesar"), commonly known as the iron wood tree, is a medium-sized, shade loving, evergreen climax tree species found in the tropical rain forests of north-east India, Western Ghats and Andamans in India. The tree is cultivated in gardens and avenues for its flowers and foliage, which are attractive, particularly in the young stages. Outside India, it is present in Bangladesh, Myanmar (Burma) and Sri Lanka. The trees generally produce fertile seeds at the age of 15-20 years, though their germination is rather slow and poor. The fruits of *Mesua* L. produce from one to four seeds, and such a variation is expected to have an effect on its germination capacity. Our personal observation is that the seedling population of "nahor" in Arunachal forests is quite low, despite the high seed output, indicating that only a small portion of the seed population is converted into seedlings. It is hypothesised that the larger the seed, the greater would the seedling biomass accumulation be during regeneration. In view of the above facts, the present "net-house" experiment was designed to test the hypothesis and to study species-specific attributes such as seed size, and carbon economy and allocation of assimilates, and to assess the influence of seed weight on the germination, growth and biomass accumulation of *Mesua ferrea* seedlings.

Materials and methods

Study site

The study was carried out in a tropical rain forest belt in the North-Eastern Regional Institute of Science and Technology (NERIST) campus (lat 27°07'N, long 93°22'E, altitude 126 m asl) in Arunachal Pradesh, India. The regional climate is cool (16 °C) and dry (relative humidity = 54%) in the winter (November-February) and warm (36 °C) and wet (relative humidity = 80-98%) in the summer (March-October), with mean annual precipitation (1800 mm) distributed fairly evenly throughout the year.

Seed production

Three plots (20 m x 20 m) located within an area of 2 km^2 were selected in a tropical forest belt in the NERIST campus to study the density of *M. ferrea* trees in the natural environment. The density of the trees was 10 -15 plants ha^{-1}. Ten "nahor" trees were selected randomly in each plot. The average height and diameter at breast height (DBH) was 8.3 m and 15.0 cm in plot I, 7.9 m and 12.2 cm in plot II and 8.7 m and 14.5 cm in plot III. The fruits were collected from even-aged trees. The appearance of a brown colour on the fruit coat was taken as an indication of maturity. Then, the seeds were separated. The fruits of each of the trees contained varying numbers of seeds (one to four) indicating a mono- to tetra-locular ovary in this species and they were therefore classified into four different classes viz., SW1, SW2, SW3 and SW4, that represented seeds from one-seeded to four-seeded fruits, respectively. The production of the SW3 and SW2 categories was greater than those of the others. The number of seeds in each category in all three plots was counted and then pooled category-wise, irrespective of the study plots. Some seeds were immediately tested for viability using 0.1% 2,3,5-triphenyl-tetrazolium chloride, and the rest were stored separately in sterile polythene bags at room temperature (minimum 25 °C, maximum 35 °C) for further use.

Experimental design in nursery

The experiment was designed to identify the differences in the germination and early growth of *Mesua ferrea* across the seed size classes. The measurements determined were (a) germination, (b) seedling height and number of leaves, and (c) biomass allocation to above- (stem, leaves) and below-ground (roots) portions. For this study, 30 seeds from each category were pretreated by soaking them in cold water for 24 h and then they were sowed individually at 5 cm depth in garden soils (Total nitrogen = 0.33%, Organic matter = 3.70%, pH = 5.2). The pots used were 30 cm in diameter and 28 cm deep, to avoid constraining the root system. The experiment was unifactorial, consisting of four categories of seeds having different seed weights with 30 replicates making a total of 120 pots. Seeds were sown on 5 October 1998 and each pot was supplied with 250 ml of tap water every alternate day to moisten the soil. The experiment was conducted in a green house wherein on average the temperature was a maximum 28 °C and minimum 20 °C, relative humidity was ca. 60% and light intensity was 24,300 lux.

Germination and seedling growth

Seedling emergence was observed after 30 days. Data were collected every 15 days over a period of 60 days from the date of sowing. Weeding was done manually and periodically. The reproductive capacity (in numbers) of each of the seed size classes was calculated using the formula: (number of seed output per tree x % germination)/100. The growth performance of recruited seedlings was assessed in terms of their height (cm), leaf number and leaf area (cm^2). At the end of the experiment (i.e. 60 days) all the seedlings were carefully uprooted and sliced using a sharp blade in the root and shoot portions and measured for their lengths. Leaf area measurements were made using a portable leaf area meter (LiCOR). For biomass measurements, the intact root system of the harvested seedlings was washed with water to remove attached soil particles. The soil from each of the pots was also sieved (mesh size 0.5 mm), and any remaining root material was recovered, washed and included with the appropriate sample. To determine the dry matter content, the root and shoot samples were oven-dried at 80 °C for 48 h. In order to determine whether the amount of carbon reserves varies with seed weight, the ash content was determined by combusting seeds in silica crucibles in a muffle furnace at 550 °C for 6 h, with 50% of the ash free mass being regarded as carbon content (Allen et al. 1974).

Statistical analysis

Data were analysed with one-way ANOVA. Tukey's test was used to compare the mean values across four seed size classes. Linear regression was calculated to determine the relationships between seed weight and seed germination and seedling growth parameters (Zar, 1974).

Results

Seed production

There was a close association between the seed production ($tree^{-1}$) and basal area of the tree (Table 1). All the trees produced fruits containing one to four seeds, but in a varied proportion. Nevertheless, the three-seeded (SW3) fruits occurred in the greatest proportion (*ca.* 43-47%), followed by the two-seeded (SW2) fruits (22-29%). The production of one-(SW1) and four-seeded (SW4) fruits was less (6-21%, Table 1).

Viability, seed weight, ash and carbon content

The percentage viability of *M. ferrea* seeds decreased from 81% in SW1 to 58% in SW4. Distinct differences were observed in the seed mass between the four seed size classes (Table 2). The ash content was significantly ($P < 0.05$) greater (3.02%) in SW1, followed by SW4 and SW2, while it was lowest in SW3. Carbon content did not vary significantly between the four seed weight classes.

Table 1. Average DBH, height and basal area and total seed production of *Mesua ferrea*.

Parameters	Plot no. 1	Plot no. 2	Plot no. 3
Tree characteristics			
DBH (cm)	15.00	12.20	14.50
Height (m)	8.30	7.90	8.70
Basal area (cm^2)	176.78	116.80	165.00
Seed production			
Total no. of seeds	98	87	95
Number of seed samples in each size class of the three plots			
SW1	15	18	12
	(15.30)	(20.69)	(12.63)
SW2	28	25	21
	(28.57)	(28.74)	(22.11)
SW3	42	39	45
	(42.86)	(44.83)	(47.37)
SW4	13	5	17
	(13.27)	(5.74)	(17.89)

Values in parentheses are the % of each fraction to total seed production.

Table 2. Germination, reproductive capacity, viability and ash content of seeds of different size classes.

Seed weight class	Fresh weight range (g)	Viability (%)	Reproductive capacity (in numbers)	Germination (%)	Ash content (%)
SW1	5.20-8.40	81	9.99	66.66	3.02
SW2	4.40-5.15	72	14.80	60.00	1.08
SW3	2 .70-4.39	68	19.59	46.66	0.31
SW4	1.50-2.50	58	3.89	33.33	2.15

Seed germination and reproductive capacity

Germination was highly correlated ($P < 0.01$) with seed fresh weight across all observation dates ($r = 0.843$-0.964). Seeds belonging to the SW1 category showed faster and better rates of germination (Table 3). At the end of the study, the order of % germination was SW1 > SW2 > SW3 > SW4. Differences in seed germination due to seed weights and number of seeds produced per fruit were highly significant ($P < 0.01$). Germination lasted for 60 days, beyond which no significant increase in seed germination could be observed. Exceptionally, in the SW3 and SW4 categories the seed germination rate was approximately 47 and 33% respectively, both on the 45th and 60th days of observation from the date of sowing (Table 3). The reproductive capacity (number per $tree^{-1}$) of *M. ferrea* was highest (19.6) in SW3 and lowest (3.9) in SW4.

Table 3. Germination and shoot growth in different seed size classes of *Mesua ferrea*.

Seed weight classes	Time (day)		
	30th day	45th day	60th day
Germination (%)			
SW1	53.33^a	60.00^b	66.66^c
SW2	33.33^a	46.66^b	60.00^c
SW3	40.00^a	46.66^b	46.66^b
SW4	26.66^a	33.33^b	33.33^c
Shoot height (cm)			
SW1	7.22^a	9.97^b	24.01^c
SW2	6.71^a	9.57^b	19.86^c
SW3	3.86^a	5.93^b	16.73^c
SW4	4.30^a	9.70^b	24.53^c

Note: The day was counted from the date of sowing.
Values having similar superscripts across each row are not significantly different at $P < 0.05$.

Seedling growth, root/shoot ratio and leaf area

In this shade-house experiment, all recruited seedlings survived at least up to 30 days from the date of germination. Measurement of shoot length shows that seedling growth was initially slow up to 15 days, and then accelerated by *ca.* 58% in the next 15 days. Such fluctuations could be observed in all seedlings irrespective of the seed weight classes (Table 3). Root length on the 60th day after germination was 12.7, 13.5, 10.4 and 14.8 cm in the seedlings recruited from the SW1, SW2, SW3 and SW4 categories, respectively, while shoot length was 24.0, 19.9, 16.7 and 24.5 cm (Table 3). There was no definite trend in plant length with respect to seed fresh weight. However, seeds with a lower dry mass (SW2 and SW3) produced seedlings with lower height growth, whereas those of higher dry mass (SW1 and SW4) produced seedlings with greater shoot growth (Table 3). The plant (root + shoot) length was highest in SW4, followed by SW1, SW2 and then SW3. The variation between seed weight and plant length was significant ($P < 0.05$). The root/shoot length ratio varied between 0.53 and 0.68 (Table 4).

The total number of leaves averaged 3, except in SW3 and SW2 where it was 2.4 and 2.7 respectively. The surface area of the first leaf at the end of the experiment was 28 cm^2 in SW1 and SW4, while it was 23 and 26 cm^2 in SW2 and SW3, respectively. The dry weight of leaf components of plants remained more or less the same, *ca.* 0.2 g. The contribution of the leaf dry mass to the total dry matter content of a 1-month old seedling was 27% in SW1 and SW4, 39% in SW2 and 60% in SW3 (Table 4).

Table 4. Plant length and root-shoot ratio and leaf attributes in *Mesua ferrea* seedlings

	Seed weight classes			
Parameters	SW1	SW2	SW3	SW4
Plant length (cm)				
Root	12.70^a	13.53^b	10.40^c	14.83^d
Shoot	24.01^a	19.86^b	16.73^c	24.53^a
Total	36.71^a	33.37^b	27.13^c	39.36^d
Root-shoot ratio				
Length basis	0.53^a	0.68^b	0.62^b	0.60^c
Dry weight basis	0.43^a	0.46^b	0.73^c	0.37^d
Leaf attributes				
No. of leaves	3.00^a	2.71^b	2.43^b	3.00^a
Leaf area (cm^2)	27.50^a	22.75^b	25.50^c	28.00^a
Dry weight	0.21^a	0.24^a	0.22^a	0.20^a
% of total dry weight	27.30^a	38.54^b	60.27^c	26.53^a

Note: Values across each row with different superscripts are significantly different at $P < 0.05$

Biomass and carbon content

Dry matter yield in seedlings was also significantly affected by seed weight. The dry weights of the seedlings (both root and shoot) declined from SW1 to SW3 and increased sharply in SW4 (Table 5). The variations in total plant weight between seed weight classes were significant ($P < 0.05$). The variations in the ash content of the roots were significant ($P < 0.05$). In the case of shoots, the ash content (%) was significantly ($P < 0.05$) lower in SW2. The dry matter yield (g seedling^{-1}) was 0.78 in SW1, 0.62 in SW2, 0.37 in SW3 and 0.75 in SW4. A similar trend was observed in ash free mass (0.36-0.75 g seedling^{-1}) and carbon content (0.18-0.37 g seedling^{-1}), and the yield order was SW1 > SW4 > SW2 > SW3 (Table 5). There was a strong positive relationship between seedling dry weight and plant length and carbon content in this study along the seed weight gradient ($r = 0.984$-0.999, $P < 0.001$). Seed weight was positively correlated ($P < 0.01$) with seedling length ($r = 0.723$), biomass ($r = 0.729$) and carbon content ($r = 0.731$).

Table 5. Biomass and carbon content of seeds and seedlings (shoot and root portions) of *Mesua ferrea* 60 days after germination.

Parameters	Seed weight class			
	SW1	SW2	SW3	SW4
Seed mass (g)				
Fresh weight	6.08^a	4.72^b	3.52^c	2.49^d
Dry weight	2.17^a	2.64^b	2.77^b	1.83^c
Ash free mass	2.17^a	2.43^a	2.76^b	1.82^a
Carbon	1.08^a	1.21^a	1.38^a	0.91^a
Seedling shoot mass (g)				
Dry weight	0.55^a	0.43^a	0.25^b	0.55^a
Ash free mass	0.53^a	0.42^a	0.24^c	0.53^a
Carbon	0.27^a	0.21^b	0.12^c	0.27^a
Ash content (%)	3.63^a	2.33^b	4.00^c	3.63^a
Seedling root mass (g)				
Dry weight	0.23^a	0.19^b	0.12^c	0.20^a
Ash free mass	0.22^a	0.18^b	0.11^c	0.19^a
Carbon	0.11^a	0.09^a	0.06^b	0.09^a
Ash content (%)	4.35^a	5.26^b	8.33^c	5.00^b

Note: Values with similar superscripts across each row are not significantly different at $P < 0.05$.

Discussion

The seed germination rate was highest during the initial 30 days in SW1, which indicates that heavier seeds (presumably having larger food reserves) germinated better and faster. A similar observation was made by Tripathi and Khan (1990) while working with the subtropical forest tree species *Quercus dealbata* L. and *Quercus griffithii*, Hook. f. & Thomson ex Miq. and this was attributed to the larger food reserves in these seeds. Greater plant length and dry mass in the SW1 seedlings is attributed to larger carbon reserves that confer a competitive reproductive advantage on plants. In addition, it leads to the hypothetical conclusion that the ecological fitness of the "nahor" species under study is linked to greater maternal carry-over effects. In another sense, the following could be viewed as some of the indicators of the increased tolerance of *M. ferrea* seedlings: (i) possession of large seeds with sufficient resources for initial seedling establishment; (ii) relatively greater allocation of vegetative biomass in favour of leaf components; and (iii) low root/shoot ratio (Harmer, 1995). This is important as *M. ferrea* grows in the middle-storey of tropical rain forests where photosynthetic active radiation would be low.

All seed size classes produced seedlings with root/shoot ratios < 1. This in general indicates that *M. ferrea* had a greater proportion of dry mass allocated to the shoots. This is in concordance with the observations of Ramakrishnan et al. (1982) that late successional and/or climax species allocate more biomass to the shoot system. The proportionately greater shoot mass in SW3 would, perhaps, make the recruited seedlings more tolerant of soil drought and/or nutrient deficiency. However, to come to any sort of conclusion in this regard, evaluation studies on such physiological and morphological advantages are needed. The parameters to examine could be water-use and nutrient-use efficiency in environments that provide high and low amounts of photosynthetic photon flux (PPF) density.

Though there are distinct growth and allocation differences among the different seed size classes, two broad groups can be recognised: SW1 and SW4 (group 1) and SW2 and SW3 (group 2). The lack of a difference might also suggest that *M. ferrea* has no intermediate ecological status between these two groups. For example, group 2 showed a germination pattern over 30 days similar to that of group 1. Nevertheless, our study demonstrated that although there is considerable morphological overlap among the seedlings derived from the four seed size classes, the SW1 and SW4 categories had specific growth characteristics that allowed them to establish and grow better than SW2 and SW3. Thus, morphological patterns in the seed germination and regeneration establishment of these four seed size classes provide only a partial explanation of their coexistence.

Incidentally, whenever the environment consists mostly of favourable habitat, a parent does its best to produce only small offspring, and if the conditions are reversed, the production of larger offspring could be more advantageous (McGinley et al., 1987). However, the greater availability of safe microsites may render seed size differences of lower adaptational significance. In the case of *M. ferrea*, the observed differences in seed germination, seedling survival and growth due to seed size indicate that *M. ferrea* seeds of different sizes vary considerably in their safe-microsite requirements, which may ensure the successful colonisation of a mosaic of habitats within the forest ecosystems where they are dispersed under *in situ* conditions.

Conclusions

This study concludes the following: (a) the number of seeds produced by a single *M. ferrea* tree was positively related to its basal area; (b) the "nahor" tree produced more three- and/or two-seeded fruits than one- or four-seeded fruits; (c) the seed germination percentage showed a declining trend from the SW1 category to the SW4 category; (d) improved seed germination, and survival and growth of seedlings in SW1 and SW4 indicates that sufficient energy is contained in the seeds to promote the emergence of seedlings and their ability to sustain themselues until they grow high enough to support themselves photosynthetically, and (e) there was a positive relationship between seed mass and seedling dry matter and carbon contents. In general, the following may be considered the regeneration strategy of *M. ferrea*: (i) the production of fruits with one to four seeds vis-a-vis the occurrence of seeds of different weights showing variations in food reserves and energy content, and (ii) the differential response of these seeds and the seedlings produced by them in terms of germination and biomass allocation.

The data on the seed and seedling ecology of *M. ferrea* presented in this paper may be helpful in evolving strategies for improving the regeneration of the species *in situ* and in nursery conditions that would prove significant both in a forestry as well as in a horticultural sense, as the natural regeneration of this species through seedlings is rather poor in this region. The poor seed germination (33-66%) observed has implications for the tree's function in particular and forest management in general. The relatively higher production of three- and two-seeded fruits that had a moderate germination rate, and the greater contribution of leaves to total biomass yield, suggests that these seeds may regenerate successfully under closed canopy, and could well be an adaptive strategy of the "nahor". In addition, the species distribution is patchy in forest ecosystems and that could mainly be due to the low viability of seeds produced in larger numbers. In contrast, the single-seed category, which was less productive, had good viability and gave forth seedlings with better growth and yield.

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